OPTIMIZATION OF BIOMOLECULE IMMOBILIZATION AND PATTERNING ON GOLD SURFACES FOR LABEL FREE DETECTION OF MICROARRAYS

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Immunoassays are a common and useful means of performing biochemical analysis. The strong, specific binding of an antibody to its antigen has been widely exploited in clinical applications as well as in sensor design. The most common approach to immunoassays is to immobilize either the antibody or antigen to a solid support and to detect binding with a labeled partner. Labels for immunoassays have had a range of composition and detection mechanisms and include enzymes, fluorescent molecules, nanoparticles and radiolabels. Recently, the widespread application of DNA microarrays (gene chips) for massively parallel analysis has prompted researchers to develop multisensing immunoassays in microarray format. As well, the high throughput demands of proteomics have driven development in the fabrication and reading of microarrays of a variety of proteins. In this presentation, a facile method for creating arrays of antigen and antibody proteins on gold is demonstrated. Interactions of antibodies at these microarrays are detected by surface plasmon resonance (SPR) imaging. SPR detects changes in refractive index within a short distance from the surface of a thin metal film as variations in light intensity reflected from the back of the film and thus, does not require labeling.

Microarrays are generally prepared by the robotic pin printing of biomolecules on glass slides. Here, a simple method is presented for patterning of protein antigens at a gold surface for use in SPR imaging experiments. Microfluidic devices fabricated from polydimethylsiloxane (PDMS) were used to flow various fluids over a gold substrate in spatially defined channels. This technique was used to pattern the surface chemistry of the gold as well as to adsorb proteins from solution to the modified substrates. Figure 1 is a schematic detailing the technique for patterning three antigen proteins. The resulting protein arrays were probed with bonding partners in order to demonstrate the effectiveness of the patterning for antibody capture experiments. SPR imaging was used to aid in the optimization of array fabrication and to observe the interactions of unlabeled proteins with these microarrays.

We discuss our efforts into optimizing protein arrays via variations in surface chemistry and protein surface concentration. In one experiment, an array was prepared consisting of bovine IgG immobilized at various fractional surface coverage (θ) by adsorbing from solutions of different concentrations. Figure 2 shows an SPR image of such an array after exposure to 670 nM of anti-bovine IgG and rinsing with PBS. As shown in the cross-sectional profile, the amount of antibody binding that is detected scales with the surface density of antigen.

In other experiments, we have evaluated the effect of different IgG attachment schemes on the orientation of adsorbed IgG and its ability to bind Anti-IgG molecules. These attachment schemes include physisorption to different terminal functional groups of an alkyl thiol monolayer and affinity capture of IgG through an immobilized protein layer. Preliminary work has also begun in the photolithographic patterning of the gold on the SPR chips. The aim of this work is to lower the limits of detection in SPR imaging through elimination of background adsorption. Also, it has the potential to address channel leakage problems encountered when using PDMS microfluidic devices with non-aqueous solvents through improved adhesion of the PDMS microfluidic network. This procedure creates an array of gold spots against a glass background, which does not adsorb proteins.

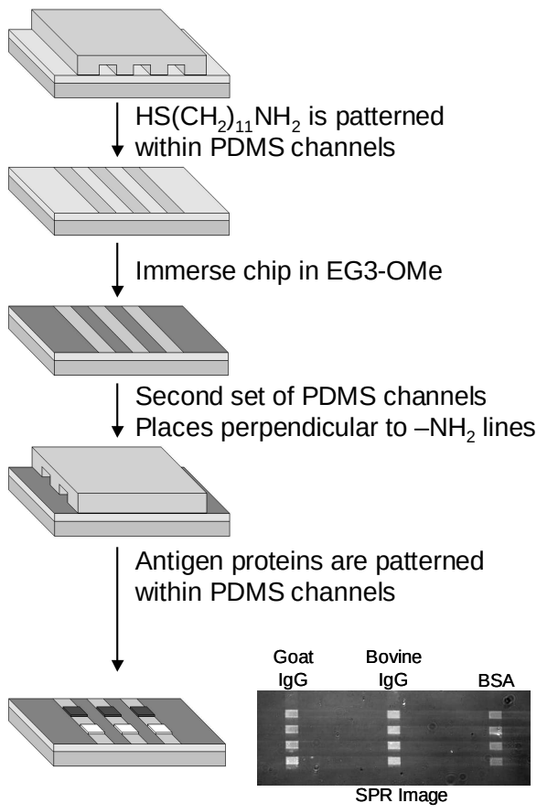

Figure 1: A schematic representation of the method for surface patterning of proteins. The SPR image in the lower right shows the resultant array.

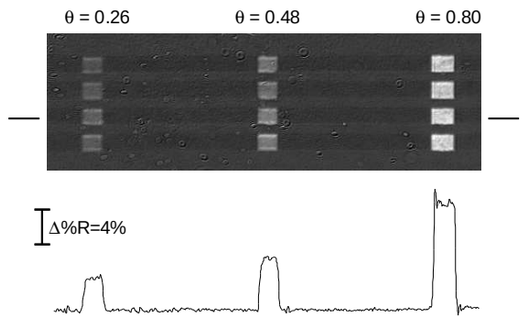

Figure 2: SPR image of a bovine IgG array following exposure to a 667 nM solution of anti-bovine IgG. The array consist of bovine IgG adsorbed at different fractional coverage (θ). The line profile shows the magnitude of the signal through a row of array elements.