

Photolithographic local surface modification of integrated evanescent wave based biosensors

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Abstract

Photolithographic local surface modification was performed on mercaptopropyl trimethoxy silane modified Mach-Zehnder interferometric sensors, by using thiol-ene coupling reaction for the selective blocking of non-sensing and reference areas. The reactive thiol groups remaining after the photolithographic step were biotinylated to enable the measurement of chromeon 642-streptavidin. The surface characterization was performed with fluorescence imaging and chromeon 642-streptavidin measurement on Mach-Zehnder interferometric sensors.

Key words: photonic sensors, local surface modification, thiol-ene coupling, streptavidin

Introduction

Evanescent wave photonic sensors are integrated optical waveguide devices which exploit the exponentially decaying evanescent field of guided light that penetrates into the surrounding media. The sensing principle relies on the change of the phase velocity of the guided light induced by the binding of analyte molecules to the functionalized surface (Fig. 1A). This sensing principle enables sensitive label-free real-time monitoring of biomolecular interactions.

In the past, different integrated photonic transducers such as Mach-Zehnder interferometers [1], gratings [2], and ring resonators [3] have been developed.

In our work, we use hydrogenated amorphous silicon based Mach-Zehnder interferometric (a-Si:H-MZI) sensors as model system for integrated evanescent wave sensors (see Fig. 1B). Integrated MZI biosensors comprise a sensing and a reference arm (see Fig. 1B). The sensing arm is modified with receptors, which specifically bind the target biomolecules. This binding induces a local refractive index change at the waveguide surface, which causes a change of the phase velocity of the propagating light. In the MZI, this change of phase velocity is translated into a modulation of the optical output power.

A common strategy to ensure that the reference waveguide arm is not affected by the surface modification is to cover it with a cladding layer (e.g. silicon dioxide, SU-8) a so-called asymmetric MZI. Although, the introduction of a

structured cladding material is easy to realize, it has some disadvantages, such as the need for cancellation strategies during refractive index changes (e.g. during the sample loading) or temperature fluctuations [4]. In order to overcome these disadvantages a local surface modification method is required that allows functionalizing of the reference and sensing arms with similar chemical compounds (a symmetric MZI) that differ in their biomolecular binding properties. For the realization of local surface modification different methods such as spotting techniques, micro contact printing or photolithography can be used. Among these methods photolithography appears most suitable because it avoids surface damages and contaminations of the waveguide layer.

In our study, we investigated photolithographic surface modification by thiol-ene coupling (TEC), which is a radical mediated reaction that leads to bonding between thiols and alkenes in high yields, is feasible in the presence of oxygen [5], and can be performed on different thiolated surfaces with common lithographic procedures [6]. In this work, we use the TEC reaction to fabricate locally modified a-Si:H-MZI sensors by photolithographic blocking of a mercaptopropyl trimethoxy silane (MPTMS) surface layer with diethylene glycol vinyl ether (DEGVE). In a next step, the non-reacted thiol groups on the sensing arm were modified with maleimide-PEG₂-biotin for the measurement of streptavidin. The resulting surfaces were characterized with fluorescence imaging and a-Si:H-MZI measurements with respect to their sensing behavior.

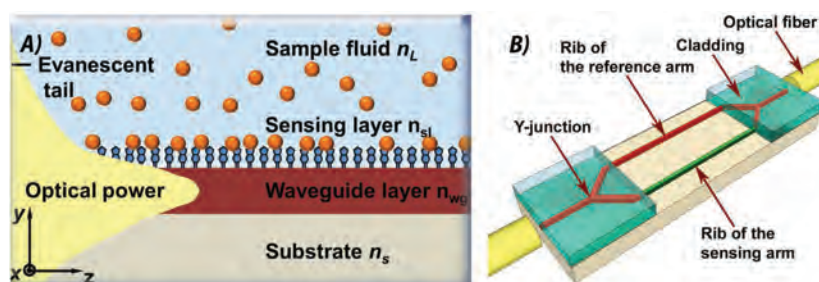


Fig. 1. A) Evanescent wave based sensing principle: binding of biomolecules to sensing layer induces local refractive index change at the surface, which changes the phase velocity of the light propagation in the waveguide; B) MZI structure: reference and sensing arms are in contact with measurement liquid. Local surface modification enables specific binding to the sensing arm.

Materials

(3-Mercaptopropyl)trimethoxysilane (MPTMS), 2,2-dimethoxy-2-phenylacetophenone (DMPA), Tetramethylrhodamine-5-maleimide (TMR5M), diethylene glycol vinyl ether (DEGVE), Dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), chromeon 642-streptavidin and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Vienna, Austria).

Methods

The samples were photolithographically processed in the double side mask aligner EVG620 (365 nm, 600 mJ/cm²) with a chrome photomask with 150 μm structures.

To characterize the surface modification different fluorescence labels were chosen to visualize surface features. Fluorescence scans were performed with the ArrayPro® TECAN fluorescence scanner from Media Cybernetics in Bethesda, USA, with 532 nm (or 633 nm) excitation wavelength and a 575 nm (or 692 nm) emission filter.

For the sensor measurements hydrogenated amorphous silicon based Mach-Zehnder interferometric (a-Si:H-MZI) optical waveguide sensors were operated with TE-polarized laser light of 1310 nm wavelength. The in- and out coupling of light was performed with optical fibers. A Y-junction in the input rib waveguide splits the light propagating into two optical paths - the measurement and the reference arm (Fig. 1B).

In this study we use a) a sensor where the reference arm is covered with a SU-8 cladding (asymmetric MZI), and b) a photolithographically modified sensor, where both, sensing and reference arm, are in contact with the sample liquid (symmetric MZI). Sensor type b) is pictured in Fig. 1B. Also this sensor type is covered with a SU-8 cladding that protects the Y-junctions and the in- and output waveguides. In both cases, the interaction length of the waveguides with the sample liquid is 10 mm. The use of these two sensor types allows a

comparison of the performance of the photolithographic surface modification.

For the measurements a temperature controlled sample holder and an automated fluidic control system was used. All measurements were performed at 20 °C and with a flow rate of 20 μl/min.

Control samples and a-Si:H based MZI sensors fabrication

The control samples consist of hydrogenated amorphous silicon (a-Si:H) films deposited on crystalline silicon wafers covered with 5 μm silicon oxide by means of plasma enhanced chemical vapour deposition (PECVD). The PECVD was performed with 2% silane gas diluted in nitrogen, 0.5 Torr pressure, 10 W power and 250°C for 10 min. The final a-Si:H film thickness was about 80 nm. With these control samples different measurements were carried out to characterize the surfaces at critical stages of the modification procedure.

For the MZI sensor fabrication the above described substrates were cut into 3x3 cm² pieces. Next, conventional optical lithography and reactive ion etching were carried out to pattern the rib waveguide structure. The last step of the MZI sensor fabrication was the creation of a structured cladding layer employing spin-coated SU-8 2002 (MicroChem, Boston, USA), which was soft baked at 95°C (1 min), exposed to UV light at 365 nm (12 s) by using a Chromium-cladding mask, post baked at 95°C (2 min), developed in SU-8 developer (60 s), rinsed in isopropyl alcohol, and finally hard baked at 150°C (15 min).

Surface modification

First, all samples were silanized with MPTMS. For this purpose, the samples were activated with oxygen plasma (3 min, with plasma system Femto from Diener, Stuttgart, Germany) and then silanized by dipping into the silanization solution (5% (v/v) MPTMS in THF with 0.4% (v/v) conc. HCl) for 1 h.

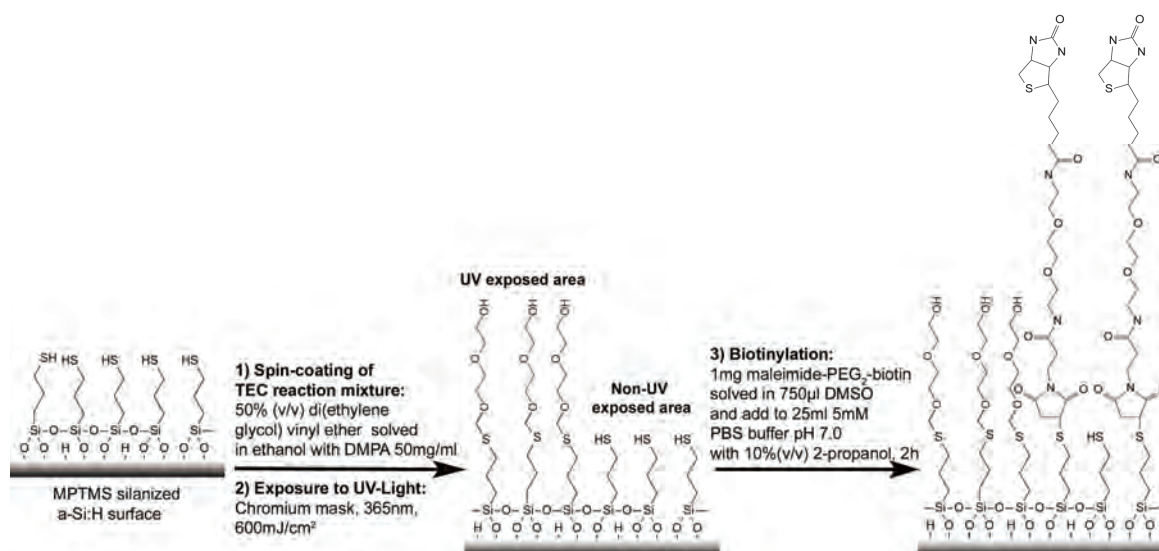


Figure 2. Reaction schema of the surface modification of MPTMS silanized a-Si:H surfaces. The steps include the spin-coating of the TEC reaction mixture, the exposure to UV-light under the use of a structured chromium mask, and the biotinylation of the reactive thiol-groups.

The further surface modification process after silanization is depicted in Fig. 2. It includes spin-coating, UV-light exposure, and biotinylation.

For the photolithographic modification the samples were spin-coated (15 s, 1500 rpm) with the TEC reaction mixture (50mg/ml DMPA, 50%(v/v) DEGVE in EtOH) and exposed to UV-light in the double side mask aligner EVG620 with a dose of 600 mJ/cm² using a chromium-mask with 150 µm wide structures. This led to DEGVE blocked areas (UV exposed areas) and surface areas with reactive thiol groups (non-UV exposed areas). In a further modification step, the reactive thiol groups were biotinylated to enable specific binding of streptavidin to the surface (see Fig. 2).

To control the reactivity of the thiol groups before and after the DEGVE blocking the fluorescence label TMR5M is used. This fluorescence label reacts with the thiol groups through the maleimide moiety, which is also used for the biotinylation reaction with maleimide-PEG₂-biotin.

In order to compare the thiol group reactivity, an MPTMS silanized sample is compared with a sample that is photolithographically blocked with DEGVE. These samples were then modified with TMR5M (100 µl TMR5M of 1 mg/ml stock solution in methanol was added to 10 ml 5 mM PBS buffer pH 7.0 with 10%(v/v) 2-propanol, 30 min). The comparison of the fluorescence intensities reveals similar values of 1193 ± 305 a.u. and 1054 ± 89 a.u. for the samples with and without DEGVE blocking, respectively. Figure 3A shows the fluorescence scan with distinctive surface features of DEGVE blocked (dark) and TMR5M modified (green) surface regions. In addition to the thiol group

activity, specific binding of streptavidin was visualized on a-Si:H control samples. For this purpose, again samples with and without photolithographic blocking step were processed and biotinylated as described above. In addition, the control surfaces were treated with BSA to block unspecific binding sites in the biotinylated surface areas (0.1 mg BSA in 15 mM PBS buffer, 10 min). Then the control samples were dipped in chromeon 642-streptavidin solution (160 nM in 15 mM PBS pH 7.2) for 30 minutes. After washing in PBS buffer fluorescence scans were performed. The fluorescence intensities have similar values of 1431 ± 81 a.u. and 1514 ± 336 a.u. for the samples with and without DEGVE blocking, respectively.

These results indicate a similar binding behavior of the fluorescence labeled chromeon 642-streptavidin to the a-Si:H control samples independent of the photolithographic blocking step. In Fig. 3B, sharp surface features of the DEGVE photolithographically blocked control sample with the immobilized chromeon 642-streptavidin are clearly visible.

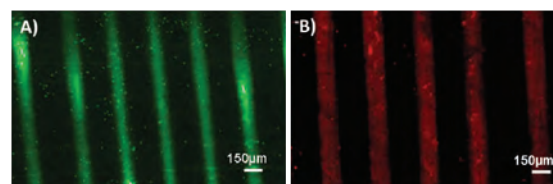


Figure 3: A) Photolithographic blocking of MPTMS silanized a-Si:H surface with DEGVE and modification of the free thiol groups with Tetramethylrhodamine 5-maleimide (TMR5M); and B) maleimide-PEG₂-biotin and chromeon 642-streptavidin.

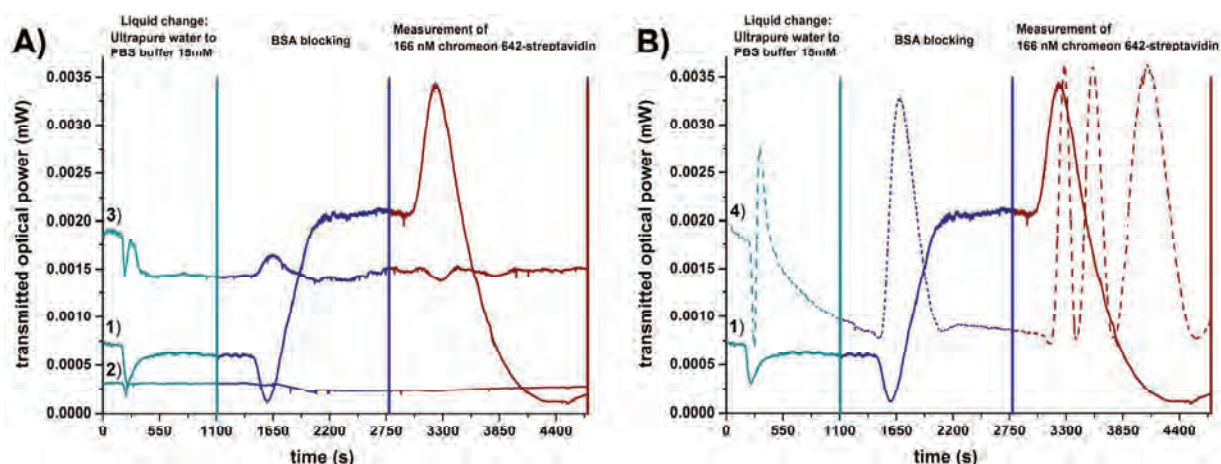


Figure 4. Measurements on different MZI-sensors: A) MZI-sensor with open reference and measurement arms (symmetric MZI): 1) photolithographically blocked with DEGVE and biotinylation of the sensing arm, 2) photolithographically blocked sensing and the reference arm with DEGVE, 3) biotinylation of the sensing and reference arm; B): 1) MZI-sensor with open reference and measurement arms: photolithographically blocked with DEGVE and biotinylation of the sensing arm, 4) MZI-sensor with SU-8 cladded reference arm and biotinylation of the sensing arm.

Measurements on a-Si:H-MZI sensors

In this section, we perform streptavidin measurements on sensors with the reference arm covered with an SU-8 cladding, and on sensors, where both, sensing and reference arm, are in contact with the sample liquid. All measurements were carried out with the same sequence of rinsing steps employing a fully automated liquid handling. Initially, the fluidic chamber is filled with ultrapure water. In the first measurement step (0-1100 s, step time 1100 s), ultrapure water is replaced by PBS buffer. In the second step (1100-2800 s, step time 1700 s), online blocking with BSA is performed. In the third step (2800-4700 s, step time 1900 s), a solution with 166 nM chromeon 642-streptavidin was rinsed through the fluidic chamber.

Figure 4A plots the streptavidin measurements on three different modified sensors with open reference arm. In this figure, curve 1) represents a measurement with local receptor modification on the sensing arm, whereas in curve 2) the whole sensor surface is blocked with DEGVE and in curve 3) the whole sensor surface is just biotinylated without photolithographic DEGVE blocking. For case 1) a clear modulation of the output signal during the BSA blocking step and the streptavidin measurement can be observed. In the blocking step the adsorption of BSA on the sensing arm is stronger than on the reference arm resulting in a phase shift with respect to the reference arm. For case 2) and 3) the output signal stays almost constant indicating the self-referencing effect of the balanced MZI configuration with both arms open. Small fluctuations in curve 2) during BSA blocking and streptavidin rinsing

can most likely be attributed to asymmetric binding due to inhomogeneous flow profiles of the liquids. This assumption is supported by the fact that also in the first rinsing step signal fluctuations occurred during the exchange of ultrapure water by PBS buffer, which has a different refractive index.

Figure 4B compares the streptavidin measurement of the uncovered sensor as plotted in Fig. 4A with the measurement on a sensor with an SU-8 cladded reference arm. For the asymmetric MZI, the change of fluids in step one (till 1100 s) is accompanied by a relative phase shift of the light propagating in the sensing arm with respect to the reference covered by SU-8 due to the refractive index change of the fluids, i.e. no self referencing takes place. In the case of BSA blocking, the signal change is more pronounced than for the symmetric MZI, where BSA adsorption occurs on both arms but with in different quantity. As a consequence, for the symmetric MZI the relative change of the effective refractive index is weaker than for the asymmetric MZI. The most significant difference between measurement results of the locally modified MZI and the MZI with SU-8 covered reference arm is an approximately five times stronger phase shift for the latter observed during the streptavidin binding step. This difference can be attributed to a lower binding efficiency of the locally modified surface. This was confirmed by fluorescence scans on the two sensor devices that were used for the evanescent measurements in Fig. 4B. The fluorescence signal of the sensing area of the asymmetric MZI without photolithographic blocking is approximately three times higher than that of

the symmetric MZI with photolithographic blocking.

Conclusion

We have investigated a local surface modification protocol using thiol-ene coupling for photolithographic blocking of surface regions where no selective binding of target molecules is desired. Fluorescence measurements on control samples indicate a good performance of the employed surface modification protocol. Evanescent wave sensing on integrated optical Mach-Zehnder interferometers with both arms uncovered revealed good self referencing properties during changes of fluids with different refractive indices as well as with respect to high specific and low non-specific binding that occurs equally on both arms. However, the streptavidin binding efficiency on sensors with photographically blocked reference arms was considerably lower than for sensors with SU-8 cladded reference arms processed without the photographic blocking. The lower binding efficiency is most likely related to handling issues during the processing. Further optimization of the photographic blocking procedure on MZI-sensors is therefore required to overcome this limitation.

Acknowledgement

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