SENSING TECHNOLOGIES ON ACTIVE AND PASSIVE Al_2O_3 GLASS

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SENSING TECHNOLOGIES ON ACTIVE AND PASSIVE Al₂O₃ GLASS

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Contents

Abstract	v
Samenvatting	vii
Chapter 1 Introduction	1
1.1 Integrated optical sensing	3
1.1.1 Sensing technologies	4
1.1.2 Whispering gallery mode sensors	6
1.1.3 Challenges of integrated optical sensing	10
1.2 Al_2O_3 for integrated sensing	12
1.3 Outline of the thesis.....	15
Bibliography	16
Chapter 2 Al_2O_3 ring resonators	27
2.1 Analysis of a waveguide ring resonator	28
2.1.1 Spectral resonances.....	29
2.1.2 Ring resonator parameters	30
2.1.3 Ring resonator sensor	32
2.2 Ring resonator design	34
2.2.1 Sensitivity	35
2.2.2 Bend losses	37
2.2.3 Coupling section	38
2.3 Fabrication.....	41
2.3.1 Sputter deposition of Al_2O_3	41
2.3.2 Lithography and etching.....	44
2.3.3 Chip preparation	45
2.4 Ring resonator characterization	46
2.4.1 Ring resonator characterization at 1570 nm	47

2.4.2	Ring resonator characterization at 1035 nm	51
2.4.3	Comparison of Al ₂ O ₃ with Si-based ring resonator platforms.....	52
2.5	Conclusion.....	53
	Bibliography	55
Chapter 3 Passive Al₂O₃ ring resonator sensors		59
3.1	Introduction	60
3.2	Passive sensor characterization	61
3.2.1	Temperature sensing.....	63
3.2.2	Bulk RIU sensing.....	66
3.2.3	Surface sensing	67
3.3	rhS100A4 biomarker sensing	69
3.3.1	Chemical protocol.....	69
3.3.2	rhS100A4 biomarker sensing	71
3.4	Conclusion.....	74
	Bibliography	75
Chapter 4 Active Yb³⁺:Al₂O₃ laser-based sensors.....		79
4.1	Introduction	80
4.2	Laser characterization.....	83
4.2.1	Lasing spectrum.....	85
4.2.2	Power characteristics	86
4.2.3	RF beat note and laser linewidth	87
4.2.4	Improving the laser performance.....	89
4.3	Active sensor characterization.....	90
4.3.1	Temperature sensing.....	91
4.3.2	Bulk refractive index sensing	91
4.4	rhS100A4 biomarker sensing	94
4.5	Conclusion.....	97

Bibliography	99
Chapter 5 Mode-splitting in a ring resonator for self-referenced biosensing	103
5.1 Introduction	104
5.2 Grating-induced mode-splitting	105
5.3 Self-referencing of a grating-integrated ring resonator	108
5.4 Grating fabrication	110
5.5 Grating characterization	113
5.5.1 Transmission measurements	114
5.5.2 Temperature and bulk refractive index variations	115
5.5.3 Modification of the grating coupling coefficient	117
5.6 Biomarker sensing	117
5.7 Conclusion	122
Bibliography	123
Chapter 6 Yb³⁺:Al₂O₃ self-referenced biosensors based on beat note detection	127
6.1 Introduction	128
6.2 Grating-integrated ring resonator laser	130
6.2.1 Grating implementation on ring laser	130
6.2.2 Microwave generation	133
6.2.3 RF stability	135
6.3 Optical beat note biosensing	139
6.4 Conclusions	143
Bibliography	145
Chapter 7 Conclusions	147
List of publications	153
Acknowledgements	157

Abstract

Soluble biomarkers obtained from human bodily fluids can act as a diagnostic tool for assessing the presence of a disease or monitoring its progression. Optical resonators are well suited for detecting disease biomarkers since they have both a high sensitivity due to a large evanescent field and high quality factors (Q) that allow monitoring minute concentrations of analyte. The research presented in this thesis concerns the investigation of both passive and active Al_2O_3 (aluminum oxide) ring resonators for biomarker detection.

To test the viability of Al_2O_3 as a photonic waveguide material for biosensing, undoped, passive Al_2O_3 ring resonators were theoretically studied and designed for optimal sensing performance. Ring resonators with a highest Q of $5.1 \pm 0.1 \times 10^5$ were achieved at a wavelength of 1570 nm for a cladding of air, which corresponds to propagation losses down to 0.42 ± 0.01 dB/cm. The shift of the resonance wavelength of the ring resonators was monitored as a function of time while the temperature or bulk refractive index of the environment of the ring was varied, showing the operation of these devices as sensors. A limit of detection of 1.65×10^{-6} RIU was obtained, equaling those of the best ring resonator sensors based on conventional photonic waveguide materials. Finally, a surface functionalization for Al_2O_3 was applied to form a bioreceptor layer of Anti-S100A4 monoclonal antibodies to detect the rhS100A4 cancer protein biomarker from urine with a limit of detection of 3 nM.

Single-mode $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ ring and disk resonator lasers were realized for light emission at a wavelength around 1030 nm. These were used as sensors by monitoring the shift of the lasing wavelength by heterodyning their emission light with that of an external laser to form an optical beat note on a photodetector. After applying a functionalization and antibody immobilization on the laser surface, biomarker detection in urine was recorded through variations of the heterodyned beat note frequency with a limit of detection down to 300 pM.

The resonances of the ring resonators contain a two-fold degeneracy that could be lifted by implementing a Bragg grating inscribed in a PMMA top cladding. Grating-integrated ring resonators exhibited mode-splitting that could be tuned by the reflectivity of the grating. The amount of mode-splitting remained invariant upon applying environmental perturbations such as temperature or bulk refractive index

variations, since these hardly affect the grating reflectivity. However, binding of antibodies and biomarkers to the grating did induce a change of mode-splitting due to the varying grating reflectivity. Passive Al_2O_3 grating inscribed ring resonators were, thus, established as self-referenced biosensors.

The same PMMA gratings were applied to $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ ring resonators to induce mode-splitting of the lasing mode, resulting in two closely separated wavelengths. Microwave signals were produced from the optical beat note formed upon lasing at both mode-split resonance wavelengths. The beat note frequency varied upon biomarker binding to the grating. Such mode-splitting based active lasers hold the potential for integration in self-referenced sensor devices that do not need expensive or complicated equipment for sensor signal generation and read-out.

Samenvatting

Menselijke lichaamsvloeistoffen bevatten biomarkers die een meetbare indicator kunnen zijn voor de diagnostisering van ziektebeelden of het volgen van een ziekteverloop. Optische resonatoren zijn zeer geschikt voor de detectie van dergelijke biomarkers vanwege hun grote gevoeligheid verkregen door de aanzienlijk veldsterkte van evanescente golven en de hoge kwaliteitsfactoren (Q) die het vastleggen van minuscule concentraties mogelijk maken. Dit proefschrift beschrijft het onderzoek betreffende het meten van biomarkers met zowel passieve als actieve Al_2O_3 (aluminiumoxide) ringresonatoren.

Passieve Al_2O_3 ringresonatoren zonder dotering zijn theoretisch bestudeerd om de haalbaarheid van Al_2O_3 als fotonisch golfgeleider materiaal voor biosensoren te toetsen. Onbedekte ringresonatoren hadden een hoogste Q van $5.1 \pm 0.1 \times 10^5$ voor een golflengte van 1570 nm, wat overeenkomt met een golfgeleider propagatieverlies van 0.42 ± 0.1 dB/cm. Om de ringresonator als sensor te gebruiken werd de verschuiving van de resonantiegolflengte als functie van tijd bijgehouden terwijl de ringresonator onderhevig was aan temperatuur- en brekingsindexvariaties. Een detectielimiet van 1.65×10^{-6} RIU was bepaald voor brekingsindexvariaties, wat gelijkend is aan de best behaalde resultaten voor ringresonator sensoren gemaakt van de conventionele fotonische golfgeleider materialen. Tenslotte werd een functionalisatieproces toegepast om het oppervlak van Al_2O_3 golfgeleiders te bedekken met een bioreceptor laag van Anti-S100A4 monoklonale antilichamen. Hiermee zijn rhS100A4 proteïne kankerbiomarkers gedetecteerd met een detectielimiet van 3 nM in urine.

Lichtemissie van een enkele longitudinale golf met een golflengte van ~ 1030 nm was gerealiseerd met $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ ringresonator en schijfresonator lasers. Deze werden gebruikt als sensor door de verschuiving van lasergolflengte nauwkeurig te volgen. Dit werd mogelijk gemaakt door de vorming van een microgolf via heterodyne detectie door het elektromagnetische veld van het geëmitteerde laserlicht te mengen met dat van een externe laser. Nadat de laser bedekt was met een laag antilichamen is biomarker binding aangetoond door het detecteren van variaties van de frequentie van de microgolf. Een kleinste concentratie van 300 pM was gedetecteerd in urine.

De resonanties van een ringresonator bevatten een tweevoudige multiplicititeit die opgeheven kon worden door een Bragg rooster aan te brengen in een PMMA bedekking over de ringresonator. Dit leverde een splitsing van de op resonanties met als resultaat twee nieuwe golflengtes, wiens spectrale afstand ingesteld kon worden aan de hand van de reflectiviteit van het rooster. De splitsing varieerde nauwelijks onder opgelegde temperatuur- of brekingsindexvariaties, aangezien deze de reflectiviteit van het rooster amper aanpassen. Echter, het binden van antilichamen en biomarkers aan het rooster varieerde diens reflectiviteit waardoor de splitsing van de twee golflengtes ook veranderde. Dit maakt een sensorwerking mogelijk die ongevoelig is voor omgevingsfactoren.

Dezelfde roosters werden aangebracht op $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ ringresonator lasers om golflengtesplitsing op te wekken van het geëmitteerde laserlicht. Microgolven werden gecreëerd door het laserlicht van beide gesplitste golflengtes met elkaar te mengen. De frequentie van de microgolf varieerde door binding van antilichamen en biomarkers aan het rooster. Een dergelijke laser maakt een meetinstrument mogelijk dat geen dure of complexe apparatuur vereist voor signaalgeneratie of het uitlezen daarvan.

Chapter 1

Introduction

Point-of-care devices for disease biomarker detection are of enormous interest for medical diagnostics and patient monitoring. Towards that aim, integrated optical sensors are excellent candidates for precise and label-free detection of biomolecules in complex matrices such as body fluids. An overview of the state of the art in integrated optical sensors and their material platforms is given. Rare-earth ion doped aluminum oxide, $RE^{3+}:Al_2O_3$, is introduced as an alternative to these material platforms because of its prospects for realizing integrated on-chip laser-based biosensors. This is followed by the outline of this thesis wherein novel sensing strategies implemented on undoped and doped (i.e., passive and active) Al_2O_3 waveguides are explored.

Cancer is one of the main causes of death in developed countries. Worldwide cancer statistics estimated that there were 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 [1]. Early diagnostics and screening for different types of cancer will permit treatment before symptoms emerge, greatly increasing the survival rate and the quality of life of patients and reducing the impact of the disease. Furthermore, the monitoring of the pre- and post-operative evolution of cancer patients is also of major significance [2]. Therefore, a *simple medical diagnostic test* near the point-of-care is highly desirable to provide physicians, patients and their care team with immediate results to aid with better clinical management decision making and to improve personalized therapy [3]. In fact, a recent survey conducted in Australia, Belgium, the Netherlands, the UK and the USA revealed that primary care clinicians listed cancer as a condition for which they want a point-of-care test [4].

Detection of cancer *biomarkers* is a good strategy for early and timely diagnostics in point-of-care devices [5]. Soluble biomarkers obtained from human biofluids can be obtained in a non-invasive manner and can provide a measurable diagnostic for assessing the presence of a disease [6]. Among the many possible disease biomarkers, the family of S100 proteins is associated with a regulatory role in a variety of cellular processes [7]. Many lines of investigation suggest that overexpression of S100 proteins is associated with tumor progression and prognosis [8,9]. S100A4 is a member of this family and it is found as a highly expressed transcript in metastatic tumor cell lines. It is up-regulated in several malignancies including bladder cancer and it was found to play a role in tumor aggressiveness [10–12]. These reports indicate the value of the S100A4 protein as a biomarker for disease progression including bladder cancer. However, S100A4 urine levels for bladder cancer are not reported in the literature. Turnier et al. showed median urine levels of S100A4 around 0.1 nM for healthy controls and median urine levels ranging from 0.5 to 1 nM for patients with lupus nephritis using a commercially available ELISA kit [13].

For a diagnostic point-of-care device to be successful in a clinical environment it should be user-friendly, low-cost and portable while delivering results within several minutes. At the same time, the device should be ultra-sensitive, highly specific, multiplexed and label-free. The system should include microfluidics for the handling of minute amounts of liquid samples, a biointerface functionalized with capture probes to detect the biomarker of interest and to convert the capture-probe to

Fig. 1.1: Point-of-care integrated optical biosensor. (a) Schematic of complete device. (b) Schematic of disposable cartridge containing integrated optical sensors. Both images obtained from [14].

biomarker interaction into a measurand, and a read-out and data analysis module to generate a measurable signal and to extract the relevant information. A modular design consisting of a fixed detection and analysis apparatus into which a disposable, biofunctionalized chip is placed could combine these requirements. This approach has the benefit of both being reusable while having a disposable low-cost sensing chip. Integrated optical biosensors are good candidates to meet the requirements listed above [15–24]. Figure 1.1 represents the schematic concept of the integrated optical biosensor for point-of-care applications developed in this thesis as part of the Glass Multiplexed Biosensor (GLAM) European project. The system comprises a disposable optical microchip, which is exposed to a drop of urine from the patient, and a portable point-of-care cartridge reader, which includes all the interrogation optics and electronics. Data processing of the collected data closes the loop, contrasting the biomarker levels of the patient with the available cancer biomarker databases. A more detailed description of the optical biosensor concept as proposed in the GLAM project is shown in Fig. 1.1 (b).

1.1 Integrated optical sensing

Photonic integrated circuits were first proposed by Miller in 1969 [25], marking the onset of the field of integrated optics. It involves the manipulation of light on a photonic chip using waveguide technologies. Since its introduction, numerous

research activities have been and are carried out on integrated photonics in both industrial and governmental settings. The technology holds the potential for a large impact on, among others, data communications [26,27], photonic information processing [28,29], quantum computing [30,31], optical metrology and sensing [32–35], and medical diagnostics [36]. Integrated optical sensing covers many different technologies on multiple material platforms. The general principle relies on the change of light propagation properties, namely intensity and phase, upon interaction with the analyte. Interest in optical sensing is driven by its attractive features including a high sensitivity, high speed, lack of electrical interference and electromagnetic insensitivity. The devices can be operated in a label-free configuration without the need for amplification or sample pre-treatment [37], leading to devices that avoid sample preparation and require minute amounts of analyte.

1.1.1 Sensing technologies

The enzyme-linked immunosorbent assay (ELISA) is one of the most commonly used and commercially available biochemistry assays to detect proteins [38,39]. It works by immobilizing an unknown amount of antigens (i.e., analyte) onto a surface, to which afterwards the detection antibody is added to form a complex with the antigen. An enzyme is then linked to the complex and developed by adding an enzymatic substance to produce a fluorescent, colorimetric or chemiluminescent signal, the reading of which indicates the quantity of antigen present in the initial sample. However, the false-positive rate in ELISA assays can be high [40,41] and it is difficult to use in human sera due to the poor blocking effect of non-specific reactions [42]. ELISA assays require tedious and laborious procedures that are time consuming and need specialized personnel [35]. In addition, ELISA offers only an endpoint readout not allowing for dynamic or real-time observations of binding kinetics. It has been shown that integrated silicon photonics can have a superior performance for the detection of the interleukin-2 biomarker compared with ELISA both in terms of real-time results, speed and precision [43], already showcasing integrated optical sensors as alternative to ELISA assays.

Another commonly found sensor platform for biomarker detection is surface plasmon resonance (SPR) [44]. A SPR is a resonant electron oscillation that can be excited when a light wave propagates along the interface between two surfaces with different sign of the real part of the dielectric function. SPRs are very sensitive to dielectric perturbations of their evanescent field [45]. However, generating a SPR

typically requires a prism to couple the incident light into the surface plasmon wave, which makes their integration into a portable sensor difficult [46]. Implementation of SPR on waveguides [47] and localized SPR (LSPR) allows for integration and miniaturization [48], but some consider that their sensitivity should be higher for relevant clinical and environmental applications [3].

In contrast to ELISA and SPR, a plethora of alternative devices and sensing approaches exists based on photonic structures integrated on small optical chips. Among these are photonic crystals [49], Mach-Zehnder interferometers [50], and whispering gallery mode resonators [51], each of which have their own respective advantages and drawbacks. Photonic crystal structures consist of periodically arranged dielectric variations with periodicity on the order of a wavelength in one-, two- or three-dimensional orientations. The dielectric variations provide light reflection at specific wavelengths resulting in a photonic band gap of forbidden optical frequencies [19]. Introducing a defect can result in a narrow resonance inside the photonic band gap that can shift upon perturbations of the dielectric environment. Monitoring the frequency of the resonance or shifts of the bandgap allow photonic crystals to be used as sensors [35,52]. For instance, photonic crystals were used to detect biomolecules [53], gasses [54] and refractive index variations in liquid [55]. Photonic crystals require complicated fabrication methodologies to define their nanoscale structure requiring electron beam lithography and/or complicated processing in three-dimensions. The need for slow complex fabrication techniques limits their large-scale production [56]. Mach-Zehnder interferometers are devices in which light is divided over two waveguide branches whose relative phase difference produces an interference pattern at the output [50]. Typically, one branch is exposed to the sensing analyte and experiences a phase shift with respect to the other branch, which can be monitored as a shift of the interference pattern or a drop of transmitted intensity [57,58] Mach-Zehnder interferometers have been used for high sensitivity temperature sensing [59], the detection of pollutants [60], refractive index sensing [61,62] and the detection of biomolecules [63,64]. However, achieving high sensitivities and low detection limits requires device lengths in the range of 5—10 mm, since the sensitivity of a Mach-Zehnder interferometer scales linearly with the difference in path length between both branches. Such long sensing path makes it difficult to achieve the circuit density required for multiplexed sensing functionalities [56]. Finally, whispering gallery resonators are cavities in which light propagates along an outer contour while the evanescent field of the light probes the

surrounding medium. The light travelling inside the whispering gallery resonator experiences constructive interference at specific wavelengths, at which it can continue to propagate inside the resonator for many roundtrips. These wavelengths are accompanied by sharp resonances in the transmission spectrum. Whispering gallery resonators can be used for sensing by monitoring shifts in the resonance wavelength [65–67], broadening [68,69] or narrowing [70] of the resonances or the appearance of mode-splitting [71–73], as shown in Fig. 1.2 (a). Whispering gallery mode resonators can have very large quality factors up to 8×10^9 , approaching the theoretical limit solely based on material losses [74]. These high quality factors allow for excellent sensitivities [75–78].

1.1.2 Whispering gallery mode sensors

Many different types of optical whispering gallery mode resonators exist. The most common resonator cavities for sensing include spheres [79], bottles [80], capillaries [81], toroids [82], disks [83] and rings [84]. Spheres are commonly made by reflow of the tip of an optical fiber resulting in extremely smooth surfaces, which, in combination with the very low material losses, lead to some of the highest optical quality factors reported [85]. Spheres have been used for temperature [86], pressure [87], biomolecule [88] and environmental [89] sensing. Furthermore, coating a high quality factor sphere with a nonlinear material can induce second harmonic generation [90], which can be harnessed for the detection of small quantities of molecules [91]. Bottle resonators are cylindrical cavities that have a varying radius along the axial direction leading to the confinement of light in the axial direction. A great deal of research efforts have been devoted to the application of these devices for sensing [92], with demonstrations of temperature sensors [93], humidity sensors [94], nanoparticle sensors [95], strain sensors [96], and biosensors [97].

Capillary resonators consist of a hollow core that can support a liquid or gas flow while the thin capillary walls support light propagation with the evanescent field overlapping with the hollow center [81]. Their sensitivities are rather low due to evanescent fields extending only a small fraction into the liquid core [98,99]. Toroid resonators are suspended on a silicon pedestal [82]. Among their sensing applications are the detection of biomolecules [100], which could be performed down to single-molecule levels [101], and single nanoparticle imaging [102]. Although the previously listed resonators all have very high quality factors in the range 10^6 – 10^{10} , and excellent sensing results have been achieved using them, they

Fig. 1.2: Examples of whispering gallery mode biomarker sensors. (a) Sensing principles: resonance shift (i), resonance broadening (ii) and resonance splitting (iii). (b) Pedestal toroidal resonator [82]. (c) Silica spherical resonator [77]. (d) SOI disk resonators [103]. (e) SOI racetrack resonator [104].

all suffer from a complicated three-dimensional geometry that does not allow simple, monolithic integration on a chip and requires difficult light-coupling schemes involving fragile, tapered fibers [105]. Ring resonators are a class of whispering gallery mode resonators that can easily be integrated on a chip on a large scale using complementary metal-oxide-semiconductor (CMOS) compatible fabrication technologies [106]. Examples of whispering gallery mode resonators are shown in Figs 1.2 (b)–(e).

Ring resonators are circular cavities formed by looping optical waveguides onto themselves [84]. Light coupling is achieved through a bus waveguide. This permits

a robust coupling that, unlike the previously mentioned resonators, does not require a fiber taper or prism and it is well-suited for integration with microfluidics. Furthermore, ring resonators are fabricated by either electron beam lithography or photolithography and they do not require a laser reflow process, enabling their compatibility with large-scale fabrication. Disk resonators are a special class of ring resonators, where the waveguide forming the cavity lacks an inner sidewall. This results in smaller sidewall roughness losses, and thus higher quality factors, but also reduced evanescent field overlap with the sensing region and thus lower sensitivities. Both ring and disk resonators have lower quality factors than the three-dimensional whispering gallery resonators due to larger fabrication induced losses, such as sidewall roughness resulting from the etching process [107–109] and the lack of a reflow process. By optimizing the microfabrication process, Q -factors close to 10^8 have been reported in Si_3N_4 ring resonators [110]. Both disk and ring resonators have been demonstrated on various material platforms including polymer [111,112], indium phosphide [113,114], lithium niobate [115–117], tellurium oxide [118], and the silicon based materials silicon nitride [119,120], silicon oxynitride [121–123] and silicon-on-insulator (SOI) [104,124–126].

The performance of a ring resonator sensor is characterized in terms of its sensitivity, i.e., the response of the sensor to the applied perturbation, and the limit of detection (LOD), i.e., the smallest perturbation that can be reliably detected [76]. Typically, the resonance wavelength shift is monitored upon the introduction of an analyte using a finely tunable laser or a high-resolution optical spectrum analyzer. Then, the sensitivity of ring resonators is defined as the resonance wavelength shift per applied perturbation, often expressed in terms of shift per refractive index unit (i.e., nm/RIU) or shift per thickness of a deposited layer of biomaterial (i.e., nm/nm). The LOD is limited by the wavelength noise present in the system. The LOD , in RIU, is given by three times the noise divided by the sensitivity of the system. Mechanical vibrations on the coupling to the chip, disturbances in the microfluidic flow, laser wavelength and intensity fluctuations, and thermal noise all contribute to the wavelength noise and care must be taken to eliminate these sources or to reduce them as much as possible. The bulk refractive index LOD is a useful metric for comparing different sensors, since it incorporates both the sensitivity of the device to dielectric perturbations to its surroundings and the noise present in the sensor. The following paragraphs present an overview of the best sensing results reported in the literature

in terms of sensitivity and *LOD* for the best developed ring resonator sensing platforms, SOI and silicon nitride.

SOI is the most dominant photonic platform for integrated optical biosensing. Its main attractive features include its large refractive index contrast of ~ 3 and its compatibility with CMOS fabrication technologies. Its high refractive index contrast allows tight bend radii down to $1.5\ \mu\text{m}$ [127] and, therefore, a high integration density. Its CMOS compatibility makes it possible to use electronics fabrication facilities for device fabrication [128]. SOI is transparent in the infrared for wavelengths above $1100\ \text{nm}$ and applications of this technology are most commonly found at telecommunication wavelengths (i.e., around $1550\ \text{nm}$) [129]. Although quality factors as high as 2.2×10^7 have been demonstrated for large core devices with an oxide cladding at $1550\ \text{nm}$ [126], the high refractive index of SOI increases the scattering losses in small core waveguides [107,130]. Furthermore, water absorption losses are high at this wavelength range [131], limiting the obtainable quality factors to $\sim 10^4$ when operating in an aqueous environment. Nevertheless, excellent sensing results were obtained on the SOI platform, including, for single ring resonators: (i) bulk refractive index sensitivities of $\sim 50\text{--}250\ \text{nm/RIU}$ [103,125,132] depending on the polarization and waveguide dimensions used, (ii) bulk refractive index *LODs* down to $7.6 \times 10^{-7}\ \text{RIU}$ [133], (iii) the label-free detection of bladder cancer biomarkers from urine down to the μM concentration range [134], (iv) the sensing of a clinically relevant cancer biomarker down to a *LOD* of $25\ \text{ng/ml}$ [135], (v) the simultaneous detection of five biomarkers to demonstrate the possibility of concurrently performing multiple immunoassays on a ring resonator platform [136], and (vi) commercialization of the sensing platform by Genalyte Inc. [137].

Another well-established ring resonator material platform is silicon nitride. Compared with SOI, it has a smaller refractive index contrast of ~ 0.5 , limiting its integration density. Its fabrication process is CMOS compatible and its transparency window covers both the visible and near-infrared for a range of $400\ \text{nm}\text{--}2.35\ \mu\text{m}$ [138], allowing device operation at wavelengths with negligible water absorption losses. Quality factors up to 67×10^6 [110] and waveguide propagation losses as low as $0.05\ \text{dB/m}$ at a wavelength of $1580\ \text{nm}$ were achieved for a low confinement silicon nitride waveguide [139]. Bulk refractive index sensitivities up to $110\ \text{nm/RIU}$ at a wavelength of $850\ \text{nm}$ were demonstrated [119,140], together with the detection of proteins such as thrombin ($37\ \text{kDa}$) down to $1\ \text{nM}$ [141] and lectins ($72\ \text{kDa}$) down to $10\ \text{pM}$ [142]. New data processing schemes based on a fast Fourier

transform have been proposed that rely on low quality factor ring resonators and larger laser scan steps that do not require finely tunable laser systems [143]. Using this methodology, a *LOD* of 8.5×10^{-7} RIU was demonstrated on a silicon nitride ring resonator [144]. Furthermore, a novel sensing scheme was developed based on camera imaging of multiple coupled-resonators where changes in elastic-light scattering of the mode-field intensity distribution were linked to small refractive index changes of the cladding, omitting the need for complex interrogation using tunable lasers [145]. In a first attempt, a refractive index change of 1.3×10^{-4} RIU was experimentally detected.

Although excellent sensing results have been achieved using ring resonators, novel sensing schemes are still being introduced to improve their performance. This is done by either increasing the sensitivity of the sensor or by reducing its noise to improve the *LOD*. The sensitivity can be improved by using ring resonators in the Vernier configuration [146–149], for a record bulk refractive index sensitivity of 24300 nm/RIU [150]. However, the wavelength shift is determined from the peak of an envelope in the transmission spectrum that has a large uncertainty. This, in combination to a higher sensitivity to noise sources, compensates for the ultra-high sensitivities achievable and the attainable *LODs* are not significantly improved over those obtained in single ring resonators [149]. Another approach is based on subwavelength gratings [151,152] and slot-waveguides [153,154], which exhibit a larger overlap of the evanescent field with the sensing region, resulting in an improvement of several times the sensitivity of a plain single ring resonator [104]. The larger optical overlap with the sensing region does come at the cost of a reduced quality factor due to increased scattering losses and absorption losses, resulting in increased wavelength determination uncertainties and, therefore, worse *LOD*. Both methods of enhancing the sensitivity do not offer a big advantage towards achieving a smaller *LOD* [155]. Alternatively, the *LOD* can be improved by reducing the noise by using reference ring resonators to monitor ambient thermal fluctuations [156], thermal sensitivity cancellation [157], or self-referenced sensing schemes based on mode-splitting [158–162], although until the developments within the GLAM project [162], this last type of sensors have only been utilized for (nano)particle detection and not for biosensing applications.

1.1.3 Challenges of integrated optical sensing

Despite the many successful demonstrations of sensing on integrated optical devices, many challenges remain before they can find applications in point-of-care medical

applications. Even though large sensitivities and low *LODs* were demonstrated, only the highest quality factor whispering gallery mode resonators were able to reach the ultimate sensitivity levels of single molecule or nanoparticle detection. It remains an ongoing challenge to continuously improve both the sensitivity and the *LOD* to achieve unprecedented sensitivity integrated on-chip, since in many cases the target biomarkers are present in minute concentrations in body fluids. Furthermore, low-cost integration on-chip of the sensor is essential, together with simple packaging technologies that provide stable alignment, protect the device against damage, allow simple chip and device handling, and can be integrated with microfluidics for analyte delivery.

Another challenge is formed by the noise present in the sensing systems. Noise levels should be minimal for detection of the smallest analytes. This requires eliminating or reducing noise contributions from various sources such as environmental fluctuations (i.e., thermal, refractive index, particles), mechanical vibrations and laser noise.

Other challenges are present in the chemical treatment and formation of the bioreceptor layer necessary to capture the biomarkers. This layer must be selective to only the specific substance of interest. Surface chemistry is essential to provide the inert ring resonator surface with functional groups selectively reactive to the biomarker of interest. A common method is to immobilize either component of an antigen-antibody pair onto the surface of the ring. The used surface chemistry should then provide a strong and stable bond of the capture sites on the ring to prevent detachment and sensitivity degradation, while also maintaining bioreceptor activity. The used surface chemistry protocol should be reproducible to yield identical results for repeated measurements. Since ring resonators only monitor a shift of resonance wavelength but they do not provide a discrimination mechanism to decouple all the origins of the associated dielectric perturbations that induce said shift, selectivity is important to ensure that the shift belongs solely to the analyte of interest. The surface should then be treated with a blocking method to make it inert to non-specific fouling of molecules.

Point-of-care sensors should be able to operate near the patient site without permanent dedicated space or highly trained personnel. Ideally, the packaged and microfluidic integrated device can be placed in an instrument possessing the light sources, detector, electronics and data analysis. Currently, most ring resonator sensors employ either a finely tunable laser or a high resolution optical spectrum

analyser. However, one approach to circumvent this is by using an active ring resonator and forming a low-frequency beat note either on the chip itself [163,164] or by using a reference laser [100]. This reduces the complexity of the module, which only requires a pump to achieve lasing and a photodiode for detection. The generated electric signal on the photodiode can easily be analysed in the electronic section of the sensing module, thus enabling the desired device operation.

The few demonstrations of active sensors rely on complicated three-dimensional optical cavities that are not suited for on-chip integration, hampering their widespread use as multiplexed biosensors [165–168]. Optical sensing based on active devices requires a material that can overcome this limitation. Aluminum oxide, Al_2O_3 , is a photonic material that can be monolithically integrated at the wafer level and that offers both optical guiding and active functionalities. Despite its many attractive features, this material has been rarely explored for integrated optical sensing [164].

1.2 Al_2O_3 for integrated sensing

This thesis investigates Al_2O_3 as a material platform for integrated optical biosensors for the detection of disease biomarkers. Al_2O_3 is a dielectric material found in both crystalline and amorphous phase. Al_2O_3 has attractive features including a large transparency window extending from the UV to the mid-infrared (i.e., 150–5500 nm) [169,170]. It can easily be deposited on silicon oxidized wafers allowing for monolithic integration and wafer-scale processing. Optical waveguiding was demonstrated in amorphous Al_2O_3 at various wavelengths within its transparency window, including waveguides and devices at 371 nm [171], 1020 nm [172], 1550 nm [173] and 2000 nm [174]. Low optical losses down to 0.12 ± 0.02 dB/cm [175] and 0.04 ± 0.02 dB/cm [176] were reported for planar waveguides fabricated with both reactive sputtering and atomic layer deposition, respectively. After waveguide definition with reactive ion etching, propagation losses down to 0.21 ± 0.05 dB/cm were demonstrated at a wavelength of 1550 nm [177]. The low loss over a wide wavelength range enables devices that operate at wavelengths outside the telecommunications band where water absorption losses are low or negligible, making them well-suited for biosensors. The refractive index contrast with the SiO_2 cladding of ~ 0.2 RIU, although smaller than for silicon-based materials, still allows for dense integration on-chip with bend radii down to tens of micrometers.

Rare-earth ion doped Al₂O₃, RE³⁺:Al₂O₃, has a high trivalent rare-earth ion solubility [178] that is much larger compared with that of the silicon-based photonic material platforms [179,180] or silica-based glass [181]. For instance, a dopant concentration of $\sim 2 \times 10^{20} \text{ cm}^{-3}$ for Er³⁺:Al₂O₃ was possible with only moderate luminescence quenching [182]. Rare-earth ion doping was demonstrated for active operation at a variety of wavelengths, including ~ 0.88 , ~ 1.06 and $\sim 1.33 \text{ }\mu\text{m}$ for Nd³⁺:Al₂O₃ [183], $\sim 1.03 \text{ }\mu\text{m}$ for Yb³⁺:Al₂O₃ [184], $\sim 1.55 \text{ }\mu\text{m}$ for Er³⁺:Al₂O₃ [185–189], ~ 1.8 – $1.9 \text{ }\mu\text{m}$ for Tm³⁺:Al₂O₃ [190] and $\sim 2 \text{ }\mu\text{m}$ for Ho³⁺:Al₂O₃ [174]. Most lasers realized on RE³⁺:Al₂O₃ are either channel RE³⁺:Al₂O₃ distributed feedback lasers [191–192] or RE³⁺:Al₂O₃ ring lasers [185,188]. Furthermore, it is possible to monolithically integrate RE³⁺:Al₂O₃ with passive photonic functions in SOI [186], silicon nitride [193–197] and undoped Al₂O₃ [198]. Examples of RE³⁺:Al₂O₃ devices are shown in Fig. 1.3.

The active functionality of RE³⁺:Al₂O₃ allows for laser-based biosensors. However, before this work, there was only a single report of optical sensors using doped or undoped Al₂O₃, which was the realization of a dual-wavelength Al₂O₃ distributed feedback laser whose evanescent field was used to detect the presence of glass microspheres of diameters ranging between $1 \text{ }\mu\text{m}$ and $20 \text{ }\mu\text{m}$ [164]. The detection was not selective, as the device was sensitive to any microsphere brought into its close proximity. This particle sensor was based on the generation of an on-chip beat note whose frequency was monitored upon particles approaching the evanescent field of the laser cavity. This is an excellent example of leveraging the active functionalities of this material for a novel sensing scheme that permits a simple readout module. Furthermore, high quality factor undoped Al₂O₃ ring resonators have been recently demonstrated [199], although they were not utilized for biosensing applications and they required rather complicated multi-layered fabrication technologies. In this thesis, both undoped and ytterbium doped Al₂O₃ was developed for use in optical biosensors based on ring resonators, with the objective of developing active, self-referenced biosensors interrogated by a simple readout module based on the detection of an optical beat note. To achieve this goal, sensors on this material platform need to be developed and tested, which will be performed on passive, undoped devices to assess the basic performance of the platform, followed by using active, doped devices for active sensing technologies and beat note detection. The technology developed during this work constitutes a series of first proof-of-concept demonstrations that pave the way

Fig. 1.3: Examples of $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ devices. (a) $\text{Er}^{3+}:\text{Al}_2\text{O}_3$ spiral amplifier [182]. (b) $\text{Er}^{3+}:\text{Al}_2\text{O}_3$ ring resonator laser [185]. (c) Integration of $\text{Er}^{3+}:\text{Al}_2\text{O}_3$ with the passive SOI platform [186]. (d) Integration of undoped Al_2O_3 with the SiN passive platform [193]. (e) Integration of $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ ring resonator laser with SiN passive platform [185]. (f) $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ distributed feedback laser used for particle sensing [164]. (g) Al_2O_3 ring resonator sensor developed in this thesis.

towards the development of a low-cost sensor that could eventually find its way out of the lab and into a clinical setting.

1.3 Outline of the thesis

This thesis concerns the design, fabrication and characterization of integrated optical biosensing technologies for the detection of protein biomarkers on the novel Al_2O_3 photonic platform. Both undoped and doped Al_2O_3 were investigated, the former to assess the sensing performance of the Al_2O_3 platform and the latter to use it for active and beat note sensing technologies. Due to its relevance as a disease biomarker, the S100A4 protein was used throughout this thesis for the testing of the developed integrated optical sensing technologies. The rest of the thesis is organized as follows. Chapter 2 presents the development of Al_2O_3 ring resonators. The theoretical analysis and design was performed for two operation wavelengths, 1035 nm and 1570 nm. The optimized devices were fabricated and their performance was tested. Chapter 3 details the use of undoped Al_2O_3 ring resonators as integrated optical sensors. Bulk refractive index and temperature sensitivities were tested, together with the development of a surface functionalization protocol to immobilize a bioreceptor layer of anti-S100A4 monoclonal antibodies to detect the rhS100A4 protein from a complex matrix.¹ Chapter 4 describes the realization of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonator lasers that were used for active sensing. Their laser performance in an aqueous cladding was characterized, together with a demonstration of active biosensing functionalities.² Chapter 5 explores the possibility of integrating a grating on an undoped Al_2O_3 ring resonator to induce mode-splitting. A proof-of-concept demonstration of biosensing using the developed device is presented.³ Chapter 6 describes the generation of radio frequency signals by integrating gratings on active ring resonator lasers. A biosensor based on beat note detection is successfully demonstrated.⁴ The thesis concludes with a summary of the work together with an outlook of future work in Chapter 7.

¹ The results were published in the OSA Optics Express journal in the paper titled “ Al_2O_3 microring resonators for the detection of a cancer biomarker in undiluted urine” [200].

² The results were published in the OSA Optics Letters journal in the paper titled “ $\text{Al}_2\text{O}_3:\text{Yb}^{3+}$ integrated microdisk laser label-free biosensor” [201].

³ The results are described in the manuscript “Mode-splitting in a microring resonator for self-referenced biosensing”, which has been submitted for publication.

⁴ The results are described in the manuscript “ $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ self-referenced biosensors based on beat note detection”, which is being prepared for submission.

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Chapter 2

Al₂O₃ ring resonators

Ring resonators are circular optical cavities formed by looping a waveguide onto itself. Constructive interference allows long light circulation inside the resonator at specific wavelengths, which are accompanied by sharp resonances in the transmission spectrum of the ring resonator. Tracking these resonances allows the use of ring resonators for optical sensing. This chapter describes the theoretical analysis, geometrical design, fabrication and characterization of Al₂O₃ ring resonators in the context of optical biosensors. This was done at two central operation wavelengths of 1035 and 1570 nm. A highest quality factor of 5.1×10^5 and waveguide propagation loss down to 0.42 dB/cm was achieved for a radius of 200 μm at a wavelength of 1570 nm for a cladding of air.

Ring resonators are a fundamental component of integrated optics and they are found in a wide variety of applications. They are circular cavities that support optical waves travelling along its perimeter [1]. Whenever the light travelling inside the cavity accumulates a phase of an integer times 2π after completing one roundtrip, constructive interference allows the light to continue propagating inside the resonator. This only occurs at specific wavelengths that are accompanied by sharp resonances in the transmission spectrum of the ring resonator. One of the first mentions of a ring resonator in the scientific literature was the introduction of a pillbox resonator by Marcatili in 1969, who published a detailed study and simulation of a ring resonator for usage as a bandpass filter [2]. This bandpass filter consisted of a waveguide that loops onto itself, the ring resonator, coupled to two bus waveguides, as shown in Fig. 2.1. (a). Since then, many different types of ring resonators were demonstrated on numerous material platforms, including silicon-on-insulator (SOI) [3], silicon nitride (SiN) [4], InP [5] and polymer waveguides [6].

Ring resonators are frequently found in optical sensing applications because of their high sensitivity and low *LOD* to changes in refractive index [7–9]. Such optical sensors are often found in the notch filter configuration, as shown in Fig. 2.1. (b), where the ring is coupled to a single bus waveguide. This Chapter describes the analysis, design, fabrication and characterization of ring resonators based on a notch filter configuration for Al_2O_3 integrated optical biosensors.

2.1 Analysis of a waveguide ring resonator

Ring resonators can be modelled by a variety of methods, including the finite-difference time-domain methods that fully solves Maxwell's equations, as can be

Fig. 2.1: Schematic of ring resonators. (a) Bandpass filter proposed by Marcatili [2]. The bandpass filter selects only a discrete set of frequencies f_i that are filtered out of the input signal. (b) Model of a ring resonator coupled to a single bus waveguide: the notch filter.

done for the ultra-compact SOI ring resonators [10]. However, since the refractive index contrast of Al_2O_3 on a SiO_2 substrate is rather small (~ 0.2), the considered structures are too large to be simulated with this method due to tremendous computational costs. They can be modelled semi-analytically using coupled mode theory and the transfer matrix method to derive a theoretical transmission function, the parameters of which can be extracted from waveguide mode calculations using commercially available software [1,3,11]. This approach was employed here.

2.1.1 Spectral resonances

The basic geometric configuration of a ring resonator is shown in Fig. 2.1 (b). It consists of a circular waveguide cavity with radius R that has a bus waveguide placed tangential to it. The ring resonator has a circular shape with circumference $L=2\pi R$, although various other shapes are possible including folded or hairpin resonators that have increased path lengths [12] or racetrack resonators for more control of the coupling section [13]. In the following treatment, only a single-mode transverse electric (TE) or transverse magnetic (TM) waveguide mode travelling inside the ring resonator is considered, although it can easily be extended for the case of a multi-mode waveguide or a disk resonator that supports multiple radial waveguide modes [14,15]. The spectral response of the ring resonator can be described based on the coupling coefficients of the electric field in the directional coupler, the optical attenuation inside the ring resonator and the phase accumulation of the waveguide mode propagating along the perimeter of the ring resonator.

Following the diagram of Fig. 2.1 (b), the scalar incident field on the bus waveguide $E_{\text{in},1}$ can couple to the ring through either evanescent coupling or by a multimode interference coupler. $E_{\text{out},2}$ is the fraction of incident light coupled into the ring resonator, $E_{\text{out},1}$ is the fraction of incident light transmitted through the bus waveguide, and $E_{\text{in},2}$ is the field within the ring resonator after completing a roundtrip. The corresponding unitary transfer matrix of a lossless and reciprocal directional coupler is given by [16]:

$$\begin{pmatrix} E_{\text{out},1} \\ E_{\text{out},2} \end{pmatrix} = \begin{pmatrix} t & k \\ -k^* & t^* \end{pmatrix} \begin{pmatrix} E_{\text{in},1} \\ E_{\text{in},2} \end{pmatrix} \quad \text{Eq. (2.1)}$$

where t is the transmission coefficient and k the coupling coefficient of the field amplitude. After coupling into the ring resonator, the light propagates along the

perimeter accumulating a phase θ and a field attenuation of a (i.e., $E_{in,2}=ae^{j\theta}E_{out,2}$), where θ is given by:

$$\theta = \frac{2\pi L n_{\text{eff}}}{\lambda}, \quad \text{Eq. (2.2)}$$

where n_{eff} is the effective refractive index of the waveguide mode and λ is the wavelength. The waveguide losses originate from several sources, including material absorption due to structural defects and absorption inside the waveguide material, coupling to radiation modes due to the curvature of the waveguide and scattering of the light due to surface roughness on the surface of the waveguide [17]. The field loss coefficient, a is given by:

$$a = 10^{-\frac{\alpha L}{20}}, \quad \text{Eq. (2.3)}$$

where α is the total sum of the material, bend and scattering losses in units of dB/m. The field leaving the bus waveguide, $E_{out,1}$, can be derived from Eq. 2.1 and is given by:

$$E_{out,1} = E_{in,1} \frac{-a + te^{-j\theta}}{-at^* + e^{-j\theta}}. \quad \text{Eq. (2.4)}$$

Upon normalizing the incident field $E_{in,1}=1$, the power transmission function of the ring resonator is given by [1]:

$$T = |E_{out,1}|^2 = \frac{a^2 + |t|^2 - 2a|t|\cos(\theta + \varphi)}{1 + a^2|t|^2 - 2a|t|\cos(\theta + \varphi)}. \quad \text{Eq. (2.5)}$$

where $t=|t|e^{j\varphi}$, with φ the phase of the directional coupler. Whenever the waveguide mode acquires a phase of $\theta+\varphi=2m\pi$, with m an integer determining the azimuthal order of the resonance, the ring is on resonance and the transmission spectrum contains a sharp minimum at the corresponding resonance wavelength $\lambda_{\text{res}}=n_{\text{eff}}L/m$. Then, the light in the ring resonator can circulate for many roundtrips, limited by the internal cavity loss and by the outcoupling of the cavity.

2.1.2 Ring resonator parameters

Figure 2.2 shows the calculated wavelength-dependent transmission spectrum of a ring resonator containing several resonances. The transmission spectrum is characterized by the free spectral range (*FSR*), defined as the spacing between two

Fig. 2.2: Ring resonator transmission function containing several resonances.

consecutive resonances, and the full width at half maximum (*FWHM*) of the resonances. The *FSR* can be derived as [18]:

$$FSR = \frac{\lambda^2}{n_g L}, \quad \text{Eq. (2.6)}$$

where n_g is the group index of the waveguide mode and is given by:

$$n_g = n_{\text{eff}} - \lambda \frac{dn_{\text{eff}}}{d\lambda}. \quad \text{Eq. (2.7)}$$

For a single resonance, its *FWHM* can be derived as [18]:

$$FWHM = \frac{(1 - a|t|)\lambda^2}{\pi n_g L \sqrt{a|t|}}. \quad \text{Eq. (2.8)}$$

There exists two measures of the sharpness of a ring resonator, the finesse F and the quality factor Q . The former is a measure of the sharpness relative to the spacing of the resonances, the latter is a measure of the sharpness relative to the resonance frequency. They are given by:

$$F = \frac{FSR}{FWHM}, \quad \text{Eq. (2.9)}$$

$$Q = \frac{\lambda}{FWHM}. \quad \text{Eq. (2.10)}$$

Both F and Q relate to the number of roundtrips that an optical mode can propagate before it is fully lost due to the internal or coupling losses. More specifically, F

represents, within a factor of 2π , the number of roundtrips that the field completes before its energy is reduced by a factor $1/e$, Q represents the number of oscillations of the field before its energy is reduced by a factor $1/e$. A distinction is often made between loaded and unloaded, or intrinsic and external Q . The former is the one given by Eq. 2.10, the latter is the Q when coupling loss is not considered. Throughout this thesis, Q refers to the loaded quality factor taking into account coupling losses. Finally, the extinction ratio ER describes how deep the resonance is. The ER equals the ratio between the highest and lowest value of the transmission spectrum over a distance of a single FSR :

$$ER = \frac{T_{\max}}{T_{\min}} = \left(\frac{(a + |t|)(1 - a|t|)}{(a - |t|)(1 + a|t|)} \right)^2. \quad \text{Eq. (2.11)}$$

The performance of a ring resonator is mostly determined by two parameters, the loss coefficient, a , and the transmission coefficient, $|t|$, which are both functions of wavelength. They can be extracted from measurements by determining both the $FWHM$ and ER , followed by first solving for the product $a/|t|$ in Eq. 2.8 and then substituting the result in Eq. 2.11. This results in a quadratic equation, the roots of which are given by a and $|t|$. However, the coupling coefficients appear symmetric in both Eq. 2.8 and Eq. 2.11. Therefore, additional analysis is required to distinguish between the internal losses and the coupling parameter [12,19]. Typically, the analysis described above is performed for several ring resonators that have different geometries, such as variations in the directional coupler (i.e., varying $|t|$) or in roundtrip losses (i.e., varying a). Then, by keeping either a or $|t|$ constant while varying the other parameter, the values of a and $|t|$ can be decoupled. After correctly obtaining a , the propagation loss of the waveguide can be extracted through Eq. 2.3.

2.1.3 Ring resonator sensor

The resonance of a ring resonator is very sensitive to external perturbations due to the evanescent field extending outside the optical waveguide into the sensing region. A ring resonator can therefore be used as a refractive index sensor. A change of the dielectric environment surrounding the ring resonator dictates a change of the effective refractive index of the waveguide mode, Δn_{eff} , which is accompanied by a shift of the resonance wavelength, $\Delta \lambda_{\text{res}}$, as shown in Fig. 2.3. A ring resonator is very versatile to detect dielectric perturbations of various origins, including

Fig. 2.3: Sensing principle of a ring resonator. A perturbation of the dielectric environment of the ring resonator, indicated by $\Delta\epsilon$, and thus a modification of the optical path length, shifts the resonance wavelength.

temperature [20], bulk refractive index [21], molecule binding [22], strain [23], gas [24] or nanoparticles [25]. The wavelength shift of a ring resonator is given by [3]:

$$\Delta\lambda = \frac{\Delta_{\text{env}} n_{\text{eff}} \lambda}{n_g}, \quad \text{Eq. (2.12)}$$

where $\Delta_{\text{env}} n_{\text{eff}}$ is the effective index change due to an environmental change, which can be obtained from the variational theorem of waveguides and is expressed as [3]:

$$\Delta_{\text{env}} n_{\text{eff}} = c \iint \Delta\epsilon(x, y) |\mathbf{E}|^2 dx dy, \quad \text{Eq. (2.13)}$$

where c is the speed of light in vacuum, $\Delta\epsilon$ is the dielectric perturbation that is applied to either the ring resonator or its surroundings, $\mathbf{E}$ is the normalized electric field vector of the waveguide mode travelling in the ring resonator, and the integration extends over the whole cross-sectional plane perpendicular to the propagation direction. Sensitive sensing performance is obtained by optimizing the modal overlap of the electric field with the region of the sensing analyte.

The sensitivity of a ring resonator is the amount of resonance wavelength shift per amount of applied perturbation. The cases considered in this thesis are the temperature sensitivity, S_T , for temperature variations (Eq. 2.14), the bulk refractive index sensitivity, S_{RIU} , for refractive index variations (Eq. 2.15) and the surface sensing sensitivity, S_{prot} , for a layer of (bio)molecules (such as proteins) bonded on top of the ring resonator (Eq. 2.16). The latter is often expressed as a function of the thickness, t_{prot} , of the deposited layer, which is assumed to have a constant refractive index. These sensitivities are given by:

$$S_T = \frac{\Delta\lambda}{\Delta T}, \quad \text{Eq. (2.14)}$$

$$S_{\text{RIU}} = \frac{\Delta\lambda}{\Delta n}, \quad \text{Eq. (2.15)}$$

$$S_{\text{prot}} = \frac{\Delta\lambda}{\Delta t_{\text{prot}}}, \quad \text{Eq. (2.16)}$$

where ΔT is the change of temperature, Δn is the change of bulk refractive index and Δt_{prot} is the change of thickness of the deposited layer on the surface of the waveguide.

2.2 Ring resonator design

The design of Al_2O_3 ring resonators was performed at two central operation wavelengths of 1035 nm and 1570 nm. Optical sensing is possible at both wavelengths and it will be detailed in the next Chapters. Sensing at 1035 nm will be performed with active, Yb^{3+} -doped devices and sensing at 1570 nm will be carried out on passive, undoped devices.

The design starts by first determining the waveguide cross-section, which is a rectangular fully etched Al_2O_3 waveguide on a thermally oxidized silicon substrate, SiO_2 , submerged in an aqueous cladding. Figure 2.4 illustrates the waveguide cross-section with waveguide width W and height H . The main considerations for the cross-sectional design are single-mode operation for both the TE and TM polarizations and a high bulk refractive index and surface sensing sensitivities. The first consideration is necessary since higher order waveguide modes will lead to a more complex transmission spectrum which will make it more difficult to track the resonances for sensing. The second is required for getting as large resonance wavelength shifts as possible. The waveguide cross-section controls both of these.

Fig. 2.4: Al_2O_3 waveguide cross-section indicating the refractive indices (wavelength of 1035 nm).

Furthermore, the design addresses a trade-off between high sensitivity (i.e., as high overlap as possible of the electric field with the analyte) and low bend radiation losses (i.e., as high confinement within the core of the waveguide as possible). To ensure a high Q , the maximum allowed bend radiation loss given a certain cross-section is limited to 0.5 dB/cm, for which the corresponding minimal bend radius was determined. To obtain a small footprint, allowed cross-sections require a minimal bend radius of at least 100 μm . Given this constraint, the cross-section with largest sensitivity was chosen. Finally, the coupling section is designed based on the chosen waveguide cross-section and bend radius, for which the coupling gap was determined such that $a=|t|$ (i.e., critical coupling). The commercially available software Lumerical MODE Solutions was used to perform fully vectorial two-dimensional finite difference eigenmode calculations to determine the waveguide mode profiles and extract the relevant design parameters (i.e., the n_{eff} , sensitivities, bend loss and coupling coefficients given a set of W and H). The refractive indices of the materials are summarized in Table 2.1. In the following sections, the results of the design of the ring resonator used in rest of the thesis are detailed.

Table 2.1. Material parameters.

λ (nm)	Material	n
1035	Al_2O_3 ¹	1.671
1570	Al_2O_3 ¹	1.668
1035	SiO_2 [26]	1.450
1570	SiO_2 [26]	1.444
1035	H_2O [27]	1.327
1570	H_2O [27]	1.317

2.2.1 Sensitivity

The refractive index sensitivity is directly related to the overlap of the electric field of the mode with the region of the analyte. By varying the cross-section of the waveguide, the overlap of the electric field with the sensing region varies and, therefore, different sensitivities can be obtained. The sensitivities were calculated according to Eqs. 2.12—2.16 for various waveguide heights and widths. First, given a waveguide cross-section and the wavelength of interest, the number of modes supported by the waveguide as well as their respective electric mode field distributions and effective refractive indices, n_{eff} , were calculated. The group index, n_g , was then computed according to Eq. 2.7. Then, $\Delta_{\text{env}}n_{\text{eff}}$ was determined for the case of a change of bulk refractive index, and for the case in which the waveguide is

¹ Measured in-house using a Woollam M-2000UI ellipsometer.

Fig. 2.5: Sensitivities for various waveguide cross-sections at a wavelength of 1570 nm. The dots indicate the cut-off condition for single-mode operation. (a) Bulk refractive index sensitivity as function of waveguide width and height for TE polarization. (b) Bulk refractive index sensitivity for TM polarization. (c) Surface sensing sensitivity for TE polarization. (d) Surface sensing sensitivity for TM polarization.

covered by a 10 nm thick layer of refractive index 1.5 representing the binding of a protein layer. The resulting S_{RIU} and S_{prot} for both polarizations at a wavelength of 1570 nm are shown in Fig. 2.5. The sensitivity increases when the cross-section is reduced, either by width or height, due to the increase in the extent of the mode electric field outside the core of the waveguide as the waveguide dimensions are reduced. In almost all cases, the sensitivity is highest for TM polarization, which typically has a larger fraction of electric field extending in the top cladding, as can be seen in Fig. 2.6. The cross-sections considered have typical bulk refractive index sensitivities of ~ 50 – 150 nm/RIU, values that are comparable to those encountered in literature for similar ring resonator sensors based on the conventional material platforms [28–31].

Fig. 2.6: Electric field profiles of the fundamental modes of a waveguide a cross-section of $2 \times 0.7 \mu\text{m}^2$ at a wavelength of 1570 nm. Only the dominant components of the mode are visualized. (a) E_x component of the TE waveguide mode. (b) E_y component of the TM waveguide mode.

2.2.2 Bend losses

Straight waveguides usually have a symmetric distribution of the intensity of the waveguide mode with respect to the waveguide center. However, in a bent waveguide the mode shifts to the edge of the waveguide and experiences leakage loss through coupling to radiation modes [32]. Furthermore, if the cavity is in the racetrack geometry, mode mismatch losses are present at the transition from the straight to bend section, which can be reduced by shifts of the waveguide core [33] or more complex bends, such as sine bends [34]. Although the radiation loss can be suppressed by introducing a thin metal layer [35] or multiple concentric rings [36], a plain circular ring resonator is considered here since it has a simpler design and fabrication process. Then, the bend losses can be simulated by first calculating the waveguide field in polar coordinates, followed by converting the corresponding imaginary part of the effective refractive index in a loss term [37]. The following analysis is based on the TM polarization since it has the highest sensitivities, as was shown in Fig. 2.5.

Fig. 2.7: TM polarization bend loss at a wavelength of 1570 nm. (a) Waveguide height of 800 nm. (b) Waveguide height of 600 nm.

Figure 2.7 shows the bend loss at a wavelength of 1570 nm of an Al_2O_3 waveguide for TM polarization for various waveguide widths and two heights of 600 nm and 800 nm. In both cases the bend losses increase upon decreasing the bend radius. Furthermore, the bend losses are larger for the thinner waveguide, since it has a smaller confinement and larger evanescent field. Therefore, the cross-section cannot be arbitrarily reduced to achieve the highest sensitivities while also maintaining a high Q . The cross-section yielding the highest sensitivity given a maximum bend loss of 0.5 dB/cm for a minimum radius of 100 μm was found and the resulting waveguide designs are summarized in Table 2.2, together with the corresponding sensitivity. In practice, the bend radius is always chosen to be larger than 100 μm to anticipate fabrication errors and to further minimize bend losses. On the other hand, increasing the radius allows for smaller cross-sections due to the reduced bend loss, and thus could allow for higher sensitivities.

Table 2.2. Al_2O_3 waveguide design parameters for TM polarization.

λ (nm)	W (μm)	H (μm)	S_{RIU} (nm/RIU)	S_{prot} (pm/nm)
1035	1.4	0.50	73	73
1570	2.0	0.75	110	101

2.2.3 Coupling section

Given a waveguide cross-section and a ring resonator radius, the coupling coefficient can be calculated for an arbitrary directional coupler. This is done by following the coupled mode theory formalism, as described by [38,39]. By using this method, the evolution of the modal amplitudes of two waveguides along a spatial path, which can have any three-dimensional geometry including the one of the directional coupler of a ring resonator, can be calculated [40–43]. Figure 2.8 shows the geometry of this calculation. The directional coupler is discretized over a length of $2L$ in $2n$ small segments along the z -direction, each segment, z_i , having a length of Δz_i . The mode amplitudes, $A(z)$ and $B(z)$, propagate respectively in the straight waveguide and the bent waveguide. Assuming that a segment z_i has a constant separation $g(z_i)$ with coupling coefficient $K(z_i)$, the coupled mode equations of the mode amplitudes can be solved for all segments with solutions [41]:

$$\begin{pmatrix} A_i \\ B_i \end{pmatrix} = \begin{pmatrix} \cos(K_i \Delta z_i) & -j \sin(K_i \Delta z_i) \\ -j \sin(K_i \Delta z_i) & \cos(K_i \Delta z_i) \end{pmatrix} \begin{pmatrix} A_{i-1} \\ B_{i-1} \end{pmatrix}. \quad \text{Eq. (2.17)}$$

Fig. 2.8: Diagram of the straight to bent waveguide coupling in a ring resonator.

This method can be applied to all segments along the length of the directional coupler and, in the limit of $\Delta z_i \rightarrow 0$, the resulting mode amplitudes are given by [41]:

$$\begin{pmatrix} A(L) \\ B(L) \end{pmatrix} = \begin{pmatrix} \cos\left(\int_{-L}^L K(z)dz\right) & -j \sin\left(\int_{-L}^L K(z)dz\right) \\ -j \sin\left(\int_{-L}^L K(z)dz\right) & \cos\left(\int_{-L}^L K(z)dz\right) \end{pmatrix} \begin{pmatrix} A(-L) \\ B(-L) \end{pmatrix}. \quad \text{Eq. (2.18)}$$

Equation 2.17 is a more complete description of the transfer matrix of the directional coupler given in Eq. 2.1. The coupling coefficient $K(z)$ is given by coupled mode theory as [41]:

$$K(z) = \frac{\omega}{4} \iint \Delta\epsilon(x, y) |\mathbf{E}_A(x, y)| |\mathbf{E}_B(x, y)| \cos(\theta_i) dx dy, \quad \text{Eq. (2.19)}$$

where $\mathbf{E}_A(x, y)$ and $\mathbf{E}_B(x, y)$ are the normalized vector electric fields of the waveguide modes in the straight and bent waveguide respectively, ω is their angular frequency, $\Delta\epsilon$ is the perturbation in dielectric constant as experienced by the mode travelling in

the bus waveguide due to the presence of the bent waveguide and θ_i the angle between the wave vectors of the two modal fields. The integral extends over the spatial region of the dielectric perturbation.

Equations 2.17—2.19 provide a strategy for designing the coupling and transmission coefficients given a certain waveguide cross-section and ring radius. First, given a geometry of a directional coupler (i.e., radius of the ring resonator and minimum separation between ring and bus waveguide g_{minimal}), the separation $g(z)$ is calculated along the propagation direction of the directional coupler. Then, given a waveguide cross-section and corresponding mode, the coupling coefficient $K(z)$ is repeatedly calculated for all separation distances $g(z)$ along the directional coupler. By performing the integration of the matrix elements in Eq. 2.18, the transmission and coupling coefficients of Eq. 2.1 are calculated, which can be repeated for various coupler geometries. This calculation was performed for the designed waveguide geometry and is shown in Fig. 2.9. The coupled power $|k|^2$ decreases for increasing coupling gaps, since the evanescent field decays exponentially outside the waveguide core.

In order to get the deepest resonances, the internal losses of the ring resonator need to be matched to the coupling losses. This condition is known as critical coupling and it requires that $\alpha=|t|$. Given propagation losses in the range 0.5—5 dB/cm, this condition is close to being fulfilled for coupling gaps in the range 600—1000 nm for bend radii in the range 100—200 μm . For practical purposes, the design will contain

Fig. 2.9: Straight to bent waveguide coupling at a wavelength of 1570 nm for a waveguide cross-section of $2.0 \times 0.75 \mu\text{m}^2$ and a radius of 200 μm . (a) Power coupling along the propagation direction z of the directional coupler for various coupling gaps g . Solid lines represent $|k|^2$, dashed lines $|t|^2$. (b) Coupled power $|k|^2$ as function of coupling gap.

multiple identical ring resonators with a variation of coupling gaps to increase the chance of achieving critical coupling given fabrication errors.

2.3 Fabrication

Devices were fabricated using the determined design parameters. Multiple variations of the design parameters were included as de-risking strategy due to the tolerance in the fabrication of the devices. The fabrication was performed at the cleanroom of the MESA+ Institute for Nanotechnology at the University of Twente.

2.3.1 Sputter deposition of Al_2O_3

Al_2O_3 layers were deposited using a radio-frequency reactive co-sputtering process on an AJA ATC 1500 instrument, which is schematically shown in Fig. 2.10. Silicon substrates with an 8 μm thick thermal oxide layer were mounted on the substrate holder, which is in a face down configuration. The holder can be rotated and translated vertically to have a distance of 4—7 inches from the sputtering targets. Two 1 kW halogen lamps are placed above the substrate holder to heat the substrate holder to a maximum set temperature of 700 °C. The actual substrate temperature is, however, lower than the set temperature [44]. The reaction chamber can be pumped down to a pressure of 10^{-7} mTorr, which is required to reach a negligible level of OH^- contamination on the sputtered film. OH^- contamination induces luminescence quenching on layers doped with rare-earth ions [45,46] and absorption losses around 1240 and 1380 nm [47]. The sputter deposition machine has three sputtering guns that are equipped with a 2-inch Al (99.9995% purity), Er (99.95% purity), and Yb (99.9% purity) target respectively. These can be controlled individually by either DC or RF power supplies, of which two can be fired simultaneously. An Ar flow is applied to each target, where it ionizes and is accelerated towards the target, inducing sputtering of the target surface material. An O_2 flow is added to oxidize the sputtered material.

The optimum deposition parameters were mostly reproduced from previous work by [48,49] and they are summarized in Table 2.3. A substrate height of 6 $\frac{3}{4}$ inch was used due to this position resulting in layers with a high uniformity of 93% over a distance of 5 cm away from the center of the substrate. The substrate holder was heated at a set temperature of 550 °C, which translates to a substrate temperature of ~400 °C. Increasing the substrate temperature has been reported to result in lower optical losses [50], however, a threshold exists upon which crystallization occurs

Fig. 2.10: Schematic representation of an AJA ATC 1500 reactive co-sputtering system. Adapted from [48].

resulting in cracking due to stress [48,51], limiting the maximum temperature close to the used set value. After stabilization of the substrate temperature and reaching a sufficiently low chamber pressure, a RF power of 200 W was applied to the Al target together with an Ar flow of 25 sccm. The chamber pressure was then adjusted to 3.7 mTorr by controlling the pump valve connected to the reaction chamber.

Table 2.3. Deposition parameters for reactive co-sputtered Al_2O_3 layers.

Set temperature (°C)	Power (W)	Ar flow (sccm)	O ₂ flow (sccm)	Height (in)	Pressure (mTorr)
550	200	25	2.5	6¾	3.7

It is crucial that the deposited Al_2O_3 is free from scatter or absorption points to yield the lowest possible propagation losses. This requires the absence of any free Al-atoms to prevent a metallic content, which leads to high optical losses. It also requires the absence of a polycrystalline phase, which will induce volume scattering

Fig. 2.11: Sputtering of Al₂O₃ layers. (a) Bias voltage curve indicating the optimum operation point. (b) Light propagation in a high quality Al₂O₃ layer in a prism coupling setup. (c) Light propagation in a poor quality Al₂O₃ layer in a prism coupling setup. Wafers have a 10 cm diameter.

losses. The O₂ flow into the chamber controls the amount of oxidation of the sputtered Al and the stoichiometry of the deposited layer [52–55]. Whenever the O₂ flow is low, not all the sputtered Al oxidizes and the layers can contain a metallic Al content. At this point the deposition speed of the formed layer is the highest. Upon increasing the O₂ flow to a certain value, all the sputtered Al gets fully oxidized and the deposited layer has the right stoichiometry. Further increasing the O₂ flow partially oxidizes the target, resulting in a drop of the bias voltage on the Al target due to an increased secondary electron emission yield of Al₂O₃ versus Al [56]. The deposition speed is also drastically reduced, due to the lower sputtering yield of Al₂O₃. This drop of bias voltage stops whenever the O₂ flow is high enough to fully oxidize the target, at which point the deposition speed is lowest and further increasing the O₂ flow does not affect the process anymore. The optimum O₂ flow occurs just before the onset of oxidation of the target, since then the deposited layer has the proper stoichiometry and the deposition speed is the highest [52].

Measuring the bias voltage on the target provides a method of determining the settings of the deposition process, as shown in Fig. 2.11 (a). A drop of bias voltage is observed upon increasing the O₂ flow above 2.5 sccm, indicating the point where the deposited layer is fully oxidized and the onset of target oxidation. At this point the deposition speed is around 4–6 nm/min, which drops significantly for higher O₂ flows. The thickness and refractive index of the deposited Al₂O₃ layers are measured using a Metricon 2010/M prism coupling setup at a wavelength of 632.8 nm. Optical losses can be determined qualitatively using this setup by observing the propagation of the laser light coupled into the layer, as shown in Figs. 2.11 (b) and (c). Only

layers with sufficiently good optical guiding are used for further processing. Two sets of layers were deposited, one for each target wavelength. The best deposited layer for a wavelength of 1570 nm had a thickness of 801 nm and refractive index of 1.6647, for a wavelength of 1035 nm the best layer had a thickness of 556 nm and refractive index of 1.6675. In both cases, the deposited layers were slightly thicker than the designed value due to fabrication errors. Both showed no visible attenuation of laser light coupled into it.

2.3.2 Lithography and etching

The ring resonators and bus waveguides were defined in the Al_2O_3 layer using inductively-coupled plasma reactive ion etching by the method of [57]. First, the Al_2O_3 layer was coated with an OiR907-12 photoresist mask with thickness of 1.2 μm using spin-coating. It was patterned using standard UV contact lithography methods using an EVG 620 exposure tool. The used etching process was already previously optimized for selectivity and etching speed in the work by [57]. Etching was performed on an Oxford Plamalab 100 ICP reactive ion etching system, using a gas mixture of BCl_3 and HBr at a ratio of 5:2. Process parameters were a RF power of 25 W, ICP power of 1750 W, pressure of 12 mTorr and total gas flow of 35 sccm. The process had an etching speed of ~ 50 nm/min and the resulting waveguides had low sidewall roughness and an angle of 75° . A SEM image of the cross-section of an etched waveguide is shown in Fig. 2.12 (a). Due to fabrication errors the waveguide has a width of 2.15 μm at its top and height of 801 nm, which is slightly larger than the designed values of $2.0 \times 0.75 \mu\text{m}^2$. This will result in a larger confinement and thus a lower coupling coefficient and lower sensitivity than the designed values.

Fig. 2.12: Waveguide cross-section. (a) Secondary electron SEM image of Al_2O_3 waveguide cross-section without cladding. (b) Backscattering electron SEM image of cross-section of coupling gap covered in SiO_2 cladding.

The lithography and etching process introduce a mismatch between designed and fabricated coupling gap. Figure 2.12 (b) shows a SEM image of a directional coupler that is covered by a SiO_2 top cladding. The designed coupling gap had a width of 800 nm, but the resulting fabricated structure has a coupling gap of ~ 600 nm. This, together with a wider waveguide and sidewall angle, occurs because not all material in the coupling gap could be removed during the etching process. This is a systematic effect observed in all directional couplers. This effect becomes more pronounced for smaller coupling gaps. For gaps smaller than 600 nm the two waveguides remain connected. This results in more power coupling from the bus waveguide to the ring resonator, increasing the coupling losses and decreasing the Q . However, this can be simply compensated by setting the designed coupling gap at a slightly higher value.

2.3.3 Chip preparation

After etching of the devices, the residual resist mask is removed with an O_2 plasma etch followed by a wet cleaning step in 99% HNO_3 . This is followed by deposition of a SiO_2 cladding over the devices to prevent waveguide damage. However, the ring resonators should remain exposed to be addressed by liquids flowing over it for sensing. A shadow mask was used to deposit SiO_2 over the whole substrate while leaving a region around the ring resonators open. The SiO_2 cladding was deposited using an Oxford PECVD 80 system to deposit the cladding with plasma enhanced chemical vapor deposition at a temperature of 300 °C. The deposited cladding was 3 μm thick.

The chips were singulated using a Micro Ace 3 dicing machine. First, the sample was spin-coated with a thick photoresist, OiR 908-35, with a thickness of 3.5 μm to prevent both damage to the ring resonators as well as particle contamination. A

Fig. 2.13: PDMS-bonded device. (a) Optical microscope image of Al_2O_3 ring resonators inside a PDMS microfluidic channel. The rings have a radius of 200 μm . (b) Photograph of the optofluidic device with fluidic connections. The chip has dimensions of $1.2 \times 1.9 \text{ cm}^2$.

diamond dicing blade with a rotation speed of 30 krpm and transverse speed of 0.5 mm/s was used to dice chips with dimensions of $1.2 \times 1.9 \text{ cm}^2$. The end facets had minimal damage and provided good optical coupling. To enhance the coupling to the chip, a horizontal waveguide taper was used with a width of $10 \text{ }\mu\text{m}$. Afterwards, the chip was cleaned by stripping the resist in acetone, followed by a wet cleaning in HNO_3 .

In order to address the ring resonators with various liquid analytes, a PDMS microfluidic device was developed. A fluidic mold was made consisting of a rectangular microfluidic channel with cross-section of $700 \times 60 \text{ }\mu\text{m}^2$ and length 1.2 cm. PDMS RTV615 from Permacol was mixed with a curing agent in a ratio of 7:1, after which it was degassed in a vacuum desiccator for one hour and poured over the mold. The PDMS was cured in an oven for 2 hours at $80 \text{ }^\circ\text{C}$. Then, it was cut and bonded to the chip, as shown in Fig. 2.13. Oxygen plasma treatment was not used and the bonding was reversible. Metal connections were attached to the channel and connected to plastic tubes.

2.4 Ring resonator characterization

The fabricated ring resonators were characterized to obtain their optical losses and Q s. The ring resonator requires low optical losses and a large quality factor to provide sharp, narrow resonances that allow for a precise determination of resonance wavelength [58]. An optofluidic experimental setup was developed, a schematic of which is shown in Fig. 2.14 (a). The chip containing the ring resonators is placed on a sample holder and held in place by vacuum contact. A thermoelectric cooler (TEC) is mounted on the sample holder to set the stage temperature within $\pm 1 \text{ mK}$. Two

Fig. 2.14: Ring resonator characterization method. (a) Schematic of the experimental setup. (b) Optical microscope image of alignment laser light coupled into the ring resonator.

polarization maintaining fibers were butt-coupled to the chip with index-matching fluid using piezo-controlled micropositioners. To perform the fiber alignment to the chip, red light from a He-Ne laser was coupled to the device as shown in Fig. 2.14 (b). The polarization of the excited waveguide mode could be controlled by rotating the fiber connector coupled to the chip. A syringe pump was connected to the PDMS microfluidic device and used to flow various liquids over the ring resonators. The setup was contained within a plastic box to limit convective air currents and enhance temperature stabilization.

Optical characterization was performed at two wavelengths. Characterization at a wavelength of 1570 nm was performed with an Agilent 81646 tunable laser. This device can scan over a wide range of wavelength (i.e., 1460—1640 nm). Characterization at a wavelength of 1035 nm was performed with a TOPTICA CTL 1050 source that could scan the wavelength over the range of 1020—1070 nm. Both sources were scanned at step sizes of 1 pm to obtain the transmission spectrum. The laser light was guided through the fibers and chip and collected at a power meter, which was synchronized with the tuning wavelength of the laser.

The ring resonator parameters were extracted from the transmission spectra. The resonances can be sufficiently well approximated by a Lorentzian function in a narrow spectral window surrounding the resonance [58,59]. First, the resonances present in the unprocessed data had their background subtracted and were normalized to unity. The corrected spectra were fitted with a Lorentzian function, from which the *FWHM* and *ER* were obtained. These were used in Eqs. 2.8—2.11 to determine the *Q*, *F*, *t* and *a*. The propagation loss was extracted from *a* using Eq. 2.3. This was done for a variety of ring resonators, each with different radii and coupling gaps around the main designed values of Table 2.2. The following sections present the results of the analysis for the highest *Q* ring resonators for both design wavelengths.

2.4.1 Ring resonator characterization at 1570 nm

Transmission spectra were obtained in the wavelength range of 1500—1600 nm for both TE and TM polarized light for a ring resonator with a fabricated waveguide cross-section of $2.15 \times 0.8 \mu\text{m}^2$, radius of 200 μm , a designed coupling gap of 700 nm and a cladding of air. The results are shown in Fig. 2.15. Both the spectra for TE and TM polarized light contained multiple sharp resonances of only the fundamental waveguide mode, as shown in Figs. 2.15 (a) and (e). In both cases, the ring resonator

was slightly undercoupled, resulting in the resonances not reaching full extinction. Furthermore, the resonances deepen slightly at increasing wavelengths. Figures 2.15 (b) and (f) show the Lorentzian fits of the background-corrected, normalized transmission spectra of a resonance at the central operation wavelength. No asymmetries or nonlinearities were present in the resonance that could indicate thermal broadening due to an optical bistability [60] or Fano-like resonances [61,62].

The wavelength dependency of the Q was determined from the Lorentzian fits and is shown in Figs 2.15 (c) and (g). The Q declines slightly with increasing λ . As shown in Figs, 2.15 (d) and (h), whereas the loss coefficient remains almost constant as function of wavelength, the transmission coefficient shows a steady decline for both polarizations. A decrease in t equals an increase of the coupling coefficient k to the ring resonator, since they are related by $t=(1-k^2)^{1/2}$, and increases the losses due to outcoupling and reduces the Q . Since the confinement of the waveguide mode decreases for higher wavelengths, the coupling coefficient increases as function of wavelength. Furthermore, it can be seen that the loss coefficient, a , is slightly lower for TM polarization, indicating that it experiences higher losses. This can be explained from the lower confinement of the TM polarization that increases the overlap with the sidewall roughness, resulting in higher scattering losses. Furthermore, a lower confinement also results in larger bend radiation losses. Finally, the ring resonators are undercoupled since t exceeds a .

The Al_2O_3 ring resonators have a highest Q of 5.1×10^5 with a corresponding propagation loss of 0.42 dB/cm for a cladding of air. Previous work on sputtered Al_2O_3 waveguides reported propagation losses of 0.2 dB/cm for 2.5 μm wide straight ridge waveguide with an etch depth of 220 nm in a 740 nm thick sputtered Al_2O_3 film [57]. The loss of the waveguides reported here are slightly higher. The deep etch performed in this thesis increases the sidewall roughness, and thus scattering losses. However, the deep etch is necessary to allow for tight bend curvatures, which are not possible in shallow ridge waveguides due to them having typically higher bending loss. Su *et al.* [63] reported micro-trench ring resonators with Q exceeding 10^6 . The reported rings are undercoupled with extinction ratio up to 1 dB. Their propagation losses are similar to those of the Al_2O_3 ring resonators reported here, although the waveguides in this work are much simpler to fabricate and require only a single deposition, lithography and etching step [64]. Furthermore, the micro-trench ring resonator consists of a waveguide of much larger dimensions, which increases the confinement of the mode more and, in that way, reduces bend radiation and

Fig. 2.15: Ring resonator characterization around 1570 nm. (a) Full transmission spectrum of ring resonator for TE polarization. (b) Fit of resonance of (a). (c) Quality factors of (a). (d) Loss and coupling coefficients of (a). (e)–(h) Same as (a)–(d), but for TM polarization.

sidewall roughness scattering losses. A similar approach could have been employed in this thesis to further increase the Q of the devices, however, that is not desirable for sensing applications. The results presented here demonstrate that the developed Al_2O_3 ring resonators have a performance comparable to the best examples encountered in the literature.

The ring resonators were then submerged in deionized water and characterized again, as shown in Fig. 2.16. The Q dropped significantly due to the strong light absorption of water at wavelengths in the range 1400–1600 nm [65]. Quality factors of 6.2×10^4 and 4.6×10^4 were measured for TE and TM polarization respectively. The lower Q is not only due to the high water absorption losses, but also to the increased out-coupling of the ring resonator since the aqueous cladding results in a less confined waveguide mode. The evanescent field now extends more into the coupling gap and increases the coupling coefficient, as shown in Figs. 2.16 (b) and (d). Furthermore,

Fig. 2.16: Ring resonator characterization around 1570 nm for an aqueous top cladding. (a) Fit of a resonance for TE polarization. (b) Loss and coupling coefficients for TE polarization. (c) Fit of a resonance for TM polarization. (d) Loss and coupling coefficients for TM polarization.

the losses decrease at higher wavelengths due to water having a lower absorption coefficient at higher wavelengths in the measurement range, as can be clearly seen by the increase of a as function of wavelength.

2.4.2 Ring resonator characterization at 1035 nm

The same experiment and analysis was performed on ring resonators designed for operation at 1035 nm. Transmission spectra were obtained in the wavelength range of 1020–1070 nm for a ring resonator with a fabricated waveguide cross-section of $1.5 \times 0.55 \mu\text{m}^2$, radius of $150 \mu\text{m}$ and a coupling gap of 700 nm . Since water absorption is almost negligible at this central wavelength, the characterization was only performed with deionized water as cladding, as shown in Fig. 2.17. Again, the spectrum contained sharp resonances corresponding with only the fundamental waveguide mode. Q s of 1.5×10^5 and 1.2×10^5 were obtained for TE and TM polarization respectively. The Q s are relatively low despite the lower water

Fig. 2.17: Ring resonator characterization in water around 1035 nm. (a) Fit of a resonance for TE polarization. (b) Loss and coupling coefficients for TE polarization. (c) Fit of a resonance for TM polarization. (d) Loss and coupling coefficients for TM polarization.

absorption losses, presumably due to increased scattering losses at lower wavelengths [66].

2.4.3 Comparison of Al_2O_3 with Si-based ring resonator platforms

The performance of Al_2O_3 ring resonators is compared with the silicon-based ring resonator material platforms SiN and SOI. This is done in terms of transparency window, propagation loss and Q , refractive index, footprint, and bending radius. An overview of the Al_2O_3 ring resonator parameters is given in Table 2.4.

Table 2.4. Al_2O_3 ring resonator parameters.

λ (nm)	Cladding (-)	Polarization (-)	Q (-)	F (-)	α (dB/cm)
1570	Air	TE	5.1×10^5	408	0.42
1570	Air	TM	4.4×10^5	294	0.51
1570	Water	TE	6.2×10^4	44	3.94
1570	Water	TM	4.6×10^4	31	6.00
1035	Water	TE	1.5×10^5	99	2.35
1035	Water	TM	1.2×10^5	77	3.11

Al_2O_3 ring resonators have a transparency window spanning from the UV till the mid-IR, namely from 150 nm till 5.5 μm [54,67]. High Q s in the near-UV range have been experimentally demonstrated for Al_2O_3 deposited by atomic layer deposition [68]. Very recently, low propagation losses were demonstrated at 407 nm in a high refractive index layer sputtered with a set substrate temperature of 700 $^\circ\text{C}$ [69]. Furthermore, Li *et al.* [70] reported devices in sputtered holmium doped Al_2O_3 operating around 2 μm . The transparency window of SiN [71] is slightly smaller than that of Al_2O_3 , with a lowest operation wavelength above 400 nm. SOI is limited by its bandgap to wavelengths above 1.1 μm .

The lowest propagation loss of Al_2O_3 waveguides reported here is 0.42 dB/cm at a wavelength of 1550 nm, which occurs in a cladding of air. Very recent work by Hendriks *et al.* [69] has led to optimized slab waveguides with less than 0.1 dB/cm propagation losses (i.e., 0.025 dB/cm) for a high refractive index phase of sputtered Al_2O_3 . The propagation loss of the Si-based platforms can also be very low, with losses of 0.045 dB/m at a wavelength of 1580 nm for a very thin SiN stripe waveguide surrounded by thermal oxide [72] and 0.026 dB/cm for large core SOI waveguides [73], both with a SiO_2 cladding. Q s as high as 6.7×10^7 for SiN [74] and 3.6×10^6 for SOI [75] have been reported. However, the ring resonators used in those demonstrations are not suited for biosensing, both because their waveguide cross-section either does not permit a high sensitivity due to very high waveguide mode

confinements or the presence of a top cladding. In practice, the Q s encountered in biosensors are much lower, with typical values of 1.5×10^4 for SiN [76] and 4.3×10^4 for SOI [21]. The lower Q s follow from the high water absorption losses at the operating wavelength, which lie often around 1550 nm. It is important to mention here that, as it will be further discussed in the following Chapters, the performance of the sensors is currently not limited by the width of the resonances but by the noise of the system.

The relatively low refractive index of Al_2O_3 limits the minimum waveguide size and bend radius. The waveguide dimensions encountered on Al_2O_3 are typically larger than those of the Si-based technologies. For instance, the high refractive index of SOI allows bending radii down to 1.5 μm [77] and much higher on-chip integration density. For Al_2O_3 , the minimal bending radii is around 100 μm , meaning that the FSR cannot be very large, although this is partially compensated by the smaller group index of Al_2O_3 modes. A large FSR is beneficial for sensing due to an increased dynamic range for wavelength shifts. Advantages of the refractive index of Al_2O_3 are that sidewall roughness scattering is less important than for the Si-based technologies and less care needs to be taken for smooth waveguide sidewalls. Furthermore, fabrication of the Al_2O_3 waveguide dimensions does not require electron beam lithography and is well-suited for fabrication using contact UV lithography, although electron beam lithography could reduce the sidewall roughness and the waveguide propagation losses.

Finally, a high solubility for rare-earth ions is a material property that sets apart Al_2O_3 from the Si-based technologies, allowing novel biosensing principles to be developed based on lasers operating in an aqueous environment, which represents a major advance over the state-of-the-art provided by this thesis.

2.5 Conclusion

The analysis, design, fabrication and characterization of Al_2O_3 ring resonators is presented at wavelengths of 1035 nm and 1570 nm. The former falls within a spectral range with low water absorption loss. At these wavelengths the Al_2O_3 ring resonators are designed to have a high sensitivity for bulk and surface sensing, while supporting only a single TE and TM waveguide mode and keeping the bending losses minimal. The designs exhibit bulk refractive index sensitivities of ~ 100 nm/RIU, similar to those of the conventional material platforms. Two ring resonator designs are subsequently fabricated and optically characterized. The determined measured

waveguide propagation loss and Q of the Al₂O₃ ring resonators are comparable with the best results reported in literature, both for previous reports for Al₂O₃ as well as for the mature silicon-based SiN or SOI ring resonators designed for biosensing. A lowest propagation loss of 0.42 dB/cm, and corresponding Q of 5.1×10^5 was measured at a wavelength of 1570 nm for a cladding of air for TE polarization, which drops by a factor of ~ 10 upon submerging the ring resonator in water due to absorption losses. This work shows the potential of Al₂O₃ ring resonators for integrated optical sensors operating in an aqueous environment.

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Chapter 3

Passive Al₂O₃ ring resonator sensors

The sensing performance of passive Al₂O₃ ring resonators is evaluated at a central wavelength of 1570 nm. Temperature sensitivity, bulk refractive index sensitivity and surface biosensing experiments demonstrate that the ring resonator sensor performs best for TM polarization, due to a lesser confinement of the mode. A bulk refractive index sensitivity of ~100 nm/RIU is experimentally demonstrated, with a corresponding limit of detection of ~10⁻⁶ RIU. A surface functionalization protocol is developed to covalently immobilize Anti-S100A4 monoclonal antibodies on the Al₂O₃ surface. These allowed for the selective capture of the target biomarker rhS100A4 to the surface of the Al₂O₃ ring resonators. This protein is linked to human tumor development, and therefore is of high interest for the early diagnostics of the disease. Biomarker concentrations down to 3 nM in undiluted urine are detected using this sensor.

This chapter is based on:

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Optical resonators are very sensitive to environmental disturbances applied to their surroundings, resulting in a shift of their resonance wavelength [1–3], variations of their linewidth [4,5] or mode splitting [6–8]. Tracking of the shift of the resonance wavelength has been widely used in recent years [9–13] for many sensing applications, including the detection of biomolecules [14], chemicals [15], particles [16], pressure [17], pH [18], temperature [19], strain [20] and refractive index [21]. These were all demonstrated on a multitude of different material and resonator sensing technologies. This Chapter describes the use of Al_2O_3 ring resonators as a novel optical sensing technology platform.

3.1 Introduction

Integrated optical sensors are good candidates for the label-free detection of molecular biomarkers [22–24]. Ring resonator based biosensors have shown high sensitivity and a *LOD* compatible with clinical relevant needs [25,26]. Their operation is based on the wavelength shift of narrow resonances in their transmission spectrum induced by refractive index variations probed by the evanescent field. Upon surface functionalization with capture probes, biomarkers can bind to them inducing refractive index changes that result in detectable resonance wavelength shifts. Label-free detection of biomarkers using ring resonators has already been demonstrated in different integrated photonic platforms, including the Si-based material platforms SOI [27–29], SiON [30] and SiN [31]. However, the concentration of the biomarkers in clinical samples is often at the nanomolar level or lower, and most of these references use amplification strategies in order to achieve clinically relevant *LODs*, adding a complexity to the system associated to non-label free strategies [32–34].

Label-free operation with biological samples such as blood, urine or saliva is required for a ring resonator to be useful in clinical applications. However, the biological samples form complex matrices that must be properly processed to remove interfering substances such as proteins and metabolites. That pre-treatment is a tedious and critical step and it is not conducive to on-line processing and automation. Nonetheless, examples of biomarker detection from biological samples do exist. Label-free detection of cancer biomarkers from buffer samples containing fetal bovine serum at a detection limit of 25 ng/ml was reported using SOI ring resonators [35]. More recently, the label-free detection of bladder cancer DNA biomarkers from urine was realized, although at quite high concentrations in the μM

range, which are not always useful for monitoring or early diagnostics [36]. To use ring resonators as sensitive and selective biosensors for clinical samples with good *LOD*, efficient functionalization strategies are required that allow anchoring capture probes on the surface to selectively recognize the biomarker of interest [37]. If this condition can be met, the used material platform can be freely chosen.

Al_2O_3 is an emerging photonic material that, unlike the Si-based technologies, has not been explored yet for biosensing. One of its attractive features is its high rare-earth ion solubility that allows for gain and lasing functionality [38–41], which is of interest for active biosensing applications [42]. High- Q ring resonators were previously demonstrated on this material [43,44], although no demonstration of their application to sensing is reported in literature. Some Al_2O_3 sensor architectures do exist, although they are solely based on distributed-feedback resonators. For instance, thermal characterization of a passive distributed-feedback resonator allowed for the monitoring of resonance wavelength shifts as function of temperature [45]. Furthermore, the detection of micron-sized particles and their sizing was performed by measuring frequency differences between two longitudinal laser modes of a distributed-feedback laser, the evanescent field of which interacts with the micron-sized particles on the surface of the laser [46].

This gap in Al_2O_3 sensors is bridged by the development of refractive index sensors based on undoped Al_2O_3 ring resonators presented here. The sensors were characterized in terms of their temperature, bulk refractive index, and surface sensitivities. Subsequently, a biochemical protocol was developed to functionalize the Al_2O_3 material and immobilize Anti-S100A4 monoclonal antibody capture sites for the detection of the rhS100A4 protein from undiluted urine. As it was already mentioned in Chapter 1, the recombinant human S100A4 (rhS100A4) biomarker is part of the S100 protein family, which is associated with a regulatory role in a variety of cellular processes [47,48] and their overexpression is associated with tumor progression and prognosis [49–51], which can be of high value for monitoring cancer progression [52–54].

3.2 Passive sensor characterization

Two laser systems are available for the sensor characterization of the Al_2O_3 ring resonators, a TOPTICA CTL 1050 tunable laser operating around 1035 nm and a Agilent 81646 tunable laser operating around 1570 nm. As was demonstrated in the previous Chapter, ring resonators were successfully realized and characterized at

Fig. 3.1: Comparison of the resonance wavelength repeatability of ring resonators submerged in water. (a) Repeated measurements of resonance wavelength at central wavelength of 1035 nm. (b) Repeated measurements of resonance wavelength at central wavelength of 1570 nm. Both systems have an uncertainty of around 0.001 pm in the determination of the resonance wavelength of an individual resonance.

both operation wavelengths, but it was also demonstrated that ring resonators with an aqueous top cladding at 1035 nm have a Q that is only a factor of ~ 2.5 larger than those at a wavelength of 1570 nm. However, additional criteria should dictate which laser system to use for the sensing experiments, since such a small difference in Q will not have a significant impact on the LOD that can be reached [55], especially given the almost identical designed sensitivities of the ring resonators at both wavelengths as found in the previous Chapter. A more important factor is the variance of the resonance wavelength for repeated scans in a reference medium, which dictates the smallest analyte concentrations that can be detected [56].

The two laser systems were compared with each other in terms of variance of the resonance wavelength of a ring resonator. This was done in a flow of deionized water at a rate of 100 $\mu\text{l}/\text{min}$ at a fixed stage temperature of 21.5 $^{\circ}\text{C}$. Figure 3.1 shows the relative positions of a resonance wavelength during repeated measurements for each of the two laser systems. The laser at 1570 nm has a better stability over time, mostly because its internal wavelength referencing is more accurate than that of the TOPTICA laser system. This motivated the use of the Agilent laser system operating at 1570 nm over the TOPTICA system operating at 1035 nm for sensor characterization, although the ring resonators operate at both wavelengths. Ideally, one would use the ring resonators at 1035 nm due to the absence of water absorption loss, and thus higher Q s, with a laser system that has similar wavelength noise as the Agilent. However, such a system was not available for these measurements.

The Al_2O_3 ring resonators characterized in the previous chapter acquired structural damage over time, drastically lowering their Q . This damage only occurred on the devices without a SiO_2 top cladding and mostly result from the mechanical handling of the devices and the repeated cleaning, functionalization and PDMS bonding procedures. Therefore, another similar ring resonator with identical geometric parameters was used for the sensor characterization. This device had a slightly lower Q and higher propagation losses due to fabrication errors. Its ring resonator performance parameters are summarized in Table 3.1.

Table 3.1: Parameters of Al_2O_3 ring resonator used for sensing.

λ (nm)	Cladding (-)	Polarization (-)	Q (-)	F (-)	α (dB/cm)
1570	Water	TE	5.9×10^4	49	4.0
1570	Water	TM	4.5×10^4	38	5.7

The chosen ring resonator was characterized in terms of its bulk refractive index, temperature and surface sensitivity. In all the following sensing experiments in this Chapter, the flow rate was kept fixed at 100 $\mu\text{l}/\text{min}$ and, if not further specified, the stage temperature was set to 21.5 ± 0.001 $^\circ\text{C}$. The liquids flown over the ring resonator were switched manually by exchanging syringes connected to the syringe pump. The transmission spectrum of the ring resonator was repeatedly scanned during the sensor experiments. The evolution of resonance wavelengths as function of time was subsequently extracted from the measured spectra.

3.2.1 Temperature sensing

The temperature sensitivity of the ring resonator was determined by monitoring the resonance wavelength shift upon varying the stage temperature, T , while flowing deionized water over the ring resonator. The stage temperature was increased in steps of ~ 0.5 $^\circ\text{C}$ every 4 minutes, as shown in Fig. 3.2 (a). Upon increasing the stage temperature, the resonance wavelength shifts to higher values, as shown in Fig. 3.2 (b). The shift occurs due to a change of the effective refractive index of the waveguide mode, since a temperature increase affects the refractive indices of the waveguide and surrounding cladding materials through the thermo-optic effect. A positive shift corresponds with an increase of the effective refractive index of the waveguide mode. Figure 3.2 (c) shows the temporal response of the ring resonator when the stage temperature is repeatedly increased for both TE and TM polarizations. The corresponding measured temperature sensitivities were 8.1 ± 0.1 pm/K and 4.3 ± 0.1 pm/K for TE and TM polarizations, respectively, as

Fig. 3.2: Temperature sensitivity. (a) Measured temperature of the stage. (b) Temperature-induced resonance wavelength shift for TE polarization. (c) The resonance wavelength shift for both TE and TM polarization upon varying the stage temperature from 20.9 °C (i) to 24.6 °C (vii) in steps of $\sim 0.5\text{ °C}$. (d) Resonance wavelength shift as function of stage temperature.

shown in Fig. 3.2 (d). The TM polarization has a lower temperature sensitivity since more modal power resides in the aqueous top cladding, which has a negative thermo-optic coefficient [57]. The difference in sign of the thermo-optic coefficient of water and Al_2O_3 can potentially be exploited for the realization of athermal ring resonators with very small sensitivity to temperature fluctuations. The same principle is applied to the design of athermal ring resonators in other material systems, where the waveguide mode is tailored to have lowest temperature sensitivity by waveguide geometry design [58] or by covering the waveguide with a material with opposite thermo-optic coefficient [59].

The temperature sensitivity of the Al_2O_3 ring resonator is rather low compared with those typically encountered in SOI biosensors with an aqueous top cladding [60]. This follows from the low thermo-optic coefficient of Al_2O_3 of $1.86\times 10^{-5}\text{ RIU/K}$ [45], which is almost a factor of 10 lower than that of silicon [59]. Because of this,

SOI ring resonators with a SiO_2 top cladding are often found as temperature sensors [61,62].

Fig. 3.3: Bulk refractive index sensitivity. (a) NaCl concentrations flown over the ring resonator. (b) Resonance wavelength shift for bulk refractive index variations of the aqueous top cladding for TM polarization. (c) The resonance wavelength shift for both TE and TM polarization upon varying the NaCl concentration from 0.05 (i) to 0.3 wt% (vi) in steps of 0.05 wt%. (d) Resonance wavelength shift as function of bulk refractive index variation. (e) Single measurement of resonance wavelength noise for TM polarization over a time interval of 2 minutes.

3.2.2 Bulk RIU sensing

To determine the bulk refractive index sensitivity, NaCl dissolved in deionized water was prepared in concentrations ranging from 0.05—0.30 wt%, which were flown over the ring resonator. Figure 3.3 (a) shows the NaCl concentrations flown over the ring resonator as a function of time. Deionized water was flown over the sensor after each NaCl concentration. The refractive index of deionized water can be varied by the addition of different amounts of NaCl (in wt%) as 0.0018 RIU/wt% [57]. An increase in NaCl concentration results in an increase of the effective index of the waveguide mode, and thus a red shift of the resonance wavelength, as shown in Fig. 3.3 (b). The resonance wavelength shift over time was recorded upon flowing the different NaCl solutions for both TE and TM polarizations, which is shown in Fig. 3.3(c). The drop of the resonance wavelength down to the water baseline corresponds with the flow switching back to deionized water. This shift to the baseline is very reproducible and occurs rapidly after switching the flow. Figure 3.3 (d) shows the determined bulk refractive index sensitivities, which were 70.8 ± 0.6 nm/RIU and 102.3 ± 0.5 nm/RIU for TE and TM polarizations respectively. These values match the design values within 10%. Deviations from the designed sensitivity might arise from slightly different refractive indices and waveguide cross-sections due to fabrication variability. The sensitivities could be improved by increasing the ring resonator radius and simultaneously decreasing the waveguide cross-section, to obtain a larger evanescent tail while keeping the bend radiation loss low. As was already discussed during the waveguide design, TM polarization has a larger bulk refractive index sensitivity, because the TM waveguide mode is less confined and its evanescent tail extends further into the top cladding.

The ring resonator response is subjected to wavelength and intensity noise, which impose an uncertainty on the determination of the resonance wavelength during the sensing experiment. Noise sources include intensity and wavelength fluctuations of the tunable laser, thermal noise on the chip, and instabilities and refractive index variations of the flow due to dirt and contaminations within the microfluidic channel. These result in a fluctuation of the resonance wavelength when repeatedly scanning the resonance. This noise can be lowered by repeatedly acquiring spectra, fitting their resonance wavelengths, followed by averaging the position of the resonance wavelength [55]. The noise is the standard deviation of the fitted resonance wavelength and it is shown for a time interval of 2 minutes, or equivalently in this case, 15 repeated measurements, in Fig. 3.3 (e). This time corresponds with the

duration of the ring resonator being exposed to the NaCl solution. The standard deviation of the resonance wavelength for TM polarization for repeated measurements taken during a 2-minute flow of deionized water was determined as $\sigma=0.036$ pm. A more representative figure of the noise during the whole sensing experiment is the average over all the 2-minute intervals of various NaCl solution flows over the ring resonator. In that case, a standard deviation of $\sigma=0.054$ pm was determined for TM polarization.

The *LOD* of a bulk refractive index sensor is the smallest amount of refractive index change that can be reliably detected [56,63,64]. The *LOD* is given by three times the uncertainty divided by the sensitivity, which for bulk refractive index sensing is given by:

$$LOD = \frac{3\sigma}{S_{RIU}}. \quad \text{Eq. (3.1)}$$

Combining the measured bulk refractive index sensitivities with the measured detection noise of 0.054 pm yields a lowest *LOD* of 1.65×10^{-6} RIU for TM polarization. The demonstrated *LOD* of the Al₂O₃ ring resonator is similar to the best reported results achieved with SOI and SiN ring resonators for refractive index sensors, which have *LOD* values of $\sim 10^{-6}$ RIU [14,31,65]. The same holds for its sensitivity. This result shows that this first demonstration of Al₂O₃ ring resonator refractive index sensing already has a performance comparable with the more mature silicon-based technologies. Furthermore, various methods exist that could further lower the *LOD*. They rely on either improving the sensitivity by employing Vernier filters [66], slot-waveguides [67] or sub-wavelength grating waveguides [68], or by lowering the noise by laser-frequency locking for an enhanced signal-to-noise ratio [16]. However, these require advanced design and fabrication techniques or experimental setups that are beyond the scope of the work presented in this thesis. Furthermore, attention needs to be paid to the thermal noise of those devices to ensure that not only the sensitivity, but also the *LOD* is improved.

3.2.3 Surface sensing

Surface sensing was tested for both TE and TM polarizations. This was done using the neutravidin (Thermo Scientific NeutrAvidin 31000, molecular mass 68 kDa) and biotinylated bovine serum albumin (BSA, Pierce Bovine Serum Albumin, Biotinylated, 29130, molecular mass 66.4 kDa) biomolecules. Neutravidin was

Fig. 3.4: Surface sensing experiment with neutravidin and biotin. The liquids flown over the ring resonator were PBS 10 mM (i), neutravidin at a concentration of 167 nM (ii) and biotin at a concentration of 83 nM (iii).

immobilized onto the surface of the ring resonator by physical adsorption. The formed layer acts as a capture site for the second biomolecule through biorecognition of the biotin by the neutravidin. Both biomolecules were prepared in a solution of 10 mM phosphate buffered saline (PBS, Gibco DPBS 21600010). PBS helps maintain the pH of the analyte constant at a value of 7.4 and it has a similar osmolarity and ion concentration as the human body, mimicking the environment that biomolecules encounter in the complex media of interest.

Figure 3.4 shows the response of the ring resonator during the surface sensing experiment. First, a flow of PBS was introduced over the ring resonator and maintained for 30 minutes to establish a stable resonance wavelength. Only the last ten minutes of the initial flow were recorded and shown. Next, PBS containing the neutravidin biomolecule was introduced at a concentration of 167 nM. Both polarizations show a strong shift upon biomolecule introduction, indicating the adsorption of biomolecules onto the surface of the ring resonator. The resonance wavelength shift starts to flatten after 10 minutes, indicating that during this time the surface of the ring resonator was almost fully covered with a monolayer of the biomolecule or that an equilibrium between attaching and detaching was reached. PBS was then flown again over the ring resonator. During this step, the resonance wavelength experienced a small negative shift, indicating that a small amount of the adsorbed neutravidin detaches from the surface. Finally, biotinylated BSA in 10 mM PBS was flown over the ring resonator, which induced another shift of the resonance wavelength. Due to the high binding affinity between neutravidin and biotin, the resonance wavelength shift saturates after a short time and all available capture sites

for BSA are occupied. For both biomolecules, the shift is higher for TM polarization due to its evanescent field having a larger overlap with the sensing region directly above the surface of the waveguide. This result is also in agreement with the larger sensitivity expected for TM polarization, as found in the waveguide cross-section design. However, the shift induced by the binding of the BSA is ~60% smaller than that of the adsorption of the neutravidin, whereas both molecules have similar molecular masses. This dissimilarity was also observed before in the literature and attributed to inefficient binding and reduced close packing of the BSA during its capture by the neutravidin due to steric hindrance [31,69].

TM polarization has both a higher surface sensitivity and bulk refractive index sensitivity. Furthermore, it exhibits a lower thermal sensitivity, which is beneficial for a smaller temperature-induced noise. Because of these results, rhS100A4 biomarker sensing was performed at TM polarization.

3.3 rhS100A4 biomarker sensing

Al₂O₃ requires a functionalization protocol to form a bioreceptor layer that selectively captures the biomarker of interest on the surface of the waveguide. This section describes the implementation of such a protocol on the ring resonators and the subsequent detection of the rhS100A4 protein biomarker.

3.3.1 Chemical protocol

The rhS100A4 protein was produced as described by [70] at LEITAT. Hybridoma obtaining and cell subcloning was performed using standard technologies. Briefly, mice were immunized with hrS100A4 and B cells were fused with myeloma cells to obtain hybridoma cells. After cell subcloning, serum free supernatant of the selected antibody-secreting hybridoma cell line was obtained and purified using protein A columns (MabSelect Sure LX; Amersham) and an ÄKTA purifier FPLC system (GE Healthcare).

A robust protocol for covalently binding antibodies to the Al₂O₃ surface was developed by IBEC. Briefly, the Al₂O₃ surface was activated with O₂ plasma and a carboxylic acid-terminated layer was deposited. After the activation of the carboxylic terminals by an EDC/NHS reaction, the chips are ready for anchoring the antibody capture sites on its surface. The surface functionalization process developed permitted the preferential binding of antibodies to the Al₂O₃ layer with respect to the surrounding SiO₂ material. Molecular recognition reactions would then guarantee

Figure 3.5: Al₂O₃ surface functionalization. (a) Schematic of the chemical protocol. (i) Surface activation with O₂ plasma. (ii) 6-phosphonohexanoic acid, 500 μM in anhydrous heptane, reacts with the Al₂O₃ surface to generate a carboxylic acid-terminated surface. (iii) Activation of carboxylic terminals by EDC/NHS and anti-S100A4 antibody immobilization. (iv) Protein biorecognition. (b) Fluorescence assay to verify the immobilization of the antibody capture probes. (i) Schematic representation of the fluorescence assay. (ii) Microscope fluorescent image of Al₂O₃ ring resonator functionalized with 6-phosphonohexanoic acid and immobilized with an antibody labelled with fluorescein isothiocyanate fluorescence dye (FITC, 495 nm/519 nm excitation/emission). Magnification 40X, acquisition time 2000 ms. The green light originating from the Al₂O₃ ring resonator indicates the binding of the fluorescent antibodies attaching to it.

that rhS100A4 proteins contained in the sample bind to the surface immobilized antibodies selectively. Figure 3.5 (a) shows a schematic outline of the procedure used to functionalize the surface of the Al₂O₃ ring resonators. First, the chip was cleaned with deionized water, acetone and ethanol to remove organic surface contaminants. Afterwards, to activate the surface for functionalization, the chip was cleaned with HNO₃ and O₂ plasma. After O₂ plasma activation, the surface was modified with 6-phosphonohexanoic acid (500 μM, anhydrous heptane) by placing a 100 μl droplet thereof onto the chip and to let it evaporate. It was then heated in an oven at 120 °C for 12 hours to form a carboxylic acid-terminated layer. Prior to antibody immobilization, the chip was immersed in a carboxylic acid activating solution (1-Ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDC), 25 mM, N-Hydroxysuccinimide (NHS), 50 mM, in 100 mM MES buffer, pH 4.7) for 2 hours, and gently washed with deionized water. To test the successful immobilization of antibodies on the surface, a fluorescence assay was carried out with an antibody labelled with fluorescein isothiocyanate fluorescence dye (10 μg/ml), as shown in

Fig. 3.5 (b). The fluorescence assay shows that the antibodies selectively bind to the Al_2O_3 waveguide surface, while bonding to the SiO_2 substrate is much smaller.

3.3.2 rhS100A4 biomarker sensing

The PDMS microfluidic channel was non-permanently bonded over the chemically functionalized ring resonators. Urine was chosen as testing medium, given the prevalence of the rhS100A4 biomarker in urine [71]. Urine was pooled from six healthy individuals. To enhance the biorecognition between the antibody and the biomarker, 10 v/v% of PBS 100 mM was added to the urine and its pH was then adjusted to 7.5 by adding NaOH. The urine was filtered through 0.2 μm pores and stored at 4 °C before use. Then, different amounts of the rhS100A4 protein were spiked to the urine samples to obtain samples with known biomarker concentrations ranging from 3000 nM down to 1 nM. The prepared urine samples were then flown over the Al_2O_3 ring resonator while monitoring their resonance wavelength shift. Figure 3.6 (a) shows the sensorgram of the experiment. The first phase consists of a washing step with PBS of the surface functionalized and activated ring resonator sensor until a stable baseline was obtained, which occurred after 30 minutes. Only the resonance wavelength shift of the last 10 minutes is shown. The second phase consists of immobilizing the anti-S100A4 monoclonal antibodies at a concentration of 50 $\mu\text{g}/\text{ml}$ in 10 mM PBS. The resonance wavelength stopped shifting after 20 minutes, indicating that no more binding events occur and that the surface is saturated with antibody capture probes or that an equilibrium between association and dissociation was reached. Then, a subsequent washing step with PBS removes all the non-covalently bound antibodies. During this step, no considerable negative resonance wavelength shift was observed, indicating that the antibodies are tightly bound to the surface and that they do not detach. The third phase consists of a blocking step with 1% BSA in 10 mM PBS to prevent nonspecific binding [72]. The large shift is due to the high refractive index of the BSA sample. A small net shift is present after the following washing step in PBS, meaning that some of the BSA bound to uncovered Al_2O_3 . This confirms that most of the surface of the ring resonator was already covered during the antibody immobilization. Then, the biosensor was exposed to a sample of blank urine that does not contain the rhS100A4 protein to establish a baseline. Again, a large bulk refractive index difference results in a larger shift. Finally, the fifth phase introduces the biomarker containing urine.

Figure 3.6 (b) shows the response of the biosensor to different concentrations of the protein in urine. For visibility, the binding curves of the two highest concentrations

Fig. 3.6: rhS100A4 biomarker sensing. (a) Full sensorgram. The flow over the ring resonator was 10 mM PBS (i), anti-S100A4 antibody at 50 $\mu\text{g}/\text{ml}$ (ii), BSA 1% in PBS 10 mM (iii), blank urine (iv), and rhS100A4 in urine at 25 nM (v). (b) Binding curves of rhS100A4 to the antibodies immobilized onto the surface of the ring resonator (c) Resonance wavelength shift as function of rhS100A4 concentration. (d) Noise during a blank urine experiment compared with the lowest concentration detected.

(400 and 3000 nM) were omitted. The initial bumps at ~ 1 – 2 minutes result from a short-time temperature overshoot caused by the dynamics of the flow system upon switching the liquids. However, the signal returns back to the initial baseline after the overshoot and it does not hamper the sensing experiment. After 8 minutes, the total wavelength shift was recorded and plotted as function of biomarker concentration in Fig. 3.6 (c). It can be observed that the resonance wavelength shifts to higher values for all biomarker concentrations. Higher protein concentrations result in larger net shifts. Furthermore, in Fig. 3.6 (b) it can be seen that the resonance wavelength shift saturates at the highest concentrations, indicating that an equilibrium between binding and disassociation of the proteins to the antibodies has been achieved.

Fig. 3.7: rhS100A4 control experiments. (a) Effect of functionalization on the sensorgram. The flow over the ring resonator was 10 mM PBS (i), anti-S100A4 antibody at 50 $\mu\text{g/ml}$ (ii), BSA 1% in PBS 10 mM (iii), blank urine (iv). (b) Selectivity assay for anti-S100A4 antibody in urine. Control I: rhS100A4 at 400 nM without antibody immobilization. Control II: neutravidin at 400 nM in urine.

A small drift of the resonance wavelength was present during the experiment, which is most likely due to some minute non-specific binding, buildup of material on the ring resonator or a thermal drift. In this case, the uncertainty during the 8 minutes of flow of a blank urine sample was measured at ~ 0.05 pm/min. The *LOD* is the smallest protein concentration that yields a total shift that exceeds three times the total drift of 1.2 pm. A smallest total resonance wavelength shift of 1.43 pm was measured after 8 minutes of biomarker flow at a concentration of 3 nM. This concentration lies within the clinically relevant range demonstrated by [71].

Two control experiments were performed. First, antibodies were deposited onto a ring resonator to which a surface functionalization was not applied to test the necessity of the functionalization protocol, as shown in Fig. 3.7 (a). An antibody flow still produced a strong resonance wavelength shift, although a negative drift was present after switching back to 10 mM PBS and the following other liquids. The antibodies can still bind to the Al_2O_3 surface through adsorption, although the bond is not stable and antibodies detach over time. This negatively affects the measurement through a lower shift induced by protein binding since the number of antibody capture sites reduces over time. Furthermore, the presence of a negative drift that can partially compensate the shift of the biomarker binding makes the interpretation of the protein binding curves more complicated. As a conclusion, the chemical functionalization of the Al_2O_3 surface is necessary to achieve a proper covalent attachment of the antibodies for optimal sensor performance.

The second control experiment tested the selectivity of the biomarker binding to the antibodies, as shown in Fig. 3.7 (b). First, a ring resonator without functionalization and antibody immobilization was used to detect the rhS100A4 protein. The ring resonator was covered with BSA in a blocking step, after which the protein in urine at a concentration of 400 nM was flown over it. The resulting resonance wavelength shift of 6.3 pm is ~ 10 times smaller than that of a ring resonator with antibodies immobilized for the same protein concentration, which experienced a shift of 59.9 pm. In this case, the resonance wavelength shift can still occur due to adsorption or non-specific binding, although it is significantly reduced. This result indicates the necessity of immobilizing antibodies for capturing the rhS100A4 biomarker. Second, a ring resonator that underwent functionalization and antibody immobilization was subjected to a urine containing the neutravidin protein, which should not be able to bind to the antibody through molecular biorecognition. The neutravidin protein was at the same mass concentration in urine as the 400 nM rhS100A4 biomarker sample. The resulting resonance wavelength shift for the sample containing neutravidin was at 3.1 pm 20 times smaller than that for the rhS100A4 protein. Again, non-specific binding could still occur, although it is strongly reduced. As a final remark, during the establishment of the blank urine baselines in Fig. 3.6 (a) and (b), a very small drift of the resonance wavelength was present. This means that almost none of the biomaterial present in the complex matrix of urine binds to the immobilized antibodies, further confirming the selectivity of the antibodies to the biomarker of interest. These results indicate that the Al_2O_3 ring resonators immobilized with anti-S100A4 antibodies are selective in detecting the rhS100A4 biomarkers.

3.4 Conclusion

To conclude, the passive Al_2O_3 material is shown to be well-suited for integrated optical sensing. Its bulk refractive index sensitivity of ~ 100 nm/RIU and LOD of $\sim 10^{-6}$ RIU are comparable with those of the more mature SOI and SiN technologies. A robust surface functionalization protocol was developed that permits a good coverage of the Al_2O_3 surface with antibodies. The label-free detection down to 3 nM of the rhS100A4 biomarker, associated with cancer and human tumor development, was demonstrated in whole urine without the need of either sample pre-treatment or signal amplification steps and within less than 10 minutes.

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Chapter 4

Active $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ laser-based sensors

Laser-based sensors hold the promise of an ultra-low intrinsic limit of detection due to their narrow linewidths. However, the widespread use of these devices for biosensing applications has been hindered by their complexity and lack of robustness. This work demonstrates active sensing with integrated on-chip disk and ring resonator lasers. $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ was utilized because of its low optical losses as well as its emission at a wavelength around 1000 nm, where absorption losses of water are negligible. Single-mode laser emission on devices with an aqueous top cladding was demonstrated for both disk and ring resonator lasers. By employing a heterodyne detection scheme to form a beat note between the laser emission of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers and an external reference laser, minute shifts of lasing wavelength can be detected and monitored, allowing for active, laser-based sensor operation. A limit of detection of 300 pM of the human cancer protein biomarker rhS100A4 in urine was experimentally demonstrated, showing the potential of the proposed devices for active biosensing applications.

This chapter is based on:

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Chapter 3 introduced the Al_2O_3 ring resonator platform for passive sensing applications. However, unlike most optical resonator sensing platforms, Al_2O_3 permits on-chip active functionality through rare-earth ion doping [1–6]. In fact, by carefully controlling the dopant concentration, waveguide design and resonator geometry, Al_2O_3 resonators can be used as lasers [7–9]. Many reports have shown examples of ring resonator lasers in Al_2O_3 doped with different rare-earth ions, including Er^{3+} [10] and Yb^{3+} [11] doping and distributed feedback lasers based on Er^{3+} [12], Ho^{3+} [13] and Yb^{3+} [14] doping. Nd^{3+} -doped sapphire planar waveguides were also demonstrated for lasing [15]. Despite these excellent efforts in laser development on the Al_2O_3 material platform, there exist almost no demonstrations of laser-based active sensors [16]. This chapter describes the development of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonators lasers with an aqueous top cladding. Whereas ring resonators have two sidewalls, disk resonators lack an inner sidewall, which is beneficial for lasing because they have lower sidewall roughness losses and a higher confinement of the lasing mode. By applying the same chemical functionalization protocol introduced in Chapter 3, active optical biosensing of the rhS100A4 biomarker was demonstrated at unprecedented low concentrations [17].

4.1 Introduction

Passive optical resonator sensors have been widely studied in the last decades due to their high sensitivity in the label-free detection of biomolecules [18–20]. These biosensors hold promise of their integration into portable, sensitive, low cost, multiplexed and easy-to-use devices that, within minutes, can detect biomarkers from bodily fluids for medical applications. Label-free detection relies on the sensitivity of the resonators to changes experienced by their evanescent field due to the attachment of the target biomarkers, which, amongst other effects, induces shifts in the resonance frequency [21]. However, biosensors based on the detection of frequency shifts exhibit an intrinsic resolution limit given by the linewidth of the resonances [22], and they require complex interrogation schemes with either finely tunable lasers or high resolution optical spectrum analyzers (OSA) to continuously monitor the location of the resonance wavelength, which hampers their implementation outside the laboratory.

In contrast to passive resonators, recent reports highlight the potential advantages of employing active, laser-based devices [23–30] for optical biosensing. As shown in Fig. 4.1 (a), these have much narrower linewidths than their passive counterparts and

dramatically decrease the intrinsic *LOD*, as now smaller variations of the lasing wavelength can be detected [31]. Furthermore, an active device can use a simple, cheap interrogation setup to monitor the laser wavelength shift by heterodyning with an external reference laser [32] or by monitoring variations of the beat note generated by the self-heterodyning of the split-modes of the laser due to the attachment of nanoparticles or viruses [33–35]. Aforementioned demonstrations require an optical pump to achieve lasing, an electrical spectrum analyzer (ESA) to record the heterodyne beat notes that typically have low frequencies in the kHz—GHz range, and, in some cases, an external reference laser at fixed wavelength. This constitutes a simpler setup than the traditional wavelength scanning and tracking of the resonance of a passive ring resonator. Furthermore, using this scheme leads to a much smaller resolution compared to what is achievable with an optical spectrum analyzer (OSA). However, up to date, the benefits attributed to active, laser-based devices have been mostly demonstrated on complicated three-dimensional optical cavities not suitable for on-chip integration, therefore hampering their use as multiplexed biosensing platforms. Advancing sensing functionalities based on active optical resonators would require an integrated photonics material platform that can overcome this limitation.

Al_2O_3 is an emerging photonic material that allows both sensing functionalities [36] and rare-earth ion doping for monolithically integrated lasers [37]. By using the Yb^{3+} dopant, lasing can be realized at a wavelength around 1000 nm where water absorption is negligible, making it possible to operate in an aqueous environment. Surprisingly, there is only one report exploiting the active optical properties of Al_2O_3 as a sensing material, where glass microspheres with diameters of 1—20 μm were detected and their size determined by using a dual-wavelength distributed feedback laser [16]. However, the detection was not selective, as the device was sensitive to anything brought in its close proximity. So far, the biosensing capabilities of active, laser-based $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ devices have not yet been reported.

In this thesis, on-chip integrated $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonator lasers are demonstrated. To be compatible with state-of-the art biosensing procedures, the lasers were combined with microfluidics and operation in an aqueous environment was demonstrated. The lasers were optically characterized and single-mode lasing emission around 1030 nm was demonstrated. The devices can be used as sensors by monitoring the change of the optical path length of the resonator cavity in the same way as it was shown in the previous Chapter. To resolve these small variations, and

Fig. 4.1: Principle of heterodyne active sensing. (a) An active resonator can monitor minute variations of lasing wavelength that cannot easily be resolved by its passive counterpart. (b) Schematic of experimental setup to monitor those minute variations by performing a heterodyne beating with an external laser.

to improve the wavelength resolution typically achieved with an OSA [32], a radio frequency (RF) beat note signal is created by heterodyning the emission spectrum of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ laser with that of an external reference laser emitting at an almost identical wavelength [38,39], as shown in Fig. 4.1 (b). By combining the emission of both lasers, the electric fields of both the on-chip $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ laser and the reference laser at a detector can be written as the following sum [38]:

$$\mathbf{E}(t) = \mathbf{E}_1 \cos(2\pi f_1 t) + \mathbf{E}_2 \cos(2\pi f_2 t), \quad \text{Eq. (4.1)}$$

where f_1 and f_2 are the optical frequencies of the two lasers and $\mathbf{E}_1$ and $\mathbf{E}_2$ are the corresponding vector electric fields. A photodetector responds to the electric field impinging on it with a photocurrent proportional to the intensity of the light, or the electric field squared $|\mathbf{E}^2(t)|$, which is given by [38]:

$$\begin{aligned} |\mathbf{E}(t)|^2 &= \frac{1}{2} |\mathbf{E}_1|^2 [1 + \cos(4\pi f_1 t)] + \frac{1}{2} |\mathbf{E}_2|^2 [1 + \cos(4\pi f_2 t)] \\ &+ |\mathbf{E}_1 \cdot \mathbf{E}_2| [\cos(2\pi(f_1 + f_2)t) + \cos(2\pi(f_1 - f_2)t)]. \end{aligned} \quad \text{Eq. (4.2)}$$

The optical frequencies of the laser fields are on the order of 3×10^{14} Hz at a wavelength of $\sim 1 \mu\text{m}$, meaning that the first three terms in Eq. 4.2 oscillate at too high frequencies for a detector system to respond to them. However, the difference frequency in the last term, $f_1 - f_2$, can be made sufficiently small to allow detection

by a photodetector by selecting an appropriate external laser operating at a wavelength close to that of the on-chip laser. In that case, the measured signal is proportional to the time-average of Eq. 4.2 and is given by [38]:

$$\langle |E(t)|^2 \rangle = \frac{1}{2}|E_1|^2 + \frac{1}{2}|E_2|^2 + |E_1 \cdot E_2| \cos(2\pi(f_1 - f_2)t). \quad \text{Eq. (4.3)}$$

The photodetector will then produce a sinusoidal current with beat note frequency $f_{\text{beat}} = f_1 - f_2$, which can be analyzed with an ESA if the beat note has a value in the ~kHz—GHz range. By keeping the external laser at a fixed frequency, the shift of the wavelength of the on-chip laser due to environmental variations is directly translated into a shift of the beat note frequency between the on-chip laser and the reference laser.

4.2 Laser characterization

$\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonator lasers were fabricated following the methods detailed in Chapter 2. In order to provide the ring resonators with optical gain, Yb^{3+} dopants were introduced in the Al_2O_3 layers by co-sputtering from both an Al and Yb target. A 550 nm thick $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ dielectric layer was deposited onto a thermally oxidized silicon wafer. The refractive index of the sputtered layer was measured as 1.6783. The co-sputtering parameters were RF powers of 200 W and 35 W applied to respectively the Al and Yb targets, an oxygen flow of 2.5 sccm into the reaction

Fig. 4.2: Optical microscope photographs of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers. (a) Ring resonator laser. (b) Disk resonator laser. (c) Disk resonator laser under pump illumination at a wavelength of 976 nm. The green luminescence originates from upconversion of Er^{3+} impurities in the Yb-target that are sputtered into the Al_2O_3 layer.

chamber, a total argon flow of 30 sccm, a substrate temperature set to 650 °C (~450 °C on the wafer), and a pressure of 3.7 mTorr. The applied RF power is expected to yield a dopant concentration on the order of $\sim 5 \times 10^{20} \text{ cm}^{-3}$ [9], although this value was not optimized for the waveguides used here. The high substrate temperature is possible due to the presence of the rare-earth ions that prevent crystallization and allow maintaining an amorphous layer at elevated deposition temperatures [7]. The disk and ring resonators were then patterned using standard UV contact lithography (Olin OIR 906-12 photoresist and EVG 620 exposure tool). The patterned structures were etched using reactive ion etching (Oxford Plasmalab System 100 inductively coupled plasma reactive ion etcher) with a plasma of BCl_3 and HBr at a ratio of 5:2, using a total power of 25 W. Several devices were fabricated with disk and ring radii between 50 and 200 μm and coupling gap variations between 0.6 and 1.0 μm , according to the design parameters presented in Chapter 2. The bus and ring waveguides had widths between 1.0 and 2.2 μm . Then, a 3 μm thick SiO_2 cladding was deposited using PECVD (Oxford Plasmalab 80 Plus) at 300 °C with a shadow mask to keep the disks and rings exposed to the environment, while covering the rest of the chip with the cladding layer. Next, chips of $1.2 \times 1.9 \text{ cm}^2$ were diced (Micro Ace 3) and PDMS microfluidic channels with a cross-section of 600 by 70 μm^2 were bonded onto the chips by simply sticking them to the chip without the use of an oxygen plasma treatment. Finally, the PDMS channels were filled with either deionized water or synthetic urine (Surine Negative Control). The resulting disk and ring resonators are shown in Fig. 4.2. Since the Yb target does not have perfect purity, some Er contamination is present in the target and it gets introduced into the Al_2O_3 layer in minute amounts during the co-sputtering process. Er-mediated energy transfer upconversion results in the green luminescence visible in Fig. 4.2 (c).

After fabrication, the laser spectrum, power characteristics and laser linewidth of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonators were characterized in a flow of deionized water. Many of the fabricated lasers exhibited lasing upon optical pumping, but only the best performing lasers were selected for in-depth characterization and were used in the sensing experiments. Selection criteria were stable, single-mode lasing operation and high output power.

4.2.1 Lasing spectrum

The $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonators were optically pumped with a fiber laser diode (Thorlabs BL976-SAG300) with wavelength of 976 nm and linewidth of 0.5 nm. A single-mode fiber was butt coupled to the chip using index matching liquid to couple the pump power to the chip. The on-chip devices exhibited spontaneous and stimulated emission upon optical pumping at an incident power of 27 mW (i.e., power measured after the input fiber, prior to fiber-to-chip coupling losses). The laser emission was collected by the bus waveguide in both the forward and backward directions, as shown in Fig. 4.1(b). The backward lasing light was collected by the same fiber used for launching the pump light and separated using a 980/1060 nm wavelength demultiplexer (Thorlabs WD202G-FC). The separated light was guided to an OSA with 0.1 nm resolution (Hewlett Packard 70950B) for analysis of the

Fig. 4.3: Lasing spectra of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonator lasers exposed to a flow of deionized water. The devices were optically pumped at 976 nm at an incident power of 27 mW. On-chip laser emission was TE-polarized. (a) Example of multimode ring resonator laser, radius of 150 μm and coupling gap of 0.6 μm . (b) Single mode ring resonator laser, radius of 150 μm and coupling gap of 0.7 μm . (c) Single mode disk resonator laser, radius of 100 μm and coupling gap of 0.6 μm . (d) Zoom of the lasing mode in (c), showing the side-mode suppression ratio (SMSR).

lasing spectrum. Lasing occurred in many devices, with both single- and multimode laser operation being observed. Figures 4.3 (a) and (b) show multimode and single-mode lasing, respectively, for two ring resonators with identical radius of $150\text{ }\mu\text{m}$ and waveguide cross-section of $0.55 \times 1.8\text{ }\mu\text{m}^2$, but different coupling gaps of $0.6\text{ }\mu\text{m}$ and $0.7\text{ }\mu\text{m}$. For the former, the multiple lasing modes are separated by the FSR of the ring resonator. However, no clear correlation between coupling gap, or other geometrical parameters, and single- or multimode laser behavior was found for the devices. Figure 4.3 (c) and (d) shows single-mode lasing operation of a disk resonator with a radius of $100\text{ }\mu\text{m}$ and coupling gap of $0.6\text{ }\mu\text{m}$. Both single-mode lasers had a side-mode suppression ratio ($SMSR$) of more than 25 dB and the laser emission was TE polarized. The spectral resolution of the OSA of 0.1 nm was too large to determine the linewidth of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers.

4.2.2 Power characteristics

The disk and ring resonator presented in Figs. 4.3 (b)—(d) were the best performing devices in terms of output power and stable single-mode lasing spectrum. Their power characteristics were determined with a power meter (Hewlett Packard 81536A). Figure 4.4 shows the output laser power as a function of incident pump power of the disk and ring resonator lasers. The laser threshold and slope efficiency with respect to incident pump power (i.e., power exiting the pump fiber) prior to coupling to the chip were determined. Slope efficiencies of ~ 0.1 and $\sim 0.2\%$ were measured, together with lasing thresholds of $\sim 7\text{ mW}$ and $\sim 17\text{ mW}$ incident power, for respectively the disk and ring resonator. Currently, these power characteristics

Fig. 4.4: Power characteristics of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers, both exposed to a flow of deionized water. Pump power refers to the incident power on the chip prior to fiber-to-chip coupling losses, lasing power refers to measured power at the power meter after fiber-to-chip coupling loss. (a) TE-polarized disk resonator laser operating at 1024 nm. (b) TE-polarized ring resonator laser operating at 1055 nm.

are limited by the low fiber-to-chip coupling efficiency of 20%, which was measured with a passive transmission measurement at a wavelength of 1035 nm. The coupling efficiency can be enhanced in future works by implementing a waveguide-to-fiber mode converter using a combination of vertical and horizontal tapers to increase the fiber-to-chip coupling efficiency, and achieve, thus, larger slope efficiency and lower lasing threshold. Furthermore, using a passive-active integration scheme could reduce pump absorption in the bus waveguide and enhance the efficiency [1,40,41]. Correcting for both the fiber-to-chip coupling loss of the pump light and the chip-to-fiber coupling loss of the laser light yields an on-chip slope efficiency of 3% and threshold of 1.2 mW for the disk resonator laser and an on-chip slope efficiency of 5% and threshold of 3.4 mW for the ring resonator. Often, the power characteristics reported in literature are corrected for the fiber-to-chip coupling losses [11,12,42].

4.2.3 RF beat note and laser linewidth

A 10 kHz linewidth tunable laser (TOPTICA CTL 1050) was utilized as external reference laser to achieve a low frequency (i.e., below 10 GHz) heterodyne beat note. This frequency is sufficiently low to be detected by a RF ESA (Hewlett Packard E4407B). The wavelength of the external laser was set close to the single-wavelength laser emission of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk and ring resonator lasers and it was set to continuous mode operation. The external laser driving current was set to 80 mA. The two laser light fields were combined by a 50/50 splitter (Thorlabs PN1064R5F2) and guided to a photodetector (New Focus 1014) for analysis with the ESA, as shown in Fig. 4.1 (b). The resulting RF spectra of the generated beat note of the disk and ring resonator lasers are shown in Fig. 4.5 (a) and (c), respectively. As shown in Fig. 4.5, the RF spectra contain two closely separated peaks, which are due to mode-splitting of the lasing modes. This mode-splitting is produced by coupling of the clockwise (CW) and counterclockwise (CCW) propagating laser fields inside the resonator mediated by sidewall roughness scattering around the perimeter of the resonator [43–45]. Due to the random nature of the coupling mechanism mediated by the roughness, the magnitude of the splitting varies from device to device, but it is typically below 200 MHz. In these particular devices, the split-mode frequency difference is respectively 40 and 220 MHz for the disk and ring resonator lasers, as shown in Fig. 4.5 (b) and (d).

The laser linewidths of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers were determined from the linewidth of the heterodyne beat notes of the split-modes in the RF spectra. The linewidth of the measured beat note equals the combined linewidths of the external laser and the

Fig. 4.5: Beat note spectrum analysis. Spectral resolution is 100 kHz and acquisition time is 50 ms. (a) RF spectrum of a single mode disk resonator laser heterodyned with the external laser. Laser operation was at 1024 nm and TE polarization. (b) Self-beating RF spectrum of both split-modes of the disk resonator laser in (a). (c) RF spectrum of single mode ring resonator laser heterodyned with the external laser. Laser operation was at 1055 nm and TE polarization. (d) Self-beating RF spectrum of both split-modes of the ring resonator laser in (c).

$\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers [46]. The two individual peaks are fitted with a Lorentzian function to determine their 3 dB linewidth. Given that the external laser has a linewidth of 10 kHz, its contribution to the measured linewidth can be neglected. A linewidth of ~200—300 kHz was found for the disk resonator laser, whereas the ring resonator laser had a measured linewidth of ~1 MHz. To confirm the linewidth measurement, the linewidth of the direct self-beating between the two split lasing modes was recorded, whose results are shown in Figs. 4.5 (b) and (d). In both cases, the direct beat note has a 3 dB linewidth that is twice the value determined by heterodyne beating with the external laser. Considering the lasing wavelength of around 1020—1060 nm, the measured linewidth corresponds to a quality factor of 1.2×10^9 and 3×10^8 for respectively the disk and ring resonator lasers. The measured linewidths are around two orders of magnitude higher than the record linewidths

obtained in rare-earth ion doped Al_2O_3 lasers, which are several kHz [42,47]. Those lasers had much higher output powers, resulting in an already narrower linewidth based on the Schawlow-Townes bound on the linewidth, which scales inversely with the laser power [48]. Furthermore, the devices presented in this work experienced higher losses, resulting in additional linewidth broadening, and do not have a cladding, making them more susceptible to environmental noise.

4.2.4 Improving the laser performance

Reliable and reproducible laser fabrication requires control over the lasing spectrum and its characteristics. However, the current generation of lasers have no spectral filtering mechanism besides the radius of the resonator, which determines the position of the lasing wavelengths inside the gain bandwidth of the material. The selection of lasing wavelength and its single- or multimode nature is a complex process that depends on many factors, including the gain bandwidth of the material, sidewall roughness of the resonator, and the spectral dependency of the power coupling. Both the sidewall roughness and wavelength dependency of the power coupling to the ring introduce wavelength-dependent losses that determine which resonances exhibit lasing. Without a more advanced spectral selection scheme, the number of modes and their wavelength remains a stochastic process. Single-mode operation could be achieved by using more complicated device architectures, such as, among others, multiple coupled ring resonators [49], intra-cavity feedback using a reflector and phase control [50], or by modifying the resonator cavity with structural perturbations [51] or a grating [52]. Such strategies are currently being developed for the next generation of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers.

The measured slope efficiencies and output powers of the lasers in this work are lower than other demonstrations of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers [11,37]. Higher output powers lead to narrower linewidths and better signal-to-noise ratio of the lasing modes, and are thus desired. Several parameters of the lasers in this work are responsible for their low power. First, the waveguide cross-section is designed to be small to enhance sensitivity (when used as a sensor). This results in small confinement in the core area and, therefore, less available gain per pump power travelling in the waveguide. Correspondingly, the dopant concentration could be further optimized for the used cross-sections. Furthermore, the device operates in an aqueous environment instead of being submerged in a SiO_2 cladding, resulting in a larger refractive index contrast and thus larger scattering losses, again reducing the available net gain. As observed in Chapter 2, the background losses of Al_2O_3 ring

resonators at a wavelength of 1035 nm was around 2.5 dB/cm, whereas those of the rib waveguides used in the lasers of [37] are a factor of ~ 10 lower. Additionally, the passive resonances of the lasers were scanned around their lasing wavelength with a tunable laser (TOPTICA CTL 1050). It was found that, at the lasing wavelength, the devices have a cold cavity Q of $1\text{--}2 \times 10^5$ and that they are undercoupled. Increasing the coupling coefficient to achieve critical coupling at the pump wavelength could also be beneficial for the lasing performance due to an increase of the enhancement factor of the pump light circulating in the disk resonator. As already mentioned, active-passive integration or a horizontal and vertical taper could improve the power characteristics. Other approaches include lowering the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ dielectric slab losses and reducing the sidewall roughness.

4.3 Active sensor characterization

Just like its passive counterparts, the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers can experience a change of lasing wavelength through modifications of the n_{eff} of the mode travelling inside the resonator. This is illustrated in Fig. 4.6, where the lasing spectrum of a disk resonator is shown for three different liquid top claddings. As the refractive index of the liquid increases, the lasing wavelength shifts to higher values, indicating that the disk can be both tuned and used as a sensor. This shift occurs due to the combination of both elongating the optical path length of the cavity, and mode hopping of the lasing mode to higher azimuthal modes. The resolution of the OSA only permits observable shifts of 0.1 nm or larger, making the heterodyne method necessary for sensing smaller lasing wavelength shifts. This is done by creating a low frequency beat note (1—10 GHz) with the external reference laser, recording the RF spectra repeatedly at a

Fig. 4.6: Shift of the wavelength of the lasing mode of a $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk resonator subjected to flows of differing refractive index. Laser operated in TE polarization.

rate of 15 scans per minute at a resolution of 1 MHz, extracting the frequencies of the peaks in the RF spectra and monitoring their locations over time as function of the applied perturbation.

Both the best performing ring and disk resonator lasers were used for the active sensor characterization. However, the ring resonator proved to be less stable than the disk resonator and it could not be used for determining its temperature sensitivity due to a drop of the laser power over time, followed by the disappearance of lasing at all. This might have originated from waveguide damage accumulated over time. Nevertheless, its bulk refractive index sensitivity could be measured before this damage occurred.

4.3.1 Temperature sensing

The temperature sensitivity of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk laser was determined by monitoring the beat note frequency while keeping the reference laser at a fixed wavelength and varying the stage temperature. The temperature was increased from 22.9 °C to 25.4 °C in steps of 0.5 °C every 2 minutes. The beat note spectra for different stage temperatures are shown in Fig. 4.7 (a). Upon increasing the stage temperature, the beat note frequency shifts to higher values. Figure 4.7 (b) shows the temporal response of the beat note, although only the lower frequency peak is plotted for enhanced visibility, since the two split-modes lie close to each other in frequency (40 MHz). The disk resonator exhibited a temperature sensitivity of 1.72 ± 0.03 GHz/K, as shown in Fig. 4.7 (c). In terms of wavelength sensitivity, this equals to 5.73 pm/K, which is slightly lower compared with the passive ring resonator. The sensitivity of both split-modes was almost identical, since they experience the same environmental changes. In fact, this mechanism was already exploited for self-referenced sensing applications to eliminate environmental noise experienced by the resonator [33,34].

4.3.2 Bulk refractive index sensing

The bulk refractive index sensitivity of the disk resonator and ring resonator lasers were characterized by exposing them to different concentrations of NaCl dissolved in deionized water. Figures 4.8 (a) and (d) show the temporal response of the heterodyned beat note of both lasers to the applied bulk refractive index variations. Again, only the lower frequency peak of the split-mode is shown for enhanced visibility. Figures 4.8 (b) and (e) show the bulk refractive index sensitivities of respectively the disk resonator and ring resonator. A sensitivity of

Fig. 4.7: Temperature sensitivity of a $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk resonator laser. Laser emission was at 1024 nm and TE polarization. (a) Shift of beat note frequency for varying temperatures of the stage from 22.9 °C (i) to 25.4 °C (vi) in steps of 0.5 °C. Spectral resolution is 1 MHz. (b) Temperature induced beat note frequency shift for the lower frequency split-mode in (a). (c) Temperature slope sensitivity of both split-mode frequencies. The curve of mode 2 was vertically shifted by 0.5 GHz for increased visibility.

5.67 ± 0.23 THz/RIU was measured for the disk resonator. The sensitivity of the ring resonator was almost twice larger. The wavelength sensitivity equals 18.9 nm/RIU for the disk resonator and 34.6 nm/RIU for the ring resonator. The sensitivity of both split-modes of a resonator is again almost identical. The bulk refractive index sensitivity of the disk resonator is lower compared with the sensitivity of the ring resonator due to the absence of one of the waveguide sidewalls, which leads to a smaller overlap of the evanescent field with the surrounding medium. The sensitivities are lower than those of the passive devices. The main reason is that the cross-section of the waveguides are now rather large compared with the wavelength, resulting in a smaller fraction of optical power in the evanescent field. As discussed earlier, a large cross-section is required for efficient pump light absorption and conversion.

Fig. 4.8: Bulk refractive index sensitivity of $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers. Spectral resolution is 1 MHz. (a) Shift of the lower frequency split-mode upon flowing various NaCl concentrations over the disk resonator laser operating at 1024 nm and TE polarization. Applied NaCl concentrations were from 0.0 wt% (i) to 0.5 wt% (vi) in steps of 0.1 wt%. (b) Bulk refractive index slope sensitivity for both split-modes. The higher frequency mode has an offset of 0.5 GHz for enhanced visibility (c) Noise of both split-modes in (a). (d–f) Same as (a–c), but for a ring resonator laser operating at 1055 nm and TE polarization. Applied NaCl concentrations were from 0.0 wt% to 0.1 wt% in steps of 0.025 wt%. Smaller values were used due to the larger sensitivity. There is no offset on the higher frequency mode in (e) due to splitting being quite large for the ring resonator.

The active sensors experience noise in their beat note frequency, which arises mainly from fluctuations of the temperature of the chip, power fluctuations of the pump diode, the microfluidic flow and fluctuations of the frequency of the external reference laser due to temperature or atmospheric pressure variations within its cavity. It is difficult to decouple all noise sources, but it can be quantified by the standard deviation in the beat note frequency. Figures 4.8 (c) and (f) show repeated measurements of the beat note frequency of both split-modes in a flow of deionized water for a duration equal to the time that the resonator is exposed to the NaCl flow. The uncertainty in the beat note frequency was determined as $\sigma=7$ MHz for the disk resonator and $\sigma=11$ MHz for the ring resonator, or $\sigma=0.023$ pm and $\sigma=0.037$ pm respectively. The *LOD* is the smallest bulk refractive index variation that can reliably be detected and is given by the uncertainty and sensitivity [22,53,54]. The *LOD* equals 3.7×10^{-6} RIU and 3.2×10^{-6} RIU for the disk resonator and ring resonator respectively. These *LODs* are similar to previous reports on passive ring resonator sensors, as well as the *LOD* of passive Al_2O_3 ring resonators presented in the previous Chapter. However, the active, laser-based sensor is currently being limited by the noise and therefore not benefiting yet from the much smaller intrinsic *LOD* achievable in active sensors due to the increase in the *Q*-factor by ~ 3 orders of magnitude with respect to a passive Al_2O_3 ring resonator.

Fully exploiting the benefits of the narrow linewidth and the high intrinsic *LOD* would require eliminating the noise sources present in the current system. A filter could be inserted in the flow line prior to entering the chip to remove any dirt and to provide a more homogeneous flow for lower noise, although the complex composition of a clinical matrix such as urine, which is used for the following biosensing experiments, will inherently lead to variations in the flow or drift. Fluctuations of the incident pump power can also affect the stability of the system, together with frequency and amplitude noise of the reference laser.

4.4 rhS100A4 biomarker sensing

The disk resonator laser was used to detect the rhS100A4 protein cancer biomarker. The ring resonator was omitted due to stability problems and the disappearance of lasing, as discussed earlier. Similar to the previous Chapter, specific molecular recognition was achieved by immobilizing monoclonal antibodies onto the surface of the disk resonator.

The approach suitable for Al_2O_3 surfaces previously designed by [36] was employed here. However, the protocol was modified slightly for a more convenient and simpler biosensing experiment. More specifically, after applying the functionalization by evaporating a droplet of 6-phosphonohexanoic acid on the sample, it was heated in an oven at 120 °C for 16 hours, after which the activation was performed for 4 hours (i.e., in a mixture of 25 mM EDC and 50 mM NHS in a 100 mM MES buffer). Furthermore, the antibody immobilization was not performed in flow as in Chapter 3, but it took place by placing a droplet of 10 mM PBS containing antibodies at a concentration of 200 $\mu\text{g}/\text{ml}$ on the functionalized and activated chip for 18 hours at 4 °C. This was followed by rinsing the chip with 10 mM PBS and deionized water, after which the PDMS microfluidics were bonded non-permanently and 1% BSA in 10 mM PBS was flown over the device for 1 hour to curb non-specific binding. The microfluidic channel was then flushed with 10 mM PBS. rhS100A4 detection was performed in synthetic urine (Surine™ Negative Urine Control) as a model of a complex body fluid. A total of 100 ml of urine was used, to which 10 ml 100 mM PBS was added. Then, the pH was adjusted to 7.5 by adding NaOH. The resulting sample was then filtered through 0.2 μm pores. Finally, 1 ml urine samples were spiked with the rhS100A4 biomarker at known concentrations between 100 pM and 3 μM . The antibody immobilization and BSA surface blocking steps were not recorded because of the former taking place in the fridge and the latter experiencing a too large beat note frequency shift that would exceed the range of the ESA (24 GHz). Blank urine and urine containing the rhS100A4 were flown over the device at a flow rate of 40 $\mu\text{l}/\text{min}$ for 20 minutes, while keeping the stage fixed at a temperature of 21.5 ± 0.001 °C.

The beat note frequency shift due to biomarker binding is shown in Fig. 4.9. This was only visualized for the shift of the lowest heterodyned frequency of the split-mode, although both modes experienced equal shifts. A positive beat note frequency shift occurs that flattens over time, indicating that a dynamic equilibrium between binding and disassociation of the proteins to the antibodies is reached. Furthermore, the total amount of beat note frequency shift after 20 minutes increases with biomarker concentration. Here, the uncertainty is measured as the standard deviation of the beat note frequency of each of the split-modes during the flow of blank urine samples, as shown in Fig. 4.9 (c). The bumps present in the first few minutes of the data originate from temporarily stopping the flow system upon switching between liquids, which induces a short time temperature overshoot that is accompanied by a

Fig. 4.9: rhS100A4 biomarker sensing with $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk resonator laser. Laser emission was at 1024 nm and TE polarization. Spectral resolution is 1 MHz. (a) Shift of the lower frequency of the split-mode upon biomarker binding at low protein concentrations and (b) high protein concentrations. (c) Noise upon flowing blank urine samples. The standard deviation is the average of the 4 blank urine samples. (d) Beat note frequency shift as function of rhS100A4 concentration.

short increase of the emission frequency of the disk resonator laser. In all cases, the signal dropped back to the initial baseline. An average standard deviation of $\sigma = 30$ MHz was measured for the blank urine samples, which includes temperature drifts, fluctuations of the frequencies of both the disk resonator laser and the reference laser and potential disturbances to the biosensor due to variation of the refractive index of the synthetic urine flowing over the biosensor.

The *LOD* is defined as the concentration that yields a shift of beat note frequency that exceeds three times the uncertainty present in the blank synthetic urine samples. A *LOD* of 300 pM of rhS100A4 in synthetic urine was experimentally demonstrated with a total frequency shift of 162 ± 13 MHz, which exceeds three times the noise of 90 MHz in blank synthetic urine samples. This limit of detection represents one of the lowest reported in the literature for label-free detection in a complex matrix such

as synthetic urine, and it is one order of magnitude lower than the *LOD* that was achieved for the same protein biomarker using a passive ring resonator biosensor [36]. This is remarkable when considering that the passive sensor had a higher sensitivity. Several factors were different between these two experiments that could explain this difference. First, the biochemical functionalization and immobilization protocol applied to the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk resonator was slightly adjusted, which has now longer functionalization, activation and immobilization times. Especially the last step could lead to a better antibody coverage of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ surface. Furthermore, the biomarker flow time is more than twice larger for the active biosensing experiments, allowing more binding events to occur during the exposure to the analyte and thus larger total beat frequency shifts per biomarker concentration. Finally, the noise of the active biosensing experiment, 30 MHz or 0.1 pm is much lower compared to the passive experiment (1.2 pm). This could be due to improvements to the experimental setup, or the narrower linewidth of the beat notes that offers an improvement of the determination of their shift compared to the broad, passive resonances tracked in the passive biosensing experiment.

However, the measured *LOD* is still far from the intrinsic *LOD* dictated by the laser linewidth. In fact, the current system is dominated by noise, both due to temperature fluctuations as well as variations in the frequency of both the on-chip and external reference laser. Regardless, this result shows the possibility of using an active laser-based sensor to detect a clinically relevant cancer biomarker from a complex liquid, such as urine, at low detection limits.

4.5 Conclusion

To conclude, the first proof-of-concept of active sensors based on an $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ disk resonator and ring resonator laser was presented. Both disk resonator and ring resonator lasers were integrated on-chip and exhibited narrow-linewidth, single-mode lasing while operating in an aqueous environment. A heterodyning detection scheme using an external reference laser operating at a wavelength very close to the emission wavelength of the on-chip $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ laser was used to monitor lasing wavelength shifts. A bulk refractive index sensitivity and limit of detection comparable to those obtained on the passive Al_2O_3 ring resonator sensors was achieved. Molecular recognition of rhS100A4 biomarkers, associated to human cancer development, was achieved in synthetic urine by binding to a bioreceptor layer of antibodies immobilized onto the disk resonator surface. A *LOD* of 300 pM

in synthetic urine was experimentally demonstrated. This falls within the concentration range being reported as clinically relevant [55], therefore demonstrating the potential of this active platform for label-free biosensing. These novel results pave the road towards the realization of biosensing platforms based on active, laser-based devices that are easy to integrate in point-of-care instruments equipped with portable, simple and relatively low cost readout schemes.

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Chapter 5

Mode-splitting in a ring resonator for self-referenced biosensing

A ring resonator has a two-fold degeneracy corresponding with two counterpropagating modes, one in the clockwise and the other in the counterclockwise propagation direction. A Bragg grating integrated on the surface of the ring provides coupling between the clockwise and counterclockwise travelling modes lifting the degeneracy of the resonance modes of the pristine ring resonator. The amount of mode-splitting is directly related to the reflectivity of the grating and it is principally affected by structurally modifying the grating. Environmental perturbations to the surroundings of the grating, such as temperature and bulk refractive index variations, hardly affect the amount of mode-splitting. This principle allows the realization of a self-referenced sensing scheme based on the detection of variations of the mode-splitting induced by structural changes to the grating. In this work, a poly(methyl methacrylate) (PMMA) Bragg grating is integrated onto a ring resonator. It is shown experimentally that the amount of splitting of a resonance varies minimally under temperature and bulk refractive index perturbations. However, attaching a layer of biomolecules inside the grating does affect its reflectivity and, therefore, the amount of mode-splitting present. This result represents the first demonstration of an integrated mode-splitting sensor, paving the road towards the realization of truly self-referenced biosensors.

This chapter is based on:

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As it was shown in the previous Chapters, Al_2O_3 ring resonators can provide label-free biosensing of disease biomarkers from complex matrices down to clinically relevant levels. Currently, most ring resonator sensors exhibit a limit of detection limited by environmental noise. The devices respond not only to the biomarker of interest, but also to all dielectric perturbations experienced by the ring resonators, including non-specific binding events, variations in temperature and in the bulk refractive index of the fluid matrix, such as urine or plasma. Different techniques to reduce the influence of these sources of noise have been proposed and implemented in the literature, such as the use of reference rings [1,2], multi-mode differential sensing scheme [3] and frequency-locking for ultra-fast scanning that allows to eliminate slow drifts and improve the signal-to-noise ratio [4]. Recently, a novel, self-referenced approach based on the splitting of the resonance modes of an optical resonator has gained increasing attention [5–8]. This chapter describes the efforts of implementing a similar sensing scheme on passive integrated Al_2O_3 ring resonators.

5.1 Introduction

Optical resonators support two degenerate modes sharing the same resonance wavelength and traveling in the clockwise (CW) and counterclockwise (CCW) directions. Mutual coupling between the CW and CCW modes lifts their degeneracy resulting in a doublet splitting of the original resonant wavelength [9–11], as shown in Fig. 5.1. The coupling can be provided by a scattering medium or a reflector along the ring resonator perimeter. The amount of mode-splitting is directly related to the strength of the coupling between the CW and CCW modes. Individual binding events of nanoparticles and even single viruses have been detected using ultra-high quality factor resonators by monitoring the magnitude of the mode-splitting [5,12,13]. Since the variations of the mode-splitting are due to the magnitude of the scattering (i.e., coupling between the CW and CCW modes), these sensors are, to first-order, insensitive to environmental variations such as temperature fluctuations or variations in bulk refractive index [6]. The mode-splitting induced by the introduction of reflective elements inside the resonator has been investigated [14–18]. Fiber Bragg gratings were introduced into fiber ring resonators to induce controlled mode-splitting [19,20]. This configuration was demonstrated as a strain sensor. Strain applied locally to the grating varies both its reflectance and the Bragg wavelength leading to changes in the magnitude of the mode-splitting [21,22].

Most implementations of mode-splitting sensors, whether they are based on the ultra-high quality factor resonators or on gratings on fibers, are not well-suited to be monolithically integrated on a chip. For instance, the ultra-high quality factor resonators are often based on three-dimensional structures formed from SiO_2 fiber tips or pedestal toroids that do not lend themselves for simple integration and read-out on a chip, limiting their potential usage for sensing applications. However, controlled mode-splitting has also been demonstrated in ring resonators on silicon-on-insulator (SOI) by either self-coupling [23,24] or by means of a grating imposed on the ring resonator [25–27]. Very recently, several theoretical demonstrations of self-referenced biosensing using grating-induced mode-splitting in ring resonators have been proposed [7,8].

In this thesis, the first experimental demonstration of an integrated self-referenced biosensor based on mode-splitting is presented. A Bragg reflector grating made of poly(methyl methacrylate) (PMMA) was nanofabricated on the surface of an undoped aluminum oxide (Al_2O_3) ring resonator [28,29]. The Al_2O_3 surface was selectively functionalized to bind antibodies to capture the rhS100A4 protein. The insensitivity of the proposed sensor to environmental perturbations such as temperature and bulk refractive index variations was characterized. Finally, biosensing of the rhS100A4 biomarker using this device was successfully demonstrated.

5.2 Grating-induced mode-splitting

The considered structure is a bus waveguide coupled to a ring resonator with an integrated Bragg grating. The reflectivity of the grating provides coupling between the clockwise and counterclockwise propagating modes, resulting in mode-splitting, which is absent in the case of a pristine ring. A semi-analytical model combining fully vectorial 2D eigenmode calculations (Lumerical MODE solutions) with the transfer matrix method and coupled mode theory [15,21] was utilized.

A schematic of the grating-integrated ring resonator model is shown in Fig. 5.1 (b). The field entering the bus waveguide via the input port excites the resonant modes of the ring via the directional coupler, which, assuming negligible loss, can be modelled as [30]:

Fig 5.1: Ring resonator geometry demonstrating the propagating electric fields. (a) Pristine ring resonator. Inset shows degenerate resonance. (b) Ring resonator with grating. Inset shows mode-splitting.

$$\begin{pmatrix} E_{out,1} \\ E_{out,2} \end{pmatrix} = \begin{pmatrix} t & k \\ -k^* & t^* \end{pmatrix} \begin{pmatrix} E_{in,1} \\ E_{in,2} \end{pmatrix}, \quad \text{Eq. (5.1)}$$

with t and k the fractions of coupled and transmitted field amplitudes respectively. Equation 5.1 is a short version of Eq. 2.18, which contains the full description of the coupling section. A lossless grating can be modelled by the scattering matrix as [16]:

$$\begin{pmatrix} E_{out,3} \\ E_{out,4} \end{pmatrix} = \begin{pmatrix} \rho & \tau \\ \tau & \rho \end{pmatrix} \begin{pmatrix} E_{in,3} \\ E_{in,4} \end{pmatrix}, \quad \text{Eq. (5.2)}$$

where τ and ρ are the fractions of the amplitude of the electric field transmitted and reflected by the grating. These can be expressed as [21,31]:

$$\tau = \frac{\Theta}{\Theta \cosh(\Theta l_b) + j\Delta\beta \sinh(\Theta l_b)}, \quad \text{Eq. (5.3)}$$

$$\rho = \frac{jK \sinh(\Theta l_b)}{\Theta \cosh(\Theta l_b) + j\Delta\beta \sinh(\Theta l_b)}, \quad \text{Eq. (5.4)}$$

where l_b is the length of the grating and Θ , $\Delta\beta$ and K are given by [21]:

$$\Theta = \sqrt{K^2 - \Delta\beta^2}; \Delta\beta = \beta - \frac{\pi}{\Lambda}; K = \frac{\Gamma(n_h^2 - n_l^2) \sin(\pi D)}{\lambda n_{\text{eff}}}, \quad \text{Eq. (5.5)}$$

where β is the propagation constant of the optical mode travelling inside the ring resonator, n_{eff} its wavelength-dependent effective refractive index, λ the wavelength, D the filling fraction of a single grating period, Γ the modal overlap of the optical mode with the grating region, n_h and n_l the alternating refractive indices of the materials inside the grating, in this case PMMA and water, and Λ the Bragg grating period [31]. The Bragg wavelength of a first order grating is given by:

$$\lambda_b = 2n_{\text{eff}} \Lambda. \quad \text{Eq. (5.6)}$$

The spectral transmission function of the Bragg grating-integrated ring resonator can be derived as [16]:

$$T = \left| \frac{t - a\tau(1+t^2)e^{j\theta} - a^2te^{2j\theta}\det(S)}{1 - 2at\tau e^{j\theta} - a^2t^2e^{2j\theta}\det(S)} \right|^2, \quad \text{Eq. (5.7)}$$

where a and θ are respectively the attenuation of the electric field and the accumulated phase by the mode of the resonator after completing a single round trip and S is the scattering matrix of the grating (Eq. 5.2). Whenever the reflected amplitude of the optical mode is sufficiently large, the resonance mode splits into two non-degenerate peaks at separate wavelengths λ_1 and λ_2 . The mode-splitting, $\Delta\lambda = \lambda_2 - \lambda_1$, can be derived from Eq. 5.7 and is directly linked to the reflectivity of the grating:

$$\Delta\lambda = \frac{\lambda^2}{\pi n_{\text{eff}} L} \arctan \left(\sqrt{\frac{|\rho|^2}{1 - |\rho|^2}} \right), \quad \text{Eq. (5.8)}$$

which, in the case of a weak grating, reduces to [15]:

$$\Delta\lambda = \frac{\lambda^2}{\pi n_{\text{eff}} L} \sqrt{\frac{|\rho|^2}{1-|\rho|^2}}. \quad \text{Eq. (5.9)}$$

5.3 Self-referencing of a grating-integrated ring resonator

The change of mode-splitting due to environmental and structural variations of the grating was investigated. Figure 5.2 shows the unperturbed grating. The effect of the grating strength on the magnitude of the induced mode-splitting was studied by varying the height of the grating teeth (Fig. 5.2(b)). The effect of environmental perturbations on the mode-splitting, such as temperature and bulk refractive index variations, were studied by either varying the refractive indices n of the different materials according to their thermo-optic coefficient (dn/dT) or by varying the refractive index of the surrounding medium (Fig. 5.2(c)). For both grating height variations and environmental perturbations (i.e., temperature and bulk refractive index

Fig. 5.2: Effect of perturbations applied to the grating. (a) Unperturbed grating with height h_1 and reflectivity r_1 . (b) Increasing the grating height to h_2 results in an increased reflectivity r_2 . (c) The reflectivity of grating with height h_1 is almost invariant under temperature and bulk refractive index variations.

variations), the effective refractive index of the guided mode inside the resonator, n_{eff} , and the mode overlap of the mode with the grating region (Γ) vary. Then, the non-degenerate resonance wavelengths can be derived from the device transfer function, T (Eq. 5.7). The analysis is performed at a central wavelength around 1630 nm (to avoid water absorption) and includes waveguide and material dispersion. The latter was linearized around the central wavelength based on full dispersive material data. The material parameters, including first-order material dispersion and the thermo-optic coefficient, dn/dT , of the SiO₂ bottom cladding, Al₂O₃ waveguide, PMMA grating and deionized water top cladding, are summarized in Table 5.1. The temperature was varied in steps of 2.5 K. The bulk refractive index

Fig. 5.3: Self-referencing in grating-integrated ring resonator. (a) Transmission spectra of a grating-integrated ring resonator for varying height of the PMMA grating on the surface of the resonator. (b) Wavelength shift of both non-degenerate modes for increasing grating heights, h , of the PMMA grating. (c) Wavelength shift of both non-degenerate modes as well as their mode-splitting as a function of changes in the refractive index of the aqueous top cladding. (d) Wavelength shift of both non-degenerate modes as well as their mode-splitting as a function of device temperature. In both (c) and (d), the mode-splitting remains almost completely insensitive to changes of refractive index of the environment or changes in the temperature of the device.

of the aqueous top cladding is varied in steps of 0.001 RIU. The waveguide cross-section has a width of 2.2 μm and a thickness of 750 nm. The ring resonator has a radius of 150 μm and a waveguide propagation loss of 0.5 dB/cm was assumed. The PMMA grating has a grating period of 535 nm, a filling factor of 0.5, and covers 4/5 of the ring resonator perimeter. The grating teeth have a height of 25 nm and the refractive index of PMMA, 1.481.

Table 5.1. Material parameters at a wavelength of 1630 nm.

Material	n	$dn/d\lambda$ (μm^{-1})	dn/dT (K^{-1})
SiO ₂	1.443 [32]	-0.01	1×10^{-5} [33]
Al ₂ O ₃	1.668	-0.005	$1.9 \times 10^{-5} \text{ K}^{-1}$ [34]
H ₂ O	1.317 [35]	-0.025	$-4 \times 10^{-5} \text{ K}^{-1}$ [36]
PMMA	1.481 [37]	-0.01	-1.3×10^{-4} [38]

Figures 5.3 (a) and (b) show that the magnitude of the mode-splitting of the grating-integrated ring resonator only varies significantly when the reflectivity of the grating varies, as is the case when the height of the grating is varied. Mode-splitting only occurs from the onset of a specific grating height, since then the coupling rate exceeds the loss rates (i.e., the mode-splitting exceeds the resonance linewidth broadening due to the internal and external losses) [39]. On the contrary, Figs. 5.3 (c) and (d) show that the mode-splitting remains almost constant for temperature and bulk refractive index variations, with a sensitivity to bulk refractive index variations of -1.4 nm/RIU and to temperature variations of 0.05 pm/K. Such small sensitivity to environmental and temperature changes is due to the very small variation of the reflectivity of the grating under such perturbations, since the mode overlap of the evanescent field with the grating is then hardly affected. These results demonstrate theoretically that a grating-integrated ring resonator can be utilized as a self-referenced sensor provided that the sensing mechanism involves altering the structure of the grating.

5.4 Grating fabrication

The fabrication of the grating-integrated ring resonator starts with the Al₂O₃ ring resonators presented in Chapter 2. Grating-integrated ring resonators were realized for wavelengths of 1630 nm and 1035 nm. Device parameters include a ring radius of 150 μm , coupling gap of 0.8 μm and a waveguide cross-section of either

Fig. 5.4: Fabrication of the grating-integrated ring resonator. (a) Process flow. (b) SEM image of the developed PMMA grating. The dotted lines indicate the waveguide sidewall. (c) AFM scan of the PMMA grating with period of 535 nm on top of a 0.8 μm tall Al₂O₃ waveguide. (d) Height profile of the PMMA grating along the dotted line in (c). (e) and (f) Same as (c) and (d), but for a grating with period of 333 nm on top of a 0.55 μm tall Al₂O₃ waveguide.

$2.2 \times 0.8 \mu\text{m}^2$ for operation at 1630 nm, or $2.2 \times 0.55 \mu\text{m}^2$ for operation at 1035 nm. After obtaining the pristine ring resonator chips, the dies were cleaned using a HNO₃ bath, followed by rinsing with deionized water and O₂ plasma cleaning.

A PMMA Bragg grating was printed over the device using electron beam lithography. This method was chosen due to its simple fabrication process that only requires patterning and development of the grating, without any subsequent deposition or processing steps. Furthermore, the PMMA layer can easily be removed and the process repeated non-destructively, contrary to other implementations relying on etching of a grating along the sidewall of the waveguide [25,26]. Figure 5.4 (a) illustrates the process flow. First, a PMMA layer (NANOTM 950PMMA A4) was spun at 3.5 krpm for 1 minute. Then, a ~5 nm thick chromium coating was sputter coated using a homebuilt sputter coater at a power of 200 W and base pressure of 6.6×10^{-3} mBar. This layer prevents charging during the electron beam exposure, since both the substrate and the sample are non-conductive. Alternatively, a conducting electron beam lithography resist could be used. Then, the grating pattern is exposed on top of the resist with a RAITH150 Two electron beam lithography tool. A dose of $180 \mu\text{C}/\text{cm}^2$ was used, together with an acceleration voltage of 10 kV. The fabricated grating parameters were a period of either 535 nm or 333 nm, corresponding to a Bragg wavelength of 1630 nm or 1035 nm, a grating length of 4/5 of the ring circumference and a fill fraction of 0.35 on the mask. After exposure and patterning, the Cr layer was removed by wet etch with a mixture of perchloric acid and ceric ammonium nitrate (TechniEtch Cr01) for 10 seconds, followed by a rinse in deionized water. The exposed PMMA was subsequently developed with a mixture of MIBK:IPA at a ratio 1:3 for 40 seconds, after which the device was rinsed with IPA for 20 seconds and blown dry with N₂. The resulting gratings were imaged with SEM and AFM, as shown in Figs. 5.4 (b)–(f).

The two different waveguide cross-sections have a different resulting PMMA grating height. The waveguide for operation at 1630 nm has a PMMA thickness of ~30 nm on top of the waveguide, the waveguide for operation at 1035 nm has a PMMA thickness of 110 nm. During the spinning process the waveguide acts as a bump on the substrate. The higher the bump, the thinner the resulting PMMA layer on top of the waveguide compared to the PMMA layer on the substrate. Both heights can be fine-tuned by choosing the appropriate PMMA resist and spin speed. Both resulting gratings have a fill factor of ~0.5 and some roughness is present on them.

Finally, a PDMS microfluidic device with channel dimensions of 600 μm width and 70 μm height was non-permanently bonded without an oxygen plasma treatment onto the chip and connected to a syringe pump to address the device with various

liquids. The channel was then filled with deionized water to submerge the grating-integrated ring resonator.

5.5 Grating characterization

To optically characterize the devices, their transmission spectra were continuously recorded using either an Agilent 81646 laser or TOPTICA CTL 1050 laser, corresponding with the devices with a grating period of respectively 535 nm or 333 nm. The sample was held on a temperature controlled ($\sim \pm 0.001$ °C) holder by vacuum. TE-polarized laser light was guided through single mode polarization maintaining fibers (PM1500-XP) and butt coupled to the sample using index-matching fluid. All experiments in this section were performed in a flow of deionized

Fig. 5.5: Grating-induced mode-splitting of a ring resonator at 1630 nm in deionized water. The grating had a period of 535 nm, height of 30 nm, duty cycle of 0.5 and 4/5 coverage of the ring circumference. (a) Transmission spectrum of a ring resonator covered in PMMA before grating patterning and development. (b) Zoom and fit of a degenerate mode from (a). (c) Transmission spectrum of the ring resonator after grating patterning and development. (d) Zoom and fit of the maximum mode-splitting in (c).

water at a flow rate of 100 $\mu\text{l}/\text{min}$ and a stage temperature of 21.5 $^{\circ}\text{C}$, if not otherwise specified.

5.5.1 Transmission measurements

The acquired transmission spectra were normalized to unity and the resonances were fitted with either a single or double Lorentzian function, depending on whether mode-splitting was present or not. Figure 5.5 shows the transmission spectra of the same device designed for operation at 1630 nm covered in PMMA before and after grating fabrication. Before grating fabrication, the spectrum of the ring resonator contains multiple degenerate sharp resonances. Mode-splitting of the resonance modes is clearly observed after grating fabrication. The magnitude of the mode-splitting varies along the bandwidth of the grating due to the variation of the reflectivity of the grating with wavelength. The measured maximum mode-splitting lies around a wavelength of 1627 nm and amounts to ~ 410 pm. The other resonance modes in the vicinity of 1627 nm experience also mode-splitting due to the relatively large bandwidth of the grating, which extends multiple FSRs of the ring resonator. The mode-splitting decreases as the wavelength is detuned from the Bragg wavelength.

The same experiment was repeated on the waveguide designed for operation at 1035 nm with a grating period of 333 nm. Again, the transmission spectrum obtained before grating fabrication had multiple degenerate resonances, one of which is shown in Fig. 5.6 (a). The grating induces mode-splitting. A highest mode-splitting of ~ 425 pm was realized, as shown in Fig. 5.6 (b).

Fig. 5.6: Grating-induced mode-splitting of a ring resonator at 1050 nm in deionized water. The grating had a period of 333 nm, height of 110 nm, duty cycle of 0.5 and 4/5 coverage of the ring circumference. (a) Transmission spectrum of degenerate resonance. (b) Transmission spectrum of resonance experiencing the maximum mode-splitting.

Both devices had a lower Q and higher propagation loss after grating fabrication. This most likely resulted from scattering losses from additional roughness of the grating.

5.5.2 Temperature and bulk refractive index variations

The variation of the mode-splitting due to environmental perturbations was measured. Figure 5.7 shows the results for the device designed for operation at 1630 nm. First, its temperature was varied while monitoring the wavelengths of the non-degenerate resonance modes. Upon increasing the temperature of the stage, the whole transmission spectrum shifts to higher wavelengths, as shown in Fig. 5.7 (a). However, as both wavelengths shift to higher values, their mode-splitting remains

Fig. 5.7: Effect of environmental perturbations to the surroundings of the grating for a device designed for operation at 1630 nm in deionized water. The grating had a period of 535 nm, height of 30 nm, duty cycle of 0.5 and 4/5 coverage of the ring circumference. (a) Wavelength shifts of non-degenerate resonances upon applying temperature variations to the device. (b) Shift of the right peaks in (a), together with the temperatures applied to the chip and the mode-splitting of the resonance. (c) Measured wavelength shifts of the non-degenerate resonances for temperature variations applied to the device. (d) Measured wavelength shifts of the non-degenerate resonances for bulk refractive index variations applied to the top cladding.

almost constant, as shown in Fig. 5.7 (b). There are still some variations present in the magnitude of the mode-splitting with a standard deviation around the mean of 0.5 pm, but these are more than a factor 100 lower than the wavelength shifts of the individual degenerate modes. This is illustrated by the almost identical slope sensitivities of both peaks, as shown in Fig. 5.7 (c). The same analysis was repeated by flowing various solutions of deionized water with different NaCl concentrations to vary the refractive index of the liquid. Again, almost identical slope sensitivities were obtained, as shown in Fig. 5.7 (d). The mode-splitting experienced a temperature dependency of -0.04 pm/K and a bulk refractive dependency of -0.33 nm/RIU.

Figure 5.8 presents similar tests for a device designed for operation at 1035 nm. This device is a different one from the one presented in Fig. 5.6. It exhibits a mode-splitting of ~240 pm. Temperature variations yielded a shift of both resonances that have almost identical slope sensitivities, as is shown in Fig. 5.8 (a). Solutions of NaCl in deionized water were flown over the device to vary the bulk refractive index, while the shift of both resonance wavelengths was monitored. Again, both resonances shifted almost equal amounts, as is shown in Fig. 5.8 (b). The mode-splitting experienced a temperature dependency of -0.12 pm/K and a bulk refractive dependency of -0.19 nm/RIU.

These results indicate that the mode-splitting of the device does not vary upon applying environmental perturbations to the surroundings of the grating,

Fig. 5.8: Effect of dielectric perturbations to the surroundings of the grating for a device designed for operation at 1035 nm in deionized water. The grating had a period of 333 nm, height of 110 nm, duty cycle of 0.5 and 4/5 filling of the ring circumference. (a) Measured wavelength shifts of the non-degenerate resonances for temperature variations applied to the device. (b) Measured wavelength shifts of the non-degenerate resonances for bulk refractive index variations applied to the top cladding.

demonstrating experimentally the self-referenced nature of the mode-splitting of a grating-integrated ring resonator.

5.5.3 Modification of the grating coupling coefficient

To demonstrate the effect of the reflectivity of the grating on the mode-splitting, the device presented in Fig. 5.6 was briefly etched with an O_2 plasma to lower the height of the teeth of the grating (grating parameters were a period of 333 nm, height of 110 nm, duty cycle of 0.5 and 4/5 filling of the ring circumference). Figure 5.9 (a) shows the AFM grating height profiles before and after O_2 plasma etch. This step reduced the grating height by almost 40 nm. Figure 5.9 (b) shows the transmission spectra before and after etch, which resulted in a decrease of mode-splitting by almost 200 pm. Since the grating now has a lower reflectivity due to the smaller overlap of the evanescent field with the grating teeth, the mode-splitting decreases. This test, combined with the previous results on the effect of environmental perturbations on the mode-splitting, shows that only changing the structure of the grating results in a significant change of the mode-splitting.

5.6 Biomarker sensing

Biosensing was demonstrated by modifying the mode-splitting through binding of biomolecules to alter the reflectivity of the grating. This was done by binding the rhS100A4 protein biomarker to antibodies immobilized onto the Al_2O_3 surface exposed by the electron beam lithography, as shown in Fig. 5.10 (a). The openings in the PMMA allow the biomarkers to bind to the Al_2O_3 in between the PMMA

Fig. 5.9: Structural modification of a grating. (a) AFM scans of grating profiles before and after etching the PMMA. Dotted lines indicate average grating heights. (b) Mode-splitting before and after grating etch.

grating teeth. A new grating with a period of 535 nm was fabricated on the device of Figs. 5.5 and 5.7, using a slightly higher PMMA spin speed of 4 krpm. The resulting grating was 25 nm tall and exhibited a mode-splitting of around 300 pm, which is now smaller because of the reduced height. A three-step protocol was developed to deposit the biomolecules inside the grating teeth. The process is illustrated in Fig. 5.10 (a). First, the sample and 200 μ l of (3-Glycidyloxypropyl)trimethoxysilane (GPTMS, Sigma Aldrich 440167) were placed in a desiccator for 4 hours to apply a silane functionalization through evaporation in gas phase. To validate the functionalization protocol, a fluorescence assay was carried out with an antibody

Fig. 5.10: Biomarker binding to the grating. (a) Surface functionalization protocol. GPTMS silanization is applied to the Al_2O_3 surface, followed by monoclonal antibody deposition and protein capture by the antibodies. (b) Microscope fluorescent image of the grating functionalized with GPTMS silane and immobilized with an antibody labelled with fluorescein isothiocyanate fluorescence dye (FITC, 495 nm/519 nm excitation/emission). Magnification 100X. (c) Change of mode-splitting upon antibodies binding to the grating. (i) 10 mM PBS, (ii) antibodies at a concentration of 200 μ g/ml in 10 mM PBS. (d) Change of mode-splitting upon biomarker capture by the antibodies immobilized on the device. (i) 10 mM PBS, (ii) 1 μ M rhS100A4 biomarkers in 10 mM PBS, (iii) 5 μ M rhS100A4 biomarkers in 10 mM PBS. The grating had a period of 535 nm, height of 25 nm, duty cycle of 0.5 and 4/5 coverage of the ring circumference.

labelled with fluorescein isothiocyanate fluorescein dye (10 $\mu\text{g/ml}$), as shown in Fig. 5.10 (b). The fluorescent signal originates mostly from the individual openings in the PMMA grating structure, indicating that the fluorescent antibodies bind mostly to the Al_2O_3 surface.

After applying the functionalization protocol, the device was exposed to a flow of 10 mM phosphate buffer saline (PBS) in order to establish a signal baseline. The amount of initial mode splitting was 297.6 pm. A solution of anti-S100A4 antibody at a concentration of 200 $\mu\text{g/ml}$ in 10 mM PBS was prepared and flown over the device as shown in Fig. 5.10 (c). All of the biomolecule solutions were flown at a rate of 30 $\mu\text{l/min}$ for 20 minutes. Upon flowing the antibodies over the device they bonded to the Al_2O_3 in the teeth of the grating, resulting in a reduction of the mode-splitting. In this case, the mode-splitting is lowered from 297.6 to 288.0 nm. This is followed by rhS100A4 protein flows at concentrations of 1 and 5 μM in 10 mM PBS, as shown in Fig. 5.10 (d). Corresponding mode-splitting variations of ~ 4 and ~ 6 pm were measured. These changes are significantly larger than the noise of $\sigma=0.5$ pm present during the bulk sensing tests, indicating that the variation of the grating structure due to biomolecule attachment can be used for biomarker detection. Furthermore, this sensing scheme has the advantage that it can be potentially used for the detection of biomarkers in liquids with unknown composition, such as encountered in clinical settings, since the sensor should react only to biomarker binding and not bulk refractive index changes.

The changes of the mode-splitting are rather small compared to the resonance wavelength shifts encountered in conventional ring resonator sensing. This is due to the fact that the mode-splitting does not measure changes of the effective index of the mode travelling inside the ring, but changes of the reflectivity of the grating due to the binding of biomarkers. Since these proteins are small and have a low refractive index of ~ 1.5 [40], their effect on the grating reflectivity is rather low. Furthermore, some of the antibodies and biomarkers can still bind to Al_2O_3 and PMMA through adsorption, lowering the relative change due to grating reflectivity modification. This sensor will perform better when used with larger target analytes, such as cancer biomarker extracellular vesicles [41], nanoparticles, bacteria or denser materials with a higher refractive index. Alternatively, a smaller ring radius can be used to increase the mode-splitting and its sensitivity [42], as it inversely scales with its length (Eqs. 5.8 and 5.9). A smaller waveguide cross-section also increases the modal overlap with the grating and yields a larger variation of the grating reflectivity

upon biomolecule binding. One might also monitor the mode-splitting at a wavelength at the edge of the reflectivity spectrum instead of at the Bragg wavelength where the reflectivity profile is relatively flat. Then, a shift of both the resonance and Bragg wavelength results in the mode experiencing another reflectivity, which is accompanied by a change in mode-splitting [42]. However, despite this scheme having an increased sensitivity, it is not self-referenced. Finally, a promising solution was proposed in [39], where the ring is fully covered by a Bragg grating and the latter is functionalized only over a quarter of the perimeter. Then, only a single pair of degenerate modes undergoes additional mode-splitting upon particle binding, allowing sensitivities comparable to conventional schemes together with self-referenced operation.

Another biosensing approach based on mode-splitting was tested. Now, the grating was formed by the biomolecules. Figure 5.11 (a) illustrates the protocol, where first a grating in PMMA is fabricated. Then, either a functionalization based on the 6-phosphonohexanoic acid or GPTMS is applied. Next, the PMMA is removed by dissolving it in acetone and followed by rinsing the sample in deionized water. This leaves behind periodic functionalized sections on the Al_2O_3 surface. This is followed by antibody immobilization, which results in a grating consisting of alternating regions of bare Al_2O_3 and antibody-immobilized Al_2O_3 . The antibody grating induces a mode-splitting, although smaller than that of the PMMA gratings, due to the antibodies being smaller. Sensing can then be performed by binding biomarkers to the antibody grating. Its increase in height increases the mode-splitting. Figure 5.11 (b) shows a calculation of the change of mode-splitting upon varying the height of a grating consisting of material with a refractive index of 1.5.

This protocol was tested on a ring resonator with a radius of $150\text{ }\mu\text{m}$ and waveguide dimensions of $1.4 \times 0.55\text{ }\mu\text{m}^2$. The grating period was 333 nm for operation around 1035 nm . Figure 5.11 (c) shows the transmission spectrum of both a pristine ring resonator without any cladding or functionalization and a ring resonator after applying the grating fabrication protocol followed by functionalization with 6-phosphonohexanoic acid, PMMA removal and antibody immobilization. The antibodies were prepared in 10 mM PBS at a concentration of $200\text{ }\mu\text{g/ml}$ and were immobilized on the functionalized device for 16 hours overnight, after which the device was rinsed with 10 mM PBS . The device without grating definition experiences no mode-splitting, whereas the latter had a maximum mode-splitting of

Fig. 5.11: Biomolecule grating. (a) Functionalization protocol. First, either the GPTMS silanization or the 6-phosphonohexanoic acid functionalization is applied to the Al_2O_3 surface, followed by removal of the PMMA by dissolving it in acetone. Then, antibodies are immobilized onto the functionalization, to which the proteins can bind. (b) Sensitivity of the mode-splitting by increasing the height of the biomolecule grating. Design parameters were a period of 535 nm, 4/5 coverage of ring perimeter, filling factor of 0.5, ring radius of 150 μm and waveguide dimensions of $2.2 \times 0.75 \mu\text{m}^2$. (c) Mode-splitting induced by antibody binding with the process described in (a).

25.3 pm, as shown in Fig. 5.11 (c). Afterwards, a flow of 10 mM PBS containing the rhS100A4 protein biomarker induced no significant change of mode-splitting, although both the mode-split resonance wavelengths did experience a positive shift. This can be attributed to the biomarkers binding to the untreated surface of the ring resonator through adsorption, without discriminating between the functionalized antibody immobilized regions and the bare, untreated Al_2O_3 regions. Selective chemistry is essential for this method to work by only allowing biomarker binding to the regions of interest.

5.7 Conclusion

This work describes a proof of principle of a self-referenced biosensor using a Bragg grating implemented on a ring resonator. The Bragg grating lifts the degeneracy of the counterpropagating modes resulting in mode-splitting, which depends on the reflectivity of the grating. Since the grating reflectivity varies very weakly for environmental perturbations such as temperature and bulk refractive index variations, the device can be used as a self-referenced sensor that has almost no response to such dielectric perturbations of the bulk medium, whereas it responds to structural changes that alter its reflectivity.

In this work, a first experimental demonstration of a self-referenced integrated biosensor based on mode-splitting was successfully realized. The amount of mode-splitting remained almost constant upon changing the temperature of the chip and the refractive index of the top cladding. However, upon depositing biomolecules inside the grating teeth the amount of mode-splitting reduced since the reflectivity of the grating was altered, demonstrating the applicability of the device as biosensor.

These results indicate the viability of using mode-splitting in resonators not only for single-molecule and small-particle detection, but also for self-referenced biosensing. Many future improvements are to be expected for enhanced sensitivity, and integration simplicity, paving the way for future sensing applications.

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Chapter 6

Yb³⁺:Al₂O₃ self-referenced biosensors based on beat note detection

Radio frequency (RF) microwave signals can enable simple-to-use, point-of-care sensors with a very simple readout module that does not rely on finely tunable lasers or high resolution spectral analyzers and that uses off-the-shelf electronics. The sensor produces a beat note signal with frequency that is related to the binding of the analyte of interest to the surface of the sensor. Previous demonstrations of this sensing principle required complicated, three-dimensional geometries and they were mostly used for single-particle or virus detection and not for biomarker sensing from complex fluids. In this work, on-chip Yb³⁺:Al₂O₃ ring lasers with an integrated grating were experimentally demonstrated as self-referenced biosensors. By imprinting a grating on the ring resonator, the degeneracy of the counterpropagating modes is lifted, resulting in simultaneous lasing at two distinct lasing wavelengths. This produces a beat note in the microwave frequency domain (<10 GHz) that can be recorded using a photodiode. Biomarker detection based on the tracking of the beat note frequency was experimentally demonstrated.

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Photonic generation of microwave and millimeter waves has received a lot of attention due to its applications in communication and sensors [1]. Generating microwaves using integrated photonics is increasingly becoming popular due to its many advantages compared with approaches based on electronics, including high-speed operation, abundant processing bandwidth, low cost, low power consumption, and the distribution of signals over long distances using low-loss, inexpensive optical fibers [2,3]. Various approaches have been demonstrated for the generation of integrated microwaves and other radio frequency (RF) signals in the MHz—GHz range, including the heterodyne beating of two lasers [4], the self-heterodyne beating of a dual-wavelength distributed-feedback laser [5] and the self-heterodyne beating of the split laser modes of active resonators [6]. Especially the latter two concepts are of particular interest, since they were both used for particle sensing demonstrations. In those devices, a particle binding event induced step changes, positive or negative, of the on-chip generated beat note frequency that could easily be detected with an electrical spectrum analyzer (ESA) [7,8]. This chapter describes a similar approach of generating microwave signals on-chip based on the combination of the $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ lasers described in Chapter 4 with the gratings presented in Chapter 5. The devices described in this chapter were utilized for the first proof-of-concept demonstration of biosensing of the rhS100A4 biomarker based on beat note detection.

6.1 Introduction

Optical sensors can only reach the status of point-of-care devices whenever they do not rely on complicated, bulky or expensive readout modules. Instead, they require simple-to-use operation using off-the-shelf equipment. These requirements disqualify most optical sensors that use finely tunable lasers or high resolution optical spectrum analysers. Attempts at reducing the cost of the readout module include the use of packaged devices integrated with cost-effective tunable lasers [9,10], the usage of broadband sources [11] and on-chip integrated spectrometers for ring resonator interrogation [12].

Another approach relies on generating a low-frequency optical beat note either on the chip itself [6,7] or by heterodyning with a reference laser [13,14]. Both have the advantage of requiring a simple readout scheme consisting of an optical pump, such as a diode laser, and a photodiode detector. The electronic signal from the photodiode

Fig 6.1: RF generation in an active ring resonator. (a) Pristine ring resonator without grating. Inset shows lasing mode of pristine ring resonator. (b) Grating-integrated ring resonator. Inset shows mode-splitting of grating-integrated ring resonator lasing mode. (c) Example of photodiode signal generated by an optical beat note.

can then be analysed using off-the-shelf electronics. Recently, sensors based on beat note detection have attracted a lot of interest due to their capability to detect individual nanoparticles. Furthermore, it was shown theoretically that grating-integrated ring resonators or active ring resonator lasers can be used as biosensors [15,16].

Figure 6.1 illustrates the concept of on-chip beat note generation using a grating-integrated ring resonator laser. Imposing a grating on a ring resonator leads to mode-splitting due to coupling of the clockwise (CW) and counterclockwise (CCW)

travelling modes [18,19]. However, in a ring resonator laser mode-splitting results in simultaneous lasing at the two closely separated wavelengths. These form a low-frequency optical beat note that can be detected using a photodiode and electrical spectrum analyzer (ESA) [4]. By properly designing the reflectivity of the grating, the signal frequency can be kept sufficiently small for analysis using simple electronics (i.e., <10 GHz). Figure 6.1 (c) shows an example of an optical beat note, which generates a sinusoidal current on a photodiode. The frequency of this signal is directly linked to the difference between the dual lasing wavelengths. Changes to the grating structure, such as particle binding, vary its reflectivity, resulting in changes of the generated beat note frequency [6,8]. This type of sensor is inherently self-referenced and thermal or environmental perturbations do not significantly affect the amount of mode-splitting [17], as was discussed in Chapter 5.

6.2 Grating-integrated ring resonator laser

Gratings were imprinted onto ring resonator lasers to induce mode-splitting. This is larger than the mode-splitting already present due to side-wall roughness of the ring resonator waveguide forming the cavity. The ring resonator was covered by a thin cladding of PMMA, onto which a grating was inscribed directly above the ring resonator waveguide. The used samples were the active ring resonators presented in Chapter 4, whereas the gratings were produced following the methods described in Chapter 5. Since the devices should operate in an aqueous environment, $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ was used for lasing operation at a wavelength around 1030 nm. All ring resonators presented in the following sections had a radius of 150 μm and waveguide cross-section of $2.2 \times 0.55 \mu\text{m}^2$. The inscribed gratings had a period of 330 nm, which matches the operation wavelength, and they contained 100 periods. All following experiments were performed in a flow of deionized water at a flow rate of 30 $\mu\text{l}/\text{min}$, or, if specified, either in a plain buffer solution or in a buffer solution containing biomolecules. The device temperature was kept constant at 25.0 ± 0.001 °C during all the experiments.

6.2.1 Grating implementation on ring laser

The grating parameters affect its reflectivity and the resulting mode-splitting that the resonances will experience. The available photodiode and ESA allow operation up to 26 GHz, which imposes a maximum of ~85 pm mode-splitting at ~1000 nm. Gratings identical to those realized in Chapter 5, but with a length smaller than 200 periods, yield lasing with mode-splitting that is within the detectable range of

130

the ESA. Ring resonator lasers were coated in PMMA, followed by the inscription of a grating using the methods described in Chapter 5. The grating-integrated ring lasers were then passively characterized with a tunable laser (TOPTICA CTL 1050) to record their transmission spectra. This was followed by optically pumping the devices at 976 nm using a fiber diode laser (Thorlabs BL976-SAG300) and measuring their laser spectra with an OSA (Hewlett Packard 70950B). Finally, the lasing light was guided onto a photodiode (New Focus 1014) and analyzed using a ESA (Hewlett Packard E4407B).

Figures 6.2 (a) and (b) show the transmission spectra of the same cold cavity ring resonator covered in a PMMA cladding, both before and after inscribing the grating. Prior to grating definition, the spectrum contains degenerate resonances that are well-fitted by a single Lorentzian function without mode-splitting present. After grating definition, most of the resonances in the grating reflection bandwidth experience mode-splitting. The largest splitting obtained was 32.5 pm at a wavelength of 1036 nm, as shown in Fig. 6.2 (b). The Q reduces slightly, presumably due to additional scattering losses imposed by either the grating or residual PMMA remnants on the waveguide surface after development. The splitting is now much smaller than the splitting demonstrated in Chapter 5, since a smaller number of periods is allowed due to the ESA operates up to 26 GHz.

The devices were optically pumped and their lasing spectrum was measured for the same ring resonator laser, with and without grating, as shown in Figs. 6.2 (c) and (d). Figure 6.2 (c) shows multimode laser operation prior to grating inscription. The lasing modes experience strong mode-competition and the emitted power of each mode fluctuates over time. All ring resonator modes within the emission bandwidth of the material experience sufficient gain to lase. There is not a mechanism that selects a preferential lasing mode. However, imposing the grating over the waveguide leads, in this case, to single-mode lasing operation with a side-mode suppression ratio ($SMSR$) of 28 dB, as shown in Fig. 6.2 (e). Presumably, this occurs due to the grating modifying the loss and gain experienced by the different modes of the ring resonator.

A large variability in the lasing performance of the devices before and after grating definition was observed. Some devices exhibited multimode lasing both before and after grating definition. Some devices did not lase at all after grating definition. Some

Fig. 6.2: Optical characterization of grating-integrated ring resonator lasers. All ring resonators had a radius of $150 \mu\text{m}$ and a waveguide cross-section of $2.2 \times 0.55 \mu\text{m}^2$. Grating parameters were 100 periods with a grating period of 330 nm. (a) Passive resonance of pristine ring laser covered in PMMA. (b) Passive resonance of a grating-integrated ring resonator laser experiencing mode-splitting. (c) Lasing spectrum of a pristine ring resonator laser covered in PMMA exhibiting multimode behavior. (d) Lasing spectrum of a grating-integrated ring resonator laser. (e) Zoom of the lasing peak in (d), showing the side-mode suppression ratio (SMSR).

operated single-mode prior to grating definition and multimode afterwards. This is probably due to a change of the feedback and losses in the device after grating

fabrication. This variability is not only bad for predictability and reproducibility of device operation, but it also affects the optical beat note frequency. Since the resulting laser wavelength is not known, the amount of mode-splitting and beat note frequency are not predictable. After inscription of the grating in the PMMA cladding, lasing can occur anywhere along the grating reflection profile. For instance, as it will be shown later on, a hop of lasing mode to another wavelength is often accompanied by a change of beat note frequency because the new lasing mode experiences a different mode-splitting. There exists some ways to remedy this irreproducibility, such as using smaller radii to achieve a larger *FSR* and to guarantee that only one, or very few, laser modes are present in the material gain-bandwidth [20], multiple rings [21–23], intracavity feedback [24,25] or structural modifications of the laser cavity [26,27]. These approaches were not implemented in this work, but are necessary to ensure stable laser operation and microwave generation.

6.2.2 Microwave generation

After optically pumping the device and establishing lasing, optical beat notes were measured on the photodiode and analyzed with the ESA. Prior to grating definition, all devices generated beat note frequencies in the range of 50–400 MHz, which was attributed to mode-splitting induced by the waveguide sidewall roughness [28–31]. Figures 6.3 (a) and (c) show the ESA spectra of the photodiode signal of optical beat notes of two distinct pristine ring resonator lasers in a PMMA cladding prior to grating definition (Fig. 6.3 (a) shows data of the same laser as in Fig 6.2). Both spectra were obtained at a resolution of 1 MHz and a scan time of 10 ms. Figures 6.3 (b) and (d) show the ESA spectra of the photodiode signal of optical beat notes of the same two lasers, now after grating definition. The former was acquired at a resolution of 300 kHz and a scan time of 12 ms, the latter at a resolution of 100 kHz and scan time of 38 ms. Both devices operated single mode. A beat note frequency of respectively 10.2 GHz and 7.14 GHz was measured after grating fabrication. These values differ from each other since both devices lased at different wavelengths and experienced a different mode-splitting. Both optical beat notes had a linewidth of ~300 kHz, similar to the lasers reported in Chapter 4. The high frequency beat notes had much lower power levels compared with the low frequency beat notes, which is due to electrical amplification of the photodiode signal that works only up to ~5 GHz. The signal could be improved by optical amplification using fiber amplifiers.

Fig. 6.3: ESA spectra of optical beat notes of two single-mode ring resonator lasers, both with and without grating inscribed in the PMMA cladding. (a) ESA spectrum of photodiode signal of the first ring resonator laser covered in PMMA. Acquisition at 1 MHz resolution and a scan time of 10 ms. (b) ESA spectrum of the photodiode signal of the laser in (a) with a grating inscribed in the PMMA cladding. Acquisition at 300 kHz resolution and scan time of 12 ms. (c) ESA spectrum of photodiode signal of the second ring laser covered in PMMA. Acquisition at 1 MHz resolution and a scan time of 10 ms. (d) ESA spectrum of the photodiode signal of the laser in (c) with a grating inscribed in the PMMA cladding. Acquisition at 100 kHz resolution and scan time of 38 ms.

Despite the current output powers being low, they are sufficiently high for tracking the frequency of the optical beat note for sensing experiments. However, larger output powers would permit a more reliable operation and a better signal-to-noise-ratio. These could be achieved by the methods described in Chapter 4, such as active-passive integration and improved fiber-to-chip coupling using either vertical waveguide tapers or a grating-coupler. The latter could also be beneficial for simpler integration into a packaged module where the pump light sources can be bonded onto the chip.

The two lasers in Fig. 6.3 operated at a single lasing mode after grating definition and only a single RF beat note was recorded. However, it was observed for

multimode lasers that multiple RF beat notes were present, each corresponding with an individual lasing mode experiencing a different amount of mode-splitting. Identifying which lasing mode corresponds with which RF beat note could be performed by forming an optical beat note with an external laser, such as was performed in Chapter 4. Then, the external laser should be scanned over all lasing modes and the corresponding mode-splitting can be determined to match with a measured RF beat note. Obviously, this could only work if the on-chip laser operated at wavelengths in the tunable range of the available external laser, which was not always the case for the grating-integrated ring resonator lasers.

6.2.3 RF stability

The ESA spectra of the photodiode signal of the optical beat notes were recorded repeatedly over time, from which the beat note frequency was extracted to monitor the temporal behavior and stability of the beat note frequency. The data was acquired at a rate of ~ 2 spectra per second. Figure 6.4 (a) shows the beat note frequency over time of the device the with beat note shown in Fig. 6.3 (b). The spectra were acquired at a resolution of 100 kHz and a scan time of 38 ms. This experiment was repeated three times during three consecutive, identical measurements of 8 minutes each, separated by 7 and 3 minutes respectively. The laser operated single mode for the duration of the experiment. Remarkably, besides some noise on the value of the beat note frequency, the measured beat note frequency contains discrete fine separations of 10—50 MHz. If looking at the most frequently occurring beat note frequency, this frequency varies only slightly with a standard deviation smaller than 1 MHz, as shown in Fig. 6.4 (b). The same behavior can be seen in Figs. 6.4 (c) and (d), which show the beat note frequency of Fig. 6.3 (d) over time. The spectra were acquired at a resolution of 100 kHz and a scan time of 38 ms. Again, the measured frequency has a set of discrete values with separations on the order of 10—50 MHz, with a small (i.e., 0.9 MHz) standard deviation of the most numerous occurring beat note frequency.

An oscilloscope (Owon Smart DS8302) was used to study the nature of the multitude of recurrent, but spaced beat note frequencies. However, since the highest sampling rate of the oscilloscope is 1 GHz, the beat notes of the grating-integrated ring resonator lasers could not be studied directly since they oscillate at frequencies exceeding the measurement bandwidth of the instrument. In order to test the temporal dynamics of the lasers, a single-mode ring resonator laser covered in a SiO_2 cladding, but without grating, was characterized as test case. Figure 6.5 (a) shows an

Fig. 6.4: Time traces of optical beat note frequencies of two grating-integrated ring resonator lasers. All spectra were obtained at an acquisition rate of ~ 2 spectra per second. Solid red lines indicate mean frequency, dashed lines indicate the interval spanned by one standard deviation. All acquisitions were at 100 kHz resolution and a scan time of 12 ms. (a) Beat note frequency of ring resonator laser of Figs. 6.3 (a) and (b). The figure shows the beat note frequencies of three consecutive measurements. (b) Zoom on most central line of the beat note frequency in (a). (c) Beat note frequency of ring resonator laser of Figs. 6.3 (c) and (d). (d) Zoom on most central line of the beat note frequency in (c).

oscilloscope measurement of the electronic signal generated on the photodiode as produced by the optical beat note on the photodiode. An oscillating signal with frequency of ~ 135 MHz is recorded, which matches the frequency of the beat note as recorded with the ESA. The laser operates quasi-continuously by switching between continuous wave and pulsed operation. The signal in Fig. 6.5 (a) corresponds to continuous wave operation, whereas Fig. 6.5 (b) shows the photodiode signal during pulsed operation. Then, the measured photodiode signal contains periodic peaks corresponding to the laser blinking on and off. Figure 6.5 (b) shows self-pulsation at a frequency of 1.4 MHz, but on longer time scales a variety of self-pulsing frequencies in the range 0.5—5 MHz were observed. Self-pulsation of $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ lasers was previously reported by [32], where the transition from quasi-continuous to continuous wave operation occurred upon increasing the pump

Fig. 6.5: Photodiode signal obtained with oscilloscope. Acquisition at 1 GHz. (a) Photodiode signal during continuous wave laser operation. (b) Photodiode signal during self-pulsed operation. (c) Short-time Fourier transform of photodiode signal during continuous wave operation. (d) Short-time Fourier transform of photodiode signal during pulsed operation. A jump in oscillation frequency occurred during the measurement.

power delivered to the laser cavity. However, they used pump powers exceeding 1 W, which is higher than the power that can be delivered by the laser used in this work. Further studies of $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ lasers should use pumping at higher powers to observe whether the self-pulsing of the laser disappears.

The photodiode signals recorded with an oscilloscope were spectrally characterized by Fourier analysis. This was done by taking the short-time Fourier transform of the photodiode signal sliced in segments of 500 ms. Figure 6.5 (c) shows the frequency spectrum of the SiO_2 -cladded laser during continuous wave operation. A single frequency was found that matches the value obtained with the ESA. However, at a later time during the self-pulsing laser operation, the beat note experienced a brief 20 MHz downward shift, after which the original frequency was restored, as is

shown in Fig. 6.5 (d). The magnitude of this jump is consistent with the frequency spacing observed in Fig. 6.4.

Finally, the laser emission of a different grating-integrated ring resonator laser was mixed with that of the external tunable laser to create an optical beat note. Figure 6.6 (a) shows the RF spectrum of the signal generated at the photodiode, containing the direct beat note generated by the mixing of the two mode-split lasing wavelengths of the on-chip laser and the beating of those two wavelengths with the external laser, denoted by Peak 1 and Peak 2. The spectra were acquired at a resolution of 3 MHz, scan time of 20 ms and an acquisition rate of ~ 2 spectra per second. The difference between the two peaks is identical to the frequency of the direct beat note. Furthermore, Fig. 6.6 (b) shows the frequencies of all three peaks in Fig 6.6 (a) over time. The lasing wavelength of the on-chip laser experiences some noise that

Fig. 6.6: Heterodyne beating of grating-integrated laser with external laser. The spectra were acquired at a resolution of 3 MHz, scan time of 20 ms and an acquisition rate of ~ 2 spectra per second. (a) Electrical spectrum of heterodyne beating. The frequency separation f_b between Peak 1 and Peak 2 matches the value of the direct beat note. (b) RF peaks in (a) tracked over time. (c) Direct beat note compared with the beat note as derived from the two peaks created by beating with the external laser.

manifests itself as variations of the frequencies of both mode-split peaks. However, both the directly measured beat note and the derived beat note calculated as the difference between both mode-split peak frequencies (Peak 1 and Peak 2) remain stable over time, as shown in Fig. 6.6 (c). Furthermore, the frequencies of both the directly measured beat note and the frequency corresponding with the mode-splitting as determined by using an external laser occurred at the same distinctly spaced frequencies.

The spacing of the beat note frequency at intervals on the order of 10—50 MHz was revealed by all three methods of beat note characterization based on direct self-beating, beating with an external laser and recording the optical beat note signal on the oscilloscope. All three measurements were performed with the same photodiode, but repeating the experiment with a different photodiode resulted in the same observations, indicating that the measured effects take place in the on-chip laser and not the external equipment (although the pump laser was not replaced). Furthermore, this effect was observed in both the grating-integrated ring resonator lasers and the pristine ring resonator lasers. For the latter, it was observed for both an aqueous and SiO₂ top cladding. Besides the observed pulsed operation of the Yb³⁺:Al₂O₃ lasers, it was previously reported that they can also experience relaxation-oscillations induced by fluctuations in pump power, cavity losses or other laser parameters [33]. They reported relaxation-oscillations at frequencies on the order of the frequency spacing observed here. It still remains unknown what the exact cause of the beat note frequency spacing is. It is possible that one or multiple of the described mechanisms contribute to the observed effect, but further study is required to disclose its origin.

6.3 Optical beat note biosensing

A preliminary attempt at biosensing using a grating-integrated ring resonator laser was performed by monitoring the beat note frequency over time for a flow of various biomolecules. A chip containing grating-integrated ring resonator lasers was functionalized using the method utilizing GPTMS as described in Chapter 5. After functionalization, the chip was bonded with a PDMS microfluidic channel and filled with a solution of 10 mM phosphate buffer saline (PBS). Upon optical pumping, one circuit exhibited single-mode lasing, which occurred at a wavelength of 1030 nm and it was accompanied by an optical beat note with frequency of ~1640 MHz. This value is lower than the beat note frequencies measured in Figs. 6.3 and 6.4, but a different device was used here and lasing occurred at a different wavelength.

Furthermore, the grating has a different reflectivity profile and, therefore, different mode-splitting as function of wavelength. This, combined with lasing at a different wavelength, can result in the lower observed beat note frequency. During the sensing experiment the ESA spectrum of the optical beat note was recorded repeatedly, from which the optical beat note frequency was extracted. The ESA parameters were a resolution of 1 MHz, scan time of 40 ms and an acquisition rate of ~ 4 spectra per second. The devices were exposed to a flow of anti-S100A4 monoclonal antibodies at 200 $\mu\text{g/ml}$ and rhS100A4 protein biomarkers at concentrations of 0.8, 4 and 20 μM , all in 10 mM PBS, and alternated by a flow of plain PBS. All liquids were flown at a rate of 30 $\mu\text{l/min}$.

Figure 6.7 (a) shows the time evolution of the beat note frequency during the flow of the various liquids. First, plain PBS was flown over the device for 23 minutes to establish a baseline of the beat note frequency. During this flow the device operated at a single lasing mode with a beat note frequency that remained stable with an uncertainty of 7.5 MHz, as shown in Fig. 6.7 (b). However, upon introducing the antibodies over the device the beat note frequency changed considerably, as shown in Fig. 6.7 (c). The beat note frequency exhibited both jumps and branching in two distinct, multiple frequencies immediately after introducing the antibodies. This occurred due to the lasing mode shifting to the next two longitudinal modes, or to the next two *FSR*-separated ring resonances, as shown in Fig. 6.7 (d). Since both lasing modes occur at higher wavelengths, and thus experience a slightly different mode-splitting, their RF beat notes occur at different frequencies. One of the two beat note frequency branches disappears and another appears after 15 minutes of antibody flow. Again, this was associated with another shift of the lasing mode, as it can be seen in Fig. 6.7 (d). Then, the second beat note frequency branch disappears quickly after establishing the third branch and the device continued to operate at a single lasing mode for the remainder of the antibody flow. Most notably, all beat note frequency branches experienced a downward shift during the antibody flow, which stabilized very quickly after switching to a flow of plain PBS.

Regardless of the complicated behavior of the beat note frequency during the antibody flow, some remarks regarding its usefulness for biosensing can be made. First, stable RF signals can be generated while exposing a grating-integrated ring resonator laser to a flow of buffer solution. Furthermore, antibody flown over the device resulted in a change of the beat note frequency, which was continuously observable and occurred for all three branches of the recorded beat note frequency.

Fig. 6.7: Biosensing with RF beat note. (a) RF beat note over time during biosensing experiment. The flown liquids are I: 10 mM PBS, II: 200 $\mu\text{g/ml}$ mAb S100A4 in 10 mM PBS, III, IV, and V: 0.8, 4, and 20 μM rhS100A4 protein biomarker in 10 mM PBS. Flow was 30 $\mu\text{l/min}$. Acquisition at 1 MHz resolution, scan time of 40 ms and rate of ~ 4 spectra per second. (b) RF beat note during a flow of plain buffer liquid to monitor the RF stability and noise. (c) RF beat note for a flow of PBS (I) and antibodies (II). Hops of the lasing mode are observed at i and ii, which are in both cases accompanied by jumps between two RF beat notes. (d) Lasing spectra during the lasing mode hops in (c). At the times when two RF beat notes are present, laser radiation is emitted at two wavelengths separated by the FSR of the ring. (e) RF beat note during a flow of PBS (I) and 0.8, 4 and 20 μM rhS100A4 protein biomarker flows, respectively (II, III, and IV). The lasing mode hops again at i and ii, which is accompanied by a change of RF beat note. (f) Lasing spectra at various times in (e).

The three branches and their discontinuous appearance and disappearance resulted from lasing at subsequent, different lasing modes that underwent mode hops during the experiment. Although these hops complicated the biosensing experiment and its analysis, noticeable and monotonous beat note frequency changes could be observed and tracked for a flow of antibodies, whereas they were absent for a flow of a plain buffer.

The optical beat note was monitored during a flow of rhS100A4 protein biomarkers. Figure 6.7 (e) shows the beat note frequency for a flow of various biomarker concentrations. Upon introducing the first biomarker concentration of 800 nM, the beat note frequency underwent a downward shift of ~200 MHz and maintained a stable value after switching back to a flow of PBS. No lasing mode hops occurred. However, immediately after introducing a concentration of 4 μ M another lasing mode appeared, accompanied by a change in beat note frequency, as shown in Fig. 6.7 (f). The newly appeared lasing mode soon became the dominant one and produced a single beat note frequency that experienced a downward shift during biomarker flow. Upon introducing the highest biomarker concentration, again a jump in beat note frequency took place, which was induced by another mode hop of the laser.

In all different flows a downward shift of beat note frequency was observed, whereas every flow of plain PBS resulted in a stable beat note frequency. These results demonstrate for the first time experimentally the use of microwave generation for biomarker detection. However, the results presented here are only a preliminary demonstration without the full characterization of its sensitivity, stability and limit of detection. In fact, its sensitivity is currently not well-defined due to the mode hops of the laser preventing continuously monitoring the beat note frequency changes for a sufficiently large set of different analyte flows. Furthermore, the exact relationship between beat note frequency changes, such as the jumps and the downward drifts, and the flow of biomolecules is not properly understood. For instance, it is not known where the biomolecules exactly bind, whether that is on the exposed alumina surface or on the PMMA top cladding. Although the fluorescence data presented in Chapter 5 suggests that the antibodies mostly bound to the bare alumina surface, it is possible that some still attached to the PMMA surface and thus still induce a shift of lasing wavelength due to elongating the optical path length, which can be accompanied by a change of mode-splitting and beat note frequency due to lasing at a different wavelength experiencing a different grating reflectivity [34]. The exact

nature of the mode hops of the laser is also not fully understood, which only occurred during the flow of biomolecules and not the flow of PBS. Chapter 3 studied the total resonance wavelength shift of similar ring resonators, which never exceeded more than 200—400 pm, whereas here the laser underwent multiple hops of each 500 pm, which corresponds to the FSR of the device. It is possible that both the high biomolecule concentrations and the binding of molecules to the laser induced changes to the loss- and gain-spectrum of the device, thus selecting a different resonance mode for lasing. Further study would be required to elucidate this and to better understand and control the lasing dynamics during the sensing experiment.

6.4 Conclusions

Ring resonator lasers with gratings inscribed in a thin top cladding of PMMA can emit lasing radiation at two closely spaced lasing wavelengths due to the induced mode-splitting of the resonance modes. It was shown that the corresponding electrical fields can form an optical beat note on a photodiode and produce microwaves with frequencies up to 10.2 GHz. The generated beat note frequencies were stable over time, but the presence of a biomolecule flow induced measurable variations of the beat note frequency.

However, as of yet the generation of microwave signals and optical beat notes is not very well controlled due to the aforementioned difficulties in controlling which modes start lasing and the mode-splitting experienced by each mode. Furthermore, the produced optical beat notes suffer from low power levels, the laser, and thus the microwave signal, is pulsed, the beat note frequency occurs at spaced values and lasing mode hops are present inducing beat note frequency hops. Study of these effects is ongoing and requires further work to be fully understood. Furthermore, precise control over which mode lases and aligning the main lasing wavelength at the peak of the grating reflection profile is required for repeatability and more consistent studies. Various performance improvements are necessary, including either optical or electrical amplification of the signals, higher laser output powers, well-defined single-mode laser operation and elimination of the small frequency jumps.

It was demonstrated that an optical beat note generated by a grating-integrated ring resonator laser can be used to detect a flow of biomolecules through changes of the beat note frequency. The significance of this demonstration is marked by the experiment only requiring simple equipment, making it well-suited for point-of-care

applications that do not rely on bulky or complicated measurement tools. Biomolecule flow was registered by a change in the beat note frequency. In a point-of-care application this could be recorded with a simple photodiode and analyzed with off-the-shelf electronics, although this was not shown here. These results bring together the sensitivity of optical ring resonators with a simple detection scheme, fabricated monolithically on a photonic integrated chip.

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Chapter 7

Conclusions

The work in this thesis proposed and demonstrated experimentally several novel sensors in the $RE^{3+}:\text{Al}_2\text{O}_3$ integrated photonics platform. Biosensing was demonstrated using the rhS100A4 protein biomarker as test analyte, with a measured limit of detection down to a clinical relevant range, namely 3 nM for the passive Al_2O_3 ring resonator sensor and 300 pM for the active Al_2O_3 laser sensor. Biosensing based on mode-splitting and beat note detection, both intrinsically self-referenced, were also experimentally demonstrated in integrated devices. This chapter summarizes the main results and conclusions of this work, together with an overview of challenges that need to be resolved before $RE^{3+}:\text{Al}_2\text{O}_3$ sensing technologies can significantly contribute to point-of-care diagnostic applications.

This thesis concerns the development of integrated optical biosensors based on ring resonators realized on the Al_2O_3 material platform and demonstrates a number of proof-of-concepts thereof. A variety of biosensing schemes was explored with a focus on detecting the cancer protein biomarker rhS100A4. The detection of minute concentrations of biomarkers from complex liquids holds great promise for the realization of sensors that can be operated in a medical setting. Furthermore, rare-earth ion doping of Al_2O_3 , $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ allows for active and laser functionalities integrated on-chip, which could potentially be used for novel sensing readout schemes and simpler devices. However, sensing applications based on the $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ material were barely explored prior to this work.

The first part of this thesis investigated the use of undoped Al_2O_3 ring resonators for biosensing to validate the suitability of this material for realizing optical biosensors. More specifically, Al_2O_3 ring resonators were theoretically studied and designed for optimal sensing performance with emphasis on both a high sensitivity and low cavity losses. Following these design guidelines, ring resonators were fabricated and optically characterized. A highest Q of 5.1×10^5 was achieved at a wavelength of 1570 nm, which corresponds to a waveguide propagation loss as low as 0.42 dB/cm for TE polarized light using air as top cladding for a radius of 200 μm . Using microfluidics, these ring resonators were used as sensors by monitoring their resonance wavelength shift as function of applied temperature and bulk refractive index variations (i.e., deionized water spiked with different wt% of NaCl). A highest thermal sensitivity of 8.1 ± 0.1 pm/K was demonstrated, together with a bulk refractive index sensitivity of 102.3 ± 0.5 nm/RIU. A noise of 0.054 pm was measured for the sensor. A bulk refractive index limit of detection of 1.65×10^{-6} RIU was determined, which equals those of the best single ring resonator sensors based on conventional material platforms. Finally, surface functionalization was developed and applied to the Al_2O_3 waveguide surface to form a bioreceptor layer of Anti-S100A4 monoclonal antibodies to capture the rhS100A4 cancer protein biomarker with high specificity. Concentrations down 3 nM were measured using this sensor.

The second part of this thesis detailed the realization of integrated on-chip $\text{Yb}^{3+}:\text{Al}_2\text{O}_3$ ring resonator and disk resonator lasers that emit light with a wavelength of ~ 1030 nm while operating in an aqueous environment. These devices were then used as biosensors. Single-mode lasing operation was achieved with a side-mode suppression ratio of around 25 dB and output powers up to several tens of μW . They had light-light power efficiencies of ~ 0.1 — 0.2% and power thresholds down to

7 mW (i.e., versus incident pump power prior to fiber-to-chip coupling loss). The disks had a better lasing stability and they were therefore utilized for sensing. Their emitted light was heterodyned with that of an external laser to form an optical beat note on a photodetector with frequencies in the range MHz—GHz. Detecting variations of this beat note frequency enabled using the on-chip laser as a sensor. Functionalized and antibody immobilized disk resonator lasers were successfully used for the detection of the rhS100A4 cancer protein biomarker in urine down to 300 pM. Despite their slightly lower sensitivity than the undoped Al₂O₃ ring resonators, the disk resonator lasers experienced lower noise, thus allowing for a lower limit of detection. In fact, the disk resonator lasers have an intrinsic limit of detection orders of magnitudes lower than those of the undoped ring resonators, but their sensing performance is ultimately limited by the noise present in the experiment.

The final part of this thesis described the implementation of gratings on ring resonators to induce mode-splitting of the initially degenerate resonance modes. It was found that a Bragg grating inscribed in a PMMA cladding provided sufficient reflection to couple both counterpropagating travelling modes, resulting in the resonance wavelength to form a doublet by mode-splitting. Furthermore, a grating-integrated ring resonator laser could produce microwave signals by forming an optical beat note upon lasing at the dual resonance wavelengths of the doublet. The amount of mode-splitting can be varied by adjusting the grating reflectivity while it remains almost invariant under environmental perturbations. This enables the implementation of self-referenced sensing for simple detection and readout schemes. Biomolecule binding to the grating, such as antibodies and protein biomarkers, resulted in a change of mode-splitting that could be observed as a variation of the optical beat note frequencies. Although its sensitivity and limit of detection were inferior to those obtained in the sensing experiments based on the shift of the resonance or lasing wavelength, this method holds the potential of being integrated in sensor devices that do not need expensive or complicated equipment.

The work in this thesis is not complete and many challenges still remain to be resolved or further explored. Since this work detailed proof-of-concepts and technology development, many improvements need to be explored, such as reproducibility of the sensor fabrication and its sensing performance and further testing of the cross-interaction between different proteins in the samples with the capture probes on the ring resonator surface. Furthermore, as was detailed in

Fig. 7.1: Bridging the gap between laboratory-based and portable sensor applications. (a) Photograph of the setup used in the experiments performed in this thesis. (b) Photograph of custom-built sensor module consisting of pump, photodetector, fibers, analysis and user interface. Only the packaged sensing cartridge containing the integrated sensing circuits is missing.

Chapter 1, integrated optical sensors need to be useful in practical applications, not solely in a lab environment. Although sensing was successfully demonstrated using both doped and undoped Al_2O_3 , the experiments were far from portable, as shown in Fig. 7.1 (a). The experimental setup is neither miniaturized nor is it simple to operate with minimal training, and it relies on expensive and dedicated equipment. To remedy this, the GLAM project worked towards the realization of modular, packaged sensor devices based on the sensing technologies presented in this thesis. Figure 7.1 (b) shows a photograph of the readout module developed by the GLAM consortium within the framework of this thesis. The device contains all the electronic equipment for registering and tracking optical beat note frequencies, together with pump light sources to generate laser light on an integrated optical chip. The only component missing is the sensor cartridge containing a $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ chip to generate the sensing signal. Realizing sensor cartridges in a reproducible manner and integrating these with the developed GLAM readout module would be a first step towards commercialization of the $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ sensing technologies. Successfully transferring $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ sensing technologies outside the laboratory requires the following steps.

First, the on-chip laser output power needs to be improved. Achieving higher output powers could be beneficial to prevent the self-pulsating behavior observed in the lasers in this thesis and to force continuous wave operation. Also, the low output power of the lasers limited the number of working devices with a beat note of sufficiently high power to be detected by the ESA. In order to increase the output power of the lasers, the fiber-to-chip coupling losses need to be reduced to guide the

pump light more efficiently to the laser cavity. Vertically tapered end facets can be useful, as is hybrid integration of a passive waveguide material with the $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ to reduce on-chip pump absorption losses. This will reduce the threshold of the laser and it will increase the available power to pump the devices, increasing the output power. In addition, the coupling coefficient of the ring lasers should be much higher for efficient coupling of the pump light and to fully exploit the enhancement factors possible in ring resonators. Increasing the coupling of the pump to the ring can be achieved by designing racetrack resonators, smaller coupling gaps or coupling regions where the bus waveguide follows the curvature of the resonator for a longer coupling length. Furthermore, doping with Nd^{3+} would allow light emission around the ~ 1060 nm transition, which experiences similar low water absorption losses. However, no reabsorption occurs due to the four-level-laser scheme of Nd^{3+} lasers, potentially leading to lower lasing thresholds and higher output powers.

Second, better on-chip laser stability is required. Currently, some of the devices suffered from mode hops and multimode lasing, behaviors that did not only change in between experiments, but sometimes even during an experiment. It is important to maintain stable laser emission not only for tracking the wavelength shift of the same lasing mode to avoid tuning the external reference laser, but also to get a stable beat note frequency when utilizing grating-integrated lasers. Furthermore, the absence of frequency filtering or feedback resulted in an arbitrary distribution of devices exhibiting single-mode lasing. Single mode lasing could be achieved more consistently by including passive components or tuning elements to force laser emission at well-defined, specific lasing modes with high reproducibility. Finally, the dynamics and noise experienced by the beat note should be studied in more detail to properly understand the observed separations of the optical beat note frequencies in order to eliminate or reduce them.

Third, both the fabrication and biochemical treatments should be performed in a more streamlined manner that is easily scalable. The gratings were fabricated on each chip individually, but a wafer-scale process is preferred. Furthermore, different materials and grating structures should be explored such that the resulting mode-splitting, and its change upon biomolecule binding, becomes more reproducible and stable. In particular, the grating should be designed with a constant reflectivity over the gain bandwidth of the Yb^{3+} so that even mode hops in a single-mode laser will not produce jumps in the frequency of the beat note. A larger ensemble of biomarkers can be tested for more versatile sensor operation to detect more analytes.

Furthermore, a multiplexed approach for fingerprint biomarker analysis could also be included, where each individual sensing circuit only responds to a specific biomarker of interest. This would require localized surface chemistry and bioreceptor immobilization on each circuit. Finally, all the functionalization could be done either in flow or during the chip fabrication, on the condition that the formed functionalization or bioreceptor layer remains functional for extended time.

Finally, the $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ sensor should be integrated on an optical chip that allows simple packaging and handling in a modular sensor device. As was already illustrated in Fig. 7, the experiments performed in this thesis do not fulfill this requirement. This condition could be met by designing a sensor cartridge that can be placed in a device containing optical connections to the cartridge for guiding pump light and collecting the signal. Such a scheme would be especially well-suited for detection based on the optical beat note, which requires solely a pump source for its generation and could be detected and analyzed with a simple readout module (Fig. 7.1(b)). Alternatively, on-chip integrated tunable lasers could be used for addressing the resonances of the ring resonators or their lasing counterparts. All these components should be miniaturized on a single chip that can be fabricated with scalable technologies.

In conclusion, the future of integrated optical sensors based on $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ faces enormous challenges that are mostly focused on laser performance, stability, miniaturization and packaging, sensor readout and scaling. Confronting and resolving these is essential for integrated optical sensors to reach a level of maturity in which they can actively and positively contribute to healthcare applications and medical diagnostics. The work on $\text{RE}^{3+}:\text{Al}_2\text{O}_3$ sensing technologies presented in this thesis represents a step forward towards that end.

List of publications

Journal articles: in this thesis

- **M. de Goede**, M. Dijkstra, R. Obregón, J. Ramón-Azcón, E. Martínez, L. Padilla, F. Mitjans and S.M. García-Blanco, “Al₂O₃ microring resonators for the detection of a cancer biomarker in undiluted urine,” *Optics Express* **27**(13), 18508—18521 (2019).
- **M. de Goede**, L. Chang, J. Mu, M. Dijkstra, R. Obregón, E. Martínez, L. Padilla, F. Mitjans and S.M. García-Blanco, “Al₂O₃:Yb³⁺ integrated microdisk laser label-free biosensor,” *Optics Letters* **44**(24), 5937—5940 (2019).
- **M. de Goede**, M. Dijkstra, L. Chang, N. Acharyya, G. Kozyreff, R. Obregón, E. Martínez and S.M. García-Blanco, “Mode-splitting in a microring resonator for self-referenced biosensing,” submitted.
- **M. de Goede**, L. Chang, M. Dijkstra, N. Acharyya, G. Kozyreff, R. Obregón, E. Martínez and S.M. García-Blanco, “Yb³⁺:Al₂O₃ self-referenced biosensors based on beat note detection,” in preparation.

Patent:

- F. Mitjans Prat, L. Padilla García, G. Kozyreff, N. Acharyya, **M. de Goede**, L. Chang, M. Dijkstra, S.M. García-Blanco, J. Ramón Azcón, R. Obregón, E. Martínez Fraiz, J. Toudert, J. Martorell Pena, V. Brankovic, V. Mihajlovic, M. Parausic, D. Tasovac, “Self-referenced sensor,” European patent application EP19382327.5 (2019).

Journal articles: not in this thesis

- **M. de Goede**, E. Johlin, B. Sciacca, F. Boughorbel and E.C. Garnett, “3D multi-energy deconvolution electron microscopy,” *Nanoscale* **9**(2), 684—689 (2017).
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