



Food Safety and Quality with Microsystems

Athens. 29/11/04

Tutorials on Biosensors

An immunosensor may be defined as:

‘a compact analytical device incorporating(an antibody or antibody fragment)..... either integrated within or intimately associated with a physicochemical transducer.’

Given the kinetics of an antibody-based binding procedure, most immunosensors are typically non-regenerable, hence most immunosensors:

‘will produce a discrete..... digital electronic signal proportional to a single analyte or a related group of analytes.’

Definitions adapted from: Turner, A.P.F., Karube, I. and Wilson, G.S. (1987) *Biosensors: Fundamentals and Applications*. Oxford University Press, Oxford. 770p.

- **Antibody**
 - Immunoglobulin, Ig, Ab
 - Protein (animal), 150 kD (150,000 MW)
 - Response to foreign substance (typically >5000 MW)
 - Specific binding affinity
 - Secreted from plasma cells

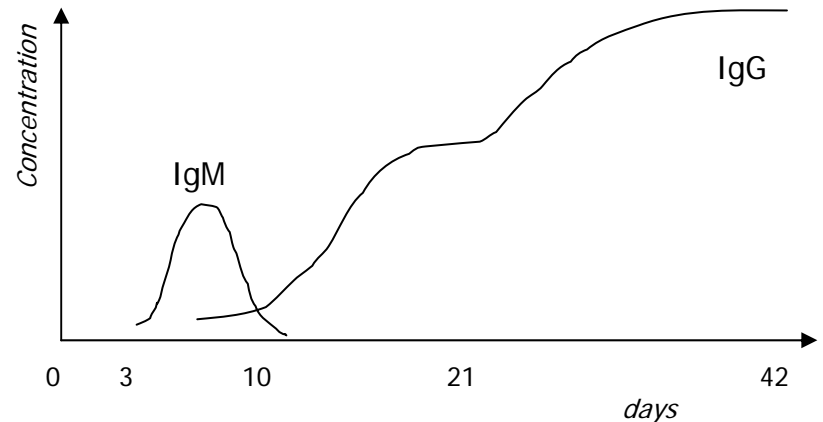
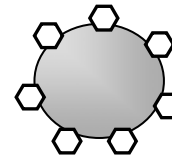
- **Antigen**
 - Immunogen, Ag
 - Foreign macromolecule (e.g. protein, polymer)
 - Elicits Ab formation
 - Ab specificity against sites on Ag (antigenic determinants)
 - Typically the 'analyte' in immunodiagnostic applications

- **Hapten**
 - Small foreign molecule (<5000 MW) – need to attach to macromolecule (carrier) to obtain a response (eg: BSA)

Antibody binding sites

- **The central proviso of any immunodiagnostic application is the *specific* binding of the target analyte (antigen) by the antibody**
- Occurs at the antibody binding site
- Similarities with enzyme active sites:
 - *Hydrophobic*/Electrostatic/H-bond/Van der Waals
 - Not covalent
 - Size (~25 Angstroms)
 - Diffusion controlled
- Differences:
 - Ab-Ag binding is reversible (equilibrium process), although complex [Ab-Ag] formation is greatly favoured
 - Variable binding affinities

- Eg: Dinitrophenyl (DNP) compound.
- Link DNP to carrier. Inject into host.
 - ~3-4 d IgM (10^3 kD)
 - ~10 d IgM decrease, IgG increase (150 kD)
 - ~3 wk IgG plateau. Administer booster dose
 - ~6 w Blood drawn (antiserum). ~ 1 mg ml⁻¹ anti-DNP antibody. Mostly IgG



- Purify: Affinity chromatography

Note: There are 5 main classes of Ig – Immunosensors overwhelmingly use the IgG type



Usually HETEROGENEOUS because:

- **Ag is macromolecular.** Usually several potential antigenic determinants, hence several possible antibody binding specificities
- Hence **polyclonal**

Variable binding affinities evident, BUT:

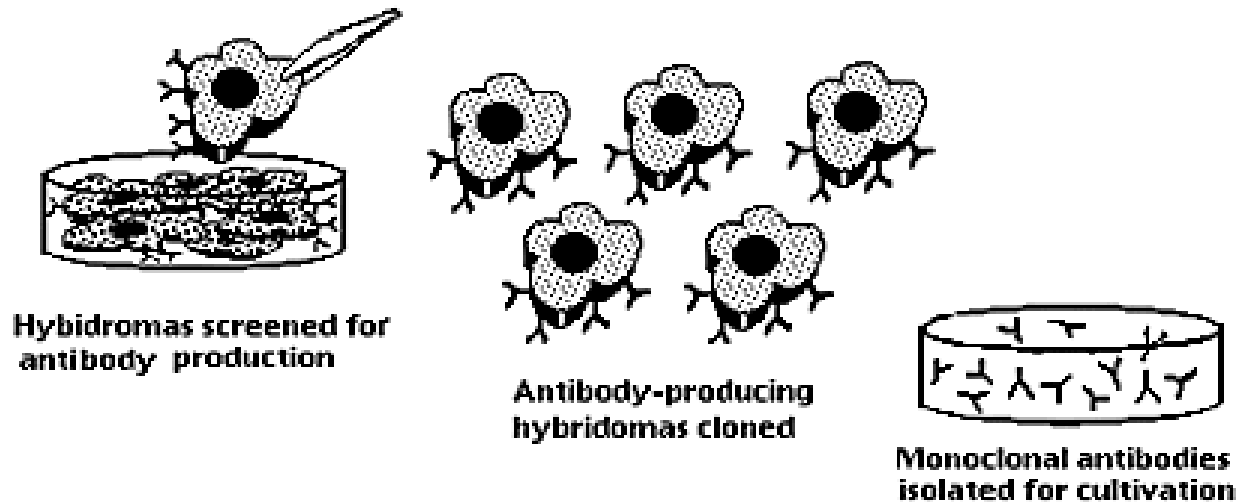
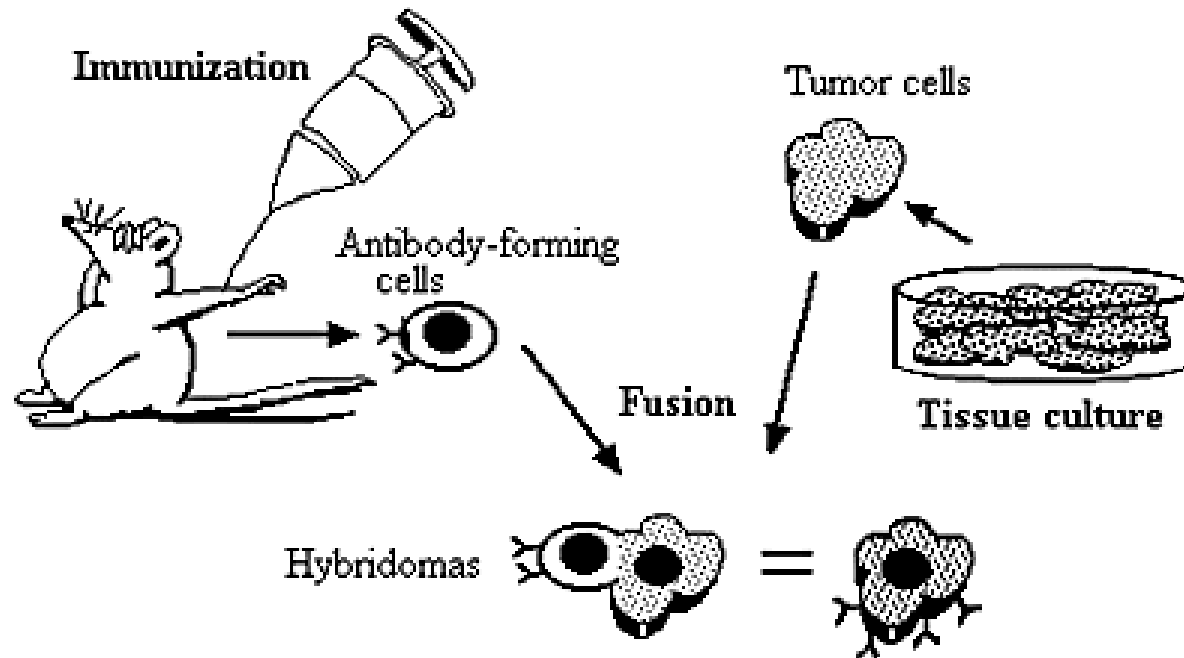
- Antibodies synthesised by a single plasma cell are **HOMOGENEOUS**
- (But note: each plasma cell will produce a single antibody binding specificity, by definition **monoclonal**)
- Polyclonal antibodies are present in the antiserum from Ag inoculated animal
- Source of antibody batch ends when animal dies, hence inter-batch variability

Monoclonal Abs

Well-defined specificity against target antigen(s)

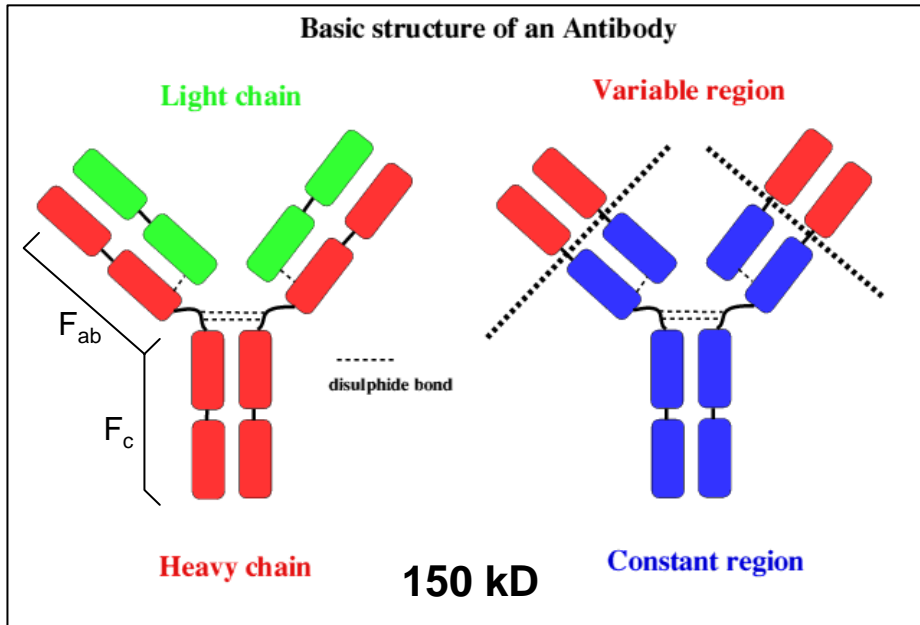
Production:

- Mouse immunised by injection of target antigen
- ~6 w, plasma cells are isolated from mouse spleen
- Individual plasma cells are isolated and fused with tumour (myeloma) cells to form **hybridomas** and cultured in separate vessels
- Rapid division of hybridoma cells and secretion of Ab
- Each hybridoma culture produces and secretes large amounts of identical Ab - generation after generation (**immortal cell line**)
- Screen for antibody of desired specificity
- Monoclonal: identical offspring of single cloned plasma cell



Monoclonal Antibody Production

IgG basic structure



Proteolytic cleavage:

- 2 F_{ab} fragments (50 kD)
- 1 Ag binding site each
- No precipitate + Ag

- 1 F_c fragment; no Ag binding
- Crystallisable - homogeneous
- Biological/structural properties

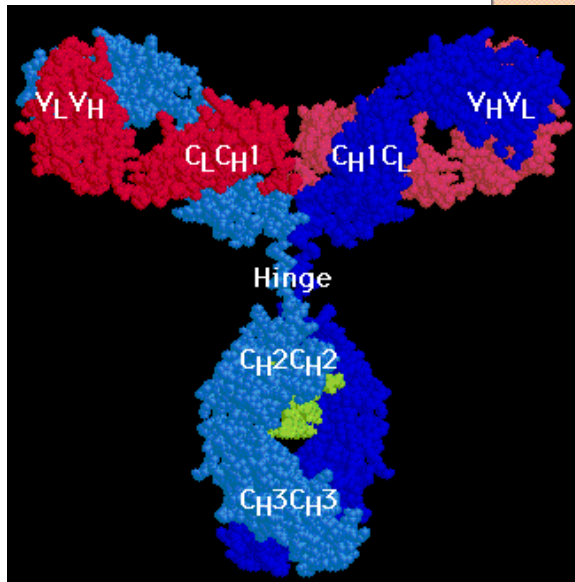
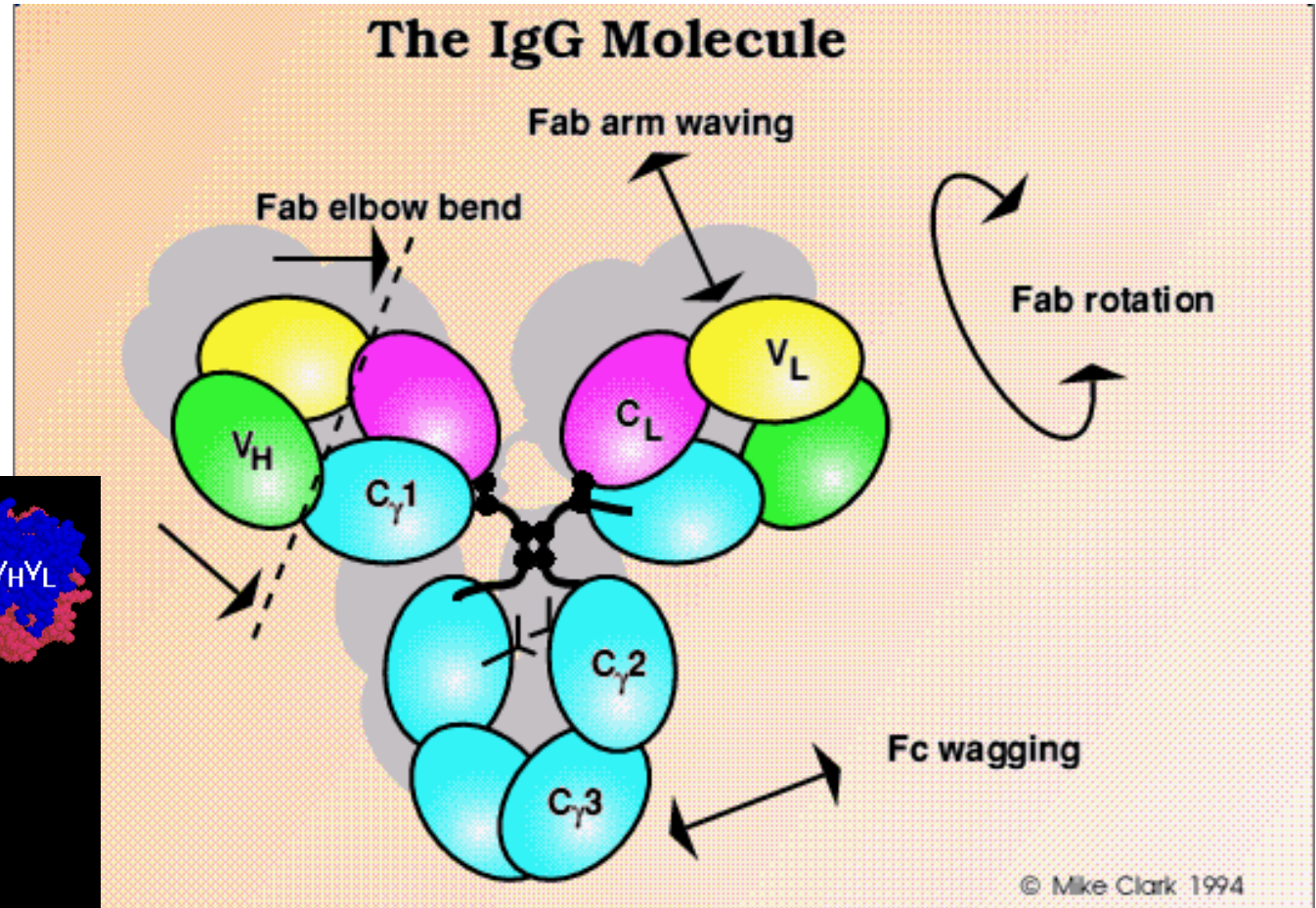
Disulphide bond cleavage:

- Mercaptoethanol
- Chromatography:
 - Light (L) chain (25 kD)
 - Heavy (H) chain (50 kD)
- Reconstitute: L₂H₂

IgG is 'Y' shaped

Ag binding near end of Fab units

Segmental flexibility enhances Ab binding to multivalent Ags



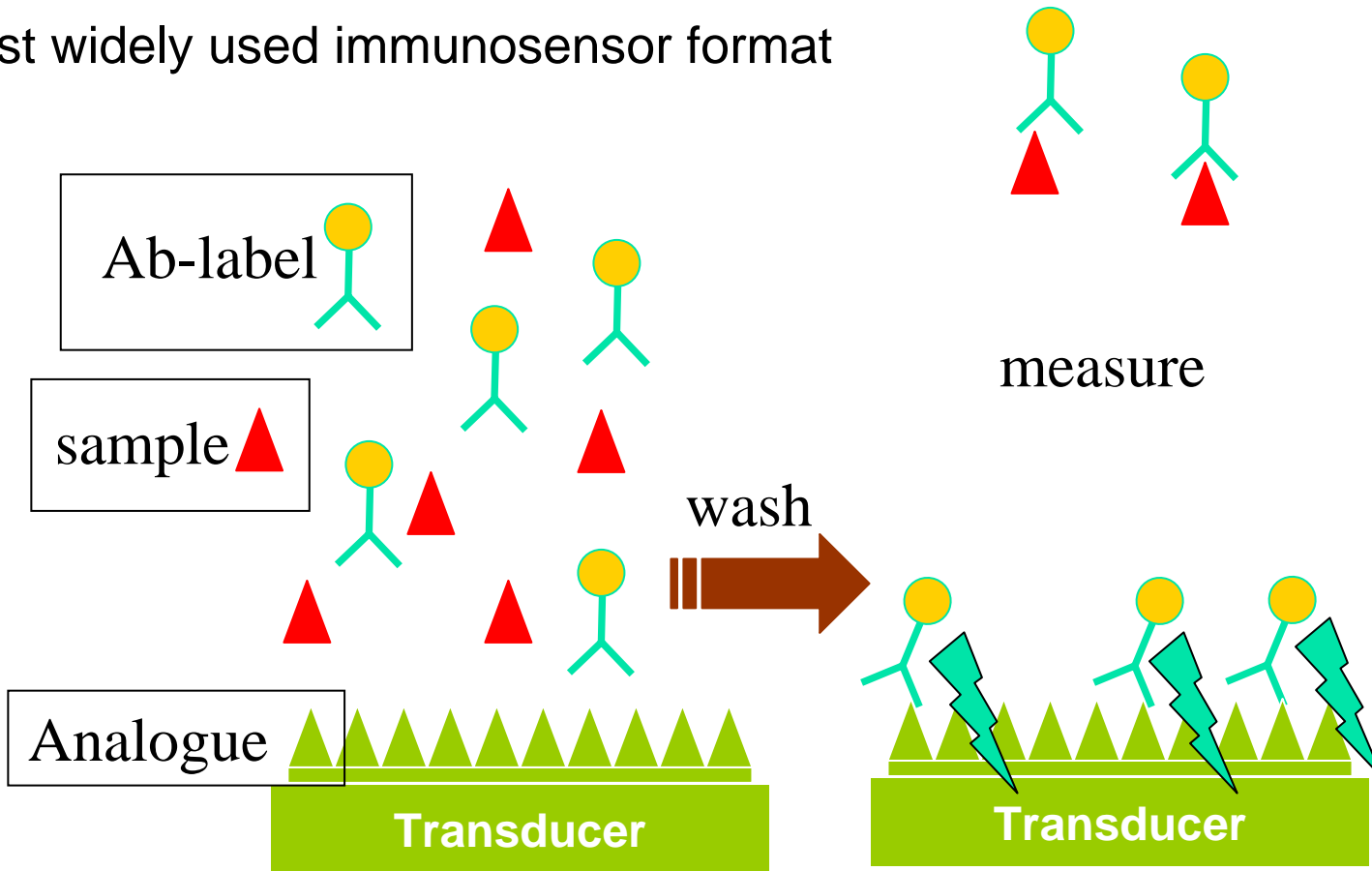
- Based on specific Ab-Ag interaction

- Benefits
 - Selectivity (uniqueness of Ab binding site)
 - Sensitivity (Ab-Ag binding affinity is generally high)
 - Economy (minute amounts of Ab required)
 - High throughput (rapid, multi-testing)
 - Can be very simple (no complex instrumentation)
 - Cheap

- May be direct (label free) or indirect (labeled tracer compound required)

Competitive indirect immunosensor

Most widely used immunosensor format



Immobilised antigen format – *immobilised antibody format also possible*

- Majority of immunosensor formats require immobilisation of an immunoreagent within, or in intimate association with the transducer:
- May be antibody or hapten-protein conjugate
- *Some physical immobilisation methods:*
 - Physical adsorption to suitable surfaces
 - electrostatic, hydrogen bonding, hydrophobic interactions etc.
 - Plastics, glass, carbon etc.
 - Gel entrapment
 - e.g. sol-gel formation, use of polyionic compounds
 - Physical entrapment by membranes
 - e.g. dialysis membrane



- *Chemical immobilisation methods*
 - Use of bifunctional agents to cross-link protein to solid-phase support (transducer) – vast number of chemistries reported
 - Example: carbodiimide , glutaraldehyde, cyanogen bromide
 - Can immobilise to magnetic beads
 - NB: Antibodies are proteins, hence have carboxyl and amino groups which can be exploited for immobilisation purposes

- *Other common methods:*
 - Formation of self-assembled monolayers
 - e.g. alkanethiols on Au, carboxylic acids on Al_2O_3 or Ag

 - Exploitation of streptavidin-biotin binding interactions
 - Streptavidin – specific inhibitor of Biotin (enzyme of the vitamin B complex) – very high binding affinity
 - Conjugated to proteins + supports for simplified immobilisation strategies

Amperometry, Potentiometry, Impedance

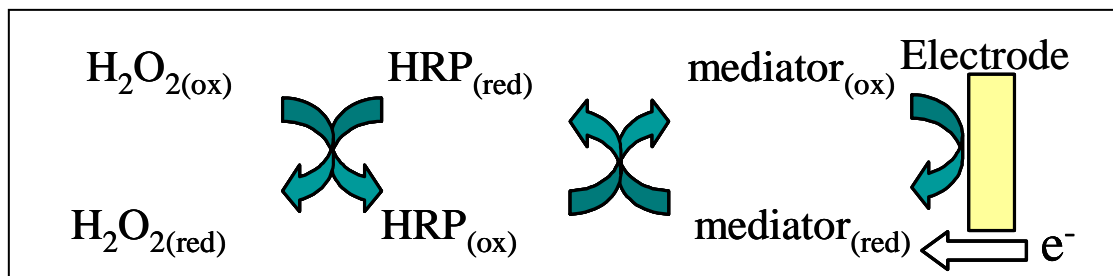
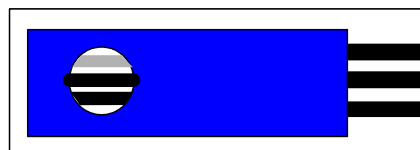
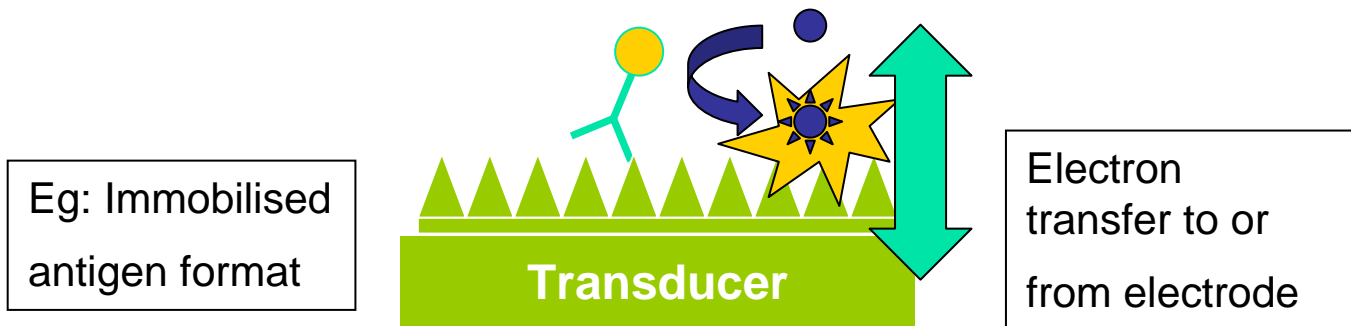
Impedance

- Typically immobilise antibody (or antigen analogue) on an electrode (gold commonly used).
- Addition of antigen (or antibody) results in a change in impedance (due to changes in resistive and capacitive properties) close to electrode surface
- AC technique, therefore impedance can be measured across a range of frequencies if desired (similar principle to optical spectroscopy)
- Direct electrochemical signal measured - no requirement for labelled immunoreagents (although labels, such as colloidal gold can be used to enhance sensitivity)
- Microelectrode/nanoelectrode structures can improve sensor sensitivity as the electric field is concentrated closer to the electrode surface (where the binding reaction occurs) when compared with conventional electrodes

Electrochemical transduction (2)

Amperometry:

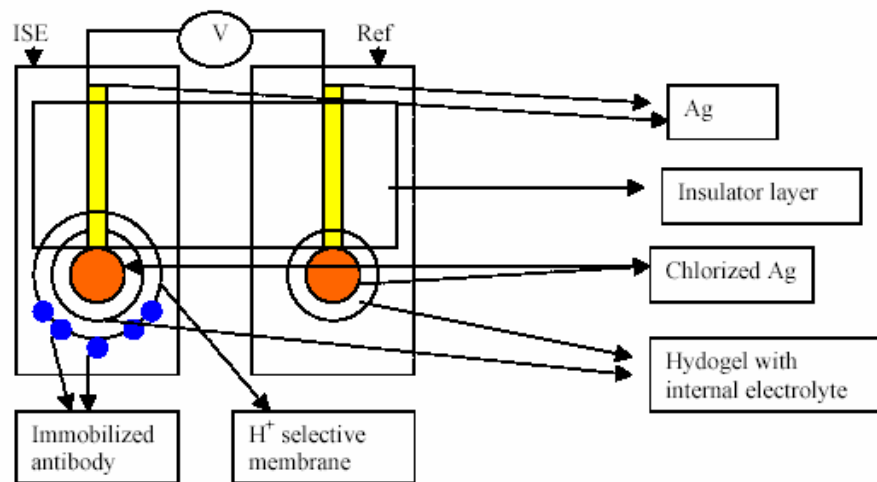
- Electroactive label; enzyme + electroactive product
- 'Working' electrode poised at a fixed potential, measure current due to redox behaviour of label
- Indirect



Electrochemical transduction (3)

Potentiometry

- Indirect detection technique – requires appropriately labelled immunoreagents
- Performed at an indicator electrode under null current conditions
- Requires a label that can change the redox state, pH or ionic strength in the vicinity of the indicator electrode
- Results in measurable potential shift that can be related to label, hence analyte concentration



e.g: pH probe with antibody immobilised at sample interface.
Label could be an enzyme that alters the local pH conditions on addition of substrate

5. Optical transduction

Generality

Label-free

SPR

Waveguide

Ellipsometry

Interferometry

Label: Fluorescence

Guided excitation

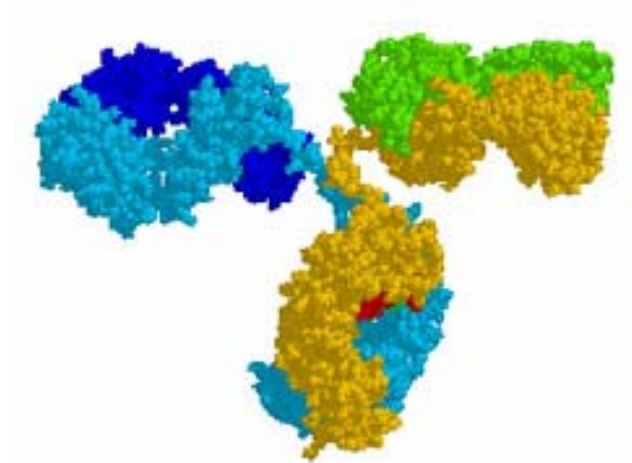
Confocal

WP1

Optical transduction



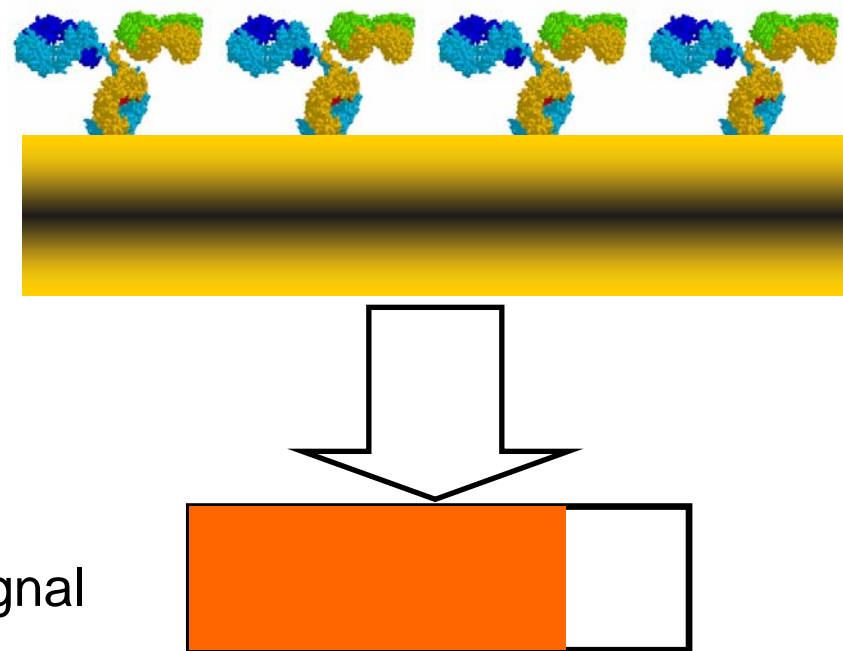
- Transform a binding event into an optical signal



Antibody

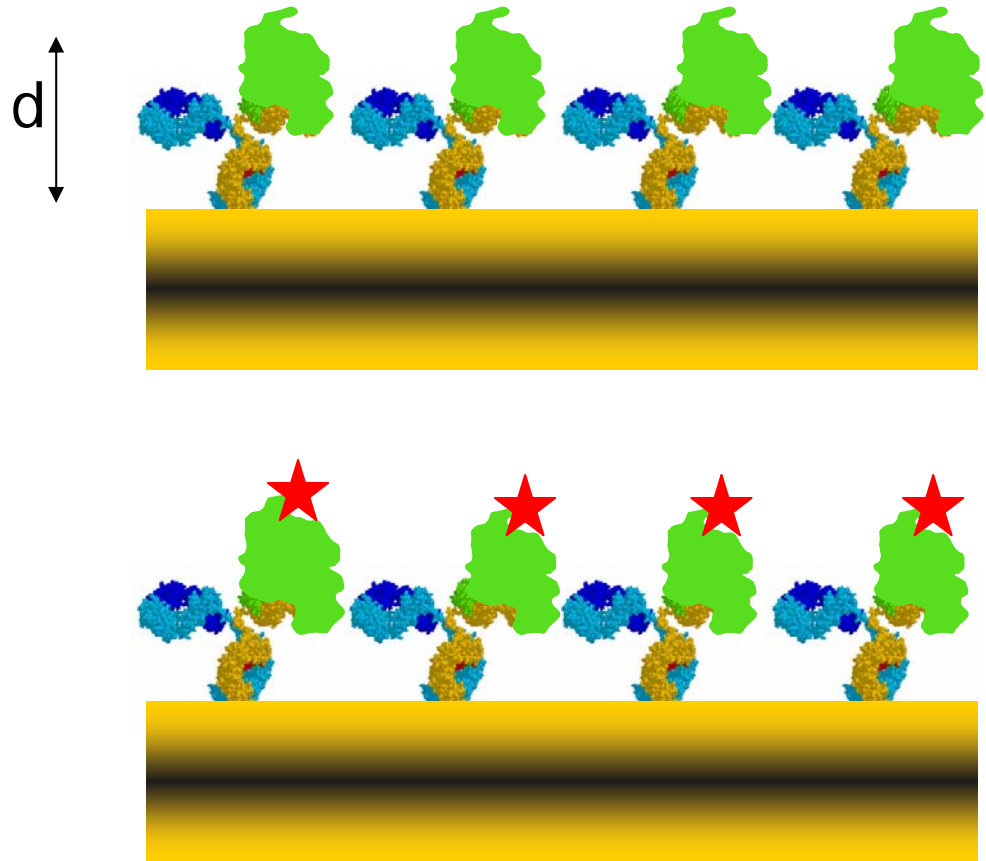
Optical transduction

- The binding of molecules will affect the optical signal
- There are several possibility to create the optical signal
 - Direct: the molecule itself will create an optical change
 - Indirect: a label will be necessary to have an optical effect

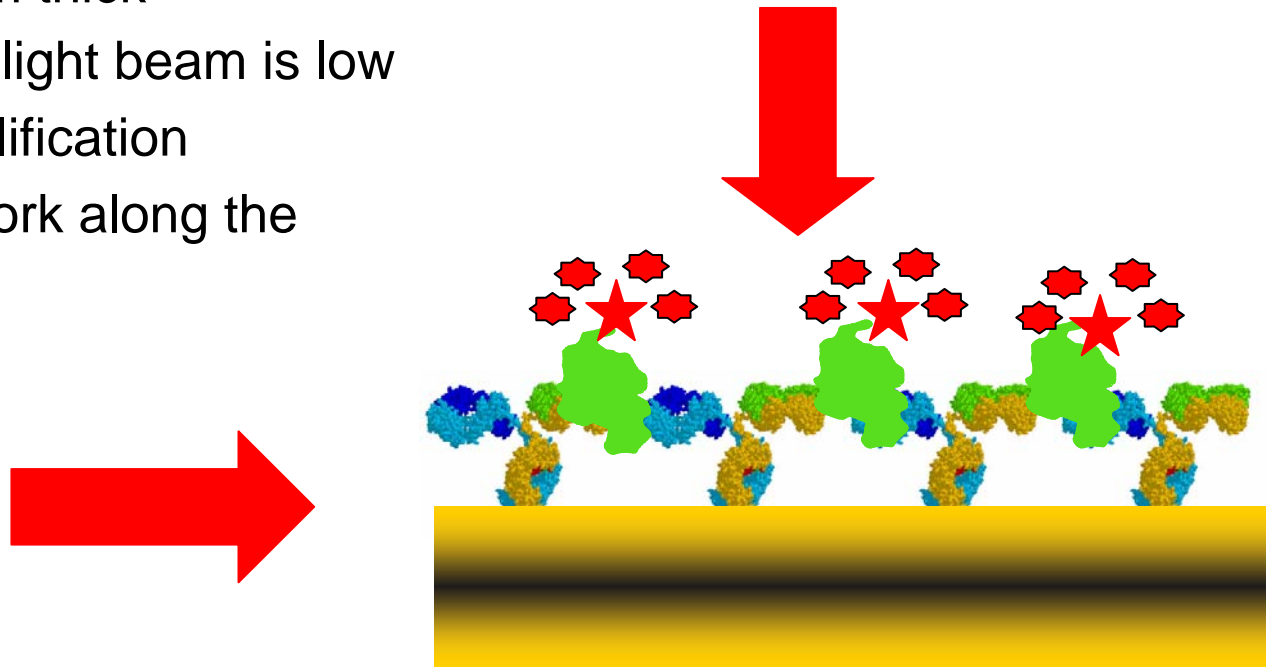


Optical transduction

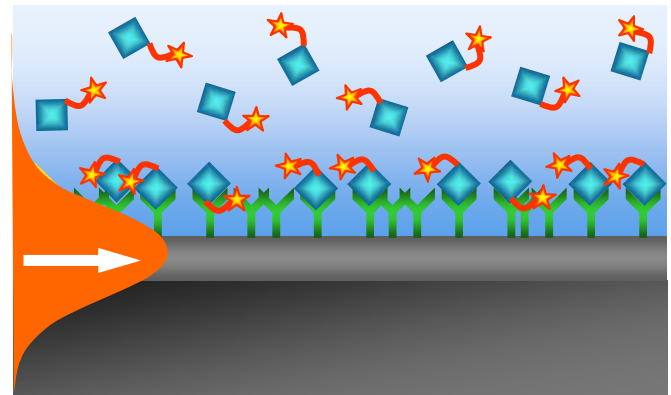
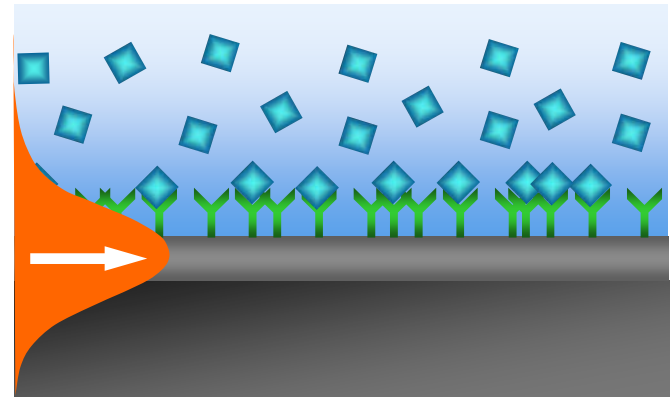
- Label-free
Binding=> n, d
- Label
Binding=>
 - Color
 - Absorption
 - Fluorescence



- Antibody layer
 - About 5 nm thick
 - Effects on light beam is low
 - Need amplification
 - Need to work along the interface



- Evanescent wave sensors:
 - *Label-free sensors*: the evanescent field probes the optical properties of the sample/ interface
 - *Fluorescence based*: fluorescent labels are excited through the evanescent field, emitted light is collected by waveguide

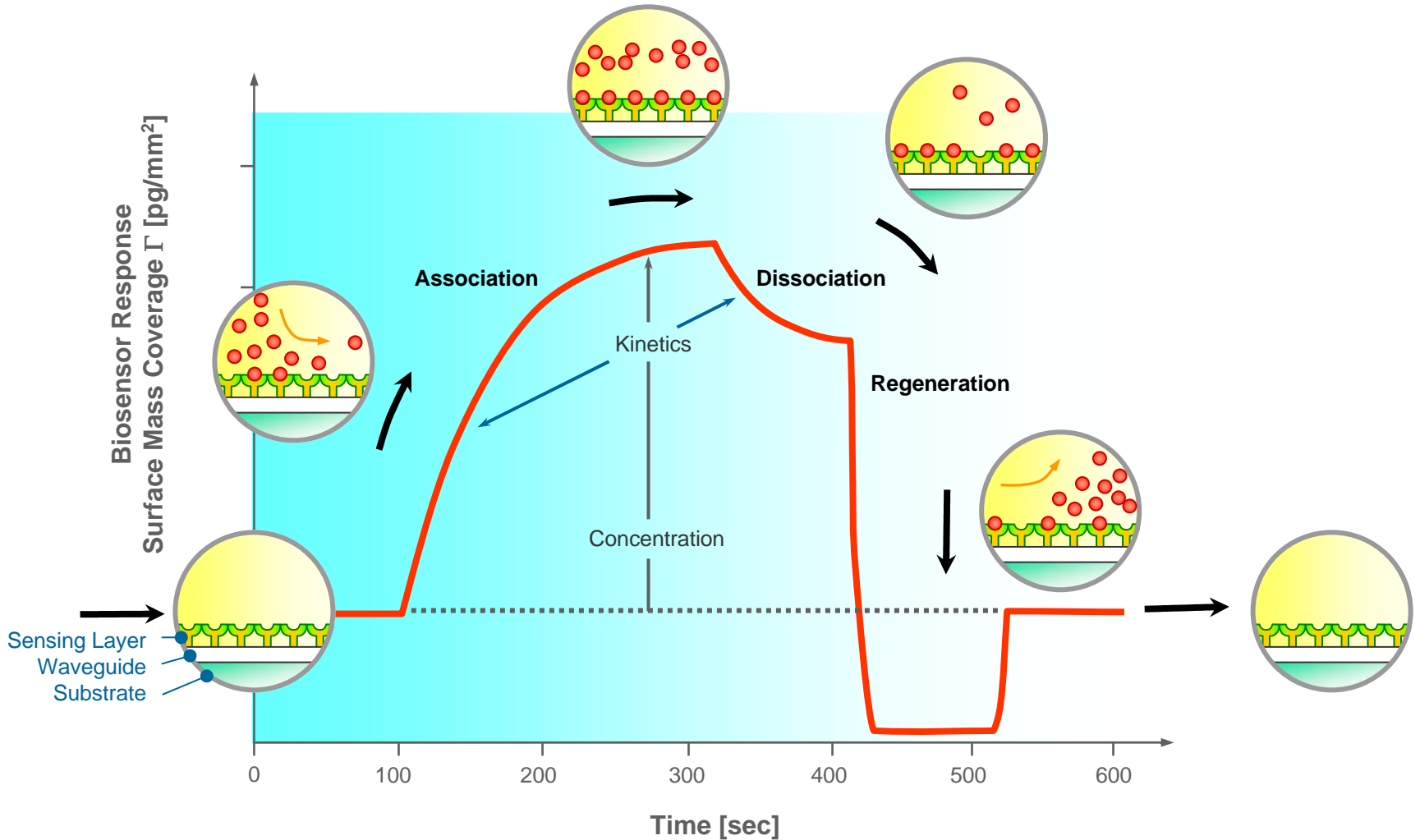


Label-free sensing

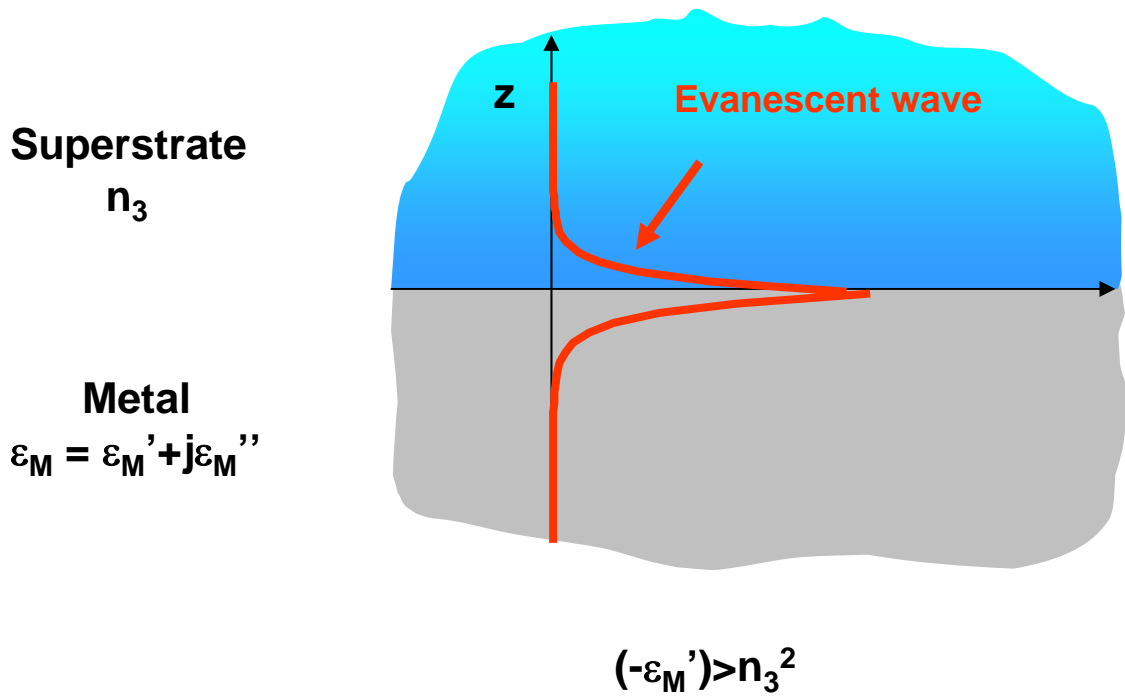
- Surface plasmon resonance
- Waveguide resonance
- Ellipsometry
- Interferometry

Typical binding cycle of an optical BioSensor

Label-free



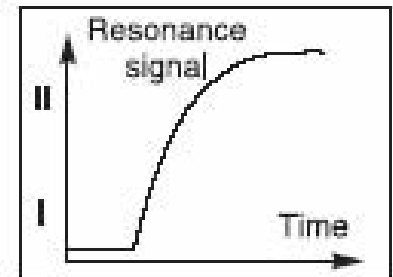
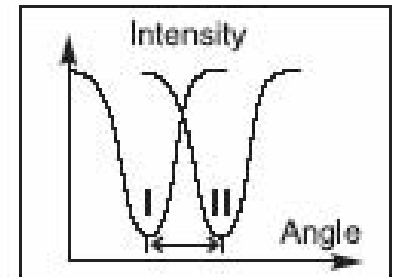
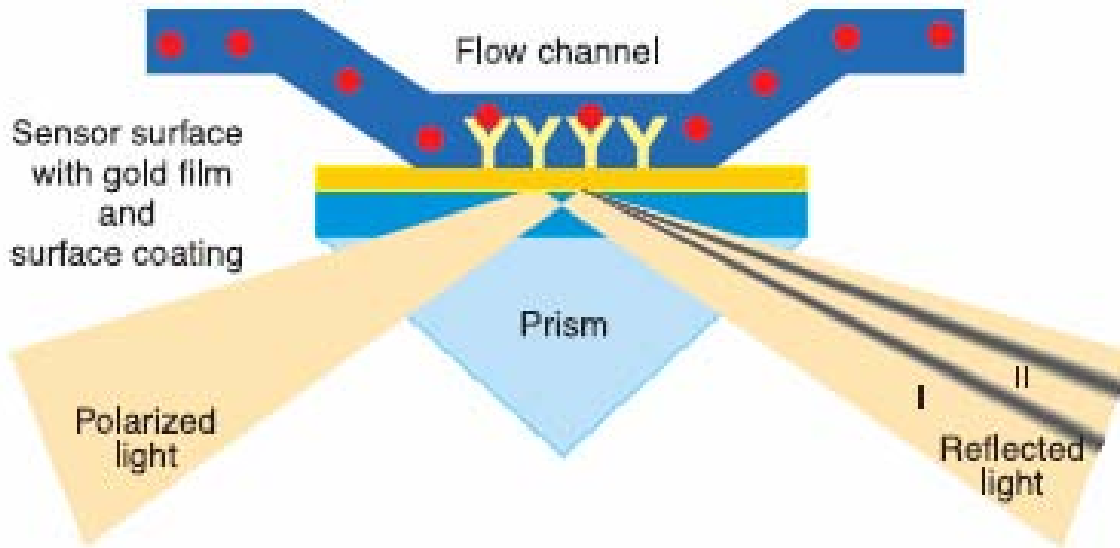
Plasmon



$$n_e = \frac{n_3}{\sqrt{\left(1 + \frac{n_3^2}{\epsilon_M'}\right)}}$$

$$d = \frac{\lambda}{2\pi n_3 n_e} \sqrt{-\epsilon_M'}$$

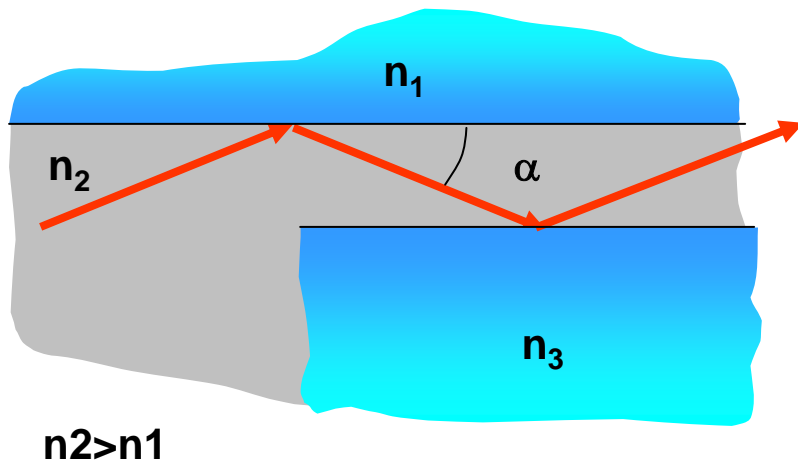
Surface Plasmon Resonance



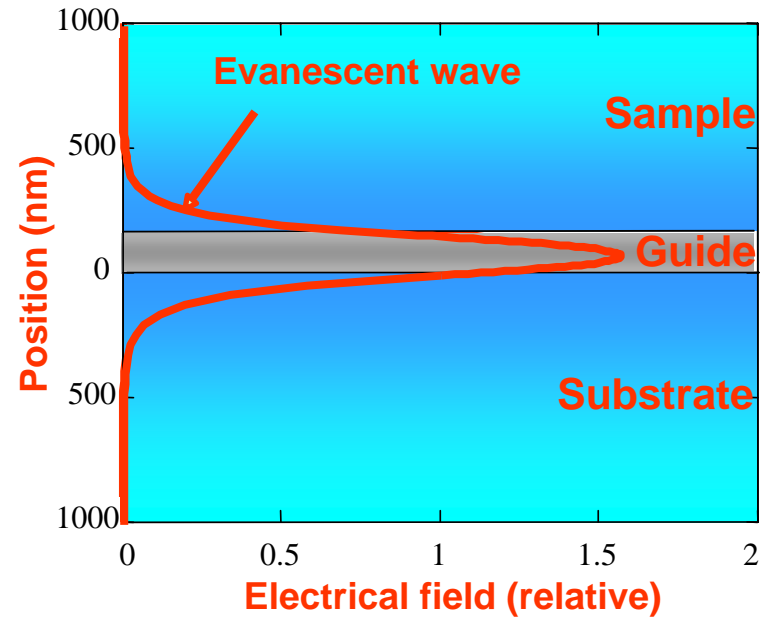
Sensorgram

Biacore

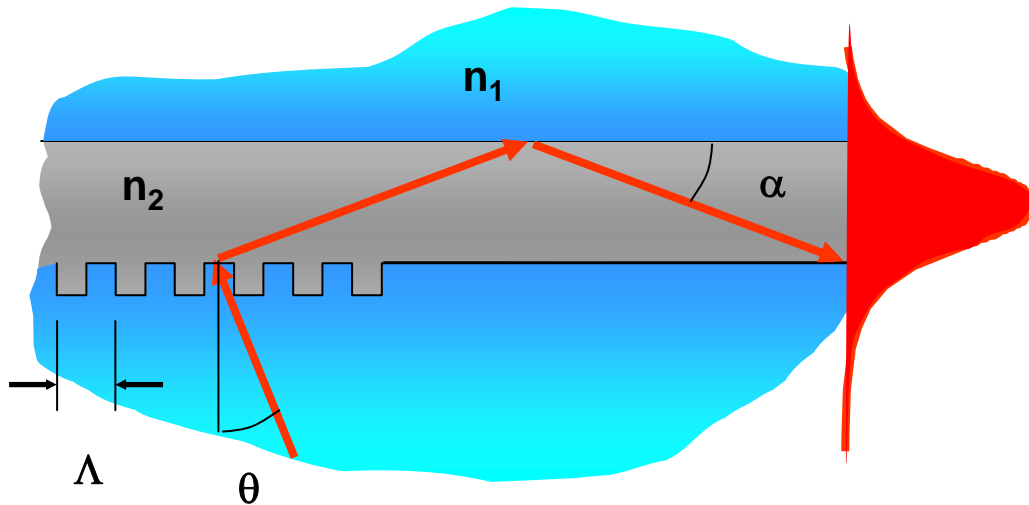
Waveguides and evanescent waves



$$n_e = n_2 \cos(\alpha)$$



Grating couplers



Guided modes are excited **when** the grating coupler resonance condition is fulfilled.

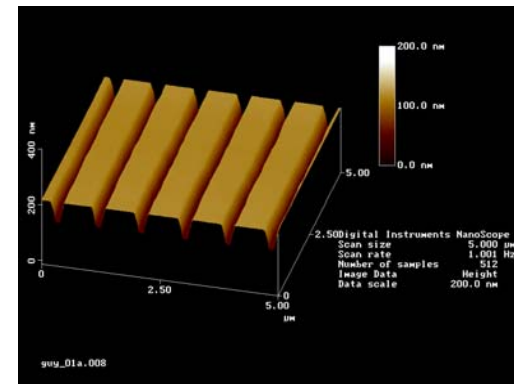
$$n_e(t) = \sin(\theta) - \lambda_r(t) / \Lambda$$

$$\lambda_r(t) = \Lambda (n_e(t) - \sin(\theta))$$

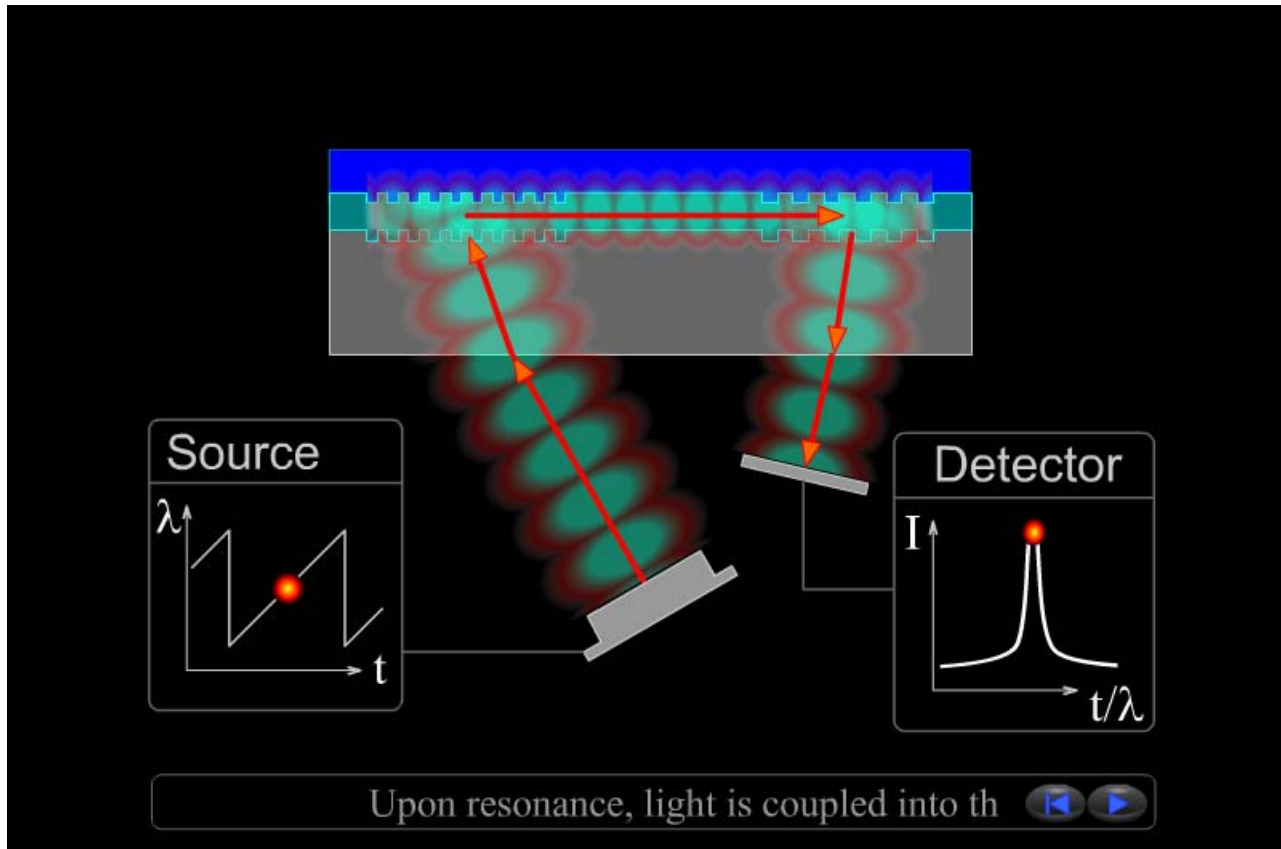
Waveguide grating parameters :

Grating nanostructure (period 360 nm; groove depth 10nm)

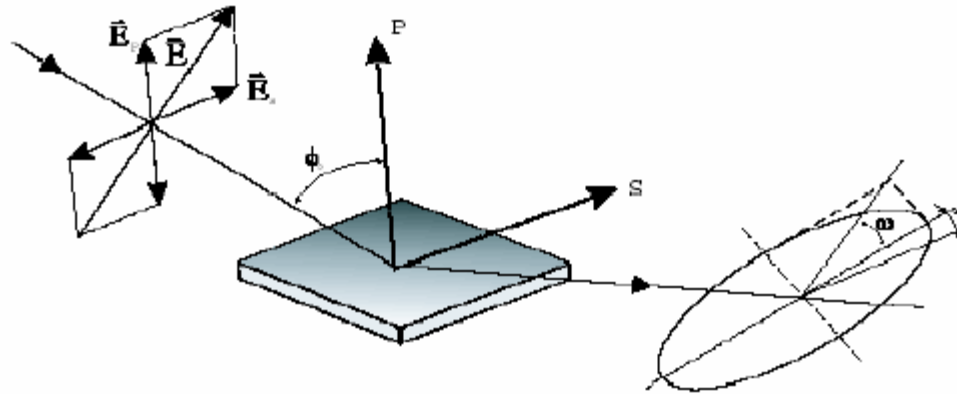
- Waveguide (high refractive index 2.1; thickness 150 nm)



Waveguide Resonance Wavelength Scanning



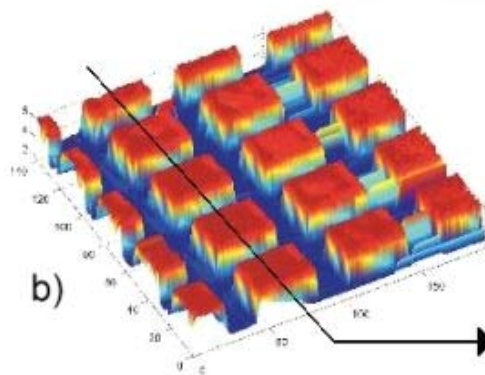
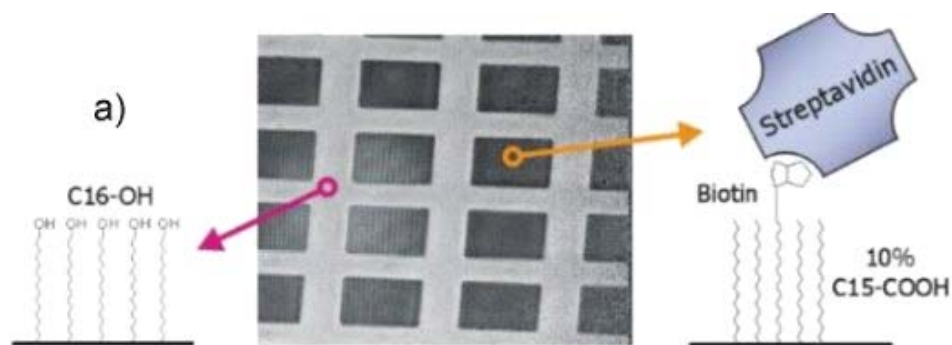
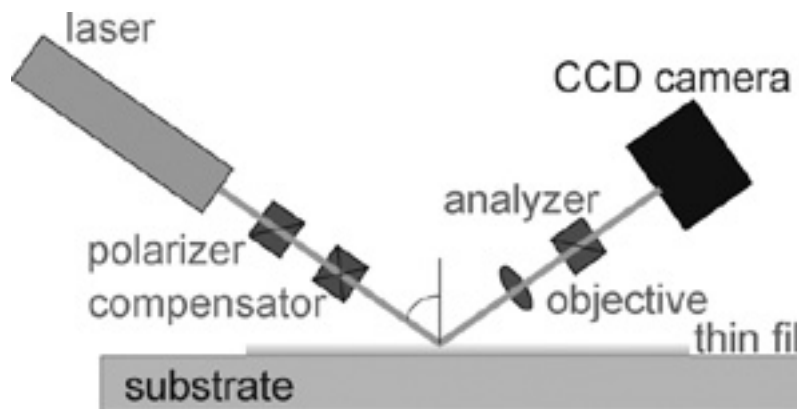
Ellipsometry



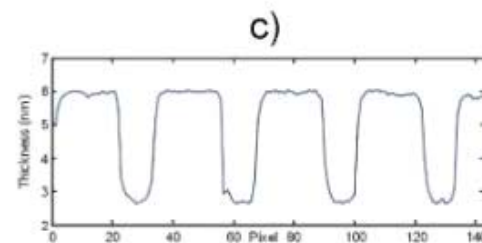
- Linearly polarized Incident light is elliptically polarized after reflection. Reflection coefficients along polarization s and p follow:

$$\frac{r_p}{r_s} = \tan \Psi . e^{i\Delta}$$

Imaging ellipsometry



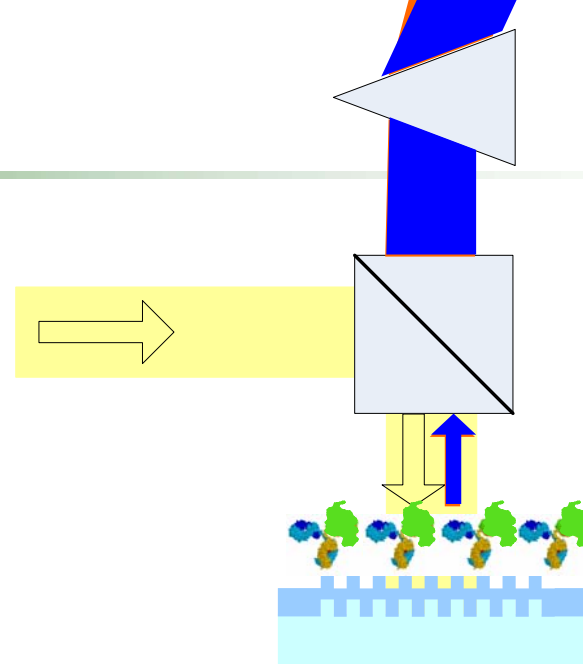
© Goran Klenkar



Other technology

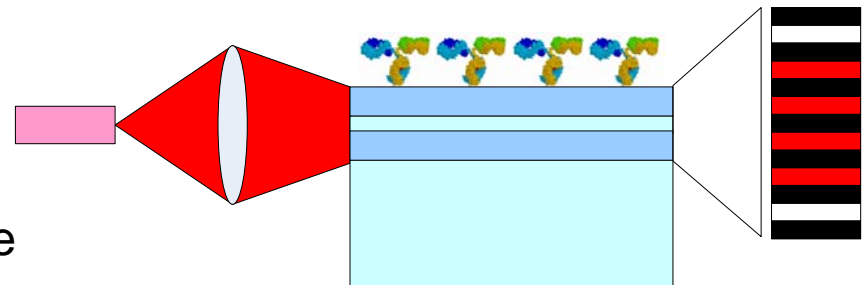
- Colorimetric resonant reflection
 - Binding=> change wavelength (color) of reflected light

SRU Biosystem



- Waveguide interferometry
 - Binding=> change optical phase between light propagating inside top waveguide versus reference buried waveguide

Farfield Sensors

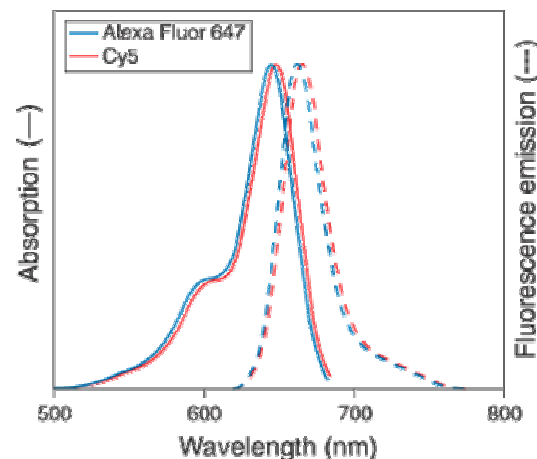
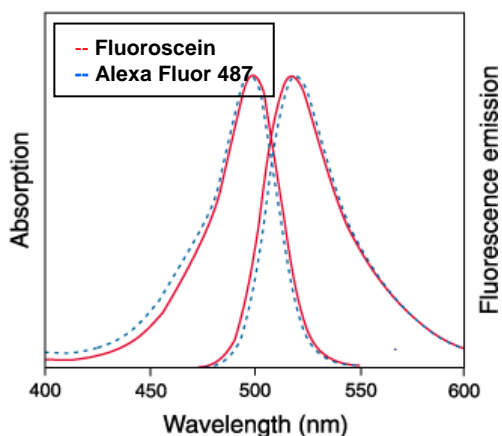


Fluorescence detection

- Waveguide excitation - direct detection
- Confocal scanner
- Waveguide excitation – waveguide detection
- Example of WP1

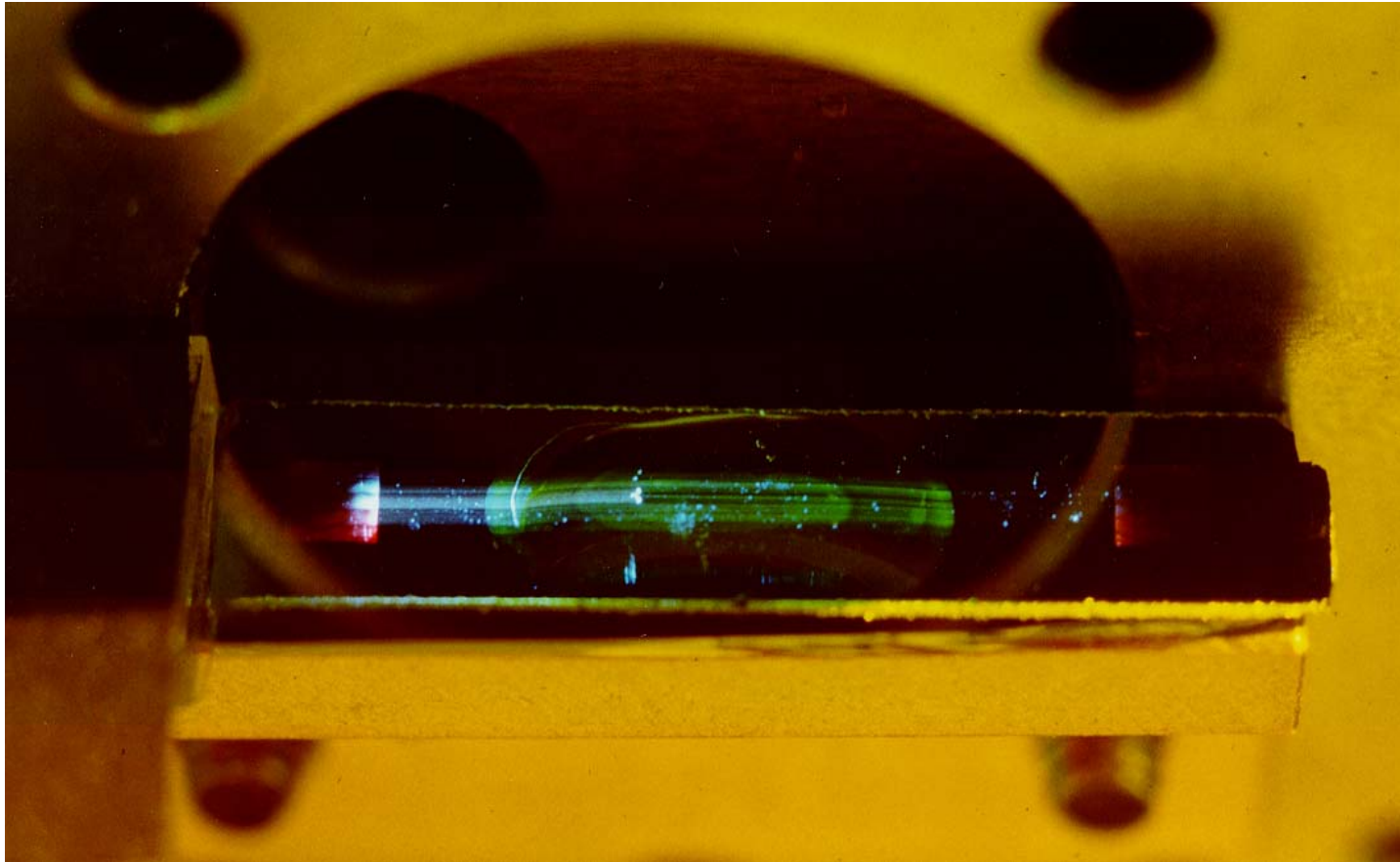
- Fluorescent molecules:
 - Absorb light
 - Emit light at a longer wavelength

Molecular Probes
www.probes.com

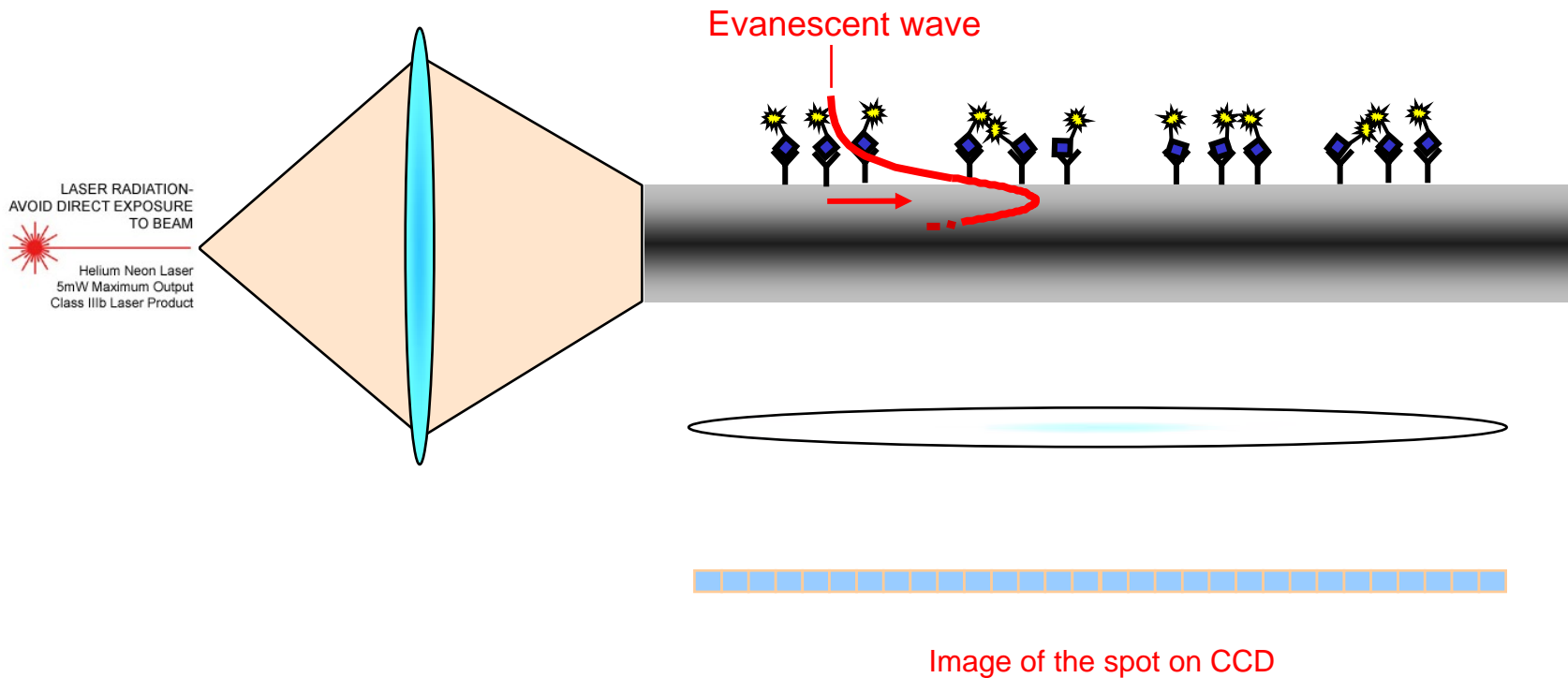


- Fluorescent molecules can be attached to antibody, antigen or DNA

View of Bound Fluorescence Excited by a Waveguide Mode

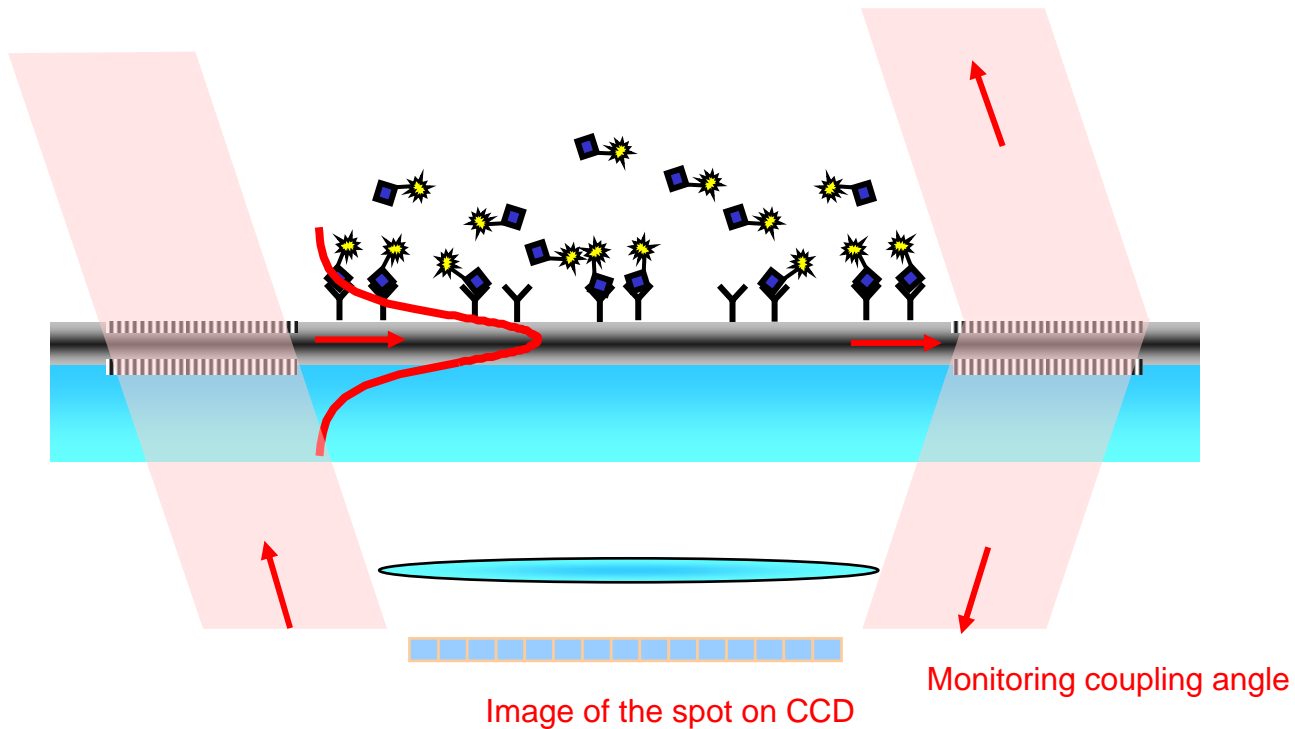


Butt coupled – Direct detection



C. A. Rowe-Taitt et al, Biosensors and Bioelectronics 18 (2000) 579-589

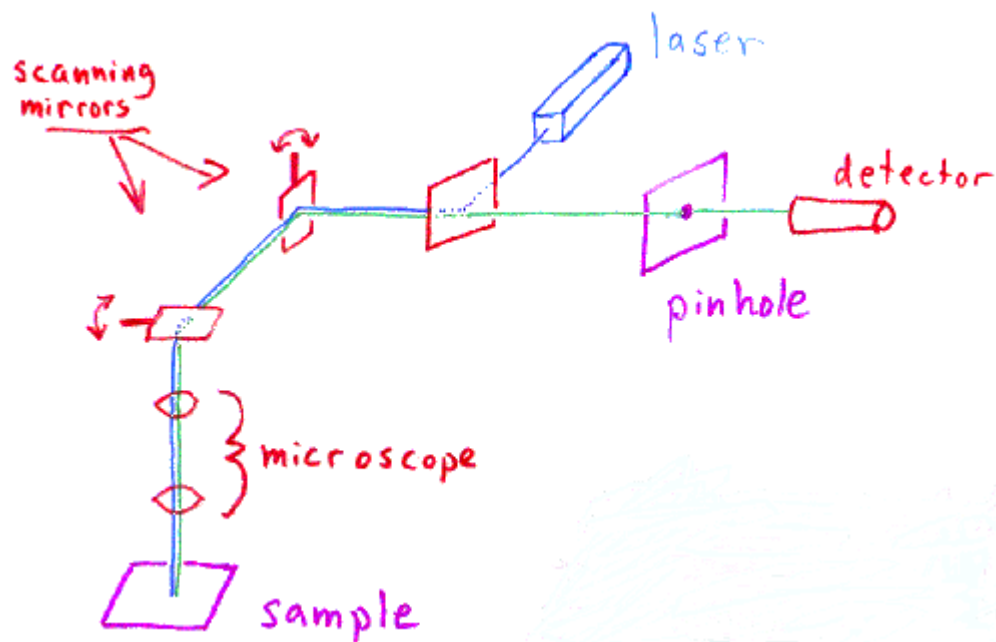
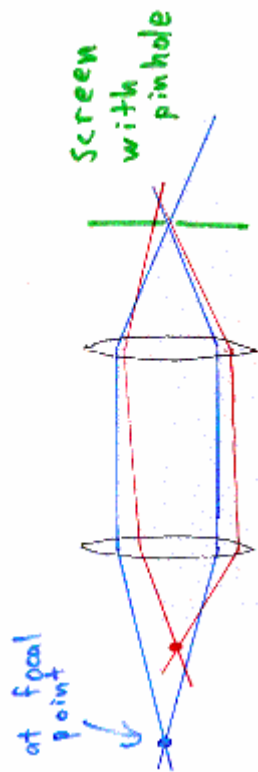
Grating coupled – direct detection



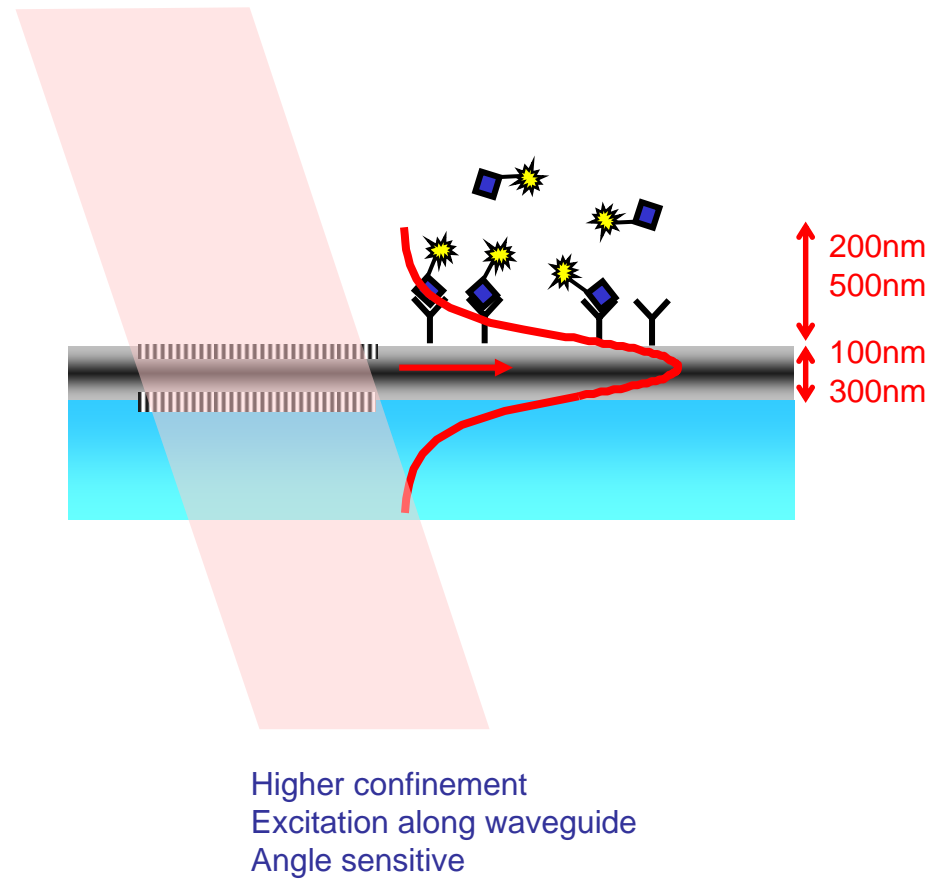
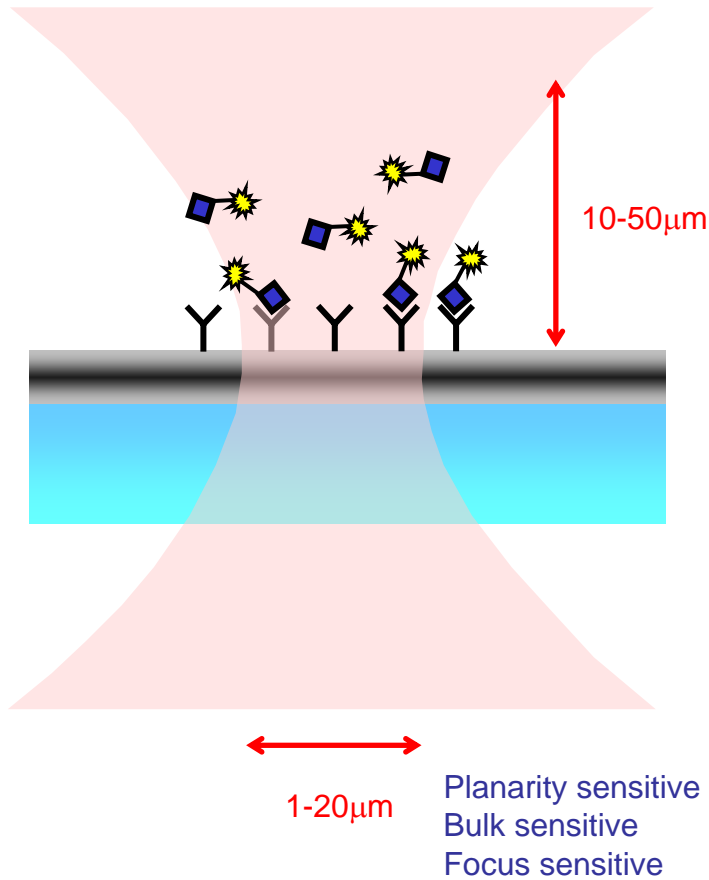
60 fold higher sensitivity (derived from the signal to noise ratio) for reader using this configuration compared to a confocal scanner

G. L. Duvenek et al. *Analytica Chimica Acta* 469 (2002) 49-61

Confocal scanner

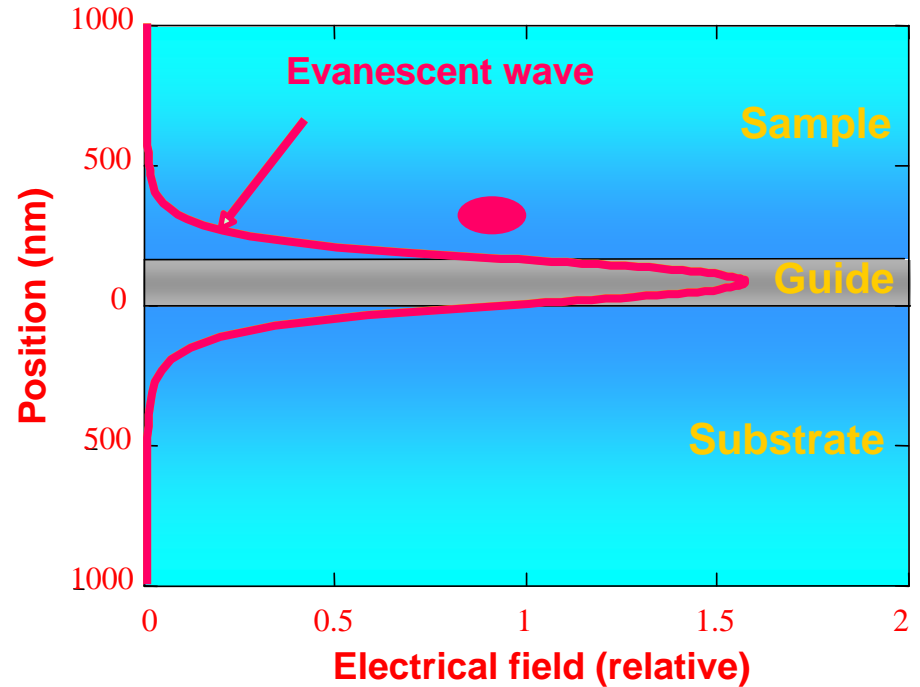
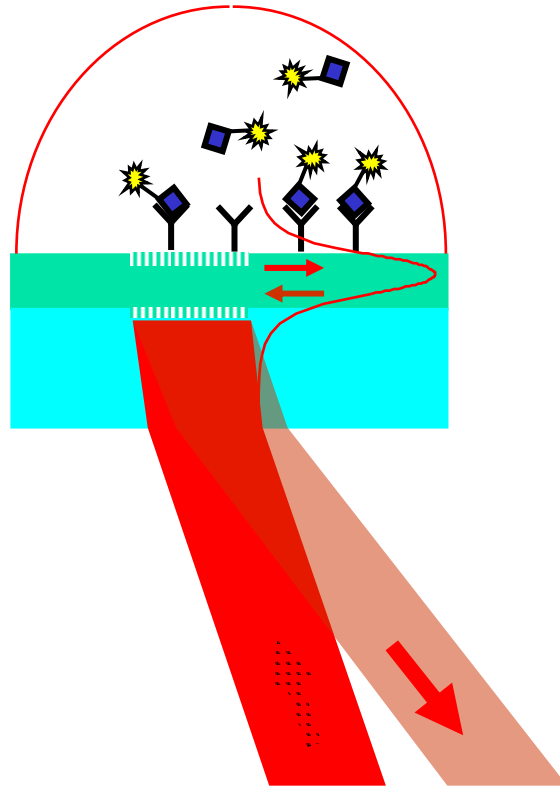


Comparison confocal scanner versus waveguide excitation



Grating coupled – Grating outcoupled (WP1)

Grating: coupling, outcoupling, beam shaping and wavelength separation



Signal (guided fluorescent light): proportional to excitation power and fluorescent molecule surface density

Label-free ↔ Fluorescence

- Label-free
 - + No labelling required
 - + Direct assay format
 - - Non-specific binding may be a problem
- Fluorescence
 - + High sensitivity
 - + Detection is specific
 - - Labelling required

- Optical
 - + Sensitivity
 - + Non-contact
 - - Optical
- Electrochemical
 - + Only electrical contact
 - + Low-cost option (disposable transducers?, low-cost metering devices, c.f. Self test medical market)
 - + Can operate in optically opaque media

- DNA sensor: identification of living organism
 - Needs specific DNA probes to detect target organism/gene
- Immunosensor: identification of chemicals
 - Needs antibody specific to determinant on target analyte
- There is no competition
 - Applications are different
 - In some cases both approaches can be used
 - Example WP3
 - toxigenic fungi is a living organism = DNA
 - Produce toxins = chemical