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Design and process development of a photonic crystal polymer biosensor for point of care diagnostics

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ABSTRACT

In this work, we report advances in the fabrication and anticipated performance of a polymer biosensor photonic chip developed in the European Union project P3SENS (FP7-ICT4-248304). Due to the low cost requirements of point-of-care applications, the photonic chip is fabricated from nanocomposite polymeric materials, using highly scalable nanoimprint-lithography (NIL). A suitable microfluidic structure transporting the analyte solutions to the sensor area is also fabricated in polymer and adequately bonded to the photonic chip.

We first discuss the design and the simulated performance of a high-Q resonant cavity photonic crystal sensor made of a high refractive index polyimide core waveguide on a low index polymer cladding. We then report the advances in doped and undoped polymer thin film processing and characterization for fabricating the photonic sensor chip. Finally the development of the microfluidic chip is presented in details, including the characterisation of the fluidic behaviour, the technological and material aspects of the 3D polymer structuring and the stable adhesion strategies for bonding the fluidic and the photonic chips, with regards to the constraints imposed by the bioreceptors supposedly already present on the sensors.

Keywords: Lab-on-a-chip, photonic crystal, biosensor, point-of-care diagnostics, polymer, polyimide, nanocomposites, nanoimprint lithography.

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1. INTRODUCTION

Label-free Immunoassay detection devices are needed as diagnostic tools in emergency medicine, whether required in an ambulance, emergency room or as part of a rapid near-patient laboratory test in a hospital environment. The ability to perform complex highly accurate biomolecular tests without the need to send patient samples to a central laboratory is highly desirable, allowing diagnosis to be rapidly achieved and to correctly identify the treatment program for the specific patient and their condition.

In Vitro Diagnostics (IVD) are conventionally associated with centralized laboratories utilizing large, complex, and often labour-intensive analytical equipment. However, the highly parallel nature of such tests has allowed cost effective application of very sensitive biomolecular analysis to clinical healthcare. Due to their relatively long test-request-to-result time such diagnostic tests have historically been used to support the 'physical diagnosis' arrived at independently by the physician and to monitor treatment, rather than directly inform the diagnosis. The emergence of rapid, miniature and easy-to-use IVD tests has seen analysis move closer to the patient, which in the correct circumstances can directly inform diagnosis and hence affect the clinical outcome. In particular, point-of-care testing has seen significant uptake in emergency medicine, where the use of some tests can directly impact key points in decision making concerning triage, diagnosis or disposition.

Due to the low cost requirements of point-of-care applications, the photonic chip is fabricated from nano-composite polymeric materials, using highly scalable nano-imprint-lithography (NIL). A suitable microfluidic structure transporting the analyte solutions to the sensor area is also fabricated in polymer and adequately bonded to the photonic chip. The general concept of the biophotonic sensor is illustrated in Figure 1.

Figure 1: General concept of the biophotonic chip. (1) The photonic structures are imprinted on a polymer layer by NIL. (2) The bioreceptors (ligands) are spotted on the 2D array of photonic transducers. (3) A microfluidic chip is bonded to the photonic chip. (4) The biochip is interrogated by an optical reader at point-of-care for stroke patient diagnosis.

In this work, we report advances in the fabrication and anticipated performance of a polymer biosensor photonic chip developed in the P3SENS project (see www.p3sens-project.eu). We first discuss the design and the simulated performance of a high Q-factor resonant cavity photonic crystal sensor made of a high refractive index polyimide core

waveguide on a low index polymer cladding. This design is the starting point providing the specifications for the material processing. We then report the advances in fabrication and characterization of doped (nanocomposite) and undoped (pure) polymer thin film by nano imprint lithography (NIL). Finally the development of the microfluidic chip is presented in details, including the characterisation of the fluidic behaviour, the technological and material aspects of the 3D polymer structuring and the stable adhesion strategies for bonding the fluidic and the photonic chips, with regards to the constraints imposed by the bioreceptors supposedly already present on the sensors.

2. BIOMEDICAL APPLICATION

P3SENS targets the development of a novel multi-analyte biochip assay from blood or serum/plasma that allows for point-of-care diagnosis and prognosis for the enhanced clinical management of stroke.

Vascular cerebral accident or stroke is caused either by the occlusion (ischemic stroke, about 80% of the cases) or rupture (hemorrhagic stroke, 20% of the cases) of a large blood vessel in the brain reducing blood supply to the affected brain tissue, which can lead to hypoxia and irreversible tissue damage. Stroke is the third largest cause of death in industrialised countries, after ischaemic heart disease and cancer and it is the most common cause of adult disability. Diagnosis and treatment of stroke currently rely on careful history taking, examination and investigation of symptoms (lack of awareness, impediment of speech, vision or motion, altered reflexes, headaches). This is normally performed either out-of-the hospital or at the emergency department and can take a significant amount of time to complete. Pre-hospital wrong-diagnosis rate can exceed 30% reducing the effectiveness of overall stroke care and in particular the rapid access to thrombolytic therapy. It is well known that tissue plasminogen activator (t-PA) treatment thrombolysis is not without risk of inducing iatrogenic haemorrhage and this risk currently restricts the time of administration of t-PA to within 3-6 hours after the onset of symptoms. Given the lack of obvious signs of ischaemic stroke on a computed tomography scan (CT-scan) and the low availability of magnetic resonance imaging (MRI) a decision to treat with thrombolytic therapy must currently be based on a 'best guess' diagnosis of stroke. Furthermore, appropriate management of stroke patients also requires incorporation of patient outcome as soon as possible in the process. Indeed, a better and early knowledge of the presumed neurological course may help to improve management with respect to therapeutic options, stroke unit allocation, rehabilitation and support to family and relatives.

As part of clinical diagnosis of a disease in a patient, it is common to measure the concentration of a protein, called a biomarker, in a biological sample - in order to diagnose the disease^[1]. Examples of such diagnostic tests include Troponin-I for detecting acute myocardial infarction and Prostate-specific antigen for the screening of prostate cancer. In stroke, there are no single IVD biomarkers that have clearly demonstrated value for diagnosis^[2] and prognosis^[3] of stroke. Therefore - because no single marker has been found so far, that, by itself has enough power to distinguish stroke patients from mimics or controls - the use of panels of candidate markers could be a powerful alternative.

In this context, there is an unmet need for quick, easy and reliable blood tests based on a panel of biomarkers. Consequently, six well-known cerebrovascular associated analytes (S100b, H-FABP, NSE, Troponin I, BNP and CRP) were selected according to the literature for further immunoassay development and implementation on the P3SENS biochip as a multi-analyte panel.

3. SENSOR CHIP DESIGN

In order to obtain the highest possible surface sensitivity, the refractive index contrast of core and cladding materials should be maximized (to limit the evanescent field extension from the surface) and the waveguide thickness should be minimized (to increase the field proportion outside the core). At the same time, the absorption and scattering in both core and cladding materials should be minimized so as to obtain the highest resonator Q-factor. The polymer layers must further be stackable, with good adhesion and good material compatibility to avoid material degradation, unwanted chemical interactions and leakage of fluids. Layer thickness uniformity should be better than 10%. The core layer patterning process (NIL) must be able to produce features as small as 200 nm with aspect ratios up to 5:1 and with good optical quality surfaces.

3.1 The material system

The optical layer stack to be constructed (of which the processing will be described in Sec. 4) consists of a core layer that is surrounded by bottom and top cladding layers (Figure 2). Polyimide (P84TM, $n = 1.64@1310\text{nm}$) was chosen as the

base material for the core due to its relatively high refractive index. Doping polyimide (PI) with nanoparticles makes it possible to further increase the refractive index to obtain a nanocomposite High Refractive Index (HRI) material with n up to 1.74@1310nm. For the bottom and top claddings two commercially available materials were tested, namely polymethyl-methacrylate (PMMA, $n = 1.48$) and CytopTM. The latter is a Low Refractive Index (LRI) material with a refractive index close to water (1.33). So far, silicon is being used as the mechanical substrate because of its good availability, reliability, smoothness and clean-room-compatibility. In the final device silicon could be replaced by a suitable polymer material. A summary of the three material combinations studied in this work is given on the right of Figure 2.

Figure 2: The photonic material stack using a HRI core (P84TM) and a PMMA or LRI polymer cladding (CytopTM). The material systems L, M, H stands for low, medium and high index contrast respectively.

3.2 The optical transducer design goals and fabrication trade-off

The proposed sensor design targets a high sensitivity to molecular binding that occurs at the surface of the core waveguide [$S \sim 0.5 \text{ pm}/(\text{pg}/\text{mm}^2)$] and a high Q-factor [$Q \sim 10,000$] for optimal resonant wavelength reading precision [$\sim 1 \text{ pm}$] in the optical interrogation instrument. The surface sensitivity is primarily a function of the core waveguide height and index contrast between the core and cladding materials that constitute the waveguide and is therefore highly dependent on the material choice and deposition depth constraints. The calculated dimensions (waveguide width and height) that maximize the sensitivity of the three different material systems are shown in Figure 3 and compared with reference SiN/SiO₂^[4] and Si/SiO₂^[5] waveguides. Very compact micro-cavity resonators based on 1D photonic crystal principles have also been demonstrated in Si/SiO₂^[6]. The sensitivity was calculated using a finite-element mode-solver, by considering a 30 nm adlayer with a refractive index 6×10^{-3} larger than that of the water buffer solution, corresponding to a mass density of $\sim 1 \text{ ng}/\text{mm}^2$ according to de Feijter's formula^[7]. For instance, an undoped PI waveguide on LRI has its optimum sensitivity value at $0.30 \text{ pm}/(\text{pg}/\text{mm}^2)$ for a cross-section of $w=0.7 \text{ μm}$ and $h=0.5 \text{ μm}$. This sensitivity is about 4 to 5 times less than a state-of-the-art SiN or Si waveguide. However, it is practically not possible to work at the sensitivity optimum because the waveguide is now very close to the cut-off wavelength and becomes leaky - resulting in large bending losses and a lower quality factor resonator. It is thus preferable to design slightly thicker waveguides at the expense of a slightly lower sensitivity.

Furthermore, fabrication constraints have to be taken into account. The main challenge originates from the combined need to deposit a thin enough polymer core layer (400-1000 nm) with suitable optical grade (thickness uniformity and smoothness) and to imprint it deeply enough to achieve the requested waveguide and transducer structures. Using rib waveguides instead of strip waveguides would help to decrease the imprinting depth while using a relatively thick core layer, but makes the control of the imprinting depth more challenging. As a consequence, the optical waveguide and transducer design presented hereafter relies on a relatively thick and fully imprinted PI layer (900 nm). In order to improve the sensitivity in the future, thinner core layers ($\sim 600 \text{ nm}$) with higher refractive index are developed in parallel to this initial material choice.

Even though the sensitivity is an important figure of merit, the most relevant quantity is the limit of detection (LOD). The $LOD = P/S$, where P [pm] is the precision in reading the wavelength and S [pm/(pg/mm²)] is the sensitivity. The LOD [pg/mm²] is the smallest change that can be observed above the noise level. The LOD can be improved both by increasing S and by decreasing P . For the latter, high-Q factor cavities (narrow line width) facilitate the precision in reading the wavelength, especially when the SNR is low or in the presence of light intensity fluctuations. Regarding the sensitivity, it should be noted that sensors that are more sensitive to surface index variations also tend to be more sensitive to undesirable temperature and bulk index fluctuations, so that an increase in S often results in an increase in P and therefore does not necessarily lead to a decrease in the LOD. On-chip temperature compensation by athermal design and differential measurements wrt. a reference channel have to be considered in order to decrease P . Unfortunately athermal designs are not possible with polymers because both water and polymers have negative thermo-optic coefficients^[8]. Referencing with a nearby channel remains therefore the best approach. It not only helps in reducing the impact of temperature fluctuations, it also reduces the impact of perturbations in the refractive index of the flowing sample/buffer. Effective referencing can be obtain by placing a high density of transducers on the chip (typical distance between transducers < 200 μ m). In conclusion, the design should not only be considered from the point of view of a single sensor, but from a combination of sensors and reading instrument with the objective to decrease the LOD.

Figure 3: Waveguide surface sensitivity for the three material systems L, M and H, compared with a SiN/SiO₂ (ref.^[4]) and a Si/SiO₂ (ref.^[5]). The values indicated with a star (*) correspond to the geometry maximizing the sensitivity for the considered material system. The other values correspond to this paper design.

3.3 Transducer design

First of all, it should be noted that a full TE band gap 2D photonic crystal (PhC) in water solution cannot be realized with the chosen materials. It would indeed require a refractive index contrast^[9] $n_{\text{core}}/n_{\text{clad}} > 1.39$, which implies a core index $n_{\text{core}} > 1.85$ when surrounded by water ($n=1.33$), whereas a maximum of $n=1.74$ is achievable with polyimide (Sect. 5). In the absence of a full band gap, only a leaky 2D PhC is possible and one cannot have a band-gap guided mode. A leaky waveguide PhC implies a low resonant mode life-time (i.e. a low Q factor value), resulting in a less precise resonant wavelength measurement, a decreased multiplexing capability and the need for a broader acquisition spectrum.

However, this insufficient refractive index contrast for a 2D PhC can be bypassed by using a combination of Total Internal Reflection (TIR) confinement and 1D photonic bandgap confinement. In this case, the light is confined by the 1D PhC in the direction of the light propagation (Bragg mirror), and by TIR in the other directions (vertical and lateral).

Since the sensor cavities have to be multiplexed in wavelength, it is important that each cavity supports only a few modes - and preferably only a single longitudinal mode - in order to avoid resonant peak overlap. The proposed polymer sensor is therefore based on a Fabry-Perot cavity with highly reflective 1D photonic crystal Bragg mirrors (Figure 4).

The Bragg reflectivity calculated by 3D FDTD reaches 98% and is larger than 90% over a 6 nm bandwidth (Figure 5, left). The Fabry-Perot cavity formed by the two Bragg mirrors leads to 3-4 sharp resonant peaks as calculated by the transfer matrix method (the FDTD method was not used here due to calculation time limitations). This method neglects scattering losses and slightly overestimates the sensor bandwidth so that in practice only one or two peaks should survive around the maximum of reflectivity.

Figure 4: Geometry of the side coupled cavity transducer; width = 1.6 μm ; height = 0.9 μm ; period = 0.427 μm ; duty cycle = 0.5; cavity length = 80 μm ; bragg length = 100 μm ; bragg modulation 0.22 μm ; coupling gap: 0.7-1.4 μm .

A fast harmonic analysis (FHA) of the cavity energy decay makes it possible to calculate a cavity intrinsic Q-factor of $Q_0=22,000$, neglecting side coupling and intrinsic material losses. The total Q-factor (Q), accounting for material intrinsic absorption and side coupling losses can however easily be calculated - since^[10] $Q=1/(1/Q_0+1/Q_a+1/Q_c)$, where Q_0 is the cavity intrinsic Q-factor as calculated by FDTD, $Q_a=1/(2(1-f)k_w/n_w+2fk_p/n_p)$ is the Q-factor associated with intrinsic water and polymer absorption (where f is the mode filling factor, $k_w=11 \times 10^{-5}$ and $k_p=10^{-5}$ are the water and polymer extinction coefficients respectively, and $n_w=1.33$, $n_p=1.64$ are their refractive indices)^[11], and Q_c is the Q-factor associated with the waveguide to cavity coupling, which depends on the distance, g , between them. This point is illustrated in Figure 5 (right), where the Q factor is shown as a function of Q_c/Q_0 , the inverse of the bus-to-cavity decay rate normalized to Q_0 . When $Q_c=Q_0$, for instance, an acceptable peak throughput attenuation of $\sim 35\%$ and total Q factor of ~ 7500 are obtained. These results show that the Q-factor of a sensor made out of polymer can compete with Si/SiO₂ or SiN/SiO₂ resonant cavities^[4], at the expense of somewhat larger dimensions. Indeed, the cavity length is here 80 μm in length, while an equivalent Si or SiN cavity could be 10 times smaller. It should however be mentioned that there is no real need to make the sensor so small since the limit is actually imposed by the size of the spotted bioreceptor area (typically $100 \times 100 \mu\text{m}^2$) - and too small a sensor area also gives a non-statistical response during the bioassay. Such a resonant peak configuration should make it possible to detect a central wavelength variation of 1 pm or even better.

Figure 5: (left) Bragg mirror transmission and reflection spectra calculated by 3D FDTD, and cavity reflection spectrum by the coupled mode theory and the transfer matrix method. The cavity transmission is not shown for the sake of clarity (it is the complement of the reflection). (right) Cavity quality factors and throughput attenuation as explained in the text.

Considering the previously calculated sensitivity of $0.129 \text{ pm}/(\text{pg}/\text{mm}^2)$ in material system M, a LOD of $\sim 8 \text{ pg}/\text{mm}^2$ can be expected, which is similar to that for optical waveguide light mode spectroscopy (OWLS)^[12], but is lower than state-of-the-art label-free techniques based on ring resonators (for instance values given in ref.^[4]).

3.4 Microfluidic design

The manipulation and preparation of biological samples (e.g., serum or blood) are critical features of the micro-analytical systems since dilution, mixing, transport, positioning and separation of the relevant components of the analyte solution are far from evident in micro-fluidic channels. Before the sample reaches the sensing area of the chip several preparative steps must be carried out. One has to consider the need for bubble-free sample collection, dilution with buffer solution, complete mixing to ensure reproducible sample concentration, washing the waste, etc. The sample transportation can be provided by passive or active pumping, and additional sample preparation functions could be integrated, like plasma separation from whole blood for instance. In a lab-on-a-chip analytical system these functions are integrated on a single chip and realised by passive or active microfabricated components^[13].

In our case the designed structures are intended to manage six different sample transport channels, with the same transport length between the inlets and the sensing areas ensuring reproducible sensing conditions in each channel. The structure integrates washing and three different mixing functions. The adequate mixing of the sample solution with the buffer is absolutely important to ensure evolution of a homogenous concentration distribution in the lateral cross-section of the fluidic channel to exploit all the active surface of the sensing area. Figure 6 clearly represents the improper concentration distribution of a model protein (fluorescent human serum albumin) in a phosphate buffer solution in a simple connecting channel structure (T-mixer). Note, that the mixing is very slow in microchannels due to the laminar flow condition and low Reynolds-number.

Figure 6: The concentration distribution of fluorescent serum albumin (HSA) in a simple T-mixer structure.

Several possible mixing strategies can be found in macroscale, such as molecular diffusion, turbulent diffusion, advection and Taylor-dispersion. In contrast, in the microscale, the flows are usually laminar and the component streams mix only by diffusion, creating a dynamic diffusive interface with predictable geometry (Figure 6). Turbulent flow cannot be built up in microscale due to the dominant viscosity - so in this case the mixing possibilities are limited^{[14],[15]}. The mixing condition could be improved by integration of advanced mixer structures into the microfluidic system - and in this case the chaotic advection could be an ideal mixing method considering stable and laminar flow also.

The mixing behaviour of different microfluidic structures was analysed by numerical modeling using COMSOL Multiphysics to determine the possibly achievable mixing efficiencies. For reference a simple T-mixer structure was implemented with rectangular cross-section and Herring-Bone type mixer and T-mixer staggered blocks were characterized as possible chaotic mixers. The channel width was chosen to 50 μm , the depth was determined by the SU-8 technology (according to the 20 μm layer thickness of the SU-8 2015 photoresist). For the calculations the parameters of the $0.1 \text{ mM}/\text{mL}$ human serum albumin solution and $1 \text{ μL}/\text{min}$ flow rate were applied as initial boundary conditions (for each model).

According to the model calculations the Herring-Bone type and the T-mixer staggered blocks presented highest mixing efficiency as concluded in Figure 7. The surfaces in this diagram show the difference between the evolved and the ideally mixed concentration distribution on the outlet surface considering 1 mm channel length in case of the different structures. It was clear that the two chaotic mixers are the most applicable structures and accordingly these were integrated in the transport fluidics to be realized.

According to the preliminary expectations considering the sample transport, the adequate mixing condition and washing possibilities the fluidic layouts were designed consisting different advanced chaotic mixer systems as presented in Figure 8.

Figure 7: Comparison of the modeled mixing efficiencies of the different mixer structures demonstrated by the square of the concentration deviation from the average value calculated on the outlet surface.

Figure 8: The implemented design of the proposed transport fluidic system consisting T-mixer (a), T-mixer staggered blocks (b) and Herring-Bone type mixer (c), respectively.

4. CONSTRUCTION OF THE OPTICAL LAYER STACK

The material system used for the fabrication of the photonic chip has been described in Sec. 3.2. Two methods for the application of the thin polymer films have been used namely spin-coating and doctor-blading. The optimum choice depends on the type of polymer and on the type of substrate.

A three-step approach has been considered in the development of the optical layer stack:

1. Preparation of a layer stack consisting of Si/PMMA/PI (System L).
2. Replacement of PMMA with low refractive index material: Si/LRI polymer/PI (System M).
3. Using doped PI as the core material: Si/LRI polymer/HRI polymer (System H).

The layer stack developed in step 1 will be used to implement a more conventional sensor structure with limited sensitivity due to its low index contrast. Increasing the index contrast in step 2 will improve the sensitivity of the conventional sensor as is discussed in section 3.2. Finally, step 3 aims for polymer waveguide sensors that have the highest index contrast and thus largest sensitivity. The results obtained so far in the construction of the optical layer stack are described next, starting from the bottom layer.

PMMA with a refractive index of approximately 1.48 was used as the initial bottom cladding option. When PMMA was diluted with toluene to about 10 w-%, the target thickness of at least 3 μm was achieved with both spin coating and doctor-blading. Depending on the baking conditions the PMMA film exhibited a refractive index of $n=1.48\text{--}1.49$. However deposited PMMA films showed a relatively high waviness of about ± 150 nm over millimeter-scale for both deposition methods.

Ideally the refractive index of the bottom cladding layer should be close to that of water (~ 1.33) or even smaller as described in the previous sections. Partially fluorinated materials are an option to achieve such low refractive index, either as pure coating material or in the form of nanoparticles in a composite material. Films with an appropriate thickness of a few micrometers and a refractive index of 1.33, or even lower, were successfully prepared using commercially available materials.

Therefore, PMMA was replaced by Cytop (AGC Chemicals), a commercially available amorphous fluoropolymer, for which we measured $n = 1.33$ and better uniformity and smoothness than for PMMA. Due to its low viscosity the best results are obtained by stacking multiple thin layers instead of a single μm -thick layer. Both PMMA and Cytop films could be deposited in a reproducible way.

Partially fluorinated polymers like Cytop typically exhibit a very high capillary contact angle, e.g. well above 90° for water. Therefore a suitable pre-treatment was developed for Cytop. The Cytop surface was treated by an argon plasma to increase the wettability and to ensure homogeneous coating of the core layer (PI) on top of it. Figure 9 shows the significant reduction of the contact angle from around 69° down to 12° with increasing pre-treatment time which finally allows wetting of the HRI coating solution on top of the Cytop film. The same treatment that aimed to improve the wetting of the fluorinated materials may prove essential in a sensor working on capillary action. Due to its higher polarity, PMMA was observed not to require such pre-treatments.

Commercially available PI (P84TM) was used as the first core material option. Figure 10 shows an approximately 0.5 μm thick film of this undoped polyimide on a silicon wafer. Thickness uniformity, surface smoothness and homogeneity of these films are very good. The material exhibits a refractive index of approximately 1.64 and an extinction coefficient of about 10^{-5} .

To obtain the photonic chip structure, the PI has to be deposited on top of the bottom cladding layer. In this work, thin layers of undoped PI were successfully applied on top of both PMMA and Cytop layers. Using spin coating on top of PMMA the polyimide film achieved the required thickness range (350–1000 nm) and smoothness with an approximately 8 wt% coating solution and a spinning speed of 1000–2000 rpm. With the argon plasma treatment discussed above an approximately 1 μm thick polyimide film could also be spin-coated on top of Cytop.

Figure 9: Decrease of contact angle of the HRI coating solution on Cytop (bottom cladding layer) after suitable pretreatment with argon plasma.

Figure 10: TEM image of a pure polyimide (PI) film of approximately 0.5 μm thickness on top of a silicon wafer.

To increase the refractive index one can incorporate high refractive index nanoparticles, such as metal oxides, into the core layer^{[16],[17],[18],[19]}. Since the thickness of the core layer has to be on a scale of hundreds of nanometres, the nanoparticles must be much smaller than these characteristic dimensions to obtain a uniform material with acceptable low scattering for the optical application. Several metal oxide materials like titanium dioxide and cerium dioxide with characteristically high refractive indices have been tested to increase significantly the overall refractive index of the core layer. Figure 11 shows the increase of the refractive index as a function of the amount of incorporated nanoparticles in polyimide. Around 25 wt% of particles which equals approximately 10 vol%, are necessary to obtain a refractive index of 1.7. The nanocomposite material has sufficient low coagulation of particulates and low enough absorption. Increasing the amount of particles leads to a further increase of the refractive index but also to a strong increase of the extinction coefficient which is not tolerable anymore.

Figure 11: Refractive index (left) and extinction coefficient (right) of the polyimide layer versus the amount of incorporated TiO_2 nanoparticles at a wavelength of 1.3 μm . Light grey data points on the extinction coefficient plot are below the detection limit of the instrument.

The roughness of the films increases with increasing amount of nanoparticles. The TEM image in Figure 12 shows a selected small section with a very good quality of a PI nanocomposite film on silicon. The nanoparticles can be recognized as the dark spots in the PI film. Nevertheless, the deposition of doped PI as a smooth and homogeneous sub- μm film on top of the bottom cladding layer is quite challenging and requires further process development.

As a final step in the construction of the optical layer stack, one has to deposit the top cladding on top of the core layer. Furthermore, a window above the transducers has to be opened into the top cladding, and the microfluidic layers have to be bonded on top of it. In this work, both PMMA and Cytop were successfully applied on top of PI with good adhesion.

Figure 12: TEM image of a ~ 300 nm thick PI nanocomposite film doped with TiO_2 nanoparticles on top of a silicon wafer.

5. STAMPING TOOL FABRICATION

To form the polymer waveguides and the optical sensors one has to pattern the core layer with nm-resolution and good repeatability. In this work NIL was chosen as the preferred patterning method because of its ultra-high resolution (down to 10 nm features) and relatively high throughput, especially compared to electron-beam (EBL) or focused ion-beam (FIB) lithography. The results obtained with NIL can also be later transferred into molding and roll-to-roll patterning, which offer even higher throughput when the production volumes increase.

The NIL stamps were defined using EBL which allows relatively rapid pattern definition at submicron feature size over large areas ($\sim 20 \text{ mm}^2$) on silicon. The resist used is hydrogen silsesquioxane (HSQ), a high-resolution negative inorganic e-beam resist (Figure 13). The etching of the silicon to the required depth is achieved using inductively coupled plasma in sulphur hexafluoride and perfluorocyclobutane ($\text{SF}_6/\text{C}_4\text{F}_8$) atmosphere using the HSQ pattern as an etch mask. This produces the required stamp profile for nanoimprinting the waveguide features into polymer (Figure 14).

Figure 13: (left) SEM image of ~ 250 nm thick patterned HSQ; (centre) original Bragg mirror and bus-bar design; (right) detail

Figure 14: (left) Bragg mirror and bus-bar in the fabricated NIL stamp (after silicon etching); (centre) waveguide stamp with structure height of 900nm; (right) Microscope image of an array of waveguides patterned into polymer with a NIL stamp (3 μm wide grooves separated by a 4 μm trench).

First tests carried out in this work (Figure 14) suggest that the fabricated stamps can be used to pattern undoped polyimide. Imprinting polyimide that has been doped with TiO_2 nanoparticles has also been done but to a smaller depth. Hard particles make the application of NIL somewhat more challenging when compared to the undoped material.

Figure 15 shows a schematic illustration of NIL patterning, and an AFM profile scan of the imprinted 1.2 μm thick sample. The latter shows one of the basic properties of NIL obtained with a 1070 nm deep NIL test stamp. The stamp did not penetrate fully into the polyimide layer, but the resulting polymer waveguide ridge still has the full height of the stamp feature because of material flow in the vicinity of the stamp features. The material flow occurs in the imprinted core layer, but also in the bottom cladding layer. The uplift of the unpatterned surface during the NIL process originates from this material flow and decreases as a function of the distance from the stamp features. Bank formation at the edges of the stamp features can affect the design of the photonic device, but it also allows the hot embossing characteristics to be employed in such a way that only a portion of the required depth needs to be imprinted, while the uplift finalizes the required feature height.

Figure 15: (left) Schematic cross-section of the NIL stamp and the resulting structure with nonidealities emphasised for better visualization; (right) AFM scan profile of the patterned polymer (waveguide) features showing the banks formed around the trenches.

In NIL, being a multiparametric experimental method, optimization is required for each material; viscosity, reflow temperature and the presence of underlying layers are major factors. Also, to keep the NIL stamp working long enough for high throughput requirements, the quality of anti-stiction coating on the stamp becomes important. Even more so, when high aspect ratio and fine features need to be applied. Often, residual gas in the film(s) may release and be trapped between the films creating undesirable gas pockets. Radial depth dependence, as well as mislevelling on the nanometer scale, is easily encountered - dictating that it is essential to allow for some non-ideality in the features.

6. MICROFLUIDIC PROCESSING

Conventionally applied materials in polymer micromachining are the polydimethylsiloxan (PDMS) and the SU-8 negative photoresist. These materials have excellent structural, mechanical and biological behaviour making them convenient for the application in microfluidics.

The PDMS is a silicon based organic polymer: $(\text{H}_3\text{C})_3[\text{Si}(\text{CH}_3)_2\text{O}]_n\text{Si}(\text{CH}_3)_3$ which is absolutely feasible to form microfluidic structures in bioanalytical applications due to its reliable geometric transfer, flexibility, transparency, biocompatibility and price. It could support both the large scale production and fast prototyping. In addition, it can be applied for fabricating optical devices too^[20]. Disadvantages are the hydrophobic character (adsorption of hydrophobic ligands) and the possible long term degradation. PDMS has absolutely simple formation method by moulding, polymerizing on a pre-structured moulding form and peeling from. The moulding form could be a photoresist structure that makes use of the SU-8 thick negative photoresist material.

The SU-8 epoxy based photoresist is particularly applied both as structural material of a fluidic system or as moulding replica for PDMS replication. An improved 3D multilayer formation process was developed in order to achieve reliable SU-8 structuring for the formation of high aspect ratio sidewalls and advanced functional elements (chaotic mixers) of the microfluidic system as described in Figure 16.

Figure 16: Multilayer SU-8 process for fabrication complex 3D polymer structures.

To create microfluidic channels in different polymer materials (SU-8 and PDMS) two variations of the realisation processes were developed. Both of them apply the described (Figure 16) multilayer SU-8 technology to form the moulding form for PDMS or directly the microfluidic structure. The different layers contain the different functional components of the microfluidic system as demonstrated in Table 1. The polymer based microfluidic system and its main parts are represented in Figure 17.

	1 st SU-8 layer	2 nd SU-8 layer	3 rd SU-8 layer
PDMS moulding form	fluidic channels with reservoirs and T-mixers	fluidic inlets and Herring-Bone type mixer structure	—
SU-8 microfluidics	fluidic inlets from the backside	fluidic inlets and Herring-Bone type mixer structure	fluidic channels with reservoirs and T-mixers

Table 1: The different SU-8 layers are implemented to form the substantial functional components of the microfluidic system

Both the PDMS and the SU-8 materials are transparent, making parallel microscopic investigations possible, and therefore, both materials are well suited for microfluidic applications once leakage-free bonding of these materials to the underlying optical structures has been realized. Bonding has already been demonstrated between two types of tested PI and SU-8 samples at temperatures exceeding the imidization threshold.

Figure 17: The implemented microchannel connected to the outlet reservoir tailored in SU-8 photoresist material (SEM view: a) and the fabricated PDMS fluidic chip (b) molded on SU-8 replica.

To obtain a stable and high adhesion, several different strategies have been followed: (i) plasma oxidation of the surfaces using a low temperature oxygen plasma, especially suited for bonding PDMS to oxides or polymers. (ii) use of an adhesive polymer buffer layer that can be spin-coated, rolled or stamped over the fluidic structures, and (iii) low temperature thermocompression bonding. The possible bonding strategies applicable for the materials used for the fabrication microfluidic and optical structures are summarised in Table 2. Note that the low processing temperature is a crucial requirement to ensure the technological compatibility with the biological application, with regard to the pre-immobilized bioreceptors. The bonding strength and the sustainability of overpressure are under investigation.

PDMS microfluidics	Possible bonding strategies under investigation
Top cladding layer of the optical chip:	
Polyimide	▪ Silanisation (applying organofunctional alkoxy silanes as (3-aminopropyl)-triethoxysilane (APTES)) ^[21] and
PMMA	
SU-8	▪ Low temperature oxygen plasma activation
CYTOP	
SU-8 microfluidics	Possible bonding strategies under investigation
Top cladding layer of the optical chip:	
Polyimide	▪ Low temperature compression bonding applying adhesive polymer buffer layer or
PMMA	▪ UV assisted bonding applying UV curable adhesive ^[22]
SU-8	
CYTOP	

Table 2: Possible bonding strategies for the integration and sealing of the fluidic and optical chip.

7. BIOCHEMISTRY

A panel of 6 candidate biomarkers will be used for the diagnosis of stroke. The selected biomarkers (S100b, H-FABP, NSE, Troponin I, BNP and CRP) are validated with respect to their biotinylated antibodies by ELISA and Western blot. The immobilization of the biomarker specific antibodies is based on grafting into a hydrophilic interfacial polymer layer derivatised by neutravidin. The biotinylated antibodies are immobilized through the strong and specific biotin-neutravidin linkage. The build-up of the molecular layers is followed by Optical Waveguide Lightmode Spectroscopy (OWLS)^{[23],[24]}. After the optimization of the parameters of immobilization and analyte detection, the antibodies will be spotted on different channels of the photonic Biochip.

CONCLUSIONS

We have shown by simulation that a resonant cavity sensor made of polyimide on a low index fluorinated polymer is a viable solution, leading to a Q-factor value and a sensitivity as high as 7500 and 0.129 pm/(pg/mm²) respectively. A limit-of-detection of 8 pg/mm² should be attainable.

Optimisation of the nanocomposite polymers for both inherent optical properties and processability via NIL presents a significant challenge. However, promising results have already been achieved in material development, thin film stacking and nanopatterning, as well as in combining these results. The refractive index of sub-micrometer thick polyimide films have been increased from 1.64 to 1.74 with approximately 50 wt% of nanoparticles incorporated into the polymer matrix. One important issue is to reduce the impact of the nanoparticles on the extinction coefficient, which increases with increasing particle concentration. Such high index nanocomposite material can be structured by nano imprint lithography (NIL) with feature sizes down 100 nm.

In order to expose effectively the sensor areas with the analyte solutions, polymer microfluidic networks were integrated on top of the sensor. Transparent SU8 and PDMS materials were applied to form the microfluidic channels utilising a 3D multilayer structuring process. The resulting all-polymer devices have great potential in point-of-care diagnostics applications where cost-effective solutions are critical.

Future work within P3SENS will target the fabrication of fully functional chips biofunctionalized for a panel of six target biomarkers. In stroke, there are no commercially available immunoassay devices that allow point-of-care diagnosis of

ischaemic stroke from blood or serum/plasma. Since no single marker has so far been found, that by itself has enough power to distinguish stroke patients from mimics or controls, panels of candidate markers could be a powerful alternative. Six well-known cerebrovascular associated analytes (S100b, H-FABP, NSE, Troponin I, BNP and CRP) were selected for further immunoassay development and implementation on the P3SENS biochip, as a multi-analyte panel.

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