# Surface Plasmon Resonance Imaging for Biosensor Applications

Presented by Michelle Brideau

March 31, 2005

CHEM 395

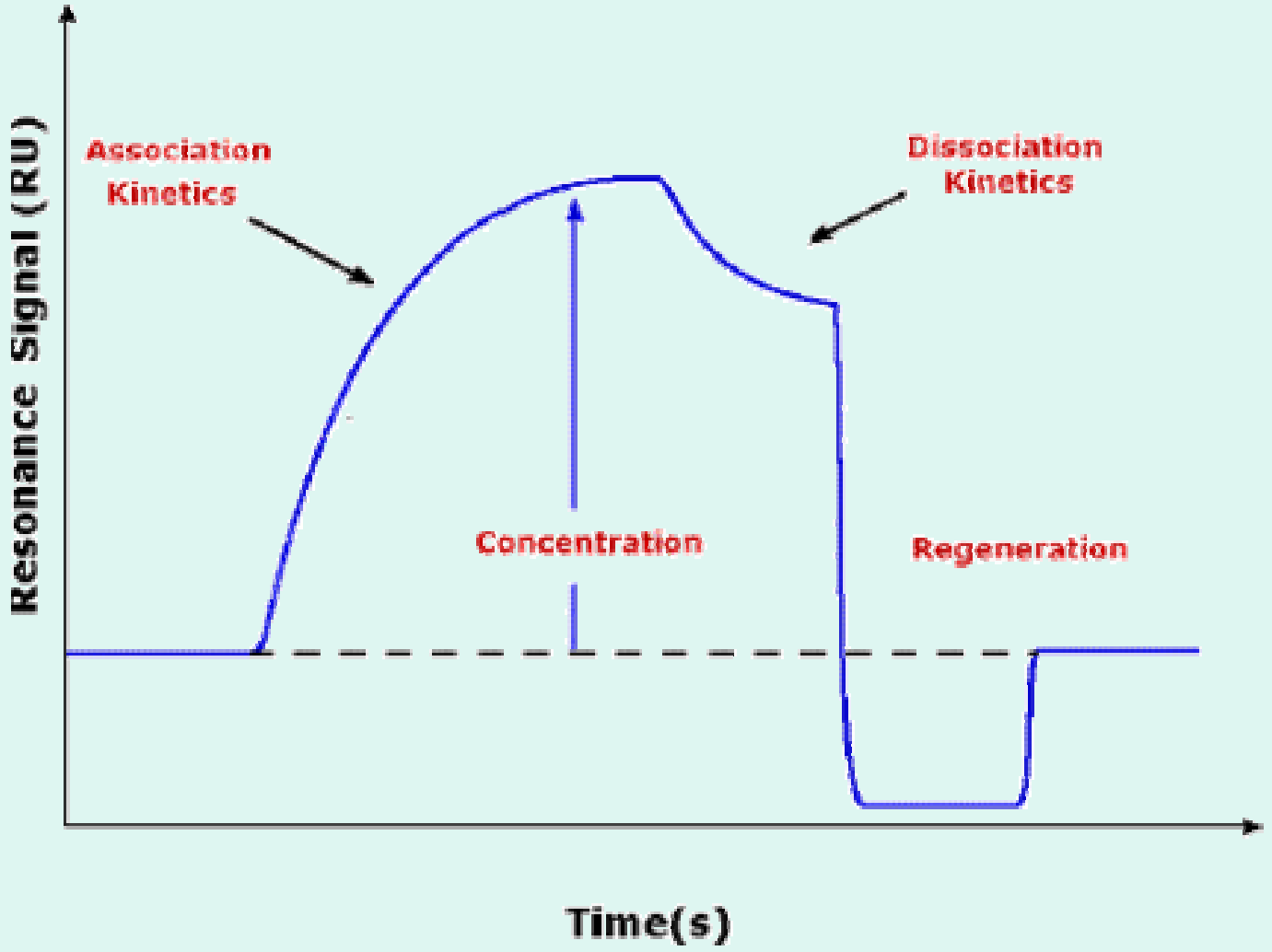
# Outline

1. Surface Plasmon Resonance
    - SPR background
    - SPR imaging
2. SPR imaging experiments
3. Conclusions
    - Advantages of SPR
    - Future applications

# Surface Plasmon Resonance (SPR)

- Label-free method
- Surface sensitive spectroscopic technique
- Used to detect the binding of biological molecules onto arrays of probe biomolecules covalently attached to chemically-modified gold surfaces

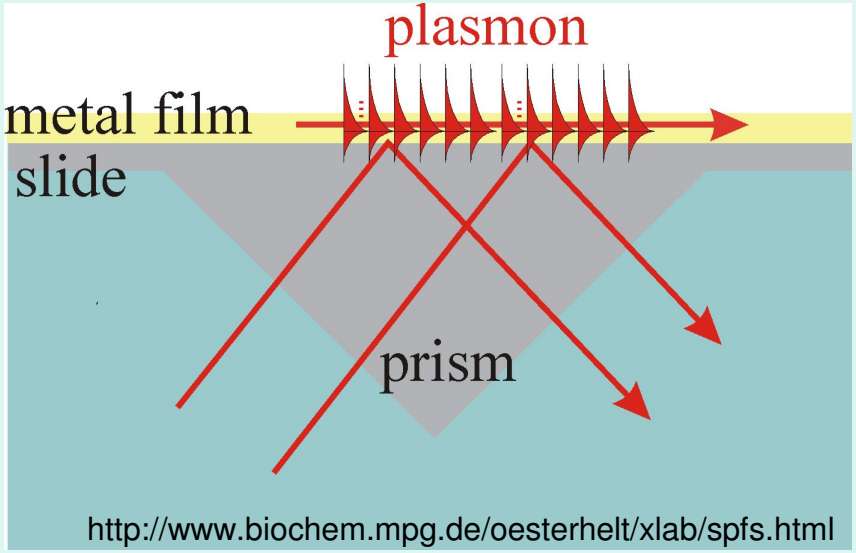
# Typical Signal from SPR Measurement




http://brahms.chem.uic.edu/~cgpage/frames.html

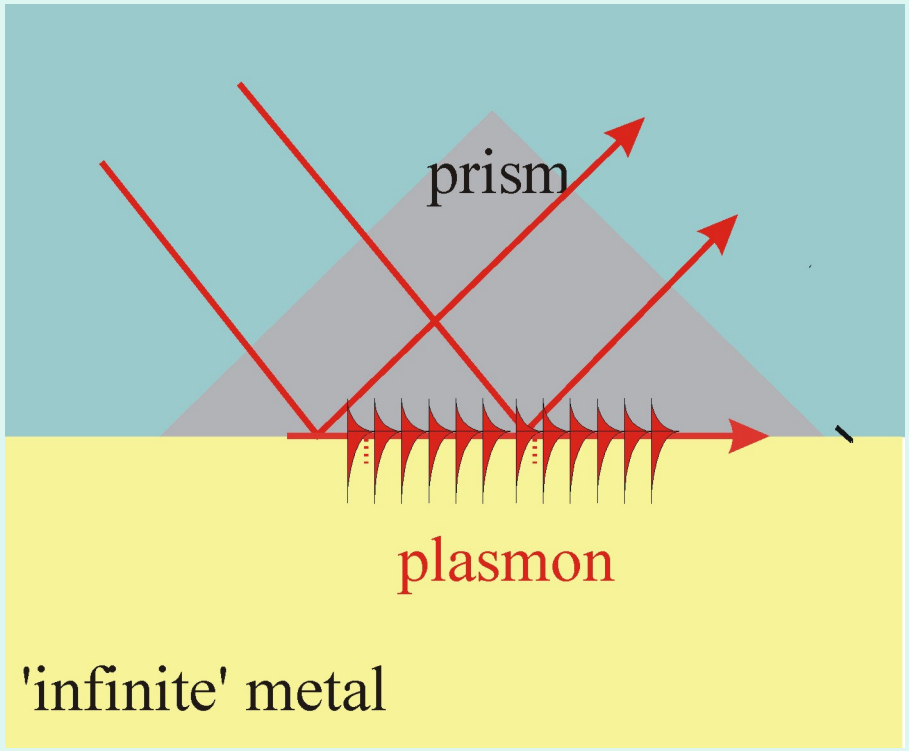
# How does SPR work?

- This Kretschmann Experimental System uses a metal film thin enough to monitor the plasmon




A plasmon can be thought of as a ray of light bound onto a surface - propagating among the surface and presenting itself as an electromagnetic field.

# Otto Experimental System (1968)




- Impossible to observe plasmon through infinite metal
- The Kretschmann system (1971) is used in designs of SPR instruments

# Metals

- Gold and silver are most commonly used. Aluminum and copper may also be suitable metals.

# SPR

- A surface-plasmon-resonance is excited at a metal-dielectric interface by a monochromatic, p-polarized light beam, such as He-Ne laser beam
- The surface plasmon is sensitive to changes in the environment near the interface and therefore has potential as a sensing probe.
- Sensitive detection method that monitors variations in thickness and refractive index in ultra-thin films

# Refractive Index

- Ratio of velocity of propagation of electromagnetic wave in vacuum to velocity in medium

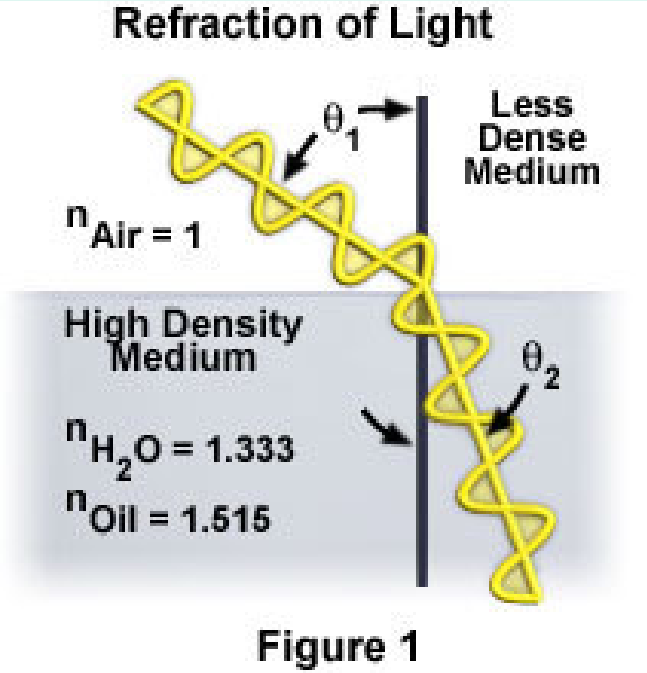


Figure 1

http://www.microscopyu.com/articles/formulas/formulasri.html

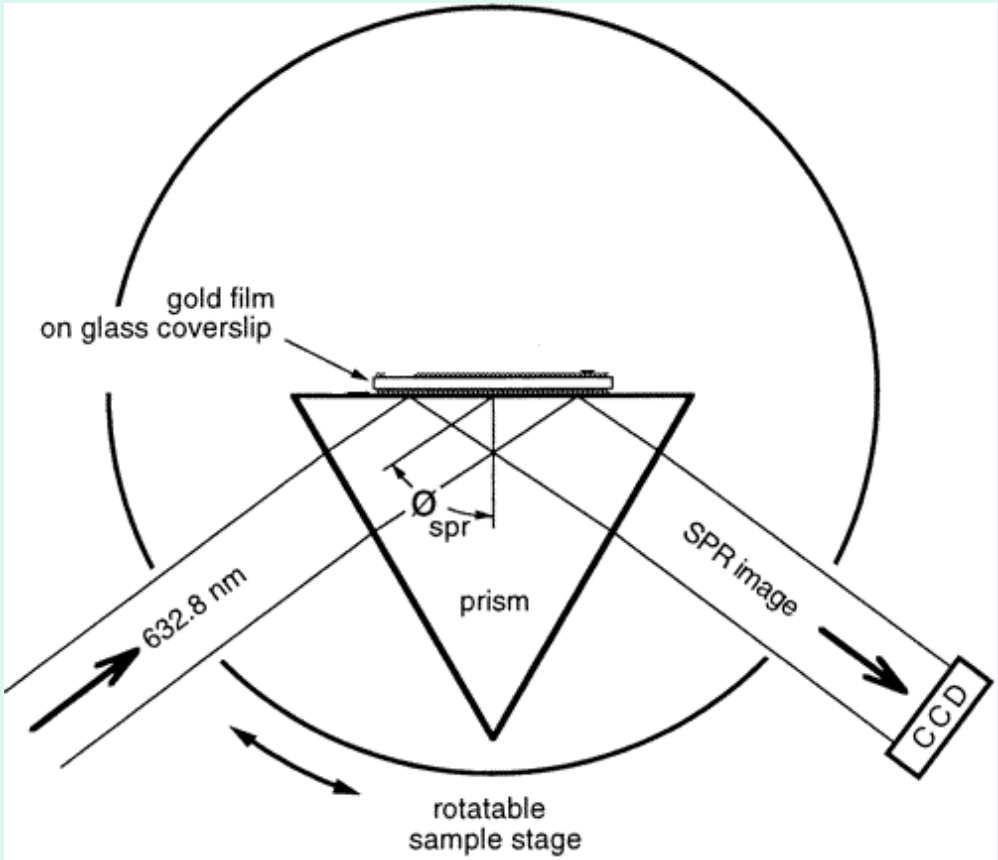
# More about SPR

- It is a non-destructive means of sensing
- Surface Plasmons have already been used for gas sensing, biosensing, immuno-sensing and electrochemical studies.

# Modifications for SPR imaging apparatus

- The p-polarized He-Ne laser beam passes through a spatial filter and can be expanded using a beam expander
- The reflected light is collected by a CCD camera to produce an “SPR image”

# SPR imaging apparatus




- Spatially-filtered, expanded, p-polarized HeNe laser beam illuminates the gold sample through a prism coupler.
- Reflected light from the gold surface, containing the SPR image, is monitored with a CCD camera.
- The angle of incidence can be changed by rotating the entire sample assembly.

A.J. Thiel et. al., *Anal. Chem.* **69** (1997), pp. 4948–4956.

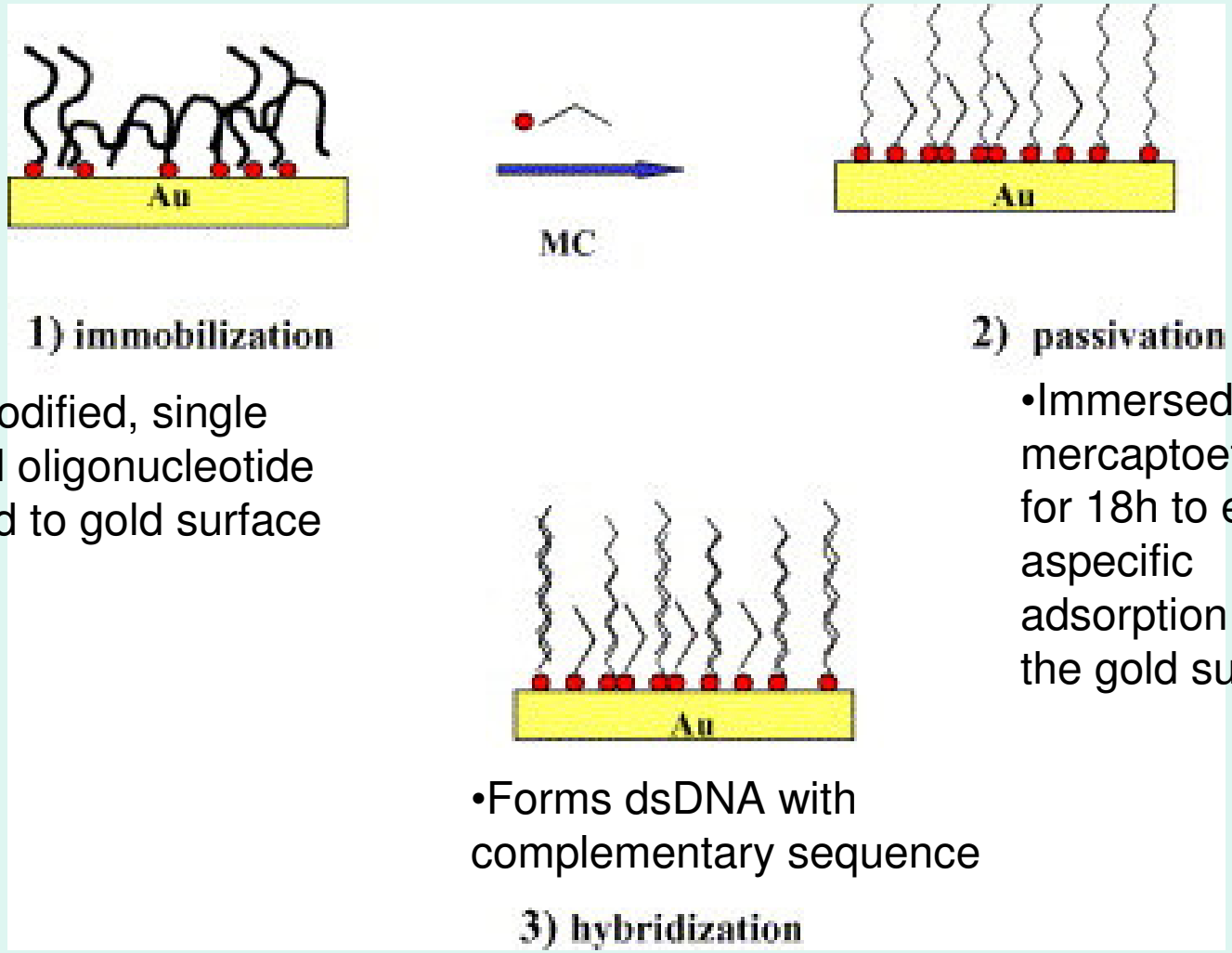
# SPR Imaging Experiment

- Investigation of adsorption processes of oligonucleotides onto gold substrates in aqueous buffer solution
- Monitored hybridization process of thiol-modified single stranded oligonucleotide anchored to gold surface with its complementary sequence
- Traditional analysis of hybridization involves labeling, such as radioactive isotopes or fluorescent molecules

# Preparation

- Glass slides (cleaned in piranha solution)
- 2nm chromium layer (adhesion layer) followed by 50nm gold film
- Gold surface spotted with HS-ssDNA (thiolated oligonucleotide probes) and left to react for at least 18h
- After soaked and rinsed in water, the probes were ready for hybridization
- Probes were immersed in hybridization buffer containing complementary oligonucleotide in Phosphate buffer system at pH 7.4

# Hybridized helices formed on gold substrate



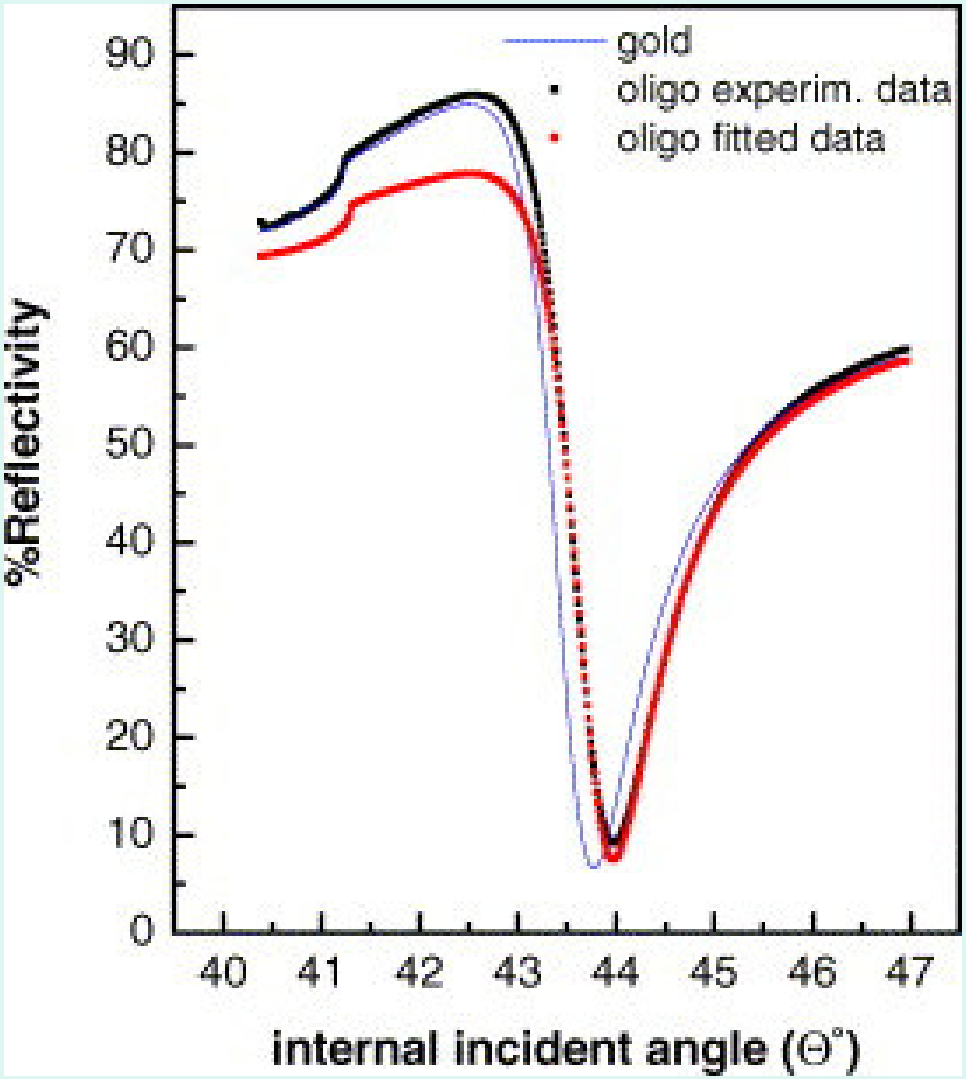

•Thiol-modified, single stranded oligonucleotide anchored to gold surface

•Immersed in mercaptoethanol for 18h to eliminate aspecific adsorption sites on the gold surface

•Forms dsDNA with complementary sequence

•R. Rella, et al. *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.

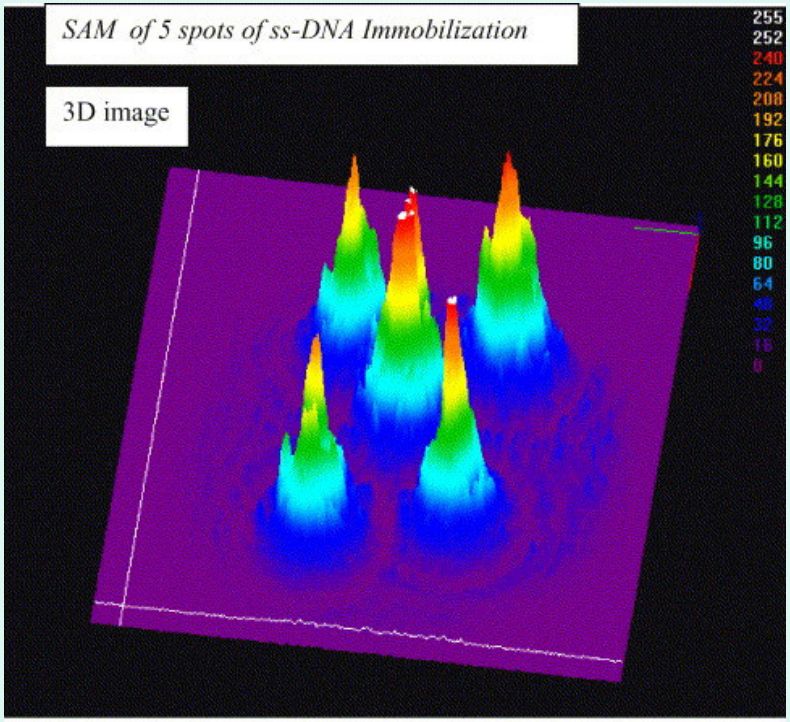
# SPR Reflectivity

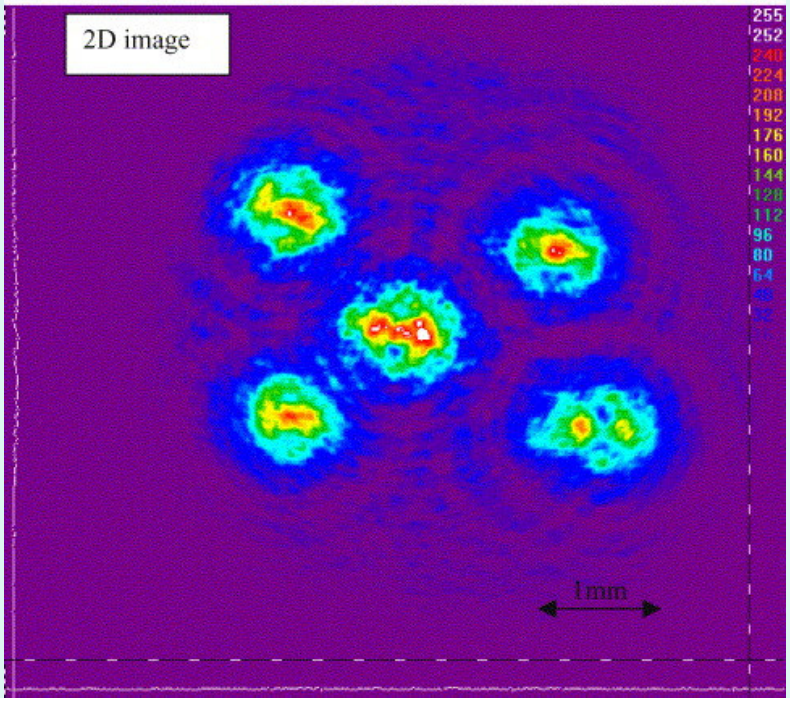



- The cusp does not change in the presence of the oligo, therefore ensures reproducibility of the scanning measurement

- 0.41 degree shift of the self assembled monolayer from the bare gold surface was used to estimate a 5nm thickness of self assembled oligonucleotide and refractive index of 1.65


- R. Rella, et al. *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.



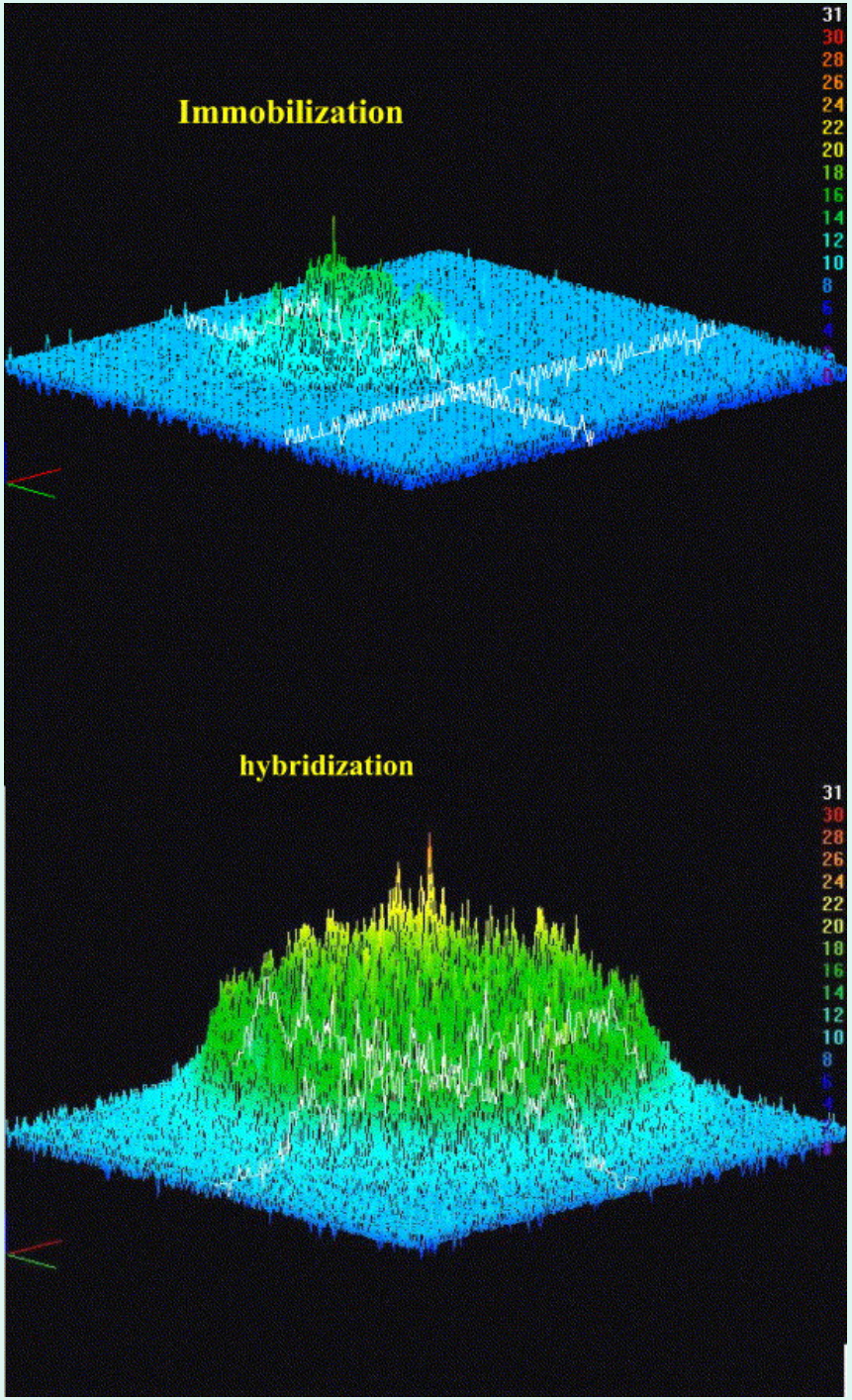




# 2D and 3D Images of ssDNA

- Shows the 5 spots of self assembled thio-oligonucleotide DNA probes immobilized on the gold surface

- Color variation indicates variation in the thickness of the self assembled monolayer (SAM)


- R. Rella, et al. *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.

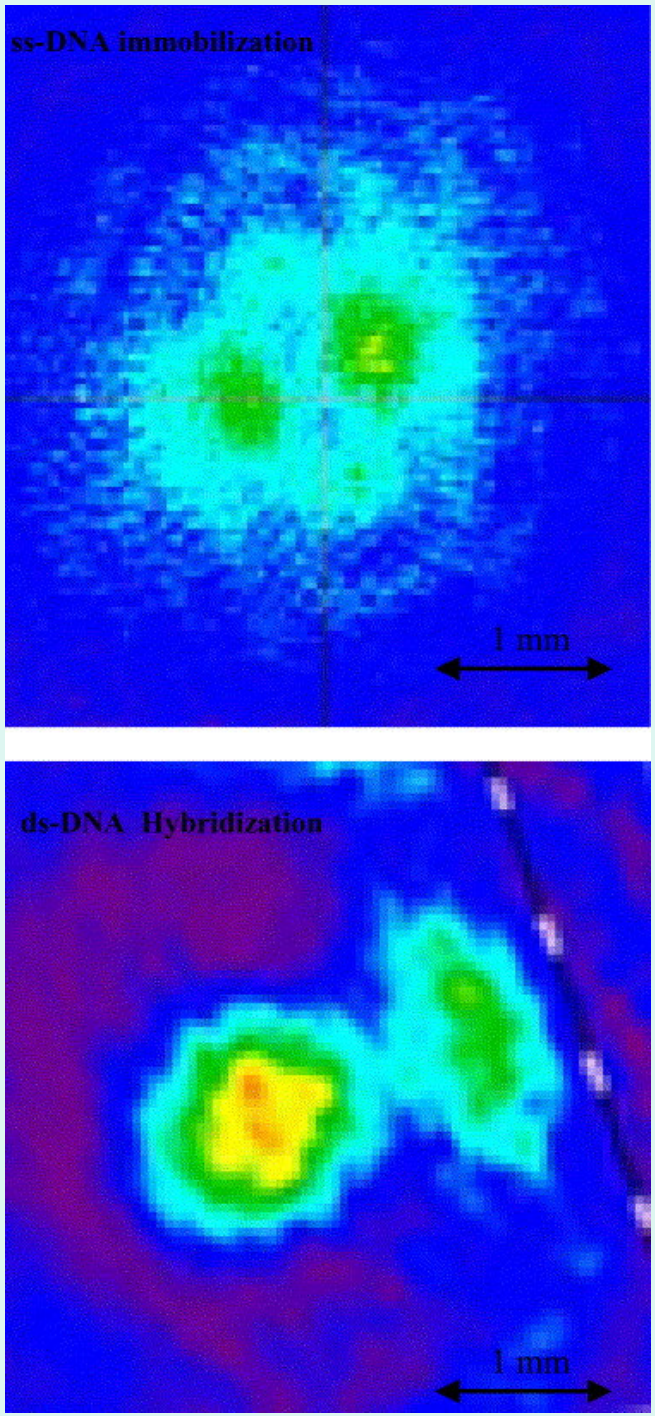



# 3D Images of ssDNA (top) and dsDNA (bottom)

- SPR images of immobilization (ssDNA) and hybridization (dsDNA) in liquid.

- Hybridization process shown by variation in resonance signal of a specific self assembled spot.

•R. Rella, et al. *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.




# 2D Images of ssDNA (top) and dsDNA (bottom)

- The increase in resonance signal corresponds to the increase in layer thickness indicative of the hybridization process

- R. Rella, et al. *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.

# Summary

- SPR imaging used to obtain images of thio-oligonucleotides linked onto a gold surface
- Detection of DNA hybridization using liquid phase SPR imaging techniques

# Advantages of SPR imaging

- Identification of binding events with label-free molecules
- High speed and sensitivity with real-time reaction monitoring
- High spatial resolutions
- Identity specific vs. non-specific adsorption processes
- In-situ capabilities

# Future Applications

- Using the technique to obtain the total number of target DNA hybridization events per unit area
- Construct patterned gold substrates and self assemble DNA probes of different nature
- Construct a DNA array suitable to detect specific DNA sequence target

Thank You

# References:


- R. Rella, J. Spadavecchia, M.G. Manera, P. Siciliano, A. Santino, G.Mita. Liquid Phase SPR imaging experiments for biosensors applications, *Biosensors and Bioelectronics*. 20 (2004), pp.1140-1148.

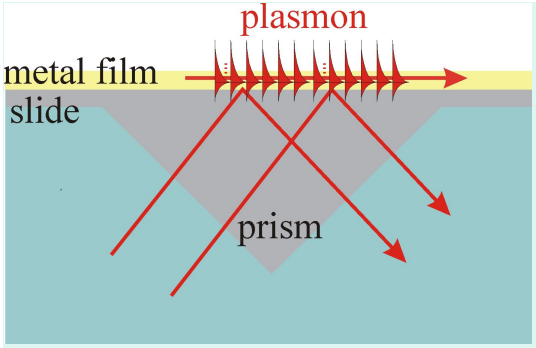
- A.J. Thiel, A.G. Frutos, C.E. Jordan, R.M. Corn and L.M. Smith, In situ surface plasmon resonance imaging detection of DNA hybridization to oligonucleotide arrays on gold surfaces, *Anal. Chem.* **69** (1997), pp. 4948–4956.

- http://arrayit.com/Products/Microarray_Platforms/SPRMP/sprmp.html
- http://brahms.chem.uic.edu/~cgpage/frames.html
- http://www.biochem.mpg.de/oesterhelt/xlab/spfs.html

# NOTES

# SIGNAL DETECTION

- The light source for SPR is a high efficiency near-infrared light emitting diode which has a fixed range of incident angles. The SPR response is monitored by a fixed array of light sensitive diodes covering the whole wedge of reflected light. The angle at which minimum reflection occurs is detected and converted to the resonance units. The SPR angle depends on several factors, most notably the refractive index into which the evanescent wave propagates on the non-illuminated side of the surface. In addition the other parameters are the wavelength of the incident light and the properties of the metal film.




# How it works

•As we change the angle of incidence of light (at a fixed frequency), at some angle the projection of the light wave vector onto the metal film becomes equal to the wave vector of SP's at the same frequency. Light energy can then be effectively transferred to the SP's.

•If we vary the thickness of the metal film, the phase difference between the reflected light and the light re-radiated by SP's will change. If we scan the angle of incidence of light onto a metal film of the right thickness, then at a certain angle the reflected light intensity will go sharply to almost zero, indicating resonant coupling to surface plasmons. This angle is always greater than the angle of total internal reflection off the prism/outer dielectric interface, and is called the attenuated total reflection (ATR) angle.

•And this angle is important, because the position and the width of this ‘resonance point’ are very sensitive to the properties of the surface and the media next to it. It makes it possible to use surface plasmon resonance techniques for chemical and biological sensing.

# SPR imaging

- SPR imaging is a surface sensitive optical technique for monitoring the adsorption of solution species into patterned molecular microarrays that have been made on chemically modified metal surfaces (gold). SPR can be used to study the specific adsorption of biopolymers (oligonucleotides, proteins, antibodies) onto DNA or protein microarrays. The microarray format makes the SPR technique a high throughput differential binding measurement system that can be used to accurately measure the specific organic and biomolecular interactions with a variety of surfaces under identical solution adsorption conditions.
- Various biomolecular interactions can be characterized including DNA-DNA, RNA-DNA, protein-DNA and protein-protein binding. As a surface-sensitive measurement, SPR is an indispensable tool for the study of in-situ biological systems of weakly bound interactions. Since the SPR imaging experiment senses the change in the local index of refraction upon adsorption onto the metal surface, no labeling of the biomolecules is required. The combined simplicity and versatility of surface plasmon resonance imaging measurements make them ideally suited to the study of ultra thin organic films. The technique is extremely sensitive, provides reasonable lateral resolution and can be used in both ex-situ and in-situ configurations to monitor the real-time binding of label free analyte molecules to metal surfaces. The two dimensional analysis of imaging data, combined with our microarray manufacturing and processing technologies on gold surfaces makes surface plasmon resonance a powerful tool for the parallel processing of multiplexed biomolecular interactions.

# Specifications of the SPR Imager

- Sensitive to less than 1 angstrom molecular thickness range
- Liquid cell attachment for in-situ measurements
- High precision coupled prism and detector rotating stage
- 
- Spectral range from 600nm to 1100nm
- Incident angle range for 40 to 70 degrees for differing prism and sampling systems
- High sensitivity near IR CCD detector with video interface
- Image acquisition interface and image processing software
- 110 or 220 volt versions