Optical Phase to Biological Mass Conversion for Label-free Interferometric Sensing Methods

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Abstract: The conversion between the detected optical phase and the biological mass accumulated on the surface is determined using a label-free interferometric technique. The results demonstrate a linear phase/mass relationship for DNA and protein microarrays.

I. Introduction

Researchers in the field of biotechnology have been using the biological microarrays for a number of applications [1]. The necessity of performing this detection in a label-free format has emerged due to the drawbacks of labeling target molecules. Variations in the collected signal due to inherent properties of fluorophores, environmental effects, and the detection parameters make the fluorescent techniques an indirect measure of molecular accumulation. In addition, the fluorescent molecules may alter the natural binding properties of the target molecules. As a result, complex calibrations are required for quantification of the signal [2].

Label-free detection techniques are amenable to dynamic and quantitative detection, thus kinetic characterization of the interactions are possible with these sensing platforms. They also have the added advantage of being simpler and cost effective. A commonly seen label-free detection method is the utilization of optical interferometry that use a layered substrate as a solid support for probe biomolecules [3-5]. These biosensors work with the same underlying principle such that the biomaterial accumulation on the surface increases the optical path difference (OPD) between the two light beams reflecting from different layers of the solid support. The OPD is detected by tracking the interference of the reflected light beams. Change in OPD is the product of biomaterial's refractive index and layer thickness. The surface accumulation is either approximated by a change in refractive index accompanied by a constant layer thickness, or by a change in layer thickness accompanied by a constant refractive index.

Over the past year, we have introduced Spectral Reflectance Imaging Biosensor (SRIB) to be used for label-free and high-throughput detection [5]. We have shown that this system is capable of acquiring dynamic binding data at a sensitivity of ~10pg/mm^2 for each spot of a 400 spot array. This technique can be utilized for monitoring various types of biomolecular interactions. During the data acquisition, we use a common approximation that defines the optical refractive index of biomolecules as ~ 1.45, and 1 nm of height change corresponds to ~1 ng/mm^2 mass density increase on the surface. This approximation has been used by many researchers over the past 2 decades, but there is a lack of direct measurement of this conversion. In the presented work, we show that this approximation holds well for a wide range of mass densities. We also show that SRIB has a linear response for a very wide dynamic range.

II. Experimental Procedure

To demonstrate the quantification capability and linearity of SRIB, we have prepared microarrays of single stranded DNA (ssDNA) and two different types of protein (IgG and BSA). The SiO_2/Si substrates were spotted with varying concentrations of ssDNA, IgG, and BSA dissolved in deionized water (di-H_2O). To determine how much material is expected

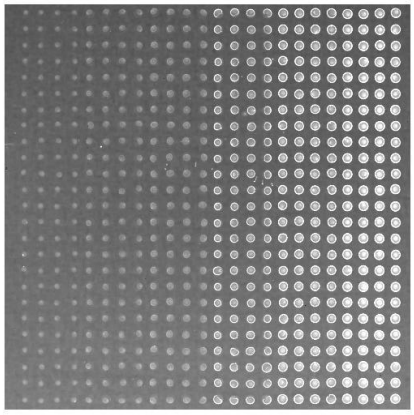

Figure 1: Increasing concentration of biological material (IgG, BSA and ssDNA) was spotted on the surface. The spotting buffer was salt-free water, therefore upon evaporation of the water, the only remaining mass was the biological mass. The amount of mass dispensed on the surface was accurately calculated by knowing the spotting concentration and measuring the volume of the spots during spotting. For each concentration, 100 replicates were spotted; spot diameters are 100um and spot-to-spot distances are 200um. SRIB image of BSA dilution sample is shown.

to be present on each spot, the volume of each spot (found through the use of the non-contact piezoelectric spotter software) and the concentration of molecules in each spotting solution are taken into consideration. The surfaces have been dried after spotting, and have not been washed, since washing would remove unbound molecules, making it impossible to predict the amount of material expected to be present on the surface. Then the samples were scanned with SRIB (Fig.1), and the total mass at each spot was calculated.

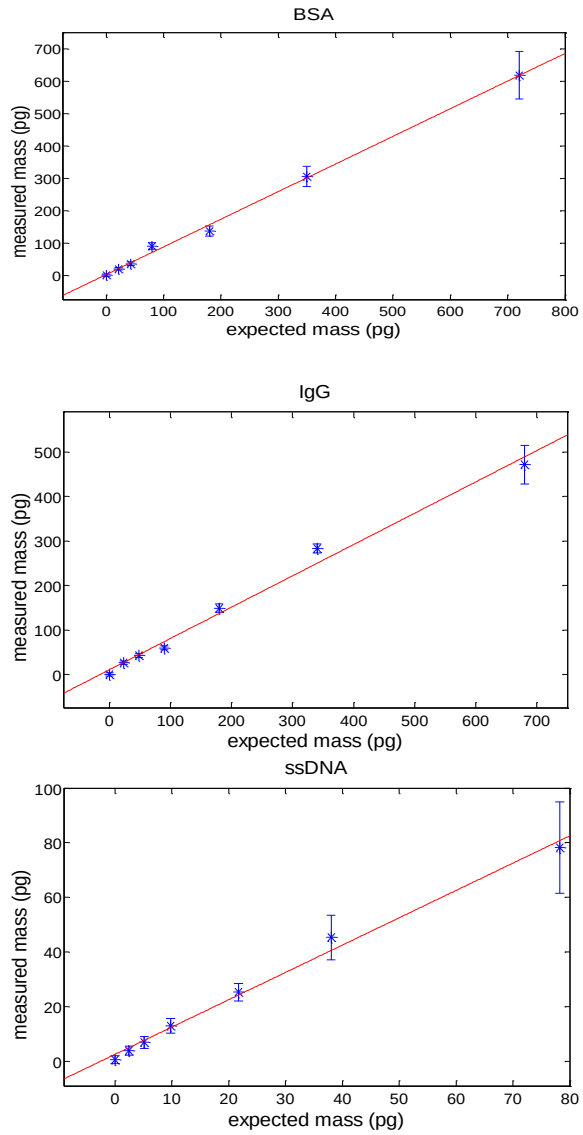

Figure 2: The spotted total mass of each spot was calculated using the known spotting concentration and measured spotting volume. The total masses were then compared with SRIB measured total mass values. As it is clearly shown, the SRIB signal changes linearly with the increasing surface adsorbed biological mass. As the total mass sensitivity is in the order of 50 fg per spot, these results indicate that SRIB is capable of performing quantitative measurements for a wide dynamic range (~4 orders of magnitude).

III. Results

Figure 1 shows the detected amount of material for ssDNA, IgG, and BSA spots versus the expected mass that was calculated by knowing the spotting concentration and volumes. All the results align very well with the linear fit, and the dynamic range larger than that would be needed for most biological applications (surface bound mass usually saturates ~100pg in a 100um spot). Using the measured slopes of the data given in Fig.1, we can calculate the detected biomaterial height to surface accumulated mass conversion (assuming a biomaterial refractive index of n=1.45) as:

For BSA: 1nm ~ 1.17ng/mm^2

For IgG: 1nm ~ 1.2ng/mm^2

For ssDNA: 1nm ~ 0.8ng/mm^2

IV. Conclusions

The results indicate that a thin layer of adsorbed biological material acts as thin transparent films. Therefore, the optical effect of biological accumulation on surfaces can be well approximated simply by phase retardation. In this work, we establish the connection between the added phase and adsorbed mass density for 3 different macro-molecules.

We have shown that previously introduced SRIB technique has a linear response for accumulation of various biomolecules on the surface, thus quantitative measurements leading to kinetic data analysis is possible in a high-throughput format (hundreds to thousands of interactions simultaneously). We also show that the commonly cited conversion of 1nm layer height ~ 1ng/mm^2 biomaterial surface mass density holds quite well for 3 different biomolecules.

V. Referances

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