SILICON BASED OPTICAL BIOSENSORS

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Introduction

Optical biosensor

Optical biosensors are a class of sensors that use the variation of the properties of a light beam as sensing mechanism. Optical detection is a well-established method in life sciences and analytical chemistry to detect products of many techniques. The ideal biosensor not only has to respond to low concentrations of analytes, but also must have the ability to discriminate among species according to the recognition molecules that are immobilized on its surface. Biosensor development is driven by the continuous need for simple, rapid, and continuous in-situ monitoring techniques in a broad range of areas, e.g. medical, pharmaceutical, environmental, defense, bioprocessing, or food technology. The advantages on using such kind of sensors are their high sensitivity and selectivity, isolation from the electromagnetic interference, the multiplexing capabilities (multiple channels/multi parameters detection), compact design, fast sensing speed, the possibility to perform real-time diagnostic, the fact that are minimally invasive for *in vivo* measurements, etc. One of the most important disadvantages is the fact that they are temperature sensitive. A general block diagram of a biosensor system is described in Fig.1. A biosensor in general utilizes a biological recognition element that senses the presence of an analyte (the specie to be detected) and with an external stimulation, creates a physical or chemical response that is converted by a transducer to a signal. The recognition element binds or reacts with a specific analyte, providing bio-detection specificity. Enzymes, antibodies or even cells such as yeast or bacteria have been used as bio-recognition elements. Stimulation, in gen-
eral, can be provided by optical, electric, or other kinds of force fields that extract a response as a result of bio-recognition. The transduction process transforms the physical or chemical response of bio-recognition (that could be a change in the refractive index, change in the phase, fluorescence signal, absorption signal, change in the transmission, ecc...), in the presence of an external stimulation, into an optical or electrical signal that is then detected by the detection unit. Thus, the main components of an optical biosensor are (i) a light source, (ii) an optical transmission medium (fiber, waveguide, etc.), (iii) immobilized biological recognition element (enzymes, antibodies or microbes), (iv) optical probes (such as a fluorescent marker) for transduction, and (v) an optical detection system. The detection system can be based on a charge coupled device (CCD), photomultiplier tube (PMT), photodiode, or spectrometer [1].

The past few years have seen great advances in lab-on-a-chip (LOC) technologies for genomic, proteomic, and enzymatic analysis [2]. LOC is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size. The need for low-cost and disposable medical devices has driven the development of these LOC systems. Disposable biosensors are required because of the irreversible sensor response after the analyte’s adsorption and the possible contamination after each examination.
Classification and state of the art of optical biosensors

Optical biosensors can be broadly classified based on the different parameters [99]: the transduction method (absorption, fluorescence, luminescence), the geometry (bio-Optrodes, surface plasmon resonance, fiber brag grating, interferometer) and based on evanescent wave (resonator, ecc). In this section we would give a general overview on the different types of optical biosensors.

The simplest optical biosensors use absorptions phenomenon to determine changes in the concentration of analytes [6]. The sensor works by sending light through an optical fiber to the bio-sample. The amount of light absorbed by the analyte is determined by measuring the light coupled out via the same fiber or a second optical fiber. Since the absorption is usually wavelength dependent and different species may have different absorption spectra, by measuring the absorption spectra via fiber optic sensor, different species and concentration levels can be determined. The major advantages of absorption based sensors are that they are simple, easy to use, and cost effective.

Fluorescence [7, 99] is commonly used in bio-optrodes. Fluorescence occurs when molecules absorb light at one wavelength and then emit light at a longer wavelength [6, 8]. Since the excitation and emission occurs only at distinct energy levels, each fluorescent molecule has a unique fluorescence spectral fingerprint, which is very important for the optical biosensor application [21]. Optical fluorescence detection methods are routinely used to achieve picomolar sensitivity, but they typically require incubation times on the order of hours [22, 23].

Time-resolved fluorescence is another phenomenon used in bio-optrodes. This method is based on the fluorescent molecule’s excited state lifetime [24].

Chemiluminescence [6, 3] is similar to fluorescence. The difference is that chemiluminescence occurs by exciting molecules with a chemical reaction (usually occurring by the oxidation of certain substances such as oxygen or hydrogen peroxide), whereas fluorescence occurs by exciting molecules via light. Thus, in the case of chemiluminescence, no external source of light is required
to initiate the reaction.

Bioluminescence \([6, 3]\) is simply chemiluminescence occurring in living organisms, which represents a biological chemiluminescent reaction process. Many organisms produce bioluminescence for signaling, mating, prey attracting, food hunting, and self-protection.

The word "optrode" is a combination of the words "optical" and "electrode". Optrode-based \([8, 3]\) fiber optic biosensors (bio-optrodes) are analytical devices incorporating optical fibers and biological recognition molecules. The basic structure of an optrode is composed of a source fiber and a receiver fiber that is connected to a sensing fiber. To achieve sensing capability, the tip of the sensing fiber is usually coated with a sensing material by the dip coating procedure. The analyte to be sensed may interact with the sensing tip by changing one or several of the parameters like, its refractive index, absorption, reflection, scattering properties; or polarization behaviors. The fiber in this case acts as a light pipe transmitting light to and from the sensing region.

Surface plasmon resonance (SPR) is another light phenomenon that has found wide use in bio-optrodes during the past decade. SPR biosensors exploit special electromagnetic waves - surface Plasmon - to probe changes in the refractive index at surfaces of metals.

In fact, surface plasmon waves are extremely sensitive to small changes in the refractive index near the sensor surface, and these changes are proportional to the sample mass on the surface. So SPR can be used in label-free biosensing \([9, 20]\). Many optical coupling schemes has been used for SPR biosensors, such as the most widely used Kretschmann prism configuration \([10]\), grating \([11]\),

![Figure 2](image-url)
channel waveguide [12], light pipe [13], and optical fiber [14] schemes.

SPR biosensors have become a central tool for characterizing and quantifying biomolecular interactions both in life science and pharmaceutical research. The commercialized SPR sensors, as exemplified by the instrument from Biacore [15], are typically based on prism coupling and have a $DL$ between $1 \cdot 10^{-6}$ to $1 \cdot 10^{-7}$ RIU and a mass surface density $DL$ around $1 \text{pg/mm}^2$.

The major advantages of SPR sensors includes, high sensitivity, label-free, enabled analysis for a wide range of bio systems requiring only small amounts of samples. In waveguide-based SPR biosensors, the $DL$ is usually from $10^{-5}$ to $10^{-6}$ RIU [16, 17]. For the grating coupling configuration, the $DL$ ranges from $10^{-5}$ to $10^{-6}$ RIU [18, 19].

Optical microring resonators are an emerging sensing technology that has recently been under intensive investigation [113, 25, 140, 26, 132]. Light coupled to these cavities is confined within the structure by total internal reflection, forming high quality factor (Q) resonant modes. Any interaction with the evanescent tail of the optical field affects the guided-mode, and thus changes the resonance behavior of the cavity. This change in resonance can be detected with very high sensitivity by optimizing the microcavity design and the method of observation.

Ring resonators biosensors have so far been implemented in three major configurations: (1) microfabricated ring-, disk- or microtoroid-shaped resonators on a chip [140, 27, 151, 119, 112, 121, 28, 29, 111]; (2) stand-alone dielectric microspheres [26, 30, 31, 32, 121]; and (3) capillary-based opto-fluidic ring res-

Figure 3: some examples of WGM resonators based optical sensors: a microsphere based sensor, a slot ring resonator coupled to a bus waveguide and a toroid resonator coupled to a tapered fiber [124, 134, 27].
onators (OFRR) [113, 25, 33, 34, 37, 38, 39]. An example, a silicon-on-insulator (SOI) ring resonator on an area below 100 $\mu m^2$ showed capability of detecting bulk RI change at a sensitivity of 70 $nm RIU^{-1}$ with a $DL$ of $7 \cdot 10^{-5} RIU$ [127]. Using this kind of sensors, it is possible to reach sensitivity to single binding events [140]. Recently, the slot-waveguide based ring resonator was developed, which has a larger light-analyte interaction and hence a higher sensitivity, as more light is present in the slot through which biomolecules flow [156, 132]. A slot-waveguide ring resonator showed a RI sensitivity of over 200 $nm RIU^{-1}$ and a $DL$ of $2 \cdot 10^{-4} RIU$ [156], and an estimated protein $DL$ of approximately 20 pg mm$^{-2}$ [132].

There are some types of Interferometer-based biosensors:

The Mach-Zehnder interferometer (MZI) is characterized by a coherent, single mode, single polarization light at the input that it is split into a reference arm and a sensing arm; finally the arms recombine. A change in the RI at the surface of the sensor arm results in an optical phase change on the sensing arm and a subsequent change in the light intensity measured at the photodetector. The first biosensing demonstration using an integrated MZI was performed by Heideman et al. [40]. Waveguide technologies improved in the years that followed these pioneering demonstrations: Brosinger et al. [41] and Schipper et al. [43]. The measured RI DL was around $10^{-5} RIU$, and the authors demonstrated sensitive detection of an IgG molecular monolayer [41]. Further advancements to the integrated MZI functionality have been incorporated by Blanco et al. [74].

Young’s interferometer (YI) differs from the MZI for the fact that the two arms to form interference fringes on a detector screen, such as a CCD. The first demonstration of an integrated YI for sensing was demonstrated by Brandenburg and Henninger [44]. A follow up to this work using the same design concept yielded a RI $DL$ of $10^{-7} RIU$ [45]. A commercial product is currently available based on the integrated YI technique from Fairfield Scientific [46].

In the Hartman interferometer (HI) configuration, functionalization molecules are patterned in strips on top of a planar waveguide [47]. Light is coupled into and out of the planar waveguide using gratings. Integrated optics is placed
near the output of the chip to create interference between pairs of functionalized strips. A couple of examples in [48, 49].

A separate class of interferometry-based biosensors exist, called backscattering interferometry (BI). Typically, a BI system consists of a coherent single wavelength laser focused onto a small sensing area and a detector to analyze the reflected intensity. Sub-wavelength structures on the sensing surface result in interference at the detector. Capture of proteins onto a selected part of the illuminated structure change the intensity on the detector due to a phase change of the reflection from this surface. This produces a sensing transduction signal. An example of use of the BI technique is the biological compact disk (BioCD) immunoassay [50, 51], which has been commercialized by QuadraSpec [52].

Photonic crystal (PC) biosensors are a novel type of label-free optical biosensing platform. In these kinds of devices, on the transmission (or reflection) spectrum, the defect mode appears as a relatively sharp peak within the bandgap [53, 54, 55, 56, 57]. Since spectral position of the defect mode is highly sensitive to the change in the local environment around the ”defect”, it can be used as the sensing transduction signal when the RI changes as a result of the binding of the molecules to the defect. Due to the strong light confinement provided by the photonic bandgap and the flexibility of adjusting the defect mode wavelength across the photonic bandgap by finely tuning the structural parameters, PC biosensors have received tremendous attention since the first demonstration of detecting RI change in both waveguide [56] and microcav-
ity laser [57] configurations. Mandal and Erickson recently demonstrated a novel optofluidic sensor array based on the use of arrays of 1D photonic crystal resonators and a single bus waveguide, which can perform highly parallel, label-free detection of biomolecular interactions [58]. The simulation results predict that the $DL$ is on the order of tens of attograms, and detection of $7 \cdot 10^{-5}$ RIU was demonstrated experimentally.

Another configuration of PC biosensor is based on photonic crystal fiber (PCF), which is a fiber optic cable with air holes in the cross-section. PCF is a promising sensing platform for two reasons: first, the air holes in the fiber can act as a simple fluidic channel to deliver the biological samples, second, the unique light guiding mechanism of photonic crystal fiber ensures a strong light-matter interaction because of the large light-sample overlap [60, 62, 55].

Fiber Bragg Gratings (FBGs), while developed as a tool for the telecommunications industry, have flourished as a versatile sensor with a wide breadth of applications. They are currently among the most popular of all fiber-based optical sensors for analyzing load, strain, temperature, vibration, and RI [63]. Some examples: M.C.P. Huy et al., detected $6 \cdot 10^{-6}$ RIU [64], T.L. Lowder et al. detected $DL$ of $10^{-5}$ RIU [65, 66].

Evanescent wave sensors utilize the electromagnetic component of the reflected light at the side surface between the fiber core and the fiber cladding. The evanescent wave can interact with analytes within the penetration depth; thus, by immobilizing biological material within this region, the absorption of prop-
agating light or generation of fluorescence during the binding of analytes can be detected. The increase in evanescent field forms the basis for increased sensitivity. In addition, several investigators have attempted to increase the penetration depth of the evanescent field and facilitate mode coupling by bending [68, 69], tapering [70, 71], altering the light launching angle [72], and increasing the wavelength [73]. The major advantage of using evanescent wave is the ability to couple light out of the fiber into the surrounding medium, which offers a large interaction surface.

In this thesis the evanescent wave approach was analyzed in three different devices: for sensing labelled molecules in a bioreactor, for sensing unlabelled molecules with micro disks resonator and for sensing using the slot waveguide configuration.

**Evanescent wave biosensors**

Several types of evanescent wave biosensors have been demonstrated on silicon or silica platforms, including fiber optic sensors [3], planar waveguide sensors [5], and, more recently, ring resonator, disk resonator and silicon wire waveguide sensors [75]. The ring and disk resonators achieve high sensitivity detection through the design of very high quality factor resonant structures that allow the evanescent field to interact with biomolecules over multiples cycles of the confined wave.

Sensor designs are categorized on the basis of the five parameters that completely describe a light wave that are: amplitude, wavelength, phase, polarization state and time-dependent waveform. In this work an evanescent wave biosensor that investigates the luminescence signal due to the presence of marked proteins was studied. In particular, these marked proteins are pumped by the evanescent wave from the waveguide on top of which the proteins are deposited.

This geometry offers adjustable sensitivity because the parameters of the sensing region can be varied. In addition, photobleaching is reduced considerably because of the low optical power of the evanescent wave [76].
These devices provide a promising means for detection of target molecules and for the real-time monitoring of solutions, gases, or reactions that occur near the device surface. Moreover waveguide evanescent field sensors provide significant reduction in sensor size as compared for example to fiber sensors, making them amendable to integration for more robust operation, more rapid and multiplexed analysis with high channel count assays, and feasibility for volume production.

**Label free biosensors with disks resonators**

Optical biosensors can use the label-free detection protocol, in which biomolecules are unlabeled or unmodified. This is a great advantage; in fact labels can interfere with the assay, besides the fact that the analyte requires a previous preparation [77]. Various optical label-free biosensors utilize the refractive index change as the sensing transduction signal. Some biosensing platform could include: surface plasmon resonance, interferometers, waveguides, fiber gratings, ring resonators, and photonic crystals [78].

In this work microdisk resonators coupled to waveguides are studied as platform for a label-free biosensors. In a disk resonator, the light propagates in the form of whispering gallery modes (WGMs), which result from total internal reflection of light along the curved boundary between the high and low refractive index media. The evanescent field of the WGM responds to the binding of biomolecules. The important thing to be noticed is that, in contrast to the straight waveguide, the effective light-analyte interaction length in a disk resonator sensor is not determined by the sensor’s physical size, but rather by the number of revolutions of the light supported by the resonator, which is characterized by the resonator quality factor, or the Q-factor. The effective length, \( L_{eff} \), is related to the Q-factor by: \( L_{eff} = Q\lambda/2\pi n \), where \( \lambda \) is the resonant wavelength and \( n \) is the refractive index of the ring resonator [79]. Consequently, despite its small physical size, a disk resonator can achieve sensing performance similar or superior to a waveguide but using orders of magnitude less surface area and sample volume. Moreover, due to the small
size of disk resonators, high density sensor integration becomes possible [77].

The WGM spectral position, the resonant wavelength, $\lambda$, is related to the refractive index through the resonant condition: $\lambda = \frac{2\pi r n_{eff}}{m}$, where $r$ is the disk radius (or in case of a ring resonator is the ring outer radius), $n_{eff}$ the effective refractive index experienced by the WGM, and $m$ is an integer. $n_{eff}$ changes when the refractive index near the disk resonator surface is modified due to the capture of target molecules on the surface, which in turn leads to a shift in the WGM spectral position. Thus, it is possible to obtain both quantitative and kinetic information about the binding of molecules near the surface.

Optical microring or microdisk resonators devices offer the advantage that they can be fabricated by standard technologies and are readily integrated into multiplexed detection systems. Label-free micro resonator-based detection is reported in the nanomolar range with response times $\sim 1 min$ [78].

**Why silicon based?**

Silicon is the eighth most common element in the universe by mass, but very rarely occurs as the pure free element in nature. In particular, silicon is second most abundant element in the earth’s crust after oxygen, making up 27.7% of the crust by mass [80]. Silicon photonics (or better CMOS Photonics) is a viable way to develope a small number of integration technologies with a high level of functionality that can address a broad range of applications. CMOS technology is the dominant semiconductor technology; it is possible to achieve low cost due to the mass production, integration of different components in compact devices, packaging of the devices in small dimension, ecc.

Silicon is transparent to infrared light with wavelengths above about 1.1$\mu m$; silicon also has a very high refractive index, of about 3.5 [81]. Moreover silicon possesses an insulator oxide ($SiO_2$) with a refractive index of 1.45; so devices realized in silicon and silicon dioxide have a very high refractive index contrast, condition needed to guide light in a structure such as waveguides (with tipical dimension in the micrometers range). In integrated circuits, a wafer of
monocrystalline silicon serves as a mechanical support for the circuits, which are created by doping, and insulated from each other by thin layers of silicon oxide (which is easily produced by exposing the element to oxygen under the proper conditions). Silicon has become the most popular material to build both high power semiconductors and integrated circuits [82]. The silicon photonics world is also researching to achieve an all silicon integrated optical circuit: this structure should be able to produce, guide and detect optical signals.

Why polymer based?

Because of their chemical and physical properties, polymers may be tailored over a wide range of characteristics; the use of polymers is finding a permanent place in sophisticated electronic measuring devices such as sensors. During the last years, polymers have gained tremendous recognition in the field of artificial sensor in the goal of mimicking natural sense organs. Better selectivity and rapid measurements have been achieved by replacing classical sensor materials with polymers involving nano technology and exploiting either the intrinsic or extrinsic functions of polymers [129]. Semiconductors, semiconducting metal oxides, solid electrolytes, ionic membranes, and organic semiconductors have been the classical materials for sensor devices. Nowadays, polymers are more and more used as gas sensors, pH sensors, ion-selective sensors, humidity sensors, biosensor devices, etc [130].

Overview of the thesis and collaborations

The work during these three years was focused on the optical characterization of two different kind of biosensors, and in the study and realization of a polymer based sensor.

In the first chapter the results achieved on the silicon based optical biosensor based on the detection of marked proteins are reported. This work was done within the Naomi project financially supported by Provincia Autonoma di Trento. This project tackles the challenge to develop miniaturized protein
chips, focusing on the combination of nano and micro technologies to deliver compact, reliable and potentially low cost diagnostic tools, with improved analysis performances.

In the second chapter the results achieved on the silicon based label-free optical biosensor are reported. Also this work was done within the Naomi project. The label-free approached is based, in this work, on the detection of variations in the transmission of optical microdisks. In particular the work was focused in the detection of shifts of the resonance wavelength of the micro resonator, due to the presence of protein on top of the microdisk.

In the third chapter the results achieved in the study and realization of a polymer based sensor are reported. This work was done within the Go.P.Si. project financially supported by Provincia Autonoma di Trento. This project aims to realize an hybrid sensor using a particular kind of optical waveguide (the slot waveguide) and to demonstrate the possibility of achieving high sensitivity also using low index material, comparable with the one of the well known high index materials such as for example silicon.

Finally, the conclusions of the work are reported.
Introduction
Chapter 1

Silicon based photonic layer for biosensing: marked proteins based approach

NAOMI project concerns the development of a photonic layer capable to discriminate among different protein families to be used into a multispectral protein-chips. In this work, two different approaches were followed: the first one consists in using a low losses channel waveguide to excite, with the mode evanescent field, a reactor filled with the targeted proteins and then collect the luminescence; the second one is the so called label free approach, that in this work consists in probing the transmission resonance shifts of integrated disk resonators due to protein trapping. In this chapter the first approach based on marked protein is presented.

1.1 Marked proteins based approach

The aim of the fluorescence-based approach is the development of the photonic layer for the protein-chips. It is possible to obtain information on the quantity and type of proteins by marking them with chromophores in the visible range. A photonic layer to drive light from an external source to the sensing area to
excite the chromophores, collect the emission, filter the excitation light and channel the optical signal to the detector layer was designed, fabricated and tested.

The overall layout of the final design for the photonic layer is sketched in Fig.1.1. Integrated channel waveguides made in Silicon Oxynitride (SiON) can be considered the fundamental component of the photonic layer. The idea is to realize a channel with multiple sensing areas (the brighter regions in Fig.1.1), where the waveguide (darker regions in Fig.1.1) provides the basis for the photonic circuits to excite the bioreactors. The source is an external laser which is coupled into the waveguides by developing a suitable tapering of the input waveguide. This waveguide tapering consist in reduction of both width and thickness of the input waveguide; in fact the idea is that the input waveguide has sufficiently big dimension in the starting part, in order to have an easy alignment of the light signal from the source inside the disposable photonic layer. Since the light-sample interaction region is composed by a 2D array of reactors, an optical network is designed to drive the laser to each one of these reactors. The luminescent signals is then collected by a photodetector array placed on top of the photonic layer. For developing this photonic layer, the starting point was to study the material by a structural characterization and an optical characterization of the slab waveguides, then to investigate separately all the components of the final device: tapers, splitters

Figure 1.1: sketch of the final photonic layer composed by a multiple sensing channel.

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1.2 Experimental setup description

Two new custom-tailored setup was installed at the Nanoscience laboratory in Trento to perform propagation losses and transmission measurements of the two different photonic layer. Both the experimental set-ups allow to control the polarization state of the signal coupled in the waveguide and to analyze the intensity, the transmission spectrum and the far-field mode shape of the transmitted light mode.

In this section, the experimental configuration developed for sensing the photonic layer for marked protein approach is presented. The laser sources used were: a pigtailed laser diode at the wavelength of 670nm, a GaAlAs pigtailed laser diode at the wavelength of 830nm, a diode-pumped solid-state laser working at the wavelength of 473nm, a superluminescent light emitting diode working at 840nm of central wavelength with a bandwidth of 40nm, a Ti : Sa laser (tunability from ~ 650nm to 1100nm) that emits pulses of few ps or of few fs at 86MHz of repetition frequency [83].

The first setup configuration is used to study the luminescence of marked proteins within a waveguide coupled bioreactor. In particular this setup could be used to collect the luminescence signal from the top of the bioreactor or the transmitted signal of the device under investigation. The input setup stage, consists in a laser source coupled with a taper optical fiber that focuses the light to a spot of 5μm in diameter. The input signal is injected in the waveguide by means of a 6 axis nanometric translational stage and a microscopic vision system. The output setup stage is composed by an objective lens (20X of magnification and 22mm of working distance) coupled with the 12XUltra – zoom by Navitar in order to achieve a field of view of the order of magnitude of the bioreactor area (50x50 μm²). This ultra-zoom and the objective lens are mounted on a 3 axis translational stage. The output signal is then coupled into a multimode optical fiber using a prealigned wide area fiber collimator. This output setup stage can be mounted either vertically,
to collect the signal from top of the bioreactor, or horizontally, to collect the transmitted signal from the edge of the device (see Fig.1.2). Finally the signal is coupled in a monochromator (SOPRA DMS2 monochromator), connected to a water-cooled photomultiplier tube, by means of a doublet lenses system in order to match the numerical aperture of the optical fiber ($NA = 0.275$) and of the monochromator ($NA = 0.058$). For a precise and accurate injection of the solutions within the bioreactor, a commercial micro-infiltrating system has been used (FemtoJet by Eppendorf), provided with an ultra-fine glass capillary that allows to target the small sensor site [103].

![Image](image1.png)

Figure 1.2: experimental setup for testing the device for the marked protein approach.

### 1.3 First set of channel waveguides

The first set of three multilayer waveguides (sample 1, 2 and 3) was prepared by Plasma Enhanced Chemical Vapour Deposition (PECVD) technique by Fondazione Bruno Kessler of Trento (FBK) on 4-inch silicon substrates due to the higher flexibility that should offer this technique. PECVD is a technique that allows the deposition of $Si$, $SiO_2$, $SiN_x$ and $SiO_xN_y$ films with variable composition, keeping the substrate temperatures at around $\sim 300^\circ C$. The
other deposition technique available at the FBK is the Low-Pressure Chemical Vapor Deposition (LPCVD), that allows depositing different kind of oxides up to a maximum thickness of about $2\mu m$. The choice of PECVD instead of LPCVD technique is dictated to the possibility of having cracks of the thin films, if a critical thickness is exceeded, due to considerable large residual stress with LPCVD. This problem is especially severe in $Si_3N_4$ films deposited by LPCVD which have an enormous tensile stress of $1.2\, GPa$ (if deposited on bulk silicon) and tend to crack, if the thickness exceeds $200\, nm$. Otherwise $Si_3N_4$ would be an ideal material for the waveguide core due to its transparency in the UV-Visible range. Indeed $Si_3N_4$ waveguides with propagation losses between $1-3\, dB/cm$ were prepared at FBK in the past [84]. However, properties of these films prepared by PECVD change during thermal annealing due to the release of hydrogen and rearrangement of chemical bonds, which influences both the stress and the transparency of the resulting films. So finally the choice of the material was to use the $SiO_xN_y$ that is a good compromise in order to achieve low losses and to avoid the formation of cracks on the surface.

### 1.3.1 Fabrication process

The fabrication process consists essentially of (1) the deposition of a $3\mu m$ thick $SiO_2$ bottom cladding ($n \sim 1.45$), (2) deposition of $\sim 200/250\, nm$ thick core layer of the waveguide in $SiON$ ($n \sim 1.80$), (3) thermal annealing of the wafers at $1100\, ^\circ C$ in $N_2$ for 1h, (4) define waveguides with photolithography (layer-1), (5) transfer pattern to wafer by reactive ion etching (RIE) to form ridge waveguides; all waveguides were etched more than the thickness of the $SiON$ layer, (6) define facets of waveguide with photolithography (layer-2) in order to get waveguide of 6 different lengths ($5600$, $10500$, $14800$, $20900$, $386004$, $49600\, \mu m$, respectively), (6) transfer pattern to wafer by a double RIE process: first the $SiON$ and $SiO_2$ layers are etched in an oxide-etcher, then the silicon substrate was etched to a depth of $\sim 30\, \mu m$ in a deep reactive ion etcher; Fig.1.3 shows a sketch of the lateral profile of the waveguide facets and a SEM (Scanning Electron Microscope) front image of the facet; (7) deposit
on one of the wafers (WG2) 50nm of PECVD nitride (see table 1), (8) dicing of wafers.

1.3.2 Optical characterization

Propagation losses were measured on the first set of waveguides, channel waveguides 10µm wide fabricated on wafers 1,2, and 3. The attenuation was measured at two wavelengths: at 670nm at Nanoscience Laboratory of Trento (NL lab) using the cut back method and at 488nm at the "Nello Carrara” Institute of Applied Physics of Florence that is part of the National Research Council (CNR-IFAC) using the photometric detection of scattered light intensity. The cut-back method is commonly used to calculate the attenuation in optical fibers and is accurate only if the input coupling condition is kept constant [86]. The total power at the output ($I_{out}$) is measured for different waveguide lengths ($L_i$) and both coupling losses from the input source ($\alpha_c$) and propagation losses ($\alpha_l$) can be calculated according to the following relation:

$$I_{out} = I_{in} \cdot \alpha_c \cdot e^{(-\alpha_l \cdot L_i)}$$

where $I_{in}$ is the power from the input source [87]. Due to the photolithography definition of the facet of the sample, it was not possible to approach the taper fiber to the device. Thus, the cut-back method was not possible. In fact, as shown in Fig.1.3, the etched depth of the sample is about 30µm while the taper fiber has a 125µm diameter in the last stripped part; moreover the measurement was not possible also because the waveguide facet is about 200µm far from the edge of the sample and the working distance of the taper fiber is of the order of magnitude of few tens of micrometers. In order to be able to perform this losses measurement, the silicon should be further etched down to about 100µm. For the above reason all other loss measurements were performed using the method based on the direct detection of the light scattered by the waveguide ($P_s$), which is supposed to be proportional to the guided power $P$ (with the same constant of proportionality along the whole guide length) [88]. The light is collected from the top of the waveguide (typically at
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Figure 1.3: sketch of the lateral profile of the waveguide facet. The silicon substrate is etched to a depth of \( \sim 30\mu m \) in a deep reactive ion etcher (left) SEM image of the facet of a waveguide: the width of the waveguide is approximately \( 10\mu m \), and the facet shows the typical ripples from the RIE process in the Si-substrate. The facet of the waveguide is extremely smooth (right).

\[ P_s(z) = P_{s0} \cdot e^{(-\alpha_i z)} \] (1.2)

This measure does not depend on the way the light is coupled into the waveguide but care should be taken in selecting the starting point (\( z = 0 \)) in order to avoid spurious effect (like additional scattering from input light propagating outside the waveguide). Fig.1.4 shows the linear fit in logarithmic scale for the light scattered from a waveguide in sample 2. The wavelength is 670 nm and propagation losses are around 30 dB/cm. In Fig.1.4 the exponential decay of the scattered light at 488 nm is shown for a 10 \( \mu m \) wide waveguide of sample 1. The source is the 488 nm line of an Ar\(^{3+}\) laser coupled to a single mode fiber, which is then butt-coupled to the waveguide. The scattered light is collected using a Vidicon camera. The attenuation is extremely high, up to 60 dB/cm, and the light is very rapidly absorbed, as shown in the inset picture. Typically the decay is not a single exponential and only the last part of the propagation is monitored (besides, the very short propagation may strongly affect the measurement error). Similar results were obtained for sample 3. In conclusion these waveguides exhibit extremely high material attenuation (30 dB/cm at 670 nm of working wavelength and 60 dB/cm at 488 nm of working wave-
Figure 1.4: scattered power vs length in a 10µm waveguide of wafer 2. The wavelength is 670nm. The scale is logarithmic (left); scattered power vs length in WG1 at 488nm. The inset shows a picture of the short propagation of blue light close to the guide input (right).

length). As expected, decreasing the working wavelength, the losses increase; in fact the contribution of the Rayleigh scattering behaves as $\lambda^{-4}$.

### 1.4 Second set of waveguides

Because of the large propagation losses exhibited by the first run of waveguides, a second series of waveguides was prepared in order to optimize the fabrication process and to achieve smaller losses values. In particular planar waveguides were fabricated, which consist essentially of three layers of materials with different dielectric constants, and have a width much greater than their thickness. Because of this peculiarity they can be approximated with the slab waveguide model, which considers that they extend infinitely in the direction parallel to their interfaces [92]. The advantages of this simpler structure is that the fabrication process is simplified (there is no etching to define the waveguide channels) and light can be easily coupled in by prism coupling so that material losses can be easily evaluated. The disadvantage is that the waveguide is not directional (it does not confine the light in the lateral direction) and that losses introduced by the sidewall roughness in the final waveguide cannot be estimated. So, after this material optimization, a
set of channel waveguides should be needed in order to determine the effective propagation losses.

1.4.1 Optical and structural characterization

The losses measurements of the slab waveguides where performed at CNR-IFN with a Metricon 2010 system and using a gadolinium-gallium-garnet (GGG, \( n = 1.965 \) at 633\( nm \)) prism. The scattered light is collected by a fiber array and attenuation measurements can be performed at 632.8, 1319 and 1542\( nm \). Film thickness and refractive index can then be calculated when at least two modes (or 1 TE and 1 TM) are found. All the results are summarized in Tab.2. Atomic Force Microscopy (AFM) was performed on all samples at NL in order to assess the SiON surface quality. All samples show a structured surface characterized by small grains as shown in Fig.1.5. The surface morphology seems to be independent on the film refractive index and thickness, as well as on the thermal treatment. The sample surface is generally homogeneous (several points have been probed for each slab waveguide) and average roughness is about 2.5\( nm \). In two heat-treated samples (7a and 8a), surface cracks

Figure 1.5: AFM image of a 10\( \mu m \) x 10\( \mu m \) portion of the surface of a slab SiON waveguide. A structured surface characterized by small grains can be clearly seen (left). 2D profile of the grains over a 5\( \mu m \) length (right).
are clearly visible as shown in Fig.1.6 where an AFM image and a profile of a crack is shown as an example. Cracks have indeed different features and can be clearly seen also with the optical microscope over the whole surface of these wafers.

Figure 1.6: AFM image of a portion of the surface of sample 8a exhibiting a crack (left) and its profile (right).

1.5 First test-vehicle

After the analysis on the best fabrication process performed on the slab waveguide, the next step was to realize a test-vehicle. The idea of realizing the test-vehicle of the photonic layer was twofold: (1) realize an optical platform similar to the one used in the final microsystem to optimize the technology and to identify the main obstacles without running through a complete fabrication process; (2) create a test-device which allows to perform different experiments (functionalization, optical characterization, integration with microfluidics and detector, ecc...) necessary before assembling or developing the architecture of the whole system. It was used a very similar structure of the one presented in the work of Cooper [93].

As shown in Fig.1.7, light is coupled into the input waveguide (10μm wide and 250nm thick, this width value was chosen in order to achieve a wide area bioreactor) and then transmitted to the bioreactor (10x50μm in size); the op-
tical signal can be detected from the transmission waveguide (having the same dimension of the input waveguide) or through the 90° bent waveguide (15 or 25 \( \mu m \) wide and the same thickness as it is patterned from the same SiON layer of the other waveguide) or from top of the reactor. A Y splitter provides a reference channel for a reproducible optical alignment of the sample, both in case of fluctuation of the power of the sample and in case of different alignment of the taper fiber. In fact it was noticed that once coupled the light signal from the taper fiber in the waveguide, it was possible to distribute more light in the reference waveguide or in the transmission waveguide, just moving the taper along the y-direction (parallel to the facet of the waveguide). This happen because the focused spot diameter of the taper fiber (2.5\( \mu m \)) is much smaller that the input waveguide width (10\( \mu m \)). The reference waveguide was used in particular to try to couple the same amount of signal in the transmission waveguide, in order to have comparable measurements.

Materials chosen for the buffer-layer and top cladding of the waveguides are silicon-oxides based glasses and borophosphosilicate glass, respectively, with refractive indices \( n \) (at the wavelength of 630\( nm \)) of 1.45 and 1.48 and a thickness of 3\( \mu m \) and 1\( \mu m \) respectively. For the core layer SiON material was used with refractive index \( n \) (at 630\( nm \)) in the range 1.8 – 2.0.

Figure 1.7: sketch of the waveguide structure of a single sensing channel (left) and the layout of the silicon chip containing several identical sensing channel (right).
1.5.1 Fabrication process

An overview of the layout is given in Fig.1.8. The fabrication process of the first generation test-vehicle (TV) consists essentially of: (1) deposition by PECVD of a 3µm thick SiO$_2$ bottom cladding; (2) deposition by PECVD of the SiON waveguide core (300nm thick, $n = 1.8$); (3) thermal annealing of the wafers at 1100°C for 1 hour; the SiON thickness reduces to 240nm; (4) photolithographic definition of the waveguides (layer-1); (5) transfer of the pattern to the wafer by reactive ion etching (RIE) to form channel waveguides with different waveguide widths; all waveguides were etched into the bottom cladding (the over etched depth is about 130nm); (6) deposition by LPCVD of the SiO$_2$ top cladding by using BPSG (1µm thick); (7) reflow process in order to planarize the top cladding; (8) photolithographic definition of the reactor (layer-2); (9) transfer of the pattern to the wafer by RIE; different etching times allowed to achieve different etching depths; (10) deposition of a 50nm thick SiN$_x$ on the whole wafer; (11) definition of the cutting line and DRIE etching of the wafer until the silicon substrate; (12) dicing of wafers.

Three different samples have been realized (TV1, TV2 and TV3), corresponding to different depths of the bioreactors. The idea was to investigate the
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The efficiency of the interaction between different extensions of the evanescent field (due to different bioreactor depth that is different waveguide thickness under the bioreactor) with the bioreactor filled. Despite the complexity of stopping the etching process within the core waveguide, the technological partner was able to produce three different photonic layers (illustrated in Fig.1.9) which consist in: one device in which the core waveguide under the bioreactor was completely etched (TV1), one device in which the core waveguide under the bioreactor was not etched (TV2) and one device in which the core waveguide under the bioreactor was partially etched (TV3). The latter device is the only one case in which it is possible to analyze the interaction between the mode evanescent field and the bioreactor content (see Sec.1.5.2).

![Figure 1.9: three different etching depths of TV samples.](image)

1.5.2 Structural characterization

The TV samples have been structurally characterized by SEM, AFM microscopy and interferometric measurements, in order to study the bioreactor shape and depth. The latter parameter, related to the waveguide thickness at the sensor surface, strongly influences the interaction of the biological sample with the mode evanescent field and, hence, the sensitivity of the device.

The goal of AFM analysis (a Solver P47H-PRO microscope by NT-MDT has been used) was twofold: first, to observe the profile of the patterned channel waveguide. The channel height is found to be $0.38 \pm 0.02 \mu m$, as shown in the cross-section profile of Fig.1.10. Since the SiON layer deposited by PECVD is $0.240 \pm 0.002 \mu m$ in thickness, this means the etching extends of about $0.14 \pm 0.02 \mu m$ across the SiO$_2$ buffer layer. Second, AFM images of TV samples were acquired also to study the bioreactor shape and depth. As an ex-
ample, the 3D-AFM image of the bioreactor etched in TV3 sample is reported in Fig. 1.11. The bioreactor is a 50x50 \( \mu m^2 \) square etched in the transmission channel (10\( \mu m \) wide). The higher etching depth at the corners of the square is due to the higher etching rate of BPSG with respect to the SiON waveguide core. The bioreactor depth is calculated as the difference between the height of the waveguide outside and at the center of the etched area (the double-step clearly observable in the intersection between the 10\( \mu m \) wide waveguide and the 25\( \mu m \) wide waveguide is an artifact due to the scanning direction and of the fact that these steps are close to the z-limit of the AFM system used. In fact analyzing a smaller area in the step region, it was possible to see that no step is present). Results of AFM characterization performed on several bioreactors of TV samples strongly disagree with the values expected from fabrication (see Tab. 1.1). In particular, AFM data indicate that over- and under-etching of the SiON layer have been achieved in TV1 and TV2 samples, respectively, while an intermediate structure has been realized in TV3 sample. To get deeper insight on the bioreactor structure, interferometric measurements have been performed at the FBK to estimate the residual silicon oxide present under the bioreactor, after the etching. Since the initial thickness of the silicon oxide layers is known by SEM measurement, it is possible to calculate the bioreactor depth by subtracting the residual oxide thickness (see Tab. 1.1).

TV1 sample exhibits a complete etching of the BPSG top cladding while a
Figure 1.11: 3D-AFM image of a bioreactor in TV3 sample (similar images have been obtained for TV1 and TV2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bioreactor Depth [$\mu m$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFM</td>
</tr>
<tr>
<td>TV1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>TV2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>TV3</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

Table 1.1: depth of the bioreactor in TV samples obtained by AFM and interferometric measurements. The values expected from fabrication are also reported (with a 10% error due to the etching process).

A residual $SiO_2$ buffer layer of about $2\mu m$ is still present below the reactor. This confirms the over-etching of the waveguide core, as expected from the nominal structure and AFM measurements.

In TV2 sample the etching removed part of the $BPSG$ cladding, without reaching the $SiON$ layer. Thus, contrarily to expectations but in agreement
with AFM results, this sample exhibits an under-etching of the waveguide core. TV3 sample shows a complete etching of the BPSG top cladding while the SiO$_2$ underneath it remains unaffected. This means that the etching process has been stopped somewhere within the SiON core layer.

The results of the AFM structural characterization do not perfectly match the nominal structures expected from fabrication. Nevertheless these depth values will explain the optical behavior of the sensor channel (see Sec.1.5.3) and the losses values measured are in agreement with the losses values calculated by FDTD simulations performed on the photonic structure using the depth values measured by AFM (see Sec.1.5.5).

1.5.3 Optical Characterization of the first test-vehicle

Sensing measurements have been carried out filling the bioreactor with different liquid solutions; the first step was to study the bioreactor optical losses as a function of the refractive index of the filling solutions. In particular, three different configurations have been studied: empty reactor ($n = 1$), reactor filled with a diluted water/glycerol solution 1 : 2 vol (estimated $n = 1.37$) and reactor filled with glycerol ($n = 1.47$). All the optical characterizations were performed at the wavelength of 670 nm.

Bioreactor losses mean the ratio of light coupled after the bioreactor and the light that comes just before it; to calculate the light just before the bioreactor, the first step was to characterize the splitting ratio of the device, in order to validate the use of the reference channel. In fact a great variation of the splitting ratio between the transmission and the reference channel was observed for identical structures on the same chip, measured in the same experimental conditions. This clearly prevents from using the second arm of the splitter as reference channel for quantitative measurements. An explanation has been found in the dependence of the splitting ratio on the alignment condition between the tapered fiber and the large input channel. Moreover, fabrication tolerances have to be considered. This is detrimental from the experimental point of view since results obtained for the very same sensor structure can not be compared.
An average splitting ratio was estimated using some, nominally identical, TV2 samples, the one where the bioreactor etched only partially the top cladding layer. In fact, in this case the bioreactor content does not interact with the signal in the waveguide. In particular, the reverse-coupling configuration was used, that consists in the injection of the input signal from the reference waveguide and from the transmission waveguide and in detecting the output signal from the input waveguide (see Fig. 1.12). The splitting ratio was calculated as the ratio \( \frac{I'_R}{I'_T} \), where \( I'_R \) and \( I'_T \) are the light intensities measured from the input waveguide after injecting from the reference and from the transmission waveguide, respectively. Results show that \((54 \pm 9)\%\) of the input signal goes to the bioreactor, and \((46 \pm 9)\%\) goes in the reference waveguide.

Knowing this splitting ratio is possible to go back to the light that comes just before the bioreactor \( (I_Y) \) and to calculate the bioreactor losses as the ratio \( \frac{I_T}{I_Y} \), where \( I_T \) is the light after the bioreactor. The results are summarized in Tab. 1.2. Both to confirm the losses values measured and to optimize the modeling of the bioreactor, a systematic numerical investigation was performed. A commercial electromagnetic simulation software by Lumerical, implementing a parallel 3D FDTD method to solve Maxwell equations was used. In the simulations, the multilayer of the three different TV samples, with the same geometrical dimension values measured by AFM and the same refractive indices values, has been considered, including the three different liq-
uid solutions used to fill the bioreactor (see Fig.1.13). Since the experimental measurements have been carried out using unpolarized light, the simulated propagation modes can be treated as a combination of fundamental TE and TM modes of the waveguide. Hence, the overall optical transmission across the bioreactor can be calculated as the arithmetic mean of the transmission values obtained from two independent simulations, one for the TE and one for the TM modes. The results are reported in Tab.1.2.

The bioreactor losses were calculated for the TV1 and TV3 samples using the

![Figure 1.13: field profile (side view) simulated for TV3 with different filling solution: a) air (n = 1), b) water and glycerol (n = 1.37) and c) glycerol (n = 1.47).](image)

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>BIOREACTOR LOSSES VALUES [dB]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIR $n = 1$</td>
</tr>
<tr>
<td>TV1</td>
<td>No Signal</td>
</tr>
<tr>
<td>TV2</td>
<td>REF</td>
</tr>
<tr>
<td>TV3</td>
<td>6.9 ± 2.1</td>
</tr>
</tbody>
</table>

Table 1.2: Bioreactor losses values measured and simulated for the three different samples and for the three different filling solutions.

TV2 sample as reference, that is the sample in which the reactor content does
not interact with the waveguide. As expected, increasing the refractive index of the filling solution, the losses values decrease; in fact reducing the refractive index mismatch, between the waveguide ($n = 1.8$) and the bioreactor content ($n = 1 - 1.37 - 1.47$), the optical modes confinement increases. Moreover the bioreactor losses are higher in TV1 than in TV3 sample, to the point that the transmission is even inhibited in the case of empty bioreactor ($n = 1$) of the TV1 sample. This behavior is easily understandable reminding the sample cross-sections; as shown in Fig.1.9, in the TV1 sample the waveguide layer was completely etched and the etching extends hundreds of nm down into the buffer layer, instead in the TV3 sample the waveguide layer was only partially etched. In these conditions, the propagation of the light is prevented in the TV1 sample and it is only decreased in the TV3 sample, where the evanescent field tail is increased by the lower waveguide thickness. Moreover the bioreactor losses were confirmed by the FDTD simulation, validating the simulation method adopted.

### 1.5.4 Sensing measurements of the first test-vehicle

The next step was to use TV3 to optically excite and collect the fluorescence signal emitted by dye molecules in solution within the reactor. The fluorescence photons coupled into the transmission channel are collected at the exit of the chip and spectrally analyzed by means of a monochromator connected to a photomultiplier tube. Since the TV3 sample exhibits low propagation losses around 670nm, *Fluka 93662 Fluorescent Red NIR 700* has been selected as fluorescent dye ($\lambda_{ex} \sim 670\text{nm}; \lambda_{em} \sim 735\text{nm}$ in 0.1M phosphate $pH$ 7.0). Fluorescent Red NIR 700 was first diluted in Dimethyl Sulfoxide (*DMSO*) to form a 10mM solution. This solution was diluted by gradually adding a 0.1M phosphate $pH$7.0 buffer solution to obtain different dye concentrations in the range $10 - 250\mu M$.

As shown in Fig.1.14, both *DMSO* and the phosphate buffer exhibit a very low absorption coefficient around 670nm. All details about Fluorescent Red NIR 700 are presented in App.3.7, together with the emission spectra acquired.
Figure 1.14: absorption spectra of DMSO and 0.1 M phosphate pH 7.0 buffer as a function of wavelength.

as a function of the dye concentration, using a commercial fluorescence spectrometer Cary Eclipse by Varian.

Fig.1.15 presents two transmission spectra obtained measuring the output signal from the transmission waveguide in the range 700 – 900nm, before and after infiltrating the bioreactor with a 100µM dye solution. Dye excitation and fluorescence collection have been performed through the input and the transmission waveguide channel, respectively; no signal was detected from the 90° bent waveguide. When the reactor is empty (black line in Fig.1.15), only the tail of the laser source at about 700nm appears on the weak background signal. As soon as a drop of solution fills the bioreactor volume (red line in Fig.1.15), the light guided by the waveguide structure excites the dye molecules. As a result, a fluorescence band centered at about 750nm is transmitted out of the
photonic chip. The spectra in Fig.1.15 is a proof of concept that this device

can works as photonic layer for a lab-on-chip biosensor.

The next step was to monitor the luminescence behavior at a fixed wavelength (740 nm that is the maximum of the emission wavelength was considered) as a function of time after the reactor filling. The result obtained for the normalized intensity is shown in Fig.1.16: at time zero the drop was deposited on the reactor. After a delay time the luminescence starts with a threshold-like behavior, and after a certain time the luminescence decreases until it vanishes. This measurement was repeated for different concentration values; some data are reported in Fig.1.17. In Tab.1.3 some data are reported: the delay time, the rise time and the duration of the luminescence for different concentration values. A variation in the delay time was noticed, but not in the luminescence intensity. The delay time seems to be correlated with the
Figure 1.16: behavior of the luminescence vs time for a drop of filling solution of 60 $\mu M$ of concentration at fix wavelength value of 740 nm.

Figure 1.17: behavior of the luminescence vs time for a drop of dye solution of different concentration (left) and a zoom of the same graph to focus on the different delay time due to the different concentration of the dye solution (right).

dye concentration value. In particular, disregarding the results obtained for the 90$\mu M$ and 100$\mu M$ concentration values, assuming to had some problems
in the measurements, it is possible to see a decreasing of the delay time as the concentration values increase. The rise time oscillates from the value of 5s to 11s, so more than 100%. An hypothesis could be that nominally identical reactors show different wettability properties.

These two results suggest that there could be some threshold in the luminescence detection, for example that a certain amount of excited DYE molecules has to be reached close to the top of the wg, to be able to detect a signal. Another possibility could be that there are some dynamics of wettability of the bioreactor.

As concern the luminescence duration, a great dependence from the volume deposited on the reactor was noticed (reactor volume 2.5pL). With the micro-infiltrating system used, there wasn’t the possibility to control the volume deposited in the bioreactor. In fact the volume deposited with this system depend on the wettability properties of the surface; in principal for identical wettability properties the same amount of liquid solution is deposited using the same parameters of the system (that means the same pressure supply).
1.5.5 Numerical simulation

Once the experimental evidence of fluorescence excitation/transmission using the silicon photonic chip was obtained, the next step was to investigate and optimize the efficiency of an evanescent wave based photonic layer (like in the case of TV3 sample) and compare it with the efficiency of a direct light excitation one (like in the case of TV1 sample)[108] [107]. In particular the excitation and the collection efficiencies were first investigated separately. Then the total efficiency of the photonic structure was analyzed. The refractive indices considered in these simulations are the same as in the previous case, except that now a water solution was considered as bioreactor content, with refractive index of 1.33; this value corresponds to the dye solution used in the experiments. In fact the major part of the biological solutions that could be investigated using this kind of photonic layer are aqueous solutions.

The first investigation dealt with the calculation of the confinement factor of the light within the bioreactor. Assuming that the confinement factor is almost constant along the waveguide direction (x-axis in Fig.1.18) to mimic the response of the TV3 structure, the average confinement factor over a 10 nm thick water layer was calculated as a function of the distance $d$ from the bottom of the bioreactor. The calculated confinement factor values have been normalized to the power of the mode propagating in the waveguide under the bioreactor. As expected, in the presence of an evanescent field, the confinement factor decreases exponentially as the distance from the bottom of the bioreactor increases.

The same analysis was performed also to mimic the response of the TV1 structure, considering a bioreactor depth a little bit larger than for the real TV1 sample. This time, the mode confinement factor was calculated as a function of the distance $x$ from the waveguide edge (see Fig.1.19), over a 10 nm thick layer positioned in the middle of the waveguide SiON core (vertically). The calculated confinement factor values have been normalized to the input power of the mode propagating in the input waveguide. As clearly visible in Fig.1.19, the confinement factor is not constant along the x-axis, but it rapidly decreases, reducing by about one half of its initial value after a few hundred
Figure 1.18: average confinement factor for different distances $d$ from the bottom of the bioreactor to mimic the response of the TV3 structure at the wavelength of 670 nm.

of nanometer along the $x$ direction. This is the first proof of the inefficiency of dye excitation within the bioreactor when a propagating light approach is used (case of the TV1 sample) instead of an evanescent field one (case of TV3 sample).

The performances of TV3 and TV1 structures were investigated also for the collection of the fluorescence signal from the transmission waveguide. The goal is to quantitatively determine the amount of fluorescence signal coupled back to the waveguide as a function of the position of the emitters inside the bioreactor. The emission of a single dye molecule excited through the evanescent field has been modeled by introducing an isotropic, unpolarized and incoherent point dipole source in the FDTD simulation. Since the FDTD software allows setting only point dipoles with fixed orientation and polarization (no randomly
oriented dipoles are available), several simulations with different dipole orientations have been combined, considering the average transmission at a fixed dipole position.

First, the transmission of an incoherent and isotropic single dipole located at the center of the bioreactor was studied, changing its position $d$ from the bottom surface of the bioreactor, along the $Z$ direction, for the TV3 structure (Fig.1.20). The minimum separation distance considered in this study is $10\text{nm}$, dictated by the grid resolution used in the simulation. The collection efficiency values were calculated at the position $T$ (vertical black line in the inset of Fig.1.20), $10\mu\text{m}$ apart from the bioreactor along the $X$ direction. As expected, increasing the distance $d$ between the dipole and the bottom of the bioreactor, the collection efficiency decreases, due to the less effective inter-
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Figure 1.20: waveguide transmission obtained for an incoherent and isotropic single dipole located at the center of the bioreactor for the TV3 structure at 740 nm (dye emission wavelength). The inset shows the sketch of the simulated photonic structure.

The second step was to focus on the effect of the dipole position inside the bioreactor along the waveguide axis (X direction, see inset of Fig.1.20), at a fixed distance $d$ from the reactor bottom surface. The collection efficiency was calculated at the same position $T$ considered in the simulation presented above (see inset of Fig.1.20). As before, the minimum separation distance $d$ between the dipole and the bottom of the bioreactor is 10 nm. Fig.1.21 presents the results obtained for the TV3 structure. As shown in the graph, the coupling of the fluorescence signal into the transmission waveguide shows a smooth dependence for position along the X axis. The transmission rapidly increases.
only when the dipole approaches the right side of the bioreactor due to direct coupling of light through the waveguide edge. Furthermore, the collection efficiency is strongly influenced by the overlap between the evanescent field and the dipole, decreasing rapidly as the distance \( d \) of the dye molecule from the bioreactor bottom surface increases. The same analysis was performed for the

Figure 1.21: transmission in the output waveguide (on the right of the reactor) as a function of dipole position along the waveguide axis (\( X \) direction) of the TV3 structure at 740nm (dye emission wavelength). Each curve corresponds to a different distance \( d \) between the dipole and the bottom surface of the bioreactor, in the range 10 – 300nm.

TV3 structure, in which no waveguide is present under the bioreactor. In particular, we investigated the fluorescence signal transmission to the right side of the bioreactor, for different dipole positions along the \( X \)-direction (from \( X = 25\mu m \) -center of the bioreactor- to \( X = 49\mu m \) -right side of the bioreactor). The vertical position of the dipole along the \( Z \) axis is maintained fixed in correspondence of the central section of the SiON core layer. The results obtained are reported in Fig.1.22. The comparison of these results with the ones presented in Fig.1.21 confirms the major role played by the evanescent field in promoting the coupling of the fluorescence signal into the transmission waveg-
uide. In fact, in the case of the TV1 structure, the absence of the evanescent field causes the transmission to be lower than 1% over most of the \( X \) dipole positions, making it even negligible for \( X < 25\mu m \) (see Fig.1.22). Only in close proximity of the right side of the bioreactor (45\( \mu m < X < 50\mu m \)) the transmission increases up to 6%, due to a direct coupling of the fluorescence signal through the waveguide edge.

Finally, in order to evaluate and compare the overall performance of our silicon photonic chips, the excitation and collection simulations performed for the two structures were combined together. The procedure consists in calculating the arithmetical mean of the different coupling efficiency values (shown in Fig.1.21) related to the different \( x \) axis position of a single dipole, for every

![Figure 1.22: transmission values in the output waveguide (on the right of the reactor) as a function of dipole position along the waveguide axis (\( x \)-axis) at a fixed \( Z \)-position (central-section of the waveguide vertically) for the TV1 structure at 740\( \text{nm} \) (dye emission wavelength).](image-url)
distance $d$ from the bottom of the bioreactor. Then, these averaged coupling efficiency values were multiplied for the confinement factor value (shown in Fig.1.18) corresponding to the same distance $d$. Finally also the optical losses due to the first interface between the input waveguide and the bioreactor were taken into consideration. These losses have been estimated to be $2.56 \, dB$ for the TV3 structure. The results are reported in the graph of Fig.1.23 as a function of the distance $d$. As expected, the closer the active dye molecules to the bioreactor surface, the higher the overall optical performance of the device. The maximum value calculated for the excitation/collection is about 5% of the total power present in the input waveguide. The same calculation was per-

![Figure 1.23: total performance of the TV3 structure for different distances $d$ of the active dye molecules from the bottom of the bioreactor (evanescent field approach).](image)

formed for the TV1 structure, as well. The procedure followed to calculate the
total excitation/collection was the same as before; but in this case we obtain only one value for one fixed $Z$ position. In fact for the TV1 structure only the central-section of the waveguide core was considered. In this case, the losses of the structure due to the first interface between the input waveguide and the bioreactor were not considered. In fact the confinement factor was calculated with respect to the total input power. The overall optical performance of the TV1 structure is 0.3%, that is one order of magnitude lower than in the case of the TV3 structure. Again, this is due to the fact that in sample TV1 no evanescent field can be exploited for the excitation of the dye molecules as well as for the collection of their fluorescence signal. To note also that for the propagating light approach, as shown in the graph in Fig.1.24, when the confinement factor presents higher value, the coupling efficiency is lower and vice versa. On the contrary, in the evanescent field approach the confinement factor and the coupling efficiency are almost constant along the waveguide direction ($x$ axis). This experimental and theoretical investigation pointed out that the efficiency of the evanescent field approach is one order of magnitude higher than the one achievable by the simple propagating light approach. Moreover it was shown that the excitation/collection efficiency increases with decreasing the dye molecules distance $d$ from the bottom of the bioreactor.

1.6 Second test vehicle

The second test vehicle was realized in order to work with shorter wavelength; the new working wavelength is 473 nm. The advantage in using a shorter working wavelength lies in the increased availability of more efficient commercial dye solution and chromophores. Moreover, the spad detector that will be used for the final device is more sensible at these wavelengths [101]. The layout of the second test vehicle is exactly the same as for the first one. As for the first test vehicle, light is coupled into the input waveguide (10 $\mu$m wide and $\sim$ 950 nm thick) and then transmitted to the bioreactor (10x50 $\mu$m in size); the optical signal can be detected from the transmission waveguide (having the same dimension of the input waveguide) or from top of the reactor.
A Y splitter provides a reference channel for a reproducible optical alignment of the sample. Materials chosen for the buffer-layer and top cladding of the waveguides are silicon-oxides based glasses and borophosphosilicate glass, respectively, with refractive indices $n$ (at the wavelength of 630 nm) of 1.45 and 1.48 and a thickness of 1.7 µm and 550 nm respectively. For the core layer SiON material was used with refractive index $n$ of 1.637 (at 630 nm).

### 1.6.1 Fabrication process

The fabrication process consists essentially of (1) the deposition of a $\sim 2\mu m$ thick $SiO_2$ bottom cladding (PECVD); (2) deposition of the 1µm core of the waveguide in SiON (PECVD) with refractive index 1.64; (3) thermal annealing of the wafers at 1100°C in $N_2$ for 1h; (4) definition of the waveguides with
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photolithography (layer-1); (5) transfer pattern to wafer by reactive ion etching (RIE) to form channel waveguides with different waveguide width; all waveguides were etched into the bottom cladding (the over etched depth is about 250\text{nm}); (6) deposition of top cladding SiO\textsubscript{2} by using BPSG6\% (LPCVD) with 1100\text{nm}; (7) reflow the channel waveguide in order to planarize top cladding; (8) repetition of the step (6) and (7) again; (9) removal excess BPSG6\% on top of waveguide in order to better control the reactor etching depth; leave 550\text{nm} BPSG6\% top cladding; (10) definition of the reactor with photolithography (layer-2); (11) transfer pattern to wafer by reactive ion etching (RIE) to form reactor; with different etching time (80, 105, 130 and 155 seconds), the resulting etching depths are different; (12) deposition of 50\text{nm} thick SiN\textsubscript{x} on whole wafers; (13) definition of the cutting line with photolithography (layer-3); (10) transfer pattern to wafer by a double RIE process: 1st the SiN\textsubscript{x}, SiON and SiO\textsubscript{2} layer are etched until silicon substrate, 2nd the silicon substrate was etched to a depth of \sim 70\mu\text{m} in a deep reactive ion etcher; (11) dicing of wafers. The waveguide profile of the second test vehicle is shown in Fig.1.25

Figure 1.25: waveguide profile of the second test vehicle.
1.6.2 Structural characterization

This second test vehicle was measured by mechanical profilometer. The first measurement was performed in two different positions of the same reactor (as shown in Fig. 1.26). Regarding the Fig. 1.25, the height of top cladding BPSG6% measured at reactor center is thicker than that measured at reactor edge which is nominal top cladding thickness. Fig. 1.27 shows the reactor edge profile for different etching time. The heights of SiON waveguide within the reactor are about 850, 720, 500 and 300 nm respectively for etching time 80, 105, 130, 155 seconds.

1.6.3 Optical characterization of the second test vehicle

For this second test vehicle a detailed optical characterization was not performed, as for the first one. In fact the work was mainly focused on the sensing measurement of functionalized bioreactor, as presented in the next section.

1.6.4 Sensing measurement on the second test vehicle

The first step was to fill the bioreactor with a drop of dye solution and monitor the luminescence from the transmission waveguide, as for the test vehicle.
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Figure 1.27: reactor edge profile for different etching time, so for the different sample.

Amino-methyl-fluorescein has been selected as dye solution, with the maximum excitation wavelength at $\lambda_{ex} \sim 488\text{nm}$ and the maximum emission wavelength at $\lambda_{em} \sim 520\text{nm}$. All details about amino-methyl-fluorescein are presented in App.3.7.

The luminescence behavior at a fixed wavelength (520nm that is the maximum of the emission wavelength for the amino-methyl-fluorescein was considered) versus time after the reactor filling was detected (see Fig.1.28); also in this case, no luminescence signal from the 90° bent waveguide was detected. At time zero the drop was deposited on the reactor; after a delay time the luminescence starts with a threshold like behavior and after a certain time the luminescence decreases to zero.

After the experimental evidence of fluorescence excitation/transmission filling the bioreactors of the second test vehicle with a dye solution drop, the next step was to test the functionalized bioreactors. The functionalization was performed by the biophysics group at the NL laboratory. It consists in the introduction of an organic bioactive layer on top of the inorganic sur-
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Figure 1.28: behavior of the luminescence vs time for a drop of a filling solution of 100µM of concentration at the fixed wavelength of 520nm.

faces. This layer plays the important role of recognizing and capturing specific biomolecules and to make them detectable. In particular Naomi project is focused in the recognition of VEGF proteins: vascular endothelial growth factor, that stimulates the growth of new blood vessel and play a central role in pathologies such as tumors, cronical ischemia, retinopathy.

The functionalization consist first in (1) cleaning the surface to be functionalized: sonication in trichlorethylene, acetone and isopropanol (5min each), drying in nitrogen. (2) Etching in 2% HF for 1 minute, rinse in water and drying in nitrogen; this step is used to remove oxide and to enrich the surface NH and NH₂ groups. (3) Functionalization in toluene +80mM Br – dodecanoicacid (in reflux at 110°C), then washed in trichlorethylene, acetonitrile, water and methanol (sonication for 5min each), drying nitrogen. This step is used to cover the surface of carboxyl groups (−COOH). (4) Acti-
vation of carboxyl groups with $1\text{-ethyl}-3-(3\text{-dimethylaminopropyl})\text{carbodiimide hydrochloride (EDC)}$ and $N\text{-hydroxy-sulfosuccinimide (NHSS)}$ in water solution (sonication for 30 min), then rinse and dry in $N_2$ quickly. This step is used for reacting the carboxyl groups with the amino groups of the protein. (5) Deposition of fluorescent marked-protein (as a first test bovineserumalbumin (BSA) was used: the functionalization steps are exactly the same) in water solution (phosphate buffer pH 8) on the bioreactor by means of a microspotter. After 2 hours, washing in aqueous solution 3 times (5 min each) and finally in water. Drying in $N_2$.

A fluorescence microscope was used to verify the success of the functionalization (see an example in Fig.1.29). As it is possible to see in the right part of

![White light, wide field microscopy](example1.png) ![Fluorescence microscopy](example2.png)

Figure 1.29: optical microscope image of a bioreactor using white light (left) and fluorescence microscope image using light at the wavelength of 480 nm (right).

Fig.1.29, the reactor was functionalized and the marked proteins are present in it.

A systematic set of measurement of functionalized bioreactor were performed, in order to detect the luminescent signal from the transmission waveguide and also from top of the bioreactor. It was not possible to detect any transmitted luminescence neither from the dry bioreactor nor from wet bioreactor. In fact it was tried to wet the functionalization area, depositing a water drop on top of the bioreactor (with the micro infiltrating system) in order to try to increase the luminescent efficiency of the fluorophores. Additional analysis are needed

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in order to understand what are the problems.

### 1.7 Vertical tapered waveguide

A vertical tapered waveguide will be need in the development of the final photonic layer (as shown in Fig.1.1). The tapering consists in the reduction of the thickness of the input waveguide to reach the proper thickness in the bioreactor area. A set of tapered channel waveguide, without bioreactor, were produced in order to test and optimize the structure.

#### 1.7.1 Channel waveguide

The first step was to produce a set of channel waveguide, with the same fabrication process that will be used to prepare the vertical taper waveguide, in order to test and optimize the structure.

The fabrication process consist in: (1) the deposition of a 2µm thick $SiO_2$ bottom cladding (PECVD); (2) thermal annealing of the wafers at 1000°C for 1h; (3) deposition of the core of the waveguide in $SiON$ (PECVD) with different index and different thickness; (4) definition of the waveguides with photolithography (layer-1); (5) transfer pattern to wafer by reactive ion etching (RIE) to form channel waveguides with different waveguide width (from 1µm to 10µm); all waveguides were etched until the bottom cladding; (6) thermal annealing of the process wafers at 1100°C for 1h; (7) deposition of top cladding $SiO_2$ by using $BPSG6\%$ (LPCVD) for 1h ($\sim 720nm$); (8) reflow the channel waveguide in order to planarize top cladding; (9) repeat the step (7) and (8) again; (10) removal of the excess top cladding and leave 550nm $BPSG6\%$ on top of waveguide, which keep the same thickness of top cladding as that of Run TV2-3 (Reactor test); (11) definition of the wafer cutting line with photolithography (layer-2) and get waveguide of 6 different lengths (5600, 10500, 14800, 20900, 38600, 49600 µm, respectively); (12) transfer pattern to wafer by a double RIE process: 1st the $SiON$ layer is etched in an oxide-etcher, 2nd the silicon substrate was etched to a depth of $\sim 150µm$ in a deep
reactive ion etcher; (13) dicing of wafers.

Four samples labeled from TV1W1 to TV1W4 were fabricated, in which TV1W1 and TV1W2 have SiON core with high refractive index 1.85 and thickness of $\sim 0.9\mu m$, and TV1W3 and TV1W4 have SiON core with low refractive index 1.63 and thickness of $\sim 0.26\mu m$. The optical characterization of the channel waveguide with 10$\mu m$ width of the sample TV1W3 was performed at the wavelength of 633$nm$, because at 473$nm$ there was no detectable output signal. The losses measurements were performed with the cut back method (see Eq.1.1); for this purpose, a set of waveguides with different lengths was fabricated and tested. In Fig.1.30 is reported the result of the cut-back, that shows a coupling losses of $(13.2 \pm 0.5)dB$ and a propagation losses of $(6.4 \pm 0.5)dB/cm$.

![Figure 1.30: losses of the channel waveguide of the sample TV1W3 at the wavelength of 633$nm$ calculated using the cut-back method.](image)

\[ Y = 6.4 \times X + 13.2 \]
1.7.2 Fabrication process

The tapers were fabricated on silicon wafers with standard silicon microfabrication technology at FBK. Following the deposition of a 2.0μm thick silicon oxide cladding on Si substrates, we deposited the SiN layer of 1.0μm for the waveguide core with the appropriate composition for obtaining $n = 1.63$ after thermal annealing. First linear waveguides were defined via proximity mode lithography and transferred to the SiON layer by reactive ion etching. In this way, strip waveguides with 10μm width and 1μm height were obtained. The etched waveguides were annealed at a temperature of 1100°C in N$_2$ atmosphere as for the planar waveguides. During the fabrication of the tapers, the key process consists in an appropriate combination of grayscale lithography and dry isotropic etching. Gray-scale lithography utilizes locally modulated exposure doses to develop the 3D structure in the photoresist. Differential exposure doses lead to multiple depths of exposed photoresist across the surface. From the differential exposure doses, a gradient height photoresist structure corresponding to the designed silicon structure will remain once developed [109]. Projection printing with conventional chrome on glass (COG) mask was used to generate the gray-scale pattern. Two different pixel patterns were tested - composed by lines or square dots (2D), for gray scale generation. Fig.1.31 shows pixels details for the 6μm pitch length. When the photoresist layer is subjected to the subsequent silicon oxynitride waveguide etching step, the thinner regions of the photoresist are fully removed by the etcher. This exposes the underlying silicon oxynitride earlier than that covered by the thicker regions of photoresist. Therefore the depth to which the underlying silicon oxynitride is etched is determined by the thickness of the photoresist after being developed and the etch time. In this way, three dimensional patterns are transferred from the resist to the underlying silicon oxynitride layer.

HiPR6517HC photoresist was chosen for this work. This resist has low contrast and thus is well suited for grayscale level since the thickness of the resist after exposure is nearly linear with respect to the exposure dose. The parameters for the grayscale lithography were first optimized to obtain a taper
in photoresist as long as the taper on the mask. Taking into account that the etch-rate of photoresist is roughly twice the one of silicon oxynitride - on the reactive ion etcher used - the ideal height of the photoresist is $2\mu m$ to maximize the taper length. The length of the resulting tapers is $80\mu m$, $125\mu m$, and $200\mu m$ for a pitch of $4\mu m$, $5\mu m$, $6\mu m$, and $7\mu m$, respectively. The thickness of the evanescent waveguides was measured using height profiles at different positions over the tapers. A thickness of $220 \pm 20nm$ was obtained at the end of the vertical tapering.

### 1.7.3 Vertical taper waveguide

A schematic of the test chip is shown in Fig.1.32; in particular this schematic is reproduced for every pitch length considered (pitch is the distance between two different depths of photoresist), $4\mu m$, $5\mu m$, $6\mu m$, and $7\mu m$. Every test chip contains 4 series of 7 waveguides in silicon-oxynitride ($10\mu m$ width) with the same pitch length: waveguide 1 is a channel waveguide without taper, used as reference, waveguide 2 is a waveguide with 3 double taper composed with square dots, waveguide 3 is a waveguide with 3 double taper composed with lines, waveguide 4 is a waveguide with 1 double taper composed with square dots, waveguide 5 is a waveguide with 1 double taper composed with lines, waveguide 6 is a waveguide with 1 single taper composed with square dot and waveguide 7 is a waveguide with 1 single taper composed with lines. Four different device were realized: TA1 with the initial height of the waveguide after the thermal annealing is $0.97\mu m$ (refractive index 1.85), while the
height of the evanescent taper at the end of the taper is about 0.15\( \mu m \), and
without top cladding, TA2 and TA3 with the same specific of TA1 but with
220 nm thick top cladding of BPSG \( SiO_2 \) and PECVD \( SiO_2 \), respectively,
TA4 with the initial height of the waveguide after the thermal annealing is
0.89 \( \mu m \) (refractive index 1.63), while the height of the evanescent taper at the
end of the taper is about 0.20 \( \mu m \), and without top cladding, TA5 and TA6
with the same specific of TA4 but with 130 nm thick top cladding of BPSG
\( SiO_2 \) and PECVD \( SiO_2 \), respectively.

![Figure 1.32: scheme of the test chips used in experiment.](image)

1.7.4 Optical characterization of the taper waveguide

The first analysis was performed at the wavelength of 473 nm. At this wave-
length all samples, except the TA4, didn’t work. In fact using the maximum
power of the laser (350 mW) no signal was measured at the output of the
waveguide (see an example in Fig.1.33).

The next step was to perform a structural characterization, done by FBK
analysing the devices by means of a SEM, in order to investigate the possible
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Figure 1.33: optical microscope top-view image of a channel waveguide in the TA1 sample; after few hundred micrometers all the injected signal was lost. A very important reflection in the input facet was noticed.

causes of non-operation of the devices. As reported in Fig.1.34, the profiles of the input and of the output facet of the waveguide are not vertical. These effects are due to the etching step by RIE performed deep in the substrate, in order to dicing the wafer without damaging the structure. So in order to understand if the non verticality of the facet could be the cause of the impossibility of inject light signal inside the taper waveguide, the input facet of the device were cutted with a scriber. As shown in Fig.1.35 the signal extends a little bit more with respect to the same sample without the vertical input facet and the reflection by the input facet seems lower. Anyway, also after cutting the input facet of the device, the sample didn’t work.

The next step was to change the wavelength of the laser used to test the structure. The new wavelength used was 633 nm. At this wavelength all samples work and the output signal is constant. Tab.1.4 and Tab.1.5 summarize the total optical losses for the samples TA1 and TA4, the two samples without top cladding. To note that ”No signal” means that no signal was detected at the output of the waveguide; ”Stain” means that there is a stain on the device that covers that waveguide.

Let’s make some consideration about these losses values. The waveguide 1 is the same waveguide for all the series characterized by different pitch lengths; since it does not content any taper. So it is possible to consider the average of the four waveguide 1 losses measured (for the four series) as the reference
Figure 1.34: profiles of the facets of the waveguides measured by SEM analysis; in the inset a SEM image of the facet profile.

Figure 1.35: optical microscope top-view image of a channel waveguide in the TA1 sample with the input facet cutted in order to obtain a vertical profile.

to estimate the losses of the taper. In fact, if the coupling losses of different waveguides are very close to each other, it is possible to extract the losses of the tapers by subtracting the losses of a waveguide with taper from the ones of a waveguide without taper dividing the obtained value by the number of
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</tr>
<tr>
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<tr>
<td>7</td>
<td>-35.4</td>
<td>-37.5</td>
<td>-34.8</td>
<td>Stain</td>
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Table 1.4: total losses values for the sample TA1 measured at the wavelength of 633nm.

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Table 1.5: total losses values for the sample TA4 measured at the wavelength of 633nm.

For the sample TA4 serie 4µm, the average losses of the channel waveguide is (16.6 ± 0.5) dB. Subtracting the losses of the waveguide 1 (channel waveguide without taper) from the losses of the waveguide 3 (that has 3 tapers defined by pixel line) it is possible to obtain the losses due only to the three tapers.
Finally, dividing this value by three (the number of inserted tapers) it is possible to obtain the losses due only to one taper (see the calculation in Eq.1.3, the values used are the ones reported in Tab.1.5).

Similarly, the optical losses of a single taper can be obtained from another waveguide: subtracting the losses of the waveguide 1 (channel waveguide without taper) from the losses of the waveguide 5 (that has 1 taper defined by pixel line) (see the calculation in Eq.1.4, the values used are the ones reported in Tab.1.5). In fact:

\[
\frac{WG_3 - WG_1}{3} = \frac{18.7 - 16.6}{3} = 0.7 dB \tag{1.3}
\]

and

\[
\frac{WG_5 - WG_1}{1} = \frac{17.4 - 16.6}{1} = 0.8 dB \tag{1.4}
\]

The two values obtained for the taper losses are consistent within the error of the measurements (±0.5 dB). It is possible to do the same analysis for the waveguide defined using square dots in the fabrication process (see Eq.1.5 and Eq.1.6). Also in this case:

\[
\frac{WG_2 - WG_1}{3} = \frac{19.6 - 16.6}{3} = 1.0 dB \tag{1.5}
\]

and

\[
\frac{WG_4 - WG_1}{1} = \frac{17.5 - 16.6}{1} = 0.9 dB \tag{1.6}
\]

Also in this case the two values of one taper losses are consistent within the error of the measurements (±0.5 dB).

Also for sample TA4 serie 6µm, the same calculations were repeated, considering as losses of waveguide 1 the average value 16.6 ± 0.5 dB. The results obtained for the pixel line fabrication method are summarized in Eq.1.7 and Eq.1.8:

\[
\frac{WG_3 - WG_1}{3} = \frac{24.5 - 16.6}{3} = 2.6 dB \tag{1.7}
\]

and

\[
\frac{WG_5 - WG_1}{1} = \frac{19.2 - 16.6}{1} = 2.6 dB \tag{1.8}
\]

Again, these values are consistent. Interestingly, the losses are largest for the longest taper (6µm pitch). Since the simulations indicate that the losses for
long tapers (100µm to 300µm), as in our case, depend poorly on the taper length, it means that the losses are dominated by the scattering losses along the taper. In this case a longer taper would result in larger losses. Indeed, the whole tapered region of the taper with a pitch of 6µm is about 2.5 times longer than the one obtained with a 4µm pitch.

1.8 Conclusion

In this first part of the work, after a series of material optimizations, waveguides that work in the visible range were demonstrated. Moreover, the evanescent approach was analyzed in order to be used in an optical biosensor. A series of numerical investigations were performed in order to compare the efficiency of an evanescence based system (that resulted to be 5%) and of a propagating light approach (that resulted to be lower than 1%).

In addition, vertical taper waveguides were demonstrated, in order to reach the proper thickness to have mode evanescent field, in the bioreactor area. The sensing measurements are not yet completed, because of the long time required by every fabrication process (about four months) and the downtime problems of the technological partner. Despite this fact, the proof of validity of using this structure for biosensing was achieved depositing a drop of dye solution inside the bioreactor for both the working wavelength considered. For the moment it was not possible to detect the luminescence signal from functionalized bioreactors.
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Chapter 2

Silicon based photonic layer for biosensing: label-free approach

This chapter presents the other approach to biosensing considered in Naomi project. This is the label free approach. The sensing mechanism used in this work is a surface sensing method. Sensing is based on microdisk resonators coupled to bus waveguides. The disk surface is treated in order to have receptor sites that can selectively bind specific molecules. The molecule attached on the surface of the resonator changes the effective index of the guided mode and this results in a shift of the resonant wavelengths. The analysis is based on probing these transmission resonance shifts.

2.1 Introduction

A microdisk resonator is an optical cavity in which a continuous internal reflection of only certain wavelengths of light along the disk perimeter can be supported. These resonances (modes), related to specific wavelengths, occur when the round-trip phase acquired by the guided wave equals to multiples of $2\pi$. The resonant wavelengths are then determined by the formula:

$$M\lambda_c = n_{eff}L$$

(2.1)

where $\lambda_c$ is the resonant wavelength, $n_{eff}$ is the effective index of the guided mode, $L$ is the circumference of the resonator, and $M$ is an integer. According
to Eq.2.1, small variations in $n_{\text{eff}}$, like in the case of presence of molecules on top of the disk, lead to a detectable change in $\lambda_c$. In fact:

$$Q = \frac{\lambda_c}{\delta \lambda_c}$$  \hspace{1cm} (2.2)

so according to Eq.2.1

$$\frac{1}{Q} \sim \frac{\delta \lambda_c}{\lambda_c} = \frac{\delta n_{\text{eff}}}{n_{\text{eff}}}$$  \hspace{1cm} (2.3)

By using very high Q-factor resonators, an enhanced sensitivity for the detection of very low concentrations of molecules can be achieved. All these effects can be used to create very sensitive biochemical sensors. In a real device, the resonator is optically coupled to a bus waveguide; this last serves both for the optical input and output from the device. Ideally, the sensitivity of a microdisk based sensor is determined by the Q factor of the microresonator. Small change in the effective index ($\delta n_{\text{eff}}$) can be detected by measuring the resonance shift $\delta \lambda_c$. In fact, from Eq.2.3, it is possible to notice that the minimum detectable effective index change is inversely proportional to the Q factor of the device. In a real device, the limitation is due to the non-specific binding that can result in the detection of different proteins with respect to those we are interested in.

### 2.2 Experimental setup description

This section presents the experimental configuration developed for the label-free approach. This second setup is used to investigate the transmission of waveguide coupled microresonators. It uses optical fiber both for injecting and for collecting the signal (see Fig.2.1). In particular the input setup section consists in a laser source coupled with a tapered optical fiber. In the middle between the optical source and the sample, it is possible to add a polarizer, in order to control the polarization. With a 6 axis nanometric translational stage the input signal in the taper fiber is coupled into the waveguide by means of a microscope vision system. The device is placed on a 3 axis positioning system. Finally the output signal is analyzed with a photodiode for intensity measurements or with an optical spectrum analyzer (OSA) for spectral analysis.
Generally speaking, coupling between the microdisk and the waveguides can be achieved vertically or laterally (see Fig.2.2). In laterally coupled structures, the input/output waveguides and the disks are all coplanar. Narrow coupling gaps are required in the lateral geometry, which taxes photolithography and results in large variations in device performance. Furthermore, in the lateral geometry, waveguide and microdisk have to be realized with the same material and have to have the same thickness. In fact they are patterned from the same layer. Finally all the waveguides would be air clad and exposed to the surface treatments. The microdisk resonators used in this work are vertically coupled to input/output waveguides. In vertical coupling, the waveguides and the microdisk can be realized by different materials and could have different thicknesses. Finally, waveguides are buried and remain isolated and protected from the effects of surface treatment.

2.3 Device description

The chip size is 5mm x 2cm; the bottom cladding is composed by 3µm thick SiO$_2$ layer ($n = 1.45$), the waveguide is made of SiN ($n = 2$) (for work in
Chapter 2: silicon based photonic layer for biosensing

Figure 2.2: lateral (left) and vertical (right) coupling between microdisk and bus waveguide.

The IR range) or SiON ($n = 1.8$) (for work in the visible range) 250nm thick and 2.5µm of width; as top cladding of the waveguide there is a BPSG layer, the thickness of this layer depends on the gap needed for the vertical coupling; finally there is a SiN layer in which the microdisks are realized. On the device there are two equal series of 6 microdisks: three with 40µm of diameter and three with 50µm of diameter. In each series, composed by three microdisk, there are three different alignment conditions between the disk’s edge and the waveguide center. Calling $\Delta$ this distance between the microdisk’s edge and the waveguide center, the three conditions studied are: $\Delta = 0$ means that the edge of the disk is aligned with respect to the center of the waveguide, $\Delta = -1\mu m$ means that the edge of the disk is moved 1µm from the waveguide center, $\Delta = -2\mu m$ means that the edge of the disk is moved 2µm from the waveguide center (see Fig.2.3). These different conditions of alignment are used to investigate the best coupling condition, in order to excite only the first order mode family. The first devices have been designed and realized by APP-FBK, chemically treated and optically characterized at the NL laboratory of UniTN. The devices were characterized in waveguide transmission measurements.
2.4 First test in the near-IR range

The tests were performed in the near IR region, where two tunable lasers were available: the first one works from 1500 to 1600 nm and the second one from 1260 to 1630 nm, both with a picometer tuning step.

The experimental setup consists in coupling the laser signal into a tapered optical fiber mounted on a piezo-electric stage. The input signal was butt coupled into the bus waveguide. A fiber-polarizer has been employed between the laser and the input fiber to select the polarization, while a polarizer filter has been used in the collection system. The latter consists in an infrared CCD camera coupled to an optical zoom for the alignment and a photo-detector for measuring the intensity at the output of the bus waveguide; the scan over the spectrum was made tuning the IR source.

2.4.1 Sensing measurements on sample W20

Regarding the surface preparation of the resonators, it consists in a four stage process: cleaning of the sample, functionalization, activation and protein deposition.

The transmission spectrum was measured at three different moments of the
process. First the transmission spectrum of the disk without any treatment. A second measurement was done after the deposition of the receptor sites, that is after functionalization and activation, and, finally, a last measurement was performed after the protein deposition.

The first samples (W20) measured with this light source, is composed by SiN microdisk coupled to SiON waveguide. The dimension of the structures are always the same, waveguide with 2.5\( \mu m \) width and 250\( nm \) thick and the microdisk with 40 or 50\( \mu m \) of diameter and 330\( nm \) thick. The distance between the disk and the waveguide, the gap, is the variable parameter. For this sample W20 the gap value is 725\( nm \).

In Fig.2.4 and Fig.2.5 are reported the transmission spectra of the 40\( \mu m \) and 50\( \mu m \) diameter microdisk resonator respectively, detected in the horizontal polarization (TE-polarization). From these spectra it was possible to calculate the free spectral range (FSR) and the Q-factor (Q) of the microresonators; the results are summarized in the Tab.2.1. As expected, for the sample W20 the FSR is bigger for the smaller microdisk: \( FSR_{40} = (10.0 \pm 0.2)nm > (8.0 \pm 0.2)nm = FSR_{50} \). In fact from Eq.2.4

\[
FSR = \frac{\lambda^2}{n_g L}
\]  

where \( \lambda \) is the working wavelength, \( n_g \) is the group refractive index and \( L \) is the cavity length, it is easy to see that for the same \( \lambda \) and \( n_{eff} \):

\[
FSR_B < FSR_s \Rightarrow \frac{\lambda^2}{n_{eff} L_B} < \frac{\lambda^2}{n_{eff} L_s}
\]

where \( L_B \) is the longer cavity length and \( L_s \) is the shorter cavity length.

After these characterizations of the resonators, the functionalized structures were tested. Some results are presented in Fig.2.6 and Fig.2.7, in which the graphs were normalized (subtracting the oscillating baseline) in order to emphasize the resonance peaks. As expected, a blue shift after the functionalization of the disk was detected, which is due to the etching done during the cleaning process (It was not possible to test the reproducibility of the etching step, because sample W20 was the only one sample available). In fact if the thickness of the microresonator decrease, also the \( n_{eff} \) of the mode supported
by the microdisk decreases and, from Eq.2.1, also the $\lambda_c$ decreases. Instead the bonds between the biomolecules and the receptor sites on top of the disk induce a red shift of the resonance peak. In fact if on top of the microresonator there is a layer of biomolecules (instead of air), the $n_{eff}$ of the mode supported by the microdisk increases and, from Eq.2.1, also the $\lambda_c$ increases. In particular a red shift of $0.6 \text{nm}$ was detected in the case of a microdisk with $40 \mu m$ of diameter and $\Delta = -1 \mu m$ of misalignment (Fig.2.6) and a red shift of $0.9 \text{nm}$ in the case of microdisk with $40 \mu m$ of diameter and $\Delta = -2 \mu m$ of misalignment (Fig.2.7).

After these analysis, it is possible to investigate the minimum concentration value of proteins detectable with these microdisk resonators. As shown in Eq.2.6, the minimum shift in the resonance peak wavelength ($\Delta \lambda$) detectable
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Figure 2.5: transmission spectra of the 50µm disk resonator; for the measurements horizontal polarization was considered.

is proportional to the relative minimum variation in the refractive index ($\Delta n$), due to the presence of the proteins:

$$\frac{\Delta \lambda}{\lambda} \sim \frac{\Delta n}{n}$$ \hspace{1cm} (2.6)

The minimum shift in the resonance wavelength ($\Delta \lambda$) detectable with this system is dictated by the minimum tunability step of the laser that is 10pm. Thus, the minimum variation of the refractive index detectable is of the order of $1 \cdot 10^{-5}$. As the concentration value of the protein detected by the system is directly proportional to the refractive index variation, it is possible to estimate the minimum concentration value detectable with these resonator, using the minimum detectable refractive index variation. In this case, the concentration of the protein deposited on the surface is not exactly known; this was only a proof of the validity of the system (the protein was tagged before deposition.
Table 2.1: FSR and Q factor of the 40 and 50 µm diameter microdisk resonators of the sample W20.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>DISK</th>
<th>FSR</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>W20</td>
<td>d = 40µm Δ = 0µm</td>
<td>10.1 ± 0.2</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1050</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>940</td>
</tr>
<tr>
<td>W20</td>
<td>d = 40µm Δ = −1µm</td>
<td>9.9 ± 0.2</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1340</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>W20</td>
<td>d = 40µm Δ = −2µm</td>
<td>10.0 ± 0.2</td>
<td>7000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4400</td>
</tr>
<tr>
<td>W20</td>
<td>d = 50µm Δ = 0µm</td>
<td>8.0 ± 0.2</td>
<td>1170</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1260</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1970</td>
</tr>
<tr>
<td>W20</td>
<td>d = 50µm Δ = −1µm</td>
<td>8.0 ± 0.2</td>
<td>4900</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4400</td>
</tr>
<tr>
<td>W20</td>
<td>d = 50µm Δ = −2µm</td>
<td>7.9 ± 0.2</td>
<td>6400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4400</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6100</td>
</tr>
</tbody>
</table>

on the microresonator, in order to have a confirmation of their presence on the microdisk after their deposition, by means of a fluorescence microscope image. The initial protein solution has a concentration of 10µM; but after the attachment of the cromophores on the proteins, the solution was passed in a chromatographic column, in order to filter out the cromophores that didn’t bond with the proteins. In this stage the solution was certainly diluted. Thus, it is not possible to do a good estimation of the minimum concentration of tagged protein detectable with this device. An overestimation, considering as concentration value this 10µM, reveals as minimum concentration detectable
Figure 2.6: transmission spectra of a 40\( \mu \)m disk resonator and \( \Delta = -1\mu m \) of misalignment, detected in three different moments of the process: sample without any treatment (black line), after the deposition of the receptor sites (red line) and after the binding of the biomolecules with the receptor sites (blue line).

Figure 2.7: transmission spectra of a 40\( \mu \)m disk resonator and \( \Delta = -2\mu m \) of misalignment, detected in three different moments of the process: sample without any treatment (black line), after the deposition of the receptor sites (red line) and after the binding of the biomolecules with the receptor sites (blue line).

of the order of 1\( \mu M \). These results are a proof of the validity of using these microdisk resonators as a label-free biosensor.
2.5 Second test in the visible range

2.5.1 Superluminescent light emitting diode

After some trials with different white lamps which result to do not have enough power, this second test in the visible range was performed using a superluminescent light emitting diode. It has a central wavelength of $840\text{nm}$, a bandwidth of $40\text{nm}$ and a total power of $40\text{mW}$ over all the band. In Fig.2.8 is reported the spectrum of the superluminescent light emitting diode detected with the OSA; in particular the comparison between two different acquisition conditions is reported: both measurements were performed without the point averaging, but one was detected with the resolution of $1\text{nm}$ (black line) and the other one with the resolution of $0.05\text{nm}$ (red line). In this last acquisition with the best resolution ($\text{Res} = 0.05\text{nm}$) it is possible to see an oscillation of the signal that could hide the resonance peaks of the microdisks. But an high resolution value would be necessary to be able to detect the resonance peaks and the possible shift due to the presence of biomolecules.

2.5.2 Sample W26

For this sample, the $\text{SiON}$ waveguide geometrical dimensions are the same as before ($2.5\mu\text{m}$ width and $250\text{nm}$ thick); the $\text{SiN}$ resonators layer is $290\text{nm}$ thick and the gap value is $300\text{nm}$ thick. On the device channel waveguides without microdisk are present, too.

No resonances in the transmission signal were observed. Thus, it is reasonable to assume that the microdisks are not coupled to the waveguide. The problems could be due to the vertical gap between the waveguide and the resonator, that could be too large and do not allow the mode evanescent field to interact with the resonator. Thus, different vertical gap values should be tested. Another problem could be the micro resonator material; in fact the microdisks are realized in $\text{SiN}$ and the waveguides in $\text{SiON}$. An hypothesis could be that the $\text{SiN}$ does not supports guided modes at the working wavelength. Thus, it should be of interest to try to couple light in a $\text{SiN}$ channel.
Figure 2.8: output spectra of the superluminescent light emitting diode detected with the OSA; in particular with two different acquisition conditions: with the resolution of 1\,nm (black line) and with the resolution of 0.05\,nm (red line). Both measurements were performed without the point averaging.

waveguide (fabricated with the same parameters of the resonator layer), in order to understand if it is a problem of the material. This is discussed in the following section (Sec.2.5.3).

### 2.5.3 Sample W7

The next step was to test a sample with both microdisks and waveguides realized in the same material, SiN. The resonator layer is 290\,nm thick and the gap value for this sample is 520\,nm (waveguide geometrical dimensions as before).
For this sample was not possible to couple any signal at the output of the waveguide. No light at these wavelength was guided by SiN waveguide 250\textit{nm} thick and 2.5\textit{µm} width. These results confirm the hypothesis that the problem of the sample W26 is the material of the micro resonators that does not support any guided modes at these wavelengths.

### 2.5.4 Sample W23 and W24

After these analysis, samples with the microdisks and the waveguide both realized in SiON were tested; in particular these samples were tested before and after the annealing of SiON layer. Again the waveguide structural dimensions are the same as before; the geometrical parameters of resonator layers are summarized in the Tab.2.2. No resonances were observed for both W23 and W24.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Resonator layer thickness [\textit{nm}]</th>
<th>Gap thickness [\textit{nm}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>W23 before annealing</td>
<td>273</td>
<td>120</td>
</tr>
<tr>
<td>W23 after annealing</td>
<td>245</td>
<td>120</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>267</td>
<td>216</td>
</tr>
<tr>
<td>W24 after annealing</td>
<td>241</td>
<td>216</td>
</tr>
</tbody>
</table>

Table 2.2: geometrical parameters of the resonator layer for the samples W23 and W24 before and after the annealing step.

Samples before the annealing of the resonator layer. In the graph in Fig.2.9 are reported two examples of transmission spectrum of the microdisk of 40\textit{µm} diameter of the sample W23 annealed. These are unpolarized measurements, recorded with a resolution of 0.2\textit{nm} using the OSA. In Fig.2.9 it is possible to see some peaks, that could be assumed to be the resonance peaks. In fact, the free spectral range (FSR) of these peaks is consistent with the FSR values expected (see Eq.2.4).

In order to calculate the FSR and the $Q$, measurements with the best possible resolution are needed. Due to the noise of the spectra recorded at the highest
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Figure 2.9: transmission spectra of the microdisk of 40µm diameter of the sample W23 annealed. The black line correspond to the transmission spectrum of the microdisk with alignment value $\Delta = -1\mu m$ and the red line correspond to the transmission spectrum of the microdisk with alignment value $\Delta = -2\mu m$. For these measurements were not used a polarizer selector, and were detected using a resolution of 0.2nm of the OSA.

resolution, in some cases was not evident to recognize the resonance peaks in these spectra. The procedure followed to analyze the spectra was to acquire the entire output spectrum with lower resolution (0.2nm) and after that acquire only a part of the spectrum (for example a 10nm wide spectrum) with the best resolution (0.05nm) and use the latter to do the calculation of $FSR$ and $Q$. An example of transmission spectrum detected with the best resolution, but in which the resonance peaks positions do not appear so clearly, is reposted in Fig.2.10. In the figure the peaks positions are indicated by the vertical dots lines, but they where extrapolated by the entire spectrum shown.
Figure 2.10: zoom on the transmission spectra of the microdisck of 40µm diameter and ∆ = −1µm of the sample W23 annealed. The vertical dots line indicates the resonance peaks positions. For these measurements were not used a polarizator selector, and were detected using a resolution of 0.05nm of the OSA.

As it is possible to see in Tab.2.3 for both the samples before the annealing of the microdisk layer, it was not possible to recognize the resonance peaks. The annealing process allows to see some resonance peaks on the microdisk. In the annealing process the geometrical parameter of the other layers does not change. In fact the annealing temperature used in previous steps of the fabrication process was equal to this one. Thus the parameters that could change are the resonator layer thickness (that could decreases) and its refractive index (that could increases). As expected, for the sample W24 annealed a FSR value bigger for the smaller microdisk than for the larger was obtained:
\[ FSR_{40} = \sim (2.7 \pm 0.2) \text{nm} > \sim (2.0 \pm 0.2) \text{nm} = FSR_{50}. \] Moreover, as expected, for the 40\,\mu m diameter disk with \( \Delta = -1\mu m \) of the sample W23 (gap = 116\,nm) annealed, lower Q-factor values were founded respect to the disk with the same parameter of the sample W24 annealed but having higher gap (= 220\,nm). In fact, higher is the coupling between microresonator and waveguide and lower is the Q-factor. The proof of validity of this device for biosensing is not yet achieved for these working wavelengths, due to the problems of light source. In fact, resonant peaks position shifts of the order of few hundreds of picometers are expected; thus measurements with the best resolution of the OSA (0.05\,nm) are required. But, as it was shown in Fig.2.8, the light source shows oscillations in the output spectrum that could hide the resonance peaks.

### 2.6 Conclusion

As concern the label-free approach, it was proved the validity of this device in the IR-range for biosensing. Moreover, the minimum tagged proteins concentration value detectable with the system investigated was estimated (W20) to be at least of the order of 10\,\mu M.

As concern the region around 800\,nm, after the study of different structures, it was found a device in which the light is coupled into the microresonator. In this case, due to the problems with the light sources, the biosensing measurements were not performed.
<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>DISK</th>
<th>FSR</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>W23 before annealing</td>
<td>$d = 40 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 before annealing</td>
<td>$d = 40 \mu m \Delta = -1 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 before annealing</td>
<td>$d = 40 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 before annealing</td>
<td>$d = 50 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 before annealing</td>
<td>$d = 50 \mu m \Delta = -1 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 before annealing</td>
<td>$d = 50 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 40 \mu m \Delta = 0 \mu m$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 40 \mu m \Delta = -1 \mu m$</td>
<td>3.2 ± 0.2</td>
<td>2000</td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 40 \mu m \Delta = -2 \mu m$</td>
<td>3.1 ± 0.2</td>
<td>1400</td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 50 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 50 \mu m \Delta = -1 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W23 annealed</td>
<td>$d = 50 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 40 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 40 \mu m \Delta = -1 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 40 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 50 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 50 \mu m \Delta = -1 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 before annealing</td>
<td>$d = 50 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 40 \mu m \Delta = 0 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 40 \mu m \Delta = -1 \mu m$</td>
<td>2.7 ± 0.2</td>
<td>4250</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 40 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 50 \mu m \Delta = 0 \mu m$</td>
<td>2.0 ± 0.2</td>
<td>4000</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 50 \mu m \Delta = -1 \mu m$</td>
<td>1.9 ± 0.2</td>
<td>2000</td>
</tr>
<tr>
<td>W24 annealed</td>
<td>$d = 50 \mu m \Delta = -2 \mu m$</td>
<td>NM</td>
<td>NM</td>
</tr>
</tbody>
</table>

Table 2.3: FSR and Q factor for the sample W23 and W24 before and after the annealing of the microdisk layer.
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Chapter 3

Polymeric slot waveguides based sensor on Si substrate

GOPSI project aims to realize a polymeric slot waveguide based sensor on Si substrate. Application of polymeric materials in integrate photonic were very limited till few years ago. In fact, this research field was dominated by inorganic dielectric or semiconductor that have a higher refractive index. However, a part some restrictions related to the refractive indices that limit their use in some applications, polymeric materials offer several advantages over the dielectric that are normally used for photonic structures [129]: polymeric materials have lower fabrication cost, using the so called soft-lithographic methods (for example nanoimprint lithography, E-beam lithography, etc); very important is the compatibility of these materials with the most common fabrication process such as the CMOS technology; polymers show a great flexibility given by the huge numbers of functionalization reactions that, through the addiction of suitable functional groups, can lead to polymers with tailored properties.

3.1 Slot waveguide

A slot waveguide is a kind of optical waveguide composed by a thin layer (the slot region) placed between two channel waveguides (the walls) that result cou-
pled together; moreover the slot region has lower refractive index value with respect to the wall one. The particularity of this geometry is to strongly confine light in the, usually, sub-wavelength slot region. This phenomenon occurs only in the case of the polarization mode whose electric field is perpendicular to the slot interfaces, due to the electric-field discontinuity at the slot-wall interface. In fact if the slot width is smaller than the decay length of the field, the overlap of the optical field evanescent tails increase within the slot, enhancing the field intensity in this region with respect to the field confined in the walls [131]. Finally there are two kinds of slot waveguides: vertical slot waveguide and horizontal slot waveguide (see fig.3.1). The choice is based on

![Diagram of vertical and horizontal slot waveguides](image)

Figure 3.1: vertical slot waveguide field profile for the quasi-TE polarization (left) and horizontal slot waveguide field profile for the quasi-TM polarization.

the great amount of field portion in the slot region that could interact with the environment. Slot waveguide is a perfect candidate for sensing scheme, in fact the greater is the field portion able to interact with the surround, the greater will be the sensor response to a variation in the environment [135]. Moreover, the choice of a vertical slot waveguide allows to inject a solution inside the slot region, using a micro-infiltrating system.


### 3.2 Schematic of the sensor

The project aims to realize a polymeric vertical slot waveguide (SWG) on a silicon substrate. The polymeric material used was the poly(methyl methacrylate) (PMMA), a commercial E-beam photoresist. Because of its low refractive index \( n = 1.49 \), it can not be deposited on a silicon substrate \( n = 3.5 \). So in between of these two layers, for decoupling them, will be need a lower refractive index layer. For its low refractive index value the porous silicon was chosen [136]. The schematic of the sensing device to realize is shown in Fig.3.2: three different geometries were considered: single, double and triple slot waveguide, in order to maximize the interaction region with the environment.

![Schematic of the sensing device](image)

Figure 3.2: schematic of the sensing device: single slot waveguide (left), double slot waveguide (top right) and triple slot waveguide (bottom right).

### 3.3 Numerical optimization of the geometry

The first step was to investigate the confinement factor \( (CF) \) of the mode inside the slot region for all the three configurations, varying the widths of the wall and of the slot regions, but considering constant layer thickness of 700nm. The \( CF \) was calculated as the fraction of the mode power flux in the
slot region $R$, see Eq.3.1.

$$CF = \frac{\int_R P_Z(s) ds}{\int_\infty P_Z(s) ds}$$

For all the simulation the refractive index values are: $n_{air} = 1$, $n_{PS} = 1.2$ and $n_{PMMA} = 1.5$ (where $n_{PS}$ refers to porous silicon). The wavelength considered is $\lambda = 600\text{nm}$.

All the simulations were two dimensional and have been carried out using a fully vectorial mode finder commercial package [137]. In Fig.3.3, 2D maps of the CF, in single and multiple slot waveguide $500\text{nm}$ thick, versus both slot $(g)$ and wall $(W_{int})$ widths are reported. In double and triple SWG systems the scan over the wall width concerns only the internal dielectric volumes (slot coupling), while the external volumes were kept fixed at $300\text{nm}$ width. The single SWG suffers from a cut-off region that strongly limits its effective use, due to the stringent fabrication tolerances required to achieve a guided mode with so small effective index and with reasonably propagation losses. Double and triple SWG do not show the cut-off region and are then fundamentally much more robust and easy to fabricate. The enhancement in the field concentration grows sublinearly with the slot number and, for both double and triple WG. Single and double SWG have the same maximum of CF of about
0.18, whereas triple SWG reaches a value of 0.20. This increase in the CF in triple slot system could be assigned to the symmetry of the fundamental mode profile that put its maximum in the centre of the WG, in contrast with the double SWG were the central part is composed by a dielectric material.

Fig. 3.4 - 3.5 - 3.6 show 2D maps of CF, in single and multiple slot waveguide (SWG) 700nm thick, versus both slot \((g)\) and wall \((W_{int})\) widths. As it is

Figure 3.4: CF for single slot in case of (a) first, (b) second and (c) third mode \((h = 700nm, W_{ext} = 200nm)\). Superimposed to the 2D maps there are a few contour lines reporting the mode effective index. Color bar limits range from \(CF_{min} = 0.01\) to \(CF_{max} = 0.28\) \((\lambda = 600nm)\).

Figure 3.5: CF for double slot WG in case of (a) first, (b) second and (c) third mode \((h = 700nm, W_{ext} = 200nm)\). Superimposed to the 2D maps there are a few contour lines reporting the mode effective index. Color bar limits range from \(CF_{min} = 0.02\) to \(CF_{max} = 0.39\) \((\lambda = 600nm)\).
Figure 3.6: CF for triple slot WG in case of (a) first, (b) second and (c) third mode \((h = 700\, \text{nm}, W_{\text{ext}} = 200\, \text{nm})\). Superimposed to the 2D maps there are a few contour lines reporting the mode effective index. Color bar limits range from \(CF_{\text{min}} = 0.01\) to \(CF_{\text{max}} = 0.45\) \((\lambda = 600\, \text{nm})\).

possible to see in Fig.3.4 - 3.5 - 3.6, in most of the cases, the slot waveguides support more than one optical mode. The presence of higher order modes is characteristic of structures with such large heights. The highest sensitivity is reveald in structures working with the fundamental mode, due to their higher \(CF\) in the slot region. These graphs constitute fundamental guidelines for designing single mode, high sensitivity polymer SWG. In double and triple SWG systems, the scan over wall widths \((W_{\text{int}})\) concerns only the internal dielectric walls, equivalent to change the inter-slot coupling effect, while the external ones are kept as fixed as \(W_{\text{ext}} = 200\, \text{nm}\). Therefore, \(CF\) is the sum of the contributions due to all slots. The white areas of Fig.3.4 - 3.5 - 3.6 refer to the cut-off regions, where guided modes cannot exist. As it is intuitive, the use of multiple SWGs produces a better total electromagnetic field confinement inside the slots [138], [139]. Single SWG is characterized by the most extended cut-off region. Due to the very low modal effective index, relatively high propagation losses should be expected in these structures. On the other hand, both double and triple SWGs have smaller cut-off regions, greater \(CF\) values and slightly greater effective indices. Thus, they are potentially more robust waveguides. Scattering from the slot surfaces can have a detrimental role, nevertheless nearly ideal structures with extremely low roughness and vertical sidewalls were already demonstrated in polymeric materials [141],
at submicron length scale, so that it is reasonable to assume very high quality of the samples realized in polymeric materials. As expected, increasing the thickness of the SWG, the $CF$ increase too. In addition, it is of interest to compare the relative enhancement of $CF$ value in SWG with a different number of slots. This parameter is defined in Eq.3.2:

$$R_{rel} = \frac{CF_M(g, W_{int}) - CF_m(g, W_{int})}{CF_m(g, W_{int})} \times 100 \quad (3.2)$$

where $M$ and $m$ refer to WG with greater and smaller number of slots, respectively, and $(g, W_{int})$ are the slot and wall widths. In general, single SWG is the worst system in terms of energy confinement properties, apart in a small region near the cut-off where single SWG shows a slightly larger confinement with respect to the double SWG (of about 5% see Fig.3.7). Small negative values appear also in two small regions in the 3 vs 2 graph of Fig.3.7. These counter intuitive behaviours are due to the energy redistribution between the different materials in the SWG, due to the long modal decay length in low index ($LI$)

Figure 3.7: a comparison between the confinement properties of the fundamental mode of single, double and triple SWG 500nm thick. 3vs1 compare the triple versus the single SWG (left), 3vs2: double against single (center) and 2vs1: refer to the double to single comparison (right). The three graphs share a common colorbar.
dielectrics. The same comparison was done also for the fundamental modes of the single, double and triple SWG 700 nm thick (see Fig. 3.8). By comparing these cases, it is observed that $R_{rel}$ grows proportionally to the effective index, but the trend is sublinear and negligible enhancement of S is expected in guided-wave structures with more than 3 slots. These calculations show that the maximum $R_{rel}$ is of the order of 50% for double SWG and 70% for triple SWG (considering only the fundamental mode). Unfortunately, these large values are achieved in a region of low sensitivity (corresponding to large slots and wall widths) and reflect the stronger mode confinement achievable in WG with larger cross section. Similar comments can be made for higher order modes. In these cases $R_{rel}$ increase up to an order of magnitude between multiple and single slots geometries, but only if very narrow slots are considered. In fact, for higher order modes, $R_{rel}$ decrease exponentially with the increase of the slot width.

As it is clear from Fig. 3.4 - 3.5 - 3.6, low index materials allow the fabrication of wide slots (up to 200 nm) without any strong reduction of $CF$. In fact, the decay length of the field in the slot region is proportional to the refractive index mismatch between the waveguide core and cladding materials; in case of LI slot waveguide the refractive index mismatch is smaller and the evanescent
field tail is longer than in case of high index (HI) materials, allowing to have wider slot region for LI slot waveguide. This is a great advantage if compared to high index materials. In fact, if applications for sensing liquids are considered, then larger slots will ease the liquid transport through the WG and the very narrow slots required by HI systems can be unworkable due to mass transport limitation on short timescales.

In conclusion, better behaviour in terms of sensitivity as well as larger range of available WG parameters (better fabrication tolerances) are achieved for SWG structures working with fundamental mode, with increasing the number of slots. However, these more complicated structures with respect to the single SWG are not always justified, although the sensing area is increased when a larger number of slots are included and they can still work with the first mode. Moreover, the reduction of sensitivity in SWGs working with second, third (or higher) modes is also clearly shown and does not allow a practical use of these propagation modes for sensing in many applications.

Fig.3.9 and Fig.3.10 reports the comparison between LI and HI SWG with the slot filled with liquid (without any loss of generality, the refractive index for the filling liquid was set to 1.33, that could represent for example water). The exact dependence of $CF$ on slot width is strongly dependent on the actual SWG geometry and a complete analysis should require a multi-parametric geometrical optimization that is out of the scope of this work. Nevertheless, a few geometries that roughly optimize this key parameter have been found and are here analyzed. The refractive index values used in these analysis are: 3.5 for the core material refractive index of the HI SWG, 1.45 as core material refractive index for LI SWG; the same refractive index for the top and bottom cladding were used for both HI and LI SWG: 1 as top cladding and 1,2 for the bottom refractive index (that correspond for example to the refractive index of the porous silicon). The porous silicon is not an usual bottom cladding for HI SWG; so in addition was investigated also a case with 1.45 as bottom cladding refractive index for the HI material (dot lines in both Fig.3.9 and Fig.3.10). In Fig.3.9 the comparison between single SWGs realized in LI and HI materials is shown. As for Fig.3.4, $\lambda = 600nm$ for LI SWG and $\lambda = 1550nm$
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Figure 3.9: dependence of $CF$ on slot width in $LI$ and $HI$ single SWG. Details of the simulated geometries are described in the legend (if not indicated the substrate refractive index, 1.2 is to be consider). The wavelength considered is $\lambda = 600\text{nm}$ and $\lambda = 1550\text{nm}$ for $LI$ and $HI$ materials, respectively.

for $HI$ SWG are assumed, respectively. The $CF$ trend as a function of slot width is opposite for these two material systems both for single and triple geometry. For the single SWG only the fundamental modes were considered because second order modes appear on a few $LI$ geometries, having a very low effective index not practical for sensing purposes. In $HI$ SWG, wide slots can lower the $CF$; in fact the refractive index mismatch between the slot and the core material is very high, and the evanescent tails of the mode decrease very rapidly. On the contrary, in $LI$ systems both mode effective index and $CF$ grow as the slot becomes larger. $HI$ SWG shows higher confinement in narrow slots but the highest value is achieved in $LI$ SWG with wide slot with the $\sim 64\%$ of the energy of the fundamental mode with effective index of 1.29 inside the slot region (for the single $LI$ SWG with wall width of $180\text{nm}$, slot width of $400\text{nm}$ and $700\text{nm}$ thick) respect to $\sim 61\%$ of the $HI$ SWG with effective index of 1.59 (for the SWG with wall width of $220\text{nm}$, slot width of $80\text{nm}$ and $250\text{nm}$ thick [135]), as expected. Moreover, for the $HI$ SWG with 1.45 as bottom cladding refractive index, the $CF$ is lower, reaching as
maximum the value of 55% of energy of the fundamental mode with effective index of 1.72 inside the slot region. Confinement factor values higher than this are achievable for LI SWG with a slot width larger than 280nm (wall width of 180nm and 700nm thick) that presents a CF value of 56% for the fundamental mode with effective index of 1.3. Fig.3.10 reports the comparison among triple SWGs. The general trends are similar to that for single SWG, but in this case also higher order modes were considered. From the comparison between the two data panels, it can be noted that fundamental modes are much more enhanced in HI SWG, rather than in LI systems, with a relative improvement of the order of 50%. On the contrary, the multimodal behaviour of triple LI SWG allows the achievement of very high CF. In the case of the same refractive index for the bottom cladding (1.2) for both HI and LI SWG, the CF values are about the same: 80% for the fundamental mode of the HI SWG with a effective index of 1.7 (for the SWG with 200nm of external wall width and 160nm of internal wall width, 60nm of slot width and 250nm thick; shown as red square in the Fig.3.10) and 78% for the fundamental mode of the LI SWG with effective index of 1.3 (for the SWG with 180nm of external wall width and 100nm of internal wall width, 400nm of slot width and 700nm thick; shown as green square in the Fig.3.10). Considering 1.45 as the bottom refractive index, LI SWG is compared to HI SWG with 1.2 as substrate refractive index, and the CF values are 80% for the fundamental mode of the HI SWG and 78% for the fundamental mode of the LI SWG, respectively.
cladding for the HI SWG, the maximum value of CF achieved is lower: 72% of the fundamental mode with effective index of 1.9 (for the SWG with 220nm of external wall width and 200nm of internal wall width, 60nm of slot width and 250nm thick; shown as cyan asterisks in the Fig.3.10).

### 3.4 Realization of the structure

As concern the realization of the sample, the first step consist in the development of the bottom cladding of the slot waveguide realized in porous silicon. The technique adopted consists in the electrochemical etching of silicon p-type with a resistivity of $R = 0.002 - 0.005 \, \Omega \, cm$. The polymer film was deposited using the spin coating technique [161], after a calibration of the film thickness versus the angular speed of spinning. The slot waveguide were patterned using the E-beam lithography technique.

#### 3.4.1 Porous silicon layer

Porous silicon samples were fabricated by electrochemical etching of doped (0.002 – 0.005 $\Omega \, cm$ resistivity) p-type silicon. Single monolayer samples were grown by applying a constant current density on a $1 \, cm^2$ circular surface or on a 4 – inch wafer. In particular different samples were analyzed which were realized applying different current densities (from 50 to 80 mA/cm²), in order to obtain the lowest refractive index (see a scheme of the setup used in Fig.3.11). For the electrochemical etch was used a solution of 3 : 7 vol of hydrofluoric acid ($HF$) 48% and ethanol, respectively. To completely oxidize the structures after the etching of the porous silicon and consequently decreasing the refractive index of the layer, an annealing treatment was performed. In particular this annealing consist of 2 h for reaching the annealing temperature of 900 °C and 3 h at 900 °C in an air atmosphere. In Tab.3.1 the refractive index values obtained for different current density applied were summarized.

The lowest refractive index value obtained was 1.18 (as shown in Tab.3.1); this value was obtained for 50mA/cm² of current density applied. The duration of the etching influences only the thickness of the porous silicon layer.
Figure 3.11: electrochemical cell used for the etching of the porous silicon.

<table>
<thead>
<tr>
<th>Current $[mA]$</th>
<th>Thickness $[nm]$</th>
<th>Etching Time $[min]$</th>
<th>Refractive Index Not Oxidized</th>
<th>Refractive Index Oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>5900</td>
<td>3</td>
<td>1.46</td>
<td>1.28</td>
</tr>
<tr>
<td>70</td>
<td>4000</td>
<td>3</td>
<td>2.08</td>
<td>1.73</td>
</tr>
<tr>
<td>60</td>
<td>5200</td>
<td>3</td>
<td>1.60</td>
<td>1.28</td>
</tr>
<tr>
<td>50</td>
<td>4600</td>
<td>3</td>
<td>1.46</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Table 3.1: summary of the refractive index values obtained varying the current density applied.

The thickness of the porous silicon layer was measured from scanning electron microscope (SEM) images. The refractive index of the PS layer was calculated from the reflectance spectrum acquired using a Cary 5000 UV-Vis-NIR spectrophotometer. In the case of a thin film on the surface of another material the reflection from the two interfaces (air-thin film and thin film-substrate) produces some interference fringes. They can be used to determine the thickness of the film in question, assuming that refractive index and angle of incidence are both known, or, vice-versa, to determine the refractive index of the film in question, assuming that the thickness and angle of incidence are both known [144]. Using Eq.3.3 it is possible to calculate the refractive index of, for exam-
where \( d \) is the film thickness in \( nm \), \( \theta \) is the beam incident angle (that in this case is fixed at 12\(^\circ\) ), \( E_{MAX} \) and \( E_{MIN} \) correspond to the maximum and minimum value of energy (in \( cm^{-1} \)) that define the wavenumber region used and \( m \) are the number of fringes inside the wavenumber region.

In Fig.3.12 is reported an example of reflectance spectrum obtained with a PS sample realized applying a current density of 60\( mA \).

Figure 3.12: reflectance spectrum of a PS sample obtained applying a 60\( mA \) current density; it is possible to see the interference fringes.
3.4.2 Polymeric layer

The polymer chosen to realize the slot waveguide is the Poly(methylmethacrylate) (PMMA AR-P 679.04 dissolved in ethyl lactate by ALLRESIST), that has a refractive index of 1.4914 at 587.6nm [145]. It is far and away the most popular e-beam resist, offering extremely high-resolution, ease of handling, excellent film characteristics, and wide process latitude. The exposure causes scission of the polymer chains. The exposed (lighter molecular weight) resist is then developed in a solvent developer.

The PMMA layer was deposited by spin coating, a system used to deposit thin film starting from liquid solution. It is a technique that consist in the deposition of an excess amount of solution on the substrate which is then rotated at high speed in order to spread the fluid by centrifugal force (see Fig.3.13). In detail, the sample is hold on the chuck by a vacuum pump; under rotation the sample is usually clean (under maximum rotation speed of 6000 rpm) using first acetone and finally isopropyl alcohol, that is the PMMA stopper. After cleaning, the polymeric solution is deposited on the substrate and is spinned using the right rotation speed in order to achieve the desired film thickness. Finally a baking at 160 °C for 15 min was performed after deposition of PMMA. A calibration of PMMA film thickness achieved as a function of the rotation speed value used was performed on silicon substrate.

Figure 3.13: spin coater used to deposit the PMMA layer.
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(see Fig.3.14). The film thickness was measured with AFM. As it is possible to see from the graph in Fig.3.14, the resultant film thickness decreases with the rotation speed increasing, with an exponential decay behaviour.

3.5 E-beam lithography

For patterning the slot waveguide, e-beam lithography technique was used. In particular was used a Jeol 6100 and a raith elphy quantum. In Fig.3.15 it is possible to see the first geometry patterned and the second one; due to the proximity effect it was necessary to decrease the dose (number of electrons per unit area $\mu C/cm^2$) in the parts closer to the waveguide. In fact the PMMA layer in those regions receives also a small contribution from the part of electrons directed to the slot region and back scattered from the substrate due to

![PMMA calibration curve](image)

Figure 3.14: calibration curve of PMMA film thickness versus the rotation speed.
the proximity of those two regions. To be noted that the smaller slot width was patterned drawing only one line with the SEM, that corresponds to about 80nm (the smaller slot width achievable with this setup). The same correction due to the proximity effect was used also for the multiple slot geometry. After several testing mask, the dose values choose for patterning the slot waveguide are: dose1 = 0.900$\mu C/cm^2$, dose2 = 0.450$\mu C/cm^2$ and dose3 = 0.338$\mu C/cm^2$. Finally the exposed PMMA layer was developed for about 50s in Entwickler AR600 – 55 and washed for 50s in the stopper AR600 – 60. In Fig.3.16 are shown some SEM images of the obtained waveguide. In Fig.3.17 are shown some AFM images of the obtained waveguide.

3.6 Strip loaded slot waveguides

The problem of these slot waveguide patterned with the e-beam lithography is that the setup used in this work does not dispone of a nanometric realignment system, and the maximum area drawable per time is 400$\mu m^2$. These dimensions are definitely too small to be handled and measured. It is important to underline the fact that a resolution of the order of 100nm in re-alignment over pre-existent structure require complicate facilities (as interferometers stages), whereas the micron range resolution needed by our approach can be achieved.
using much simpler equipment. So two ways were considered: the first was to pattern the slot waveguide in such a way that one end of the slot is on the edge of the sample (Fig.3.18 left) and using the residual layer of PMMA as a planar waveguide to inject the light in the slot waveguide (Fig.3.18 right). It was possible to realize this kind of structure, but for the moment no measurements have been done successfully. In fact, due to the very small dimensions of the waveguide and of the layer of residual PMMA, it was not possible to focalize the collecting system on the output of the slot waveguide.

Another way was to couple the light from the end of the slot waveguide that is in the edge of the sample, fill the slot region with some dye solution and to try to detect the luminescence signal from top of the sample. This time the problem was that the filling solution does not stay in the slot region, but penetrate the bottom cladding layer of porous silicon. Therefore, the porous silicon layer was made hydrophobic, silanizing it (results are
Figure 3.17: AFM images of PMMA double slot waveguide patterned with e-beam lithography technique. From this image it seems that the PMMA layer was not etched for all the thickness of the layer. This is an artefact due to the fact that the AFM tip (Whysker type) fails to enter till the all deep of the slot region, due to its size bigger than the slot width.

show in Fig.3.19). The silanization procedure consist in vapour deposition of \((1H, 1H, 2H, 2H – \text{Perfluorooctyl})\text{Trichlorosilane}\) by Alfa Aesar in atmospheric pressure. After the silanization some problems in the adhesion of the PMMA layer on the silanized surface were encountered.

The second hypothesis considered was to pattern the slot waveguide on top of a bus waveguide; the idea is to realize the bus waveguide in a \(SU – 8\) polymer layer, which can be defined by conventional UV-lithography. This geometry is equivalent to a classical strip loaded one, with the slot region acting as the loading layer. In order to maximize the energy confinement factor in this
structure, is needed that the thickness of the bottom guiding layer (SU − 8 layer) is reduced to the minimum. Some numerical simulations were performed in order to investigate this geometry. For all the WG a slot width of 100nm, a wall width of 300nm and WG thickness of 500nm and 700nm are considered. As expected, the confinement factor inside the slot volume decrease proportionally with the SU − 8 layer thickness (see Fig.3.20 and Fig.3.21), but for thin layer of SU8 (< 100nm) this reduction affects for only few percent the energy into the slot. In this simulation a SU − 8 layer of 5µm width is used. As expected, both energy confinement inside slots and walls decrease as the SU − 8 thickness increases (thickness varied from 40nm to 140nm, bottom x axis). In Fig.3.20 the scatter dataset indicates the values for a strip loaded slot waveguide (SLSWG) centered over the SU − 8 layer, while the dotted lines
Figure 3.20: energy confinement factor inside single and multiples SLSWG versus the $SU - 8$ layer thickness (coloured data). (left) single slot, (center) double slots, (right) triple slots. For comparison the same energy confinement factor for a slot waveguide (not strip loaded) versus the slot asymmetry is sketch with the black solid line. For all the WG a slot width of 100$nm$, a wall width of 300$nm$ and a WG thickness of 500$nm$ were considered. Red data: slot values, green data: walls values, blue data: $SU - 8$ layer.

Figure 3.21: energy confinement factor inside single and multiples SLSWG versus the $SU - 8$ layer thickness (coloured data). (left) single slot, (center) double slots, (right) triple slots. For comparison the same energy confinement factor for a slot waveguide (not strip loaded) versus the slot asymmetry is sketch with the black solid line. For all the WG a slot width of 100$nm$, a wall width of 300$nm$ and a WG thickness of 700$nm$ were considered. Red data: slot values, green data: walls values, blue data $SU - 8$ layer.

dataset report the values simulated for a SLSWG shifted by 1.5$\mu m$ to respect the center of the $SU - 8$ layer. The confinement factors are identical for both simulated geometries and this validate the use of strip loaded geometry for

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SWG fabrication in LI materials. For comparison the confinement factor of a classical SWG is reported against the misalignment of the slot to respect the center of a symmetric WG (ytop x axis). The difference between the black line at zero nm of shift and the red dataset gives the decrease of the confinement factor due to the presence of the SU – 8 layer. This SU-8 bus waveguide was not realized due to problems related to the porous silicon substrate.

3.7 Conclusion

In this chapter the use of low refractive index materials was discussed, as suitable candidates to fabricate slot waveguides for sensing applications. An investigation on the sensitivity of single and multiple structures was performed. As expected structure operating with the fundamental mode show higher sensitivity respect to the higher order modes. The great advantage in using LI material is that it is possible to realize wider slots without any reduction of the CF (HI material require very narrow slot regions); in this way, if sensing liquids are considered, larger slots facilitate the liquid transportation. LI single and multiple slot structures with CF values similar to the CF obtained for the HI material were individuated. This is of particular interest for practical use of LI materials, such as polymer, for sensoristic application; in fact polymers can be either surface functionalized (processed in order to give the necessary chemical selectivity to the sensor) or easily infiltrated (including active media inside, enabling the realization of complex WG arrays).

Finally, the LI slot waveguides were fabricated, but not yet characterized due to their too small dimensions. Some hypothesis, in order to solve this problem, were investigated; it was consider to realize a strip loaded structure. Also in this case, some calculation in order to validate the possibility to realize this structure with our e-beam lithography were performed, but for the moment it was not yet possible to realize them, due to some problems in realizing the bus waveguides related to the porous silicon substrate.
General conclusion

In this thesis, optical characterization and modeling of photonic structure for sensing application were performed. The experimental work was focused on the analysis of the optical properties of two different devices that can be implemented in lab-on-chip systems: bioreactors (fluorescence-based approach) and micro-resonators (label-free approach). Finally, in the last part of my thesis, an alternative approach to sensing was described which makes use of low index materials. This part of the thesis was focused not only on the modeling and on the characterization of the structures, but also on the fabrication of them, by means of the e-beam lithography. The main intent of this last part of the work was to show that low index materials can rival with performances of high index dielectrics.

In the first part, two different approaches for integrated optical biosensing were analyzed and characterized. First, it was developed and characterized an evanescent field based photonic layer to be used in a lab-on-chip device for biosensing. A growing interest in this field was observed during the last years, due to the increasing demand of lower cost, faster and real time analysis capabilities, more compactness of the systems and minimizing the analyte amount to be handled. A planar lightwave circuit, composed of both channel and vertical taper waveguides was demonstrated in the visible range. The efficiencies of two approaches were calculated and compared: the efficiency of this evanescent field based system resulted to be 5% and the one of a propagating light approach resulted to be lower than 1%. Thus, it was demonstrated the advantage of using an evanescent field based system, in term of sensing efficiency, with respect to a
propagating light approach. The proof of validity of this device was achieved using a macroscopic amount of dye solution (a drop in the bioreactor), but not yet for functionalized bioreactors. This work will continue in the future, in order to validate this device as biosensing of functionalized bioreactors.

In the next part of the work, it was analyzed a micro-resonators based structure for biosensing. Whispering gallery mode biosensors are become of great interest for the high sensitivity that they could achieve (single binding events) and for the possibility of performing label-free analysis (label can structurally and functionally interfere with the assay); this latter peculiarity allows to reach real time analysis without previous preparation of the analyte. Micro-resonators vertically coupled to a bus waveguide working with wavelengths around 800 nm were demonstrated. The proof of validity of using this device for biosensing was achieved in the near-IR region, where it was possible to detect shifts in the resonant peaks position, due to the presence of biomolecules. Moreover, the minimum concentration value of proteins detectable with these microdisk resonators was investigated and it results to be of the order of 1 μM.

The development of a silicon based lab-on-chip system is a part of a large research project (NAOMI) which aims at develop a powerful platform for biological assays using systematic, coordinated and multidisciplinary processes. Thus the realization of each project run require months. This fact reduced the number of samples available for optical characterization and the work will continue in the future, to validate both the device biosensing properties of the functionalized bioreactors and to improve the sensitivity of the micro-disk approach.

In the last part of the work, it was studied and developed a slot waveguide based sensors realized using low index materials, within Gopsi project. Low index materials, such as polymers, offer a number of advantages with respect to high index dielectrics. Not only they show low cost, reproducible and CMOS
compatible fabrication processes, but they have the capabilities to be tailored over a wide range of characteristics. Another important features of low index material is that, after a proper design of the structures, they can rival with performances of high index dielectrics.

In fact, it was shown that it is possible to realize low index device for sensing with sensitivity comparable to the one achievable with high index materials. In addition, it was also shown that, with low index materials, it is possible to realize structures with wider slot regions, without any reduction of the confinement factor (high index materials require very narrow slot regions in order to have high CF values). In this way, if sensing liquids are considered, larger slots facilitate the liquid transportation. This is of particular interest for practical use of LI materials for sensoristic applications. Single and multiple low index slot waveguides were fabricated, by means of the e-beam lithography, but not yet characterized due to their too small dimensions. Some hypothesis, in order to solve this problem, were investigated and the solution seems to be very close.

As a final outcome, this work shows as different kind of biosensing approaches (evanescent-field based sensing using evanescent waveguides or slot waveguides and refractive index based sensing using micro-resonators) could be tailored in order to answer to specific analysis needs and to achieve integrated photonic sensors.
Conclusion
Appendix

Slab waveguide measurements

Samples 9 and 10 do not support any mode at 632.8, 1319 e 1542nm while sample 8a does not allow light coupling with the prism because of the degraded surface. Also sample 7a contains surface cracks, and thought it was possible to couple the light into the waveguide, the propagation losses were quite high (> 20dB/cm). Waveguides 9a snd 10a have lower index and also lower losses. Absorption losses in $SiN_x$ or $SiON$ with large refractive index (close to 2) are generally due to $Si − Si$ bonds. Increasing the ratio of $N_2O$ with respect to $SiH_4$ during the deposition results in a lower refractive index, but also in a significant reduction of the material absorption losses (because of the reduction of $Si − Si$ bonds). Thicker samples (sample 8 vs 7, and 10a vs 9a) have about half of the losses of the thinner ones, probably because the modes are more confined and less affected by surface scattering losses. It is worth noticing that in waveguide 10a the propagation losses are rather low and that the value of 1.2dB/cm is very close to the project target value.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness [µm]</th>
<th>632.8nm</th>
<th>1319nm</th>
<th>1542nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. modes</td>
<td>Refractive Index</td>
<td>Losses [dB/cm]</td>
<td>No. modes</td>
</tr>
<tr>
<td>7</td>
<td>0.3 ± 0.1</td>
<td>1(TE) 1.80 ± 0.01 11.0 ± 0.3</td>
<td>1(TE) 1.6 ± 0.3 0(TM)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.6 ± 0.1</td>
<td>2(TE) 1.80 ± 0.01 7.0 ± 0.3</td>
<td>1(TM) 1.77 ± 0.01 0.7 ± 0.3 2(TM)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NM</td>
<td>0(TE)</td>
<td>NM</td>
<td>0(TE)</td>
</tr>
<tr>
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<td>NM</td>
<td>0(TE)</td>
<td>NM</td>
<td>0(TE)</td>
</tr>
<tr>
<td>7a</td>
<td>0.3 ± 0.1</td>
<td>1(TM) 1.80 ± 0.01 21.0 ± 0.3</td>
<td>1(TM)</td>
<td>NM</td>
</tr>
<tr>
<td>8a</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>9a</td>
<td>0.3 ± 0.1</td>
<td>1(TM) 1.64 ± 0.01 4.0 ± 0.3</td>
<td>0(TM)</td>
<td>NM</td>
</tr>
<tr>
<td>10a</td>
<td>0.6 ± 0.1</td>
<td>1(TM) 1.63 ± 0.01 1.2 ± 0.3</td>
<td>0(TM)</td>
<td>NM</td>
</tr>
</tbody>
</table>

Table 2: thickness, number of modes, film refractive index and propagation losses measured for the slab waveguides from sample 7 to 10 and from sample 7a to 10a. (NM means Not Measurable).
Absorption and emission spectra of Fluka 93662 Fluorescent Red NIR 700

Fluorescent RED NIR 700 is a fluorescent dye for the near infrared area with empirical formula $C_{39}H_{44}N_2O_6S$ and molecular weight 668.84 g/mol. The maximum excitation wavelength is $\lambda_{ex} = 672\text{nm}$ and the emission wavelength is $\lambda_{em} = 735\text{nm}$ in 0.1M phosphate solution $pH$ 7.0. Extremely long wavelength fluorescence is especially well suited for applications where sample autofluorescence might be critical or penetration of tissue or other matrix is required. In Fig.23 are reported the emission and the excitation spectra for different concentration of the solution. From the datasheet of the DYE was expect the following properties: $\lambda_{ex} = 670\text{nm}$ $\lambda_{em} = 735\text{nm}$ in 0.1M phosphate $pH$ 7.0. Instead, using a commercial spectrofluorimeter (Varian Cary Eclipse), was found an $\lambda_{ex} = 700\text{nm}$ and an $\lambda_{em}$ dependent from the concentration value (that can arrive until 780nm). At the wavelength of 670nm the DYE is excite not at the maximum absorption value, but in a region with about half of the efficiency; but this is the laser line closer to the maximum of the excitation wavelength of the dye solution available at the NL lab.
Figure 23: fluorescence spectra of RED NIR 700 in 0.1M phosphate \( pH \) 7.0 as a function of dye concentration. All spectra have been acquired with a commercial fluorescence spectrometer Cary Eclipse by Varian.

Amino-methyl-fluorescein

4′ – (aminomethyl)fluorescein is is a fluorescent dye for the near infrared area with empirical formula \( C_{21}H_{16}ClNO_5 \) and molecular weight 397.8141. Carboxylic acids of proteins and other water-soluble biopolymers can be coupled to this molecule in aqueous solution using water-soluble carbodiimides such as EDAC (E2247). In Fig.24 is shown the chemical structure of the amino-methyl-fluorescein.

Figure 24: chemical structure of 4′ – (aminomethyl)fluorescein.
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