



Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #7

Checking Probes-layer quality (RM+SPR+SEM+AFM)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs §5.2.1-2)

- Resonant Mirror
- Surface Plasmon Resonance
- Scanning Electron Microscopy
- Atomic Force Microscopy
- Scanning Tunneling Microscopy

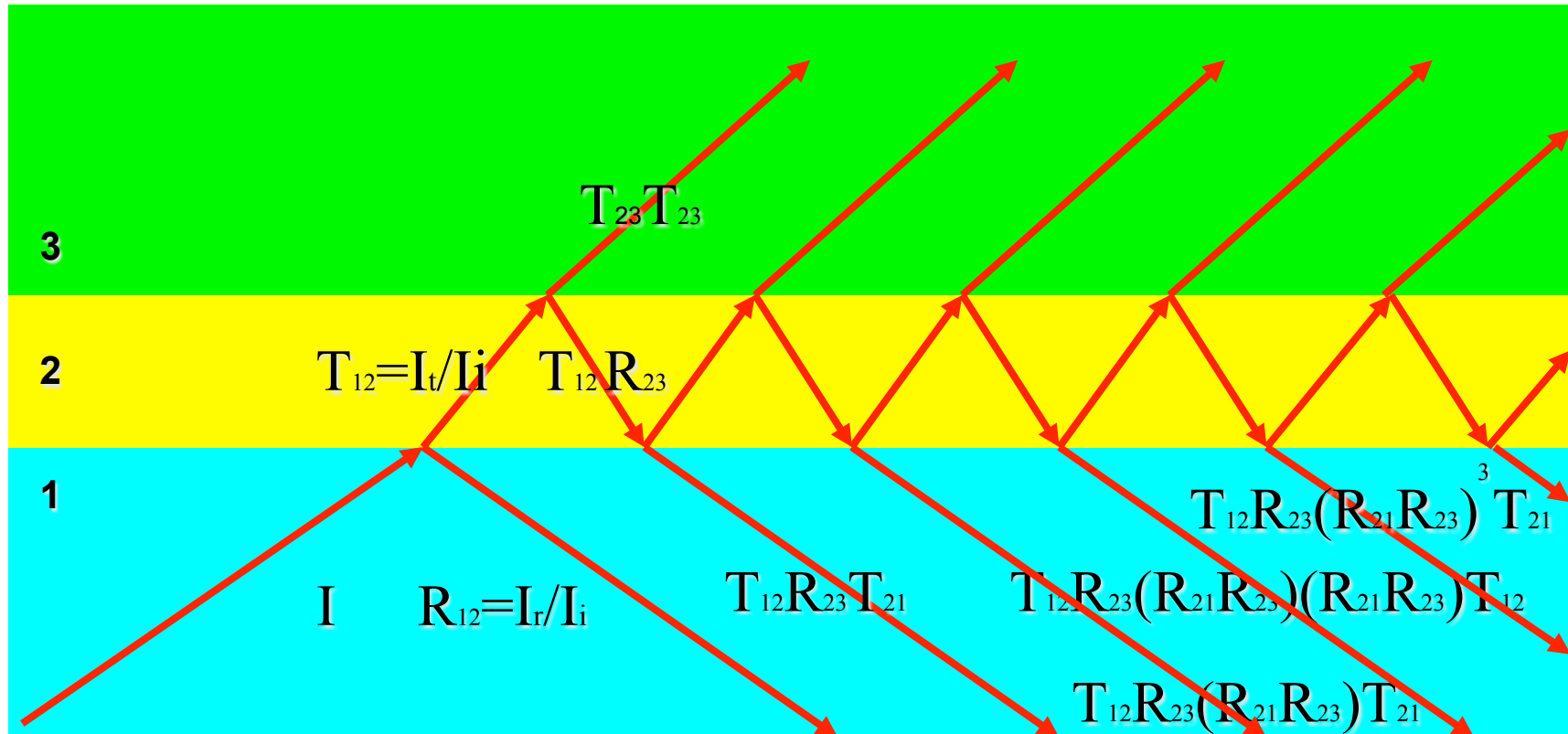
How to characterize the Probes Immobilization?

We have seen what are the mechanisms of self-assembly!

How to monitor the self-assembly process?

How to check the film quality?

Three-layers Reflection



$$R = R_{12} + T_{12}R_{23}T_{21} + T_{12}R_{23}T_{21}(R_{21}R_{23}) + T_{12}R_{23}T_{21}(R_{21}R_{23})^2 + \dots$$

Three-layers Reflection

$$R = R_{12} + T_{12}R_{23}T_{21} + T_{12}R_{23}T_{21}(R_{21}R_{23}) + T_{12}R_{23}T_{21}(R_{21}R_{23})^2 + \dots$$

$$R = R_{12} + \sum_{n=0}^{\infty} T_{12}R_{23}T_{21}(R_{21}R_{23})^n$$

$$R = R_{12} + T_{12}R_{23}T_{21} \sum_{n=0}^{\infty} (R_{21}R_{23})^n$$

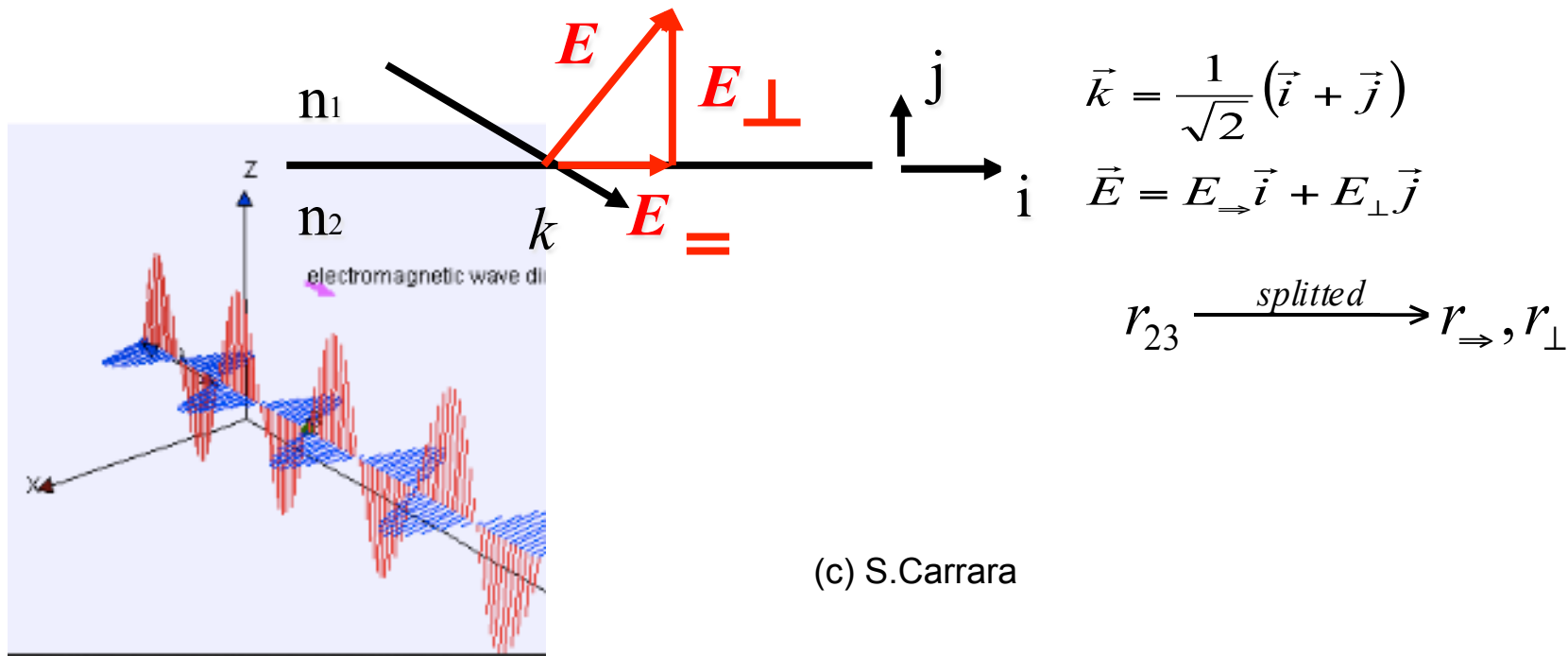
By the geometric series

$$\sum_{n=0}^{\infty} x^n = \frac{1}{1-x}$$

$$R = R_{12} + T_{12}R_{23}T_{21} \frac{1}{1 - R_{21}R_{23}}$$

The Reflection coefficient and the Electrical Field

$$R = R_{12} + \frac{T_{12} R_{23} T_{21}}{1 - R_{21} R_{23}} \quad R_{23} = |r_{23}|^2 \quad R_{23} = \frac{I_r}{I_i}; r_{23} = \frac{E_r}{E_i}$$



The Fresnel Coefficients

$r_{23} \xrightarrow{\text{splitted}} r_{\Rightarrow}, r_{\perp}$

$r_{23\Rightarrow} = \frac{n_3 \cos(\vartheta_2) - n_2 \cos(\vartheta_3)}{n_3 \cos(\vartheta_2) + n_2 \cos(\vartheta_3)}$

$r_{23\perp} = \frac{n_2 \cos(\vartheta_2) - n_2 \cos(\vartheta_3)}{n_2 \cos(\vartheta_2) + n_2 \cos(\vartheta_3)}$

$t_{23} \xrightarrow{\text{splitted}} t_{\Rightarrow}, t_{\perp}$

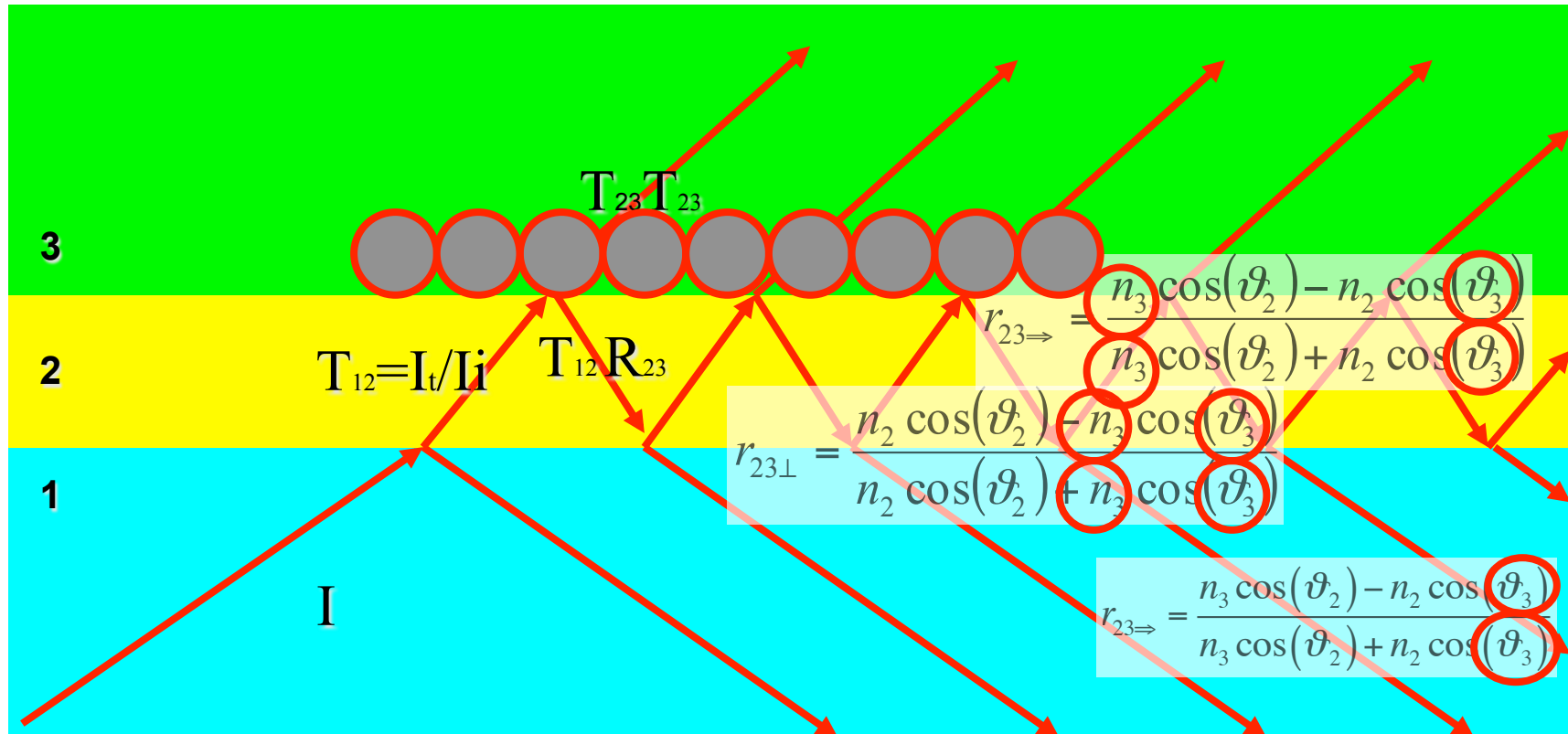
$t_{23\Rightarrow} = \frac{2n_2 \cos(\vartheta_2)}{n_3 \cos(\vartheta_2) + n_2 \cos(\vartheta_3)}$

$t_{23\perp} = \frac{2n_2 \cos(\vartheta_2)}{n_2 \cos(\vartheta_2) + n_3 \cos(\vartheta_3)}$

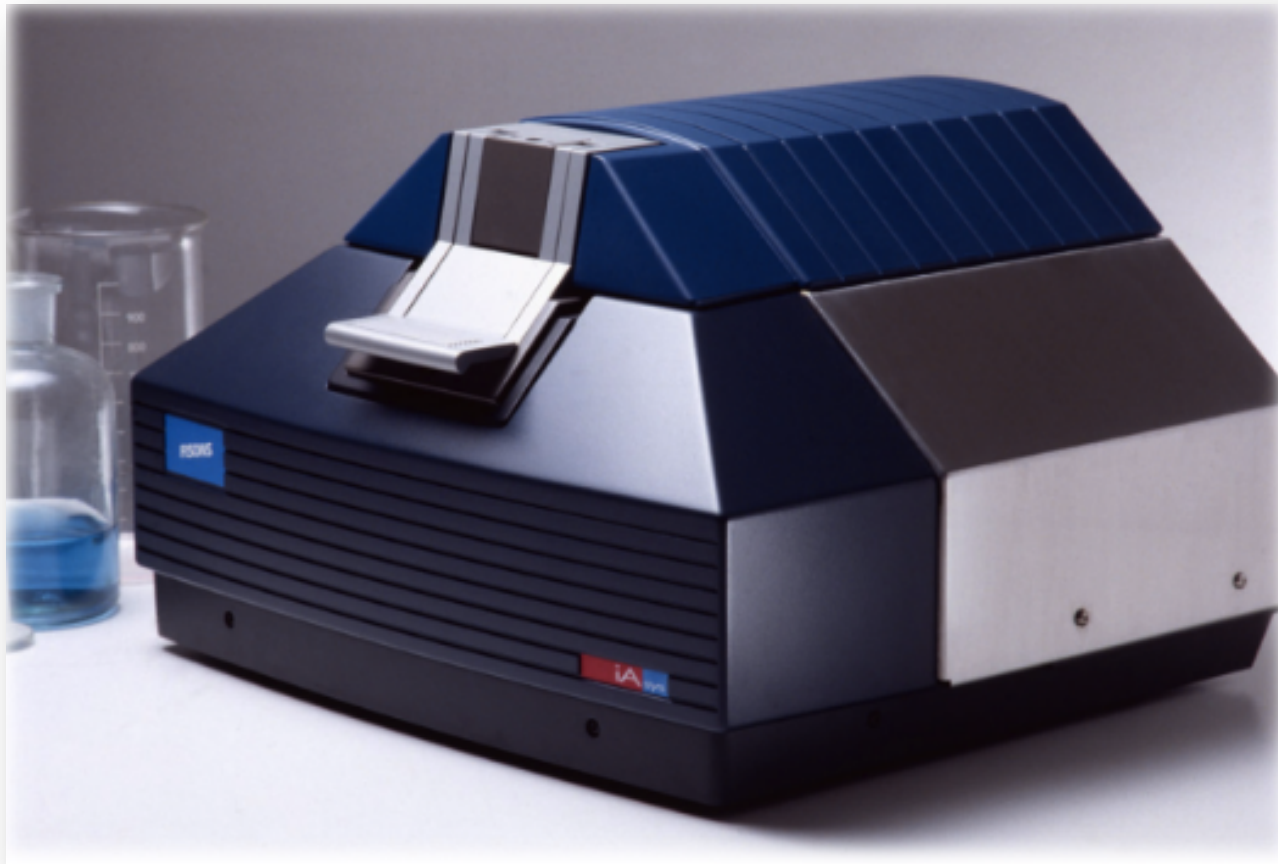
$n_2 \sin(\vartheta_2) = n_3 \sin(\vartheta_3)$

Snell's Law

Three-layers Reflection



Product

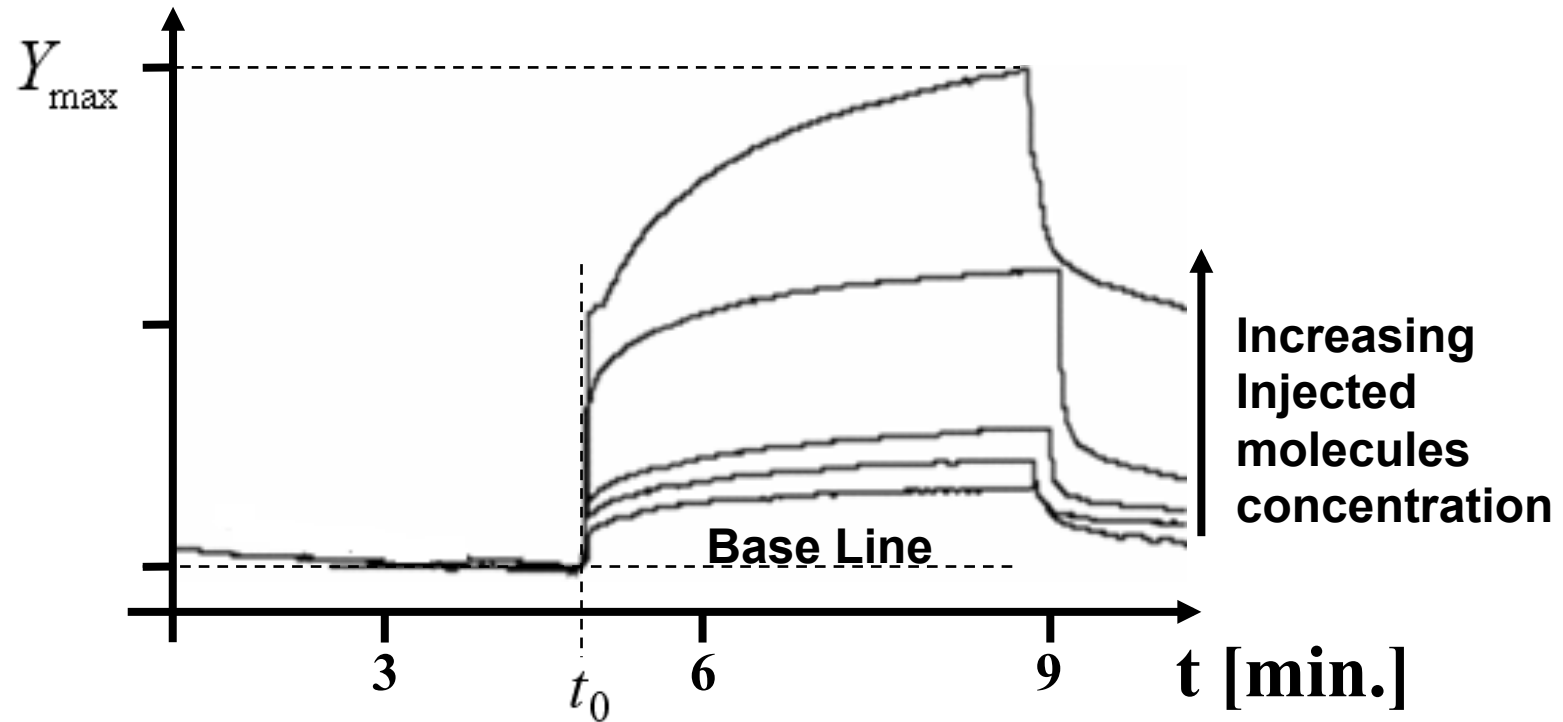


***IAsys plus* Affinity Sensor**

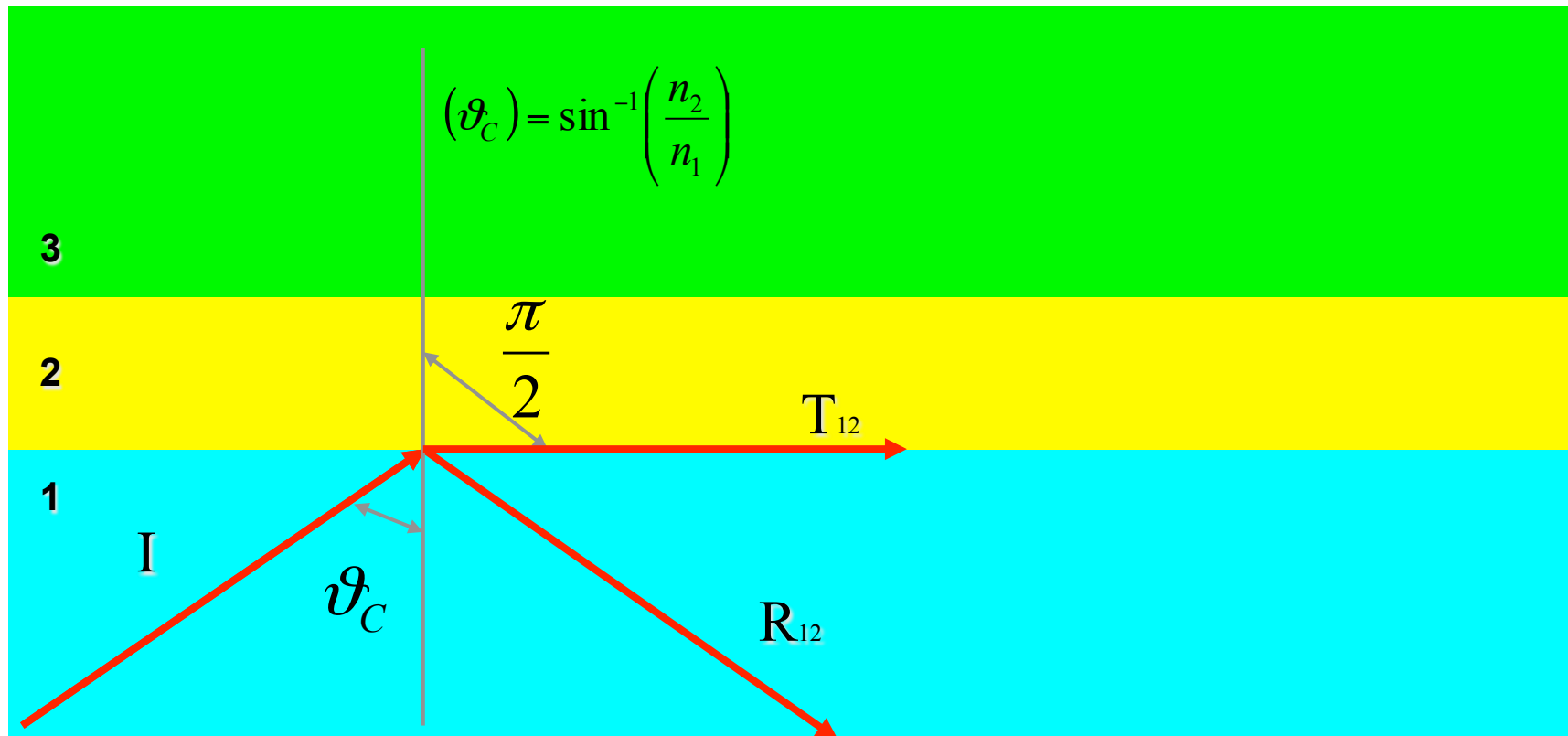
(c) S.Carrara

Yield Monitoring

Yield = Percentage of covered surface



Three-layers Reflection

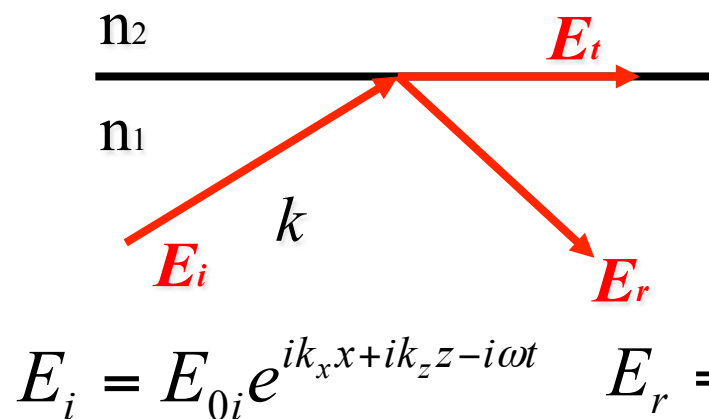


The Propagation along the interface

$$E_t = E_{0t} e^{ik_x x + ik_{pz} z - i\omega t}$$

$$k_{pz} = \sqrt{k_0^2 \epsilon_p - k_x^2}$$

$$\begin{cases} k_0 = \omega / c = 2\pi / \lambda \\ k_x = k_0 \sqrt{\epsilon_p} \sin(\vartheta) \\ n_2 = \sqrt{\epsilon_p \mu_p} \end{cases}$$



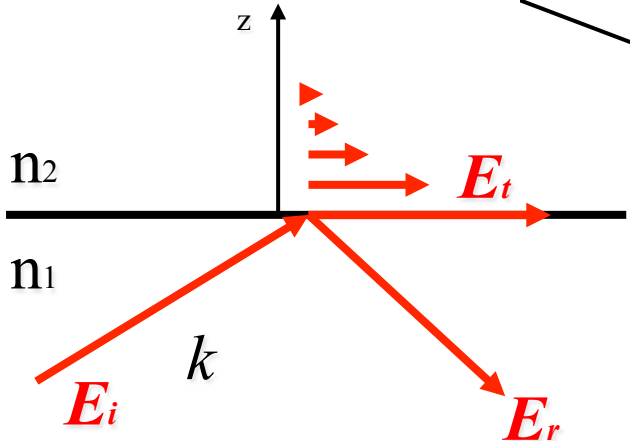
$$E_i = E_{0i} e^{ik_x x + ik_z z - i\omega t} \quad E_r = E_{0r} e^{ik_x x - ik_z z - i\omega t}$$

The Evanescent wave

$$E_t = E_{0t} e^{ik_x x + ik_{pz} z - i\omega t}$$

$$k_{pz} = \sqrt{k_0^2 \epsilon_p - k_x^2}$$

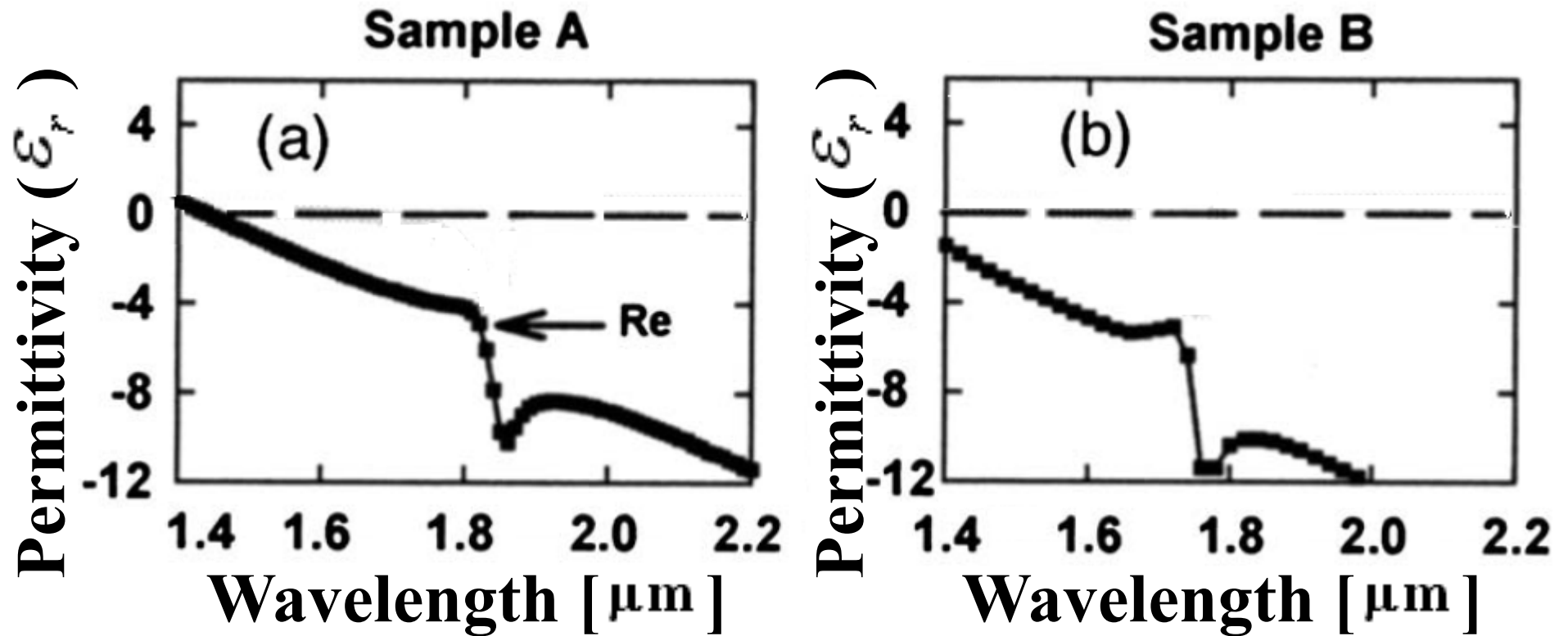
$\epsilon_p \leq 0$

$$E_t = \left(E_{0t} e^{-|k_{pz}|z} \right) e^{ik_x x - i\omega t}$$


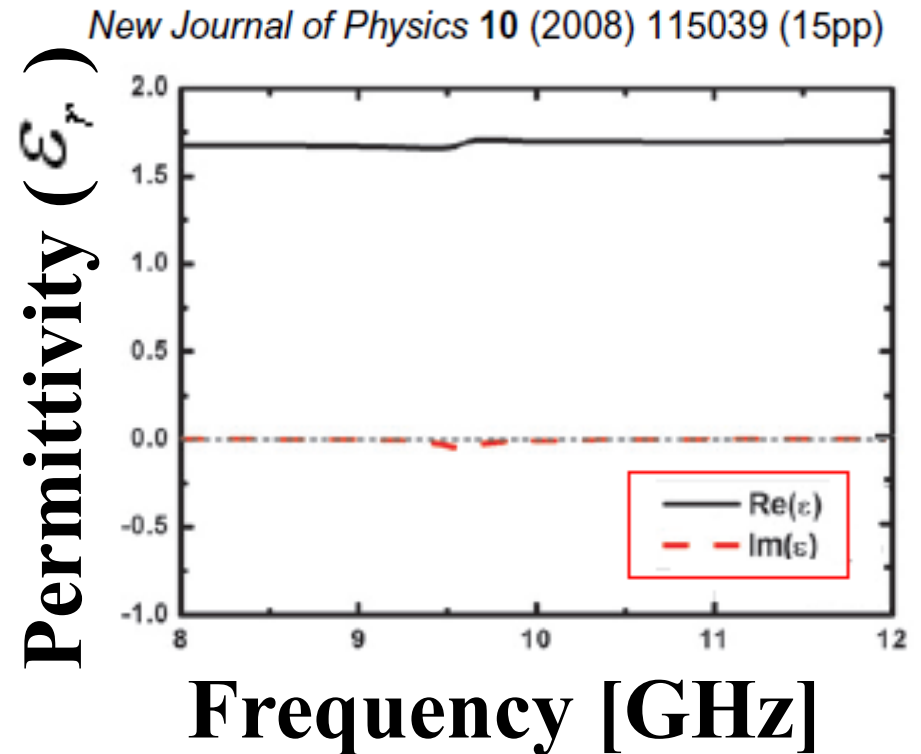
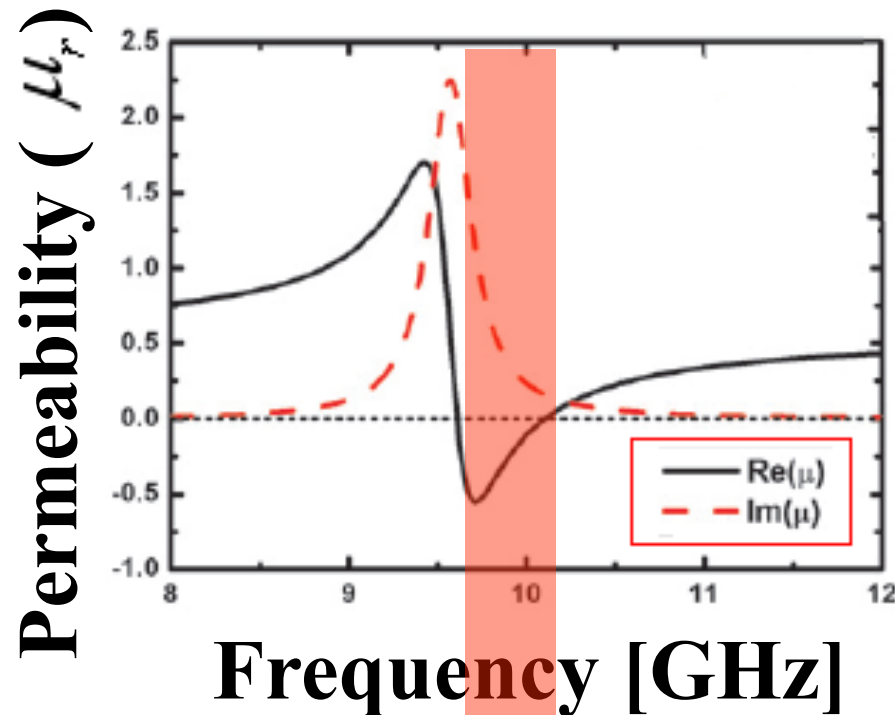
The diagram illustrates the physical context of the equations. It shows a horizontal interface between two media with refractive indices n_1 (bottom) and n_2 (top). An incident wave vector k is shown in medium n_1 , reflecting back as E_r . A transmitted evanescent wave E_t is shown in medium n_2 , decaying exponentially with distance z from the interface. Red arrows represent the electric field vectors E_i , E_r , and E_t .

Negative Permeability of metals

Zhang *et al.* J. Opt. Soc. Am. B/Vol. 23, No. 3/March 2006 pag 434



Negative Permittivity in metallic clots



New Journal of Physics **10** (2008) 115039 (15pp)

Negative dielectric constant

$$E_t = \left(E_{0t} e^{-|k_{pz}|z} \right) e^{ik_x x - i\omega t}$$

$$\epsilon_p \leq 0$$



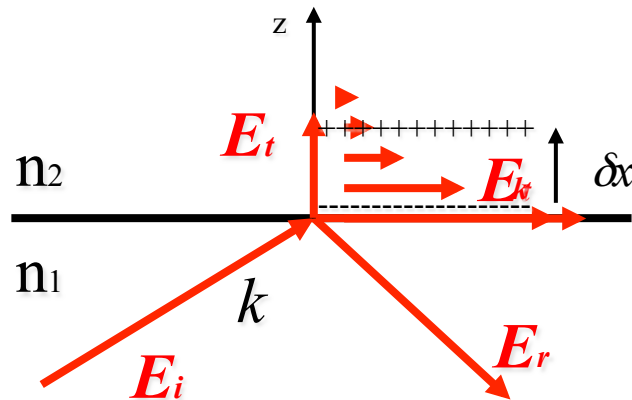
$$F = ma = m \frac{\partial^2 \delta x}{\partial t^2}$$

$$F = eE_t = -eE_{\delta x} = -e \frac{\sigma}{\epsilon_0}$$

$$F = -e \frac{en_e \delta x}{\epsilon_0}$$

$$\frac{\partial^2 \delta x}{\partial t^2} + \frac{e^2 n_e \delta x}{m \epsilon_0} = 0$$

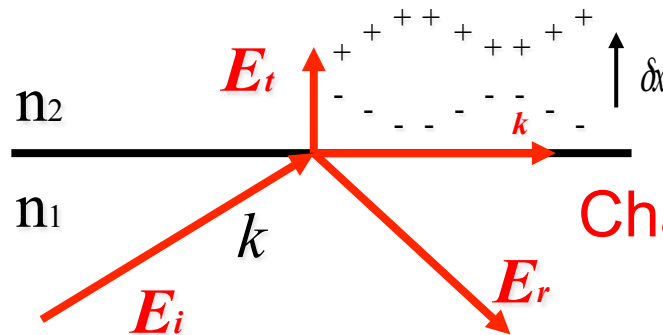
$$\omega_p^2 = \frac{e^2 n_e}{m \epsilon_0} \leftarrow \frac{\partial^2 \varphi}{\partial t^2} - \frac{e^2 n_e}{m \epsilon_0} \varphi = 0$$



Electronic Waves

$$\frac{\partial^2 \delta x}{\partial t^2} + \omega_p^2 \delta x = 0 \longrightarrow \delta x = \delta x_0 e^{-i\omega_p t} = \frac{\epsilon_0 E_{0t} e^{-|k_{pz}|z_p}}{e} e^{-i\omega_p t}$$

The Plasmon!



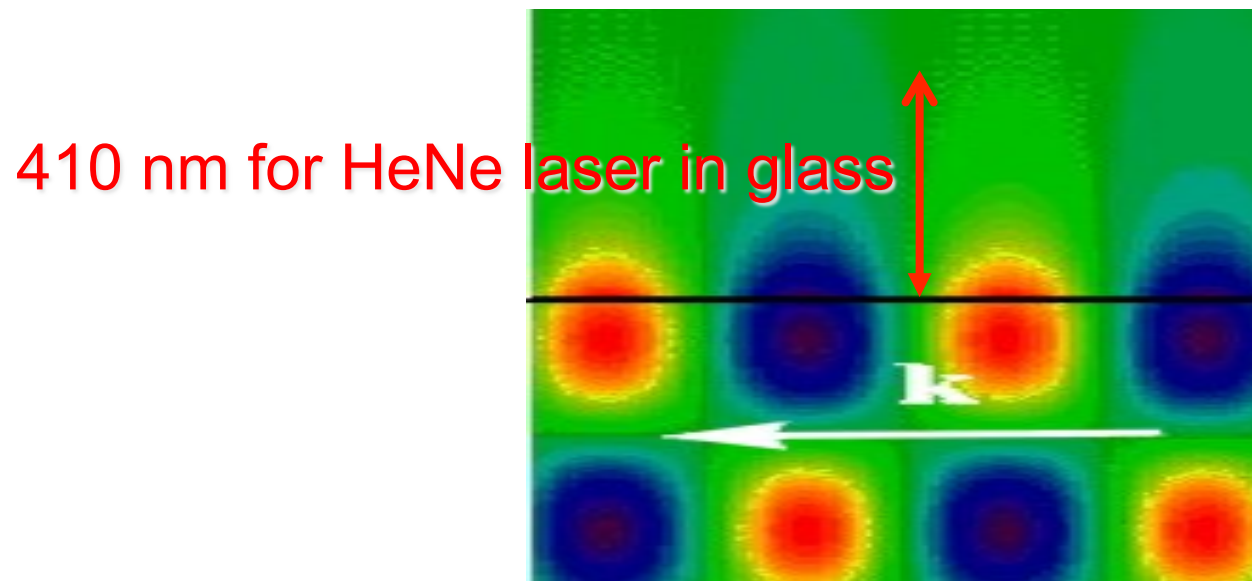
Characteristic of the metal

$$\omega_p^2 = \frac{e^2 n_e}{m \epsilon_0}$$

$$k_{pz} = \sqrt{k_0^2 \epsilon_p - k_x^2}$$

Characteristic of the evanescent wave

Simulation of Evanescent wave propagation



Penetration of the Evanescent Wave

For an amplitude of 1/3 of the value in $z=0$, we have

$$E_{ot}e^{-|k_{mz}|z} = \frac{1}{3}E_t|_{z=0} = \frac{1}{3}E_{0t} \longrightarrow e^{-|k_{mz}|z} = \frac{1}{3} \approx \frac{1}{e}$$

By extracting z :

$$z = \frac{1}{|k_{mz}|} = \frac{c_0}{2\pi f \sqrt{|\mu_r \epsilon_r|}} = \frac{\lambda}{\sqrt{|\mu_r \epsilon_r|}}$$

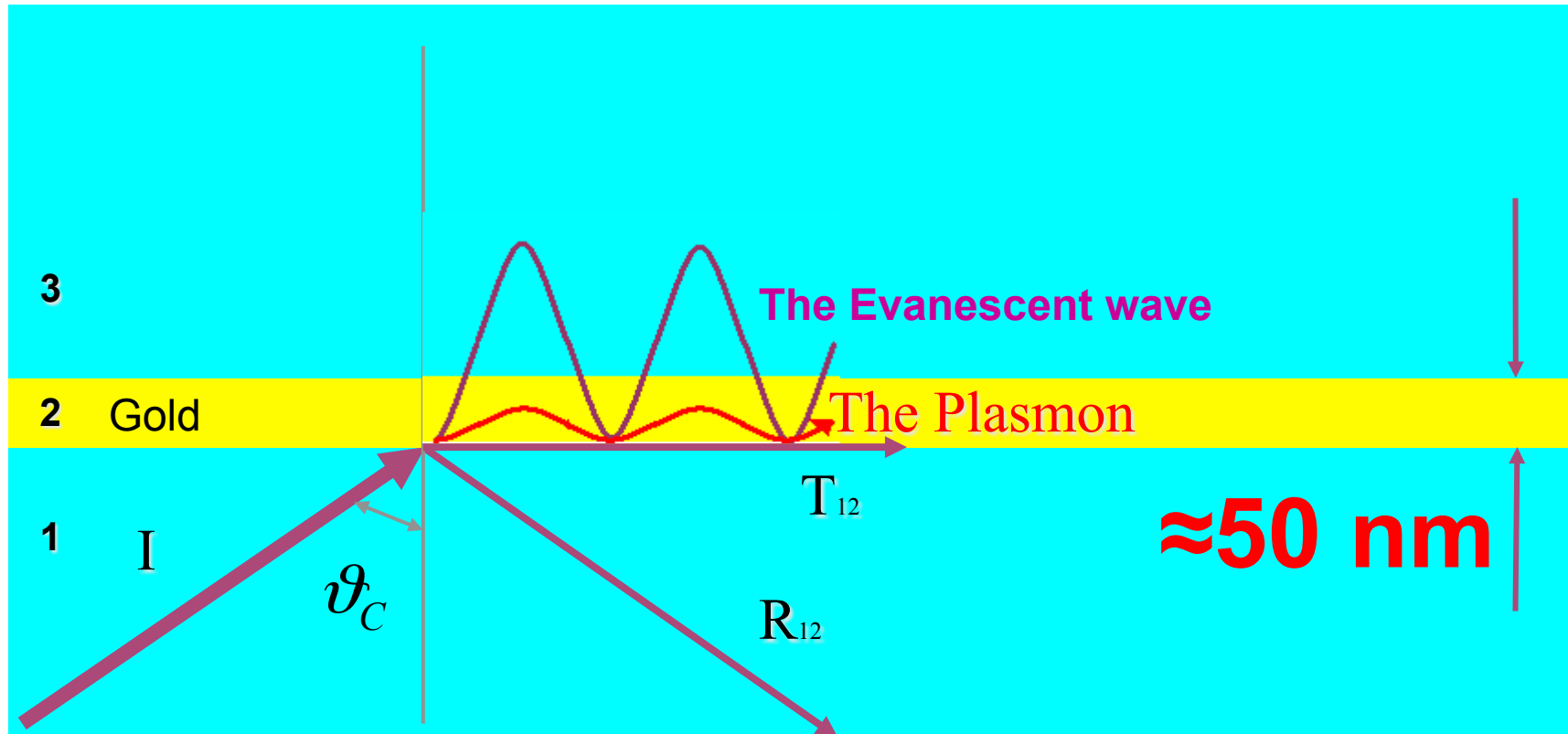
For gold, with relative magnetic permeability close to one and relative electric permittivity equal to 6.9, we obtain a thickness of

$$z_{gold} = \frac{1}{|k_{mz}|} = \frac{\lambda}{\sqrt{|\mu_r \epsilon_r|}} = \frac{400 \text{ nm}}{\sqrt{6.9 \cdot 1}} \approx 152 \text{ nm}$$

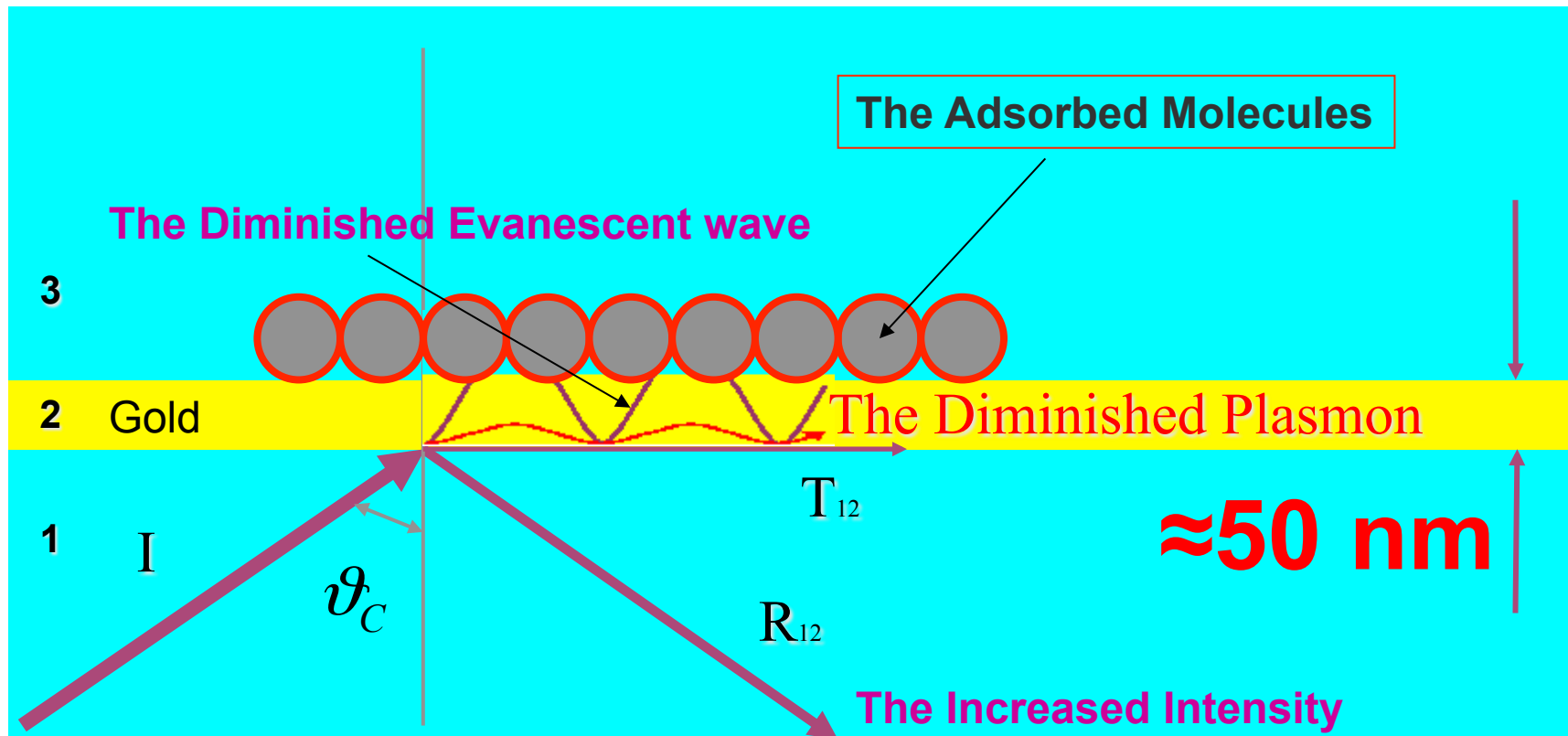
For nickel, with permeability and permittivity equal to 100 and 10 (respectively), we get:

$$z_{nickel} = \frac{1}{|k_{mz}|} = \frac{\lambda}{\sqrt{|\mu_r \epsilon_r|}} \approx \frac{400 \text{ nm}}{\sqrt{100 \cdot 10}} \approx 12 \text{ nm}$$

Penetration of the Evanescent Wave



Perturbation of the Evanescent Wave

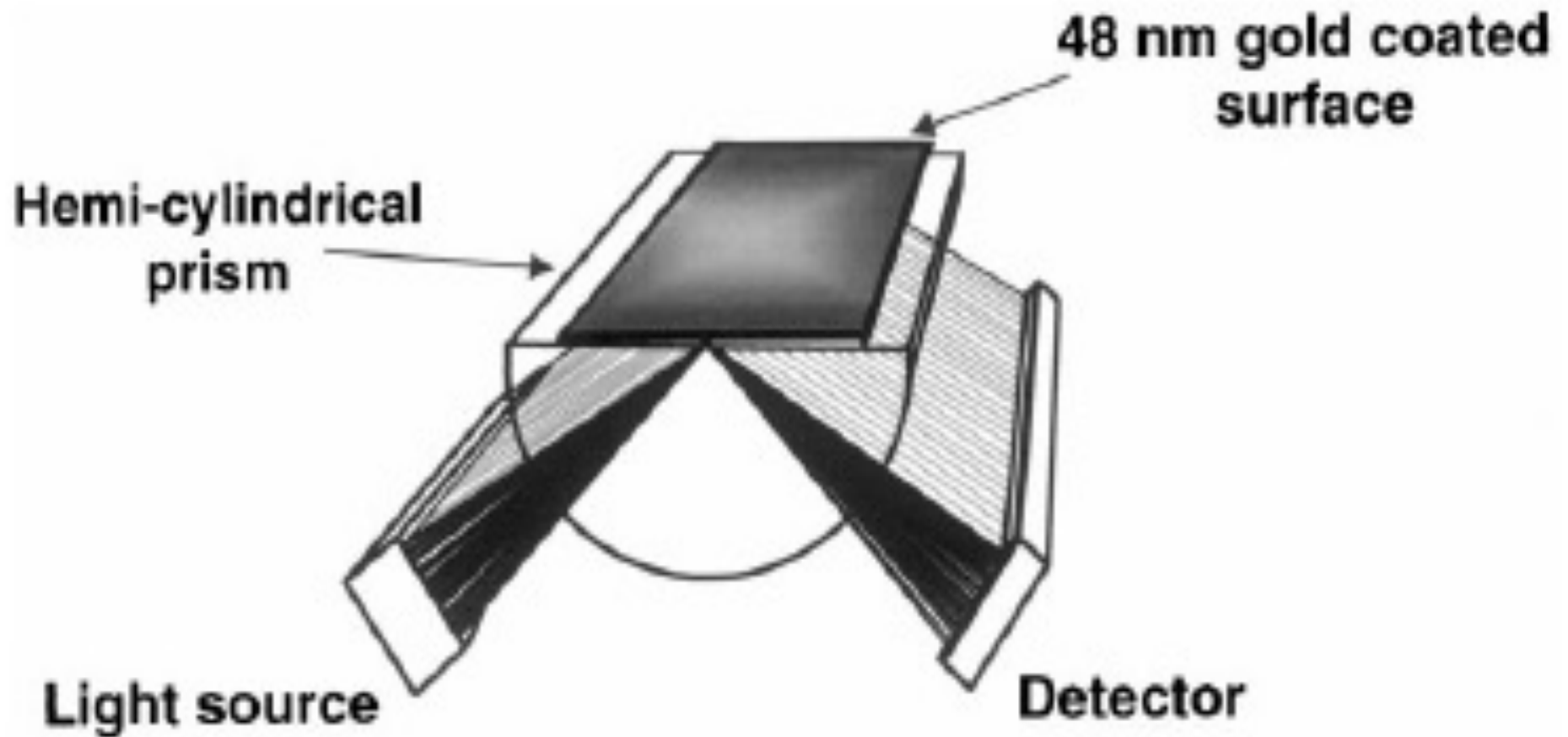


A Plasmon Resonance based Biosensor

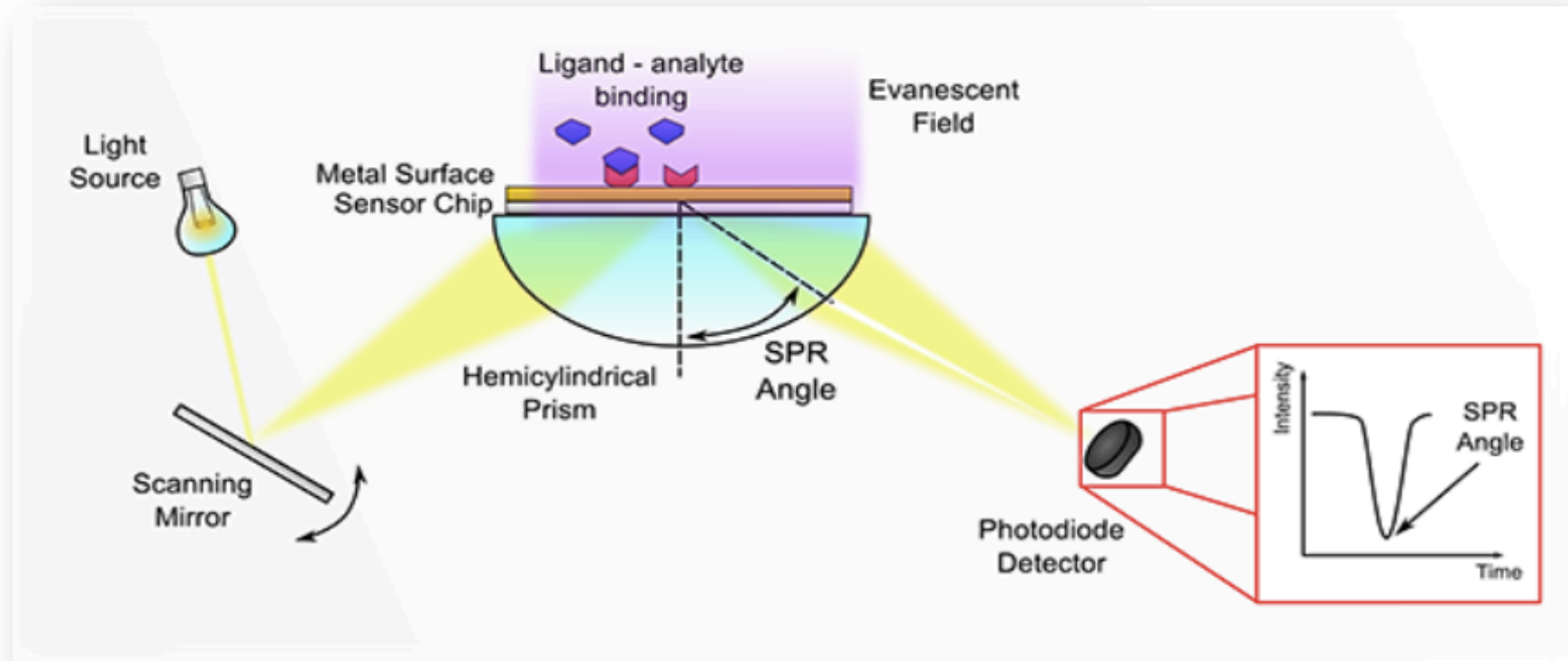


BIACORE 3000

The sensing Area

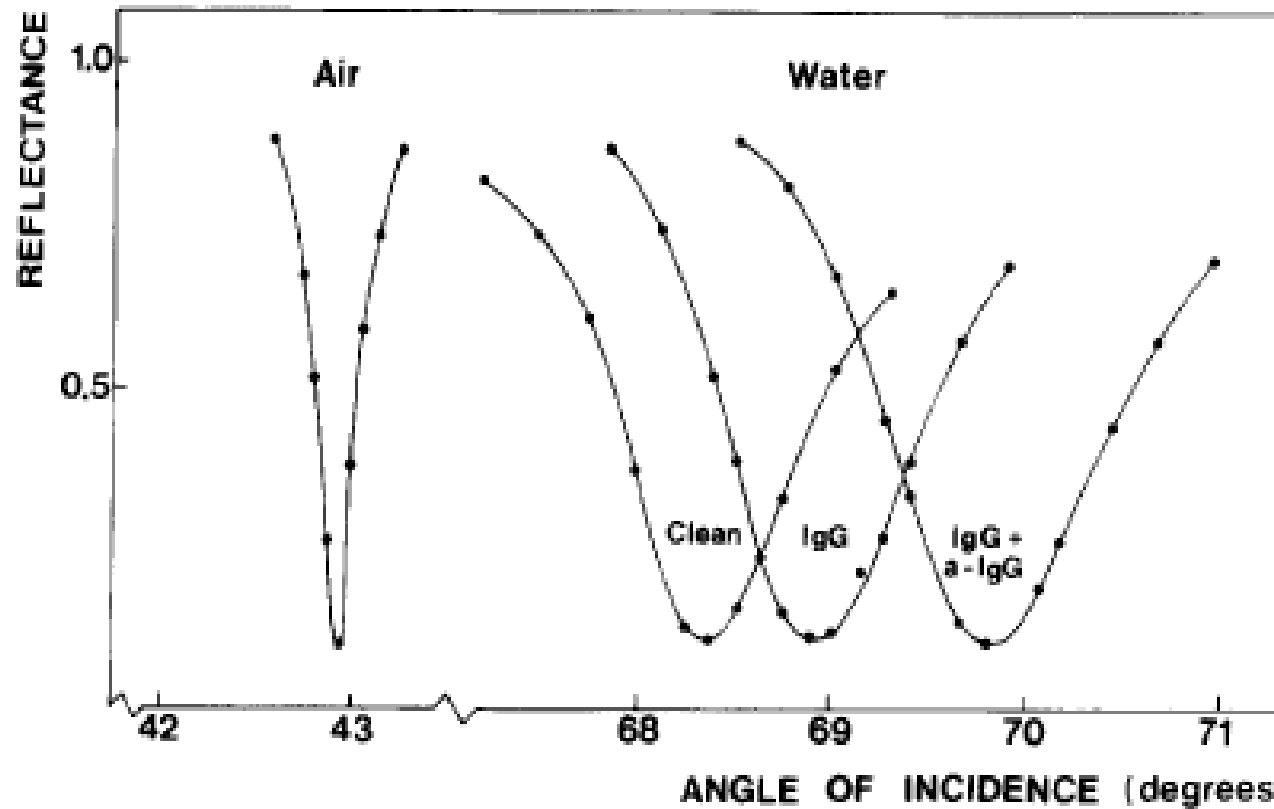


Working Principle



The presence of the complexes onto the gold surface will change the angle of resonance for the formation of the Surface Plasmons. This shift is proportional to the ligands amount bonded onto the surface

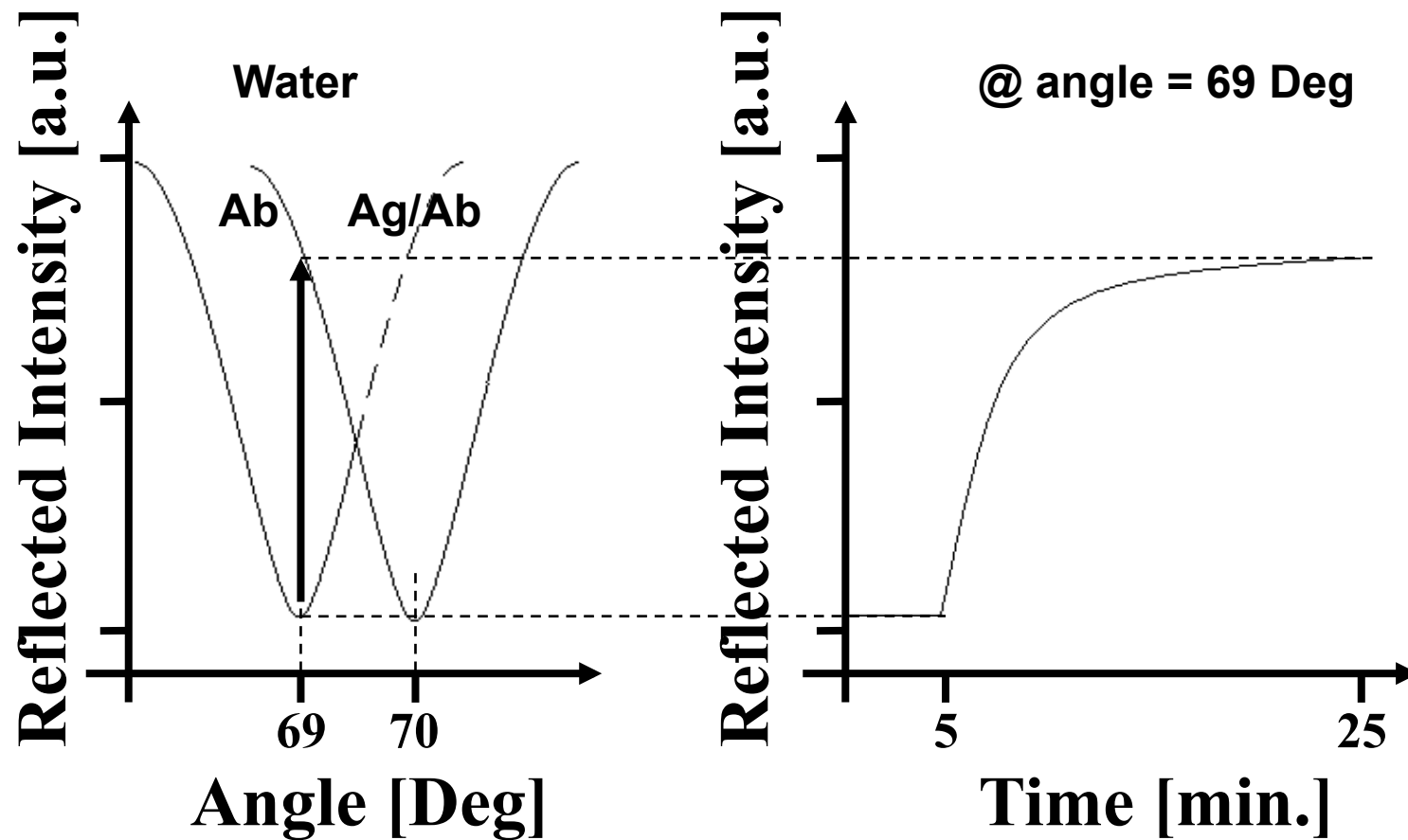
Characterization of Monoclonal antibodies



Bo Liedberg, et al, Biosensors & Bioelectronics 10 (1995) i-iv

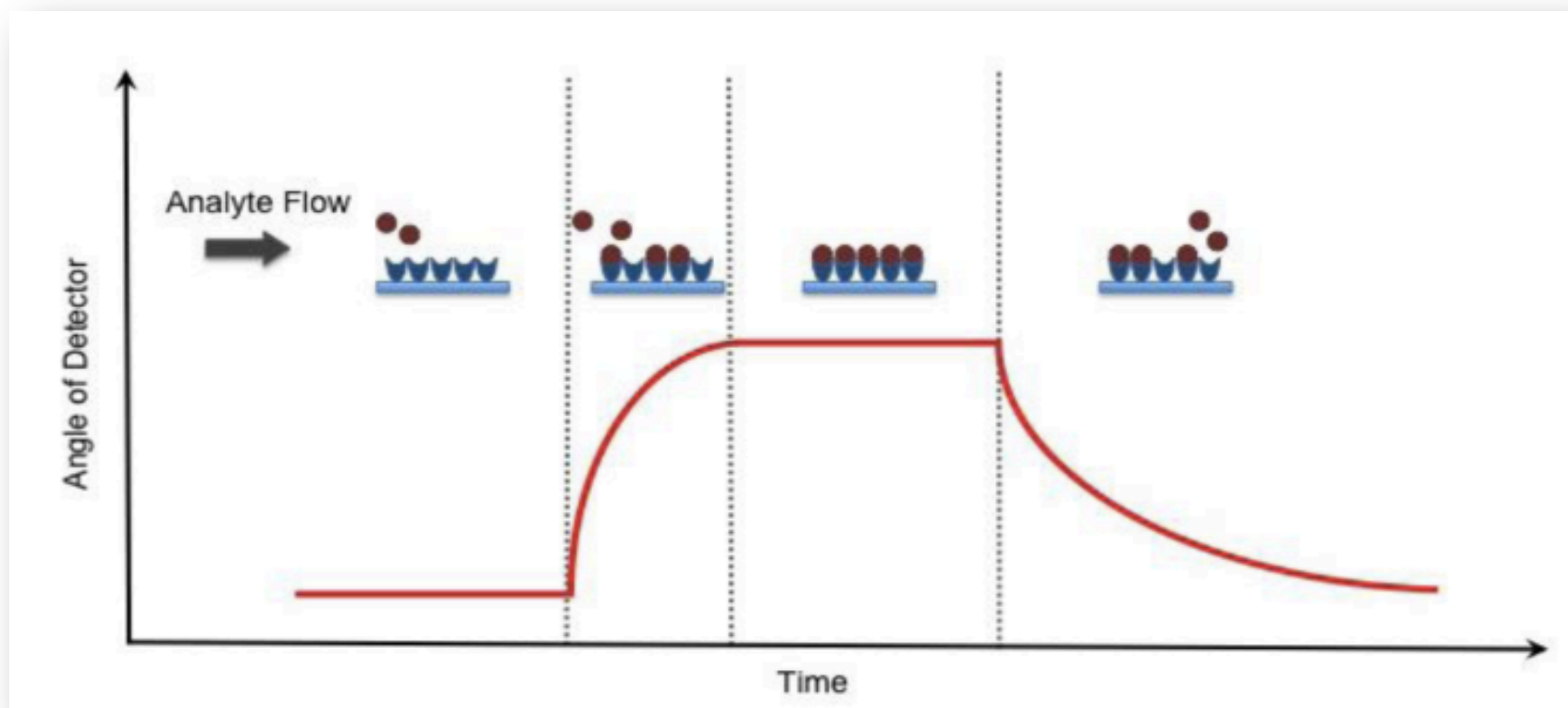
Shift in the resonant angle to sense IgG adsorption

Kinetic Studies



The kinetics could be reached by means of the change of the reflected intensity at fixed angle

Detection of Binding Events



Molecular uptake as monitored by SPR

SPR on SAM

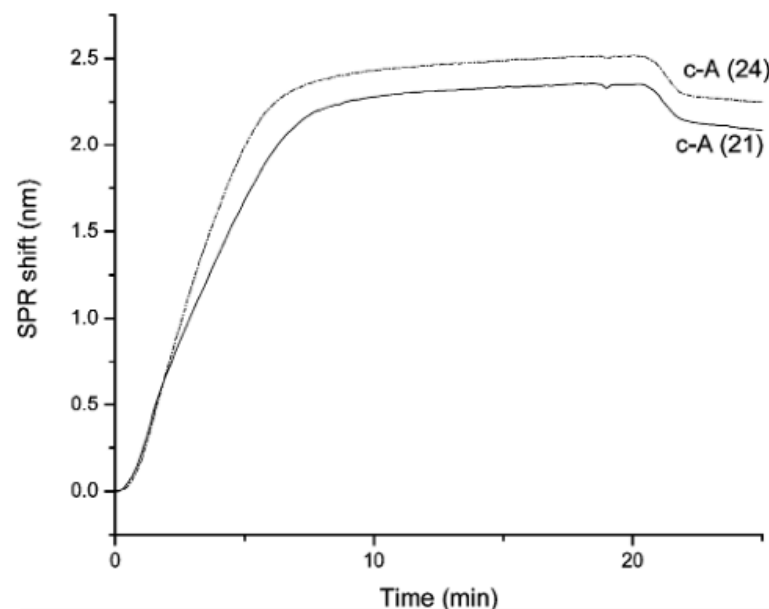


Figure 7. Comparison of the hybridization of a truncated complement (c-A 21) with the hybridization of the full-length complement (c-A 24). The ssDNA/OEG surface was prepared from a solution with a DNA mole fraction of 0.02.

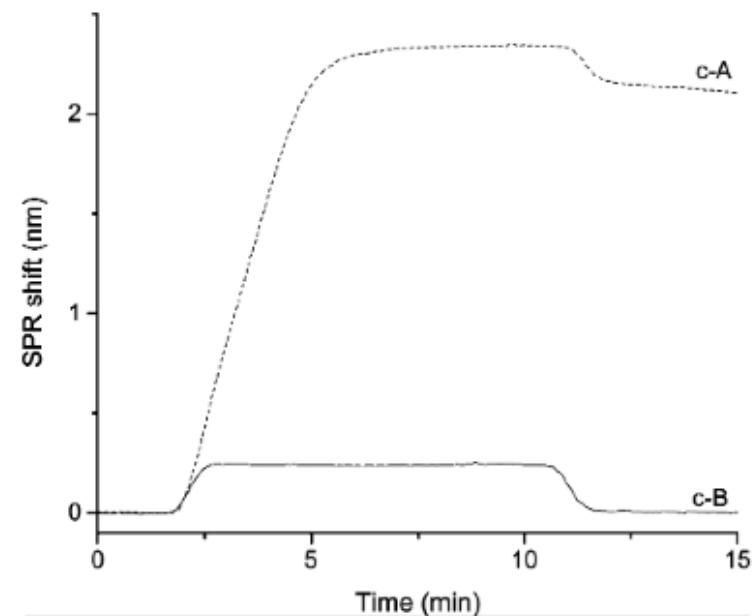


Figure 2. Control experiment to test DNA specificity. Both c-A and c-B were flowed over a sequence A ssDNA/OEG SAM, but only the complementary DNA hybridized. The ssDNA/OEG surface was prepared from a solution with a DNA mole fraction of 0.02.

Langmuir, Vol. 22, No. 10, 2006

DNA hybridization as monitored by SPR

SPR on SAM

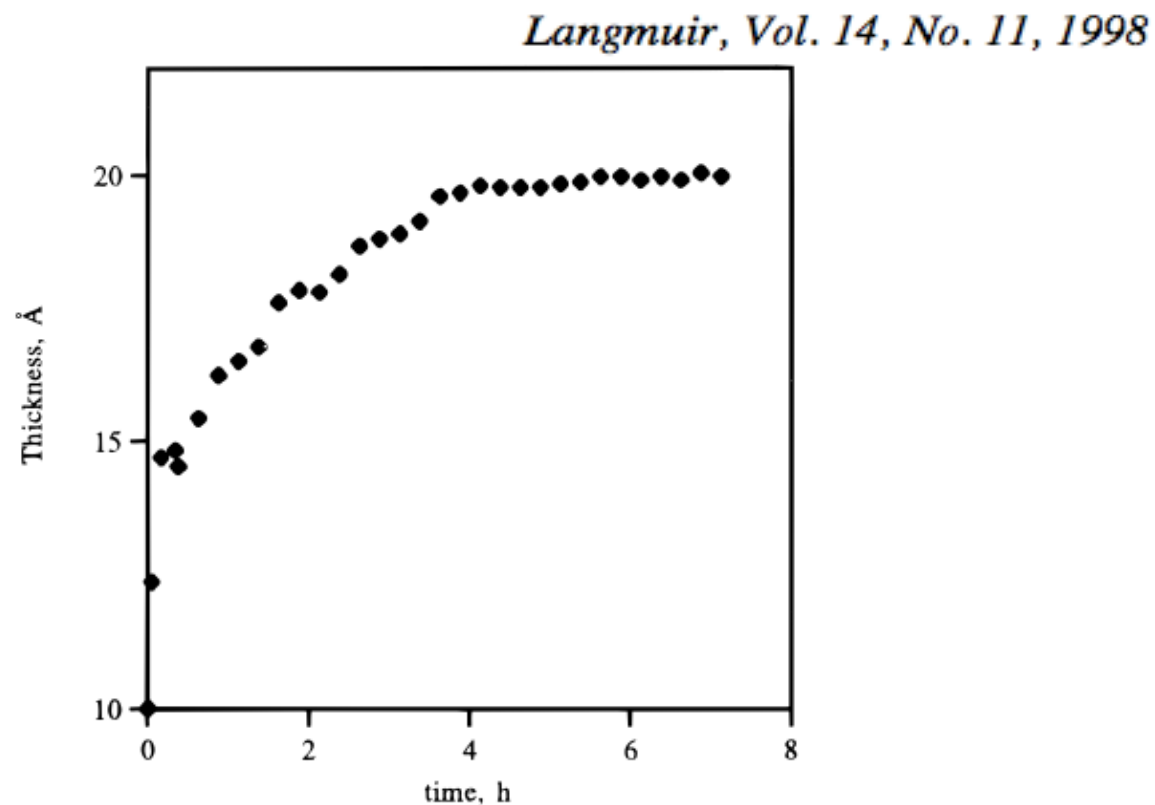


Figure 7. Increase in SPR measured thickness of the monolayer of disulfide **1** upon treatment with 0.1 mM ethanol solution of octadecanethiol.

Thiols SAM formation as seen by SPR

(c) S.Carrara

How to characterize the Probes Immobilization?

We have seen what are the
mechanisms of self-assembly!

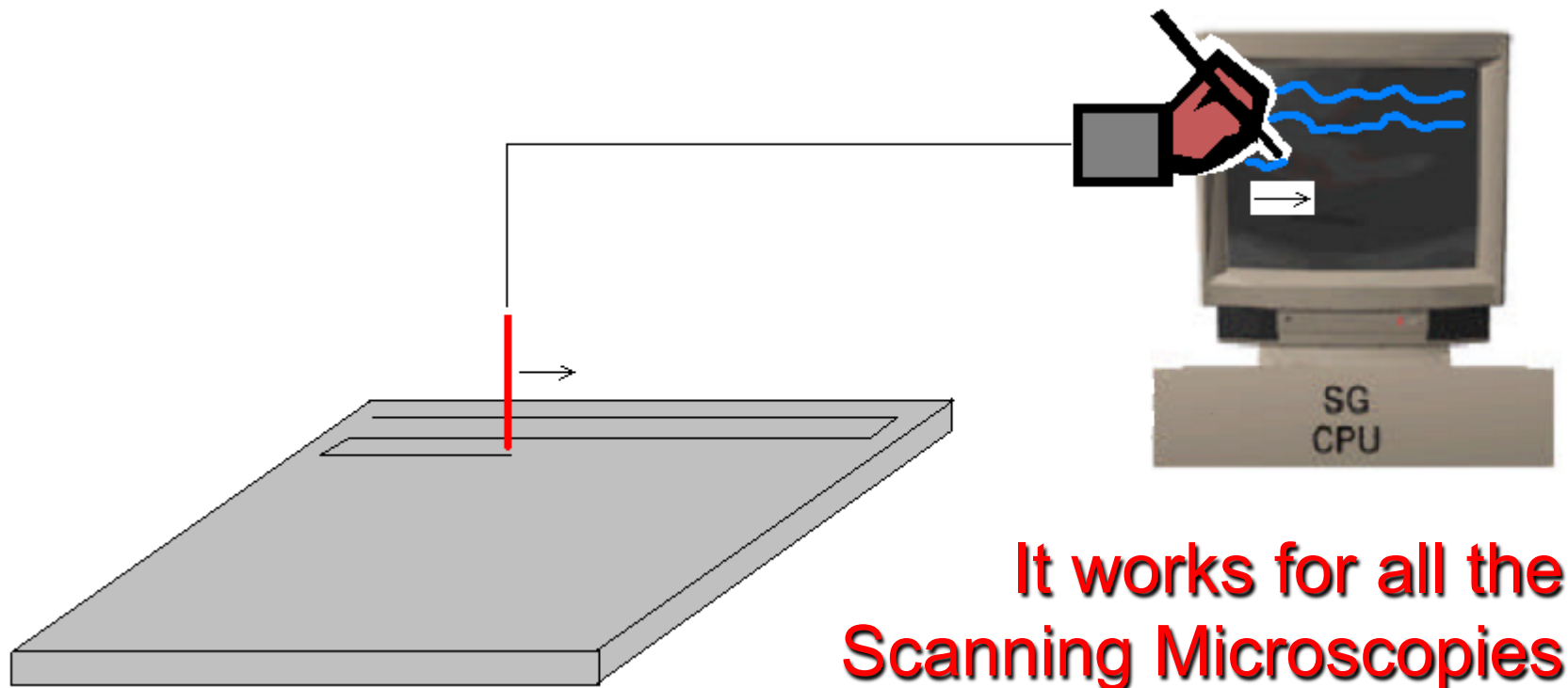
How to monitor the self-
assembly process?

How to check the film quality?

Four different Microscopies

1. Transmission Electron Microscopy (**TEM**)
2. Scanning Electron Microscopy (**SEM**)
3. Atomic Force Microscopy (**AFM**)
4. Scanning Tunneling Microscopy (**STM**)

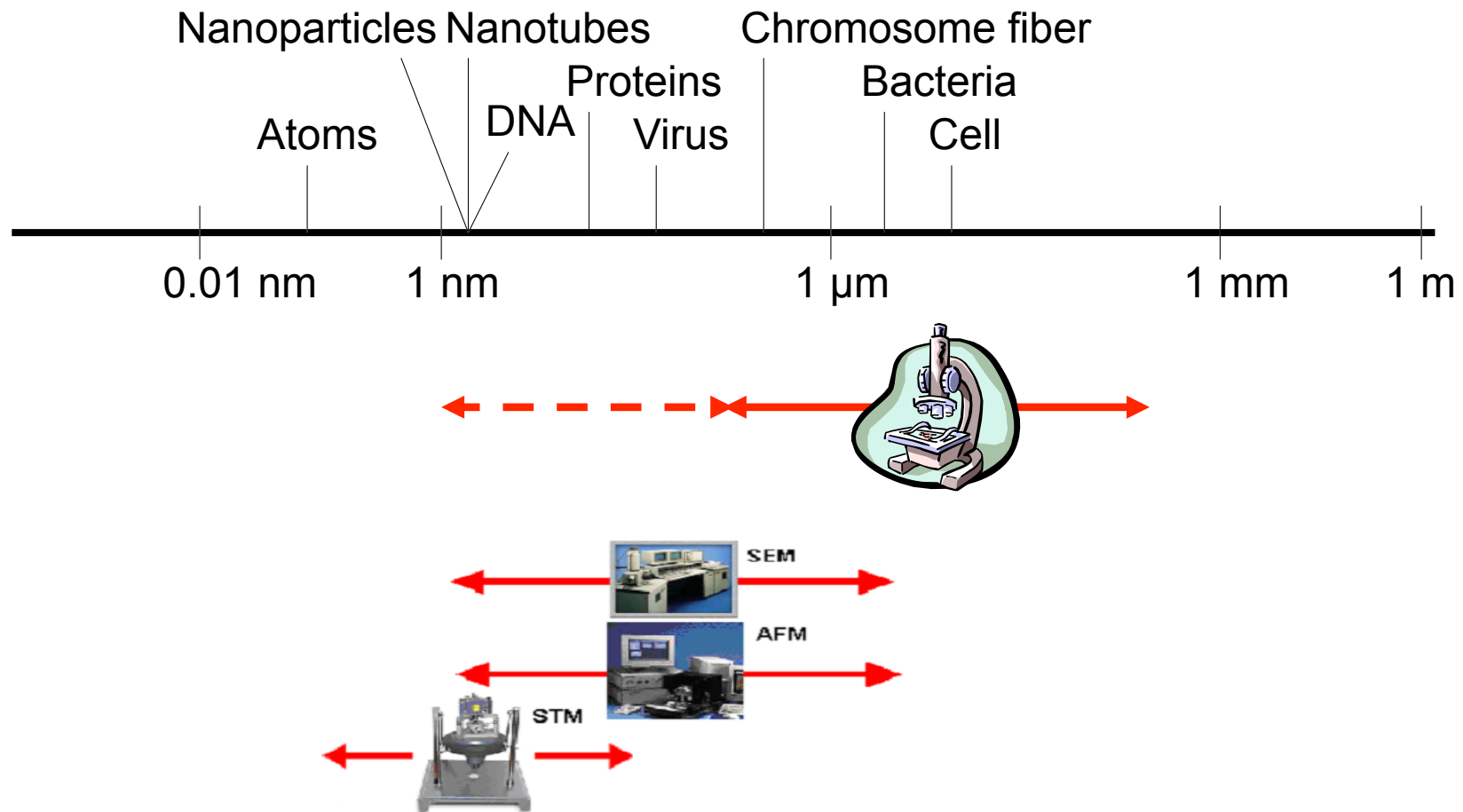
All are Scanning Microscopies



**It works for all the
Scanning Microscopies**

The sample scanning generates an image visualized
with computer graphic tools

SEM Microscopy



Electron Microscopies

Scanning
Electron
Microscopy

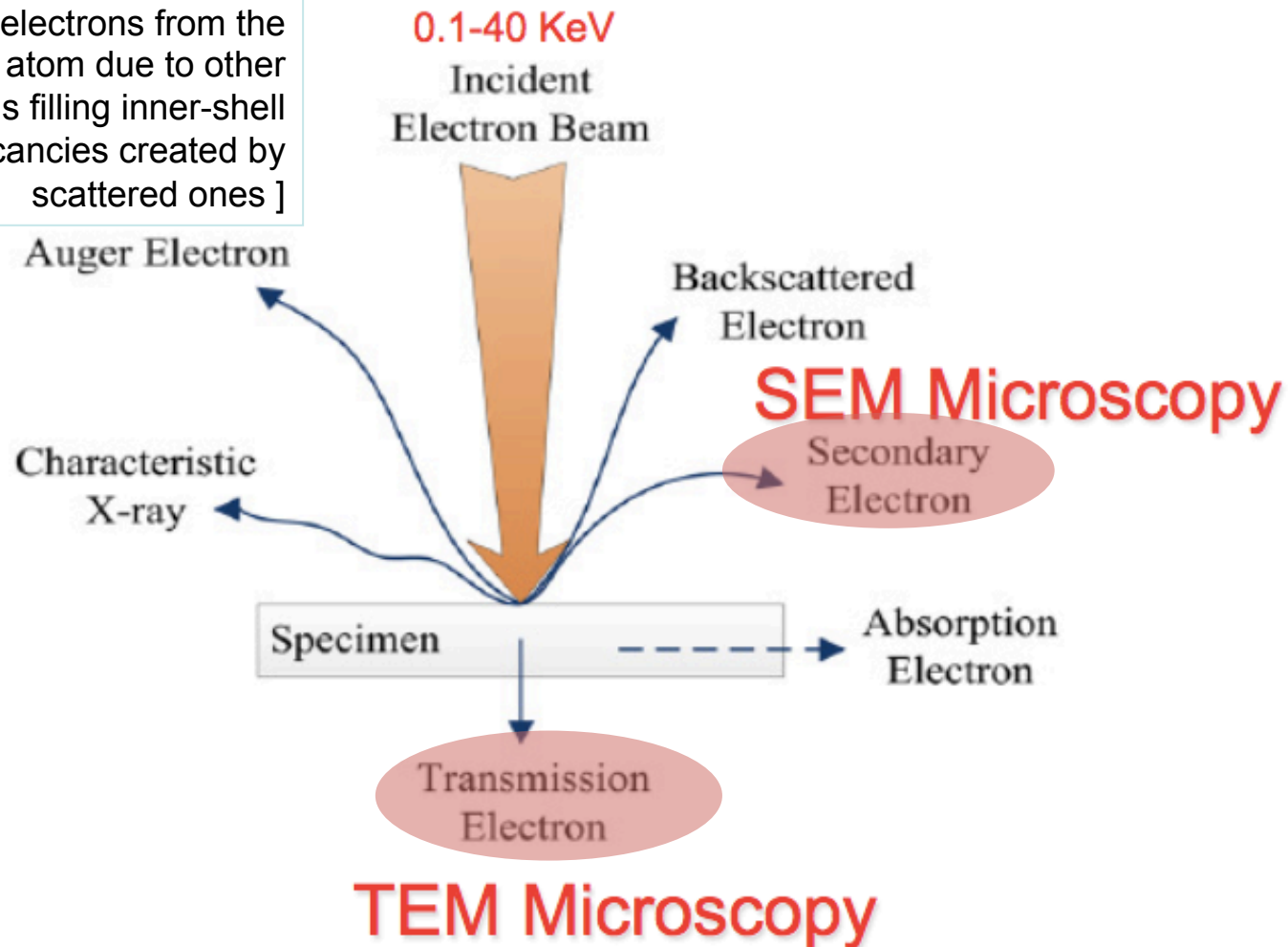


Transmission
Electron
Microscopy

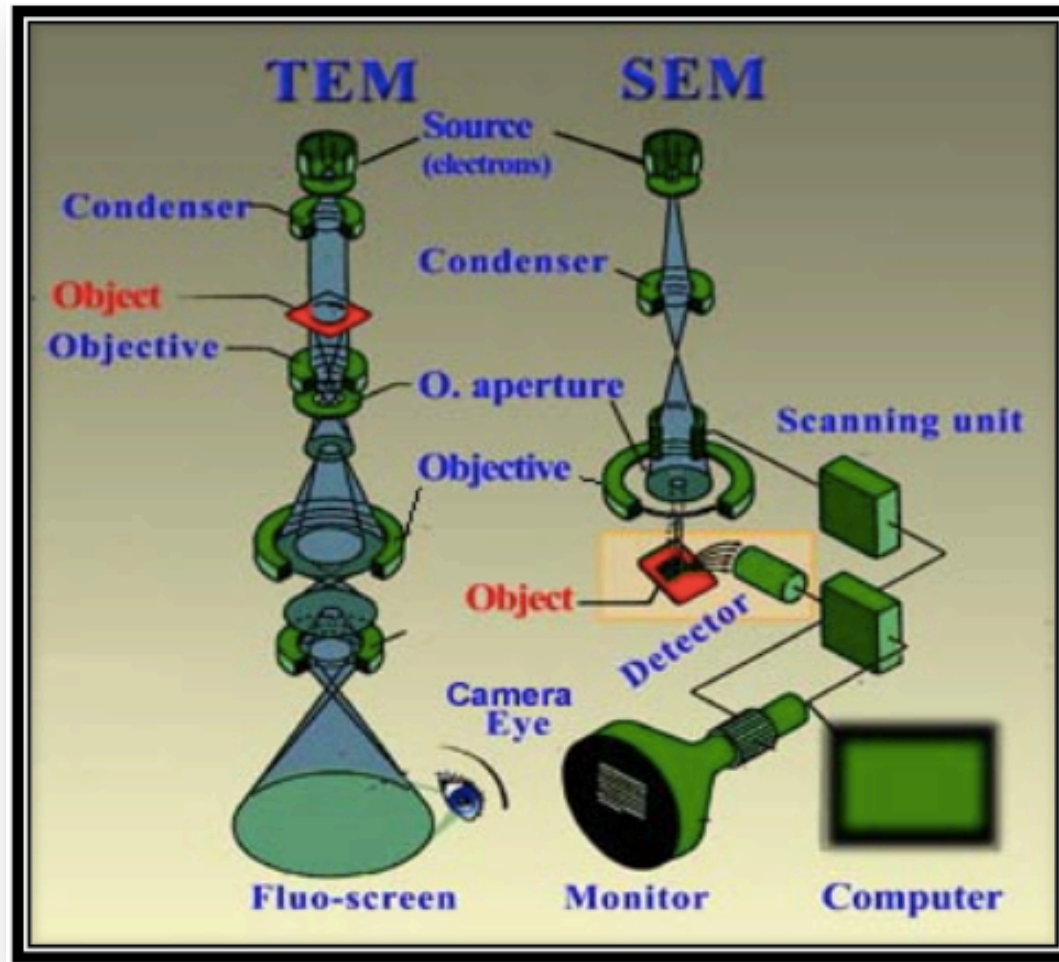


Electron Microscopy Principle

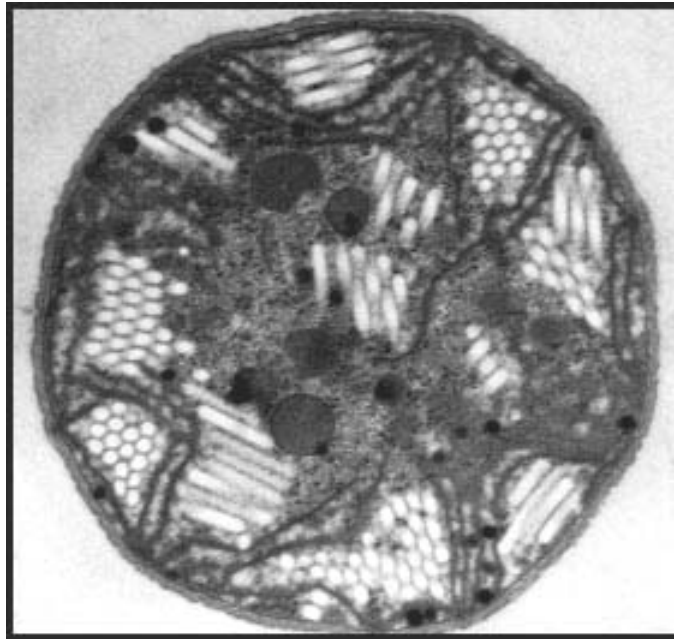
[Emitted electrons from the same atom due to other electrons filling inner-shell vacancies created by scattered ones]



Electron Microscopies

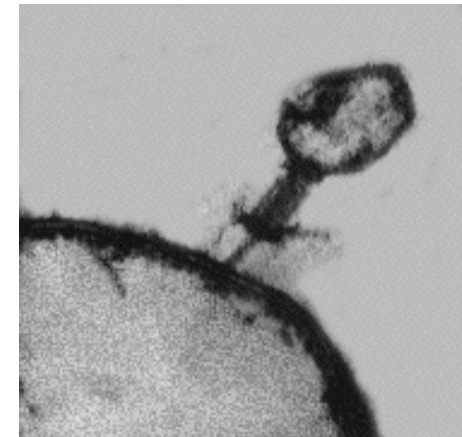
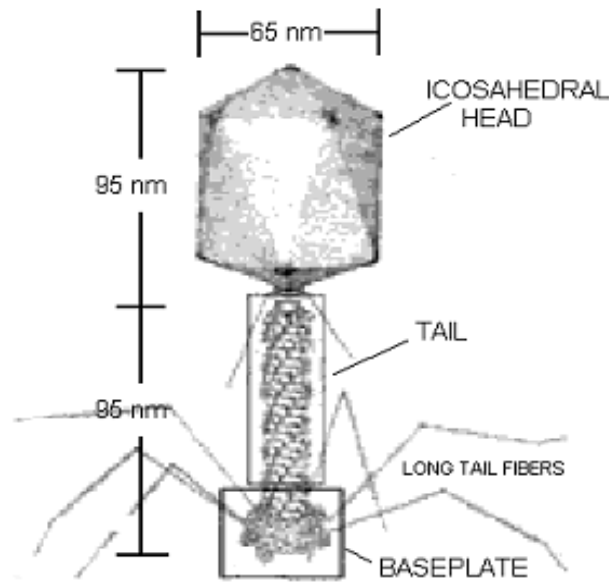


TEM Imaging



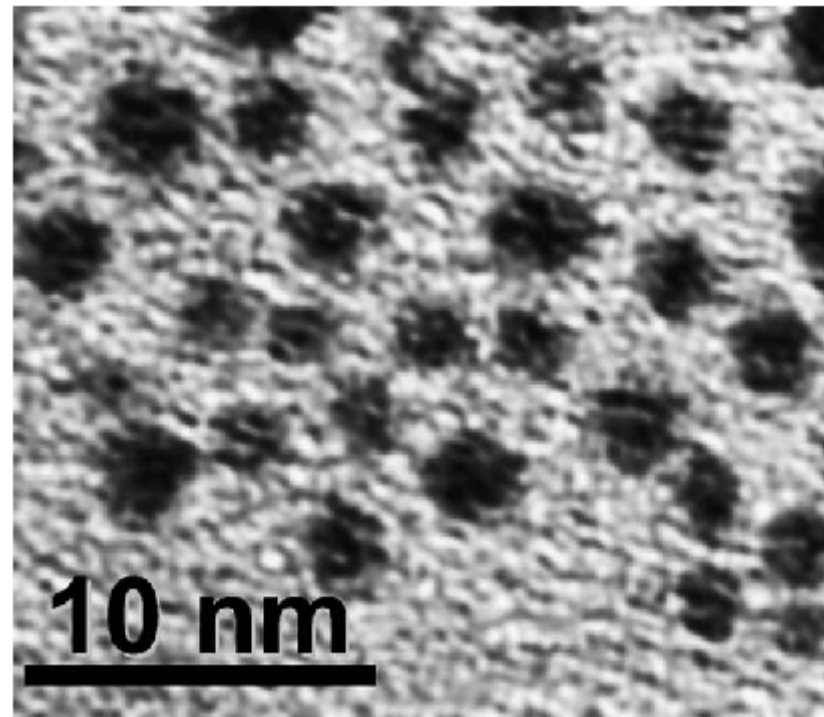
Cell of Cyanobacteria Microcystis by TEM

TEM Imaging



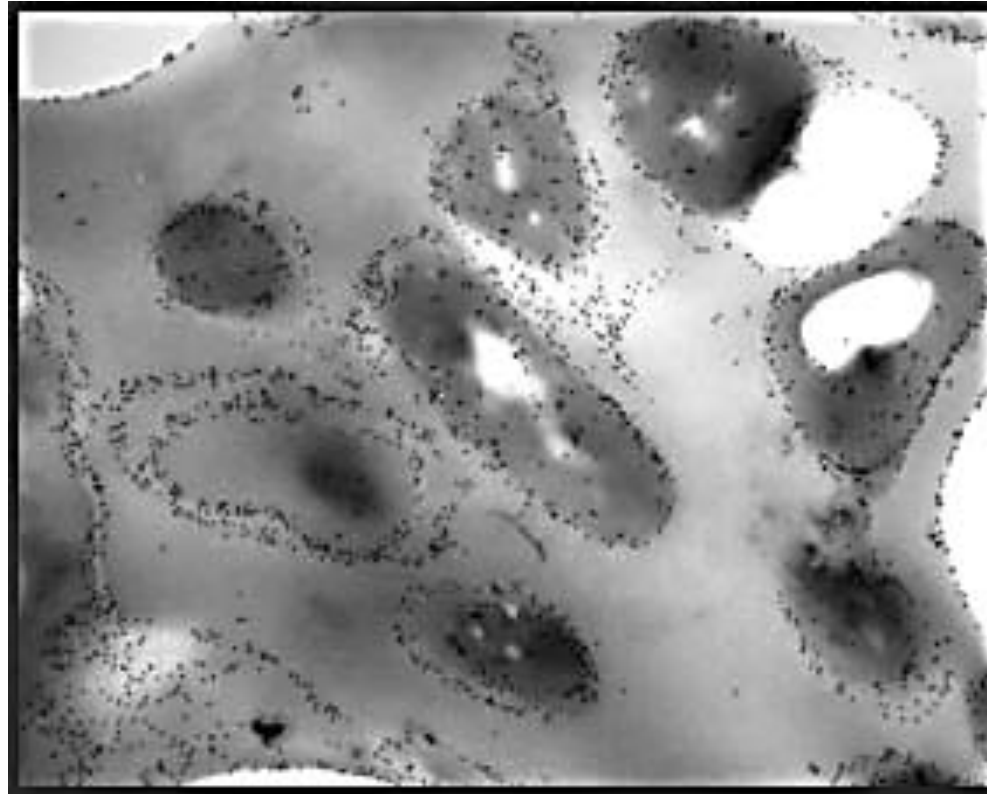
Virus T4 Bacteriophages

SEM Imaging



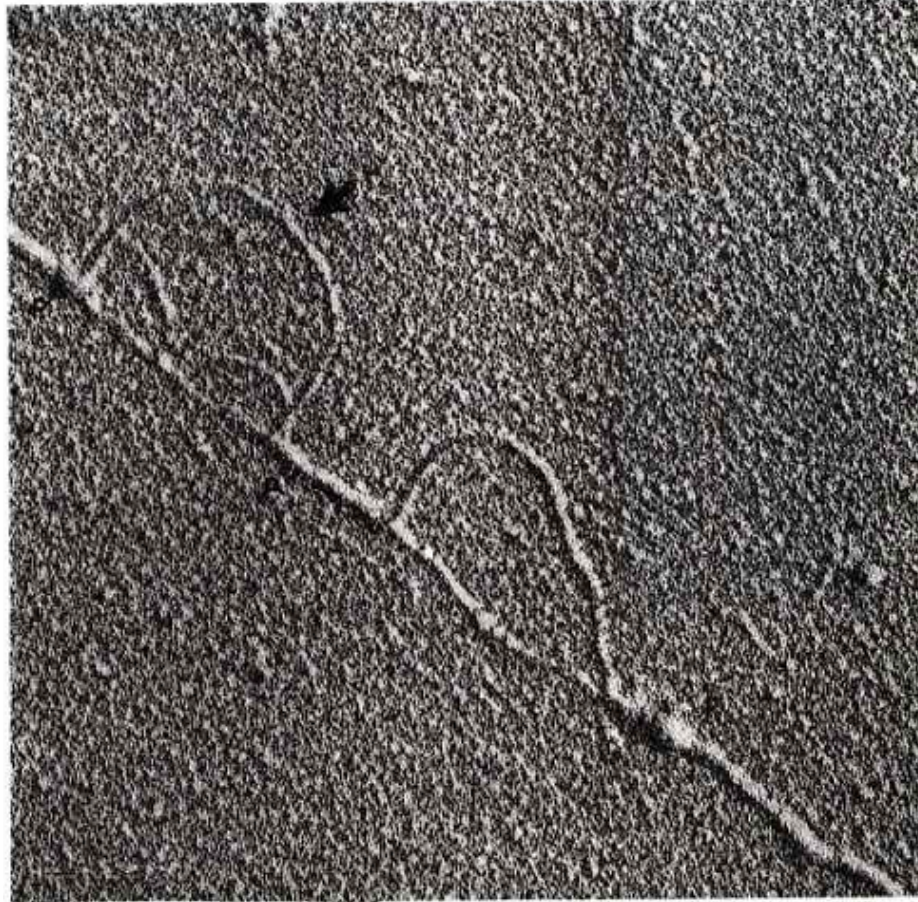
Gold nanoparticles made off gold core and a thiols-shell to stabilize the particle

SEM Imaging



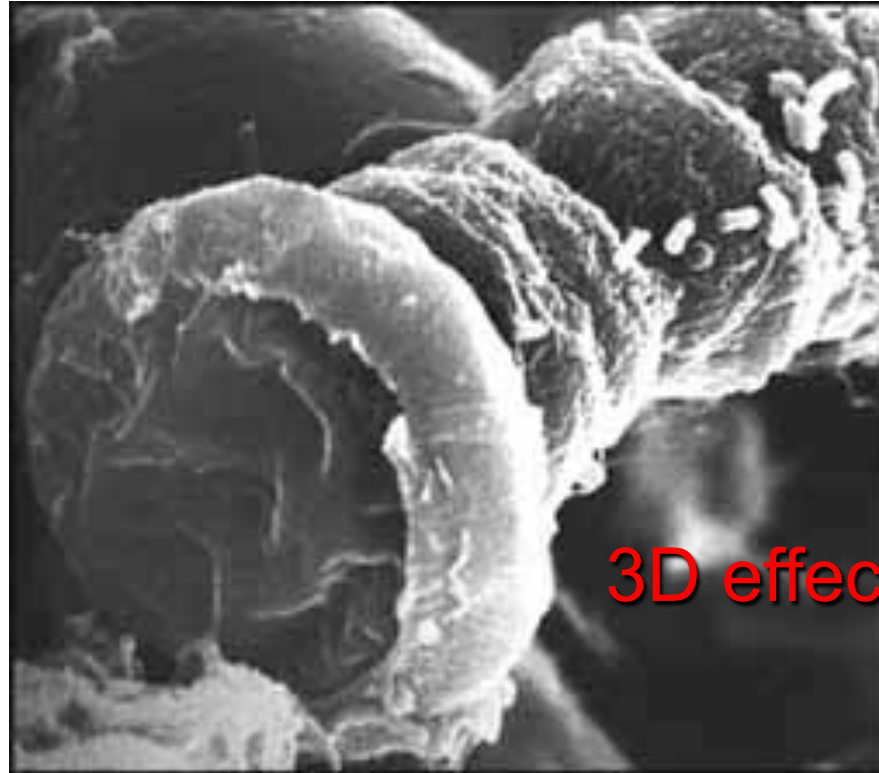
Antigens localized on the surface
of bacteria cells

SEM Imaging



Isolated Chromatin (DNA-macromolecules including hystons and not-histons proteins) of about 30 nm and with small filament

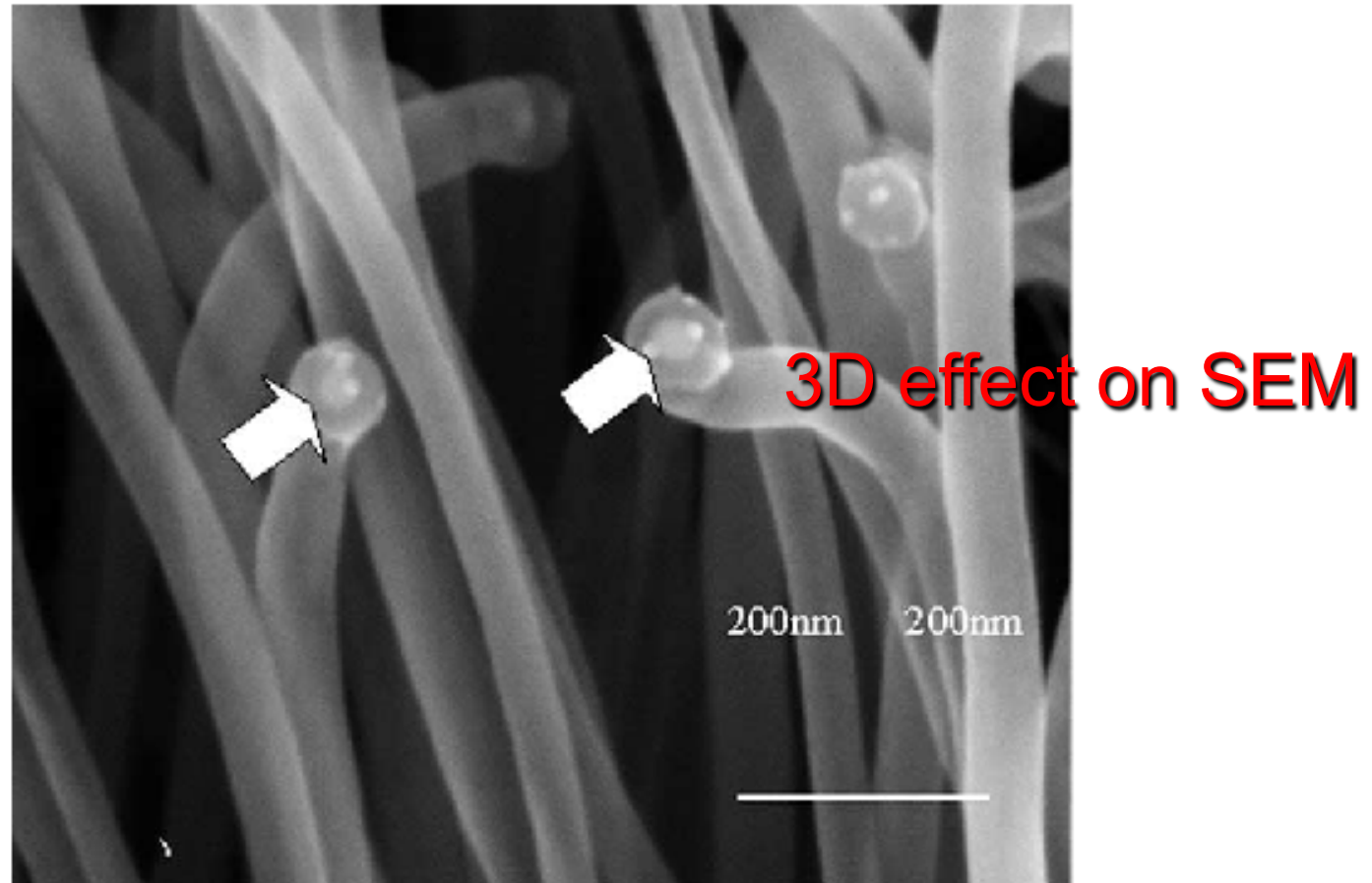
SEM Imaging



3D effect on SEM

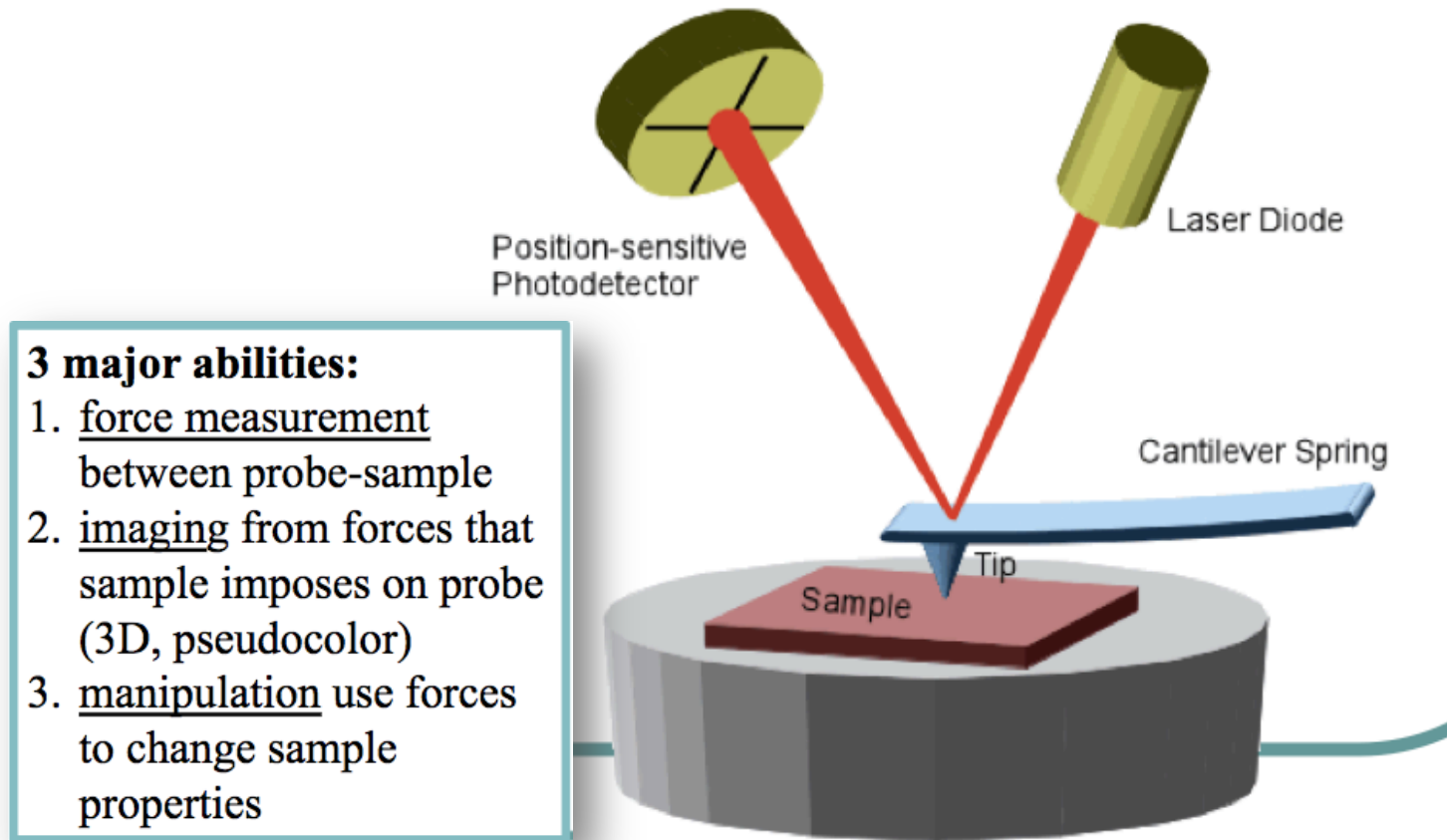
End part of Nodularia
(cyanobacteria in filament shape)

SEM Imaging



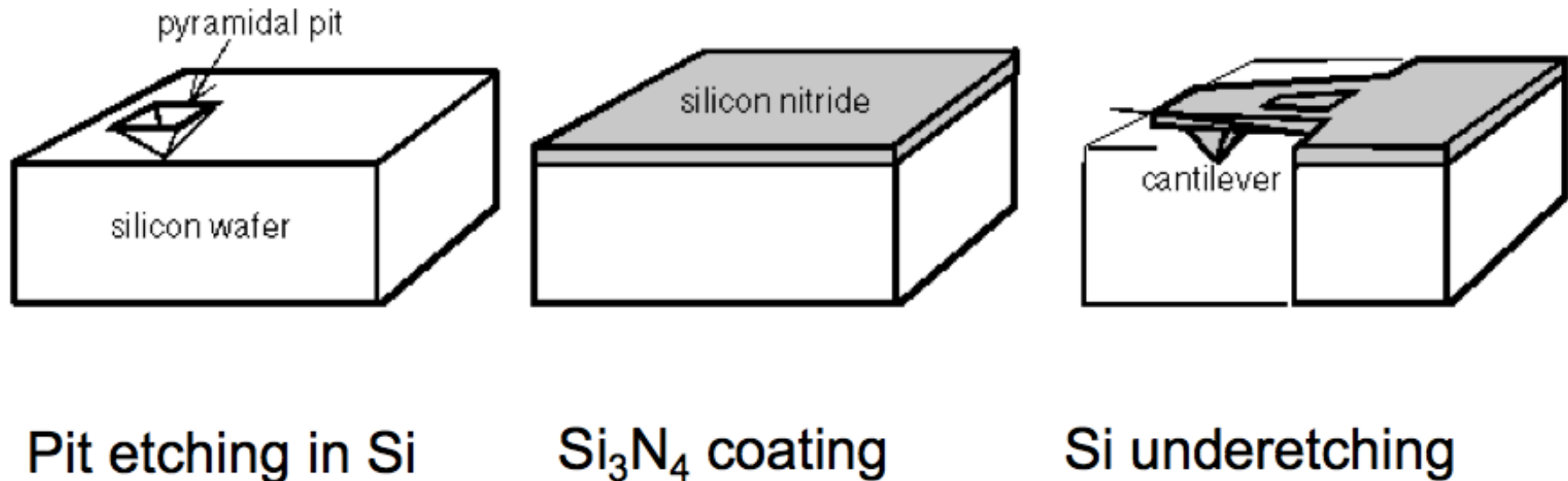
Carbon Nanotubes with a metallic nanoparticles located at the apical part

AFM detection principle



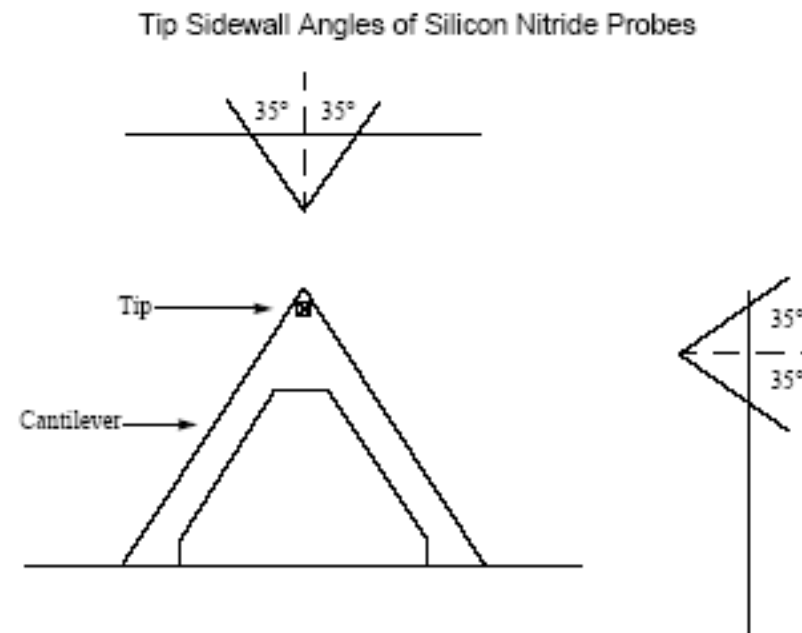
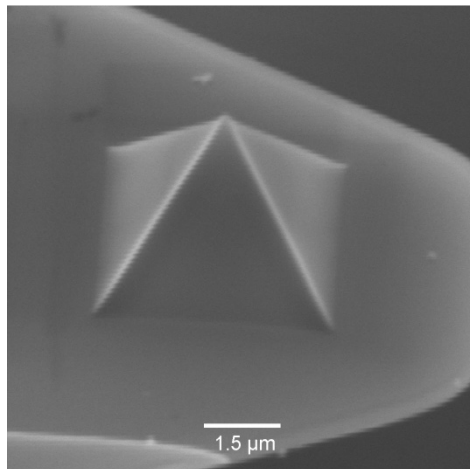
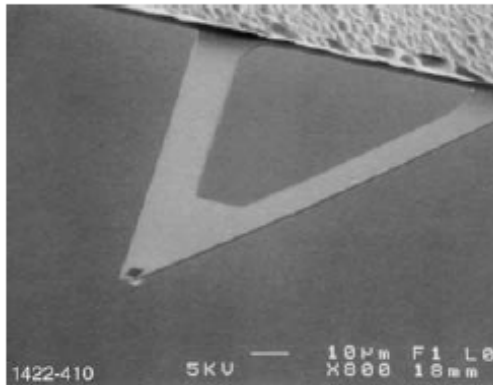
Detection principle is obtained by monitoring the bending of the cantiliver that host the tip

AFM Tip fabrication



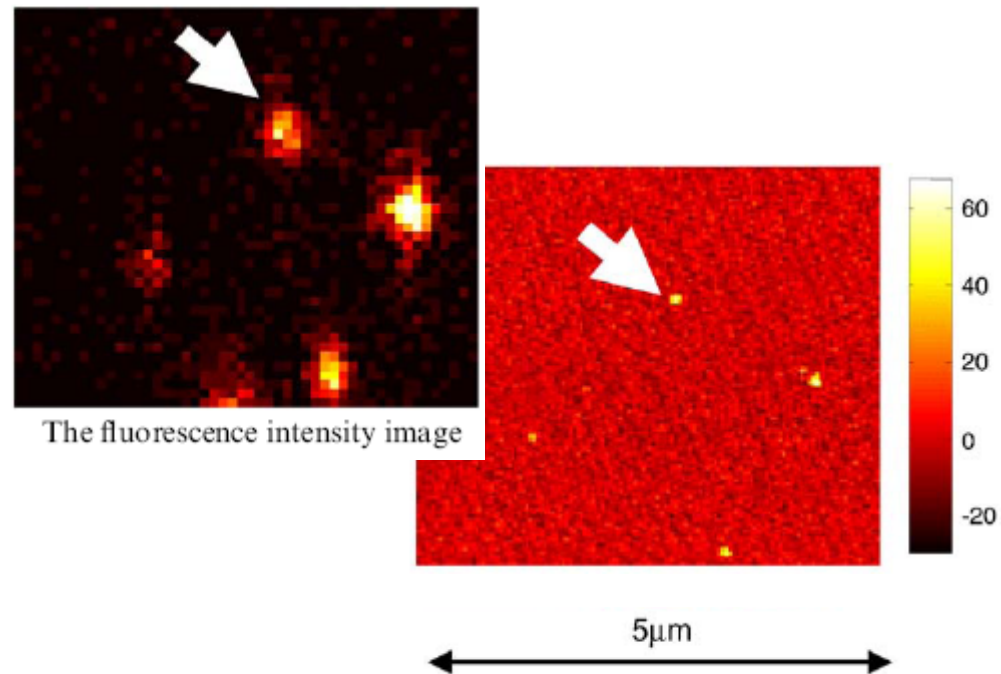
AFM tips are typically sculpted on silicon wafers

SEM on Silicon nitride probes



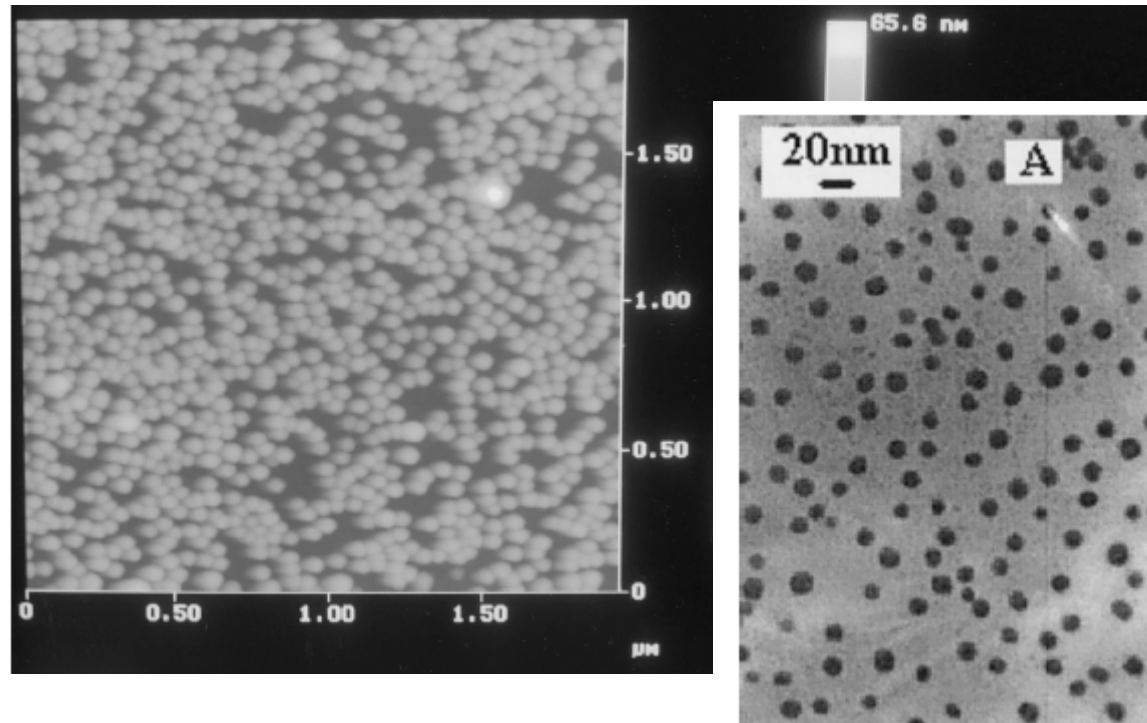
(credit by Cambridge University)

Optics versus AFM



Fluorescent Nanoparticles with average size of about 40 nm

AFM Imaging



TEM images

Gold nanoparticles made off gold core and a
thiols-shell to stabilize the particle

AFM Imaging

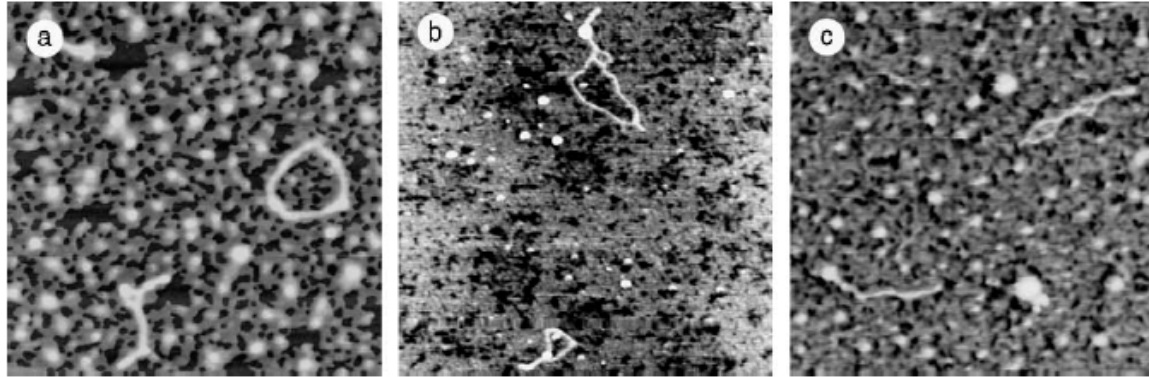


Fig. 1. Representative AFM images of plasmid DNA on the surface of newly cleaved mica at room temperature: open circular structure (a) ($2\ \mu\text{m} \times 2\ \mu\text{m}$), supercoiled structure (b) ($2.5\ \mu\text{m} \times 2.5\ \mu\text{m}$) and relaxed supercoil (c) ($2\ \mu\text{m} \times 2\ \mu\text{m}$).

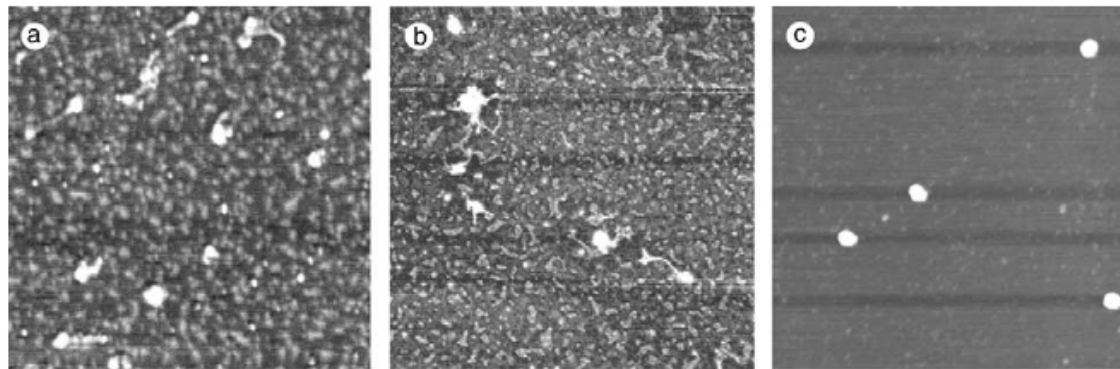
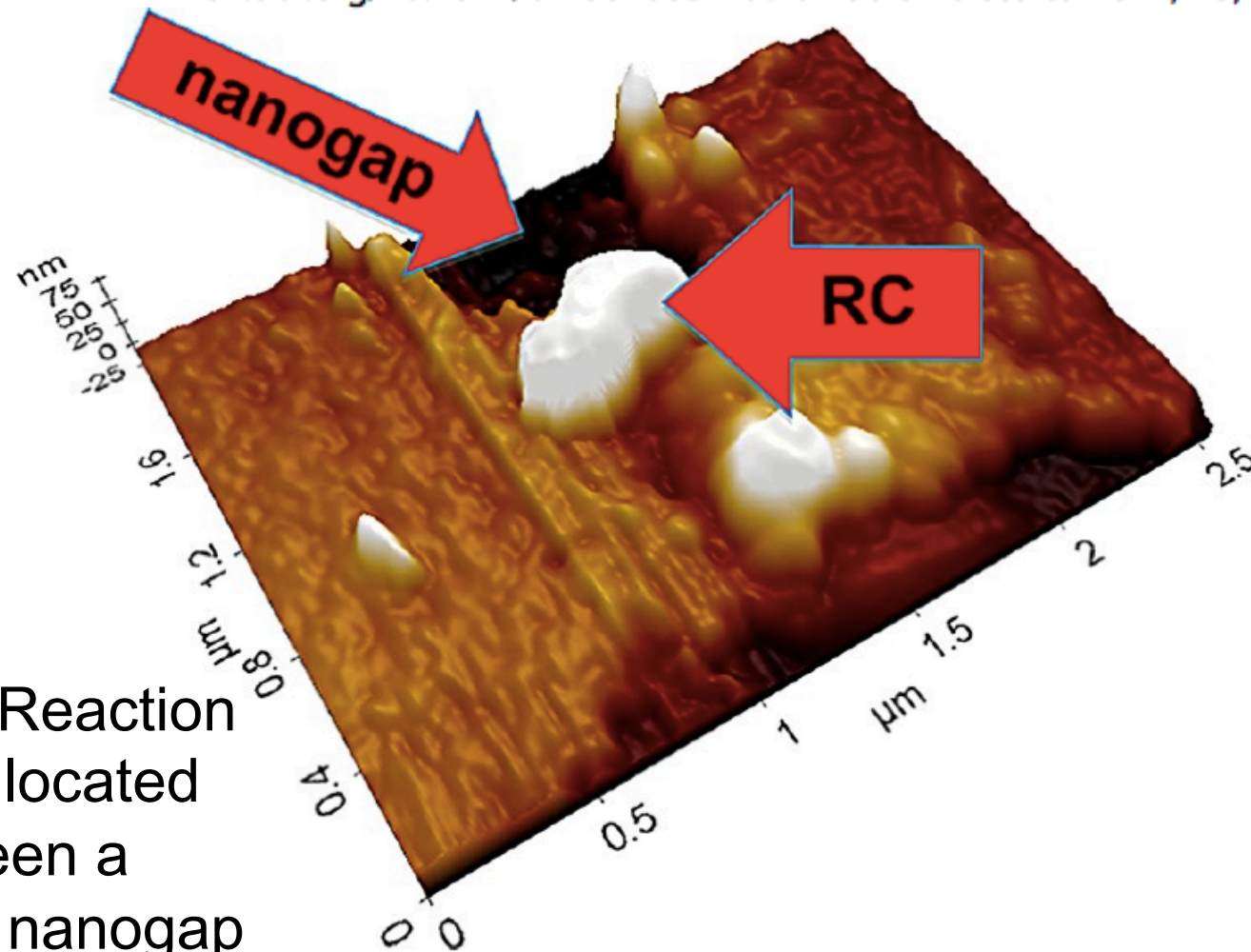


Fig. 2. Representative AFM images of plasmid DNA on the surface of mica after heated at $80\ ^\circ\text{C}$ (a), $84\ ^\circ\text{C}$ (b) and $90\ ^\circ\text{C}$ (c). In both (a) and (b) circular structure disappear and formed globule structure with undenatured tail. (c) Only globule structure appear, which correspond to whole denaturation of DNA. Scale bar 500 nm for all images.

DNA

AFM Imaging

[dx.doi.org/10.1021/bm301063m](https://doi.org/10.1021/bm301063m) | *Biomacromolecules* 2012, 13, 3503–3509



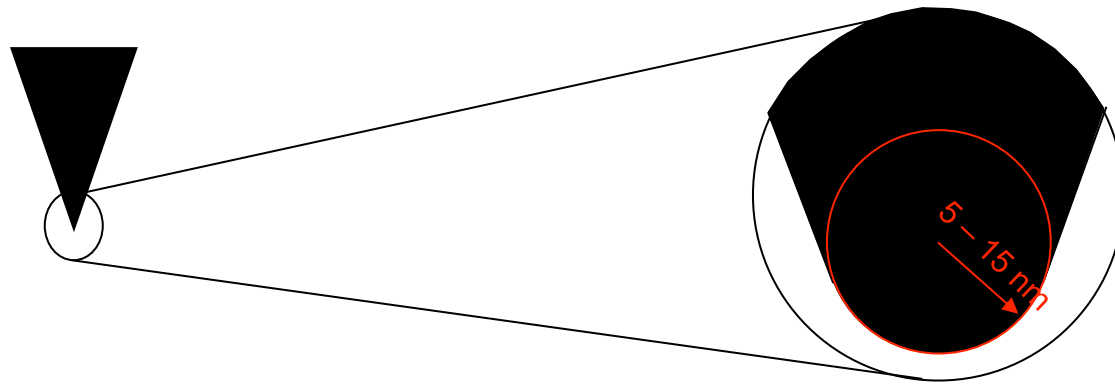
A protein (Reaction Center) located in between a metallic nanogap

(c) S.Carrara

Lateral resolution

Lateral resolution is primarily determined by the radius of curvature of the tip end.

Typical tip radii quoted by the manufacturers for tapping mode tips are around 5 – 15 nm

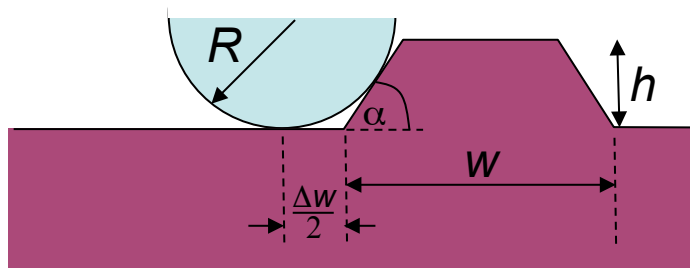


The sidewall angles of the tip will also determine its ability to probe high aspect ratio features.

Errors in width measurements

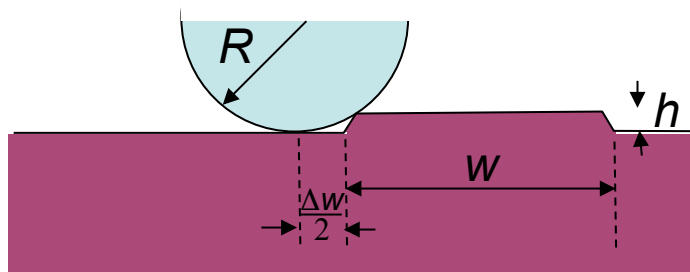
Assuming a hemispherical tip:

$$h > R(1 - \cos\alpha)$$



$$\frac{\Delta w}{2} = R \tan \frac{\alpha}{2}$$

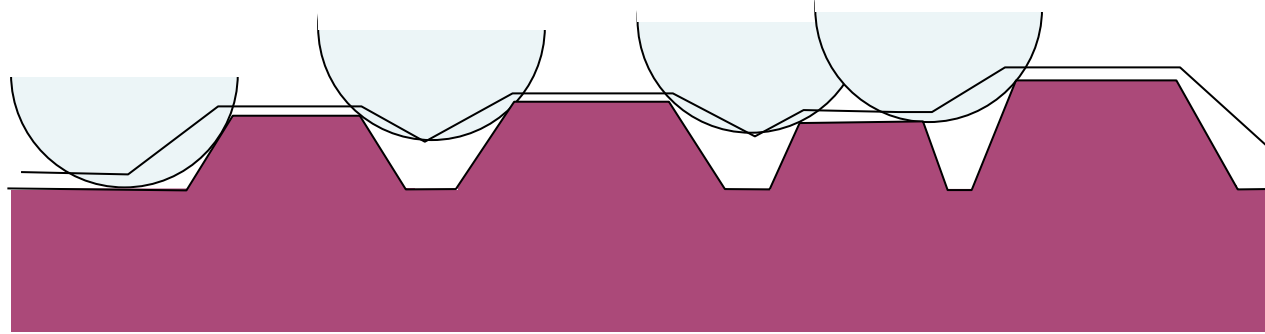
$$h < R(1 - \cos\alpha)$$



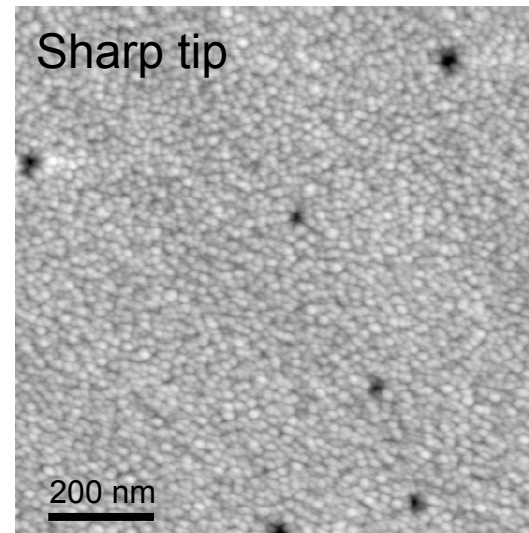
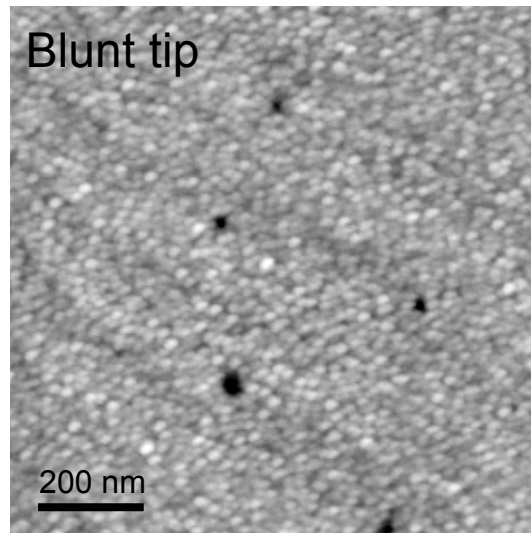
$$\frac{\Delta w}{2} = \sqrt{h(2R - h)} - h \cot \alpha$$

Not only the tip radius but the feature height and its likely shape must be known to accurately determine the true width

Dense nanostructure arrays

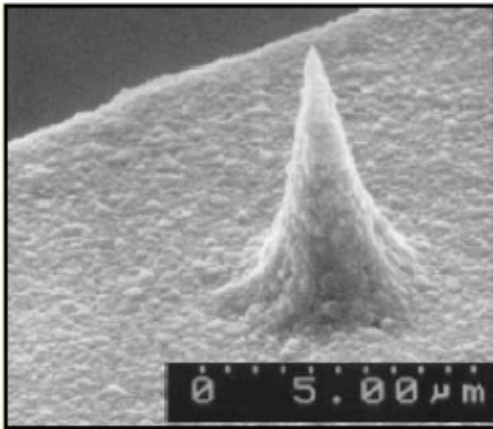


For densely packed features the tip size can also cause errors in determining the height of the islands, or the overall appearance of the surface

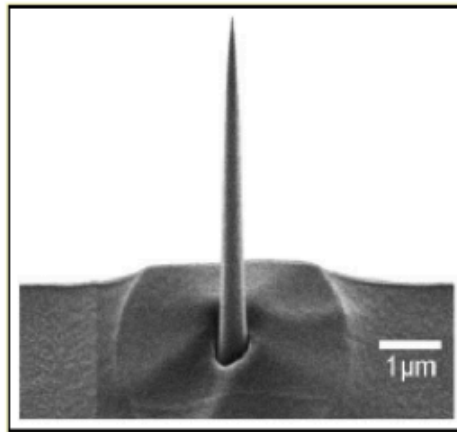


(credit by Cambridge University)

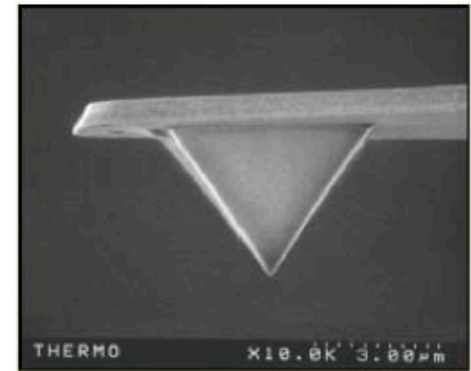
Special AFM tips



Diamond-coated tip



FIB-sharpened tip

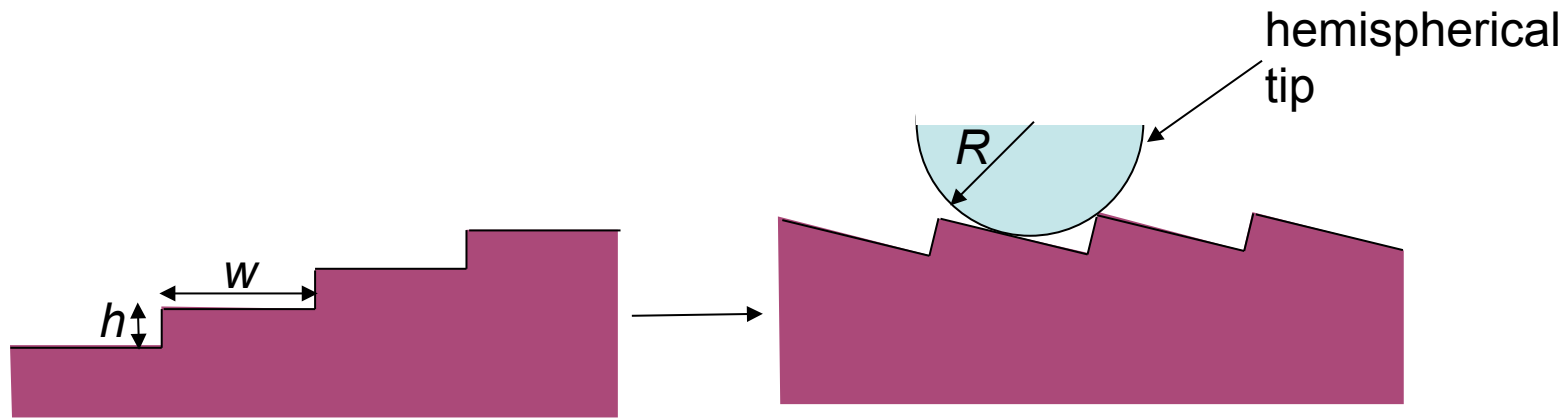


Gold-coated Si_3N_4 tip

Special tips are sometimes used to further increase the microscope resolution

(images credit by University of Utah)

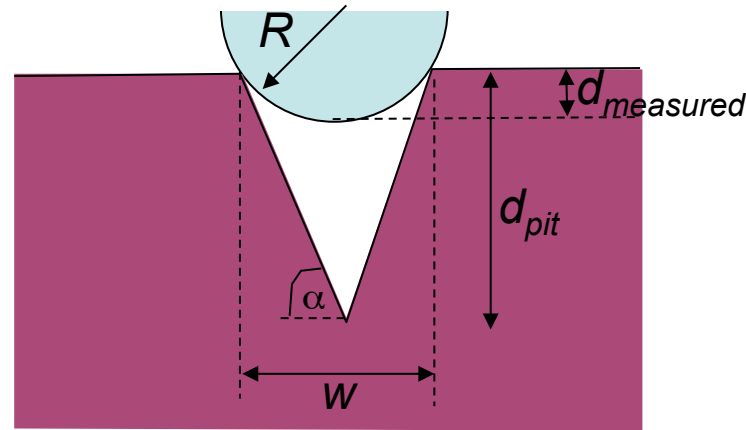
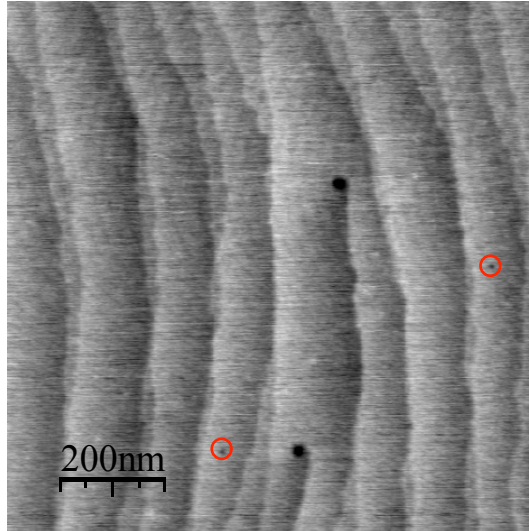
Terraced surfaces



Single atom-high steps may be routinely imaged by AFM.

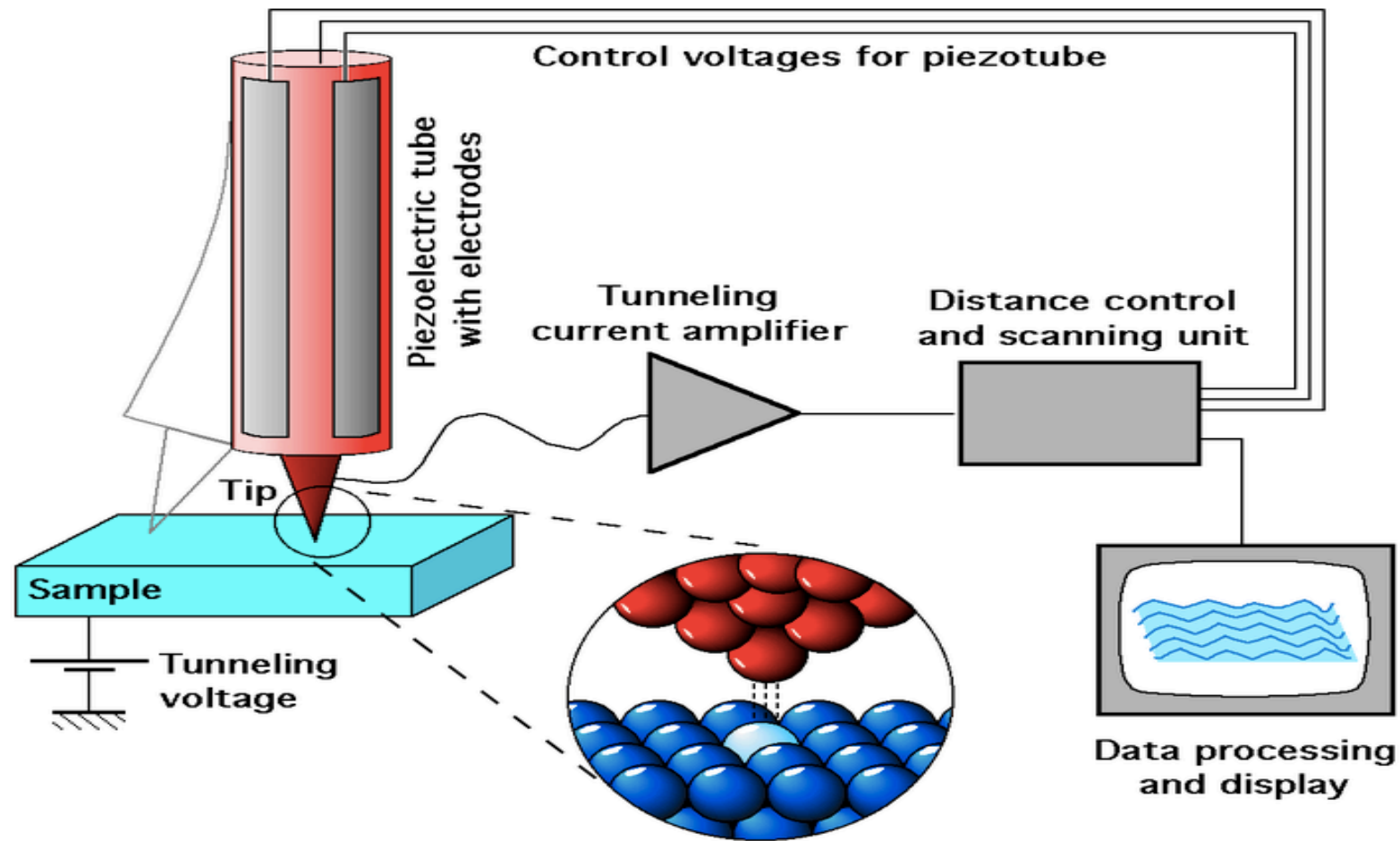
However, as step-edges become closer together (e.g. on vicinal substrates) it may become impossible to see separate terraces.

Small pits



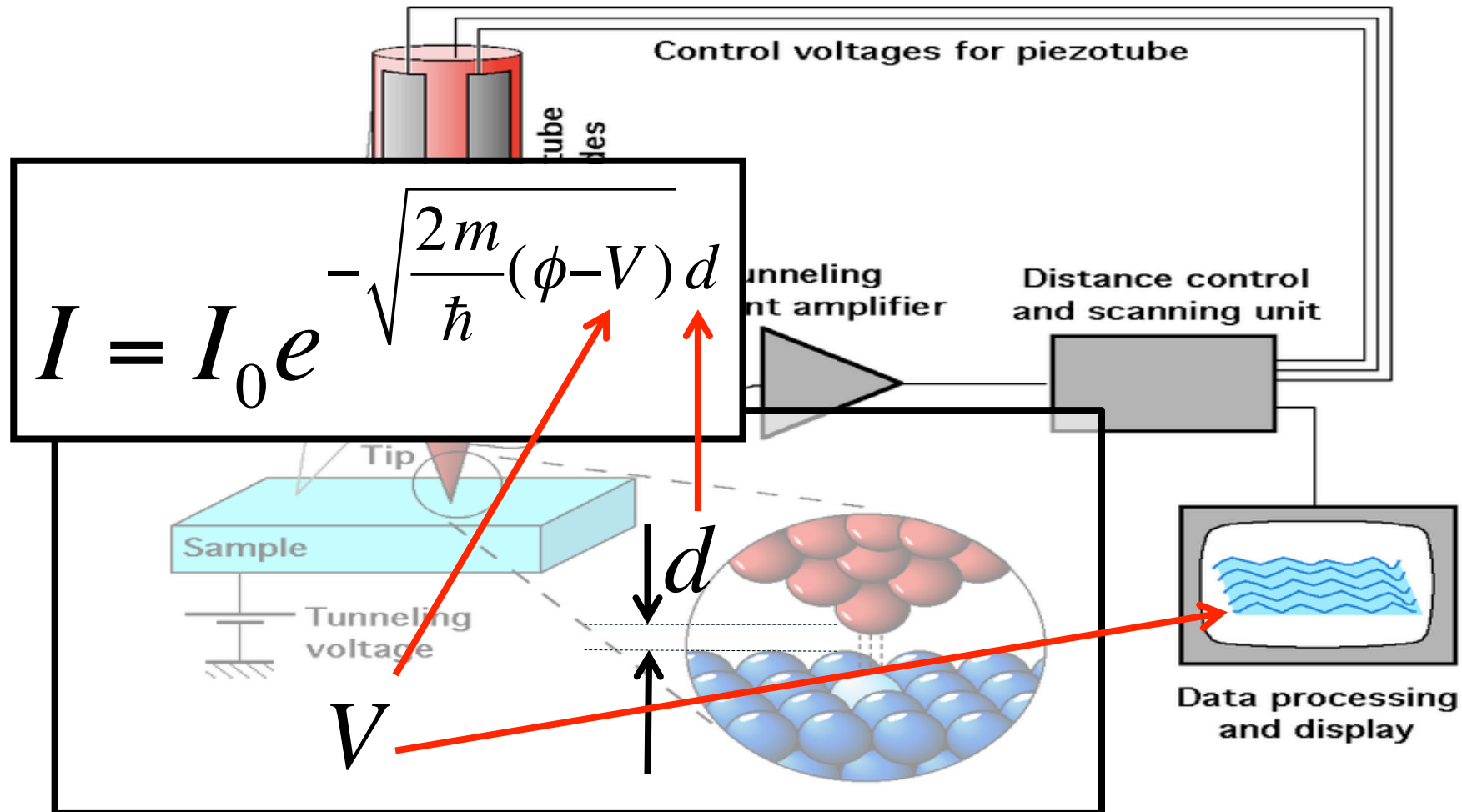
When imaging small pits, such as pits associated with threading dislocation terminations, one is unlikely to see the true pit depth. The measured width will actually be more accurate than the measured depth, which will depend on the angle α , and the tip radius. The pit will not be visible if the measured depth is less than the noise level.

STM Microscopy



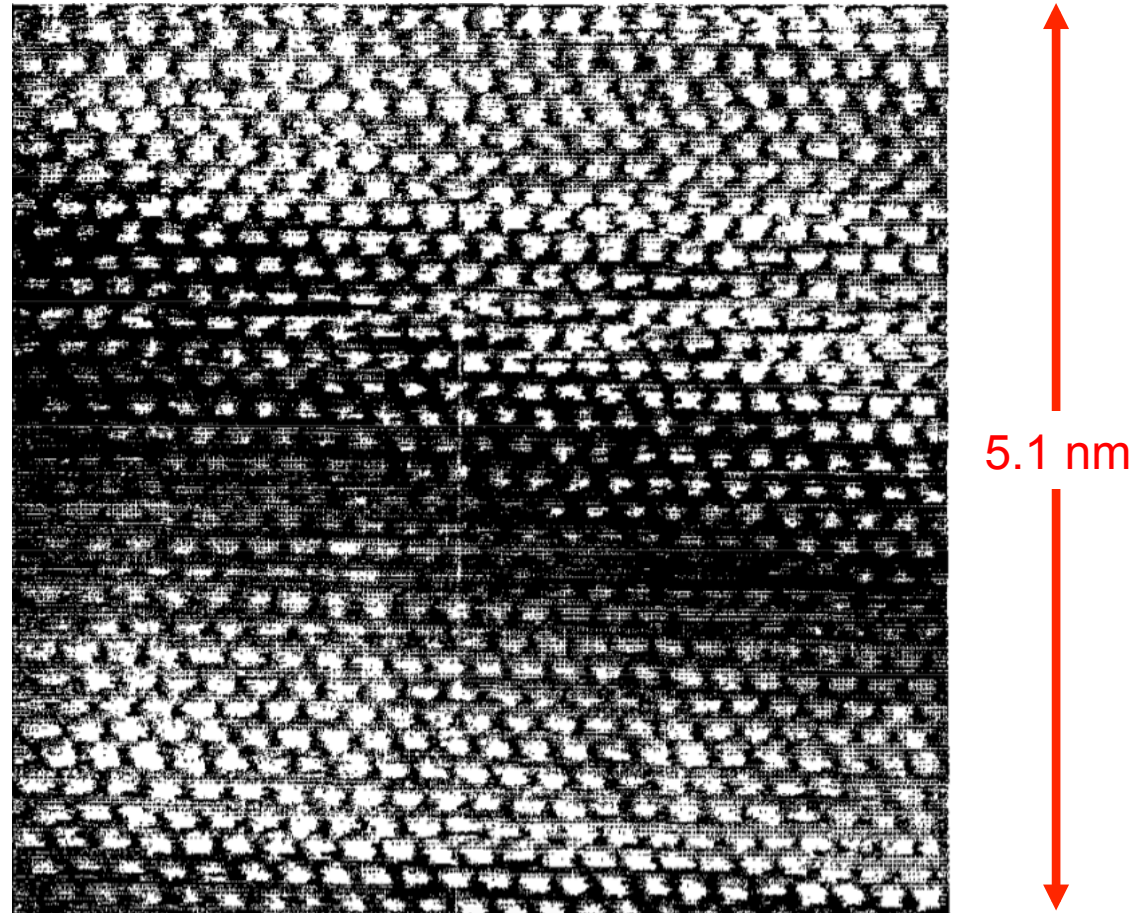
The STM Microscopy is based on tunneling currents as established in between the tip and the sample

STM Microscopy



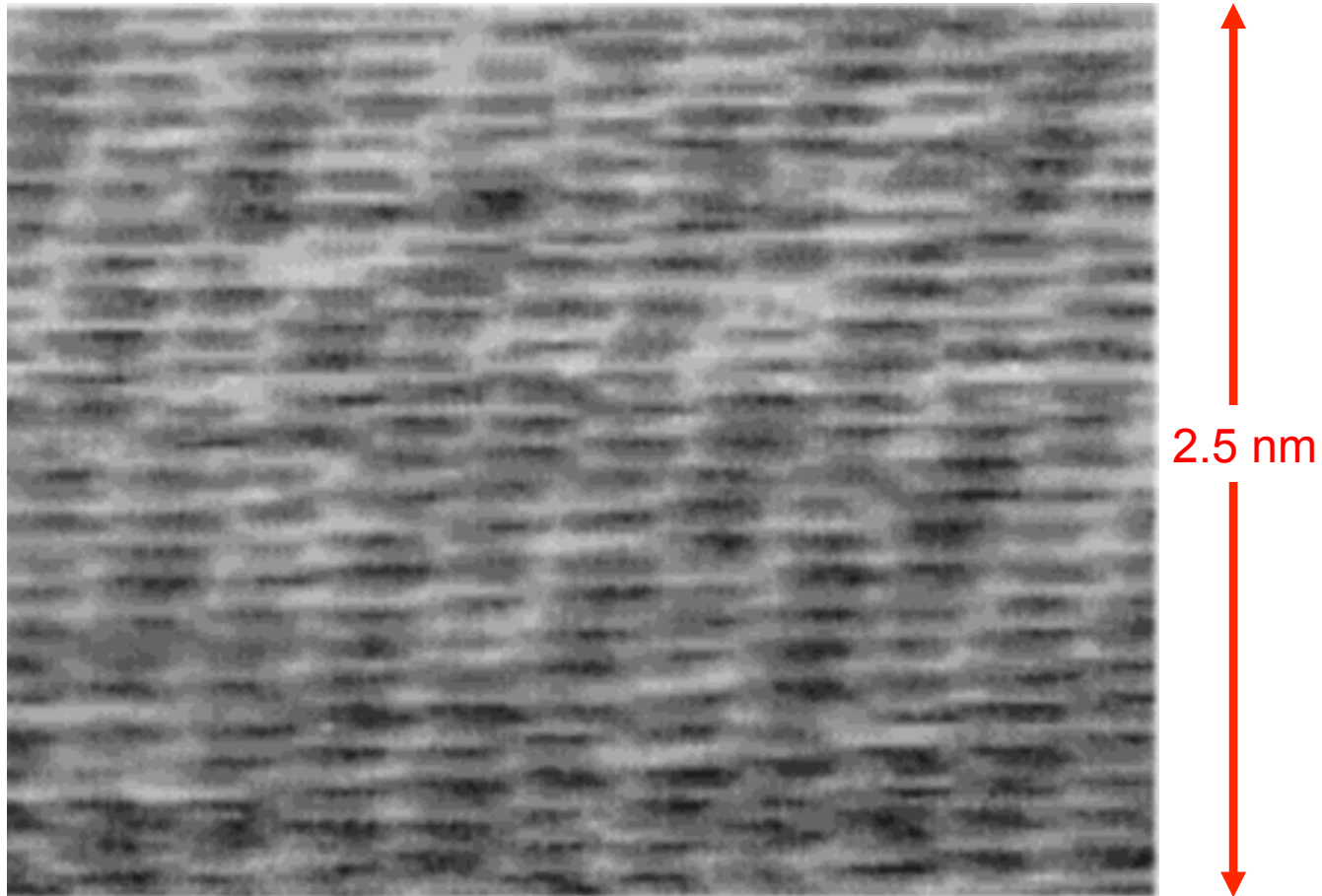
The STM Microscopy is based on tunneling currents as established in between the tip and the sample

STM imaging



Carbon atoms in the lattice structure of the highly oriented pyrolytic graphite. Image by STM in air.

STM imaging



Organic molecules (thiols) self-assembled onto highly oriented pyrolytic graphite. Image by STM in air.