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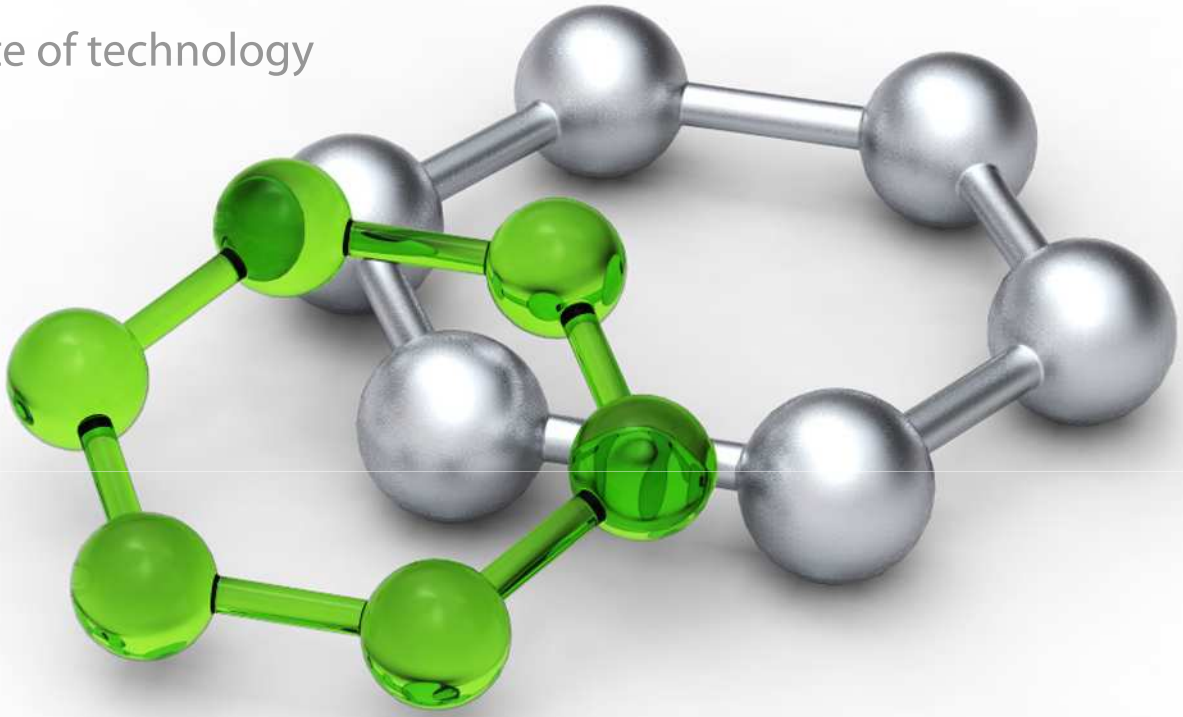
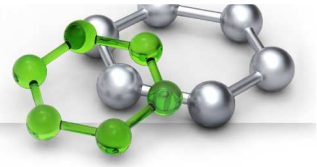


Bioanalytical Applications of Nanotechnology

Petr Skládal



Bioanalytical Applications of Nanotechnology

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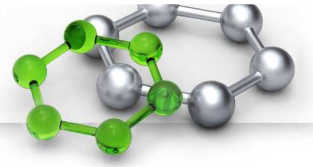
Kamenice 5, Brno-Bohunice

http://biosensor.chemi.muni.cz

http://www.nanobio.cz

Nano ?



nanotechnology

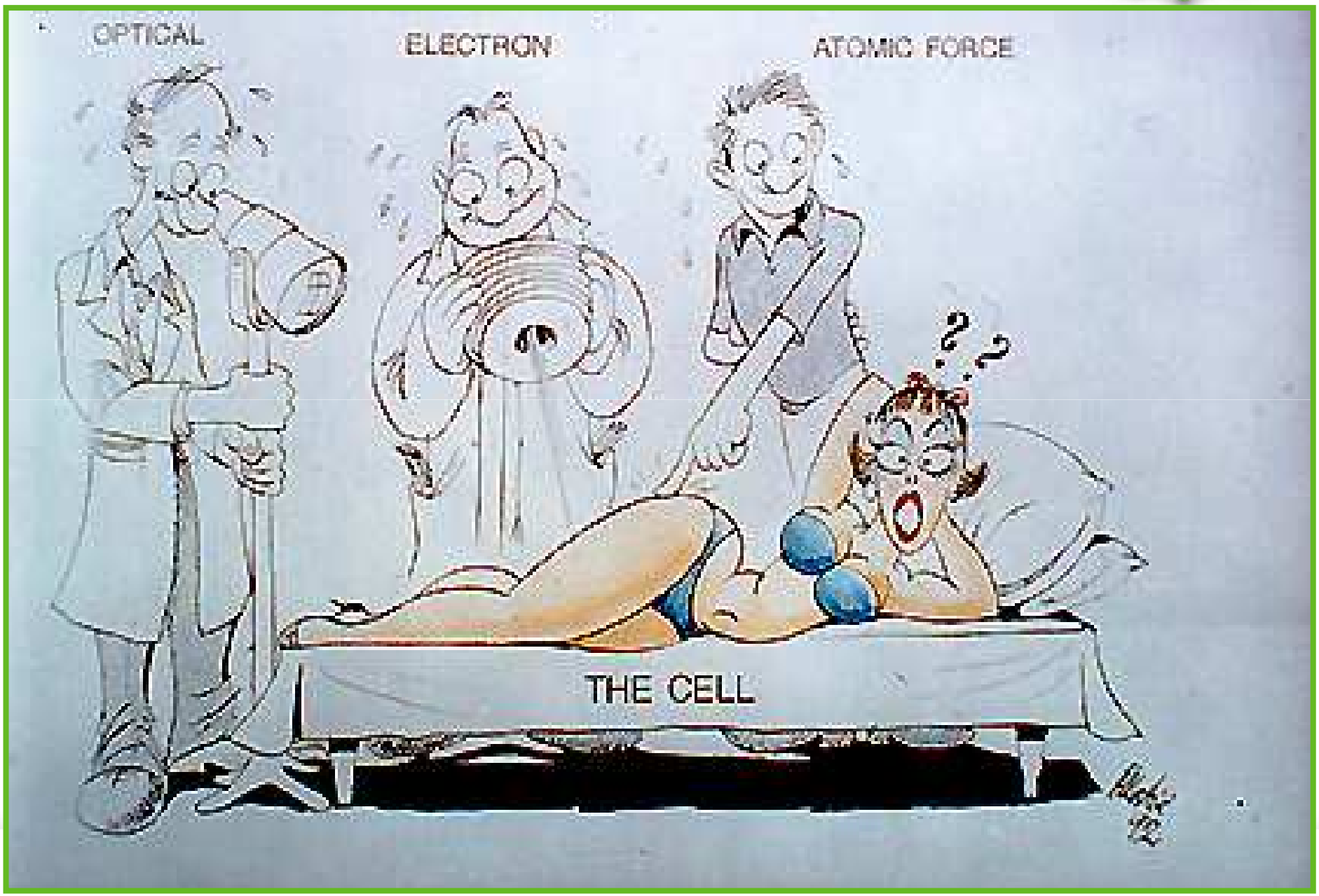
... investigates and develops structures, which at least in one dimension are sized in the nanometer range (nanoparticles, nanowires, nanolayers)

nanobiotechnology

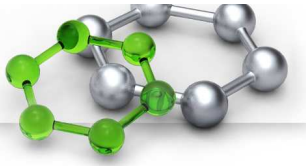
... employs nanosystems of biological origin (biomolecules, molecular complexes, viruses, subcellular components, ...) in technical systems

... applies nanotechnological approaches for investigation of biological systems, in order to obtain information not accessible using 'classic' techniques

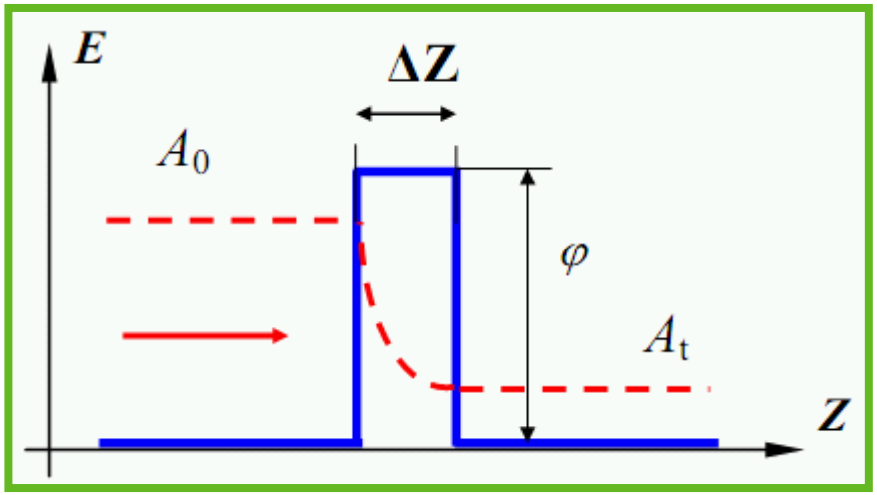
Scanning probe microscopy

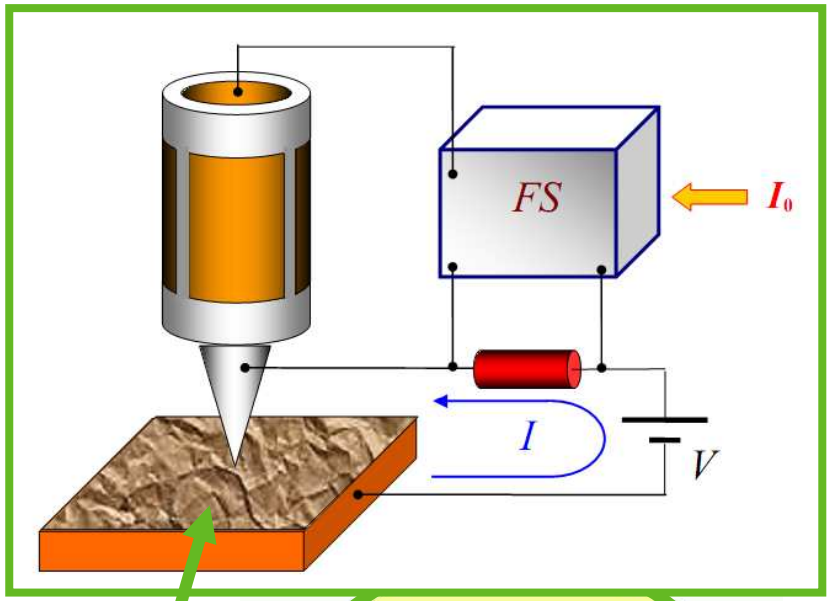


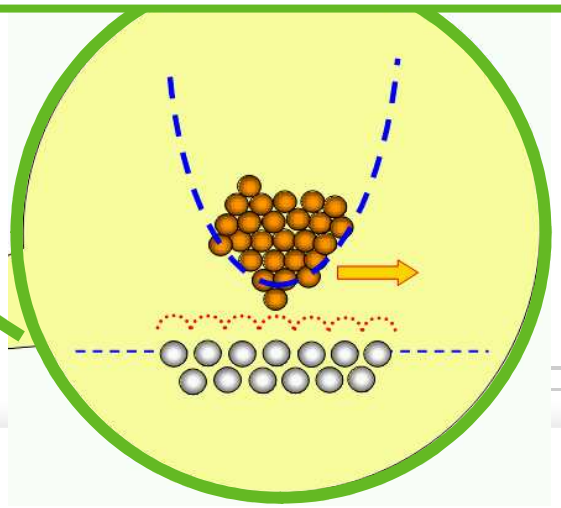
Scanning tunelling microscopy (STM)



- based on tunneling of electrons through a narrow potential barrier between a metal tip and a conducting sample in external electric field



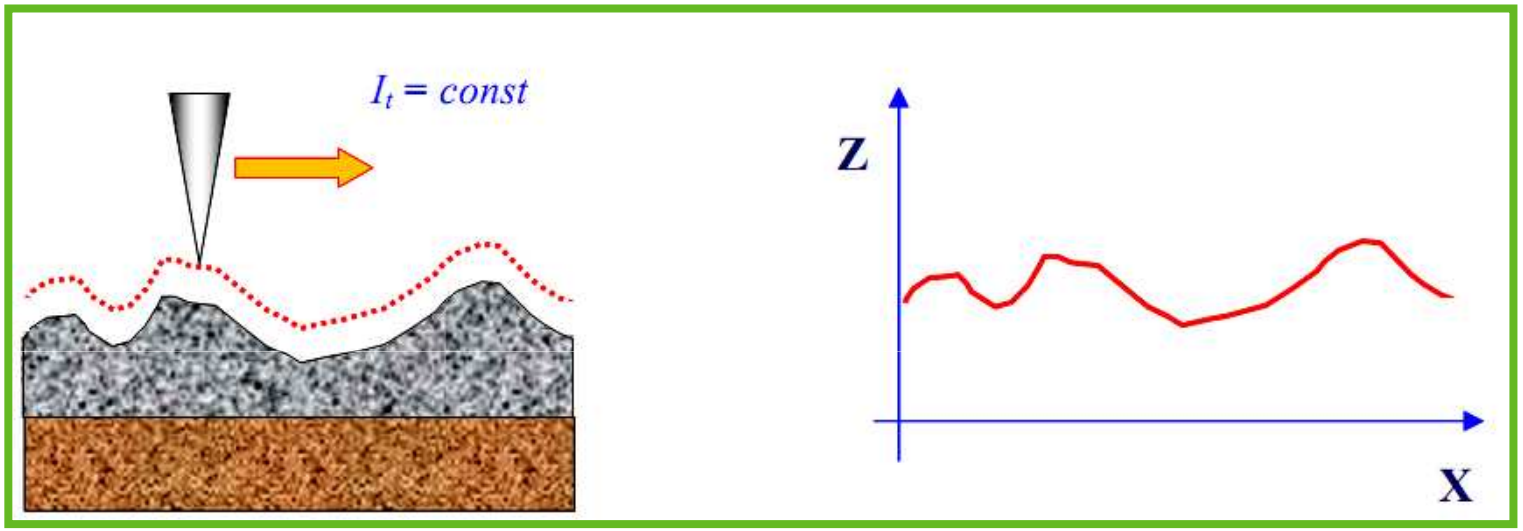


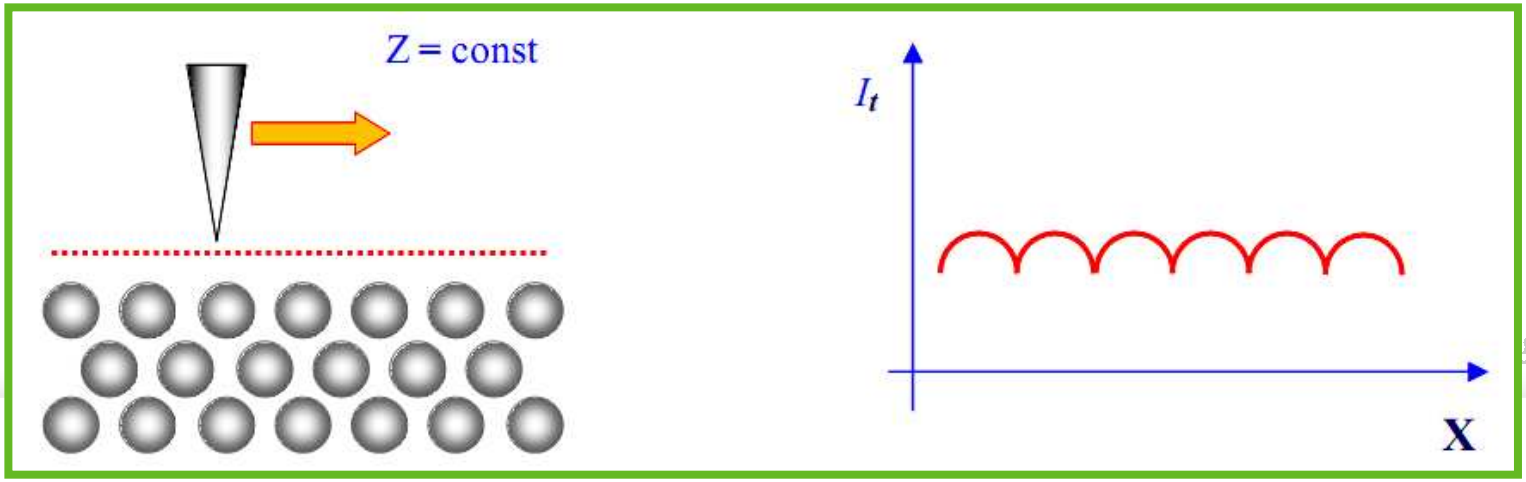


- atoms at the top of the tip and surface atoms of the sample

STM scanning modes

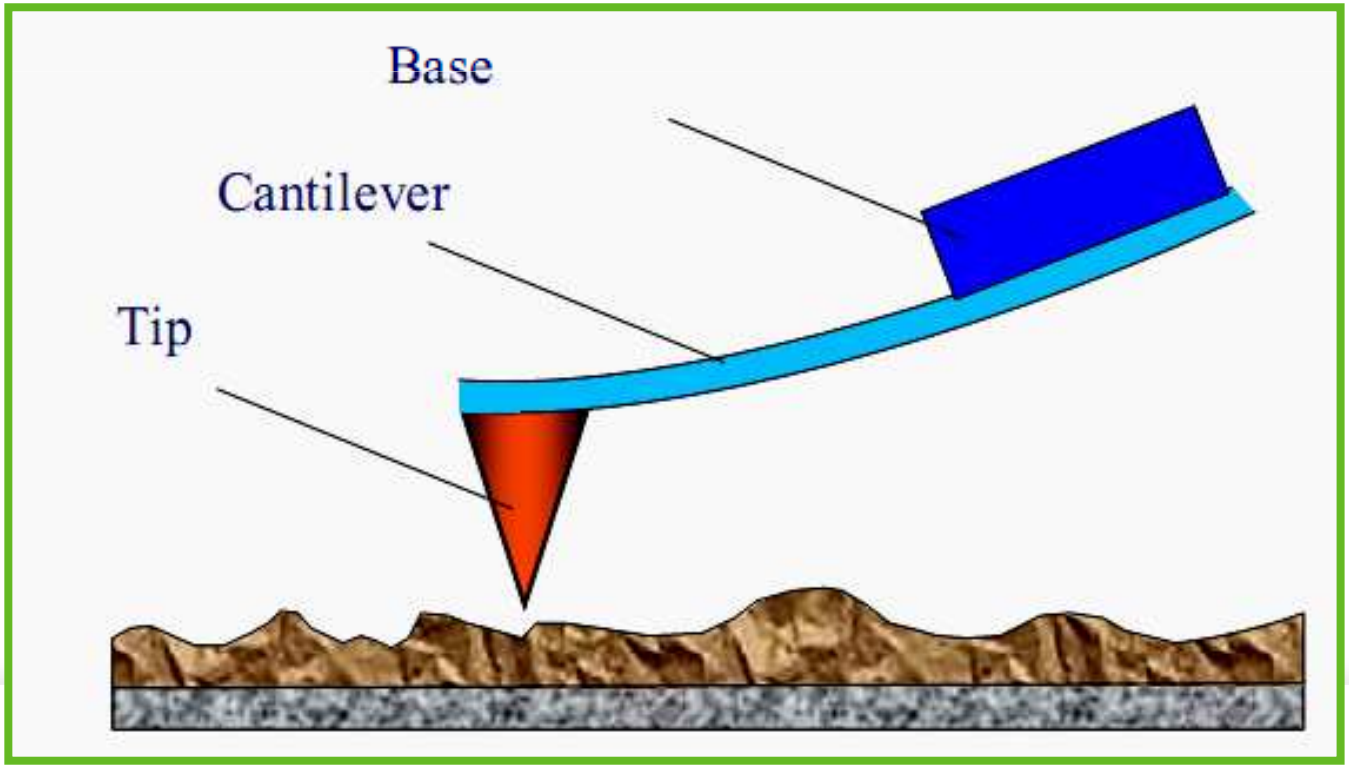
- feedback mechanism (z-positioning of the tip in order to maintain constant the required parameter) allows operation under constant current or constant distance

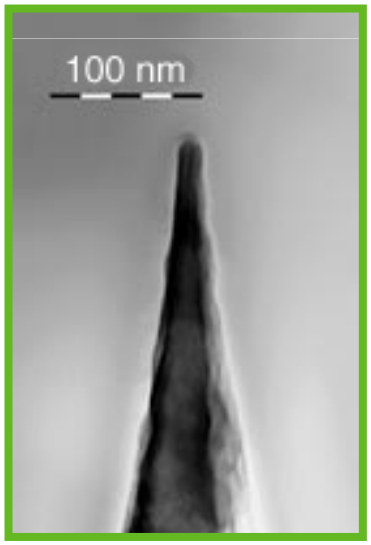


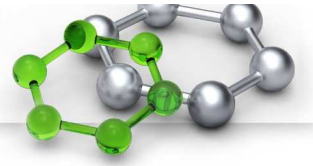


Atomic force microscopy

- measurement of the interactive force between a tip and the sample surface using special probes made by an elastic cantilever with a sharp tip
- the force applied to the tip by the surface results in bending of the cantilever, its *z*-deflection follows vertical profile of sample





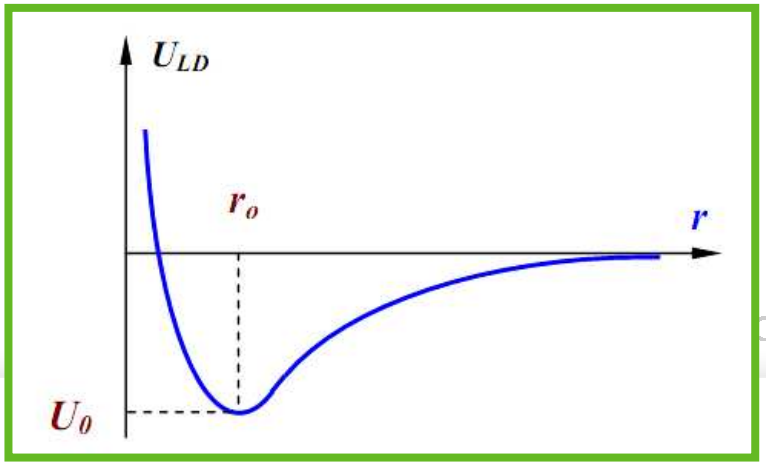


Interaction forces

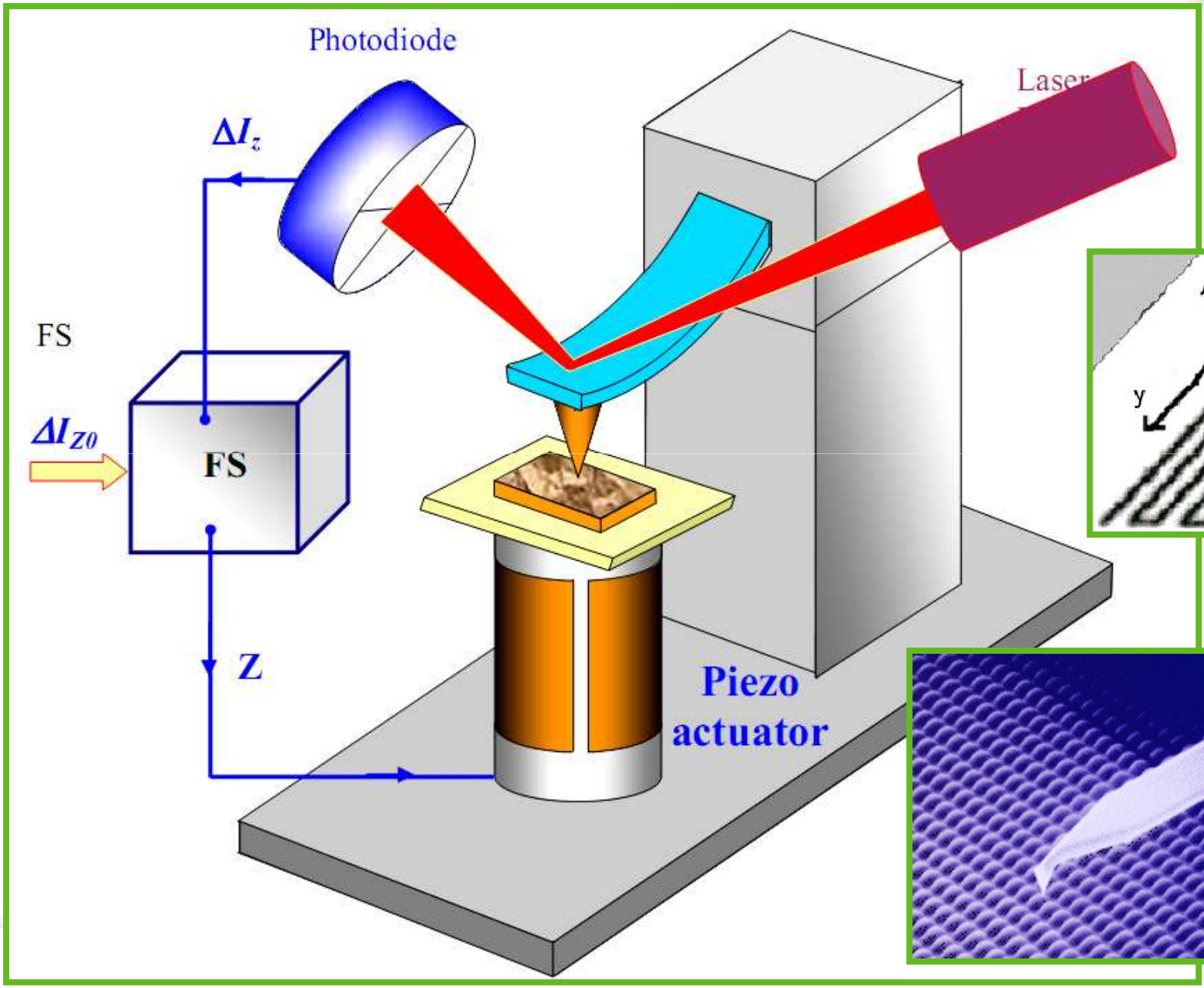
- measured by AFM can be explained by considering the van der Waals forces
  - the van der Waals potential energy of two atoms, located at a distance *r* from each other, is approximated by the exponential function
    Lennard-Jones potential

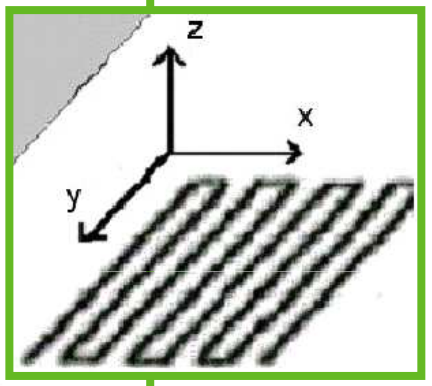
$$U_{LD}(r) = U_0\left\{-2\left(\frac{r_0}{r}\right)^6 + \left(\frac{r_0}{r}\right)^{12}\right\}$$

  - the first term of the sum describes the long-distance attraction caused, basically, by a dipole-dipole interaction
  - the second term takes into account the short range repulsion due to the Pauli exclusion principle
  - r_0 is the equilibrium distance between atoms, the energy value in the minimum

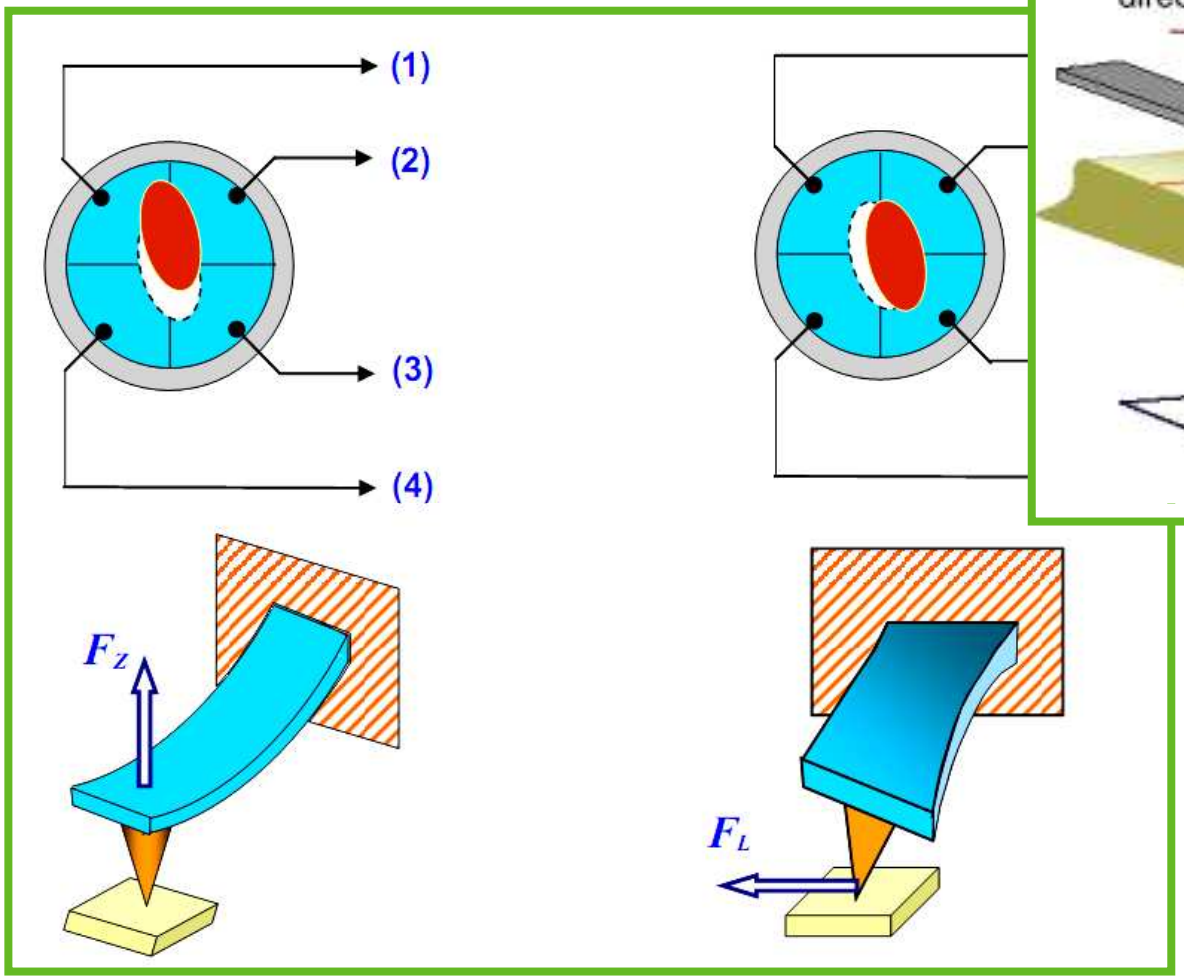


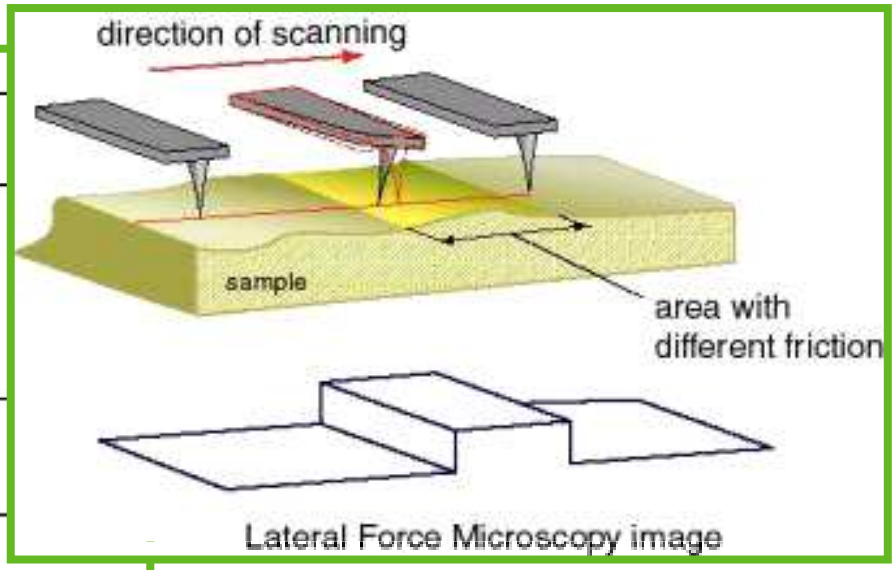
Optical lever system and feedback mechanism





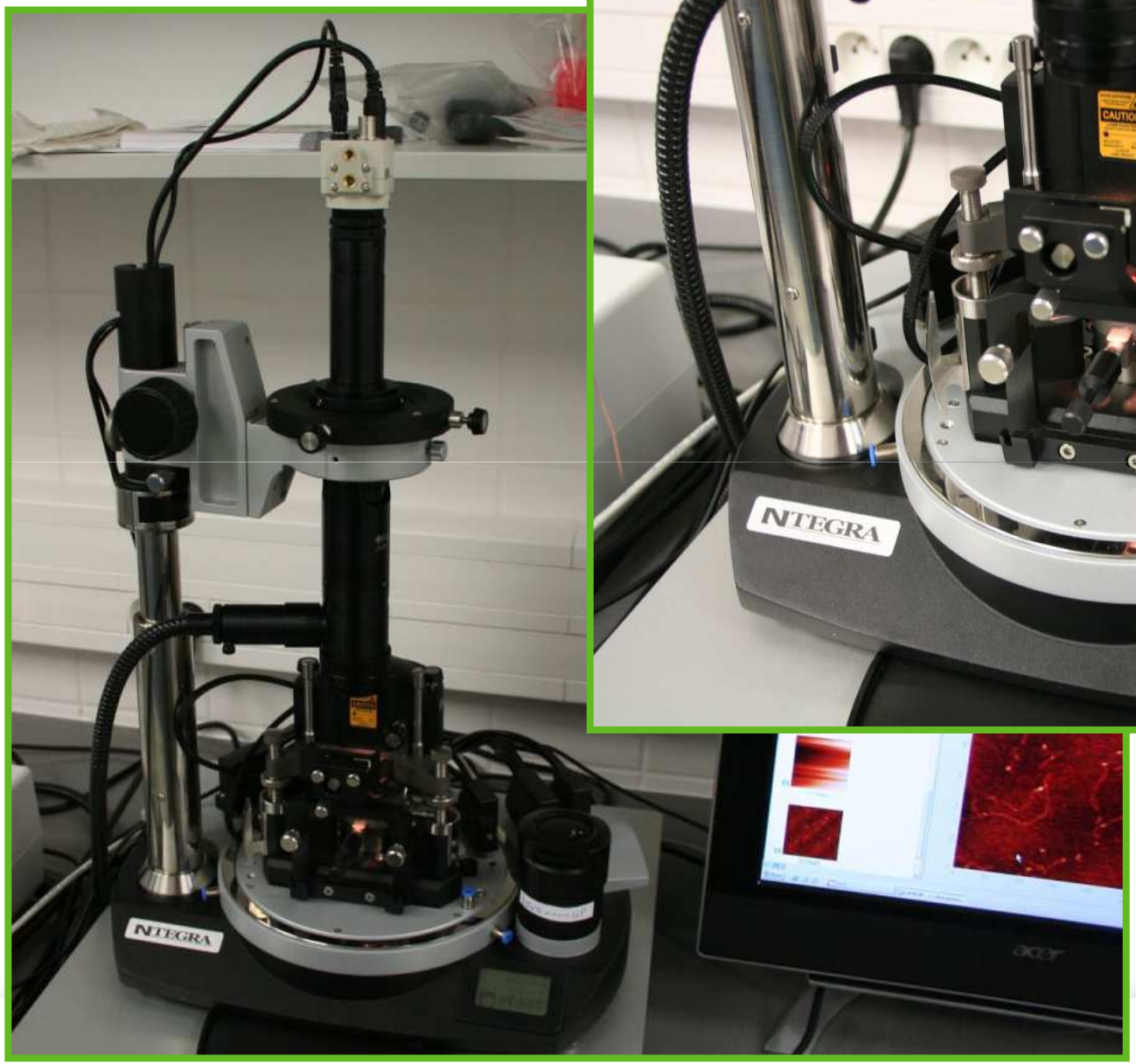
Deflections of cantilever

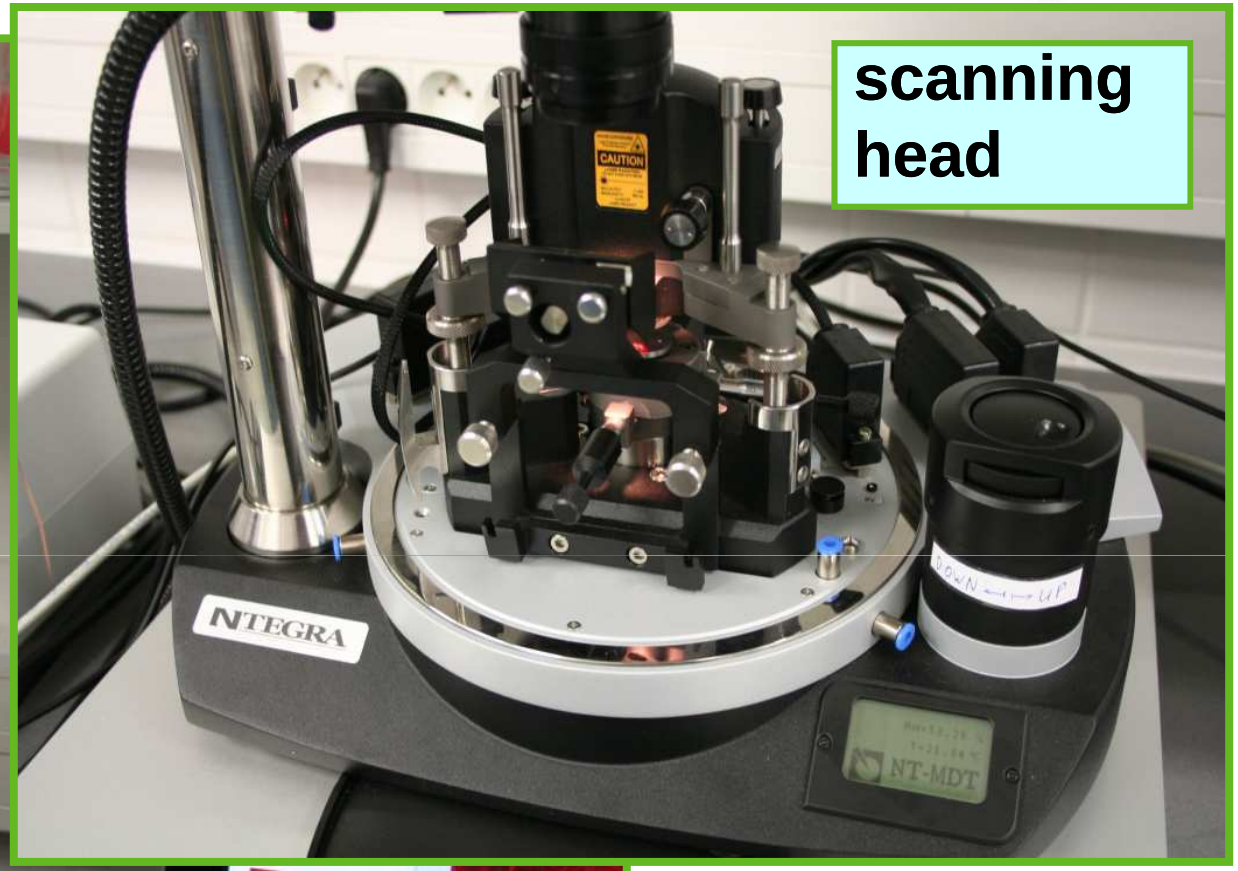




vertical = topology lateral = friction

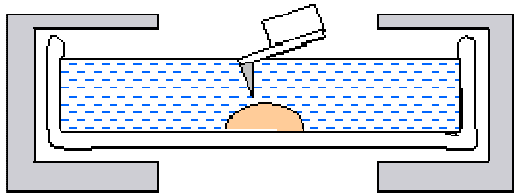
AFM system Ntegra Vita



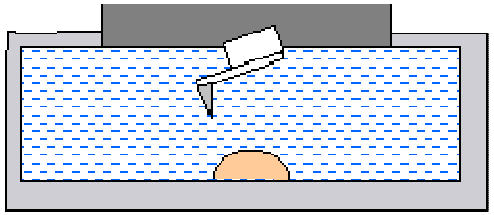


AFM works in solution, too

cells for solution scanning



opened for Petri dishes



closed flow-through, thermostated

Contact modes

NT-MDT
Molecular Devices and Tools for Nano Technology

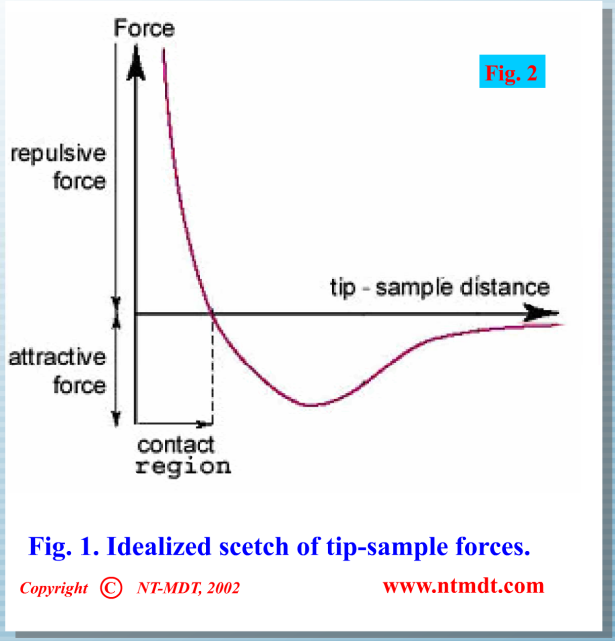


Fig. 2

Fig. 1. Idealized scetch of tip-sample forces.

 www.ntmdt.com

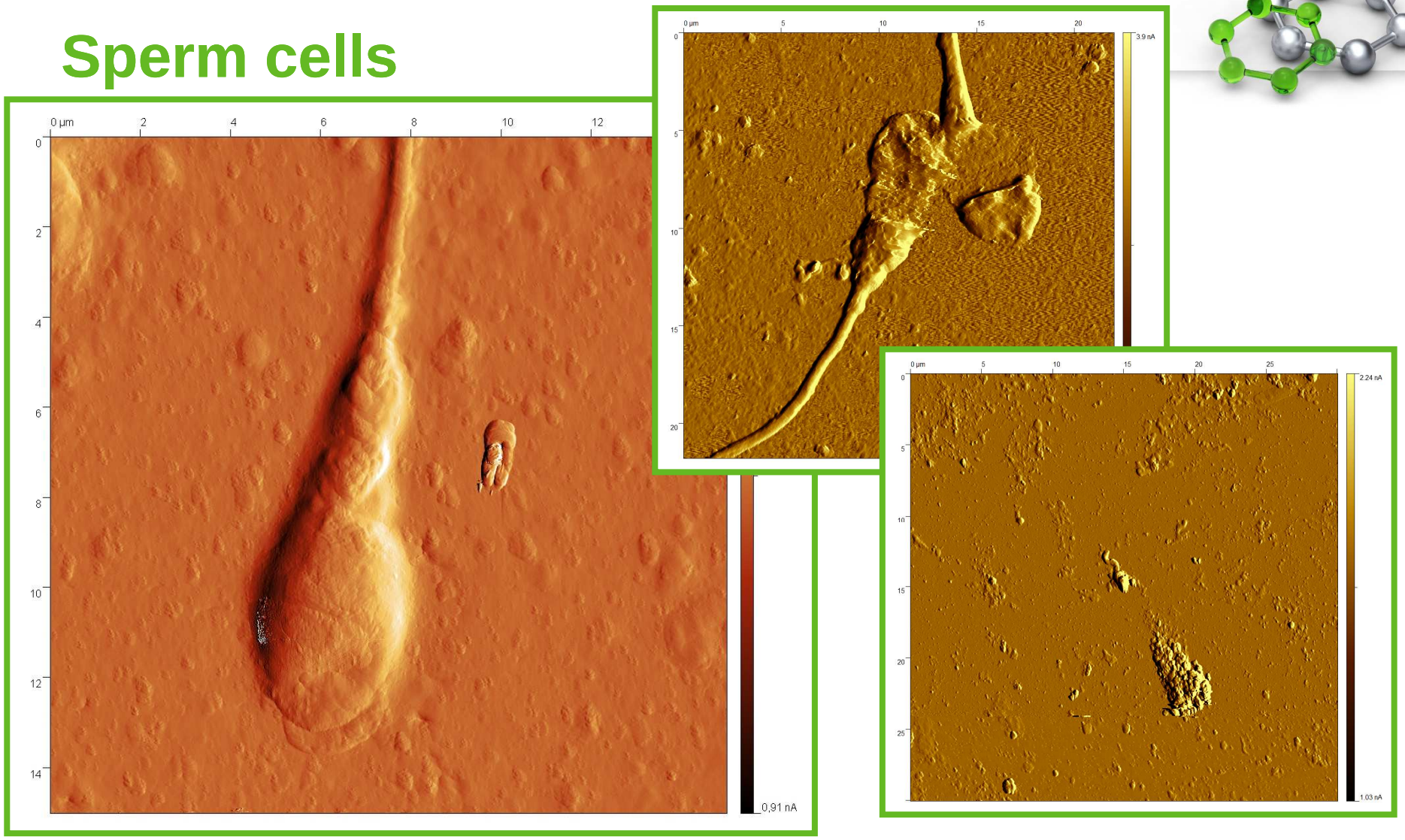
Examples

dc Contact techniques:

- Constant Height mode
- Constant Force mode
- Contact Error mode
- Lateral Force imaging
- Spreading Resistance imaging

In **Contact mode** of operation the cantilever deflection under scanning reflects repulsive force and is used as **such**, in **feedback circuitry** or in **their combination** to imagine the sample surface profile. Simultaneously with topography acquisition under scanning one can imagine some other characteristics of the investigated sample.

Sperm cells



- Jan Přibyl (NCBR) and Igor Crha (Medical Faculty) investigate sperm cells and effects of oxidative stress (hydrogen peroxide as simulator)

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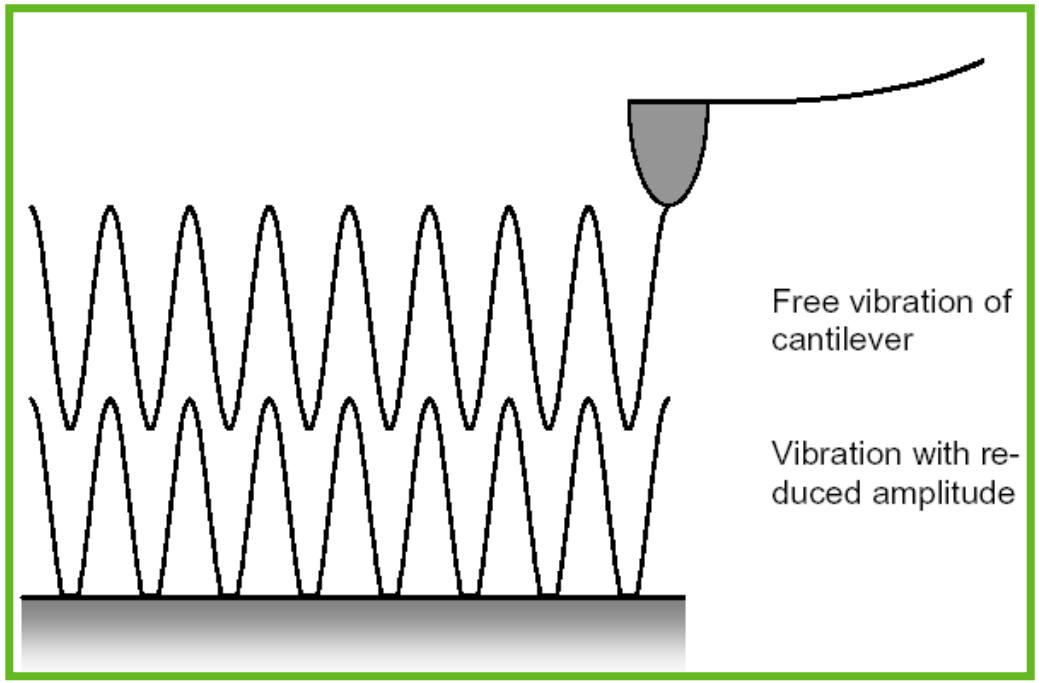
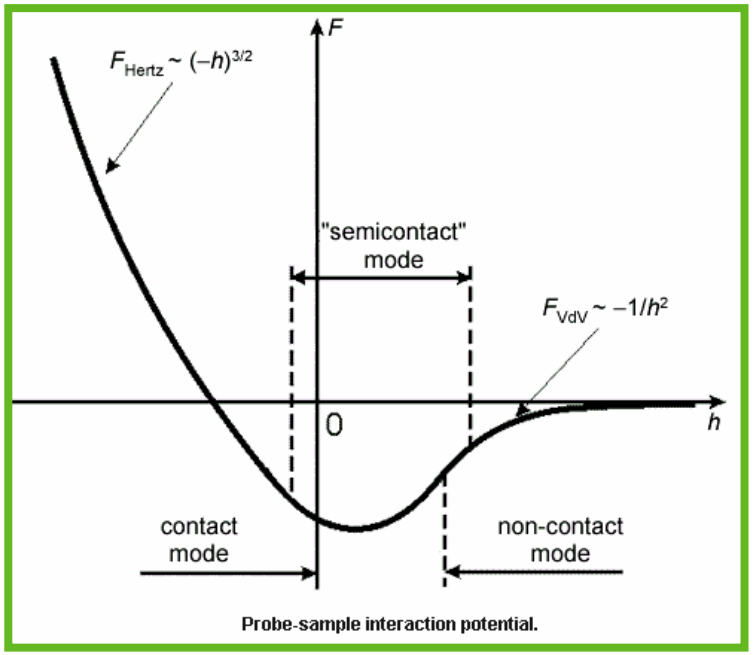
Tapping modes





Probe-sample interaction potential.

- cantilever oscillates near its resonance frequency, damping of the oscillation amplitude becomes recorded
- no effect of friction forces
- only temporary contact between tip and the surface - no damage of sample (and the tip) - suitable for **biomolecules**

Semicontact modes

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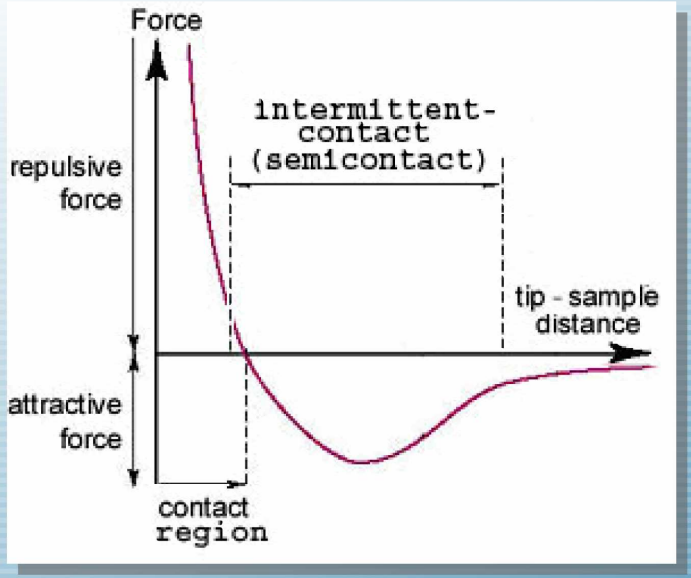

Semicontact techniques:

- Semicontact mode
- Phase Imaging mode
- Semicontact Error mode

Usage of **SFM** with oscillating cantilever was firstly anticipated by **Binnig**. Relatively small shift of cantilever oscillating frequency with sensing repulsive forces means that contact of cantilever tip with sample surface under oscillation is not constant. Only during small part of oscillating period the tip "feels" contact repulsive force. Scanning sample surface with cantilever oscillated in this manner is not non-contact, but intermittent contact (semicontact).

Non-contact modes

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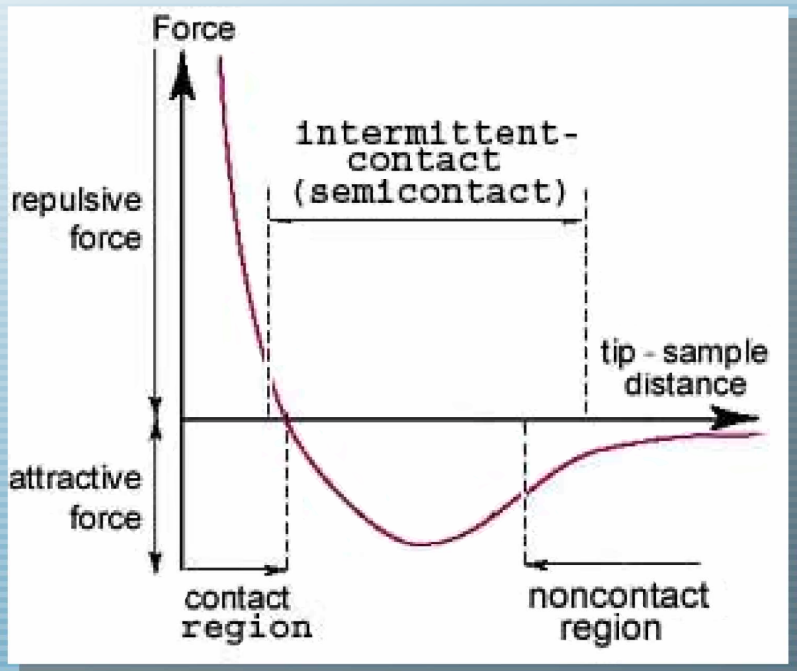
Non-Contact techniques:

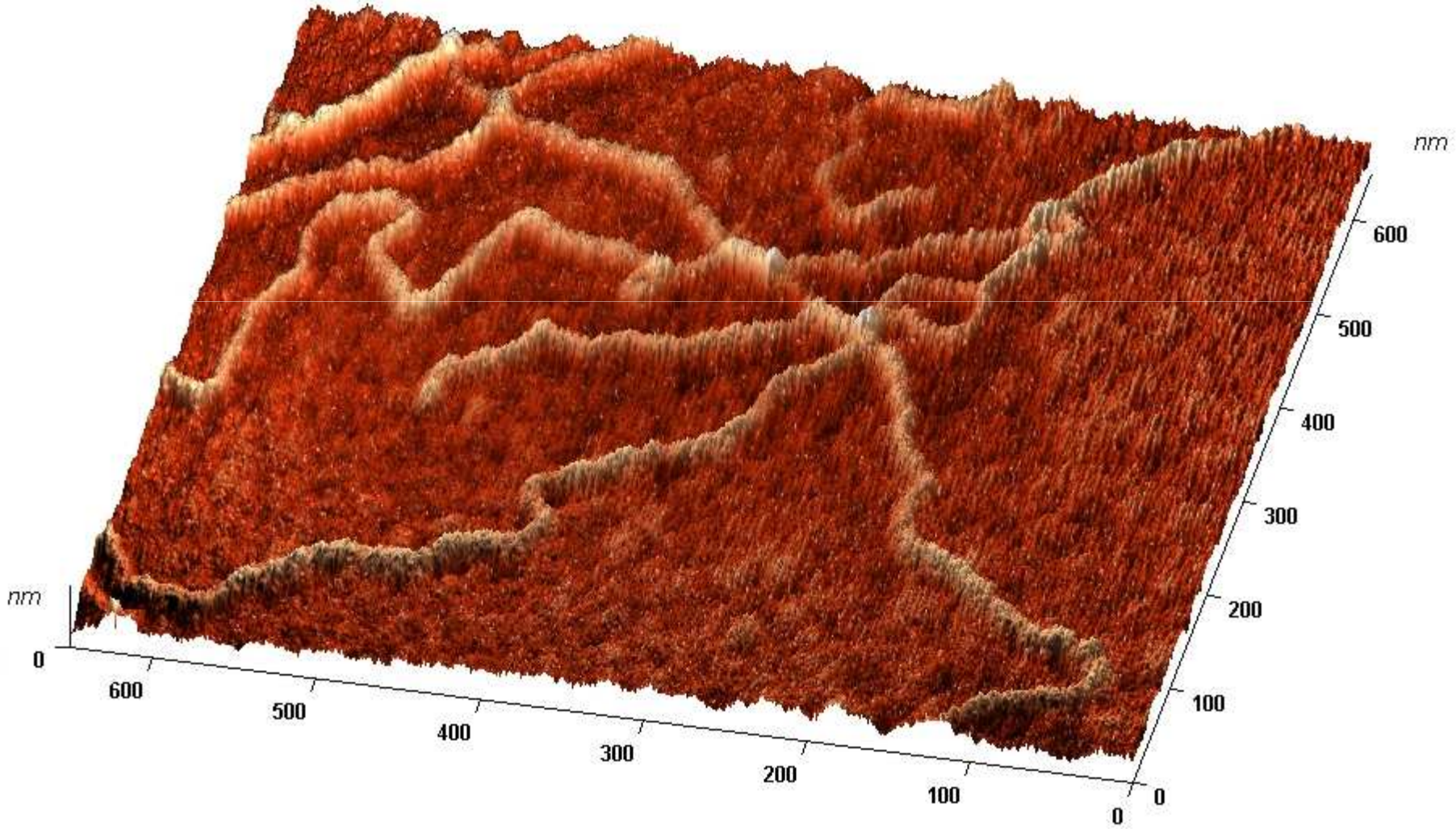
- **Non-Contact mode**
- **Frequency Modulanion mode**



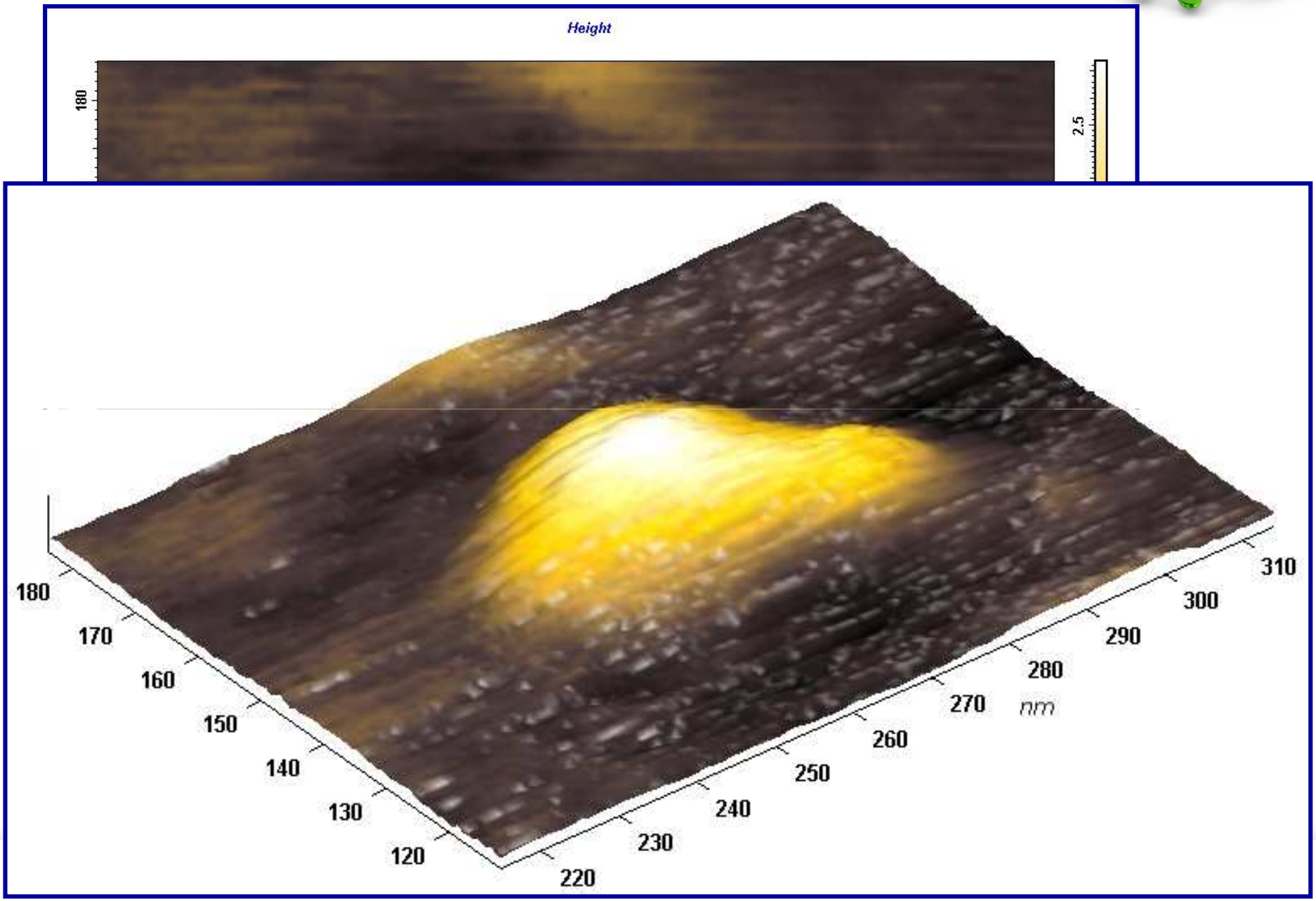
The **Non-Contact AFM** (**NC AFM**), invented in 1987, offers unique advantages over other contemporary scanning probe techniques such as contact **AFM** and **STM**. The absence of repulsive forces (present in Contact **AFM**) in **NC AFM** permits it use in the imaging "**soft**" samples and, unlike the **STM**, the **NC AFM** does not require conducting samples.

AFM imaging of nucleic acids

- calf thymus DNA on mica modified with Mg^{2+}

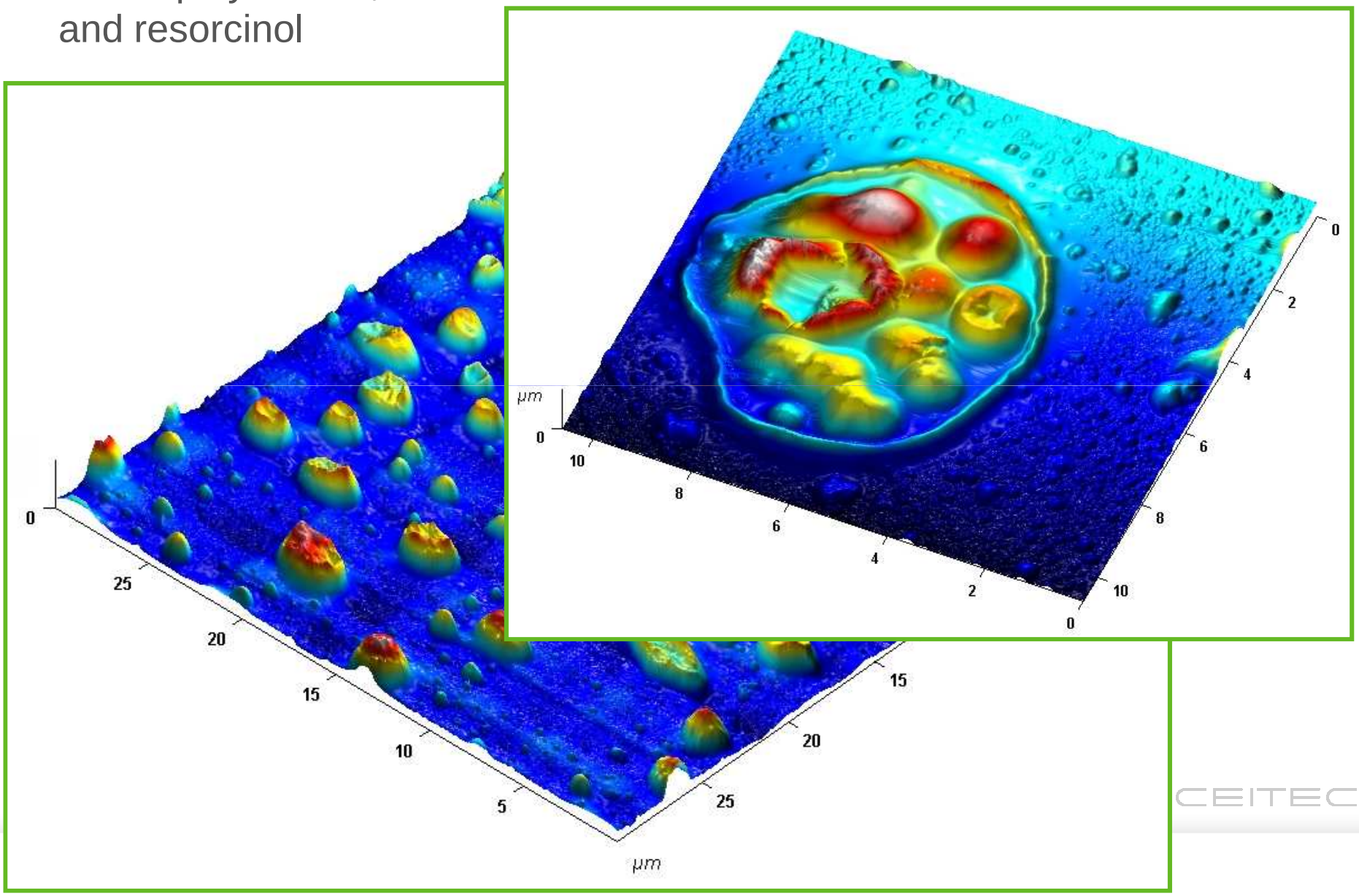


IgG details

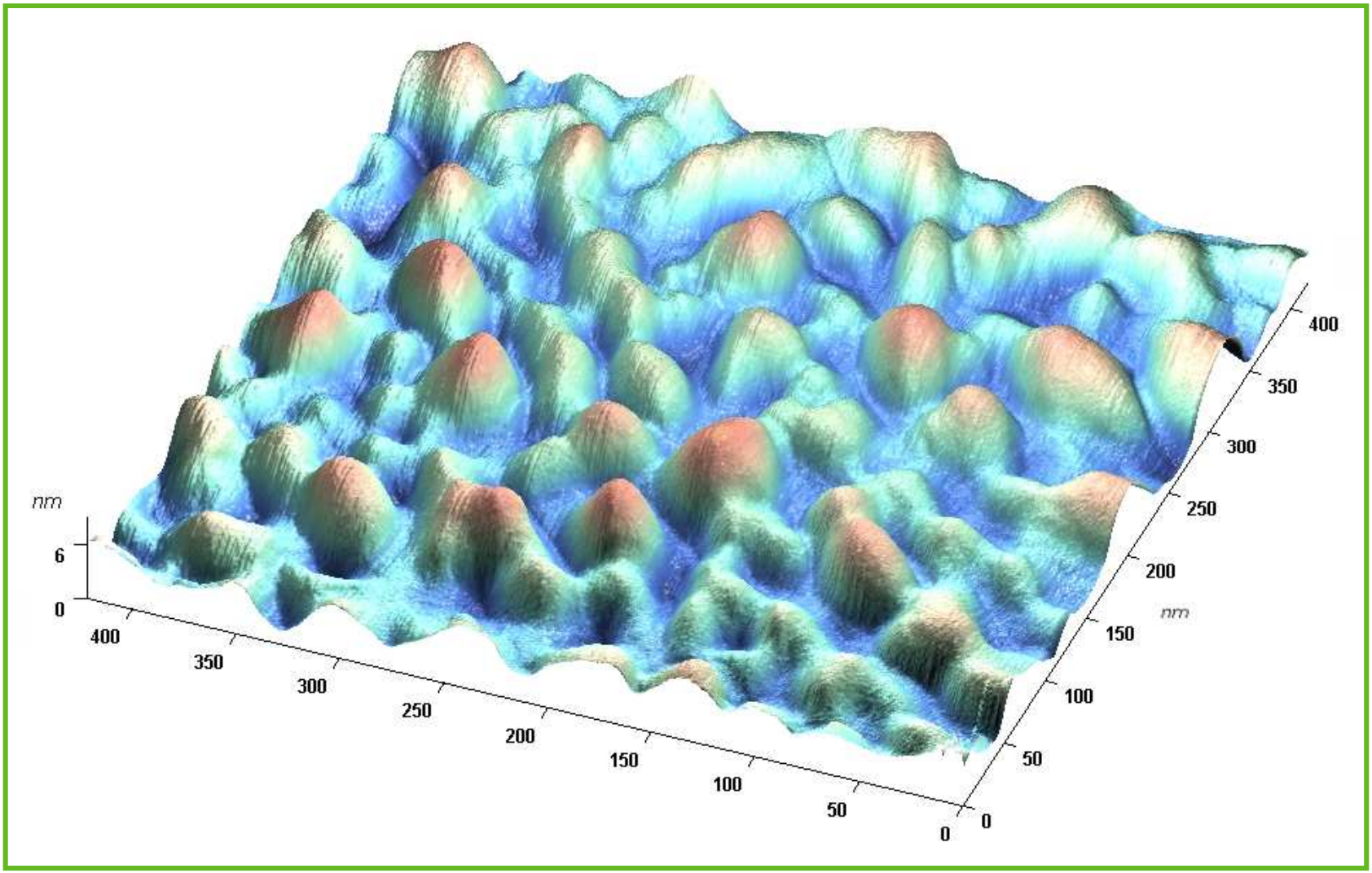

Height
180
2.5
180
170
160
150
140
130
120
220
230
240
250
260
270
280
290
300
310
nm

Biosensing surfaces

- electropolymer - 1,4-diaminobenzene and resorcinol

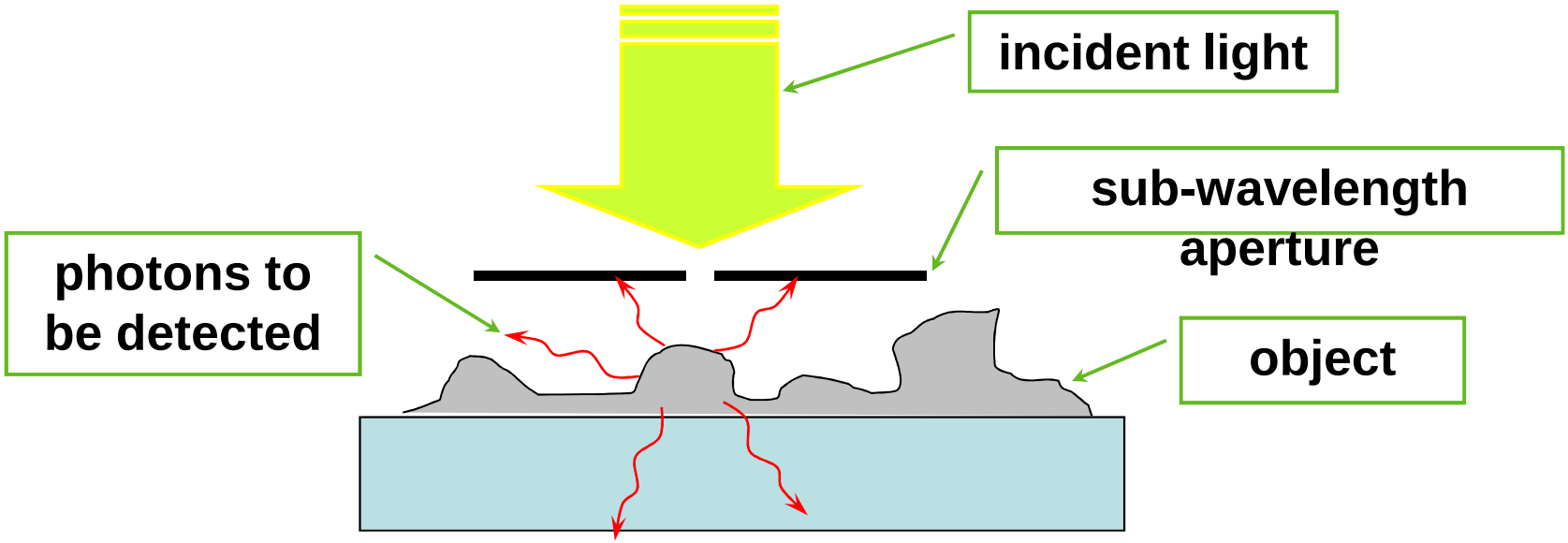


Nanolabels for imaging and analysis



- nanoparticles of gold attached to thiol-modified mica

Scanning near-field optical microscopy (SNOM)



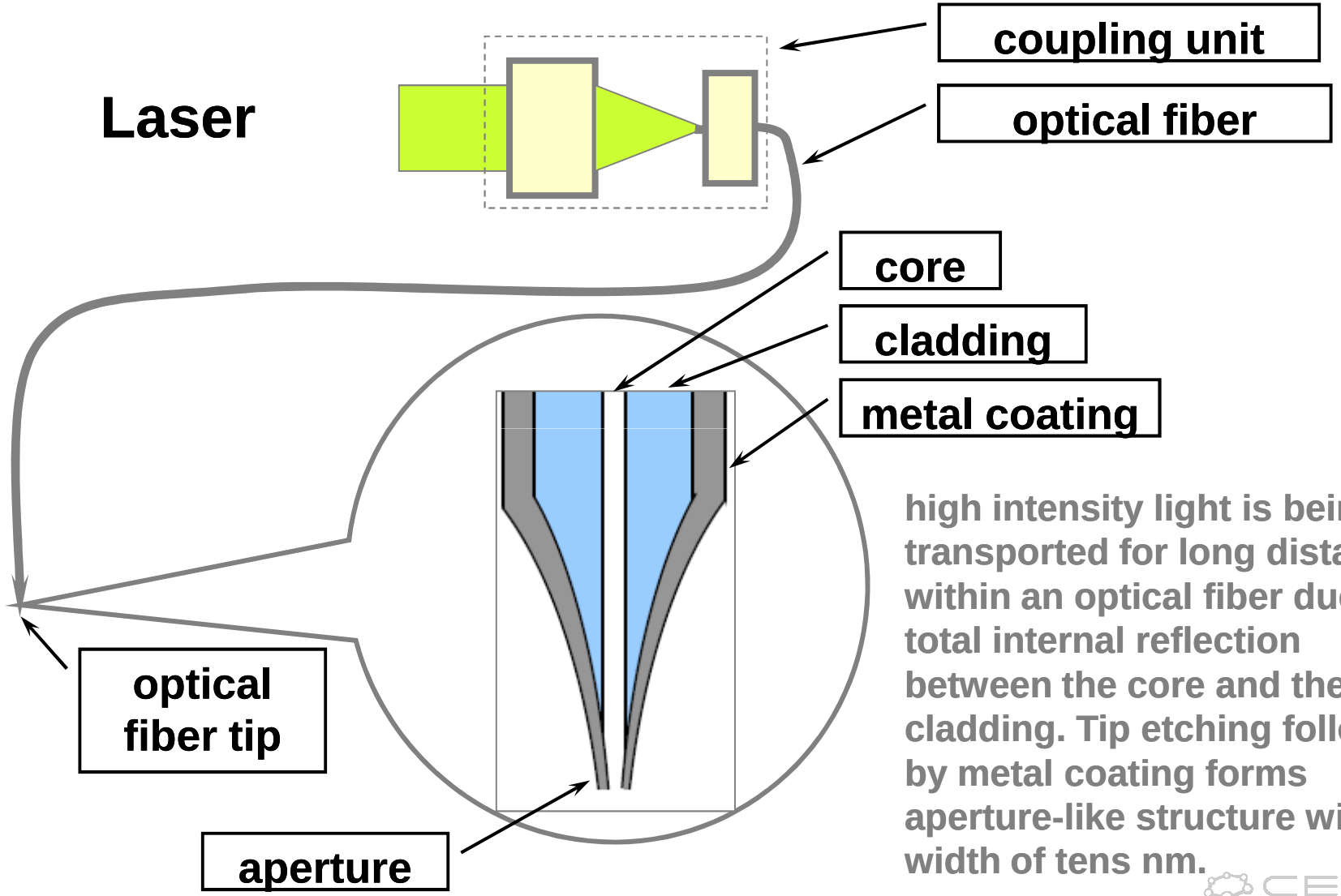
sub-wavelength aperture is approached for 1 to several nanometers to the object surface

then generated photons are caught by detector

point by point scanning gives 2D image with resolution about 1 nm (beyond the difraction limit …)

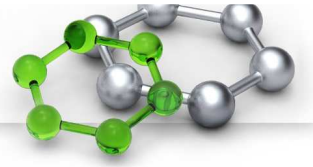
$$R \approx \frac{\lambda}{2n}$$

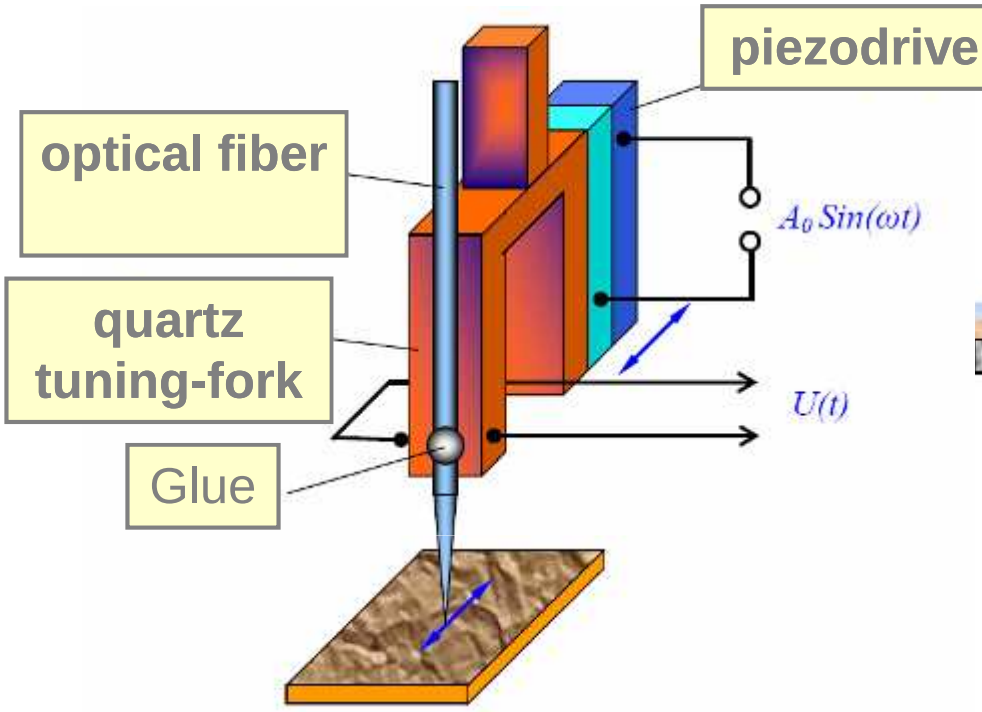
Optical fiber tip as the aperture

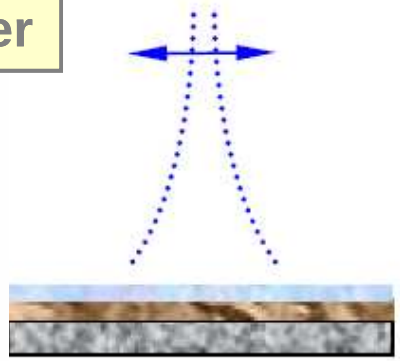


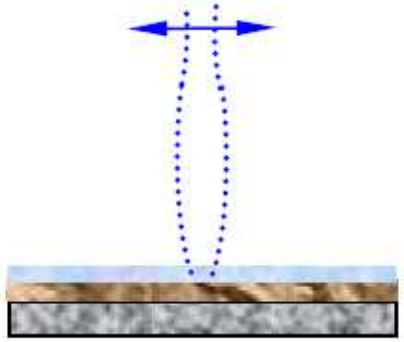
high intensity light is being transported for long distance within an optical fiber due to total internal reflection between the core and the cladding. Tip etching followed by metal coating forms aperture-like structure with a width of tens nm.

Shear-force feed-back control









probe vibrates at resonance frequency of quartz tuning-fork. Amplitude and phase of such vibration significantly change when the probe tip arrives the closest proximity of the object surface. Feed-back control mechanism fixes parameters of new state providing precise height positioning of the tip. Thus sear-force topography image of the surface can be obtained simultaneously with near-field optical one.

SNOM scanning near optical field microscopy

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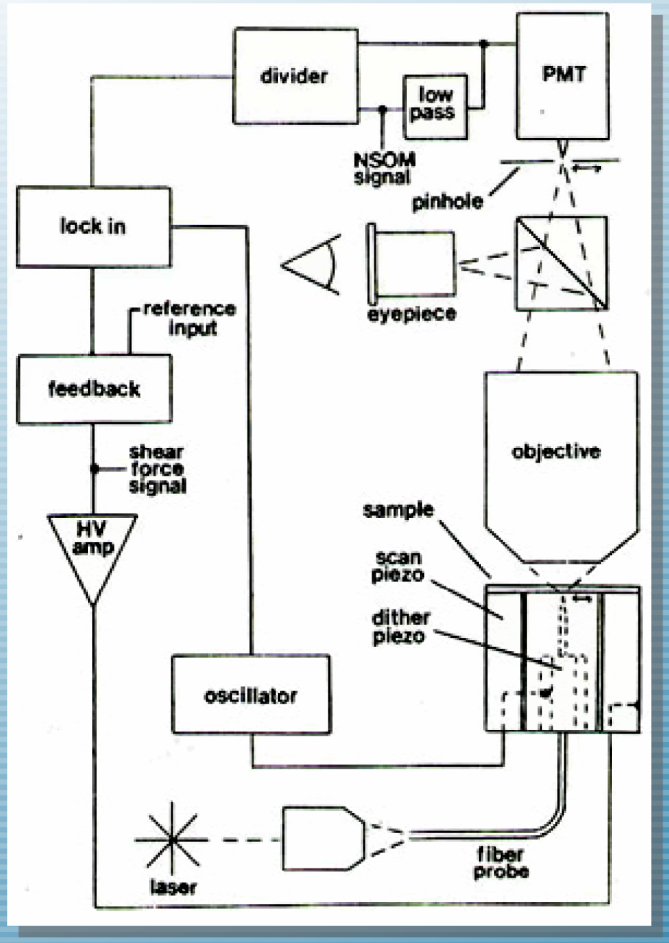


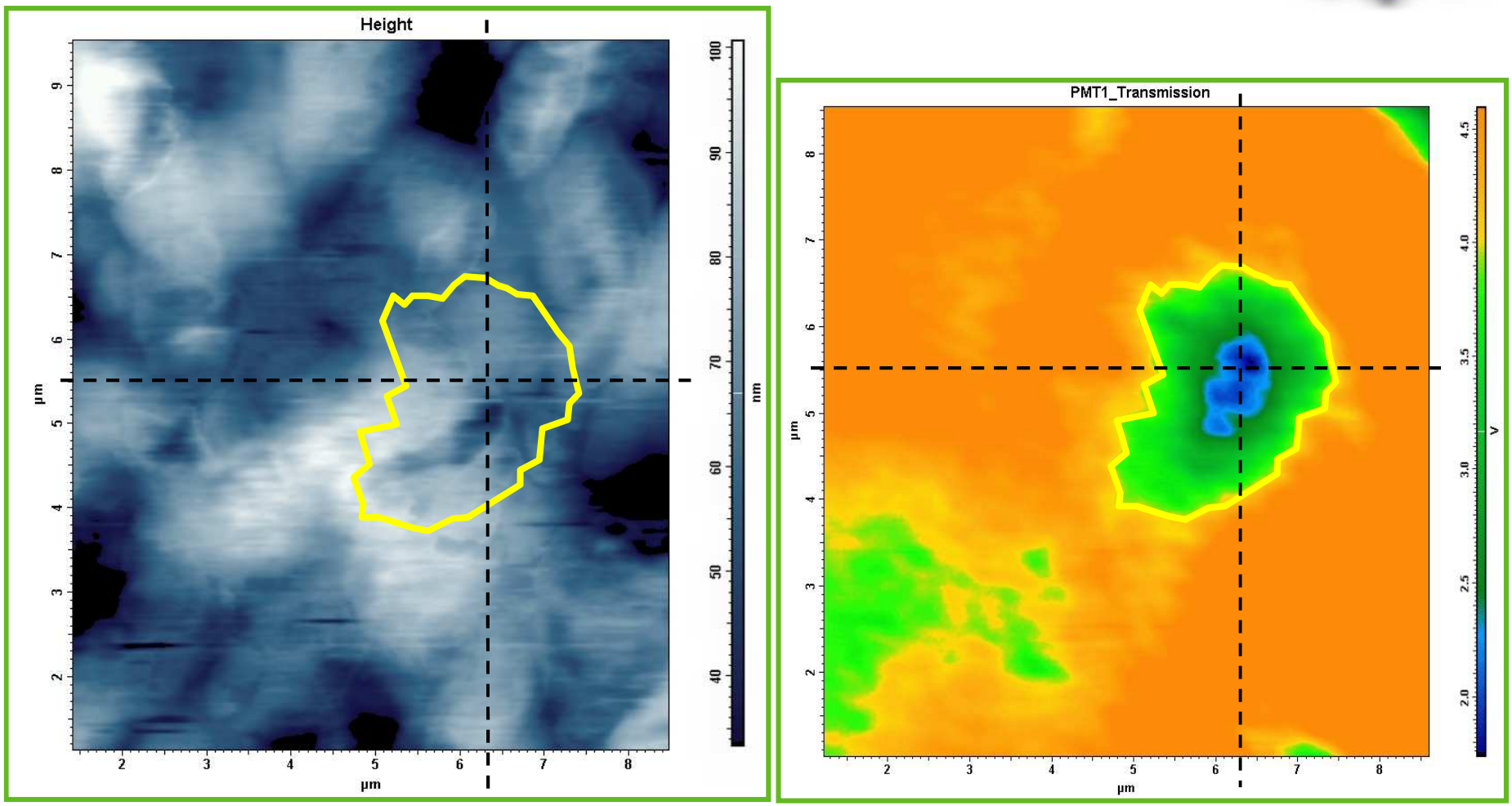
SNOM:

- Shear Force Microscopy
- Transmission mode
- Reflection mode
- Luminescence mode

Scanning Near-Field Microscopy (SNOM)

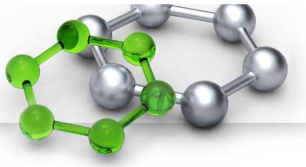
The resolving power of classical optical microscopes is restricted by **Abbe's** diffraction. However, it is possible to overcome this limit. If a subwavelength hole in a metal sheet is scanned close to an object, a super-resolved image can be built up from the detected light that passes through the hole. Scanning near-field microscopy based on this principle was first proposed by **Synge** in 1928.

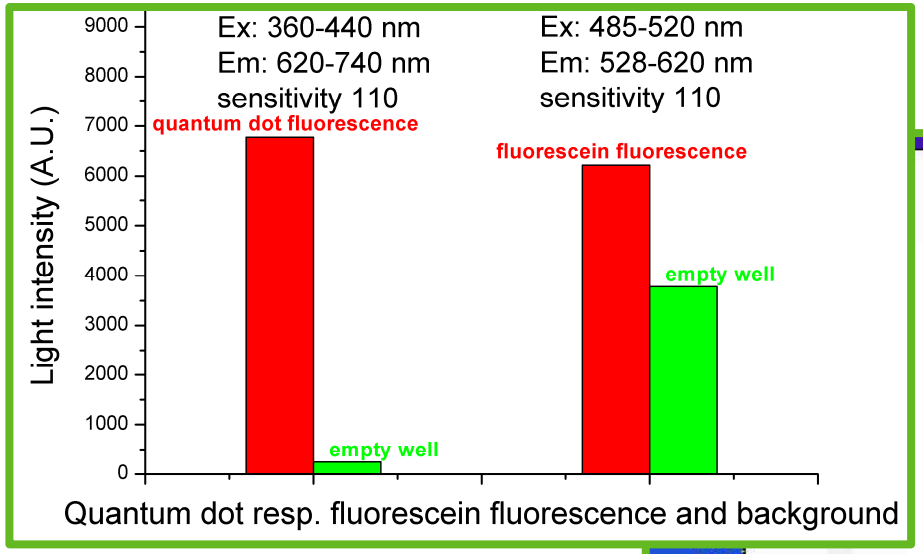
SNOM

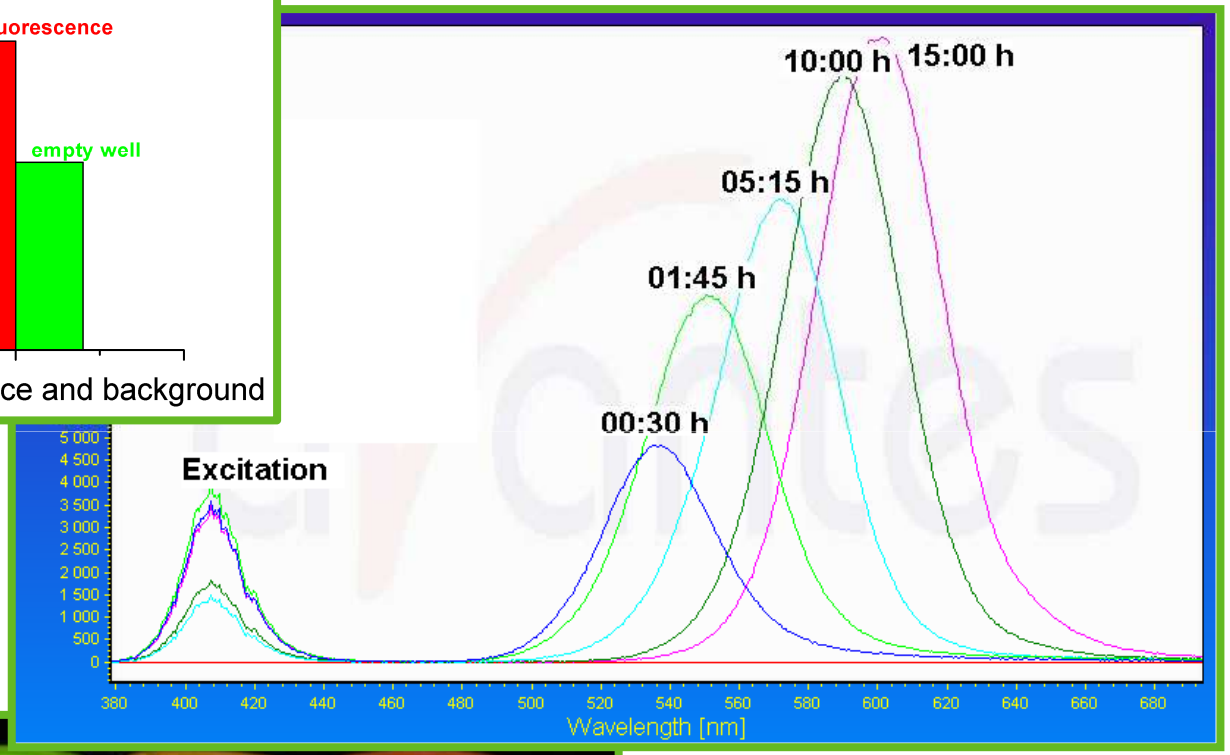


- height (left, AFM mode) and optical (right, SNOM mode) images of electropolymerized film

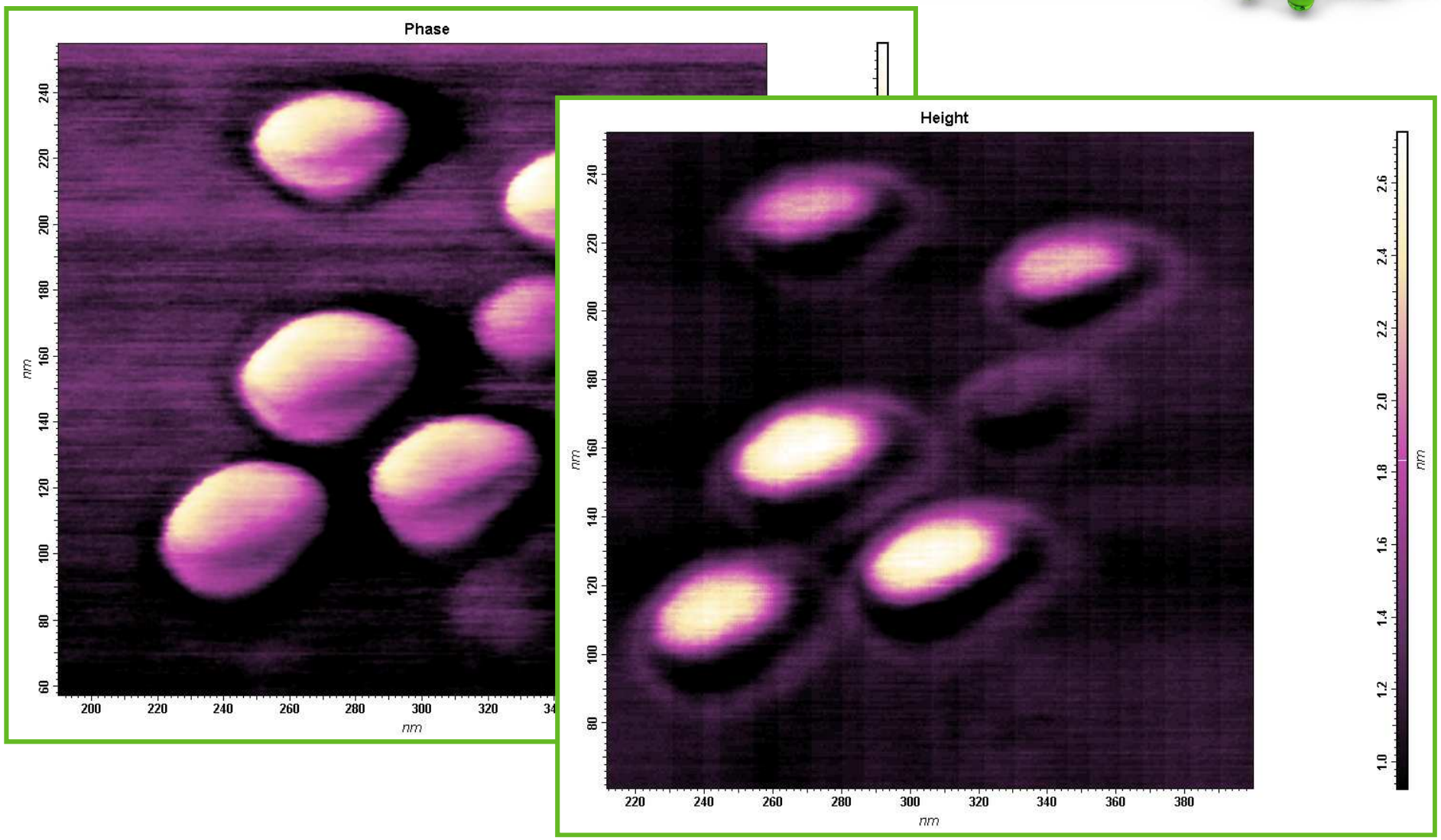
Quantum dots





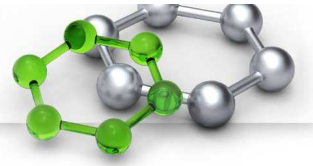


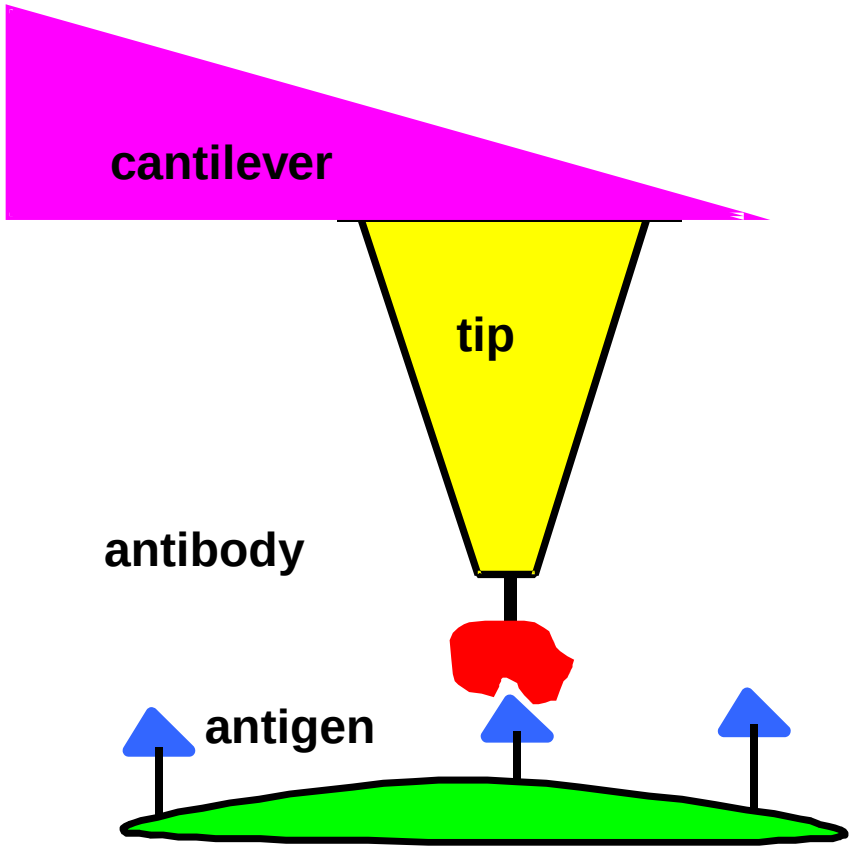
Quantum dots (QD)



- QD - teluride core, CdS shell, glutathione or silacate
  - functionalized for conjugation with biomolecules - nanolabels

Nanobiointeractions

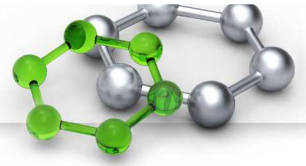


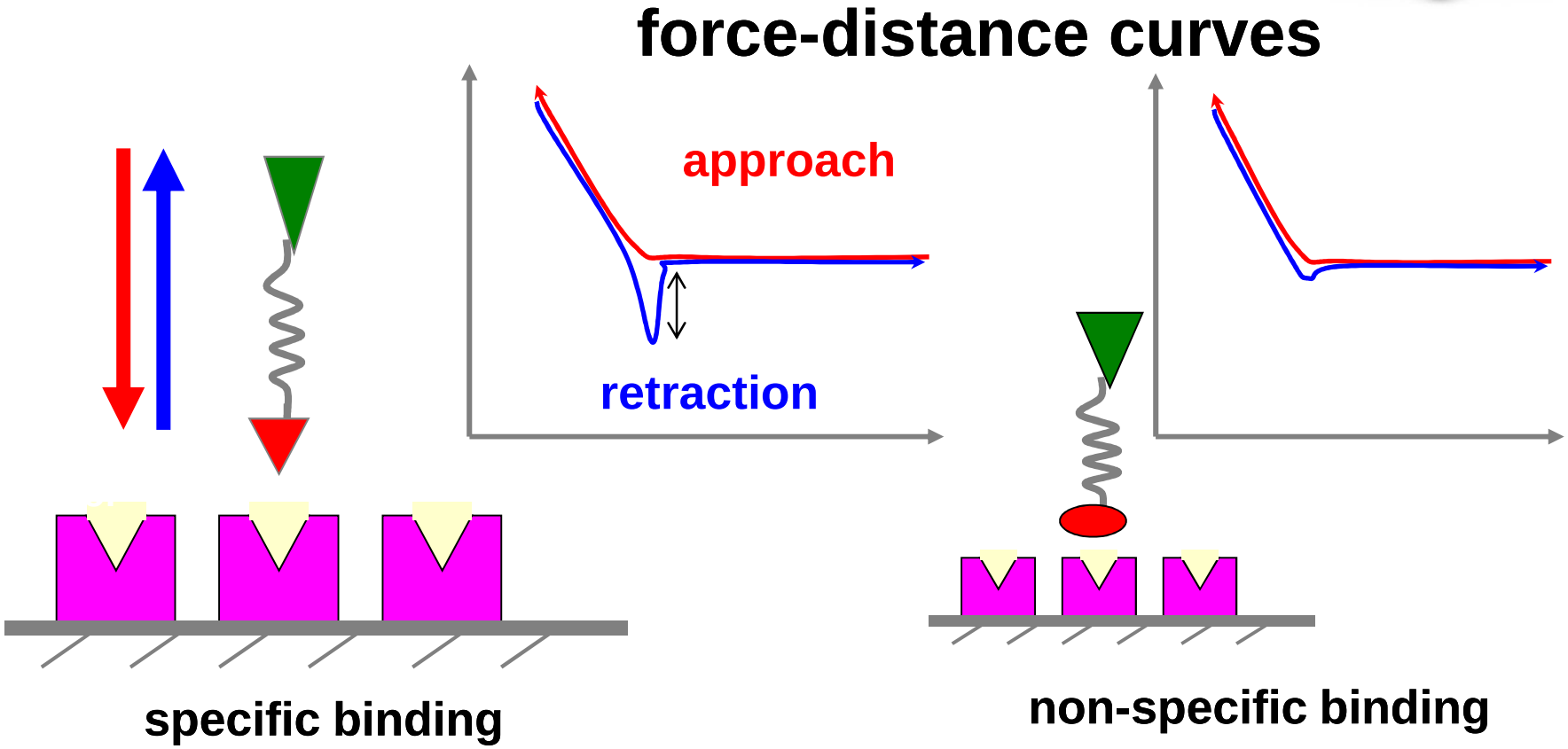


- characterization of biocomplexes between complementary molecules
- the modified tip is contacting the individual molecules on the sampled surface
- direct measurement of biointeraction forces (required to break down the affinity complex)

SPM biosensors

Ligand-receptor binding forces

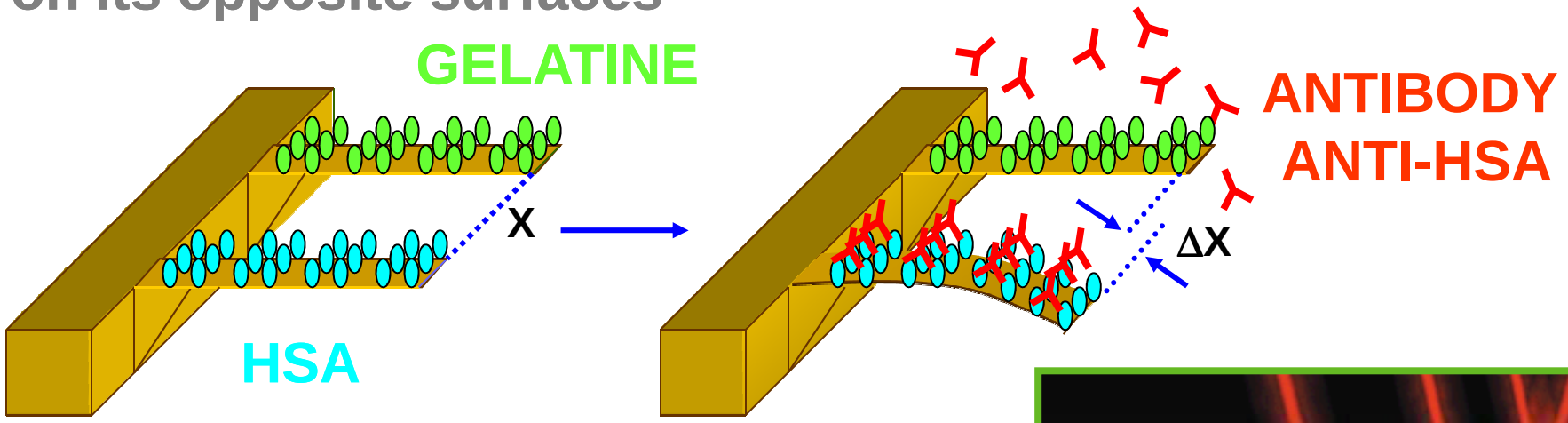


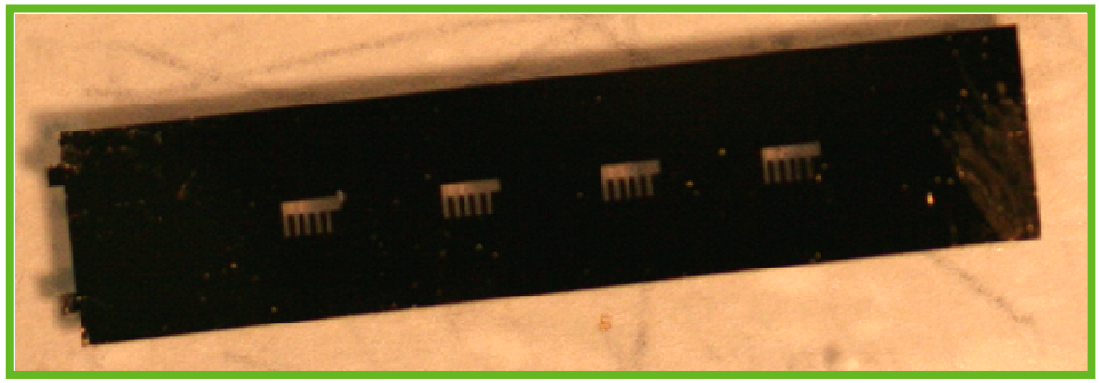


- tip with covalently linked ligand approaches the surface with specific receptor
- the formed affinity complex requires an additional force to rupture the bond and retract the cantilever

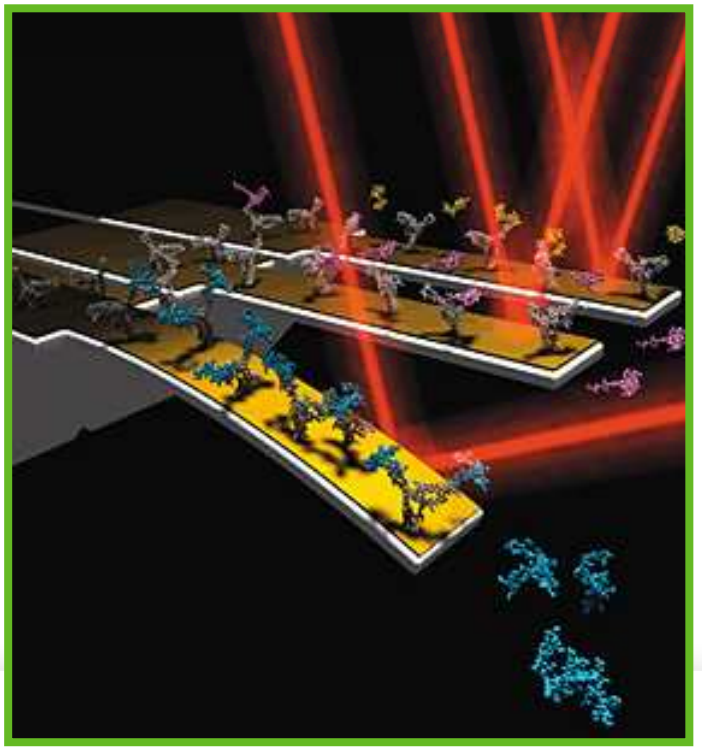
Nanomechanical (bio)sensors

cantilever (no tip) bends due to different surface tension on its opposite surfaces



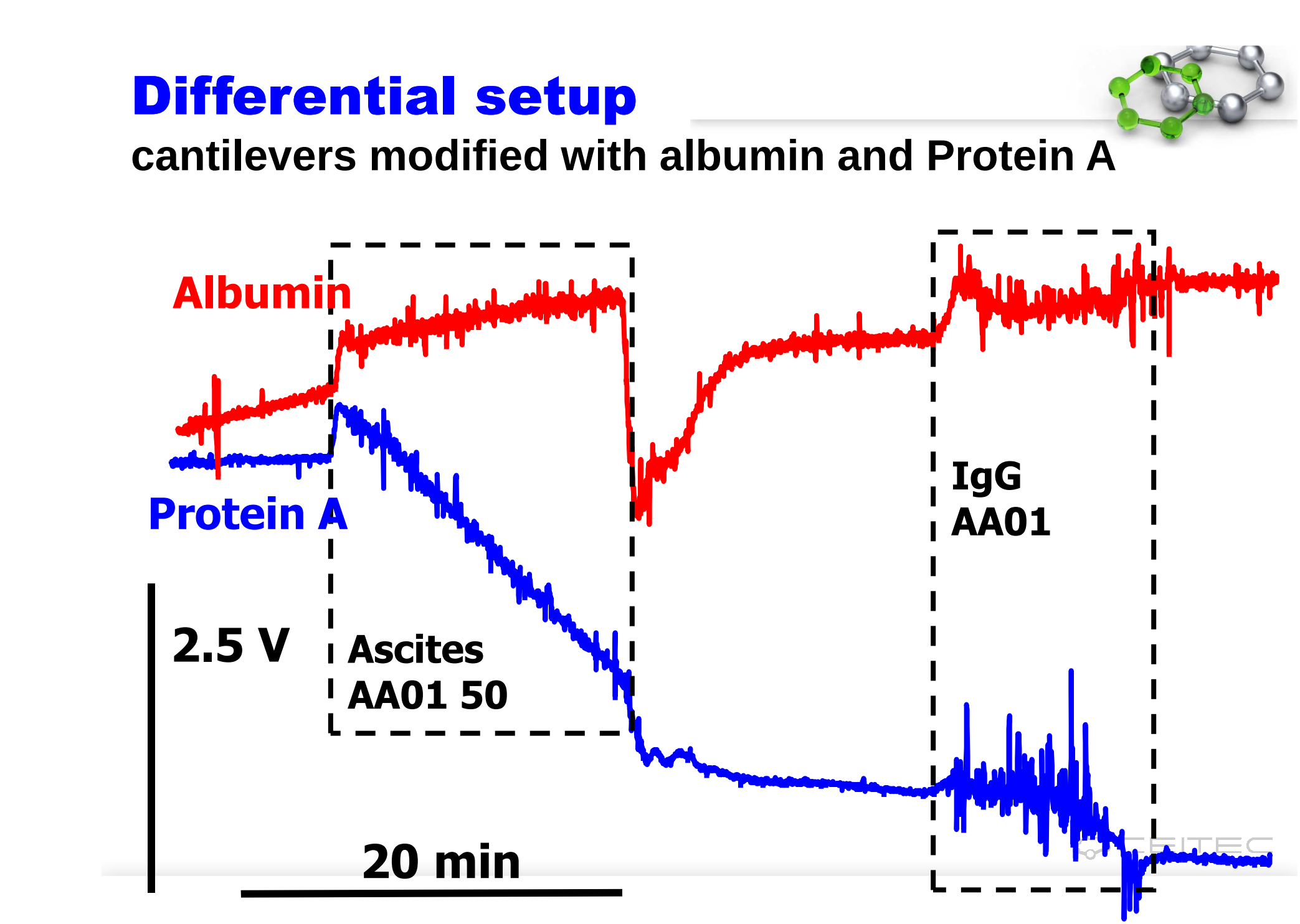


arrays of miniature cantilevers

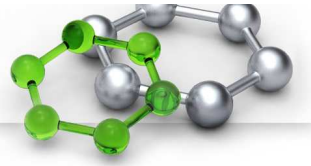


Differential setup

cantilevers modified with albumin and Protein A

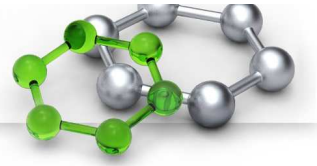


Nanobiotechnology for Health



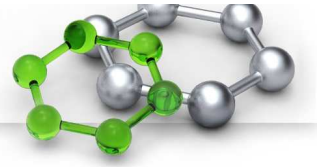
- scanning probe microscopies for biomedical and biomolecular imaging
- modified scanning probe tips, functionalized nanoparticles and nanopatterned surfaces
- novel miniature biosensors for clinical point-of-care assays and in vivo monitoring

Technologies



- imaging of bioobjects – atomic force microscopes (AFM), scanning near field optical microscope (SNOM), electrochemical AFM scanning, and scanning tunnelling microscopy (STM)
- AFM-based nanolithography - nanomanipulations and nanopatterning of (bio)surfaces resulting in nanoassemblies (dual probe AFM/SNOM)
- complementary data and affinity kinetics - surface plasmon resonance (SPR) systems Biacore T100, ProteomXR, automated microcalorimetry
- precise deposition of biomolecules - micro- and nanodeposition systems - novel nanoarray sensors with native and artificial recognition elements

Come and try nanotechniques now …



- Nanobiotechnologies and biosensors for biointeraction studies - openning up the modern technology to researchers in biology
- OPVK project no.: CZ.1.07/2.3.00/09.0167
- visit ***www.nanobio.cz***

esf evropský sociální fond v ČR | EVROPSKÁ UNIE | MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY | OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

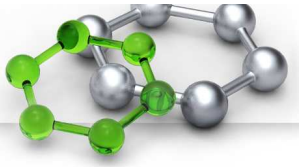
… or wait for CEITEC start-up

- we participate on Structural biology
- core facility Nanobiotechnology and Biointeractions

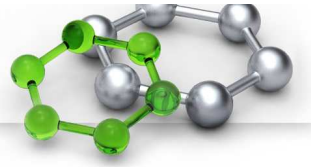
THANK YOU FOR YOUR ATTENTION!

CEITEC

Visualization and modification of biological objects including tissues, cells, cellular structures, and biomolecules.

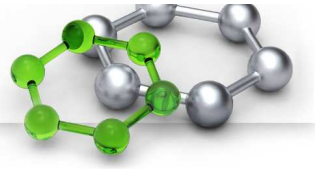


- application of scanning probe microscopies for imaging of normal and abnormal tissues, cells, cellular structures and individual biomolecules to achieve early detection of health disorders
- design of novel labelling techniques based on nanoparticles (magnetic NPs, quantum dots) for bioanalytical systems and optical imaging
- development of nanomechanical biosensors for highly sensitive detection of clinical markers, with potential for in vivo / in situ monitoring in real time
- fabrication of biosensing nanoarrays for multiparallel sensing of complex mixtures of biomolecules - proteomic and metabolomic profiles related to diseases or pathogenic infections



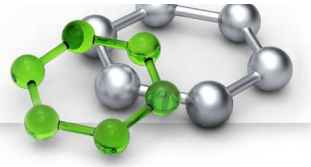
1 Imaging of biosurfaces and biomolecules using scanning probe microscopies

- atomic force microscopy (AFM) in both dry state and in liquids, non-contact (tapping) mode
- modified tips for scanning of surface hydrophobicity and specific target molecules and cellular surfaces (e.g. tips modified with antibodies)
- conductive tips with applied potential will be used for bioelectrochemical studies
- repeated scans for movement and morphologic changes of cells (VideoAFM)
- supplementary information on cells and celular elements - scanning near optical field microscopy (SNOM, overcoming the diffraction limit) both in reflection and transmission modes, combined with fluorescence and dual-probe set-up
- atomic resolution at molecular level - scanning tunelling microscopy (STM)



2 Nanobiointeractions and measurement of forces within biocomplexes

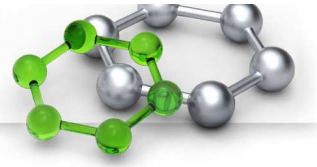
- binding of two individual complementary molecules (antibody-antigen, ligand-receptor, hybridization of oligonucleotide) will be studied using one partner bound to the solid support and the other linked to the scanning tip
- contact mode - force-distance curves measured over the studied surface will allow identification and quantification of the target biomolecules
- the force data obtained at the nanolevel will be carefully correlated with the results obtained at the macrolevel using surface plasmon resonance techniques providing information about kinetics of affinity interactions in real time



3 Nanomanipulation and nanolithography of biological objects

- AFM-lithography - tip of the cantilever in the contact with scanned object can be used to manipulate cells on the surface
  - forcing cell contacts, making small holes (“nanoneedle”) or scratches (“nanoscalpel”) in the cell surface.
- the surface-adhered cells will be forced to interact with each other or with extracellular objects – nanoparticles, liposomes, lipid/DNA complexes and viruses using mechanical pushing with the AFM tip
- patterning of biolayers formed with the help of self-assembling
  - several nanolitographic techniques (scratching, plowing, dip-pen, local electrochemical reaction – dissolution or deposition of materials, electropolymerisation)
- nano(bio)sensing arrays and other functional nanoobjects will be constructed

4 Nanobiosensors and biosensing nanoarrays



- cantilever as nanomechanical transducer
  - bending due to affinity interaction on one of its sides
- nanoarrays – biochips consisting from sets of specific recognition proteins
  - monoclonal / recombinant antibodies, engineered receptors and enzymes, artificial peptide folds designed by molecular modelling)
- incubated with clinical samples and afterwards the binding pattern will be read with the help of AFM (SNOM, STM) either directly or after suitable amplification
  - magnetic nanoparticles, quantum dots
- validation using “larger” micrometer-sized array elements
  - evaluated using multichannel SPR, fluorescence scanning and scanning electrochemical microscopy (SECM)