

# Surface Plasmons and Its Applications

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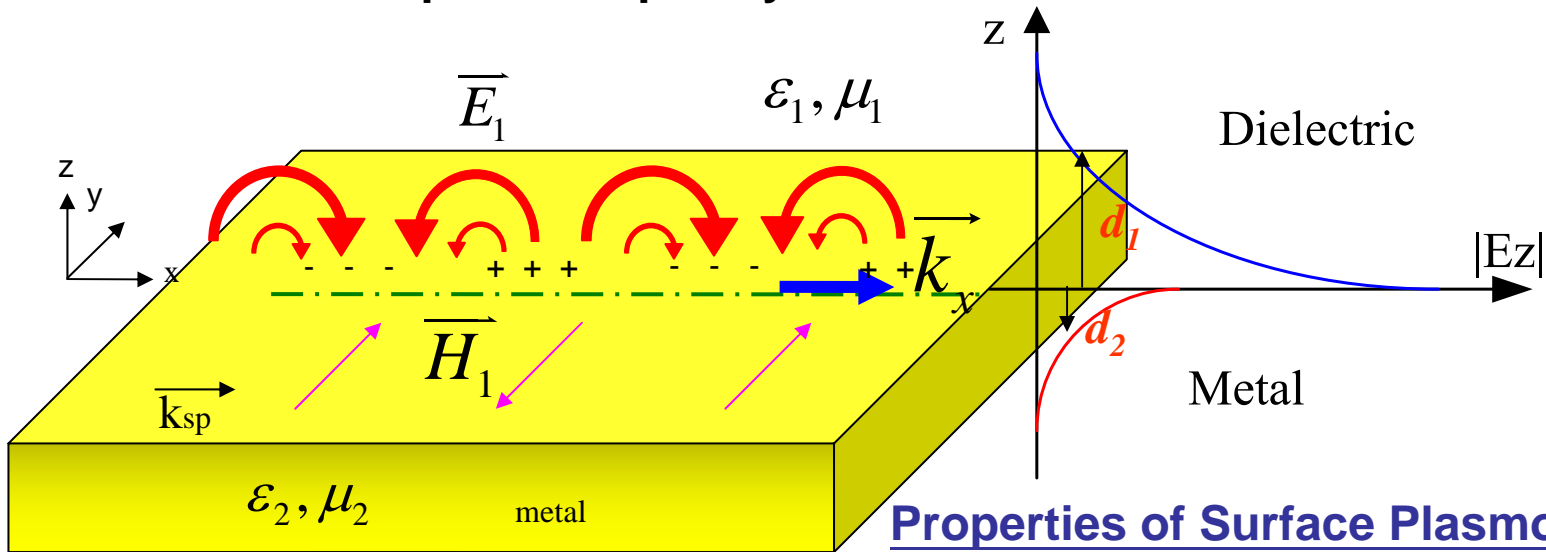
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## Outline

1. Introduction to surface plasmons
2. Ways to excite surface plasmons
3. Applications of surface plasmonic resonance
4. Introduction to nano-plasmonics
5. Applications of nano-plasmonics

# Surface plasmons

Surface plasmons are collective oscillations of electrons on metallic surface with the optical frequency.



## Properties of Surface Plasmon wave

1. A **TM-mode** guided optical wave
2. Optical wave is **evanescent** on the metallic surface and most optical intensity is on the **dielectric** part.

$$k_x = k_0 n_{sp} = k_0 \sqrt{\frac{\epsilon_1 \epsilon_2}{(\epsilon_1 + \epsilon_2)}}$$

$$E_1 = E_0 \exp(-k_0 z \sqrt{n_{sp}^2 - \epsilon_1}) = E_0 \exp(-z / d_1)$$

$$E = E_0 \exp(-k_0 z \sqrt{n_{sp}^2 - \epsilon_2}) = E_0 \exp(-z / d_2)$$

$$\epsilon_2 = -12 \text{ (gold)}, \epsilon_1 = 1.32^2 \text{ (water)}, \lambda_0 = 800 \text{ nm} \Rightarrow n_{sp} = 1.435, d_1 = 232 \text{ nm}, d_2 = 34 \text{ nm}$$

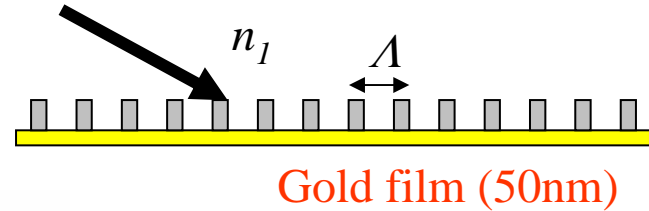
The dielectric constant of metal is negative and much larger than  $\epsilon_1$ .

# Traditional Methods to Generate Surface Plasmons

Light incident directly from dielectric part



## 2. Grating coupling

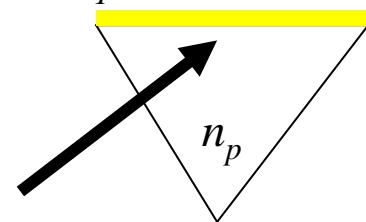


$$k_x + k_a = k_{sp}$$

$$k_0 \sin \theta + \frac{2\pi}{\Lambda} = k_0 n_{sp}$$

## 1. Prism coupling

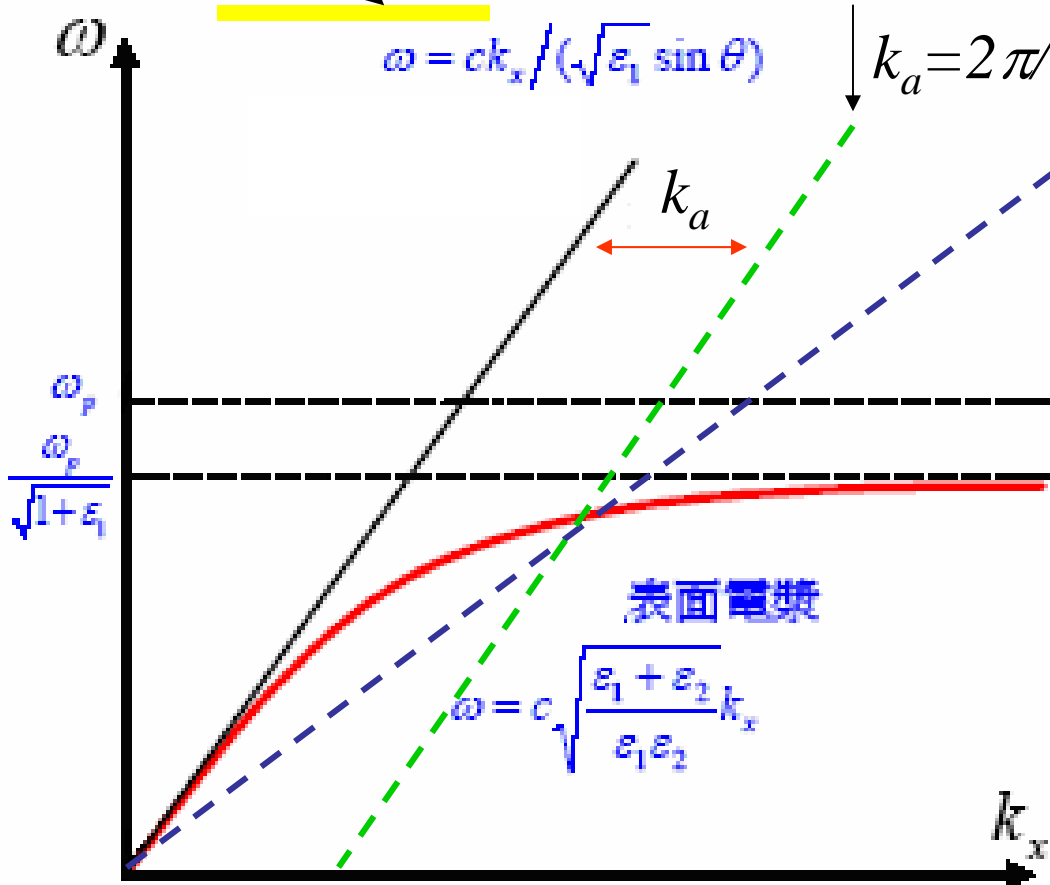
$n_1$  Gold film (50nm)



$$n_1^2 = \epsilon_1 \Rightarrow \epsilon_p = n_p^2$$

$$\omega = ck_x / (n_p \sin \theta) = ck_x / n_{sp}$$

$$\Rightarrow n_p \sin \theta = n_{sp}$$



# Surface Plasmon Resonance for Biodetections

## Advantages:

1. Non-labeling
2. Real-time monitoring
3. High surface sensitivity
4. Water environment

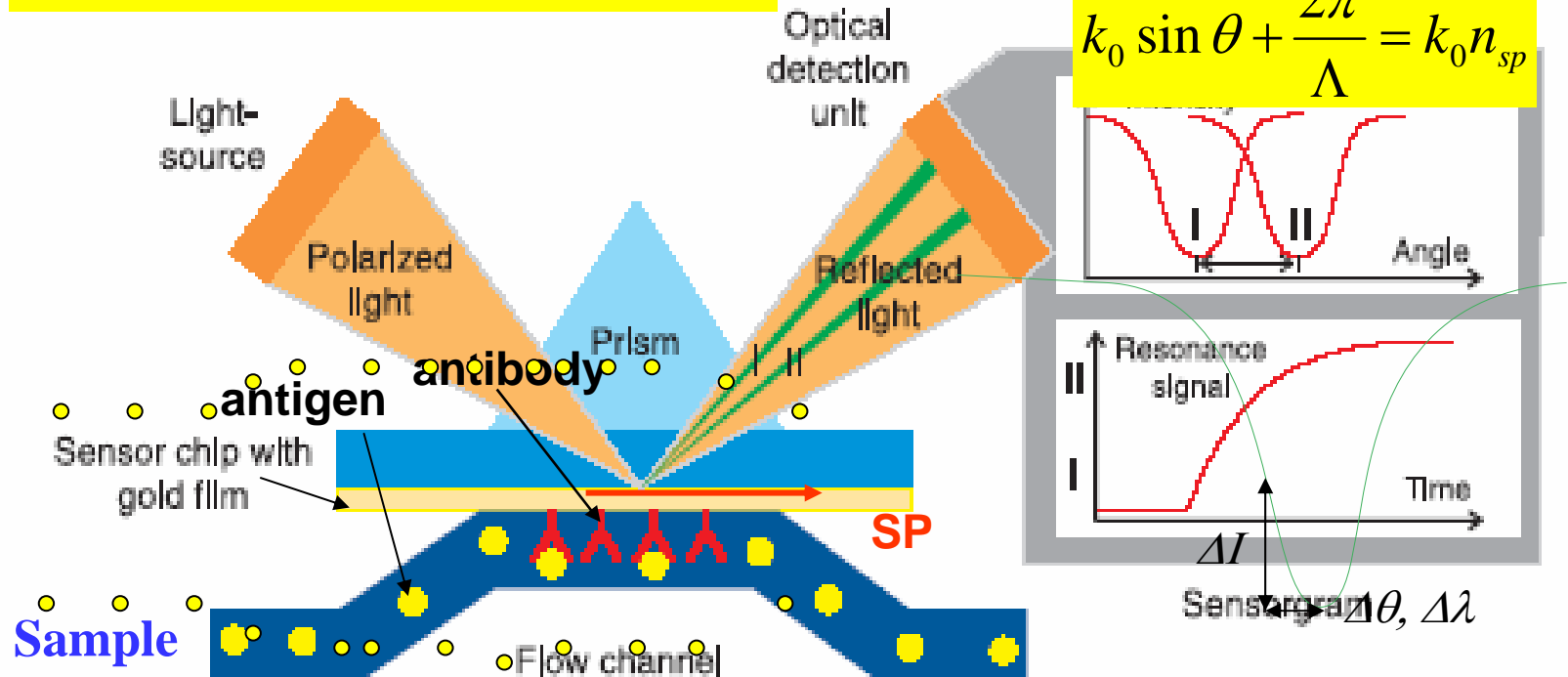
TM polarized light

$$\omega = ck_x / (n_p \sin \theta) = ck_x / n_{sp} \\ \Rightarrow n_p \sin \theta = n_{sp} \sim n$$

$$k_x + k_a = k_{sp}$$

$$k_0 \sin \theta + \frac{2\pi}{\Lambda} = k_0 n_{sp}$$

SP



## Disadvantages of Conventional SPR:

bulky, expensive, difficult for high-throughput detections and requires a large amount of sample solution.

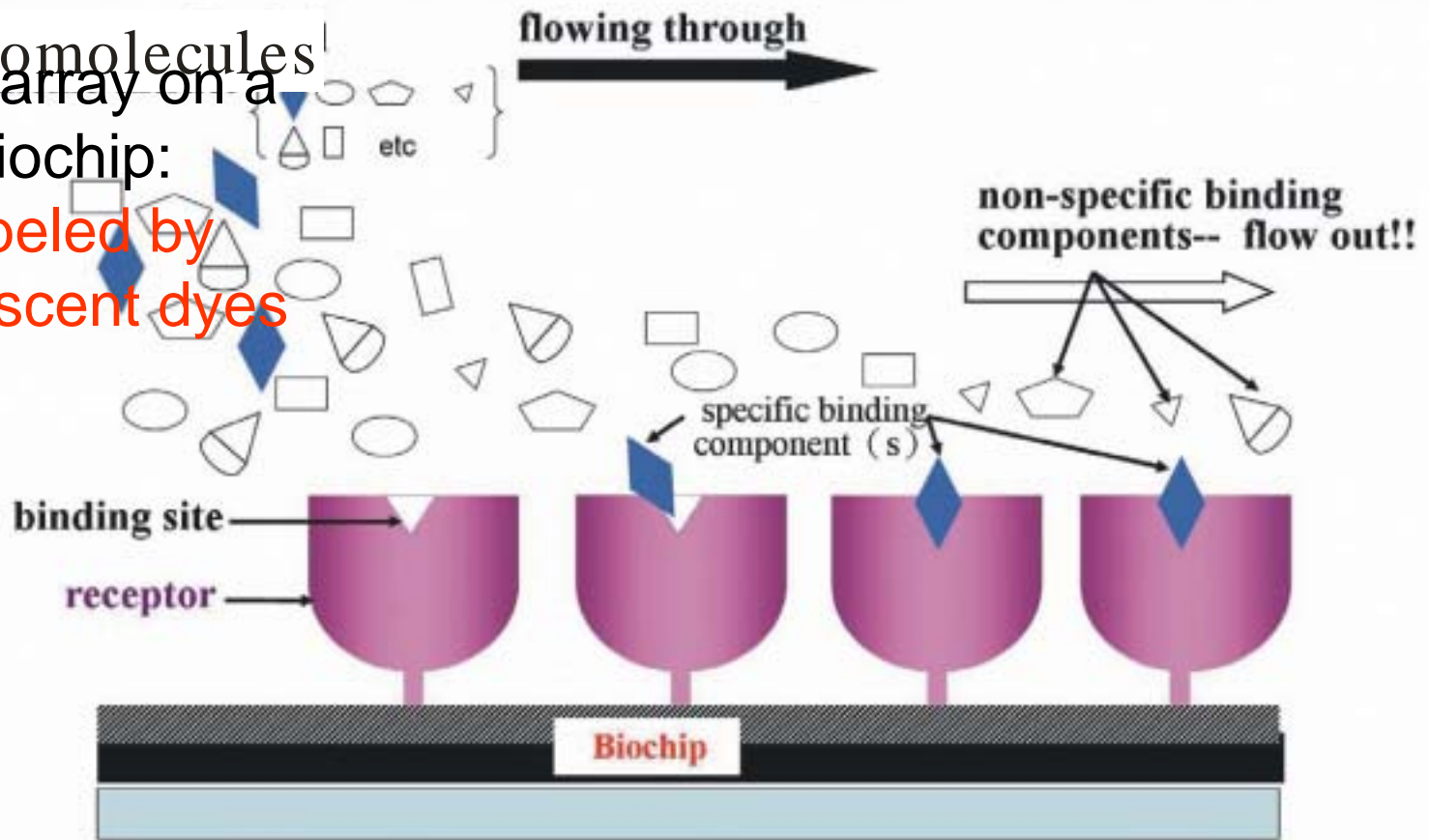


Our studies: Using surface plasmonic effect in a metallic nano-gap. It will have the advantages of

- (1) Higher sensitivity
- (2) Smaller sample volume
- (3) Easier for high throughput detections

# Biomolecular Interactions Studied by Specific Binding

Biomolecules  
Microarray on a  
Biochip:  
Labeled by  
fluorescent dyes



# 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

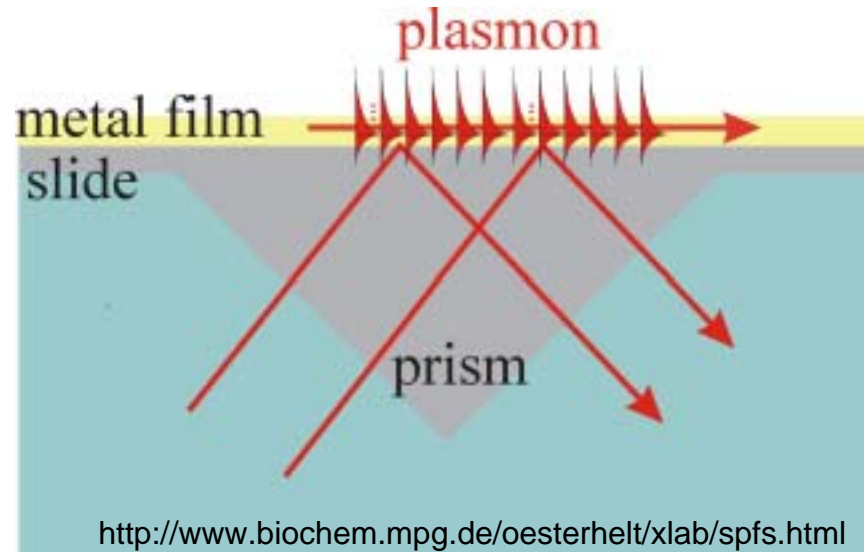
# More about SPR

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



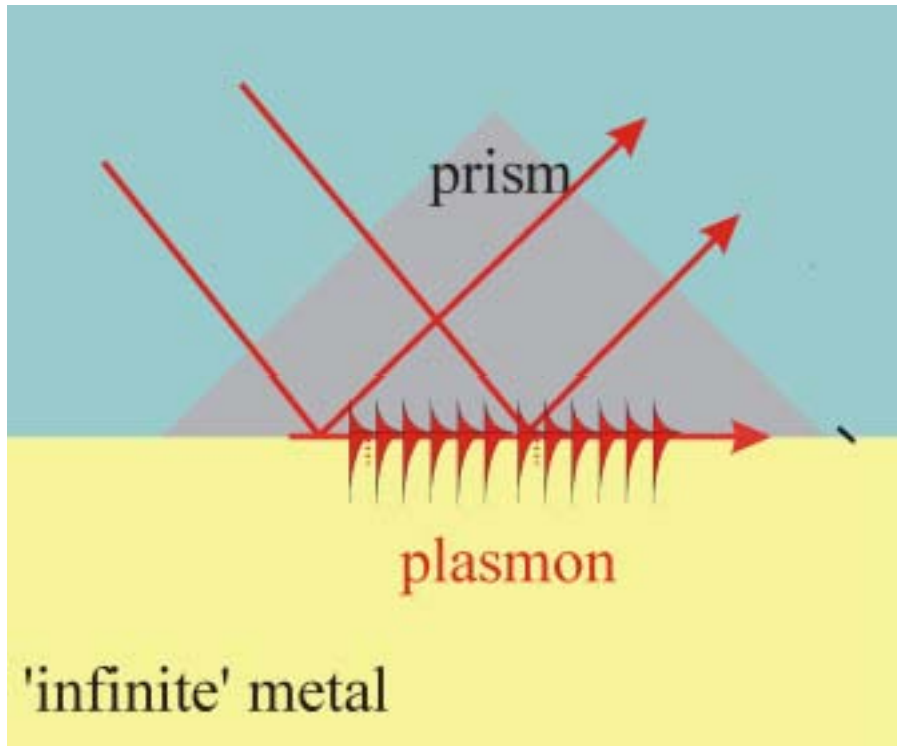
# How does SPR work?

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



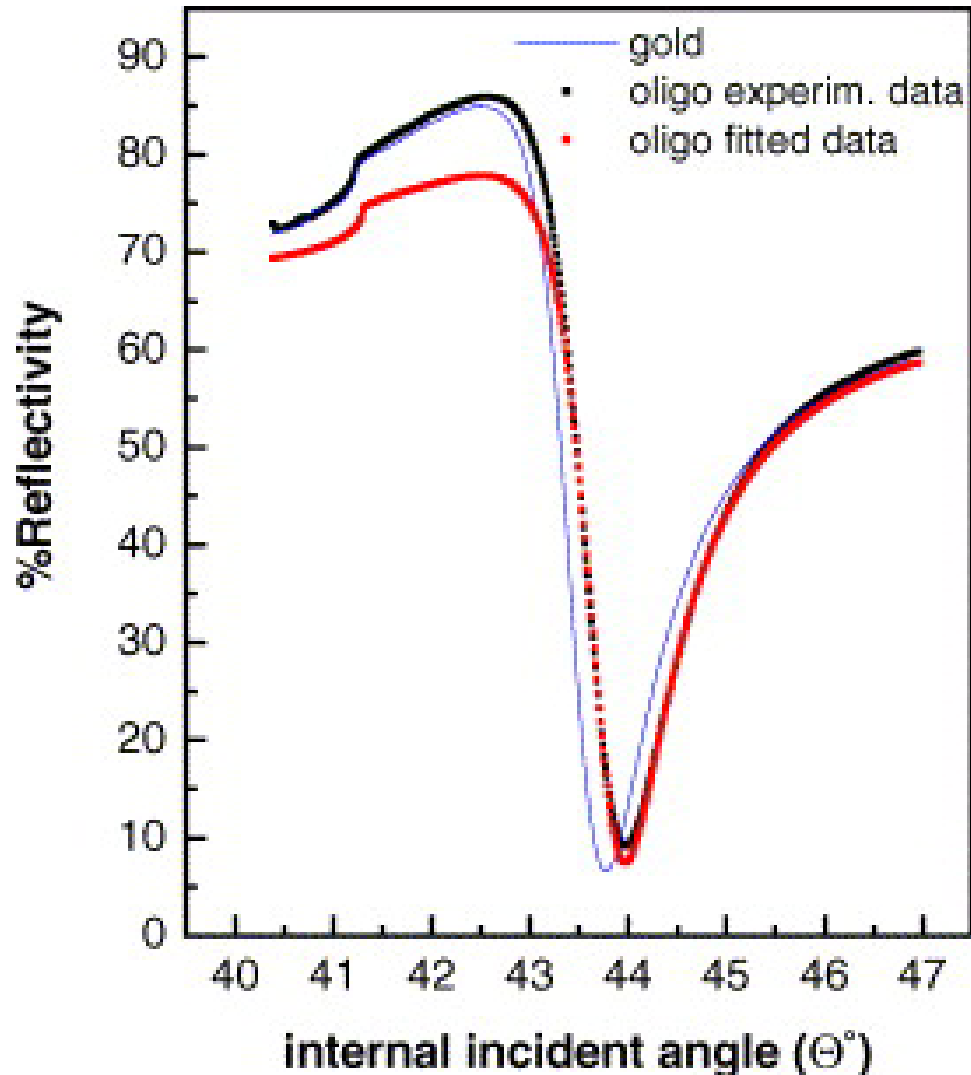
A plasmon can be thought of as a ray of light bound onto a surface - propagating along the surface and presenting itself as an electromagnetic field.

# Otto Experimental System (1968)



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

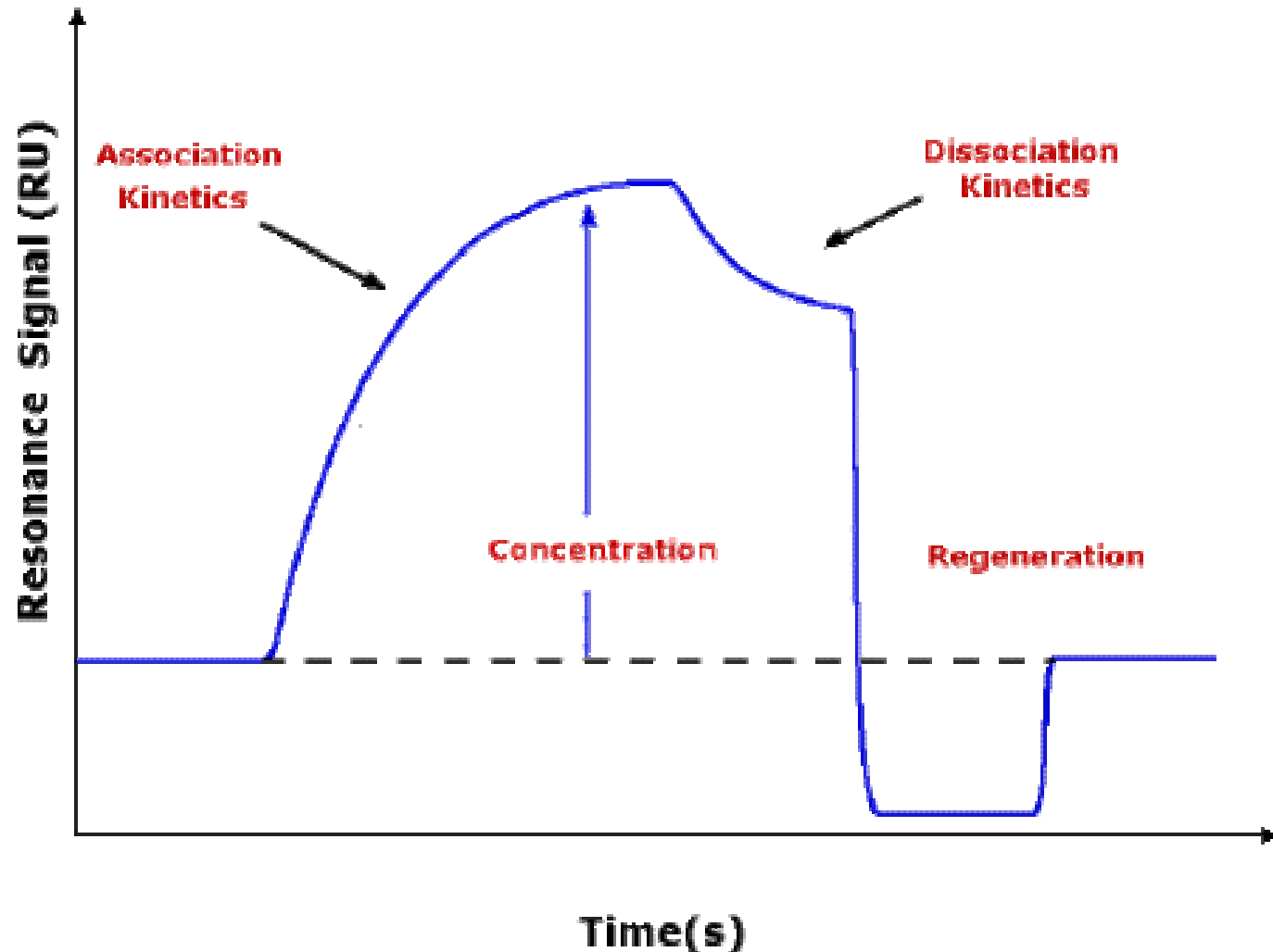
# SPR Reflectivity

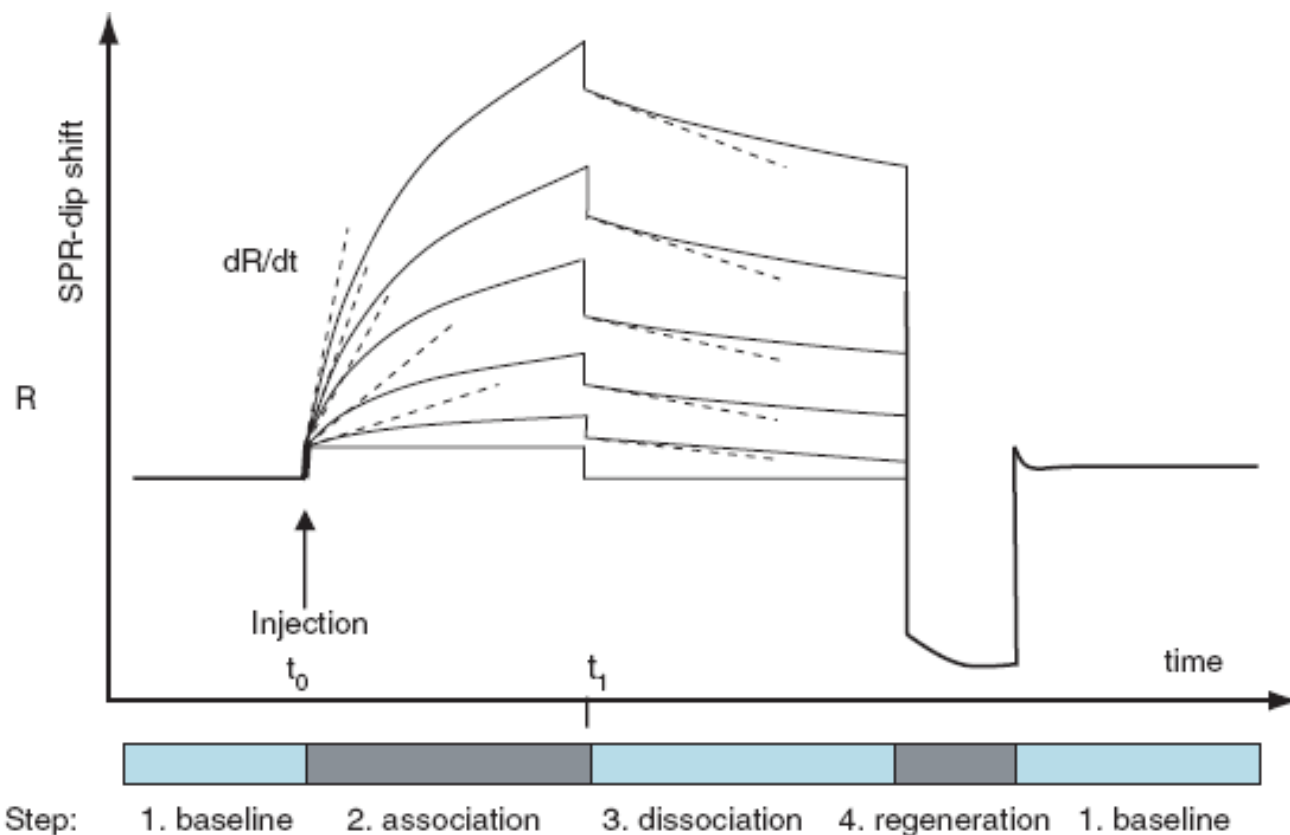


- The cusp does not change in the presence of the oligo, therefore ensures reproducibility of the scanning measurement

- 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

# Typical Signal from SPR Measurement





**Figure 1.5** Typical overlay plot of sensorgrams from serial diluted analyte concentrations. Just after injection at  $t_0$  a sample specific binding of the analyte occurs and mass transport to the surface is rate limiting and linearly dependent on the concentration. From the slopes of a positive control ( $dR/dt$ ), the concentration of an unknown sample can be determined. During the association phase the number of unbound ligand molecules decreases and dissociation takes place. The off-rate constant or dissociation constant ( $k_d$ ) can be determined after injecting dissociation buffer at  $t_1$ . See for more details chapter 4 and 5 of this book.

# Metals

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

The most prominent benefit of direct detection using SPR biosensor technology is the determination of kinetics of (bio)molecular interactions. Reaction rate and equilibrium constants of interactions can be determined, *e.g.* the interaction  $A + B \rightarrow AB$  can be followed in real time with SPR technology, where A is the analyte and B is the ligand immobilized on the sensor surface.

Table 1.1 contains the most relevant kinetic parameters, the association and dissociation constants, for the simplest case  $A + B \rightarrow AB$ . The association constant is the reaction rate of complex (AB) formation, giving the number of complexes formed per time at unit concentration of A and B. As soon as the complex AB is formed, its dissociation can commence. The dissociation rate constant describing this process expresses the number of AB complexes

**Table 1.1** Definitions of the most relevant kinetic parameters: the association and dissociation constants.

	<i>Association rate constant, <math>k_a</math></i>	<i>Dissociation rate constant, <math>k_d</math></i>
Definition	$A + B \rightarrow AB$	$AB \rightarrow A + B$
Description	Reaction rate of AB formation: number of AB complexes formed per unit time at unit concentration of A and B	Dissociation rate of AB: number of AB complexes dissociating per unit time
Units	$\text{l mol}^{-1} \text{s}^{-1}$	$\text{s}^{-1}$
Typical range	$10^3\text{--}10^7$	$10^{-1}\text{--}5 \times 10^{-6}$

**Table 1.2** Definition of the equilibrium association and dissociation constants.

	<i>Equilibrium association constant, <math>K_A</math></i>	<i>Equilibrium dissociation constant, <math>K_D</math></i>
Definition	$[AB]/[A][B] = k_a/k_d$	$[A][B]/[AB] = k_d/k_a$
Description	Affinity to association: high $K_A$ , high affinity to associate	Stability of AB: high $K_D$ , low stability of AB
Unit	$\text{l mol}^{-1}$	$\text{mol l}^{-1}$
Typical range	$10^5\text{--}10^{12}$	$10^{-5}\text{--}10^{-12}$

# SPR

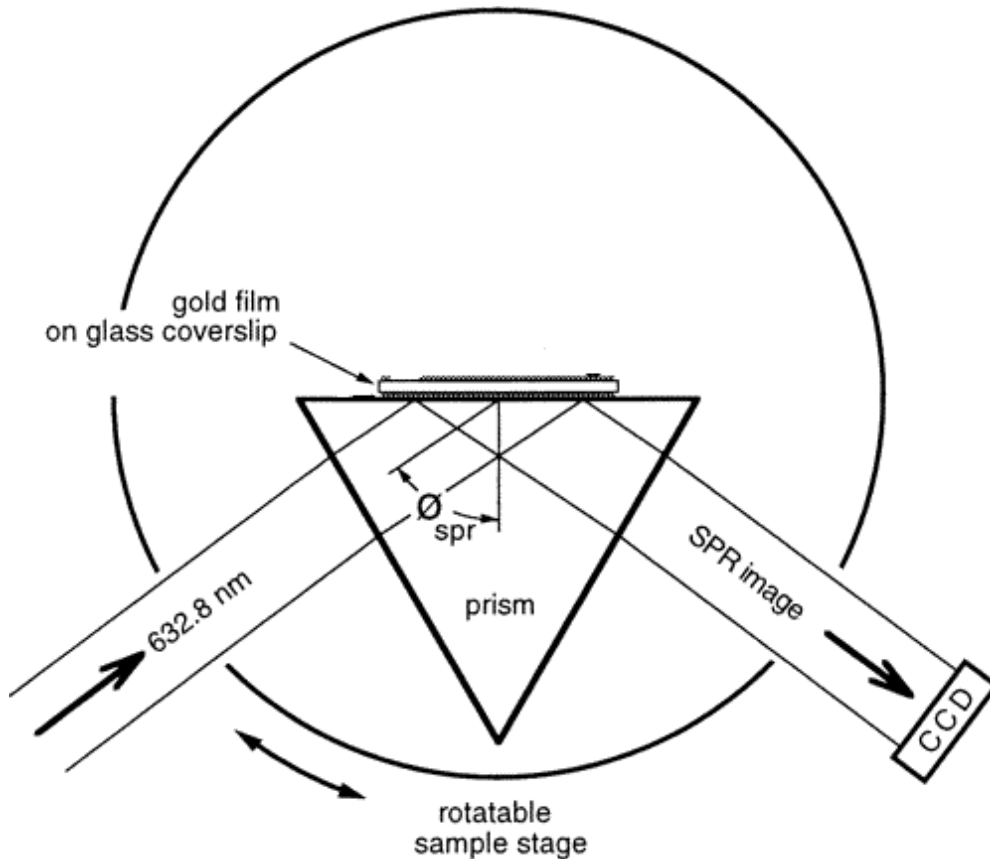
- A surface-plasmon-resonance is excited at a metal-dielectric interface by a monochromatic, p-polarized light beam, such as He-Ne laser beam
- The surface plasmon is sensitive to changes in the environment near the interface and therefore has potential as a sensing probe.
- Sensitive detection method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



# 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.

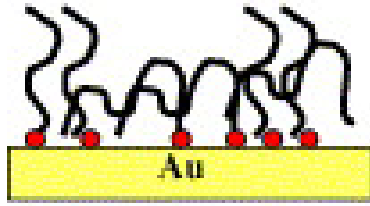
# SPR Imaging Experiment

- 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

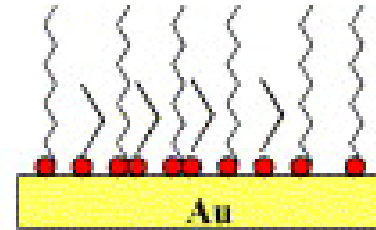
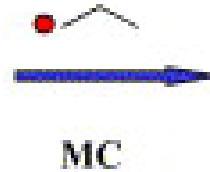
- Glass slides (cleaned in piranha solution)
- 2nm chromium layer (adhesion layer) followed by 50nm gold film
- Gold surface spotted with HS-ssDNA (thiolated oligonucleotide probes) and left to react for at least 18h
- After soaked and rinsed in water, the probes were ready for hybridization
- Probes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

# Hybridized helices formed on gold substrate



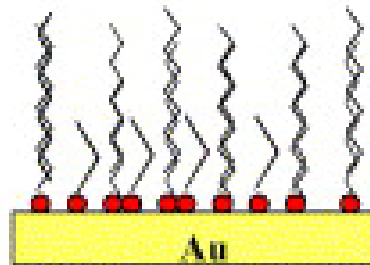
## 1) immobilization

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



## 2) passivation

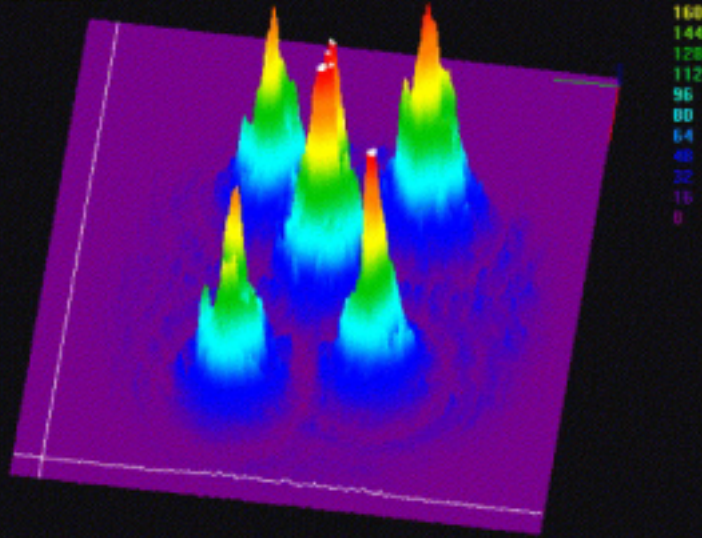
- Immersed in mercaptoethanol for 18h to eliminate aspecific adsorption sites on the gold surface



- Forms dsDNA with complementary sequence

## 3) hybridization

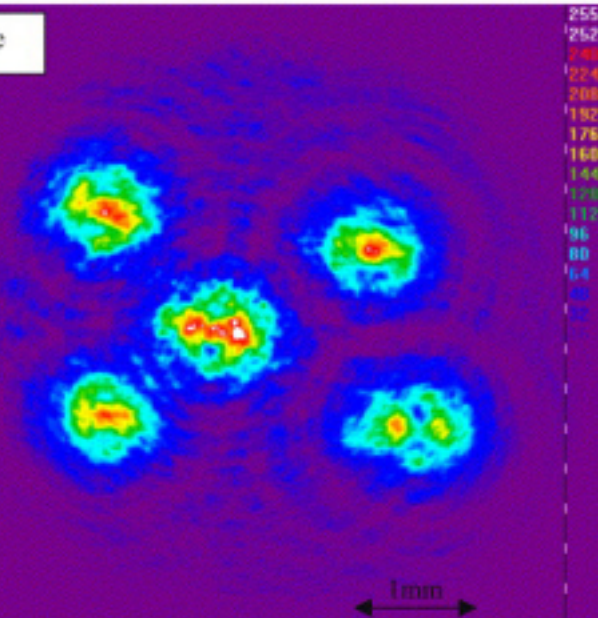
3D image



# 2D and 3D Images of ssDNA

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

2D image



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

## Disadvantages of fluorescent labeling:

- (1) Difficult for protein labeling
- (2) Bio-activity may be affected
- (3) Fluorescent labeling are expensive and time-consuming
- (4) Fluorescence quenching

## Our goal: To develop label-free detection technology with following advantages :

- (1) High sensitivity
- (2) Small sample volume
- (3) Water environment detection
- (4) High Throughput, real-time detection  
( microarray )