Molecular Immobilization on a Gold Surface Using a Microfluidic Device

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Abstract/Introduction:

In this study, surface plasmon resonance (SPR) imaging and microfluidics were combined to immobilize single stranded deoxyribonucleic acid (DNA) on a patterned gold surface, detect DNA hybridization, and measure molecular binding kinetics in a real-time, label-free manner. Single-stranded thiolated DNA (T-DNA) was first immobilized on a gold surface; thereafter, the complementary DNA (cDNA) strand and the non-complementary DNA (ncDNA) strand were injected through a polydimethylsiloxane (PDMS) flow cell [1]. Because there are multiple channels in the PDMS flow cell, different solutions were injected through each channel, rendering a multi-array sensor—the PDMS flow cell reduced the volume of the sample solution needed (which increases the diffusion rate) and decreased the overall size of the biosensor. The hybridization was detected through an SPR imaging technique, comprised of a collinear system in which an incident light is shone underneath the sensor [2]. The sub-wavelength nanoholes can efficiently convert incident photons into collective oscillations of conduction electrons, known as surface plasmon (SP) waves at the gold surface. These surface plasmon waves propagate throughout the surface, and re-radiate as a sharp transmission of light at the other surface end. This transmission was detected by a couple charge device (CCD) camera and was shown as an intense transmission peak (peak position depends on the periodicity of the array and the refractive index surrounding the gold surface). If there were molecules present on the surface of the array, there would be a decrease in transmission intensity, resulting in a shift of the peak. Fluorescently tagged cDNA was used to verify the hybridization of DNA using a fluorescence array scanner. This technique can be used, in part, for gene mutation screenings as well as molecular interaction studies. The results showed that hybridization was possible through a microfluidic device and that a transmission peak could be detected through the biosensor.

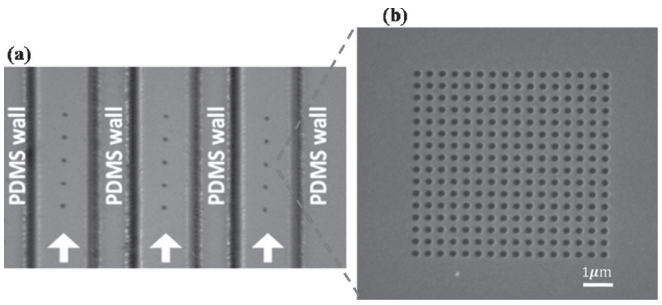

Figure 1: (a) Bright field image of a PDMS channel. (b) SEM image of nanohole array milled by a focus ion beam (200 nm diameter and 400 nm period).

Experimental Procedure:

Using an electron beam evaporator, 5 nm of chromium, followed by 100 nm of gold, were deposited onto a clean glass slide, and placed in an ozone oven for ten minutes. Next, a 3 × 5 array of 16 × 16 nanoholes, (200 nm diameter and 400 nm periodicity), was milled onto the surface of the slide using a focused ion beam (Figure 1).

The fabricated microfluidic device used photolithography to construct three channels (each channel 100 μm wide and 50 μm deep) (Figure 2a) by spinning SU-8 photoresist onto a blank four inch silicon wafer at 2000 rpm for 30 seconds. The wafer was then prebaked, soft-baked, exposed to ultraviolet light under a patterned chrome mask, baked again, and developed [1]. Next, it was coated with a self-assembled monolayer of alkanethiols using a vacuum chamber. A 10:1 mixture of PDMS and curing agent was poured onto the wafer, and cured. Finally, six holes (1 mm diameter), were punctured into the inlets and outlets.

After preparing the reagents in Table 1, 30 μl of T-DNA were pipetted onto the surface of the slide over the nanoholes, incubated in a 37°C humid chamber, and rinsed. The T-DNA area was then treated with 90 μL of mercaptohexanol, incubated, and washed. The PDMS flow cell was coated with bovine serum albumin, incubated, and rinsed. A glass cover slide was placed over the PDMS and gold slide and secured by mechanical bonding, where tubes (0.05 inches in diameter and 3 inches long) were inserted into the inlets and outlets (Figure 2a and b). Finally, fluorescent/complementary-DNA (F/cDNA) was injected into the right channel, fluorescent/non-complementary-DNA (F/ncDNA) into the left channel, and the center channel was left empty. After a 24 hour incubation period in the humid chamber, the fluorescent labels were detected using a fluorescent microarray scanner.

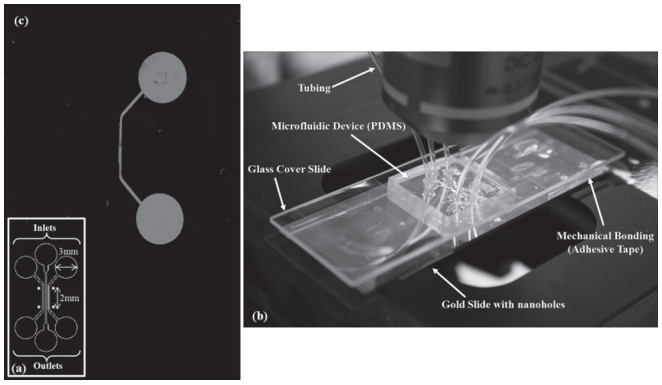

Figure 2: (a) Schematic of PDMS flow cell. (b) Complete SPR sensor. (c) Result from fluorescence array scanner. Right channel (F/cDNA), Left channel (F/ncDNA), Center channel (empty).

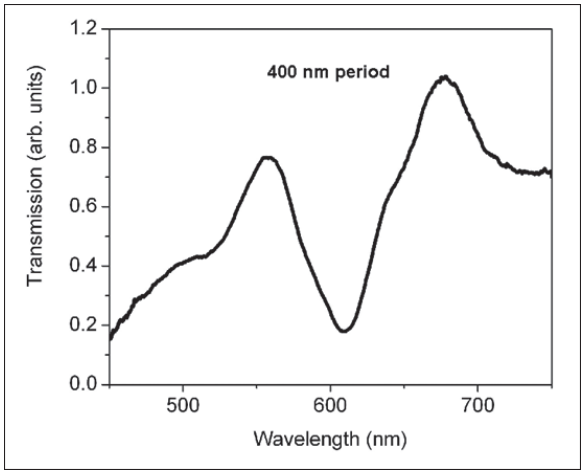

Figure 3: Transmission spectrum. The sharp peak shifts when DNA binds to the surface.

Results and Conclusions:

After several trials, DNA hybridization in a PDMS flow cell was successful. Figure 2c shows the fluorescence of the positive control channel. The main problem encountered for the first several trials revolved around the F/cDNA leaking out of its channels and into other channels; this error could be attributed to air bubbles or impurities between the PDMS flow cell and gold slide, or the flow rate of F/cDNA. Since DNA is hydrophilic, it is more prone to spread on the surface rather than stay within the channel walls. This leakage problem was corrected using a contact aligner to apply the flow cell to the gold surface—a uniform pressure over a wide area was applied onto the PDMS and the gold slide ensuring a tight seal. Figure 3 shows a transmission spectrum of the nanohole arrays without any molecules on the surface. The two peaks are due to the different periodicities between the adjacent hole, and the diagonal hole.

Future work would include the injection of DNA and complementary DNA through the flow cell while simultaneously measuring the transmission to see if the imaging system could detect the hybridization.

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References:

[1] Sia, S.K., Whitesides, G.M.; "Microfluidic devices fabricated in Poly(dimethylsiloxane) for biological studies"; Electrophoresis, 24, 3563 – 3576 (2003).

[2] Lesuffleur, A., Im, H., Lindquist, N. C., Lim, K. S., and Oh, S.; "Laser-illuminated nanohole arrays for multiplex plasmonic microarray sensing"; Opt. Express. 16, 219-224 (2008).

Stock solution	T-DNA	F/C-DNA	F/N-CDNA	DNA
100 mM Tris-HCl	12.5 mM	10 mM	10 mM	10 mM
10 mM EDTA	1.25 mM	1 mM	1 mM	1 mM
5 M NaCl	0	1M	1M	1M
10 mM TCEP	1.25 mM	0	0	0
20 μM T-DNA	1.25 mM	0	0	0
20 μM F/C-DNA	0	1 μM	0	0
20 μM F/NC-DNA	0	0	1 μM	0
20 μM DNA	0	0	0	1 μM
DI-UV Water	*	*	*	*

*Table 1: Reagents used * Volume varies depending on total solution volume.*