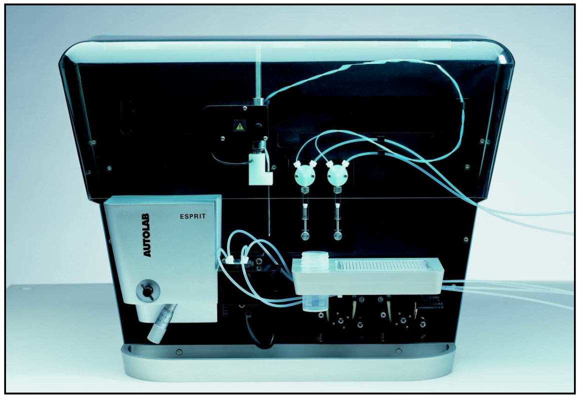


Surface Plasmon Resonance with the Autolab SPR instruments

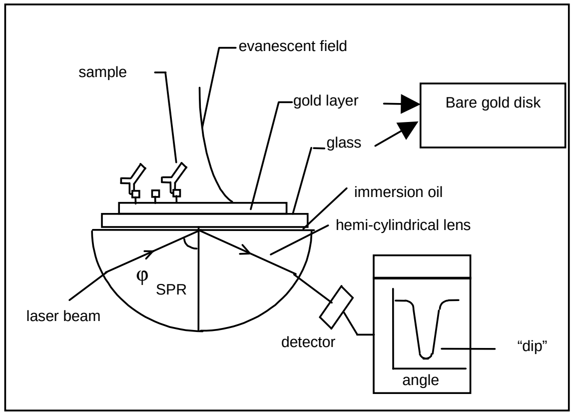
Introduction

Surface Plasmon Resonance is a surface sensitive optical technique that can be used to study nano thin (organic) layers on noble metal films. The technique is based on measuring the change in refractive index due to, for example binding of an organic layer on a metal surface.

Theory

The surface plasmon resonance effect is based on the interaction between electromagnetic waves of incident light and free electrons in a conducting surface layer. When light is reflected at the interface of two dielectric media, total internal reflection will take place above the so-called critical angle. At the same time, the light will generate an evanescent field. This electromagnetic field has maximum intensity at the surface of the dielectricum. In case a metal layer is present in the evanescent field, the field can be enhanced and the free electrons on the outer surface (also called: surface plasmons) can resonate with the field. This is called surface plasmon resonance. In cases where this resonance effect takes place, light will be transferred to surface plasmon waves and one will thus see a decrease in the amount of reflected light. The angle at which this decrease occurs is called the resonance angle, see figure 1. Because the wave vector of light in air is always smaller than the wave vector in the noble metal surface, a

Figure 1: Schematic view of SPR principle.



prism is needed between the sample and air to make sure that the wave vectors in both media can be the same. The SPR effect is useful because any change in the dielectric constant (or the refractive index) will result in a change in the resonance angle.

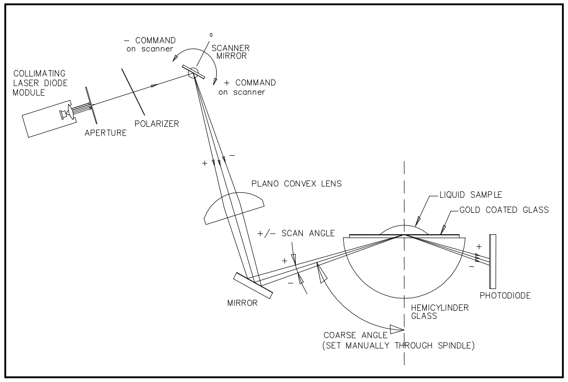
Methods

There are a few different ways to measure the resonance angle. Most well known are the methods:

1. Used by Biacore in which a convergent light beam is used. This beam results in a number of incidence and reflecting angles. The resonance angle then shows up on the detecting photo-diode as a dark spot. Advantage of this method is that no mechanical parts are needed to control the angle of incidence. Disadvantage is that the resolution in refractive index is usually only around 10^{-3}.
2. Used in the Autolab SPR instrument (see figure 2) in which the reflection is measured as a function of the incident angle of the light beam. The incident angle is changed by using a vibrating mirror. The advantage of this system is that within a short time a broad range of incident angle can be measured. In the vibrating mirror set-up, the angular shift is measured for a non-coated gold sensor surface with a resolution of approximately 0.05 millidegrees, corresponding to a refractive index resolution of approximately $1*10^{-6}$. For a coated gold sensor surface, the angular shift is measured with a resolution of approximately 0.1 millidegrees.
3. Where instead of changing the angle of incidence, the wavelength of the light is changed. This method is used in an optical fibre setup.

The SPR effect is only available on metals in which the electrons behave as a free electron gas, meaning that their motions are independent of the charge that they leave behind when moving. This limits the choice of metal to copper, silver, aluminium and gold.

Figure 2: Autolab SPR optical principle.



Gold is by far the most used in biological applications. The thickness of the metal layer depends very much on the optical constants of the bulk material as well as on the wavelength of the light. The Autolab SPR instrument uses 670 nm as a wavelength resulting in an optimum thickness for the gold layer of about 50 nm. There is a linear relationship between the amount of bound material and shift in SPR angle. The SPR angle shift in millidegrees is used as a response unit to quantify the binding of macromolecules to the sensor surface. The response also depends on the refractive index of the bulk solution. A change of 120 millidegrees represents a change in surface protein of approximately 1ng/mm2, or in bulk refractive index of approximately 10^{-3}.

The detection principle limits the size of the analyte that can be studied. If the molecular weight of the compound is below 5000 Dalton, then the change in refractive index upon binding to the sensor surface is too low to be detected directly. Only special modified layers in combination with specific small molecules will show the direct binding. The penetration depth of the evanescent wave of 300-400 nm also determines the size of macromolecules or particles that can be studied. Particles larger than 400 nm cannot be measured totally. As a result, the signal is not linear related to the amount of bound particles. Under these circumstances it is possible to study the binding qualitatively, but a quantitative or kinetic analysis cannot be performed.

Applications

Since the SPR effect is only sensible to changes that take place in a few hundred nm from the surface most of the studies involve adsorbed molecules, meaning that in cases where for example antibody-antigen interactions are studied, one of them will be immobilized on the surface. With the SPR disc that comes with the Autolab SPR instrument the following options are possible:

Bare gold surfaces:

- Macromolecular interaction measurements can be performed by coating the ligand electrostatically to the surface, followed by adding the analyte. After coating, a blocking compound is usually necessary to prevent aspecific interactions. This method is especially suitable for detection of large particles as cells and viruses.
- Biomolecular interaction measurements with biotinylated macromolecules. Firstly, the gold sensor surface is coated with biotin, followed by binding with streptavidin. Secondly, biotinylated molecules are allowed to bind with unoccupied binding sites of streptavidin (stoichiometry streptavidin-biotin interaction is 1:4). Thirdly, binding of the analyte can be measured.
- Biomolecular interaction measurements with thiol containing compounds. Gold shows a strong interaction with sulphur. By applying this property for peptides, self-assembled receptor layers are developed.
- Direct measurement of low molecular weight compounds by response enhancement with latex particles. Low molecular weight compounds can be attached to carboxy modified latex by a carbodiimide coupling reaction Direct binding of low molecular weight compounds coupled to latex particles can be determined using a coated ligand at the sensor surface.
- Measurements on conducting polymers using electropolymerization.

Another option is the use of a so-called sensor-chip (Pharmacia AB, Sweden) which is a wafer covered with a dextran coated gold-layer. The dextran coating is very suitable to study macromolecular interactions. Three methods are used to couple ligands:

- Immobilization of the ligand. The ligand is coupled covalent either by amine functional groups or by thiol functional groups to the dextran layer.
- Non-covalent binding of biotinylated ligand to a streptavidin-immobilized sensor chip. Due to the severe biotin-streptavidin interaction, it is possible to regenerate the surface without breaking the non-covalent biotinstreptavidin

bond. This method is suitable to couple synthetic DNA molecules to the surface.

- Immobilization of capturing antibodies. Capturing antibodies are used when the activity of antibodies is reduced by the immobilization procedure. Detection of antigens is achieved in three steps. Firstly, immobilization of the capturing antibody (for example anti-Rabbit Anti Mouse-Fc). Secondly, binding of the second antibody (a mouse antibody) by the capturing antibody. Thirdly, specific binding of the antigen by the second antibody. Regeneration of the surface will usually break all non-covalent interactions.

These methods have been used to study biomolecular interactions intensively. Examples of biomolecular interactions are:

- peptide-antibody interaction
- protein-antibody interaction, epitope mapping
- protein-DNA interaction
- protein-polysaccharide interaction
- protein-virus interaction
- protein-cell interaction
- protein-T cell receptor interaction
- antibody-antibody interaction, capturing antibody
- DNA-DNA interaction

Electrochemical Surface Plasmon Resonance (ESPR)

Through the combination with an Autolab Potentiostat, the Autolab SPR instrument is very well suited for ESPR experiments, where SPR phenomena are studied in-situ during an electrochemical experiment. This combination of techniques has been described by a number of different groups for example to study the changes in a thin organic film upon change of the electrostatic field (or potential of the electrode). This technique is thus well suited for biosensor development, the development of modified electrodes, electropolymerization, etc.

Experimental example

In the figure below a cyclic voltammogram is shown of toluidine blue in 0.1 M KCl solution. The experiment was performed in the cuvet of an Autolab SPR instrument, where the gold layer of the glass was used as working electrode. A Pt wire was used as counter electrode and a miniature Ag/AgCl electrode as a reference. The change in the SPR resonance angle (or the refractive index) indicating the adsorption of the Toluidine molecule in the oxidized state is shown in the blue curve. It is clearly visible that upon oxidation of the toluidine molecule adsorption on the surface takes place (positive change in SPR angle). In the reduced state, the toluidine molecule is back in solution again.