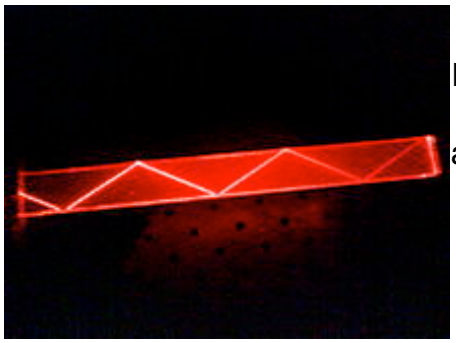
Bioanalytical chemistry

7. Surface plasmon resonance

Total Internal Reflection Fluorescence (TIRF) Microscopy

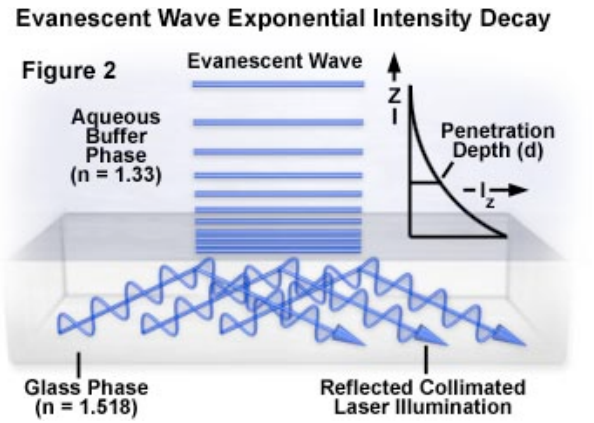


http://en.wikipedia.org/wiki/File:TIR_in_PMMA.jpg

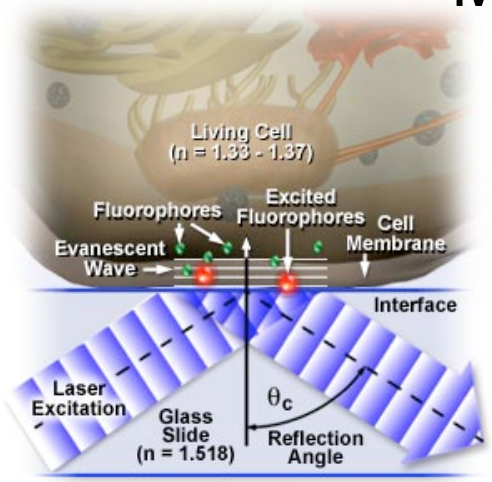
Light is totally reflected when it strikes an interface at an angle greater than the critical angle (defined as the angle above which total reflection occurs, recall Snell's law)

http://snowflakemorayeel.wordpress.com/category/pets/

- When light is internally reflected, a standing wave known as an **evanescent wave** is formed at the boundary
- The strength of this wave decays exponentially with distance from the boundary



Total Internal Reflection Fluorescence (TIRF) Microscopy

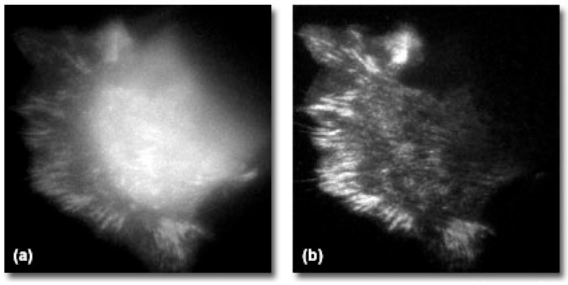


The energy of the evanescent wave is equal to the energy of that reflected light. Accordingly, the evanescent wave can be used to excite appropriate fluorophores to the excited state.

This allows the selective excitation of fluorophores that are in close proximity (i.e., within 200 nm) to the interface. For example, if a cell is attached to the surface, only those fluorophores in the membrane, or very close to the membrane, will be excited and observed in the fluorescence image.

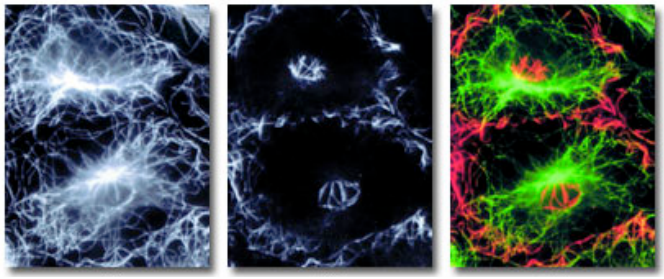
focal adhesions

widefield TIRF



tubulin

widefield TIRF Overlay

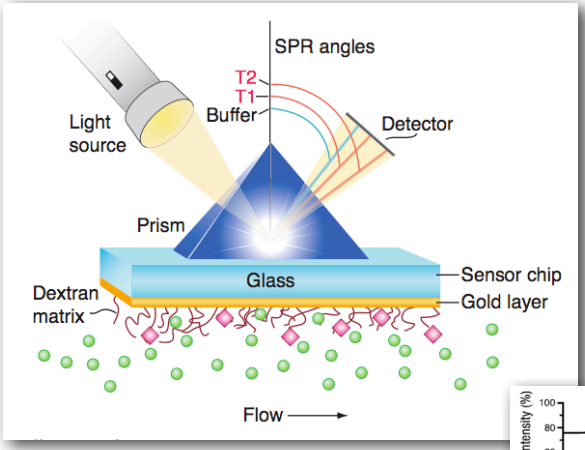


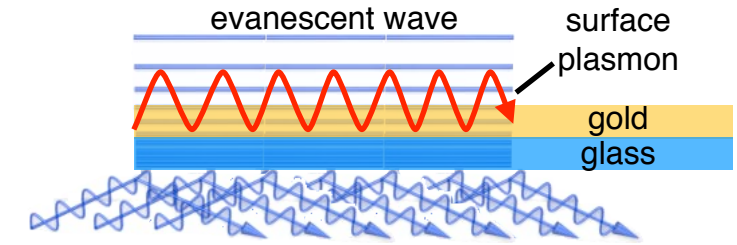
- TIRF microscopy is one of the most sensitive fluorescence microscopy techniques and is routinely used for single molecule imaging. The big advantage is that the fluorescence background is very low since fluorophores further from the surface are not being excited.
- Wilson, W.D., Analyzing Biomolecular Interactions, Science 295, 2002, 2103-2105.

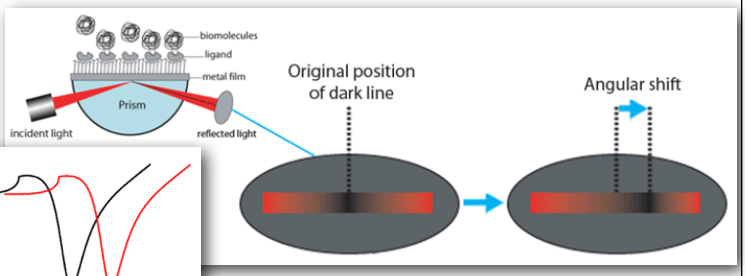
Surface plasmon resonance

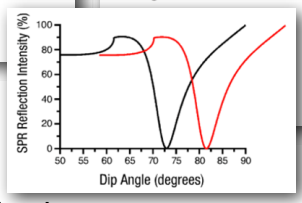
SPR is another technique that makes use of the evanescent wave formed when light is reflected from a surface.

However, SPR is not typically used for imaging, but rather for characterizing or detecting intermolecular interactions.





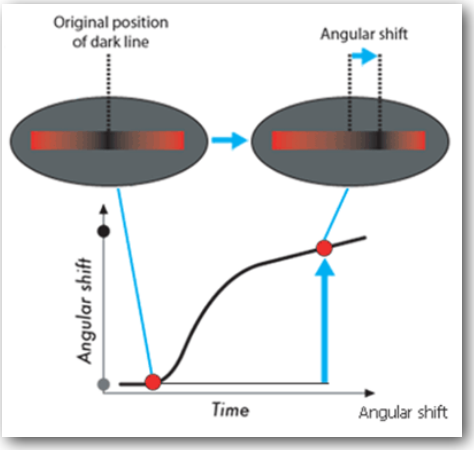


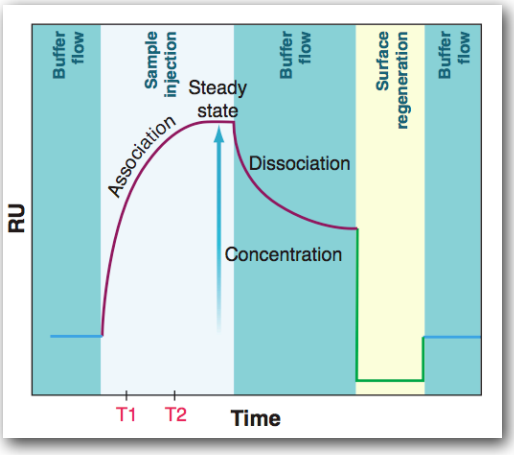


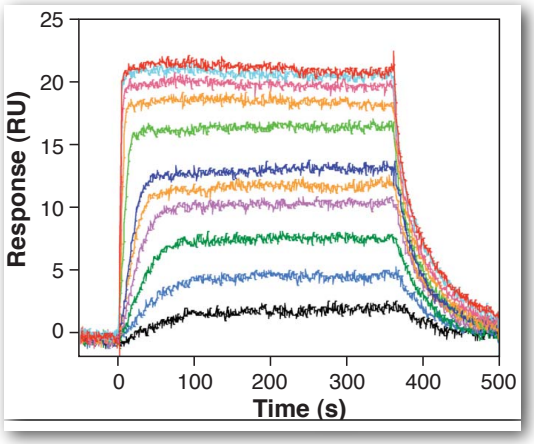
If the correct wavelength and angle is chosen, a resonant wave of excited electrons (plasmon resonance) is produced at the gold surface, decreasing the total intensity of the reflected wave. The angle of the SPR is sensitive to the layers attached to the gold. Binding and dissociation of ligand is sufficient to change the SPR angle.

- Surface Plasmon Resonance (SPR) is a technique to measure protein-protein or protein-ligand immobilized on a surface. SPR uses a sensor chip consisting of a 50 nm layer of gold on a glass surface. The receptor protein or the ligand can be attached to this surface and a liquid containing the ligand is flowed over the binding surface. The detection system consists of a polarized light beam that passes through a prism on top of the glass layer. The light is totally reflected but another component of the wave called an evanescent wave, passes into the gold layer, where it can excite the Au electrons. If the correct wavelength and angle is chosen, a resonant wave of excited electrons (plasmon resonance) is produced at the gold surface, decreasing the total intensity of the reflected wave. The angle of the SPR is sensitive to the layers attached to the gold. Binding and dissociation of ligand is sufficient to change the SPR angle.
- Q: How binding and dissociation of ligand can change SPR angle? Does just evanescent wave and gold layer are responsible for that/?
- A: The simplest model for understanding how SPR works is just to consider that the angle of reflectance depends on the refractive index on the solution immediately adjacent to the gold-coated surface. When more molecules move into close proximity to the surface (i.e., due to a binding interaction with an immobilized receptor), the local index of refraction changes due to the effective change in molecular concentration. This explanation is sufficient for understanding and interpreting SPR data, but it is oversimplified. For example, this explanation does not explain why there it is actually a 'dark band' that is changing its angle of reflectance. An important aspect of SPR (but one that is harder to explain) is the presence of a surface plasmon at the gold/solution interface. The dark band results from absorption of the incident light when the wavelength and angle is "matched" with the surface plasmon. Without going into too much detail, we can say that properties of the surface plasmon are dependent on the refractive index of the solution, so when the refractive index changes, so does the angle at which incident light is absorbed.
- Suggested reading: Wilson, W.D., Analyzing Biomolecular Interactions, Science 295, 2002, 2103-2105.
- http://www.biacore.com/technology/spr_technology.lasso#
- http://www.biosensingusa.com/biosensing_instrument_technology.html

Surface plasmon resonance







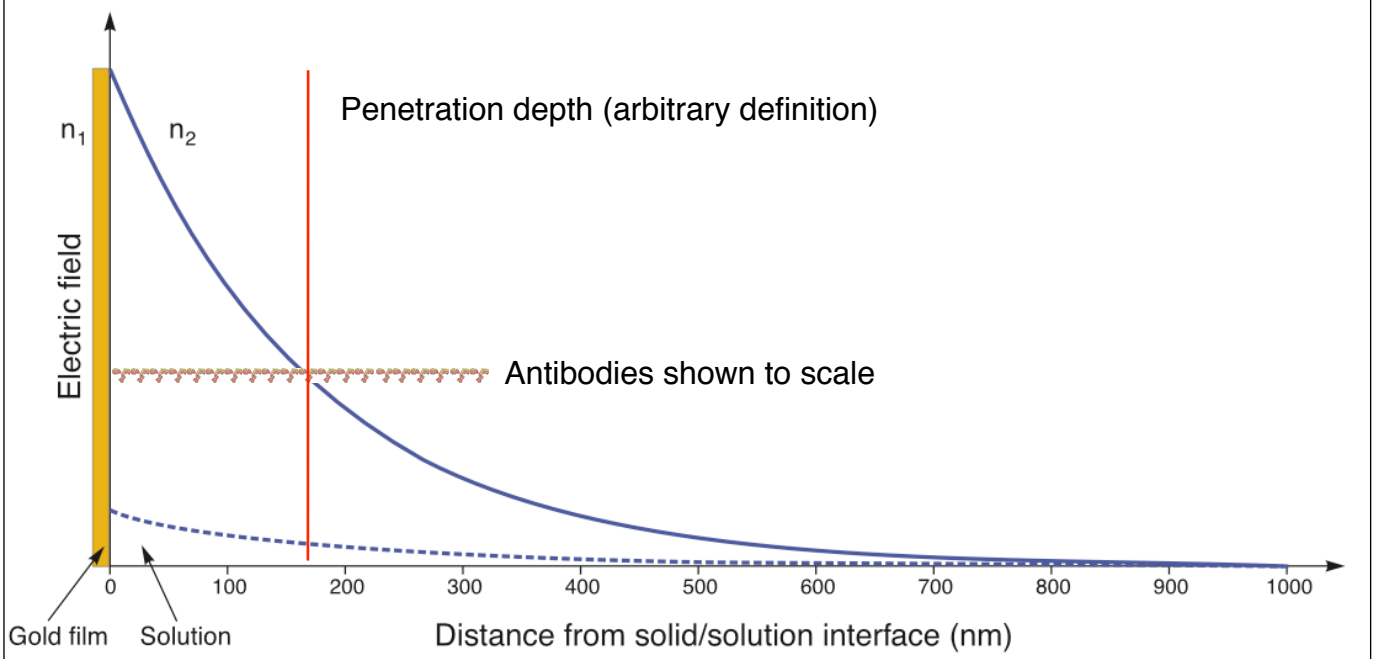
In principle, you could get a rough estimate of the K_d by plotting response (RU) as a function of sample concentration. However, this is not how it is typically done.

Rather, the association and dissociation phases of the RU vs time trace are fit in order to extract the k_{on} and k_{off} kinetic constants.

recall that $K_d = \frac{k_{off}}{k_{on}}$

- http://www.biosensingusa.com/biosensing_instrument_technology.html#
- http://www.biacore.com/technology/spr_technology.lasso#

Penetration depth of the evanescent wave on a SPR chip with a gold film



- Defined arbitrary penetration depth = 1/e (.37) * maximum intensity
- This arbitrary cutoff occurs around 150-200 nm
- This is equivalent to ~10 antibodies stacked side by side (shown to scale)
- The dimensions of an antibody are ~13 x ~16 nm
- The point is that the further you get from the surface, the weaker the SPR effect will be. However, it is difficult to imagine a case where the signal could drop below ~50% due to distance from the chip.
- The dashed line represents the strength of the evanescent wave in the absence of the gold film. The solid line is the strength with the gold film.

A SPR test for biotin in food samples

How does this test work? Why not just use immobilized avidin?

http://www.biacore.com/pdf/products/foodkits/Biotin_Kit_PIS.pdf

Biotin Kit

Routine assay for concentration measurements

Determination of biotin in food samples

Vitamins are added as supplement to various health and nutritional foodstuffs and their addition to certain cereal products is required in several countries.

The concentration of vitamins is determined in the quality and production steps during food processing, as part of legislative control of fortification. Food companies need to gain accurate data on the concentration of vitamins added as supplement not only to meet consumer's requirements for labeling, but also to closely control vitamin dosage in production to minimize wastage.

Biotin, also known as vitamin H, is an important nutritional factor and food additive, playing a major role in the metabolism of both humans and animals alike. Biotin acts as a coenzyme and participates in the process of gluconeogeonesis, biosynthesis of fatty acids and the metabolism of amino acids. The traditional method for analyzing biotin is by using a microbiological assay, which is slow, taking up to two days to obtain results. As demands on cost-efficiency increase in food production companies, new analytical methods for natural or added compounds such as biotin are needed.

The combination of Biotin Kit with Biacore® Q offers a rapid and reliable quantification technique. The Biotin Kit contains all the necessary reagents and materials for the biotin assay. Biacore Q uses SPR-based biosensor technology, to provide a rapid vitamin assay technique with highest accuracy and superior precision.

Biacore's SPR technology provides a rapid method for measuring biotin in hours rather than days. Biacore's SPR technique is usually unaffected by matrix effects which interfere with many conventional detection methods used in food analysis.

Optimized for Surface Plasmon Resonance (SPR) biosensor technology

Biacore's SPR technique exploits the high affinity of a Biotin antibody to measure biotin content in samples, using Biacore Q system. The sensor chip is first prepared by immobilizing biotin onto the surface of the chip.

Food samples are mixed with a fixed amount of Biotin antibodies and injected over the sensor chip surface. The remaining free biotin antibodies are determined by measuring the binding to the sensor chip. The SPR response obtained is inversely related to the amount of biotin in the sample. Sample analysis is fully automated and the sensor chip is regenerated after each analysis, ready for the next sample to be tested.

- Q. Testing for biotin in food - is the reason that we don't use avidin or streptavidin immobilized and capturing biotin because we have no way of attaching an unknown quantity of biotin post translationally with an antigen in order to use antibodies against that antigen? or is it also because of the cost of using avidin/streptavidin? or because of the difficulty of reusing the surface again in another sample due to the very low Kd of the avidins and biotin?
- A. One reason is that biotin is a small molecule and would produce only a small change in SPR signal when it binds to the avidin or streptavidin. The second reason is that it would be nearly impossible to reuse the chip, due to the high affinity of biotin for avidin.