ON-CHIP ELECTROKINETIC SAMPLE FOCUSING FOR MICROARRAY-BASED BIOMOLECULAR INTERACTION ASSAYS
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ABSTRACT
Imaging surface plasmon resonance (iSPR) is a label-free microarray based technique in which multiple biomolecular interactions can be monitored simultaneously. In this work, we present an integrated electrokinetic focusing microfluidic chip and iSPR system suitable for large-scale microarray applications.

KEYWORDS: Biomolecular interactions, electrokinetic focusing, imaging surface plasmon resonance

INTRODUCTION
Label-free measurement techniques, such as surface plasmon resonance (SPR) and iSPR, are emerging as important complementary methods to conventional bioassays because real-time binding kinetics information can be determined[1]. Recent advances in iSPR are becoming increasingly important to label-free microarray-based assay applications, where multiple biomolecular interactions can be measured simultaneously. However, conventional iSPR microarray assay approaches rely on protein printing techniques for immobilization of the ligand to the gold imaging surface[2]. The combination of microfluidics with iSPR allows the in-situ immobilization of ligands and subsequent analyte transport for the biomolecular interaction assay[3]. In this work, we present an integrated microfluidics and iSPR platform, where ligand immobilization and subsequent sample solutions can be precisely positioned with an electrokinetic focusing technique.

ELECTROKINETIC FOCUSING
Electrokinetic focusing is a valve-less electroosmotically driven technique used for controlling the sample stream in a microfluidic chip[4]. The microfluidic chip arrangement (figure 1) uses the center inlet for sample introduction and upper and lower inlets for guiding. Electrical voltages \( V_u \) and \( V_l \) control the y-direction position and width of the sample. Voltage \( V_c \) is used to transport the sample in the x-direction with the required flow velocity. The biomolecular interactions of the sample and immobilized ligand are measured at each gold island using an iSPR system. The electrokinetic focusing chip consists of two layers, a bottom glass layer with 24 patterned gold islands (200 µm on a side), and a poly(dimethylsiloxane) (PDMS) top layer consisting of the microchannels, inlets, outlet and interaction chamber. In order to avoid transport limitation effects in the SPR measurement, and subsequent kinetic...
analysis, sample flow rates above ~ 20-30 µL/min are required[1]. These flow rates require transport voltages $V_c \sim 150$ V and $V_{inj} \sim 1000$ V for sample guiding. These large electric fields across the interaction chamber lead to gold degradation, a common problem when combined with electrochemical applications[5]. A thin (10 nm) layer of silicon dioxide (SiO$_2$) deposited directly on the clean gold surface using plasma enhanced chemical vapour deposition is used to electrochemically isolate the gold islands during sample focusing.

EXPERIMENTAL RESULTS AND DISCUSSION

For demonstration, a sample (3% glycerol in 5mM HEPES buffer and Rhodamine-B) was focused to rows 1 through 4 using predetermined voltages $V_l$, $V_c$ and $V_u$ for each row. Figures 2(a)-(d) show fluorescence microscopy images of the focused flow at each row. Figures 2(e)-(h) show measured iSPR sensorgrams during sample focusing.

Figure 2. (a)-(d): Fluorescence microscopy images demonstrating sample stream focusing (3% glycerol in 5 mM HEPES + Rhodamine B) over 6 gold islands. Inset plot shows the uniform intensity profile of the focused sample stream. (e)-(h): iSPR measurements of 3% glycerol in 5 mM HEPES focused at each row (columns shown with different colours). Inset plot shows the average response of the 3% glycerol focusing over the gold islands with standard error. Electrical focusing voltages ($V_u$, $V_c$, $V_l$).
Biomolecular interactions of the well-characterized interactant pair (human IgG – antihuman IgG) were measured during sample focusing. First, human IgG (400 µg/ml) was immobilized by physical adsorption on all SiO$_2$ coated gold islands using electroosmotic flow (EOF) from the center inlet. Figure 3(a) shows the SPR angle shift following IgG immobilization. Rows 1 through 3 have a similar ligand density while row 4 has a lower surface concentration due to a nonoptimal immobilization procedure. Figure 3(b) shows an example of a SPR the biomolecular interaction measurement following the introduction of 20 µg/ml of antihuman IgG diluted in PBST buffer as it was focused to each row for 300 s followed by the dissociation for 1500 s. The guiding stream is 5 mM HEPES buffer. The source for the high noise level in our data is unknown and is currently under investigation.

**CONCLUSIONS**

An integrated electrokinetic sample focusing system with iSPR was demonstrated for the real time IgG-antihuman IgG biomolecular interaction measurement. The results are encouraging but more research is needed to integrate the technology for large-scale, high throughput bioassays.

**REFERENCES**