

# Surface plasmon resonance



## Abstract

Biacore's SPR technology is a label-free technology for monitoring biomolecular interactions as they occur. The detection principle relies on surface plasmon resonance (SPR), an electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions. The resonance is a result of energy and momentum being transformed from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the film from the reflected light. The phenomenon SPR was initially observed by Turbadar [1] although it was the works by Otto [2], Kretschmann and Raether [3], Agerwal [4], and Swalen [5] that brought understanding and showed the versatility of the technique.

In Biacore systems, SPR is used to monitor interactions occurring in a biospecific surface on a metal layer by measuring changes in the solute concentration at this surface as a result of the interactions. For reviews about SPR the reader is referred to Welford [6] and Raether [7].

In order to describe SPR, it is helpful to start with the phenomenon of total internal reflection (TIR) which occurs at an interface between non-absorbing media. When a light beam propagating in a medium of higher refractive index meets an interface at a medium of lower refractive index at an angle of incidence above a critical angle (see Mirabella [8], de Mello [9]), the light is totally reflected at the interface and propagates back into the high refractive index medium (see Figure 1).

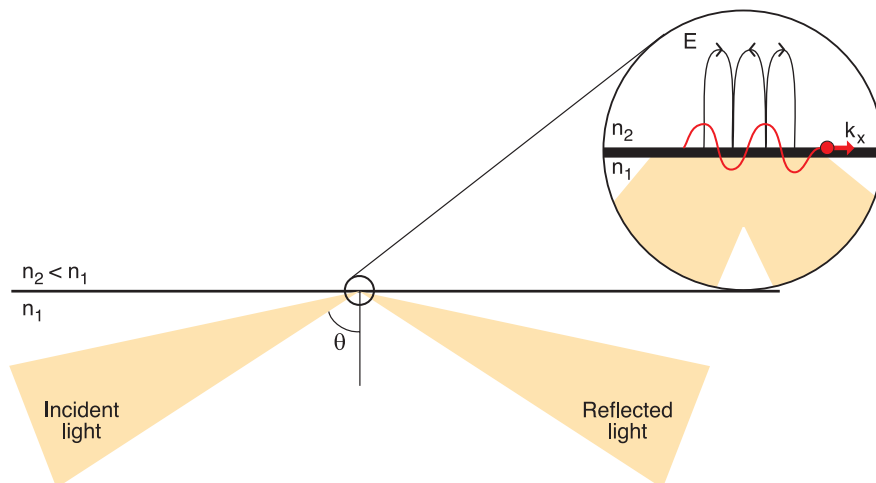


Figure 1. TIR for non-absorbing media. Light propagating in a medium of refractive index  $n_1$  undergoing total internal reflection at the interface with the medium of a lower refractive index  $n_2$ . The evanescent field,  $E$ , is a non-transverse wave having components in all spatial orientations, decreasing in field intensity with penetration into medium of  $n_2$  (Mirabella 7).  $\theta$  is the angle of incidence.

Although the fully reflected beam does not lose any net energy across the TIR interface, the light beam leaks an electrical field intensity called an evanescent field wave into the low refractive index medium. The amplitude of this evanescent field wave decreases exponentially with distance from the interface, decaying over a distance of about one light wavelength from the surface (Figure 4). If the lower refractive index media has a non-zero absorption coefficient, the evanescent field wave may transfer the matching photon energy to the medium. This is exploited in internal reflection spectroscopy (IRS) as reviewed by Mirabella [8]. The penetration depth of the evanescent field wave is usually defined as the distance over which the wave decays to  $1/e$ , or about 37%, of its maximum intensity.

If the TIR-interface is coated with a layer of a suitable conducting material, such as a metal, of a suitable thickness the p-polarized component of the evanescent field wave, may penetrate the metal layer and excite electromagnetic surface plasmon waves propagating within the conductor surface that is in contact with the low refractive index medium (Figure 2). For a non-magnetic metal like gold, this surface plasmon wave will also be p-polarized and, due to its electromagnetic and surface propagating nature, will create an enhanced evanescent wave (Figure 4).

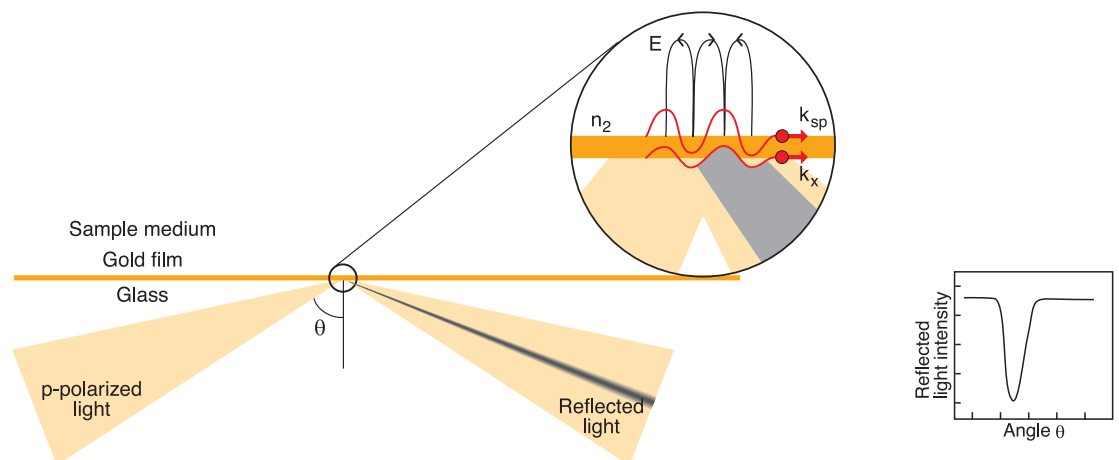
This evanescent wave has electric field components directed in all spatial

orientations during penetration into the low refractive index medium. Because the electric field penetrates a short distance into the lower refractive index medium, the conditions for SPR are sensitive to the refractive index at the gold surface.

For plasmon excitation by a photon to take place the energy and momentum of these “quantum-particles” must both be conserved during the photon “transformation” into a plasmon. This requirement is met when the wavevector for the photon and plasmon are equal in magnitude and direction for the same frequency of the waves (the wavevector is a parameter in the mathematical formula for the electromagnetic wave related to the momentum). The direction of the wavevector is the direction of the wave propagation (i.e. the light ray direction), while its magnitude depends on the refractive indices of the media that the electromagnetic field wave interacts with along its propagation path.

Since the wave vector of the plasmon wave is bound to the conductor surface, it is the wavevector of the component of the incident light which is parallel to the conductor surface that can be equal to the wave-vector of the surface plasmons ( $k_{sp}$ ,  $k_x$  in Figure 2). The magnitude of the surface-parallel wavevector,  $k_x$ , is the wavevector of the incident light times  $\sin(\theta)$ ,  $k_x = (2\pi/\text{wavelength}) * n_1 * \sin(\theta)$  (Figure 1).

Figure 2. SPR is excited by p-polarized totally internally reflected light at a glass/metal film interface, the surface plasmon enhancing the evanescent field amplitude, E. In Biacore systems which use a sensor chip, this interface takes the form of an exchangeable gold-coated glass slide. SPR is observed as a dip in the reflected light intensity at a specific angle of reflection.



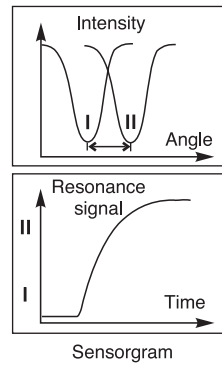
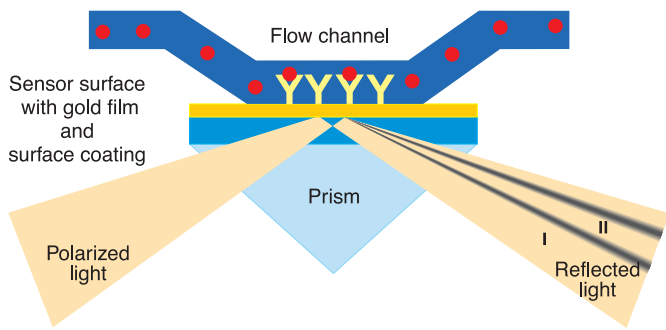


Figure 3. In Biacore systems, the incident p-polarized light is focused into a wedge-shaped beam providing simultaneously a continuous interval of light wavevectors  $k_x$ . This range covers the working range for the plasmon wavevector  $k_{sp}$  during biomolecular interaction analysis. An increased sample concentration in the surface coating of the sensor chip causes a corresponding increase in refractive index which alters the angle of incidence required to create the SPR phenomenon (the SPR angle). This SPR angle is monitored as a change in the detector position for the reflected intensity dip (from I to II). By monitoring the SPR-angle as a function of time the kinetic events in the surface are displayed in a sensorgram.

The wavevector of the plasmon wave,  $k_{sp}$ , depends on the refractive indices of the conductor,  $n_{gold}$ , (being a constant complex number) and the sample medium,  $n_2$ , as  $k_{sp} = (2 * \pi / \text{wavelength}) * (n_{gold}^2 * n_2^2 / (n_{gold}^2 + n_2^2))^{0.5}$ . In both expressions the wavelength is the value for the light wave in vacuum. Thus, an increased refractive index of the sample,  $n_2$ , penetrated by the plasmon enhanced evanescent field increases the wavevector of the plasmon wave.

The wavevector of the light  $k_x$  can be tuned to equate the plasmon wavevector by varying either the angle of incidence,  $\theta$ , or the wavelength of the light, Figure 2. The dielectric equations describing this dependence and the application of this technology are discussed in detail by Swalen [5], Kovacs [10], Kretschmann [11], Liedberg [12], Jönsson [13] and Davies [14].

The wavevector and energy match enables a resonant absorption of energy via the light-evanescent wave field, a plasmon

excitation (SPR) causing a characteristic drop in the reflected light intensity. For a given wavelength of incident light, SPR is seen as a dip in the intensity of reflected p-polarized light at a specific angle of incidence (Figure 2).

In Biacore systems which use sensor chips, monochromatic light is focused in a wedge-shaped beam on the TIR interface and the angle of minimum reflectance intensity is determined using a two-dimensional detector array (see Figure 3). The low refractive index medium is the surface coating of the sensor chip and the “surrounding” sample solution.

Biomolecular interactions occurring at the sensor surface change the solute concentration and thus the refractive index within the evanescent wave penetration range. The angle of incidence required to create the SPR phenomenon (the SPR angle) is therefore altered and it is this change which is measured as a response signal.

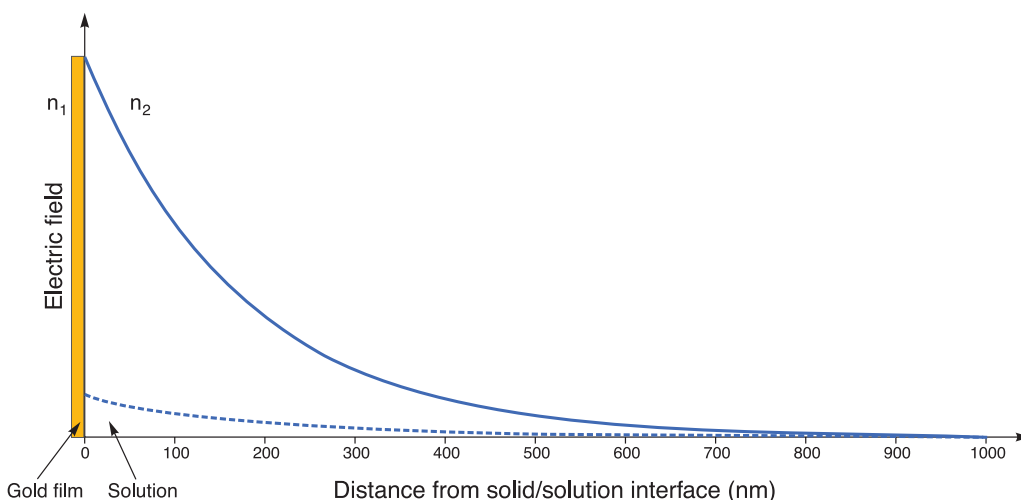


Figure 4. Relative evanescent electric field amplitude (E) versus distance to solid/solution interface (nm). Continuous line for SPR-evanescent wave (gold film), dashed line for non-absorbing TIR (no gold film).

In general, different proteins have very similar specific refractive index contributions, i.e. the refractive index change is the same for a given change in concentration (see Polymer Handbook<sup>15</sup>). Values for glycoproteins, lipoproteins and nucleic acids are of the same order of magnitude. SPR thus provides a mass detector which is essentially independent of the nature of the interactants. Most importantly, the technique requires no labelling of the interacting components,

and the possibility of a mass detector is realized. Moreover, since it is the evanescent field wave and not the incident light that penetrates the sample, measurements can be made on turbid or even opaque samples. Sensitivity considerations for SPR have been discussed by Kooyman<sup>16</sup>, and linear correlation between resonance angle shift and protein surface concentration has been shown by Stenberg<sup>17</sup>.

## References

1. Turbadar, T., Proc. Phys. Soc. (London) 73; 40 (1959).
2. Otto, A., Z. Phys. 216; 398 (1968) and Phys. Stat. Solidi. 26; 199 (1968).
3. Kretschmann, E. and Raether, H., Z Naturf. 230; 2135 (1968).
4. Agarwal, G.S., Phys. Rev. B8; 4768 (1973).
5. Swalen, J.D., J. Mol. Electron. 2; 155 (1986).
6. Welford, K., Opt. Quant. Elect. 23; 1 (1991).
7. Raether, H., Physics of Thin Films 9; 145 (1977).
8. Mirabella, F.M. and Harrick, N.J., "Internal Reflection Spectroscopy: Review and Supplement" (Harrick Scientific Corporation) 3 (1985).
9. de Mello, A.J. in "Surface Analytical Techniques for Probing Biomaterial Processes" (Davies, J., Ed., CRC Press, Boca Raton, New York) 1 (1996).
10. Kovacs, G. in "Electromagnetic Surface Modes" (Ed. Boardman, A.D., Wiley) 143 (1982).
11. Kretschmann, E., Z. Phys. B241; 313 (1971).
12. Liedberg, B., Sensors Actuators 4; 299 (1983).
13. Jönsson, U. et al., BioTechniques 11; 620 (1991).
14. Davies, J. and Faulkner, I., in "Surface Analytical Techniques for Probing Biomaterial Processes" (Davies, J., Ed., CRC Press, Boca Raton, New York) 67 (1996).
15. Polymer Handbook 3rd ed., VII; 469 (1989, Wiley).
16. Kooyman, R.P.H. et al. Anal. Chim. Acta 213; 35 (1988).
17. Stenberg, E., et al. J. Coll. Interface Sci. 143; 513 (1991).