Surface Plasmons and Their Bio-Applications

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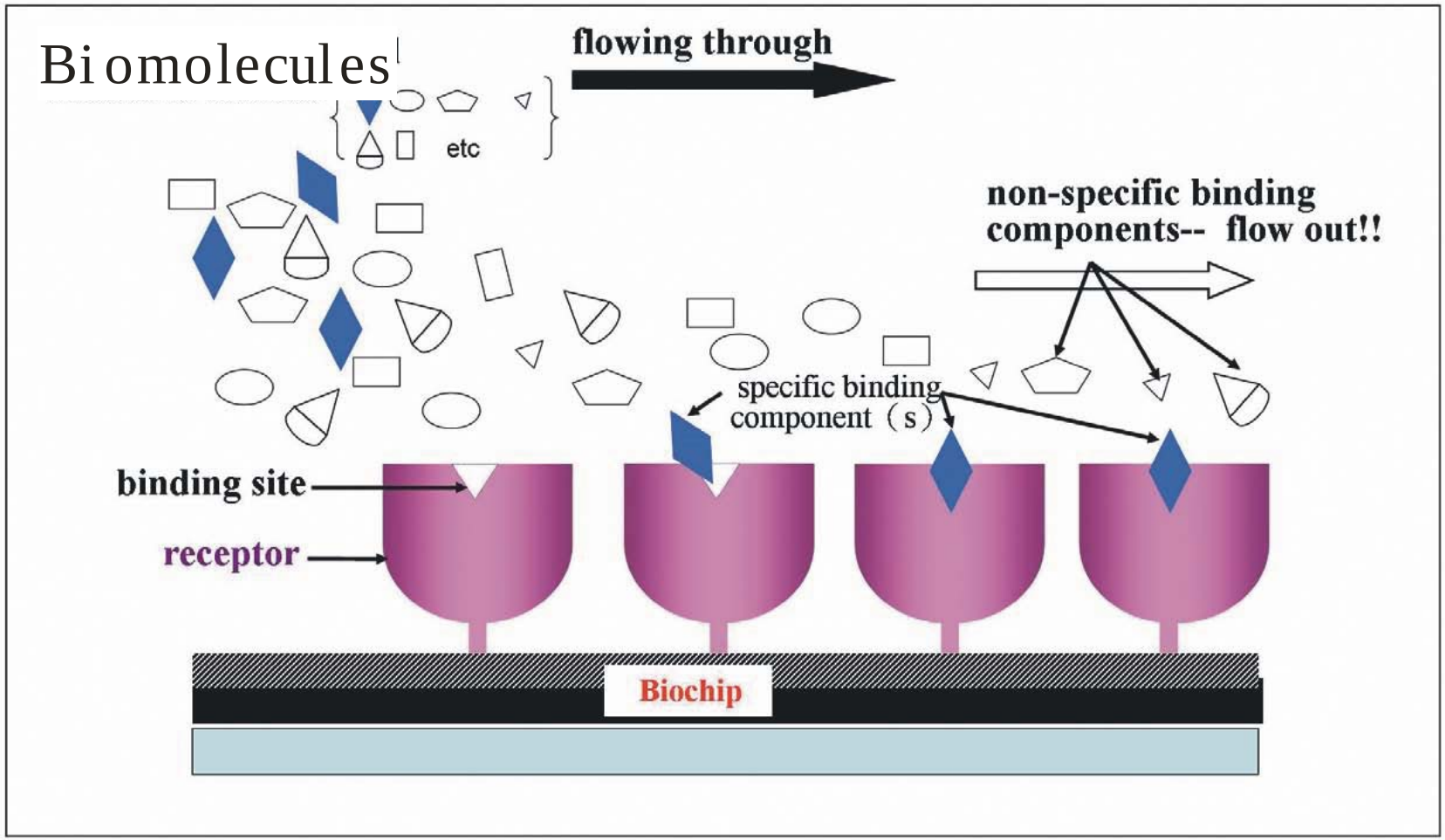
Outline

1. Introduction to Immunoassay
2. Surface Plasmons
3. Excitation of Surface Plasmons
4. Surface Plasmon Resonance Sensors

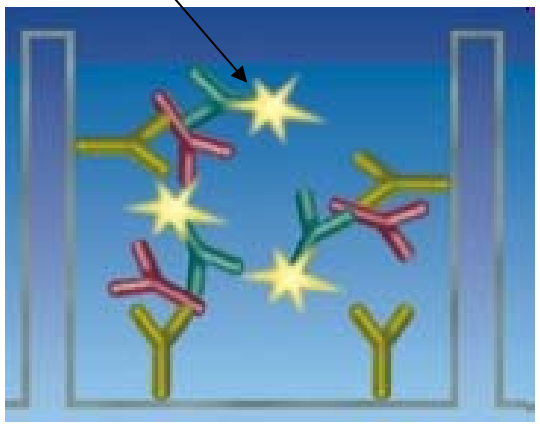
Immunoassay by using specific binding between biomolecules

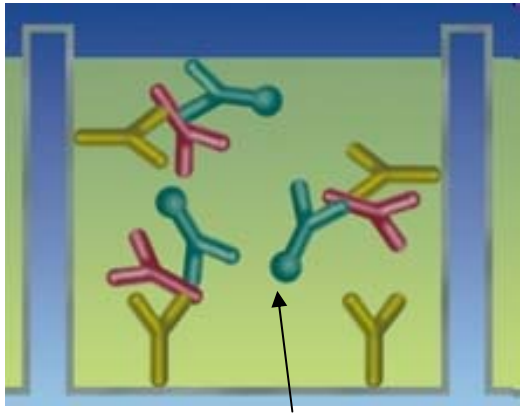


Immunoassays combine the principles of chemistry and immunology enabling scientific tests for a specific and sensitive detection of the analytes of interest. The basic princple of these assays is the specificity of the antibody-antigen reaction. Though being very specific and sensitive immunoassays are easy to perform which has contributed to the widespread use and tremendous success.

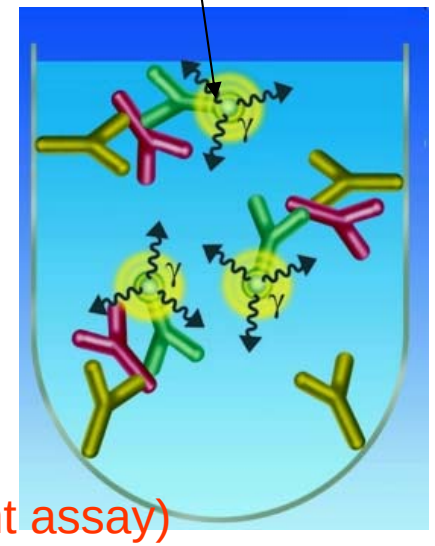
RIAs (Radioimmunoassay) and enzyme immunoassays like ELISA (Enzyme-linked immunosorbent assay), LIA (luminescent immunoassay), and FIA (fluorescent immunoassay) are widely used in research, drug discovery and diagnostics for highly specific and cost efficient detection of analytes not detectable with other techniques.

LIA (Luminescence immunoassay)



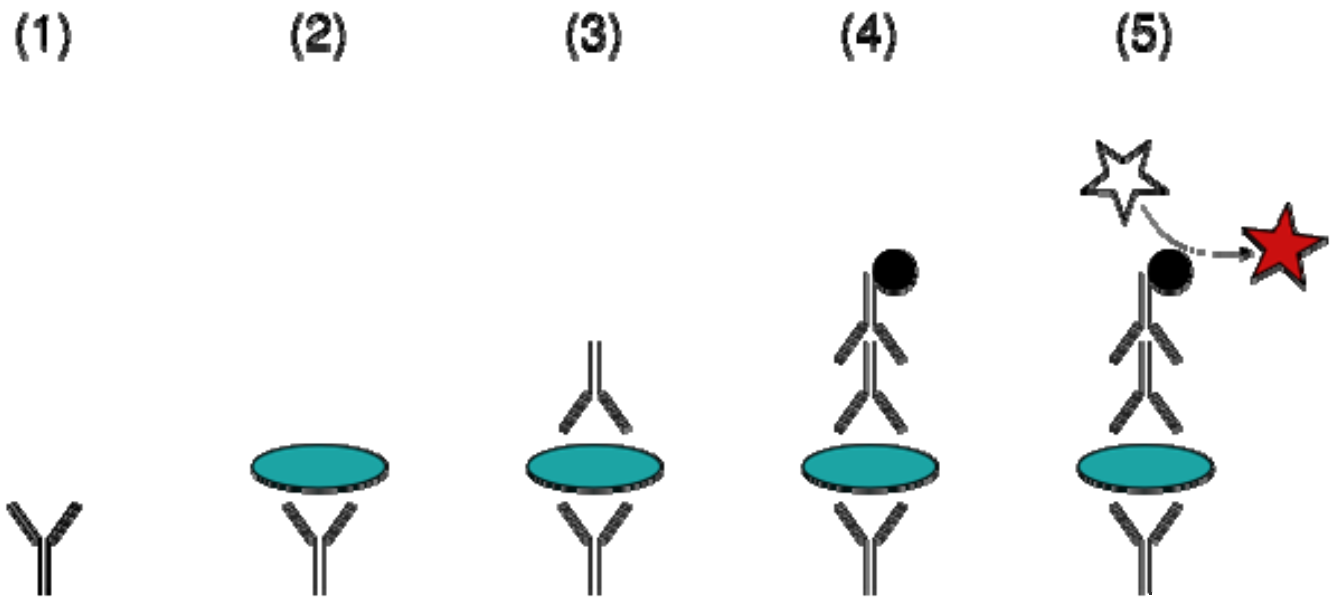


Radio-Immunoassay



ELISA (Enzyme-linked immuno-sorbent assay)

ELISA (Enzyme-Linked ImmunoSorbent Assay)

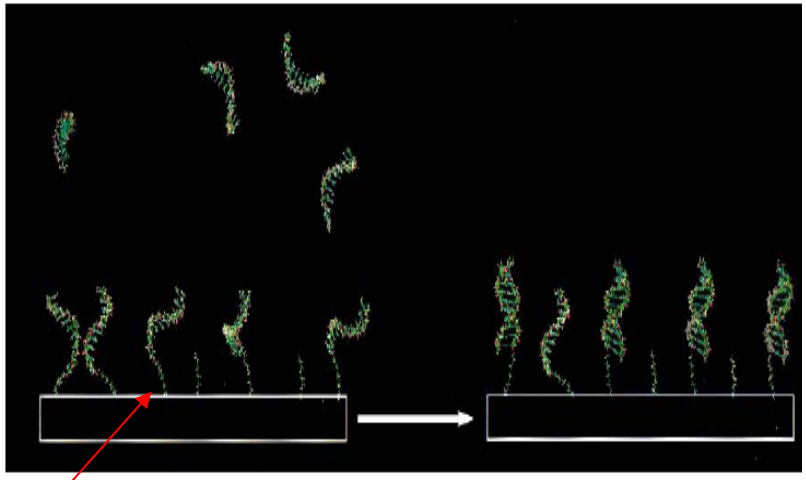


A sandwich *ELISA*. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

Applications for Immunoassays:

- Tumor Markers, e.g. AFP, CEA, hCG, PSA …
- Cardiac Markers, e.g. CK-MB, CRP, Digoxin, Myoglobin …
- Cell based Assays, e.g. cell cytotoxicity …
- Allergy, e.g. histamines, egg, milk, allmonds …
- Growth Deficiency, e.g. hGH
- Enzyme activity
- Hormone and Steroid Screening, e.g. T4, fT3, TSH …
- Drug Abuse Screening, e.g. amphetamines, cocaine, LSD …
- Immunological Screening
- Infectious Diseases, e.g. Chlamydia, CMV, Hepatitis, Rubella …
- Veterinary, e.g. bacterial infection, fertility, drugs, BSE …
- Food and Beverages, e.g. pathogens, toxins…
- Water Analysis, e.g. bacterial contamination, toxins, heavy metalls …
- Agriculture, e.g. endotoxins, pesticides …
- Environment, e.g. industrial chemicals, pesticides, surfactants …

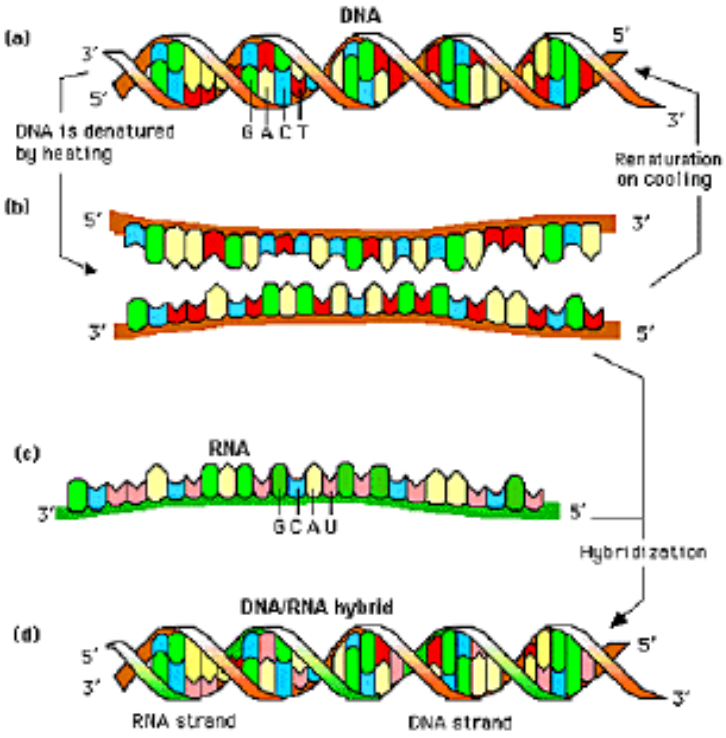
DNA assay



Immobilization

Use poly L-lysine and 3-aminopropyltriethoxysilane coated slides.

Hybridization

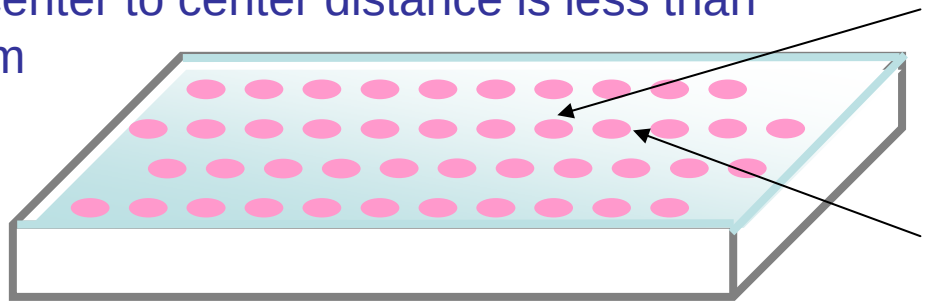


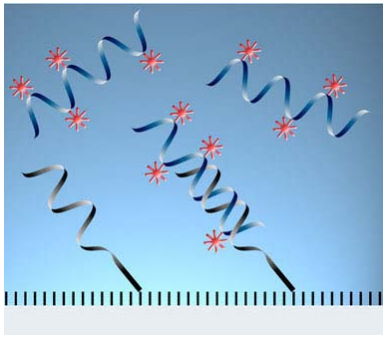
Nucleic Acid Hybridization

Micoarray chips

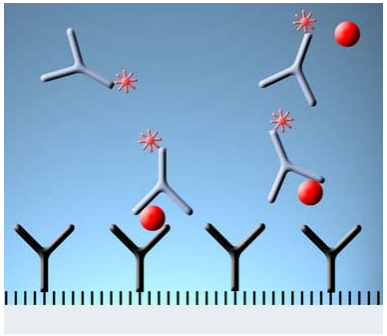
Microarray chips are collections of miniaturized test sites arranged on a solid substrate that permits many tests to be performed at the same time in order to achieve higher throughput/speed.
The Biochip technology allows biomedical researchers to study entire genes, gather information about expression, gene mutation patterns and to then integrate to an ordered array of known DNA immobilized onto a silicon chip. The total biochip market size is projected to grow to about $2.7 billion in 2007.

Miniaturization: Each dot is less than 150 μm in diameter .
Spot center to center distance is less than 200 μm





DNAs



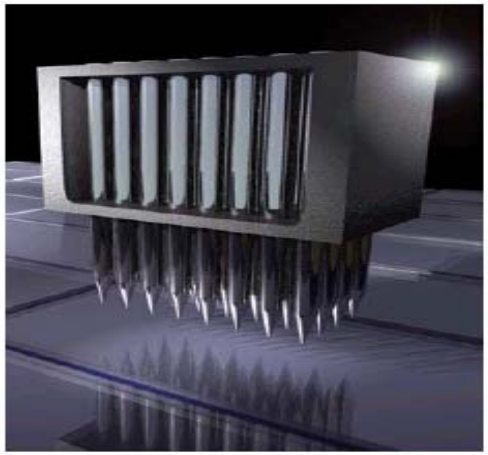
Proteins

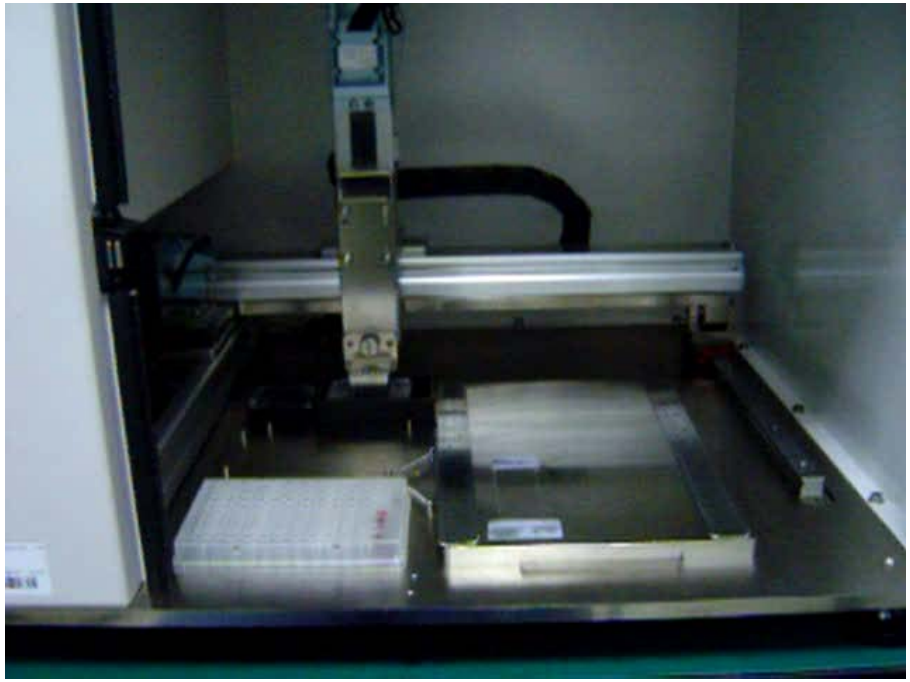
At least 2,500 spots per cm2. >35,000 spots per slide (25 mm x 75 mm)
Parallel processing → High throughput

Gene expression assays

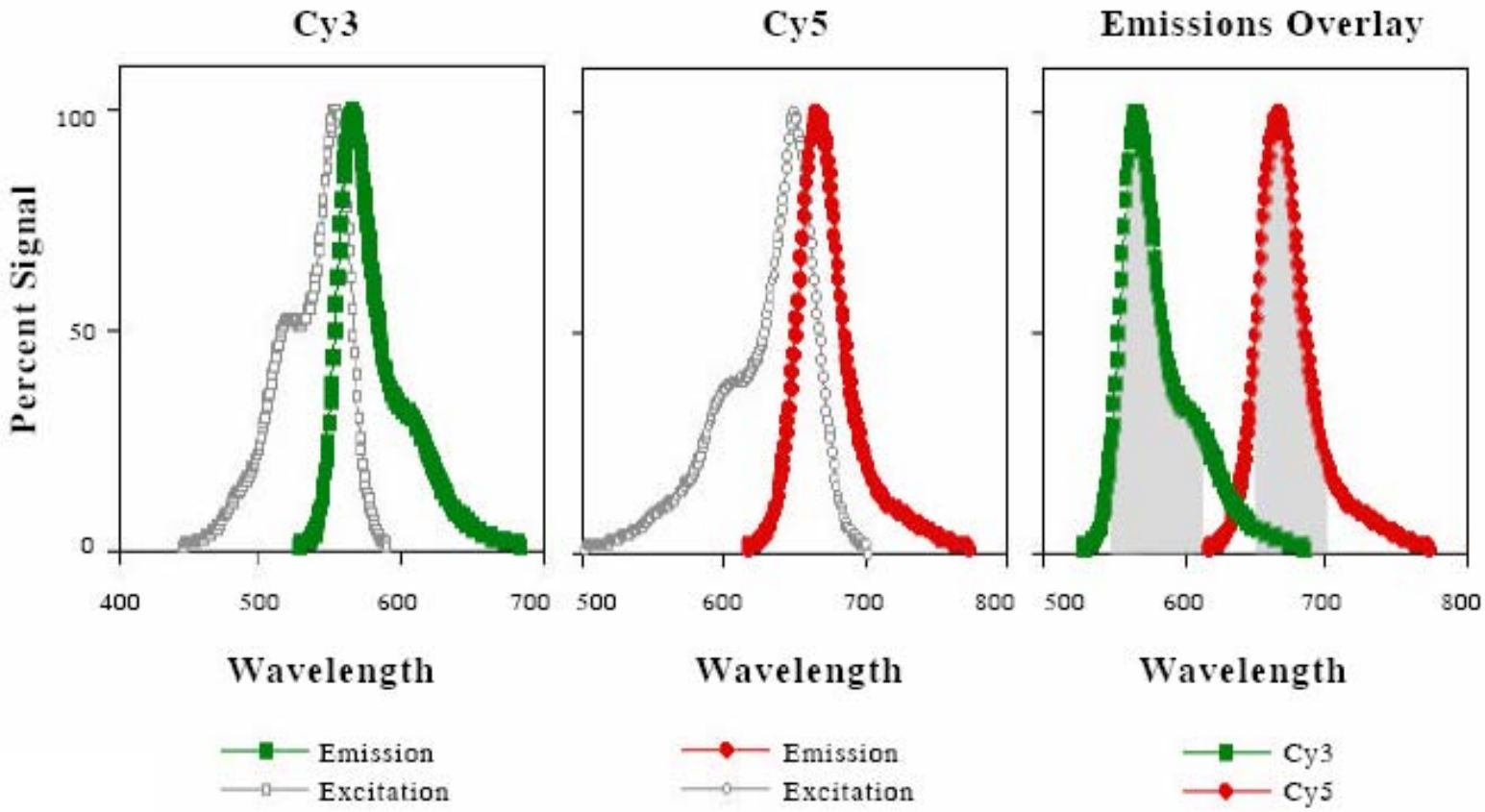
- Spotted cDNA arrays (Brown/Botstein);
- Short oligonucleotide arrays (Affymetrix);
- Long oligonucleotide arrays (Agilent Inkjet);
- Fibre optic arrays (Illumina);
- Serial analysis of gene expression (SAGE);
- ...

Spotted cDNA microarray



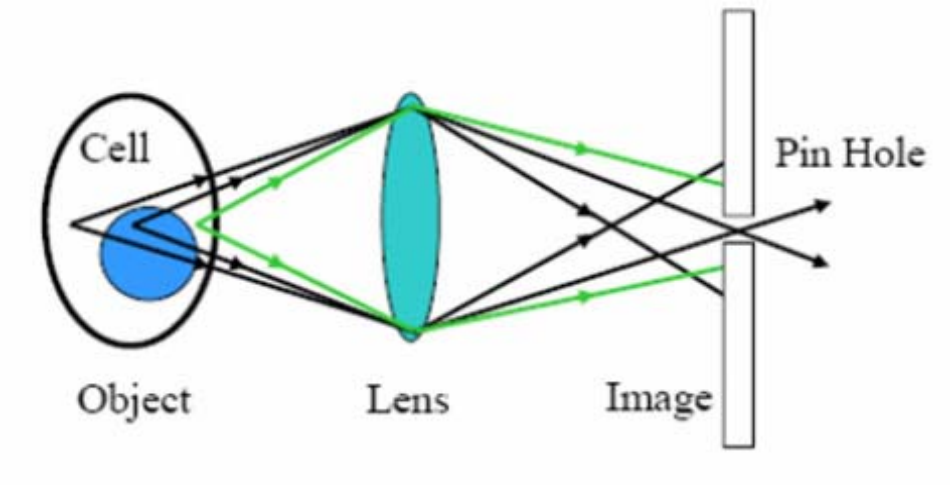


Excitation and Emission Spectra for Cy3 and Cy5

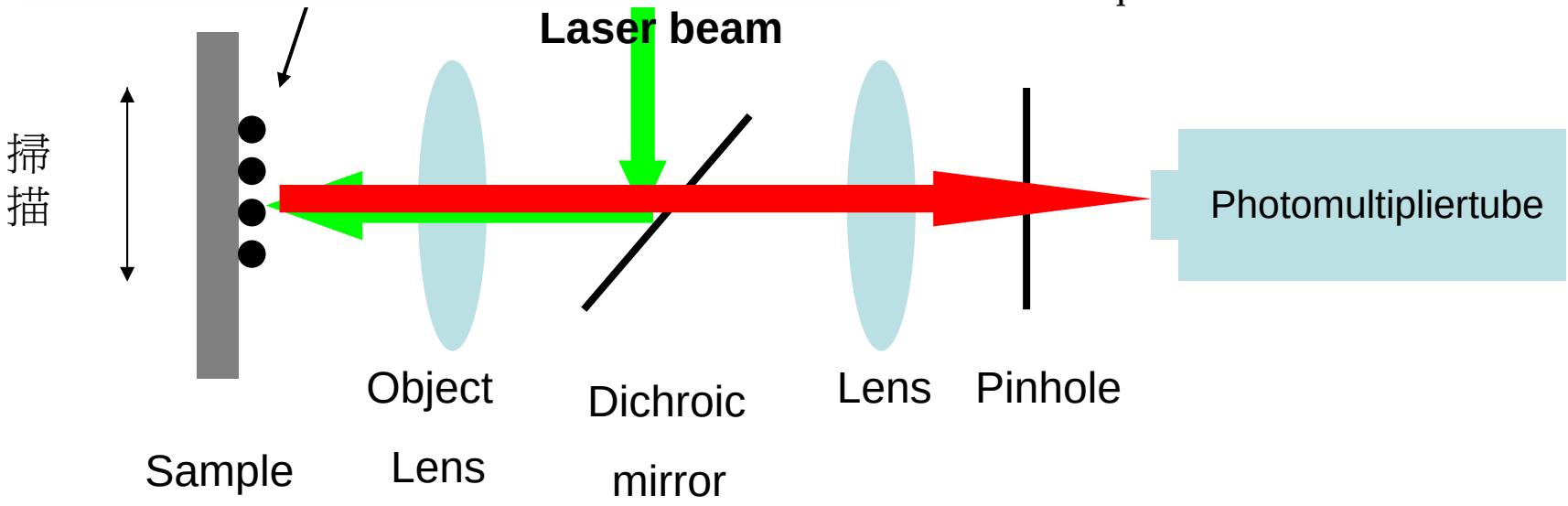


Optical Bio-Detection

Laser Confocal Scanning

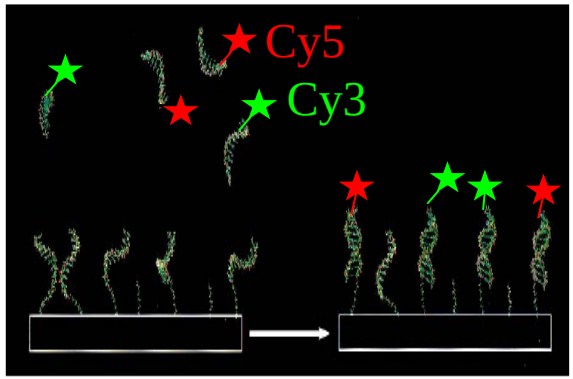


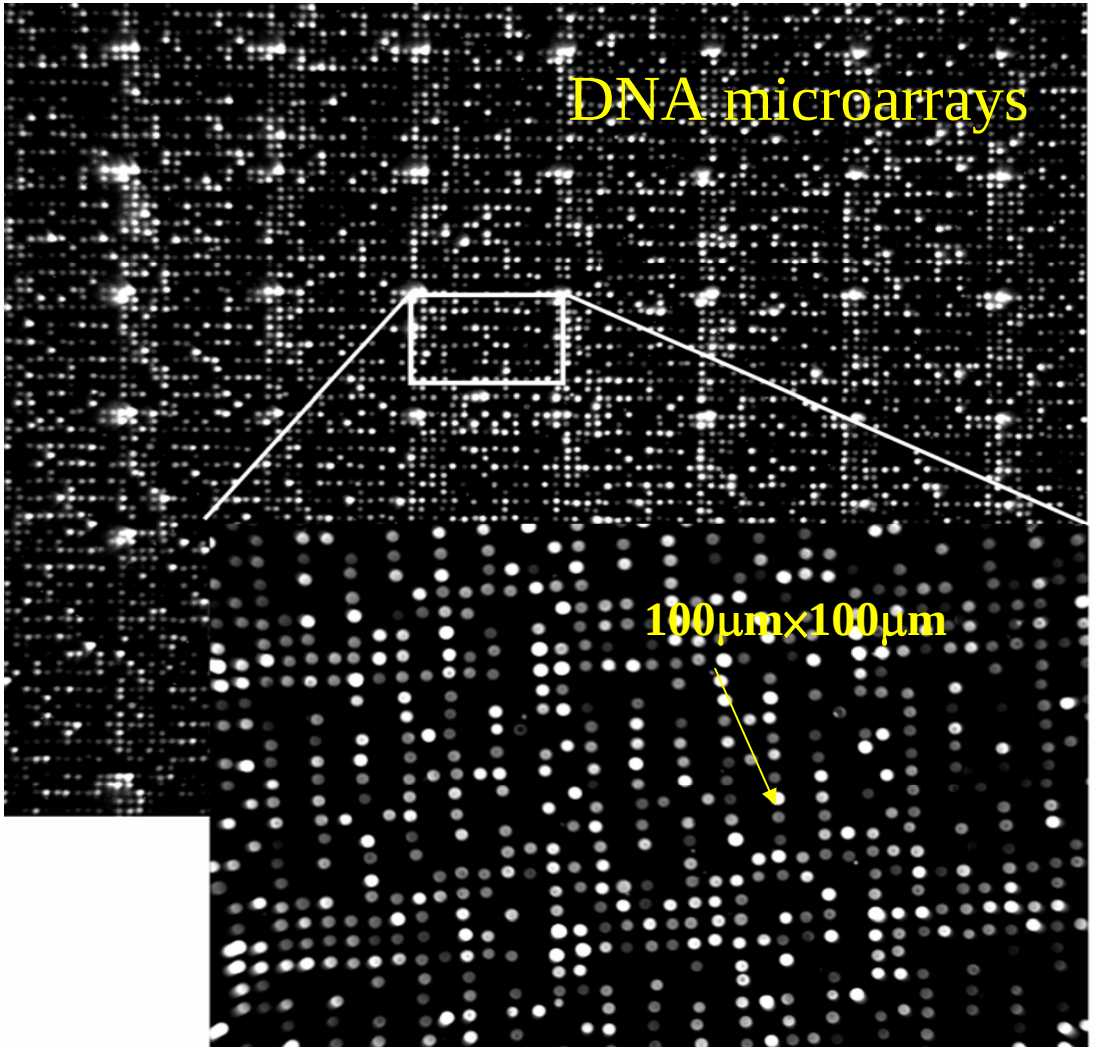
advantages: high spatial resolution and sensitive detection at the focal point.
Drawbacks: needs to scan the sample points by points. It takes a long time for scanning all the microdots. Furthermore, the confocal laser scanning method will cause position errors when multiple scans are required.



Biomolecular Interactions Studied by Specific Binding

Microarray on a Biochip:
Labeled by fluorescent dyes



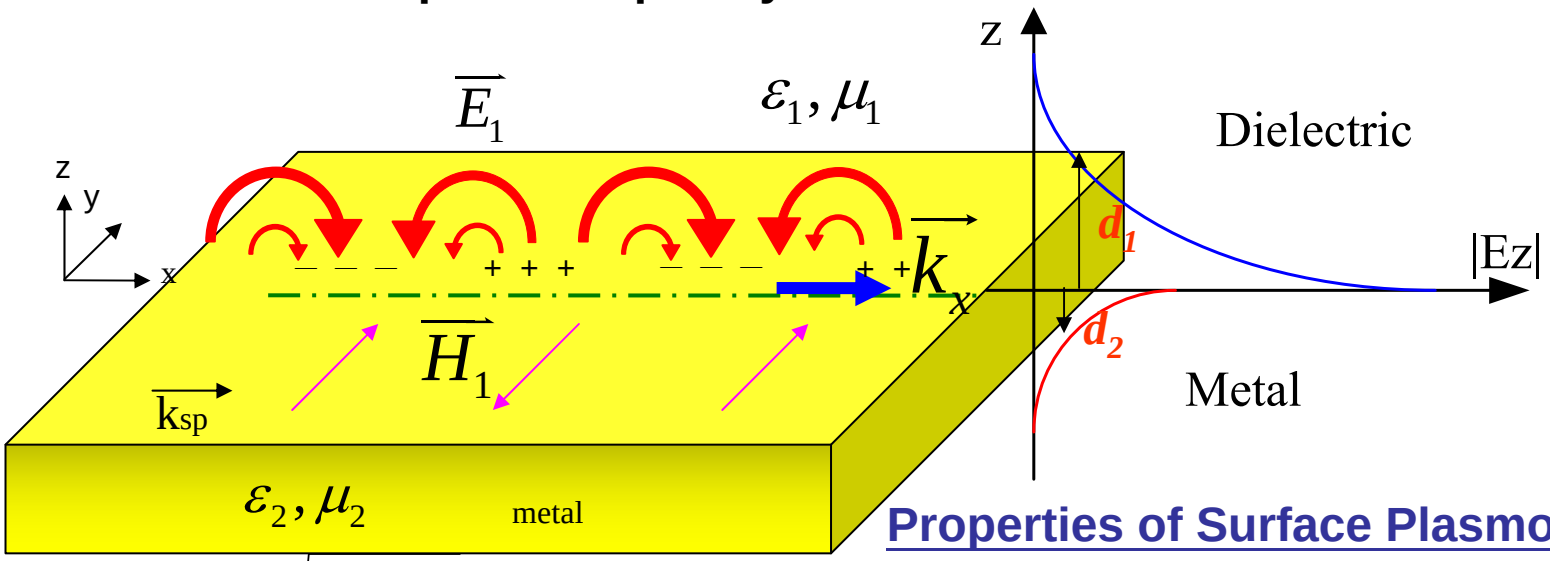


The important parameters for the assays

1. Sensitivity → Detection technology, Amplification..
2. Specificity → Bioaffinity, biomarker
3. Sample preparation → Label-Free
4. Cost → Label-Free
5. Throughput, speed → Microarray Image

Surface plasmons

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



$$k_x = k_0 n_{sp} = k_0 \sqrt{\frac{\varepsilon_1 \varepsilon_2}{(\varepsilon_1 + \varepsilon_2)}}$$

$$E1 = E_0 \exp(-k_0 z \sqrt{n_{sp}^2 - \varepsilon_1}) = E_0 \exp(-z/d_1)$$

$$E = E0 \exp(-k_0 z \sqrt{n_{sp}^2 - \varepsilon_2}) = E_0 \exp(-z/d_2)$$

Properties of Surface Plasmon wave

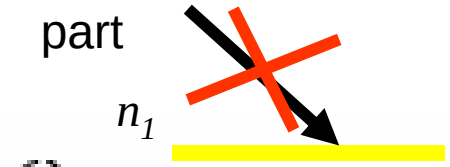
1. A **TM**-mode guided optical wave
2. Optical wave is **evanescent** on the metallic surface and most optical intensity is on the **dielectric** part.

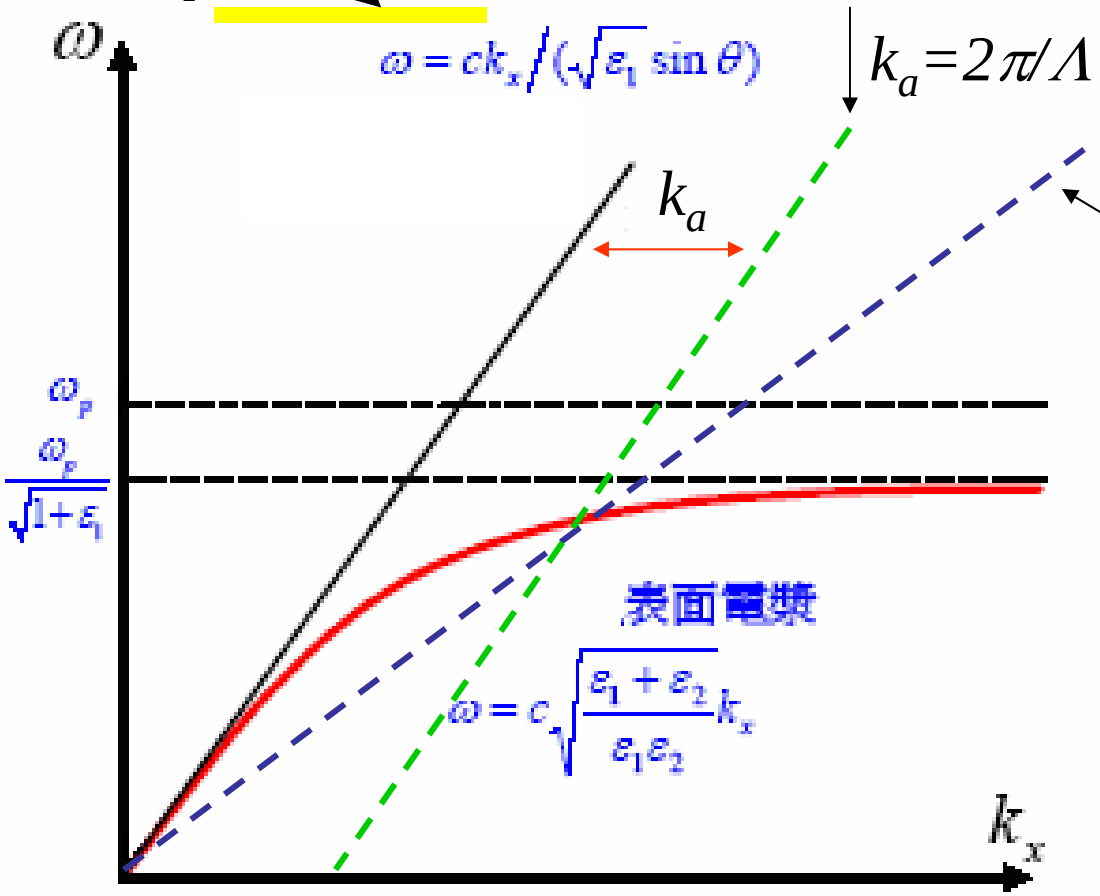
ε_2=-12 (gold), ε_1=1.32^2 (water), λ_0=800nm => n_{sp}=1.435, d1=232nm, d2=34nm

The dielectric constant of metal is negative and much larger than ε_1.

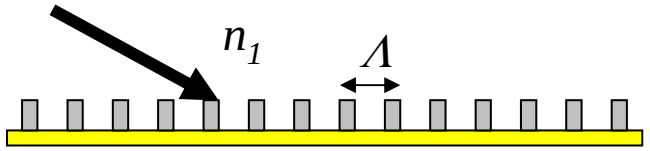
Traditional Methods to Generate Surface Plasmons

Light incident directly from dielectric part





2. Grating coupling



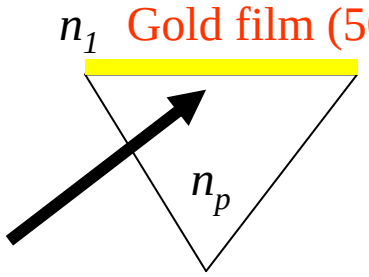
Gold film (50nm)

$k_x + k_a = k_{sp}$

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

$k_a = 2\pi/\Lambda$

1. Prism coupling



$n_1^2 = \varepsilon_1 \Rightarrow \varepsilon_p = n_p^2$

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

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

Surface Plasmon Resonance for Biodetections

Advantages:

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

TM polarize

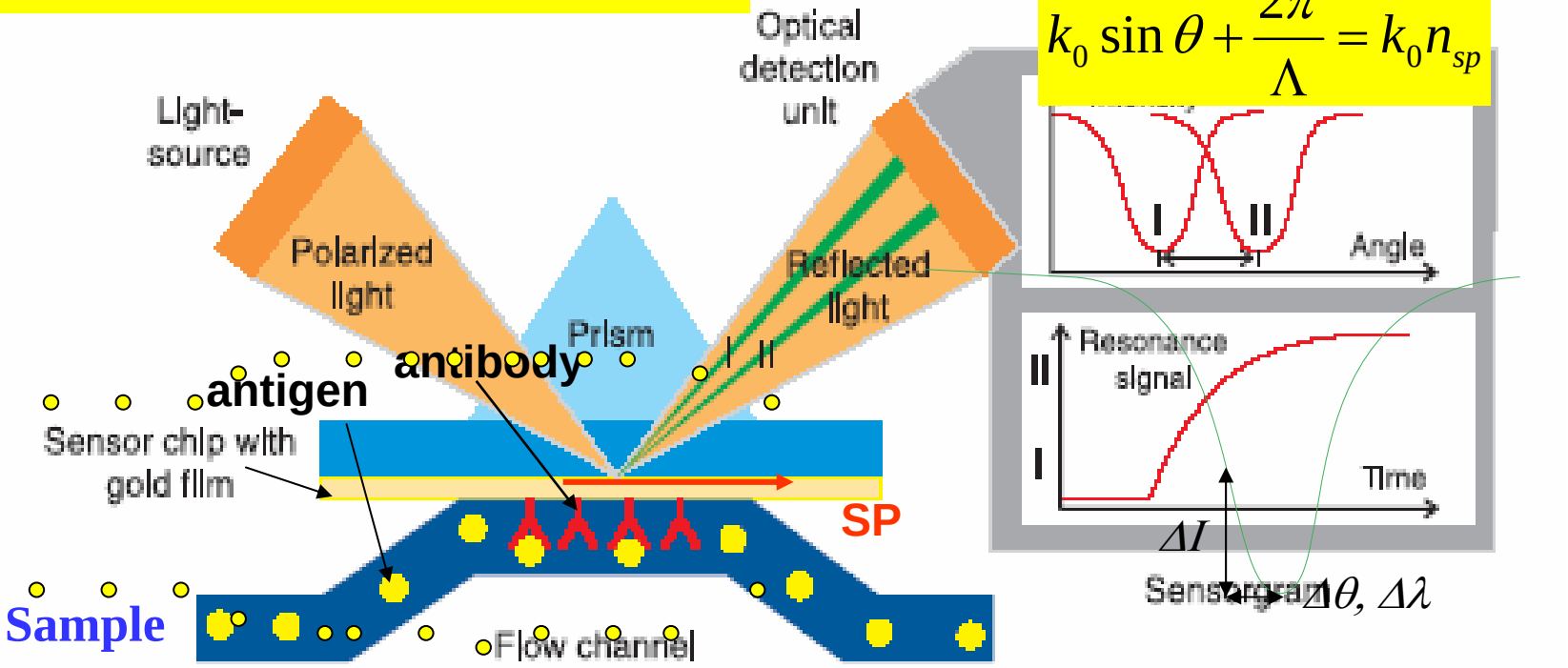
$\omega = ck_x/(n_p \sin\theta) = ck_x/n_{sp}$

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

$k_x + k_a = k_{sp}$

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

SP



Transducer Sensitivity

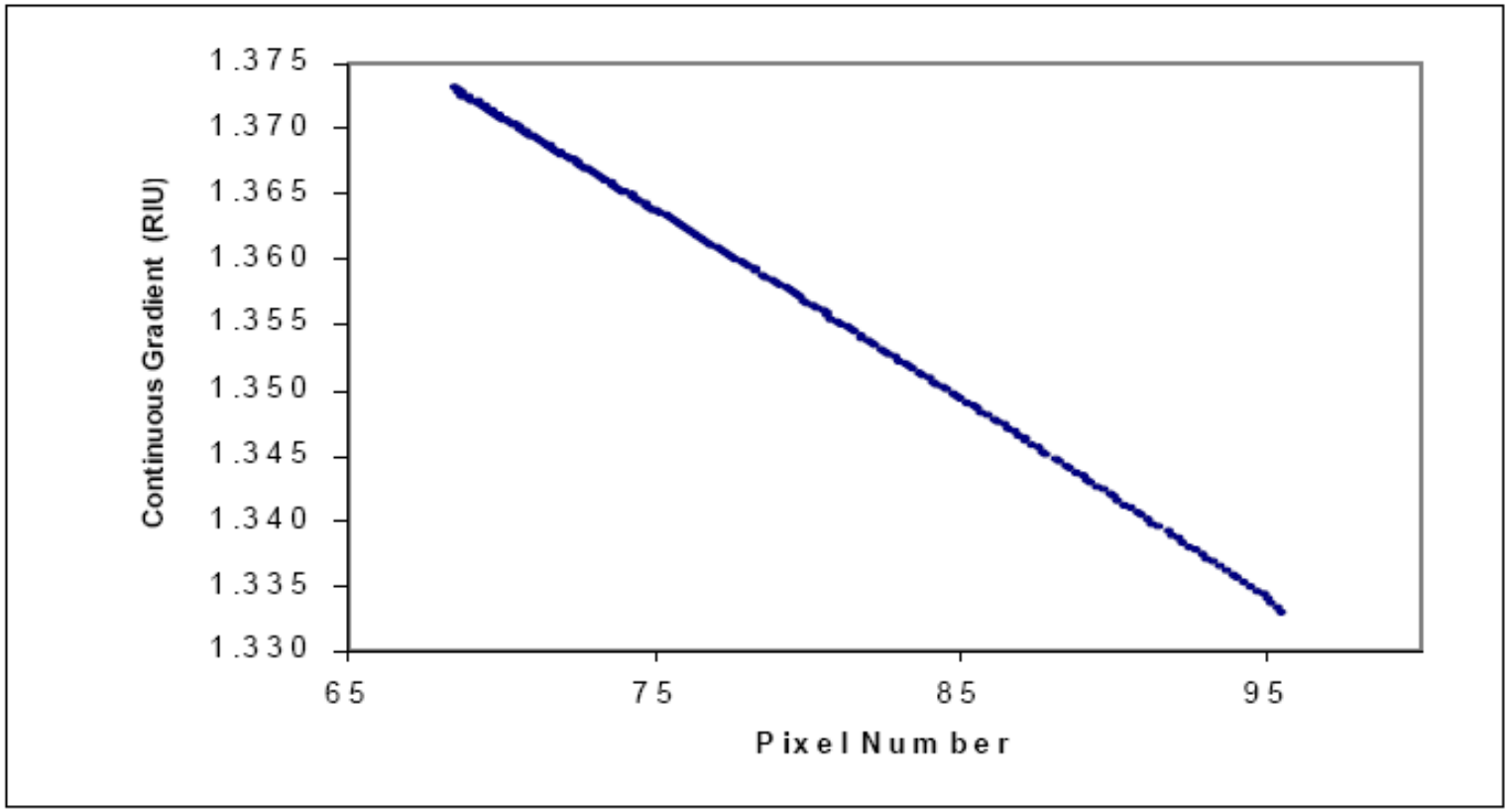


Figure 2: Plot of the pixel position of the SPR minimum versus refractive index.

Figure 3. Plot of refractive index versus SPR angle. The slope of a regression line is the sensitivity (11 x 10^{-3} RIU/degree) where R^2 = 0.9997. In practice a quadratic expression is fitted to the data when converting pixel position to SPR angle, or refractive index, since the SPR phenomenon has a slight non-linearity.

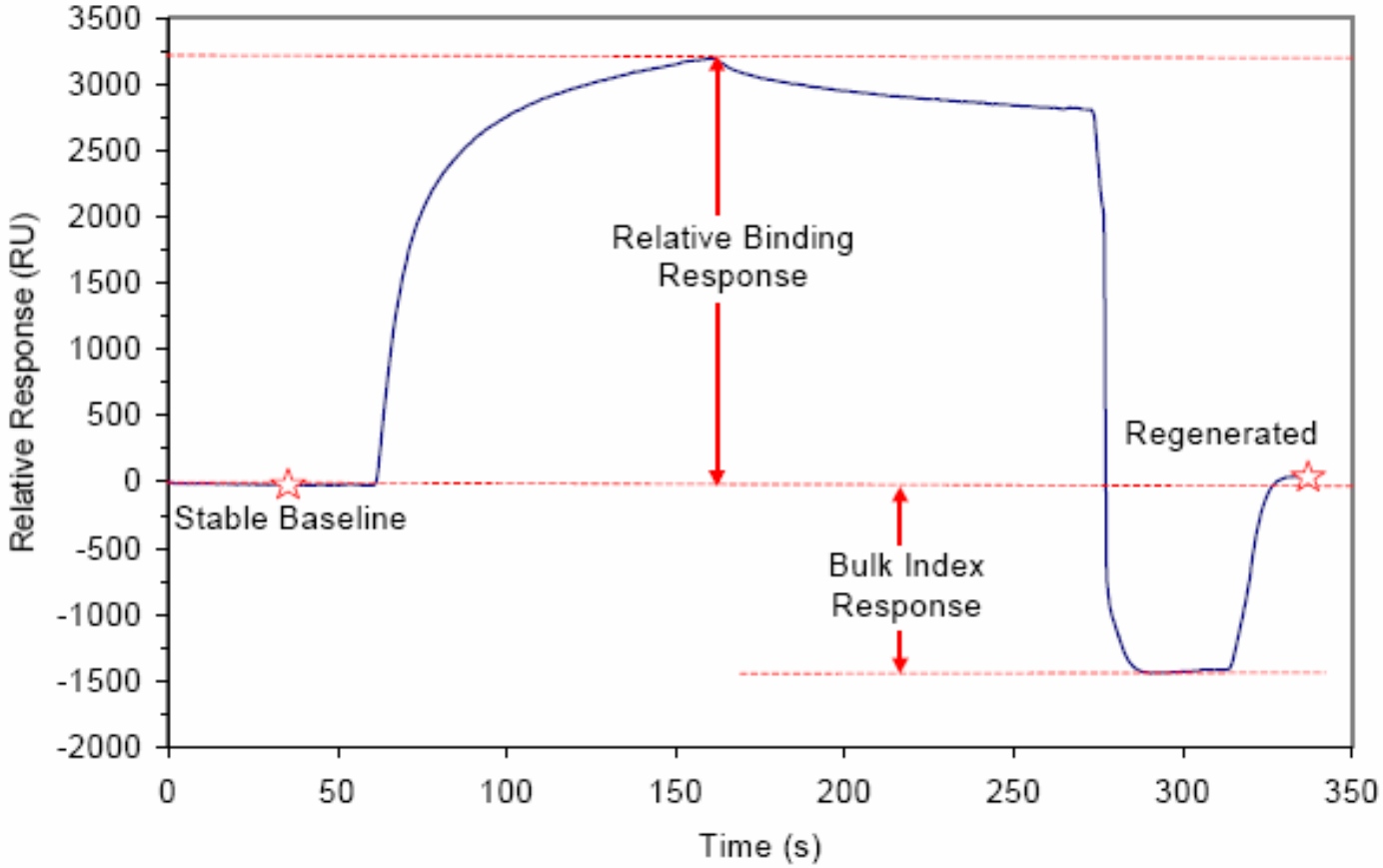


Figure 4. Binding response curve for the interaction of an antibody with immobilized protein A. The difference in response before and after sample injection is the relative binding response and it is this value that is measured during concentration analysis. The units here are in RU, where 1 RU = 1 x 10^{-6} refractive index units (μRIU). The data update rate for this plot was 0.6 Hz. The baseline noise was 1.5 x 10^{-6} RIU and baseline drift was < 1.0 x 10^{-6} RIU/ min. Note: As expected the bulk index variation between the constant flow buffer and the regeneration solution causes a step in the response. This occurs rapidly and does not impact on data quality.

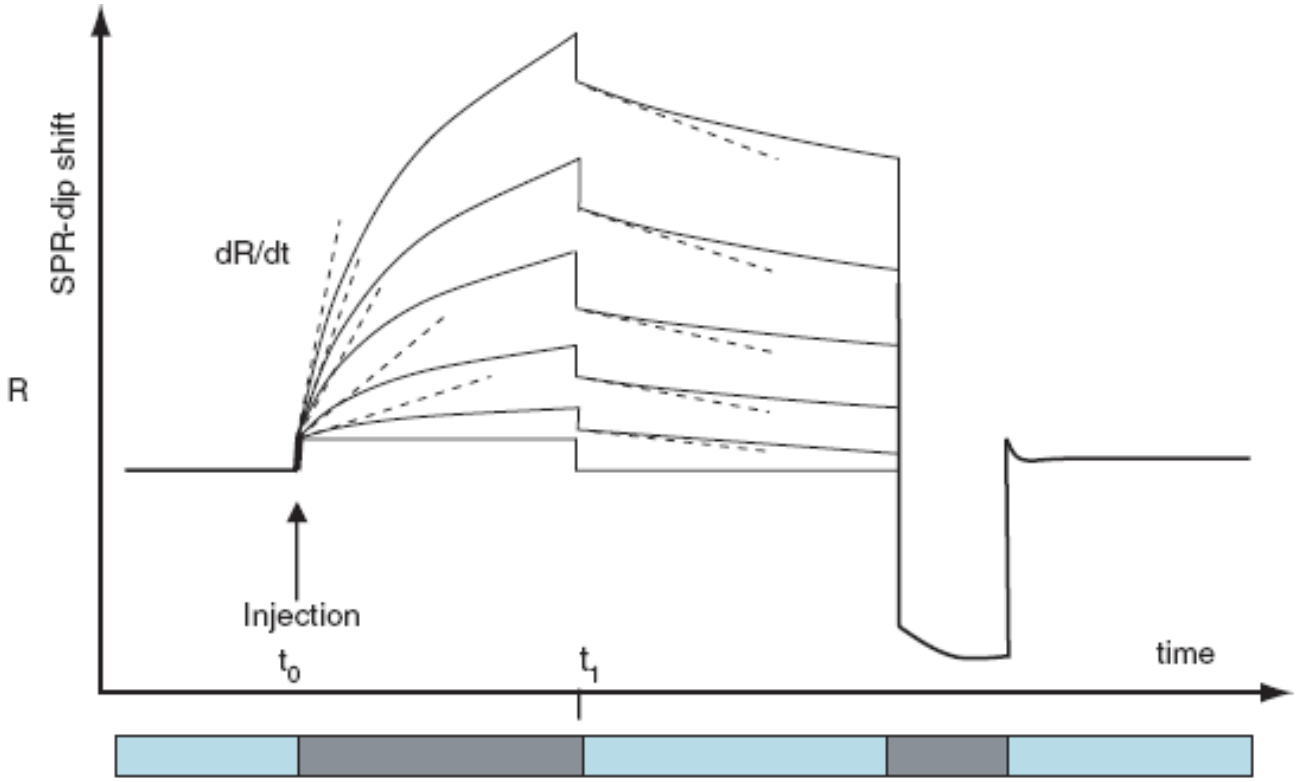


Table 1.2 Definition of the equilibrium association and dissociation constants.

	Equilibrium association constant, K_A	*Equilibrium dissociation constant, K_D*
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	$1\,mol^{-1}$	$mol\,l^{-1}$
Typical range	10^5–10^{12}	10^{-5}–10^{-12}

Comparisons of SPR sensor

Theoretical sensitivity to variations in the refractive index of analyte and resolution of model SPR sensing structures: (a) prism-based system (BK7 glass—gold 50 nm thick—analyte with the refractive index of 1.32); (b) grating-based system (grating with the pitch and depth of 800 and 70 nm, respectively—gold—analyte with the refractive index of 1.32)[a]; optical constants of gold were taken from [16]

Detection approach	Angular interrogation		Wavelength interrogation		Intensity measurement	
Optical system used for excitation of SPW	Sensitivity (deg RIU^{-1})/ Resolution (RIU)[b]		Sensitivity (nm RIU^{-1})/ Resolution (RIU)[c]		Sensitivity (% RIU^{-1})/ Resolution (RIU)[d]	
	$\lambda = 630$ nm	$\lambda = 850$ nm	$\lambda = 630$ nm	$\lambda = 850$ nm	$\lambda = 630$ nm	$\lambda = 850$ nm
Prism coupler-based SPR sensor	191 5×10^{-7}	97 1×10^{-6}	970 2×10^{-5}	13 800 1×10^{-6}	3900 5×10^{-5}	15 000 1×10^{-5}
Grating coupler-based SPR sensor	43 2×10^{-6}	39 2×10^{-6}	309 6×10^{-5}	630 3×10^{-5}	1100 2×10^{-4}	4400 5×10^{-5}

[a] The following SPR instrumentation accuracies were assumed:
[b] 1×10^{-4} deg for angular resolution [10].
[c] For wavelength interrogation, 0.02 nm [32].
[d] For the intensity measurement, 0.2% of the optical power [33].

J. Homola et al. / Sensors and Actuators B 54 (1999) 3–15

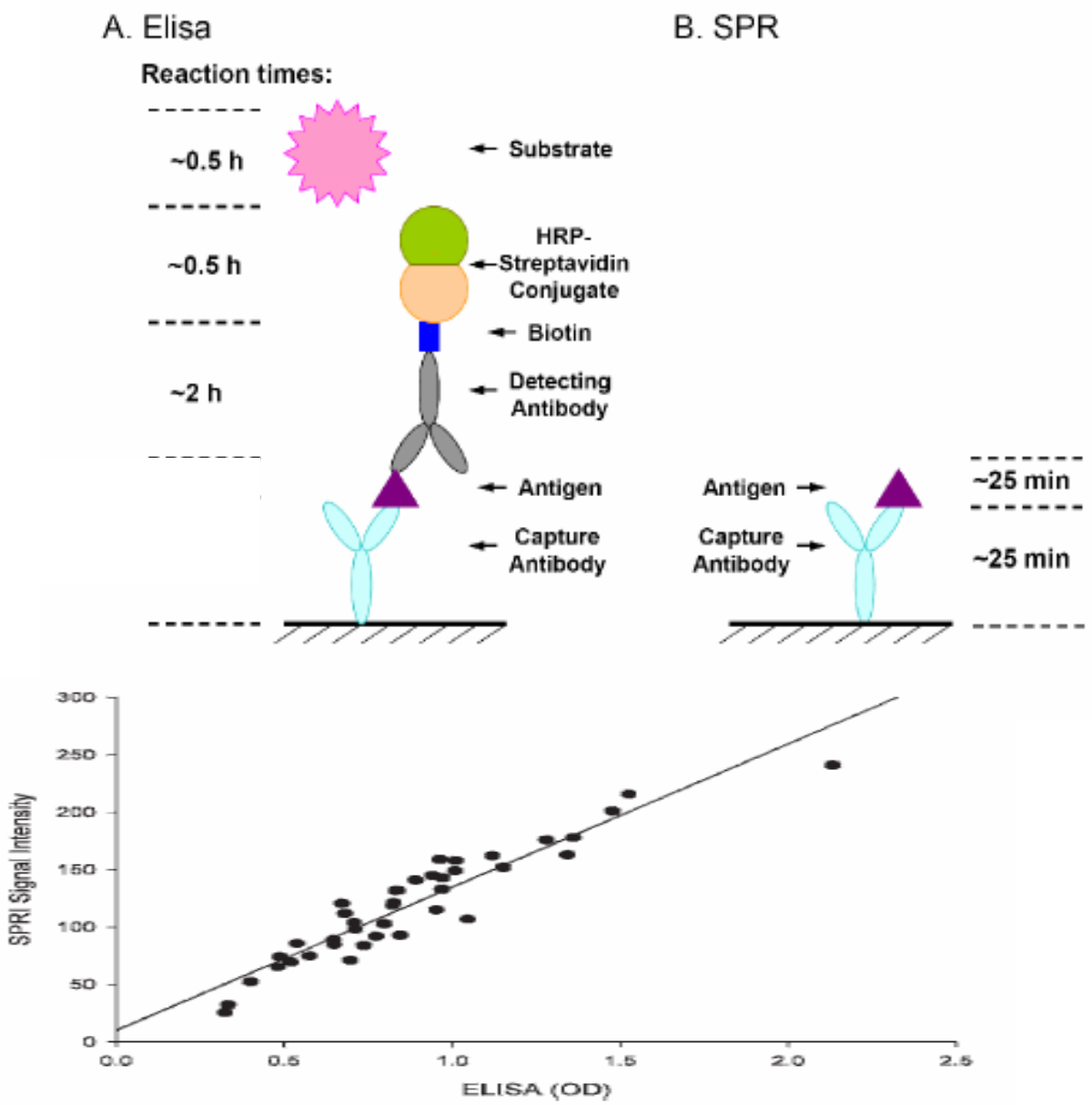
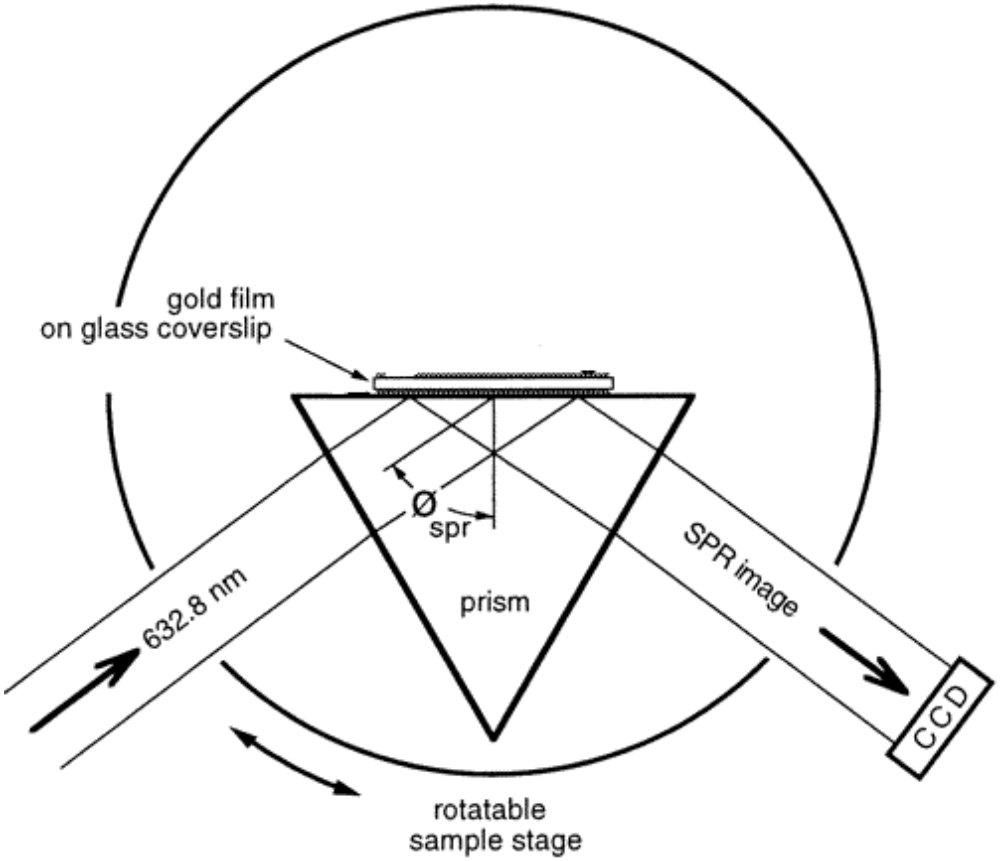


Figure 2. Correlation between the antibody OD values of the iridovirus in the rock bream sera determined by ELISA and signal intensity determined by SPRI (Pearson correlation coefficient, $n = 40$, $r = 0.939$, $P < 0.01$).

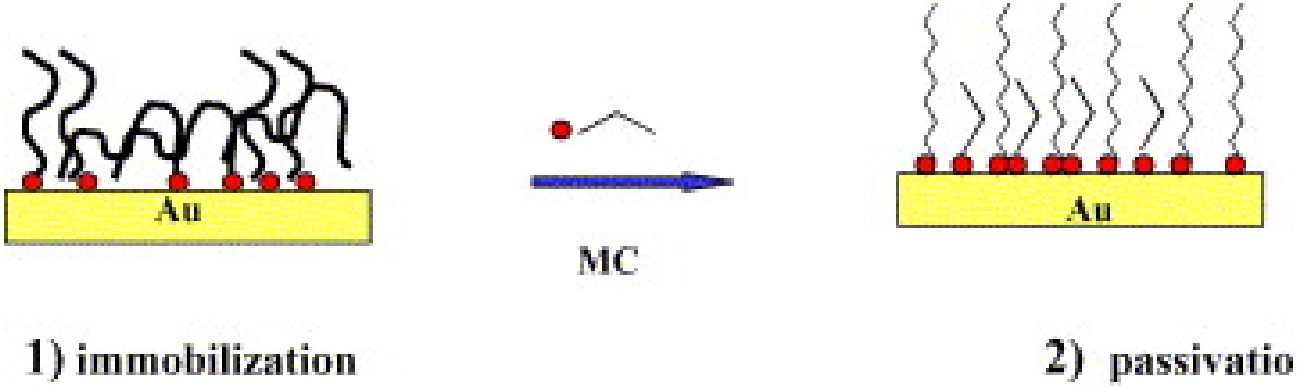
SPR imaging apparatus



- 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.

Hybridized helices formed on gold substrate

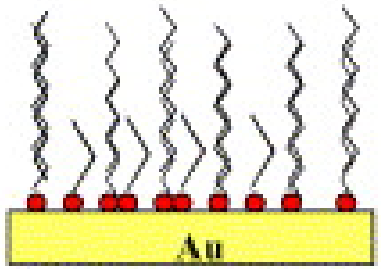


1) immobilization

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

2) passivation

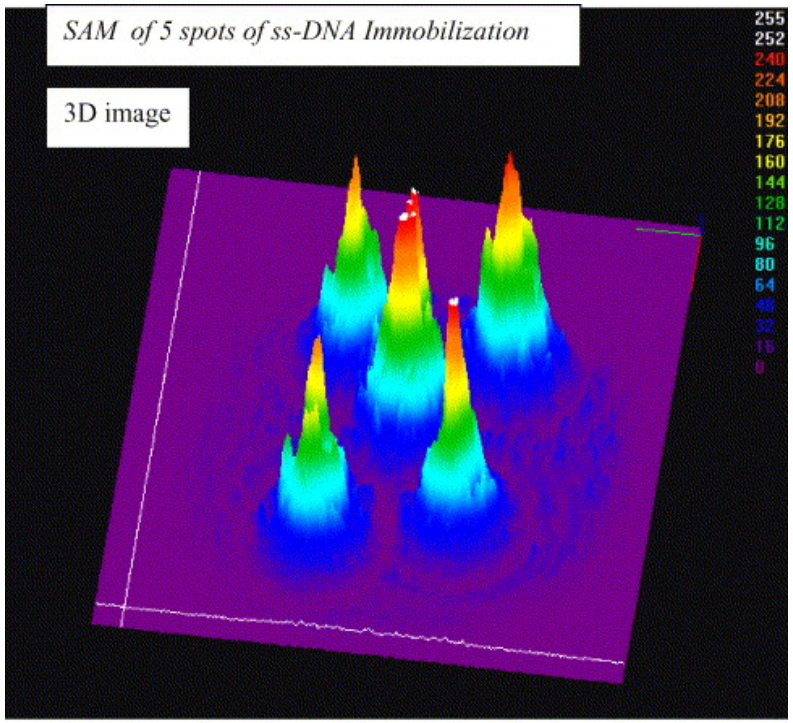
•Immersed in mercaptoethanol for 18h to eliminate aspecific adsorption sites on the gold surface

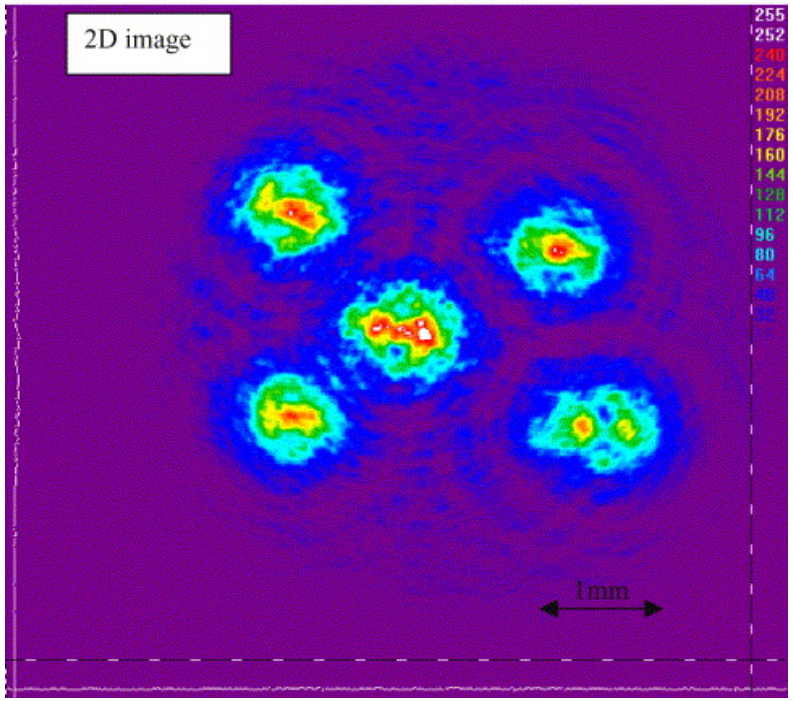


•Forms dsDNA with complementary sequence

3) hybridization

•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.

Advantages of SPR :

1. Label-Free, reducing cost and time
2. Quick tests, kinetic studies available
3. Comparable sensitivity with ELIS

Price $150,000 (US)

Price $9,995 (US)

Disadvantages of Conventional SPR:
bulky, expensive, limited high-throughput detections (not chip-based, prism needed), requires a large amount of sample solution.