Elettra
Sincrotrone
Trieste

School on Biophysical Approaches to Macromolecules and Cells: Integrated Tools for Life Sciences and Medicine

Loredana Casalis
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The Abdus Salam
International Centre
for Theoretical Physics

IAEA

NM-AIST, Arusha, Tanzania 9-20 September 2019

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What is biophysics?

Biophysics is the field that applies the theories and methods of physics to understand how biological systems work, i.e. the mechanics of:

- how the molecules of life are made
- how different parts of a cell move and function
- how complex systems in our bodies—the brain, circulation, immune system, and others— work.

(ref. Biophysical Society)

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What is biophysics?

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Scientists from math, chemistry, physics, engineering, pharmacology, biology, biotechnology and materials sciences explore and develop new tools to understand how biology—all life—works. They design cutting-edge technologies and develop methods to overcome disease, but also to eradicate global hunger and produce renewable energy sources.

Aim of the school

Introduce young researchers to the latest developments in the field of molecular biophysics (structural biology, nano-biophysics, nano-medicine, computational methods) and their revolutionary impact on biotechnology, pharmacology, drug delivery and early diagnostics of wide spread diseases.

Forge links between the different communities, to acquire a shared "language" and background and foster the emerging biophysical sciences in Africa.

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What do Biophysicists do

Therefore biophysicists work on:

- Data Analysis and Structure (DNA sequencing and correlation with diseases, protein structure, analysis of huge quantity of data)
- Computer Modelling (see and manipulate the shapes and structures of proteins, viruses, and other complex molecules to develop new drug targets, or understand how proteins mutate and cause tumours to grow)
- Molecules in Motion, Cell-Cell Interactions (understand how molecules move inside the cells, how cells interacts with other cells and extra-cellular environment)
- Bioengineering, Nanotechnologies, Biomaterials (biomechanics applied to understanding of diseases; design of functional nanomaterials for drug delivery and prosthetic applications)

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SILVIA ONESTI, Structural Biology

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ALI HASSANALI, Computational Biology and Data Science

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LOREDANA CASALIS, NanoBiophysics, NanoMedicine

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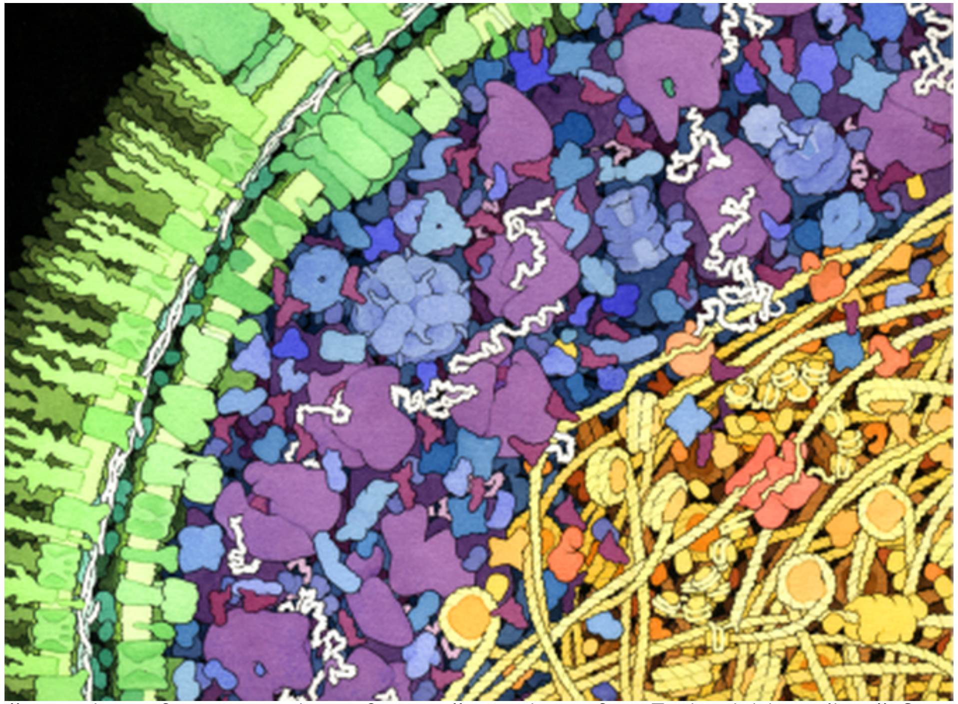
Biomolecular interactions

We now know that the interior of cells is very crowded and structurally organised: protein interactions inside the cell cannot be understood without concepts of diffusion, viscosity, elasticity



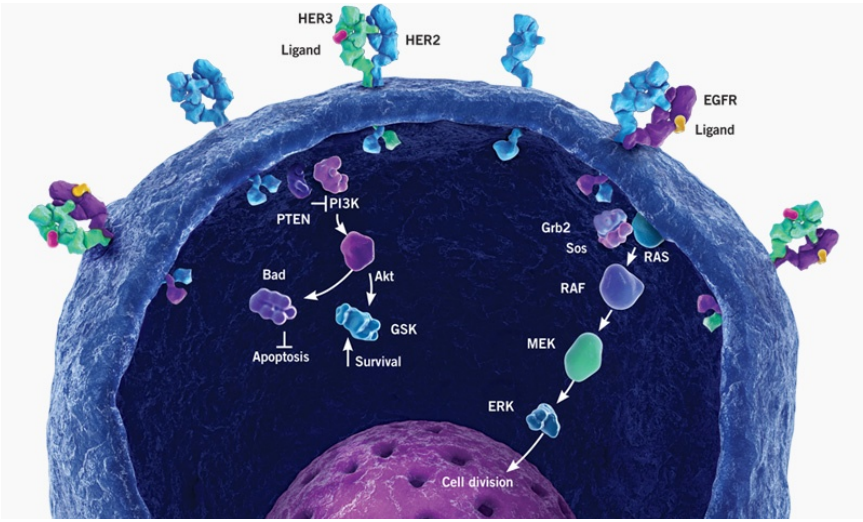
llustration of cross-section of a small portion of an Escherichia coli cell. Source: David S. Goodsell, The Scripps Research Institute

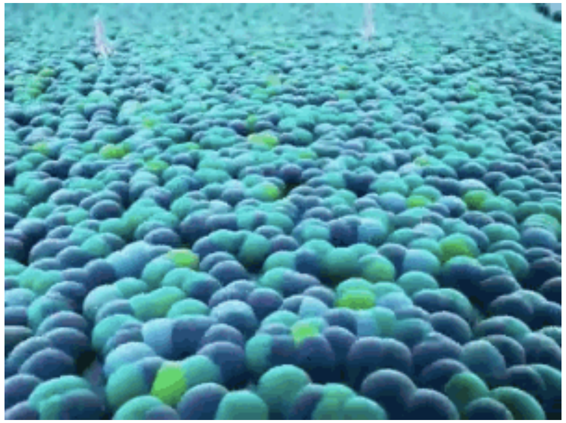
The internal cell crowding is non-uniform, there are gradients of macromolecules and other species which enhance the transport at the nanoscale (ACS Nano, DOI 10.1021/acsnano.9b02811).

Self-assembling of membrane-free membrane-less compartment behave as liquid droplet and form in response to environmental stress/regulatory processes.

Biomolecular interactions

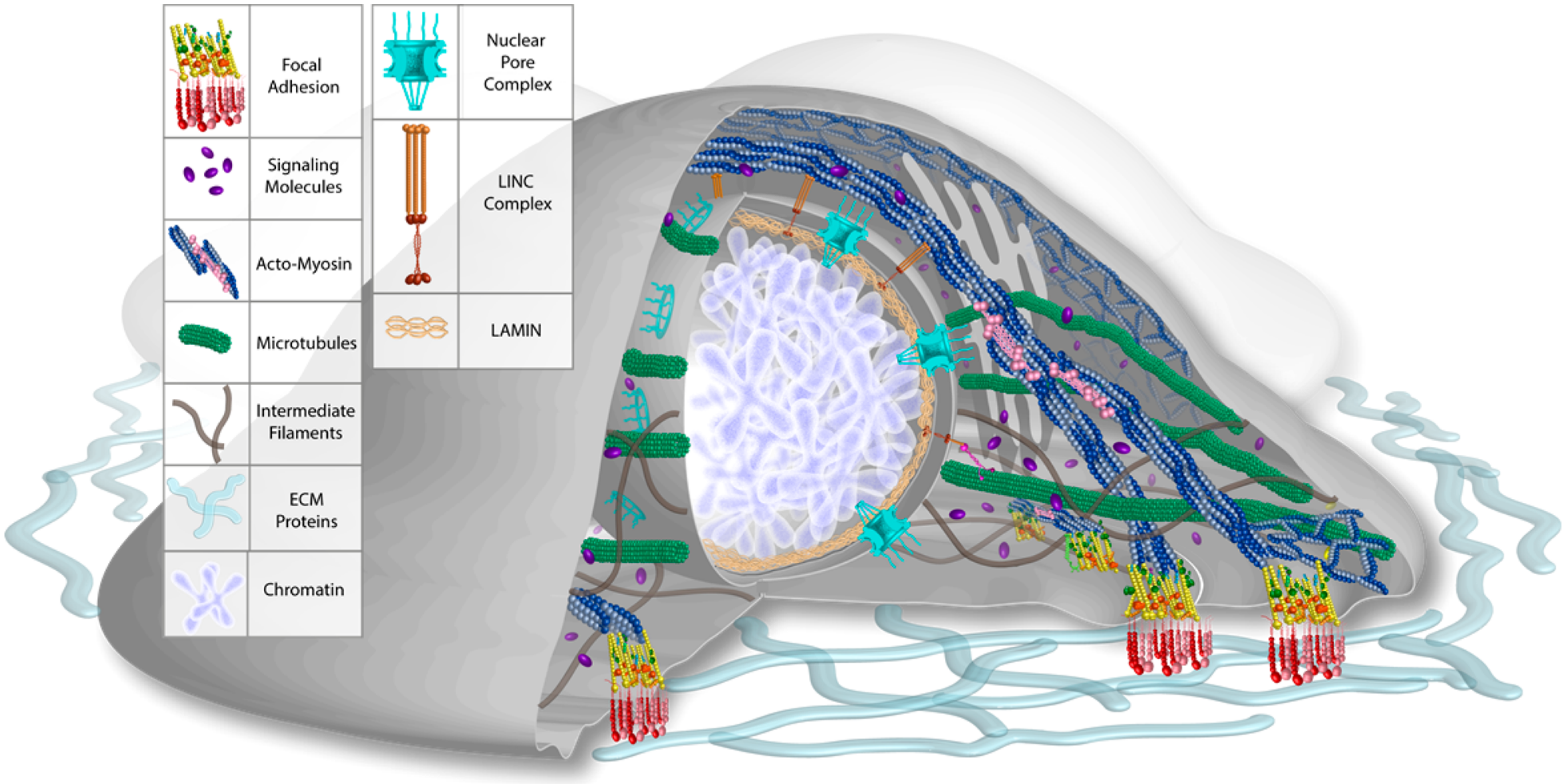
Also, we know that cell membranes play a crucial role in cell-cell, cell-environment communication. How much do we know about molecular interactions at the cell membrane?





llustration of cross-section of a small portion of an Escherichia coli cell. Source: David S. Goodsell, The Scripps Research Institute

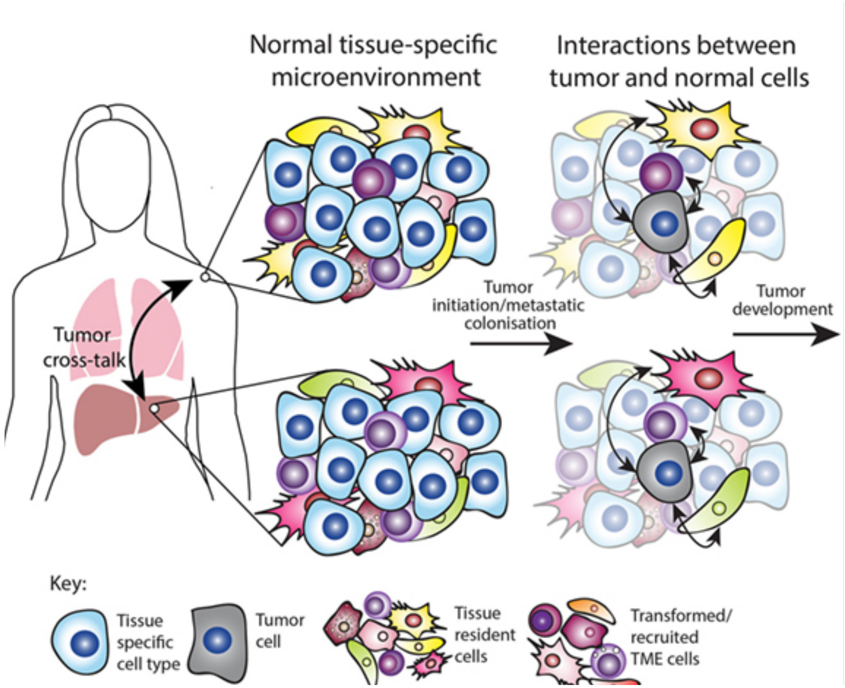
Cell mechanics



Cells respond to extracellular matrix (ECM) cues generating and transducing mechanical forces into biochemical signals and genomic pathways which affect cell properties.

Such forces define tissue architecture and drive specific cell differentiation programs. In adults perturbation of ECM (stiffness, mutations) cause pathologies in different organs, including ageing and malignant progression.

Cell-cell interactions and diseases



In cancer, entire tissues get corrupted: healthy and diseased cells coexist.
If we understand cell-ECM interaction and how the cell regulates genes, we might be able to change the microenvironment to favor only the healthy ones.

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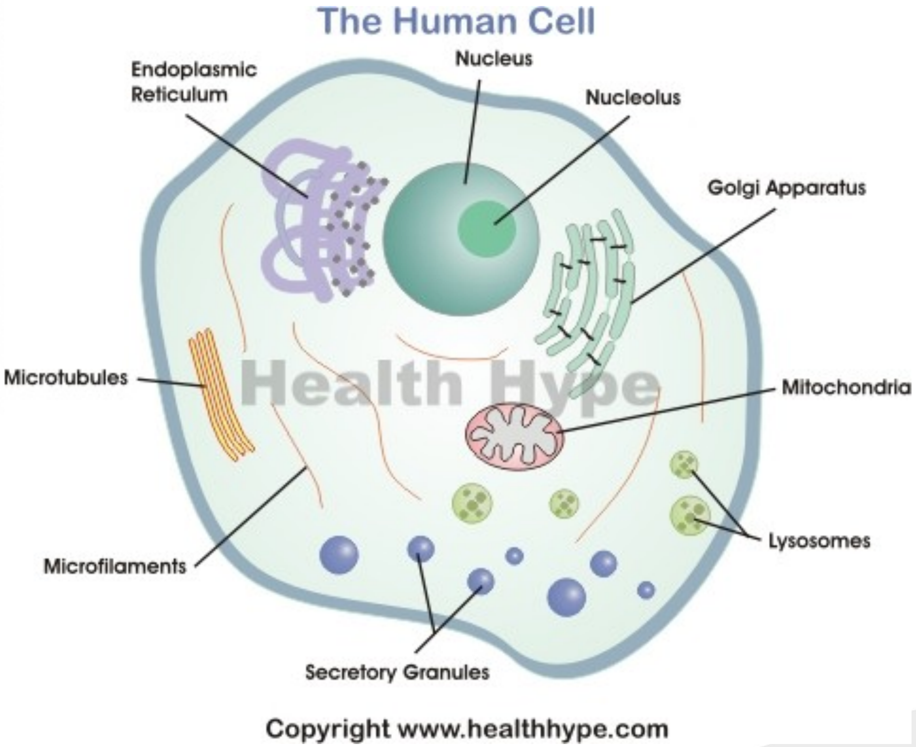
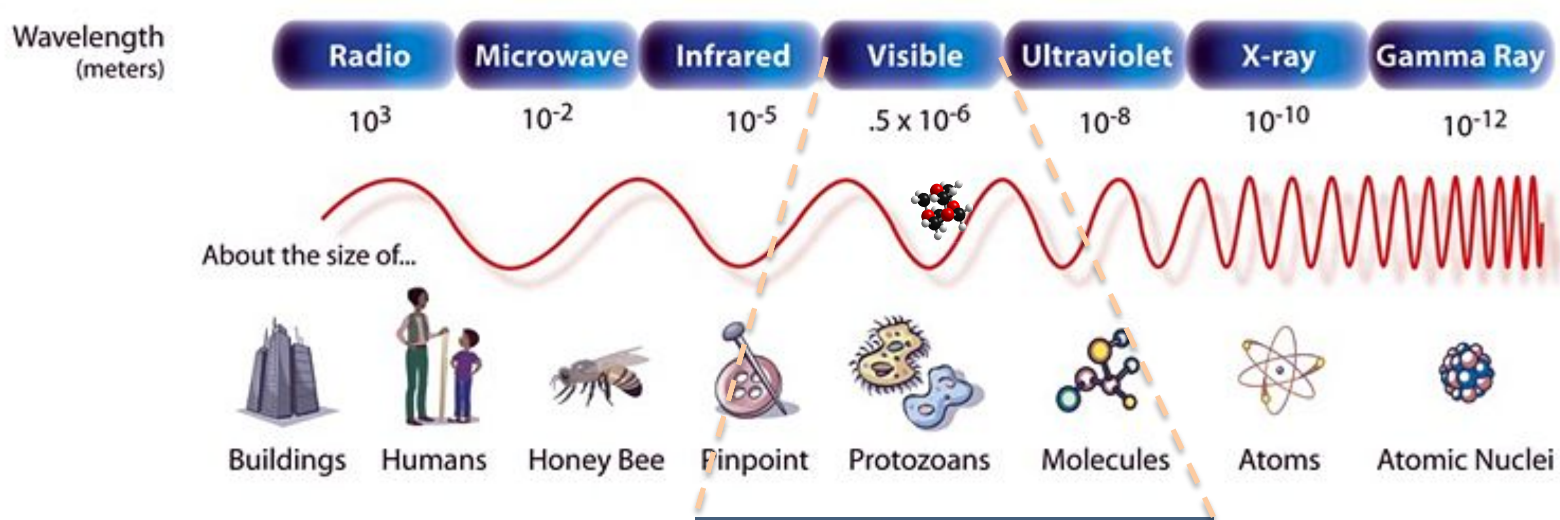
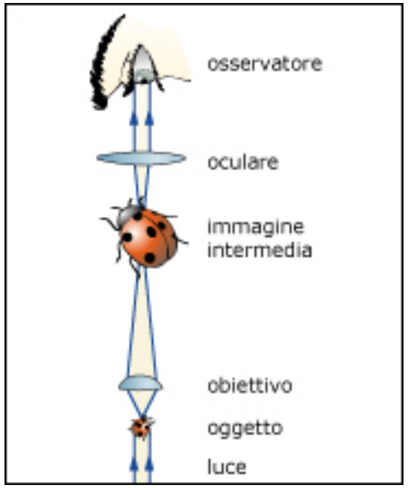
How to study molecular interactions?



New tools are needed to help discriminate between healthy and diseased cells and understand molecular processes inside cells

New tools required for molecular resolution: optical microscopy and beyond



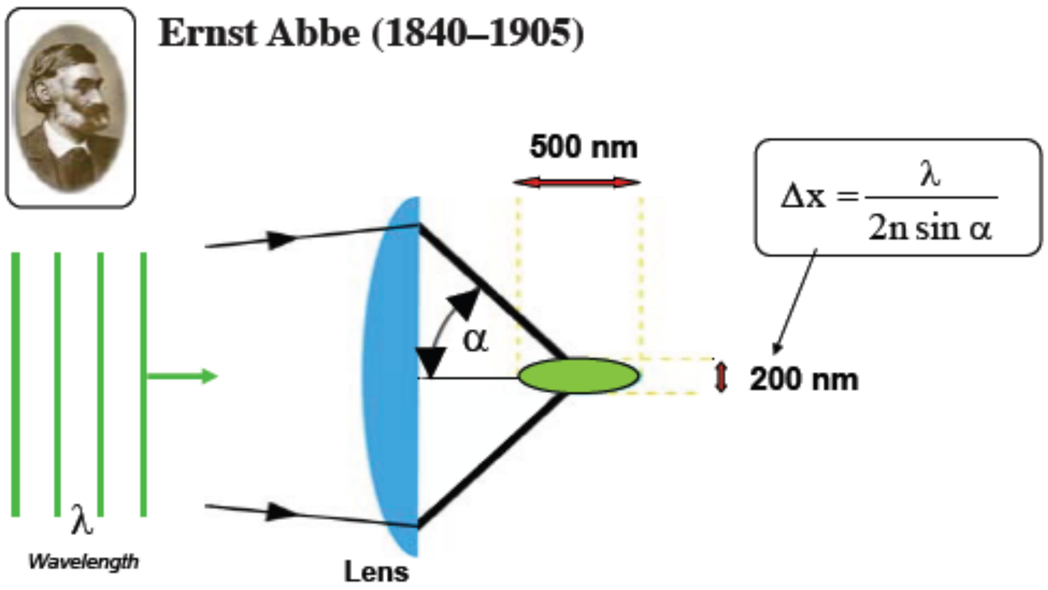


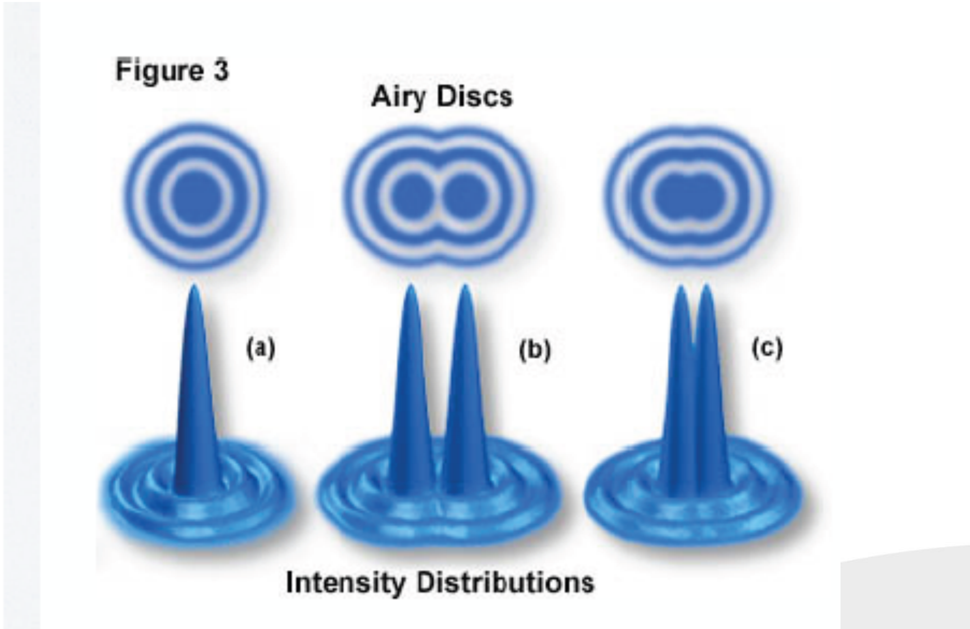
To visualize an object we need wavelenghts shorter than the object dimensions.

Visible light: 400-600 nm

New tools required for molecular resolution: optical microscopy and beyond

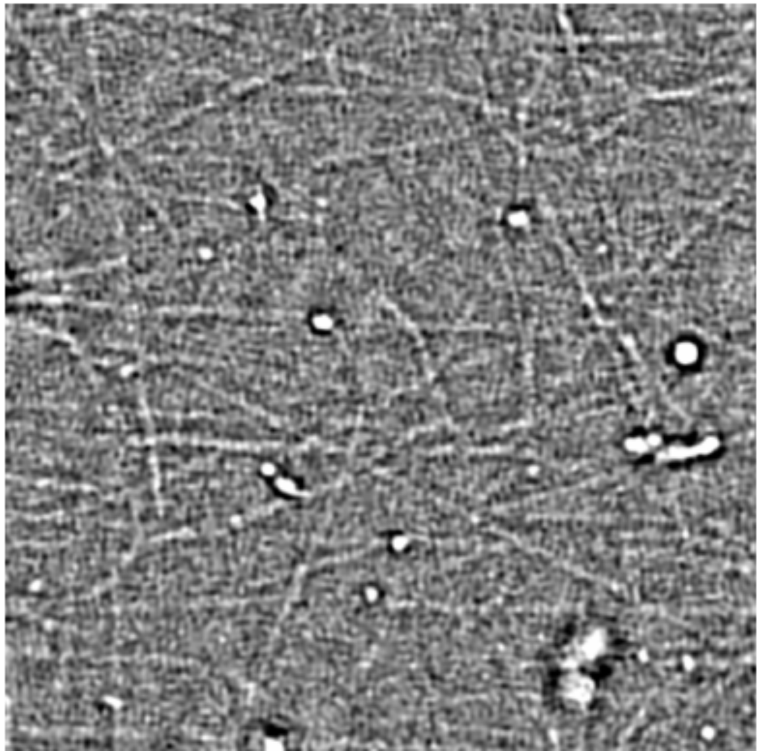
Resolution R: the smallest resolvable distance between two objects





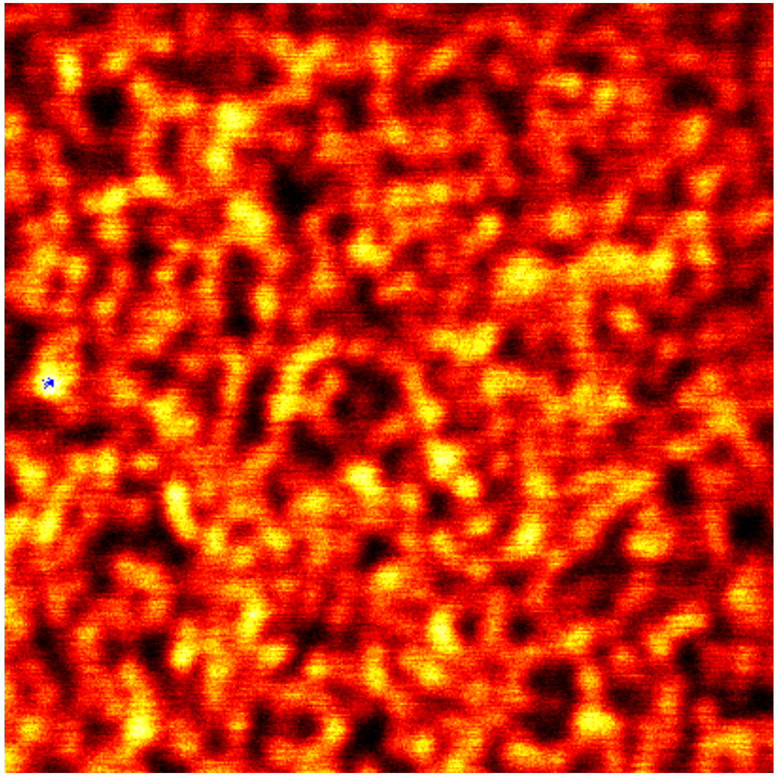
Optical Microscopy: resolution

…but can resolve smaller object…



25 nm dia. tubes

…depending on their relative distance…

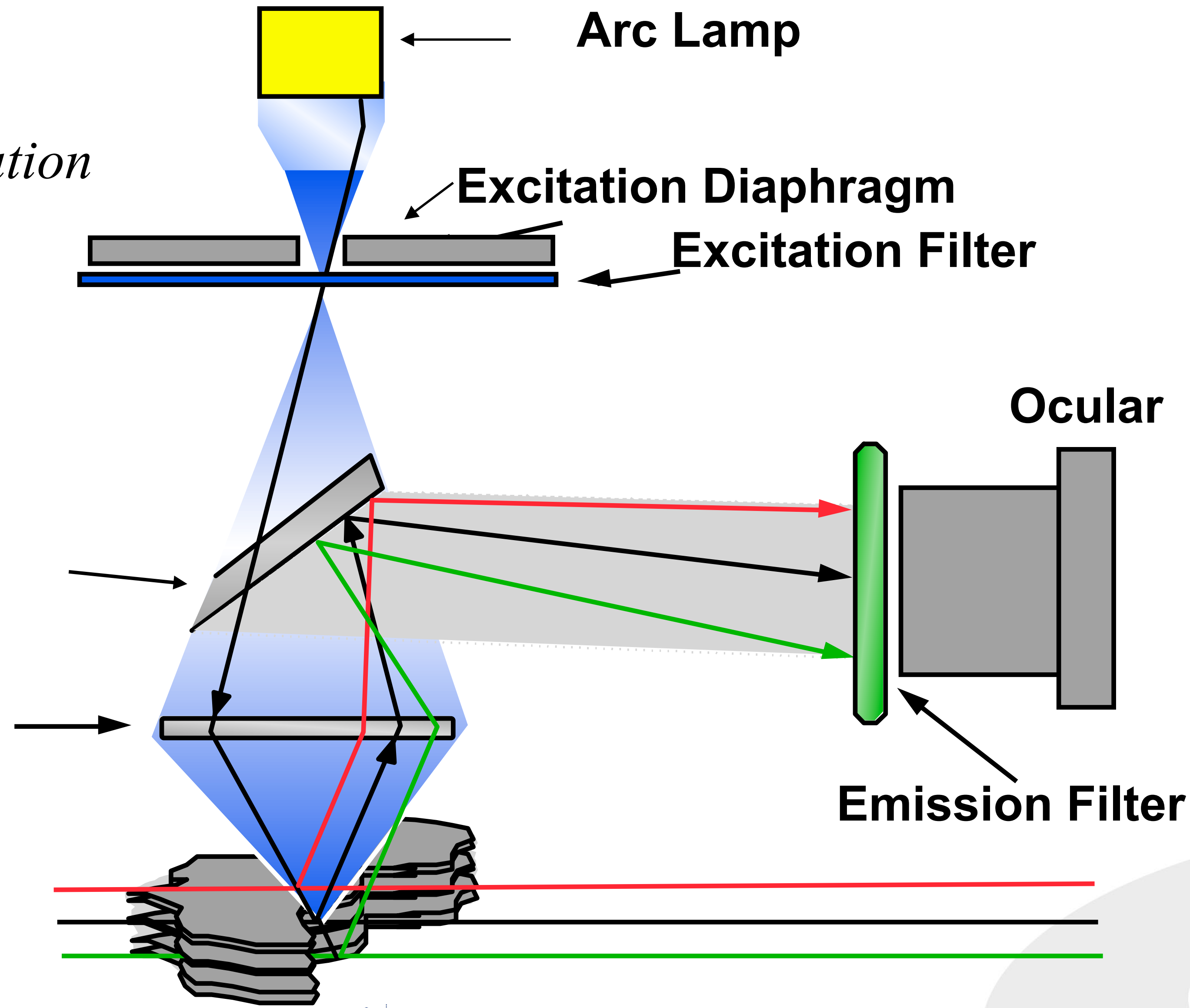


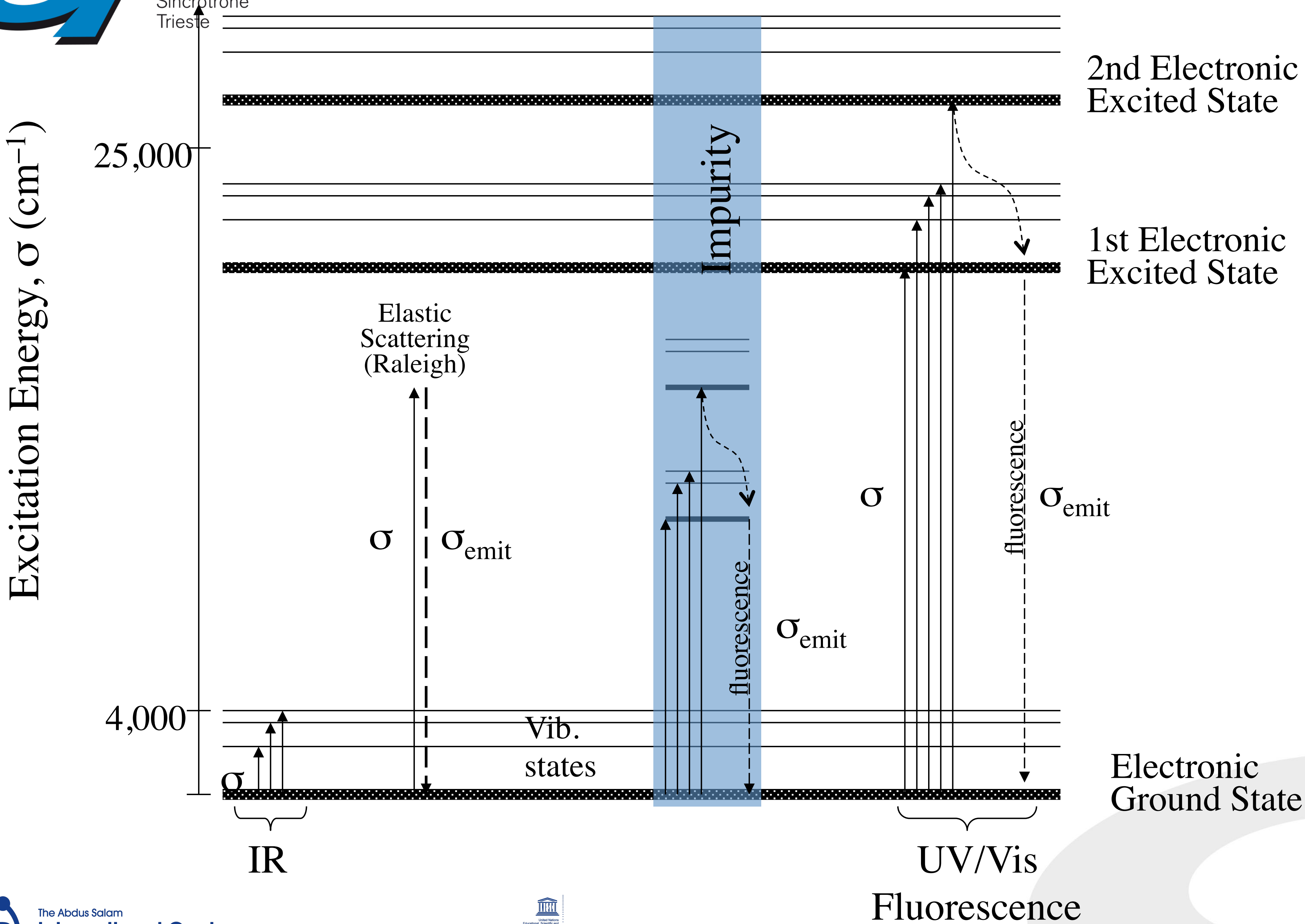
40 nm polystyrene beads

Courtesy of Iwan Schaap

Fluorescence Microscopy

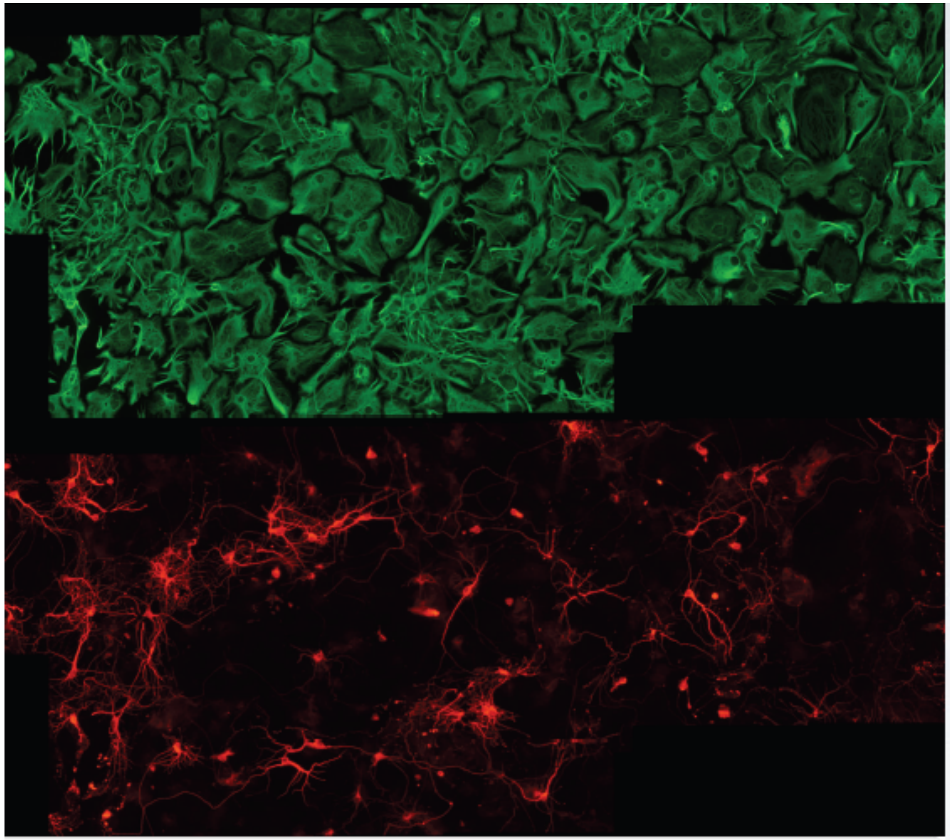
EPI-Illumination

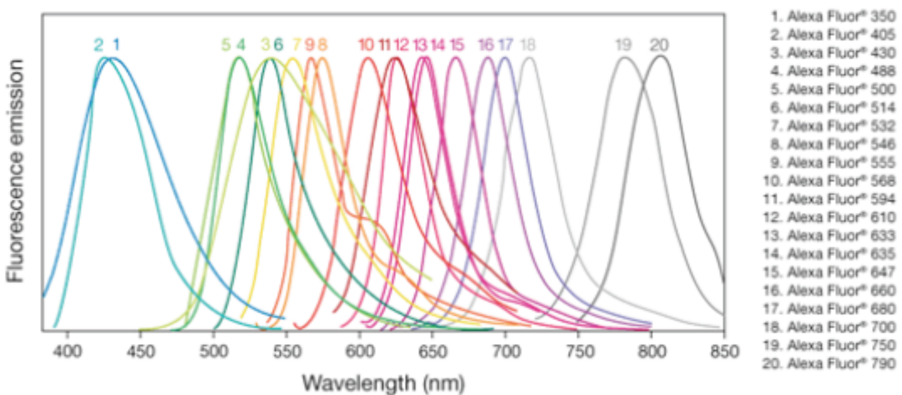




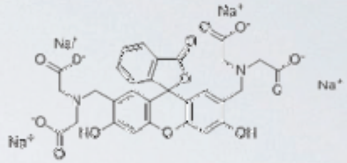
Excitation Energy, σ (cm⁻¹)
25,000
4,000
2nd Electronic
Excited State
1st Electronic
Excited State
Electronic
Ground State
Impurity
Elastic
Scattering
(Raleigh)
σ
σemit
fluorescence
Vib.
states
IR
UV/Vis
Fluorescence

Fluorescence Microscopy

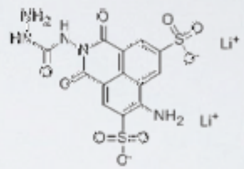




Direct

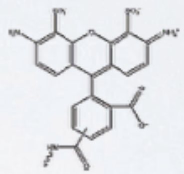


Calcein (495/515 nm)



Lucifer Yellow (428/540 nm)

Indirect



Alexa 488 (488/525 nm)

Genetic

Green Fluorescent Protein (GFP) (488/510 nm)

STED microscopy

I st physical concept to break the
diffraction barrier in
far-field
fluorescence microscopy

S.W. Hell & J. Wichmann (1994), *Opt. Lett.* 19, 780
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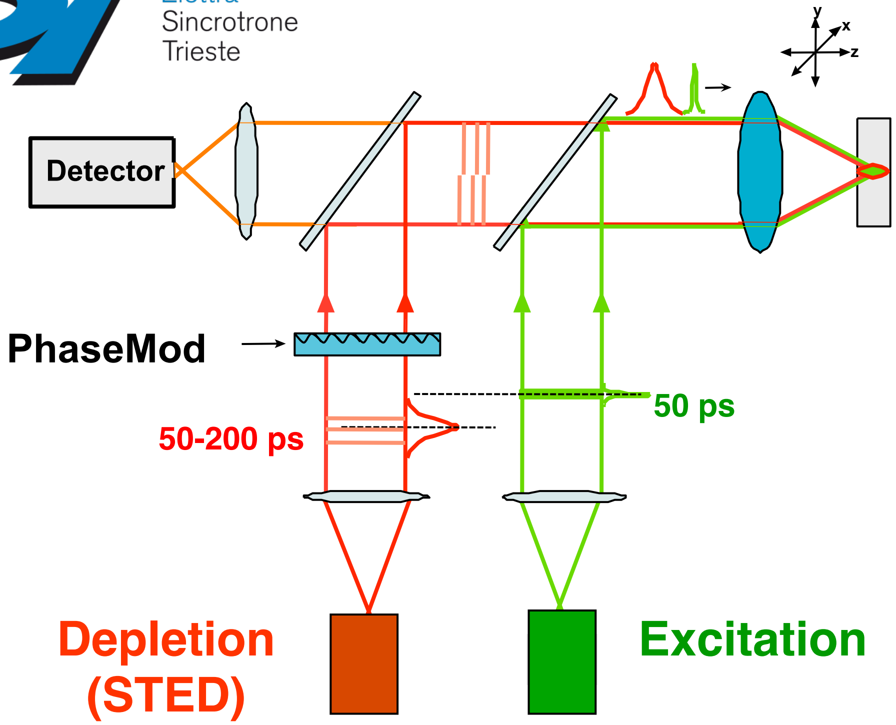
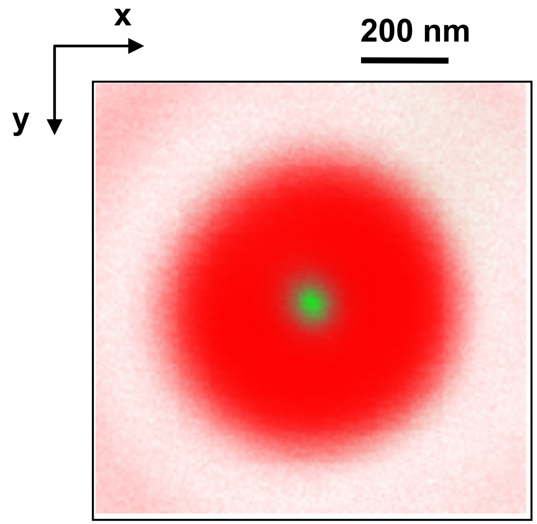
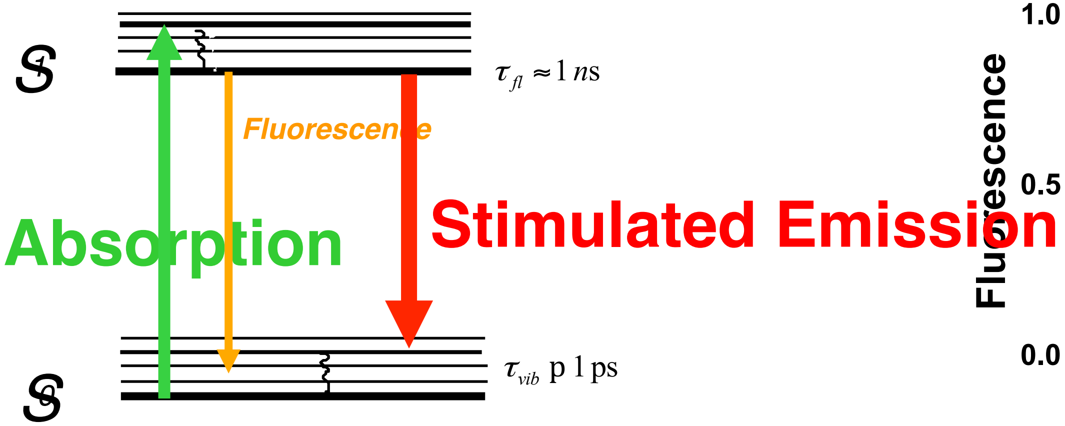
Nobel Laureate Chemistry 2014

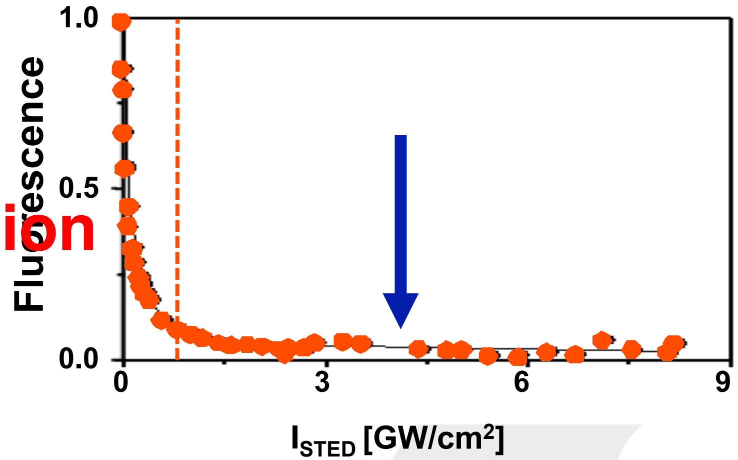
S.W. Hell & J. Wichmann (1994), *Opt. Lett.* **19**, 780.





The stronger the STED beam the narrower the fluorescent spot!





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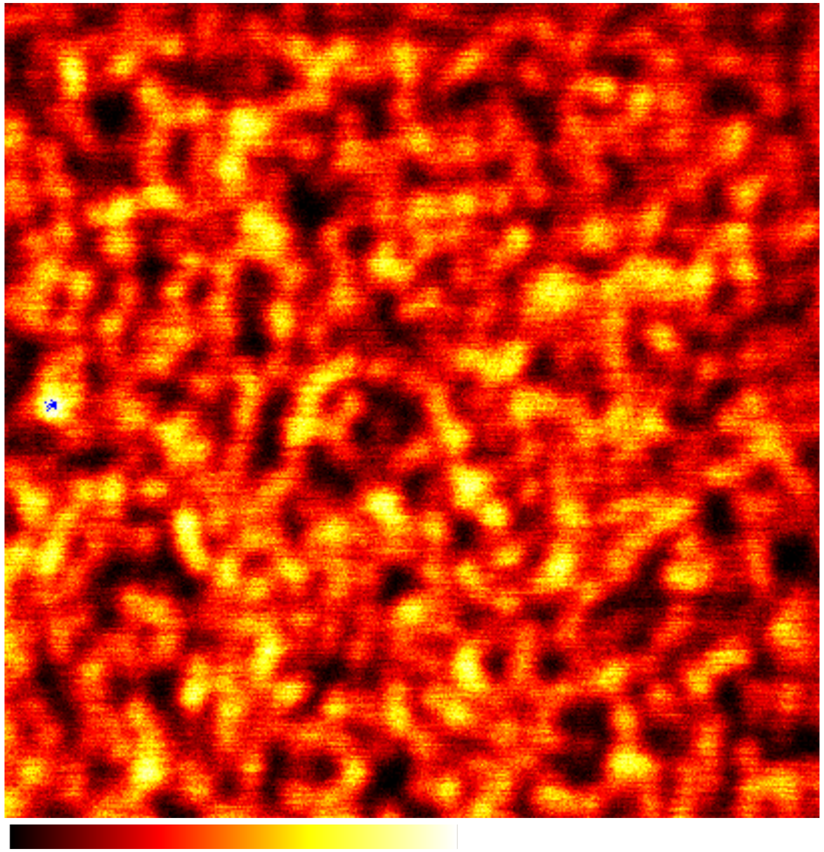
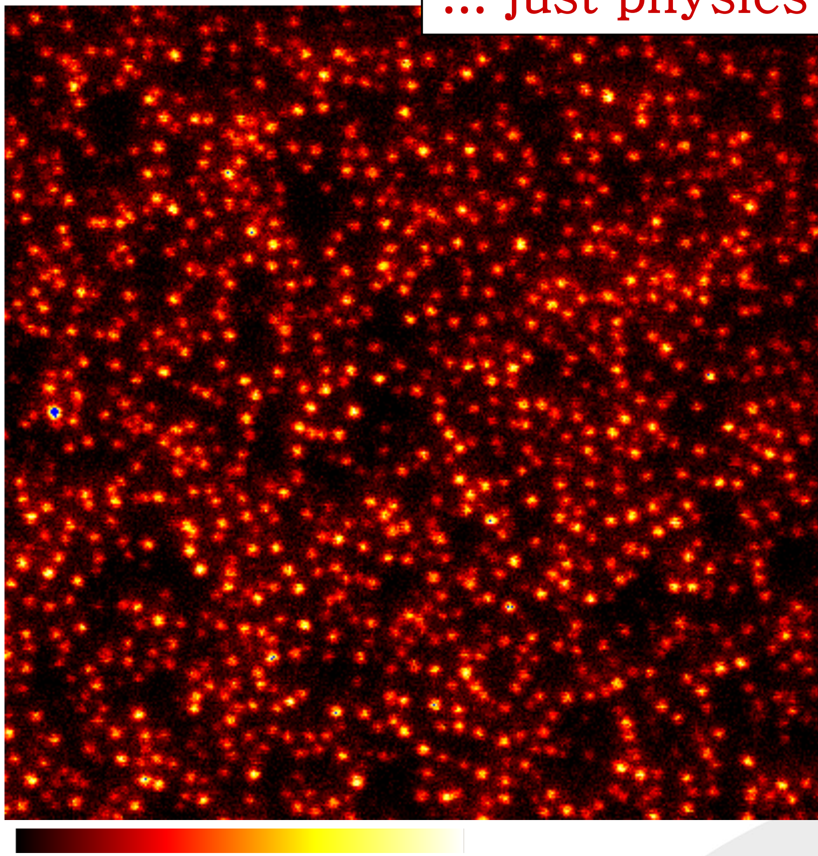
Imaging 40 nm fluorescence beads:

Confocal



STED

... just physics !♪



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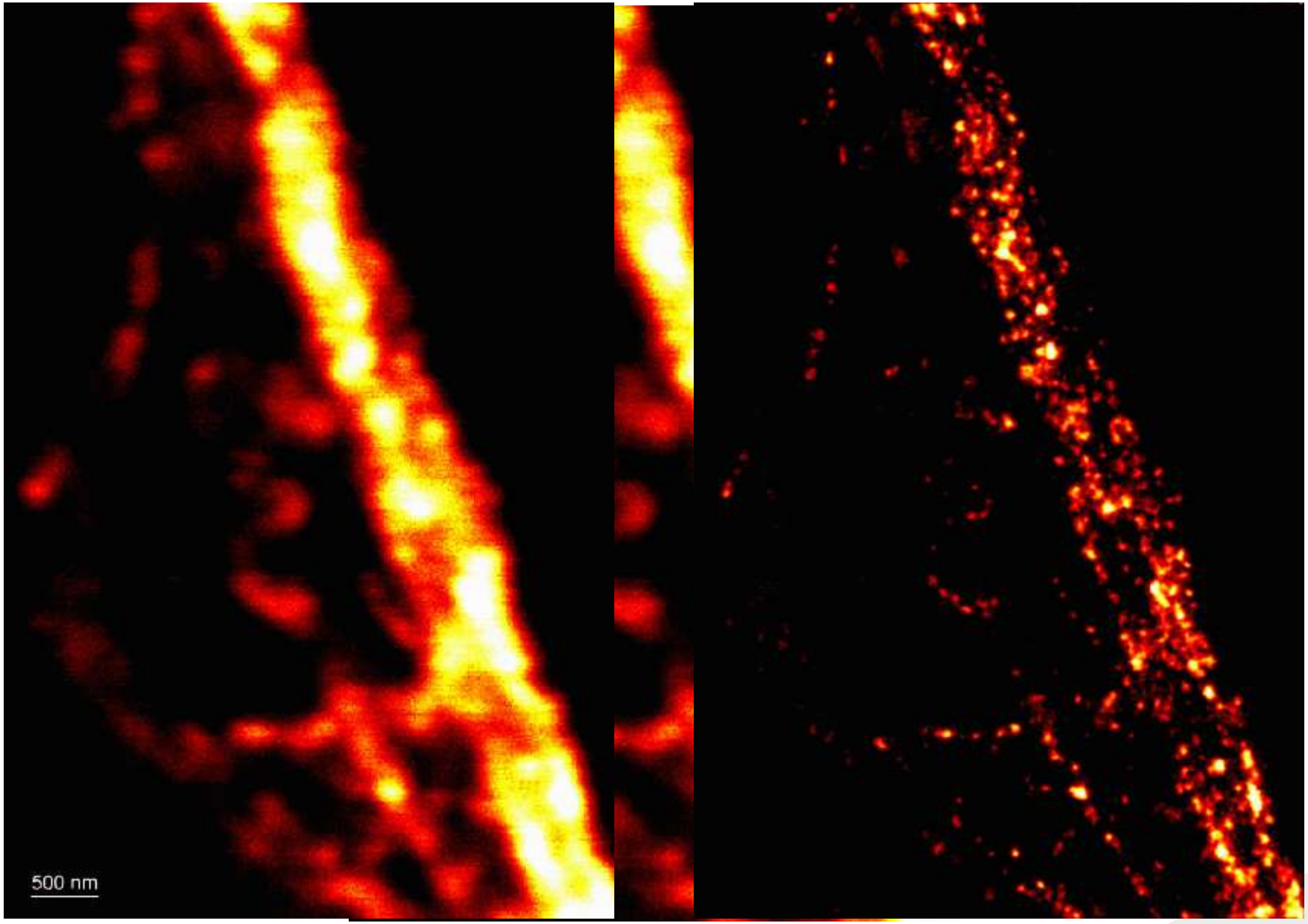
Heavy subunit of neurofilaments in neuroblastoma

Confocal



STED

G. Donnert, et al. (2006), *PNAS* 103, 11440.

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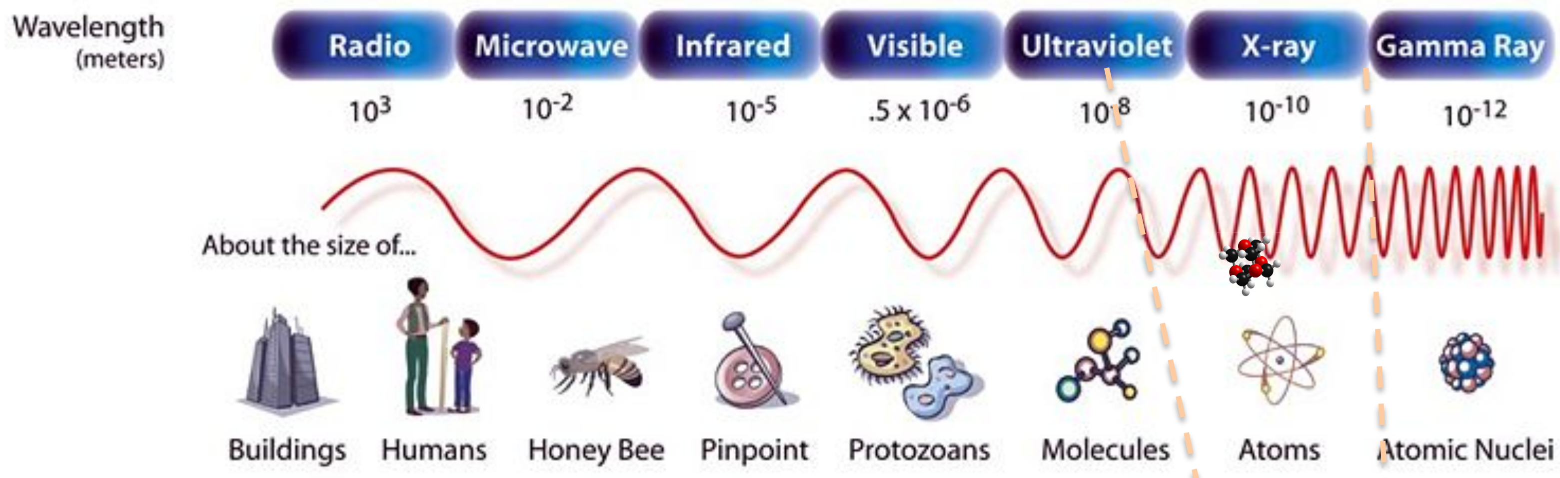
Synchrotron radiation facilities are sources of X-rays



- X-ray Protein Diffraction
- Small Angle X-ray Scattering
- (Neutron Diffraction)

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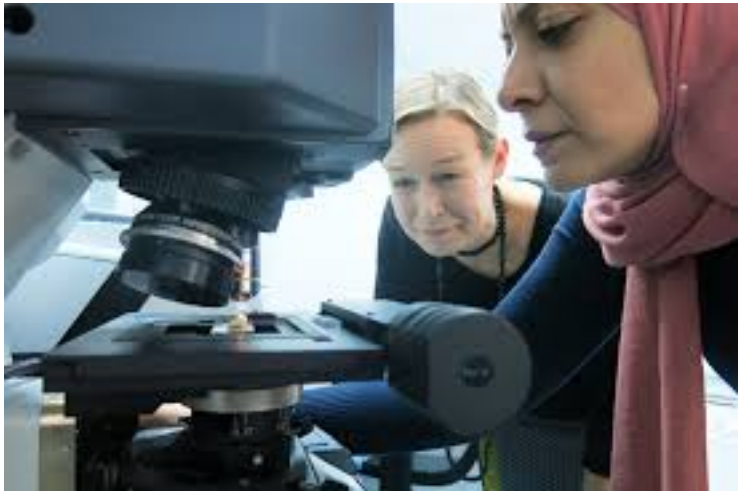
Big Science requires facilities: the case of SESAME, Jordan
(http://sesame.org.jo/sesame_2018/)

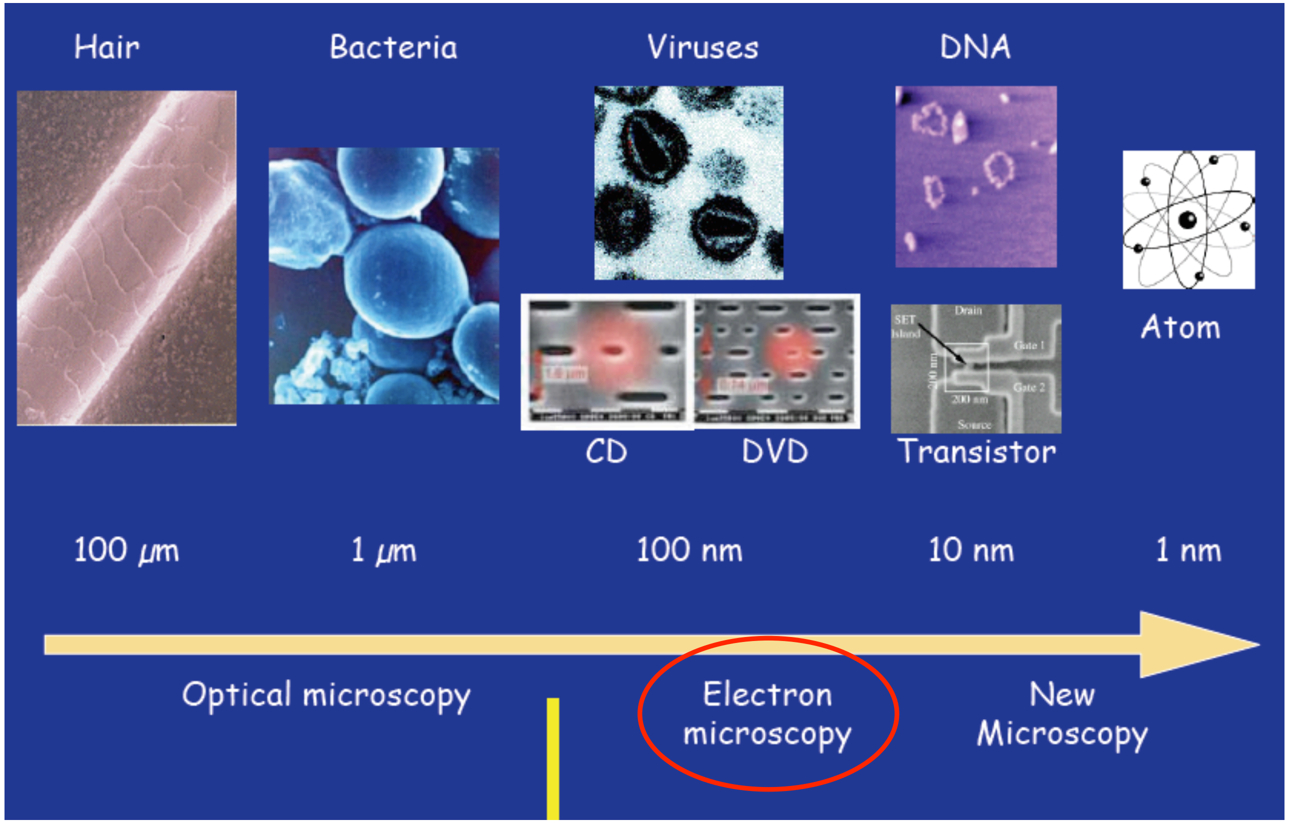


Member states of SESAME!
Science4Peace

Coming next: African Light Source (Ghana?)

The Abdus Salam
International Centre
for Theoretical Physics

Other microscopy techniques



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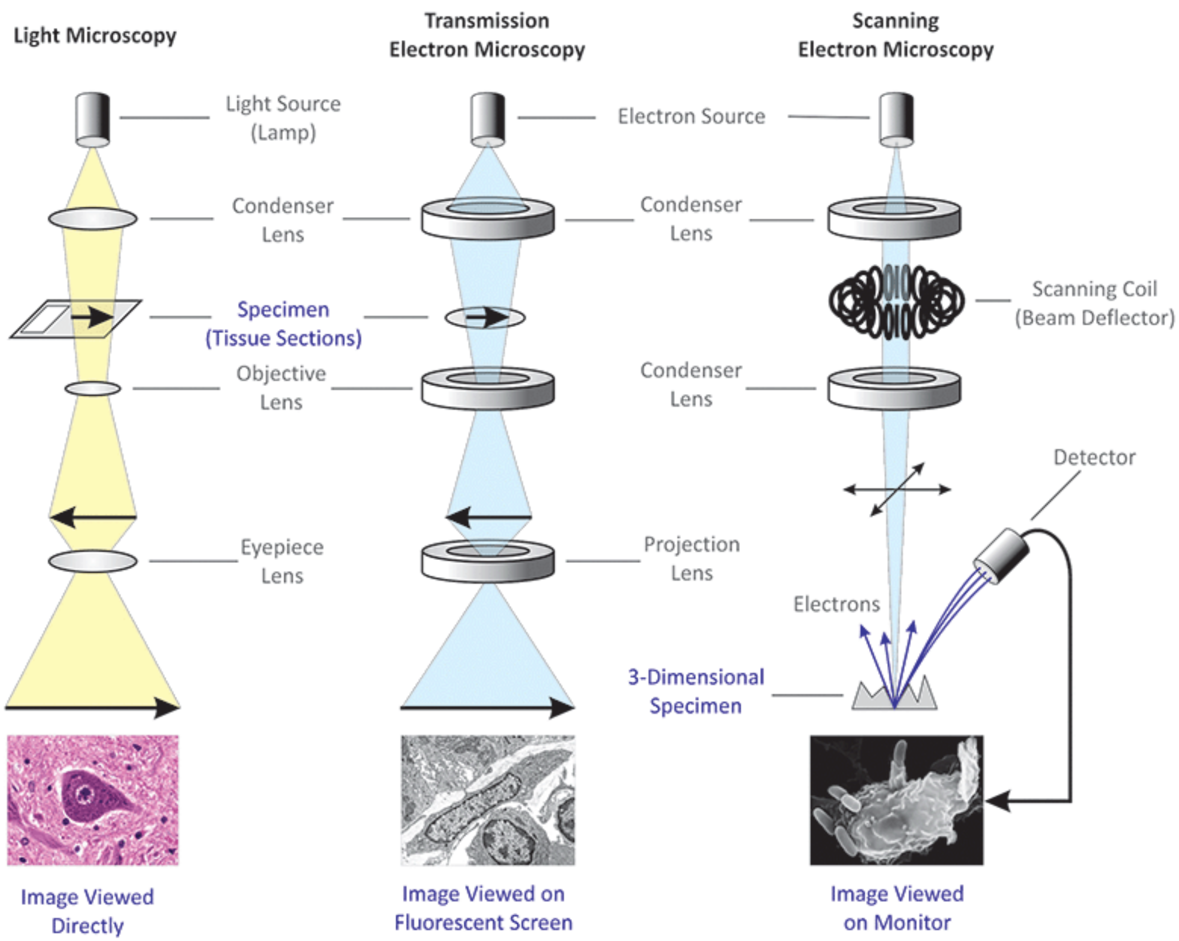
Other microscopy techniques



Electromagnetic microscopes provide a high resolution (nm fractions) magnified **2-D image** of an object surface.

Height and depth of the surface features are not supplied.

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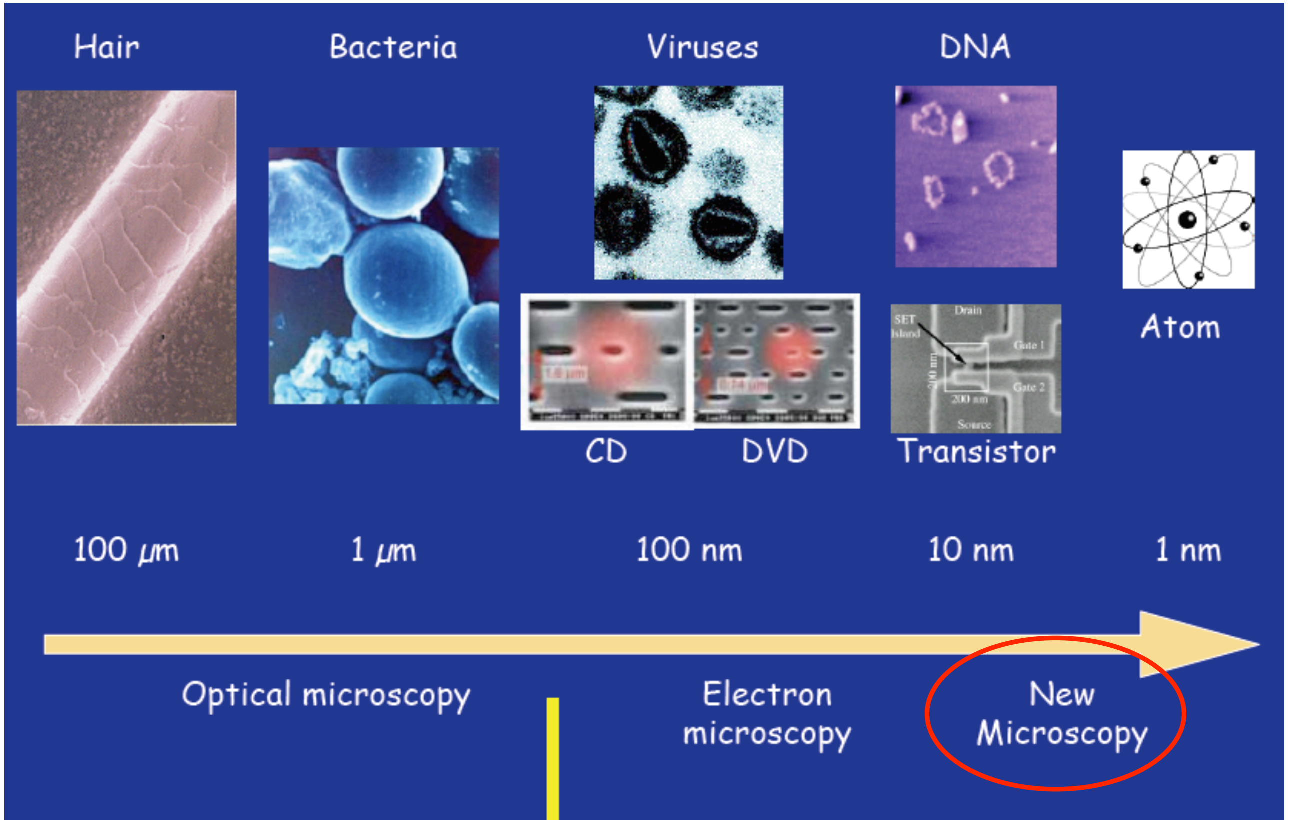
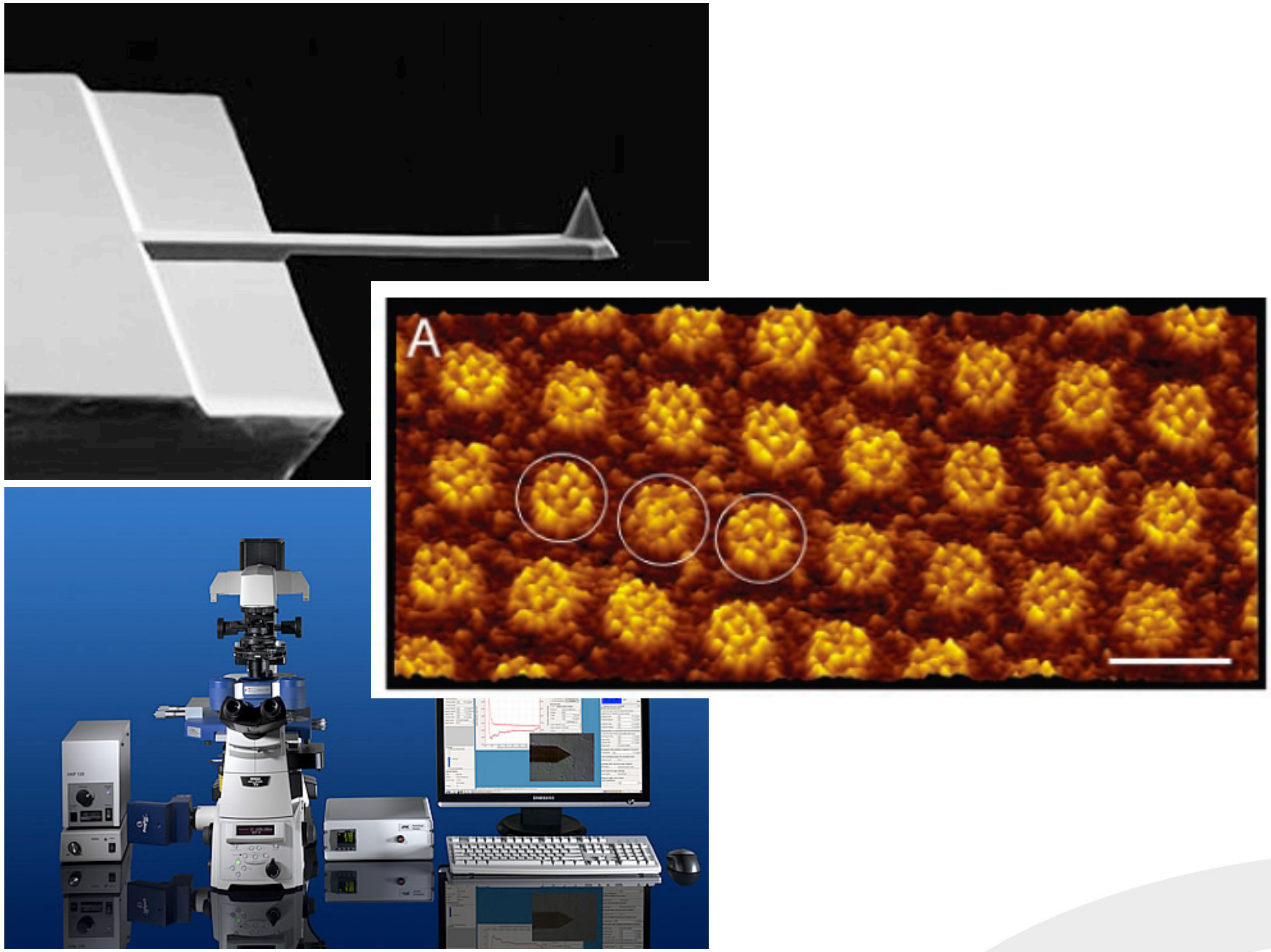
Other microscopy techniques



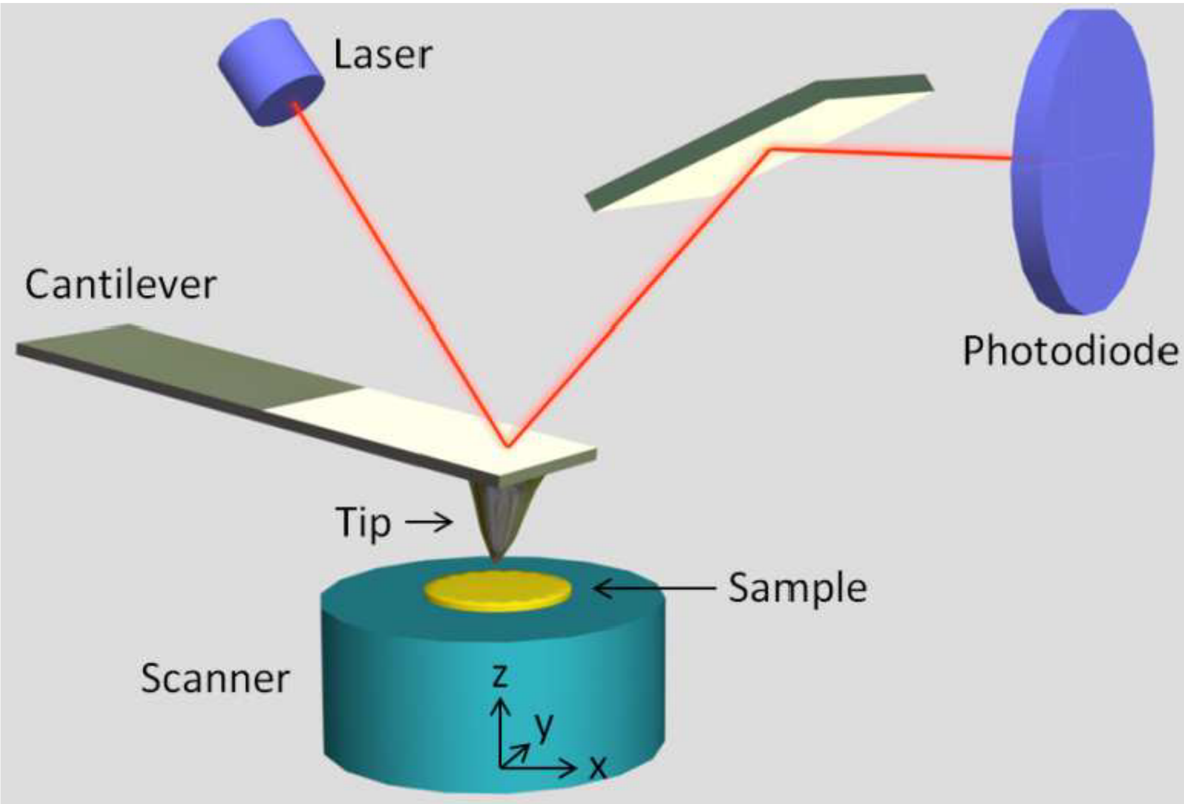
Atomic Force Microscopy (AFM)

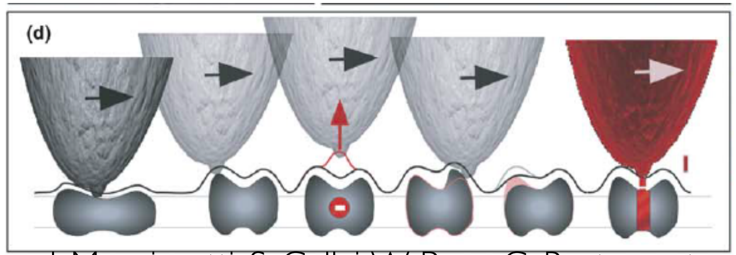
A nanomechanical probe



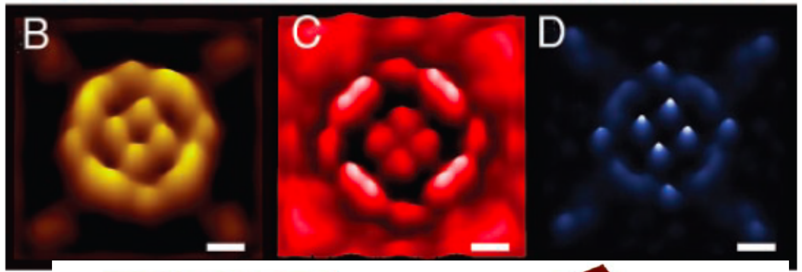
S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

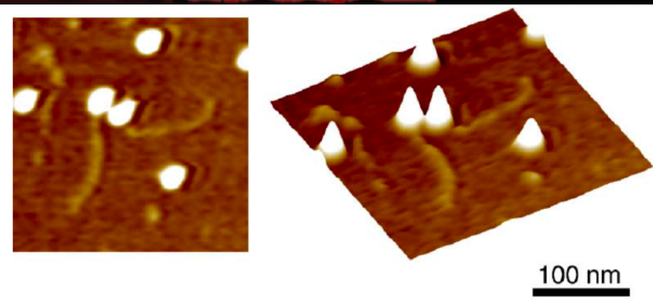
Atomic Force Microscopy (AFM)





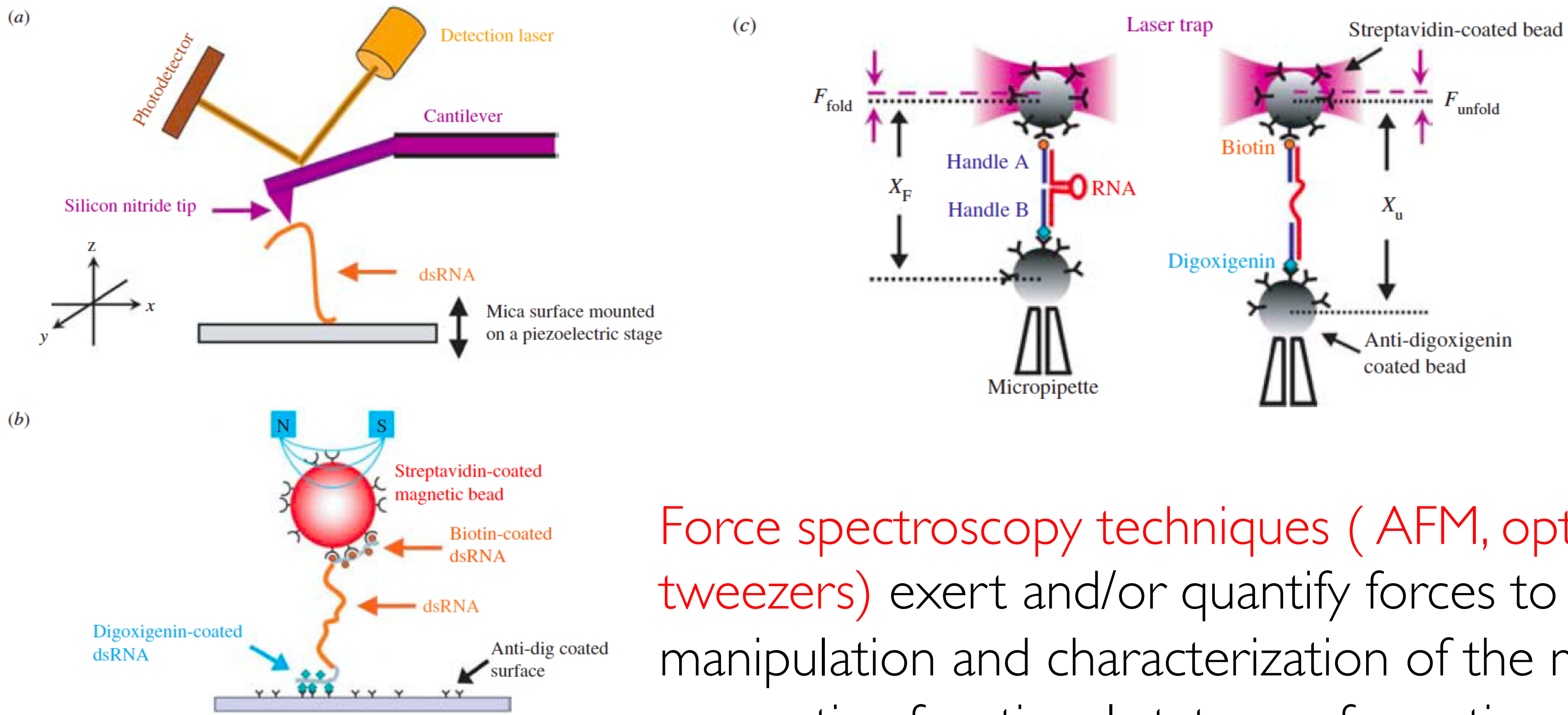
I. Mangiarotti, S. Cellai, W. Ross, C. Bustamante, C. Rivetti, L. Mol. Biol. 385, 748 (2009)





AFM does not rely on EM radiation to create an image.
It is a mechanical imaging instrument that derives the 3-D profile (topography) and the physical properties of a surface by measuring the INTERACTION FORCES with a scanning, nanometer sized probe.

Single (macro)molecule force spectroscopy: pulling



Force spectroscopy techniques (AFM, optical tweezers) exert and/or quantify forces to allow manipulation and characterization of the mechanical properties, functional state, conformations and interactions of biological systems to molecular resolution.

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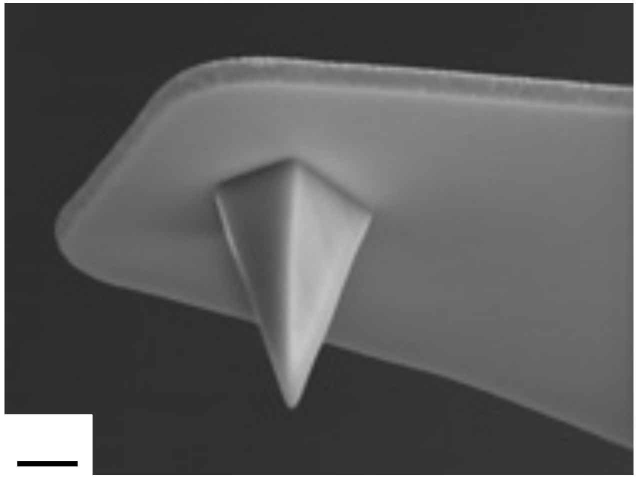
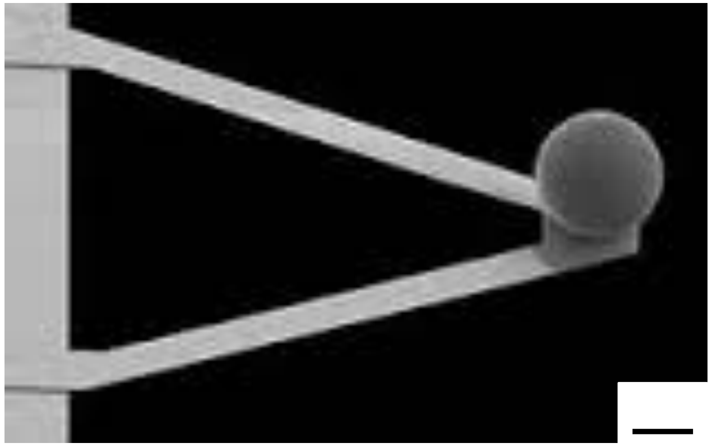
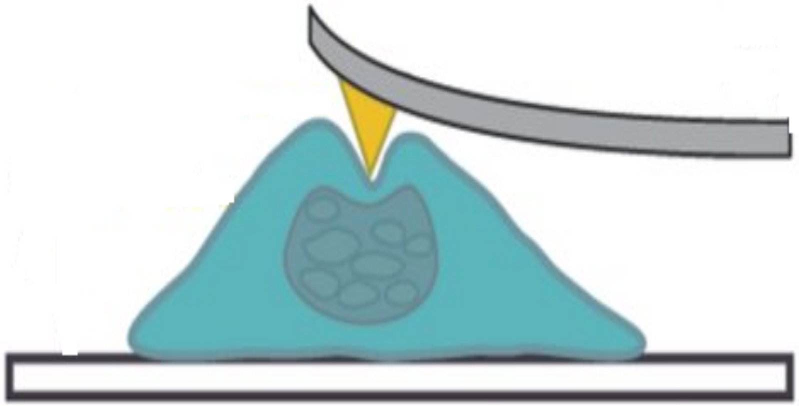
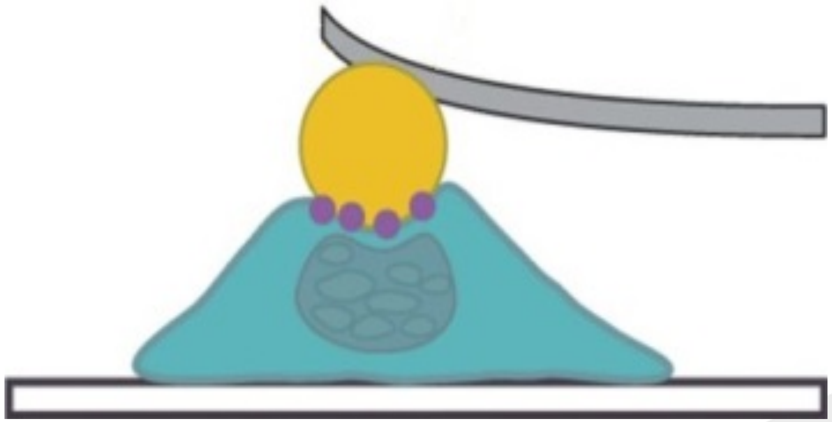
AFM Force-Spectroscopy on cell/tissue (pushing-pulling)

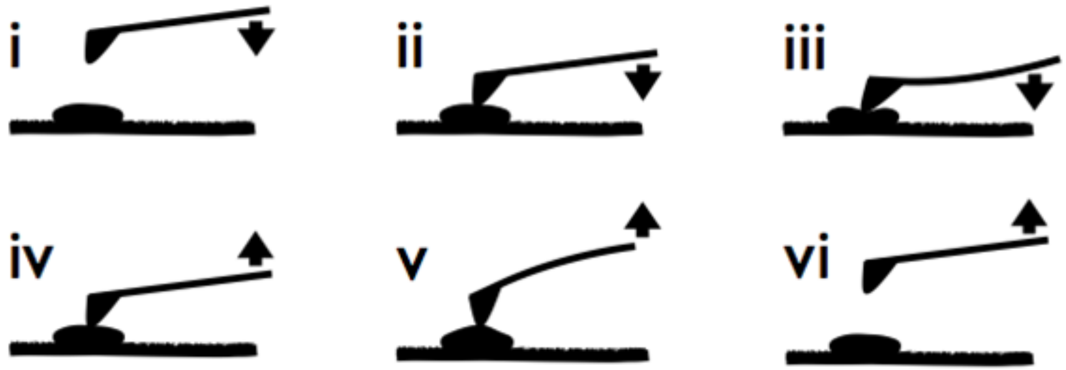


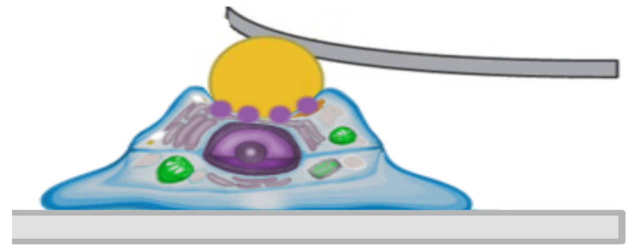






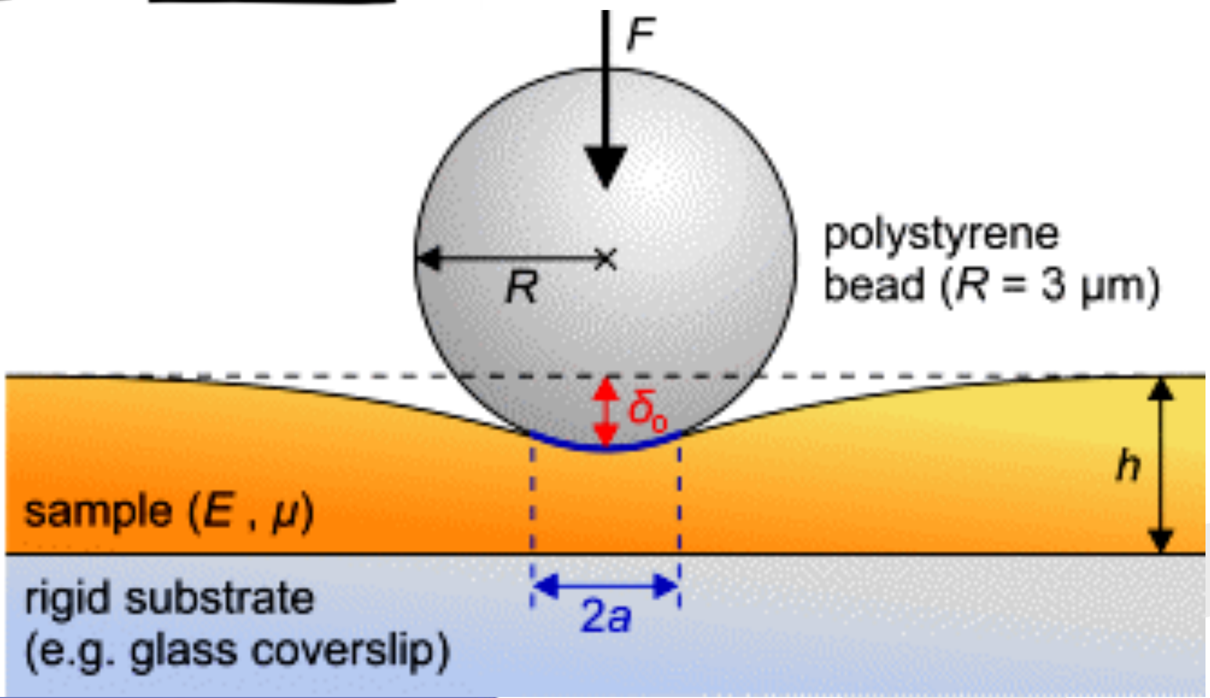
AFM Force-Spectroscopy
measure (bio)material compliance





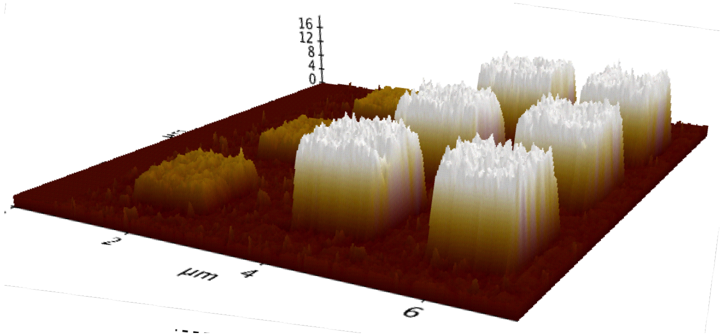
$$F = \frac{4}{3} \frac{E}{1-\mu^2} \sqrt{R \delta_0^3}$$

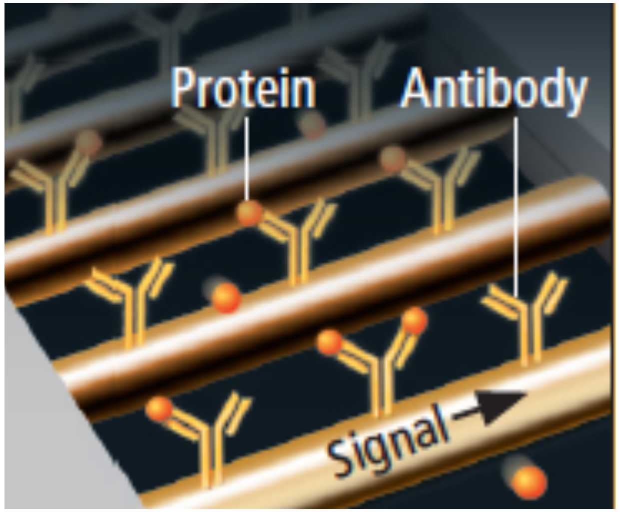
F ... applied force
R ... radius of the probe
δ_0 ... indentation of the sample
E ... elastic modulus
μ ... POISSON's ratio

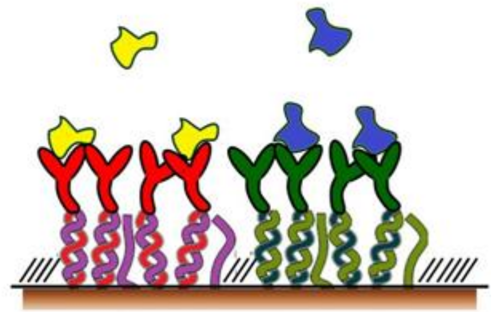


AFM-based Nanoarrays (Nanomedicine)

- Protein nanoarrays for disease detection and monitoring

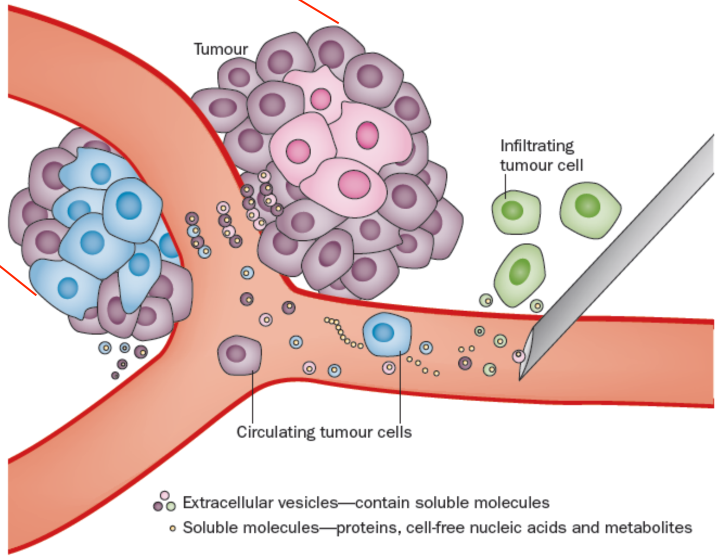


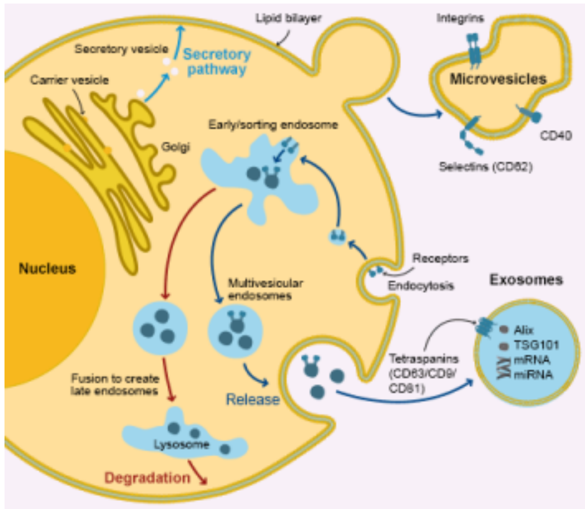




J.R. Heath et al., Sci. Am. 2009

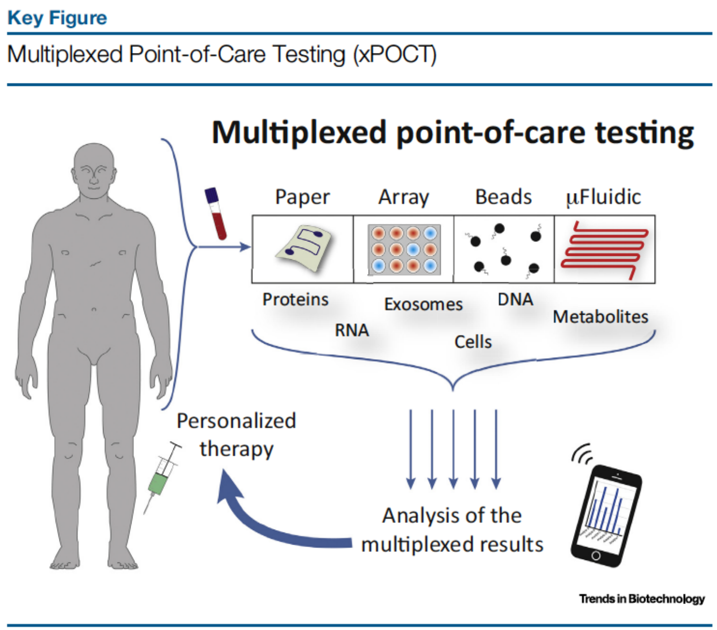
Deviced for nanodiagnostics





Challenges

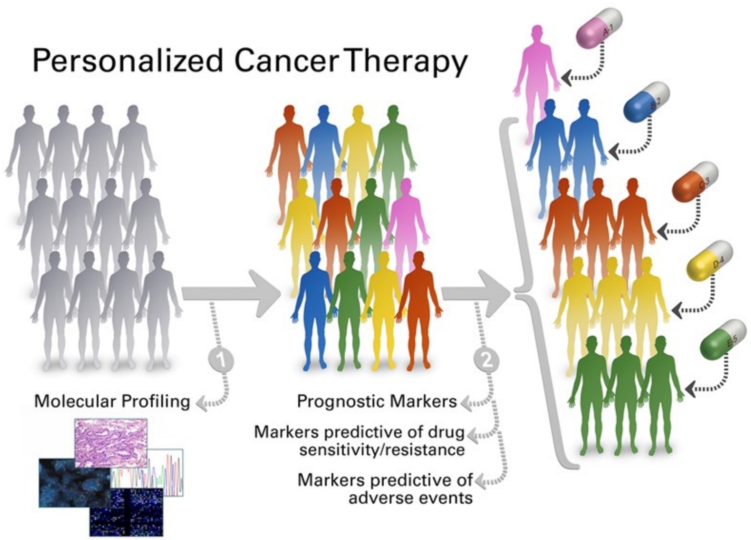
- ✓ Low cost diagnostics for population screening (early detection of tumours, neurodegenerative diseases)



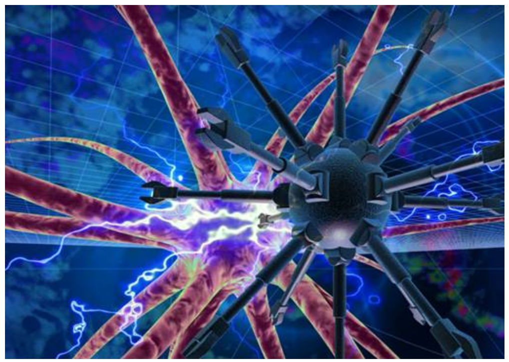
C. Dincer et al., CellPress 2017

- ✓ Personalized therapy

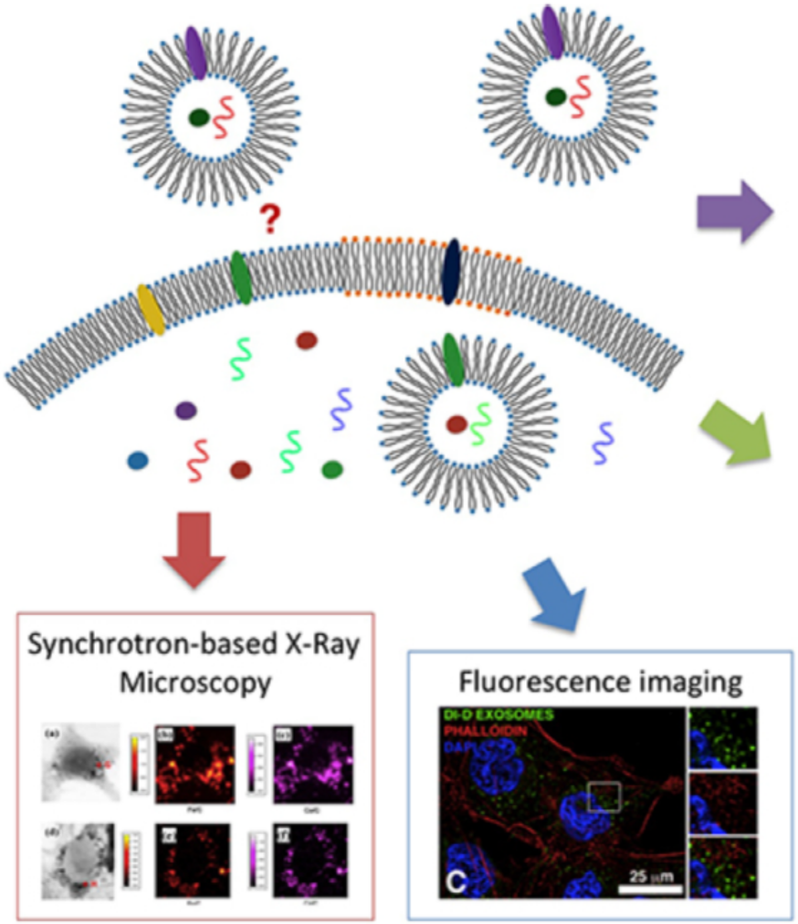
  Digitalization, artificial intelligence, big data

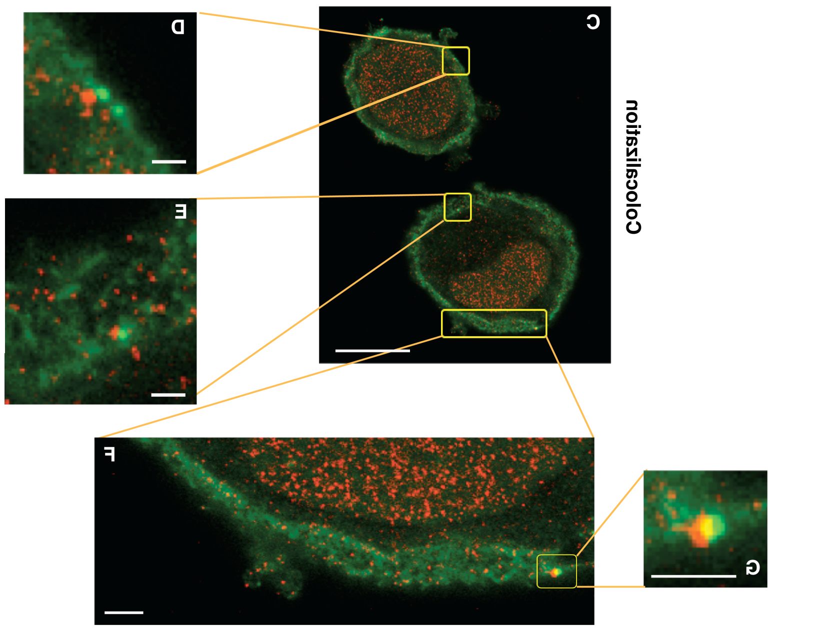


pct.mdanderson.org



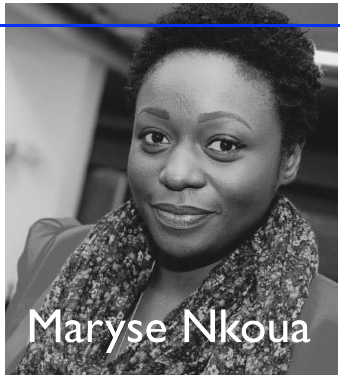
Multi-technique approach





Aknowledge programs to sustain science in Africa, as ICTP Diploma Program, and the ones that promote women in science

https://twas.org/, https://owsd.net/



ORGANIZATION FOR WOMEN IN SCIENCE FOR THE DEVELOPING WORLD

Diploma ICTP CM, PhD Nanotech Univ. Trieste, (2015).
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Thank you!

The Abdus Salam International Centre for Theoretical Physics