

## Overview of Surface Plasmon Resonance

Common  
Revision A.00

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The SensiQ line of instruments are surface plasmon resonance (SPR)-based biosensing platforms that enable high end functional characterization of biomolecules.

SPR is an optical phenomenon that provides a non-invasive, label-free means of observing binding interactions between an injected analyte and an immobilized biomolecule in real time. The SPR effect is sensitive to binding of analyte because the associated increase in mass causes a proportional increase in refractive index, which is observed as a shift in the resonance angle. A flow injection analysis configuration is commonly employed in which the analyte of interest, solvated in a buffer solution, is transported across the sensing surface, where it interacts with the immobilized biomolecule. When combined with appropriate surface chemistry, microfluidics and software, this technique is unmatched in its range of applications including:

- Affinity analysis
- Kinetic analysis
- Concentration assays
- Active concentration assays
- Binding stoichiometry
- Thermodynamic analysis
- Study of interaction mechanisms
- Dependence of interaction on environmental conditions
- Routine screening
- Ligand-fishing
- Epitope mapping

## Surface Plasmon Resonance

SPR transducers are usually constructed by using prism coupling of incident light onto an optical substrate that is coated with a semitransparent noble metal under conditions of total internal reflection. First, a condition of total internal reflection (Figure 1) must exist at the interface. Total internal reflection will exist until the incident angle decreases to  $\theta_c$  (critical angle), at this angle; some of the light is refracted across the interface. At the point of reflection at the interface, an evanescent field (standing wave) will penetrate the exit medium to a depth in the order of 1/4 of the incident light wavelength.

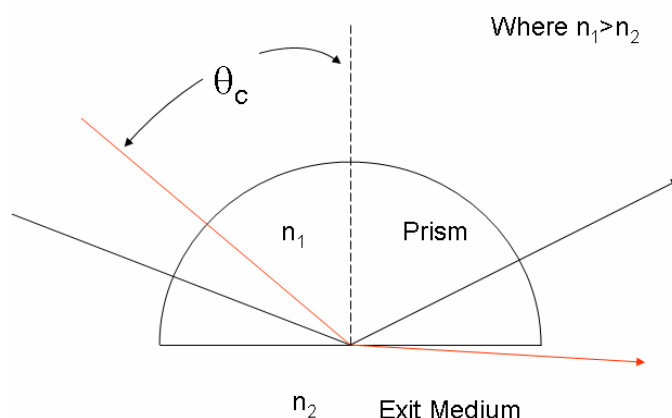


Figure 1: Total internal reflection at a prism interface.

$n_1$  and  $n_2$  are the refractive indexes of the prism and exit medium, respectively. The refracted beam at the critical angle is shown in red. All light is reflected at incident angle greater than  $\theta_c$ .

If a semitransparent noble metal film is placed at the interface (Figure 2), then under conditions of total internal reflection surface plasmon resonance can occur. This is commonly known as the Kretschmann configuration.

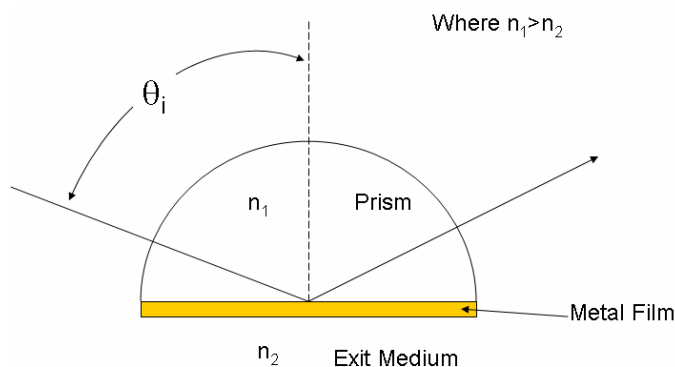


Figure 2: SPR using a prism coupling configuration.

A condition of surface plasmon resonance will occur when the following conditions are satisfied. The incident wave vector is given by the following expression:

$$K_i = \left( \frac{2\pi}{\lambda} \right) n \sin \theta_i$$

where  $K_i$  is a component of the incident light wave vector parallel to the prism interface,  $\theta_i$  is the incident light angle,  $\lambda$  is the wavelength of the incident light and  $n$  is the refractive index of the prism.

The wave vector of the plasmon mode is described by the following expression

$$K_p = \left( \frac{2\pi}{\lambda} \right) \sqrt{\frac{\epsilon_1 \epsilon_2}{\epsilon_1 + \epsilon_2}}$$

where  $K_p$  is the surface plasmon wave vector and  $\epsilon_1$  and  $\epsilon_2$  are the dielectric permittivity constants of the metal film and the dielectric exit medium, respectively.

Surface plasmon resonance occurs when

$$K_i = K_p$$

The intensity of the reflected light will decrease where surface plasmon resonance exists thereby giving rise to a well defined minimum in the reflectance intensity. If the incident angle is fixed and polychromatic and light is reflected from the surface, then light will be adsorbed by the resonance at particular wavelengths giving rise to a typical plasmon resonance minimum in the reflectance spectrum. If monochromatic light is reflected from the surface over a range of incident angles then a similar reflectance minimum will occur with respect to the incident angle. The reflectance minimum that results from plasmon resonance is caused by the phase difference of the surface mode relative to the incident photon field. In fact the phase difference below the resonance is  $0^\circ$  and approaches  $180^\circ$  above the resonance. Therefore the photons reflecting from the metal-prism interface undergo destructive interference with the photons emitted by the excited plasmons (being  $180^\circ$  out of phase just above the maximum resonance) and this result in the characteristic reflectance minimum. Figure 3 shows a typical SensiQ SPR minimum.

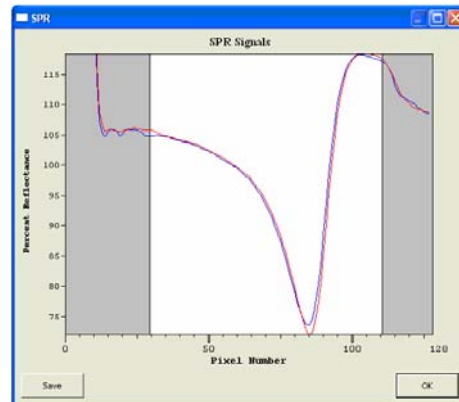


Figure 3: SensiQ Dual Channel SPR Minima.

For biosensing, it is this change in the refractive index of the dielectric exit medium at the gold surface that is of interest. Therefore, if the refractive index of the prism is constant then a change in the resonance condition may be related to changes in the refractive index of the exit medium. In this way, it is possible to monitor the accumulation of biofilms on the gold surface in order to measure binding of biomolecules to a surface that has been coated with an affinity ligand. This SPR configuration may be considered a surface sensitive refractometer where the sensitivity depth is defined by the penetration depth of the evanescent field (~200nm).

## Binding Response Curves

The increase in mass associated with a binding event causes a proportional increase in refractive index which is observed as a change in response (Figure 4). This response is defined in response units (RU) where 1 RU is equivalent to 1  $\mu$ Refractive index unit, which represents approximately 1pg of protein/mm<sup>2</sup>. During an injection, the injected analyte ( $A_0$ ) is transported to the interaction surface by convection and diffusion where its concentration ( $A$ ) may change due to complex formation. This change in concentration is minimized by ensuring a high mass transport coefficient ( $k_m$ ) hence the use of nL scale flow cells. Immobilizing a low concentration of ligand is also extremely effective in ensuring that mass transport of analyte does not become limiting. The response increase during injection of analyte ( $A$ ) is due to the formation of analyte-ligand complexes ( $AB$ ) at the surface. The decrease in response after analyte injection is due to dissociation of the complexes. A binding interaction model may be fitted to this data allowing the kinetic and/or affinity constants to be determined. If particularly fast interactions are recorded, a model that compensates for mass transport limitation (i.e. two compartment model) may be fitted. Bulk index effects and non-specific binding are removed by real-time reference curve subtraction.

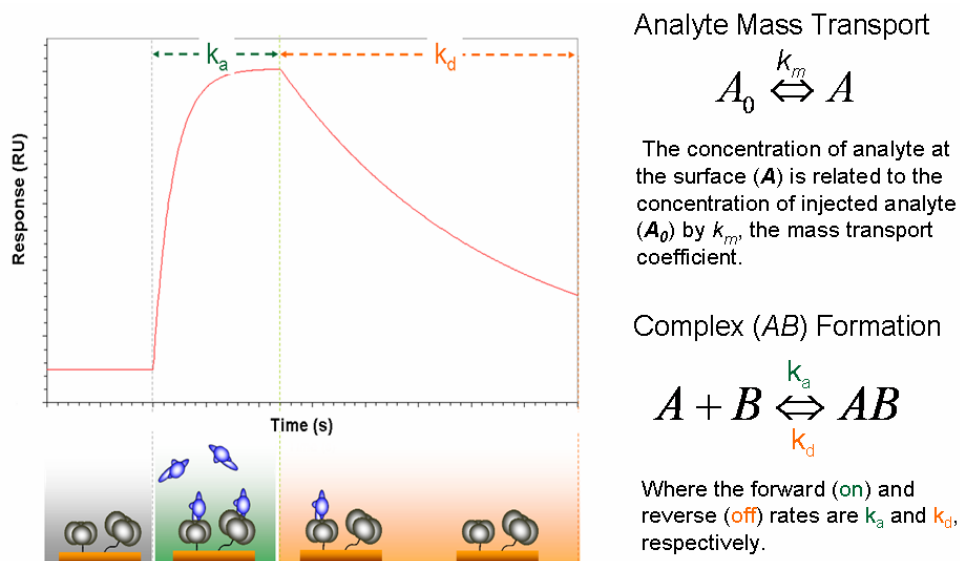


Figure 4: Interpretation of Binding Response Curves.