COMBINED LAB-ON-A-CHIP AND MICROARRAY APPROACH FOR BIOMOLECULAR INTERACTION SENSING USING SURFACE PLASMON RESONANCE IMAGING

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ABSTRACT

Surface plasmon resonance imaging (SPR) is a well-established label-free detection technique for real-time biomolecular interaction measurements. An integrated LOC sensing system with fluidic control for sample movement to specific locations on microarray surface in combination with SPR imaging is demonstrated by the measurements of human IgG and anti-IgG interactions from 24 patterned regions.

Keywords: SPR, Biomolecular Interaction, Microarray, Lab-on-a-chip

1. INTRODUCTION

Lab-on-a-chip (LOC) systems are rapidly becoming vital for the miniaturization of analytical techniques for various applications, such as genomics and proteomics [1]. LOC systems with integrated fluidic transport capability combined with SPR imaging provides a powerful technique to perform label-free multi-analyte interaction measurements in microarray formats [2]. Multi-analyte detection in microarray formats is very important in the field of medical diagnostics and drug discovery. The goal of this article is to develop a multi-analyte immunoassay based on a SPR imaging platform and integrated microfluidics with fluidic transport for sample addressing.

2. PRINCIPLE OF SURFACE PLASMON RESONANCE IMAGING

SPR imaging, a typical system is shown in figure 1a, is based on the excitation of surface plasmons of a thin metal layer (~50 nm), most commonly gold, by excitation with p-polarized light. With the excitation source at a certain angle of incidence the free electron oscillation reaches a maximum and an evanescent field is enhanced at the dielectric-gold

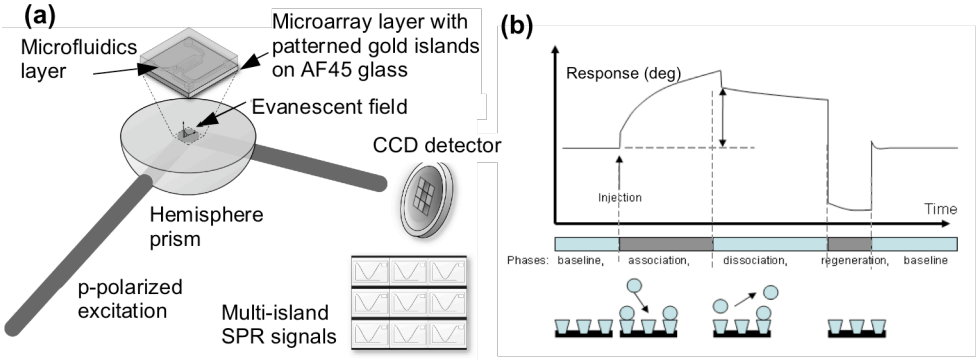

Figure 1. (a) Integrated LOC and SPR imaging; (b) sensorgram showing different phases of biomolecular interaction.

interface. The amount of substance adsorbed at the surface during immobilization and

antibody-antigen binding results in a measurable change in the surface plasmon resonance condition. In this case, the change in angle is measured temporally upon binding, shown qualitatively in the sensorgram in figure 1b. The baseline phase establishes a starting point before introducing the target analyte to the prepared surface. The association phase shows an increasing sensor response as the injected target analyte is injected and binds to the immobilized probe on the sensor surface. The dissociation phase results following the injection of a running buffer and non-specifically bound molecules dissociate from the surface. The regeneration phase proceeds with the injection of low pH buffers to remove bound target analytes. Following the regeneration phase the sensor response should return near the starting baseline signal.

3. SYSTEM DESIGN

The sensing system consists of a commercially available surface plasmon resonance imaging system (iSPR, IBIS Technologies, b.v., Hengelo, The Netherlands) integrated with a LOC system with multiple sample inlets and outlets and electrodes for electrokinetic fluidic transport. Figure 1(a) shows a schematic of an integrated system.

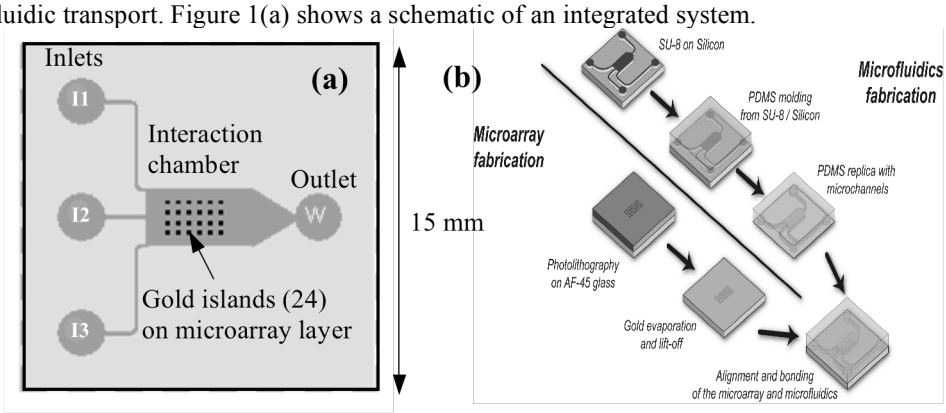

Figure 2. (a) top microfluidic layer with inlets I1-I3, waste outlet (W), microchannels and interaction chamber. The microarray layer contains the patterned gold islands within the interaction chamber. (b) Two-path microfabrication process diagram used for manufacturing the LOC system. The microarray and microfluidic layers are aligned and bonded together realizing the final composite structure.

The schematic of the LOC system is shown in Figure 2(a). The microfluidic layer consists of an interaction chamber that is interconnected with three inlets I1-I3 for a buffer solution, samples and reagents. The outlet is to remove waste (W) following an analysis operation. The microarray layer consists of gold regions, aligned to the interaction chamber of the microfluidic layer. The gold sensing surface can be a continuous layer or patterned regions as shown in figure 2a. For multi-analyte sensing applications each gold region can consist of a different immobilized probe and necessary experimental controls. Different methods are available for probe immobilization including non-contact protein printing [3] or in-situ immoblization using microfluidic networks [4].

4. MICROFABRICATION

The biochip consists of two layers, as shown figure 2(b). The top layer is made of poly di-methyl siloxane (PDMS) (Sylgard 184, Dow Corning, USA) that contains the channel structures, interaction chamber, inlets and outlet. The SPR gold was deposited on

the bottom layer that consists of AF45 glass refractive index (n=1.52) matched to the hemispherical prism. The microfabrication is performed in two separate processes. The PDMS layer is fabricated using a silicon wafer with patterned SU-8 (MicroChem Corporation, USA) structures to form the microfluidic structures. The lower microarray layer consists of gold islands for SPR imaging formed using a conventional lift-off procedure. The two layers are finally aligned and bonded with the aid of oxygen plasma activated surfaces. In the case that the probe molecules are first immobilized on the gold, then only the top microfluidic layer surface is treated with the oxygen plasma activation step.

5. RESULTS AND DISCUSSION

The microfabricated biochip was placed in a custom fixture with 10 µL cuvettes directly coupled to the inlet ports. The glass microarray layer was then placed in direct contact with the hemispherical prism of the SPR imaging system. Fluidic control is performed using electroosmotic flow with Pt wires immersed in the solution and connected to a high voltage power supply. The applied voltages were controlled with a LabView program. To demonstrate the concept 1mg/mL human IgG probe molecules are loaded into the sample

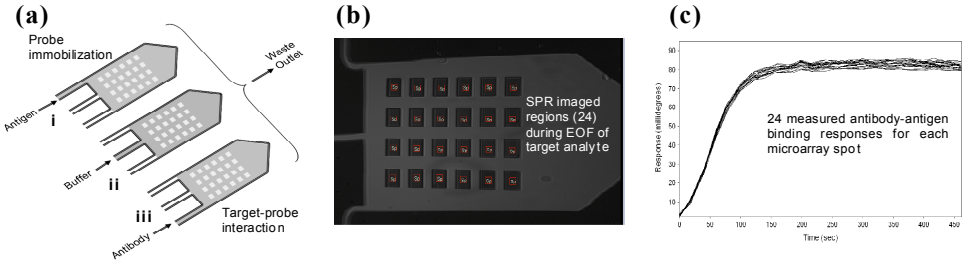

Figure 3. (a) Three step process for i: probe immobilization, ii: surface cleaning with buffer, and iii: antibody-antigen interaction. (b) SPR image of our chip with 24 gold islands; (c) Sensorgram of 24 human IgG and anti-human IgG interactions using EOF.

cuvette. The probes are then transported to the interaction chamber by applying 100 V between inlet port I1 and the exit port W. The antigens are immobilized by direct adsorption onto the gold surfaces. The second inlet reservoir I2 is then filled with PBS buffer (pH 7.2) and transported through the system. The 10 mg/mL anti-human IgG is then loaded into inlet I3 and transported to the immobilized probes electroosmotically with an applied voltage of 50 V. The general procedure is shown in figure 3(a). The real-time biomolecular interaction was measured by SPR imaging, shown in figure 3(b) and (c). We were able to control the flow of anti-IgG over the IgG patterned gold islands in the biochip and detect the binding of anti-IgG to the immobilized IgG on the gold islands with SPR imaging.

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