

TECHNICAL NOTE 122:

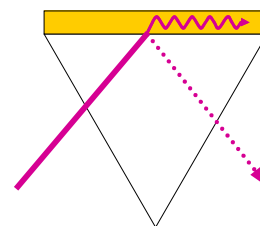
INTRODUCTION TO SPR and SPR IMAGING

Surface Plasmon Resonance (SPR) is an optical method that can be used to monitor molecular events on surfaces. SPR detection does not require fluorescent or other chemical labels. Since even subtle chemical modifications of proteins can profoundly change protein function, such label-free measurements are the preferred approach for protein function studies. There are also significant benefits for genomic applications.

SPR imaging (SPRi) facilitates label-free detection in an array format, greatly improving throughput and experimental robustness. This introduction provides a brief background on the SPRi technique.

Characteristics of Surface Plasmon Resonance

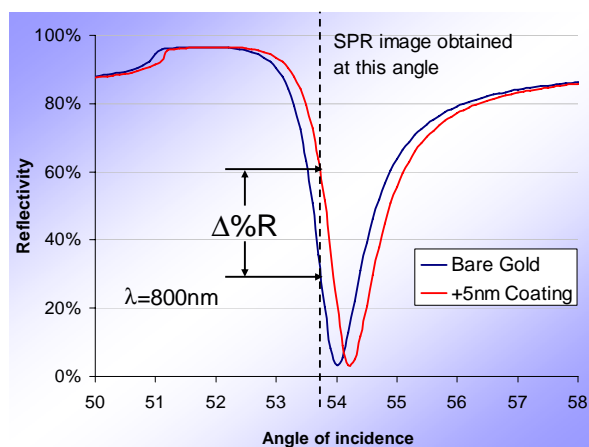
Under just the right conditions, photons in a beam of light incident upon a glass-gold interface “resonate” with the electrons in the gold. As a result, instead of the light simply reflecting off the gold surface, photon energy is converted to a wave of “plasmons” in the gold (see sketch at right). Under ideal conditions, very little light is reflected. The resonance is achieved by “coupling” the photons to the electrons in the metal using either a prism (as illustrated) or grating. Using a piece of plain glass such as a microscope slide will not work. GWC’s SPRiMager®II uses prism-coupled SPR because it is more sensitive than grating-coupled SPR.



SPR can be used to detect events on a surface because adding material to the surface changes the resonance, thereby changing the percent of light reflected. The method is quantitative—the change in reflected light is proportional to the change in mass on the surface.

™ Prism-Coupled SPR

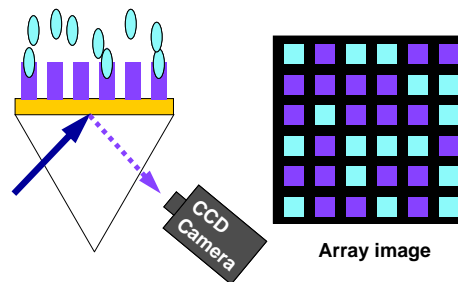
Among many factors affecting the SPR response is the angle of incident light. Traditional instruments measure the SPR response over a range of angles (blue curve in the chart below). When material adsorbs to the gold surface, the curve shifts to the right (red curve in the chart). Traditional instruments determine the shift in “SPR angle” (angle of minimum reflectivity) when material adsorbs to the surface.



SPR imaging

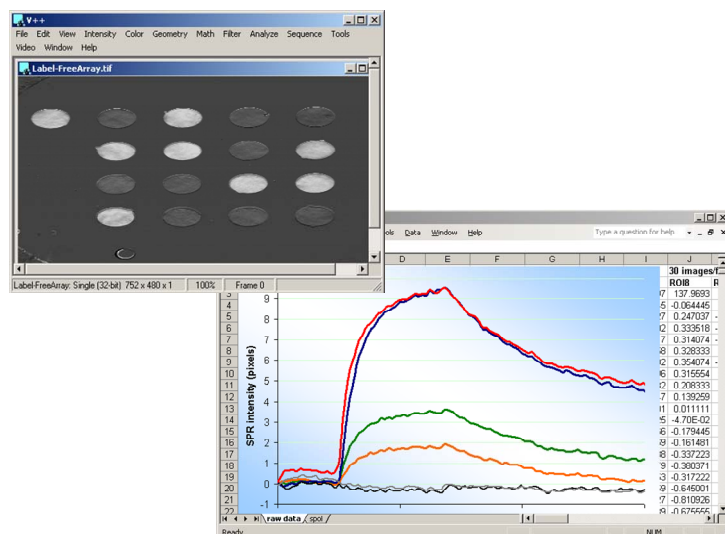
GWC’s SPR imaging platform eliminates the complexity of angle scanning while adding the benefits of array analysis. The SPRiMager®II takes SPR measurements at a fixed angle of incidence, and collects the reflected light with a CCD camera. Changes on the surface are recorded as reflectivity changes, $\Delta\%R$. Thus SPR imaging collects measurements for all elements of an array simultaneously. This arrangement provides for superior experimental robustness over systems that use independent channels for parallel measurements: different elements of the array can be reserved for positive and negative controls whose measurements are collected at the same time and under the same condition as experimental samples.

The cartoon at right illustrates how reflectivity differences across the array yield information on molecular events on the array. The blue rectangles on top of the gold represent molecular probes—e.g. antibodies. The pale blue ellipses represent analyte—e.g. antigens. On the array image, the darkest areas lie between the molecular probes, as these areas have the least mass. The mid-blue areas where the probes are located have more mass, hence more reflectivity. The pale blue areas where analyte has bound to probe. The relationship between mass changes on the surface and changes in reflected light is linear for a change of up to 10% reflectivity.



Real Time Monitoring

Data analysis for the SPRImager®II involves quantifying changes in reflectivity between array images collected at different times, such as before and after binding of analyte to probes. Quantification is performed on “difference images”. For example an image collected before binding (the “reference image”) is subtracted from an image collected after binding. The software is usually set to collect images at regular intervals throughout the course of an experiment, and difference images are calculated and displayed as the data are collected. Simultaneous display of the current array image and the chart quantifying reflectivity changes is one of the most powerful capabilities of the SPRImager®II.



For the experiment shown at left, an array was made with antibodies raised to the same antigen, alongside control antibodies that do not recognize the antigen. The antigen was then flowed over the array, and binding to antibodies was monitored on the SPRImager®II. The array was then washed in buffer to monitor dissociation of antigen.

The array image shown is the end-point of the experiment, the result of subtracting the initial reference image from the last image collected. The brighter the spot, the greater the mass of antigen bound to antibody. The Excel® chart shows the raw data obtained for reflectivity changes on the array during the experiment. To

complete the analysis, the control curves for non-binding antibody (bottom pair in the chart) are subtracted from the curves for the experimental antibodies to generate accurate reflectivity changes corrected for any nonspecific binding.

Versatility of the SPRImager®II Platform

Since the SPR response occurs regardless of the chemical composition of molecules binding to the surface, SPR imaging can be applied to analysis of virtually any biomolecular interaction, as well as many other types of chemical and surface interactions. GWC’s web site provides examples of the use of the SPRImager®II for a variety of applications in proteomics and genomics research.

For more information and for protocols for specific applications, please contact your GWC Technologies representative.

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