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TESIS DOCTORAL

Silicon Photonics Mach-Zehnder Biosensor with Coherent Detection for Point-of-Care Devices

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CERTIFICAN:

Que D. Jonas Leuermann, Ingeniero en Tecnología Electrónica, Informática y de la Comunicación, ha realizado en el Centro Andaluz de Nanomedicina y Biotecnología (BIONAND), y en el marco del programa Marie Skłodowska-Curie "NanoMedPhd", ambos adscritos a la Universidad de Málaga, el trabajo de investigación correspondiente a su TESIS DOCTORAL, titulada:

Silicon Photonics Mach-Zehnder Biosensor with Coherent Detection for Point-of-Care Devices

En este trabajo se describe la implementación de un sistema de biosensado fotónico basado en un interferómetro Mach-Zehnder integrado con lectura coherente con aplicaciones al diagnóstico en el punto de atención. Para ello se realizó un análisis teórico del límite de detección del sistema en función de las diferentes fuentes de ruido. Este análisis se confirmó parcialmente en experimentos que alcanzaron límites de detección de estado-del-arte. Además, se demostró la capacidad de biosensado con la detección de la proteína C-reactiva, alcanzando también límites de detección excepcionales. Finalmente se realizó una prueba de concepto orientada al diagnóstico de la alergia a la amoxicilina mediante la detección de los anticuerpos IgG e IgE correspondientes.

El trabajo es completamente original, y ha dado lugar a la publicación de tres artículos en revistas de impacto, que avalan de manera exclusiva esta tesis. Por todo ello, consideran que esta Tesis es apta para su presentación al tribunal que ha de juzgarla. Y para que conste a efectos de lo establecido en el Real Decreto 99/2011, de 28 de enero, por el que se regulan las enseñanzas oficiales de doctorado, AUTORIZAN la presentación de esta Tesis en la Universidad de Málaga.

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Realizada bajo la tutorización de ÍÑIGO MOLINA FERNÁNDEZ y dirección de ROBERT HALIR (si tuviera varios directores deberá hacer constar el nombre de todos)

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Silicon Photonics Mach-Zehnder Biosensor with Coherent Detection for Point-of-Care Devices

Jonas Leuermann



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*“Wer will was Lebendigs erkennen und beschreiben,
sucht erst den Geist herauszutreiben,
dann hat er die Teile in seiner Hand,
fehlt, leider, nur das geistige Band.
Encheiresin naturae nennt’s die Chemie,
spottet ihrer selbst und weiß nicht wie.”*

Faust - Der Tragödie erster Teil, Johann Wolfgang von Goethe



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Jonas Leuermann
Málaga - Spain, April 2021

Acronyms

ACRP	Anti-CRP antibody.
ADC	Analogue-Digital-Converter.
APTES	(3-aminopropyl)-triethoxysilane.
APTMS	(3-aminopropyl)-trimethoxysilane.
AWGN	Additive White Gaussian Noise.
BMI	Bimodal Interferometer.
BS3	bis(sulfosuccinimidyl)suberate.
BSA	Bovine Serum Albumin.
CA	Contact Angle.
CBS	Casein Blocking Solution.
CCD	Charge Coupled Device.
CMOS	Complementary Metal-Oxide-Semiconductor.
CMZI	Coherently Read Mach-Zehnder Interferometer.
CRP	C-Reactive Protein.
CSNR	Complex-Signal-to-Noise-Ratio.
DAQ	Data Acquisition.
DendAmox	Amoxicillin Haptenised Dendrimer.
DSP	Digital Signal Processing.
ECL	External Cavity Laser.
EDFA	Erbium Doped Fibre Amplifier.
ELISA	Enzyme-Linked Immunosorbent Assay.
ETA	Ethanolamine.
FA	Fibre Array.
FITC	Fluorescein Isothiocyanate.
FPL	Fabry-Perot Laser.
FSR	Free-Spectral Range.
GUI	Graphical User Interface.
HOL	Handheld Fibre Test Optical Light Source.
HRI	Homogeneous Refractive Index.
IC	Integrated Circuits.
LFA	Lateral Flow Assay.
LOC	Lab-on-Chip.
LOD	Limit of Detection.
LPN	Laser Phase Noise.
LSPR	Localised Surface Plasmon Resonance.

MMI	Multi-Mode Interference Coupler.
MN	Mechanical Noise.
MZI	Mach-Zehnder Interferometer.
PAMAM	Poly(amidoamine).
PBS	Phosphate Buffer Saline.
PCR	Polymerase Chain Reaction.
PD	Photodiode.
PDF	Probability Density Function.
PDMS	Polydimethylsiloxane.
PEG	Polyethylene Glycol.
PIC	Photonic Integrated Circuit.
POC	Point-of-Care.
PSD	Power Spectral Density.
RIA	Radioimmunoassay.
RIN	Relative Intensity Noise.
ROI	Region Of Interest.
SEM	Scanning Electron Microscope.
SG	Signal Generator.
SMF	Single-Mode Fibre.
SMZI	Spectral Mach-Zehnder Interferometer.
SNR	Signal-to-Noise-Ratio.
SOI	Silicon-on-Insulator.
SPR	Surface Plasmon Resonance.
SWG	Sub-Wavelength Grating.
TE	Transversal Electrical.
TIA	Transimpedance Amplifier.
TIR	Total Internal Reflection.
TM	Transversal Magnetical.
VOA	Variable Optical Attenuator.
WSL	WSL-100.
XPS	X-Ray Photoelectron Spectroscopy.

Nomenclature

Mathematical Number Sets

$\mathbb{N}$	Natural numbers without zero
$\mathbb{N}_0$	Natural numbers with zero
$\mathbb{Z}$	Whole Numbers
$\mathbb{R}$	Real Numbers
$\mathbb{C}$	Complex numbers

Mathematical Constants

π	Pi	3.1415926535...
e	Euler's number	2.7182818284...
j	Imaginary unit	$\sqrt{-1}$

Mathematical Definitions

a, A	Scalar
$\vec{a}, \vec{A}$	Vetor
$\mathbf{a}, \mathbf{A}$	Matrix
$\vec{A} \cdot \vec{B}$	Scalarproduct of vector $\vec{A}$ and $\vec{B}$
$\vec{A} \times \vec{B}$	Crossproduct of vector $\vec{A}$ and $\vec{B}$
$\nabla \cdot \vec{A}$	Divergence of vector $\vec{A}$
$\nabla \times \vec{A}$	Rotation of vector $\vec{A}$
∇^2	Laplace operator performed on vector $\vec{A}$ in Cartesian coordinates

Physical Constants

c	Speed of light in a vacuum inertial system	$\approx 299\,792\,458 \text{ m s}^{-1}$
h	Plank constant	$\approx 6.62607 \times 10^{-34} \text{ J s}$
ε_0	Permittivity constant	$\approx 8.854 \cdot 10^{-12} \text{ F m}^{-1}$
μ_0	Permeability constant	$4\pi \cdot 10^{-7} \text{ H m}^{-1}$

Other Symbols

$\vec{E}, \vec{H}$	Electric and magnetic field	$V\ m^{-1}, A\ m^{-1}$
n	Refractive index	RIU
n_{eff}	Effective refractive index	eRIU
φ, ψ	Phase shift	rad
λ	Wavelength	m
f	Frequency	Hz
ω	Angular frequency	rad Hz
L	Length of a waveguide	m
S_{WG}	Waveguide sensitivity	eRIU/RIU
S	Sensitivity	rad/RIU, rad/(ng/mL)
σ	Noise	V or rad
LOD	Limit of detection or detection limit	RIU or ng mL ⁻¹
SNR	Signal-to-noise-ratio	dBc
cSNR	Real-valued signal-to-noise-ratio of a complex signal	dBc
α	Propagation/absorption losses	Np cm ⁻¹ or dB cm ⁻¹
IL_{oss}	Insertion losses	dB
P	Optical power	W
η	Coupling efficiency	dB
G	Gain of the transimpedance amplifier	V A ⁻¹
R_{sp}	Photodiode responsivity	A W ⁻¹
NEP	Noise equivalent optical power	W/ $\sqrt{\text{Hz}}$
ϑ_{un}	Undersampling noise floor amplification factor	
ϱ	Voltage or current spectral density	V/ $\sqrt{\text{Hz}}$ or A/ $\sqrt{\text{Hz}}$
PSD	Power spectral density	V ² Hz ⁻¹
V_{DAQ}	Data acquisition voltage range	V
B	Bandwidth of a digital low-pass filter	Hz
i	Current	A
v	Voltage	V
I	In-Phase signal (real part of a complex signal)	V or A

Q	Quadrature-Phase signal (imaginary part of a complex signal)	V or A
T	Time period or temperature	s or °C
F	Flow velocity	$\mu\text{L min}^{-1}$



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Acronyms

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1. Introduction

A biosensor is a “compact analytical device or unit incorporating a biological or biologically derived sensitive recognition element integrated or associated with a physio-chemical transducer” [1]. This definition already indicates that a discussion of biosensors is a complex topic as it combines two different research fields: biochemistry and engineering. This thesis aims to develop an optical biosensing system based on silicon photonics chips for nanodiagnostics. Thus, this work will involve both integrated photonics and biochemistry, both of which are highly diverse fields on their own. In an attempt to make this manuscript more accessible to audiences from both fields, this chapter introduces some key concepts on both the integrated optics and the biochemistry involved in diagnostics in Sections 1.1 and 1.2. The general operation of label-free biosensors will be described in Section 1.3 with an assembly and performance overview for optical biosensors in Section 1.4. Different optical evanescent field biosensors will be reviewed in Section 1.5, and a final description of the goals and contributions of this thesis will be outlined in Section 1.6.

1.1. Silicon Photonics

In 1841, Jean-Daniel Colladon demonstrated that light could be trapped in a stream of water, guided and redirected by total internal reflection (TIR) [2]. More than a hundred years later, the field of photonics was born, using dielectric arrangements, for example an optical fibre, to guide light via TIR over a large range of distances, from millimetres up to thousands of kilometres, with telecommunications as one of its main fields of application. For the 21st century photonics is expected to be a key enabling technology, contributing to the economy with a similar impact as the development of the microelectronic industry in the 20th century [3].

The complementary metal-oxide-semiconductor (CMOS) fabrication technology, established by the microelectronic industry in the early sixties, enabled the production of miniaturised electrical integrated circuits (IC). Over the following decades more and more highly integrated digital and analogue circuits could be fabricated on-chip inexpensively on a large scale, leading to an exponential growth of computational power, resulting in the technology platforms which serve and uphold our modern society. The material that underlies this technological revolution is one of the most common minerals on earth: silicon.

Both silicon and its native oxide, silicon dioxide (quartz), are transparent in the near-infrared, and the geometries required for guiding light are, in principle, compatible with CMOS fabrication techniques, giving rise to the field of silicon photonics, which studies the integration of light sources, waveguides, and detectors based on silicon. The high index contrast between the waveguide core material (silicon) and the cladding (silicon oxide) allows redirecting the guided light with tight curvatures radii, enabling high device density, so that many different functionalities can be integrated on a single chip. The possibility of integrating photonic and electronic functionalities together makes this technology platform very attractive, especially in the field of telecommunications. Other applications such as spectroscopy [4] and biosensing [5] are other emerging fields which demonstrated excellent performance using photonic integrated circuits (PICs), some of them using other CMOS compatible materials such as silicon nitride [6]. Indeed, silicon photonics is a promising candidate for biosensing applications and is expected to experience a rapid economic growth within the next decade [7].

1.2. Nanomedicine Diagnostics

As far back as mankind can remember diseases have accompanied human history and evolution. Some Diseases are caused by pathogens. Pathogens, a term hard to define, generally refers to microorganisms which infect and corrupt its host with a temporary or life-long illness, causing anything between mild repercussions and death. Those microorganisms can be bacteria, viruses, protozoan, or even fungi, to cite a few [8]. However, pathogens are not the only thread in this sense: other sources of diseases can be parasites which are larger organisms infecting its host, toxins, or genetic malfunction. Even the hosts immune system itself can affect its well-being, a situation which is often referred to as autoimmune disease [9]. Its not until recently in human history, that pathogens, parasites and others could be identified as the origin of diseases, using tools such as optical microscopes. It is thanks to the inventive and scientific spirit of human kind that technology was, and still is, developed which enabled the first medical diagnostics.

1.2.1. General Overview

Diagnosis is the first step towards a comprehensively successful disease treatment and therapeutics. Without the knowledge and information-gathering diagnostic tools, therapeutics would be groping in the dark and may even add more complications. Treatment of illness would rely only on flawed human experience. In this context, nanomedicine, an even more recent development in human history, applies nanometre-sized tools to gather understanding and knowledge, diagnose and treat illnesses with the final goal of improving the patient's quality of life [10]. Nanotechnology based diagnostic tools became, and still are, an important part of our society and already improved the well-being of countless individuals. Although those tools apply those very small structures, normally in the range of nano- to micrometres, they are normally installed in a comparatively large apparatus which is typically necessary to support the detection and interpretation of the gained information.

Nowadays many medical diagnostics are predominantly performed and analysed in especially equipped laboratories. There, often expensive bulky equipment and/or complicated biochemical procedures are used and applied by specially trained personnel. Long waiting times, already beginning from the moment a sample is taken and arrives at the corresponding laboratory, typically accompany the diagnostic process. Depending on the amount of requests the laboratory must handle, additional delays and postponements may apply apart from the time the actual diagnostic procedure requires. The latter is commonly determined by the nature of applied diagnostic procedure. Most rely on the detection of a so called biomarker in a biological body fluid, for example blood, serum or urine [11]. For diagnostics, biomarkers are disease specific, validated and confirmed, indicators, which, if detected, provide insight to a distinct pathogen, parasite, or toxin affecting the patient, depending on the illness causing origin [12]. A biomarker can be a high diversity of biomolecules, such as antigens, antibodies, DNA, mRNA, and enzymes [13]. Ideally, it is already present in a detectable concentrations at an early stage of the illness, making a fast diagnosis feasible for accurate therapeutic decisions [14]. Body fluids are normally highly complex samples, containing large amounts of various proteins which could interfere with specific detection process if not handled properly.

1.2.2. Common Diagnostic Techniques

Enzyme-Linked Immunosorbent Assay A common example for a biomarker based detection procedure is the enzyme-linked immunosorbent assay (ELISA). ELISA is an indirect label-based analyte recognition scheme, because the presence of the target biomarker, the analyte, is not directly, but rather in connection with the label. There are different types of ELISA, such as direct-, competitive-, indirect-, indirect competitive-, and sandwich ELISAs, each of

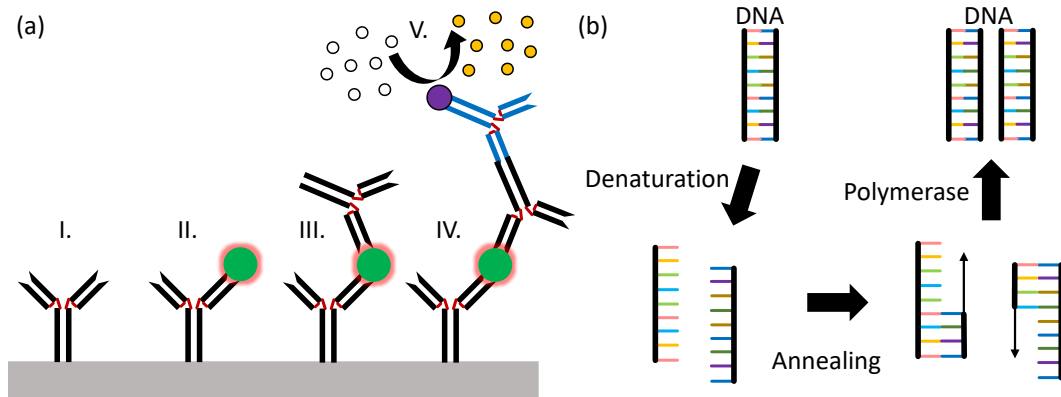


Figure 1.1.: (a) Different steps of a sandwich ELISA. (b) The basic repetitive steps for the PCR.

which has advantage and disadvantageous [15]. The typical process of an exemplary sandwich ELISA [16] is shown in Fig. 1.1(a). A capture antibody, which specifically recognises the target analyte, is immobilised on the surface of a vile (I.), for example a microtiter plate. Next, the sample of the patient is placed on the surface, enabling the antibody recognition layer to “capture” the analyte from the sample (II.), if present, via affinity binding to the corresponding antigen. A washing steps removes the rest of “not-captured” sample. An antibody, affine to the already recognised antigen, is brought in attaching to the same (III.), if present. From this the name sandwich ELISA derives, the target analyte encapsulated between two antibody layers. Another antibody is then introduced which specifically recognises the prior launched antibody step (IV.). It is important for the consecutive detection process that the antibody contains a label, for the case of ELISA an enzymatic label, covalently linked to it [17]. The role of the label can be better understood explaining the detection process (V.): another washing step removes the unbound enzyme-labelled antibodies, and by subsequently adding enzyme-specific chemical to the surface leads to very particular chemical reaction. Depending on the enzyme, this reaction can for example result in a change of colour. The intensity of the colour alteration relates the amount of analyte recognised by the surface. ELISA is a very sensitive (in fact the sandwich ELISA is the most sensitive ELISA up to $2 \rightarrow 5$ times higher than the other ELISA types), but time intensive method, requires several steps until the analyte related signal can be detected [18] and is a more qualitative detection approach. Other similar popular methods are the radioimmunoassay (RIA) and fluorescent immunoassay (e.g. ImmunoCAP), applying radioactive- and fluorescent labels, respectively.

Polymerase Chain Reaction Another widespread nanodiagnostic tool for a DNA-biomarker-based detection is the polymerase chain reaction (PCR) amplification method, illustrated schematically in Fig. 1.1(b). Since its establishment in 1983 [19], it has been widely used for diagnostic purposes. The target, from which only trace amounts are required, can be any specific DNA fragment from a variety of different samples, e.g. blood, skin, hair, saliva, or microorganism to name a few [20]. The basic principle is the repeated replication, including the duplicates, of a the DNA fragment. Necessary for the entire procedure are the target fragment, primers, nucleotides, and DNA polymerase. All steps are typically temperature driven, and the precise regulation is crucial for a successful performance. First, the DNA fragment is denatured, that means the two complementary DNA strands are separated from each other. In the second step, called annealing or hybridisation, the primer binds to a specific region of the single strand target DNA fragment. Necessarily, there is a second primer, targeting the complementary strand. After that the DNA polymerase starts to extend the bonded primer along the DNA strand using the surrounding nucleotides until the entire DNA fragment is reproduced,

i.e. the two DNA strands are complemented, and the amount of target fragments increased. By repeating this cycle the amount of target DNA fragments increases exponentially, given sufficient amount of primers, nucleotides, and polymerase. Of course, the amount of DNA fragment replication will saturate once the necessary resources are not present in the required quantity. Typically tens of cycles are necessary to reach a sufficiently high DNA fragment concentration [21]. There are various types of PCR tests, such as quantitative real-time PCR (qRT-PCR), reverse-transcriptase PCR, multiplex PCR, or nested PCR, just naming a few. In a qRT-PCR the amount of replicas can be quantitatively monitored over time. Adding a fluorescent dye label to the nucleotides or primers enables the detection of the target DNA fragments during the process (real-time), besides the elimination of post PCR DNA fragment detection.

1.2.3. Point-of-Care

Normally, both ELISAs and PCRs are very sensitive, specific and can be designed to have a high-throughput. The problem with most of the aforementioned methods is the time they require, the level of knowledge and experience the personnel needs to have to perform the techniques, a controlled environment, and most of them only offer qualitative or semi-quantitative results. The time is a critical issue in this matter, mainly due to the latency by sending the sample to be analysed to the specially equipped laboratory. Fast diagnostic based decisions may be hindered because of this slow infrastructure. The accurate realisation of the methods requires special training and most likely a certain degree of experience to suppress common mistakes during the procedures which otherwise may result in false-positives. Many diagnostic devices require a controlled environment, preparation of the sample and the label, and often a bulky read-out. Precise quantitative results could help the medical technicians performing a more accurate diagnosis, for more data-based driven decisions regarding therapeutic treatment. Additionally, the price for each test is not very cheap. Most of those issues are targeted by so called point-of-care (POC) devices. The idea is to have a device which can be used directly for primary care, i.e. diagnostic at the patients site with a fast (a few minutes), light but still robust, label-free and specific, plug and play, quantitative result giving device, and cheap as defined by the World Health Organization [22]. The epidemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) starting at the beginning of 2020 showed the need for portable, fast, reliable, and low detection limit diagnostic tools for quick data-based decision, not only for treatment, but also to monitor and control the spread of an infectious disease. [23]. The precise measurement of distinct IgG or IgM anti-body concentration could help to determine the current status of an infection or even immunity [24]. The possibility to isolate individuals, instead of the entire population of a country, may have less serious negative impact on their economy, while still helping to suppress the viral spread.

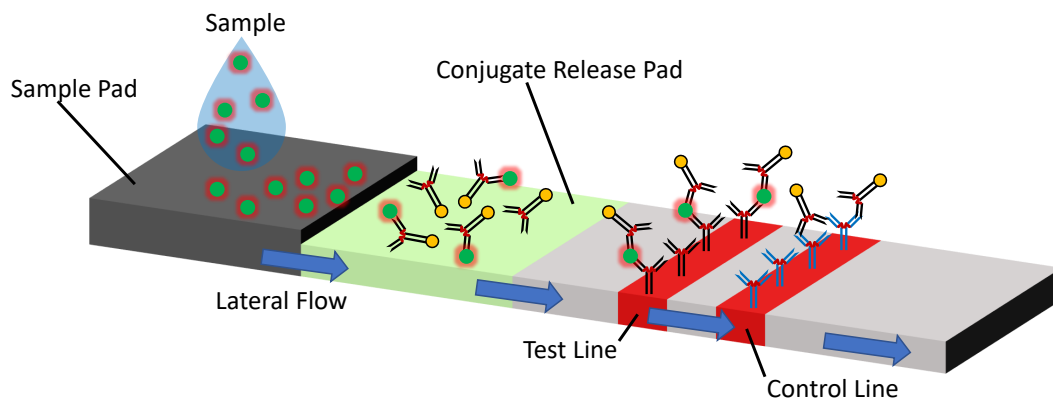


Figure 1.2.: Working principle of a lateral flow immunoassay test.

Lateral Flow Assay One popular example for a POC solutions is the commonly known pregnancy test [25]. It is a lateral flow assay (LFA) test, producing a qualitative result in minutes without the need for bulky control equipment [26]. LFAs are highly flexible on the applied sample, i.e. urine, sweat, saliva, serum, plasma, blood, target many different biomarkers, such as pathogens, chemicals, toxins, water pollutants, and have various fields of application, e.g. medicine, quality control, food production, or environmental safety [26]. The working principle is shown in Fig. 1.2. In case of the LFA pregnancy test, here more precisely an immunoassay, the urine sample, containing a distinct antigen, is introduced to the sample pad and flows towards the conjugate release pad. There the corresponding specific antibody, labelled with coloured or fluorescent particles, binds to the antigen, if present, and propagate together towards the detection test and control line. Similarly, the test line specifically detects the antigen via immobilised antibodies, whereas the control line specifically detects the unbound antibodies from the conjugate pad, again via immobilised antibodies [27]. For a pregnancy test this is a sufficient detection scheme (yes or no), but for other applications a quantitative result would be more advantageous. There are concepts facing this issue, but by at the same time complicating the read-out [28].

Almost every aforementioned detection method have some of the following issues: requires labelling, has to be carried out in laboratories, is time consuming, requires trained personal, is expensive, is not flexible enough regarding the target analyte (just one type of test per device), exhibits reduced throughput, or offers only qualitative or semi quantitative results. A more recent development are label-free biosensors which will be discussed in the next section.

1.3. Quantitative Label-Free Biosensors

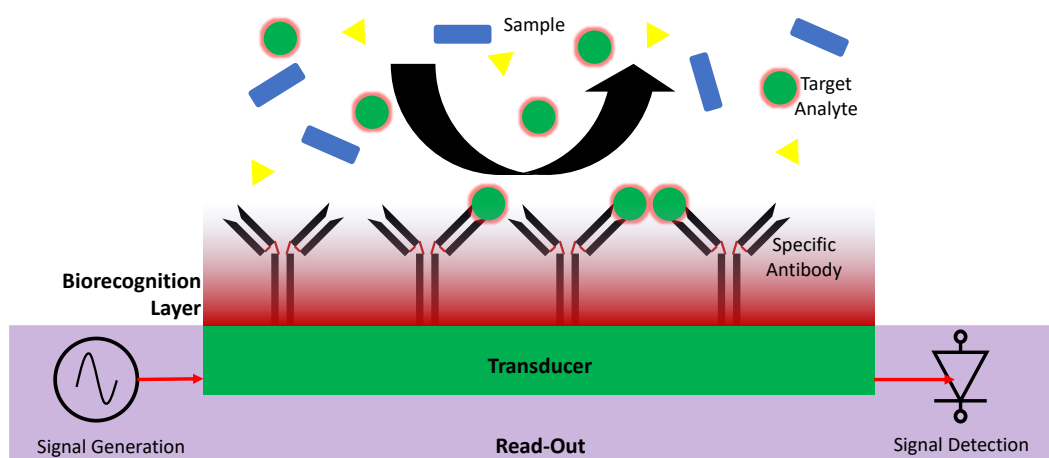


Figure 1.3.: Fundamental biosensor working principle including biorecognition layer, transducer, and read-out.

A label-free biosensor directly detects the target analyte without any need of subsequently applying labelled molecules to add a measurable quantity as it is the case for ELISA, PCR, or LFA. Typically, these biosensor can be subdivided into three main parts as shown in Fig. 1.3: the biorecognition element or layer, the transducer, and the read-out [29]. The working principle is as follows. A sample, possibly containing the target analyte, is brought close to the sensor surface. Functionalised on top of the sensing structure there is a layer of the biorecognition element. This biorecognition element attracts, ideally, just one specific molecule, the target analyte. If the sample contains this target, there is a possibility that it attaches to the layer. Ideally, no other molecule is able to do so. This process of attachment causes a change of

a physical parameter of the transducer. The transducer is normally a certain material in a more complex arrangement, e.g. forming an electrical transistor, a mechanically vibrating system, or an optical guide. The read-out applies a signal to the transducer and records its response, for example in form of a change of resonance frequency or intensity in real-time. The alteration of the transducer due to biorecognition produces a change in the response detected by the read-out. From this process, the difference between labelled and label-free detection becomes more apparent. Label-free biosensors do not require an extra assay step to add a measurable quantity into the detection procedure, for the presence of the analyte itself on top the transducer material already causes a quantitative change, detectable by the read-out [30]. This is a desirable feature as the number of labelled molecules during the label process typically cannot be reliably controlled [31].

The read-out consists of three parts: The signal generator, the detector, and the digital signal processing. The first can for example be an electrical or optical light source which is applied to the transducer. The transducer reacts to this signal according to its physical parameters including the alterations caused by the biorecognition process. The detector converts the modified signal into an electrical signal which is subsequently digitalised. This digital signal is then processed, e.g. that can include filtering, resampling, and other mathematical operations, by a data processing unit which calculates the transducer transfer function. Monitoring this function over time results in quantitative estimation about the concentration of the recognised target analyte.

Most biosensors are somehow integrated on a chip and enable highly multiplexed detection, i.e. several different tests at the same time, from which the concept for lab-on-chip (LOC) derives. The idea is to have a biosensing system which can perform several tests on the same sample in real-time. Monitoring in real-time the health status of a patient is a desirable feature for future biosensors [32], but was already successfully demonstrated for some applications [33]. Optical surface plasmonic resonance biosensors are a well-known candidate for LOC solutions. However, integrated photonic biosensors offer probably an even higher potential for this type of biosensing systems. Both are discussed in the next section.

In the following the biorecognition process and two non-optical transducer techniques will be shortly discussed to broaden the readers view on this topic.

1.3.1. Biorecognition Functionalisation

In order to convert a physical sensor into a biosensor a biochemical recognition element has to be added into the equation. This element strongly depends on the analyte to be detected. The biorecognition molecule can have various forms such as enzymes, antibodies, antigens, receptors, nucleic acids, or even whole cells and tissues [34]. Often a certain kind of protein is the targeted goal, for which for example a distinct antibody exists. This antibody is specifically designed, often by the human immune system, to recognise its counterpart (the antigen), attach to it, and to trigger an immune response. A biosensor, here in-vitro, only tries to do the first two steps. Two common ways to create this recognition layer is to attach the counterpart onto the surface via physical adsorption or a covalent bond. Immobilising this biorecognising element onto the transducer is the first step towards a functional biosensor. A second step becomes necessary due to the insufficient or imperfect way of the prior immobilisation. In real case scenarios no 100% surface coverage is obtainable. This free area may offer space and opportunity for non-target molecules to attach to the transducers surface and trigger a false-positive sensor response. Therefore, before the sensor can be used for a real case application, this remaining area must be reduced or disabled. To do so, an anti-fouling layer has to be established, typically by physical adsorption of an highly non-reactive biomolecule. The procedure to realise this anti-fouling layer is often referred to as “blocking”.

1.3.2. Non-Optical Transducer Biosensing Techniques

There is a vast variety of transducers available which can be applied for biosensing. For sake of clarity and brevity, just a few examples of non-optical transducers will be given here.

Four categories of non-optical transducers can be typically found: electrochemical, piezoelectric, magnetic, and thermometric [35]. Among them electrochemical biosensors are the most common due to their high sensitivity, reproducibility, and low-cost realisation capability [36]. Electrochemical biosensors can be typically characterised as potentiometric [37], amperometric [38], or conductometric [39] depending on the way of signal extraction [40]. The probably most popular example of a successfully commercialised biosensor is the in-vivo electrochemical glucose sensor for diabetes patients [33]. The detection principle is based on the measured glucose-enzyme interactions [41]. Glucose interacts among others with the functionalised enzyme layer and produces hydrogen peroxide [42] which then oxidises on a catalytic anode and induces an electron transfer, proportional to the glucose molecule concentration [43]. Already in 2004, glucose sensors dominated the biosensing worlds market by 85% [44].

Another more recent demonstrated electrochemical biosensor uses a graphene supported field effect transistor and was able to directly detect SARS-CoV-2 from nasopharyngeal swab specimens from COVID-19 infected patients [45]. For that a layer of SARS-CoV-2 spike antibodies was functionalised on the gate of the field effect transistor. Any viral SARS-CoV-2 pathogen attaching to this layer would introduce a change in current which can be related to the viral concentration of the patient sample.

Surface acoustic wave biosensor, a piezoelectric type, were also investigated in the last decades [46]. The attachment of the target analyte would change the mechanical properties of the sensing structure. This alteration would then be converted into an electrical signal proportional to the amount of analyte recognised by functionalised biomolecule layer.

1.4. Optical Biosensors - An Overview

1.4.1. General Assembly

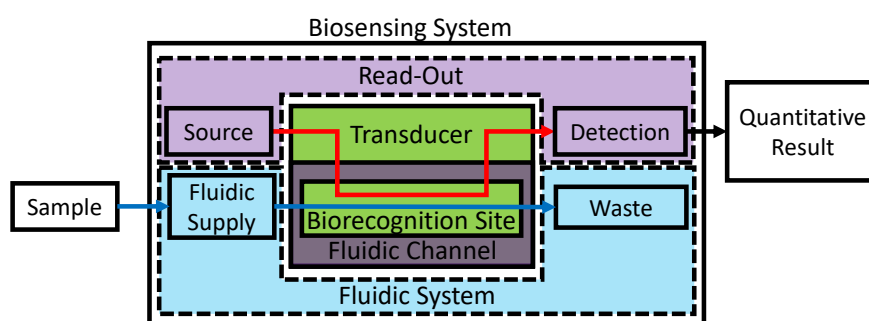


Figure 1.4.: General Assembly of biosensing system with biorecognition layer, transducer, read-out, and fluidic system

Before elaborating on the performance characteristics and the different optical transducer detection approaches, a general idea of the entire optical biosensor system and the detection procedure has to be established. The biosensor itself, including the read-out, is only one necessary part of a complete apparatus. Most optical biosensors have a separate system which transports the patient sample to the biorecognition site of the sensor: the (micro)fluidic system. Typically, a sealing device, for example a specially designed polydimethylsiloxane (PDMS)

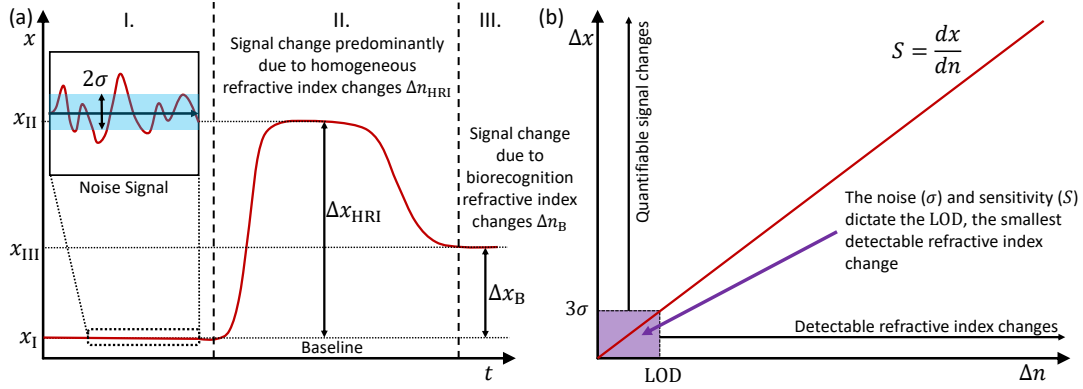


Figure 1.5.: (a) Typical signal evolution for, first (I.), a buffer flowing over the sensor establishing the baseline x_I , second (II.), a sample entering and passing the sensor surface. Finally (III.), the sample leaves the channel. The inset in I. shows the noise level (σ) of the signal. For homogeneous refractive index (HRI) (Δn_{HRI}) detection, i.e. no biorecognition layer functionalised, the signal Δx_{HRI} in time slot II. is of special interest. For biosensing, HRI alterations are irrelevant as only refractive index changes in the biorecognition layer (Δn_{B}) will cause a permanent binding signal Δx_{B} in time slot III. (b) The signal uncertainty (3σ) dictates the smallest quantifiable signal change Δx . In combination with the sensitivity (S), it marks the smallest detectable refractive index change Δn , the detection limit (LOD).

material, creates a small fluidic channel (in the range of micro to millimetres) right above the sensing structure(s). Connecting one end to the fluidic supply and one end to a waste deposit enables a continuous liquid flow over the sensors. The flow velocity F is typically in the order of microlitres per minute and the sample of the volume V_{inj} is typically below a millilitre. This is illustrated in Fig. 1.4.

The typical steps for a detection assay are shown in Fig. 1.5(a). The assay starts (I) by flowing a buffer medium over the sensor which should ideally give a constant signal, the baseline x_I . However, this observed baseline is not entirely constant, but superposed by a zero-mean time-varying signal, the noise. There are several origins from which the noise may occur, for example from the electric detection devices or mechanical vibrations. The baseline noise is typically characterised by its standard deviation (σ). In the next step (II.), the sample is introduced, possibly causing a signal change due to the ongoing biorecognition process or homogeneous refractive index (HRI) alterations (Δn_{HRI}). The latter is of interest when the bulk detection limit is determined, for which no biorecognition layer is typically functionalised prior to the experiment. The maximum signal change Δx_{HRI} during time slot II. corresponds to the applied HRI change of the sample. In the last step (III.), to minimise the influence of other effects, e.g. non-biorecognition, the buffer medium is brought in again, washing away the sample and re-establishing the initial condition. In the case of biosensing, if any change of the physical parameter Δn_{B} , in the biorecognition layer, caused a signal alteration Δx_{B} to the baseline, this directly relates to the amount of analyte “captured” by the biorecognition layer. In case of pure bulk sensing, the signal should ideally go back to baseline, i.e. $x_{\text{III}} = x_I$.

1.4.2. Performance

This section is dedicated to the performance characteristics of biosensors in general and to create a common ground for the coming discussion in the state-of-the-art (see Section 2.4).

There are many performance characteristics for a biosensor, but only a few which can be

compared between all types [47]. For example, the sensitivity $S = dx/dn$, which is defined by how strongly the signal x reacts, may it be a change in resonance wavelength or phase change, to changes of a physical parameter n , e.g. change of the homogeneous refractive index surrounding the sensing structure, is a sensor type specific parameter and can only be compared within this context. The sensitivity is often given in rad per physical parameter change for a fixed wavelength interferometric sensing scheme or nm per physical parameter change for a spectral transmission based sensing scheme. The experienced measurement uncertainty/noise (σ), in units of nm or rad, is also a sensor-specific performance parameter. The detection limit, defined as

$$\text{LOD} = \frac{3\sigma}{S}, \quad (1.1)$$

indicates the smallest amount of physical parameter change which can be accurately detected. As opposed to sensitivity or noise, it can be readily compared across different sensor types. Figure 1.5(a) shows a qualitative signal shape x over time for an alteration of the physical parameter n where the inset shows the noise σ of the signal x . Figure 1.5(b) shows the linear relation between the signal alterations (Δx) and physical parameter change (Δn). The detection limit is a result of the measured uncertainty level (3σ) and the sensitivity (S). In general, the goal is to minimise (the lower the better) the LOD, by maximising the sensitivity (the higher the better) or minimising the noise level (the lower the better). For bulk sensing, i.e. homogeneous refractive index (HRI) sensing, the physical parameter monitored is the refractive index surrounding the sensor, so that the LOD is expressed in refractive index units (RIU). When talking about biosensing performance, the LOD will be given in smallest detectable concentration of the analyte, i.e. mass per volume (ng mL^{-1} or pg mL^{-1}).

When considering the design of a photonic biosensing system, both the sensitivity and noise have to be considered carefully. In many situations the sensitivity is essentially determined by the design of the transducer, whereas the noise is introduced due to thermal fluctuations and the read-out system. Many publications for photonic biosensors in literature devote their main focus often in maximisation of the sensitivity, but usually do not reach excellent detection limits, because the noise is too high. As an example, for some sensor types the best detection limits are achieved by sensor with relatively low sensitivity, but proper noise handling, for this see [48] with $S = 163 \text{ nm/RIU}$ and $\text{LOD} = 7.6 \times 10^{-7} \text{ RIU}$ and [49] with $S = 2170 \text{ nm/RIU}$ and $\text{LOD} = 1.6 \times 10^{-5} \text{ RIU}$.

When targeting lab-on-chip and/or point-of-care devices the cost of the final device is also an important factor. The silicon photonics integrated chips can be manufactured very cheaply, at least in larger scales. The entire device, i.e. control elements, read-out, etc. can still be quite expensive, depending on the used sensor type. For POC devices not only the price per device is important, but also other parameters, such as size, weight and robustness, to enable the operating personnel to use it for primary care. Therefore, a miniaturised and robust solution is required.

1.5. Architectures for Evanescent Field Sensing

In this section, some types of label-free optical biosensors are presented and discussed. Optical sensors have the advantage that they are robust against electromagnetic interference along the sensing site [50]. Optical sensors can be based on measuring: optical absorption, Raman spectroscopy, and refractive index. Refractive index type optical biosensors are based on the principle of evanescent field sensing and are currently promising candidates for lab-on-chip and point-of-care devices for which they are discussed in the following. There are two popular evanescent field sensing types: plasmonic and dielectric waveguide mode sensing. In both cases, light is applied to specific arrangement of materials, with distinct parameters (qualitatively shown in Fig. 1.6). For the former (Fig. 1.6(a)), the applied light excites a surface plasmonic

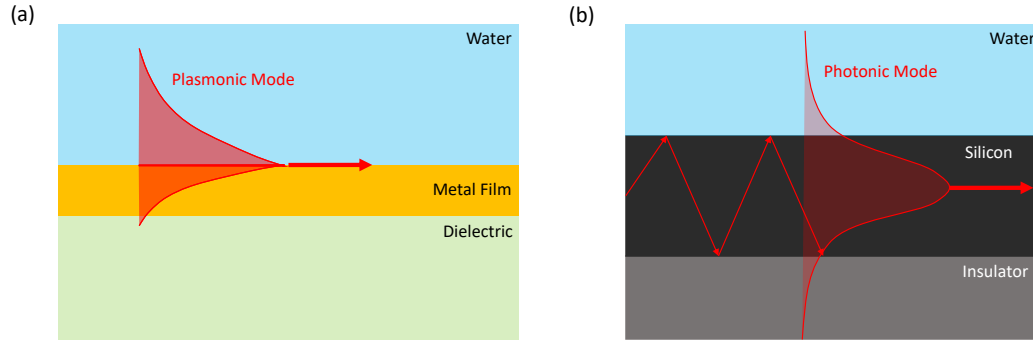


Figure 1.6.: Qualitative representation of a plasmonic (a) and a dielectric waveguide mode (b).

charge oscillation which supports the propagation of the optical mode along the surface. For the latter (Fig. 1.6(b)), the incident light excites a dielectric waveguide mode, a certain electromagnetic field profile which typically enables this light to propagate within this dielectric material arrangement via total internal reflection (TIR) at a distinct velocity determined by its effective refractive index, the ratio between the velocity of light in vacuum and the mode phase velocity. The mode phase velocity propagation constant depends on many factors, however, for sensing, a liquid dielectric material, for example water, is assumed to surround the sensing structure, enabling the transport of the desired analyte. Some part of the mode field profile penetrates the liquid dielectric material as shown schematically in Fig. 1.6(b). This part of the electromagnetic field is called the evanescent field. On top of the sensing device the biorecognition layer is functionalised to capture the analyte. By this process, the refractive index surrounding of the sensing structure is modified, modulating the effective refractive index of the optical mode; this is why this type of sensing is often referred to as evanescent field sensing.

Since this thesis focuses on photonic mode based sensing, the plasmonic type sensors are only described shortly in the following.

1.5.1. Surface Plasmon Resonance Sensor

Plasmonic based biosensors utilise the interaction between electromagnetic waves and delocalised electrons, i.e. in the conduction band, on a metal surface to measure the biological recognition of an analyte. A surface plasmon is an electric charge oscillation within the surface of a thin layer metallic structure which can be excited by light. The incident light induces the free electric surface charges to oscillate according to the wavelength and incidence angle of the light. The surface plasmonic resonance mode is excited when the light meets the plasma frequency condition, i.e. the resonance frequency of the surface electrons, such that the light couples its energy into the electric electron charge density oscillation. This means that most of the light will be absorbed by the surface and thus minimises the reflection. The resonance frequency depends on the interface between the metal and the dielectric, to be more precise on its dielectric constant. Therefore, any change in refractive index close to the interface of the dielectric-metal surface translates to a change of the plasmon resonance frequency. This effect is used for detecting changes in refractive index, for example caused by the attachment of the analyte to the biorecognition layer on the metal surface. Due to the way of excitation, only TM plasmonic modes are able to propagate, which dictates the incident light to have the same polarisation. Using light in the visible sets certain requirements to the metals dielectric constant, which is why only distinct metals can be used in this case, for example gold or copper [51].

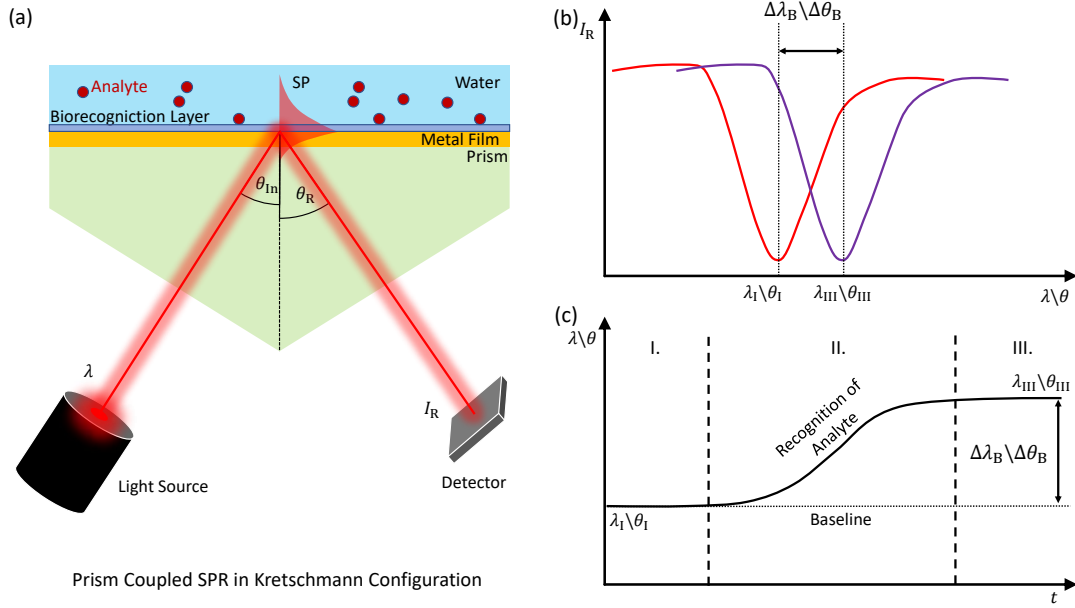


Figure 1.7.: (a) Schematic view of a Kretschmann configured SPR biosensor. (b) The reflected light intensity as a function of the different operation modes, wavelength or incident angle modulation, to determine the plasmonic mode over time in (c).

There are two common types of plasmonic sensors: surface plasmon resonance (SPR) and localised surface plasmon resonance (LSPR). The first is characterised by a homogeneous sensing structure in which a propagating plasmonic resonance mode is excited. SPR is a very mature technique, already applied to numerous fields of application in molecular biology, pharmacy, environmental- and nanomedicine diagnostics [52, 53, 54]. In LSPR small nanometre-sized structures are applied for sensing exciting localised, i.e. confined in the nano structures, plasmonic resonance modes. In comparison to SPR, this is a more recent developed technique. It has the major advantage of having a more simple and easier realisable read-out than the SPR, in addition to a higher integratability, multiplexability, and reproducibility [55]. Those advantages make LSPR more suitable for LOC and POC devices [56].

For SPR, there are three different popular ways to excite the propagating plasmonic resonance mode: prism [57], grating [58], or waveguide coupling [59]. The first uses a prism to excite the plasmonic mode at an angle θ_{In} (see Fig. 1.7(a)), and is the most widely used type of coupling mechanism [60]. The second, uses a periodic grating at the metal-insulator interface which couples the light into the plasmonic mode. The last approach uses underlying integrated photonic structures to couple light into the plasmonic section.

Both a monochromatic or a broadband light source can be used within a SPR detection scheme. The latter, however, often requires a spectral filter element. There are two popular operation modes from which the refractive index change can be calculated. For example, assuming a tunable monochromatic light source, covering a wavelength range $\lambda_{min} < \lambda < \lambda_{max}$ with constant power, illuminating the region-of-interest (ROI) on the surface from a certain angle θ_{In} , the reflected light intensity at the angle $\theta_R(\lambda)$ can be monitored [61]. Alternatively, a broadband white light source can be applied and a spectrum analyser is used for the detection of the reflected light. For the other mode, this time assuming a fixed wavelength monochromatic light source with wavelength λ_0 , the angle of incident can be modulated while measuring the intensity of the reflection at a fixed angle. Both detection schemes are shown in Fig. 1.7(b), showing the qualitative reflectivity response I_R as a function of wavelength λ or angle of incident θ_{In} . The dip in the response corresponds to plasmonic resonance, absorbing the light. Any modification

of the refractive index close to the metal film modifies the position of the minimum observed in those responses and can serve, therefore, as quantitative indicator for the amount of analyte attached to the biorecognition layer over time (see Fig. 1.7(c)).

Localised surface plasmon resonance sensors work very similar, substituting the flat metal film by small three-dimensional nanoparticles or nanostructures. The confined plasmonic modes are not only very sensitive to the used material, shape, and size, but also to the surrounding dielectric constant which is the physical parameter altered in optical biosensing [62]. Its evanescent field rapidly declines and is, therefore, only sensitive to changes in refractive index close to the surrounding [63]. Among others, this enabled the detection of single molecules [64, 65].

Plasmonic based sensors have demonstrated detection limits of around $10^{-6} \rightarrow 10^{-5}$ RIU, and typically a few nanomolar to picomolar, depending on the type of analyte [56].

1.5.2. Waveguide Based Sensors

Optical sensors based on dielectric waveguides make use of dielectric material arrangements to achieve total internal reflection to enable propagation of the applied light within the structure as indicated in Fig. 1.6(b). The simplest example for a photonic waveguide is a slab in which light may enter the guiding material on the one side with a specific angle and is totally reflected at the boundaries with the surrounding material, also often referred to as cladding. Applying silicon-on-insulator photonic wire waveguides for biosensing is a relative recent development [66]. Depending on the material properties and the structural dimensions, often in the range of the wavelength of the incident light, only a few electromagnetic field profiles may be able to propagate in the waveguide, called waveguide modes. Part of the field penetrates the surrounding, exponentially decreasing with the distance to the guiding material surface, similarly to the plasmonic modes. Partially substituting the direct surrounding with a liquid enables the interaction of the mode with the analyte. Functionalising a biorecognition layer on top of the guiding core, will result in an alteration of the refractive index by any attachment of the analyte to this layer that is within the range of the evanescent field. The alteration of the refractive index modifies the effective refractive index of the mode, and equivalently the propagation velocity of the mode applied for sensing. The effective refractive index change cannot be read directly but must be transferred into another quantitative measure, often some sort of intensity variation. Depending on the way of extraction, different requirements for the optoelectronic read-out are necessary.

In the following the most popular photonic integrated waveguide based sensor type are briefly discussed, together with their accompanying advantages and disadvantages.

Ring Resonators Ring resonator based sensors use a waveguide structure which essentially feeds back its propagating light into itself after covering a certain distance, passing the same section typically thousands of times. However, only distinct wavelengths are able to propagate in the resonator due to interference of the light along the propagation direction: the resonance wavelengths λ_i with $i \in \mathbb{N}$. Analyte recognised by the functionalised biorecognition layer on top of the photonic resonator alters the effective refractive index, hence, the optical length of the sensing mode, and therefore shifts the resonance wavelengths by $\Delta\lambda_i$ [67]. Normally, light of different wavelengths is launched into the resonator via a neighbouring waveguide using waveguide coupling. The situation is shown in Fig. 1.8(a).

There are two popular ways to extract the resonance wavelength information. The first generally requires a tunable high-quality narrow-linewidth laser, often an external cavity laser. Its light is guided to the waveguide coupling section where, depending on the coupling strength and resonance condition, the light is either being ignored by the resonance structure, i.e. it passes the ring and the entire optical power is transmitted to the detector, or it is ideally completely

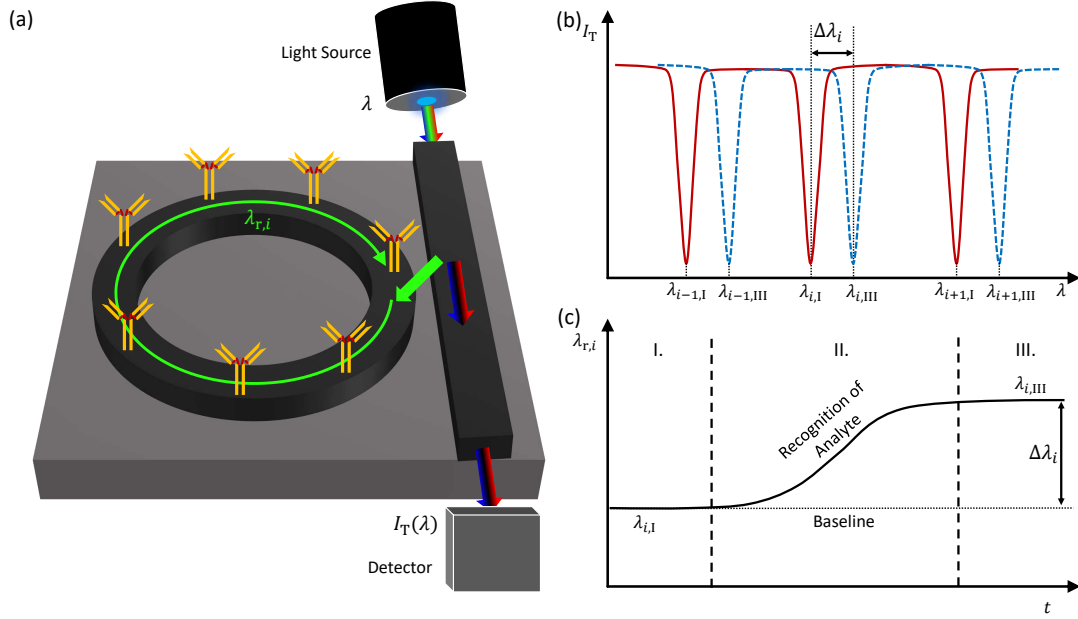


Figure 1.8.: (a) Typical ring resonator based sensing structure. (b) Optical transmission through the structure, and (c) time resolved change of resonance wavelength as basis for the amount of analyte attached to the surface.

absorbed by the resonator. By sweeping over a certain wavelength range, the transmission spectrum of the resonator is obtained, its minima positions displaying the corresponding resonances (see Fig. 1.8(b)). Continuously scanning over the optical spectrum, determining the minima, and displaying them over time (see Fig. 1.8(c)) quantitatively indicates the amount of analyte being recognised by the functionalised layer. The second approach operates similarly, but applies a broadband light source, such as a light emitting diode (LED), to the resonator. All constructive and destructive interferences inside the resonator happen at the same time. The optical output is then fed into an optical spectrum analyser, which can be an actual high quality spectrum analyser, or a wavelength multiplexer with consecutive separated photodiodes. Ring resonators have demonstrated detection limits of around 10^{-6} RIU [48].

One main disadvantage of this type of sensing is the requirement of a typically expensive high quality laser source, or an spectrum analyser. This does not only dominate the price for LOC devices but also also becomes impractical for POC solutions, where price and simplicity of the read-out are important factors. Furthermore, they do not reach the detection limits of interferometer type sensors which are typically one to two orders of magnitudes better.

The ring resonator type of sensor is probably the most mature photonic integrated sensors technology right now. Lab-on-chip devices are already commercially available, for example by Genalyte [68] offering the Maverick platform [69] which can operate up to 128 different sensing structures per chip quasi simultaneously in real-time. High throughput SARS-CoV-2 serological IgG and IgM antibody detection has been successfully demonstrated on this platform and offers an attractive alternative to the current verifications methods [70].

Photonic Crystals This kind of sensor often integrates some sort of photonic micro cavity based on photonic crystals. Photonic crystals are two-dimensional periodic dielectric structures. They emulate the interaction between electrons and a crystals lattice and their behaviour, just in this case between photons and the periodic dielectric arrangement. Without going into detail, their precise design can be used to tailor their photonic bandgap and enables the design

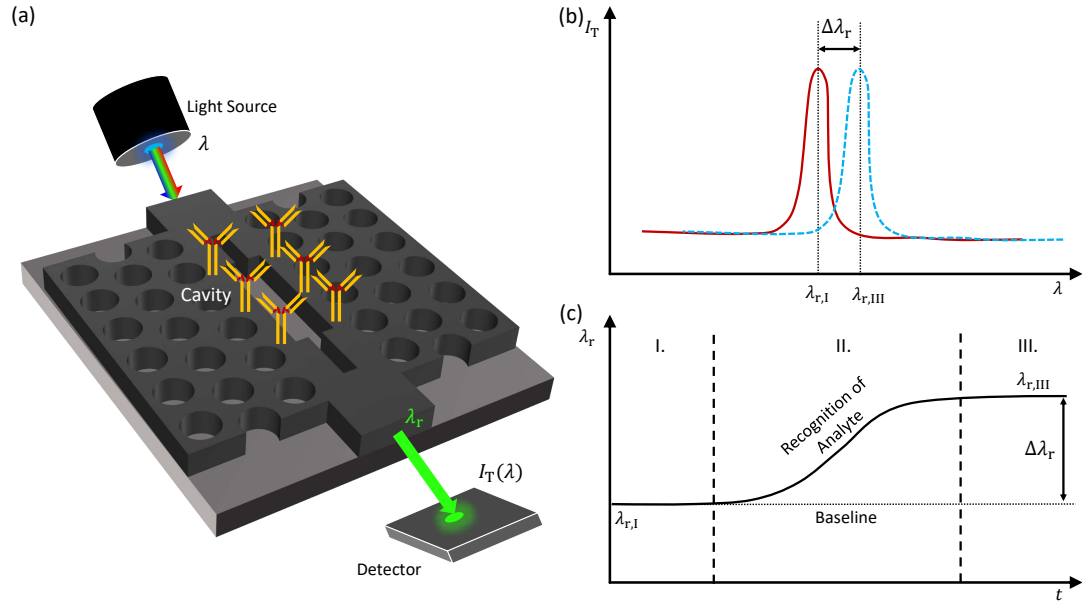


Figure 1.9.: (a) Typical photonic crystal slot based sensing structure. (b) Optical transmission through the structure, and (c) time resolved change of resonance wavelength as basis for the amount of analyte attached to the surface.

of cavities with extremely-high quality factors, i.e. having a very narrow-linewidth resonance mode. Therefore, their read-out is very similar to previous presented ring resonating structures. A sketched illustration of one possible realisation is shown in Fig. 1.9(a). There are many different design implementations [71]. The read-out normally consists of a sweepable high-quality laser and a photodiode at the output. However, there are concepts which substitute the laser by a broadband light source [72]. The qualitative transmission spectrum is shown in Fig. 1.9(b) being very similar to ring resonators, with resonance wavelength λ_r . If functionalised for biorecognition purposes, the attachment of the desired analyte will cause a change $\Delta\lambda_r$ of the resonance wavelength of the photonic crystal resonator which, when displayed over time, indicates the amount of recognised analyte (see Fig. 1.9(c)) [73].

Many photonic crystal based sensors have been demonstrated to work in the telecom wavelength band which also enables small footprints. No additional read-out calibration is required for this type of sensor. They demonstrated mean limit of detections down to approximately 10^{-5} RIU [74]. Analogously to their ring resonator counterparts, their main disadvantage is the necessity of an expensive high quality laser making a POC application challenging. However, for LOC solution they are very attractive due to their small occupied area.

Young Interferometer This type of interferometer uses the wave nature of light to create a spatial pattern of constructive and destructive interferences. As in the case of all interferometers, the on-chip coupled light is split into two different optical paths, the reference and sensing arm. The latter is exposed to the surrounding liquid and the analyte, whereas the first is buried in insulator and, therefore, is not affected by the refractive index changes caused by the analyte (see Fig. 1.10(a)). The light at the output of the sensing arm experiences a phase shift difference compared to the reference arm, depending on the biorecognition of the functionalised layer. Light in both optical paths is shined onto an off-chip detection element, creating a unique interference pattern, which changes with the biorecognition process (see Fig. 1.10(b)) [75, 76, 77]. This shift, plotted over time indicates the amount of attached analyte (see Fig. 1.10(c)).

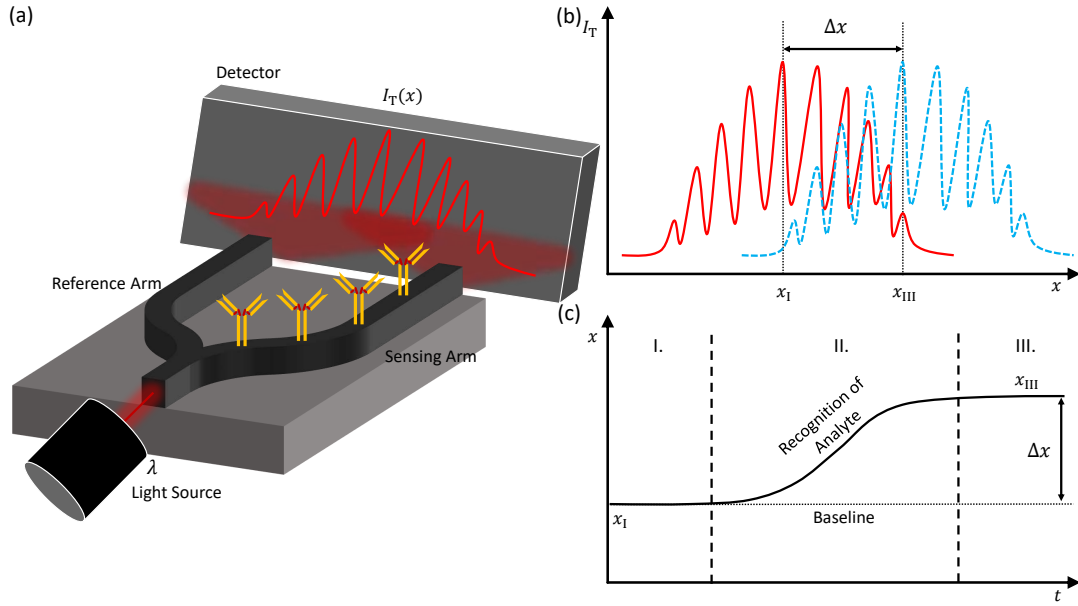


Figure 1.10.: (a) Schematic of a Young interferometer based sensing structure. (b) Spatial transmission spectrum through the structure, and (c) time resolved change of spatial shift as basis for the amount of analyte attached to the surface.

These sensors have demonstrated very-low detection limits, down to 4×10^{-8} RIU, and can be used with less expensive white optical sources [78]. Even though this simplifies the read-out, which is preferable for LOC solutions, the detection scheme can become problematic for multiplexing, i.e. applying the source to several sensing structures and detect all interference patterns at a time due to the occupied space.

Mach-Zehnder Interferometer This interferometer is very similar to the Young type, but with the difference that the recombination of sensing and reference arm is realised on-chip. For this often a simple combiner, for example a Y-Junction, is applied which combines both incoming modes and depending on the experienced phase difference $\Delta\varphi$ results in destructive or constructive interference [79]. Traditional Mach-Zehnder configurations only require a fixed wavelength light source. An exemplary representation is shown in Fig. 1.11(a). The received intensity interference signal is a periodic function of the phase (or effective refractive index) difference as shown in Fig. 1.11(b). Monitoring the intensity signal over time and extracting the phase shift indicates the amount of analyte bound to the surface due to biorecognition of the functionalised surface (see Fig. 1.11(c)) [80].

Traditional Mach-Zehnder interferometers have the advantage of probably having the most simple read-out, just requiring a fixed wavelength optical source and photon detector. However, this modesty comes with the price of possible sensitivity fading, i.e. no intensity change for alterations in effective refractive index, and phase ambiguity, not knowing in which direction the effective refractive index is actually moving. This was overcome by adding a modulation to the read-out, e.g. modulating the emitted wavelength of the optical source, or the effective refractive index of the reference arm [81]. Either way, this complicates the read-out and/or the fabrication procedure of the chip. Very low bulk detection limits, down to 10^{-7} RIU, were demonstrated with modulated and non-modulated MZI detection schemes [82].

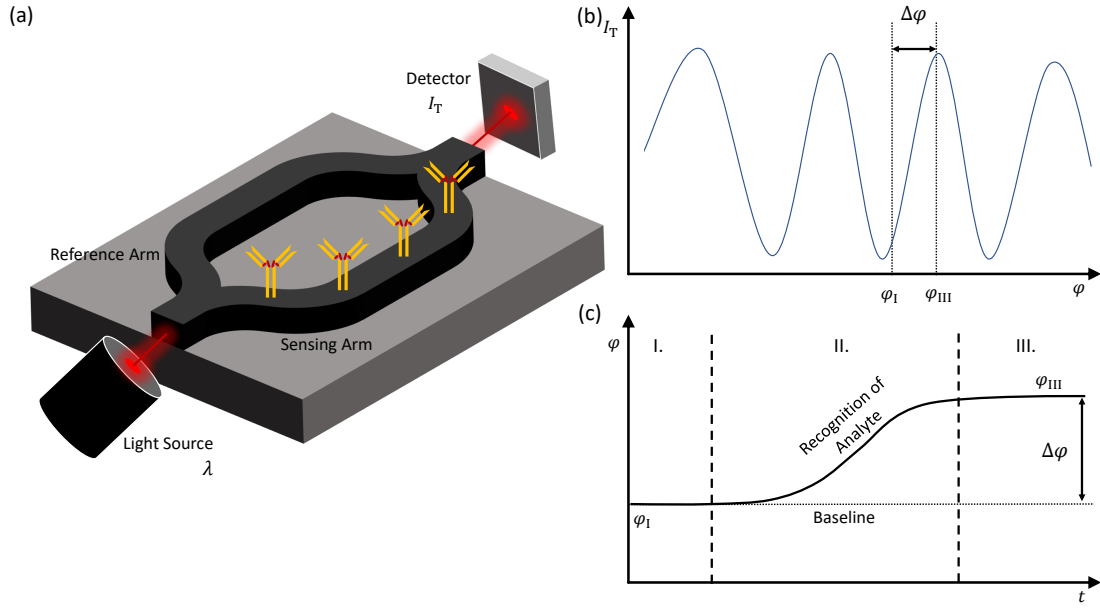


Figure 1.11.: (a) Typical integrated Mach-Zehnder interferometer with Y-Junctions for splitting and recombining to create the interference signal. The intensity to phase difference relation in (b), and its evolution over time to monitor the attachment of the analyte on the surface via biorecognition in (c).

Spectral Mach-Zehnder Interferometer Unbalanced Mach-Zehnder interferometers, that is interferometers with different optical path lengths in the reference and sensing arms, can be combined with a sweepable narrow-linewidth or white optical light source, as shown in Fig. 1.12(a) [83]. Measuring a sufficient range of the transmission spectrum can be used to detect refractive index changes in the sensing arm. Due to the asymmetric optical paths each recombined signal, for a distinct wavelength, experiences a more constructive or destructive interference [84]. The obtained minima λ_i in this transmission correspond to destructive interference at the recombination section. Ideally, the neighbouring minima are equidistant, forming the free-spectral range (FSR) λ_{FSR} (see Fig. 1.12(b)) [85]. In case an analyte gets recognised by its counterpart, the minima are shifted to higher or lower wavelengths by $\Delta\lambda_i$, thereby providing a quantitative indication of the amount of analyte detected by the functionalised biorecognition layer over time, as illustrated in Fig. 1.12(c).

There are two ways to extract this shift in minima. The first, as mentioned above, is the usage of a high quality sweepable narrow-linewidth laser and a simple photo detection device. This is of course counterproductive if a low-cost POC device is desired. Alternatively, a cheaper sufficiently-broadband optical source can be used and at the same time applying a optical spectrum analyser [86]. This can be done by either utilising an external spectrum analyser or by integrating an optical multiplexer on-chip with consecutive photodiodes for detecting distinct ranges of the SMZI transmission spectrum [87]. Detection limits around 10^{-6} RIU were demonstrated [86].

Bimodal Interferometer In the last decade a relatively new concept for an interferometric sensor was presented, called bimodal interferometer (BMI) [88]. Light travelling in a single-mode waveguide hits the sensing waveguide section in which a second-order mode can propagate as shown in Fig. 1.13(a) for a vertical bimodal sensor [89]. At the interface between the single-mode and the sensing waveguide optical power couples into the ground- and second-order mode. Both modes have different effective refractive indices and, therefore, propagate with difference

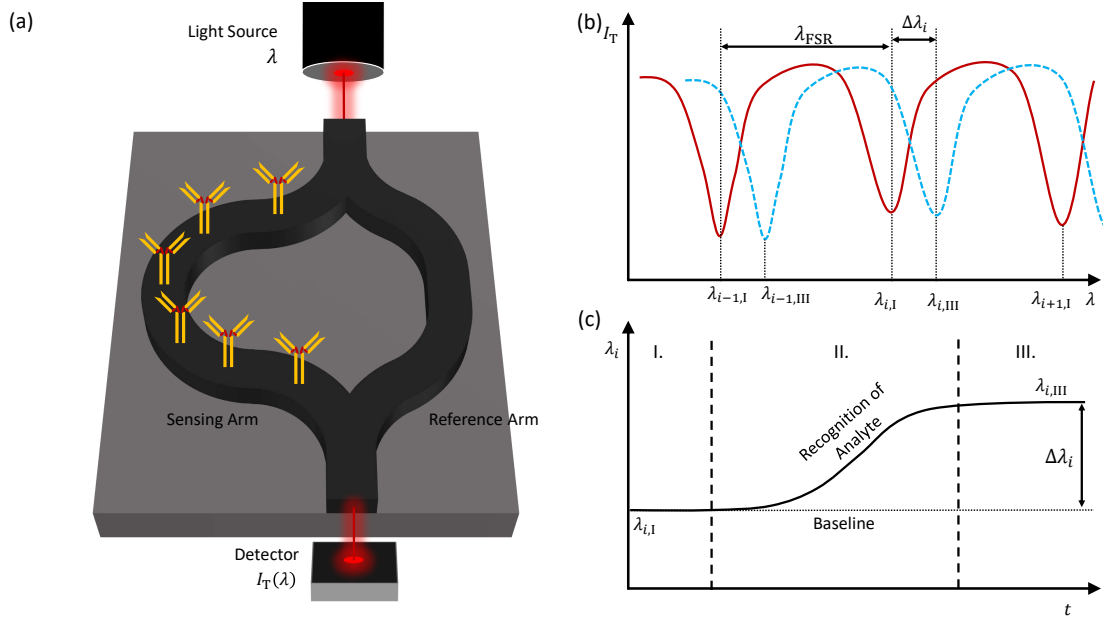


Figure 1.12.: (a) Asymmetric Mach-Zehnder interferometer for spectral transmission extraction, shown in (b). The unbalanced arms create a transmission spectrum with various minima λ_i , with $i \in \mathbb{N}$, due to destructive interference in distances λ_{FSR} , the free-spectral range (FSR), to one another. (c) Shifts of the minima due to the attachment of analyte can be monitored over time to indicate the amount of the same.

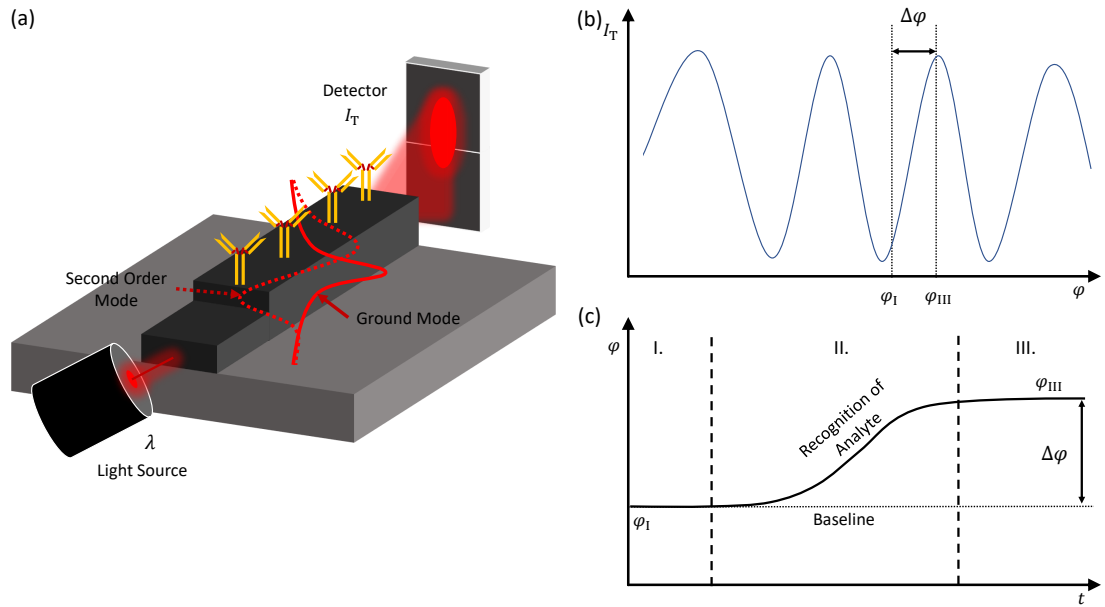


Figure 1.13.: (a) Vertically excited bimodal waveguide section for sensing. The effective refractive index difference change between both modes results in the interference signal in (b) and indicates the amount of recognises analyte by the surface over time in (c).

velocities. Due to the evanescent tails of both modes into their surrounding both experience an alteration in effective refractive index if a refractive index change is caused by the attachment of the target analyte to the bimodal waveguide. However, the second-order mode will be more affected by any change by virtue of a higher mode delocalisation from the guiding core medium [90]. After propagating through the sensing section, both modes will have a distinct optical power and phase, and phase difference $\Delta\varphi$ to each other. They are then shined on the detector which receives an interference pattern, depending on the power and phase difference of the modes. For a vertical bimodal waveguide the phase difference is encoded into the obtained power and position of the interference pattern maxima. By using two separate photodiodes to detect the parts of the pattern, one will receive more, the other less optical power, from which a differential interferometric signal can be recovered (see Fig. 1.13(b)). As in the MZI the phase difference can be extracted and be related to the amount of analyte detected (see Fig. 1.13(c)).

Bimodal interferometers can be used with a fixed wavelength scheme and two identical separated photodiodes to detect the interference pattern distribution. As with the non-modulated MZI sensor sensitivity fading and phase ambiguity are possible. An optical source modulation scheme, called all-optical modulation, has proven to successfully avoid both drawbacks with detection limits down to range of 10^{-7} RIU [91]. Nevertheless, this is a complication of the read-out. Here, an additional read-out calibration is necessary to enable the correct phase extraction.

Other types of BMI have been proposed, such as horizontal excited BMI [92], transmission pattern read horizontal excited subwavelength BMI [93, 94], or trimodal BMI [95]. The latter extracts the introduced effective refractive index difference from the excitement of the ground (reference) and third-order (sensing) mode.

Coherently Detected Mach-Zehnder Interferometer Coherent receivers have been vastly used in telecommunication to extract the contained information in phase and amplitude [96]. Coherent techniques for complex (amplitude and phase) reflection coefficient measurement based in a 120° MMI couplers, where originally proposed in [97], in the frame of the six-port metrology approach. Coherently detected Mach-Zehnder interferometers for sensing was demonstrated in [98]. Instead of a simple recombining element, e.g. a Y-Junction, as in the case of the traditional MZI, a 2×3 MMI may be applied (shown in Fig. 1.14(a)), where the two input ports are the reference and the sensing arm, and splits the incoming light into three different interferometric signals, 120° phase shifted to each other (see Fig. 1.14(b)), depending on the applied phase difference $\Delta\varphi$ between the inputs. The three interferometric signals enable. As in the case of a normal MZI the phase difference monitored over time can be related to the amount of analyte detected (qualitatively displayed in Fig. 1.14(c)).

1.5.3. General Observations

One problem which most of the aforementioned photonic or plasmonic detection schemes have in common relates to the read-out system, i.e. light source and signal detection. Either they use an expensive high quality laser and a very simple detection element, e.g. a simple photodiode, or a cheaper laser and a more sophisticated detection scheme while typically worsening the detection limit. This can include for example an modulation scheme for correct signal extraction, or an optical filtering element and a large amount of individual optical signals to detect. The latter could also become unfavourable for highly multiplexed sensor detection because every optical signal requires a distinct physical space.

Point-of-care devices require a simple and cheap realisation which is why a cheap non-modulated optical with a small and simple detection scheme would be advantageous.

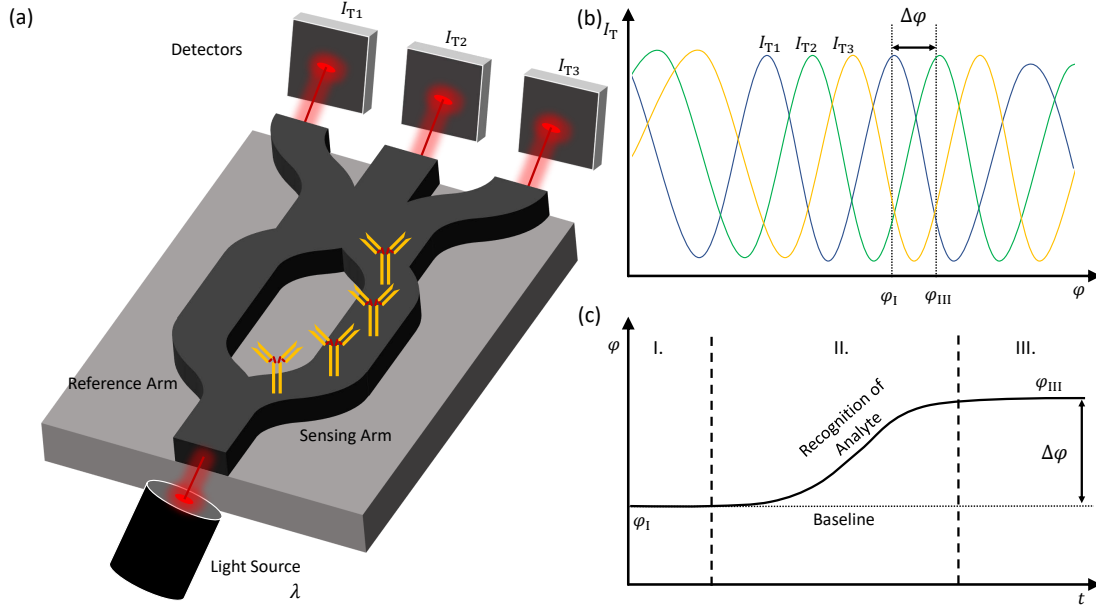


Figure 1.14.: (a) Coherently detected Mach-Zehnder interferometer with three interferometric output ports. (b) The intensity-phase diagram of the three optical outputs. All three interferometric signals are 120° phase shifted to one another. (c) The shift of extracted phase due to the attachment of analyte can be monitored over time to indicate the amount of the same.

1.6. Goals and Contributions of this Thesis

The main goal of this thesis is to demonstrate the applicability of coherently detected Mach-Zehnder interferometers (CMZI) for label-free biosensing purposes in the context of point-of-care oriented devices. Beyond that, within this framework, the CMZI refractive index sensor is demonstrated in the context of biosensing for the first time in literature and for β -lactam based antibiotic allergy detection. Taking into account that a first generation of chips was already available when the thesis was initiated three sub-goals are defined:

1. Develop and optimise the fluidics and read-out system. Based on the experimental read-out system, a theoretical assessment of the achievable detection limit will be presented, highlighting the parameters which can be modified to reach the lowest LOD. This contribution is published in [99]. Until the date of its publication, few publications could be found in literature targeting interferometer-based biosensor noise optimisation, none at all for coherent based detection. Using this insight, an experimental LOD optimisation procedure is then developed and demonstrated with excellent detection limits down to almost 1×10^{-8} RIU. This contribution is published in [100].
2. Demonstrate the CMZI's biosensing capability for the first time in literature. The surface of the sensing waveguide is biochemically treated to functionalise its surface to be specific for C-reactive protein detection. In an additive immunoassay, very good detection limits of around 180 pg mL^{-1} were obtained. Furthermore, to demonstrate the CMZI's capability to operate with inexpensive optical sources, a favoured option for POC devices, a very cheap handheld Fabry-Perot fibre test laser was feed into the sensing system and accomplished outstanding bulk detection limits of 10^{-7} RIU and 1.8 ng mL^{-1} for specifically detection CRP. These results are published in [101].
3. Provide a proof of concept for allergy diagnosis with the CMZI. Proceeding to a more

application based environment, the sensor is used for the detection of IgG and IgE amoxicillin antibodies. The presence of those in a patient sample can indicate a possible allergy towards amoxicillin, a penicillin for bacterial infectious disease treatment. A fast and reliable test platform may help future medical personnel for fast diagnostics. For this purpose the sensing surface was biochemically prepared with amoxicillin haptenised poly(amidoamine) (PAMAM) second generation dendrimer to be particularly selective to those antibodies. Detection limits of around 1 ng mL^{-1} were achieved for both, IgG and IgE type antibodies. The results are not yet published.

Based on this, the structure of this thesis is the following:

Chapter 1 The Introduction, this current chapter, giving a general overview of integrated photonic biosensors and relevant related topics.

Chapter 2 Short introduction to the fundamental photonic theory related to coherently detected Mach-Zehnder interferometric biosensor design including a theoretical derivation for its laser noise immunity, followed by a state-of-the-art description of photonic integrated biosensors.

Chapter 3 Here, sub-goal one is addressed: Description of the developed setup, its system level description, the contributing noise sources, and their minimisation, followed by an exemplary performance optimisation procedure for homogeneous refractive index measurements.

Chapter 4 Biomolecule detection performance with C-reactive proteins in an direct primary additive immunoassay using both a high-quality narrow-linewidth laser and an inexpensive hand-held laser source for demonstrating point-of-care compatibility, covering sub-goal two.

Chapter 5 Sub-goal three: Specific detection of IgG and IgE amoxicillin antibodies in an direct primary additive immunoassay for quantitative penicillin allergy detection applications.

Chapter 6 The final summary of the obtained results, their conclusion and future prospects.

Appendix A Appendix with additional and complementary content.

Appendix B Spanish summary of the presented thesis.

Appendix C Curriculum Vitae of the author.

2. Integrated Photonics for Biosensing

In this chapter the parallels between photonics for telecommunications and biosensing will be first highlighted (Section 2.1). In Section 2.2, some necessary foundations of light propagating in dielectric waveguides is given to establish a basic understanding of how they physically operate, and how their structural manipulation can help to find a proper design for biosensing purposes. Additionally, other important photonic structures are shortly reviewed. In the following Section 2.3 the typical and the coherently detected Mach-Zehnder interferometer are discussed. Based on this a more elaborated state-of-the-art will be presented and discussed in Section 2.4.

2.1. From Data Transmission to Diagnostics

In the recent decades integrated silicon photonics has become of mayor interest in the biosensing community. Not only due to its compatibility with the CMOS manufacturing process, potentially enabling inexpensive mass production, but also thanks to possible miniaturised sensing structures which are advantageous for lab-on-chip solutions. Furthermore, integrated photonic biosensors have demonstrated ultra-low detection limits. Before being adapted for sensing purposes, it has been mainly applied to optical telecommunications, i.e. long-haul connections (between continents), fibre-to-the-home and data centre interconnects to name a few. Advances in this field over the last 30 years have lead to an exponential grow of the worldwide data rate [102].

Nowadays, almost every data link relies on optical communications, including wireless data links such as 5G: even though the access network uses radio waves propagating through free-space, the links between the antennas and the core network are fibre based. A typical fibre data link is shown in Fig. 2.1(a). The transmitter uses an electro-optical modulator, which is fed by the optical laser source, to send data to the receiver. The output of the modulator has to pass through the optical channel, this may be a fibre or free-space propagation, before it is detected on the receiver side by an optical-electrical converter, i.e. a photodiode. The optical channel adds a perturbation to the optical signal disturbing it in such a way that the data reconstruction on the receiver side is altered. Different optical effects may be the cause of this modification, such as chromatic and modal dispersion, propagation losses, polarisation mixing etc. In order to compensate these impairments, digital signal processing is used on the receiver side. Generally speaking, the goal in telecommunication is to increase the data rate by decreasing the channel perturbation or its impact [82].

Photonic sensing (and photonic biosensing) is very similar to this concept but with the difference that there is no transmitter. In sensing the time varying channel perturbation is the information the receiver wants to observe – the channel acts as a “transmitter”. Figure 2.1(b) illustrates this scenario. Two effects of the perturbation are often of most interest for the receiver: alterations in the absorption and propagation velocity. Both provide indications about physical modifications of the optical channel. Technically speaking, the objective in sensing is to amplify the channel perturbation and detect it at the receiver side. Due to this high similarity between both applications, many aspects and techniques can be transferred from optical communications to biosensing such as the coherent detection scheme which is applied to the investigated Mach-Zehnder interferometer.

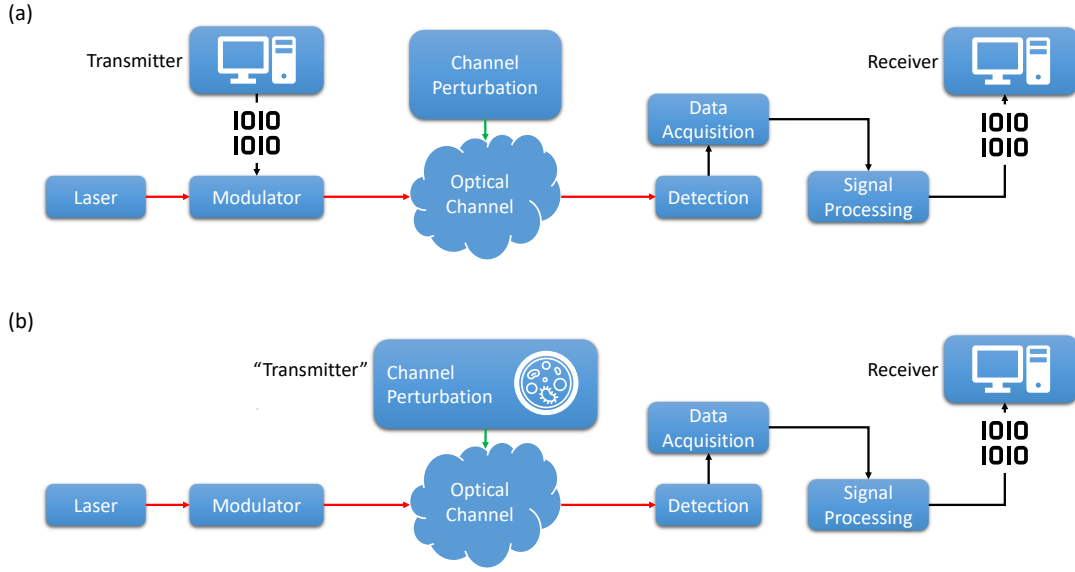


Figure 2.1.: (a) Showing a conceptional flow diagram of optical data links. (b) Schematic flow diagram of optical sensors.

2.2. Some Fundamentals of Photonics

Generally, photonics deals with the generation, emission, amplification, transmission, and detection of light in the visible, and near- to mid-infrared. In the context of the investigated integrated photonic biosensor within this thesis, the focus lies on the transmission, i.e. characterisation of light propagation through the sensor and other supporting elements.

2.2.1. Electrodynamics

In 1873, James Clark Maxwell published his work "Treatise of Electricity and Magnetism" [103], forming the foundation of modern electrodynamics and predicting the existence of electromagnetic waves. Within this theory he connected electric and magnetic phenomena into one electromagnetic set of fundamental laws, often referred to as "Maxwell's equations":

$$\nabla \cdot \vec{\mathbf{D}} = \rho, \quad (2.1)$$

$$\nabla \cdot \vec{\mathbf{B}} = 0, \quad (2.2)$$

$$\nabla \times \vec{\mathbf{E}} = -j\omega\vec{\mathbf{B}}, \quad (2.3)$$

$$\nabla \times \vec{\mathbf{H}} = \vec{\mathbf{J}} + j\omega\vec{\mathbf{D}}. \quad (2.4)$$

$\vec{\mathbf{D}}$ and $\vec{\mathbf{B}}$ being the vectors of the electric displacement and magnetic induction, $\vec{\mathbf{E}}$ and $\vec{\mathbf{H}}$ the electric and magnetic field vectors, ρ the electric charge density, $\vec{\mathbf{J}}$ the electric current density vector, $j = \sqrt{-1}$ the imaginary unit, and $\omega = 2\pi f$ with f the oscillation frequency of the electromagnetic wave. Complementary information about the influence of materials and their boundary conditions can be found in the Appendix A.1.1.

Electrodynamics describes the propagation of electromagnetic waves, such as radio waves, visible light, X-ray, and gamma radiation. The only difference between them is their oscillation frequency f . In many photonic applications, light, in the visible ($f = 384 \rightarrow 789$ THz) to

near-infrared ($f = 100 \rightarrow 384$ THz), propagates through media where there are no charges, i.e. $\rho = 0$ and $\vec{\mathbf{J}} = 0$. This condition simplifies Eqs. (2.3)-(2.4) in an homogeneous and isotropic medium to

$$\nabla^2 \vec{\mathbf{E}} - \mu \varepsilon \omega^2 \vec{\mathbf{E}} = 0, \quad (2.5)$$

$$\nabla^2 \vec{\mathbf{H}} - \mu \varepsilon \omega^2 \vec{\mathbf{H}} = 0. \quad (2.6)$$

with $\mu = \varepsilon_r \varepsilon_0$ the dielectric and $\mu = \mu_r \mu_0$ the permeability tensors, and the constants $\varepsilon_0 \approx 8.854 \times 10^{-12}$ F/m and $\mu_0 = 4\pi \times 10^{-7}$ H/m the permittivity and permeability, respectively, of vacuum.

2.2.2. From Plane Waves to Guided Modes

So far, many sets of $\{\vec{\mathbf{E}}, \vec{\mathbf{H}}\}$ satisfy Eqs. (2.5) and (2.6). One set of these equations is called monochromatic plane wave, i.e. waves of one frequency and plane phase front [104]. In photonics, dielectric materials, i.e. having a permeability $\mu_r = 1$, are used to create what is called a photonic waveguide in which such a plane wave, supported by total internal reflection (TIR), propagates in a certain direction. Assuming a constant monochromatic excitation, each component of $\vec{\mathbf{E}}$ and $\vec{\mathbf{H}}$ can therefore be described by

$$\Psi = A e^{j(\omega t - \vec{\mathbf{k}} \cdot \vec{\mathbf{r}})}, \quad (2.7)$$

using phasor notation. A the amplitude, t the time, $\vec{\mathbf{k}}$ the wavevector, and $\vec{\mathbf{r}}$ the position in space. Wavefronts with constant phase ($\vec{\mathbf{k}} \cdot \vec{\mathbf{r}} = \text{const}$) propagate in $\vec{\mathbf{k}}$ direction. In a lossless homogeneous medium, the absolute value of the wavevector becomes

$$|\vec{\mathbf{k}}| = \frac{2\pi}{\lambda} \frac{c}{v} = \frac{2\pi}{\lambda} n \quad (2.8)$$

with $\lambda = c/f$ the wavelength (distance between neighbouring phase fronts of the same phase), $c \approx 299\,792\,458$ m s $^{-1}$ the speed of light in vacuum, v the propagation or phase velocity of the wave, and $n = \sqrt{\varepsilon_r \mu_r}$ the refractive index.

One important aspect for light propagation in a waveguide is called total internal reflection (TIR). Referring to Figure 2.2, we consider two materials with refractive indices n_1 and n_2 and a planar wave propagating from the first material towards the second material at an angle θ_1 to the surface normal. The absolute value of its wavevector $|\vec{\mathbf{k}}_1|$ can be expressed as

$$|\vec{\mathbf{k}}_1|^2 = \left(\frac{2\pi}{\lambda} n_1 \right)^2 = \beta_{1,z}^2 + \beta_{1,x}^2. \quad (2.9)$$

When touching the surface some part of the light is transmitted into material 2 at an angle θ_T , whereas the rest is reflected back into material 1 at an angle $\theta_R = \theta_1$, as shown in Fig. 2.2(a). Their wavevectors $\vec{\mathbf{k}}_T$ and $\vec{\mathbf{k}}_R$ derive the same way as $\vec{\mathbf{k}}_1$. The continuity of the waves at the interface of material 1 and 2 implies that $\beta_{T,z} = \beta_{R,z} = \beta_{1,z} = (2\pi/\lambda) n_1 \sin(\theta_1)$, i.e. incident, reflected and transmitted wave propagate the same velocity in z -direction. Snell's law can be directly derived from this condition:

$$\frac{n_1}{n_2} = \frac{\sin(\theta_T)}{\sin(\theta_1)}. \quad (2.10)$$

The x -component of the transmitted wave's k -vector plays a crucial role for the reflection:

$$\beta_{T,x} = \frac{2\pi}{\lambda} \sqrt{n_2^2 - n_1^2 \sin^2(\theta_1)} \quad (2.11)$$

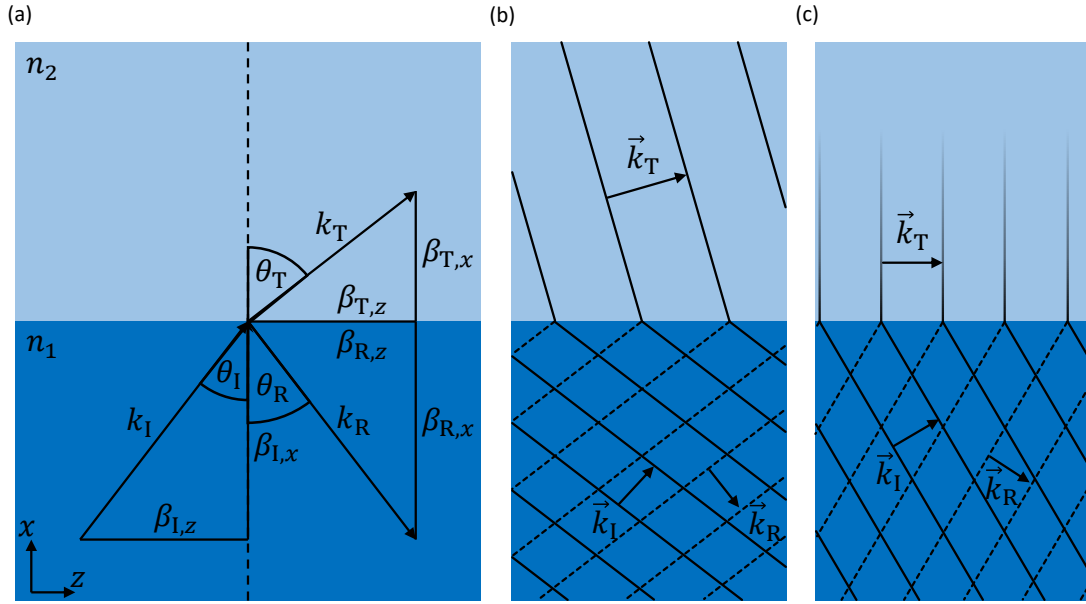


Figure 2.2.: (a) The k -diagram of an infinitely large planar incident wave (k_I) in medium n_1 . k_R is the resulting plane wave reflection, and k_T the transmission in medium n_2 . (b) The incident k_I wave and the excited transmitted plane wave k_T , the black lines showing the phase fronts with distance 2π . (c) Incident phase fronts in n_1 excite exponentially decaying wave in n_2 , due to TIR.

For angles $0 \leq \theta_I < \theta_{\text{TIR}}$, $\beta_{T,x}$ has a real solution, for which the transmitted wave travels in x and z direction, as shown in Fig. 2.2(b). In case $\theta_{\text{TIR}} \leq \theta_I \leq \pi/2$, Eq. 2.11 only has a complex solution with a real part $\Re(\beta_{T,x}) = 0$, which results in an exponentially decaying electromagnetic field along x - all the incident power is thus reflected back (see Fig. 2.2(c)). Angle θ_{TIR} is therefore called the angle of total internal reflection.

Until now, an infinitely-large planar wave in an homogeneous and isotropic materials has been assumed. However, optical waveguides are structures in which light is confined in a finite region while travelling in propagating direction, therefore occupying an effectively small area or volume. The refractive index distribution of a simple example of a waveguide structure is shown in Fig. 2.3(a), in which a two-dimensional dielectric optical guide, consisting of two different materials with refractive indices n_c , and n_s , is used. The material with refractive index n_c is called the waveguide core, whereas its surrounding material with n_s is often referred to as the cladding of the waveguide. The whole structure is homogeneous along the propagation direction z . To obtain TIR in the core, n_c has to be larger than n_s . The refractive index of the whole device is

$$n(x) = \begin{cases} n_s, & |x| \geq d/2 \\ n_c, & |x| < d/2 \end{cases} \quad (2.12)$$

Light travelling through it can have various forms, in fact, an electromagnetic wave in this structure is a superposition of these forms:

$$\left\{ \vec{E}, \vec{H} \right\} (x, z, t) = \sum_m \eta_m \left\{ \vec{E}_m, \vec{H}_m \right\} (x) e^{j(\omega t - \beta_m z)} \quad (2.13)$$

assuming the power of the waves to be normalised, i.e. $\int \vec{E}_m \times \vec{H}_m dx = 1$ and $\sum_m \eta_m = 1$. Each electromagnetic field profile $\left\{ \vec{E}_m, \vec{H}_m \right\}$ propagating in z -direction which is a solution to Eqs. (2.5) and (2.6) is called a guided mode. Parameter $\beta_{m,z}$ is the propagation constant

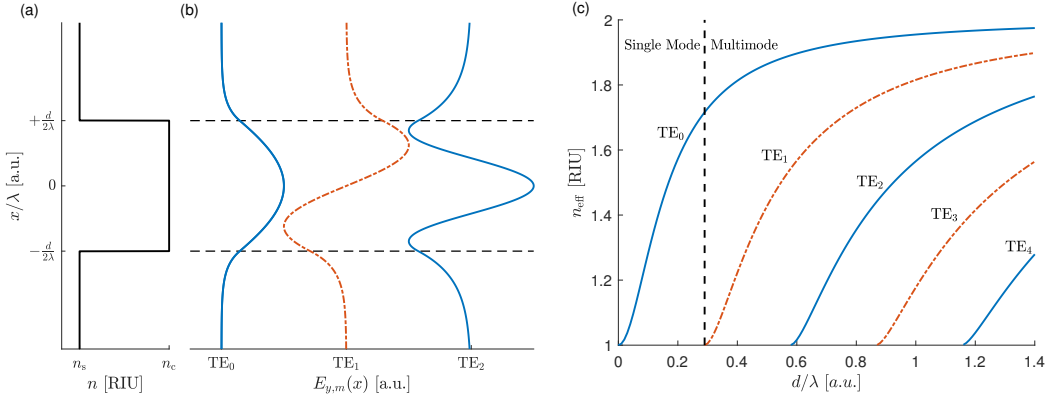


Figure 2.3.: (a) 2D slab waveguide refractive index distribution along the width axis x , (b) the field profiles of the first three modes, and (c) the effective refractive index diagram showing single mode and multimode condition. Blue lines are odd and orange dashed lines are even modes.

of m -th order mode. There are two categories of these modes, transverse electric (TE) and transverse magnetic (TM). In this 2D case, TE means that the electric field only has a x component, whereas TM means the same for the magnetic field. For simplicity, only TE modes will be derived for the structure in Fig. 2.3(a). Eq. (2.13) can be derived to

$$\left[\frac{\partial^2}{\partial x^2} + k_0^2 n(x)^2 - \beta_{m,z}^2 \right] \vec{E}_m(x) = 0, \quad (2.14)$$

which has to be solved for each region separately, i.e. core and both surroundings, connected by the boundary conditions. The parameter $k_0 = 2\pi/\lambda$ is called wavevector in vacuum. The corresponding magnetic field can be calculated using Maxwell's Eq. (2.4). For an exponentially decay of the electric field, implying $\lim_{|x| \rightarrow \infty} |\vec{E}_m(x)| \rightarrow 0$, outside the core $k_0^2 n(x)^2 - \beta_{m,z}^2$ must be negative while inside the core it has to be positive to maintain propagation. Therefore, guided modes must satisfy

$$k_0 n_s < \beta_{m,z} < k_0 n_c \Leftrightarrow n_s < n_{\text{eff},m} < n_c \quad (2.15)$$

with $n_{\text{eff},m}$ the effective refractive index, in effective refractive index units (eRIU), of mode m , indicating its phase velocity in comparison to the speed of light in vacuum.

A mathematical approach to solve for propagatable modes can be found in Appendix A.1.2. However, another approach can be done by just considering TIR along propagation direction and a constructive interference condition along the waveguide cross section. The light propagating in an infinitely long straight waveguide bounces back and forth in the guided core with an incident-/reflective angle of $\theta_I = \theta_R > \theta_{\text{TIR}}$. In order to be guided, i.e. without any losses, within this structure, the light reflected from both side walls need to create a constructive interference pattern. This implies that the propagation constants along the cross sections, $\beta_{I,x}$ and $\beta_{R,x}$, take multiples of π after travelling the distance d , the waveguides width, in x -direction, i.e. $\beta_{I,x}d = \beta_{R,x}d = m\pi$. Due to this restrictions it can be shown that for every value of d , at a given wavelength λ , there is only a discrete number of M guided modes possible, i.e. $m \in [0, M-1]$. For the considered symmetric waveguide structure there is always at least one valid solution to the problem, with increasing d the number of possible mode increases accordingly. The electric field distributions of such as structure, supporting three TE modes, is shown in Fig. 2.3(b). Figure 2.3(c) shows the effective refractive indices for each mode as a function of the waveguide width d . Structures supporting just the fundamental

mode, i.e. $M = 1$, are called single mode waveguides, whereas for $M > 1$ they are commonly referred to as multimode waveguides. It is worth highlighting that modes of the same photonic structure are orthogonal to each other, meaning that no mode can be expressed as a linear combination of other modes, this includes TE and TM polarised modes. Single mode waveguides or fibres are often demanded by application as enabling multimode propagation would decrease performance. However, for some application, the existence of various modes travelling in a photonic structure enables the manipulation of amplitude and phase of the propagating light. Later in this chapter some of these structures will be described in more detail. Real photonic devices, however, are not limited to two dimensions and, with the exception of very simple geometries, no analytical solution exists for Eqs. (2.5) and (2.6). Therefore, different methods can be used to calculate the modes numerically such as finite difference time domain, finite element method, beam propagation method, and eigenmode expansion. Several commercial software tools exist to solve Maxwell's equations. For instance, RSoft is a finite difference based solver, while FIMMWAVE/FIMMPROP relies on the eigenmode expansion method. However, the design and simulation of applied photonic structures itself are not within the main focus of this work and, hence, are not discussed in the following.

2.2.3. Photonic Design

The most relevant properties and design for the photonic structures used throughout this work are shortly discussed in this section, however, the full design and optimisation process are not within the main scope of this work.

Photonic integrated chips can be manufactured with the CMOS fabrication line. This makes a mass production not only possible, but also very cheap. A general description of the fabrication process for PICs can be found in the Appendix A.1.3. In telecommunications SOI, i.e. silicon (Si) waveguides are defined on top of a silicon dioxide (SiO_2) layer, are often used, but in biosensing silicon nitride on insulator is also an attractive candidate due to the lower propagation losses from the near-infrared to the visible regime [105, 106]. The latter is applied for the discussed sensors in this work.

In Fig. 2.4(a) the cross section of a typical strip waveguide is shown. The silicon nitride core with refractive index n_c , has a thickness H_{WG} , a width W_{WG} , and a length L_{WG} (along propagation direction z). The immediate surrounding consists of silicon dioxide with a refractive index $n_i < n_c$, which includes the top cladding and the bottom cladding with thickness H_{BOX} . This geometry is commonly referred to as a completely buried waveguide – the intensity profile of the fundamental TE mode intensity, i.e. $\propto |\vec{E} \times \vec{H}^*|$, is overlaid onto the waveguides geometry. The mode parameters can be considered constant because the waveguide environment does not change. Indeed, the fundamental mode travels along the propagation direction z with a constant effective refractive index n_{eff} . As previously discussed, the mode profile inside the core depends on the mode order, in this case half a sinusoidal oscillation, whereas the profile in the surrounding is exponentially decaying along the cross section. A sensing waveguide is rather similar (see Fig. 2.4(b)), but some of the surrounding material is removed, in this case the upper cladding, thereby exposing the waveguide to the environment. Usually this environment is a liquid, flowing over the sensors surface, containing the analyte to be detected. For simplification purposes, first we assume this fluid to have a homogeneous refractive index $n_s < n_c$.

In the following the design of sensing waveguides will be discussed in more detail. Apart from that, other necessary photonic devices will be introduced, such as tapers, bended waveguides, MMIs, and gratings which are essential for most sensor architectures.

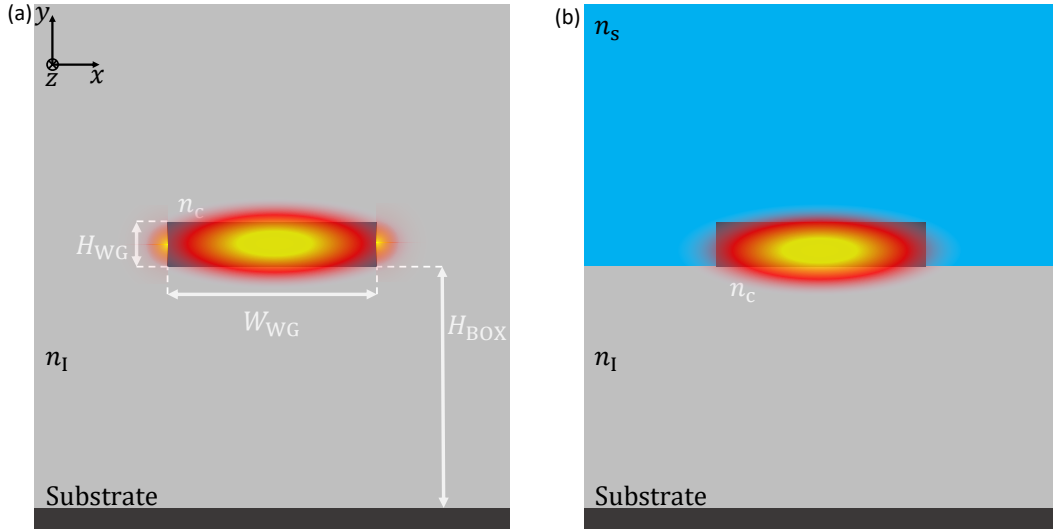


Figure 2.4.: (a) Common strip waveguide buried in insulator and its corresponding fundamental mode. (b) Sensing Waveguide, exposed to its environment and the resulting supported fundamental mode.

2.2.3.1. Sensing Waveguide Designs

The difference between a conventional interconnecting waveguide and a sensing waveguide becomes apparent when considering the mechanism that is used to detect changes in the waveguides environment.

For the sensing waveguide, the exponential decay of the evanescent field depends on the refractive index of the surrounding liquid n_s . The distance, from the edge of the core to the point where the mode intensity has decayed to $1/e$ of the value at the edge, is called penetration depth. For commonly used strip waveguides, this value can be around 50 - 100 nm for the fundamental TE mode [107], increasing with higher mode number. Refractive index changes within this penetration depth have a direct impact on the mode velocity. Therefore, alterations of the close environment of the core will lead to a modification in propagation speed (and Δn_{eff}), which can be expressed as a phase shift

$$\Delta\varphi = \frac{2\pi}{\lambda} L \Delta n_{eff}(\Delta\gamma) \quad (2.16)$$

along a sensor with length L , with $\Delta\gamma$ a change of the physical parameter γ , triggering a refractive index change within the direct surrounding of the sensing waveguide. Additionally, the substance may cause a change in attenuation $\Delta\alpha$. But typically, in refractive index biosensing, alterations in $\Delta\alpha$ are very small and, therefore, can be neglected. How strongly the effective refractive index alters due to this parameter variation is defined as waveguide sensitivity, i.e. $S_{WG} = \partial n_{eff} / \partial \gamma$ [108]. In bulk sensing the waveguide sensitivity is defined by homogeneous alterations of n_s , the refractive index of the surrounding: $S_{WG} = \partial n_{eff} / \partial n_s$. For this case, perturbation theory suggests the electromagnetic field profile unchanged due to this modification, hence, the change of the effective refractive index Δn_{eff} of the corresponding mode would linearly relate to Δn_s . The waveguide sensitivity can be approximated by

$$S_{WG} = \frac{\partial n_{eff}}{\partial n_s} \approx \frac{n_s}{n_{eff}} \int_{A_s} |\vec{E}|^2 dA, \quad (2.17)$$

with A_s the region which experience the homogeneous refractive index change, and $\vec{E}$ the normalised electric field profile of the TE mode [88, 109]. The phase change of the sensing

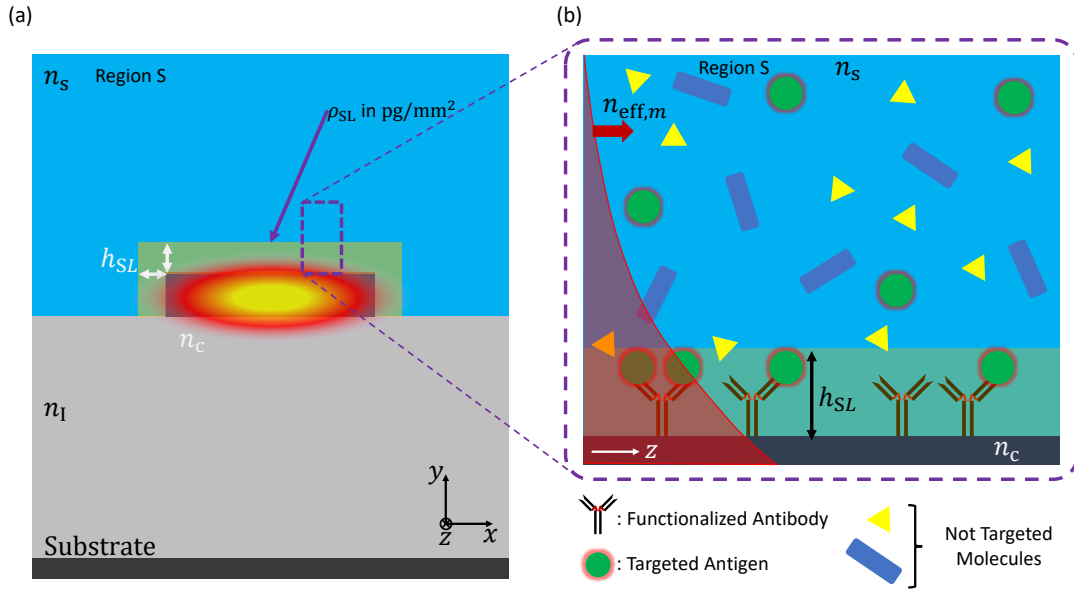


Figure 2.5.: (a) Sensing waveguide, showing the biomolecular layer on top of the core. (b) Zoomed inset of the surface layer. Due to the functionalised recognition protein, here an antibody, only its counterpart, the corresponding antigen, may attach to the surface permanently. Other molecules just bypass the structure with the flow of the liquid.

waveguide adapts to

$$\Delta\varphi = \frac{2\pi}{\lambda} L S_{\text{WG}} \Delta n_s. \quad (2.18)$$

In biosensing applications, the change in region S is not homogeneous, but more concentrated to the region adjacent to the waveguide interfaces, typically within a distance of a few nanometres since biomolecules, like proteins, often have a diameter of a few to tens of nanometres. An illustration for biosensing is shown in Fig. 2.5(a). A small layer, of thickness h_{SL} , surrounds the waveguide. This layer consist typically of a special immobilised bioreceptor molecule to which only one specific target molecule can attach to, e.g. an antibody and its antigen (see Fig. 2.5(b)) [50]. One way to define a (surface) waveguide sensitivity here is to assume a thickness growing analyte layer with constant refractive index on top of the waveguide due to biorecognition:

$$S_{\text{WG}} = \frac{\partial n_{\text{eff}}}{\partial h_{\text{SL}}}. \quad (2.19)$$

However, this approach is more simulative than practical. A more applied method is to define the sensitivity as the change of effective refractive index due to a small sample analyte concentration passing the sensing structure, i.e

$$S_{\text{WG}} = \frac{\partial n_{\text{eff}}}{\partial C_{\text{inj}}}. \quad (2.20)$$

In comparison to the prior definition, this parameter can be measured easily in experiments, as demonstrated in Chapter 4, but its quantity depends on the sample volume and time it needs to pass. There is a high correlation between bulk and surface sensitivity, however, it may vary with the waveguide design, i.e. if HRI sensitivity can be doubled by optimising the design, it does not necessarily mean that surface sensitivity increases by the same amount. In order to increase the light-matter interaction of the sensing mode different material arrangements and

designs can be used to maximise the waveguide sensitivity S_{WG} , typically by delocalising the sensing mode from the core material. However, it will later be shown that for the optimisation of the detection limit of our interferometric sensors the essential factor is the ratio between the waveguide sensitivity and the losses (see Section 3.4.1).

Technology Platform Choosing an appropriate material platform does play a significant role in the design process of the sensing waveguides, but also for all the other components and devices integrated on chip. Some important factors here are the materials for core, underlying cladding, and substrate, the minimum feature size, and the wavelength range of interest. High index contrast materials, such as SOI, help to maximise the waveguide sensitivity for core thicknesses around 200 nm, nonetheless, silicon nitride on insulator performs well or even better for thicknesses $\gtrsim 300$ nm [66]. The minimum feature size does not influence the waveguide sensitivity per se, but potentially limits photonic design process in some way. The waveguide sensitivity does slightly vary with the used wavelength, but in a small wavelength range this change is negligible. However, the applied spectral content should not exceed the transparency window of the technology platform (see appendix Fig. A.2). If we neglect changes in the wavelength sensitivity, then the total phase shift due to variations in effective refractive index becomes larger for smaller wavelengths (see Eq. 2.16). Therefore, sensors operating in the visible potentially enable higher signal shifts than those operating in the infra-red. Complementary information may be found in Appendix A.1.3.

Transverse Electric- or Magnetic Polarisation An important decision for the sensing waveguide design is not only the structural design and the chosen materials, but also the polarisation of the propagating sensing mode. Typical waveguide cross-sections in SOI have larger widths than heights which means that the TM mode is typically more delocalised than the TE mode which increases the light-matter interaction [66, 110]. A thick enough bottom oxide layer is then required to avoid substrate leakage.

Slot Waveguides A typical slot waveguide consists of two rectangular structures running parallel in a small distance to each other. Even though they appear to be two very close waveguides, from the modal point of view they are one structure, designed for single mode operation. Slot waveguides are typically designed for TE polarised light. Due to the boundary condition between the surrounding and the core material along the horizontal axis and the proximity of both cores, a large amount of the modal power is pushed into the gap between them [111]. The light confinement between the core materials dominates both HRI and surface sensitivity. Slot waveguides have demonstrated high bulk and surface sensitivities in both simulation and experiment, up to 0.83 RIU/RIU or 1.6 mRIU/nm [112, 108].

Subwavelength Grating Waveguides A subwavelength grating (SWG) is different to the typical ridge, slab, or slot waveguide. While those have a cross section which is ideally unchanged along the propagation direction, SWG are periodic structures with a periodicity Λ , that is smaller than the wavelength propagating through them. The most simple structure would consist of equally sized rectangulars arrayed periodically along propagation direction. For $\lambda/\Lambda > 2n_{BF}$, according to Bloch-Floquet theory [113, 114], there exists a guided mode with an effective refractive index n_{BF} . For sensing SWGs behave as homogeneous metamaterial with a refractive index described by the Rytov equations [115]. The shorter the length of the rectangulars, the lower the effective refractive index, thus deconfining the mode field into the surrounding. Therefore, a higher surface and homogeneous sensitivity can be accomplished. SWG have demonstrated sensitivities up to 0.45 RIU/RIU and 2.0 mRIU/nm in experiment [108, 116, 117].

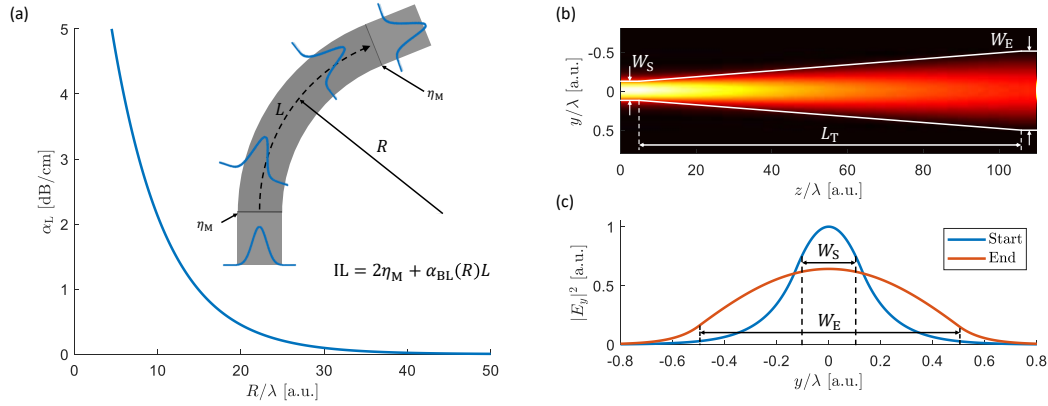


Figure 2.6.: (a) Losses introduced due to a bended waveguide section. (b) Linear taper design and the intensity mode profile along propagation direction. (c) Mode profile at the start and end of the taper section.

2.2.3.2. Other Relevant Photonic Structures

Photonic sensing waveguides act as transducer between the biochemical modifications and the optical signal, but several other photonic components are necessary to read-out the sensor signal. In the following, some structures are presented and explained in a compact way, explaining their purpose, and a abbreviated description of their working principle.

Curved Waveguides So far, only straight waveguides have been considered. However, placing one photonic structure after another would possibly stretch the total dimension beyond the chip size or may not be even feasible. Curved waveguides are used to redirect the propagation direction on chip. A straight waveguide may be considered a bent waveguide with a bending radius $R \rightarrow \infty$. Let us now assume that R has a finite value as illustrated in Fig. 2.6(a). Light propagating along the outer part of the curve opposite bend direction has to travel faster than the light along the inner part of the curve to maintain its mode pattern. The velocity of the wavefront along its cross section can be expressed as a function of the distance to the pivot of the bend: $v(r) \propto r/R \cdot c/n_{\text{eff}}$. This leads to a velocity mismatch, as some of the mode's electromagnetic field would have to propagate faster than $c/n_{c,s}$ the speed of light in the core or the surrounding medium, depending on r , which is by definition not possible. The mode's exponentially decaying field theoretically penetrates the surrounding up to infinity, even though being there infinitely small. Therefore, there is always some part of the field at a position r for which we would have $v(r) > c/n_{c,s}$. This fraction of light is radiated, so that every bent mode is a leaky mode, i.e. a mode with intrinsic losses apart from scattering or absorption [104]. As the bent radius is decreased radiation becomes stronger. Bending losses for a given bending radius R are typically indicated in dB/cm or dB/90°, the former for a length L the latter for a 90° curved waveguide. In Fig. 2.6(a) an example for bending losses is shown. The tighter a bend, the more densely can devices be integrated. However, a trade-off has to be found between losses and integration density.

Taper Tapers are transition structures to modify the mode size of the connecting waveguide, for example for multimode interference couplers and grating couplers as we will discuss below. An exemplary taper is shown in Fig. 2.6(b). Typically, the single-mode waveguide cross section is enlarged or downsized to the required width, however, possibly enabling multimode propagation. To avoid higher order mode excitation, the taper is widened symmetrically and sufficiently

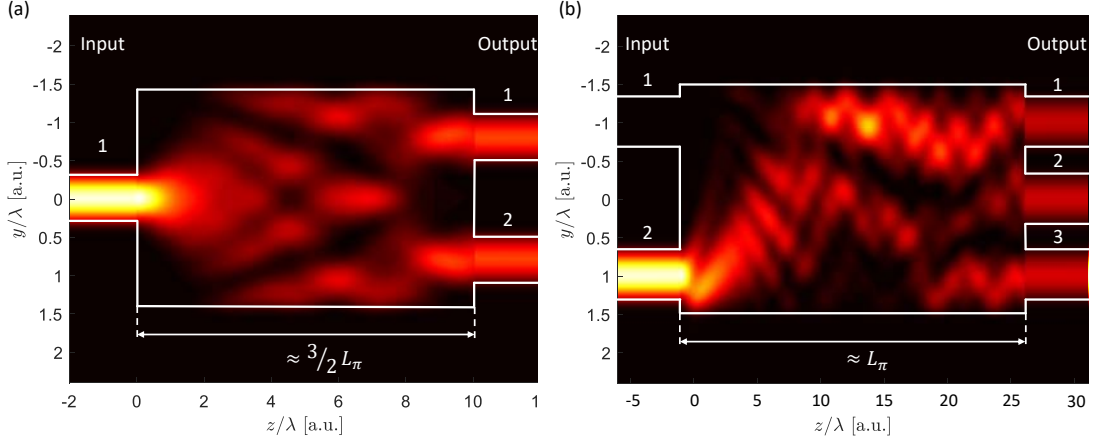


Figure 2.7.: Light propagation in an 1×2 (a) 2×3 (b) MMI coupler.

slowly, such that power stays in the ground mode. The transition between the different cross section is often realised linearly. Unfortunately, this ground mode expansion/reduction, results in unavoidable radiation. These losses become higher with shorter taper length, so that a compromise between taper length (affecting integration density), and signal attenuation has to be found.

Multimode Interference Couplers Multimode couplers can be used to split light into different paths, varying in power and phase, e.g. a simple 50:50 power splitter (see Fig. 2.7(a)) or the 2×3 120° coupler in Fig. 2.7(b). Their operation is based on the self-imaging principle, which describes how a certain electromagnetic field profile, at the beginning of a multimode section, supporting M modes, develops while travelling in propagation direction and reproduces after periodic distances [118]. An important parameter of an MMI is its half-beat length (L_π), defined by the relation

$$2(n_{\text{eff},0} - n_{\text{eff},1})L_\pi = \lambda, \quad (2.21)$$

where $n_{\text{eff},0}$ and $n_{\text{eff},1}$ are the effective refractive indices of the first and second mode, respectively. It can be shown that as long as $M > N + 1$, there will be N replicas of the input field after propagation distances

$$L = 3 \frac{p}{N} L_\pi, \quad p \in \mathbb{N}_{\text{odd}}, \quad (2.22)$$

with $\mathbb{N}_{\text{odd}}$ being natural odd numbers without zero. The simplest design is the power splitter shown in Fig. 2.7(a). Its transfer function can be written as

$$\mathbf{H}_{1 \times 2} = \frac{1}{\sqrt{2}} \begin{pmatrix} 1 \\ 1 \end{pmatrix} e^{j\varphi_{1 \times 2}} \quad (2.23)$$

with $\varphi_{1 \times 2}$ being a constant phase shift. This matrix represents the amplitude of the fundamental mode in each output waveguide, when light is launched into the input waveguide. In the following, this parameter is assumed to be zero, to simplify coming derivations, and due to the fact that it does not have any effect for the subsequent results.

The same self-imaging principle can be applied to design a 2×3 MMI which is a key element of the proposed coherent architecture. A typical design for a 2×3 MMI coupler is presented in Fig. 2.7(b). The purpose of this device is to split the incoming light from one port equally to all three outputs, but with a 120° phase shift between them, which is achieved at the distances

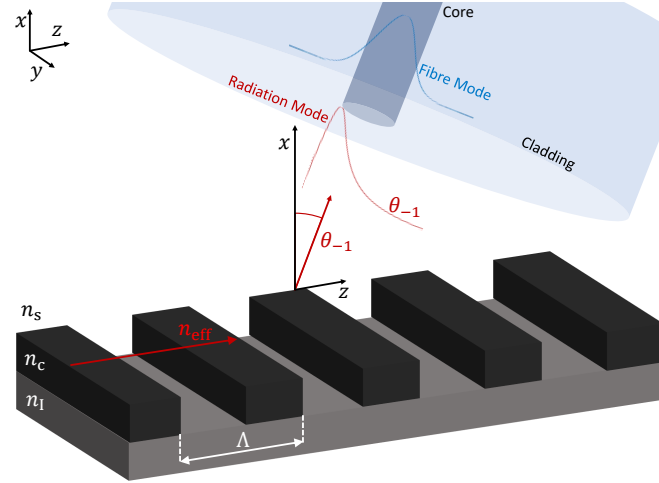


Figure 2.8.: Radiation Mode (red) of the periodic grating and the Gaussian fibre mode (blue).

$L = p/3 \cdot L_\pi$. The transfer matrix for the upper port can be written as

$$\mathbf{H}_{1 \times 3} = \frac{1}{\sqrt{3}} \begin{pmatrix} 1 \\ e^{j120^\circ} \\ e^{-j120^\circ} \end{pmatrix} e^{j\varphi_{2 \times 3}} \quad (2.24)$$

with $\varphi_{2 \times 3}$ a constant phase shift. The other input port has a similar transfer function, however, due to symmetry in reverse order. Hence, the overall transfer matrix becomes [119]

$$\mathbf{H}_{2 \times 3} = \frac{1}{\sqrt{3}} \begin{pmatrix} 1 & e^{-j120^\circ} \\ e^{j120^\circ} & e^{j120^\circ} \\ e^{-j120^\circ} & 1 \end{pmatrix} e^{j\varphi_{2 \times 3}}. \quad (2.25)$$

Higher order MMIs, such as the 2×3 and 2×4 , are typically used in telecommunications to coherently detect an optical signal, i.e. measuring the optical amplitude or power, and phase. A 2×4 MMI is the preferred choice for high speed communications, as amplitude and phase information can be easily obtained by analogue means from the four outputs. A 2×3 MMIs is advantageous for low speed applications as phase and amplitude can be digitally obtained from the three outputs while having lower losses and higher bandwidth than the 2×4 counterpart. The advantage of coherent detection in sensing will be discussed in detail in Section 2.3.2.

Insertion losses, due to mode coupling are a common source for performance degradation and are therefore an important optimisation parameter during the design process. Additionally, through imperfections during the fabrication, the received power for each output may vary, resulting in called imbalance between the output powers.

Grating Coupler Grating couplers are geometrically similar to SWGs, indeed the main difference is the higher period Λ in comparison to the wavelength λ . However, the application is entirely different. Grating couplers transfer the horizontal propagating waveguide mode into a radiating vertical beam and vice versa. This beam can be detected by a CMOS camera above the grating, or as in this work, be coupled into a fibre array, a solid block with periodically placed fibres. Let us consider the case presented in Fig. 2.8, showing the 3D side-view schematic of a simple grating coupler. Operating this structure beyond the SWG condition, i.e. $2\Lambda > \lambda/n_{\text{eff}}$, leads to diffracted plane waves radiated at angles $\theta_m \in [-\pi/2, \pi/2)$ with m the diffraction order. Gratings are often designed to support just one diffraction order ($m = -1$).

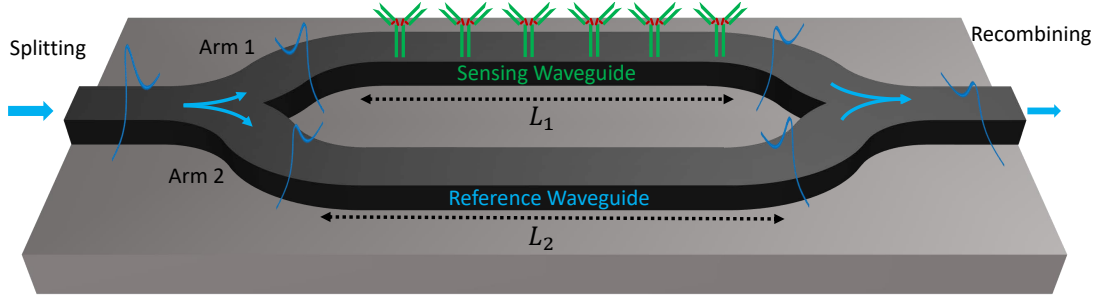


Figure 2.9.: Integrated Mach-Zehnder interferometer realisation for biosensing.

Another important factor is the mode matching of the diffraction order beam and the mode of the fibre, illustrated in Fig. 2.8. The profile of the radiated light (profile in red) has to be designed to match with the fibre mode (profile in blue) [120]. A basic grating, as the one shown in Fig. 2.8, radiates most of the light at the beginning of the grating, and the radiated power decays exponentially along the propagation direction of the grating. Therefore, the coupling efficiency is not very high for this type of grating. However, modifying the grating structure with developed design rules, can lead to highly efficient couplers [121, 122]. Several alternatives can be found in literature to achieve this [123, 124, 125].

2.3. Mach-Zehnder Interferometric Sensing Architectures

Interferometers are widely used in science and modern technology. They use the wave properties of light to generate constructive or destructive interference of light beams which can then be used to transmit or receive information. Interferometers are used for a wide variety of applications, such as measuring distance [126], wavelength, linewidth of a laser [127], surface roughness [128], refractive index changes [129], chromatic dispersion [130]. Even gravitational waves have been detected with a highly sensitive Michelson interferometer [131]. Furthermore, optical filtering [132], modulation [133], and spectral phase interferometry [134] can be realised with interferometers.

In biosensing, when the analyte binds to the surface of the sensing waveguide, the phase velocity of the propagating mode is altered. This change can, however, not be directly detected, but has to be converted to an intensity change via an appropriate sensing architecture. In this section, two interferometric sensing structures are discussed: the directly detected Mach-Zehnder (MZI) and the coherently detected MZI.

2.3.1. Directly Read Mach-Zehnder Interferometer

This type of interferometer was invented by Ludwig Mach [135] and physicist Ludwig Louis Albert-Zehnder [136] independently, from whom its name was derived. As an example, a common realisation of an integrated MZI is shown in Fig. 2.9. Here, the incoming mode is split into Arm 1 and Arm 2 via a Y-junction, or by any other suitable component such as a 1×2 MMI as done in our biosensing platform. Its function is to split the input power equally according to Eq. 2.23. Generally, both arms are single mode waveguides, made of a similar waveguide cross section with effective refractive indices $n_{\text{eff},1}$ and $n_{\text{eff},2}$. Typically, both arms have different lengths L_1 , L_2 and are constant once fabricated. The phase change obtained by

both arms right before the recombination section can be written as

$$\varphi_1 = \frac{2\pi}{\lambda} n_{\text{eff},1} L_1 \quad (2.26)$$

$$\varphi_2 = \frac{2\pi}{\lambda} n_{\text{eff},2} L_2. \quad (2.27)$$

The transmission matrix of the inputs to the outputs of both arms can be expressed by

$$\mathbf{H}_{\text{rs}} = \begin{pmatrix} e^{-j\varphi_1} & 0 \\ 0 & e^{-j\varphi_2} \end{pmatrix}. \quad (2.28)$$

In sensing one arm, here Arm 2, acts in the role of the reference, i.e. its effective refractive index $n_{\text{eff},2}$ is considered constant for no changes of refractive index in the reference waveguides environment take place. However, Arm 1, the sensing waveguide, is exposed to possible changes of its direct surrounding, for example by biorecognition of a functionalised biomolecule layer (see Fig. 2.9), altering the effective refractive index $n_{\text{eff},1}$. For simplicity we assume here that the recombination is also done by a Y-junction, however, in reverse, which means its transfer function is $\mathbf{H}_{1 \times 2}^T$, the transposed of $\mathbf{H}_{1 \times 2}$. Depending on the observed phases φ_1 , φ_2 at its two inputs, the output of the MZI will result in a constructive or destructive interference. For typical operation, a fixed wavelength source is often applied to interferometric structures. Let λ_0 be the wavelength and P_0 be the power of the light at the input of the splitting section. The lights amplitude and phase at the output of the interferometer can be calculated by

$$A_{\text{out}} = \mathbf{H}_{1 \times 2}^T \mathbf{H}_{\text{rs}} \mathbf{H}_{1 \times 2} \sqrt{P_0} \quad (2.29)$$

$$= \sqrt{P_0} e^{-j\frac{\varphi_1 + \varphi_2}{2}} \cos\left(\frac{\varphi_2 - \varphi_1}{2}\right). \quad (2.30)$$

Ideally, behind the output a photodiode would convert the incoming light into an photocurrent i which is proportional to the squared magnitude:

$$i \propto |A_{\text{out}}|^2 = P_0 \left(\frac{1 + \cos(\Delta\varphi)}{2} \right), \quad (2.31)$$

where $\Delta\varphi = \varphi_2 - \varphi_1$ is the phase difference between the two arms. The relation between phase shift and intensity is connected via the cosine function, and its extraction is therefore not always unambiguous. In telecommunications, this way of measuring the phase shift is called direct detection. Many biosensors have been realised with a direct detection read-out, suffering from mayor drawbacks:

- The least significant, from which all sensors suffer, regardless if interferometric or resonant, is due to the periodicity, in this case of the cosine function. There are an infinite number of solutions of phase differences to each intensity value, i.e. $i \propto \cos(\Delta\varphi_0 + 2\pi m)$ with $m = 0$ the actual solution. However, as long as the sampling interval of the recorded intensity is small enough, such that it is guaranteed that the actual phase difference between two samples is smaller than π , the phase can be tracked, and there is no ambiguity. In Figure 2.10(a) the green dots indicate the mathematical correct but physically wrong solutions to the problem.
- If an entire phase shift, of at least π cannot be observed, or the intensity output range is not known, no phase extraction may be realised. Without knowing this range there are, in principle, infinite solutions to Eq. (2.31).
- Another more serious flaw of this system is the called directional ambiguity. The cosine function is defined over an 2π -interval, and within this range there are two solutions for each intensity. Their derivatives have the same magnitude, but in opposite direction, meaning

$$\left. \frac{\partial i}{\partial \varphi} \right|_a = - \left. \frac{\partial i}{\partial \varphi} \right|_b. \quad (2.32)$$

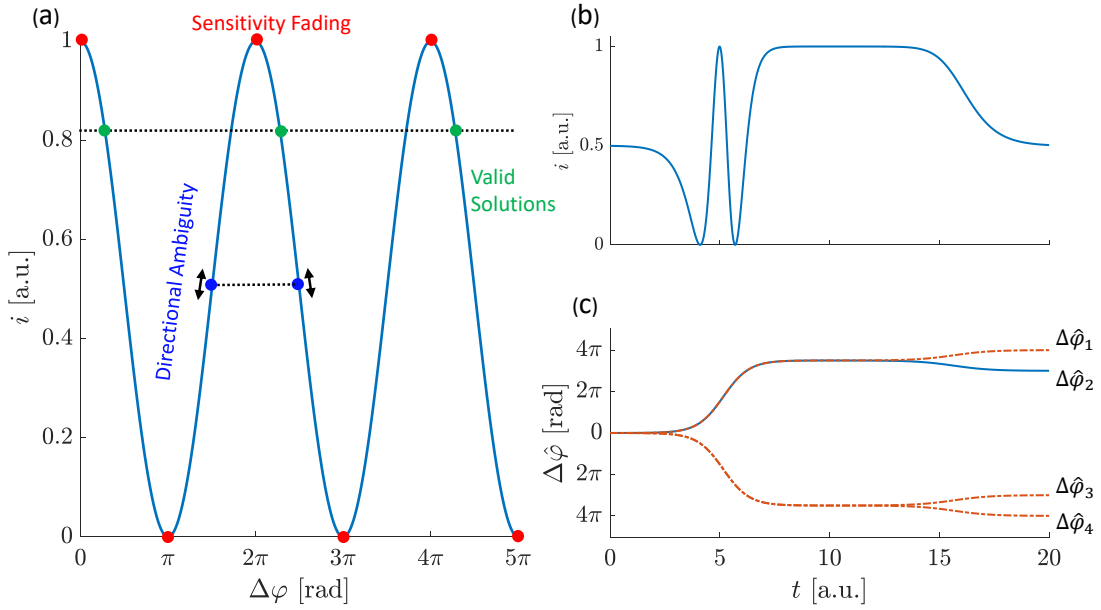


Figure 2.10.: (a) Intensity-phase diagram of an direct read Mach-Zehnder interferometer showing exemplary sensitivity fading, directional ambiguity and solution ambiguity. (b) Intensity changes due to blue phase shift in (c). Orange dashed lines are also mathematically valid solutions.

This ambiguity is highly problematic. Let us consider a direct read MZI, which we just started to read out. In case something is altering the phase in the sensing arm, the extracted phase could either increase or decrease, both being correct mathematical solutions. However, in sensing it is important to know if the phase change is positive or negative, if the targeted analyte attached or anything was removed from the surface. Figure 2.10(a) demonstrates this ambiguity (blue points).

- Sensitivity fading is an additional highly problematic factor. Generally, sensitivity is defined as

$$S = \left| \frac{\partial i}{\partial \varphi} \right| \quad (2.33)$$

and is an important characteristic parameter for biosensors. In this case, it is calculated to be $S \propto |\sin(\Delta\varphi)|$, and has maximums of 1 at $\Delta\varphi = m + \pi/2$, and minima of 0 at $\Delta\varphi = m + \pi$ with $m \in \mathbb{Z}$. This means that with a varying phase signal close to one of this minima, a correct reconstruction may not be obtainable or not even possible. The fading points (red) are shown in Fig. 2.10(a).

To illustrate some of the shortcomings of this interrogation scheme, Fig. 2.10(b) shows the output of an MZI when the phase change $\Delta\hat{\varphi}_2(t)$, shown in blue in Fig. 2.10(c), takes place. However, phase variations $\Delta\hat{\varphi}_1(t)$, $\Delta\hat{\varphi}_3(t)$ and $\Delta\hat{\varphi}_4(t)$, shown in orange in Fig. 2.10(c) yield the exact same output signal. Hence given only the MZI output additional information about what caused the phase change is needed to identify the actual phase shift. It is important to note that this problem does not arise from the interferometric nature of the MZI, but are due to the way of detecting the interferometric signal.

In the last decades different kinds of strategies to circumvent both sensitivity fading and directional ambiguity have been developed [82]. One alternative adds a modulation signal to the direct detection scheme which enables the identification of the correct phase development [47]. This may be realised by modifying the interferometric structure itself. Those techniques are

very similar to interferometric intensity modulation schemes in optical telecommunications, and can be implemented in a variety of ways: The electro-optical effect describes how the propagation speed can be modified by applying an electric field across the waveguide. Depending on direction and strength, the mode velocity can be reduced or accelerated. The same way the magneto-optic effect can be used. Both were successfully demonstrated for sensing and circumventing the aforementioned problems [137, 81]. Those are modulation schemes which are directly applied to the MZI. Using the modulation capable effects requires a change of the interferometer itself by adding sections in one or both arms in which the optical modification takes place. However, this does not come without a price. Integrating modulation schemes complicates the fabrication by either adding more steps and/or utilising materials which are not compatible with the manufacturing process. Furthermore, electrical connections have to be established, for which additional control elements and connections are required. Another approach is to modulate the light source periodically. All-optical phase modulation is a strategy where the laser driving direct current is superposed by an alternating signal, resulting in a periodic wavelength modulation. Through various signal processing steps realised on the detected photocurrent, the phase of the sensor can be extracted with neither fading of sensitivity nor directional ambiguity [138]. A frequency comb optical signal with additional optical demultiplexer has also demonstrated constant sensitivity and unambiguity [139]. Instead of modulating, the MZI can also be used with a wavelength scanning source and a photodetector, or a white light source and a spectrum analyser (or optical demultiplexer with various photodiodes [87]). Just as in the case of a ring resonators, the phase shift information is translated into spectral information [140]. Even though sensitivity fading and directional ambiguity are circumvented, there are special requirements for this case, for example both arms are not allowed to be balanced because the free spectral range would be infinitely large, hence, the sensitivity would be zero, regardless of their lengths (see Fig. 1.12) [141]. However, this approach requires equipment which typically is more expensive, in addition to losing the advantage of a simple read-out.

In this context, Mach-Zehnder interferometer based refractive index sensors can benefit from another read-out technique than directly detecting its signal, called coherent detection. Neither are additional steps required during fabrication, nor are extraordinary materials needed. Furthermore, no modulation scheme is necessary to extract the phase difference without sensitivity fading or directional ambiguity. However, the number of optical outputs, which have to be detected in a distinguishable manner, increases. This is the approach used in this thesis, and will be described in more detail in the following section.

2.3.2. Coherently Read Mach-Zehnder Interferometer

To coherently read an interferometric signal, the output of the MZI has to be modified. Instead of using a simple combiner, e.g. a Y-Junction or a 1×2 MMI, a complex read-out must be established [96]. This can be realised by a MMI structure which has more than two outputs, e.g. a 2×3 MMI [119] as illustrated in Fig. 2.11. From those outputs a complex signal representation of the two MZI arm optical signal difference can be calculated. As in direct detection, the k -th output signal i_k is proportional to the cosine of the applied phase difference $\Delta\varphi$ between both arms, but additionally each arm k experiences a phase shift of $\varphi_k = \varphi_{k-1} + 2\pi/K$, with $\varphi_1 = \text{const}$ a constant parameter. As mentioned in Section 2.2.3.2, a 2×3 MMI splits the incoming mode of each arm equally to its outputs, having a 120° phase shift to each other (see Eq. (2.24)). The output modes phase information gets lost once they are detected by a photodiode, resulting in a current $i_k \propto P_k$. Assuming lossless propagation in both arms, and a

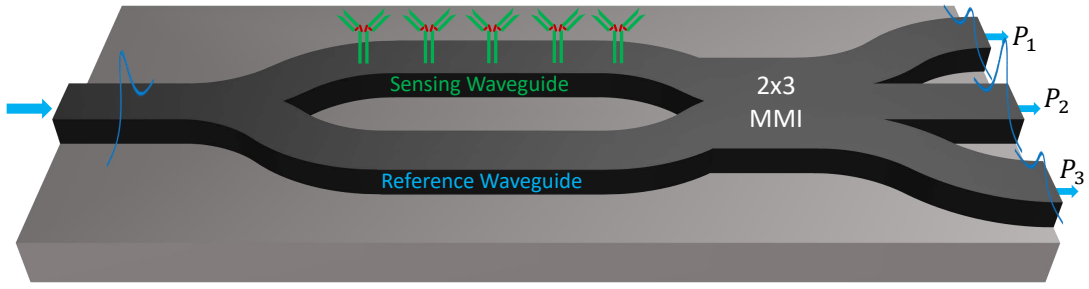


Figure 2.11.: Coherently read Mach-Zehnder interferometer realised with a 2×3 MMI.

phase shift $\Delta\varphi$ between them, each current can be expressed as

$$\vec{i} = \begin{pmatrix} i_1 \\ i_2 \\ i_3 \end{pmatrix} \propto \left\| \mathbf{H}_{2 \times 3} \mathbf{H}_{rs} \mathbf{H}_{1 \times 2} \sqrt{P_0} \right\|^2 \quad (2.34)$$

$$i_k \propto \frac{P_0}{3} \left[1 + \cos \left(\Delta\varphi + \frac{2\pi}{3}(k-2) \right) \right], \quad (2.35)$$

where $\|\mathbf{S}\|^2$ is a matrix operator which squares the absolute value of each entry $s_{m,n}$ of $\mathbf{S} \in \mathbb{C}^{M \times N}$, i.e. $|s_{m,n}|^2$. Each entry of $\vec{i}$ is an interferometric signal, like the conventional case (see Eq. (2.31)), with all the aforementioned disadvantages like sensitivity fading etc. However, they can be used to reconstruct the complex interferometric signal

$$C = A \cdot e^{-j\Delta\varphi} \quad (2.36)$$

of the interferometric recombination between sensing and reference arm which projects the phase change $\Delta\varphi$ onto the unit circle in the complex plane. As in the direct detected case, this is also a periodic transformation, but with the difference that it is proportional to $e^{-j\Delta\varphi}$, and not $\cos(\Delta\varphi)$, from which the phase can be read linearly, i.e. $\Delta\varphi = \arg(C)$. Figure 2.12(a) shows the three photocurrents according to the applied phase change $\Delta\varphi$. A direct detected signal cannot give information in which direction it is moving, whereas a coherent 2×3 MMI detection scheme does provide a unique intensity-phase relation. With the help of $\vec{\mathbf{H}}_{i2C}$, the interferometric retransform vector, the actual complex-valued interferometric signal can be reconstructed. A simple scalar product between the transposed of the vector $\vec{\mathbf{H}}_{i2C}$ and the PD current vector $\vec{i}$ results in the complex signal C [119]:

$$C = \vec{\mathbf{H}}_{i2C}^T \cdot \vec{i} = \begin{pmatrix} -\frac{1}{2} + j\frac{\sqrt{3}}{2} \\ 1 \\ -\frac{1}{2} - j\frac{\sqrt{3}}{2} \end{pmatrix}^T \cdot \vec{i}. \quad (2.37)$$

The complex signal can be decomposed into two components, the in-phase signal (I) on the real axis, and the quadrature-phase signal (Q) on the imaginary axis:

$$I = i_2 - \frac{i_1}{2} - \frac{i_3}{2} = A \cos(\Delta\varphi), \quad (2.38)$$

$$jQ = j\frac{\sqrt{3}}{2} (i_1 - i_3) = jA \sin(\Delta\varphi). \quad (2.39)$$

Similar to the three signal case, they form a unique intensity-phase relation shown in Fig. 2.12(b), this time two sinusoidal signals 90° phase shifted to each other. The complex signal can be expressed as their sum:

$$C = I + jQ = A \cos(\Delta\varphi) + jA \sin(\Delta\varphi) \quad (2.40)$$

$$= Ae^{j\Delta\varphi}. \quad (2.41)$$

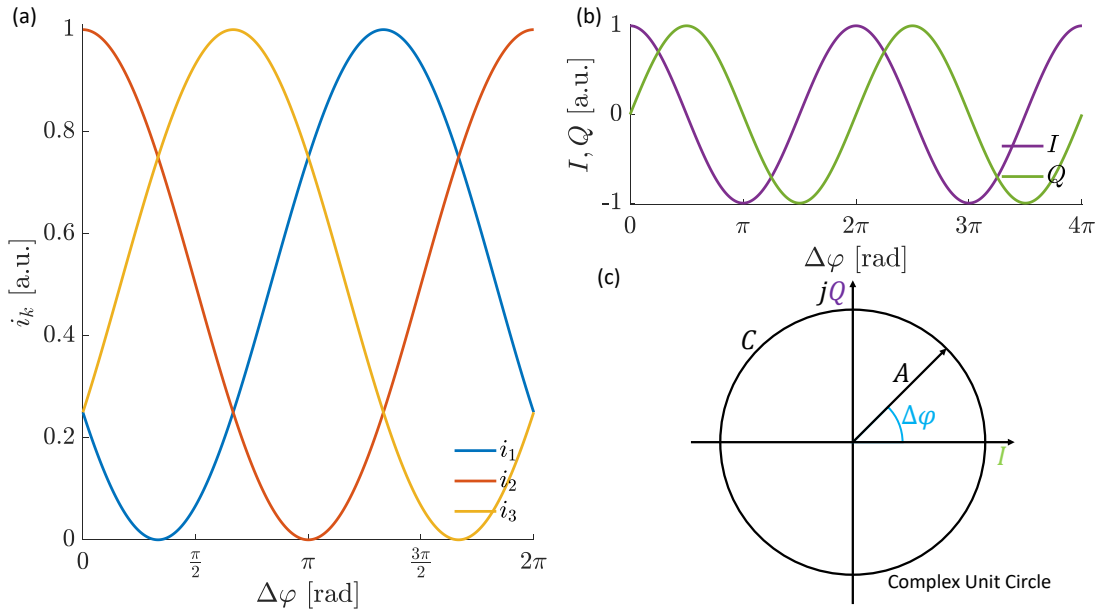


Figure 2.12.: (a) The three resulting interferometric signals from the coherent 2×3 MMI based read-out. For every possible phase $\varphi \in [0, 2\pi)$ there is a clearly defined corresponding detected current i_k constellation. (b) The in- (I) and quadrature-phase (Q) signals calculated from i_k . (c) The complex signal C as a complex weighted sum of the in- and quadrature-phase signal resulting in a linear relation to the applied phase change $\Delta\varphi$.

Due to imperfections in the design, fabrication, or other distortions a calibration for correct phase extraction is necessary. However, this will be discussed in more detail in the system level discussion in Chapter 3.

2.4. State-of-the-Art

The purpose of this chapter is to evaluate the performance of the different types of photonic integrated sensors, developed over the last decades, and their advantages/disadvantages. Furthermore, it serves as a base for the contribution of this work to the current state-of-the-art. To do so, a common ground has to be found on which they can be compared to each other. Before we take a deeper look into the different sensors, the performance parameters have to be understood in a proper manner, meaning how they should be interpreted. First, we may consider the parameters which can be benchmarked between all sensor types.

One of the most important parameters is the limit of detection, revealing the smallest refractive index unit or concentration which can be reliably detected. The lower the detectable smallest refractive index change, the better the LOD, and, therefore, the sensors performance. In clinical applications, the target analyte, may it be an antibody, DNA, or a virus, is normally present in tiny concentrations. Therefore, the sensor system should be designed in a way that the LOD is lower than the lowest concentration that has to be detected. In general that means: the lower the LOD the better. Typically, the first test of a sensor is to determine its HRI (or bulk) LOD, which is done by injecting solutions, differing in concentrations and with known refractive indices, for example sodium chloride, if operated in near-infrared, or hydrochloric acid, in the visible regime. From the obtained phase shifts the sensitivity can be directly extracted via linear fitting the sensor response against the refractive index change. Considering the extracted sen-

sitivity and the obtained noise, the LOD can be calculated according to Eq. (1.1). In contrast to the HRI sensing, there is no standardised method to measure the sensor's biomaterial detection limit. Many different biomolecules, mostly proteins, are utilised to validate its specificity and detection limit. In literature, many different types of target analytes can be found, not always to demonstrate sensors performance per se, but geared towards a specific application. Furthermore, additional parameters for performance assessment have to be considered in this case, namely the flow velocity (F), injection volume (V_{inj}) of the sample, or sample exposure time ($T = V_{\text{inj}}/F$), the time the sample needs to pass the sensor. The occupied area (A), or dimensions, of the sensor is also of considerable importance as for multiplexed detection it is necessary to have a high sensor density on the photonic chip. However, compact sensors can deteriorate the LOD due to sensitivity reduction.

Due to their distinct nature, there are some parameters which may not be used to compare between sensors types, such as total sensitivity (S), waveguide sensitivity (S_{WG}) or noise (σ), for the way of extraction/detection is too diverse. For the quality of a sensor from a system level point of view it is essential to consider their relation to each other, that is the detection limit defined as $\text{LOD} = 3\sigma/S$, for noise and sensitivity are not always decorrelated. However, within one type category, e.g. resonators or interferometers, they might be a valid argument and their optimisation is a necessary and important part in the design process.

There are also some characteristics which may influence the design process but do not necessarily determine the sensor quality, for example the used wavelength range λ_0 , or polarisation. Still, they can be important for future sensor generations and may show current trends.

A photonic sensor should also be evaluated in terms of the optical source it requires to properly operate. Some sensors require an narrow linewidth optical tunable light source, others just need a narrow-linewidth fixed wavelength laser. Some can be used with just a broadband light source, and/or require an optical demultiplexer/filter. The optical power of the source and the one detected by the read-out system which resulted in the observed LOD can also be an interesting benchmark parameter. A similar LOD obtained with less optical power enables less powerful, and thus less expensive, light sources to operate the sensing chip. It might also be relevant to see if an external signal generator is needed for proper signal extraction of the read-out, this includes modulation of the light source and/or the photonic sensor itself. Most of those requirements are correlated to the cost of the utilised optical source, and, therefore, the complete biosensing system. Especially POC devices require cheap realisations, hence, it is preferable to avoid tunable light sources, which price normally start from a few thousand to tens of thousand of dollars or euros. Non-tunable optical sources are typically much cheaper, varying from a few ten to some hundreds of dollars or euros.

In the following, we will discuss two popular integrated sensor types, resonators and interferometers. Among the resonant devices we will consider the sub-types rings, and photonic crystals, whereas among the interferometric sensors we include the following sub-type configurations: Young, Mach-Zehnder, Bimodal, spectral Mach-Zehnder, and coherently read Mach-Zehnder. Major advantages and disadvantages will be highlighted to get an insightful view on the current state-of-the-art. For this purpose, Tables 2.1, 2.2 and 2.3 show a collection of some publications and the aforementioned parameters, whenever available. The entries are first structured by type, i.e. resonator and interferometer, and then by sub-type. The next paragraphs are based on the presented information.

2.4.1. Resonators

Ring resonators have become very popular not only due to their good HRI detection limits ($10^{-5} \rightarrow 7 \times 10^{-7}$ RIU and $\sim 10 \text{ ng mL}^{-1}$ for CRP detection [142]) but mostly due to their multiplexability. This is enabled by their small size, down to $30 \times 30 \mu\text{m}^2$, but also to their capability to extract the signal without prior read-out calibration, as the extracted resonance

shift is a relative measure to prior measurements [48]. Most of them operate in the near-infrared (C-Band) and with TE polarised light, which is why they can be manufactured with the typical CMOS-fabrication line in SOI. The high refractive index difference between silicon and the dioxide enables the high integration density. Already more than 10 years ago, highly multiplexed sensing chips with 128 rings were successfully demonstrated [48, 143]. In the commercialised photonic sensor from GENALYTE around 128 rings can be read-out periodically [68]. Depending on prior functionalisation, each ring, or a group of rings, can be utilised to target a different analyte. Furthermore, due to their small size, offline functionalisation of each ring requires a minimum volume of the reagents. This makes them especially suitable for LOC solutions.

However, this comes literally with a high price. Most ring resonators are operated with an expensive tunable external cavity laser. Other approaches try to substitute the tunable laser by a white or broadband light source with optical filtering/multiplexing before detection. This decreases the costs of the optical source by a factor of around $10 \rightarrow 100$, but also requires an spectral analysing element [49].

Photonic Crystal resonators are similar to rings in terms of detecting and extracting the resonance shift. However, until now they just demonstrated moderate LODs (typically $\sim 10^{-5}$ RIU, down to 3.6×10^{-6} RIU [144] and $\sim 10 \text{ ng mL}^{-1}$ for streptavidin binding [145]), even though their sensitivity ($S > 500 \text{ nm/RIU}$) is often remarkably higher than the once of rings ($S < 500 \text{ nm/RIU}$) due to their high quality factors [146]. Nevertheless, they typically share the same dimensions and do not require a read-out calibration, but also share some of their disadvantages. Most importantly they are operated with a precise tunable laser which would result in expensive realisations. To the authors knowledge, only a few photonic crystal sensor has been demonstrated with cheaper broadband optical sources.

2.4.2. Interferometers

Young interferometers are a minority of the photonic integrated biosensors, to the authors knowledge, just one has been successfully demonstrated. Nevertheless, its ultra-low detection limit (4×10^{-8} RIU and 58 pg mL^{-1} for IgG [78]) is one of the lowest of all sensor types. Furthermore, the utilised white light source can contribute to a cheaper device realisation.

Young interferometers do not recombine the reference and sensing modes on chip and route their interference signal to a PD. Instead both arms guide the light separately off chip and let them create an interference pattern which then is detected by CCD camera. From this the change in refractive index on the sensor surface can be detected by tacking changes in the pattern itself. The way the readout is implemented, none of the typical interferometer associated problems appear, i.e. sensitivity fading and phase ambiguity. Additionally, no read-out calibration is necessary. However, this may become inconvenient if multiplexed detection is desired, as an interference pattern typically requires more physical space on the detection camera than just one single light spot.

BiModal interferometers are a relatively recent development in the last decade [88]. Instead of separating the light into two different arms, it is split into two modes within the same waveguide. The sensitivity arises from the sensitivity difference between the sensing (the higher order) and reference (the lower order) mode. This way, the interferometer becomes less affected by thermal variations. As young interferometers, the light is often not recombined on chip but is edge coupled and the interference pattern is detected by two position dependent PDs. They have been demonstrated to have low detection limits ($10^{-6} \rightarrow 4 \times 10^{-7}$ RIU and 7 ng mL^{-1} for CRP detection [91]) and also high sensitivities.

Table 2.1.: PART 1 - A selection of the state-of-the-art bulk sensing experiments performed by different types of spectral-analysing integrated photonic sensors found in literature. The abbreviations “Pol”, “BB”, “OT”, and “OF” stand for polarisation required, broadband light source applied, optical tunable source required, and optical filtering required, respectively. Whenever possible, missing values were calculated on the basis of the provided information.

	Year	Type	Sub Type	LOD [RIU]	S [$\frac{\text{nm}}{\text{RIU}}$]	S_{WG} [$\frac{\text{eRIU}}{\text{RIU}}$]	σ [pm]	$\approx A$ [μm^2]	$\sim \lambda_0$ [μm]	Pol	BB	OT	OF
[48]	2010	Res.	Ring	7.6×10^{-7}	163		0.04	30×30	1.55	TE	No	Yes	No
[147]	2017			2.5×10^{-6}	113	0.35	0.09	300×300	1.55	TE	No	Yes	No
[49]	2011			1.6×10^{-5}	2170		11.6	100×200	1.55	TE	Yes	No	Yes
[148]	2016			2.0×10^{-6}	490	0.85	0.33	30×30	1.55	TE	No	Yes	No
[149]	2017			8.5×10^{-7}	93		0.02		0.85	TE	Yes	No	Yes
[150]	2010		PhC	1.0×10^{-5}	510		1.7		1.55	TE	No	Yes	No
[151]	2009			7.8×10^{-6}	1538		4		1.55	TE	No	Yes	No
[144]	2010			3.6×10^{-6}	175		0.6		1.55	TE	No	Yes	No
[87]	2018	Interf.	SMZI	6.0×10^{-6}					0.85		Yes	No	Yes
[86]	2018			3.0×10^{-6}	3000		3		0.85		Yes	No	Yes

Table 2.2.: PART 2 - A selection of the state-of-the-art bulk sensing experiments performed by different types of non-spectral-analysing integrated photonic sensors found in literature. The abbreviations “Pol”, “BB”, “OT”, and “OF” stand for polarisation required, broadband light source applied, optical tunable source required, and optical filtering required, respectively. The * stands for modulation applied. The result of this work is highlighted in **bold font**. Whenever possible, missing values were calculated on the basis of the provided information.

	Year	Type	Sub Type	LOD [RIU]	S [$\frac{\text{rad}}{\text{RIU}}$]	S_{WG} [$\frac{\text{eRIU}}{\text{RIU}}$]	σ [mrad]	$\approx A$ [μm^2]	$\sim \lambda_0$ [μm]	Pol	BB	OT	OF
[147]	2017	Interf.	CMZI	2.8×10^{-8}	13051	0.35	0.12	250×250	1.55	TE	No	No	No
[100]	2019			1.4×10^{-8}	4900	0.22	0.02	500×500	1.55	TE	No	No	No
[152]	1998			5.0×10^{-6}	1156		6.3	150×1500000	0.79	TM	No	No	No
[153]	2013		MZI	5.4×10^{-6}	5856	0.21	11	500×1000	1.56	TE	No	No	No
[154]	2008			1.0×10^{-7}				50×1500000	0.63	TM	No	No	No
[155]	2008			1.8×10^{-7}	15287		0.9		0.63	TM	No	No	No
[78]	2007		Young	4.0×10^{-8}		0.23			0.68	TM	Yes	No	No
[156]	2019		BiM	3.0×10^{-6}	13509		14		0.66	TE	No	No*	No
[91]	2015			4.0×10^{-7}	15771	0.11	1.42		0.66	TE	No	No*	No
[139]	2019		SMZI	3.0×10^{-7}	3100	0.16	0.38		1.55	TE	Yes	No*	Yes
[157]	2014			2.8×10^{-6}	1958		1.2	25×600	0.75	TE	Yes	No	Yes

Table 2.3.: A selection of the state-of-the-art biosensing experiments performed by different types of integrated photonic sensors found in literature. The abbreviations “Pol”, “BB”, “OT”, and “OF” stand for polarisation required, broadband light source applied, optical tunable source required, and optical filtering required, respectively. The * stands for modulation applied. Three different target molecules are represented: C-reactive protein (CRP), streptavidin (StA), and IgG antibody. The result of this work is highlighted in **bold font**. Whenever possible, missing values were calculated on the basis of the provided information.

	Year	Type	Sub Type	Target	LOD [$\frac{\text{ng}}{\text{mL}}$]	$\approx A$ [μm^2]	F [$\frac{\mu\text{L}}{\text{min}}$]	T [min]	V [μL]	$\sim \lambda_0$ [μm]	Pol	BB	OT	OF
[142]	2011	Res.	Ring	CRP	10	30×30				1.55	TE	No	Yes	No
[48]	2010			StA	0.004	30×30	13.2	8	106	1.55	TE	No	Yes	No
[158]	2005			StA	0.006		500	25	12.5	0.63	TE			
[67]	2008			StA	5.2	110×110		5		1.55	TM	No	Yes	No
[5]	2007			StA	10	10×10				1.55	TE	No	Yes	No
[159]	2010			StA	30	15×15				1.55	TM	No	Yes	No
[110]	2010			IgG	3	100×100	33	80	2640	1.55	TM	No	Yes	No
[160]	2015			IgG	7100	250×250	25	25	625	1.55	TE	No	Yes	No
[145]	2014		PhC	StA	10	40×40			60	1.55	TE	No	Yes	No
[91]	2015	Interf.	BiM	CRP	7					0.66		No*	No	No
[153]	2013		MZI	StA	0.001	500×1000	8.5	45	383	1.56	TE	No*	No	No
[161]	2015			IgG	0.48		20	5	100	1.31		No	No	No
[101]	2020		CMZI	CRP	0.18	500×500	20	15	120	1.55	TE	No	No	No
[101]	2020			CRP	1.8	500×500	20	15	120	1.55	TE	Yes	No	No
[78]	2007		Young	IgG	0.058			30		0.68	TM	Yes	No	No
[87]	2018		SMZI	CRP	19.5					0.85		Yes	No	Yes
[162]	2017			CRP	2.1	25×600		15		0.75		Yes	No	Yes

To ensure constant sensitivity and no phase ambiguity, the narrow-linewidth fixed light source, until now just in the visible range, is slightly wavelength modulated (called all-optical phase modulation). Concepts for parallel detection have been proposed by splitting the light of the source equally to the sensors and then being detected by a CMOS camera [163]. Unfortunately, all-optical phase modulated BiModal sensors, in this realisation, have some drawbacks. First, the optical paths of sensing and reference mode are by definition unbalanced which potentially enables laser phase noise to interfere with the extracted phase. Second, even though the light source is “fixed”, it has to be modulated with the right parameter to ensure correct phase extraction, which does require more control elements, potentially complicating the system designs. In addition, for the presented BiModal sensors the edge coupling requires the facet of the sensing chip to be polished, which is a time consuming process. Furthermore, only straight sensing sections are supported, as bends would be more prone to bending losses and modal crosstalk, meddling the phase reconstruction. Typically those sections are very long. This can make dense sensor integration and, therefore, parallel detection more challenging. Either the microfluidic channels have to be parallelised, which would increase the need for more analyte volume to maintain the LOD, or the channel has to be very long and include bendings which would make the development of the entire microfluidic system challenging. Additionally, a read-out calibration is necessary to detect correct phase shifts.

Spectral realisation, i.e. driven by a tunable laser, of the BiModal sensor are currently investigated to circumvent arising problems. Instead of utilising the first two modes for sensing, a trimodal waveguide was proposed [95] to increase sensitivity and shorten the length (1 mm) of the sensor, however, operating in the near-infrared (C-Band). Another promising approach, again using the first two modes, has been realised with SWG [94] obtaining high sensitivities (2270 nm/RIU) for a very short sensing length of $\sim 125 \mu\text{m}$, also in the C-Band. Both suggestions recombine the interferometric signal on-chip, and, therefore, can be detected by simple PDs. Furthermore, no read-out calibration is required for proper operation. However, tunable lasers are highly expensive compared to cheap broadband sources. Theoretically, a white source may be used with this sub-type, but requiring optical multiplexing/filtering and a more sophisticated detection system.

Spectral Mach-Zehnder interferometers are in a way similar to resonators as they look for modifications in the transmission spectrum. In the visible regime they demonstrated moderate limit of detection ($\gtrsim 10^{-6}$ RIU and $\sim 2.1 \text{ ng mL}^{-1}$ for CRP detection [162]). All of the presented sensors are operated by white light source. After the recombination section the light is guided to an integrated optical multiplexer, separating the incoming light by wavelength which then can be detected by a CMOS camera or some PDs. This way parallel detection could be realised. SMZIs become more sensitive the more unbalanced the arms are designed, decreasing the free spectral range. However, as the optical source has such a broad linewidth, the FSR cannot be increased as much as possible but has to be matched to the linewidth of the laser to guarantee proper transmission curve extraction.

A comparatively low detection limit of 3×10^{-7} RIU was recently demonstrated in the near-infrared by using a frequency-comb laser, with three transmission peaks and an off-chip optical multiplexer [139]. The spectrum is realised by modulating a narrow-linewidth laser in an adequate manner. Here, the optical wavelength difference has to be adapted to the FSR of the sensor, i.e. reference and sensing arm have to be unbalanced, such that the observed phase difference for each of the three interferometric signals are 120° shifted to each other. Similar to the CMZIs a complex signal is extracted, resistant to sensitivity fading and phase ambiguity.

As almost every interferometric sensor, the physical sensor size is quite large in comparison to the resonant types. For both realisations, white or frequency-comb, the FSR has to be paired to the characteristics of the optical source. Furthermore, due to the unbalanced arms, the sensor is more vulnerable to phase noise of the source.

Mach-Zehnder interferometers were among others the first developed interferometers which were used for sensing. The modulated here is referenced to the Mach-Zehnder itself, and does not include any modulation of the optical source. Not modulated MZIs, despite their simple realisation with a fixed wavelength source and a simple PD, have demonstrated low detection limits (down to 1×10^{-7} RIU [154] and $\sim 480 \text{ pg mL}^{-1}$ for IgG antibody detection [161]), however, suffering from sensitivity fading and phase ambiguity if a modulation signal is not added via the reference arm. This approach, however, complicates the fabrication process and adds more control elements to the sensing system. Any intensity modulation or instability of the laser power transfers, alters the interferometric signal and increases the noise level. Using unbalanced arms further amplifies the optical phase noise. Furthermore, those sensors typically have a larger sensing area, which can complicate sensor density and multiplexed operation. Interestingly, in literature, the MZIs reported for the visible wavelengths do not report necessarily smaller footprints than those reported in the near-infrared. Nevertheless, just one PD is needed and parallel detection could be implemented with a CMOS camera.

Coherently Read Mach-Zehnder interferometers attracted attention in the last years as they demonstrated ultra-low detection limits down to the approximately 10^{-8} RIU [100, 147], but already demonstrated proof-of-concept in 1998 [152], and were further developed in [98]. For HRI sensing, this makes them the best sensors reported yet. Coherent detection is well-known from telecommunications, and many concepts related can be transferred easily. Instead of recombining the two arms to one output, they split the light to three different outputs. Therefore, three PDs are needed to reconstruct the phase without any sensitivity fading or phase ambiguity. By definition CMZIs are immune to variation of intensity. Additionally, by balancing reference and sensing arm, they can be made immune to optical source phase noise [99]. This makes them particularly suitable for use with cheap laser sources. No modulation in any form is required and no additional materials or steps have to be added in the fabrication process. Almost any reported MZI (including CMZI), requires a large sensing area, however, by an appropriate, reference and sensing waveguide design, e.g. minimised spiral distribution, the actual footprint might be reduced, potentially enabling highly multiplexed operation. Going in this direction, CMOS cameras may help to aid the detection. However, no parallel detection has been demonstrated so far. A read-out calibration is required to guarantee correct phase extraction.

2.4.3. Trends and This Work

The ideal biosensing system would have ultra-low detection limits, operated by a cheap light source, without requiring any type of modulation, while having a compact sensor size, high multiplexability, no read-out calibration, and simple detection of the optical signal(s). In the last decades the aforementioned sensors have demonstrated moderate (10^{-6} RIU) to ultra-low detection limits (10^{-8} RIU), the latter by CMZIs [100, 147]. In general, interferometric sensors outperform resonators in terms of detection limit, and cost of the complete read-out system, i.e. cheap light source and detector. However, they cannot compete with their small area, sensor density, and high multiplexability. Table 2.4 shows the capabilities of the different sensor types: The evaluation of the sensors is based on the previous presented data, and their discussion. Of course, this is not a fixed picture, for future research may advance and change these characteristics. However, some have not changed much over the last decade, e.g the detection limit of resonant sensors, whereas interferometers showed a clear trend to ultra-low detection limits. Interferometers have also been demonstrated to almost maintain those limits with very cheap and broadband light sources, which typically make up for a lot of cost of a final sensing device. Resonators do not require any form of modulation, but require an expensive tunable laser, while many interferometers require a modulation to overcome some limitations. Nevertheless, the best LODs were demonstrated with non-modulated MZIs. The smallest required area is

Table 2.4.: Advantages/Disadvantages of the different sensor types. The abbreviations “Mod.”, “Comp.”, “Multi.”, “Calib.”, “Det.” stand for modulation required for proper operation, compactness of the sensing structure, multiplexability demonstrated, calibration of the read-out needed, and detection scheme is simple, respectively. “Mod.” and “Calib” take binary values “Yes” (highlighted in red) or “No” (highlighted in green), where the first is an disadvantage, the latter an advantage. The other categorise are rated according to the following system: “++” (Excellent in strong green), “+” (Good in light green), “=” (Medium in yellow), and “-” (Poor in red). An empty entry in grey means not demonstrated or no data available.

Type	Sub-Type	LOD	Light Source	Mod.	Comp.	Multi.	Calib.	Det.
Res.	Ring	=	-	No	++	++	No	+
	PhC	-	-	No	+	+	No	+
Interf.	Young	+	+	No	-		No	-
	BiM	+	+	Yes	-		Yes	+
	SMZI	=	+	No	+	+	No	-
	MZI	+	+	Yes	=	+	Yes	+
	CMZI	++	++	No	=		Yes	+

still obtained with resonators, where some interferometers cannot compete with due to their required length and sometimes the incapability to redirect its light while sensing. Yet, some interferometric concepts, i.e. SMZI and CMZI, showed improved integratability. Parallel detection coincides with the sensor compactness and the final detection system. If a sensor is small, many can be placed close to one another, and by using a CMOS camera, many different sensors may be read at the same time. Almost every sensor type can be multiplexed and detected in that way. Spectral sensor type, using a broadband source, however, may occupy more physical detection space per sensor for they have to optically multiplex their wavelength content. All sensors requiring a tunable laser, do not suffer from a read-out calibration, whereas the rest does. This means that if future sensing devices tend to cheaper broadband light sources, a necessary calibration seems to be inevitable. A calibration does not complicate the read-out itself, but may complicate the injection and fluidic system, as a calibration solution, causing a phase change of at least 2π , has to be injected. The accompanying increase in time for each detection may still be short ($\sim 1 - 3$ min), depending on the volume and flow velocity.

Many different (micro)fluidic systems were demonstrated over the last decades, each having advantages and disadvantages. Nonetheless, its development and implementation does not depend on the sensor type, which is why it is not in the focus of this work or state-of-the-art. Comparison of biomolecule detection between the different sensor types is rather difficult, since no standardised method is set by the biosensing community. Therefore, it is difficult to draw reliable conclusion based on that. Only sensor types using the same method may be analysed in the same context.

In this work, it will be demonstrated that balanced CMZIs are promising candidates for future LOC and POC devices. Here, a theoretical discussing and detailed noise analysis will help to minimise the uncertainty of the signal extraction. Not only do we demonstrate that they are immune to optical source noise, i.e. RIN and LPN immunity, such that they can be used with one of the cheapest laser types (Fabry-Perot) but the detection system can be designed in a rather simple way. Additionally, this work will expand CMZIs research from HRI sensing to, for the first time, CRP (see Chapter 4), and IgG and IgE amoxicillin detection (see Chapter 5).

Until now, CMZIs show moderate compactness in comparison to their resonant counterparts, however, future research may help closing this gap. Furthermore, CMOS cameras or line CCDs may support coming multiplexed detection concepts. All together, this may pave the way to ultra-low, highly multiplexed, cheap lab-on-chip, and/or point-of-care devices based on coherently detected Mach-Zehnder interferometers.



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3. System Level Optimisation

The photonic integrated sensor chip is an important part of the entire biosensing system. It is the interface between the biological recognition of the target analyte and a corresponding quantitative physical parameter alteration which is then detected by the read-out. The sensitivity of a biosensor is mainly determined by photonic design and the biochemistry applied to the biorecognition layer. However, the noise level is mainly given by the design of the read-out which includes optical source, detection scheme, and signal processing. The detection limit depends on both sensitivity and noise level which is why a system level is important to gain insight on how it can be optimised.

Therefore, in Section 3.1 we will present the entire implemented setup with a description of each element. In Section 3.2, we will derive analytical expressions for the LOD of the CMZI considering some relevant noise sources based on the implemented real setup. For the sake of simplicity in this section we will not consider signal range limitations by the photodiodes or the data acquisition board. We use the insight gained from the analytical expressions to develop a optimisation strategy for HRI detection experiments in Section 3.3. In section 3.4 we return to the theoretical analysis and include the signal range limitations of the data acquisition board to study its impact on the limit of detection.

3.1. Experimental setup

The implementation of the biophotonic setup started in 2017, and was expanded and enhanced over the following years. A complete schematic is shown in Fig. 3.1, showing all important components and devices. A detailed characterisation of each element will follow after a more general setup description.

The designed sensing waveguides translate the biorecognition due to the sample into a physical parameter change, the effective refractive index, affecting the waveguides optical propagation velocity, i.e. causing a phase shift $\Delta\varphi$ after travelling the distance L_s of the sensing waveguide. A fluidic channel is placed on top the sensing structures. The channel inlet is connected to the injection system and the outlet to a waste deposit. The injection systems is connected to a syringe pump which maintains a constant flow velocity of the buffer, typically ultra-pure deionised water (Milli-Q). A small volume of the sample containing the analyte can be injected into the buffer flow via a valve system. An optical source, in this chapter a fixed narrow-linewidth external cavity laser (ECL), is connected to an optional erbium-doped fibre amplifier (EDFA), amplifying the incoming optical signal. A fibre polarisation rotator is used to align the polarisation with on-chip grating couplers. A variable optical attenuator (VOA), connected to a signal generation (SG) can be used to modulate the light at a speed of a few hundred Hertz. Like the EDFA, the VOA and its SG are optional elements which are not necessary for detection process itself, however, they will be important for the LOD optimisation. Light then enters the fibre array (FA), which contains eight fibres, each having an APC connector on the one side, while the other side consists of a solid block, in which the the eight fibres are placed on one line, equally distanced by 127 μm . Leaving the fibre, the light then illuminates the input grating of the desired sensing structure, which consists essentially of a coherently detected Mach-Zehnder interferometer (see Fig. 2.11). Each of the three sensor outputs couples back into the fibre-array via a grating coupler. Consecutively, each beam is collected by an individual fibre, guiding its

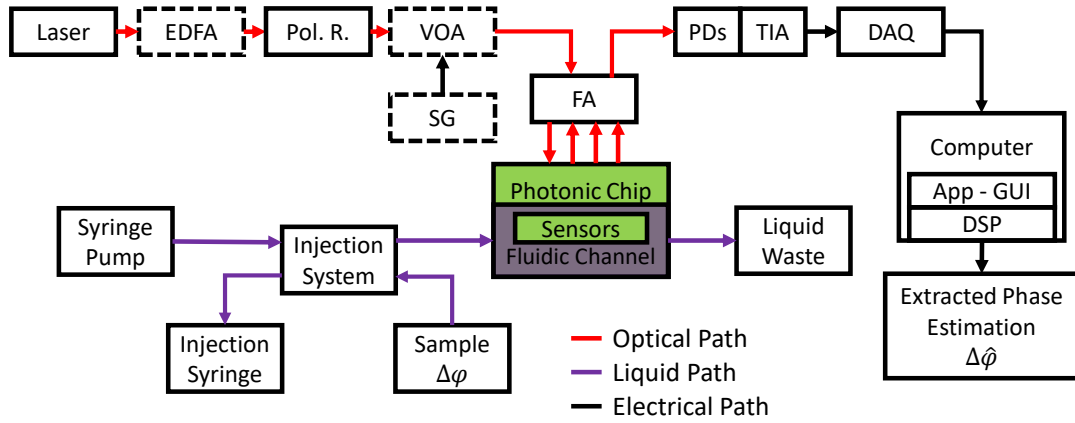


Figure 3.1.: Rough schematic of the implemented setup for the CMZI sensor, indicating the necessary elements and their connection to each other. List of Abbreviations: erbium-doped fibre amplifier (EDFA), polarisation rotator (Pol. R.), variable optical attenuator (VOA), signal generator (SG), fibre array (FA), photodiodes (PDs), transimpedance amplifier (TIA), data acquisition (DAQ), application - graphical user interface (App - GUI), digital signal processing (DSP).

light to the subsequent separated PDs. Converting the optical powers to electrical currents, they experience an amplification and a linearly conversion into voltages by the transimpedance amplifiers (TIA). The ensuing quantisation of the three voltages is realised by a data acquisition board (DAQ), sending the obtained data to the connected computer. An application manages the first digital signal processing (DSP) steps and saves the resulting information on the hard drive. More advances signal processing on this data calculates a precise estimation $\Delta\hat{\phi}$ of the phase change between the sensing and reference waveguides. Figure 3.2 shows the read-out and chip stage of the first implemented setup configuration on which the experiments in this chapter were performed.

Optical Source In the following experiments the WSL-100 external cavity laser from SANTEC will be used. It is a tunable monochromatic source the entire C-Band. The output power can be varied between $P_0 = 7 \rightarrow 15.5$ dBm ($5 \rightarrow 35$ mW), and has a linewidth $\delta\lambda < 80$ fm (a bandwidth $\delta f < 100$ kHz at $\lambda_0 = 1.55$ μ m).

Erbium-Doped Fibre Amplifier The optical amplifier is the C-Band Booster Amplifier EAD-500-C from IPG Photonics. The output power can range from $18 \rightarrow 27$ dBm ($63 \rightarrow 501$ mW) for an input power range from $-3 \rightarrow 10$ dBm ($0.5 \rightarrow 10$ mW).

Polarisation Rotator To align the polarisation state with the TE polarisation required by the grating couplers the FPC562 Manual Paddle Fibre Polarisation Controller from Thorlabs has been installed. By adjusting is three levers, twisting the embedded fibre, the correct polarisation can be manually reached.

Variable Optical Attenuator The V1550A from Thorlabs was implemented into the setup to be able to attenuate or even intensity modulate the light before entering the sensing chip. The optical transmission can be electrically controlled by applying a voltage. The optical

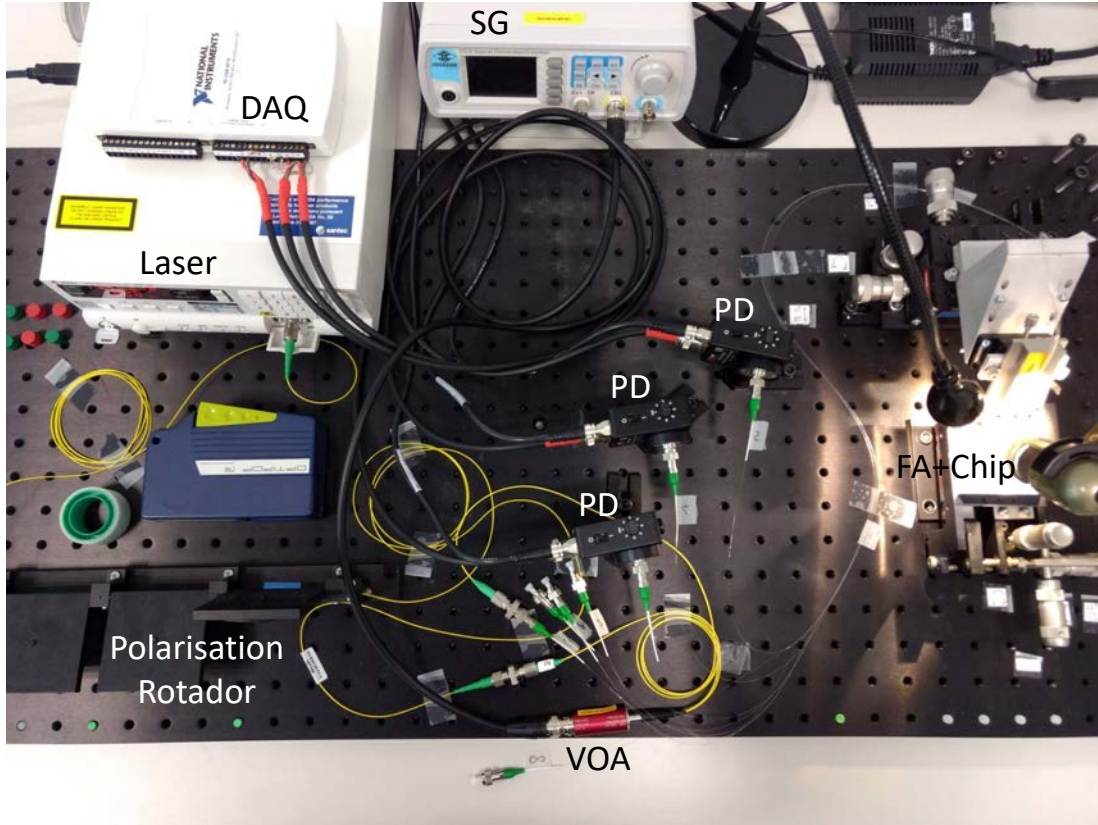


Figure 3.2.: A photo of the established setup with which the system level optimisation in this chapter was realised.

attenuation follows a strictly monotonously increasing graph, a minimum attenuation of 1.5 dB and a maximum of > 25 dB.

Signal Generator In order to supply the VOA with the necessary modulation voltage, it was connected to the JDS6600-15M Digital Control Dual-Channel DDS Signal Generator from JunCe Instrument. It can create advanced waveforms, however, here we just use a sinusoidal signal with frequencies up to around 1 kHz.

Fibre Array The array contains eight single mode fibres, which have all an APC connector on one end. The other ends are contained in a ferrule, lined up at equidistant intervals of $127\mu\text{m}$. The ferrule and the fibres are angle-polished at $\sim 33^\circ$, so that the resulting beam will not radiate in the fibre propagation direction but according to Snell's law at the fibre-air interface (see Eq. (2.10)).

Photodiodes with Integrated Transimpedance Amplifier The photodiodes used for the detection of the three optical outputs of the sensing structure are the PDA10CS-ES InGaAs Switchable Gain Detectors from Thorlabs. Their optical detection and amplifying range is from $0.9 \rightarrow 1.7\mu\text{m}$. Within this range, the responsivity (R_{sp}), the PD's electrical current response to the incoming optical power, varies, but is around 1.05 A W^{-1} at $1.55\mu\text{m}$. The TIA high-impedance gain (G) has eight configurations, namely 0, 10, 20, 30, 40, 50, 60, 70 dB, corresponding to 1.51×10^3 , 4.75×10^3 , 1.50×10^4 , 4.75×10^4 , 1.51×10^5 , 4.75×10^5 , $1.50 \times$

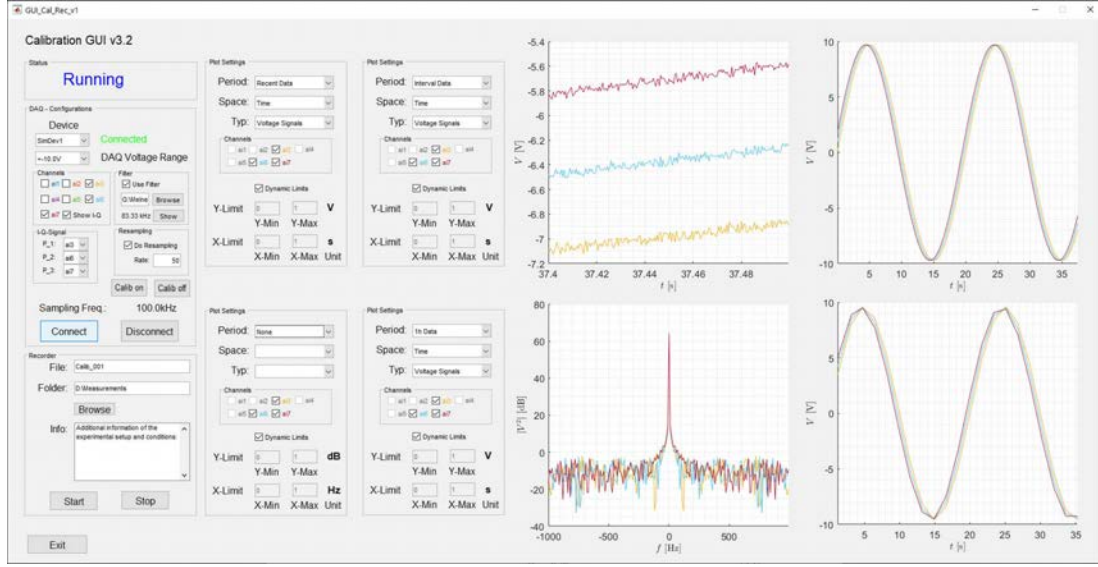


Figure 3.3.: A screenshot of the developed recording and pre-processing application in MATLAB.

$10^6, 4.75 \times 10^6 \text{ V A}^{-1} \pm 2\%$, respectively, according to the specifications. Approximately, 5 dB gain difference is obtained between each configuration. Nevertheless, measured gains for the three photodiodes are shown in the Appendix A.2, Table A.1. The resulting photo voltage is approximately given by $v \approx GR_{\text{sp}}P_{\text{opt}}$. The bandwidth of the PD ($\gg 1 \text{ kHz}$ for all gain settings) typically exceeds the bandwidth of the desired signal ($\ll 10 \text{ Hz}$), but can play a role in combination with noise aliasing effects (see Section 3.2.1.4). The noise equivalent power (NEP) spectral density for all gain settings is typically below $10^{-11} \text{ W}/\sqrt{\text{Hz}}$, for gain settings higher than $\geq 20 \text{ dB}$ it can be approximated by $2 \times 10^{-12} \text{ W}/\sqrt{\text{Hz}}$. The output voltage is between $0 \rightarrow 10 \text{ V}$

Data Acquisition Board The digital quantisation is realised by the NI USB-6210 Bus-Powered M Series USB Device from National Instruments. It can detect up to eight differential channels with an analogue-to-digital resolution of $N = 16$ bit. The input range can be set to $V_{\text{DAQ}} = \pm 0.2, \pm 1.0, \pm 5.0, \pm 10.0 \text{ V}$, the small signal bandwidth is around $B_s = 450 \text{ kHz}$, and the sampling rate is 250×10^3 samples per second, which is divided between all active channels. That means that the sampling frequency for each active channel is $f_s = 250/K \text{ kHz}$, where K is the number of all active channels. Using just one sensor only requires three PDs, i.e. $K = 3$. The channels have no prior low-pass filter, therefore, all signals within the bandwidth of the DAQ (B_s) are going to be quantised. Undersampling/aliasing effects due to the violation of the Nyquist–Shannon sampling theorem will be discussed in Section 3.2.1.4.

Application - Graphical User Interface and First Digital Signal Processing The GUI interface for recording and pre-processing the acquired data is a home written implementation in MATLAB, shown in Fig. 3.3. It enables the user to properly connect and adjust the DAQ settings, and also filter, resample and save the acquired data. Four different plotting sections can be separately configured for displaying the data. Each plot can show three different time slots, the most recently 100 ms, the last minute, or the last hour. The presented data in these intervals may be the recorded voltage signals of the DAQ, the resulting complex signal, and its extracted phase. Furthermore, the fast-fourier transformation of the most recently data can be displayed.

Before the application starts its data processing the use is able to configure the DAQ and digital signal processing parameters which apply to the recorded and partially to the displayed data. The DAQ device can be chosen in a drop-down list as well as its available voltage ranges. Next the user selects the electrical channels (here “ai1” to “ai7”) which have to be monitored (Channels), if a complex signal should be calculated (Show I-Q), and on which channel bases (I-Q Signal). In the case of the utilised DAQ the sampling frequency adapts with the amount of monitored channels. In the filter section the user can decide if a digital filter should be applied to the received data from the DAQ. The filter information has to be given in form of a “*.mat” file containing the digital filter coefficients. Those coefficients can be generated by MATLAB internal functions, its graphical user interface for Filter generation, or manually by the user. In the next step it can be decided if the filtered, or non-filtered, data should be resampled, e.g. a factor 50 means that just every 50th sample is being saved for display or recording. As the amount of unfiltered and non-resampled information can be huge, several gigabytes for a recorded hour, a sufficiently filtered and resampled signal can save a lot of memory. Here, a resampling factor of 50 means an approximated amount of half a gigabyte for four hours of recorded data.

There are two operation modes: displaying the read data on-the-fly or displaying it while saving it continuously into the memory. The two operation modes are event based triggered, that means every time the DAQ sends a package of recorded data the GUI will react to it according to the current operation mode.

The first operation mode is started by pushing the “Connect” button, which establishes a connection with the DAQ. The DAQ sends every 100 ms the voltage signals of the last interval of the same length. In the first operation mode, activated by the Connect button, the DAQ is connected to the GUI and takes the current event data, filters and resamples them according to the previous set parameters, and saves them in a temporary cache (Event Data) in which the last ten events are stored which is one second long. Based on this ten entries every second a mean value for each channel is calculated and saved to another temporary memory (Minute Data), capable to store around 1 min of mean values. To give a better overview of the developing signal a third cache (Hour Data) is implemented which stores the mean values of a 3 s long time interval of the Minute Data memory. Approximately one hour of the last 3 s mean values can be stored here. Based on this three memories, the complex signal and its amplitude can be calculated and extracted. For a correct representation of the calculated complex signal, there is a “Calibration” button, taking the Minute Data to fit them to the complex unit circle. The calibration algorithm will be discussed in closer detail in Section 3.2.1.2. The parameters of the Plot Settings can be individually configured to show the desired data. This mode can be terminated by pushing the “Disconnect” button, which closes the connection to the DAQ.

The second operation mode is in principle just an extension of the first. For this the recorder parameters have to be set which are file name, folder name, and additional information. Once the user pushes the “Start” button the recording of the filtered and resampled data starts by writing them into the RAM of the computer. While recording the user can enter additional information in form of text (ASCII), e.g. information about the performed experiments which may later help for the interpretation of the recorded data. Once the user pushes the “Stop” button the entire recorded data plus the additional text information are stored in the folder and under the file name indicated in the Recorder parameter section. Then the connection to the DAQ is terminated.

A operation flow chart of the event data triggered data processing, recording, and displaying is shown in the Appendix A.3 in Fig. A.8.

Syringe Pump The NE-300 Infusion Syringe Pump from Micrux Technologies is utilised to maintain a given flow velocity (F) in the fluidic channel. It can set flow velocities from the lower range of some μLh^{-1} up to a few mLmin^{-1} . For the purpose of this work, the flow

velocity will be typically between $10 \rightarrow 30 \mu\text{L min}^{-1}$

Injection System - Six-Port Valve System For managing the sample injections into the fluidic flow, a 6-Port Valve System C22-6186 from Valco Instruments Co. Inc. [164] was installed. The working principle is shown in Fig. 3.4. There are two possible working modes, the first lets the pump push the buffer to the channel through the sample tube, a polytetrafluoroethylene tube with an inner diameter of $d_{\text{in}} = 810 \mu\text{m}$ and length of $L_{\text{ST}} = 18 \text{ cm}$, i.e. an approximate sample volume of $V_{\text{ST}} \approx 120 \mu\text{L}$, including the connections within the valve system. When switched to position two, the valve system enables the sucking syringe to draw a liquid sample into the sample tube, while the pump maintains its flow to the fluidic channel. Turning back to the first position injects the placed sample into the fluidic flow to the fluidic channel and, therefore, to the photonic sensors.

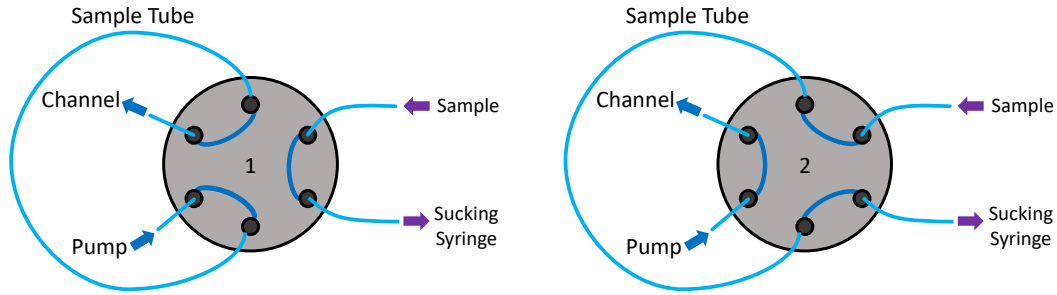


Figure 3.4.: Schematic representation of the utilised six-port valve system for inject different samples into the fluidic flow. Five different tubes are connected, the sample tube, which loads the desired sample, and four tubes connecting to the channel, pump, sample reservoir and sucking syringe. Stage 1 enables the pump to push the liquid in the sample tube to the channel. Changing to stage 2 enables the operator to suck a sample into the sample tube while the syringe pump continues pushing its liquid to the channel. Switching back to stage 1 finally injects the sample in direction of the channel.

Fluidic Channel The fluidic channel is imprinted into a polydimethylsiloxane (PDMS) block, schematically shown in Fig. 3.5. The PDMS block is presented from the down-side, which is placed on top of the chip and sensors, sealing the fluidic channel. Polydimethylsiloxane is a advantages material fluidic applications in small scale and can be used to form complex microfluidic structures [165]. It has successfully demonstrated to be integrable with photonic biosensor technology for point-of-care applications [166].

Here, the input port is connected to the six-port valve system, whereas the output connects to a waste reservoir. The height of the channel is around 0.5 mm, the length around 9 mm, and a width of approximately 1 mm. Due to these relatively large dimensions in comparison to other fluidic channels, they will not be referred to as microfluidic channels.

Later Setup Modifications The entire biosensing setup was moved from its original implementation laboratory to the basement of the Bionand building to suppress mechanical vibrations and movements. In addition, the TC1020CK Nexus Optical Table, dimensions $1 \text{ m} \times 2 \text{ m} \times 210 \text{ mm}$ with 700 mm tall active isolator legs, was installed to further reduce mechanical influences. Figures A.3 and A.4 show the newer implementation, which however was used for later experiments (see Chapters 4 and 5). Additional images of the established setup can be found in the Appendix A.3

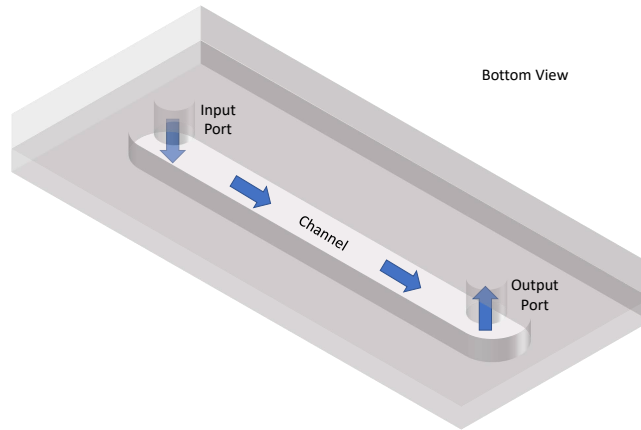


Figure 3.5.: Bottom view of the PDMS fluidic channel which is placed on top of the chip/sensing windows. The sample is pumped through the input- to the output port by via the channel which is formed by the indentation in the PDMS and the surface of the chip.

Photonic Chip The used photonic chip in this work contains different realisations of the CMZI sensing structure described in Section 2.3.2. The layout of the chip is shown in Fig. 3.6(a). The chip, with dimensions of $\approx 5 \text{ mm} \times 10 \text{ mm}$, was implemented on a silicon wafer with a $2.5 \mu\text{m}$ silicon dioxide BOX and 300 nm silicon nitride film, fabricated at the Instituto de Microelectrónica de Barcelona, Centro Nacional de Microelectrónica (IMB-CNM), CSIC [167] through a Multi Project Wafer approach offered by VLC Photonics.

The upper area is the coupling section, responsible to redirect the incoming light from above the chip into the waveguides, and vice versa, via grating couplers, see Fig. 3.6(b). The gratings (scanning electron microscope (SEM) picture shown in appendix Fig. A.6) are designed so that they radiate the waveguide mode, or collect the incoming beam, at an angle of 52° to the chips surface normal. Comparing to typically designed gratings in telecommunication with around $5^\circ \rightarrow 10^\circ$ this is a large angle. However, this was the only possible design considering the material of choice, silicon nitride on insulator, and the available minimum feature size of 600 nm by the manufacturer. The lower fibre array angle of 33° results from Snell's law. The grating length was chosen to be oversized ($L = 105 \mu\text{m}$) to make sure to radiate all incoming light and reduce possible back reflections. The entire grating and fibre coupling section were simulated using the FullWAVE FDTD Simulation Software from RSoft. The coupling was maximised to match the fibre mode with an efficiency $\eta_{\text{CE}} \approx -5.7 \text{ dB}$ (0.27), however, measurements reveal a much lower mean coupling of $< -10 \text{ dB}$ (< 0.1). This means, due to the double coupling (in- and out of the chip), more than -20 dB (99%) of the light of the optical source are lost, which is why in many experiments the EDFA is installed to amplify the input signal. The taper connected to each grating helps to minimise the losses due to mode conversion from the grating mode to the connected single mode waveguide.

From there the input light is guided to the splitting section, realised by the 1×2 MMI shown schematically in Fig. 3.6(c), dividing the light to the spiralled reference and sensing waveguides (see Fig. 3.6(d)). Silicon nitride waveguides demonstrated propagation losses below 1 dB/cm from the visible [168, 169] to below 1.5 dB/cm in the near infrared [105, 106]. Waveguide sensitivity of the single mode sensing section was computed with the FIMMWAVE mode solver from Photon Design [170]. In the first step, the TE effective refractive index of the fundamental mode TE polarised mode (shown in Fig. 3.7) was calculated for a water cladding, with a refractive index of $n_{\text{H}_2\text{O}} = 1.3162$ for $\lambda = 1.55 \mu\text{m}$. In the second step, the refractive index

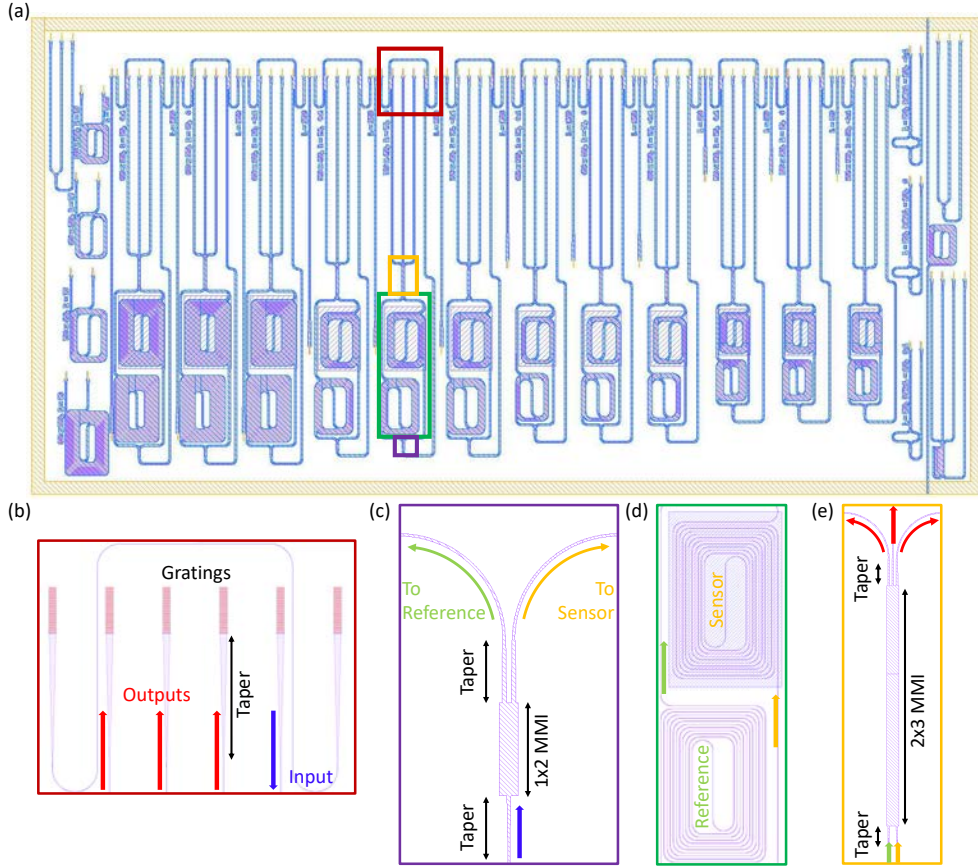


Figure 3.6.: (a) The layout of the utilised photonic integrated sensing chip. All twelve CMZI structures are found in the middle, whereas on the left and right side there are test structures for characterisation purposes. (b) The grating couples for coupling the light in (blue - Input) and out (red - Outputs). (c) The 1×2 MMI for splitting the input light equally to the reference (light green) and sensing (light orange) path. (d) Sensing and reference waveguides in their spiral form to minimise foot print. (e) The designed 2×3 MMI to create the three interferometric output signals (red) which are guided back to the output gratings section.

of the water was slightly increased. A linear fit between the results was used to extract the waveguide sensitivity of $S_{WG} \approx 0.22 \text{ eRIU/RIU}$. The 2×3 MMI, shown in Fig. 3.6(e), recombines the light of both paths and splits the interferometric signal to the three output waveguides which are then guided to the output gratings shown in Fig. 3.6(a).

There are twelve CMZI sensing structures defined on chip. From here on, each sensor receives an identification number ranging from $1 \rightarrow 12$, to simplify referencing. In all presented experiments, the fifth sensing structure was used for detection. This structure should have the optimal length to reach for the lowest detection limit. The discussion of optimal length will be derived in context of the noise sources and their influence onto the LOD in Section 3.2. Each of the twelve different balanced CMZIs are designed unique but similar. They can be divided into four different groups, differing in length of the sensing section and bending radius: $\{L_s \approx 20 \text{ mm}, R_s = 50 \mu\text{m}\}$, $\{L_s \approx 8.8 \text{ mm}, R_s = 75 \mu\text{m}\}$, $\{L_s \approx 5 \text{ mm}, R_s = 75 \mu\text{m}\}$ and $\{L_s \approx 6.3 \text{ mm}, R_s = 50 \mu\text{m}\}$. In each group the waveguide width varies by $0.1 \mu\text{m}$, i.e. $W_s = 1.1 \mu\text{m}$, $1.0 \mu\text{m}$, and $0.9 \mu\text{m}$, from left to right. The occupied area of the waveguide spirals is around $400 \times 400 \mu\text{m}^2$. A SEM picture, showing the reference arm buried in silicon dioxide

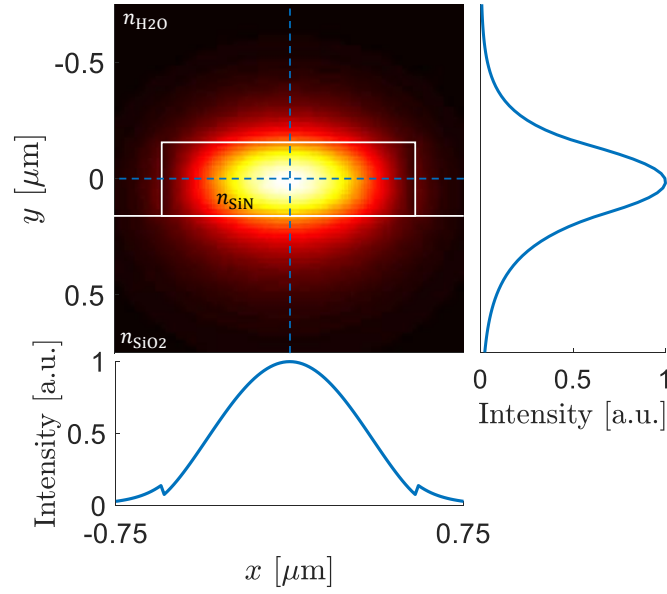


Figure 3.7.: Intensity profile of the TE_0 mode of the sensing waveguide applied in this work.

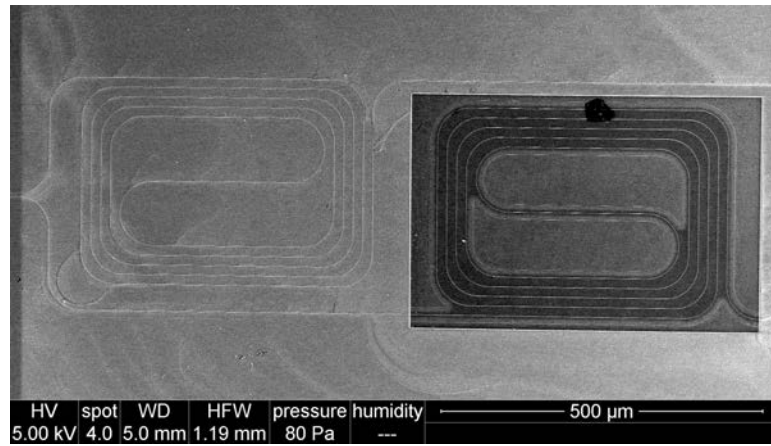


Figure 3.8.: SEM picture of on of the reference- (left) and sensing arm (right). The sensing window, the removed silicon dioxide above the sensing waveguide structure makes the differentiation simple.

(left) and sensing arm (right), of sensor 7 is shown in Fig. 3.8. Test structures for the different spirals can be found mostly on the left-, and for the MMIs mainly on the right side of the chip.

List of the Relevant Parameters Summarising the presented components for the following the system level analysis and detection limit optimisation, Table 3.1 contains all relevant parameters and their possible values.

3.2. System Level Analysis

In this section a detailed system model for the presented sensing setup is presented to get analytical mathematical expressions for optimising the LOD, and gain understanding of how

Table 3.1.: List of system level relevant parameters.

Parameter	Symbol	Value	Unit
<i>Laser Source + EDFA</i>			
Optical Power	P_0	$7 \rightarrow 27$	dBm
Wavelength	λ_0	1.55	μm
<i>Photodetection</i>			
Responsivity	R_{sp}	1.05	A W^{-1}
Gain	G	$1.51 \rightarrow 4750$	kV A^{-1}
Noise Equivalent Power	NEP	$\leq 2 \times 10^{-12}$	$\text{W}/\sqrt{\text{Hz}}$
<i>Data Acquisition</i>			
Max. Sampling Rate	$f_{\text{s,max}}$	250	kHz
Number of Channels	K	3	
Number of Quantisation Bits	N	16	
Sampling Rate	f_{s}	$f_{\text{s,max}}/K$	kHz
DAQ Input Range	V_{DAQ}	$\pm 0.2, \pm 1.0, \pm 5.0, \pm 10.0$	V
<i>Chip</i>			
Grating Coupler Efficiency	$\bar{\eta}$	-10	dB
Waveguide Sensitivity	S_{WG}	~ 0.2	eRIU/RIU

to find and eliminate limiting factors from different noise sources. Doing so, we will consider absorption of the light propagating in the sensing arm, the calibration algorithm to minimise the error in the final phase extraction, and the different noise sources contributing to the extracted phase uncertainty.

For the sake of simplicity, whenever we analyse the LOD in this section, we will assume that signal power can be varied continuously, thus ignoring the saturation of photodiodes or DAQ. For the latter, this particular aspect will be taken into account in Section 3.4.

3.2.1. General Expressions for Signal and Noise at the Sensor Output

3.2.1.1. Signal Analysis

Figure 3.9 shows a detailed system level representation of the setup. The presentation of the elements may differ from the simplified presentation in Fig. 3.1 for its focus lies on the signal propagation and noise source characteristics. Temperature changes on the photonic integrated chip can have a substantial influence on the extracted phase signal by adding noise to the system or may even falsify the extracted phase itself. Controlling the temperature distribution on-chip is therefore an important task. However, in this work no temperature control was implemented. Therefore, for the entire analysis of this thesis, temperature influences will be neglected, but should be targeted in future work. The different elements will be examined in detail in the following.

The laser couples its monochromatic light of a wavelength $\lambda_0 = 1.55 \mu\text{m}$ into a single-mode fibre (SMF), connected to the polarisation rotator, adjusting the light to TE polarisation. The following optional EDFA optically amplifies the optical power. Amplified or not, the mode enters the, again optional, VOA with a power P_0 . The power launched into the FA is called here $P_{\text{in}}(t)$. Hence, the optical amplitude $A_{\text{in}}(t)$, with $P_{\text{in}}(t) = |A_{\text{in}}(t)|^2$, can be expressed as $A_{\text{in}}(t) = [\sqrt{P_0} (1 + r(t)/2)] e^{j\phi_{\text{SPN}}(t)}$, $r(t)$ being the relative intensity noise amplitude and $\phi_{\text{SPN}}(t)$ the phase noise caused by the Laser and EDFA. The FA placed above the gratings

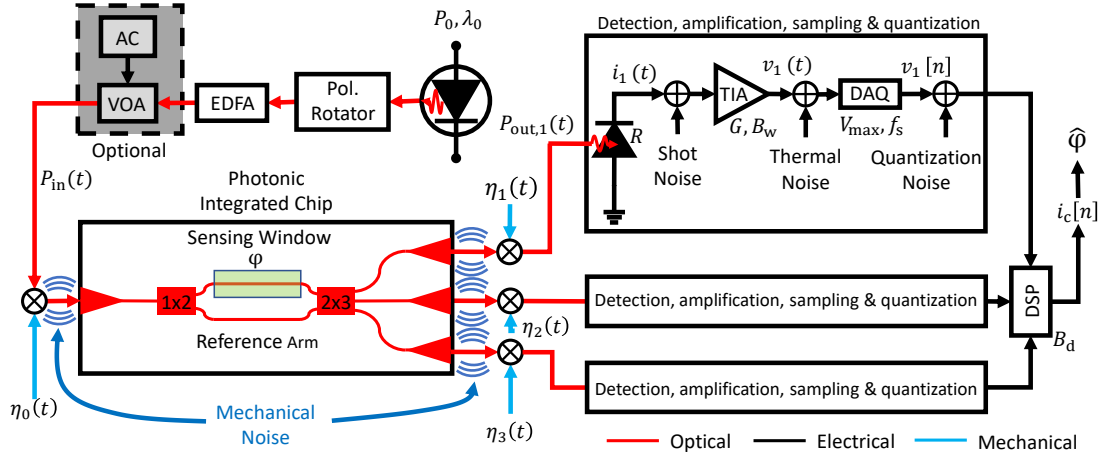


Figure 3.9.: System level representation of the entire setup, including the points of impact of the various noise sources, i.e. laser noise, mechanical noise, shot noise, thermal noise, quantisation noise, and baseband noise. (I will change the figure a little bit.)

couples light into the sensor with a coupling efficiency $\eta(t)$. This is also a time varying function, depending on the relative position of the FA and grating to each other. Mechanical movements of the relative position results in an unwanted modulation of the coupling efficiency, i.e. $\eta_0(t) = \bar{\eta}_0 + \Delta\eta_0(t)$, where $\bar{\eta}$ is the mean coupling efficiency and the sub-index 0 refers to the input grating – this issue will be discussed in detail in Section 3.2.4. The consecutive 1×2 MMI splits the power equally into the sensing and reference arm. In Chapter 2 we showed that the phase shifts $\Delta\varphi$ introduced by the sensing path depends on the effective refractive index of the mode. Considering absorption of the light in the sensing arm, the following optical transfer functions can be derived:

$$H_s = e^{-\alpha_s L} e^{j \frac{2\pi}{\lambda_0} L_s n_{\text{eff},s}}, \quad (3.1)$$

$$H_r = e^{j \frac{2\pi}{\lambda_0} L_r n_{\text{eff},r}}, \quad (3.2)$$

for sensing and reference path, respectively, with α_s the optical attenuation constant due to absorption. Generally, losses occur in both arms due to scattering and bendings, and can be approximated by a factor $e^{-(2\alpha L + \alpha_{90^\circ} N_{90^\circ})}$ for the optical signals in the reference or sensing arm, where α are the propagation/scattering losses in the arm due to fabrication errors or imperfections in Np/cm, α_{90° the 90° -bend losses and N_{90° the number of 90° -bends in the arm. Even though the parameters are slightly different for sensing and reference arm, they are assumed to be equal, so that their combined effect is a reduction of the detected voltages by a constant factor. Later these losses will be accounted for by increasing the insertion losses parameter IL_{oss} which will be defined later within this chapter.

The sensing arms attenuation is predominantly a result of the absorption in the surrounding cladding, which is for most cases water. Water, being highly transparent in the visible, especially for red colour ($2\alpha_{\text{H}_2\text{O}} = 0.0061 \text{ Np cm}^{-1}$ at $\lambda = 0.7 \mu\text{m}$ [171]), is highly absorbing in the near-infrared ($2\alpha_{\text{H}_2\text{O}} = 11.819 \text{ Np cm}^{-1}$ at $\lambda = 1.55 \mu\text{m}$ [172]). The absorption constant of the sensing mode can be roughly approximated by $\alpha_s \approx \Theta \cdot \alpha_{\text{H}_2\text{O}} + (\Theta - 1) \cdot \alpha_{\text{SiN}}$ with Θ the ratio of the overlap integral of the sensing mode in the surrounding and the entire overlap of the mode, and $\alpha_{\text{SiN}} \ll \alpha_{\text{H}_2\text{O}}$ the absorption constant of silicon nitride. The parameter Θ is also approximately the waveguide bulk sensitivity, which derives the absorption constant of the propagating mode to

$$\alpha_s \approx S_{\text{WG}} \alpha_{\text{H}_2\text{O}}, \quad (3.3)$$

which is in this case approximately 1.2 Np cm^{-1} ($\approx 0.52 \text{ dB cm}^{-1}$). Of course, absorption can also be caused by molecules attached on the surface, for example the functionalisation or the target analyte itself. Nevertheless, it will be much smaller in comparison to the buffer absorption, as they only appear in close proximity to the surface.

An ideal 2×3 MMI recombines the light, and splits it to its three optical outputs, according to Eq. (2.25). All three paths guide their light to the output gratings, radiating light to the FA. Similar to the first coupling, the efficiencies can be described by $\eta_k(t)$ ($k = 1 \rightarrow 3$). To simplify the mathematical expressions, let us consider the same mean coupling efficiency $\bar{\eta}$ for each grating. The optical output powers $P_{\text{out},k}(t)$ are guided to ideally identical PDs with responsivity R_{sp} , linearly converting the optical powers to the photocurrents $i_k(t)$. At this point, shot noise will be added to the detected currents. The TIA linearly transfers the currents to a voltage representation $v_k(t)$ with a conversion factor G , the transimpedance gain. Here, thermal or Johnson noise contributes to the converted voltage. The DAQ samples the voltage signals $v_k(nT_s) = v_k[n]$ with $n \in \mathbb{N}_0$ and $T_s = 1/f_s$ adding quantisation noise in the process. Expressions for the contributions of all three noise sources at the outputs of the DAQ will be derived in Section 3.2.2, since all processing is taking place in this domain. Alternatively, they could be easily transferred to the outputs of the PDs as this is common practice in telecommunications.

Digital signal processing calculates the complex voltage $v_c \propto \exp(j\Delta\varphi)$ according to Eq.(2.37), and the aforementioned signal flow:

$$v_c = \frac{GR_{\text{sp}}\Pi_{\text{loss}}\bar{\eta}^2 P_0}{2} H_s H_r^*. \quad (3.4)$$

The parameter Π_{loss} accounts for insertion losses, for example due to the VOA, fibre connection losses, or waveguide propagation losses. Here, it typically has a value of around $-3 \rightarrow -4 \text{ dB}$. Considering the transfer functions of Eqs.(3.1) and (3.2) the expression

$$v_c = \frac{GR_{\text{sp}}\Pi_{\text{loss}}\bar{\eta}^2 P_0}{2} e^{-\alpha_s L} e^{j\frac{2\pi}{\lambda_0} \Delta L_{\text{opt}}} \quad (3.5)$$

may be derived. The optical length difference ΔL_{opt} is defined as the mode path difference $L_s n_{\text{eff},s}(n_s) - L_r n_{\text{eff},r}$. In this sensor design, reference and sensing path share the same length $L = L_s = L_r$. The effective refractive index of the reference mode may be assumed constant, since the silicon dioxide cladding isolates the waveguide from the liquid flowing over it. The sensing mode, however, depends on the refractive index of the cladding material (n_s) via the waveguide sensitivity S_{WG} . The phase of the complex voltage is directly proportional to $n_{\text{eff},s}(n_s)$, which, to first order, is directly proportional to changes in the cladding refractive index: $n_{\text{eff},s}(n_s) = n_{\text{eff},s}(n_{s,0}) + S_{\text{WG}} \Delta n_s$, with $n_{\text{eff},s}(n_{s,0})$ the effective index when the buffer is running over the waveguide. For sensing, the absolute value of the phase is not of interest, only the relative change $\Delta\varphi = S_{\text{WG}} \Delta n_s$ is important. Therefore, the optical length difference can be simplified to $\Delta L_{\text{opt}} = L \Delta n_{\text{eff},rs} \propto S_{\text{WG}} L \Delta n_s$. Only caring for relative phase shifts Eq. (3.5) becomes

$$v_c = \frac{GR_{\text{sp}}\Pi_{\text{loss}}\bar{\eta}^2 P_0}{2} e^{-\alpha_s L} e^{j\frac{2\pi}{\lambda_0} S_{\text{WG}} L \Delta n_s}, \quad (3.6)$$

The total sensitivity of the scaled unit circle describing complex electrical signal (see Eq. (2.36) and (3.6)), which describes how it reacts to HRI changes in n_s , is generally defined by

$$S_c = \left| \frac{\partial v_c}{\partial n_s} \right| \quad (3.7)$$

In our case, a balanced CMZI, the sensitivity of the complex voltage derives to

$$S_c = \frac{\pi GR_{\text{sp}}\Pi_{\text{loss}}\bar{\eta}^2 P_0}{\lambda_0} e^{-\alpha_s L} S_{\text{WG}} L. \quad (3.8)$$

For the system level discussion this factor is very useful, for experiments and comparison with other architectures, however, the phase sensitivity S_φ may be more appropriate, here defined as

$$S_\varphi = \left| \frac{\partial \arg(v_c)}{\partial n_s} \right| = \frac{S_c}{|v_c|} = \frac{2\pi}{\lambda_0} S_{WG} L \quad (3.9)$$

which is the commonly known bulk sensitivity for interferometric refractive index sensors.

3.2.1.2. Calibration

Up to this point, all devices were assumed to be perfect and equal to their kind. However, in reality, each may have slightly different characteristics. These characteristics can for example be phase errors or imbalance of the different outputs of the MMIs, varying grating coupling efficiencies, slightly dissimilar PD responsivities, or TIA gain variations. Those parameter alterations affect the extracted complex signal by shifting, rotating and even scaling the in- and quadrature-phase signal parts differently. Therefore, a calibration procedure is required to maintain a correct phase shift extraction. Until now, the mean coupling efficiency for each grating η_k has been considered equal to $\bar{\eta}$. However, in a practical setup the grating structures and the fibres in the FA are not necessarily aligned perfectly to each other. This results in individual mean coupling efficiencies $\bar{\eta}_k$. Therefore, each detected interferometric signal v_k will exhibit a different amplitude detection range

$$v_k = \bar{\eta}_{0k}^2 \xi_k \frac{V_0}{2} \left(\frac{1 + e^{-2\alpha_s L}}{2} + e^{-\alpha_s L} \cos(\Delta\varphi + \varphi_k + \delta\varphi_k) \right), \quad (3.10)$$

where $V_0/2$ is a normalised expression for $GR_{sp} \Pi_{\text{loss}} P_0/3$. Parameters ξ_k and $\delta\varphi_k$ account for imperfections in design and fabrication of the MMIs and differences in responsivity and gain for each path, and $\bar{\eta}_{0k}^2$ is the mean value of $\eta_0(t)\eta_k(t)$. All these deficiencies result in a complex signal which is unlike a scaled unit circle. In fact, the transformation from Eq. (2.37) converts the electrical signals to a shifted, rotated, and scaled ellipse, exemplary shown in Fig. 3.10. However, through a linear transformation it can be converted back to the unit circle, i.e. $C_{\text{cal}} = \mathbf{H}_{\text{Elip2Circ}} \cdot \vec{\mathbf{H}}_{\text{v2C}}^T \cdot \vec{\mathbf{v}}$. Finding the entries of $\mathbf{H}_{\text{Elip2Circ}}$ is called elliptical calibration [98]. The different calibration steps are quite simple [98]:

1. Remove the elliptical complex shifts, i.e. $((\max(\Re\{C\}) - \min(\Re\{C\}))/2$ and $(\max(\Im\{C\}) - \min(\Im\{C\}))/2$.
2. Rotate the ellipse, removing the angle create by the imaginary (or real) axis and the graph going through the two fixed points. This is an optional step.
3. Scale the in-, and quadrature-phase signal such that $(\max(\Re\{C\}) - \min(\Re\{C\})) = (\max(\Im\{C\}) - \min(\Im\{C\}))$.

The order of the steps is interchangeable. To perform a proper elliptical calibration, at least one full ellipse must be available, i.e. $\Delta\varphi$ needs to vary at least from $0 \rightarrow 2\pi$. In an experiment, this is typically done by injecting a sample containing a different composition than the running buffer, such that the observed HRI change causes this phase variation. There are at least two effects, which complicate the calibration process and, therefore, the correct phase change extraction. First, the dependency of the attenuation constant α_s on the refractive index change causing injection. Let us assume a high sodium chloride concentrated injection. Due to the high amount of salt in the water, the attenuation constant is lowered (NaCl is transparent in the visible and near-infrared [173]). The non-calibrated signal, despite rotating in the ellipse, would experience a lower amplitude and move to a lower elliptical "orbit", which can make the elliptical fit difficult. Furthermore, a linear transformation might not be the right approach to calibrate the sensor, for non-linearities in the detection system, the responsivity or gain, may not be linear but quadratic. In case of the PD, the corresponding photocurrent might change

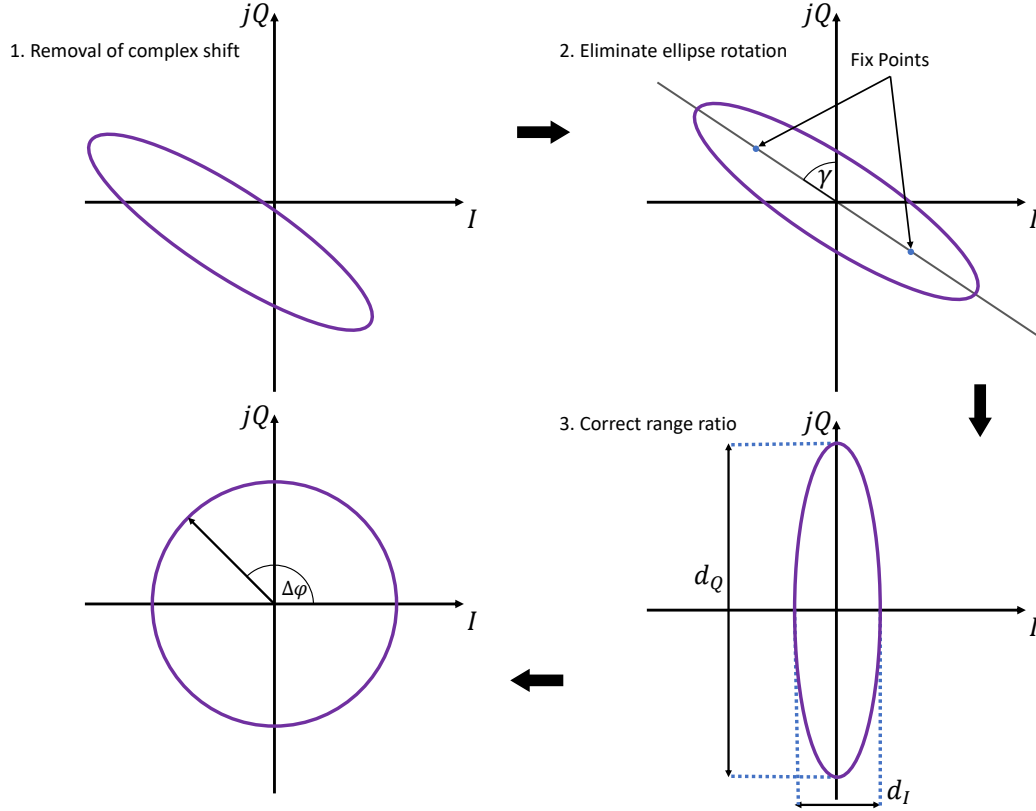


Figure 3.10.: Demonstration of the applied calibration algorithm. 1. The mean complex shift (ellipse centre) is removed. 2. The elliptical angle is removed from the calculation. 3. The ratio between the minima and maxima of the in- and quadrature-phase signal are balanced. Ideally, the final complex signal shape describes the unit circle.

according to $i \propto R_{\text{sp},\text{I}} P_{\text{opt}} - R_{\text{sp},\text{II}} P_{\text{opt}}^2$ with $R_{\text{sp},\text{I}} \gg R_{\text{sp},\text{II}} > 0 \text{ A W}^{-1}$ the new responsivity constants. For lower optical power the second term might be negligible, however, reaching for the lowest possible LODs, the optical source may be chosen stronger in terms of emitted power, pushing the dynamic range of the sensor to the non-linear regime. In addition, relative intensity noise from the optical source might cause crosstalk into the phase extraction. Typically, the effects of non-linearities are much higher for the extracted amplitude than the phase, for which a simple elliptical calibration may be sufficient. For complex sensing, i.e. detecting phase and attenuation changes in the sample, a highly precise calibration becomes of utmost importance. An alternative approach, addressing this subject, can be found in [174].

3.2.1.3. Noise in the Complex Plane

The different noise sources contribute to the complex signal in very distinct manner. However, let us assume the additive white Gaussian noise (AWGN) sources with powers σ_k^2 for the detected signals $k = 1, 2, 3$, respectively, of the CMZI. In an ideal environment the components for detection are completely equal, that means that the noise power caused by the electrical devices (in the PDs, TIAs, DAQ) is identical in each detected channel. By considering σ_e^2 to be the entire noise power of all electrical detection components of all channels, the noise of each channel derives to $\sigma_k^2 = \sigma_e^2/3$, assuming three identical channels, as shown in Fig. 3.11(a). The noise in each channel can be added by extending Eq. (3.10) by a random zero-mean real-valued AWGN

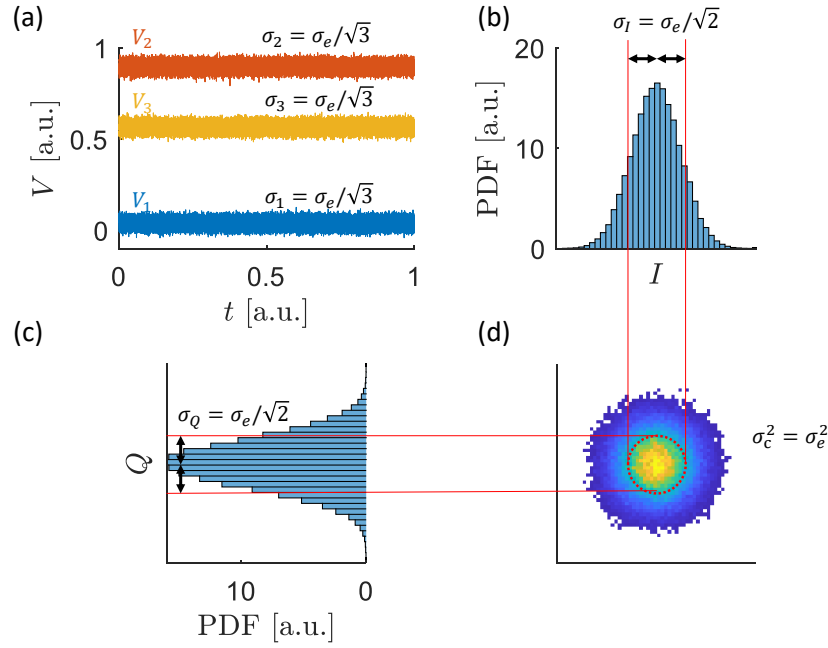


Figure 3.11.: Conversion of white-Gaussian noise from the PDs of the CMZI (a) to the in-phase (b) and quadrature-phase (c) signal and the resulting complex signal (d), showing the resulting probability density distribution.

function $\mathcal{N}_k(\sigma_k)$ with the standard deviation as its input. Then by following the definitions of the in- and quadrature-phase signal in Eqs. (2.38) and (2.39), substituting i_k by v_k , the noise power for both derives to $\sigma_I^2 = \sigma_Q^2 = \sigma_e^2/2$. That means that the noise power of all components is equally spread to the real-axis (in-phase) and imaginary-axis (quadrature-phase), as it is demonstrated in Fig. 3.11(b) and (c) by showing their probability density functions (PDF). The resulting complex signal, the sum of in- and quadrature phase, in its entirety can be formulated as

$$v_c = \frac{GR_{\text{sp}}\Pi_{\text{loss}}\bar{\eta}^2 P_0}{2} e^{j\frac{2\pi}{\lambda_0} S_{\text{WG}} L \Delta n_s} + \mathcal{N}_c\left(\frac{\sigma_c}{\sqrt{2}}, \frac{\sigma_c}{\sqrt{2}}\right) \quad (3.11)$$

with

$$\mathcal{N}_c(\sigma_I, \sigma_Q) = \mathcal{N}(\sigma_I) + j\mathcal{N}(\sigma_Q) \quad (3.12)$$

a random zero-mean complex-valued AWGN function. Function $\mathcal{N}$ is a random zero-mean real-valued AWGN function. Parameter σ_c is the standard deviation of the complex signal v_c (see Eq. (3.11)) with its in-phase and quadrature-phase standard deviations components σ_I and σ_Q . Its power directly derives from the sum of the in- and quadrature-phase noise power to $\sigma_c^2 = \sigma_I^2 + \sigma_Q^2 = \sigma_e^2$.

Assuming no refractive index changes in the sensing arm the complex-signal-to-noise-ratio (CSNR), i.e. the real-valued signal-to-noise-ratio of the complex signal v_c (see Eq. (3.11)), can be defined as

$$\text{cSNR} = \frac{|\bar{v}_c|^2}{\text{SD}^2(v_c)} = \frac{|\bar{v}_c|^2}{\sigma_c^2} = \frac{G^2 R_{\text{sp}}^2 \Pi_{\text{loss}}^2 \bar{\eta}^4 P_0^2}{4\sigma_c^2} \quad (3.13)$$

where $\bar{v}_c$ it the mean value of v_c and $\text{SD}(x)$ the common standard deviation formula for discrete random variables, defined by

$$\text{SD}^2(x) = \frac{1}{W} \sum_{w=1}^W |x_w - \bar{x}|^2 \quad (3.14)$$

with W the number of samples, and $\bar{x}$ the mean of x . The detection limit defined in Eq. (1.1) can be approximated, therefore, to

$$\text{LOD} \propto \frac{\sigma_c}{S_c} = \frac{1}{\sqrt{c\text{SNR}}}. \quad (3.15)$$

3.2.1.4. Undersampling Noise Power Spectral Density Amplification

During the analogue-to-digital conversion of the DAQ, the noise power spectral density seen by the voltage signals v_k at the input of the DAQ can experience a serious amplification at the DAQ's output if its bandwidth is much higher than the DAQ's sampling frequency f_s .

The Nyquist-Shannon sampling theorem states that a continuous signal, or noise, can be appropriately digitally represented if its frequency content, here represented by its bandwidth B_n , is lower than $f_s/2$. This is qualitatively shown in Fig. 3.12(a), where the sampling frequency f_s is much higher than the bandwidth B_n of the received signal ($B_n < f_s/2$). It is assumed

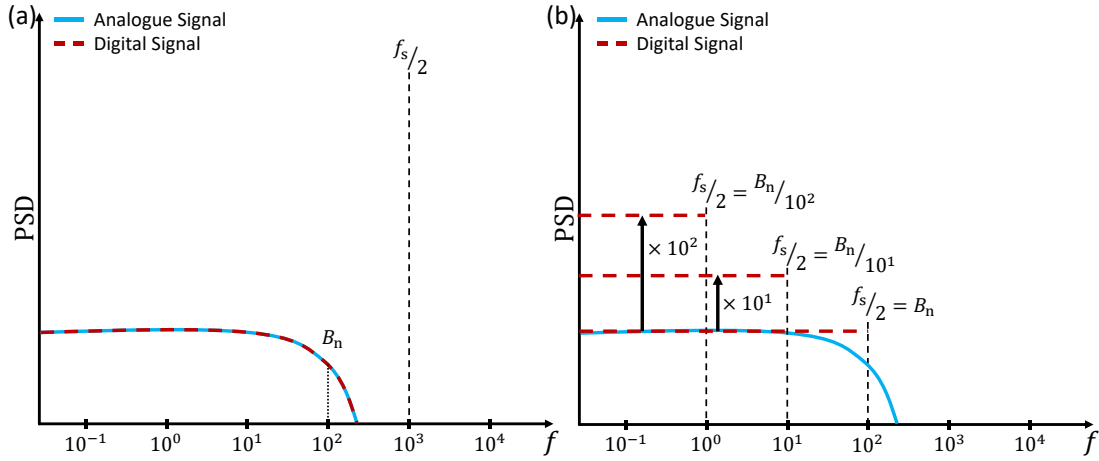


Figure 3.12.: (a) Power spectral density of an analogue signal (blue line) at the input of an ideal DAQ and its digital representation (dark red dashed line) at the output of the DAQ with $B_n < f_s/2$. (b) The same, but with $B_n \geq f_s/2$, a violation of the sampling theorem, therefore, causing an artificial increase of the power spectral density of the digital representation at the output of the DAQ.

that the continuous signal (blue line) has almost no frequency content above its bandwidth B_n due to prior electrical low-pass filtering of for example the TIA. Therefore, the power spectral density of the digital representation (dark red dashed line) matches with continuous signal. Here we assume that the DAQ has an infinitely large quantisation resolution, i.e. the number of quantisation bits $N \rightarrow \infty$, and, hence, adds no noise in the process. In case the bandwidth is higher than the sampling frequency ($B_n > f_s/2$), the noise power spectral density content of the continues signal above $f_s/2$ is folded into the frequency region $f \in [0, f_s/2)$ of the digital signal representation. This is a sampling theorem violation as the sampled continuous signal will not be appropriately represented by its digitisation. In this case, the increase of the power spectral density within $f \in [0, f_s/2)$ can be approximately accounted for by the undersampling factor

$$\vartheta_{\text{un}} = \frac{2B_n}{f_s} \quad (3.16)$$

which is multiplied to the actual power spectral density of the analogue signal after quantisation. A qualitative example for this is shown in Fig. 3.12(b) where the continuous signal (power spectral density in blue line) is sampled in multiple fractions (dark red dashed lines) of the

signal's bandwidth B_n . The undersampling factor becomes unity in case of no sampling theorem violation.

In this work, the bandwidth of the TIA is larger than the one of the DAQ's inputs ($B_s = 450 \text{ kHz}$) for gain settings below 40 dB. Quantising the required three channels at a time sets the sampling frequency to $f_s \approx 83.3 \text{ kHz}$. In this case the undersampling factor can be approximated to $\vartheta_{un} \approx 10.8$. For gain settings equal or larger than 40 dB the TIA's bandwidth will dominate. This results in undersampling factors of $\vartheta_{un} \approx 7.7$, 2.2 or 1 for 40 dB, 50 dB > 60 dB TIA gain settings, respectively. The sampling theorem violation could be avoided, i.e. $\vartheta_{un} = 1$, by implementing an electrical low-pass filter at the input of each channel of the DAQ with a bandwidth lower than $f_s/2$. However, this was not established within this thesis.

3.2.1.5. Conversion of In-Phase and Quadrature-Phase Noise into Phase Noise

Before we can analyse the different noise sources, we have to find a relation between the noise in the electrical domain, and the noise in the detected phase. Let us assume the situation in Fig. 3.13. The perfectly calibrated complex signal $C = I_s + jQ_s$ and the superimposed random

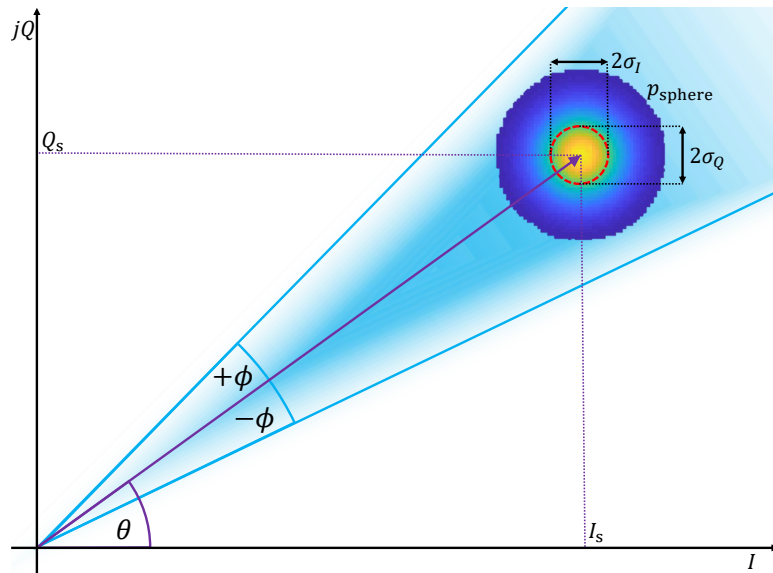


Figure 3.13.: The relation between the detected electrical additive-white-Gaussian noise in the complex plane, with standard deviations σ_I , σ_Q , and probability density function p_{sphere} , and the phase uncertainty within the angle $\theta \pm \phi$ for a complex signal at $C = I_s + jQ_s$.

complex-valued AWGN, with standard deviations σ_I in in-phase and σ_Q in quadrature-phase, are shown. For simplicity, let us assume that this complex noise is equally distributed into real and imaginary part, i.e. $\sigma = \sigma_I = \sigma_Q$. The probability density function in spherical coordinates of the IQ-signal can therefore be formulated as

$$p_{\text{sphere}}(r, \omega) = \frac{1}{2\pi\sigma^2} e^{-\left(\frac{r^2 - 2rR \cos(\omega - \theta) + R^2}{2\sigma^2}\right)}, \quad (3.17)$$

with the amplitude $r^2 = I^2 + Q^2$, angle $\omega = \angle(I + jQ)$, signal $R^2 = I_s^2 + Q_s^2$, and signal angle $\theta = \angle(I_s + jQ_s)$. The probability function of Eq. (3.17) can be written as

$$P(\phi) = \int_0^\infty \int_{\theta-\phi}^{\theta+\phi} r \cdot p_{\text{spher}}(r, \omega) d\omega dr, \quad (3.18)$$

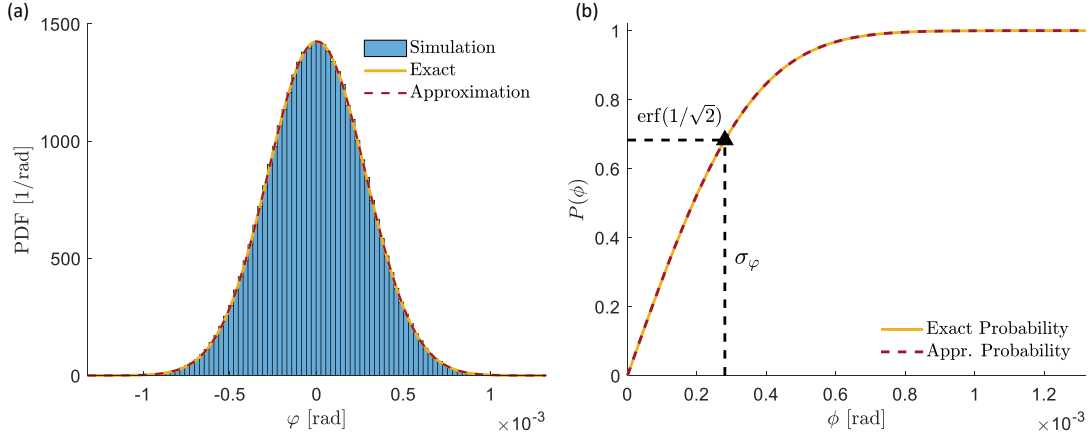


Figure 3.14.: (a) The theoretical (red) and a simulated case probability density function (light blue histogram) of the extracted phase of the complex signal with $R \gg \sigma$. (b) The exact (blue) and approximated (red) theoretical probability functions for the same complex signal

giving the probability of the complex signal noise to be within the angle interval $\pm\phi$, $\phi \in [0, \pi)$. The question which now has to be solved is, how do we have to choose the integration angle ϕ such that the probability function in Eq. (3.18) is equal to σ -confidants, i.e. $P(\phi = \sigma_\phi) = \text{erf}(1/\sqrt{2}) \approx 0.68$. That means that around 68% of the detected phase uncertainty lies between $[\theta - \sigma_\phi, \theta + \sigma_\phi]$. To solve this problem, the integrals in Eq. (3.18) may be switched, due to Fubini's theorem. Integrating over r derives the probability function to

$$P(\phi) = \int_{-\phi}^{\phi} \frac{e^{\frac{R^2}{2\sigma^2}(\cos^2(\varphi)-1)}}{2\pi\sigma} \left[\sqrt{\frac{\pi}{2}} R \cos(\varphi) \left(1 + \text{erf} \left(\frac{R \cos(\varphi)}{\sqrt{2}\sigma} \right) \right) + \sigma e^{-\frac{R^2 \cos^2(\varphi)}{2\sigma^2}} \right] d\varphi, \quad (3.19)$$

with substituting $\varphi = \omega - \theta$, $d\varphi/d\omega = 1$, therefore, the integrands become $\pm\phi$. Knowing that for strong signals ($R \gg \sigma$) the exponential expression in last term in parentheses converges to zero, and the error function to one, a more simplified probability function is obtained:

$$P(\phi) \approx \int_{-\phi}^{\phi} \frac{R}{\sqrt{2\pi}\sigma} \cos(\varphi) e^{\frac{R^2}{2\sigma^2}(\cos^2(\varphi)-1)} d\varphi. \quad (3.20)$$

Solving for this probability function is now straightforward:

$$P(\phi) \approx \left[\text{erf} \left(\frac{R}{\sqrt{2}\sigma} \sin(\varphi) \right) \right]_0^\phi \approx \left[\text{erf} \left(\frac{R}{\sqrt{2}\sigma} \varphi \right) \right]_0^\phi = \text{erf} \left(\frac{R}{\sqrt{2}\sigma} \phi \right). \quad (3.21)$$

As mentioned above, σ -confidence (σ_ϕ) or the standard deviation is reached when approximately 68% of detected random events lies in the interval $\pm\sigma_\phi$. This value is reached when the input of the error function reaches $1/\sqrt{2}$ which leads to the following equation:

$$\sigma_\phi \approx \frac{\sigma}{R}. \quad (3.22)$$

This matches with the intuitive geometrical interpretation of the problem: “Displacement = Radius $\times$ Angle”, which is here $\sigma = R \times \sigma_\phi$ from Eq. (3.22).

Figure 3.14(a) shows the extracted phase density function of a simulated scenario (blue bars) for a random complex-valued AWGN within the context of a large CSNR. The extracted phase probability density function fits almost perfectly to the precise analytical expression for the probability density function (yellow) given in the integrand of Eq. (3.19) and its approximation

(red) (see Eq. (3.20)). Besides, Fig. 3.14 (b), displays the exact probability function (yellow) and the approximation (red), graphically justifying its accuracy. Still, this approximation is only valid for large CSNR ($\text{cSNR} > 20 \text{ dB}$), which is typical true for Mach-Zehnder based interferometric biosensors. As σ is the detector standard deviation in I and Q , we can express this in terms of CSNR where R^2 is the complex signal power and $\sigma_c^2 = \sigma_I^2 + \sigma_Q^2 = 2\sigma^2$ the complex signal noise:

$$\sigma_\varphi \approx \frac{1}{\sqrt{2}} \frac{\sigma_c}{R} = \frac{1}{\sqrt{2}} \frac{1}{\sqrt{\text{cSNR}}} \quad (3.23)$$

For AWGN in the complex domain, just $1/\sqrt{2}$ of the complex signal noise power distributes to the noise extracted from the phase. Hence, the detection limit becomes

$$\text{LOD} = \frac{3}{\sqrt{2}} \frac{\sigma_c}{S_c}. \quad (3.24)$$

3.2.2. Electrical Noise Sources

In this work, four different kind of electrical noises are investigated: thermal noise (σ_{TN}^2), shot noise (σ_{SN}^2), quantisation noise (σ_{QN}^2) and flicker noise (σ_{FN}^2). Their combined contribution to the complex noise is their sum: $\sigma_c^2 = \sigma_{\text{TN}}^2 + \sigma_{\text{SN}}^2 + \sigma_{\text{QN}}^2 + \sigma_{\text{FN}}^2$. A short description of their origin and mathematical treatment will be presented in this section.

3.2.2.1. Johnson/Thermal Noise

Thermal or Johnson-Nyquist noise is often described as an additive white Gaussian noise, i.e. its power is equality distributed over the entire bandwidth. Its origin lies in thermal movements of electrons and holes in electric circuits. Typically, the detected random electrical noise caused by this is AWGN with zero mean and a standard deviation of the square root of the noises power. The detected power is usually $\propto T_K$ the temperature (in Kelvin) of the electronic device. Here, the noise contribution of a single PD can be expressed as

$$\sigma_{\text{TN,PD}}^2 = \varrho_{\text{TN,PD}}^2 B \quad (3.25)$$

where ϱ_{TN} is the voltage (or current) spectral density in $\text{V}/\sqrt{\text{Hz}}$ (or $\text{A}/\sqrt{\text{Hz}}$) and is a function of temperature, but is constant as we assume thermal equilibrium, and B the bandwidth of an digital low-pass filter applied to v_k . However, in the case of the photodiodes the current spectral density is substituted by noise-equivalent power (NEP) in $\text{W}/\sqrt{\text{Hz}}$ which is the optical noise equivalent spectral density of the detected electrical noise. The voltage spectral density of one PD can therefore be written as

$$\varrho_{\text{TN,PD}} = GR_{\text{sp}} \text{NEP} \sqrt{\vartheta_{\text{un}}}. \quad (3.26)$$

A typical value for the power spectral density in this work is $\varrho_{\text{TN,PD}}(G = 20 \text{ dB}) \approx 0.1 \mu\text{V}/\sqrt{\text{Hz}}$. The total thermal noise contribution to the complex reconstructed signal is

$$\sigma_{\text{TN}} = \sqrt{K} \varrho_{\text{TN,PD}} \sqrt{B}, \quad (3.27)$$

with K the number of quantised channels. Assuming thermal noise to be dominant, i.e. $\sigma_c \approx \sigma_{\text{TN}}$, we can rewrite Eq. (3.24) as

$$\text{LOD}(L) = \sqrt{K} \frac{3}{\sqrt{2\pi}} \frac{\lambda_0}{S_{\text{WG}}} \frac{\text{NEP} \sqrt{\vartheta_{\text{un}} B} e^{\alpha_s L}}{\Pi_{\text{oss}} \bar{\eta}^2 P_0 L}, \quad (3.28)$$

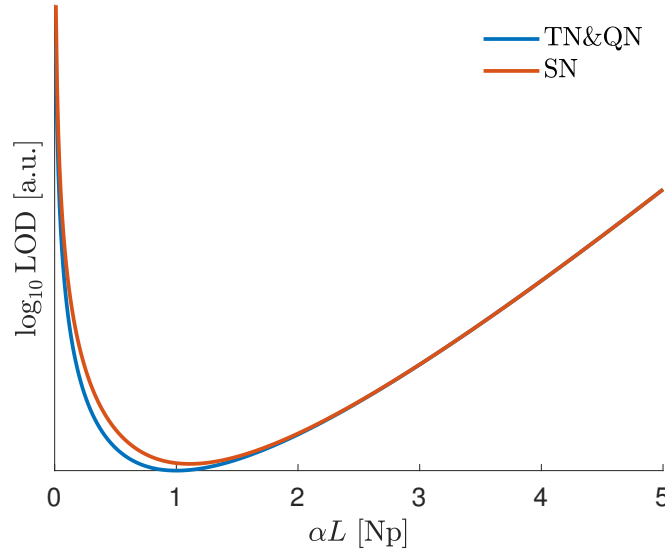


Figure 3.15.: Qualitatively logarithmically-plotted LOD as a function of absorption αL for the different detector noise sources.

which has a minimum/optimum for $\alpha_s L = 1$:

$$\text{LOD}|_{\text{opt}} = \min_L \text{LOD} = \sqrt{K} \frac{3}{\sqrt{2\pi}} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \frac{\text{NEP} \sqrt{\vartheta_{\text{un}} B}}{\text{IL}_{\text{oss}} \eta^2 P_0}. \quad (3.29)$$

$\text{LOD}(L)$ and its minimum are qualitatively shown in Fig. 3.15 (blue line). The variable K , the number of channels used, is still shown to highlight the influence of the number of detection devices on the detection limit. Accentuating the optimisation of the detection limit, it is worth highlighting that in this case the LOD scales with the inverse of the source power P_0 and linearly with the square root of the low-pass filter bandwidth B . Knowing that the sensing signals have a very low bandwidth, the filter integration time ($\propto 1/B$) can typically be increased to some seconds.

Comparing to a direct detection scheme, i.e. $K = 1$, the signal is only half as strong and the noise power in the phase reconstruction is three times as high, but also equally distributed in the complex plain, which means only half of it is relevant for phase extraction. In that comparison, direct detection schemes may have a LOD which is $\sqrt{3} \approx 1.73$ times better its coherent counterpart, but suffering from sensitivity fading and phase ambiguity. In addition, thermal noise is typically much smaller than the other noise sources.

3.2.2.2. Shot Noise

Inherent to the discrete nature of light, shot noise is often referred to as one of the fundamental limits. Its contribution is zero if no light is detected (i.e. also no signal), however, a higher amount of photons per time unit will cause a random photocurrent due to the time varying absorption events in the PD which is proportional to mean value of detected photons. Generally, the current spectral density, directly at a PD, can be written as

$$\varrho_{\text{SN,PD}} = \sqrt{2qi\vartheta_{\text{un}}} = \sqrt{2qR_{\text{sp}}P\vartheta_{\text{un}}} \quad (3.30)$$

q the elementary charge, and P the incident optical power, as it is typically modelled as a Poisson white noise. A typical value for the power spectral density in this work is $\varrho_{\text{SN,PD}} \approx 0.4 \mu\text{V}/\sqrt{\text{Hz}}$.

The total amount of shot noise is calculated by integrating over the bandwidth limited spectral density, i.e.

$$\sigma_{\text{SN,PD}}^2 = 2qR_{\text{sp}}P\vartheta_{\text{un}}B \quad (3.31)$$

In the case of our sensor, we have three PDs, whose shot noise is directly contributing to the calculated complex signal. Each PD observes a different level of received optical power, depending on the effective optical path difference between reference and sensing arm. However, as the shot noise of each PD is directly proportional to optical power, and the sum of all detected optical powers is constant (meaning the sum of the power in reference and sensing arm), the shot noise in the complex signal is constant and independent of the how many PDs are used. Consequently, the total amount of shot noise seen in the complex domain can be calculated and expresses as

$$\sigma_{\text{SN}}^2 = G^2 q R_{\text{sp}} \Gamma L_{\text{oss}} \bar{\eta}^2 P_0 \left(|H_r|^2 + |H_s|^2 \right) \vartheta_{\text{un}} B \quad (3.32)$$

$$= G^2 q R_{\text{sp}} \Gamma L_{\text{oss}} \bar{\eta}^2 P_0 (1 + e^{-2\alpha_s L_s}) \vartheta_{\text{un}} B. \quad (3.33)$$

Assuming shot noise to be dominant, i.e. $\sigma_c \approx \sigma_{\text{SN}}$, we can rewrite Eq. (3.24) to

$$\text{LOD} = \frac{3}{\sqrt{2\pi}} \frac{\lambda_0}{S_{\text{WG}}} \frac{\sqrt{q\vartheta_{\text{un}}B}}{\sqrt{R_{\text{sp}}\Gamma L_{\text{oss}}\bar{\eta}^2 P_0}} \frac{e^{-\alpha_s L}}{L} \sqrt{1 + e^{-2\alpha_s L}} \quad (3.34)$$

which reaches its minimum/optimum for $\alpha_s L \approx 1.11$:

$$\text{LOD}|_{\text{opt}} = \max_L \text{LOD} \approx \frac{1}{1.11} \frac{3}{\sqrt{2\pi}} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \frac{\sqrt{q\vartheta_{\text{un}}B}}{\sqrt{R_{\text{sp}}\Gamma L_{\text{oss}}\bar{\eta}^2 P_0}}. \quad (3.35)$$

In contrast to the thermal or quantisation noise (see Section 3.2.2.3) the LOD scales with the inverse square root of the source power P_0 , but also with $\sqrt{B}$. Interestingly, the resulting LOD is equal to the same configuration with a direct detection scheme as the detected signal is half, and the shot noise power is the same for both, but also just half of if is interfering the phase reconstruction. $\text{LOD}(L)$ and its minimum are qualitatively shown in Fig. 3.15 (orange line).

3.2.2.3. Quantisation Noise

All digital signals are series of discrete values, each represented by a certain number N of bits. The higher the number of bits the more different states can be quantified, that is 2^N . A typical analogue-to-digital converter (ADC) has a defined voltage range in which it can operate, normally the same amount in the negative as in the positive direction, e.g. $\pm V_{\text{DAQ}}$. Hence, the quantisation step of the discrete values is $\Delta V_{\text{qs}} = 2V_{\text{DAQ}}/2^N$. The random signal $v(t)$ is detected in time steps (T_s), defined by the sampling frequency ($T_s = 1/f_s$) of the DAQ, generating the discrete signal $v[z] = v[zT_s]$ ($z \in [0, Z-1]$, $Z \in \mathbb{N}$) which can take the discrete values $-V_{\text{DAQ}} + n\Delta V_{\text{qs}}$, $n \in [0, N-1]$. The amount of noise added by the quantisation process is constant and equally stretched over the entire Nyquist-spectrum ($0 \rightarrow f_s/2$) [175]. The resulting white power spectral density can therefore be formulated as

$$\varrho_{\text{QN,PD}}^2 = \Upsilon_{\text{QN}}^2 \Delta V_{\text{qs}}^2 \frac{2}{f_s}. \quad (3.36)$$

Parameter Υ_{QN} is the unitless Data Acquisition (DAQ) quality factor, which would be $\sqrt{1/12} \approx 0.3$ for a perfect ADC, but in our case was determined to 0.7, based on the specifications of the manufacturer. A typical value for the power spectral density in this work is $\varrho_{\text{QN,PD}} \approx 1 \mu\text{V}/\sqrt{\text{Hz}}$. Applying the digital low-pass filter (bandwidth B) in the digital signal processing results in the following quantisation noise power in each photodetected signal [175]:

$$\sigma_{\text{QN,PD}}^2 = \varrho_{\text{QN,PD}}^2 B = \Upsilon_{\text{QN}}^2 \Delta V_{\text{qs}}^2 \frac{2B}{f_s}. \quad (3.37)$$

The used DAQ in this setup shares its maximum sampling frequency $f_{s,\max}$ equally between all active channels, i.e. $f_s = f_{s,\max}/K$. Since the total amount of quantisation power by all channels the same in the complex signal domain, the total amount of quantisation noise becomes

$$\sigma_{QN} = \sqrt{K} \sigma_{QN,PD} = K \Upsilon_{QN} \Delta V_{qs} \sqrt{\frac{2B}{f_{s,\max}}}, \quad (3.38)$$

which is scaling with the amount of active channels. Similar to the previous discussed noise origins, the LOD becomes

$$\text{LOD} = \sqrt{K} \frac{3}{\sqrt{2\pi}} \frac{\lambda_0}{S_{WG}} \frac{\Upsilon_{QN} \Delta V_{qs} \sqrt{\frac{2B}{f_s}} e^{\alpha_s L}}{GR_{sp} \text{IL}_{oss} \bar{\eta}^2 P_0 L} \quad (3.39)$$

which has a minimum/optimum for $\alpha_s L = 1$:

$$\text{LOD}|_{\text{opt}} = \max_L \text{LOD} = \sqrt{K} \frac{3}{\sqrt{2\pi}} \frac{\alpha_s \lambda_0}{S_{WG}} \frac{\Upsilon_{QN} \Delta V_{qs} \sqrt{\frac{2B}{f_s}}}{GR_{sp} \text{IL}_{oss} \bar{\eta}^2 P_0}. \quad (3.40)$$

It can be similarly optimised as in case of thermal limiting noise. However, quantisation noise is the only noise, in this work, which is independent of the amplifier. That means the LOD can be optimised by also applying a higher TIA gain. Furthermore, the sampling frequency and number of quantisation bits become an additional factor, whose increment has a positive impact on the detection limit. $\text{LOD}(L)$ and its minimum are qualitatively shown in Fig. 3.15 (blue line).

3.2.2.4. Flicker Noise

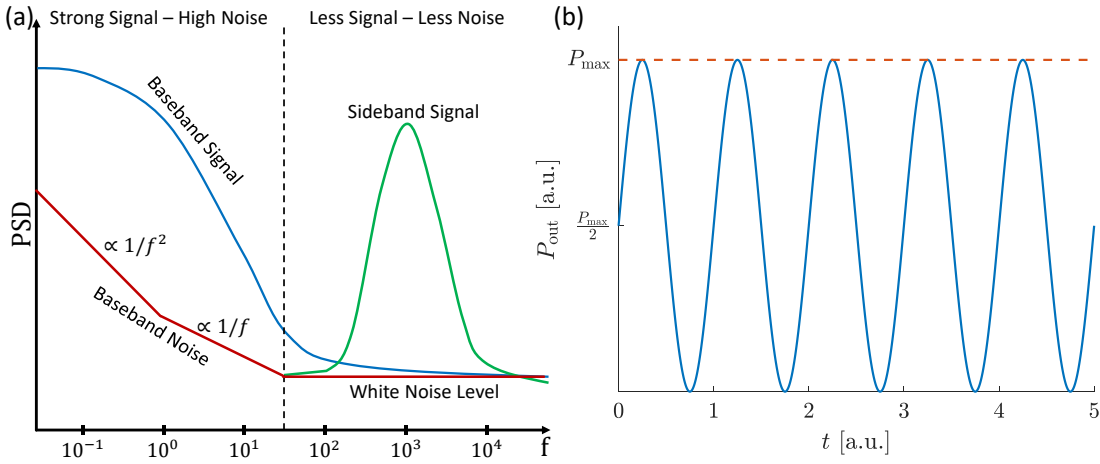


Figure 3.16.: (a) The signal received in the baseband sees a higher noise “floor” than the signal in a sideband. (b) A perfect optical intensity modulation which transfers $1/8$ of $P_{\max}$ into the desired sideband at the modulation frequency f_{mod} .

Not all electrical noise sources are spectrally flat. Especially at low frequencies, the noise spectral density becomes usually highly frequency dependent. Normally it scales with $1/f$, $1/f^2$, $1/f^3$, ..., or in general $1/f^a$ (with $a > 0$), and, from here on, is referred to as baseband or flicker noise. Depending on the frequency range around $f = 0$, different sources may dominate, as shown in Fig. 3.16(a). For higher frequencies, white noise typically starts to dominate. The precise detection of baseband noise may be a difficult task for huge time scales for measuring

could be necessary and, consequentially, the complications for precisely maintaining experimental conditions in this time interval. The origin of $1/f^a$ noise is not always clear and also can be a superposition of various factors. It may be caused by the generation (and recombination process) in the PDs, and/or TIA, or impurities in semi-conductor. Whatever reason it is, for the signal-to-noise ratio it may be the limiting source. A photo-detected optical signal is usually mapped into the frequency range around $f = 0$, called the baseband signal. If the bandwidth of the signal is around the frequency range of the baseband noise, the SNR will be lower than in the case in which the signal were in the white noise regime (see Fig. 3.16(a)). A very rough approximation for the baseband noise can be formulated to

$$\sigma \approx \sqrt{\int_f \text{PSD}_{\text{BB}}(\propto 1/f^a) df} \quad (3.41)$$

It has to be mentioned that for frequencies below a certain frequency threshold, the power spectral density becomes constant and is not a function of f , otherwise the integral would diverge to infinity. Furthermore, in a very small and low frequency range (e.g. bandwidth $B < 2 \text{ Hz}$), the PSD could be assumed constant which would lead to an detection limit of

$$\text{LOD} \approx \frac{3}{\sqrt{2}} \sqrt{K} \frac{\sqrt{\text{PSD}_{\text{BB}}} \sqrt{B}}{S_c} = \frac{3}{\sqrt{2}\pi} \frac{\lambda_0}{S_{\text{WG}}} \frac{\sqrt{\text{PSD}_{\text{BB}}} \sqrt{B}}{GR_{\text{sp}} \Gamma_{\text{loss}} \bar{\eta}^2 P_0} \frac{e^{\alpha_s L}}{L} \quad (3.42)$$

which has a minimum/optimum for $\alpha_s L = 1$:

$$\text{LOD}|_{\text{opt}} = \min_L \text{LOD} = \sqrt{K} \frac{3}{\sqrt{2}\pi} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \frac{\sqrt{\text{PSD}_{\text{BB}}} \sqrt{B}}{GR_{\text{sp}} \Gamma_{\text{loss}} \bar{\eta}^2 P_0}. \quad (3.43)$$

In biosensing the changes due to biorecognition of the sensor are causing very slow varying electrical signals (typically $f \ll 10 \text{ Hz}$), and baseband noise is, therefore, most likely the limiting factor. Hence, shifting the signal to a higher frequency range with a lower spectral noise density, might be advantageous. Shifting the baseband signal to higher frequencies, in which baseband noise is negligible, does however come with a price. To shift the based signal the optical power of the source is intensity modulated, here realised with the VOA. Since no negative powers are physically possible, there will be always a positive mean optical power superimposed with the modulation. The best possible modulation is shown in Fig 3.16(b) with

$$P_{\text{out}}(t) = P_{\text{max}} \left(\frac{1 + \sin(2\pi f_{\text{mod}} t)}{2} \right), \quad (3.44)$$

f_{mod} the modulation frequency, and P_{max} the optical input power of the VOA. A non-modulated signal would have a detected electrical power $P_e \propto (GR_{\text{sp}} P_{\text{max}})^2$. The electrical power detected ($P_{e,\text{mod}} \propto 1/T \cdot \int_T |GR_{\text{sp}} P_{\text{out}}(t)|^2 dt = P_{e,\text{DC}} + P_{e,\text{AC}}$) of the optical modulated signal (Eq. (3.44)) can be split into a direct current power part ($P_{e,\text{DC}}$) and alternating current power part ($P_{e,\text{AC}}$). For the later signal extraction only the alternating current power part is of interest, which can be calculated to $P_{e,\text{AC}} = 1/8 \cdot P_e$. Accounting for this signal reduction, the extracted complex signal v_c of the non-modulated case can be multiplied by the parameter $\sqrt{\eta_{\text{ME}}} = \sqrt{1/8}$, with η_{ME} the modulation efficiency. That means that an electrical signal power reduction of at least 9 dB is introduced by the modulation, probably even higher for real applications. To still observe a signal-to-noise-ratio increase in the passband signal, the AWGN level has to be 9 dB lower in the modulated frequency range.

3.2.3. Light Source Noise

There are two different noise sources which have their origin in the generation of the light used for sensing, optical source relative intensity noise and laser phase noise (RIN and LPN). In this

work, we consider both noises at the output of the EDFA, right before entering the VOA (see Fig. 3.9). In contrast to the detector noises, RIN causes correlated electrical signal at detection, and LPN can be minimised by design of the sensor. Here it will be theoretically demonstrated that CMZIs can be made highly immune to noise from the light source.

3.2.3.1. Relative Intensity Noise

Intensity variations of the generated light can have multiple origins, depending on the utilised laser. They may result from thermal caused length modifications of the laser cavity, supply current noise, spontaneous emission in the gain medium etc. The RIN influenced optical amplitude which is entering the fibre array can be written as

$$A(t) = \sqrt{P_0} \left(1 + \frac{r(t)}{2} \right) \quad (3.45)$$

with $|r(t)| \ll 1$ accounting for variations of the laser amplitude and, therefore, the power is given by:

$$P(t) = |A(t)|^2 = P_0 (1 + r(t))^2. \quad (3.46)$$

Once the light reaches the 1x2 MMI, it is split into reference and sensing arm. The time delay of the reference and sensing arms is τ_r and τ_s , respectively. That means that the light experiences the transition

$$a_{r,s}(t) \propto \sqrt{\frac{P_0}{2}} H_{r,s} \left(1 + \frac{r(t - \tau_{r,s})}{2} \right), \quad (3.47)$$

for the light at the output of the reference and sensing arm, respectively. The complex interference signal calculates as the product $a_r a_s^*$ to

$$v_c(t) \approx \frac{GR_{sp} \Pi_{loss} \bar{\eta}^2 P_0}{2} e^{j \frac{2\pi}{\lambda_0} \Delta L_{opt}} e^{-\alpha_s L} \left(1 + \frac{r(t - \tau_s) + r(t - \tau_r)}{2} \right) \quad (3.48)$$

assuming the mix term $r(t - \tau_s)r(t - \tau_r) \ll 1$ [99]. As the amplitude variation terms can be assumed uncorrelated after passing reference and sensing arm the RIN noise term after quantisation can be derived to an AWGN

$$\sigma_{RIN} = \frac{GR_{sp} \Pi_{loss} \bar{\eta}^2 P_0}{2} e^{-\alpha_s L} \sqrt{\frac{\varrho_{RIN}}{2}} B \quad (3.49)$$

with ϱ_{RIN} a spectral density accounting for $r(t)$ [99]. However, the phase term of Eq. (3.48) is not influenced by the amplitude variation $r(t)$ function. This means that RIN just has impact on the amplitude and not the extracted phase $\angle v_c$. This means that the coherently detected sensor is immune to RIN noise.

3.2.3.2. Phase Noise

Optical phase noise is typically generated by spontaneous photon emission in the gain medium of the laser or EDFA. Temperature changes or mechanical vibrations can alter the cavity length of the laser. This variations may cause the optical phase to be superposed by a random walk. Since the frequency of the light is the derivative of the phase, this noise spreads out the ideally infinitely narrow spectrum. The more phase noise the wider the light is distributed to other frequencies. The spread is characterised by the linewidth $\delta\lambda$ in metre, defined by the 3 dB-decline around its centre wavelength λ_0 . The linewidth can also be expressed in bandwidth δf in hertz, which can be converted from the linewidth via $\delta f = \delta\lambda \cdot c / \lambda_0^2$ and vice versa, if $\delta\lambda \ll \lambda_0$. Just considering the phase noise, the optical field can be written as

$$A_0(t) = \sqrt{P_0} e^{j\delta\psi(t)}, \quad (3.50)$$

with $\delta\psi(t)$ accounting for the undesired phase variations of the light generating device. Letting this signal propagate through both arms results in light with

$$a_{r,s} \propto \sqrt{\frac{P_0}{2}} H_{r,s} e^{j\delta\psi(t-\tau_{r,s})}, \quad (3.51)$$

for reference (a_r) and sensing (a_s) arm. Parameters τ_s and τ_r are the times the optical signal needs to pass the sensing and reference paths, respectively. The complex interferometric signal $\propto a_r a_s^*$ results in an detected complex signal

$$v_c \propto \frac{P_0}{2} e^{j\frac{2\pi}{\lambda_0} \Delta L_{\text{opt}}} e^{-\alpha_s L_s} e^{\Delta\psi(t)} \quad (3.52)$$

with $\Delta\psi(t) = \delta\psi(t-\tau_s) - \delta\psi(t-\tau_r)$, being the phase difference caused by the recombination of optical signal with phase $\delta\psi(t)$ after propagating through the sensing ($\delta\psi(t-\tau_s)$) and reference ($\delta\psi(t-\tau_r)$) arm. As stated in [176], the standard deviation of $\Delta\psi(t)$ is given by

$$\sigma_{\Delta\psi} = 2\Delta\tau \sqrt{\pi\delta f B} \quad (3.53)$$

with

$$\Delta\tau = |\tau_s - \tau_r| = \frac{|L_s n_{g,s} - L_r n_{g,r}|}{c_0} \quad (3.54)$$

the absolute time difference the light needs to pass both paths. According to Eq. (3.22) electrical complex noise can thus be written as

$$\sigma_{PN} \approx |v_c| \sigma_{\Delta\psi} \propto P_0 e^{-\alpha_s L_s} \Delta\tau \sqrt{\pi\delta f B} \quad (3.55)$$

Phase noise caused by the source is typically very small for sources with a small linewidth, e.g. external cavity-, or distributed feedback (DFB) laser. However, white sources, with linewidths up to several nm may cause an observable noise signal. Fortunately, by balancing the reference and sensing arm, i.e. equalising their optical length ($\Delta\tau = 0$), even optical sources with such large linewidths can be used, ideally without performance loss. It is also reported that by balancing the arms a higher temperature independence can be obtained [177].

3.2.3.3. Effects of Source Noise

Figure 3.17(a) demonstrates the influence of RIN and LPN on an unbalanced CMZI. The generated complex signal (blue), from which the phase is extracted, describes a circle in the IQ-plane. The RIN just affects its amplitude, generating a white Gaussian noise along the amplitude of the complex signal. The LPN only modifies the phase, generating a nearly white Gaussian noise along this direction of the complex signal, leaving the amplitude untouched. Depending on the strength of signal, RIN, and LPN, the noise probability density function will be nearly Gaussian, or, with increasing LPN, result in a highly unsymmetrical distribution (the coloured 2D plot). In Fig. 3.17(b) the probability density function of the superposition of source- with additive white Gaussian noise is demonstrated for (1) just additive-, (2) additive- and relative intensity-, (3) additive- and phase-, and (4) additive-, relative intensity-, and phase noise. This figure just serves to give the reader a feeling of the different noise sources and their effect on the complex signal. In real case scenarios the signal is typically much higher than shown here, making the observed noise more Gaussian shaped.

It should be highlighted that RIN and LPN immunity are not characteristics only applying to the coherent detection scheme. Laser phase noise can always be minimised by choosing a balanced interferometer and RIN can be removed by using a balanced detection scheme, for example by using a 2×2 MMI and two PDs and calculating the real-valued interferometric signal by $\sim (P_1 - P_2)/(P_1 + P_2)$. This is not possible for a simple detection scheme with

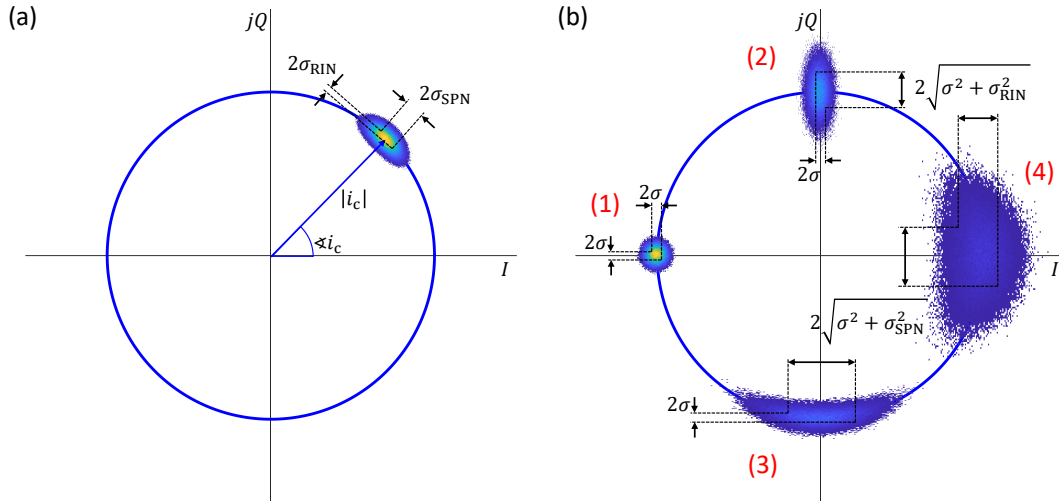


Figure 3.17.: (a) The effect of laser phase noise and relative intensity noise on the detected complex signal. RIN is always orthogonal to the desired signal ($\angle i_c$) whereas LPN is always parallel to it. (b) Graphical representation of how the noise sources mix together: (1) Pure electrical detector noise, (2) detector and RIN noise, (3) detector and Laser Phase Noise (LPN), and (4) detector and laser noise appear together.

just one optical output signal as it will always be affected, and ultimately limited, by RIN. However, a coherent read-out will always have the advantage of extracting the phase signal without sensitivity fading or phase ambiguity while being immune to spectral or intensity noise by the light source.

3.2.4. Mechanical Noise

Mechanical vibrations in the setup can cause movement of the fibre array used to inject and extract light from the chip (see Fig. 3.9). As illustrated schematically in Fig. 3.18, this causes a spurious variation of the fibre-to-chip coupling efficiency, from now on just referred to as mechanical noise (MN). Due to its unique nature, the impact of MN is difficult to circumvent, as discussed in the following.

Until now, the fibre-to-chip coupling efficiencies η_k were assumed constant with their product $\eta_0 \eta_k = \bar{\eta}^2$ also a constant. However, in real measurement setups, mechanical movements and vibrations are a common side effect, due to moving parts in the setup, installed electrical devices (e.g. ventilator), or even mechanical modes of the building. The resulting spurious modulation of the coupling efficiencies cannot be modelled as an additive noise – it rather results in a multiplication of the desired signal by the time-variant coupling efficiencies:

$$v_k \propto \eta_0(t) \eta_k(t) \left(\frac{1 + e^{-2\alpha_s L}}{2} + e^{-\alpha_s L} \cos(\Delta\varphi + \varphi_k) \right) \quad (3.56)$$

The coupling efficiency is a function of the position of the FA relative to the position of the gratings, i.e. $\eta_k(\hat{x}(t), \hat{y}(t)) = f(\vec{E}_{\text{smf}}, \vec{E}_{\text{rad}})$, where $\hat{x}(t)$ and $\hat{y}(t)$ are zero mean mechanical movements in $x - y$ directions and $\vec{E}_{\text{smf}}$, $\vec{E}_{\text{rad}}$ the electromagnetic field profiles of the single modes fibre and radiated beam of the grating, respectively. Both profiles are a function of the relative movements to each other, but by choosing the chip, and therefore the gratings, as inertial frame, we can model $\vec{E}_{\text{rad}}$ as a $\hat{x}(t), \hat{y}(t)$ independent function. The exact calculation

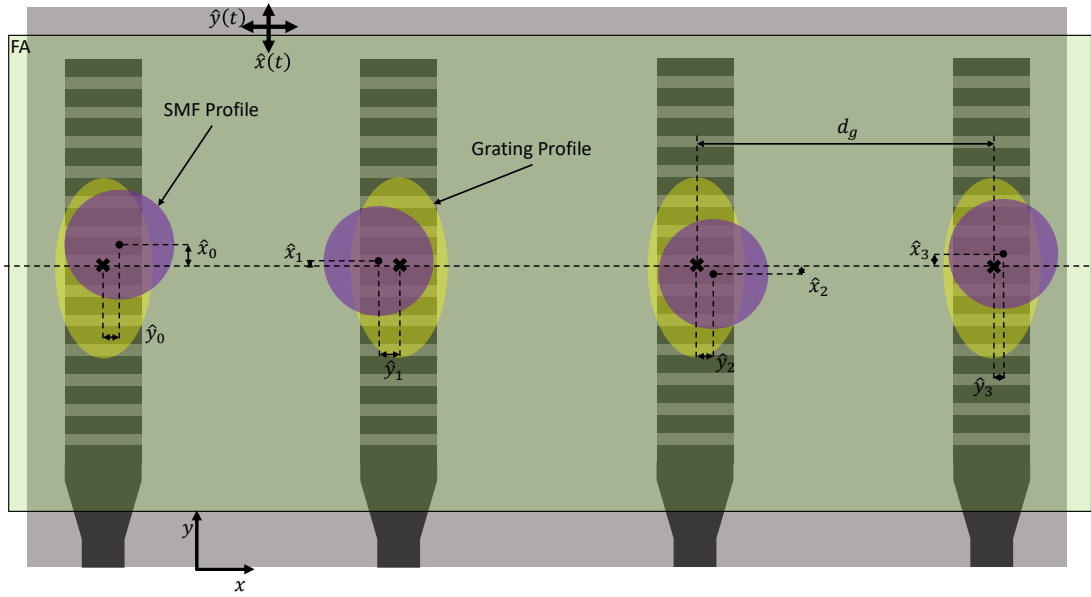


Figure 3.18.: Graphical illustration of how fabrication errors in the distances of the FA and gratings cause individual coupling efficiency modulations caused by mechanical movements, resulting in mechanical noise (MN.)

for MN are very complicated and it is difficult to derive a sophisticated model. However, to gain an understanding, we can make the assumption that both grating radiated beam and SMF mode have a normalised 2-dimensional symmetrical Gaussian TE field profile, i.e.

$$E_{\text{smf},x}(x,y) = E_{s0} e^{-\frac{(x+(\hat{x}(t)-\hat{x}_0))^2}{2\kappa_{\text{smf}}^2}} e^{-\frac{(y+(\hat{y}(t)-\hat{y}_0))^2}{2\kappa_{\text{smf}}^2}} \quad (3.57)$$

$$E_{\text{rad},x}(x,y) = E_{r0} e^{-\frac{x^2}{2\kappa_{\text{rad}}^2}} e^{-\frac{y^2}{2\kappa_{\text{rad}}^2}} \quad (3.58)$$

where TE means that their electric fields only have a component in x -direction. The mode field diameters in the x and y directions are given by κ_{smf} and κ_{rad} , and E_{s0} and E_{r0} the fields for the SMF and the radiated beam of the grating, illustrated in Fig. 2.8(b). The coupling efficiency between a fibre and a grating can be approximated by

$$\eta_k(t) \approx \left| \int \vec{E}_{\text{smf},k}(t) \vec{E}_{\text{rad}}^* dA \right|^2. \quad (3.59)$$

Solving this equation results again in an 2-dimensional symmetrical Gaussian function. The expressions $\hat{x}(t) - \hat{x}_0$, $\hat{y}(t) - \hat{y}_0$ are the MN describing terms and as each fibre has a different offset $\hat{x}_0, \hat{y}_0$, all modulation functions are unique to some extent, as indicated in Fig. 3.18, even with the same observed mechanical displacement $(\hat{x}(t), \hat{y}(t))$. Figure 3.19 is an exemplary demonstration how mechanical movements affect the coupling efficiencies of two identical gratings, however, just in one-dimension. (a) presents the coupling efficiency function for different displacements, whereas (b) shows the same mechanical displacement for the two gratings, but with different offsets $\hat{x}_0, \hat{x}_1$. The resulting efficiency modulation is shown in (c), confirming that the same mechanical displacements can result in totally different modulations. However, η_k may be correlated to η_n ($k \neq n$), but not necessarily in the way as it is for RIN. Furthermore, the mean of $\eta_k(x(t))$ is not necessarily $\eta_k(\hat{x})$, especially, for offsets in regions around $\eta_k(x=0)$ (see orange graph in Fig. 3.19(c)).

The origin of the offsets can have many different causes, e.g. no exact FA positioning, inaccurate grating positions due to fabrication, or FA manufacturing imprecision. The important fact here

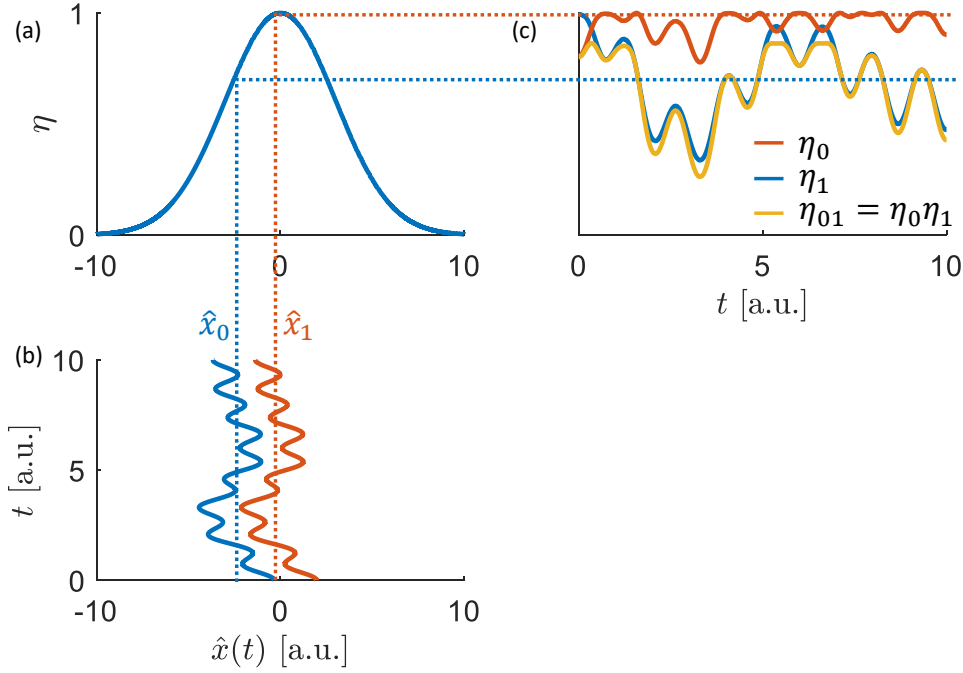


Figure 3.19.: (a) The coupling efficiency η of the light coupling from the FA to the grating coupler and vice versa. (b) The same mechanical movement $\hat{x}(t)$ of the FA causes different efficiency modulations η , due to different offsets $\hat{x}_0$ and $\hat{x}_1$. (c) The coupling efficiencies η_0 and η_1 cause a unique multiplicative noise $\eta_{01}(t)$ received by the first photodiode.

is that due to the multiplicative nature, the resulting electrical noise scales with P_0^2 , which means that if MN limits the LOD, nothing can be done for optimisation except attenuating the mechanical vibrations. In the coming detection limit optimisation procedure (see Section 3.3.2.2), the main mechanical mode for this setup is in the frequency region of some tens of Hertz, but also stretches down to the baseband and only vanishes below the AWGN floor for frequencies higher than 10 kHz. Here, especially problematic is the spectral content in the baseband, i.e. $f \approx 0$ Hz, as the desired signal of the phase shift is found there. It will interfere with the phase reconstruction by lifting the noise floor to a considerable high level, lowering the CSNR. Even modulation of the optical source itself, see previous chapter, cannot be used to shift the signal to lower noise spectral density regimes, as the efficiency modulation of the signal is a convolution in the frequency domain, i.e. MN would be copied to the modulation frequency domain.

It is worth highlighting, that if the light from gratings $k = 1, 2, 3$ were detected by a CCD line or CMOS camera, i.e. not coupled via FA, $\eta_k(t)$ would become constant, and MN, therefore, irrelevant, for its nature would be equal to RIN, to which CMZIs are, by definition, immune.

3.3. Optimisation of Homogeneous Refractive Index Sensing

In this section an exemplary bulk detection limit optimisation is performed based on the system level analysis of the prior sections. First, the protocol is presented (Section 3.3.1) on which basis the LOD parameter was extracted, followed by sub-sections (in Section 3.3.2) indicating the different steps applied to minimise the observed detection limit to approximately 1×10^{-8} RIU.

This section is based on the publication [100], a direct result from this doctoral thesis, and will, therefore, share many similarities.

3.3.1. Homogeneous Refractive Index Sensing Protocol

The detection limits for the optimisation were obtained by using the following HRI protocol. Milli-Q water was sucked into the syringe pump and then flown into the fluidic system with a velocity $F = 20 \mu\text{L min}^{-1}$. Injections of sodium chloride, diluted in the buffer, with increasing concentrations C were prepared, and injected via the valve system. The refractive indices of the chloride solutions are shown Table 3.2, and were calculated using with the equation

$$n_{\text{NaCl}}(C_{\text{w/w}}) = -0.08 \cdot C_{\text{w/w}}^3 + 0.074 \cdot C_{\text{w/w}}^2 + 0.162 \cdot C_{\text{w/w}} + 1.3162, \quad (3.60)$$

which is an experimentally determined polynomial [178], where $C_{\text{w/w}} \in [0, 0.25]$ is the mass percentage ($\%_{\text{m}}$) of sodium chloride in Milli-Q. The maximum phase change caused by the

Table 3.2.: Refractive index and refractive index changes in comparison to Milli-Q for the injected sodium chloride solutions at $\lambda = 1.55 \mu\text{m}$.

	n [RIU]	Δn [RIU]
Milli-Q	1.3162	
NaCl 0.5 M (3% _m)	1.3211	4.9×10^{-3}
NaCl 1.0 M (6% _m)	1.3262	1.0×10^{-2}
NaCl 1.5 M (9% _m)	1.3313	1.5×10^{-2}
NaCl 2.0 M (12% _m)	1.3366	2.0×10^{-2}

injections is then mapped against the corresponding calculated refractive index change Δn . The phase changes should ideally lie on the graph following $\Delta\varphi = S_\varphi \Delta n$. From $\Delta\varphi$ and Δn , the phase sensitivity S_φ is extracted. Figure 3.20 shows an exemplary experiment with (a) the observed phase changes and (b) the graph for extracting the sensitivity S_φ . The noise signal, in the inset of (a), enables us to extract the standard deviation σ_φ . The LOD is then determined as:

$$\text{LOD} = \frac{3\sigma_\varphi}{S_\varphi} \quad (3.61)$$

Using this approach the approximate sensitivity was found to be $S_\varphi = 4200 \text{ rad/RIU}$, in close agreement with the theoretical value of 4900 rad/RIU given by Eq. (3.9). As for the standard deviation of the noise signal, it is heavily dependent on the optimisation of the setup parameters, which we will discuss in the following.

3.3.2. Optimisation

To facilitate the experimental optimisation process, in each optimisation step we assume that one of the noise sources is dominant. Furthermore, we will see that it is more practical to work with proportionality relations between LODs, rather than absolute values. We therefore simplify the LOD expressions for thermal noise (Eq. (3.28)), shot noise (Eq. (3.34)) and

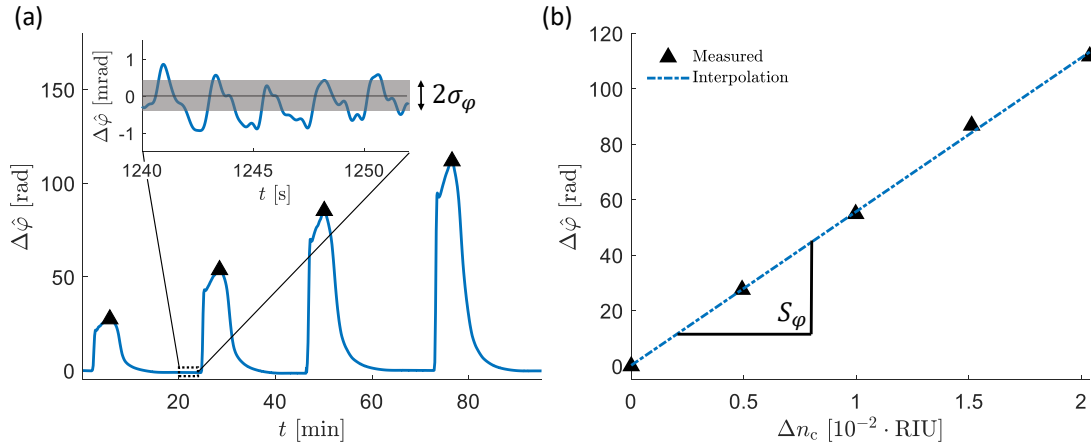


Figure 3.20.: Example of a bulk sensing experiment for the extraction of the phase sensitivity S_φ and phase noise σ_φ to estimate the detection limit $\text{LOD} = 3\sigma_\varphi/S_\varphi$. (a) Extracted phase change ($\Delta\hat{\varphi}$) for the four sodium chloride injections – the maximum changes are highlighted with black triangles. The inset shows the phase noise, with standard deviation σ_φ , when only the buffer (Milli-Q) is flowing. (b) The maximum phase changes are plotted against the corresponding refractive index change Δn_c with a linear interpolation – the slope yields the phase sensitivity S_φ .

quantisation noise (Eq. (3.39)) as follows:

$$\text{LOD}_{\text{TN}} \propto \frac{\text{NEP}}{P_0} \sqrt{\vartheta_{\text{un}} B} \quad (3.62)$$

$$\text{LOD}_{\text{SN}} \propto \sqrt{\frac{B}{P_0}} \vartheta_{\text{un}} \quad (3.63)$$

$$\text{LOD}_{\text{QN}} \propto \frac{\Delta V_{\text{qs}}}{G P_0} \sqrt{\frac{B}{f_s}} \quad (3.64)$$

For baseband (flicker) noise and mechanical noise it is clear that the detection limit is related to the power spectral density¹:

$$\text{LOD}_{\text{BN}} \propto \sqrt{\int_B \text{PSD}_{\text{BN}} df} \quad (3.66)$$

$$\text{LOD}_{\text{MN}} \propto \sqrt{\int_B \text{PSD}_{\text{MN}}(P_0) df}. \quad (3.67)$$

The initial configuration that we consider for our setup is: $\text{IL}_{\text{oss}} \eta^2 P_0 \approx 160 \mu\text{W}$, $f_s = 83.3 \text{ kHz}$, $V_{\text{DAQ}} = 5 \text{ V}$, and $G \approx 31 \text{ kV A}^{-1}$. Since the sensing signals typically change at the rate of a few Hertz, we choose an initial sampling frequency $f_s = 50 \text{ Hz}$. Figure 3.21(a) shows the noise signal (red line) and (b) its the power spectral density (red line), in units of V^2/Hz . Having

¹For a discrete signal $v[n]$ the two-sided PSD can be computed directly from the discrete Fourier-Transform $\mathcal{F}$:

$$\text{PSD} \left[f_n = \frac{n}{N} f_s \right] = \frac{|\mathcal{F}\{v\}|^2}{N f_s}, \quad (3.65)$$

with N the number of samples in v .

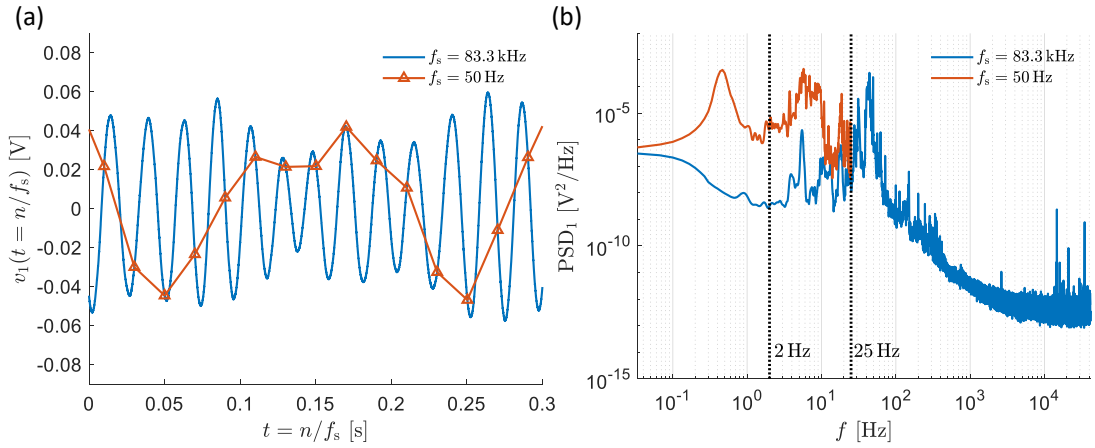


Figure 3.21.: (a) Measured variation of the electrical signal around the mean value at the first PD for different sampling frequencies (f_s): oversampling with $f_s = 83.3$ kHz and undersampling with $f_s = 50$ kHz. (b) The corresponding power spectral densities (PSD). A sufficiently high sampling frequency should be chosen to avoid artificially amplifying the noise floor in the baseband.

no prior information about the importance of the different noise sources, this would appear as a valid representation of the noise in the detection system. The observed standard deviation of the phase noise for this initial configuration is $\sigma_\varphi = 45$ mrad, yielding a detection limit of $\text{LOD} = 3 \times 10^{-5}$ RIU. In the following we examine the different noise sources in more detail.

3.3.2.1. Avoiding Spectral Aliasing

In a first step we increase the sampling frequency to the maximum possible value $f_s = 83.3$ kHz (with $K = 3$), in an attempt to reveal the true nature of the noise. The blue lines in Fig. 3.21(a) and (b) show the resulting signal in the temporal and spectral domain, respectively. We observe that the previously detected signal was undersampled, thus experiencing aliasing, and artificially increasing the noise power spectral density, as shown in Section 3.2.1.4. It also becomes clear that for higher frequencies the PSD tends towards a white noise floor. However, the PSD comparatively high below 1000 Hz. Applying a digital low-pass filter with a bandwidth $B = 25$ Hz, i.e. the same bandwidth as the previously undersampled signal, results in a $\text{LOD} = 3.8 \times 10^{-6}$ RIU, an improvement of approximately 8 times. The true PSD (blue), has a strong peak around $45 \rightarrow 50$ Hz, which cannot be observed in the undersampled signal, and which is most likely a result from a mechanical vibration mode. This was verified by a mechanical characterisation of the setup: placing an accelerometer on the fibre array holder, and exciting the setup with a small impact hammer, revealed a mechanical active mode at $f \approx 50$ Hz (see appendix Fig. A.7), which unfortunately coincides with the typical $f \approx 50$ Hz electrical hum from the power supply. However, even though the mechanical mode eigenfrequency and electrical hum frequency are the same, their origins are entirely different. The electrical hum arises from the electromagnetic coupling from the AC power wires/systems in the laboratory, whereas the mechanical mode is a result of the mechanical composition of fibre array holder, table, and the entire building structure. The undersampling of the signal folds the PSD of the higher frequencies ($f > 25$ Hz) to the lower region. That means that the mechanical mode seen in the electrical domain will be observed at around 5 Hz. Experiments in the following chapters were performed with the much more elaborated setup, including the optical table for decoupling mechanical vibrations from the surrounding, which is why no similar measurement was done to determine the mechanical mode there.

In the properly sampled signal we can eliminate part of the mechanical noise by decreasing the low-pass filter bandwidth B , and thus improve the LOD (see Eq. (3.67)). Hence, we further reduce the bandwidth from $B = 25$ Hz to $B = 2$ Hz. For a white noise source the detection limit scales with $\sqrt{B}$, however, for mechanical noise this is only a rough approximation. Instead of an improvement of $\sqrt{25/2} \approx 3.5$ a LOD improvement of 2.5 was observed, yielding an LOD of 1.5×10^{-6} RIU.

3.3.2.2. Dampening Mechanical Vibrations

So far, the fibre array has been placed very close to the chip surface, but without any direct contact. However, this allows for the mechanical vibrations, thus limiting the LOD of our system. One way to suppress mechanical noise is to design the grating such that the radiated beam covers a huge area, being less sensitive to mechanical movements, but also reducing the mean coupling efficiency. Another alternative is to fix the fibre array to the chip surface. This could be done by using a glue, or by pressing the tip of the array onto the chip surface (most of the surface is covered by a protective silicon dioxide layer, preventing damage to the waveguides). In this way, the fibre array and the chip do not move at all, or at least tend to move together. Figure 3.22 compares the power spectral density of one photodiode when the fibre array is not touching the surface (blue) and when it is touching (orange). For frequencies above

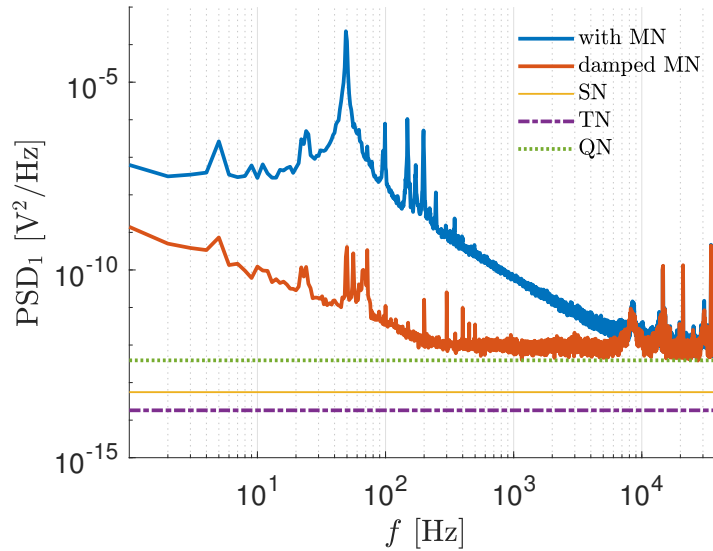


Figure 3.22.: Power spectral density of the first PD, under influence of mechanical noise (blue), with reduced mechanical noise (orange), and the theoretical noise floors for the utilised setup configuration in case of shot noise (yellow), thermal noise (purple dashed), and dominating quantisation noise (green). The mechanical noise reduced spectra converges close to the quantisation noise limit for higher frequencies, which speaks in favour for the derived model.

100 Hz, the power spectral density becomes constant and is almost as low as the theoretical quantisation noise limit (green dashed). For lower frequencies the PSD scales with $1/f^{1.3}$, indicating LOD limitation due to flicker noise. The mechanical noise peak around 50 Hz still seems to be present, however, almost six orders of magnitude lower. However, due to the digital low pass filtering, this can be neglected. At this power level, and a filter bandwidth of 2 Hz, the noise is an approximately a factor 100 lower than the previously dominant mechanical noise, which theoretically results in an LOD improvement of $\sqrt{100} = 10$. The homogeneous refractive index experiment demonstrates a factor of 4.3, that means a $\text{LOD} = 3.5 \times 10^{-7}$ RIU. The

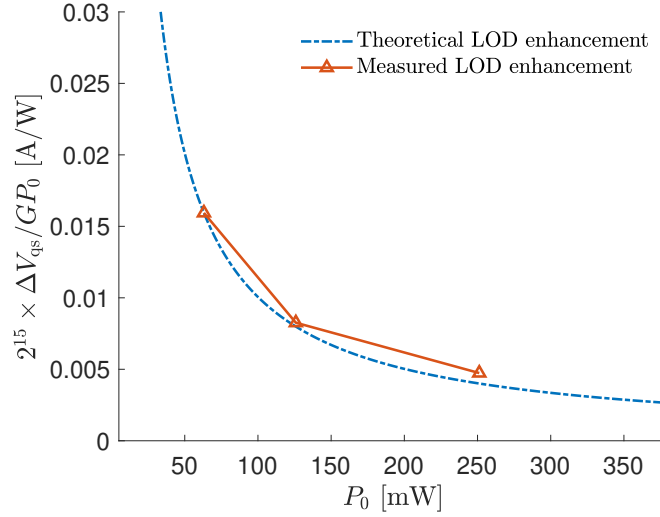


Figure 3.23.: The theoretical (blue) and measured (orange) improvement due to increase optical power from the EDFA.

difference in enhancement can have many reasons, for example different flicker or mechanical noise at different photodiodes.

3.3.2.3. Reducing Electrical Detection Noise

Figure 3.22 shows the calculated theoretical shot-, quantisation-, and thermal noise power spectral densities, where quantisation noise clearly dominates and gets close to the measured noise floor. The enhancement strategy here is very basic and can be reduced to the simple statement of “Amplifying the detected signal to using the full range of the data acquisition board.” To achieve this, we used the following steps:

1. Adapt the DAQ dynamic range (V_{DAQ}) to a higher range if possible.
2. Adjust the output power of the EDFA such that the detected electrical are as strong as possible, but still within the dynamic range. For thermal-, quantisation-, or shot noise limited systems, this should always go accompanied with an LOD improvement.
3. If the DAQ channels are not at saturation, i.e. $v_k < V_{DAQ}$, adjust to higher Gain. This will just enhance the LOD for thermal noise, due to the dependency of NEP and G , and definitely for quantisation noise, as it is independent of the gain. For shot noise, signal and noise scale both with G , and see therefore the same limitation.
4. In case DAQ saturation is reached, start again with the second step, otherwise stop here.

With dominant quantisation noise the LOD is governed by by Eq. (3.64), the observed LOD enhancement (here defined as $\Delta V_{qs} / (GP_0)$ according to the quantisation noise detection limit proportionality Eq. (3.64)) for different steps (blue triangles) is shown in Fig. 3.23. It follows the expected theoretical curve (dashed light blue). The corrected LOD becomes 7.4×10^{-8} RIU.

3.3.2.4. Avoiding Flicker Noise

To remove the influences of the residual baseband noise, an optical power modulation scheme was established by using the VOA, controlled by the signal generator, right after the EDFA (see Fig. 3.9). Baseband noise is not signal dependent and is fixed to the lower frequency

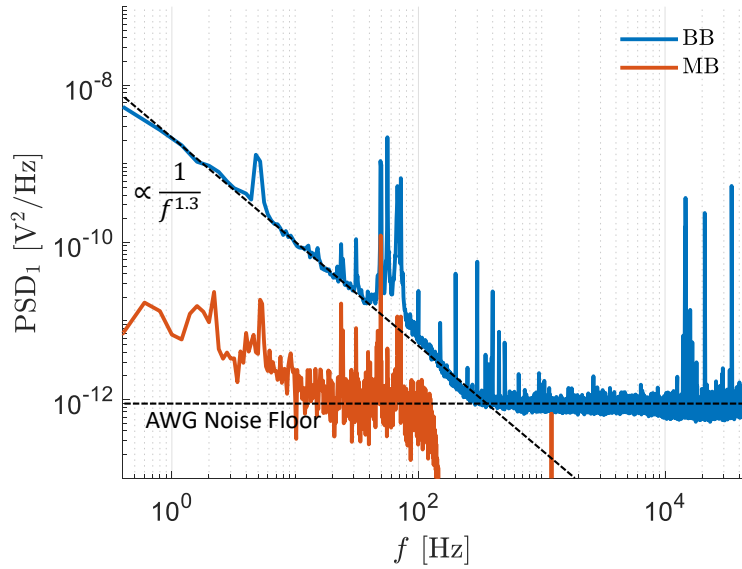


Figure 3.24.: Measured noise spectra at one PD including baseband (Flicker) noise (BB), and modulation band noise (MB). Here, the signal at the modulation frequency (600 Hz) was passband filtered, and digitally back shifted to baseband. The resulting noise floor is much lower than for BB, and reaches almost down to the AWGN floor.

range. By driving the VOA with $V_{SG}(t) = 2.5 \text{ V} + 1.5 \text{ V} \cdot \sin(2\pi f_{\text{mod}} t)$, the optical signal power linearly follows $P_0(t) \propto V_{SG}(t)$. Due to non-linearities in the VOA transfer function, higher order harmonics may occur, reducing the modulation efficiency. Figure 3.24 exemplary shows a measured power spectral density of the first channel when no changes in the refractive index are taking place. The red spectra corresponds to the uncertainty dominated by baseband ($f \in [0, 300 \text{ Hz}]$) and for additive white Gaussian noise ($f > 300 \text{ Hz}$). Signals around lower frequencies would, therefore, experience an electrical noise power floor more than 1000 larger than for the higher frequency region.

In this example, the signal has been modulated to $f_{\text{mod}} = 600 \text{ Hz}$, digitally passband filtered with a bandwidth from $f_{\text{mod}} - 150 \text{ Hz} \rightarrow f_{\text{mod}} + 150 \text{ Hz}$. The resulting signal is then multiplied with $\cos(2\pi f_{\text{mod}} t)$, shifting half of the signal content, and half of its noise, to the baseband and the other half to $2 \times f_{\text{mod}}$. As this is digital modulation, the SNR stays constant. Just considering the new baseband signal, it was filtered with a digital low-pass digital filter with $B = 150 \text{ Hz}$, which is why the spectrum decreases drastically after this frequency. However, the peak at $2 \times f_{\text{mod}} = 1200 \text{ Hz}$ can still be seen, but with a PSD around the noise floor. The resulting baseband signal, has a low noise floor for its higher frequencies ($10 \rightarrow 150 \text{ Hz}$), the AWGN floor, and a slightly increased noise floor, approximately ten times the additive white Gaussian noise floor, for frequencies below 10 Hz. Theoretically, a factor of 4 is the highest possible LOD improvement for a perfect intensity modulation (see Section 3.2.2.4). This factor is calculated as the square-root of the electrical noise power floor improvement (100) divided by the electrical signal power reduction (8).

For this optimisation procedure, a modulation frequency of $f_{\text{mod}} = 800 \text{ Hz}$ were chosen, far away from the flicker noise spectral range, and we reached a detection limit of $3.6 \times 10^{-8} \text{ RIU}$, which is an improvement factor of around 2. Assuming that the detected signal at 800 Hz suffered a 10 dB power reduction, probably even higher, the noise floor must have been at least 40 times lower.

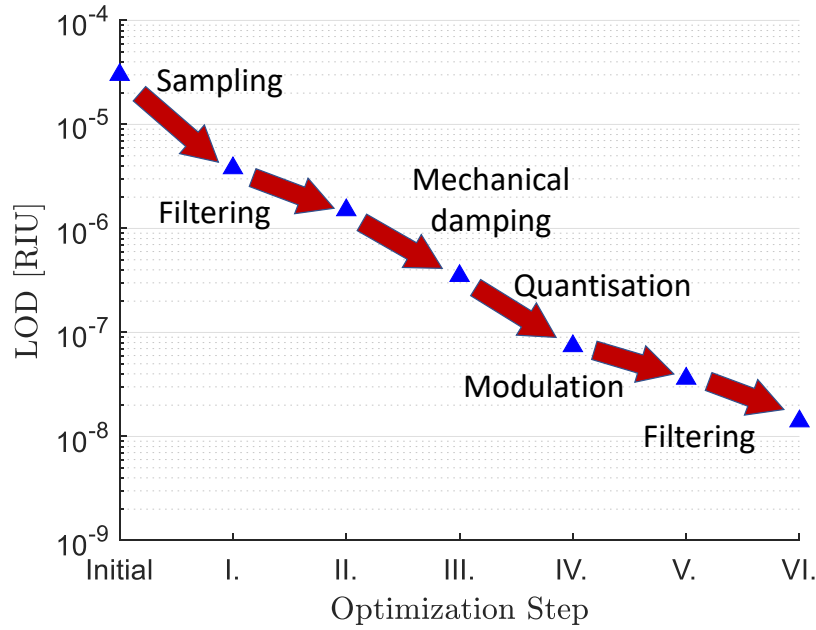


Figure 3.25.: The applied optimisation procedure showing the different detection limits for each step. Exceptional detection limits can be reached for CMZIs with proper noise handling.

Finally, as mechanical- and baseband noise are not limiting, or at least highly reduced, an aggressive increase of digital filter bandwidth from 2 Hz $\rightarrow$ 0.2 Hz, demonstrates an ultimately optimised $\text{LOD} = 1.4 \times 10^{-8}$ RIU, an enhancement factor of ~ 2.6 , which is close to the theoretical bandwidth improvement of ~ 3 , predicted by Eq. (3.64).

Figure 3.25 graphically summarises the detection limit improvement based on the presented steps. Compared to the state-of-the-art (see Section 2.4), this is a very good HRI detection limit, compared to most photonic sensors. Only two other sensors, also an CMZI [147] and a Young interferometer [78], demonstrated comparable LODs. This reinforces the idea that CMZIs are capable of ultra-low detection limits.

3.4. Theoretical Limits for Coherent Detection

In our theoretical analysis of noise and LOD in Section 3.2 we ignored saturation effects in the photodiodes or DAQ for the sake of simplicity. In this section we are going to evaluate, from a theoretical point of view, the detection limits that can be achieved when the output voltage swing is limited. In our setup this limitation is the input range V_{DAQ} of the DAQ. For our analysis we are first going to consider the sensor based on the 2×3 MMI. For this configuration the DC component of the output voltages is shown to be a limiting factor. We then analyse an alternative configuration with a 2×4 MMI and differential photodiodes which eliminates the DC component.

3.4.1. 2×3 Read-Out

In our sensor, the effective refractive index change in the sensing arm is converted into three interferometric signals which are shifted by 120° with respect to one another. All three signals

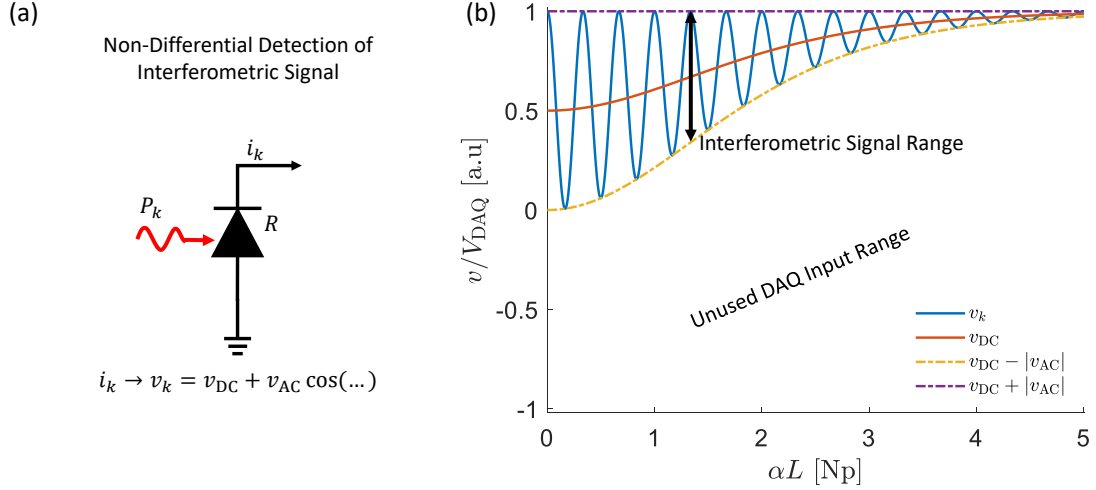


Figure 3.26.: (a) Non-differential detection of one of the individual interferometric signals of the coherent read-out realised with the 2×3 MMI. (b) The corresponding P_{max} -driven interferometric signal as a function of absorption $\alpha_s L$ in the sensing arm showing the DC-, AC part, and range of the interferometric signal. The blue curve shows qualitatively an interferometric signal. In any case, less than half of the DAQ input range are not exploitable.

are used to calculate the complex signal, which, after calibration, describes a circle in the IQ-plane. The real and imaginary part have zero mean value. Unfortunately, this is not true for the detected photocurrents and photovoltages generated at each sensor output k , which are always positive $v_k > 0$ and thus have a strong DC component, i.e. $\overline{v_k} > 0$. This implies that only half of the DAQ range can be used for detection. The situation is demonstrated in Fig. 3.26. Additional losses in the sensing arm further shrink the dynamic range for detecting the interferometric signals. In this context we can write that

$$v_k = \frac{GR\eta^2\text{IL}_{\text{oss}}P_0}{3} \left(\frac{1 + e^{-2\alpha_s L}}{2} + e^{-\alpha_s L} \cos(\Delta\varphi + \varphi_k) \right) \leq V_{\text{DAQ}} \quad (3.68)$$

meaning that the interferometric signal voltage in channel k is limited by the DAQ dynamic range. The maximum detectable optical signal is given by

$$v_{k,\text{max}} = \max_{P_0} \max_{\Delta\varphi} v_k = V_{\text{DAQ}} = GR\eta^2\text{IL}_{\text{oss}}P_{\text{max}} \frac{(1 + e^{-\alpha_s L})^2}{6} \quad (3.69)$$

which can be rewritten as

$$P_{\text{max}} = \frac{V_{\text{DAQ}}}{GR\eta^2\text{IL}_{\text{oss}}} \frac{6}{(1 + e^{-\alpha_s L_s})^2}. \quad (3.70)$$

This equation represents the maximum input power that can be used, and which thus yields the best signal-to-noise ratio for thermal and shut noise. Imagining a sensing path with infinitely large attenuation, due to the absorption constant or a very long propagation path, this maximum optical power converges to

$$P_{\text{max},\infty} = \lim_{L \rightarrow \infty} P_{\text{max}} = \frac{6V_{\text{DAQ}}}{GR\eta^2\text{IL}_{\text{oss}}}, \quad (3.71)$$

which will serve for the following observations as optical power normalisation, i.e. $P_0/P_{\text{max},\infty}$. By insertion Eq. (3.70) into Eq. (3.8) we obtain the sensitivity as a function of V_{DAQ} :

$$S_{c,\text{max}} = 6\pi \frac{L}{\lambda_0} V_{\text{DAQ}} \frac{e^{-\alpha_s L}}{(1 + e^{-\alpha_s L})^2} S_{\text{WG}} \quad (3.72)$$

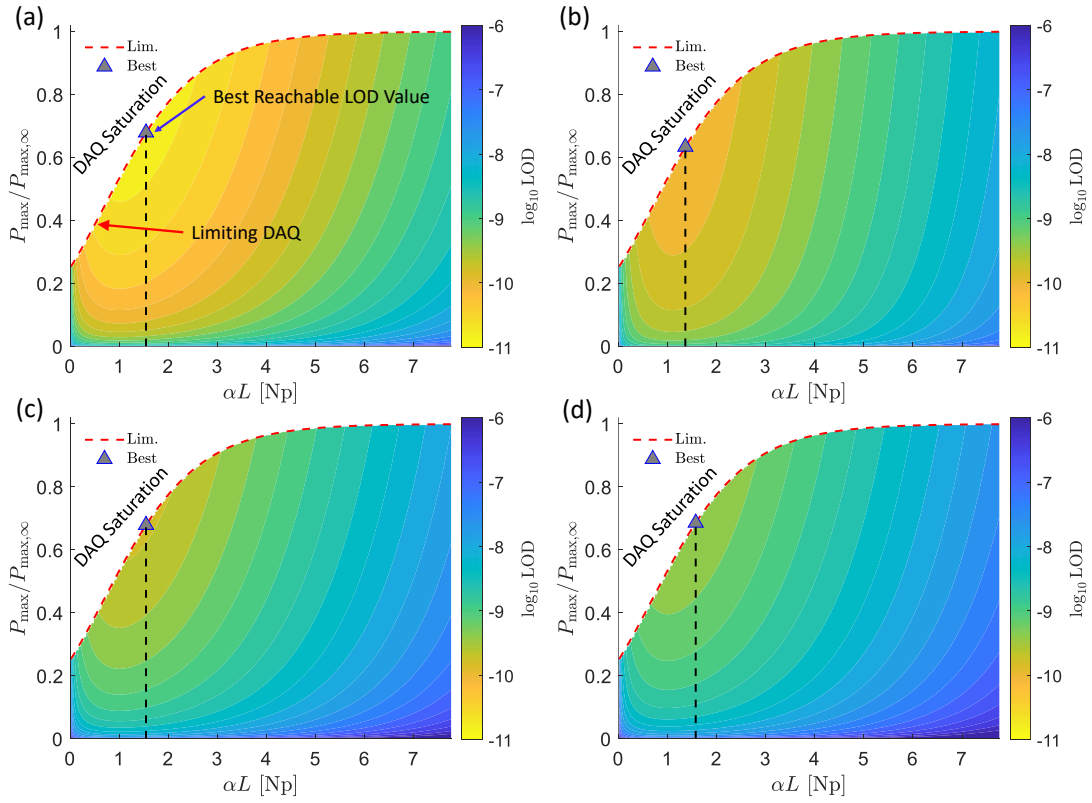


Figure 3.27.: Logarithmically plotted LOD for different normalised optical powers $P_{\max}/P_{\max,\infty}$, and absorptions αL for (a) thermal-, (b) shot-, and (c) quantisation noise for a specific setup configuration. Their superposition is shown in (d). The red dotted lines represent the limitation due to the PDs in a non-differential coherent read-out with the 2×3 MMI. The black line shows the position of the valley of the LOD with its lowest point (triangle) representing the best obtainable LOD possible.

In the following paragraphs, the best possible detection limit for three different originating noise sources (thermal noise, shot noise, and quantisation noise) will be discussed. Mechanical- and flicker noise will be assumed to be already attenuated or circumvented.

3.4.1.1. Thermal/Johnson Noise

As thermal noise does not scale with optical power, the LOD can be derived from Eqs. (3.27) and (3.72) yielding

$$\text{LOD}_{\max}(L) = \frac{1}{2\sqrt{2}\pi} \frac{\lambda_0}{S_{\text{WG}}} \sqrt{K} \frac{GR}{V_{\text{DAQ}}} \text{NEP} \sqrt{\vartheta_{\text{un}} B} \frac{e^{\alpha_s L_s}}{L_s} (1 + e^{-\alpha_s L_s})^2 \quad (3.73)$$

Compared to Eq. (3.28) the optimum length appears at $\alpha_s L \approx 1.5434$ instead of $\alpha_s L = 1$. Inserting the optimum length $L_{\text{opt}} = 1.5434/\alpha_s$ we obtain the best possible detection limit for a CMZI with a 2×3 MMI read-out:

$$\text{LOD}_{\text{best}} = \frac{4.4668}{2\sqrt{2}\pi} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \sqrt{K} \frac{GR}{V_{\text{DAQ}}} \text{NEP} \sqrt{\vartheta_{\text{un}} B}. \quad (3.74)$$

This situation is illustrated in Fig. 3.27(a).

3.4.1.2. Shot Noise

Shot noise scales with power which requires a modification of Eq. (3.33), i.e. substituting P_0 for Eq. (3.70):

$$\sigma_{\text{SN}} = \sqrt{6} \sqrt{GV_{\text{DAQ}}} \sqrt{q\vartheta_{\text{un}}B} \frac{\sqrt{1 + e^{-2\alpha_s L_s}}}{1 + e^{-\alpha_s L_s}}, \quad (3.75)$$

which is the maximum shot noise one can observe without saturating the DAQ. From Eqs. (3.75) and (3.72) we then obtain the limit of detection as:

$$\text{LOD}_{\text{max}}(L) = \frac{\sqrt{3}}{2\pi} \frac{\lambda_0}{S_{\text{WG}}} \sqrt{q\vartheta_{\text{un}}B} \sqrt{\frac{G}{V_{\text{DAQ}}}} \frac{e^{\alpha_s L_s}}{L_s} (1 + e^{-\alpha_s L_s}) \sqrt{1 + e^{-2\alpha_s L_s}} \quad (3.76)$$

for which the optimum length is $L = L_{\text{opt}} = 1.3616/\alpha_s$. The best detection limit that can be achieved is then given by:

$$\text{LOD}_{\text{best}} = \frac{\sqrt{3}}{2\pi} 3.7168 \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \sqrt{q\vartheta_{\text{un}}B} \sqrt{\frac{G}{V_{\text{DAQ}}}}. \quad (3.77)$$

The most interesting term in this equation is the last square root. Given that our optical source supports a sufficient power level, going to higher DAQ dynamic ranges and lower TIA gains, one can push the detection limit further down. The PD responsivity does not play any role in the optimisation process, neither does the sampling frequency. In short, if shot noise limits the sensors performance, do not increase gain as both signal and noise will scale the same amount, leaving the complex signal-to-noise-ratio untouched. Preferably, increase optical power P_0 to decrease gain, therefore, scaling the cSNR with P_0 [179]. This situation is illustrated in Fig. 3.27(b).

3.4.1.3. Quantisation Noise

Analogously to thermal noise, the quantisation noise limited detection limit can be derived by Eqs. (3.38) and (3.72) to

$$\text{LOD}_{\text{max}}(L) = \frac{1}{\pi} \frac{\lambda_0}{S_{\text{WG}}} \sqrt{K} \frac{\Upsilon_{\text{QN}}}{2^N} \sqrt{\frac{B}{f_s}} \frac{e^{-\alpha_s L_s}}{L_s} (1 + e^{-\alpha_s L_s})^2 \quad (3.78)$$

also with an optimal length $L_{\text{opt}} = 1.5434/\alpha_s$:

$$\text{LOD}_{\text{best}} = \frac{4.4668}{\pi} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \sqrt{K} \frac{\Upsilon_{\text{QN}}}{2^N} \sqrt{\frac{B}{f_s}}. \quad (3.79)$$

Interestingly, the LOD is independent of the DAQ voltage range, which due to the fact that $\sigma_{\text{QN}} \propto V_{\text{DAQ}}$, if most of the DAQ range is used. This means that if quantisation noise limits the sensors performance, higher optical power, gain or responsivities do not lower the optimum LOD. However, the detection limit can still be improved by the DAQ quality factor, the number of quantisation bits and sampling frequency. This situation is illustrated in Fig. 3.27(c).

3.4.1.4. Combined noise

Figure 3.28 shows all three optimum detection limit functions for thermal-, shot-, and quantisation noise for the corresponding attenuation $\alpha_s L$, for an optimally used detection range. As also visible in Fig. 3.27, the minimum LOD is actually not at around $\alpha_s L \approx 1$ but $\alpha_s L \approx 1.5$. However, it is up to the biosensing system designer to decide if it makes sense to make the sensor length larger, even though the LOD increase is not substantially larger, but occupying more physical space on the chip.

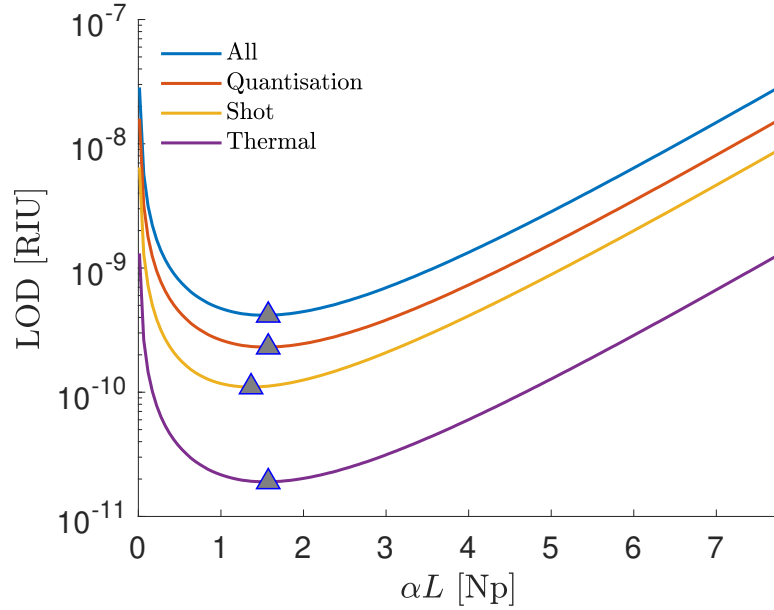


Figure 3.28.: The detection limits from Fig. 3.27 for the different (quantisation, shot, and thermal) and their superposition (All) of noise sources as a function attenuation αL assuming maximum possible usage of the DAQ input detection range. The triangles mark the positions of the best detection limits reachable for each case.

3.4.2. 2x4 Read-Out

The difference between the 2×3 - and 2×4 MMI coupler is that the last has four optical outputs each with a 90° interferometric phase shift compared to its neighbour. If we use differential photodiodes, i.e. two photodiodes with almost the same characteristics, and connected such that the output current is $\propto i_a - i_b$, the difference between the two detected currents of photodetector a and b, as shown in Fig. 3.29(a). The current is then amplified with gain G of the TIA and sampled and digitalised by the DAQ, just using two channels ($K = 2$). The two resulting voltages follow

$$v_k = GR\eta^2 \Gamma_{\text{loss}} P_0 e^{-\alpha_s L_s} \cos(\Delta\varphi + \varphi_k) \quad (3.80)$$

with $\varphi_k = [0, 90^\circ]$ for $k = 1$ and $k = 2$. Hence, v_1, v_2 are already the I and Q signals, with an ideally zero mean-value that can cover the entire DAQ range, as qualitatively demonstrated in Fig. 3.29(b). Requiring

$$v_{k,\text{max}} = \max_{P_0} \max_{\Delta\varphi} |v_k| = V_{\text{DAQ}} \quad (3.81)$$

a simple equation for the maximum allowed optical power can be derived:

$$P_{\text{max}} = \frac{V_{\text{DAQ}}}{GR\eta^2 \Gamma_{\text{loss}}} e^{\alpha_s L_s}. \quad (3.82)$$

There is no $P_{\text{max},\infty}$ for this case for $P_{\text{max}} \propto \exp(\alpha_s L)$, nevertheless, the $P_{\text{max},\infty}$ from the 2×3 case (see Eq. (3.71)) will serve as normalisation for comparability. The Eq. (3.82) implies that the optical power could be easily adjusted to compensate for absorption losses. However, what is not accounted for in this increase of optical power is: the possible saturation or non-linearity of the photodiodes, non-linear waveguide absorption, or even damage of the photonic structures on-chip. Assuming those effects neglectable, the maximum reachable electrical sensitivity becomes according to Eqs. (3.82) and (3.8):

$$S_{c,\text{max}} = \frac{2\pi}{\lambda_0} LV_{\text{DAQ}} S_{\text{WG}} \quad (3.83)$$

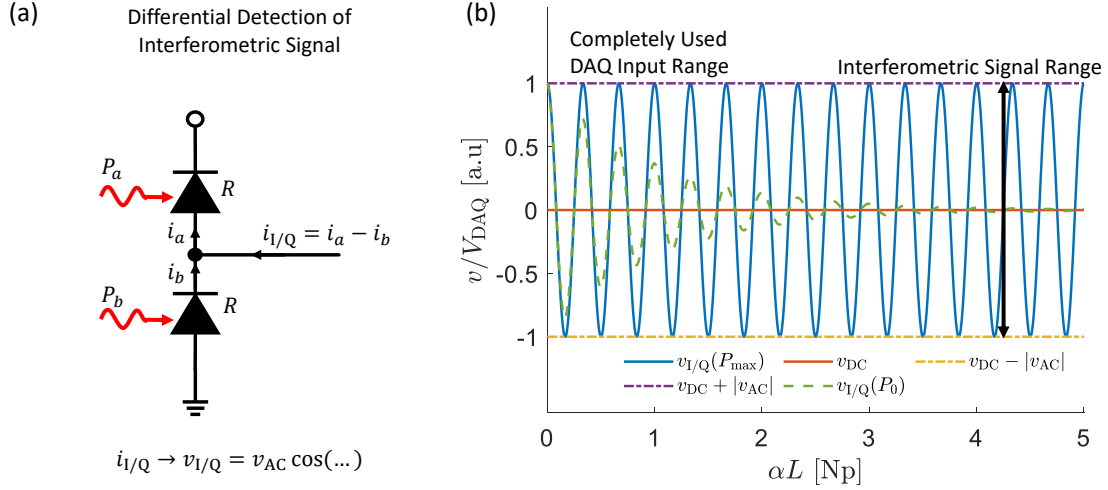


Figure 3.29.: (a) Differential detection of two of the individual interferometric signals of the coherent read-out realised with the 2×4 MMI. (b) The corresponding P_{max} -driven interferometric signal as a function of absorption $\alpha_s L$ in the sensing arm showing the DC-, AC part, and range of the interferometric signal. The blue curve shows qualitatively an interferometric signal. The entire DAQ input range is always exploitable. The green dashed line shows an qualitatively interferometric signal without power adaption to P_{max} .

which, interestingly, scales with just the length L instead of mixing terms of exponential functions of L , as it is the case for the 2×3 read-out. The phasic sensitivity S_φ and the electrical sensitivity become interchangeable with $S_{c,max} = S_\varphi V_{DAQ}$, which is actually a easy form of Eq. (3.9). As in the previous section, thermal-, shot-, and quantisation noise will be investigated independently, and some optimisation rules are derived.

3.4.2.1. Thermal/Johnson Noise

Being independent of optical power, the thermal noise does not set a limit to the detection limit derived by Eqs. (3.27) and (3.83):

$$\text{LOD}_{\max}(L) = \frac{3}{\sqrt{2\pi}} \frac{\lambda_0}{S_{WG}} \sqrt{K} \frac{GR}{V_{DAQ}} \text{NEP} \sqrt{\vartheta_{un} B} \frac{1}{L}. \quad (3.84)$$

As for the minimal observable detection limit, there is no optimum length, if thermal noise were the limiting uncertainty source. Making the sensor incredibly long, increasing sensitivity, and compensating the obtained sensor losses with a higher optical power of the source could be used to reach for the lowest detection limits. Here, low gain and high optical power is the ground rule. This situation is illustrated in Fig. 3.30(a).

3.4.2.2. Shot Noise

As in the case of the 2×3 read-out, the shot noise (Eq.(3.33)) for maximum optical power from Eq. (3.82) has to be adapted:

$$\sigma_{SN} = \sqrt{2} \sqrt{GV_{DAQ}} \sqrt{q \vartheta_{un} B} \sqrt{\cosh(\alpha_s L_s)}. \quad (3.85)$$

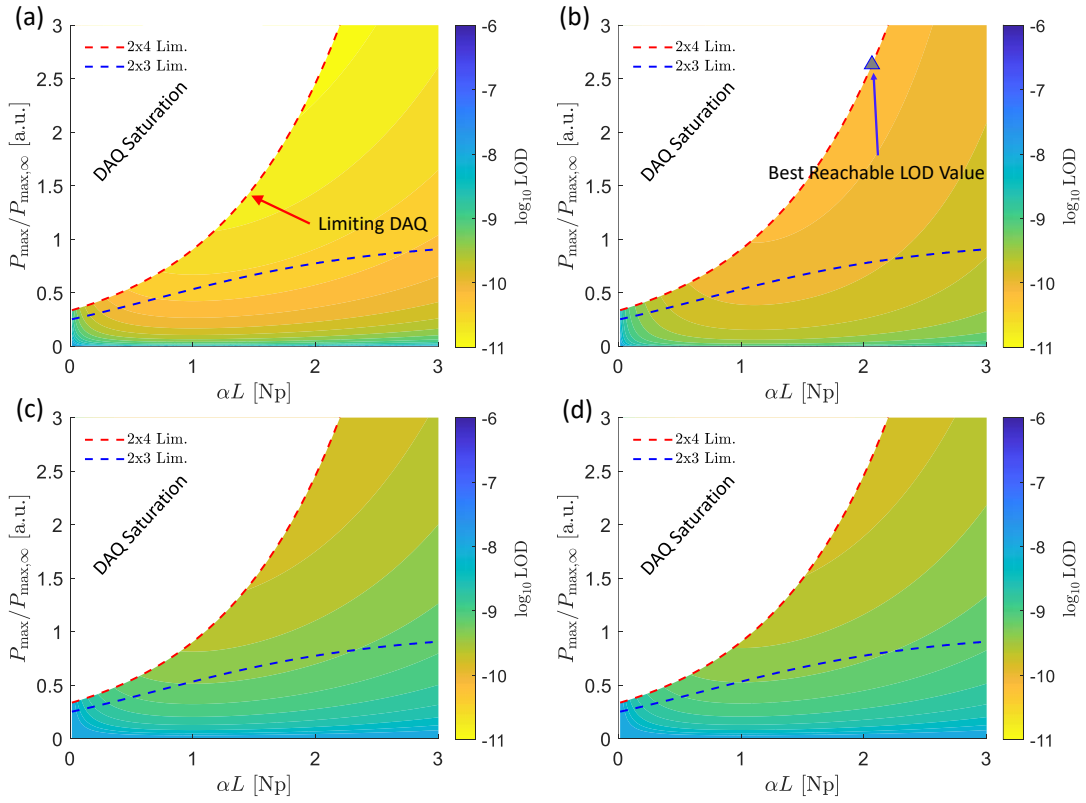


Figure 3.30.: Logarithmically plotted LOD for different normalised optical powers $P_{\max}/P_{\max,\infty}$, and absorptions αL for (a) thermal-, (b) shot-, and (c) quantisation noise for a specific setup configuration. Their superposition is shown in (d). The red dotted lines represent the limitation due to the DAQ in a differential PD detected coherent read-out with a 2×4 MMI. For comparison, the blue dotted line, the limitation from the 2×3 MMI case, is also shown. Here, shot noise is the only source, which is limited by an optimal LOD.

According to this and Eq. (3.83), the detection limit becomes

$$\text{LOD}_{\max}(L) = \frac{3}{2\pi} \frac{\lambda_0}{S_{\text{WG}}} \sqrt{q\vartheta_{\text{un}}B} \sqrt{\frac{G}{V_{\text{DAQ}}}} \frac{\sqrt{\cosh(\alpha_s L)}}{L} \quad (3.86)$$

Here, there is an optimum length $L_{\text{opt}} = 2.0653/\alpha_s$, calculating the best possible observable detection limit to

$$\text{LOD}_{\text{opt}} = \frac{3}{2\pi} \frac{\alpha_s \lambda_0}{S_{\text{WG}}} \sqrt{q\vartheta_{\text{un}}B} \sqrt{\frac{G}{V_{\text{DAQ}}}}, \quad (3.87)$$

as shown in Fig. 3.30(b). Again, lower gain of the TIA and high optical power of the source will reach to the lowest LOD range.

3.4.2.3. Quantisation Noise

Similar to thermal noise, quantisation noise does not depend on the detected optical signal strength. Hence, using Eqs. (3.38) and (3.83) the detection limit derives to

$$\text{LOD}_{\max}(L) = \frac{3}{\pi} \frac{\lambda_0}{S_{\text{WG}}} \sqrt{K} \frac{\Upsilon_{\text{QN}}}{2^N} \sqrt{\frac{B}{f_s}} \frac{1}{L}. \quad (3.88)$$

Again, no maximum length can be found in this case, and the detection limit gets better, the longer the sensor arm, as long as the source can compensate for the losses. This situation is illustrated in Fig. 3.30(c). As in the 2×3 case, it does not matter if this is done via the optical power or the gain of the TIA. Furthermore, in this particular case the sampling rate, scaling with $1/K$, will become higher, as one channel less is required. Thus the LOD will become at least $3/2$ better as in the 2×3 case for the same optimal physical sensor arm length.

3.4.2.4. Combined noise

Figure 3.30(d) shows the detection limit for the superposition of the three noise sources in (a)-(c). Again, it is up to the biosensing system designer to decide if it makes sense to make the sensor length larger, occupying more physical space on the chip. However, in this case, substantially lower detection limits could be achievable due to the proportionality to $1/L$, if shot noise is considerably low.

3.4.3. General Observations

Table 3.3 shows the proportionality factors of the LODs for 2×3 non-differential and 2×4 differential read-out based on the derived Eqs. (3.74), (3.77), (3.79), (3.84), (3.87), and (3.88). We have further simplified these equations by assuming that the intrinsic waveguide losses are much smaller than the effective absorption losses due to the water cladding, so that α_s can be approximated by $\alpha_{\text{H2O}} S_{\text{WG}}$ from Eq. (3.3). The factor S_{WG} then cancels out in Eqs. (3.74), (3.77), (3.79), (3.84), (3.87), and (3.88). From the table it becomes obvious that the optimum detection limit, i.e. for $L = L_{\text{opt}}$, for the 2×3 does not depend on the waveguide sensitivity (S_{WG}) as long as it has a reasonable value for light-matter interaction. The waveguide sensitivity mainly depends on the delocalisation of the mode from the core. Hence, optimal detection limit is independent of the applied mode polarisation (TE or TM) and sensing waveguide design, for example subwavelength or slot. However, the corresponding optimal sensing arm length L_{opt} inversely scales with the waveguide sensitivity S_{WG} . That means the higher the light-matter interaction of the sensing mode, the shorter the sensing waveguide can be designed to reach the optimal detection limit.

Table 3.3.: Detection limits for the two different coherent read-outs, non-differential 2×3 , and differential 2×4 MMI, for the three different detector noise sources.

Noise Source	2×3 ($K = 3$)	2×4 ($K = 2$)
Thermal	$0.87 \times \alpha_{\text{H2O}} \lambda_0 \frac{GR}{V_{\text{DAQ}}} \text{NEP} \sqrt{\vartheta_{\text{un}} B}$	$0.31 \times \alpha_{\text{H2O}} \lambda_0 \frac{GR}{V_{\text{DAQ}}} \text{NEP} \sqrt{\vartheta_{\text{un}} B}$
Shot	$1.02 \times \alpha_{\text{H2O}} \lambda_0 \sqrt{\frac{G}{V_{\text{DAQ}}}} \sqrt{q \vartheta_{\text{un}} B}$	$0.46 \times \alpha_{\text{H2O}} \lambda_0 \sqrt{\frac{G}{V_{\text{DAQ}}}} \sqrt{q \vartheta_{\text{un}} B}$
Quantisation	$2.46 \times \alpha_{\text{H2O}} \lambda_0 \frac{\Upsilon_{\text{QN}}}{2^N} \sqrt{\frac{B}{f_s}}$	$0.88 \times \alpha_{\text{H2O}} \lambda_0 \frac{\Upsilon_{\text{QN}}}{2^N} \sqrt{\frac{B}{f_s}}$

As thermal and quantisation noise have no best LOD for the 2×4 , the same optimal length was assumed as in the 2×3 case to increase comparability. For shot noise, however, their individual optimal lengths were assumed. The green marked entries indicate that this is actually not the best possible detection limit, for they actually scale inversely with LS_{WG} . This means that, if the system is for example clearly limited by quantisation noise, the sensor can be made much longer and/or the waveguide sensitivity increased, targeting even lower LODs, however, only until the shot noise limit is reached or other limiting factors start to appear. In any case, 2×4 differential detection lowers the LOD in comparison to the 2×3 by a factor of two, for shot and quantisation noise. Thermal noise stays constant. Differential-detected balanced CMZIs could pave the way to very ultra-low detection limits, but not without a price. Increased length means more occupied space.

3.5. Conclusions

In this chapter, the implemented setup and its different components (see Section 3.1), was investigated regarding its noise contributions from a system level point-of-view, and its consequences on the resulting detection limit for HRI experiments (see Section 3.2). Even though this kind of noise analysis is well understood and established in telecommunication, it is rare to find it in the biosensing community. Indeed, this is probably the first complete noise study with coherent read-out in this context. Within the analysis it became clear that CMZIs can be designed to be immune to laser phase and intensity noise, while circumventing sensitivity fading and phase ambiguity at the same time and, hence offering an attractive solution for point-of-care applications. These are characteristics which are not offered by other interferometers at the same time, at least not without complicating the read-out system making them less attractive for point-of-care applications.

Based on the detection limit analysis, rules of thumbs were derived to provide the designer the necessary insight and the possibility to enhance the detection limit of his sensing system depending on the type of noise that is dominant. It was shown that the sensor's sensitivity is not as important for the optimisation process as it may appear in the first place, but that sensitivity and signal-to-noise -ratio, which are correlated, must be considered equally to reach for the optimum. In Section 3.3 an experimental optimisation procedure was performed and a cutting edge limit of detection of $\sim 10^{-8}$ RIU was achieved. This demonstrates that CMZIs have the capacity to reach ultra-low detection limits.

Furthermore, in Section 3.4 the theoretical limitation of the detection limit imposed by the data acquisition, the ultimate limiting factor, for the investigated CMZI with 2×3 MMI and non-differential detection scheme was highlighted, considering the AWGN sources. It became apparent that the data acquisition dynamic input range does play a role for the optimal sensing length and the corresponding optimal detection limit. Additionally, it was derived that a CMZI with 2×4 MMI and differential detection scheme could potentially reach to even lower detection limits, by using the entire data acquisition dynamic input range.



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4. C-Reactive Protein Detection

This chapter serves two goals. The first is to demonstrate, for the first time, state-of-the-art biomolecule detection limits with the coherently detected Mach-Zehnder interferometer. The second is to show that this capability is maintained when using an inexpensive light source. As pointed out in the introduction, a significant amount of the cost of a photonic point-of-care device results from the read-out system, i.e. the light source and detection system. The price of a tunable external cavity laser, which is required for ring based sensors, normally starts from 1,000 € up to some 10,000 €. Even photonic sensors which only require a white light source, for example spectral Mach-Zehnder interferometers, typically use superluminescent LEDs, whose price is in the region of 100 € to 1,000 €. Fabry-Perot lasers are among the simplest and cheapest lasers available, and only cost some tens of euros. To the authors knowledge, only few photonic biosensors have been operated by a Fabry-Perot laser up to the publication resulting from this thesis. In this chapter, we will demonstrate that CMZIs are capable of reaching very low detection limits when operated with a high performance laser, and only suffer from a small penalty when an a Fabry-Perot laser is used. We focus on the detection of the C-reactive protein (CRP), as it is a well known biomarker for inflammation [180] and serves as a reliable comparison to other sensors for several other research groups used it for benchmark purposes.

The work described in this chapter is a result of a successful collaboration between four different research groups. It was started with the national research stay of the author of this thesis at the Nanobiosensors and Bioanalytical Applications group, lead by Laura M. Lechuga, from the Institut Català de Nanociència i Nanotecnologia of the Universitat Autònoma de Barcelona. During this time the author familiarised with a complete biosensing setup, including all its components, and with biofunctionalisation protocols in general. The initial CRP, corresponding antibody, and blocking agent for our performed experiments were also provided by this group, as well as later guidance in the signal interpretation. The offline functionalisation protocol of the CRP antibody was adapted from protocols from The Bailey Lab, lead by Ryan Bailey, from the University of Michigan - Ann Arbor (United States of America), where the author performed his international research stay. The actual experimentally obtained results of this chapter are product of the close collaboration with the Biomimetic Dendrimers and Photonic Laboratory, lead by Ezequiel Perez-Inestrosa, from the Universidad de Málaga.

The following structure is used to support the findings. In Section 4.1 we first present some biochemical fundamentals, and then describe the functionalisation protocol used for CRP detection. The biosensing experiments with the high-performance light source are detailed in Section 4.2. The experiments are repeated in Section 4.3 with a Fabry-Perot source. Finally, a short conclusion is given in Section 4.4.

4.1. Biosensor Functionalisation

The key difference between the bulk sensing experiments we have discussed in the previous chapters and the detection experiments described here is specificity: detecting a specific analyte entails ignoring any refractive index change that is not associated with said analyte. In general specificity can be defined as sensitivity of analyte detected divided by the sensitivity of unspecific binding, i.e. signal shift due to adhesion by other molecules to the surface. Specificity is achieved by chemically functionalising the surface, i.e. adding a biorecognition layer which

only captures the desired analyte. For example to detect a certain antigen, the surface is functionalised with the corresponding antibody. Figure 1.5(a) in Chapter 1 illustrates the detection process. Initially, only running buffer is flowing over the sensor, and a stable baseline signal is observed. When the sample containing the analyte starts to flow over the sensor a strong signal is observed. This signal corresponds to the bulk refractive index change of the sample compared to the running buffer. Since the sensor surface is functionalised, the analyte contained in the sample will gradually attach to the biorecognition layer, forming an additional thin layer on the waveguide surface. Once the sample is again replaced by the running buffer, the bulk sensing part of the signal fades, and only the smaller signal associated with the newly adhered analyte remains. It is the difference between this observed signal level and the baseline that quantifies the concentration of analyte in the sample. As already mentioned in Section 2.2.3.1, a practical definition of the waveguide sensitivity is the change of effective refractive index as a result of the injected analyte concentration (C_{inj} in ng mL^{-1}), defined in Eq. (2.20). Using this equation, the phase sensitivity can be generally defined according to Eq. (3.9) to

$$S = S_\varphi = \frac{2\pi}{\lambda_0} L \left. \frac{\partial n_{\text{eff}}}{\partial C_{\text{inj}}} \right|_{C_{\text{inj}}=0} \quad (4.1)$$

in $\text{rad}/(\text{ng/mL})$ where $|_{C_{\text{inj}}=0}$ indicates that the surface has initially no analyte attached to it.

4.1.1. Immobilisation Strategies

In the following two different ways of creating the biorecognition layer are discussed, physical adsorption, and covalent binding. Both strategies are used, however, for different purposes. Figure 4.1 shows an example of both approaches with an antibody acting as biorecognition element for its corresponding antigen, the analyte.

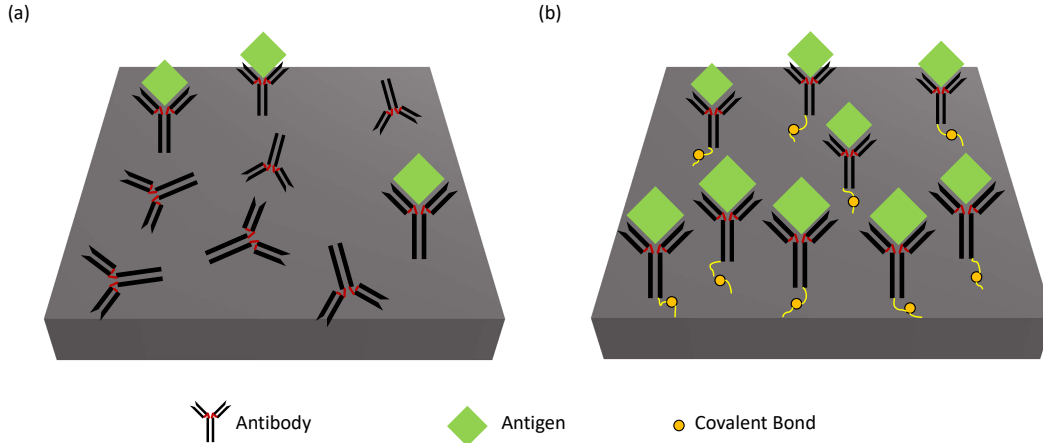


Figure 4.1.: Two different immobilisation strategies, (a) physical adsorption, and (b) covalent binding.

4.1.1.1. Physical Adsorption

Physical Adsorption is probably the simplest immobilisation technique. Bioreceptors are not attached to the surface through chemical reactions, but through weak bonds, which could be for example Van-der-Waals forces, hydrophobic interactions or hydrogen bonds [181]. This is a

very easy and cheap way to attach the recognition element on the surface. However, there are also some drawbacks, such as desorption, the reversal of adsorption [182]. That means that the applied biorecognition coating is washed away over time, also leading to non-specific adsorption of other biomolecules to the surface, possibly falsifying the measurements. This can for example happen if the pH value of the running buffer or injection changes. For protein adsorption it is important that the buffer or the injection have the right pH value to maintain its functionality. Specifically, the pH value should not coincide with the isoelectric point, where the net charge of the protein is zero, removing stabilising repulsive forces between them, making them aggregate and precipitate from the solution. This also maximises protein-protein repulsion, from both in the solution and on the surface [183]. Adsorption does not allow control of the antibody orientation which is typically associated with poor binding: as the binding to the surface is not due to a chemical reaction with the surface, all orientations of the molecule are equally possible, even those where the recognition side, the region of the molecule responsible for the recognition of the target, points down towards the surface, thus, hindering the recognition process (see Fig. 4.1(a)). Furthermore, it is not possible to separate the target molecule from the recognition element without removing it from the surface. Nonetheless, physical adsorption based photonic sensors have demonstrated reliable biomolecule detection with low detection limits [86].

4.1.1.2. Covalent Binding

In this case, the surface and the biorecognition molecule share at least one electron pair, thus establishing a strong chemical bond. This binding technique is more complex than physical adsorption, making it more time-consuming and expensive. However, often the benefits outweigh these disadvantages. The first advantage is that the orientation of the biorecognition molecules can be controlled. Antibodies are attached to the surface via the “stem” (the F_c region), so that the “branching” recognition sites (the F_{ab} region), face towards the target antigen (see Fig. 4.1(b)). However, this is not done in this work. As a second advantage, the recognition layer becomes more stable as it is permanently bonded to the surface. Strong changes in pH would, therefore, not wash the layer away, avoiding a loss of sensitivity. The surface can also be recovered after use for several detection cycles [184]. Recovering in this context means that the captured analyte is removed from the surface without negatively influencing the underlying functionalisation.

4.1.2. Biochemical Functionalisation and Detection Concept

In this chapter the different steps for functionalising a the silicon nitride surface of the sensors. The first group of steps (oxidation, silanisation) is about preparing the surface for covalent binding. The second group of steps (cross-linking, antibodies, blocking) includes the biorecognition molecule attachment, here, the anti-C-reactive protein antibody (ACRP), and specificity finalisation by blocking the residual available non-functionalised spots. Now the surface is enabled to specifically bind the target molecule, which is in this case the C-reactive protein.

4.1.2.1. Oxidation

Biomolecules cannot be directly covalently immobilised on a metal or semiconductor surface. First the surface has to be oxidised adding hydroxy groups ($-OH$) to the surface, as shown in Fig. 4.2(a) [185]. Typical ways to do that is to put the chip into a plasma chamber, using ultra-violet light, or exposing the chip to piranha solution. The latter approach is used in this thesis. The piranha solution not only serves as oxidiser, but also as cleaning agent, removing any remaining organic material from prior experiments.

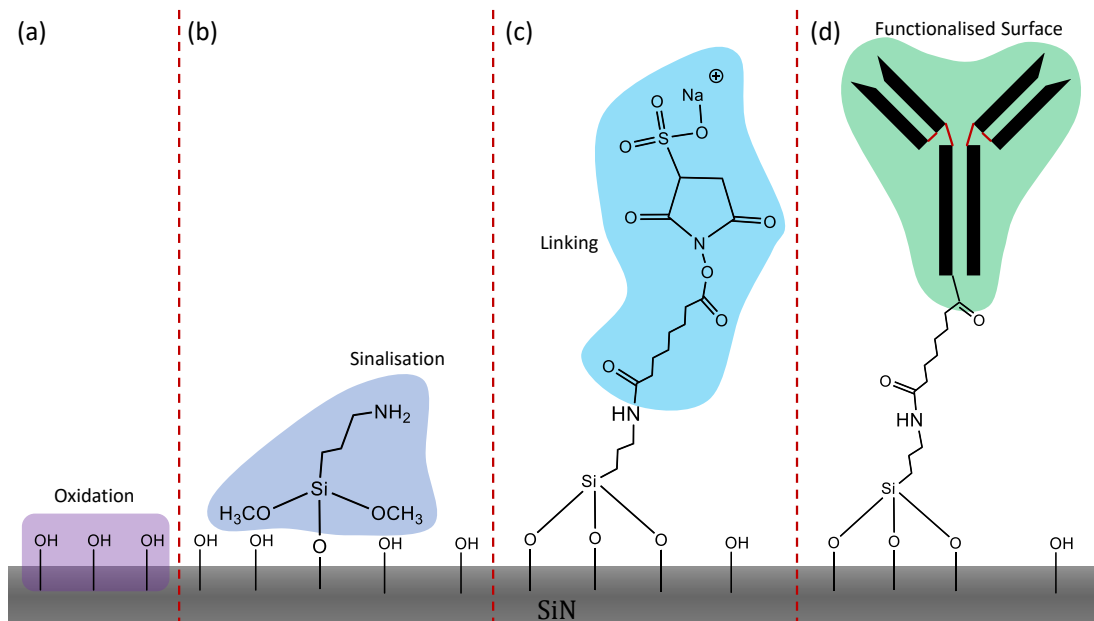


Figure 4.2.: Graphical representation of the covalent IgG CRP functionalisation immobilisation procedure. (a) The oxidation of the silicon nitride surface, (b) the silanisation with APTMS, (c) applying the BS3 crosslinker, and (d) the final covalent attachment of the ACRP to the surface of the sensing waveguide.

4.1.2.2. Silanisation

Adding an organofunctional group to the surface, i.e. a chemical termination to which a biomolecule may attach, is an important step of the functionalisation process. For oxidised metals and glasses this can be realised via silanisation. There are different types of silanes, which can be classified by their organic termination group, e.g. amino-, glycidoxy-, and mercaptosilanes. Typical candidates within the amino group are (3-aminopropyl)-triethoxysilane (APTES), (3-aminopropyl)-trimethoxysilane (APTMS). Common silanes within glycidoxy group are 3-(glycidyloxypropyl)-dimethylethoxysilane (GOPES) [186]. Figure 4.2(b) shows an APTMS molecule attaching to the oxidised semiconductor surface. In this case the alkoxy group for reacting with the hydroxy group on the surface is methoxy ($-\text{OCH}_3$), creating a $\text{Si}-\text{O}-\text{Si}$ covalent bond between the semiconductor surface and the silane. The resulting amino group (NH_2) terminated surface is then ready for bonding organic components.

The silan used for the experiments in this thesis is APTMS, from Alfa Aesar, product number A11284.

4.1.2.3. Cross-Linking

A cross-linker reagent serves as a conjugation between two molecules, in this case the silanised surface and the anti-CRP antibody (ACRP). Here, a bifunctional crosslinker agent is used to establish this connection. They often consist of a symmetrical spacer arm with reactive groups on both ends. When both terminating reactive groups are the same, it is called homobifunctional crosslinker [187]. In this work, the bis (sulfosuccinimidyl) suberate (BS3) crosslinking agent is used to bind the anti-CRP to the silane as shown in Fig. 4.2(c). BS3 is a homobifunctional, water-soluble molecule, with two activated carboxylic acid groups which enable amine-to-amine linking. Amine molecules are often available in proteins. Its spacer arm length is around 1.1 nm.

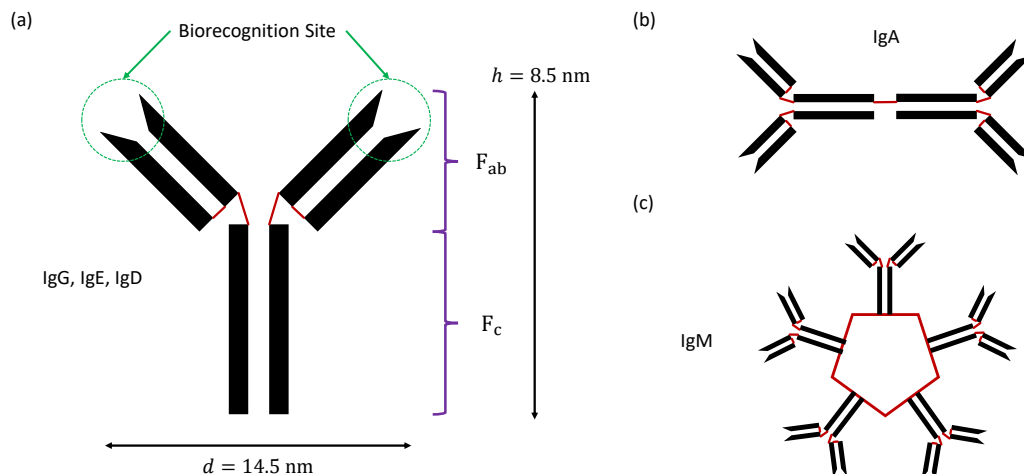


Figure 4.3.: Different types of immunoglobuline antibodies showing the monomer antibodies (IgG, IgE, IgD) in (a) with their biorecognition sites (F_{ab}) and the type differencing F_c region. (b) The dimer IgA and (c) the IgM pentamer as more complex monomer configuration.

The BS3 for these experiments was purchased from Thermo Fisher Scientific, BS3 (bis (sulfosuccinimidyl) suberate), catalogue number 21580, in powder form.

4.1.2.4. Antibodies

Before specifically talking about anti-CRP antibodies, a brief general introduction to antibodies will be given here. The immune system produces antibodies to deactivate, cluster, or mark pathogens or their products (such as toxins). There are many different kind of antibodies, having a wide range of different purposes. There are five classes of antibodies: immunoglobulin G (IgG), IgE, IgD, IgM, and IgA. The first three are monomeric antibodies, having a general structure as in Fig. 4.3(a), whereas IgA (b) and IgM (c) form dimers and pentamers, respectively. Monomers have two binding sites (the F_{ab} region), the upper ends of the Y-shape, whose chemical composition determines the target molecule, often referred to as antigen [188]. The lower end is often called F_c region and is determining the five above mentioned classes, hence, telling the immune system how to proceed with the attached antigen [189]. For example, phagocytes are cells (a subtype of white blood cells or leucocytes) looking for antibodies who attached, deactivated, or clustered pathogens. Once found they attach to the antibody F_c region, drawing both antibody and pathogen inside via phagocytosis and breaking the pathogen down into its molecular components.

The anti-CRP used for functionalising the surface is an IgG antibody for the C-reactive protein. There are a lot of amine groups in an IgG antibody to which the linking molecule could bond to, on an average IgG molecules have about 80 lysine residues (free amino groups) of which 20 are present in solvent accessible sites. There is a likelihood that the biorecognition region (F_{ab}) point towards the analyte [190], this is shown in Fig. 4.2(d). Anti-CRP has a molecular mass of $M_{ACRP} = 150 \text{ kDa}$, a height $h_{ACRP} = 8.5 \text{ nm}$, and a arm diameter of around $d_{ACRP} = 14.5 \text{ nm}$ [191].

The anti-CRP used in this work was purchased from HyTest, Monoclonal mouse anti-human C-reactive protein (high sensitivity), catalogue no. 4C28, in liquid form in a concentration of 5.8 mg mL^{-1}

4.1.2.5. Blocking

Once some or most of the sensor's surface is occupied with the desired recognition layer, the number of remnant free spots has to be sufficiently minimised. Areas which are not covered by the layer are prone to physical adsorption which means that any kind of molecule or protein may attach and cause a temporal or permanent shift in effective refractive index of the sensing mode. Hence, untreated surfaces may lead to false-positive results [192, 193]. To circumvent this, a protein that is strongly prone to physical adsorption but otherwise non-reactive protein is used prior to the detection experiment, blocking most of the non-functionalised areas. As its function is not to recognise any other molecule, the attached orientation is not relevant.

Here, bovine serum albumin (BSA) is applied as blocking agent. Its molecular mass $M_{BSA} = 66.5 \text{ kDa}$ and dimensions $V \approx 14 \times 4 \times 4 \text{ nm}^3$ are lower than of those of ACRP [194], possibly not negatively influencing the efficiency of the recognition layer.

The BSA was purchased from MERCK, Sigma-Aldrich, Bovine Serum Albumin, reference no. A2153, in form of powder.

4.1.2.6. C-Reactive Protein

The C-reactive protein is a commonly found protein in a patient's blood or serum. Its concentration is correlated to the level of inflammation, often triggered by tissue injuries and infections. It binds to dead cells to activate the complement cascade, a mechanism of the immune system, in order remove them [195]. A concentration of $\sim 1 \mu\text{g mL}^{-1}$ is often associated with a healthy patient, whereas higher concentrations indicate an inflammation. Permanent high CRP concentrations in serum may correlate with a cardiovascular disease [196] or even an increased probability of getting diabetes [197].

For biosensing it is often used as a performance benchmark. The lower the concentrations which can reliably be detected, the better the performance. The state-of-the-art in Table 2.3 lists some detection limits which were reached with this method. Very good values reported are 7 ng mL^{-1} [91] and 2 ng mL^{-1} [162]. The C-reactive protein has a molecular mass of $M_{CRP} = 120 \text{ kDa}$ [198], and a radius of 5 nm [199].

The CRP used for the following experiments was bought via BBI Solutions, Human C-Reactive Protein, code no. P100-0, in liquid form with a measured concentration of 2.31 mg mL^{-1} .

4.1.3. Functionalisation Protocol

Following the previously described steps, this chapter presents the protocol with which the sensor structures were functionalised for each CRP detection experiment shown in this chapter. In the literature many different ways to attach the biorecognition layer on top of the sensing waveguide can be found. They can be classified into on- and offline functionalisation. The chemistry is essentially the same but differs in the environment in which they are performed. For the first, the oxidised chip with the sensing structures is installed into the setup, i.e. the fluidic channel is placed above the sensing area, enabling the buffer to flow, and the injection system to introduce samples into the flow. Silanisation, cross-linking and targeting protein attachment are then done by injecting samples of the necessary components. The detection signal enables the operator to see how each steps adds up to the desired layer, or to detect if something is going unexpectedly wrong and the experiment has to be stopped or restarted. This is a quantitative measure, giving information about the quality of the functionalisation itself. Especially for deriving new techniques, this can be of considerable value. However, one disadvantage is the amount of volume used for each step. In some systems, a certain minimum flow has to be maintained, in addition with the tube volume, guiding the fluidic flow, this necessary volume

can be quite high. Injection volumes are typically in the range from $100 \rightarrow 1000 \mu\text{L}$. The more volume is needed, the more sample mass for each step is necessary to uphold the target concentration. Some biochemicals and proteins are very expensive, which is one reason why offline functionalisation was established for these experiments. Another possible drawback is that the entire chip surface exposed to the channel is functionalised by the same amount. The detection of very small concentrations of the target molecule could, therefore, become more cumbersome. Furthermore, all sensors in the same channel receive the same functionalisation, which is not necessarily wrong, but if the goal is to have various different functionalised sensing structures for different medical or biochemical tests, as desired for a lab-on-chip solution, this is probably not the best method to accomplish it. Performing the functionalisation offline (without insertion the chip into the setup) reduces the amount of sample volume needed for each functionalisation step. Each sensing structure can be treated differently by placing just one drop (typically around $1 \rightarrow 10 \mu\text{L}$) on top of it. This not only saves reagents (and money), it also enables the operator to functionalise each sensor individually. This way lab-on-chip devices are easier to realise. Genalyte, the first photonic biosensor system manufacturing company [68], applies this technique to have up to 128 different test on one chip. The sensing ring structures have a diameter of around $30 \mu\text{m}$, i.e. an area of $30 \times 30 \mu\text{m}^2$, which is much smaller than the used structures in this thesis ($\approx 500 \times 500 \mu\text{m}^2$). Therefore, their needed sample volume for each sensor is much lower, normally lower than $1 \mu\text{L}$. However, there is no quantitative data to evaluate the quality of the functionalised layer. The functionalisation of ACRP is a very established protocol [200] and its biochemistry solid, i.e. even if perfect conditions during the process are not reached, the functionalisation often turns out to be well enough [201], which is why the following offline protocol was chosen.

Offline Anti-C-Reactive Protein Antibody Functionalisation Protocol

1. Pre-cleaning:
 - i Sonicated the chip in acetone for 5 min.
 - ii Sonicated the chip in isopropyl alcohol (isopropanol or 2-propanol) for 5 min.
 - iii Sonicated the chip in methyl alcohol (methanol) for 5 min.
2. Amine functionalisation:
 - i Bathed the chip in piranha etch (piranha solution), a mixture of sulfuric acid (H_2SO_4), water, and hydrogen peroxide (H_2O_2), for at least 15 min. Piranha solution removes organic materials from the surface and oxidises it, i.e. adding OH groups to the sensors surface.
 - ii Washed in Milli-Q for at least 1 min.
 - iii Washed in acetone for 3 min while slightly shaking.
 - iv Amine-functionalised, NH_2 surface termination, with (3-aminopropyl)trimethoxysilane (APTMS). Chip emerged in 2 mL 5% APTMS for for 3 min while slightly shaking.
 - v Washed in acetone for 3 min while slightly shaking.
 - vi Washed in isopropanol for 3 min while slightly shaking.
 - vii Dried with air stream (Nitrogen would be better).
3. Antibody functionalisation:
 - i Prepare amine-to-amine linker solution: Add $700 \mu\text{L}$ of 2 mM acetic acid to 2 mg bis(sulfosuccinimidyl)suberate (BS3). The BS3 stays active around $20 \rightarrow 30$ min.
 - ii Spot a drop ($\sim 10 \mu\text{L}$) onto the desired sensor spiral and let it incubate for 3 min.

- iii Dried with air stream.
 - iv Prepare antibody solution with a concentration of $C_{\text{ACRP}} = 0.25 \text{ mg mL}^{-1}$ in phosphate-buffered saline (PBS) 10 mM (pH 7.4).
 - v Spot a drop ($\sim 10 \mu\text{L}$) onto the prior amine-functionalised sensor spiral and let it incubate for 60 min.
 - vi Dried with air stream.
4. Blocking:
- i Prepare the blocking agent solution, bovine serum albumin (BSA) with a concentration of $C_{\text{BSA}} = 10 \text{ mg mL}^{-1}$ in PBS 10 mM (pH 7.4).
 - ii Cover the chips surface in the blocking solution for 60 min.
 - iii Dried with air stream.
 - iv If necessary, temporarily store chip in PBS 10 mM (pH 7.4), to maintain the functionality.

The last step, the specificity (or blocking) of the surface is actually an optional step. It was done offline to shorten the experimental time with the sensing setup.

4.2. C-Reactive Protein Detection Performance

This section deals with the actual biosensing experiments for the CRP performance verification. Each experiment was carried out in triplicate. Section 4.2.1 describes the detection protocol, and results are discussed in Section 4.2.2. The installation procedure is described in Appendix A.5.

4.2.1. Detection Protocol

1. Inject a read-out calibration solution of PBS 20 $\rightarrow$ 100 mM (pH 7.4). A HRI change causing a phase shift at least as large as 2π has to be prepared for this. Flow velocity may be increased to minimise experiment time, as for the HRI calibration the exposure time is not important.
2. Inject twice the BSA blocking solution ($C_{\text{BSA}} = 10 \text{ mg mL}^{-1}$) to block any remaining spots after offline blocking. Wait until phase reaches equilibrium.
3. Inject a control sample, containing BSA, having the same concentration as the highest CRP injection ($C_7 = 4000 \text{ ng mL}^{-1}$).
4. Inject increasing concentrations of C-reactive protein, diluted in PBS 10 mM (pH 7.4): $C_i = 10, 50, 200, 500, 1000, 2000, 4000 \text{ ng mL}^{-1}$. Each time wait until phase reaches equilibrium.

4.2.2. Experimental Results

In this section we discuss the step-by-step signal for just one of the three experiments, since they are all very similar. The final results of all experiments is shown at the end of this section.

The calibration step (1.) of the detection protocol, in Section 4.2.1, just serves to calibrate the complex signal according to the procedure described in Section 3.2.1.2. It is a necessary step, but not relevant for the following detected binding signals, which is why it is not shown

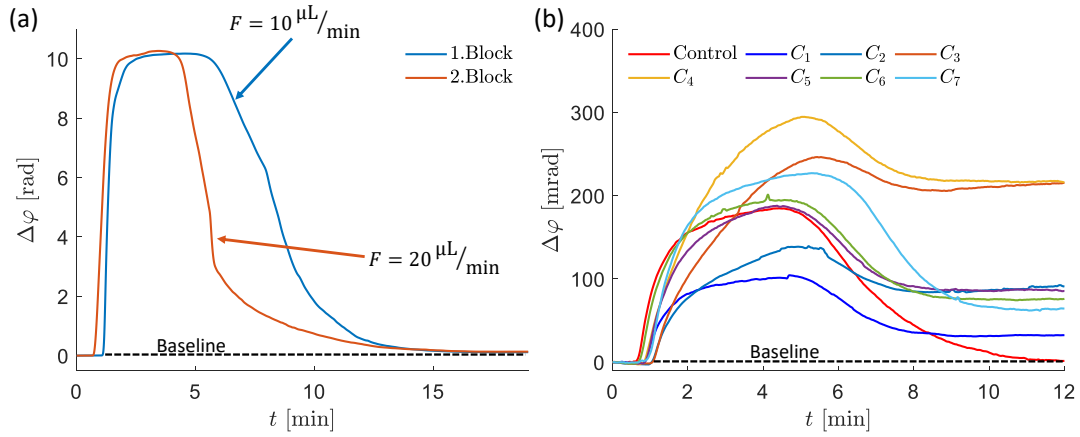


Figure 4.4.: Phase shifts from the first CRP additive immunoassay experiment. In (a) the two blocking steps are shown, resulting in almost no observable blocking of the surface. The control and CRP injections are shown in (b), the control, BSA with 4000 ng mL^{-1} , showing almost no binding signal, whereas the CRP demonstrates to be sensitive to the lowest concentration of $C_1 = 10 \text{ ng mL}^{-1}$.

here. The blocking step 2 is observable in Fig. 4.4(a). This online step blocks potential free spots left by the offline procedure or spots where the offline block has been removed due to the installation process. Unfortunately, the installation process can take some additional time, up to $10 \rightarrow 15$ min, if there are complications during the placement of the fluidic channel. Therefore, while the surface is exposed to air, antibody and blocking agent may experience reduction, and hence a loss in sensitivity and specificity. For the last the online block serves as compensation. Here, the first online block resulted in a almost negligible phase shift ($\Delta\varphi \ll 1 \text{ rad}$). First, the flow velocity was set to $10 \mu\text{L min}^{-1}$, and was increased to $80 \mu\text{L min}^{-1}$ after 8 min for ten minutes. This was done to suppress the ongoing diffusion of the high blocking concentration in direction of the buffer syringe. The longer the sample is on its way to the channel, the more time the blocking agent has to diffuse, that means distribute itself along the tube, increasing the exponentially decaying tale. Therefore, more time is needed to flow the sample through the channel. For flow velocities of $10 \mu\text{L min}^{-1}$ the blocking injection can already take up to 45 min before reaching equilibrium, which is a lot time. Ideally, flow velocities below $10 \mu\text{L min}^{-1}$ would maximise the blocking efficiency due to a longer exposure time. However, the time required to remove the agent completely from the fluidic injection system would be comparatively long. Additionally, there is a chance that for lower flow velocities the exponentially decaying tail would reach and contaminate the buffer syringe. Therefore, to minimise experiment time, a second blocking solution of the same concentration was injected at a flow velocity of $20 \mu\text{L min}^{-1}$, which here caused a negligible binding signal. This means that the offline blocking already sufficiently blocked the remaining not-functionalised surface. This is not always the case, as for other experiments both online blocking steps might be necessary.

The actual phase response to the control and CRP injections, in steps (3.) and (4.), is shown in Fig. 4.4(b). In every injection the phase first goes up due to HRI changes in the passing sample compared to the flowing buffer. In the case of the control, the phase goes back to baseline after a couple of minutes, indicating the specificity of the surface: no permanent binding can be read from the observed data. The CRP injections, however, do not go back to baseline, their signal settles at a certain phase level. The amount of binding signal depends on two things, the concentration and the binding history of the functionalised surface, i.e. how much free binding spots remain available. The first injection ($C_1 = 10 \text{ ng mL}^{-1}$) has no prior binding history, which is why it is more likely for the CRPs in the injection to be caught by their functionalised antibody counterpart. Hence, the observed binding signal here represents the most sensitive

part, as the amount of available ACRPs is maximal. All subsequent injections depend on the previous injections. Here, the first injection already occupied some of the available attachment spots. Let us assume that, the probability of attaching to an ACRP is the same for each CRP. That means that if the same concentration as the previous were injected, the resulting phase signal would be smaller because of less available spots and same binding probability. Assuming protein repulsion forces of closely attached CRPs would even reduce the binding probability, making the binding history even more important. To account for this the cumulative sum and phase shifts for the binding process are introduced:

$$C_{\text{cum},k} = \sum_{i=1}^k C_i, \quad (4.2)$$

$$\Delta\varphi_{\text{cum},k} = \sum_{i=1}^k \Delta\varphi_i. \quad (4.3)$$

Those equations approximate the real binding response curve by assuming that the observed binding signal $\Delta\varphi$ for C would be the same if C would be broken down into smaller injections with C_n , ($C = \sum_n C_n$), and their observed phases $\Delta\varphi_n$, with $\Delta\varphi = \sum_n \Delta\varphi_n$. This is a common approximation of the sensors binding response with an additive immunoassay. However, the real binding response would be observable if the surface were recovered after each injection, i.e. removing the CRPs from the surface without diminishing the functionalisation layer. However, in the framework of this thesis, this approach was not investigated. From Fig. 4.4(b) it can be clearly seen that after a certain level, even with increasing concentrations, the phase response is becoming lower each time, meaning that the sensors sensitivity tends towards zero for there are less and less available spots for antibody-antigen interactions. The functionalised surface gets closer towards saturation where almost no interaction spots are left. The sensitivity used for determining the detection limit is for a surface without any binding history, i.e. $C_{\text{cum}} = 0$. For CRP calibration the observed cumulative response $\Delta\varphi_{\text{cum}}(C_{\text{cum}})$ is fitted to the non-linear function

$$y = \frac{Ax}{B+x}, \quad (4.4)$$

with x and y the cumulative concentration and phase response [202], respectively. A and B are the fitting parameters. The observed data (triangle) and the estimated CRP response curve (line) for this case are shown in Fig. 4.5 (Experiment 1 in purple). The cumulative and fit data for the other two experiments are also shown in blue and orange. The R^2 value for each fit is > 0.99 , which confirms the validation for this fitting model. From this fit, the sensitivity can be extracted by

$$S = S|_{C=0} = \left. \frac{\partial y}{\partial x} \right|_{x=0} = \frac{A(B-1)}{B^2}. \quad (4.5)$$

The noise level for each experiment was determined by calculating the standard deviation of a one minute long interval, in which pure buffer medium was pumped through the fluidic channel. Table 4.1 shows the observed sensitivity (S), noise (σ), and detection limit (LOD) for

Table 4.1.: Observed system performance parameters extracted from CRP additive immunoassay with high quality ECL source.

Experiment	$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	$\sigma \text{ [}\mu\text{rad]}$	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
1	2.81	174	186
2	4.68	198	127
3	2.17	179	248
Mean	3.22	184	187
Fit	3.15		175

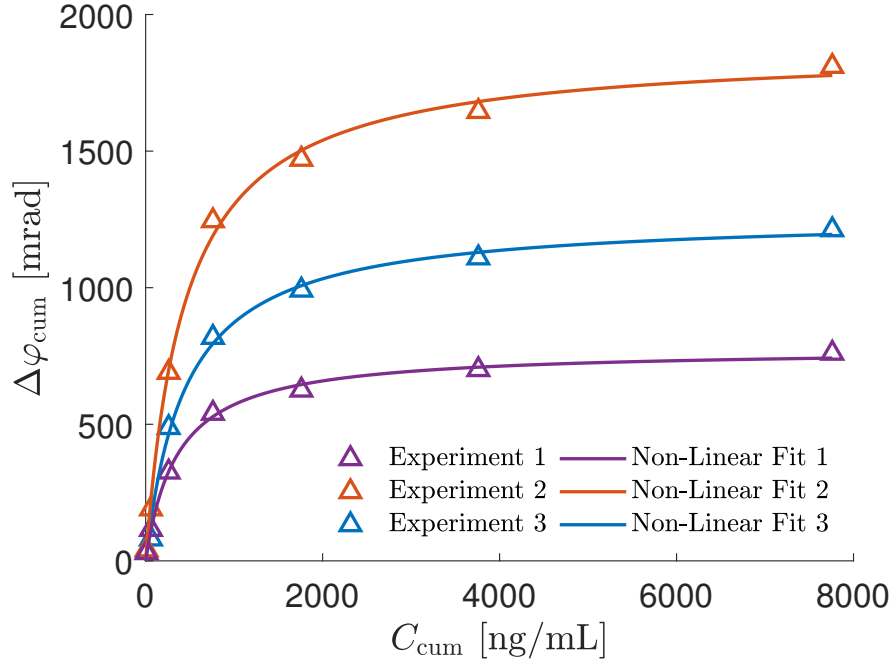


Figure 4.5.: Data and estimated calibration curves for each CRP detection experiment.

the three experiments. The presented mean values are based on the three observed experimental parameters. The fit value for the sensitivity was calculated by calculating the mean cumulative phase change for the cumulative concentrations, followed by fitting them to the non-linear function in Eq. 4.4. The LOD of the fit is based on this sensitivity and on the mean noise. The obtained noise level are very close together. A noise optimisation was realised, but not as profound as in the previous Chapter 3, i.e. no optical intensity modulation was applied. The sensitivity ranges around $3.22 \text{ mrad}/(\text{ng}/\text{mL})$ not varying by more than 50%. For future LOC and POC devices it is necessary to have a reproducible sensitivity to give quantitative estimations about the targeted biomolecules in serum, blood, or urine. Nevertheless, even though there is a variation, these results are promising, but require future optimisation of the functionalisation process. One reason may be the experience of the operator. Even though the functionalisation routine is relatively robust, the first couple of experiments will most likely not be as good as later ones. The gained experience will probably lead to an improvement over a longer time period. Another reason why the sensitivity values are differing could result from the spiral shape of the sensing arm and the way of offline functionalising. The drop which should ideally cover the complete sensing waveguide may be hindered doing so by the hydrophobicity of the structure. This may lead to regions where no functionalisation takes place, therefore, lowering the saturation and sensitivity level. However, the resulting detection limits (175 pg mL^{-1}) is significantly better than the current state-of-the-art of CRP for primary binding, which is typically a few ng mL^{-1} (see Section 2.4).

4.3. Experiments with a Fabry-Perot Laser Source

In the previous section ultra-low detection limits for CRP detection were demonstrated. Also, it has been theoretically shown that CMZIs are immune to relative intensity noise, even capable of minimising source phase noise, by balancing reference and sensing arm in terms of time delay difference (see Section 3.2.3). Hence, it should be possible to obtain the same or at least similar detection limits with a laser source, which has a comparably large spectral linewidth. Typically,

large optical linewidths disturb the extraction of the phase change, especially for sensing systems relying on the resonance wavelength detection. Spectral Mach-Zehnder interferometers have demonstrated medium to low detection limits, applying very broadband superluminescent diode and optical filtering. Looking at the prices of superluminescent diodes (typically in between $100 \rightarrow 1000\text{€}/\$$), they are much more affordable than tunable external cavity lasers (ECL) which cost up to several tens of thousands of euros/dollars. Still, in comparison to the costs of an mass-fabricated photonic chip, the fluidic components etc., it still accounts for a large part of a photonic biosensing device. Fabry-Perot diodes are probably the most simple form of a laser. The fabrication is typically very cheap, $10 \rightarrow 1000\text{€}/\$$, due to their simple working principle. They are similar in the generation of their spectral content as external cavity diodes, with the different that not only one but several resonance wavelengths exist. In the framework of this work, it will be demonstrated, that balanced, or slightly unbalanced, coherently read Mach-Zehnder interferometers are not only compatible with such lasers, but also obtain at the same time state-of-the-art detection limits.

4.3.1. Fabry-Perot Lasers

Figure 4.6(a) shows a simplified representation of a FPL, which is essentially a simple optical resonator. It mainly consists of two mirrors, one completely reflective (100%), the other not

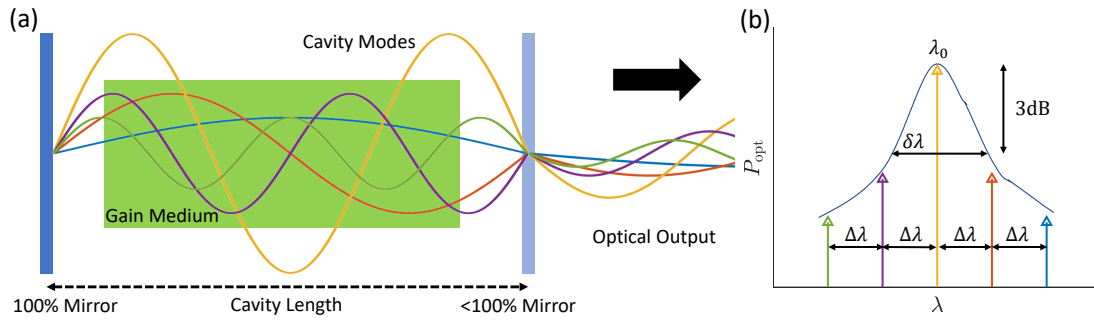


Figure 4.6.: (a) Rough schematic of a Fabry-Perot laser with the cavity defining mirrors and active medium resulting in (b) the typical Fabry-Perot laser spectrum with centre wavelength λ_0 and various additional excited modes at distances $\pm m \cdot \Delta\lambda$, forming a spectrum with the representative linewidth of $\delta\lambda$.

entirely reflective ($< 100\%$), and an optical active medium. The length of the Fabry-Perot cavity is defined by the distance between the mirrors. Certain wavelengths, the cavity modes, can now resonate between both mirrors due to their reflections, forming standing waves. The active medium serves as an amplifying medium of the supported cavity modes. Figure 4.6(b) presents the qualitative spectra for this idealised case. As in ring resonators, the free-spectral-range here is the wavelength distance ($\Delta\lambda$) of the neighbouring cavity modes. The central wavelength is typically the light which is amplified the most by active medium. An approximated “linewidth” ($\delta\lambda$) of the FPL can be given by for example a Gaussian fit to the cavity mode power and extracting the 3dB attenuation from the maxima. In a real FPL each peak would have an individual linewidth, which depends on several factors, such as the reflectivity of the mirrors, the gain and losses in the cavity, etc. Additional disturbances due to mode hopping, the cavity modes exchanging energy, may also occur. Fabry-Perot diodes are temperature sensitive, for the optical length of the cavity is modified, however, they are normally not used for establishing a tunable laser. For many applications in telecommunications, they should not be used as their introduced noise in amplitude and phase can be quite large, making them not suitable for high throughput, or long-distance data links. For the purpose of the optical robustness verification, a handheld fibre test laser was purchased at Amazon for approximately 35€. From here, it

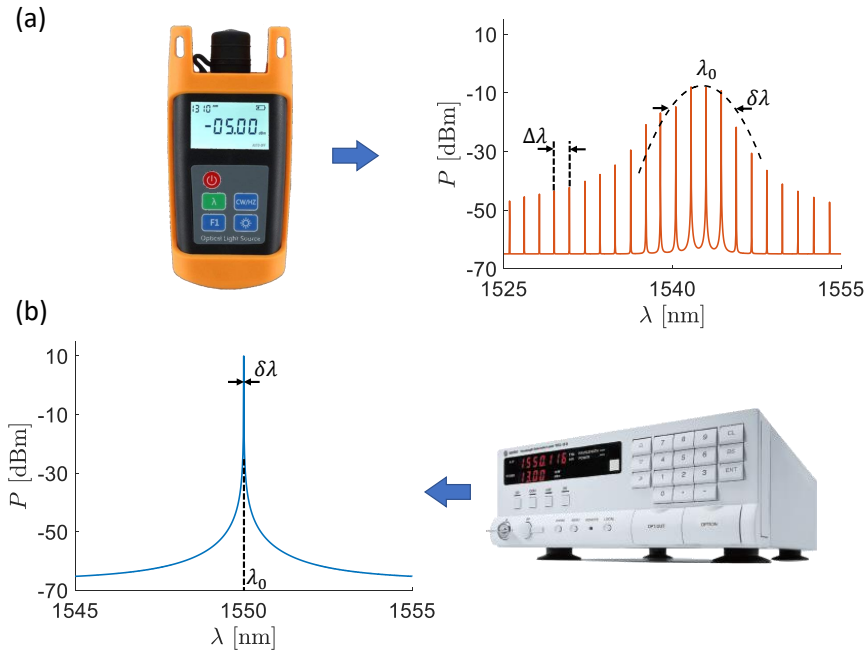


Figure 4.7.: (a) Utilised handheld Fabry-Perot fibre test laser with the reconstructed optical output spectrum. (b) The high quality tabletop external cavity laser used in most of the experiments in this thesis, and its narrow-linewidth spectrum.

will be referred as handheld fibre test optical light source (HOL). It can operate in the C- and O-Band with an minimum output power of -5 dBm, measured -3.5 dBm, including the entire output spectrum. The laser is shown in Fig. 4.7(a). Its spectrum was recorded by an optical spectrum analyser. The measured peaks were extracted, such as their corresponding power, and convoluted with a sharp Lorentzian function, resulting in the approximated spectrum right beside the image of the device. The peak wavelength is around $\lambda_0 \approx 1.543 \mu\text{m}$, the wavelength spacing $\Delta\lambda = 1.36$ nm and an approximated linewidth of $\delta\lambda = 3.6$ nm (bandwidth $\delta f \approx 450$ GHz). In direct comparison, the utilised external cavity laser (see Section 3.1) and its narrow linewidth output spectrum and image of the bench top device are shown in 4.7(b). This laser will be referred to as WSL from now on. The bandwidths of FPL is at least 4.5×10^6 larger than of the ECL. Theoretically that means, according to Eq. (3.55), that the introduced phase noise (σ_{PN}) scales by the square root of this factor, i.e. 2121. Most biosensors would experience a performance degradation if used with a FPL. This is qualitatively demonstrated for a typical ring resonator in Fig. 4.8. For a narrow linewidth tunable optical source, the wavelength response for one sweep is shown in blue. The resonance peak is clearly visible, which makes its extraction comparatively easy. If we were using a tunable Fabry-Perot laser for this purpose, having the spectra shown in Fig 4.7(a), the resonators response would look like orange graph as shown Fig. 4.8(a). The extraction of the resonance peak response would be a challenging task as the observed spectral response is a superposition of the several wavelengths. For a perfectly balanced CMZI, i.e. the delay difference ($\Delta\tau$) between sensing and reference arm is zero, the interferometric signals at the output of the 2×3 MMI are wavelength independent, and so is the extracted phase from the calculated complex signal. This is shown in Fig. 4.8(b). Of course this is only true as long as the group indices of sensing and reference mode scale the same way in the utilised wavelength range. Furthermore, this assumes that there are no other spectral dependencies. However, in this setup configuration most photonic components are wavelength dependent, such as the gratings, MMIs, even the photodiodes, detecting the optical signals have a wavelength depending responsivity. Nevertheless, as long as the optical

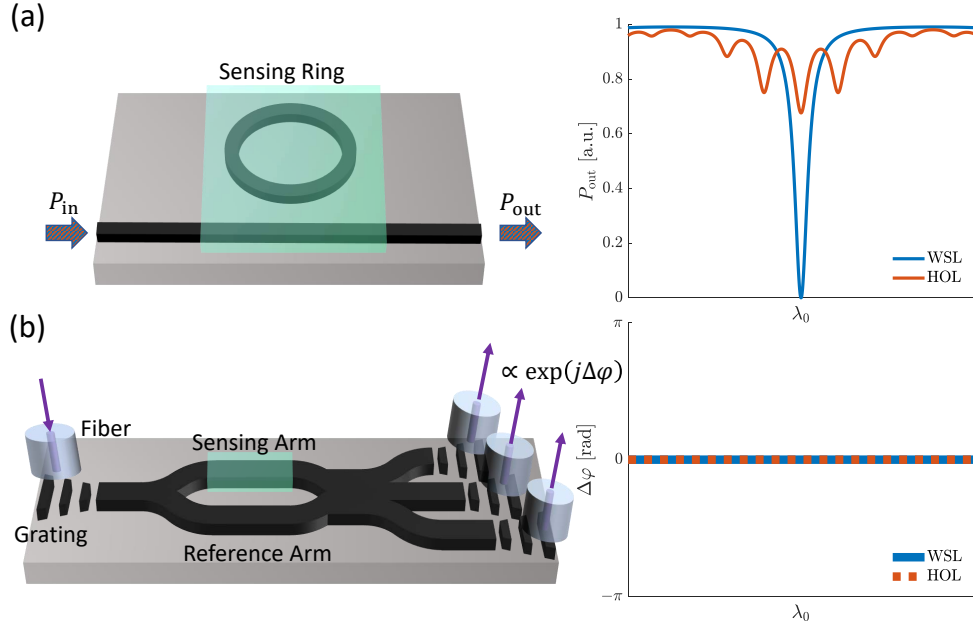


Figure 4.8.: (a) Typical ring resonator configuration and the corresponding output transmission spectrum for applying the ECL (blue) and the FPL (orange). (b) The CMZI and the corresponding extracted phase response for balanced arms by applying the ECL (blue) and the FPL (orange).

content is sufficiently distributed, these influences may not drastically increase the amount of noise introduced to the system. In the following to sections, bulk sensing experiments results for both lasers types are presented for the same experimental conditions, i.e. optical power level, gain, etc. Furthermore, the lasers were used for CRP detection benchmark experiments under similar conditions, making a direct comparison feasible. Both sections are based on the publication [101], a direct result from this thesis.

4.3.2. Homogeneous Refractive Index Experiments

The first step for verifying the performance of a sensor, before detecting biomolecules, are HRI benchmark tests. The investigated sensor already demonstrated ultra-low detection limits (see Section 3.3), but for comparability between the performance of the sensor utilised with both optical sources, the experiments will be repeated, performed under equal or at least similar conditions.

For the following HRI experiments the same HRI experimental protocol as in Section 3.3 was applied. Four types of experimental conditions were investigated. Two with the WSL and the other two with the HOL. Each of those were performed with a low power level and a high power level, in triplicate. The characteristics of the power levels are defined by the optical power reaching the photodiodes, i.e. ≈ -30 dBm for low, and ≈ -10 dBm for high power level, respectively. For the first, the VOA was used to minimise the optical output power of the WSL to the HOL. For the latter the EDFA was installed to lift both outputs to the desired power level.

The execution of the experiments was done the following manner. First the chip was installed according to the protocol in Appendix A.5 using the HOL as optical source in low power configuration. Then the HRI experiment protocol was executed. In the next step, the HOL was substituted by the WSL source. Necessary adaption steps, like polarisation and power

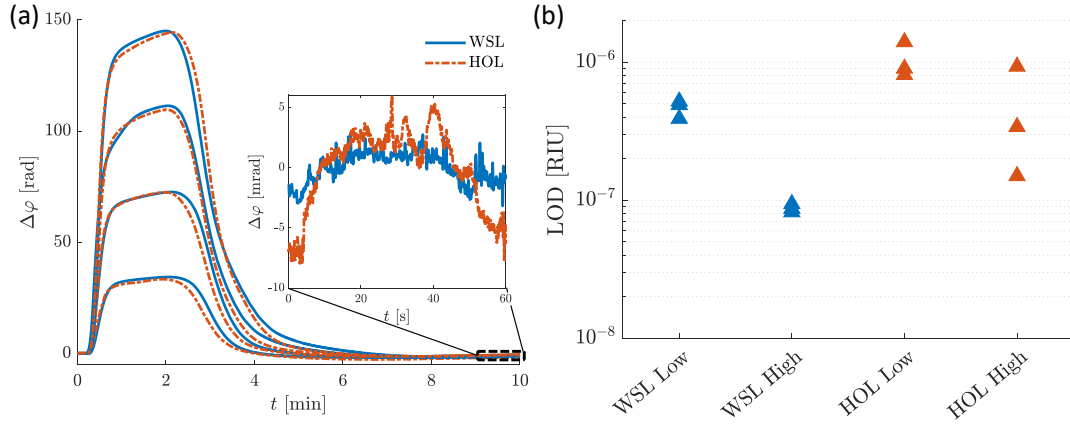


Figure 4.9.: (a) One of the low power HRI experiments performed with the ECL (blue) and FPL (orange) showing the four increasing sodium chloride injections overlaid to each other. The inset shows the noise for both laser types. (b) The statistical representation of the obtained LODs for the different laser at the two different power level.

adjustment, were realised to reach the desired power level, followed by the HRI experiment protocol. This entire procedure was done in the same manner for the high power configuration.

Figure 4.9(a) shows the introduced phase changes for one experimental run, i.e. with WSL and HOL, for low power configuration (≈ -30 dBm optical power at the PDs). The phase plateaus correspond to the 3 %_m to 12 %_m sodium chloride injections. These graphs demonstrate that even though the spectra of both optical sources are entirely different, the extracted phase response is highly similar. The usage of a Fabry-Perot laser, therefore, reproduces the results obtained by the external cavity laser to a high degree of similarity. The extracted phase sensitivities for those experiments are both ~ 7100 rad/RIU. Without looking into the noise level, there would not be any characteristic to distinguish them from one another. However, if we choose a more precise resolution for the noise signal, shown in the inset of Fig. 4.9(a), it is possible to see the differences. The phase noise for the 1 min time intervals was calculated to be $\sigma_\varphi \approx 1.2$ mrad and $\sigma_\varphi \approx 3.2$ mrad for WSL and HOL, respectively. The resulting detection limits are 5.1×10^{-7} RIU and 1.3×10^{-6} RIU. Both parameters from this experiment, sensitivity and noise, already indicate that the optical light sources are interchangeable without a significant impact on performance.

In Fig. 4.9(b) the calculated LODs are shown for each of the four configurations, each data point (blue/orange triangle) representing a performed HRI experiment. Table 4.2 presents their calculated mean detection limits. There are some noteworthy details in the presented

Table 4.2.: Detection limits in RIU for all homogeneous refractive index sensing experiments, categorised by optical source and power level. Each LOD represents the average value of three experiments.

Opt. Source	Low Power	High Power
WSL	5×10^{-7}	9×10^{-8}
HOL	1×10^{-6}	5×10^{-7}

data. Even though reaching similar sensitivities, looking at the distribution, it is obvious that the LODs obtained with the WSL are less scattered than for the HOL. That means that the noise level introduced by the HOL is not always the same and is subject to major fluctuations. Examining the mean LODs, we observe a general higher noise level from WSL to HOL (a

factor of $2 \rightarrow 3$ for the same power level), but in both configurations we see an detection limit improvement going from low to high power level (a factor of 6 and 3 for WSL and HOL, respectively). This may be explained by the simpleness of the utilised HOL. There is no sufficient temperature control of the FPL cavity, and, therefore, experiences a higher wavelength drift. Furthermore, even though the arm length of both, reference and sensor, are equal, the modes group index difference is not, $\Delta n_{g,rs} = |n_{g,r} - n_{g,s}| \approx 0.01$ (determined in simulation with FIMMWAVE [170]). Therefore, the arms are slightly unbalanced. As an additional factor, the wavelength dependencies of the different installed devices could act as and amplifier for the caused spectral noise. This includes for example the gratings or the photodiodes responsivities.

Despite the considerable performance drawbacks by using a FPL instead of a narrow linewidth ECL, it is astonishing that the difference is not higher considering the huge differences in the optical spectra. This results already indicate that (almost) balanced CMZIs are not only capable of being prone to noise caused by the light source, they may also be a candidate for POC devices with state-of-the-art LODs, maybe even reaching the ultra-low detection limits, while being much more affordable than the other part of the integrated photonic sensor family.

4.3.3. C-Reactive Protein Experiments

The next step for the verification of the sensor's performance is the detection of a biomolecule which can be compared to other biochemical sensors. In this case, the CRP benchmark was already demonstrated in Section 4.2 for the WSL. To ensure comparability, these experiments were performed the same manner. More precisely this means establishing the same signal power at the PD (≈ -10 dBm) with the HOL, supported by the EDFA. The following experiment was executed in triplicate.

The chip was functionalised and pre-blocked as described in Section 4.1.3, installed according the protocol in Appendix A.5 using this time the HOL, then followed by the CRP detection experiment using the protocol in Section 4.2.1, but with just one online blocking step. The

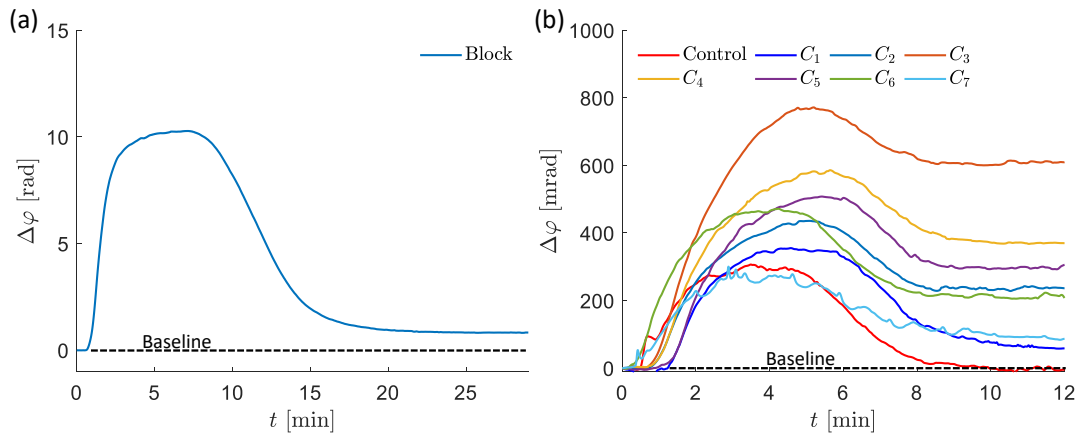


Figure 4.10.: Phase shifts from the first CRP additive immunoassay experiment with the FPL on low power. In (a) one blocking steps is shown, resulting in almost no additional blocking of the surface. The control and CRP injections are shown in (b), the control, BSA with 4000 ng mL^{-1} , showing almost no binding signal, whereas the CRP already demonstrates to be sensitive to the lowest concentration of $C_1 = 10 \text{ ng mL}^{-1}$.

online blocking step is shown in Fig. 4.10(a), showing a binding signal of ~ 1 rad of the BSA ($C_{\text{BSA}} = 10 \text{ mg mL}^{-1}$) to the surface via physical adsorption. No second blocking step was

necessary. With increasing experience in ACRP functionalisation, blocking and following CRP detection, the second blocking step became less and less efficient, i.e. almost no binding signal observable, so it was excluded here. Figure 4.10(b) shows the introduced phase shifts for the injections with the control, BSA, and the CRP concentrations of 10, 50, 200, 500, 1000, 2000, 4000 ng mL⁻¹ for $C_{1 \rightarrow 7}$, respectively.

The control injection (red), BSA with a concentration of 4000 ng mL⁻¹, shows a negligible permanent phase shift which indicates no detectable binding of the Bovine Serum Albumin (BSA) to the sensors surface. Hence, the surface can be considered to be sufficiently blocked. All CRP injections introduced a permanent phase shift after 8 → 10 min, even the first and lowest concentration of $C_1 = 10$ ng mL⁻¹, a concentration 400 times lower than the control, resulted in a considerably (≈ 60 mrad) change. Equally to Section 4.2, the cumulative injected concentrations and phase shifts were calculated, from which then the sensitivities were extracted via non-linear fit for all three experiments. The mean and standard deviation for the cumulative phase shifts for all three experiments are shown in Fig. 4.11 (orange errorbar) along with the corresponding non-linear fit (orange line). The sensitivities, noise, and LODs for each

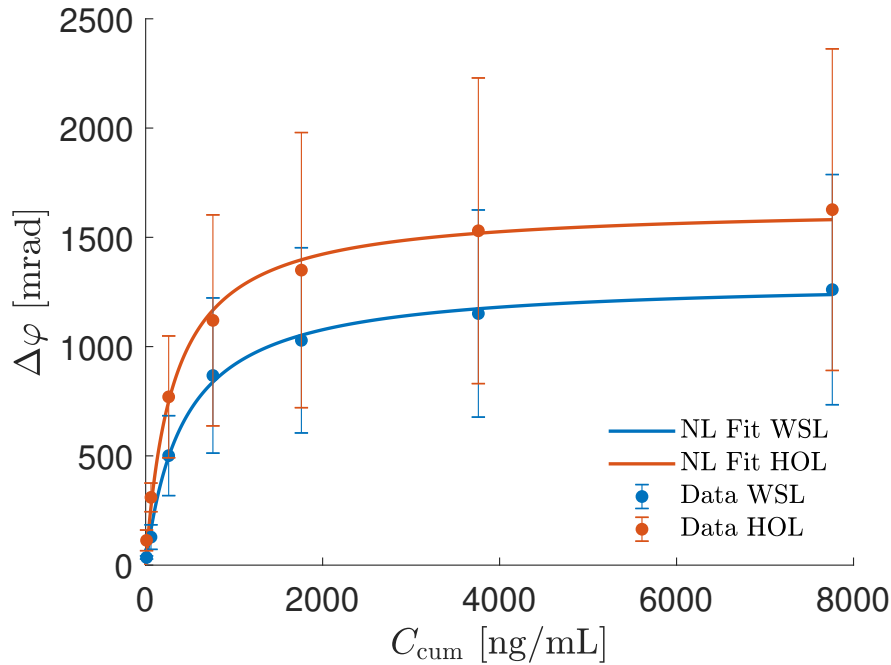


Figure 4.11.: CRP calibrations for the additive immunoassay for both ECL (orange) and FPL (blue). The dots represent the mean and the errorbars the standard deviation of the corresponding cumulative data point. The lines are the non-linear fit through the mean data.

experiment is shown in Table 4.3, including the mean and fit values. The mean-standard deviation plot and non-linear fit for the experiments done in Section 4.2 are also shown in Fig. 4.11 (blue errorbar, and line, respectively), for the purpose of comparability. Interestingly, the sensitivity values reached with the HOL are higher than with the WSL (see Table 4.1), almost a factor of 2 in between their mean values. As already mentioned for the online blocking, we gained a lot of experience in functionalising and blocking of silicon based surfaces along the experiments done. Therefore, it is not surprising that the later experiments obtained a slightly higher sensitivity. Nevertheless, they are all in the same order of magnitude. Further improvement of the functionalisation steps may lower the sensitivity deviation.

As expected, the obtained noise values are higher than for the WSL, their means differing by

Table 4.3.: Observed system performance parameters extracted from CRP immunoassay with low quality FPL source.

Experiment	$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	$\sigma \text{ [}\mu\text{rad]}$	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
1	5.74	3152	1648
2	6.30	3137	1495
3	4.92	3612	2200
Mean	5.65	3300	1781
Fit	5.27		1879

a factor of approximately 16, which is higher as expected from the HRI experiments indicated. However, the environmental situation of the sensor is entirely different and difficult to assess or predict. Therefore, in this context these are not bad results, for the resulting detection limits are around 1879 pg mL^{-1} , which is compared to the state-of-the-art (see Table 2.3) similar, or even lower, than the best listed LOD (2 ng mL^{-1} [162]).

4.4. Conclusion

Considering both HRI- and CRP results, we believe it is verified that (almost) balanced CMZIs are capable of reaching ultra-low detection limits, lower than the current state-of-the-art ($\sim 1 \times 10^{-8} \text{ RIU}$ and 184 pg mL^{-1}) using a high quality, and expensive, laser. Even if operated with an inexpensive Fabry-Perot laser, cutting edge state-of-the-art detection limits can still be obtained. For that reason, we think that balanced CMZIs are a promising and elegant solution for future point-of-care and lab-on chip devices.

5. Amoxicillin Immunoglobulin Detection

In the previous chapters we presented very good bulk limits of detection for the coherently detected Mach-Zehnder interferometer, as well as state-of-the-art results for C-reactive protein detection with both a high performance laser source and an inexpensive Fabry-Perot laser. In this chapter, we experimentally detect immunoglobulin G and E antibodies for amoxicillin - these antibodies indicate an allergy against this commonly used antibiotic. However, as promising as the results seem to be, they are preliminary and still require a lot of future research to make a useful contribution to amoxicillin allergy detection. The presented procedure does not distinguish between IgG and IgE antibodies which is a highly important specificity feature. But their controlled combined or individual detection is certainly the first step towards this goal.

The work described in this chapter is a result of a successful collaboration between four different research groups. It was started with the international research stay of the author of this thesis at The Bailey Lab, lead by Ryan Bailey, from the University of Michigan - Ann Arbor (United States of America). During his stay, most of the offline anti-amoxicillin functionalisation protocol was developed. Later, in collaboration with the Biomimetic Dendrimers and Photonic Laboratory, lead by Ezequiel Perez-Inestrosa, from the Universidad de Málaga this procedure was further improved and confirmed in experiment by detecting IgG and IgE amoxicillin antibodies individually. Both, IgG and IgE antibodies, were provided by Maria Jose Torres and María Isabel Montañez Vega from the World Allergy Organization Center of Excellence at the IBIMA-Hospital Regional Universitario de Málaga.

The chapter's structure is explained in the following. First, the motivation for amoxicillin allergy diagnostics is discussed, also showing current detection methods in Section 5.1. Then, in Section 5.2, the generation of the biorecognition layer is presented. This is followed by specificity confirmation and IgG and IgE amoxicillin antibody detection experiments (Section 5.4). The final Section 5.5 contains a short conclusion.

5.1. Motivation

Amoxicillin belongs to the group of aminopenicillins of the penicillin family. Before the discovery of penicillin in 1928 by Alexander Fleming there was no treatment available for bacterial infectious diseases such as streptococcus, diphtheria, pneumonia, or gonorrhoea. Even though Fleming discovered the antibacterial behaviour of penicillin he was not able to purify it into a stable compound [203]. It took more than ten years until the purification and antibacterial effect was demonstrated in experiment [204]. From there on, many different type of penicillin, and other antibiotics, were discovered and synthesised.

Antibiotics can have two different bacterial inhibition effects. The first is bacteriostatic in which the antibiotic molecule binds to bacteria, hindering the reproduction of bacteria and thereby slowing down the bacterial spread. The second is bactericide, where it attaches to the bacteria in order to eliminate it for example by enzyme-mediated lysis which is caused by the destruction of the bacteria's cell wall [205].

It is assumed that around 10% of the worlds population can be diagnosed, with nowadays techniques, to be allergic to penicillin [206]. However, being diagnosed does not necessarily

mean that one is actually allergic. It could be that only 90% of those diagnosed have an actual allergy to penicillin and only 0.03% show serious health threatening reactions [207]. Patients diagnosed to be allergic to penicillin are given alternative antibiotics such as quinolones or cephalosporins. On the other hand, the increase of bacterial antibiotic resistance in the last decades has been linked to this mistreatment of a wide range of antibiotics [208]. It is, therefore, desirable to have reliable diagnostic tools for the determination of allergic behaviour to certain penicillins in order avoid amplifying the bacterial resistance to antibiotics.

Allergic reactions are exaggerated immune responses to non-microbial environmental antigens [209] and are mediated by a host of different types of cells and molecular messengers. The hallmark of allergic reactions is production of IgE antibodies by activated B-cells after allergen recognition, and binding of the IgE to F_c receptors on mast cells and basophils. This is the first phase of allergic reaction formation called sensitisation and occurs either after first or repeated exposure to the antigen. Persons with allergies produce high levels of IgE in response to environmental allergens, whereas normal individuals generally produce other Ig isotypes, such as IgM and IgG, and only small amounts of IgE. IgE is the isotype responsible for sensitising mast cells and specifically recognises antigens for immediate hypersensitivity reactions. Upon subsequent exposure, the allergen in question will be recognised by the IgE coating on mast cells or basophils, thereby causing their degranulation and release of mediators responsible for causing symptoms. Release site and types of mediators determine the type, localisation and severity of reaction, which can sometimes manifest as life-threatening anaphylaxis in its worst form.

Amoxicillin is a very small molecule (≈ 365 Da [210]), and is by itself not recognisable by the immune system [211]. When the body is exposed to amoxicillin for the first time, the β -lactam ring (see Fig. 5.1(a)) attaches to a carrier protein by reacting with a free amino group, in a process called haptensisation. It is assumed that the immune reaction depends also on the

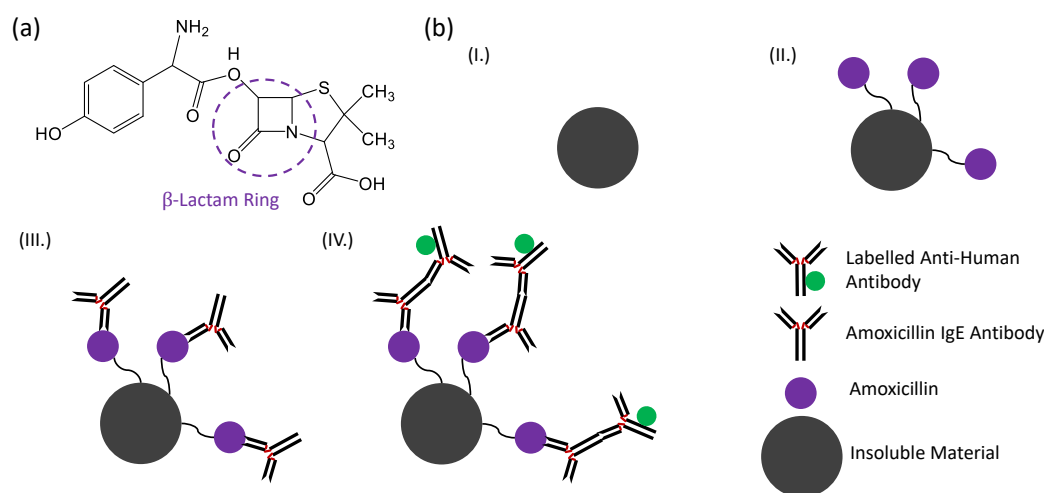


Figure 5.1.: (a) Chemical structure of amoxicillin. (b) (I.) The insoluble material serves as substrate for the detection scheme. (II.) Covalent attachment of the amoxicillin to the base. (III.) Direct detection of the IgE antibodies. (IV.) Binding of the labelled anti-human-IgE antibodies to the human IgE anti-amoxicillin antibodies for a quantitative detection via for example optical fluorescence signal of the label.

molecular composition of the haptensised protein[211]. Proteins assumed to play an important rule in this context are human serum albumin [212], transferrin, immunoglobulins [213], but also monocytes, macrophages, and B-lymphoma cells were supposedly identified as possible carriers [214].

Once the amoxicillin haptenised proteins are recognised, the underlying mast cells release histamine to increase the permeability of blood vessels, enabling white blood cells, monocytes/macrophages, to elicit an immune response to the perceived threat. This way of handling foreign pathogens or parasites is normally not dangerous and intended. In case of an allergic reaction, here to amoxicillin, this protection mechanism overreacts. Depending on the release spot, e.g. on the skin, or in the blood circulating system, this can lead to severe overreactions such as anaphylaxis.

To diagnose an allergy, the patients clinical history is typically important, in addition to a specific in vivo and/or in vitro IgE antibody test [215]. A popular example for in vivo are skin tests, in which small areas of the patients skin are exposed to a substance of interest. Unfortunately, this can have consequences for the patients health, in case a severe immune reaction occurs. Furthermore, it is a qualitative measurement. In vitro tests are typically performed with bloods sample and do not require direct allergen-patient interactions [216]. Radioimmunoassay test (RIA), a label-based detection immunoassay, were commonly used to identify specific IgE antibodies in the sample. By attaching the allergen candidate to an insoluble solid carrier or surface, and adding it to the sample, the allergen-specific antibodies, that includes all Ig isotypes, can attach to the allergen and/or haptenised protein, if present at all. The sensitivity of this method depends highly on the type of applied haptenised carrier molecule used for detection and the IgE antibody [217]. Up to this point no differentiation is done between the Ig isotypes, i.e. all Ig amoxicillin antibodies attach to the haptenised biomolecule corresponding to the concentration relations between each other. To distinguish between the attached Ig isotypes a second step is always necessary, in which for example an goat anti-human-IgE antibody is added, specifically binding to the IgE amoxicillin antibodies. Adding a measurable quantity for the case of the RIA, a radioactive label is attached to the anti-human IgE antibodies. The labelled antibodies are then incubated to the sample. The following washing step (only the insoluble material and its attachments remain) reveals the concentration of IgE antibodies in the patient blood. In case that at least a certain amount of antibodies of the patient attached to the allergen, the labelled antibodies, most likely, will have recognised them. By doing so, they increase the radioactive radiation level of the probe, which is proportional to the amount of the allergen specific IgE antibodies. The detection process is schematically shown in Fig. 5.1(b). A nowadays more popular approach, called ImmunoCAP, substitutes the radioactive with a fluorescent label [218], and is considered the golden standard for IgE detection [219, 220]. The quantitative result is commonly displayed in kilo units per litre (kU/L). Table 5.1 shows the quantitative classification and correlated interpretation [221] of the obtained signal strength. Patients diagnosed with classification 0-1 are considered allergically

Table 5.1.: Quantitative RIA/ImmunoCAP signal classification.

Class	Signal I [kU/L]	Description
0	$I \leq 0.1$	Below Detection Limit
1	$0.1 < I \leq 0.7$	Low Level
2	$0.7 < I \leq 3.5$	Moderate Level
3	$3.5 < I \leq 17.5$	High Level
4	$17.5 < I \leq 50.0$	Very High
5	$50.0 < I \leq 100.0$	Ultra High
6	$100.0 < I$	Extremely High

negative, whereas classes 2-6 are considered positive. The detection limit for ImmunoCAP is around $0.1 \text{ kU/L} \approx 0.24 \text{ ng mL}^{-1}$, the upper limit is ca. 100 kU/L , and only $40 \mu\text{L}$ of serum or plasma is required for accurate detection in 100 min [222].

Another quantitative detection technique using a surface plasmon resonance (SPR) has demonstrated similar detection limits, $\text{LOD} = 0.6 \text{ ng mL}^{-1}$ (0.25 kU/L) for IgE and $\text{LOD} = 4 \text{ ng mL}^{-1}$ for IgG, using gold nanoparticles functionalised with bisaminoalkyl polyamide dendrimers [223].

This optical sensor has several advantages in comparison to ImmunoCAP. First, no labelling is required as it is a direct detection approach. The binding of the IgE antibodies to the functionalised surface is directly detected via a change in the optical surface properties. Second, the detection time is lower, around 10 → 15 min. Furthermore, the functionalised surface was recoverable by a injection a low concentration of sodium hydroxide up to 40 times with good repeatability. This demonstrated that optical sensors with direct detection immunoassays are capable to efficiently detect IgE antibodies with minor penalties in detection limit and, therefore, offers an alternative to ImmunoCAP and RIA. However, SPR devices are very bulky equipment, difficult to integrate in a POC solution and to handle by untrained personal due to their read-out technique.

The integrated CMZI, in this context, is an attractive alternative for both ImmunoCAP and SPR. In the previous chapters it demonstrated state-of-the-art LODs, while the read-out can be designed to be very compact, which makes it even more applicable for future POC devices. To the authors knowledge, no silicon photonics integrated sensor has been applied for amoxicillin allergy detection yet.

5.2. Amoxicillin Haptenised Dendrimer Immobilisation

Similarly to ImmunoCAP, RIA, or the SPR detection, the allergen, in this case amoxicillin, has to be attached to a carrier molecule to be recognisable by the allergen specific IgE antibody. However, any type of allergen specific Ig antibody can recognise the haptenised molecule, but only the IgE type is of interest for allergy verification. For the carrier molecule, different factors play a role in this context. It should be affordable, and easy to synthesise. Their size should not exceed a certain level, due to possible sensitivity losses caused by the distance to the waveguide surface. Neither should it be too small to still be able to mimic a protein structure (biomimetic). Furthermore, it should have enough binding sites to be haptenised by the amoxicillin. Poly(amidoamine) (PAMAM) dendrimer, firstly discovered in 1985 [224], are an elegant candidate for this purpose. The chemical structure of a second generation PAMAM (G2 PAMAM) is shown in Fig. 5.2 indicating the G2 and G1 layer. The point-symmetrical structure is reminiscent of networks or trees from graph theory. They consist of a core element which is surrounded by four amino terminated groups (G0). On the monomeric arms other amino group terminated monomers can be attached forming the next generation (G1), i.e. eight amino groups on the outside of the structure. This amount can be doubled again by the same procedure to 16 amino groups, forming G2. PAMAM was demonstrated to be functionalisable with penicillin, mimicking haptenised protein behaviour, and being recognised by IgE antibodies [225]. Other experiments showed that PAMAM can be easily attached to silica nanoparticles [226] which are chemically similar to the utilised silicon nitride waveguides in this thesis. Furthermore, PAMAM G1 and G5 were successfully covalently attached to silicon surfaces for photonic biosensing [227].

The process of functionalising amoxicillin haptenised PAMAM dendrimers to the silicon nitride sensing waveguide surface is discussed in the following. Second generation PAMAM dendrimer terminate with 16 amino groups, therefore, any possible functionalisation has to link to at least on of these. The IgG ACRP antibody, covalently attached to the waveguide, described in Section 4.1.2, was also attached to its amine groups via an amine-to-amine crosslinker. Hence, up to the cross-linking the exact same immobilisation process can be used, including the prior oxidation, and silanisation of the surface. The PAMAM dendrimers are attached to the silane via the BS3 crosslinker as shown in Fig 5.3(a). Once the PAMAM dendrimers are attached, the amoxicillin can be introduced for dendrimer haptenisation, resulting in the desired functional surface modification for amoxicillin IgE antibody detection (shown in Fig. 5.3(b)) [226]. To guarantee the reactivity of the dendrimers' amine groups, such that they can react with the β -lactam ring of the amoxicillin, they have to be deprotonated. This is ensured by choosing the

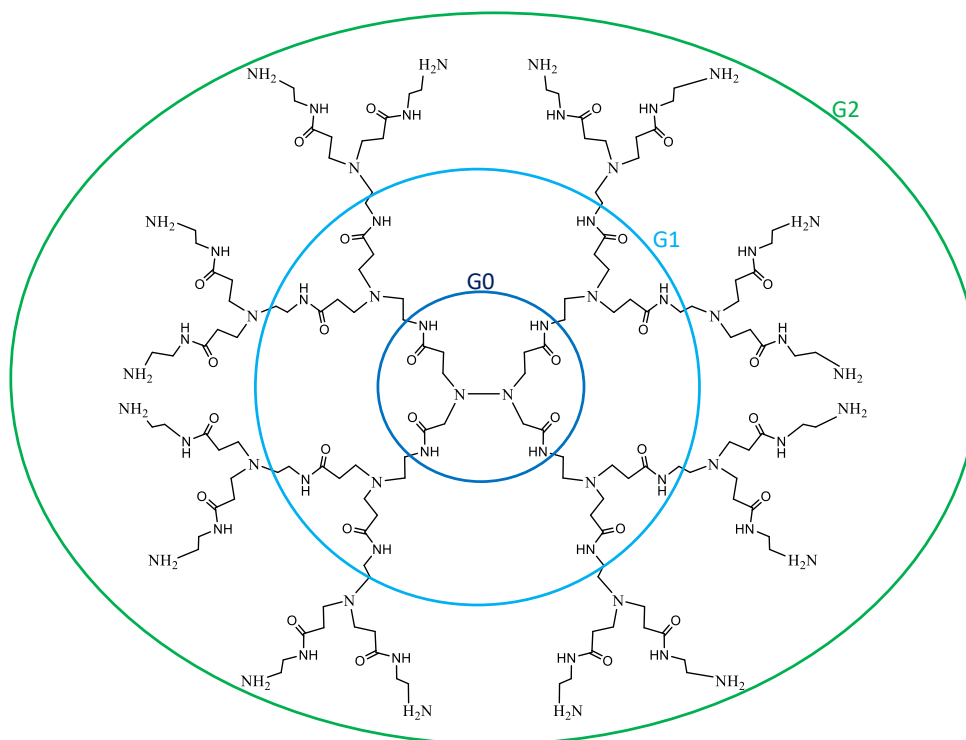


Figure 5.2.: Chemical structure of a PAMAM G2 dendrimer indicating the previous generations G1 and G0. The number of terminating amine groups A for each generation $n = 0, 1, 2, \dots$ is $A = 2^{2+n}$.

pH of the surrounding buffer relatively high, here we chose a pH 9. After this step the surface has to be blocked appropriately, such that no unspecific binding to uncovered spots occurs. The PAMAM G2 dendrimer for this work were provided by the Laboratorio de Dendrímeros Biomiméticos y Fotónica from the University of Málaga.

5.2.1. Online Functionalisation Verification

To monitor the binding process of the different functionalisation steps, the following online experiment was realised in two different setups. The first, in The Bailey Lab at the University of Michigan, Ann-Arbor, United States of America, using the already commercialised photonic biosensor diagnostic system M1 the prior version of the Maverick detection system [69]. The second is the CMZI investigated in this work. The protocols vary slightly since the underlying technologies are also different, and other chemicals may be more accessible. However, the fundamental functionalisation principle stays the same.

The experimental bases for the line of research were started during the stay at the Bailey Lab. There, the functionalisation procedure was developed and verified with the M1 diagnostic system which is why they are included in the following Section 5.2.1.1. Unfortunately, there was not enough time to extend the research to IgG an IgE amoxicillin detection, hence, no comparison in terms of performance can be done. Therefore, in order to first verify and validate the developed functionalisation protocols, those experiments were carried out again in our laboratory in Section 5.2.1.2.

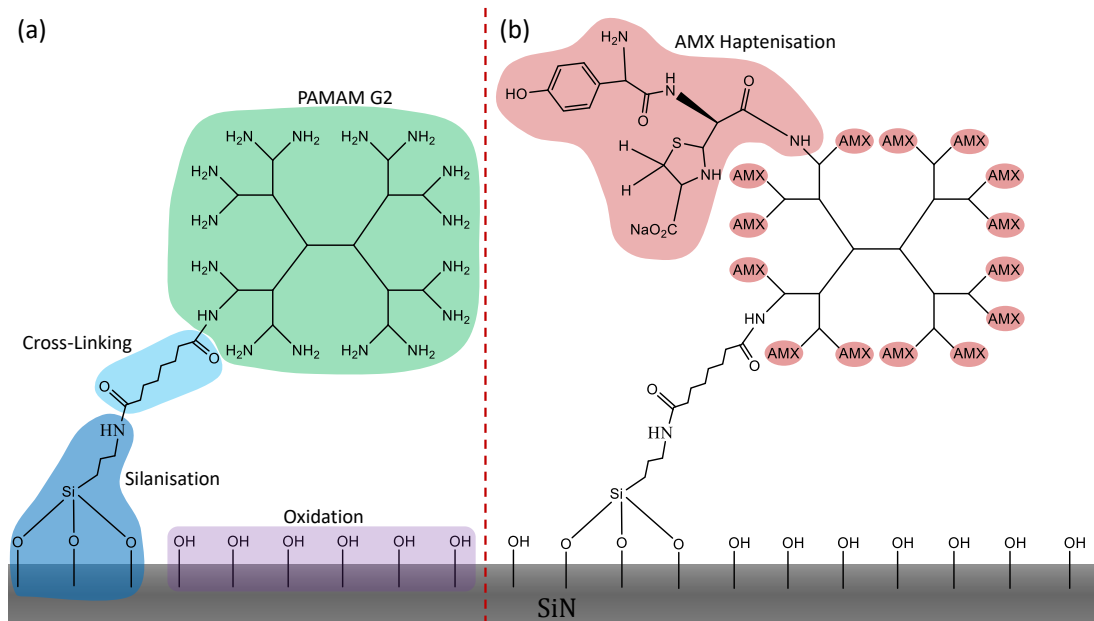


Figure 5.3.: DendAmox immobilisation using almost the same procedure, up to the crosslinking step, as in Section 4.1.2. (a) The attachment of the PAMAM G2 dendrimer to the surface via the crosslinker, and the following step (b) the amoxicillin haptenisation of the covalently immobilised dendrimers.

5.2.1.1. Genalyte M1

The photonic chips used in this detector integrate 128 different sensing ring resonators, which can be read quasi simultaneously. The material used for the waveguides is silicon instead of silicon nitride. Two fluidic channels are placed above the chip, giving access to 64 rings in each channel. More information about the biosensing device can be found in [228].

Offline Preparation The silicon photonic chip was prepared following this protocol:

I Cleaning and oxidation:

- 1 Sonicate the chip in methanol for 7 min.
- 2 Place chip in heated piranha solution for around 1 min.
- 3 Bath chip in water for 1 min.

II Silanisation :

- 1 Immerse in acetone for 2 min while shaking in an orbital shaker.
- 2 Immerse in acetone with 5% APTES, i.e. 100 μ L APTES diluted in 1.9 mL acetone, for 4 min while shaking in an orbital shaker.
- 3 Immerse in acetone for 2 min while shaking in an orbital shaker.
- 4 Immerse in isopropanol for 2 min while shaking in an orbital shaker.
- 5 Dip chip in deionised water and dry with nitrogen gas.

Online Experiment The silanised chip was then installed into the M1. There are major differences in the way the samples are provided to the sensing structures in comparison to the setup in this work. The fluidic system uses sucking pumps to introduce different sample reservoirs to the channel, this makes it very easy to change the running buffer. Furthermore, both channels can be operated independently as they suck from different reservoirs. They do not suffer from high diffusion effects typical for injection systems. The following protocol was used to verify the binding process:

Both Fluidic Channels

1. Set flow rate $F = 20 \mu\text{L min}^{-1}$.
2. Flow Milli-Q over the sensor for 5 min.
3. Flow BS3 ($C = 2.86 \text{ mg mL}^{-1}$), 2 mg solid BS3 in 700 μL 2 mM acetic acid, over the sensor for 5 min.
4. Flow Milli-Q over the sensor for 5 min.
5. Flow PAMAM G2 dendrimer, 1 mg mL^{-1} diluted in Milli-Q, over the sensor for 10 min.
6. Flow Milli-Q over the sensor for 10 min.
7. Flow carbonate buffer (pH 9) over the sensor for 5 min.

First Fluidic Channel

8. Flow amoxicillin, 10 mg mL^{-1} diluted in the same carbonate buffer, over the sensor for 30 min.

Second Fluidic Channel

8. Flow carbonate buffer (pH 9) over the sensor for 30 min.

Both Fluidic Channels

9. Flow carbonate buffer (pH 9) over the sensor for 5 min.
10. Flow Milli-Q over the sensor for 10 min.

By establishing these two slightly different fluidic protocols, with sensors in one channel exposed to amoxicillin, and sensors in the other channel exposed to the carbonate buffer, the binding process of amoxicillin to the dendrimer can be directly compared. The recorded relative resonance frequency/wavelength shifts (in pm) are shown in Fig. 5.4. The line represent the mean shift of the 64 ring sensors, the area around it in the same colour represents the standard deviation around that mean. The BS3 causes a noticeable phase shift (around $\Delta\lambda_{\text{BS3}} \approx 150 \text{ pm}$), even though the molecule is relatively small. The permanent shift due to the PAMAM dendrimer ($\Delta\lambda_{\text{PAD}} \approx 250 \text{ pm}$) also indicates good covalent attachment to the BS3. Sensors being exposed to amoxicillin experience a clear binding signal (around $\Delta\lambda_{\text{AMX}} \approx 90 \text{ pm}$), seemingly linearly increasing, in comparison to the sensors immersed in the carbonate buffer in the other channel. The two peaks which can be observed after each 10 min are due to a change of sample reservoir. The linear increase of the phase shift for the amoxicillin can be interpreted as a pure binding signal, meaning that saturation has not been reached after 30 min, for which later experiments are done with an offline functionalisation, simplifying the process itself and increasing the binding time.

5.2.1.2. Coherently Detected Mach-Zehnder Interferometer

The chip and setup described from the previous chapters was used for this online experiment.

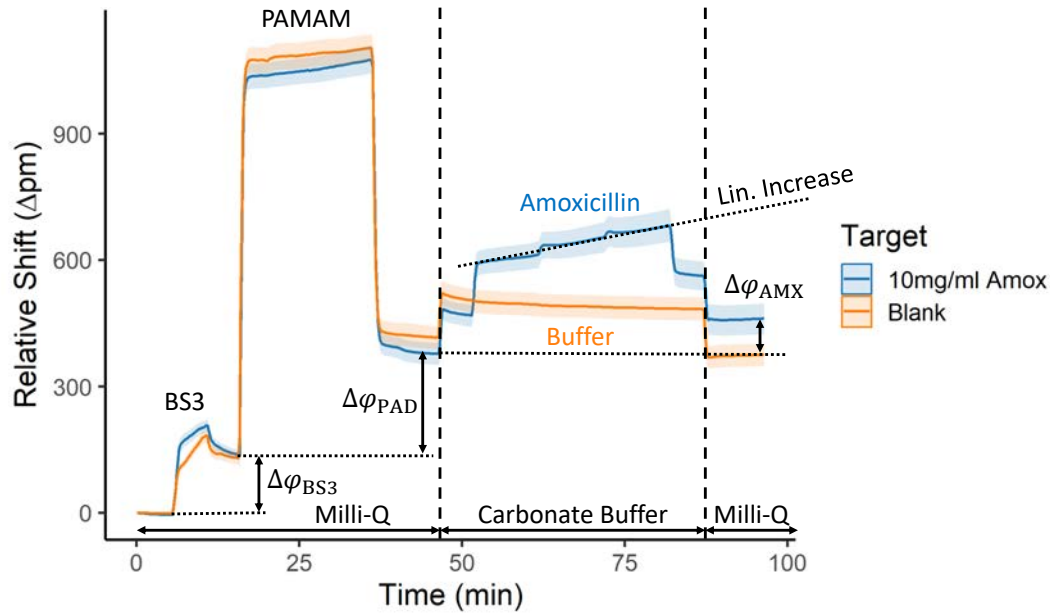


Figure 5.4.: The two channel online experiment done at The Bailey Lab, showing the different phase shift after offline APTES silanisation: crosslinking with BS3 ($\Delta\lambda_{BS3}$), PAMAM dendrimer immobilisation ($\Delta\lambda_{PAD}$), and the final amoxicillin haptenisation ($\Delta\lambda_{AMX}$). The last step is only done in one of the channels to have a direct comparison for the haptenisation process.

Offline Preparation The silicon nitride photonic chip was prepared following this protocol:

I Cleaning and oxidation:

- 1 Sonicate the chip in methanol for 7 min.
- 2 Place chip in room temperature piranha solution for around 1 min.
- 3 Bath chip in water for 1 min.

II Silanisation :

- 1 Immerse in acetone for 2 min while shaking in an horizontal shaker.
- 2 Immerse in acetone with 5% APTMS, i.e. 100 μ L APTMS diluted in 1.9 mL acetone, for 4 min while shaking in an horizontal shaker.
- 3 Immerse in acetone for 2 min while shaking in an horizontal shaker.
- 4 Immerse in isopropanol for 2 min while shaking in an horizontal shaker.
- 5 Dip chip in deionised water and dry with air gas.

Online Experiment The silanised chip was then installed into the setup. The fluidic system is based on a injection system supported by a pushing pump, and only one fluidic channel exists to expose the samples to the sensing structures. The following protocol was used to verify the binding process:

1. Fill the syringe pump with Milli-Q water.
2. Set flow rate $F = 20 \mu\text{L min}^{-1}$.

3. Flow Milli-Q water over the sensor for 5 min.
4. Inject a low concentration of sodium chloride to calibrate the read-out. Wait until the signal returns to baseline.
5. Inject BS3 ($C = 2.86 \text{ mg mL}^{-1}$), i.e. 2 mg solid BS3 in 700 μL 2 mM acetic acid, and wait until the signal settles to a new level.
6. Inject PAMAM G2 dendrimer, 1 mg mL^{-1} diluted in Milli-Q, over the sensor for 10 min, wait until the signal start to decrease, inject the same again, and wait until the signal settles to a new level.
7. Stop the pump ($F = 0 \text{ }\mu\text{L min}^{-1}$).
8. Install the carbonate buffer (pH 9.2) in the syringe pump.
9. Set flow rate $F = 20 \text{ }\mu\text{L min}^{-1}$.
10. Inject amoxicillin, 10 mg mL^{-1} diluted in the same carbonate buffer, wait until the signal start to decrease, inject the same five more times, and wait until the signal settles to a new level.

The recorded phase change is shown in Fig. 5.5. The crosslinker shows a binding signal of $\Delta\varphi_{\text{BS3}} \approx 2.4 \text{ rad}$ and the PAMAM dendrimer a shift of $\Delta\varphi_{\text{PAD}} \approx 7.7 \text{ rad}$. As the buffer was

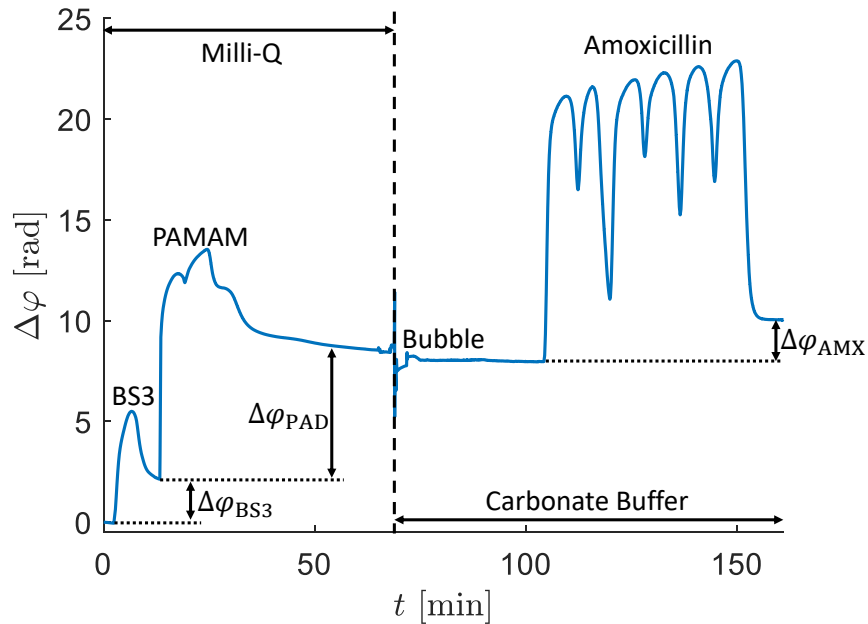


Figure 5.5.: The one channel online experiment done in our laboratory, showing the different phase shift after offline APTES silanisation : crosslinking with BS3 ($\Delta\varphi_{\text{BS3}}$), PAMAM dendrimer immobilisation ($\Delta\varphi_{\text{PAD}}$), and the final amoxicillin haptenisation ($\Delta\varphi_{\text{AMX}}$).

changed by switching the syringes of the pump, a bubble was introduced to the fluidic channel for which no reliable phase change for the transition between the buffers can be observed. For this reason no back switching to Milli-Q was attempted. The shift due to binding of amoxicillin is around $\Delta\varphi_{\text{PAD}} \approx 2.5 \text{ rad}$. As the previous experiment, this indicates a functional amoxicillin haptenised layer for following antibody recognition experiments.

5.2.1.3. Conclusion

Comparing the obtained phase-, and resonance shift from Fig. 5.5, and Fig. 5.4, respectively, both show similar binding behaviour, even though the signal shapes themselves differ due to the way of exposing the sample to the sensor. To make a rough comparison between those two measurements possible it is assumed that the peak HRI change due to the BS3 is more or less the same for both experiments, which leads to a conversion factor $\rho_{\varphi \rightarrow \lambda} \approx 35.7 \text{ pm rad}^{-1}$. Converting the observed phase to resonance shifts, $\Delta\lambda_{\varphi \rightarrow \lambda}$ is around 90, 275, 90 pm for BS3, PAMAM, and amoxicillin, respectively. Those changes are very similar to the obtained with the M1. Therefore, from a relative signal point of view, both experiments represent highly similar binding signals.

5.3. Offline functionalization

This section provides the entire offline functionalisation protocol of the amoxicillin haptenised PAMAM dendrimers in the following Section 5.3.1. Furthermore, methods such as contact angle (Section 5.3.2), X-Ray photoelectron spectroscopy (Section 5.3.3), and fluorescence microscopy (Section 5.3.4) indicate the effectiveness of the different functionalisation steps of the offline protocol.

5.3.1. Offline Functionalisation Protocol

The following offline functionalisation protocol for the DendAmox biorecognised layer was used for the sensor calibration with IgG and IgE antibodies:

I. Pre-Cleaning (in case of a new chip):

1. Sonicated in acetone for 5 min.
2. Sonicated in isopropanol for 5 min.
3. Sonicated in Milli-Q water for 5 min.
4. Dried with air.

II. Cleaning and oxidation:

1. Sonicate the chip in methanol for 7 min.
2. Place chip in heated ($T = 47^\circ\text{C}$) piranha solution for around 15 min.
3. Bath chip in Milli-Q water for 1 min.

III. Silanisation :

1. Immerse in acetone for 2 min while shaken in an horizontal shaker.
2. Immerse in acetone with 5% APTMS, 100 μL APTMS diluted in 1.9 mL acetone, for 4 min while shaken in an horizontal shaker.
3. Immerse in acetone for 2 min while shaken in an horizontal shaker.
4. Immerse in isopropanol for 2 min while shaken in an horizontal shaker.
5. Dip chip in deionised water and dry with air gas.

IV. DendAmox functionalisation:

1. Place droplet of BS3 ($C = 2.86 \text{ mg mL}^{-1}$), 2 mg BS3 solid in 700 μL 2 mM acetic acid, above the sensing structure for 3 min. Airflow dry the chip.

2. Drip a drop of PAMAM G2 dendrimer, 1 mg mL^{-1} diluted in Milli-Q, over the sensor for 1 h. Airflow dry the chip.
3. Immerse the entire chip in amoxicillin, 10 mg mL^{-1} diluted in the same carbonate buffer (pH 9.2) for 1 h while shaking. Airflow dry the chip.
- V. Conservation: Immerse chip in Milli-Q for preserving the DendAmox functionalisation. Even though conversation should be possible for some days, here, the chips were directly installed into the sensing setup to avoid any possible degradation of the functionalisation.

5.3.2. Contact Angle Indication

Contact angle (CA) measurements consist in placing a drop of water on a surface to determine its hydrophobicity or hydrophilicity of a surface. Depending on those characteristics the droplet experiences more or less attraction to the surface, forming the contact angle at the edges of the droplet with the surface and surrounding air (see Fig. 5.6(a)). Instruments like goniometers

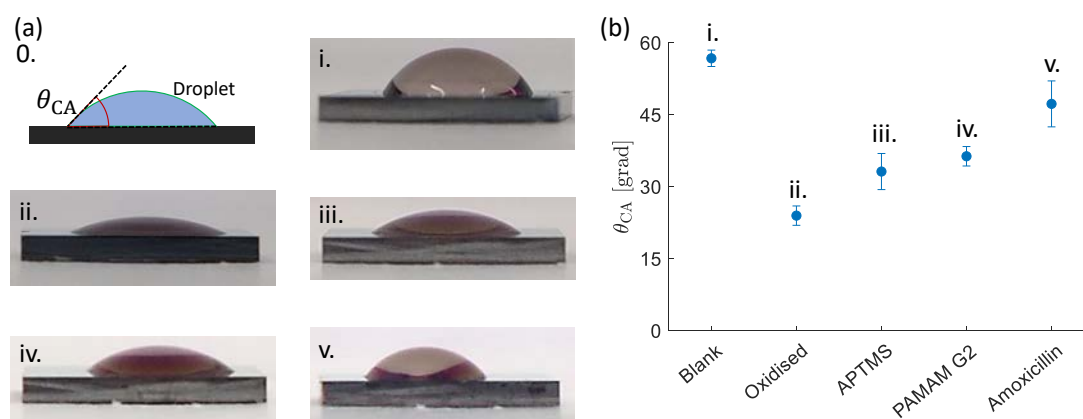


Figure 5.6.: (a) 0. The definition of the contact angle. i. The CA of a blank silicon nitride surface. ii. CA of the oxidised and iii. the following silanisation step. The final steps iv. for dendrimer immobilisation and v. amoxicillin haptenisation. (b) Contact angles obtained in the established measurement setup (see appendix Fig. A.9) for the different offline DendAmox functionalisation steps i. to v.

were designed to measure the contact angle of a purified water droplet on the surface to be measured. Normally, they consist of a stage for the surface probe, a light source for homogeneous illumination of the droplet, and a high resolution camera. Software, especially designed for contact angle measurements, extracts the contact angles of the pictures made. This way of detection is often referred to as static sessile droplet contact angle measurement. In relation with chemistry modified surfaces CA measurements can give hints about if a surface modification step worked or not. The reproducibility of the CA depends on the heterogeneity, and the cleanliness/pureness of the surface/droplet.

Even though the measuring principle is relatively simple, commercially available goniometers can cost several thousands of euros. However, for the purpose of this work, the high accuracy of a commercial system is not needed, and a self-made contact angle setup was assembled, shown in the appendix Fig. A.9. The price for the realisation of the setup was just a few tens of euros. The software used for extracting the CA was ImageJ [229] in addition with the free “Drop Shape analysis” [230] plug-in.

Four chips, with dimensions of $5 \times 5 \text{ mm}^2$, with a pure silicon nitride surface were cut from a wafer and cleaned by:

1. Sonicated in acetone for 5 min.
2. Sonicated in isopropanol for 5 min.
3. Sonicated in Milli-Q for 5 min.
4. Dried with air.

The contact angles for five different steps, blank, oxidation, APTMS, PAMAM G2, and amoxicillin of the functionalised surface are shown in Fig. 5.6(b) using a few microlitre drops of Milli-Q water. An exemplary photo of each step is shown in Fig. 5.6(a)

- i. The blank chip was measured directly after the previously described cleaning procedure. The mean contact angle of $\approx 57^\circ$ is similar to others found (63°) in literature [231].
- ii. The chips were placed in piranha solution for approximately 15 min minutes to oxidise the surface. The measured CA of $\approx 24^\circ$ matches well with other records (24°) [231]. The increase of hydrophilicity is due to the formation of a polar Si-OH groups which can interact with water on the surface [232].
- iii. The silanisation was realised according to the offline preparation protocol in Section 5.2.1.2. Ideally, the silanes should saturate the entire surface, such that no or almost non OH group is available. The APTMS introduces propyl chains which are hydrophobic and an amino group which is hydrophilic. Considering the amount of both, the surface should become less hydrophilic, which is confirmed by the measured mean CA of around 33° .
- iv. PAMAM G2 dendrimers were then attached to the silane by first covering the surface for 60 min in BS3 ($C = 2.86 \text{ mg mL}^{-1}$), 2 mg BS3 solid in 700 μL 2 mM acetic acid, followed by one hour incubation PAMAM G2 dendrimer, 1 mg mL^{-1} diluted in Milli-Q. Ideally, the number of amino terminated groups on the surface should be increased, maybe explaining the slightly increased mean CA of around 36°
- v. The haptensisation of the dendrimer was realised with immersing the chips in amoxicillin (10 mg mL^{-1} dissolved in pH 9 carbonate buffer) for 1 h. It is difficult to estimate the effect of the amoxicillin modified dendrimer (DendAmox) surface, but the CA change to 47° indicates a modification of the dendrimer.

The presented measurements are in accordance with the interpretation of a functional DendAmox surface. However, these results on their own are not conclusive, but in combination with the prior online functionalisation experiments, this perception becomes more likely.

5.3.3. X-Ray Photoelectron Spectroscopy

X-Ray Photoelectron Spectroscopy (XPS) is a photoemission spectroscopy, detecting the electron binding energy within an atom and its bonding partners. It is often used to determine which atoms are present in a small region [233]. X-Rays with a distinct frequency (f_x) are generated and shot onto a small region of interest (ROI) of the surface, typically up to $200 \times 200 \mu\text{m}^2$. The penetration depth of the rays are around $2 \rightarrow 5 \text{ nm}$ [234]. The incoming radiation irradiates electrons according to the photoelectric effect, giving them a kinetic energy E_k . A part of the released electrons propagate to an electron energy analyser where the kinetic energy is determined and the amount of electrons with this energy are counted for a short time interval. The binding energy E_{be} of the irradiated electron is can then be approximately determined by power conservation $hf_x \approx E_{be} + E_k$ [235].

One of the DendAmox functionalised chips was sent to XPS analysis to determine the composition of the chemically modified surface. Figure 5.7 shows the binding energy spectra for one ROI. The presence of sulphur is confirmed from the obtained spectra. The relative amount of sulphur (3%) is close to the theoretical calculated (5%), which indicates the existence of

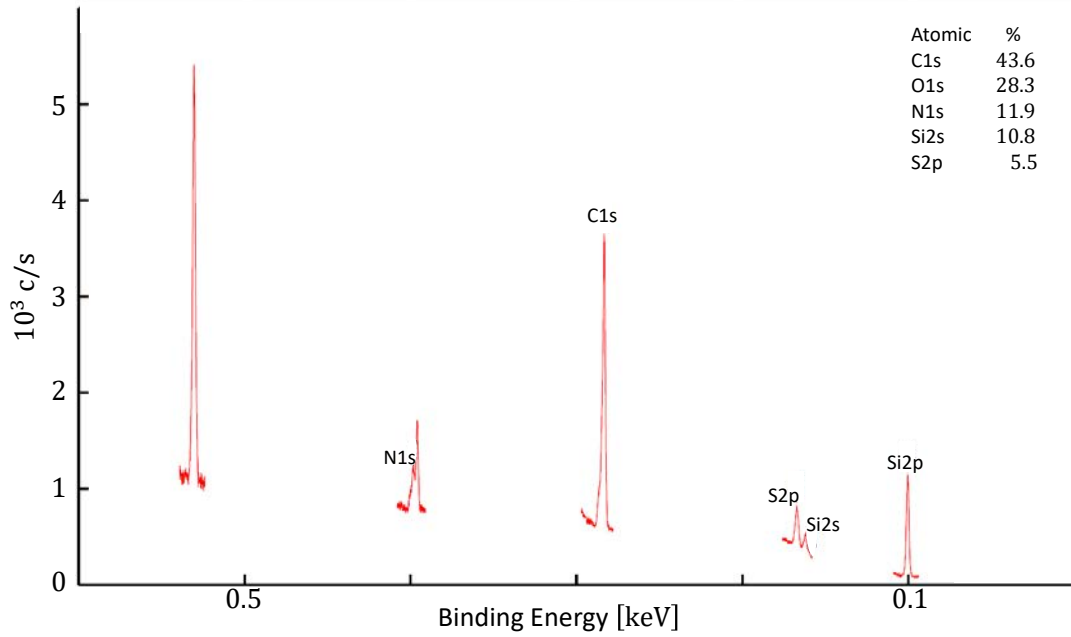


Figure 5.7.: X-ray photoelectron spectra for a DendAmox functionalised silicon nitride surface. The small peak at ≈ 180 eV indicates the presence of sulphur in the first few nanometres of the surface, a possible implication of amoxicillin.

amoxicillin on the surface and confirms the presence of a operational functionalisation layer.

5.3.4. Fluorescence Microscopy

A confocal fluorescence microscope was used to determine the distribution of the PAMAM G2 dendrimer on the surface of the sensing waveguide/window. Without going into operational details, fluorescence microscopes excite a ROI with light of a distinct wavelength [236]. In case of the presence of any material which absorbs light at this wavelength, its electrons are lifted from its ground state E_0 to a higher energy state, for example E_2 . There is a probability that the electron first jumps down to an intermediate energy level between the current and ground state, for example E_1 , and emits a photon and/or phonon with the energy difference, here $E = E_2 - E_1$, which is typically much lower than the energy of the exciting light. The electron on this level then falls down to the ground state, emitting fluorescence light of the energy $E_1 - E_0$ [237]. This radiated light is then detected by a PD or a CMOS camera. For detection of certain biological substances, such as proteins, they are often labelled with fluorescent markers absorbing at a exciting wavelength and emitting at the desired fluorescence wavelength. That way the desired protein can be monitored.

For verifying the presence of dendrimers on the silicon nitride sensing chip surface, the amoxicillin, the final step of the functionalisation protocol described in Section 5.3.1, was not attached, but substituted by a fluorescent biomarker called fluorescein isothiocyanate (FITC). It is reactive towards amine groups for which it is applied on the PAMAM dendrimers by immersing the chip in a 1 mg mL^{-1} FITC, dissolved in carbonate buffer pH9, solution for 1 h. The excitation and maximum emission wavelength are 495 nm and 519 nm [238], respectively. Another chip, cleaned with acetone, isopropanol, methanol, and piranhas solution was used as a reference to determine the level of fluorescence by the marker. Figure 5.8 shows the structural (a) and fluorescent pictures (b)+(c) of the confocal microscope. The reference, Fig. 5.8(b), shows almost no fluorescence signal in the sensing window, as expected. However, the silicon dioxide cladding,

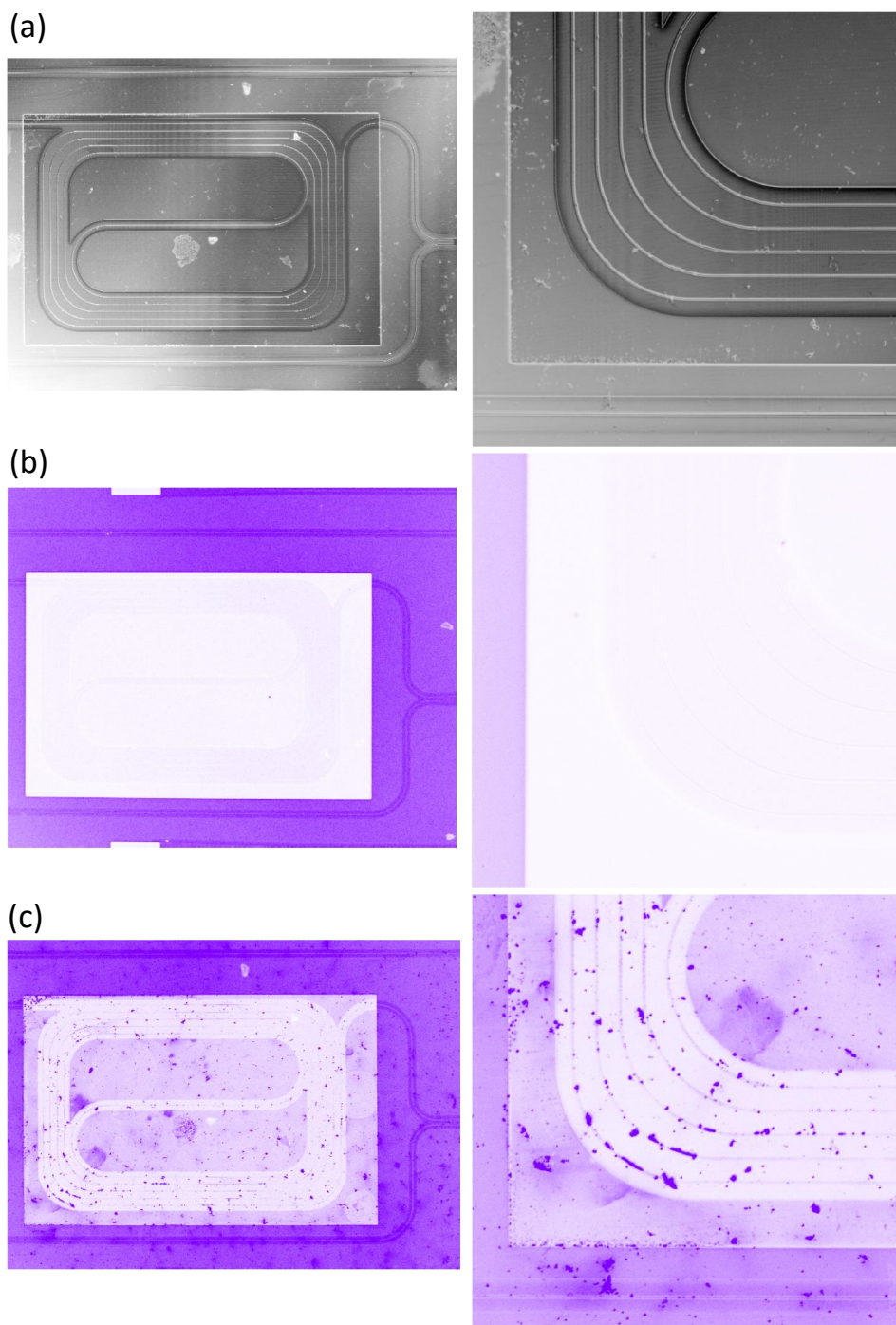


Figure 5.8.: Structural and fluorescent information of the functionalised sensing window via the confocal microscope. (a) shows the structural information of the entire sensing structure (left) and a zoomed fragment of the lower left corner (right). In (b) the fluorescent signal from the reference chip is shown. Almost no fluorescent signal is obtained within the window. In (c) the fluorescent signal from the functionalised chip is shown. A clear fluorescent signal of the FITC labelled dendrimer is obtained within this window.

covering the rest of the chip, seems to be excited and emitting a fluorescent light. This may be a result from the CMOS fabrication process. Nevertheless, as long as the ROI, the sensing window, shows almost no signal it is possible to use it for comparison. The labelled chip, Fig. 5.8(c), shows an increased fluorescence signal level. From (a) we can already determine the similarities between the structural and the fluorescent information, verifying the attachment of FITC to the surface. Looking at the zoomed picture in (c) we can clearly see the waveguide structures being imitated from the fluorescence signal. However, there are a lot of spots, where the signal is stronger than in the rest of the window.

From Fig. 5.8 we can conclude that the labelling tag is on the effective refractive index measuring part of the sensing window, but there are also factors visible which might indicate otherwise. Looking at those strong signal spots, they seem to considerably correlate with the “impurities” which can be seen in structural image (a). If not close to a waveguide, those spots do not support the functionalised biorecognition layer as they are not in direct contact with the propagating sensing mode. Even if found directly on the waveguide the introduced distance, due to the “impurities” size, from the waveguide to the layer, may not contribute to a biorecognition phase shift at all. The origin of the “impurities” can have several causes. They could be residuals from the etching process or from the previous biofunctionalisations. The oxidation due to the piranha solutions may not have removed all biomolecules, therefore, the next functionalisations may have been attached on top, supporting this clustering.

Another suspicious observation results from the fact, that the waveguide itself seems not to be uniformly functionalised for there are regions in which no fluorescent signal is obtained (see left side of Fig. 5.8(c) right upper spiral edge). In addition, the elevated silicon dioxide surrounding, which is placed in a certain distance from the waveguide, appears to be homogeneously fluorescing. This is most likely a result from a mass transfer problem, where the higher levelled surface experience a more uniformly functionalisation as the lower lying waveguide. At least one functionalisation step does potentially not reach the sensing waveguide. Almost all offline functionalisation steps are sonicated to decrease the influence from air trapped in the sensing structure, therefore, maximising the mass transfer to its surface. That includes pre-cleaning, oxidation, silanisation, and haptenisation of the dendrimers with amoxicillin. The cross-linker (BS3) and the dendrimer functionalisation step, however, are placed via droplets directly on top of the sensing structure, and are not completely immersed, therefore, cannot be sonicated. This is done in order not to waste these expensive materials. This may explain, or also be a reason, by which we see such a high variation in sensitivity in the CRP calibration experiments in Chapter 3, and also in the later IgG and IgE detection experiments. These pictures were done relatively late in the framework of this thesis, such that the obtained conclusions could not be investigated further, but will play an important role for coming experiments with this chips or will also influence the design of coming chip generations.

5.4. Experimental Verification

Here, the effectiveness of the DendAmox layer is demonstrated for both IgG and IgE anti-amoxicillin antibodies. This is done by first presenting the offline functionalisation protocol, second showing the specificity of the recognition layer by blocking the residual surface sufficiently, and by finally detecting IgG and IgE antibodies. Different types of blocking agents were tried for adding specificity to the recognition layer but only one showed a sufficient blocking efficiency. IgG amoxicillin antibodies can be found in much higher concentrations in serum of patients, and are, therefore, easier to extract for experimental purposes, which is why it was chosen as an initial target before working with the much more expensive IgE antibody, the actual biomarker used in diagnostics. Both have similar molecular weights, have similar recognition sites, but have different functions. Nevertheless, both can be used to determine the functionality of the DendAmox surface - used the first for protocol optimisation, while the

latter determines the final quality and functionality. As already mentioned at the beginning of this chapter, to distinguish between both Ig isotypes, a secondary binding step has to be done by exposing the attached IgG and IgE amoxicillin antibodies to anti-human-IgE antibodies. However, this is not done within the framework of this thesis and probably subject of future research.

5.4.1. Blocking and Specificity

To avoid non-specific physical adsorption, the non-functionalised spots on the waveguide have to be blocked, creating an anti-fouling layer, similar to the blocking in Section 4.1.2. Due to the size difference of the PAMAM G2 dendrimer, haptenised with amoxicillin, which has an diameter of approximately 3 nm [239], and the BSA, dimensions of $14 \times 4 \times 4 \text{ nm}^3$, there were concerns that using BSA as blocking agent would considerably reduce the sensitivity of the sensor by hindering the antibodies reaching their counterpart on the surface. In the case of ACRP functionalised onto the surface, this was less of a problem because the antibody has larger dimensions than the BSA blocking agent. Different blocking agents were tried to create the anti-fouling layer, such as Bovine Serum Albumin (BSA), polyethylene glycol 2k (PEG-2k), PEG-6k, PEG-10k, ethanolamine (ETA), Tween 20, and casein were tried offline or online, or in combination. Only casein resulted in a sufficient non-specific binding suppression, which is why it is the one discussed here. As the others did not block the non-functionalised surface, their performance is not displayed.

The casein was bought from Sigma-Aldrich, “Casein Blocking Buffer 10x” (B6429-500ML) a commercialised blocking agent. For our purposes it was diluted by a factor of 10, to which is referred to in the following as casein blocking solution (CBS). Casein has a molecular weight of around 25 kDa and is, therefore, smaller than BSA (66.5 kDa), and potentially offers a higher obtainable sensitivity.

5.4.1.1. Protocol

The chip was functionalised according to the protocol in the previous Section 5.3.1. Subsequently, the chip was installed in the setup using the procedure presented in the Appendix A.5. The following experimental proceeding was established:

1. Set flow velocity $F = 30 \mu\text{L}/\text{min}$.
2. Inject a read-out calibration solution of PBS $20 \rightarrow 100 \text{ mM}$ (pH 7.4). A HRI change causing a phase shift at least as large 2π has to be prepared for this. Flow velocity may be increased, to minimise experiment time, as for the read-out calibration the exposure time is not important.
3. Set flow velocity $F = 10 \mu\text{L}/\text{min}$.
4. Inject the CBS twice to block the remaining non-functionalised spots. Each time wait until phase reaches equilibrium.
5. Set flow velocity $F = 20 \mu\text{L}/\text{min}$.
6. Inject the first control sample, containing BSA, having a concentration of 4000 ng mL^{-1} . Wait until phase reaches equilibrium.
7. Inject the second control sample, containing IgG CRP, having a concentration of 4000 ng mL^{-1} . Wait until phase reaches equilibrium.
8. Inject the target sample, containing IgG amoxicillin, having a concentration of 1000 ng mL^{-1} . Wait until phase reaches equilibrium.

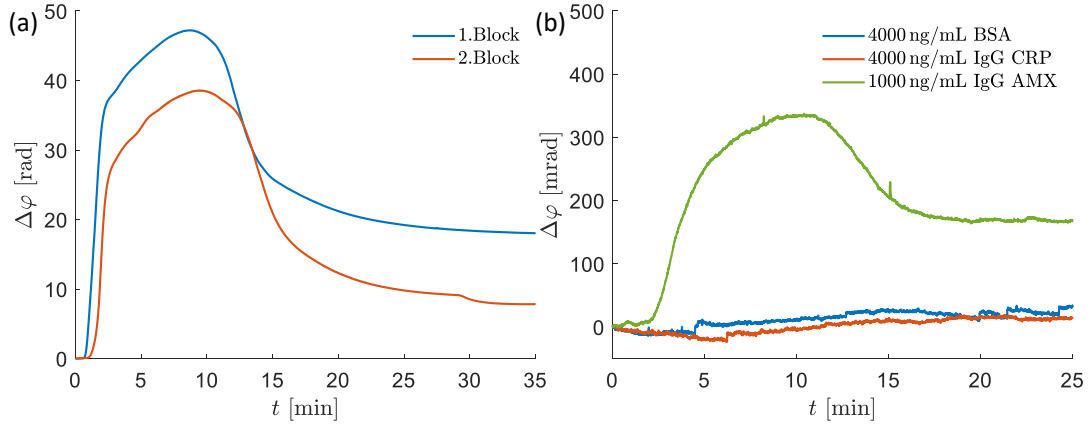


Figure 5.9.: Specificity experiment showing the CBS blocking steps in (a) of the prior offline DendAmox functionalised chip and (b) the two control sample injections (BSA and ACRP at $C = 4000 \text{ ng ml}^{-1}$) showing almost no phase response, the four times lower concentration of the IgG amoxicillin causing a comparatively significant phase shift.

5.4.1.2. Experimental Results

Following this protocol, the phase shifts in Fig. 5.9 were obtained, where (a) shows the shift due to the CBS injections, due to physical adsorption to the surface, and (b) the shifts from the controls and target samples. The read-out calibration injection is not shown as it is irrelevant for the obtained binding signals. Comparing the phase shifts, due to the CBS, with the blocking shifts observed in the CRP detection experiments (see Fig. 4.11 & 4.4 in Chapter 4), there is a much higher binding signal. This can be explained by the fact, that there was no blocking step included in the offline functionalisation protocol (see Section 5.3.1). It was excluded since it was difficult to control the spread of the CBS on the surface such that the grating couplers are not blocked as well. After a few experiments it became clear that the casein covering the input and output gratings was influencing the coupling efficiency, probably due to chemical modifications caused by the high optical power density. For long experiments this invalidated the calibration parameters, making a correct phase shift read-out unreliable or even impossible.

The observed phase shift due to control injections in Fig. 5.9(b), IgG ACRP (blue) and BSA (orange), both having a concentration of 4000 ng mL^{-1} , result in a comparatively low, phase response looking at the shift caused by the target sample (IgG anti-amoxicillin) containing just one forth of the previous control concentrations. Defining the specificity as

$$SP = \frac{\Delta\varphi_{AMX}}{\Delta\varphi_{BSA/ACRP}} \frac{C_{BSA/ACRP}}{C_{AMX}}, \quad (5.1)$$

values of ~ 42 were obtained. This indicates a functional biorecognisable layer with anti-fouling behaviour. However, the quality, in terms of noise and sensitivity of this layer, and the final detection limit are determined in the next section.

5.4.2. Immunoglobulin Amoxicillin-Antibody Detection

The final goal for determining an amoxicillin allergy is the quantitative identification of the corresponding IgE antibody concentration in a patient sample. But, due to its low availability, first the more accessible IgG antibodies was used for determining the sensor's sensitivity due to the similarities of the antibodies F_{ab} region. Once it was proven that the sensor detects

specifically IgG amoxicillin antibodies, the IgE antibody detection experiments were carried out.

The artificial production of antibodies can be realised with Hybridoma technology [240, 241]. For the generation of the IgG and IgE amoxicillin antibodies for these experiments, the Hybridoma method reported in [242] was applied. The subsequent purification of the IgE antibodies was done via affinity chromatography describes in [243], whereas the IgG type was purified by using a NAb Protein A/G Spin Kit in an affinity chromatography. For this work, the antibodies types used for the following experiments were individually supplied by the IBIMA-Hospital Regional Universitario de Málaga (a World Allergy Organisation Center of Excellence). Due to the low availability of both antibody types, the injection range had to be modified compared to the CRP experiments. The amount of increasing injections was rearranged, reduced to six, and the highest concentration was decreased to 1000 ng mL^{-1} , specifically this means $C = 10, 50, 100, 250, 500, 1000 \text{ ng mL}^{-1}$, to minimise the amount of antibodies used per experiment. However, the control injections, i.e. IgG ACRP and BSA, remain 4000 ng mL^{-1} .

The specific detection of IgG and IgE antibodies for amoxicillin is just the first step towards a functional immunoassay test for amoxicillin allergy detection in patient sample. Serum or blood samples are highly complex matrices, which is why specificity is one of the most important parameters. IgE antibodies are very rare, only 0.05% in comparison to the concentration of IgGs in blood serum [244]. Therefore, in real samples more IgG than IgE will attach to the DendAmox functionalised surface. However, only the IgE is causing an allergic reaction. To discriminate between them, a secondary binding step has to be included into the detection method. For this, typically, IgG goat anti-human-IgE antibodies are applied to the IgG and IgE amoxicillin antibodies attached to the DendAmox functionalised surface. As they only recognise the IgE type antibody, any obtained signal will be proportional to the IgE concentration found in the sensor surface. The indirect IgE immunoassay is displayed in Fig. 5.10. Step (I.)

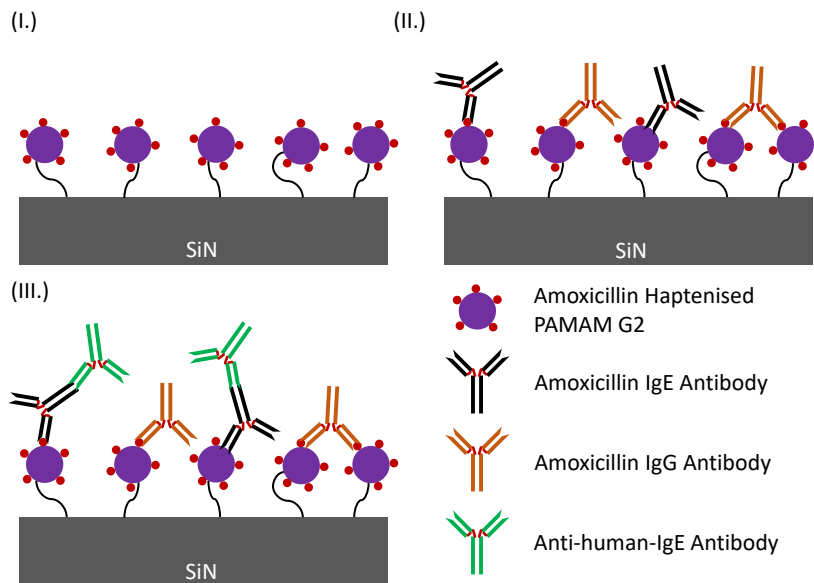


Figure 5.10.: Indirect immunoassay for quantitative detection of distinct human antibodies. Step (I.) shows the functionalised DendAmox surface, (II.) the attachment of both human IgG and IgE amoxicillin antibodies. The final step (III.), the attachment of anti-human-IgE amoxicillin antibodies to the previously attached human IgE antibodies, would give a quantitative signal for determining the concentration of IgE amoxicillin antibodies in a real human sample. This last step is not investigated in this work.

is realised by the DendAmox functionalisation, step (II.) by exposing the sensor to IgG and IgE amoxicillin antibodies, like real patient samples, which bind to the haptenised PAMAM G2 dendrimers covalently attached to the sensors surface. Step (III.) would be the final step towards real sample diagnostic, which is, however out of scope of this thesis, and requires further investigation in the future.

In the following, the full protocol of the additive immunoassay will be presented, followed by the results from the IgG amoxicillin detection experiments. Then the same is done for the IgE antibodies. Finally, a short conclusion is drawn.

5.4.2.1. Protocol

The chip was functionalised according to the protocol in the previous Section 5.3.1. Subsequently, the chip was installed in the setup using the procedure presented in the Appendix A.5. The following experimental proceeding was established:

1. Set flow velocity $F = 30 \mu\text{L}/\text{min}$.
2. Inject a read-out calibration solution of PBS $20 \rightarrow 100 \text{ mM}$ (pH 7.4). A HRI change causing a phase shift at least as large 2π has to be prepared for this. Flow velocity may be increased, to minimise experiment time, as for the read-out calibration the exposure time is not important.
3. Set flow velocity $F = 10 \mu\text{L}/\text{min}$.
4. Inject the CBS twice to block the remaining non-functionalised spots. Each time wait until phase reaches equilibrium. To shorten injection time, the flow rate can be increased after 10 min for some time.
5. Set flow velocity $F = 20 \mu\text{L}/\text{min}$.
6. Inject the control sample, containing BSA, having a concentration of 4000 ng mL^{-1} . Wait until phase reaches equilibrium.
7. Subsequently inject the six target samples in increasing order ($C = 10, 50, 100, 250, 500, 1000 \text{ ng mL}^{-1}$), just containing IgG or IgE amoxicillin antibodies, depending on the which experiment is performed. Each time wait until phase reaches equilibrium.

5.4.2.2. IgG Detection - Experimental Results

Here, the results from three experiments following the IgG additive immunoassay are presented and discussed. Three different chips were used for this purpose. In Fig. 5.11 the phase shifts for one of these experiments is presented. Figure 5.11(a) shows the blocking steps of the CBS. Even though the first blocking step causes a relatively high phase shift ($\Delta\varphi \approx 13 \text{ rad}$), the second results in a comparatively low shift ($\Delta\varphi \approx 3 \text{ rad}$) indicating a certain saturation level of the free spots on the surface. The abrupt phase change of the first block is a result of an air bubble at the output of the PDMS fluidic channel. Figure 5.11(b) shows the phase shifts due to the control (red) and IgG injections. Both the BSA control and IgG injection with 50 ng mL^{-1} are compromised by an artefact, an abrupt phase jump, which, when subtracted, results in a more precise observed shift. Hence, the control causes a negligible phase response, whereas the IgG amoxicillin causes a relatively high response. Already the shift due to the lowest concentration of 10 ng mL^{-1} is much higher than the BSA control injection of 4000 ng mL^{-1} .

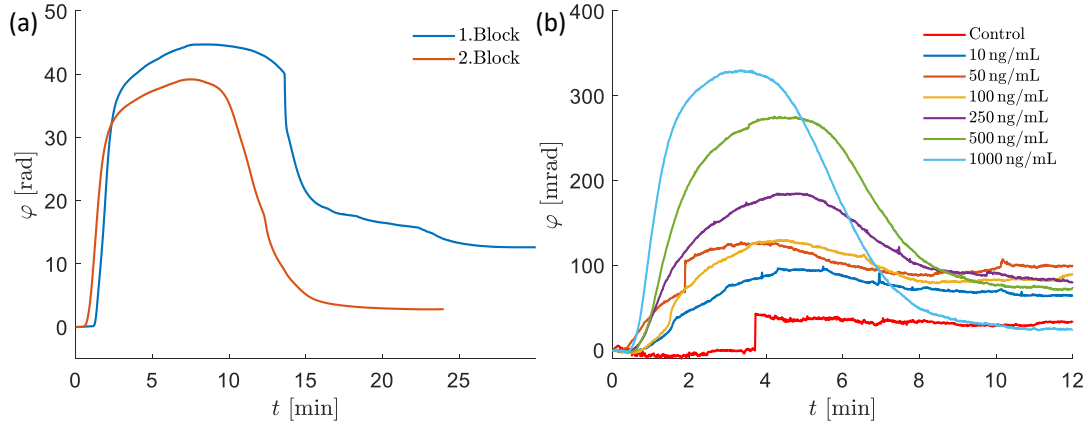


Figure 5.11.: Phase shifts from the IgG amoxicillin additive immunoassay experiment. In (a) the two blocking steps are shown, resulting in a first large and second low blocking signal, indicating block saturation of the surface. The control and IgG amoxicillin injections are shown in (b), the control, BSA with 4000 ng mL^{-1} , showing almost no binding signal (artefact subtracted), whereas the IgG amoxicillin already demonstrates to be highly sensitive to the lowest concentration of $C_1 = 10 \text{ ng mL}^{-1}$.

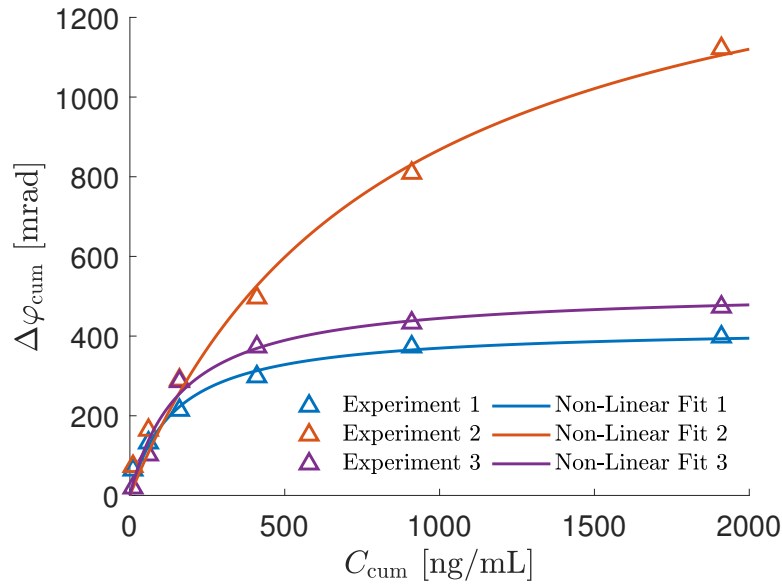


Figure 5.12.: Data and estimated calibration curves for each IgG amoxicillin detection experiment.

The calibration curves of the three applied additive immunoassay are presented in Fig. 5.12, showing the cumulative phase shifts (triangles) versus the cumulative injected concentrations, according to Eqs. (4.2) and (4.3), and their non-linear fits (lines), according to Eq. (4.4). All calibration curves show excellent fitting to the theoretical curve ($R^2 > 0.98$), however, already confirming a possibly high deviation of the binding and sensitivity. Table 5.2 presents the obtained parameter for sensitivity (S), noise (σ), and detection limit (LOD).

The sensitivities were extracted from the non-linear fitting via Eq. (4.5). The mean sensitivity and noise was calculated from the obtained experiments, the mean detection limit from those

Table 5.2.: Observed performance parameters extracted from the IgG amoxicillin additive immunoassay.

Experiment	$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	$\sigma \text{ [}\mu\text{rad]}$	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
1	2.91	1016	1046
2	1.92	322	503
3	3.12	1040	1001
Mean	2.65	793	897
Fit	2.24		1060

calculated values. The sensitivity fit was obtained by the mean cumulative phase and concentration from all three experiments, the LOD fit from this value and the mean noise. The obtained sensitivities, including mean and fit, are in the range of $2 \rightarrow 3 \text{ mrad}/(\text{ng}/\text{mL})$. The detection limits range from around $300 \rightarrow 1100 \text{ pg mL}^{-1}$ which shows the potential of detecting very low concentrations of antibodies, but due to the higher deviation in the binding curve, a slightly worse mean LOD is obtained.

5.4.2.3. IgE Detection - Experimental Results

The results obtained from the IgE detection additive immunoassays are discussed here in short, following the same protocol as the previously presented IgG experiments. Figure 5.13 shows the phase responses due to the blocking (a), and IgE with control injections (b) for one of the three performed immunoassay experiments. As in the IgG detection experiment, the first blocking step caused a shift of $\Delta\varphi \approx 13 \text{ rad}$, and the second $\Delta\varphi \approx 6 \text{ rad}$ which is considerably lower than the previous. Again, this indicates a sufficiently blocked surface for the suppression of non-specific binding. The flow velocity was increased after some minutes according to protocol to minimise diffusion and, therefore, injection time.

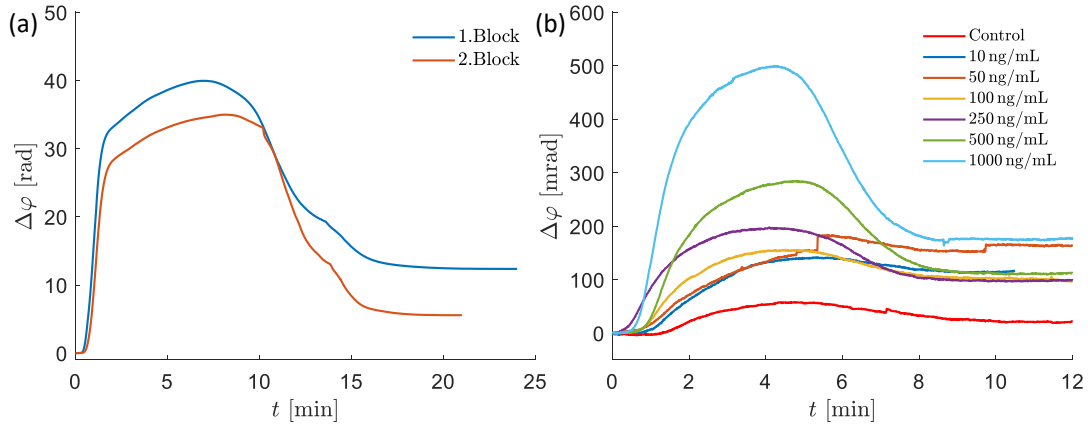


Figure 5.13.: Phase shifts from the IgE amoxicillin additive immunoassay experiment. In (a) the two blocking steps are shown, resulting in a first large and second low blocking signal, indicating block saturation of the surface. The control and IgE amoxicillin injections are shown in (b), the control, BSA with 4000 ng mL^{-1} , showing almost no binding signal, whereas the IgE amoxicillin already demonstrates to be highly sensitive to the lowest concentration of $C_1 = 10 \text{ ng mL}^{-1}$.

The phase response for the control BSA sample (red) is below 20 mrad, much lower than for the 10 ng mL^{-1} IgE with $\sim 120 \text{ mrad}$. Therefore, a highly specific surface can be assumed,

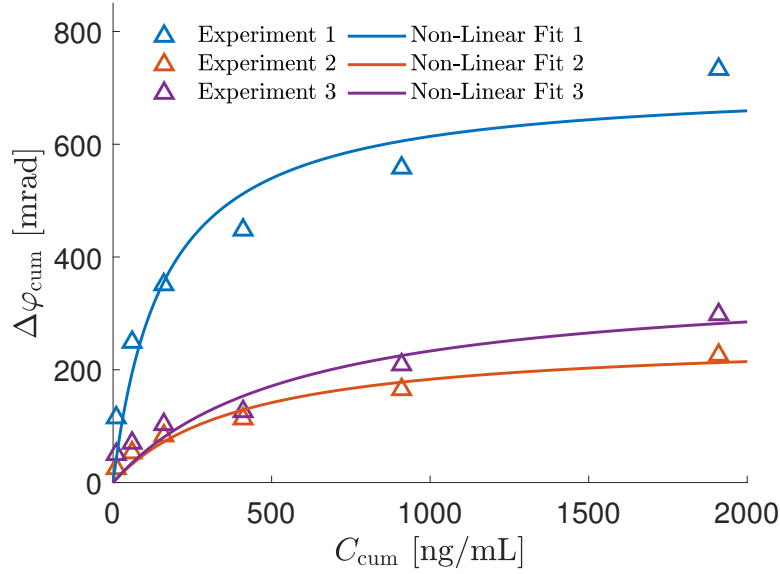


Figure 5.14.: Data and estimated calibration curves for each IgE amoxicillin detection experiment.

confirmed by the following IgE samples. Figure 5.14, shows the observed cumulative data (triangles) and non-linear fits (lines) for all three experiments. The observed R^2 values range from $0.91 \rightarrow 0.94$ and, therefore, do not fit as well to the theoretical model as the IgG binding curve. Furthermore, as for the IgG type antibodies, a high deviation in cumulative phase shift is present. Looking at Table 5.3 the same parameter are extracted from these experiments as for the IgG detection in Table 5.2. A higher sensitivity deviation than in the IgG case is

Table 5.3.: Observed performance parameters extracted from the IgE amoxicillin additive immunoassay.

Experiment	$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	σ [μrad]	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
1	4.43	557	378
2	0.62	291	1398
3	1.25	877	2105
Mean	2.1	575	821
Fit	1.91		905

obtained - it ranges from $0.61 \rightarrow 4.43 \text{ mrad}/(\text{ng}/\text{mL})$, covering a span of almost one order of magnitude. Accordingly, the detection limits ranges from around 380 pg mL^{-1} , which is close to the best observed LOD for IgG, to $\sim 2100 \text{ pg mL}^{-1}$. However the mean or fit LOD for both, IgG and IgE, are close at 1000 pg mL^{-1} .

5.5. Conclusion

In summary, our experiments for amoxicillin antibody detection show good specificity ($SP \sim 42$) and excellent best-case detection limits, down to approximately 400 pg mL^{-1} , and mean limit of detections of around 1000 pg mL^{-1} . Unfortunately, this span between best and mean case indicates that there is a consistency or reproducibility problem. Looking at the extracted sensitivities for IgG ($1.92 \rightarrow 3.12 \text{ mrad}/(\text{ng}/\text{mL})$) and IgE ($0.61 \rightarrow 4.43 \text{ mrad}/(\text{ng}/\text{mL})$) for additive

binding, it is clear that a sensor with such a high deviation cannot be used for quantitative concentration estimations. This high deviation in the response of the additive immunoassay can have several explanations. The confocal fluorescence microscopy results in Section 5.3.4 indicate that there are two different kind of spots. The first have high clustering potential on “impurities” on the sensing waveguide, whereas the second are regions where almost no fluorescence signal could be obtained on the waveguide. Therefore, the implication that the immunoassay is not reliably reproducible seems plausible. Also looking at the CRP detection experiments in Chapter 4, this seems to offer a valid explanation. Therefore, as a conclusion, CMZIs offer excellent LODs for IgE amoxicillin antibody detection, but in order to proceed to the next step towards real sample, e.g. patient blood serum, the reproducibility of the derived method has to be improved. This probably means that the offline functionalisation protocol is not yet optimised. Regarding the “impurities” within the sensing window, it should be investigated how they influence the functionalisation process, and how, if necessary, they should be removed. In general, the mass transfer of each individual step in the offline protocol has to be improved. However, these challenges are more of a biochemical nature, and, therefore, are not a direct problem resulting from the type of sensor applied. This means coherently detected balanced Mach-Zehnder interferometers are a promising integrated photonic sensor type with the potential capacity to quantitatively detect IgE antibody concentrations for allergy diagnosis.



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6. Conclusion and Prospects

Here, the initial motivations and achievements within this work are summarised and discussed in Section 6.1. Furthermore, a possible future prospect is given in Section 6.2, highlighting parts which have further potential for improvements.

6.1. Conclusion

Integrated photonic biosensors are a highly promising technology platform for medical diagnostics. In general they exhibit impressively low detection limits and offer highly multiplexed operation in real time. The sensing system is composed of the photonic chip itself, which is generally discarded after a single use, and the read-out system. The chip itself can be manufactured with standard CMOS fabrication lines which makes it very affordable. However, the design of the sensing chip dictates the type of read-out system that has to be used. Ring-resonator based chips, such as those used in the commercial Maverick system [69], require expensive high quality tuneable lasers for accurate operation. Interferometer based sensing chips have overcome initial limitations of sensitivity fading and phase ambiguity, and they have demonstrated even lower detection limits without requiring tunable sources, making them prime candidates for point-of-care devices.

There are many different types of interferometers which can be integrated in a photonic chip, but to circumvent sensitivity fading and phase ambiguity, they either use a single wavelength, narrow linewidth source with some sort of modulation scheme, or they use a broadband source with some sort of spectral analysis before detection. The coherently detected Mach-Zehnder interferometers studied in this thesis offer a compelling alternative, since they do not require any wavelength modulation, and only use a simple 2×3 MMI at the output.

In this work, first a theoretical framework for the coherently detected Mach-Zehnder interferometer sensor was investigated by showing its photonic working principle (see Chapter 2), followed by a system level description (see Chapter 3) of the setup. We studied different noise sources, such as detector noise, that is thermal-, shot-, quantisation noise, and baseband noise, furthermore laser source noise, that is relative intensity noise and laser phase noise, and finally mechanical noise caused by movements of the fibre array. Theoretical detection limits were derived gaining valuable insight into design and system level optimisation. One important aspect is the laser noise immunity of a balanced coherently read Mach-Zehnder interferometer: it was shown that relative intensity noise is by definition orthogonal to the phase extraction, whereas the laser phase noise influence can be minimised by carefully balancing reference and sensing arms in terms of optical length. This indicates that fixed wavelength light sources with relaxed linewidth requirements, such as cheap Fabry-Perot lasers, could be used with this type of sensor. In addition it was shown that baseband noise, a potential limiting source of noise, could be circumvented if an intensity modulation were added to the optical signal entering the chip. It should be mentioned that this modulation is not needed to avoid sensitivity fading and phase ambiguity, but only to shift the electrical signals, observed by the photodiodes, to a frequency region with a much lower noise floor, therefore, increasing the signal-to-noise-ratio of the complex signal and decreasing the extracted phase uncertainty. Most importantly in this context, it was shown that the multiplicative nature of mechanical noise was the main limitation, and once dampened excellent limits of detection could be achieved. Further it was shown

that the other detector noises can also be minimised for an improved limit of detection. The two different possible coherent detection schemes, the 2×3 MMI with non-differential and the 2×4 MMI with differential photodiodes, were theoretically investigated regarding the possible reachable detection limits for a thermal-, shot-, or quantisation noise limitation. Even though both can reach excellent performance, the differential approach could offer better performance values by rising the sensitivity ($S \propto L$) with increasing sensor length L and compensating for the additional introduced signal losses ($P_{\text{det}} \propto \exp \alpha_s L$) by increasing the optical signal power of the source. Furthermore, it should be highlighted that the optimum reachable detection limits, regardless of the limiting noise source, do not depend on the waveguide sensitivity (S_{WG}), the transformation constant for homogeneous refractive index changes in the surrounding buffer to effective refractive index changes of the propagating mode. However, the corresponding optimal length still depends on it ($L_{\text{opt}} \propto S_{\text{WG}}^{-1}$). Therefore, it does play an important role for sensor compactness and consequentially for the number of integrated sensors on chip. In addition to the theoretical work, an experimental demonstration for the optimisation of the existing setup was presented reaching down to detection limits of $\sim 1 \times 10^{-8}$ RIU for bulk sensing experiments with the standard verification procedure by injecting different mass percentage concentrations of sodium chloride. This excellent value describes one of the best bulk sensing detection limits reported in literature.

The biosensing performance of the coherently detected Mach-Zehnder interferometer was investigated with C-reactive protein detection in an additive immunoassay (see Chapter 4). C-reactive protein detection is often applied for determining the performance of a biosensor. An offline functionalisation protocol was applied for this purpose. Here a mean detection limit of $\sim 180 \text{ pg mL}^{-1}$ was demonstrated with a high quality external cavity laser. Applying a cheap fibre-coupled handheld fibre test Fabry-Perot laser, bought for $\sim 35\text{€}$ on Amazon, to the same sensing structure and equal detection procedure revealed a worse but still highly acceptable limit of $\sim 1900 \text{ pg mL}^{-1}$. The first limit is, to our knowledge, around an 10 fold improvement to any reported C-reactive protein detection limit in an primary binding immunoassay, i.e. direct detection scheme of the C-reactive protein. The one observed with the Fabry-Perot laser is comparable to the best reported values of around 2 ng mL^{-1} . Considering the entirely different emission spectra of the applied lasers, a factor of around 10^6 in the 3 dB bandwidth, this is still a remarkable result and at least partially proves the theoretical laser noise robustness derived in Chapter 3.

Finally, targeting a real application scenario, the specific detection of IgG and IgE amoxicillin antibodies was demonstrated. Amoxicillin is one of the most widely used penicillins worldwide, and it is estimated that around 10% of the worlds population can be diagnosed to be allergic to it to some extent. Hence, the fast and reliable detection of its antibodies may help medical examiners to make quick decision if either amoxicillin should be given to a patient or not, not only to circumvent possible allergic reactions, such as anaphylaxis, but also to anticipate the mistreatment of penicillin and the subsequent stimulation of super resistant bacteria. For this purpose an offline functionalisation protocol was developed to immobilise amoxicillin haptenised PAMAM G2 dendrimers on the sensing waveguides surface. In an additive primary binding immunoassay both IgG and IgE antibodies could be specifically detected with mean detection limits of around 1 ng mL^{-1} , and best case scenarios down to $\sim 300 \text{ pg mL}^{-1}$ and $\sim 500 \text{ pg mL}^{-1}$ for IgG and IgE, respectively. This demonstrates the high performance which can be obtained for biosensing purposes with a coherently read Mach-Zehnder interferometer based sensor.

Even though excellent detection limits in C-reactive protein and antibody detection are observed, there is still a high deviation in sensitivity and binding curves. This may be a result from mass transfer problems during the functionalisation process and requires further investigation and optimisation. A lab-on-chip or point-of-care device for medical diagnostics requires reproducible results, but in order to demonstrate the capacity of this device, the obtained results still give a valid estimation.

In short, it was demonstrated that sufficiently balanced coherently detected Mach-Zehnder interferometers offer an attractive alternative for future lab-on-chip and point-of-care devices. They can reach excellent detection limits while at the same time an inexpensive read-out system can be applied. The high sensitivity deviation is a problem, but can be addressed by improving the biochemistry.

6.2. Prospect

Based on the conclusions drawn in the previous section, some prospects are presented for further investigation and/or optimisation.

6.2.1. Chip and Read-Out Design

The read-out system, as it is presented in this work, only allows the detection of one sensing structure per experiment. During this work a complete immunoassay experiment, offline functionalisation, installation, immunoassay injections etc., took an entire day. In comparison, the highly multiplexed integrated photonic biosensing system from GENALYTE can read 128 rings quasi-simultaneously in real time. Therefore, one of the most important issues which has to be addressed in the future is the multiplexability of the photonic coherently read Mach-Zehnder interferometers and their adequate detection. Along with these challenges goes the compactness of the sensor in a very space limited chip. Of course, the nominal wavelength region of the optical light source also places a role in this discussion.

6.2.1.1. Sensor Size

The typical sensing structure or window utilised in this work is around $500\text{ }\mu\text{m} \times 500\text{ }\mu\text{m}$, that means that for a balanced sensor, the reference arm occupies almost the same size. Their combined area is therefore at least 0.5 mm^2 which is a great part of almost any photonic integrated chip. There are some design parameters which can be modified to shrink these dimensions.

The optimum length of an interferometric sensor scales, in many cases, with the waveguide sensitivity (S_{WG}), i.e. $L_{\text{opt}} \propto S_{\text{WG}}^{-1}$. By increasing the waveguide sensitivity shorter sensing waveguides can be designed without any performance degradation. Subwavelength gratings are a promising candidate for increasing S_{WG} as they delocalise the mode from the waveguide core. Waveguide sensitivities four times higher than the one used in this design ($S_{\text{WG}} \approx 0.2$) are obtainable which would decrease the required length of the current design ($L = 8.8\text{ mm}$) to 2.2 mm .

However, there is another limiting factor, the bending radius. Imagine the shortest length in the current spiral design with just one round-trip. As the bending radius is $R = 75\text{ }\mu\text{m}$ the minimal occupied dimensions are already $300\text{ }\mu\text{m} \times 225\text{ }\mu\text{m}$ with makes up a significant part of the existing design.

Another factor contributing to the dimensional growth of the spiral after the first round-trip is the half-beat length (L_{π}) of two parallel waveguides with a distance d , centre-to-centre. It determines the coupling strength between two parallel waveguides as described in Chapter 2. That means the higher the coupling length is for a given distance d , the more decoupled the waveguides are and do not optically “see” each other. Waveguide coupling within the sensing structure would result in undesired and unpredictable behaviour in the transmission line and could intervene with the correct phase extraction. Both bending losses and waveguide coupling depend strongly on the wavelength and the photonic technology.

For a recent sensing chip run of our research group, a new kind of circular sensing spiral had been designed following recommendations of [245]. There, a tighter minimum bending radii was chosen. In addition, the waveguide width of the inside and the outside spiral are different by some hundreds of nanometres, phase mismatching their ground modes. This way both, inside and outside spirals, can be placed closer together, i.e. decreasing d , while keeping L_π high.

In short, in order to decrease sensor dimensions and, hence, enable the integration of numerous sensors on chip for multiplexed detection, the three design parameters should be chosen carefully:

- Waveguide sensitivity S_{WG} , the higher the better.
- Bending radius R , the lower the better.
- Waveguide distance d , the lower the better.

6.2.1.2. Detection Scheme

The current detection scheme relies on the fibre array which is used to couple the three optical signals from the gratings of the sensor structure, to guide them to the photodiodes. Multiplexing in this scheme would require a fibre array including many fibres and an large amount of single photodiodes, which is an inadequate solution.

A possibly better solution, which has already been demonstrated in other interferometric sensors, are CMOS cameras or CCD line photodetectors, which give a two-dimensional or one-dimensional intensity signal. The detector would be placed directly above the output grating section. By carefully designing the gratings and their distances to each other, proper regions-of-interest can be defined in the intensity arrays which belong to the radiated beam profile intensities of the individual gratings. This way several sensing structures can be read simultaneously. Spectral Mach-Zehnder Interferometers have been demonstrated to be detected this way, however, requiring an optical demultiplexer to split the transmission spectrum onto different regions-of-interest. It should be highlighted that a certain amount of regions-of-interest, e.g. in [87] it is 30, is necessary in this case for each sensor. A coherently read Mach-Zehnder interferometer only needs three, and, therefore, would potentially enable a higher degree of multiplexing.

Applying CMOS/CCD detectors, the phase extraction would no longer be affected by mechanical noise for any mechanical modulation would only result in an orthogonal amplitude modulation. To amplitude variations, the coherently read Mach-Zehnder interferometer is prone by definition. Furthermore, by substituting the fibre array by free-space propagation, one grating-fibre coupling section is removed, increasing the optical power detected.

Due to the low coupling efficiency of the gratings in this work at least 99% of the induced optical power by the source is lost in these sections. The gratings were optimised for the utilised technology. Changing the technology platform to a one with more design “freedom” could enable the system work with more than 10% of the supplied optical power instead of the now used $< 1\%$.

6.2.2. Fluidic System

If multiplexed detection of various biosensors in real time were enabled, it would make sense to add a second separated fluidic channel above the chip. By doing so, some sensing structures could be used as control and allow a more reliable biomolecule detection. Changing from a pumping to a suction fluidic system would permit the operator to use different buffer mediums without increasing the possibility of introducing undesired bubbles into the channels. It would also make the exposure time of the introduced sample more predictable because diffusion is

reduced. Furthermore, the six-port injection valve would become obsolete, another source of possible bubble contaminations. Less fluidic connections and tube length additionally decreases the required pressure to maintain a certain flow velocity.

The current fluidic channel is sealed onto the chips surface by a hard plastic pressing the PDMS-channel with the help of screws onto the chip. This system is prone to misalignments and often requires several attempts before the right position is found. This unnecessarily increases the time of air exposure of the functionalised surface. Hence, it would be more efficient to design a new fluidic channel which also enables untrained and unskilled personnel to work with this sensor system. Rubber gasket systems were already demonstrated with photonic integrated sensing chips and can be designed to be self-aligning, i.e. no precise alignment by the operator is required because there is only one possible, easy to establish, configuration. The materials for the rubber and gasket system are inexpensive and can be cut by a laser cutter or be printed with a 3D printer for rubber and gasket, respectively.

6.2.3. Thermal Control

In this work, no thermal control of the photonic sensing chip was applied. However, to avoid undesired thermal caused phase shifts, which can hamper a correct phase estimation due to refractive index changes on the waveguides, the temperature of the sensing structures have to be controlled. In this work, the equilibrium of the chips temperature was realised by a sufficient amount of waiting time, such that the temperature was allowed to settle. This is of course an unsatisfactory solution. The future setup should be complemented by a properly designed and controlled temperature control of the entire chip. This can impact the fluidic system and should, therefore, be designed not separately, but in consideration of each other.

6.3. Final Words

Coherently read symmetric Mach-Zehnder interferometers demonstrated exceptional competitive performance. Their compatibility with a simple, inexpensive read-out may pave their way to future lab-on-chip and/or point-of-care devices for which their multiplexability yet has to be demonstrated. Nevertheless, as this work is a first attempt into this direction, quite promising results have been achieved.



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A. Appendix

A.1. Extension for Photonics

A.1.1. Material Equations and Border Conditions

The ∇ -operator is defined as

$$\nabla = \left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z} \right), \quad (\text{A.1})$$

with x, y, z the directions of a three-dimensional space. Therefore, representing the divergence $\nabla \cdot \vec{V}$ and the rotation $\nabla \times \vec{V}$ of a vector $\vec{V} = (x, y, z)$. To get a set of solvable Eqs. (2.1)-(2.4), they have to be completed by additive information about the relationship between $\vec{D}$ and $\vec{E}$, and $\vec{B}$ and $\vec{H}$:

$$\vec{D} = \epsilon \vec{E} = \epsilon_r \epsilon_0 \vec{E} \quad (\text{A.2})$$

$$\vec{B} = \mu \vec{H} = \mu_r \mu_0 \vec{H} \quad (\text{A.3})$$

These two equations are often called material equations. The parameter ϵ_r and μ_r are the dielectric and permeability tensors, respectively, and provide information about the electric and magnetic polarisation ability of the underlying medium. The constant $\epsilon_0 \approx 8.854 \cdot 10^{-12} \text{ F/m}$ and $\mu_0 = 4\pi \cdot 10^{-7} \text{ H/m}$ are the permittivity and permeability of vacuum. Equations (2.1)-(2.4) are solvable within a homogeneous medium. However, it is often required to solve the electromagnetic field distribution along various materials with different ϵ_r, μ_r . For this purpose, different boundary conditions can be directly derived from Maxwell's equations. Applying Gauss divergence theorem on Eqs. (2.3) and (2.4), and Stokes theorem on (2.1) and (2.2), the following continuity conditions may be derived:

$$\vec{n} \cdot (\vec{B}_2 - \vec{B}_1) = 0 \quad (\text{A.4})$$

$$\vec{n} \cdot (\vec{D}_2 - \vec{D}_1) = \sigma \quad (\text{A.5})$$

$$\vec{n} \times (\vec{E}_2 - \vec{E}_1) = 0 \quad (\text{A.6})$$

$$\vec{n} \times (\vec{H}_2 - \vec{H}_1) = \vec{K} \quad (\text{A.7})$$

where $\vec{n}$ is the normal vector from the surface between medium 1 into 2, $\vec{B}_{1,2}$, $\vec{D}_{1,2}$, $\vec{E}_{1,2}$, $\vec{H}_{1,2}$ the corresponding fields in medium 1 and 2 at the surface, σ_{cd} the surface charge density, and $\vec{K}_{\text{cd}}$ the surface current density.

A.1.2. Two-Dimensional Mode Solving for Simple Symmetric Structure

Equation (2.14) in $|x| < d/2$ is a simplified form of the general 1D Helmholtz equation and has therefore a well know real solution

$$H(x) = H_1 \sin(hx) + H_2 \cos(hx). \quad (\text{A.8})$$

Accordingly, the electric field of TE mode m has the following solution,

$$E_{y,m}(x) = \begin{cases} A_m \sin(u_m x) + B_m \cos(u_m x), & |x| < d/2 \\ C_m e^{-v_m |x|}, & x \geq d/2 \\ -D_m e^{-v_m |x|}, & x \leq -d/2 \end{cases} \quad (\text{A.9})$$

with u_m and v_m being defined as

$$u_m = \sqrt{k_0^2 n_c^2 - \beta_m^2}, \quad (\text{A.10})$$

$$v_m = \sqrt{\beta_m^2 - k_0^2 n_s^2}, \quad (\text{A.11})$$

and A_m, B_m, C_m, D_m being the field amplitude constants. An additional mode category may be introduced at this point, even and odd modes, i.e. $m = 1, 3, 5, \dots$ and $m = 0, 2, 4, \dots$, for which $A_m = 0$ or $B_m = 0$, respectively. Considering Eqs. (A.9) and (A.6) the conditions

$$\tan\left(u_m \frac{d}{2}\right) = \frac{v_m}{u_m}, \quad (\text{A.12})$$

$$-\cot\left(u_m \frac{d}{2}\right) = \frac{v_m}{u_m} \quad (\text{A.13})$$

can be derived for even and odd modes, respectively.

A.1.3. Silicon-on-Insulator Technology

One of the most important factors, even before the actual design process, is the choice of an appropriate technology platform, which means what materials are used, what shapes can manufactured, what is the minimum feature size (MFS), i.e. the smallest structure that can be accurately implemented, and what are the expected fabrication errors. In principle, photonic devices can realised in any dielectric platform in which waveguides that are small enough to support a single guided mode can be realised. However, one of the most commonly used platforms for sensing is silicon-on-insulator (SOI), a well established technology for microelectronics. In SOI, silicon (Si) waveguides are defined on top of a silicon dioxide (SiO_2) layer, which in microelectronics is referred to as insulator. The insulator itself is grown on a silicon substrate, which serves as a mechanical support. SOI is compatible with the complementary metal-oxide semiconductor (CMOS) fabrication technology. Nowadays, almost every integrated circuit (IC), e.g. simple logic elements, CPU, GPU, etc. is fabricated in a CMOS platform and its products can be found in every smartphone and computer. This capability to fabricate large amounts of chips is crucial for biosensing, where the chips are a consumable and have to be low cost. A short illustration of the different fabrication steps are shown in Fig. A.1.

1. The insulator material, with a thickness of H_{BOX} , is grown on top of a monocrystalline silicon wafer, the substrate material followed by a silicon layer with a height of H_{WG} .
2. A layer of photoresist is then spin-coated on top of it. A mask, containing the dimensional information of the photonic structures, is then placed directly on top of the resist or is projected via a lens system on the surface. Next, an ultra-violet light source ($\lambda < 380 \text{ nm}$) is used to shine through the mask on the photoresist layer, modifying its molecular structure. This step is called Lithography (shown in Fig. A.1(a)), typically being the limiting factor for the MFS.
3. In following, the modified photoresist is chemically washed away, revealing the silicon layer in form of the negative imprint of the photonic structures (A.1(b)).
4. An etching process, e.g. reactive ion etching, removes the revealed silicon material to a certain depth or completely down to the insulator, whereas the rest is still covered by the resist and is therefore protected and unaffected by the etching process (A.1(c)).

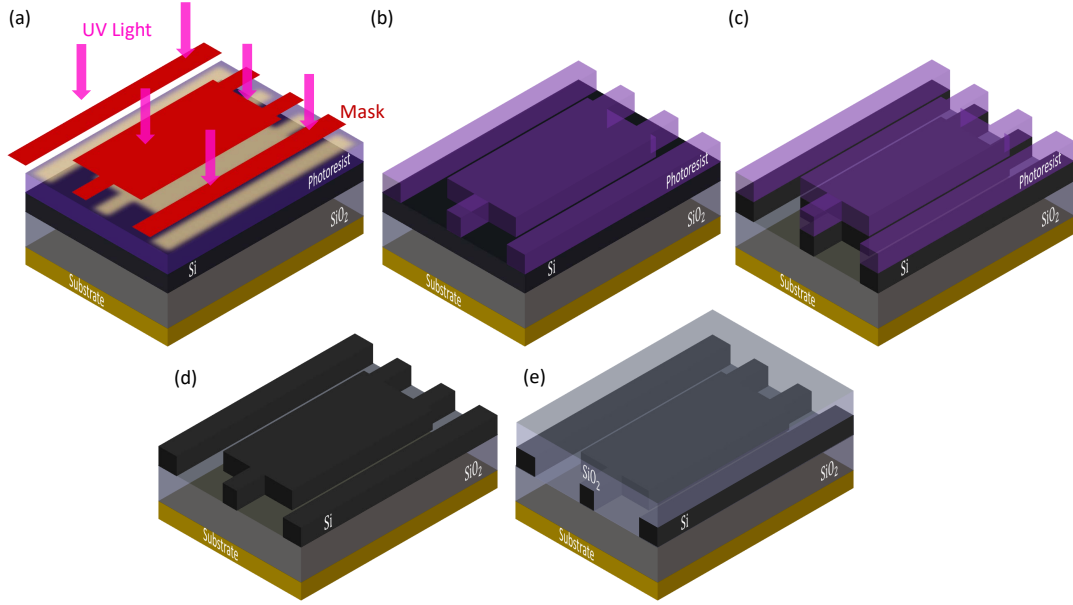


Figure A.1.: Exemplary CMOS fabrication line process. (a) Lithography step with shining UV-light onto the photoresist through the mask, containing the structural information. (b) Washing away the developed photoresist. (c) Etching of the core material. (d) Removal of the remaining photoresist. (e) Deposition of insulator material to bury separate the waveguide structures from the the direct environment. Figure adapted from [246].

5. Figure A.1(d) shows the rinsing of the photoresist, exposing the remaining silicon, the waveguide layer.
6. Typically, another insulator layer is grown on top of the optical guiding layer (see Fig. A.1(e)) serving as protection from mechanical and photonic influences, the latter to suppress modifications of the modal propagation due to changes in the close environment.
7. The steps two to four can be repeated if multiple layers of the guiding material is required. However, additional steps in the fabrication lead to higher manufacturing costs and may introduce a higher probability of chip malfunction.
8. If the modal propagation should be affected by the waveguide close environment, as it is the case in photonic sensing, an extra lithography and etching step is added to expose the waveguides in the area for dedicated sensing.

CMOS fabrication line was invented in 1963 [247] and has been developed over the last decades by the microelectronic industry, lowering the price per chip and MFS ever since. Low-cost by mass fabrication among others makes this technology platform very attractive for photonic engineering. Typically, the entire layout of the chip is much smaller ($A \lesssim 2 \text{ cm} \times 2 \text{ cm}$) than the wafer size ($d \lesssim 40 \text{ cm}$). Depending on the chip dimensions, dozens up to a few hundreds chips can be cut of a wafer. Additionally, core and insulator material can be varied enabling different advantages. Figure A.2 shows the possible core and insulator materials and their light transparency window over wavelength. Photonic telecommunications operates mostly in the near-infrared, often at $\lambda = 1550 \text{ nm}$, and is therefore dominated by silicon on silicon dioxide for their refractive indices are ≈ 3.42 and 1.42 , respectively [250, 251]. Due to their high index contrast, high mode confinement is obtained, consequentially increasing device integration density. While high index contrast helps to integrate several devices, for some situations in sensing it may not that beneficial as high mode confinement can mean lower waveguide sensitivity, i.e. light

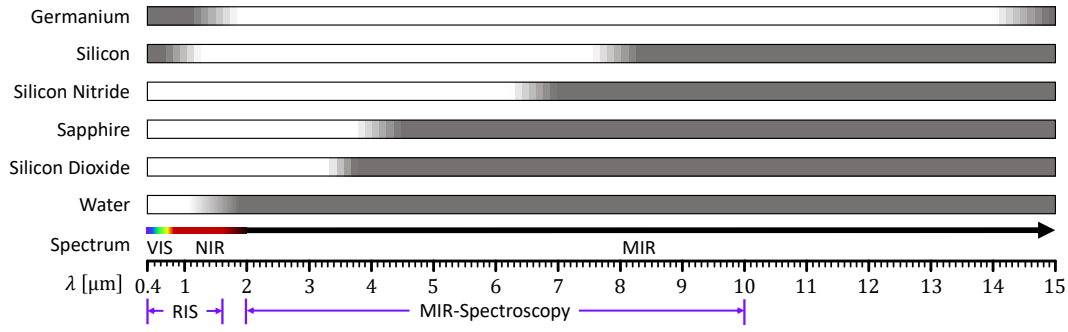


Figure A.2.: Qualitative absorption spectra of CMOS compatible materials and water. Adapted from [248][249].

matter interaction. Hence, other materials such as silicon nitride ($n \approx 2$) are also considered. Apart from index contrast, silicon nitride (SiN) is also transparent down to the whole visible wavelength regime, where silicon is completely opaque. In addition, SiN has low non-linear losses, and a low thermo-optic coefficient in comparison to silicon [252]. Buried waveguides of SiN have shown propagation losses below 1 dB/cm from the visible to below 1.5 dB/cm in the near infrared [105, 106]. Absorption in the environment becomes of interest for the design process as it decrease biosensing performance by degrading the sensors signal-to-noise (SNR). Furthermore, the buffer medium flowing over the sensors is mainly composed of water, which is transparent in the visible but becomes highly absorbent entering the near-infrared. Another argument for silicon nitride on insulator is the "optical window" of proteins at very low red to the close near-infrared regime (600 nm to 1300 nm), in which they almost do not absorb any light, minimising possible damage by the evanescent fields of the propagating modes [253, 254]. In the near infra-red, a high variety of passive and active device can be integrated such as lasers, photodetectors, interferometers, edge and grating couplers, directional couplers splitters, multi-mode interference couplers, multiplexers and demultiplexers, resonators, etc. Silicon and silicon nitride on insulator are the most commonly used platforms for refractive index sensing. For spectroscopy, however, those become less beneficial due to the absorption of silicon dioxide at higher wavelengths ($> 3.4 \mu\text{m}$). Many biochemical molecules have characteristic absorption peaks in the mid-infra-red ($2 \mu\text{m} \rightarrow 8 \mu\text{m}$) which can give information about the observed substance and its concentration. Other CMOS compatible material combinations for this purpose can be silicon on sapphire, germanium on silicon, etc. [248] or the use of suspended waveguides in silicon/germanium-on-insulator [249]. This is however not in the focus of this work.

Because of the way those photonic devices are etched into the wafer, it is difficult to fabricate structures with a geometry that varies in the vertical direction, which limits the possible design and optimisation process. For example, the etching of a round waveguide, like a fibre core on-chip, would be quite challenging, maybe even impossible with the typical CMOS-fabrication line. For this reason different design strategies have been realised by photonic engineers to maximise surface sensitivity for biosensing waveguides.

A.2. Additional Transimpedance Amplifier Information

Table A.1.: The measured (PD 1-3) and specified (Spec.) gain for the different incremental amplifying steps (0 dB $\rightarrow$ 70 dB) in dB(V/A). The real gains for 50 dB $\rightarrow$ 70 dB could not be identified.

PD	0 dB	10 dB	20 dB	30 dB	40 dB	50 dB	60 dB	70 dB
1	29.99	34.94	39.97	44.96	50.82			
2	30.71	35.71	40.74	45.72	50.60			
3	30.46	35.47	40.49	45.47	50.36			
Spec.	31.76	36.77	41.76	46.77	51.76	56.77	61.76	66.77

The measured gains for PD1 differ by factor of $\approx 1/\sqrt{2}$ from the specifications sheet, whereas PD2 and PD3 only differ by a factor of $\approx 4/5$.

A.3. Additional Setup Figures

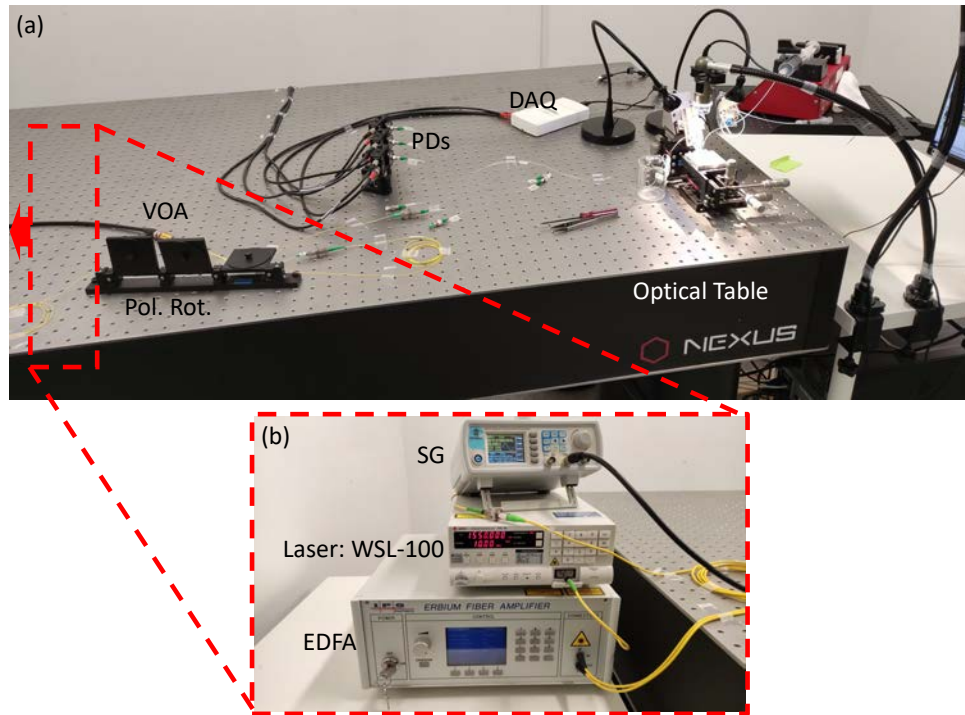


Figure A.3.: (a) The new implemented setup on the optical table to reduce mechanical vibrations of the surrounding. Polarisation rotator (Pol. Rot.), variable optical attenuator (VOA), photodiodes (PDs), and data acquisition (DAQ) are more distributed than in the prior setting. (b) The VOA controlling signal generator (SG), the WSL-100 laser source, and the erbium doped fibre amplifier (EDFA) are placed beside the optical table.

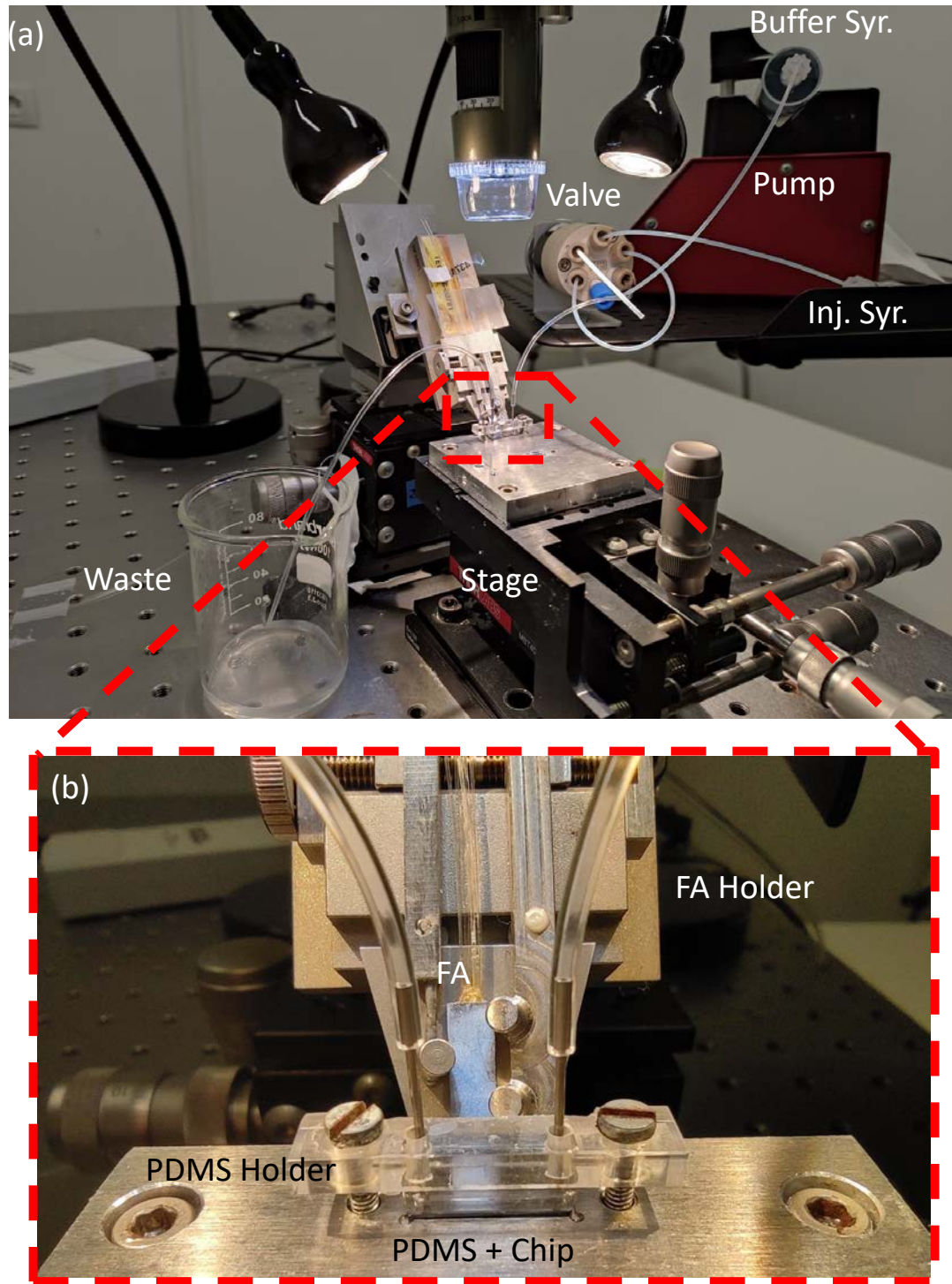


Figure A.4.: (a) The fluidic part of the new implemented setup. The pump, containing the buffer syringe (Buffer Syr.), the injection syringe (Inj. Syr.), and the valve are placed on a laptop holder which is fixed on a separate table to minimise mechanical coupling to the optical table. The waste is placed beside the stage. (b) On the stage the chip, the PDMS, and its holder are placed. The fibre array (FA), stabilised by the FA holder, brings the optical signal to the sensing chip.

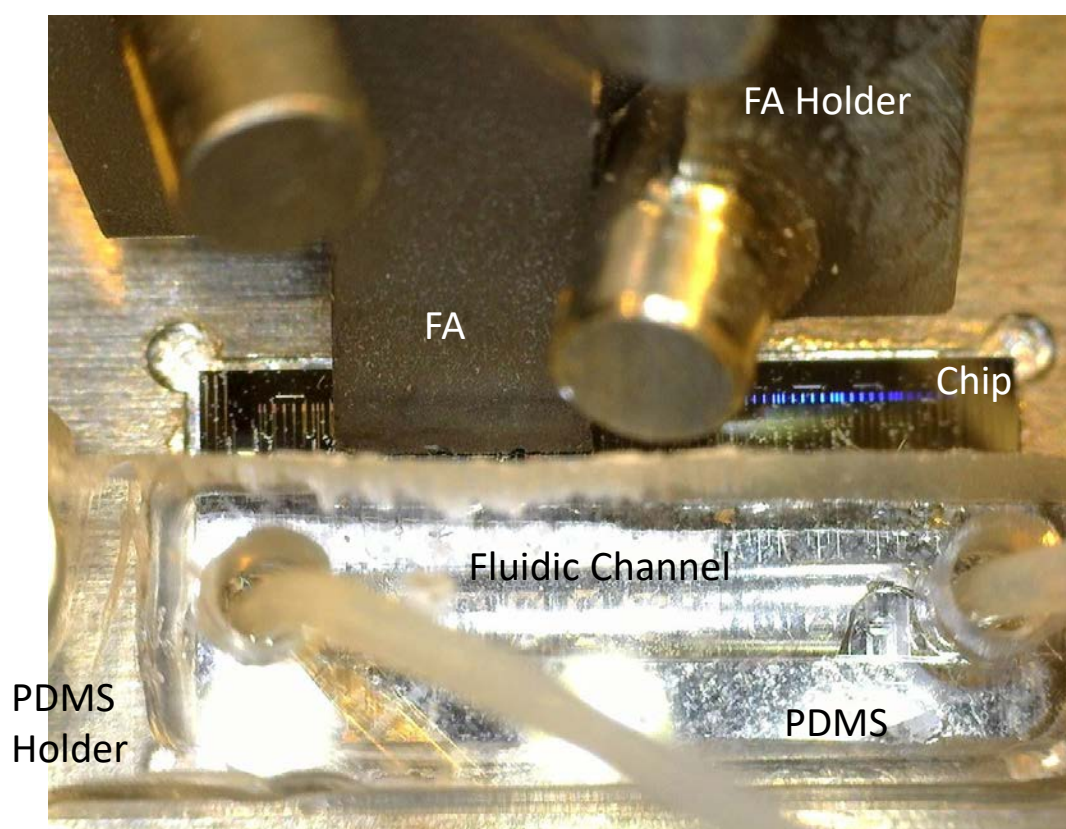


Figure A.5.: Top view of the photonic integrated sensing chip with fluidic and optical supply lines. The plastic holder presses the PDMS onto the chip completing the fluidic channel. The ends are connected via tubes to the valve system and the waste.

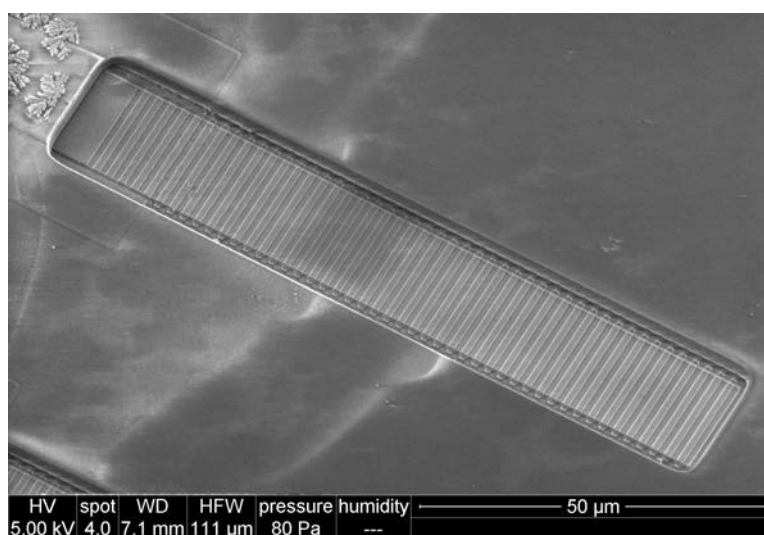


Figure A.6.: SEM picture of on of the gratings for coupling light in- and out of the chip.



Figure A.7.: Photo showing Sergio Postigo performing the mechanical mode analysis of the old setup. The hammer is used to excite the present mechanical modes measured by the acceleration sensors (connected to the white cables).

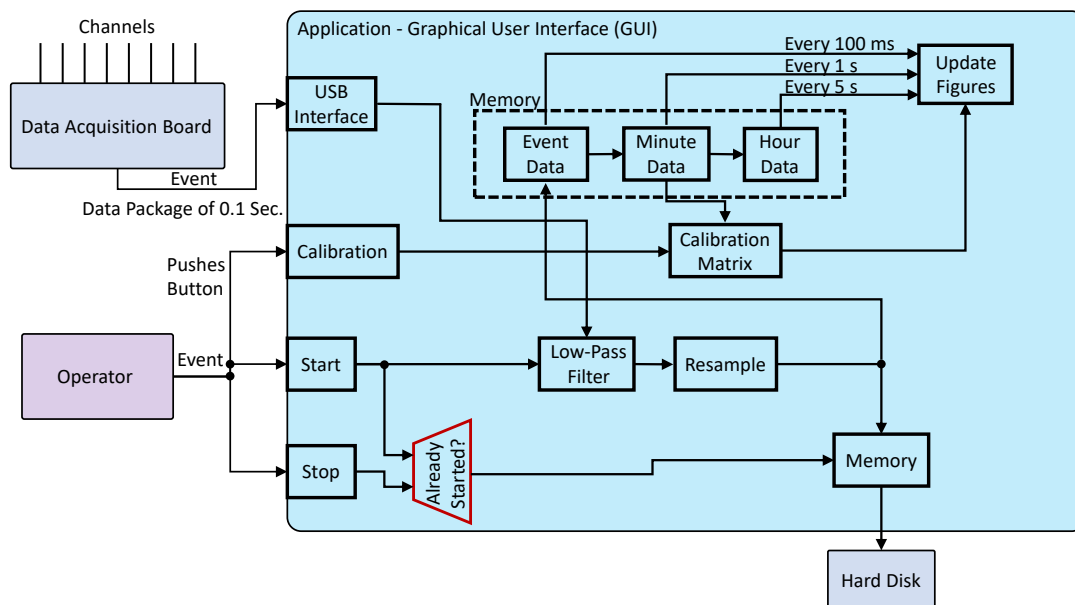


Figure A.8.: Graphical user interface (GUI) functional flow chart.

A.4. Contact Angle Measurement Setup

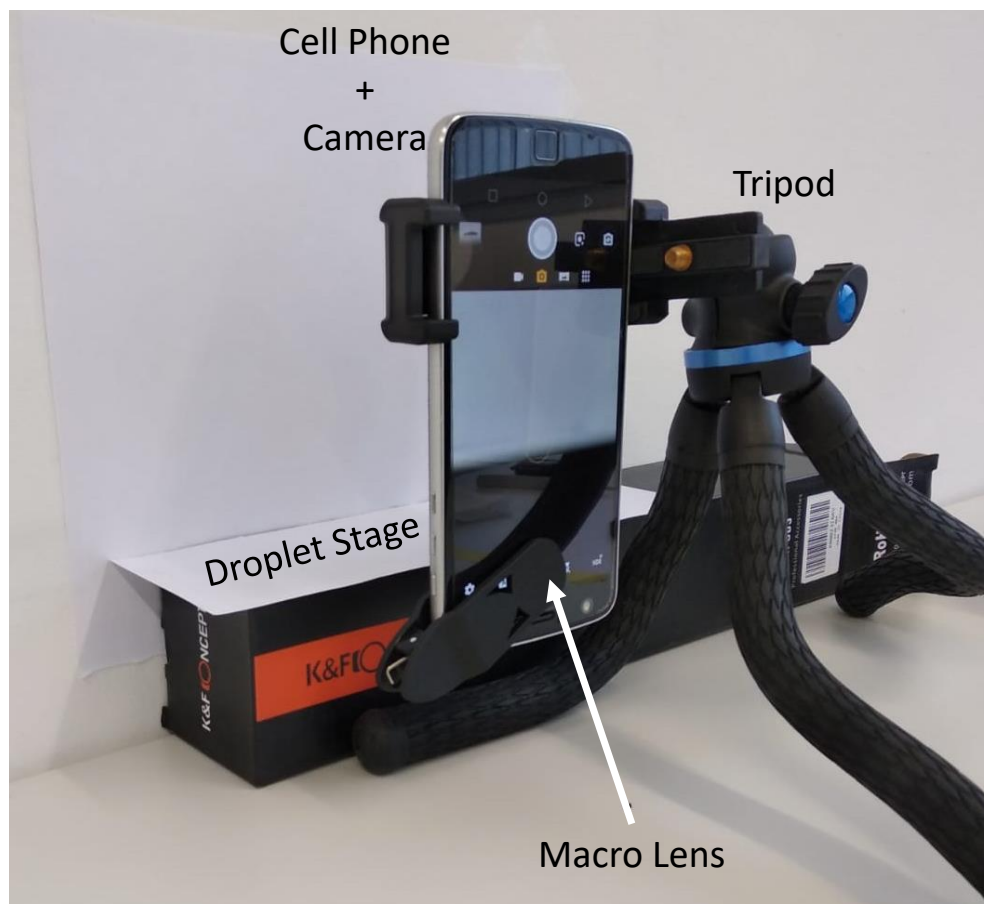


Figure A.9.: Improved contact angle measurement setup. The tripod and the macro lens were bought from amazon for a couple of euros. Due to the SARS-Cov-2 pandemic and the following lockdown, a more precise instrumentation was not available.

A.5. Installation Protocol

1. Install chip into the setup, by placing the PDMS channel on top of it, such that the fluidic channel is created right above the exposed sensor spirals. Fix the position of PDMS and chip with locating the plastic piece above and Tightening the screws. Connect the tubes for the biochemical waste and the pump-injection system. Establish a buffer, PBS 10 mM (pH 7.4), flow with a velocity of $F = 20 \mu\text{L min}^{-1}$.
2. Start laser, emitting at centre wavelength $\lambda_0 = 1.55 \mu\text{m}$ with power $P_L = 10 \text{ dBm}$.
3. Amplify the optical signal via the EDFA to around $P_0 = 20 \text{ dBm}$.
4. Set the VOA electrical input signal to $V_{dc} = 2.5 \text{ V}$
5. Set TIA gain to the 30 dB setting.
6. Start the GUI, for pre-processing and recording the data, with a digital low-pass filter, having a bandwidth $B = 0.8 \text{ kHz}$ and resampling factor of $r_s = 50$.
7. Establish connection to the DAQ with the following settings: $V_{DAQ} = 10 \text{ V}$, $K = 3$ from which follows $f_s = 83.3 \text{ kHz}$
8. Locate FA over the grating section, establish the interferometric signal in each channel, and maximise it by trying to find the best possible physical position, giving the maximum signal for all channels. For mechanical noise influence reduction the head has to touch the surface with sufficient friction, minimizing relative movements of the coupling modes.
9. Adjust polarisation to TE, which is found by the maximum resulting detected optical power in all three channels.
10. Inject a probe of sodium chloride (3‰), for HRI experiments, or PBS 20 → 100 mM (pH 7.4), for CRP experiments, to see the interferometric signal range for all three channels. If necessary adjust the detected signal by reducing (or increasing) the optical power with the VOA (in- or decreasing V_{dc} , respectively), or modifying the TIA Gain. The optimal parameters should be set in a way such that the maximum electrical interferometric signals at the DAQ input go almost up the V_{DAQ} , maximising the cSNR. Assuming QN as the most contributing noise source, the gain of the TIA should be preferably increased instead of a higher EDFA amplification, for keeping shot noise level constant.

B. Resumen en Español

Un biosensor es un “dispositivo o unidad analítica compacta que incorpora un elemento de reconocimiento biológico asociado a un transductor físico-químico” [1]. Esta definición ya indica que el biosensado es un tema complejo, que combina dos campos de investigación diferentes: la bioquímica y la ingeniería. En esta tesis pretendemos desarrollar un sistema óptico de biosensores basado en chips fotónicos de silicio para el nanodiagnóstico. Así, nuestro trabajo incluirá tanto la fotónica integrada como la bioquímica, que son campos muy diversos por sí mismos.

B.1. Introducción

La fotónica utiliza materiales dieléctricos, por ejemplo una fibra óptica, para guiar la luz mediante la reflexión interna total (TIR) a lo largo de un amplio rango de distancias, desde milímetros hasta miles de kilómetros. La tecnología fabricación de semiconductores CMOS, establecida por la industria microelectrónica a principios de los años sesenta, permitió la producción de circuitos eléctricos integrados miniaturizados. El material que subyace a esta revolución tecnológica es uno de los minerales más comunes en la tierra: el silicio. Tanto el silicio como su óxido nativo, el dióxido de silicio (cuarzo), son transparentes en el infrarrojo cercano. Esto dió origen al campo de la fotónica del silicio, que estudia la integración de fuentes de luz, guías de ondas y detectores basados en el silicio. El alto contraste de índice entre el material del núcleo de la guía de ondas (silicio) y el revestimiento (óxido de silicio) permite una alta densidad de dispositivos. De esta manera se pueden integrar muchas funcionalidades diferentes en un solo chip. El nitruro de silicio, transparente desde el infrarrojo cercano al visible, también es compatible con la fabricación CMOS y se utiliza como material del núcleo en esta tesis. De hecho, la fotónica de silicio es un candidato prometedor para aplicaciones de biosensores y se espera que experimente un rápido crecimiento económico en la próxima década [7].

B.1.1. Diagnóstico mediante Nanomedicina

El diagnóstico es el primer paso hacia el tratamiento de una enfermedad. La nanomedicina aplica herramientas de tamaño nanométrico para conocer, diagnosticar y tratar enfermedades [10]. Hoy en día, las pruebas de diagnóstico médico se realizan predominantemente en laboratorios dedicados. La mayoría de los procedimientos de diagnóstico se basan en la detección de un biomarcador en un fluido biológico corporal [11]. Los biomarcadores son indicadores específicos de la enfermedad que permiten conocer el origen de la misma [12]. Ejemplos de biomarcadores son antígenos, anticuerpos, ADN, ARNm y enzimas [13].

Una prueba de diagnóstico común es el ensayo inmunoenzimático (ELISA). ELISA es un esquema de reconocimiento indirecto de analitos basado en “etiquetas”: la presencia del biomarcador objetivo no se detecta directamente, sino en relación con una etiqueta enzimática. El proceso típico de un ensayo ELISA en sándwich [16] se muestra en la Fig. B.1(a). Otra herramienta de nanodiagnóstico muy extendida para una detección basada en marcadores de ADN es el método de reacción en cadena de la polimerasa (PCR), ilustrado esquemáticamente en la Fig. B.1(b). El analito puede ser cualquier fragmento específico de ADN de una variedad de diferentes muestras [20]. El principio básico es la replicación repetida de un fragmento de ADN

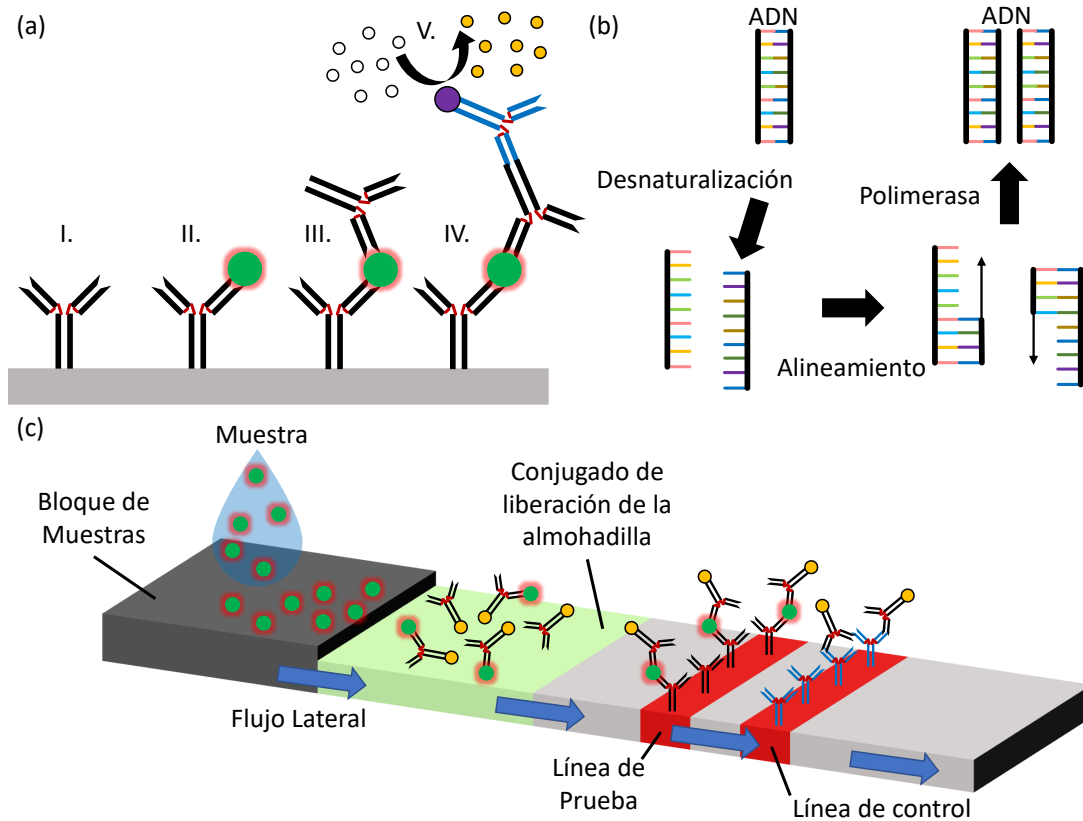


Figure B.1.: (a) Diferentes pasos de un sándwich ELISA. (b) Los pasos repetitivos básicos para el PCR. (c) Principio de funcionamiento de una prueba de inmunoensayo de flujo lateral.

antes de la detección. Normalmente, tanto los ensayos ELISAs como los PCRs son muy sensibles, específicos y pueden ser diseñados para tener un alto rendimiento. El problema con ellos es que requieren mucho tiempo, personal experimentado y un entorno controlado. Además, la mayoría de ellos sólo ofrecen resultados cualitativos o semi-cuantitativos, y el precio por prueba es bastante alto.

La mayoría de esos inconvenientes se abordan con los llamados dispositivos de punto de atención (POC). La idea es tener un dispositivo que pueda ser usado directamente en la atención primaria. Eso significa diagnosticar en el lugar donde se encuentra el paciente y usando un dispositivo económico, rápido, sin etiquetado y específico que proporcione resultados cuantitativos [22]. Un ejemplo popular de una solución de POC es la comúnmente conocida prueba de embarazo [25]. Es una prueba de ensayo de flujo lateral (LFA), que produce un resultado cualitativo en minutos sin necesidad de un voluminoso equipo de control [26]. El principio de funcionamiento se muestra en la Fig. B.1(c).

Sin embargo, casi todos los métodos de detección mencionados anteriormente presentan algunos de los siguientes problemas: requieren etiquetado, tienen que llevarse a cabo en laboratorios, requieren mucho tiempo, requieren personal capacitado, son costosos, no son lo suficientemente flexibles en cuanto al analito objetivo, presentan un rendimiento reducido u ofrecen sólo resultados cualitativos o semicuantitativos. Una novedad interesante son los biosensores sin etiqueta, que se examinarán en la próxima sección.

B.1.2. Biosensores ópticas sin etiquetas

Un biosensor sin etiqueta detecta directamente el analito objetivo sin necesidad de etiquetar las moléculas [30]. Típicamente, estos biosensores pueden ser subdivididos en tres partes principales: el elemento o capa de biorreconocimiento, el transductor y la lectura (Read-Out) [29], mostrados en la Fig. B.2: El principio de funcionamiento es el siguiente. Una muestra, que posi-

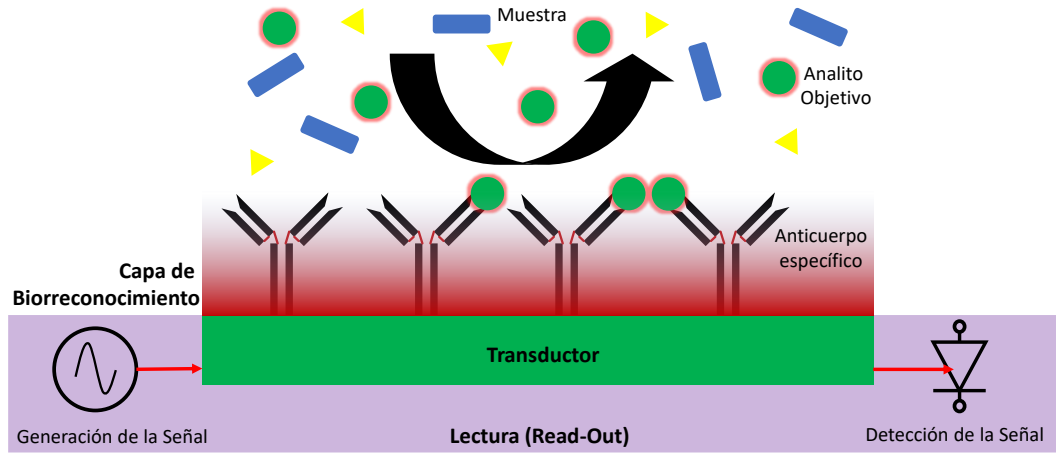


Figure B.2.: Principio fundamental de funcionamiento del biosensor, incluyendo la capa de biorreconocimiento, el transductor y la lectura.

blemente contenga el analito objetivo, se acerca a la superficie del sensor. En esta superficie hay una capa del elemento de biorreconocimiento. La función del elemento de biorreconocimiento es adherirse específicamente al analito. Si la muestra contiene este objetivo, existe la posibilidad de que este se adhiera a esta capa. Este proceso de adhesión causa un cambio de un parámetro físico del transductor. El transductor es normalmente un cierto material en una disposición más compleja, en este trabajo una guía de ondas ópticas. La lectura aplica una señal óptica a la guía de onda y registra su respuesta en tiempo real, por ejemplo, en forma de un cambio de frecuencia o intensidad de resonancia. La alteración del transductor debido al biorreconocimiento produce un cambio en la respuesta detectada por la lectura. La variación de esta respuesta a lo largo del tiempo da lugar a una estimación cuantitativa de la concentración del analito reconocido.

Una de las características más importantes para un biosensor es el límite de detección (LOD), definido como

$$\text{LOD} = \frac{3\sigma}{S}, \quad (\text{B.1})$$

y que indica la menor cantidad de cambio de parámetros físicos que puede ser detectada con precisión. El parámetro σ es el ruido de la señal detectada, y S la sensibilidad de la señal al cambio de los parámetros físicos. El LOD es uno de los pocos parámetros que pueden ser fácilmente comparados entre los diferentes tipos de sensores. En general, el objetivo de cualquier diseño de un sistema de biosensores es minimizar el LOD.

Existen muchos biosensores basados en guías de ondas fotónicas integradas, pero pueden clasificarse principalmente en sensores resonantes e interferométricos. Los primeros utilizan típicamente una fuente de luz de longitud de onda sintonizable, y por tanto costosa, y monitorizan la intensidad de la señal en función de la longitud de onda aplicada. Los máximos o mínimos en la transmisión espectral son las longitudes de onda de resonancia. Los cambios del índice de refracción del entorno de la guía de onda, causados por el biorreconocimiento, alteran las posiciones espectrales de estas resonancias. Ejemplos de sensores basados en resonancias son anillos o cristales fotónicos. Sus límites de detección están típicamente alrededor de 10^{-6} RIU.

Los sensores interferométricos utilizan una fuente de luz de longitud de onda fija donde la luz del brazo de sensado se compara con la luz del brazo de referencia. Juntos crean una señal de interferencia constructiva o destructiva. Las alteraciones del índice de refracción de la guía de ondas sensora modifican esta señal. Ejemplos típicos son los interferómetros de Mach-Zehnder (MZIs), los interferómetros espectrales, los interferómetros bimodales y los interferómetros de Young. La mayoría de ellos tienen desventajas como el desvanecimiento de la sensibilidad o la ambigüedad de la señal. Para evitar estos problemas, necesitan añadir una modulación o un elemento de filtrado óptico, complicando así la lectura. Su LOD normalmente baja a 10^{-7} RIU.

Un problema que tienen en común la mayoría de los esquemas de detección mencionados se relaciona con el sistema de lectura, es decir, la fuente de luz y la detección de señales. O bien utilizan un costoso láser de alta calidad y un elemento de detección muy sencillo, por ejemplo un simple fotodiodo, o bien un láser más barato y un esquema de detección más sofisticado, empeorando normalmente el límite de detección. Los dispositivos de punto de atención requieren una realización sencilla y barata, por lo que sería ventajoso disponer de un óptico de bajo coste con un esquema de detección pequeño y sencillo.

B.1.3. Contribución de este trabajo

El objetivo principal de esta tesis es demostrar la aplicabilidad de los interferómetros de Mach-Zehnder detectados coherentemente (CMZI) para fines de biosensado sin etiqueta en el contexto de los dispositivos orientados a los puntos de atención. Los CMZIs usan un esquema de detección coherente para reconstruir una señal interferométrica de valor complejo. La prueba de concepto de este enfoque se demostró por primera vez en [98].

Teniendo en cuenta que una primera generación de chips ya estaba disponible cuando se inició la tesis, nos centraremos en tres subobjetivos:

1. Desarrollar y optimizar la microfluídica y el sistema de lectura. Basándose en el sistema de lectura experimental, se presentará una evaluación teórica del límite de detección alcanzable. Esta contribución ha sido publicada en la revista [99]. Utilizando esta información, se desarrolla un procedimiento experimental de optimización del LOD y se demuestran excelentes límites de detección de hasta casi 1×10^{-8} RIU. Esta contribución está publicada en la revista [100].
2. Demostrar la capacidad de biosensado. La superficie de la guía de ondas de detección es tratada bioquímicamente para lograr el reconocimiento específico de la proteína C-reactiva. En un inmunoensayo aditivo, se obtuvieron muy buenos límites de detección de alrededor de 180 pg mL^{-1} . Además, para demostrar la capacidad de los CMZIs para operar con fuentes ópticas baratas, una opción preferible para los dispositivos de POC, se introdujo un láser de prueba de fibra Fabry-Perot de mano muy barato en el sistema de detección y se consiguieron unos límites de detección extraordinarios de 1.8 ng mL^{-1} . Estos resultados están publicados en la revista [101].
3. Proporcionar una prueba de concepto para el diagnóstico de la alergia. Procediendo a un entorno más basado en la aplicación, el sensor se utiliza para la detección de anticuerpos de amoxicilina IgG e IgE. La presencia de estos en una muestra de paciente puede indicar una posible alergia a la amoxicilina, una penicilina para el tratamiento de enfermedades infecciosas bacterianas. Para ello, la superficie de detección se preparó bioquímicamente con dendrímeros de segunda generación de poli(amidoamina) (PAMAM) haptenecidos con amoxicilina para ser particularmente selectivos con esos anticuerpos. Se alcanzaron límites de detección de alrededor de 1 ng mL^{-1} para los anticuerpos de tipo IgG e IgE.

B.2. El biosensor interferómetro Mach-Zehnder con detección coherente

Los biosensores interferométricos de Mach-Zehnder convencionales utilizan un elemento recombinante simple, como una unión en Y o un MMI 1×2 . Esto da como resultado una señal interferométrica que sufre de ambigüedad direccional y desvanecimiento de sensibilidad. Estos problemas se evitan leyendo coherentemente el MZI al permitir la reconstrucción de la señal interferométrica compleja.

B.2.1. Diseño

Los interferómetros de Mach-Zehnder con una sola salida no pueden reconstruir la señal interferométrica de valor complejo. Tienen que calcular el desplazamiento de fase $\Delta\varphi$ entre el brazo de referencia y el brazo sensor directamente de la señal interferométrica recibida, es decir $\sin(\Delta\varphi)$. Esta configuración tiene dos grandes inconvenientes: la ambigüedad direccional, al no saber en qué dirección cambia el desplazamiento de fase, y el desvanecimiento de la sensibilidad: desplazamientos de fase cercanos a $m\pi$, $m \in \mathbb{Z}$ resultan en un cambio casi nulo de la intensidad detectada. Muchos biosensores basados en el interferómetro de Mach-Zehnder aplican una modulación al brazo de referencia o a la longitud de onda de la fuente óptica [138] para evitar ambos problemas. Sin embargo, esto complica y encarece el sistema de sensado.

En lugar de una simple recombinación de la unión Y o de un MMI 1×2 , un MMI 1×2 puede ser usado como estructura de recombinación (mostrado en la Fig. B.3). La luz del brazo de

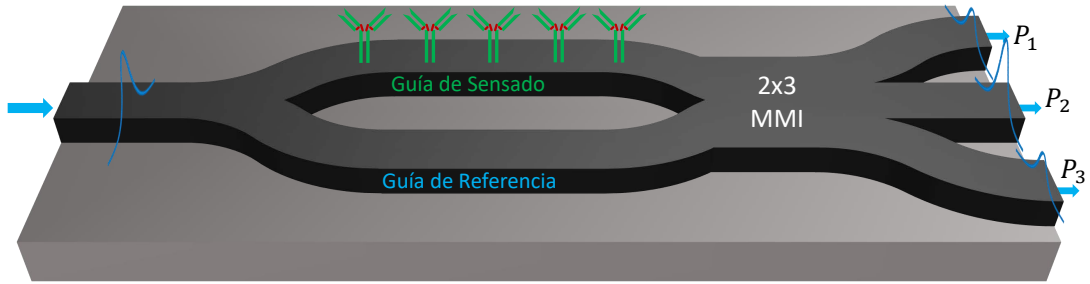


Figure B.3.: Interferómetro Mach-Zehnder de lectura coherente realizado con un 2x3 MMI.

referencia y de detección crea tres señales interferométricas desplazadas en 120° [119]. Estas pueden ser usadas para reconstruir la señal interferométrica compleja entre la guía de onda de referencia y la de detección [99]. El cambio de fase en el brazo de sensado resulta idealmente en un giro alrededor del círculo unidad $\exp(j\Delta\varphi)$ en plano complejo. De esta manera, el cambio de fase puede ser extraído directamente sin ningún desvanecimiento de la sensibilidad o ambigüedad direccional. No se requieren pasos adicionales durante la fabricación, ni tampoco materiales adicionales. Además, no se necesita ningún esquema de modulación para extraer el desfase sin desvanecimiento de sensibilidad o ambigüedad direccional. Esto es ventajoso para las soluciones de punto de atención. Sin embargo, el número de salidas ópticas aumenta a tres.

B.2.2. Análisis del nivel del sistema

La descripción a nivel de sistema de la configuración de biosensado implementada se muestra en la Fig. B.4. Contiene todos los componentes necesarios y muestra los puntos de inserción de las fuentes de incertidumbre de la señal. En este trabajo se consideraron tres clases diferentes de ruido: el ruido del detector eléctrico, el ruido de la fuente óptica y el ruido mecánico. La

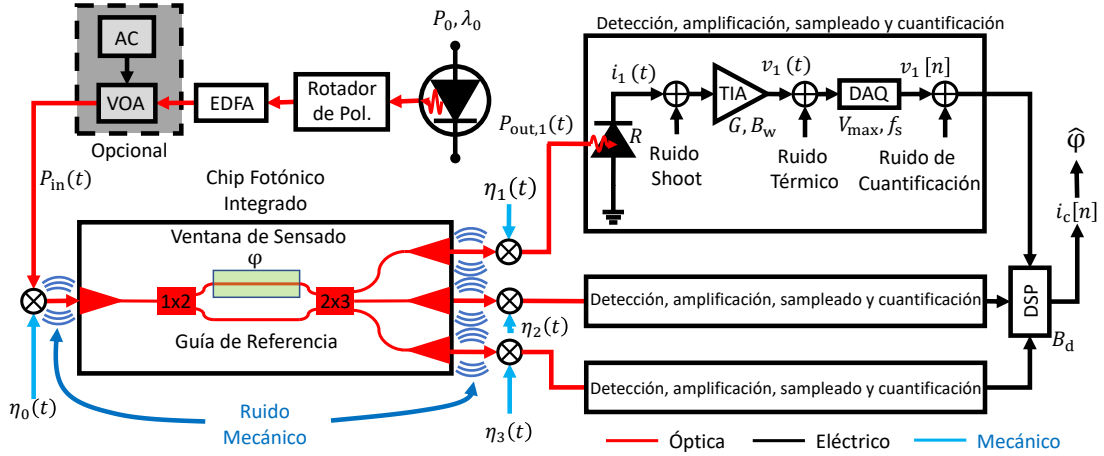


Figure B.4.: Representación a nivel de sistema de toda la configuración, incluyendo los puntos de impacto de las diversas fuentes de ruido, es decir, ruido láser, mecánico, shot, térmico, de cuantificación y de banda base.

primera incluye ruido térmico, shot, de cuantificación y de banda base. La segunda incluye ruido de intensidad relativa y ruido de fase de láser. La última considera las vibraciones mecánicas causadas por los movimientos del conjunto de fibras que dan lugar a ruido detectado en los fotodiodos.

El ruido mecánico es, con mucho, el peor ruido de este sistema, pero puede ser suprimido fijando mecánicamente las secciones de acoplamiento. Se puede demostrar que los CMZIs son, por definición, inmunes al ruido de intensidad relativa de la fuente óptica. Además, el efecto del ruido de fase del láser puede ser minimizado al equilibrar los brazos de referencia y de sensado [99]. El ruido de banda base también puede ser evitado añadiendo una modulación de la intensidad de la luz fuera del chip. En este trabajo, la influencia del ruido térmico y shot es muy pequeña. Eso deja al ruido de cuantificación como la última fuente de incertidumbre limitante. Para ese caso, se puede derivar una expresión concisa para el límite de detección:

$$\text{LOD} \propto \frac{\Upsilon_{\text{QN}} \Delta V_{\text{qs}} \sqrt{\frac{2B}{f_s}} e^{\alpha_s L}}{G P_0 L}, \quad (\text{B.2})$$

con Υ_{QN} el factor de calidad de la placa de adquisición de datos, ΔV_{qs} la resolución de voltaje, B el ancho de banda de la señal, f_s la frecuencia de muestreo, G la ganancia del amplificador de transimpedancia, y P_0 la potencia de la fuente óptica. El último término de la ecuación determina la longitud óptima del sensor a $L = 1/\alpha_s$ con α_s la constante de atenuación del modo la guía de onda de sensado. Del mismo modo, para los otros orígenes del ruido, se pueden derivar expresiones concisas que ayuden a optimizar el límite de detección.

B.2.3. Optimización experimental

Basándose en los conocimientos teóricos adquiridos, se llevó a cabo una optimización práctica de los límites de detección [100]. La figura B.5 muestra el proceso de optimización a partir de las condiciones de partida. El límite de detección inicial determinado para esta situación es $\text{LOD} = 3 \times 10^{-5}$ RIU. Se realizaron los siguientes pasos para minimizar el LOD:

- I. Incrementar la frecuencia de muestreo al máximo posible $f_s = 83.3 \text{ kHz}$ revela la verdadera naturaleza del ruido. La señal detectada inicialmente experimentó aliasing, es decir, la señal estaba submuestreada. Se hace evidente que para frecuencias más altas la densidad

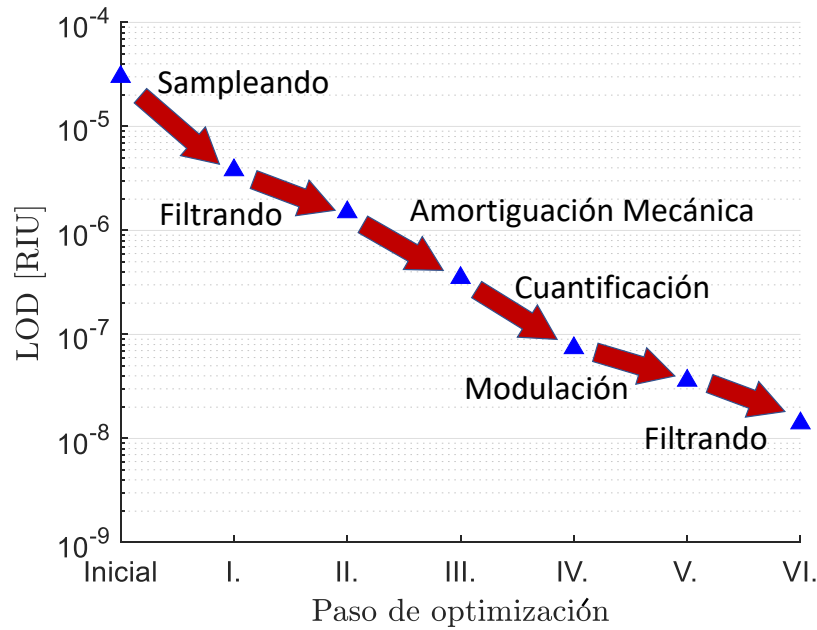


Figure B.5.: El procedimiento de optimización aplicado muestra los diferentes límites de detección para cada paso. Se pueden alcanzar límites de detección excepcionales para los CMZIs con un manejo adecuado del ruido.

- del espectro de potencia converge en un nivel de ruido blanco. Este paso resulta en un $\text{LOD} = 3.8 \times 10^{-6}$ RIU.
- II. Disminuyendo el ancho de banda del filtro paso bajo B se mejora el SNR al eliminar parte del ruido. Escoger un ancho de banda más pequeño $B = 2$ Hz también elimina parte del ruido mecánico. Esto mejora el LOD a 1.5×10^{-6} RIU.
 - III. Hasta el momento, el conjunto de fibras se colocaba por encima de las rejillas de difracción, moviéndose libremente, sin ningún contacto con la superficie. Dejar que el conjunto de fibras toque la superficie amortigua el ruido mecánico. De esta manera se obtuvo un $\text{LOD} = 3.5 \times 10^{-7}$ RIU.
 - IV. El ruido de banda base, que se supone que es casi independiente de la potencia de entrada, puede limitar nuestro límite de detección, sin embargo, aún podría mejorarse mejorando la señal detectada sin aumentar significativamente las otras fuentes de ruido de los detectores. Esta estrategia de mejora es muy básica y puede reducirse a la simple afirmación de "Amplificar la señal detectada para utilizar todo el rango del sistema de detección". La mejora observada del LOD resultó en un LOD de 7.4×10^{-8} RIU.
 - V. Para eliminar las influencias del ruido residual de la banda base, se estableció un esquema de modulación de la potencia óptica. El ruido de banda base no depende de la señal y ocupa la banda de frecuencias más baja. De esta manera se alcanzó un límite de detección de 3.6×10^{-8} RIU.
 - VI. Finalmente, una agresiva reducción del tiempo de integración de la señal de $0.5 \rightarrow 5$ s, resulta en un valor del $\text{LOD} = 1.4 \times 10^{-8}$ RIU.

Se trata de un límite de detección extraordinariamente bajo en comparación con la mayoría de los sensores fotónicos. Sólo otro sensor, también CMZI, demostró un LOD casi tan bueno como éste [147]. Esto refuerza la afirmación de que los CMZIs son capaces alcanzar límites de

detección ultra bajos.

B.3. Detección de la proteína C-reactiva

El trabajo descrito en esta sección tiene dos objetivos. Por un lado, demostrar, por primera vez, la capacidad de detectar biomoléculas del interferómetro Mach-Zehnder con lectura coherente. Por otro, demostrar la capacidad del sistema de operar con una fuente de luz muy barata.

Un alto porcentaje del coste de un dispositivo fotónico de POC resulta de la lectura utilizada, es decir, de la fuente de luz y del sistema de detección. El precio de un láser típico de cavidad externa sintonizable (ECL), que se requiere, por ejemplo, para los sensores basados en anillo, ronda las decenas de miles de Euros. Por otro lado, los láseres Fabry-Perot son de los más simples y baratos disponibles, y se pueden adquirir por unas decenas de euros. Según el conocimiento del autor, hasta la publicación resultante de esta tesis [101], ningún biosensor ha sido operado con una fuente óptica de este tipo.

La biomolécula utilizada para la detección es la proteína C-reactiva (CRP). Es una proteína que se encuentra comúnmente en la sangre o el suero de un paciente. Su concentración está correlacionada con el nivel de inflamación, a menudo desencadenada por lesiones e infecciones de los tejidos. Para la biosensores se utiliza a menudo como una prueba referencia de prestaciones. El estado de la técnica en las Tablas 2.1 y 2.2 enumeran algunos límites de detección que se alcanzaron con este método.

B.3.1. Procedimiento de Funcionalización

El procedimiento general de preparación de un chip para la detección específica de CRP se indica a continuación:

1. Oxidar el chip en solución piraña durante varios minutos. Esto no sólo limpia la superficie, sino que también añade grupos OH a la superficie de nitruro de silicio.
2. Añadir un grupo organofuncional a la superficie, es decir, una terminación química a la que puede adherirse una biomolécula. Este es un paso importante del proceso de funcionalización. En el caso de los metales y vidrios oxidados, esto puede realizarse mediante la sinalización. Aquí, se aplicó el (3-aminopropil)-tri(metoxi)-silano (APTMS), creando un enlace covalente con el grupo hidroxilo en la superficie. El grupo amino resultante (NH_2) terminado en la superficie está entonces listo para unirse a componentes orgánicos.
3. Aquí se utiliza un agente enlazador bifuncional (bis (sulfosuccinimidilo) suberato (BS3)) para establecer una conexión entre el silano y el anticuerpo que se añade en el siguiente paso. BS3 es un enlazador amino-amino y es una opción preferida porque las moléculas aminas están a menudo disponibles en las proteínas.
4. El anticuerpo anti-CRP (ACRP) utilizado para la funcionalización de la superficie es un anticuerpo IgG para la proteína CRP. Hay muchos grupos amino en un anticuerpo IgG con los que la molécula de enlace podría unirse. En promedio, las moléculas de IgG tienen alrededor de 80 grupos de aminoácidos libres de los cuales 20 están presentes en sitios accesibles a los solventes.
5. Luego se instala el chip funcionalizado en el sistema de lectura, estableciendo un flujo constante de solución salina (PBS) con un valor de pH de 7,4 sobre la estructura de detección.
6. Se inyecta una solución bloqueadora de albúmina de suero bovino (10 mg mL^{-1}) para blo-

quear la superficie que no está suficientemente funcionalizada con el ACRP. Esto suprime la unión inespecífica, es decir, la unión que no mediada por ACRP.

B.3.2. Resultados experimentales

Para los siguientes experimentos de detección se usaron dos tipos de láseres: un láser de cavidad externa costoso y un láser Fabry-Perot barato. Para asegurar la comparabilidad, estos experimentos se realizaron de la misma manera. Más precisamente, esto significa establecer la misma potencia de señal en el PD (≈ -10 dBm). El siguiente inmunoensayo aditivo fue ejecutado por triplicado para cada láser.

Se inyectaron consecutivamente concentraciones de CRP de 10, 50, 200, 500, 1000, 2000, 4000 ng mL⁻¹. Todas las inyecciones de CRP introdujeron un cambio de fase permanente después de 8 → 10 min. Luego se calcularon las concentraciones acumuladas inyectadas y los cambios de fase, de los cuales se extrajeron las sensibilidades (S) a través de un ajuste no lineal para los tres experimentos. La media (puntos) y la desviación estándar (barra de error) de los desplazamientos de fase acumulados para los tres experimentos por tipo de láser se muestran en la Fig. B.6. Los datos mostrados en azul representan los experimentos con el ECL, mientras

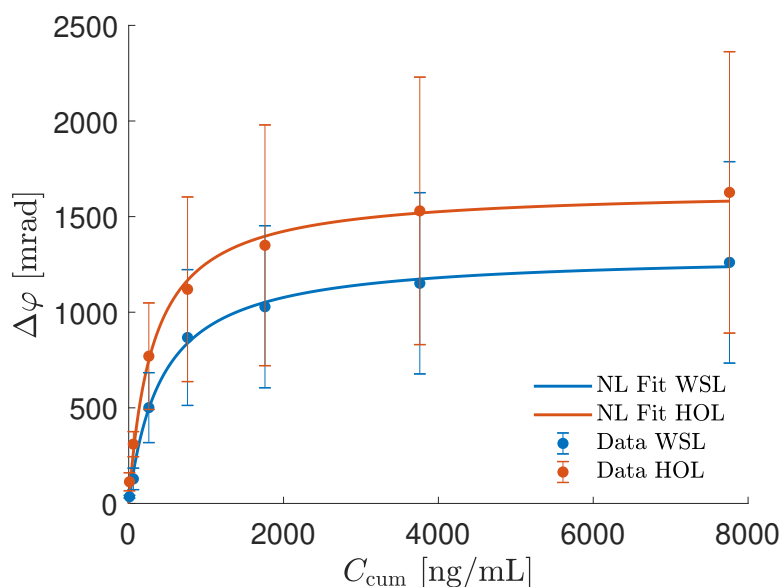


Figure B.6.: Calibraciones de CRP de la prueba de inmunidad aditiva para ambas pruebas, ECL (naranja) y FPL (azul). Los puntos representan la media y las barras de error la desviación estándar del punto de datos acumulativos correspondiente. Las líneas son el ajuste no lineal a través de los datos medios.

que los datos en naranja representan los experimentos con el FPL. Los gráficos continuos muestran los correspondientes ajustes no lineales. Las sensibilidades medias y de ajuste, el ruido y los valores medios de los experimentos triples se muestran en la tabla B.1. Las sensibilidades están todas en el mismo orden de magnitud. Sus medias difieren por un factor de 16. Esto es más alto de lo esperado. Sin embargo, la situación ambiental del sensor es totalmente diferente y difícil de evaluar o predecir. En este contexto, no son malos resultados porque los límites de detección resultantes son de alrededor de 1879 pg mL⁻¹. Comparado con el estado de la técnica (véase la Tabla 2.3) esto es muy similar, de hecho incluso inferior al mejor (2 ng mL⁻¹) del LOD listado.

Table B.1.: Parámetros de rendimiento del sistema observados extraídos de un inmunoensayo de CRP con una fuente FPL de baja calidad y alta calidad ECL.

Fuente de luz		$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	$\sigma \text{ [}\mu\text{rad]}$	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
FPL	Medio	5.65	3300	1781
	Ajuste	5.27		1879
ECL	Medio	3.22	184	187
	Ajuste	3.15		175

B.4. Detección de Amoxicilina IgG e IgE - hacia el diagnóstico de la alergia

La amoxicilina pertenece al grupo de las aminopenicilinas de la familia de las penicilinas. La respuesta alérgica a la penicilina es uno de los diagnósticos de alergia más comunes. Alrededor del 10% de la población mundial es diagnosticada como alérgica a la penicilina [206]. Sin embargo, podría ser que sólo el 90% de los diagnosticados tengan una alergia real a la penicilina. Además, sólo 0.03% muestran serias reacciones amenazantes para la salud [207]. A los pacientes diagnosticados como alérgicos a cierta penicilina se les da antibióticos alternativos. El aumento de la resistencia bacteriana a los antibióticos en las últimas décadas se supone que está relacionado con el mal uso de una amplia gama de antibióticos [208]. Por lo tanto, es deseable tener herramientas de diagnóstico fiables para la determinación del comportamiento alérgico a una cierta penicilina. Esto ayudaría a no amplificar innecesariamente la resistencia bacteriana a los antibióticos.

Un enfoque de inmunoensayo popular hoy en día, llamado ImmunoCAP, aplica etiquetas fluorescentes [218] para la detección. Se considera el estándar de oro para la detección de IgE [219, 220]. Su límite de detección es alrededor de 0.24 ng mL^{-1} [222]. Una técnica de detección cuantitativa más reciente sin etiqueta utiliza un sensor de resonancia de plasmón de superficie (SPR). Ha demostrado límites de detección similares, 0.6 ng mL^{-1} para la IgE y 4 ng mL^{-1} para la IgG. Sin embargo, los dispositivos de SPR son equipos muy voluminosos, difíciles de integrar en una solución de POC y difíciles de manejar por personal no entrenado.

En este contexto, la presentación de un CMZI integrado es una alternativa atractiva. Hasta donde saben los autores, aún no se ha aplicado ningún sensor fotónico integrado para la detección de alergias a la penicilina.

B.4.1. Procedimiento de funcionalización

La amoxicilina es una molécula muy pequeña ($\approx 365 \text{ Da}$ [210]), y por sí misma no es reconocible por el sistema inmunológico [211] o el anticuerpo correspondiente. Para ser reconocible tiene que pasar por una molécula portadora. Para ello aplicamos un dendrímero de poli(amidoamina) (PAMAM) de segunda generación (G2) en nuestro procedimiento de detección. Contiene 16 grupos amino a los que la amoxicilina puede unirse.

La preparación del sensor para la detección de anticuerpos de amoxicilina IgG/IgE es muy similar al procedimiento ya presentado en la Sección B.3.1. La unión covalente de los anticuerpos IgG ACRP a la guía de ondas también se hizo a través de sus grupos amino. Por lo tanto, hasta el enlazador se puede utilizar exactamente el mismo proceso de inmovilización. Eso significa que los pasos restantes son:

1. Incubar la superficie del sensor con un dendrímero de PAMAM G2.

2. Incubar la superficie terminada del dendrímero con amoxicilina para recibir la biomolécula haptensa para la detección de anticuerpos sensibles a la amoxicilina.
3. Bloquear la superficie no funcionalizada con una solución de caseína CBS.

B.4.2. Resultados experimentales

Los resultados de los inmunoensayos de detección de anticuerpos de amoxicilina IgG e IgE se discuten aquí. Para cada tipo de anticuerpo se realizaron tres experimentos. Siguen el mismo protocolo que para los experimentos de detección de CRP. Se inyectaron secuencialmente concentraciones de 10, 50, 100, 250, 500, 1000 ng mL⁻¹. De las concentraciones acumuladas inyectadas y las respuestas de fase, las sensibilidades se extrajeron por medio de un ajuste no lineal.

Las curvas de calibración de los tres inmunoensayos de aditivos IgG se presentan en la Fig. B.7, mostrando los desplazamientos de fase acumulados (triángulos) frente a las concentraciones acumuladas inyectadas. Los ajustes no lineales se muestran como líneas continuas. Todas las curvas de calibración muestran un excelente ajuste ($R^2 > 0.98$) a la curva teórica. Sin embargo, revelan una alta desviación de la dinámica de unión y la sensibilidad. Se extrajo un límite de detección medio de alrededor de 0.9 ng/mL. Las curvas de calibración de los tres inmunoensayos aditivos IgE se presentan en la Fig. B.7. Se analizaron de la misma manera

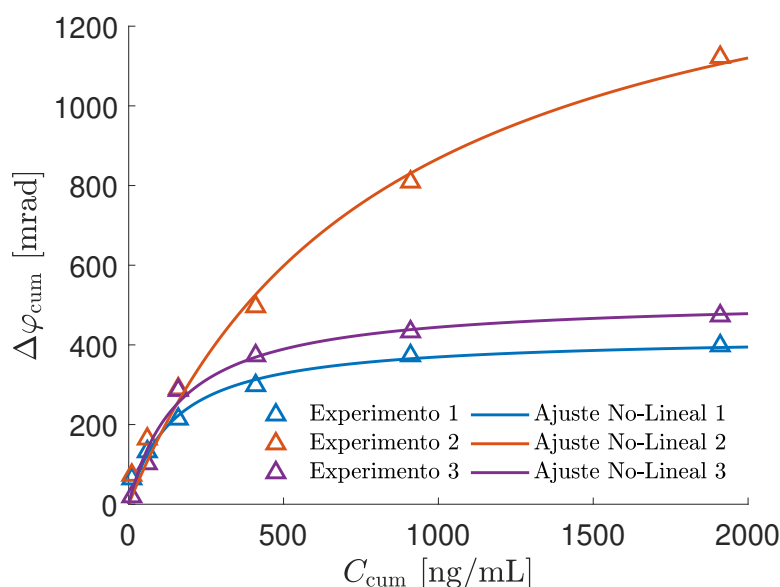


Figure B.7.: Datos y curvas de calibración estimadas para cada experimento de detección de amoxicilina IgG.

que el anticuerpo IgG. Los valores observados de R^2 van de 0.91 $\rightarrow$ 0.94 y, por lo tanto, no se ajustan tan bien a la isoterma teórica de Langmuir como la curva de unión de IgG. Se obtuvo nuevamente una alta desviación de la sensibilidad. Se extrajo un límite de detección medio de alrededor de 0.8 ng/mL.

La Tabla B.2 presenta el parámetro obtenido para la sensibilidad (S), el ruido (σ), y el límite de detección (LOD). La desviación de la sensibilidad indica que el tratamiento químico del chip aún no ha sido optimizado. Pero más allá de eso también significa que límites de detección aún más bajos también parecen posibles. Sin embargo, los límites de detección obtenidos están en el rango de lo que se obtiene con otras técnicas, como el RIA y SPR.

Table B.2.: Parámetros de rendimiento observados extraídos del inmunoensayo de aditivo de amoxicilina IgG e IgE.

Tipo de anticuerpo		$S \left[\frac{\text{mrad}}{\frac{\text{ng}}{\text{mL}}} \right]$	$\sigma \text{ [}\mu\text{rad]}$	LOD $\left[\frac{\text{pg}}{\text{mL}} \right]$
IgG	Medio	2.65	793	897
	Ajuste	2.24		1060
IgE	Medio	2.10	575	821
	Ajuste	1.91		905

B.5. Conclusiones y líneas futuras

En lo que respecta a los objetivos descritos en la introducción, se ha logrado lo siguiente:

- Primero, se investigó un modelo teórico para el sensor de CMZI. Se derivó una descripción a nivel de sistema de la configuración incluyendo diferentes fuentes de ruido, como el ruido del detector, el ruido de la fuente de láser y el ruido mecánico. Se derivaron los límites de detección teóricos, obteniendo una valiosa información sobre el diseño y la optimización a nivel de sistema. Además del trabajo teórico, se presentó una demostración experimental para la optimización de la configuración existente, llegando a límites de detección de $\sim 1 \times 10^{-8}$ RIU.
- En segundo lugar, el rendimiento de la biosensibilidad de los *acrshortcmzi* se investigó con la detección de la proteína C-reactiva (CRP) en un inmunoensayo de aditivo. Aquí se demostró un límite medio de detección de $\sim 180 \text{ pg mL}^{-1}$ con un láser de cavidad externa de alta calidad. Aplicando un láser Fabry-Perot barato ($\sim 35\text{€}$) reveló un límite de detección peor pero aún así muy aceptable de $\sim 1900 \text{ pg mL}^{-1}$.
- Finalmente, apuntando a un escenario de aplicación real, se demostró la detección específica de anticuerpos de amoxicilina IgG e IgE. Para ello se desarrolló un protocolo de funcionalización offline para inmovilizar la amoxicilina con dextrámeros en la superficie de la guía de ondas de sensado. En un inmunoensayo de unión primaria aditivo, los anticuerpos IgG e IgE pudieron ser detectados específicamente con límites medios de detección de alrededor de 1 ng mL^{-1} .

Los biosensores fotónicos integrados son una plataforma tecnológica muy prometedora para el diagnóstico bioquímico. En general, han demostrado unos límites de detección impresionantemente bajos y pueden ofrecer operaciones altamente multiplexadas en tiempo real. Los biosensores fotónicos basados en resonadores de anillo han demostrado ser una solución práctica para soluciones de laboratorio en chip, y ya existen productos comerciales [69]. Sin embargo, en su mayoría requieren un costoso láser de alta calidad para su funcionamiento preciso. Los biosensores fotónicos basados en interferómetros han superado sus anteriores limitaciones de desvanecimiento de la sensibilidad y ambigüedad de fase, ya sea añadiendo un esquema de modulación o modificando la lectura. Han demostrado límites de detección aún más bajos y han mostrado capacidad de multiplexación y son un candidato para futuros dispositivos para la atención primaria, directamente en el lugar en el que se encuentra el paciente. Estas soluciones de punto de atención requieren un dispositivo portátil y económico. Cuanto más sencillo sea el diseño de todo el dispositivo, más probable es que se reduzca su precio. La lectura, es decir, la fuente óptica, el esquema de detección y el procesamiento de la señal, suelen constituir una gran parte de un sistema integrado de biosensores fotónicos. El chip de detección fotónica en sí mismo puede ser fabricado con la línea de fabricación estándar CMOS, lo que lo hace muy asequible. El precio del resto del sistema es comparativamente alto. Especialmente, los sistemas basados en resonadores de anillo a menudo necesitan un láser de línea estrecha sintonizable como fuente óptica para extraer la longitud de onda de resonancia, lo que puede costar varios

miles hasta decenas de miles de euros. Los interferómetros sólo necesitan una fuente de longitud de onda fija para extraer con precisión el desplazamiento de fase.

El interferómetro Mach-Zehnder simétrico de lectura coherente investigado en esta tesis ofrece una solución atractiva para los dispositivos de punto de atención por varias razones. Un aspecto importante es la inmunidad al ruido del láser que incluye ruido de intensidad relativa y ruido de fase del láser. Esto significa que los láseres Fabry-Perot baratos de longitud de onda fija pueden ser usados con este tipo de sensor. Esto reduce enormemente el coste de la fuente de luz. Además, no se requiere ningún tipo de modulación para extraer la señal sin que la sensibilidad se desvanezca y sea ambigua. El proceso de fabricación permanece intacto en comparación con un típico Mach-Zehnder detectado directamente. Además, sólo se requieren tres señales de salida y pueden alcanzar excelentes límites de detección.

Sin embargo, en este trabajo todavía hay una alta desviación en la sensibilidad. Esto puede ser el resultado de problemas de transferencia de masa durante el proceso de funcionalización y requiere una mayor optimización de la bioquímica. Desde el punto de vista fotónico, lograr el funcionamiento multiplexado es una clara prioridad para la investigación futura.



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C. Curriculum Vitae

Jonas Leuermann, born 1989 in Moers (Germany), received his Bachelor and Master of Science degree at the Rheinisch Westfälische Technische Hochschule Aachen (RWTH Aachen University, Germany) in 2014 and 2016 in Electrical Engineering, Information Technology and Computer Engineering. His major field of studies within this period were Micro- and Nanoelectronics, and Information and Communications Technology, respectively. After his studies he participated as research assistant at the Institute of Integrated Photonics (IPH – RWTH) contributing to the H2020 Project Pix4Life targeting multi-wavelength laser engines for cytometry applications. He started his PhD on coherently detected photonic integrated biosensors in 2017, funded by the Horizon 2020 Marie Skłodowska-Curie Actions (No. 713721), at the Andalusian Centre for Nanomedicine & Biotechnology Bionand in cooperation with the Photonics & RF Research Laboratory from the University of Málaga (UMA, Spain). At the beginning of his work he completed a national stay at the NanoBiosensors and Bioanalytical Applications Group, led by Laura M. Lechuga, at the Institut Català de Nanociència i Nanotecnologia (ICN2) from the Universitat Autònoma de Barcelona (UAB, Spain). Later he completed an international stay at The Bailey Lab, led by Ryan Bailey, at the University of Michigan - Ann Arbor (UMich, United States of America).

A comprehensive list of his publications is given below:

Articles in International Journals

1. **J. Leuermann**, V. Stamenkovic, P. Ramirez-Priego, A. Sánchez-Postigo, A. Fernández-Gavela, C. A. Chapman, R. C. Bailey, L. M. Lechuga, E. Perez-Inestrosa, D. Collado, R. Halir, and I. Molina-Fernández, “Coherent Silicon Photonic Interferometric Biosensor with Inexpensive Laser Source for Sensitive Label-Free Immunoassays”, *Optics Letters*, vol. 26, no. 18, p. 6595, December 2020
2. **J. Leuermann**, A. Fernández-Gavela, A. Torres-Cubillo, S. Postigo, A. Sánchez-Postigo, L. M. Lechuga, R. Halir, and I. Molina-Fernández, “Optimizing the Limit of Detection of Waveguide-Based Interferometric Biosensor Device”, *Sensors*, vol. 19, no. 17, p. 3671, August 2019
3. I. Molina-Fernández, **J. Leuermann**, A. Ortega-Moñux, J. G. Wangüemert-Pérez, and R. Halir, “Fundamental limit of detection of photonic biosensors with coherent phase read-out,” *Optics Express*, vol. 27, no. 9, pp. 12616-2629, April 2019

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1. **J. Leuermann**, A. Fernández-Gavela, L. M. Lechuga, A. Sánchez-Postigo, R. Halir, and I. Molina-Fernández, “Low Limit of Detection Silicon Photonic Sensor with Extremely-Low-Cost Laser Source,” *IEEE Photonics Conference 2020*, 28 September 1 October 2020, Virtual Conference
2. A. Torres-Cubillo, **J. Leuermann**, P. Reyes-Iglesias, A. Ortega-Moñux, J. G. Wangüemert-Pérez, Í. Molina-Fernández, and R. Halir, “Towards complex refractive index sensing with a photonic integrated circuit,” *XXXV Simposio Nacional de la Unión Científica Internacional de Radio (URSI)*, 2-4 September 2020, Virtual Conference

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6. **J. Leuermann**, Í. Molina-Fernández, A. Ortega-Moñux, J. G. Wangüemert-Pérez, and R. Halir, “Comparing the fundamental limit of detection for interferometric and resonant biosensors with coherent phase read-out,” *CLEO Europe*, Munich, Germany, 23-27 June 2019
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