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Comparison of two optical techniques for label-free detection of biomolecular microarrays on solids

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Abstract

I compare two techniques for label-free detection of biomolecular reactions on solid supports in microarray format. I show that the oblique-incidence reflectivity difference (OI-RD), when operated near the Brewster angle, is as sensitivity as the surface plasmon resonance technique. The OI-RD technique is more versatile and promises higher throughputs.

Optical techniques have played an instrumental role at almost every stage of advancement in life sciences. With the advent of lab-on-chips, – microarrays of biological macromolecules and individual cells immobilized on solid supports, we are in an exciting era of life sciences when molecular-level and cellular level chemistry are being explored and characterized in a highly parallel fashion [1–3]. This approach complements conventional molecular and cellular biochemistry approaches. Given the multitude and cooperative aspects of interactions among biological molecular complexes at cellular and sub-cellular levels, parallel detection of tens or thousands of biochemical reactions in microarray format will accelerate the process of discovery. Optics-based techniques, such as fluorescence scanning microscopes are an integral part of such a high-throughput characterization process.

Fluorescence-labeling are commonly used in the detection of biochemical reactions on microarrays [1,2]. Typically, one of the reaction partners is tagged with a fluorescent molecule or a quantum dot (through either genetic engineering, such as incorporation of green fluorescence protein or a direct reaction with the host molecule). Fluorescence-labeling enables the detection of as few as a single macromolecule and is driving the field of single-molecule detection in molecular and cellular biology. However, an extrinsic tag, such as a fluorescent molecule or a quantum dot always changes properties of a host macromolecule. The significance of the change is often not known a priori. This is particularly relevant when studying properties of proteins [3]. Subtle changes in binding affinities and associated kinetics of protein molecules, by added physical properties of an extrinsic tag or through tag-induced conformational changes in protein molecules, can have a profound influence on some functions of protein molecules. Recognition of stereo-chemically modified double stranded DNA by specialized proteins in a living system is an example [4]. It is thus sensible to develop label-free detection techniques with adequate sensitivities to complement the fluorescence-based detection methods. The surface plasmon resonance (SPR) and optical ellipsometry (OE) are two optical techniques that have been explored to meet such a need. Both techniques do away with fluorescent labeling by measuring the thickness and the ''density'' of the materials on a flat solid substrate [5–13]. In this short communication, I compare the sensitivity and versatility of these two optical techniques for label-free detection of biochemical reactions on solid-supports.

In a surface plasmon resonance process, a surface-bound electromagnetic wave is excited at the interface usually between a metal (ε_m) and a transparent material (ε_s). The surface-bound wave, also known as the surface-plasmon

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polariton wave, has a well-defined wavevector along the interface $q = (\omega/c)\sqrt{\varepsilon_m\varepsilon_s/(\varepsilon_m+\varepsilon_s)}$ [14]. To excite a surface-plasmon polariton wave, one can employ the Kretschmann configuration by depositing a film of the metal (ε_m) on a glass prism (ε_g) and exposing the other side of the film to the material with ε_s, as shown in Fig. 1. When a monochromatic light beam enters from the glass prism side onto the metal film, the reflected intensity is strongly attenuated over a narrow range of incidence angle θ_g, where the wavevector of the incident beam along the surface, $(\omega/c)\sqrt{\varepsilon_g}\sin\theta_g$, matches the wavevector of the surface-plasmon polariton wave. The incidence angle θ_g at which the reflection is maximally attenuated is the surface-plasmon resonance angle θ_{SPR}, given by $\sin\theta_{SPR} = \sqrt{\varepsilon_m\varepsilon_s/(\varepsilon_m+\varepsilon_s)\varepsilon_g}$. Another way to excite a surface-plasmon polariton wave is to deposit a metal film (ε_m) on an optical grating with spatial period of a, and then expose the film to the material with ε_s. When a monochromatic light is incident onto the metal film *directly* from the medium ε_s, the surface-plasmon polariton wave is excited at the angle θ_{SPR}, given by $\sin\theta_{SPR} \cong \sqrt{\varepsilon_m\varepsilon_s/(\varepsilon_m+\varepsilon_s)} - \lambda/a$, where the specular reflectance is again maximally attenuated [15,16]. When an ultrathin film with thickness d and optical dielectric constant ε_d is deposited on the metal (e.g. a monolayer of biological macromolecules), the SPR angle shifts. In the limit that d is much less than the penetration depth of the surface-plasmon polariton wave, the shift (in radians) is given by [17,18].

$$\delta\theta_{SPR} \cong \left(\frac{2\pi d}{\lambda}\right)\left(\frac{\sqrt{-\varepsilon_m\varepsilon_s\varepsilon_g}}{\varepsilon_m^2-\varepsilon_s^2}\right)\left(\frac{\sin^2\theta_{SPR}}{\cos\theta_{SPR}}\right)\frac{(\varepsilon_d-\varepsilon_m)(\varepsilon_d-\varepsilon_s)}{\varepsilon_d}. \quad (1)$$

In the application of SPR to detection of ultrathin films on solids, one measures $(d/\lambda)(\varepsilon_d-\varepsilon_s)/\varepsilon_d$ through $\delta\theta_{SPR}$, up to a factor $\left(\sqrt{\varepsilon_s\varepsilon_g/(-\varepsilon_m)}\right)(\sin^2\theta_{SPR}/\cos\theta_{SPR})$. This factor is compeletely determined by ε_s, ε_g, and ε_m. For example, at the gold-water interface,$\varepsilon_s = 1.77$, $\varepsilon_m = -13$, $\theta_{SPR} = 71°$,

$$\delta\theta_{SPR} \approx \left(\frac{3\pi d}{\lambda}\right)\frac{(\varepsilon_d-\varepsilon_s)}{\varepsilon_d}. \quad (2)$$

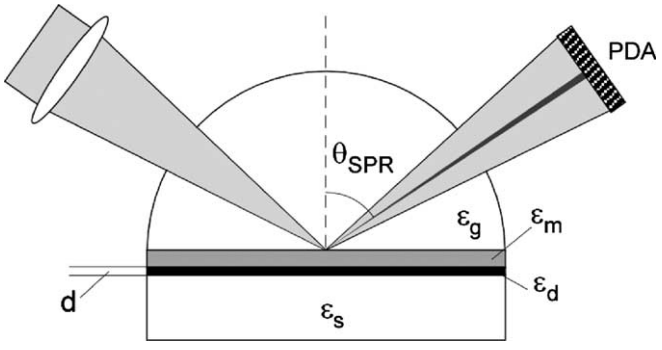

Fig. 1. Prism-coupled angle-resolved surface plasmon resonance setup in Krestchmann configuration. An ultrathin film of ε_d and d at the interface between a metal (ε_m) and a medium (ε_s) shifts the SPR angle from $\theta_{SPR} = \sin^{-1}\left(\sqrt{\varepsilon_m\varepsilon_s/(\varepsilon_m+\varepsilon_s)\varepsilon_g}\right)$ by $\delta\theta_{SPR}$ as given in Eq. (1). The shift is detected with a photo-detector array (PDA).

The SPR angle shift is often expressed in terms of resonance units (RU) with one resonance unit corresponding to 2×10^{-6} rad. The sensitivity of the SPR technique is about 1 RU.

In optical ellipsometry (OE), one measures *differential changes in phase and magnitude of the reflectivity* for p-polarized and s-polarized components of a monochromatic light [19]. The most sensitive form is the polarization-modulated nulling ellipsometry in which the changes can be directly measured as is done in the oblique-incidence optical reflectivity difference (OI-RD) technique. The latter is illustrated in Fig. 2 [19,20]. It is noteworthy that (1) by using nulling steps, one minimizes the effect of the intensity noise in the probe light source; and (2) by using the polarization modulation, one has the choice of detecting the optical reflectivity difference signals at frequencies where the electronic noise spectrum is quiet. Let a monochromatic light incident at an oblique angle θ from a transparent material with ε_0 onto the interface with another material with ε_s. Let $r_{p0} = |r_{p0}|\exp(i\phi_{p0})$ and $r_{s0} = |r_{s0}|\exp(i\phi_{s0})$ be the complex reflectivities from the bare interface, $r_p = |r_p|\exp(i\phi_p)$ and $r_s = |r_s|\exp(i\phi_s)$ the reflectivities from the interface covered by an ultrathin film with thickness d and optical dielectric constant ε_d. Let $\Delta_p \equiv (r_p - r_{p0})/r_{p0}$, $\Delta_s \equiv (r_s - r_{s0})/r_{s0}$ and define the differential change in reflectivity or the reflectivity difference as $\Delta_p - \Delta_s$. When the film thickness d is much less the optical wavelength λ, Zhu and coworkers have shown [21].

$$\begin{aligned}\Delta_p - \Delta_s &\cong \left(\frac{|r_p|-|r_{p0}|}{|r_{p0}|} - \frac{|r_s|-|r_{s0}|}{|r_{s0}|}\right) + i(\Delta\phi_p - \Delta\phi_s)\\ &\cong i\left(\frac{2\pi d}{\lambda}\right)\frac{(\varepsilon_d-\varepsilon_s)}{\varepsilon_d}\left(\frac{\varepsilon_s(\varepsilon_0-\varepsilon_d)}{(\varepsilon_s-\varepsilon_0)\sqrt{\varepsilon_0}}\right)\left(\frac{2\cos\theta\tan^2\theta}{\varepsilon_s/\varepsilon_0-\tan^2\theta}\right).\end{aligned} \quad (3)$$

The oblique-incidence reflectivity difference (OI-RD) technique *directly* measures the complex reflectivity difference, given by Eq. (3). From Eqs. (2) and (3), it is clear that

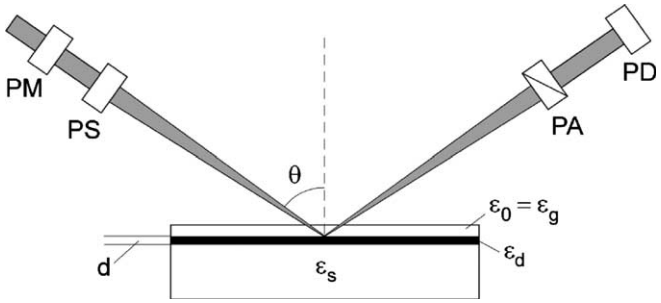

Fig. 2. Oblique-incidence reflectivity difference (OI-RD) setup. PM, polarization modulator operated a frequency Ω; PS, phase shifter; PA, polarization analyzer. An ultrathin film of ε_d and d at the interface between a transparent substrate (ε_0) and a medium (ε_s) disproportionately changes the phase and magnitude of reflectivity for s- and p-polarized light. The differential phase and magnitude changes as given in Eq. (3) are measured with a photodetector (PD) in forms of different harmonics of τ in the reflected light beam.

the OI-RD technique measures the same properties of an ultrathin film as SPR, namely, $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$. The prefactor $[\varepsilon_s(\varepsilon_0 - \varepsilon_d)/\sqrt{\varepsilon_0}(\varepsilon_s - \varepsilon_0)][2\cos\theta\tan^2\theta/(\varepsilon_s/\varepsilon_0 - \tan^2\theta)]$ depends separately on the incident angle, ε_s, ε_0, and ε_d. In the detection of *unlabeled* microarrays on transparent solid supports, ε_s, ε_0, and ε_d are all real and positive. Consequently, the primary reflectivity difference signal in response to $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$ is the *differential phase change* $\Delta\phi_p - \Delta\phi_s = Im\{\Delta_p - \Delta_s\}$. The differential change in the magnitude of reflectivity is of the order of $(d/\lambda)^2$ and thus much smaller. I particularly note that when the incidence angle approaches the Brewster angle $\theta_B = \tan^{-1}\left(\sqrt{\varepsilon_s/\varepsilon_0}\right)$, *the measurable* $\Delta\phi_p - \Delta\phi_s$ *is enhanced by the angular factor* $2\cos\theta\tan^2\theta/(\varepsilon_s/\varepsilon_0 - \tan^2\theta) \sim 1/(\theta_B - \theta)$. Since other sources of errors in the OI-RD system are not expected to change significantly when θ approaches θ_B, this strategy maximizes the signal-to-noise ratio and in turn optimizes the sensitivity to $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$. For example, with a noise background of 2×10^{-5} in an OI-RD system, one achieves the same sensitivity of 2×10^{-6} rad (i.e., one SPR resonance unit) by making the prefactor ~10 with a proper incidence angle.

I now compare the sensitivity and versatility of an SPR technique and an OI-RD technique for label-free detection of microarrays of biological macromolecules on solid supports. Clearly with the availability of Brewster angle, the polarization-modulated nulling ellipsometry is at least as sensitive as the surface plasmon resonance technique to non-fluorescent properties of an ultrathin film. The application of an SPR technique requires a transparent substrate that is coated with a metal film of a few tens of nanometers in thickness. The thickness of the metal film, typically 50 nm for gold, needs to be uniform over the illuminated region to ensure a well-defined SPR angle. This is because that at 50 nm away from a gold surface the electric field of the surface-plasmon polariton wave is still 13% of the field in the immediate vicinity of the metal surface. Consequently, a variation in thickness of the gold film leads to a spread of the SPR angle and a reduced sensitivity of SPR to $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$. The requirement of high-quality gold coating on transparent substrates can be costly and thus limit the application of the SPR technique. Furthermore, to measure the shift in the SPR angle as given in Eq. (2), one illuminates the sample surface with a converging light beam and measures the attenuated total internal reflectance at a series of incidence angles with a linear detector array. This scheme of detection inherently limits the SPR technique to a small number of features on a microarray. It is noteworthy that the grating-coupled SPR imaging technique has enabled throughputs up to hundreds of binding events simultaneously [9].

In comparison, the application of the oblique-incidence optical reflectivity difference (OI-RD) technique imposes no special requirements on solid substrates [12]. This makes the OI-RD technique compatible with all glass-slide supported microarrays and microarrays on other transparent solid substrates. Furthermore, to determine $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$, one only needs to measure the corresponding OI-RD signals at one incidence angle with one detector. As a result, this particular form of optical ellipsometry technique inherently supports high throughputs in biomolecular microarray detection. By using linear detector arrays instead of single detector, one should be able to detect $(d/\lambda)(\varepsilon_d - \varepsilon_s)/\varepsilon_d$ in real time from hundreds to tens of thousands of distinct features on a microarray.

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