HIGH-THROUGHPUT FABRICATION OF PLASMONIC NANOHOLE ARRAY SENSORS FOR LABEL-FREE KINETIC BIOSENSING

Hyungsoon Im¹, Si Hoon Lee², Nathan J. Wittenberg¹, Timothy W. Johnson¹, Nathan C. Lindquist¹, Prashant Nagpal³, David J. Norris⁴, Sang-Hyun Oh¹²*

¹Department of Electrical and Computer Engineering, ²Department of Biomedical Engineering, University of Minnesota, Twin Cities, Minneapolis, MN 55455, U.S.A., ³Los Alamos National Laboratory, Los Alamos, NM, U.S.A., ⁴Optical Materials Engineering Laboratory, ETH Zürich, Zürich, Switzerland.

ABSTRACT
In this work, we demonstrate high-throughput fabrication of periodic metallic nanohole arrays in an almost centimeter square area through simple metal deposition and peeling off processes, called template stripping [1]. A thin silica layer is used to protect the template-stripped Ag nanoholes from unwanted oxidation and also to provide a robust surface on which lipid membranes can be formed. We also demonstrate label-free optical biosensing of molecular binding kinetics with the silica coated template-stripped nanohole arrays.

KEYWORDS: Template stripping, Surface plasmon resonance, Periodic nanohole array, Biosensing.

INTRODUCTION
With success of commercial surface plasmon resonance (SPR) biosensing instruments, various types of SPR sensors have been demonstrated for biosensing applications [2]. Of particular interest are the SPR sensors based on the periodic nanohole arrays made in metallic films which exhibit the extraordinary optical transmission effect at specific resonance wavelengths [3]. The nanohole biosensors have shown a number of unique capabilities that cannot be addressed with existing instruments [4-6]. Despite the unique advantages, costly fabrication of the nanohole sensors has been a bottleneck for their widespread dissemination. Therefore, the development of new high-throughput fabrication methods is highly desired.

EXPERIMENTAL
Here, we demonstrate a new method that can fabricate large-area nanoholes by stripping self-patterned Ag films from reusable templates. Si templates with circular deep trenches are prepared using nanoimprint lithography. In the nanoimprint process, a resist layer spun on a Si wafer is imprinted by a nanoimprint stamp with circular posts. The Si wafer is subsequently etched to be a nanohole template with deep circular trenches. Figure 1a shows a scanning electron microscopy image of a Si template. Ag is then deposited on the Si template using metal evaporation. Due to the poor step coverage of evaporation, simple metal deposition creates nanohole patterns on the template as shown in Figure 1b. The metal surface is then coated with UV-curable epoxy and then covered by a glass slide. After curing the epoxy under UV light, the metallic film perforated with nanoholes, now adhered to the glass slide, was peeled off of the template to reveal the nanohole array as shown in Figure 2. The Si template can be cleaned and reused to make multiple identical samples. The template-stripped Ag nanohole array is then encapsulated by a 15 nm-thick silica layer using atomic layer deposition (ALD). The silica layer protects Ag from oxidation and improves the chemical stability of Ag nanohole arrays. As a result, polydimethylsiloxane (PDMS) microfluidic chips can be bonded on the nanohole arrays. Figure 2b shows a PDMS chip with 12 channels attached on the silica-coated nanohole array. It also enables the use of well-established surface chemistry to attach biomolecules or form lipid membranes.[6]
RESULTS AND DISCUSSION

Figure 3 shows transmission spectra measured through a template-stripped nanohole array before and after forming biotinylated lipid membrane on the silica-coated surface. The hydrophilic silica surface allows rupturing lipid vesicles and forming supported lipid membranes (SLBs) on the surface. The lipid membrane formation alters the local refractive index on the surface and red-shifts the transmission peak. By monitoring the spectral shift, it can measure molecular binding kinetics in a real-time manner. Figure 4 shows real-time measurements of specific binding between streptavidin-R-PE (SAPE) and biotinylated lipid membranes formed on the silica-coated nanohole arrays. It shows strong binding of streptavidin to the 10% biotinylated lipid membrane formed on the silica surface while only non-specific binding of streptavidin is measured from 0% biotinylated lipid membrane. It demonstrates the capability of nanohole array sensors to detect specific binding on molecules in a lipid membrane environment.

Figure 3: Experimentally measured optical transmission spectra of the fabricated nanohole arrays with 180 nm hole size and 500 nm periodicity made in a 100 nm-thick Ag film before and after the formation of biotinylated lipid membrane on the silica-coated nanohole surface. The presence of a molecular layer on the sensing surface alters the refractive index of the surface, resulting in a spectral peak shift.
CONCLUSION
In conclusion, we have demonstrated high-throughput fabrication of periodic metallic nanohole arrays. Nanohole arrays can be easily fabricated through simple metal deposition and stripping steps from Si templates. The Si templates are reusable to make multiple identical nanohole array sensors. Real-time label-free kinetic biosensing of biotinylated lipid membrane formation and streptavidin binding to the biotinylated lipid is also demonstrated using template-striped Ag nanohole arrays with a silica shell. The proposed high throughput fabrication method will lead to broad dissemination of the nanohole-based sensing platform.

ACKNOWLEDGEMENTS
This work was supported by grants from the NSF CAREER Award, NSF IDBR Program (DBI-0964216) and NIH R01 GM 692993 (S.H.O.). H.I. acknowledges a 3M Science and Technology Fellowship. S.H.L. was supported by a Samsung Fellowship. T.W.J. acknowledges an NIH Biotechnology training grant. Device fabrication was performed at the U. of Minnesota Nanofabrication Center (NFC), which receives support from NSF through the National Nanotechnology Infrastructure Network.

REFERENCES

CONTACT
*S. H. Oh tel: +1-612-625-0125; sang@umn.edu