Label-free and High-throughput Screening of Biomolecular Interactions

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Abstract:

We present a simple label-free multi-analyte detection technique that is easily scalable for high-throughput screening. We have shown a sensitivity of 20pg/mm^2 and a minimum detectable antibody concentration of 15ng/ml for a specific antigen.

Over the past decade, biological microarrays have been used by the bioresearch community to measure the binding affinities between DNA-DNA, DNA-protein, and protein-protein interactions. Using microarrays, binding between target biomolecules and tens of thousands of different probe molecules can be observed simultaneously. Most of the conventional microarray systems work by the same underlying principle whereby they illuminate the surface with a laser and observe fluorescence [1]. This requires that the target biomolecules be prepared with an additional fluorescent label (typically a dye molecule) attached. This preparation step is not only time consuming and costly, but it can be prohibitively difficult depending on the experiment [2] and may also alter the conformation of small target molecules.

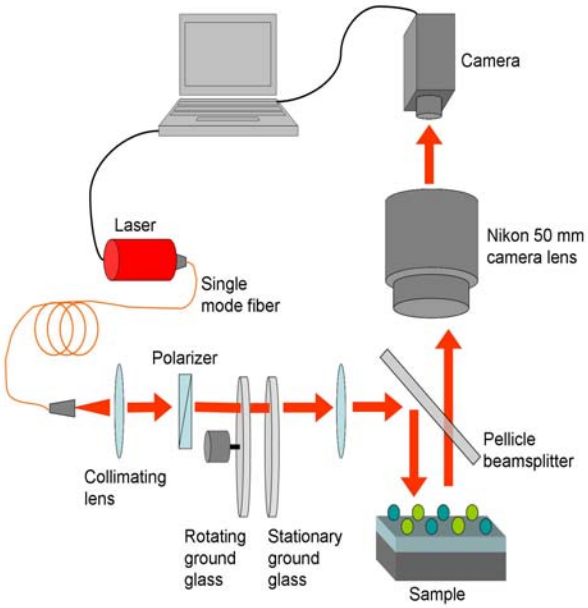

Fig.1: Laser illumination causes unwanted diffraction effects and speckle patterns on the image. These artifacts caused by the spatial coherence can be reduced by passing the illumination through a pair of diffusers.

In this work, we propose a surface profilometry technique to be used as a label-free microarray imaging device. Spectral Reflectivity Imaging Biosensor (SRIB) uses wavelength dependent reflectivity of a silicon substrate with thick thermally grown SiO_2 (10 μm), to accurately find the film thickness at tens of thousands of different spots, thus image the surface profile.

Laser beam that can be tuned from 764 nm to 784 nm is passed through diffusers to reduce the spatial coherence of the illumination, and is incident on the sample surface (Fig.1). At any fixed wavelength, reflection from the sample is imaged on a CCD camera. Then the wavelength is stepped, and another image is taken. Repeating this through the sweeping range of the laser, one can form a spectral reflectivity curve for every unit area on the sample in the field of view. The separate reflections from oxide and silicon surfaces form an interference pattern and an exact oxide thickness corresponding to each pixel is found by curve fitting to the recorded data.

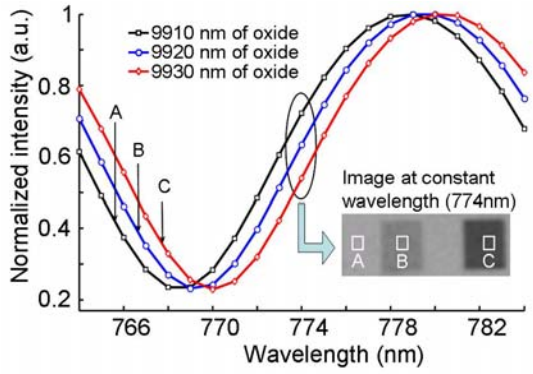

Fig.2: Different oxide thickness caused shifted reflectivity vs wavelength curves which is detectable at specific wavelengths as an intensity difference. In the image, reflectance of 3 different oxide thickness is shown.

Reflection of a single layer can be well estimated by conventional formulas [3]. The reflection coefficient for an incoming field is given by:

$$r = \frac{r_{12} + r_{23}e^{-2i\phi}}{1 + r_{12}r_{23}e^{-2i\phi}}$$

where r_{12} and r_{23} are the Fresnel reflection coefficients from the SiO_2 surface and SiO_2-Si interface, respectively, and φ is the optical phase difference between the two reflections:

$$\phi = \frac{2\pi d}{\lambda} n_{SiO_2} \cos\theta$$

Here, d is the SiO_2 thickness, n_{SiO2} is the refractive index of SiO_2, λ is the wavelength of the incident light, and θ is the incidence angle to the SiO_2-Si interface, which will be 0^o for perpendicular incidence. Curve fitting tools are used to fit this equation to the recorded data at each pixel and find the d (Fig.2). When there is accumulation of biomolecules on the surface, the total optical path difference (OPD) between the air-oxide and oxide-silicon interfaces increase. Incremental optical thickness can be approximated by a commonly used relation of 1 pm of height change corresponding to 1pg/mm^2 of biomolecule accumulation. [4]. Finally, every d value can be plotted on a grey-scale figure to image the surface profile.

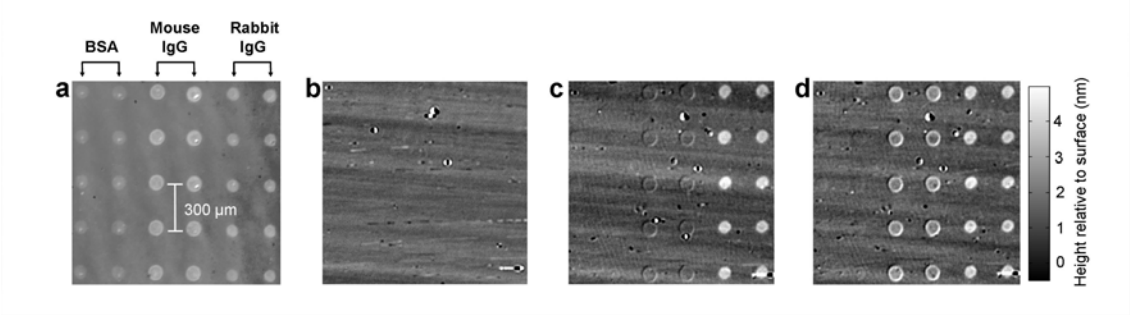

Fig.3: (a) Initial image of spotted antigens. (b) The first image is subtracted from all consecutive data to track the incremental height increase (c) After anti-rabbit IgG flow in the chamber (d) after anti-mouse IgG flow in the chamber

Using this technique, we have dynamically detected specific binding of antibodies to the spotted antigens (Fig. 3). As the data acquisition takes less than 1 min., dynamic detection was possible by continuous acquisition of data during the interactions. 30 spots of different antigens were spotted on the functionalized (with epoxysilane) surface, and this sample was placed in a flow chamber. The spotted antigens were mouse IgG and rabbit IgG, and bovine serum albumin (BSA) was spotted as control. The spotting concentrations were 5 mg/ml for all. The chamber was sealed with anti-reflection coated glass window to allow detection. First anti-rabbit IgG was introduced to the chamber at a concentration of 5 µg/ml, and specific accumulation on the rabbit IgG spots were detected (Fig 3c). Also notice the slight cross reactivity between anti-rabbit IgG and mouse IgG in Figure 3c. A flow of anti-mouse IgG followed the first incubation, and binding on the mouse IgG spots was detected. Note that the height of BSA spots remained constant during the entire experiment. During these experiments the noise floor for each spot was ~20 pg/mm^2, and a minimum concentration of 15 ng/ml was achieved for an antibody-antigen interaction.

This technique is simple, amenable to high-throughput screening and present data shows similar sensitivities to imaging SPR. As thermally grown oxide is being used, commonly used glass surface chemistries can be applied to SRIB substrates. Also, thermal oxide on silicon is ideal for label-free detection as the surface is very well polished and oxide has a uniform thickness everywhere on the surface. Our analysis show that more than 2500 different interactions can be monitored simultaneously and dynamically using this simple technique and get to a noise floor comparable to imaging SPR for each interaction.

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