Novel refractive index biosensing of microcontact printed molecules on lithium niobate

Nikhil Bhalla[1*], Shivani Sathish[1], and Amy Q. Shen[1#]

Abstract— **This work demonstrates, for the first time, the use of lithium niobate as a biosensor that detects local refractive index changes triggered by the presence of biomolecules on its surface. The sensitivity of the sensor was found to be 242±16 nm/RIU. As a case study, we immobilized proteins (IgG antibodies) using micro-contact printing to demonstrate sensing capabilities of the device. The validated proof of concept lays a foundation for developing lithium niobate based novel optical biosensors.**

I. Introduction

Lithium niobate ($LiNbO_3$) is one of the most multifaceted synthetic materials. It is uniaxial, highly birefringent and has a remarkable combination of piezoelectric, optical properties and photorefractive response [1-3]. These properties of $LiNbO_3$ have been used to develop a number of surface acoustic wave and electro-optical devices for sensing applications [4,5]. The optical properties of $LiNbO_3$ can also be exploited to develop a biosensor that detects changes in refractive index upon binding of an analyte.

In the past few decades, gold-based plasmonic sensors have been primarily used to sense local refractive index changes [6,7]. Even though gold is a biocompatible material and provides a suitable environment for the attachment of biomolecules, it relies heavily on standard thiol chemistry to bind molecules on its surface [8]. One major disadvantage of thiol chemistry is that it is prone to non-specific attachment of molecules and it is often necessary to create a complex anti-fouling surface [9,10]. In this respect materials compatible with silanes, for instance ($LiNbO_3$) provides an advantage of easily creating self-assembled monolayers (SAM) of biomolecules with high specificity. In addition, the photosensitive absorption properties of $LiNbO_3$ are subjected to less losses as compared to plasmons. This makes $LiNbO_3$ as one of the potential candidates to replace surface plasmon sensors in the course of evolution of refractive index sensors. In this work, we show a proof of concept study for measuring direct changes of refractive index of $LiNbO_3$ upon binding of biomolecules on it. This work also demonstrates how proteins can easily be immobilized on $LiNbO_3$ using micro contact printing (μ-CP) [11-14].

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[1]Micro/Bio/Nanofluidics Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, 904-0495, Japan

[*] Presenting Author: Nikhil Bhalla is a postdoc at Micro/Bio/Nanofluidics Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, 904-0495, Japan

[#]Corresponding Author: Professor Amy Q. Shen; Micro/Bio/Nanofluidics Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, 904-0495, Japan (e-mail: amy.shen@oist.jp).

II. Experimental Setup and Methods

A. Preparation of $LibNO_3$ sensor

First, 10 mm ×10 mm samples of $LibNO_3$ were diced from a 0.7 mm thick wafer. The samples were then sonicated in acetone for 10 minutes. This was followed by 5 minutes of sonication of samples in iso-propanol (IPA) to remove acetone stains. The samples were then ready for immobilization of chemical species. Subsequently, a reading of optical absorbance in air, deionized (DI) water (18.2 MΩ), acetone, ethanol and IPA was taken from the samples to calibrate their refractive index sensitivity. The samples were then exposed to silane and proteins were transferred on it using μ-CP [13-14]. Refractive index changes were recorded after silanisation and protein immobilization steps respectively.

B. Instrumentation and measurement

The measurements were done in reflection mode as shown in the schematic on figure 1. The setup was a homemade system comprised of a reflection probe (R400-7UV-VIS), halogen light source (HL-2000) and a spectroscope (USB4000-UV-VIS-ES), which were all purchased from Ocean Optics.

It was calibrated for dark and light spectrum modes, before recording signals from the scope. 200 nm of aluminum was deposited on a glass slide using e-beam evaporation, and the coated slide was used to obtain the light reference spectrum.

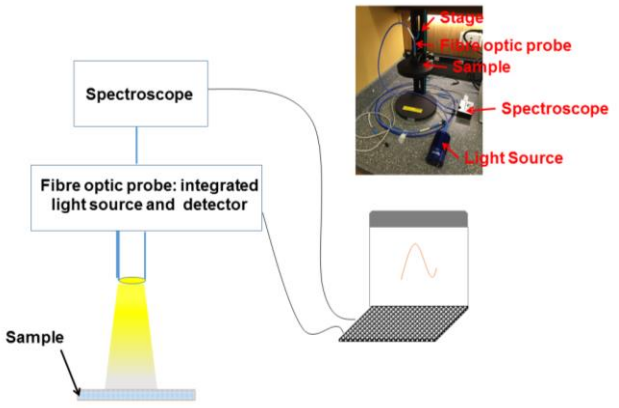


Figure 1. Schematic of the intrumentation setup for the measurements

The optical signal was then recorded in absorption mode by observing the wavelength dependence of the light absorbed

by $LiNbO_3$ via Ocean View, a cross-platform spectroscopy operating software from Ocean Optics.

C. Micro contact printing (μ-CP)

μ-CP is a technique to pattern surfaces using self assembled monolayers of silanes and thiols. To study μ-CP, two patterns were designed using AutoCAD® 2015 (Figure 1 (a) & (b)). A silicon master of this design was prepared by spin-coating DWL-40 negative photoresist onto a silicon (Si) wafer to achieve a film thickness of 25 μm. The wafer was processed using a Maskless Lithography System (Nano System Solutions, Japan). PDMS stamps were then made by pouring the PDMS: curing agent (10:1) (Sylgard® 184 silicone elastomer kit, Dow Corning) mixture onto the Si wafer followed by degassing and baking at 60°C for 4 hours.

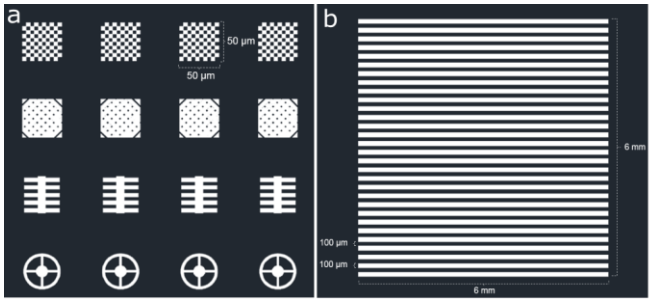


Figure 2 Design of stamps for microcontact printing

D. Protein immobilization

As a case study we choose immunoglobulin antibodies linked with fluorophores (Alexa-fluor 488 chicken anti-goat Ig-G). The $LiNbO_3$ surfaces were first silanised using 5% of 3-Aminopropyltriethoxysilane (APTES) in acetone for 1 hour in a vacuum chamber. Individual PDMS stamps, were incubated with 7.5 μl of antibody mixture (0.01 mg/ml Ig-G solution, 0.0045 mg/ml N-hydroxysuccinimide (NHS) and 0.002mg/ml1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in 1x PBS) under a plasma-activated cover slip for 10 minutes. The stamps were rinsed with sterile PBS and DI water for 10 s each, and dried with a strong puff of nitrogen gas. The inked stamp was then pressed gently onto the APTES coated $LiNbO_3$ wafer bits and incubated at room temperature for 10 minutes.

III. Results and Discussion

A. Sensor sensitivity

On shining white light, an absorbance at 486 nm was observed in lithium niobate (figure 3).

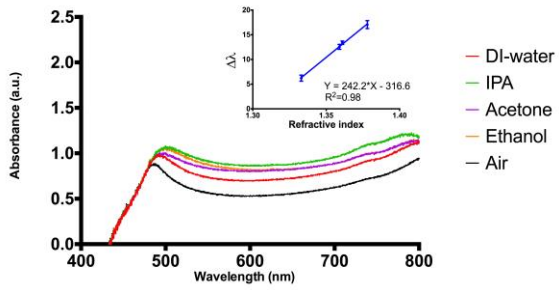


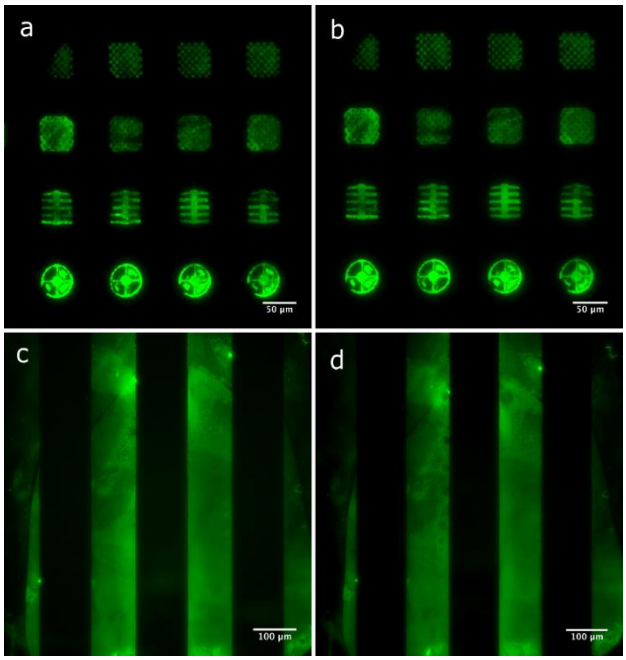
Figure 3: Optical characteristics of $LiNbO_3$ surfaces when exposed to solutions of different refractive indices: DI-water (1.3330), Acetone (1.3590), Ethanol (1.361) and IPA (1.3776)

This peak is attributed to the evanescent wave reflected back by the acoustic waves on the surface, which are actuated upon absorption of light. We observed redshifts of this peak when local refractive index of lithium niobate is changed upon exposure to solutions of known refractive index.

A shift of 12-14 nm is observed for a change of 0.0446 RIU (Refractive Index Units), for the difference between refractive indices of DI-water and IPA. The shifts of the resonance peak were linear with the change in refractive index and the sensitivity of sensor was 242±16 nm/RIU. This value is slightly better than the sensitivities of gold/silver based commercial plasmonic sensors, which varies between 110-160 nm/RIU [6, 15,16].

B. Fluorescence microscopy

Fluorescent IgG antibodies printed onto the $LiNbO_3$ wafer by μ-CP were imaged using fluorescent microscopy (Nikon Eclipse Ti-E). The images were acquired at 10 s exposure, using an epifluorescence filter to detect wavelengths of 488 nm.



(e)

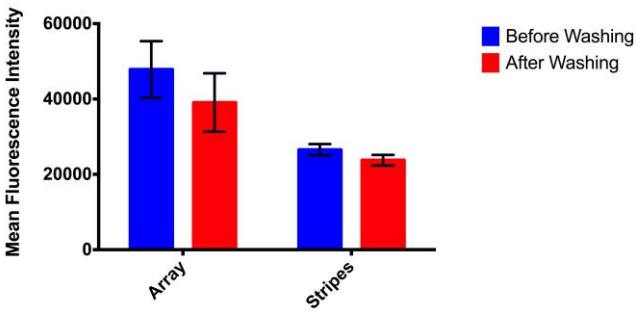


Figure 4: Fluorescence microscopy images of Alexa-fluor 488 chicken anti-goat Ig-G patterns printed onto APTES coated $LiNBO_3$ substrate (a) & (c) before wash; (b) & (d) after brief wash with a stream of double distilled water. (c & d are zoomed in versions of figure 2b)

The prints were briefly rinsed with a stream of deionised water to check for the loss of antibodies from the substrate surface. Figure (a) and (c) depicts the prints prior to the wash and, (b) and (d) after the wash. The fluorescence intensities were not significantly reduced after the wash (figure 4 e). The observed minimal decrease in fluorescence intensity is attributed to the removal of non-specifically bound antibodies from the substrate following washing. This indicates that stable covalent bonds are formed between the APTES monolayer and the antibodies printed by μ-CP on the substrate. (Note: the varying intensities of the prints in each image could be due to uneven coating of the IgG solution on the stamp prior to printing).

C. *Protein detection*

As shown in the figure 5, upon immobilization of APTES an average redshift of 10 nm in the resonance properties of $LiNbO_3$ was observed. A further redshift of 15-20 nm was observed after transfer of proteins (IgG antibodies) on silanized $LiNbO_3$ substrate. The whole experiment from sample preparation to detection took just over 2 hours. However, the detection time of the proteins was less than 5 seconds.

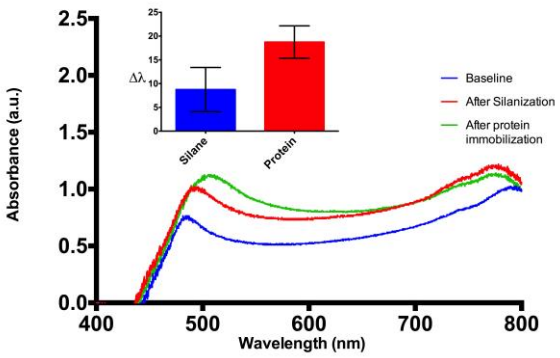


Figure 5: Change in resonance properties of $LiNbO_3$ upon silanisation with APTES and immoblisation of proteins

As emphasized in the preceding text, the interaction of electromagteic field of light with $LiNbO_3$ is responsible for creating mechanical resonanaces in the structures. The resonance is characterized by strong absortion of light at 486 nm. These mechanical resonances are damped by the mass of biomolecules that bind to the surface. As a result, full width half maximum (FWHM) increases and strong resonance shifts are observed. The increased FWHM also corresponds to an increase in the surface energy upon attachment of molecules. This is also responsible for the increase in the amount of absorption that increases with the addition of molecules.

IV. Conclusion

Optical biosensors have proven to be highly selective and sensitive systems. The evanescent field detection techniques have provided improved detection limit for microarray applications and has simplified the sample preparation because of the confined electromagnetic field illumination of the molecules at the sensor surface [15]. In this work we showed how optical properties of lithium niobate can be potentially utilized for biosensing applications. We also showed the ease of combining two techniques on the same platform i.e. fluorometric and refractive index sensing. Multiplexing techniques on a single platform often increases the reliability of measurement and makes the system robust for sensing applications [16]. This opens a great opportunity to develop novel refractive index based sensors for use in applications such as chemical sensing, drug discovery and disease diagnostics. In near future we would aim at integrating microfluidics and work towards development of an electronically automated optical immunoassay platform. In addition, μ-CP of biomolecules simplifies, accelerates, and improves the fabrication of bio-arrays. Gaining a full understanding of the transfer properties of proteins and surface energies of the substrates will enable researchers to embrace the technique of μ-CP for the production of precisely defined protein arrays with well-characterized densities of proteins on optical biosensors.

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References

[1] Preciado E, Schülein FJ, Nguyen AE, Barroso D, Isarraraz M, von Son G, Lu IH, Michailow W, Möller B, Klee V, Mann J. "Scalable fabrication of a hybrid field-effect and acousto-electric device by direct growth of monolayer MoS2/$LiNbO_3$". *Nature communications.* Pp.1-8, 2015

[2] Broderick, N. G. R., Ross, G. W., Offerhaus, H. L., Richardson, D. J., & Hanna, D. C. "Hexagonally poled lithium niobate: a two-dimensional nonlinear photonic crystal". Physical review letters, vol. 84(19), pp. 4345, 2000.

[3] Lucchetti L, Kushnir K, Zaltron A, Simoni F. Light controlled phase shifter for optofluidics. *Optics letters,* vol. 41 (2), pp. 333-5, 2015.

[4] Kerroum, I., M. E. Fall, A. Reinhardt, and F. Domingue. "Hydrogen effect on density and Young's modulus of thin films in acoustic sensors." *Sensors and Actuators B: Chemical* vol. 223 pp. 520-526, 2016

[5] Song, Yong-Ha, and Songbin Gong. "Spurious mode suppression in SH0 Lithium Niobate laterally vibrating MEMS resonators." *2015 IEEE International Electron Devices Meeting (IEDM)*. IEEE, 2015.

[6] Hammond, J.L.; Bhalla, N.; Rafiee, S.D.; Estrela, P. "Localized Surface Plasmon Resonance as a Biosensing Platform for Developing Countries. " *Biosensors* vol. 4, pp. 172-188, 2014.

[7] Bhalla, N., Formisano, N., Miodek, A., Jain, A., Di Lorenzo, M., Pula, G., & Estrela, "P. Plasmonic ruler on field-effect devices for kinase drug discovery applications. " *Biosensors and Bioelectronics*, vol. 71, pp. 121-128, 2015.

[8] Daniel, M. C., & Astruc, D. "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. " *Chemical reviews*, vol. 104(1), pp. 293-346, 2004.

[9] Sandström, Pär, Mila Boncheva, and Björn Åkerman. "Nonspecific and thiol-specific binding of DNA to gold nanoparticles." *Langmuir* vol. 19.18, pp. 7537-7543, 2003.

[10] Frederix, Filip, et al. "Enhanced performance of an affinity biosensor interface based on mixed self-assembled monolayers of thiols on gold." *Langmuir* vol. 19.10 pp. 4351-4357, 2003.

[11] Von Philipsborn, Anne C., Susanne Lang, André Bernard, Jürgen Loeschinger, Christian David, Dirk Lehnert, Martin Bastmeyer, and

Friedrich Bonhoeffer. "Microcontact printing of axon guidance molecules for generation of graded patterns." Nature protocols vol (1, 3) pp. 1322-1328, 2006.

[12] Ricoult, S. G., Sanati Nezhad, A., Knapp-Mohammady, M., Kennedy, T. E., & Juncker, D. "Humidified microcontact printing of proteins: universal patterning of proteins on both low and high energy surfaces. " *Langmuir*, vol. 30(40), 12002-12010, 2014.

[13] James, C. D., R. C. Davis, L. Kam, H. G. Craighead, M. Isaacson, J. N. Turner, and W. Shain. "Patterned protein layers on solid substrates by thin stamp microcontact printing." Langmuir vol. 14, 4, pp. 741-744, 1998.

[14] Bernard, A., Renault, J. P., Michel, B., Bosshard, H. R., & Delamarche, E. "Microcontact printing of proteins. " *Advanced Materials*, vol. 12(14), 1067-1070, 2000

[15] Brolo, A. G., Gordon, R., Leathem, B. & Kavanagh, K. L. "Surface plasmon sensor based on the enhanced light transmission through arrays of nanoholes in gold films. " *Langmuir*, vol. 20, 4813–4815 2004.

[16] Nikitin, A. G., Kabashin, A. V. & Dallaporta, H. "Plasmonic resonances in diffractive arrays of gold nanoantennas: near and far field effects." *Optical Express*, vol. 20, 27941–27952 2012.

[17] Andrade, J. D., R. A. Vanwagenen, D. E. Gregonis, K. Newby, and J-N. Lin. "Remote fiber-optic biosensors based on evanescent-excited fluoro-immunoassay: concept and progress." *Electron Devices, IEEE Transactions* vol. 32, 7, pp. 1175-1179, 1985

[18] Fernández Gavela, Adrián, Daniel Grajales García, Jhonattan C. Ramirez, and Laura M. Lechuga. "Last Advances in Silicon-Based Optical Biosensors." *Sensors* vol. 3285, 2016.