Bio & Nano Methods

- Biology, Molecular biology
  - Enzymatic procedures
  - Cell biology
  - Chemistry & Biochemistry
- Nano-scale Analysis & Manipulation
  - Electron microscopy (TEM, SEM)
  - Local probe methods (STM, AFM, SPM)
  - QCM, SPR, SERS, NSOM

Biological Methods

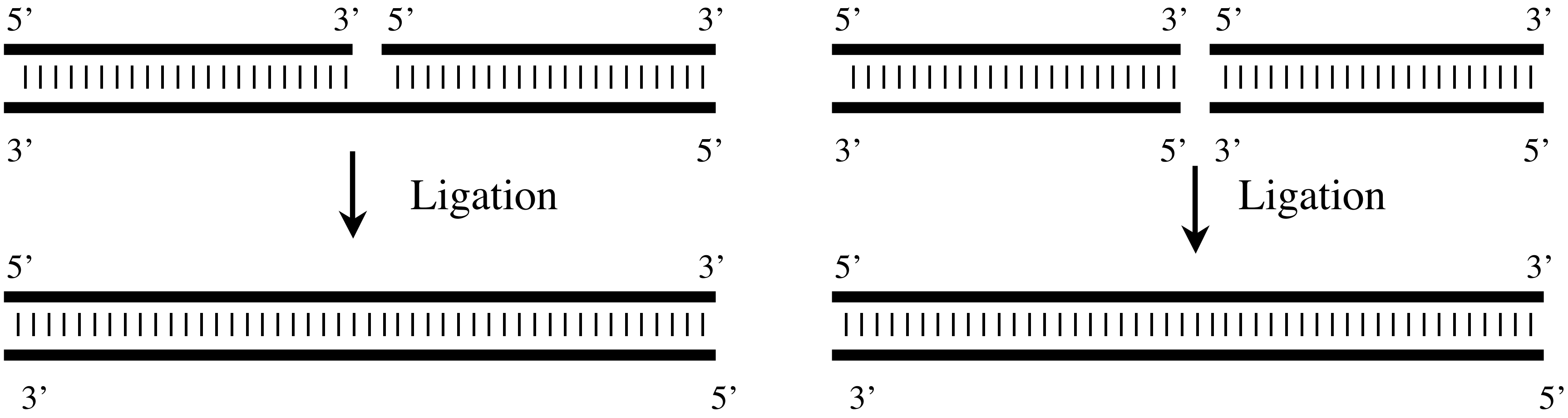
- Enzymes. Biochem. ELISA. IRMA.
- DNA/Protein manipulations.
  - PCR, random and site-specific mutagenesis.
  - Alanine-scanning mutagenesis.
  - Circular permutation.
- Cell/tissue culture methods.
- PAGE, FACS, FRET.
- Microscopy.

DNA modifying enzymes

- Ligase
- Phosphatase, Kinase
- Polymerase (DNA, RNA, RT)
- Nuclease (Endo, Exo)
- Restriction Endonucleases, Methylase
- Topoisomerase, gyrase
- DNA binding and repair enzymes

Ligase

- Repairs nicks in phosphodiester backbone.
- Overlapped or blunt-end.
- 5’ phosphate required.



Phosphatase, Kinase

- Phosphatase removes 5’ phosphate. AlkPhos. CAP.
- Kinase adds 5’ phosphate. Also catalyses phosphate exchange reaction useful for radiolabeling. PNK.

DNA Polymerases

- Replication
- PCR

- Example sources:

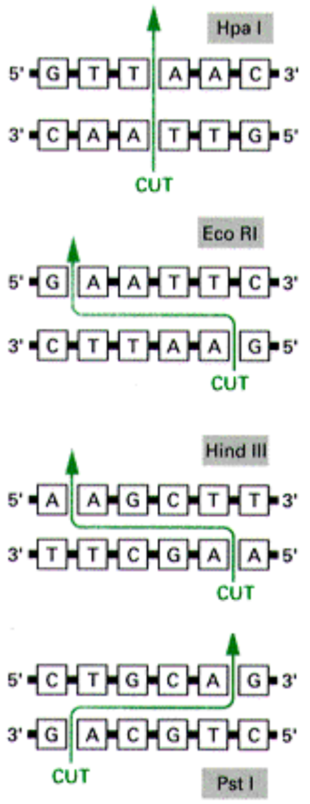
  T7, T4, thermophiles
- Also:

  reverse transcriptase

- 5’ to 3’ DNA synthesis (template and primer required).
- Proofreading:
  - 3’ to 5’ exonuclease activity
  - 5’ to 3’ exonuclease activity

Restriction endonucleases

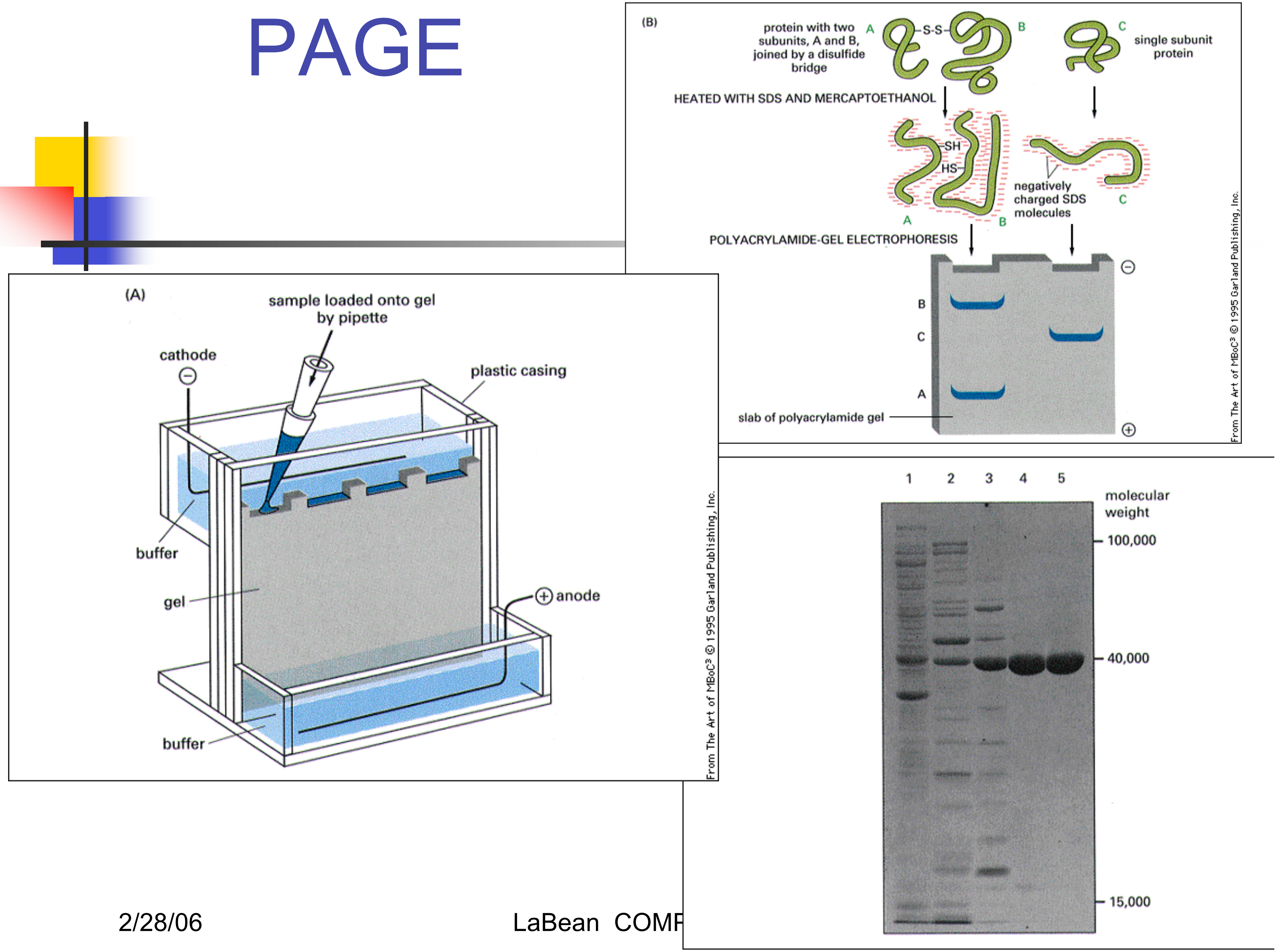
- Discovered by “restriction” of mating types in bacteria.
- “Internal cuts” of DNA phosphodiester backbone.
- Usually palindromic recognition sequences.
- Paired with methylase enzymes to protect DNA from cleavage.



Proteins that act on DNA (cont.)

- Topoisomerase (topo I)
  - Remove DNA supercoils by mechanism involving single-strand break. Tyr-phosphate transesterase.
- Gyrase (e. coli topo II)
  - Introduce supercoiling by 2-strand break mechanism
- Holliday Junction -binding protein
- SS-binding protein
- Rec A
- DNA repair enzymes

PAGE



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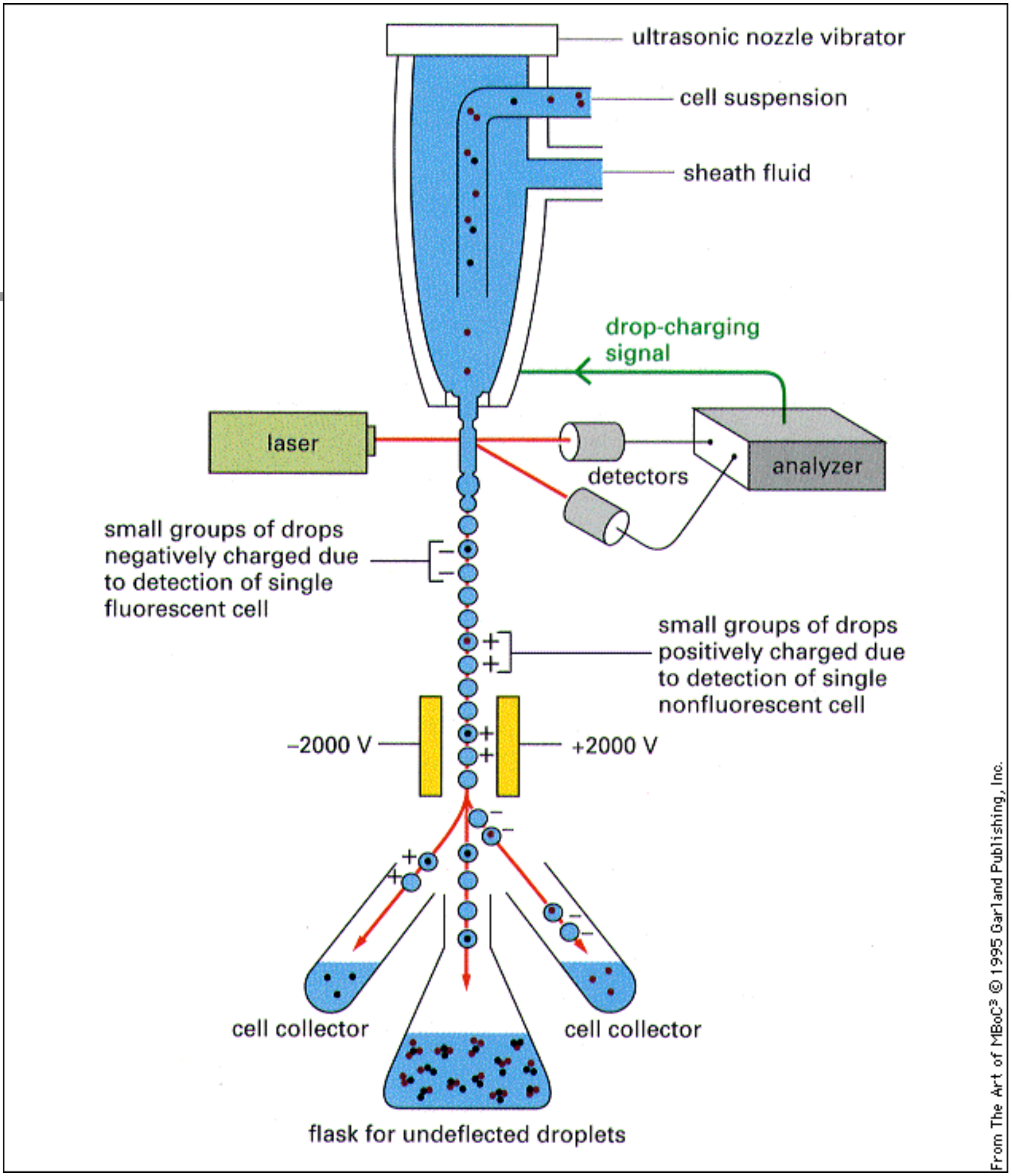
FACS



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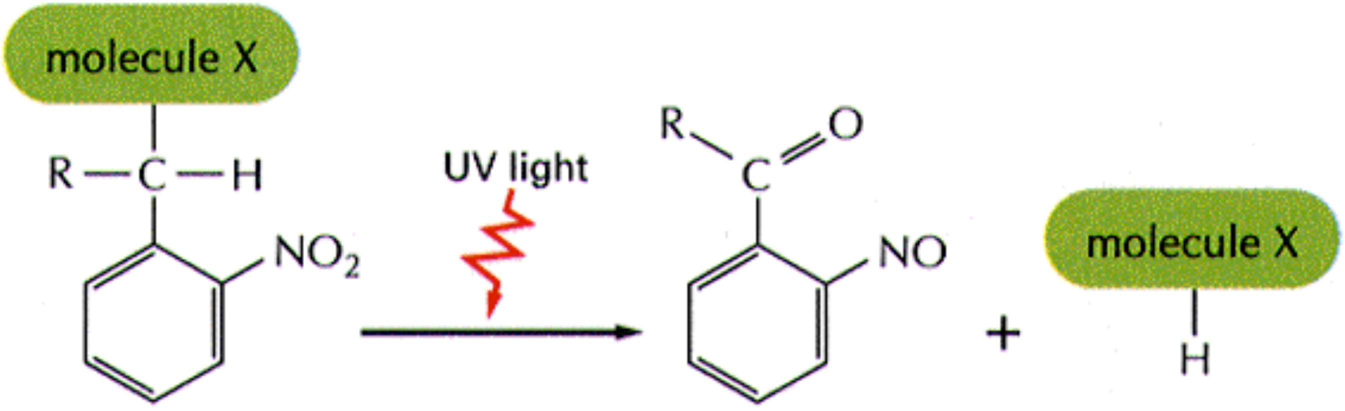
Photochemistry



inactive 2-nitrobenzyl derivative of molecule X (caged X)

2-nitrobenzaldehyde by-product

free, active molecule X

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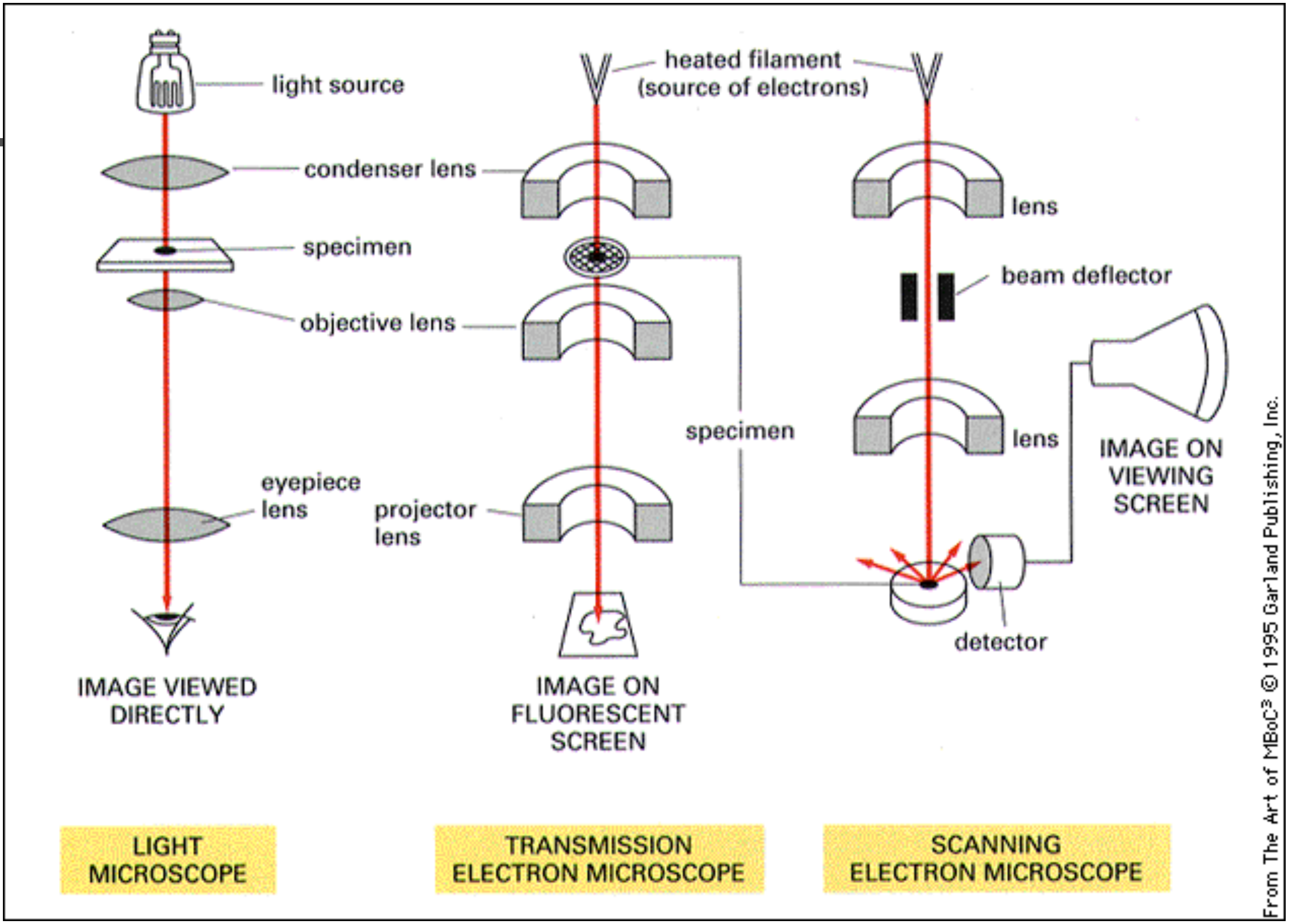
Nano & Surface Methods

- Microscopies
  - Optical, EM, SPM, NSOM
- Nanofabrication
  - Electron beam, dip-pen
  - Imprint stamping
  - Direct write (2D, 3D)
- Other
  - QCM, SPR

Optical Microscope; EM



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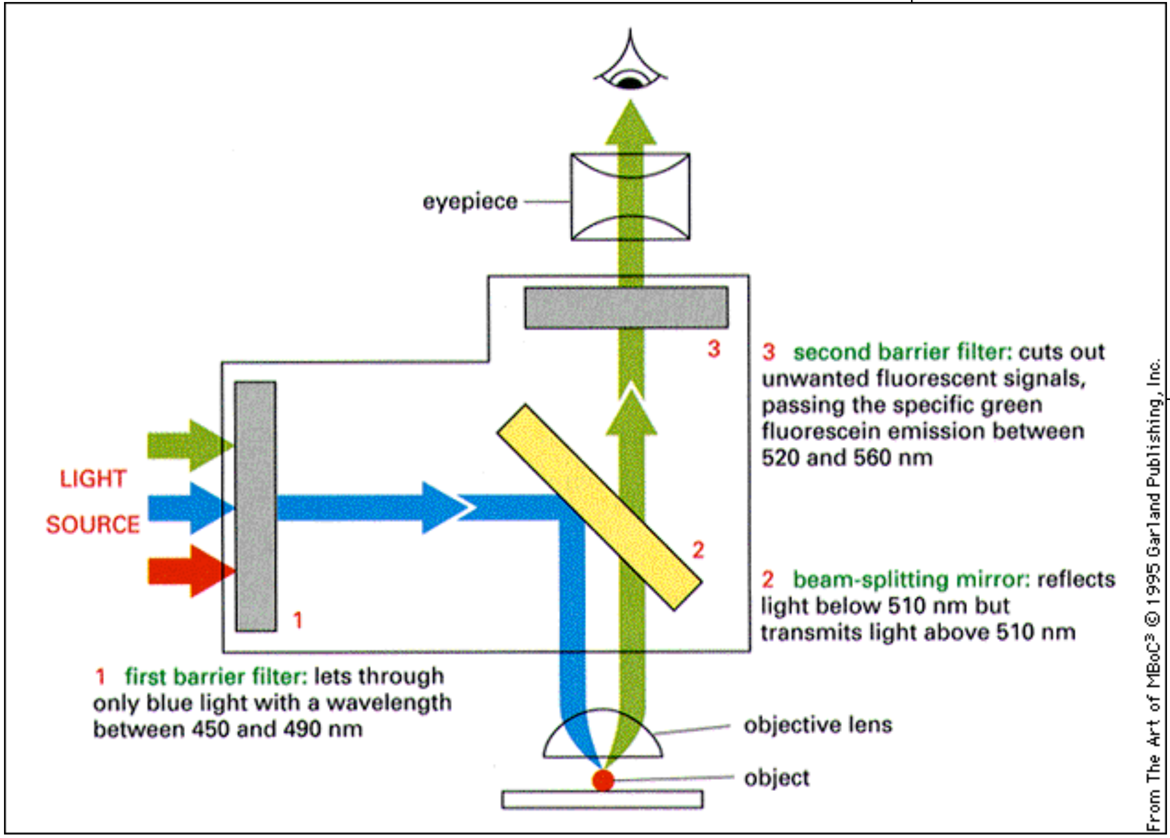
Fluorescence Microscopy



3 **second barrier filter:** cuts out unwanted fluorescent signals, passing the specific green fluorescein emission between 520 and 560 nm

2 **beam-splitting mirror:** reflects light below 510 nm but transmits light above 510 nm

1 **first barrier filter:** lets through only blue light with a wavelength between 450 and 490 nm

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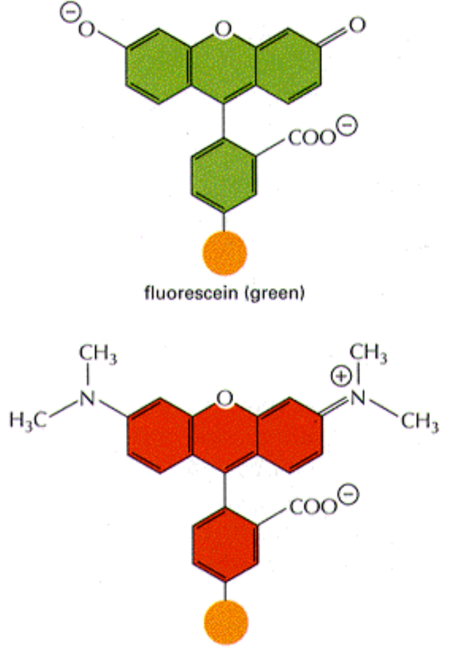

fluorescein (green)

tetramethylrhodamine (red)

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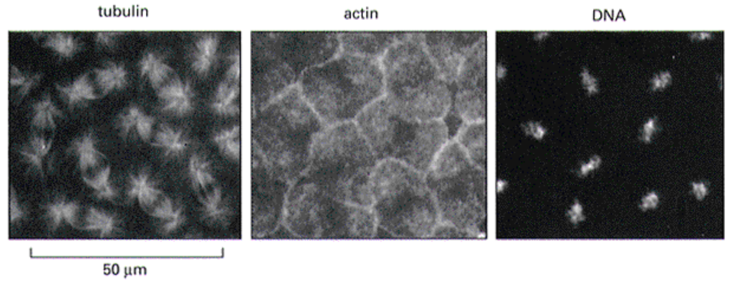

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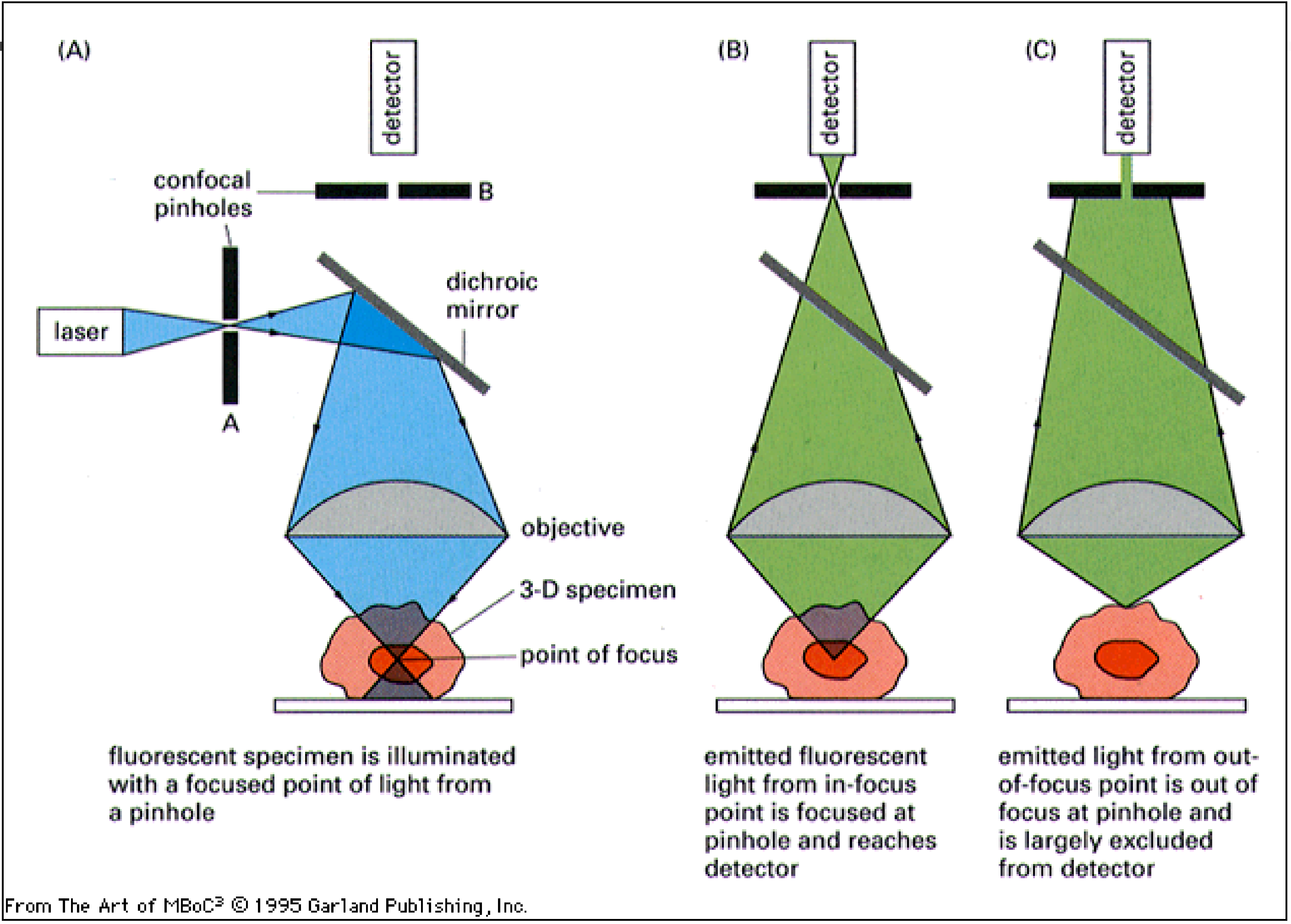
Confocal Microscopy



fluorescent specimen is illuminated with a focused point of light from a pinhole

emitted fluorescent light from in-focus point is focused at pinhole and reaches detector

emitted light from out-of-focus point is out of focus at pinhole and is largely excluded from detector

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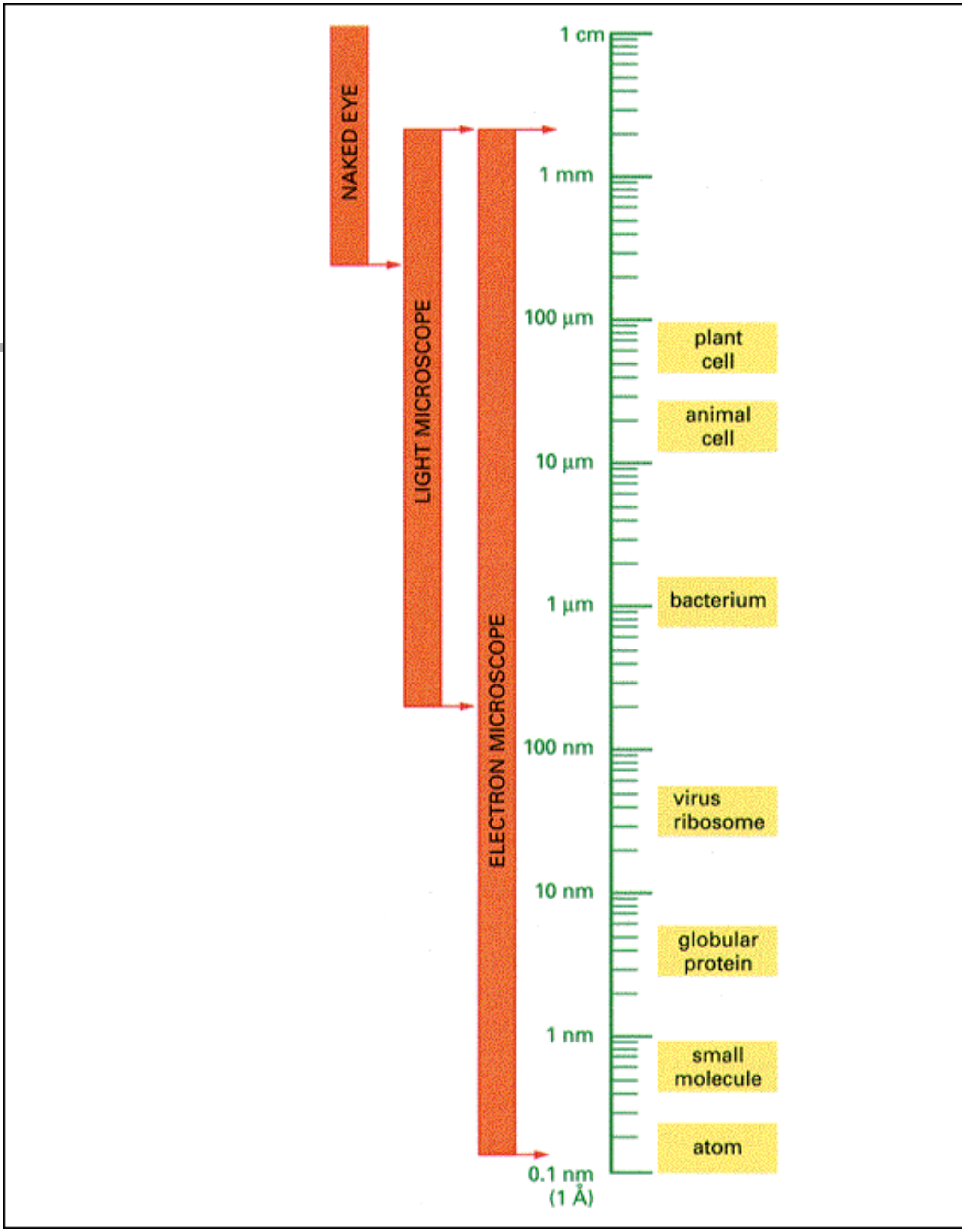
Size scale


NAKED EYE
LIGHT MICROSCOPE
ELECTRON MICROSCOPE
1 cm
1 mm
100 µm
10 µm
1 µm
100 nm
10 nm
1 nm
0.1 nm
(1 Å)
plant cell
animal cell
bacterium
virus
ribosome
globular protein
small molecule
atom

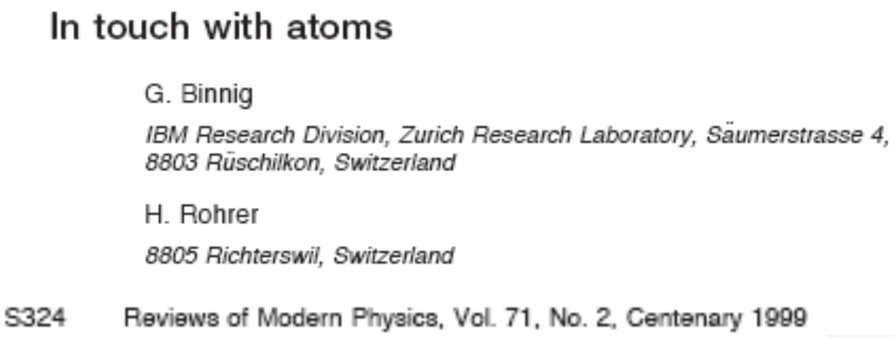
Four major tools for nanotech

STM (Scanning Tunneling Microscope)	(i) imaging (atomic scale resolution) (ii) direct electrical measurement
AFM (Atomic Force Microscope)	(i) imaging, (ii) direct electrical measurement (iii) dip-pen nanolithography
SEM (Scanning Electron Microscope)	(i) imaging, (ii) element analysis (iii) *e-beam lithography*
TEM (Transmission Electron Microscope)	(i) imaging (atomic scale resolution) (ii) element analysis

http://www.microscopy.ethz.ch/methods.htm

Sung Ha Park

STM



In touch with atoms

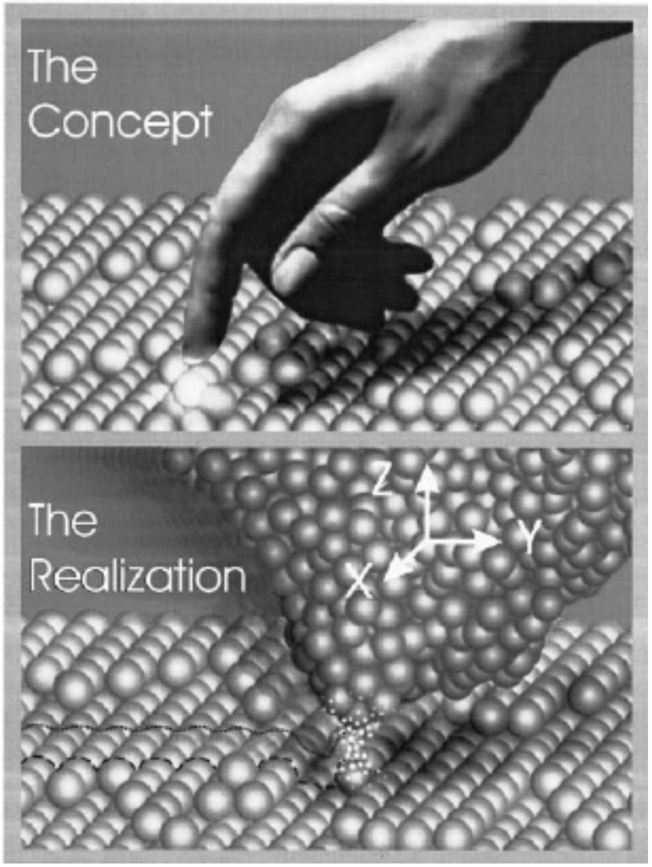
G. Binnig

IBM Research Division, Zurich Research Laboratory, Säumerstrasse 4,
8803 Rüschlikon, Switzerland

H. Rohrer

8805 Richterswil, Switzerland

S324 Reviews of Modern Physics, Vol. 71, No. 2, Centenary 1999



- Local probe. Brought together known materials and techniques.
- Tunneling junction gives atomic resolution.
- Electronic-mechanical hybrid.

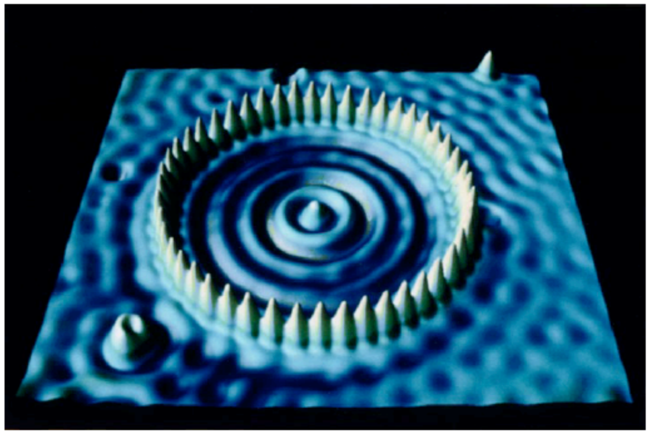
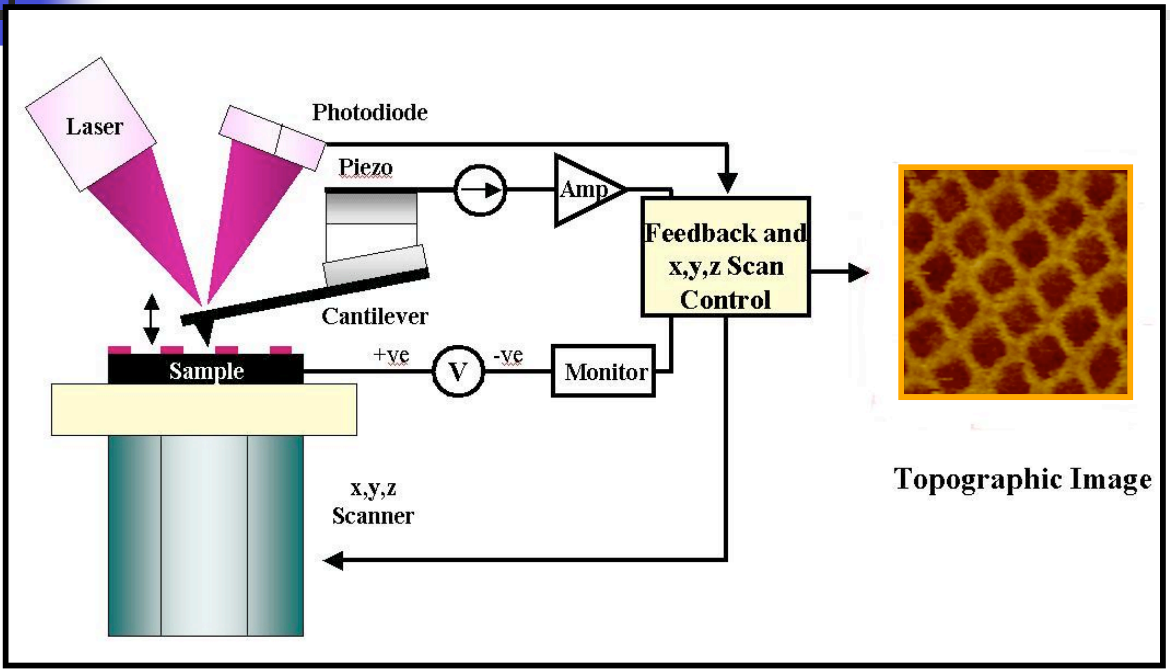
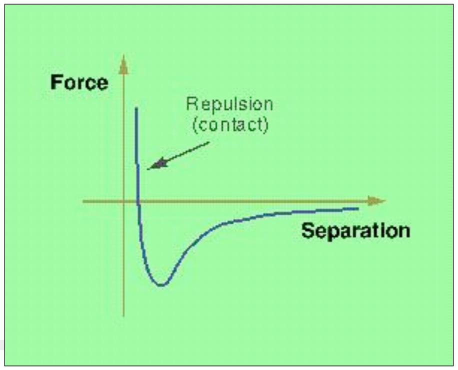


FIG. 2. (Color) STM image of a quantum corral for electrons built with 48 iron atoms on copper. The same tip is used to position the iron atoms into a 12.4-nm-diameter ring and to image them and the wave-structure interior caused by the confined surface-state copper electrons. Courtesy D. Eigler, IBM Research Center, Almaden, CA.

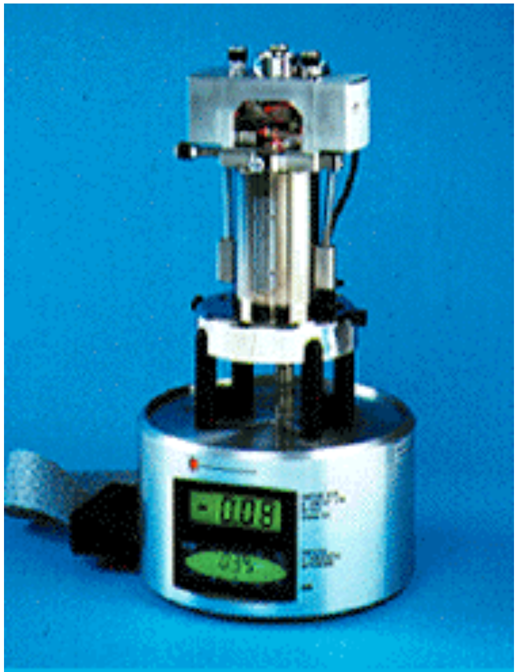
Atomic force microscopy (AFM)



Schematic diagram of AFM



- **Atomic force between AFM tip and specimen**



An AFM scanning head from Digital Instruments.

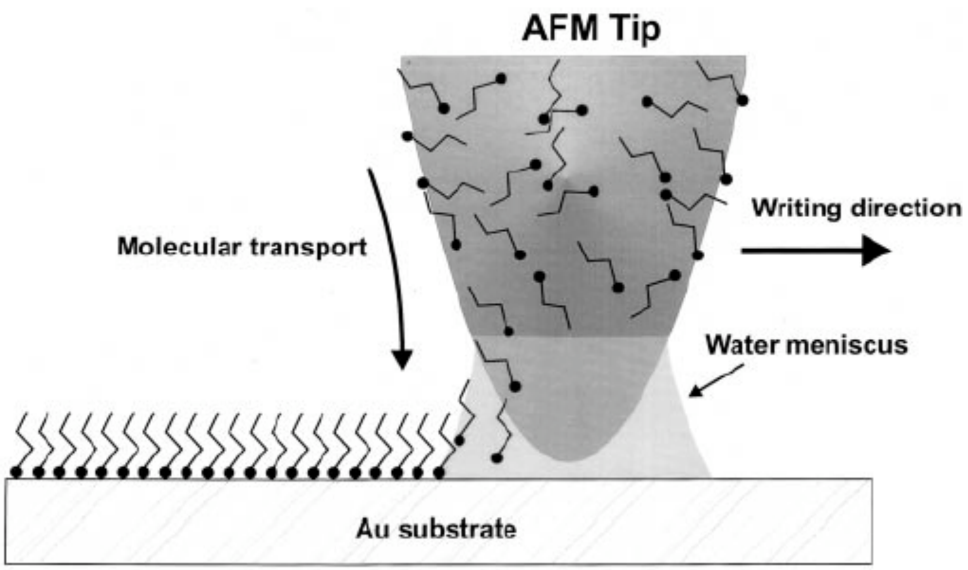
AKA: SPM

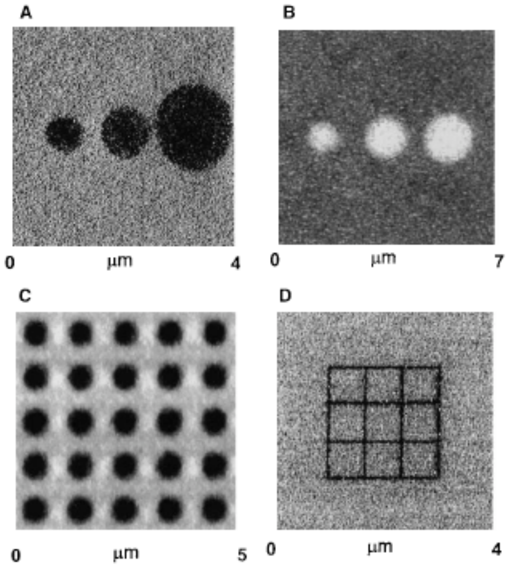
Also note: force spectroscopy, dip-pen nanolithography

Sung Ha Park

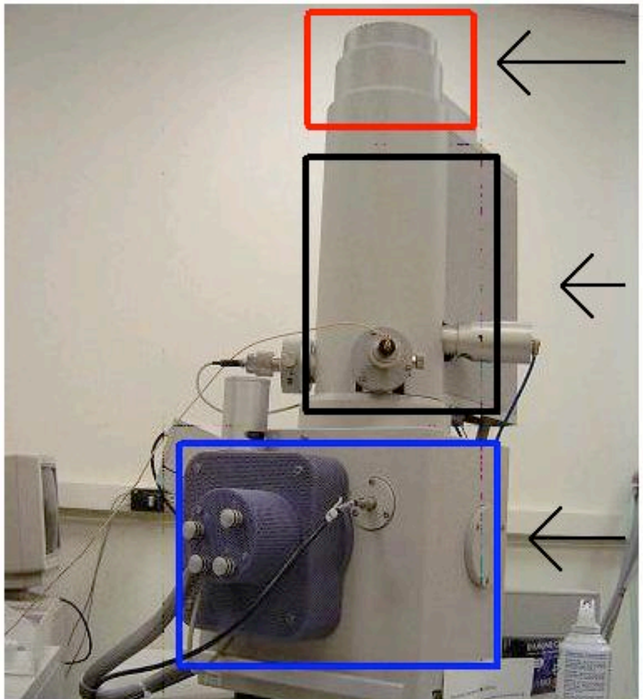
DPN (dip-pen nanolithography)

SCIENCE VOL 283 29 JANUARY 1999





Scanning electron microscopy (SEM)



Electron Gun Unit

Demagnification Unit

Detection Unit

Vac OK 3.0e-6 mBar

1.0 kV

Operate 300 pA

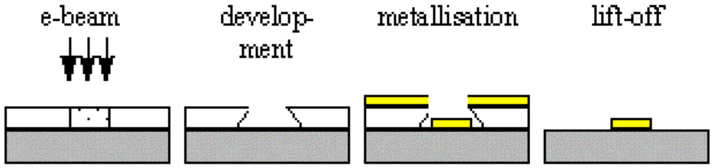
Upper IGP: 6.e-10 mBar

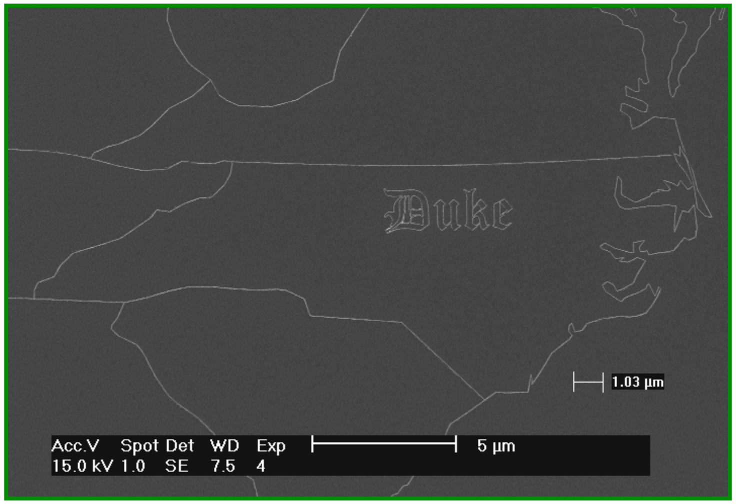
Contrast 36.1

Brightness 31.1

Acc.V 0 V Spot Magn Det CCD WD Exp 0

Electron beam lithography





Acc.V Spot Det WD Exp
15.0 kV 1.0 SE 7.5 4

TEM

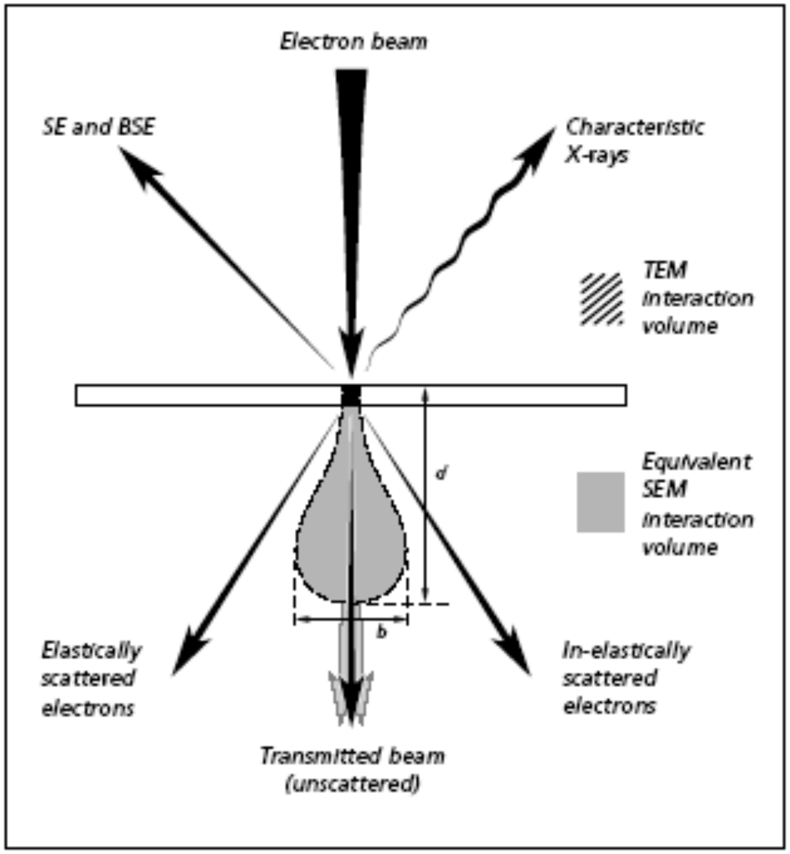


Figure 2.

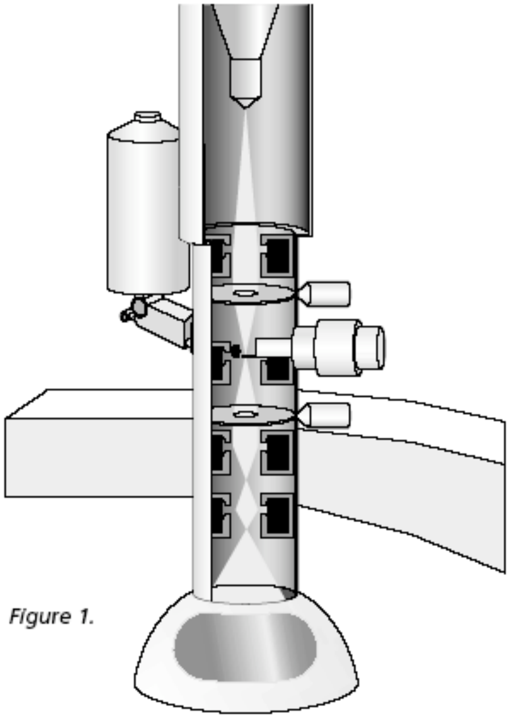
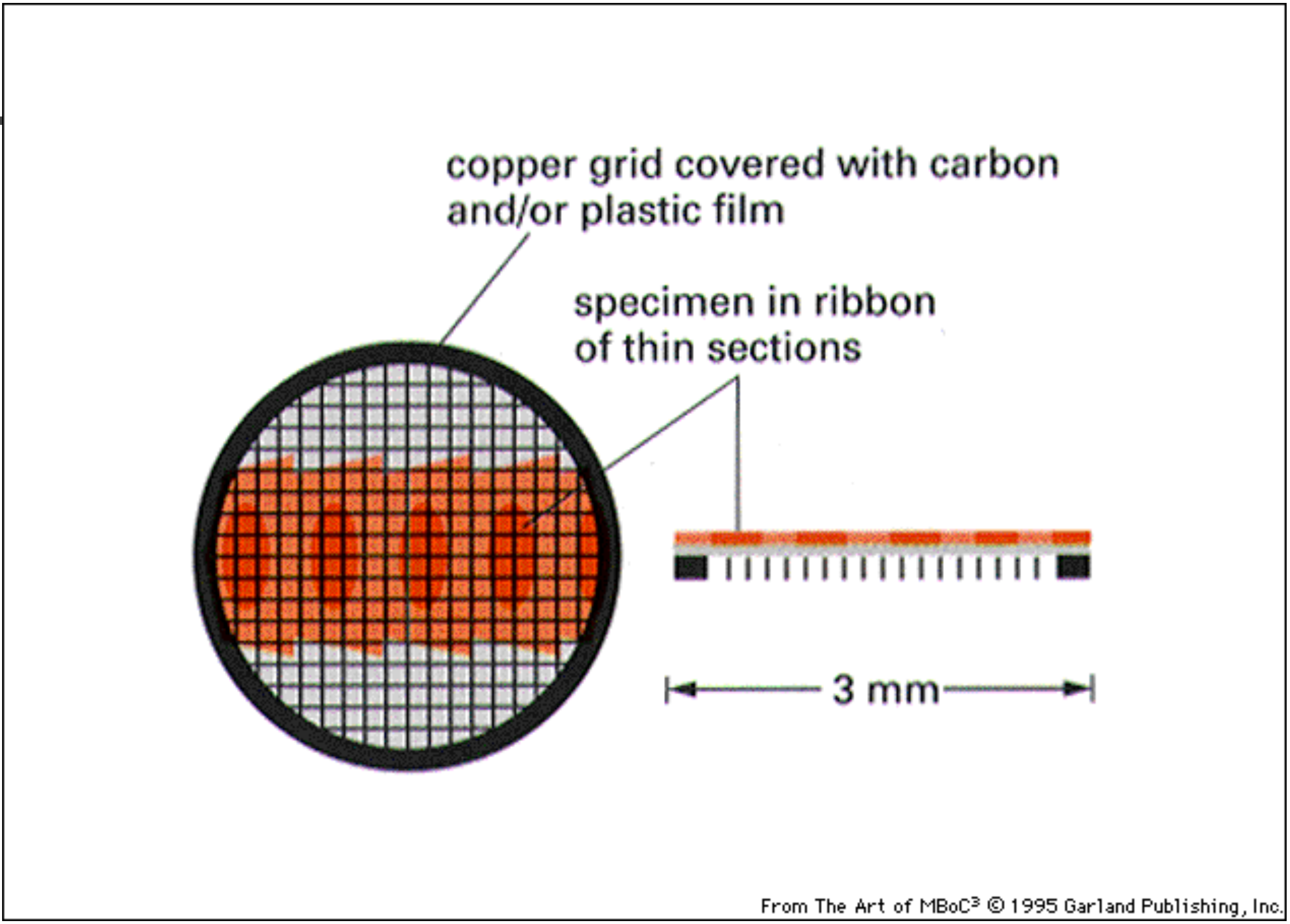


Figure 1.

TEM, sample



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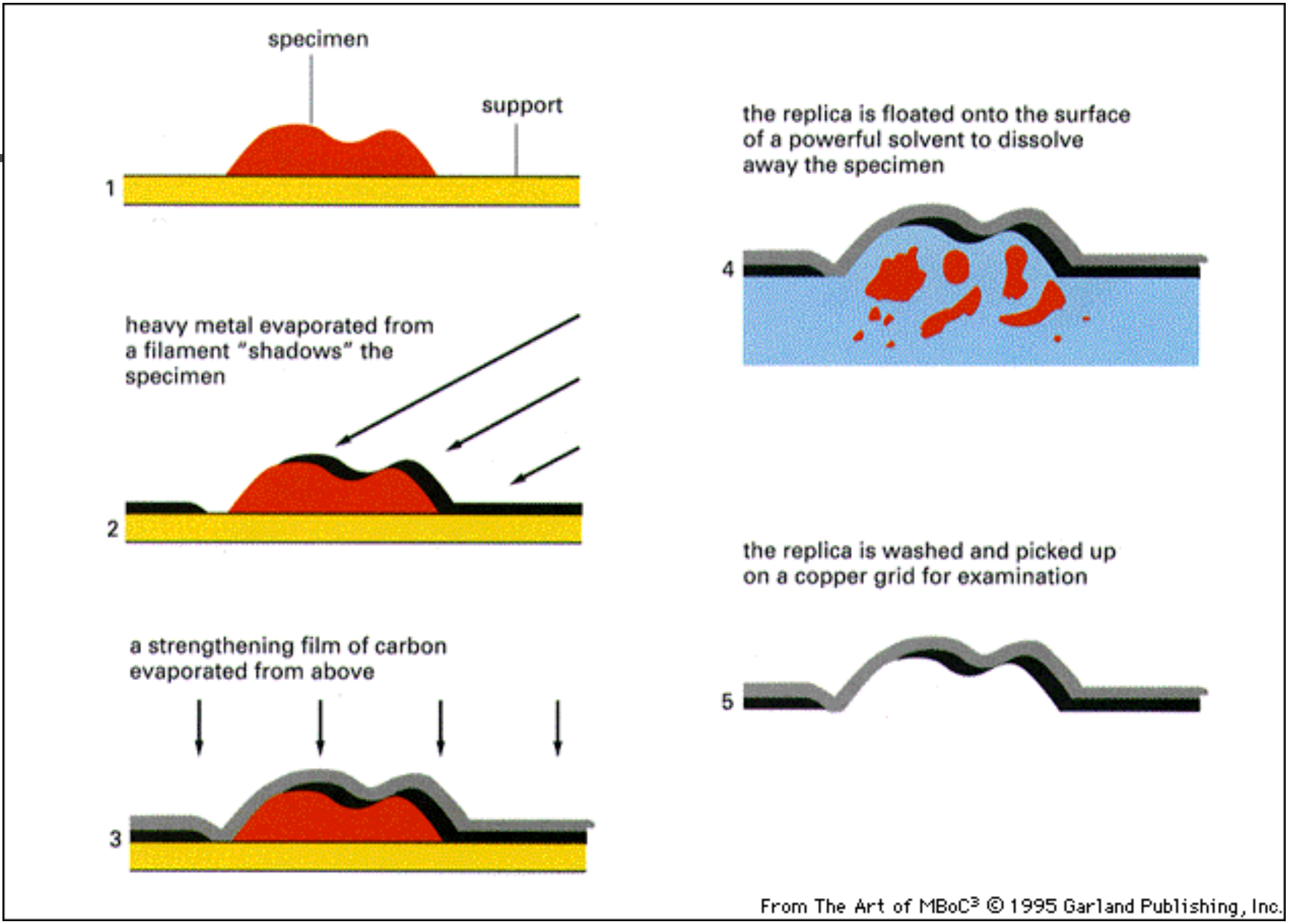
TEM, shadow



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Energy dispersive X-ray microanalysis (EDX, EDXS, EDS)

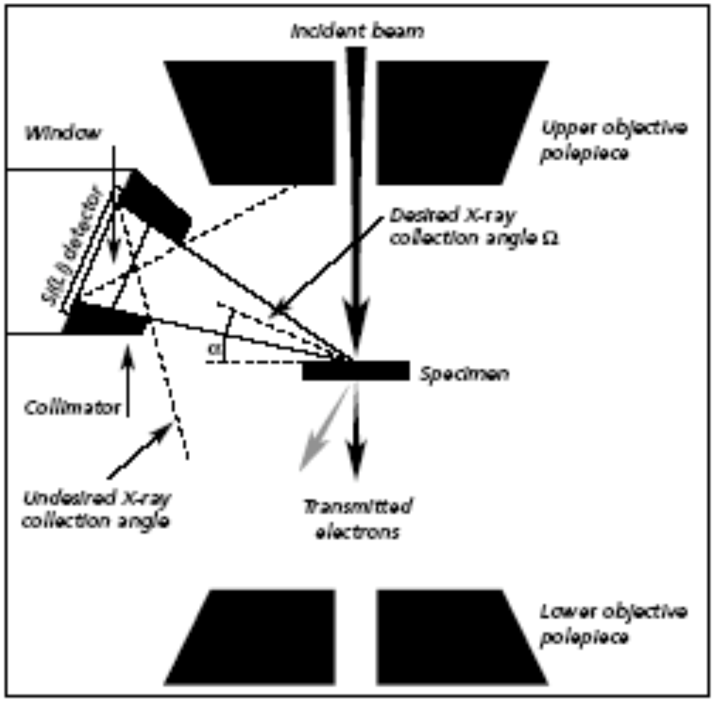


Figure 3. After Williams and Carter (1996)

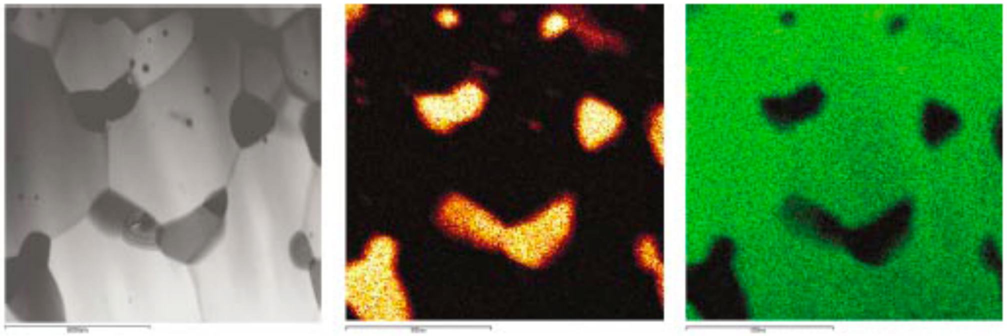


Figure 8a.

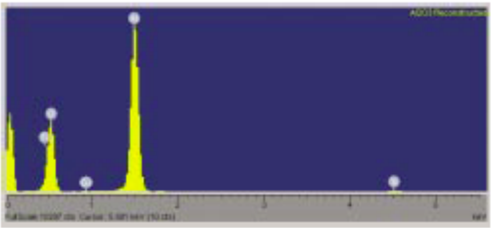


Figure 8b.

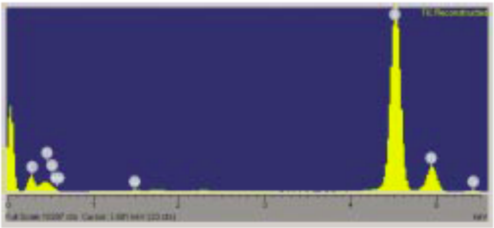


Figure 8c.

The collected datacube contains 2.5 million X-rays and was acquired for 30 minutes. Data from the SmartMap file has been used to reconstruct X-ray maps for the major elements in the sample: including titanium (orange), and aluminium (green) (Figure 8a). Representative spectra describing the different phases clearly identify the aluminum oxide (Figure 8b) and titanium carbide (Figure 8c).

EDX, example

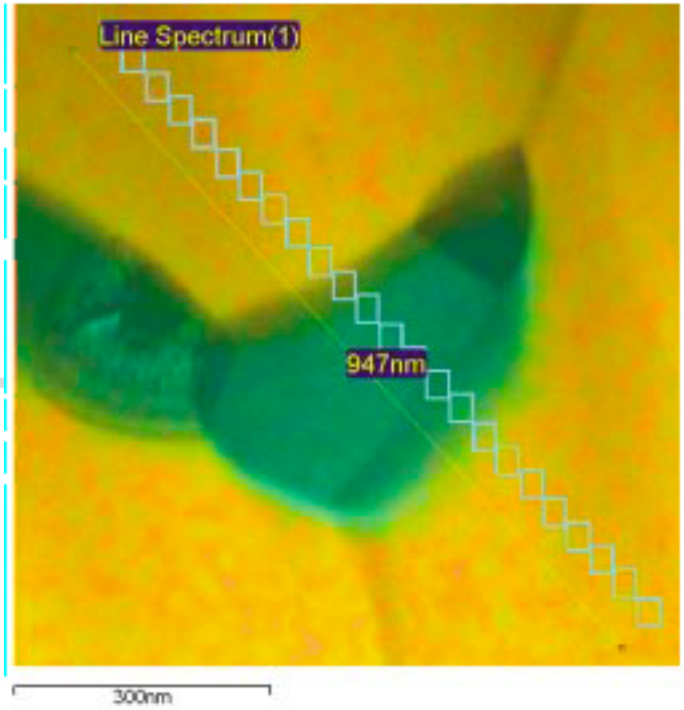


Figure 9.

Spectra have been reconstructed from 20nm square areas across a TiC grain to study the chemistry at grain boundaries in this ceramic material (Figure 9). Using the quantitative linescan software in INCAEnergyTEM, the chemistry variations across these grain boundaries can be determined (Figure 10).

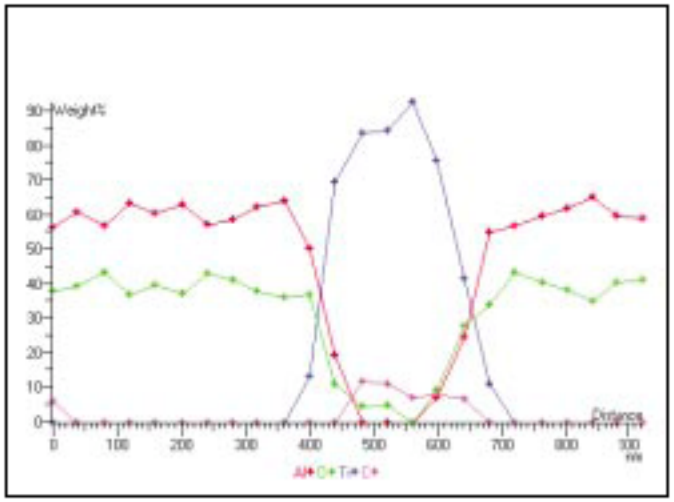
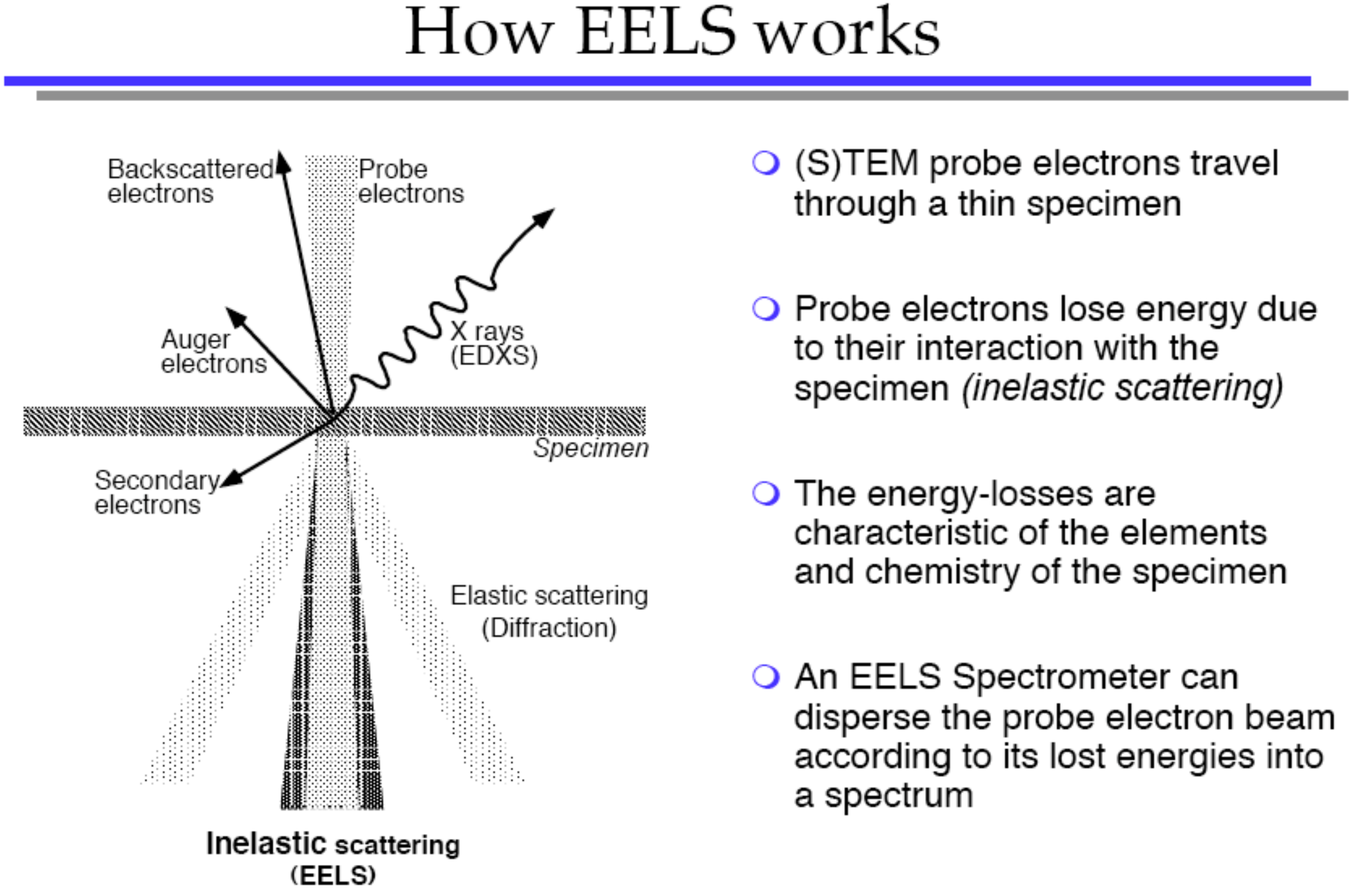


Figure 10.

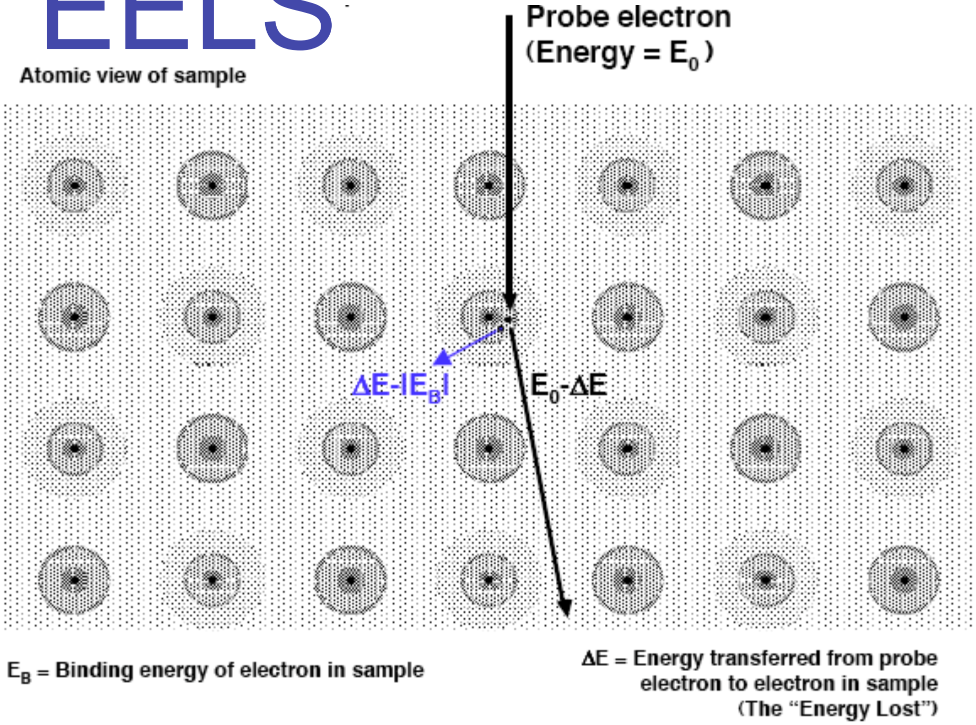
Electron energy loss spectroscopy



How EELS works

- (S)TEM probe electrons travel through a thin specimen
- Probe electrons lose energy due to their interaction with the specimen *(inelastic scattering)*
- The energy-losses are characteristic of the elements and chemistry of the specimen
- An EELS Spectrometer can disperse the probe electron beam according to its lost energies into a spectrum

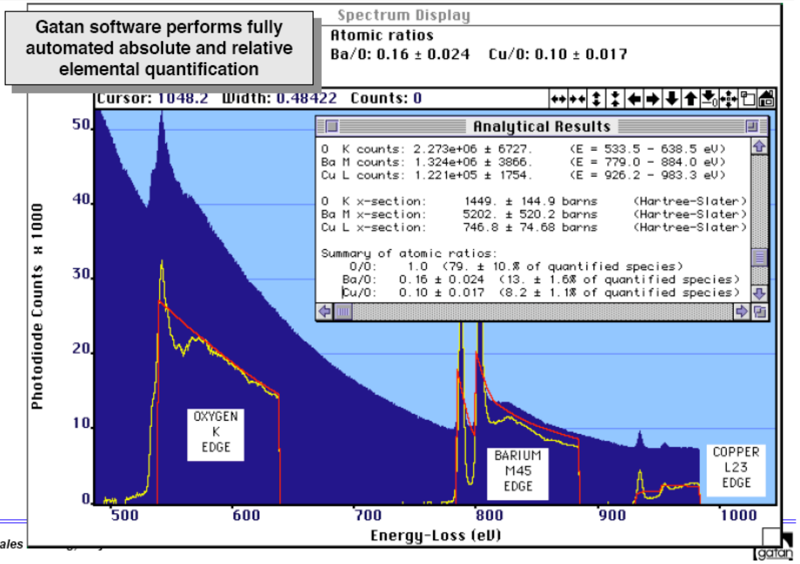
EELS



E_B = Binding energy of electron in sample

ΔE = Energy transferred from probe electron to electron in sample (The “Energy Lost”)

Automated EELS elemental analysis



Gatan software performs fully automated absolute and relative elemental quantification

Spectrum Display

Atomic ratios
Ba/O: 0.16 ± 0.024 Cu/O: 0.10 ± 0.017

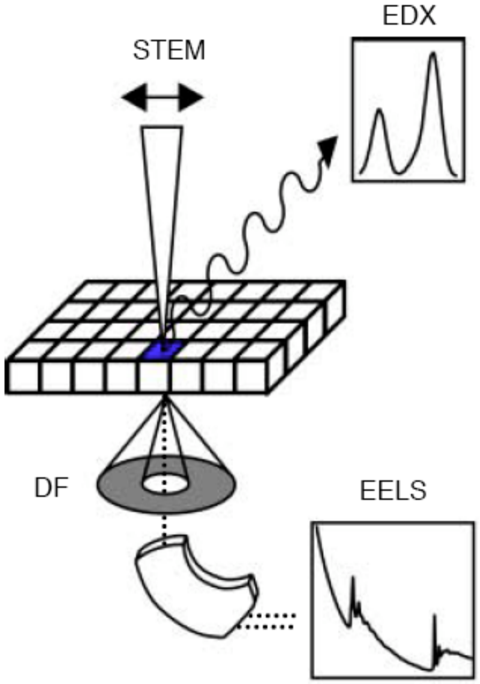
Cursor: 1048.2 Width: 0.48422 Counts: 0

Analytical Results

```
O  K counts: 2.273e+06 ± 6727.     (E = 533.5 - 638.5 eV)
Ba M counts: 1.324e+06 ± 3866.     (E = 779.0 - 884.0 eV)
Cu L counts: 1.221e+05 ± 1754.     (E = 926.2 - 983.3 eV)

O  K x-section:     1449. ± 144.9 barns     (Hartree-Slater)
Ba M x-section:     5202. ± 520.2 barns     (Hartree-Slater)
Cu L x-section:     746.8 ± 74.68 barns     (Hartree-Slater)

Summary of atomic ratios:
    O/O:    1.0  (79. ± 10.% of quantified species)
   Ba/O:   0.16 ± 0.024  (13. ± 1.6% of quantified species)
   Cu/O:   0.10 ± 0.017  (8.2 ± 1.1% of quantified species)
```

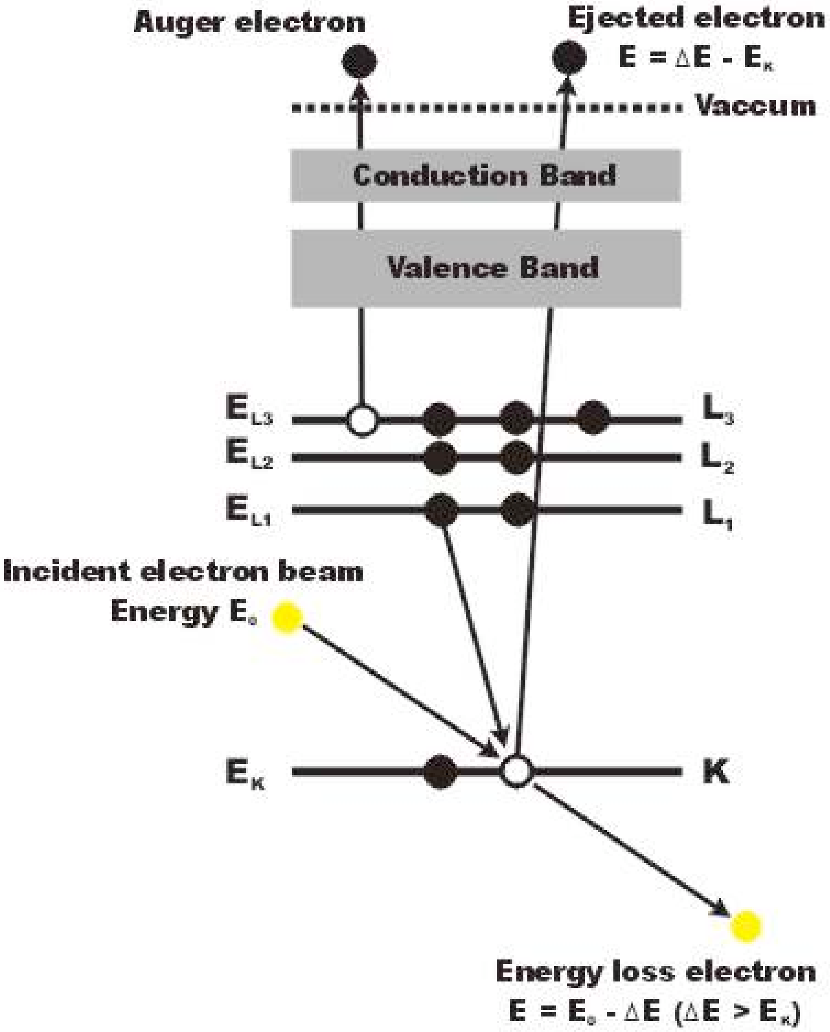


EDXS and EELS comparisons

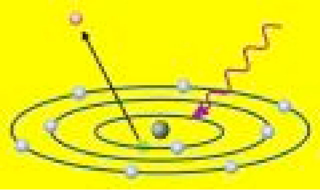
(S)TEM EDS X-ray	**(S)TEM EELS**
X-rays provide elemental information only	EELS provides elemental, chemical & dielectric information
Inefficient signal collection; inefficient low Z signal generation & detection ○ Slow mapping or poor S/N	Very efficient in all aspects ○ Higher sensitivity to most elements ○ Very fast mapping technique
X-ray spectra can contain artifact information from column and other parts of sample	EELS information is highly localized and does not contain sample or column artifacts
High detection efficiency for higher Z elements ○ Poor sensitivity to Z<10	High detection efficiency for lower Z elements ○ Poor sensitivity to a few high Z elements
Energy resolution > 120eV causes frequent overlaps	Energy resolution 0.3-2eV gives far fewer overlaps (overlaps when edges ~<30eV apart)
No sample thickness limitations	Sample thickness is important - should be less than ~100nm @ 200keV

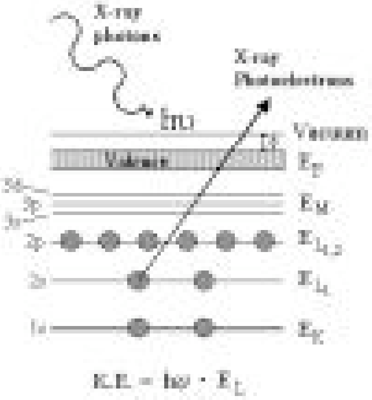
Auger Electron Spectroscopy

- 1. Ionization: A hole in an inner shell (here: K shell) is generated by an incident high-energy electron that loses the corresponding energy E transferred to the ejected electron.
- 2. Auger-electron emission: The hole in the K shell is filled by an electron from an outer shell (here: L1). The superfluous energy is transferred to another electron (here: L3) which is subsequently ejected as Auger electron.
- Since Auger electrons have an energy in the range of some 100 eV to a few KeV, they are strongly absorbed by the specimen. Consequently, only Auger electron from the surface can be measured, making Auger spectroscopy a surface method.

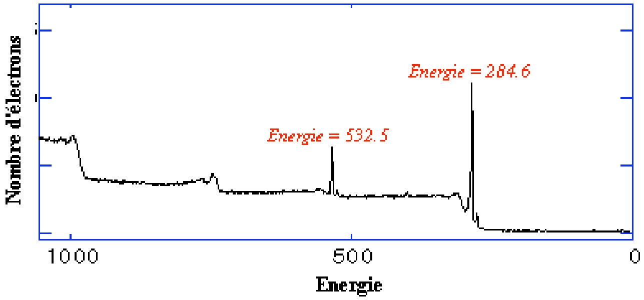


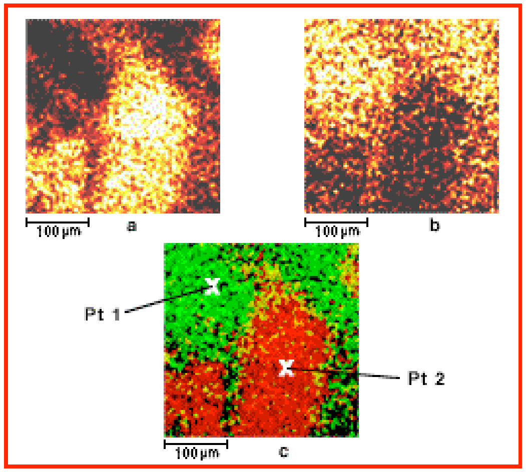
XPS





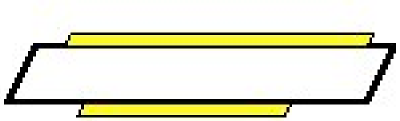
- X-ray Photoelectron Spectroscopy (XPS) is able to analyze the chemical composition of various material surfaces up to 1 nm depth.
- X-ray bombardment of surface leads to electron ejection. Detectors analyze the characteristic energy of the electrons and determine the type of atom (and its chemical state) from which it came.
- Images with tens of microns resolution.





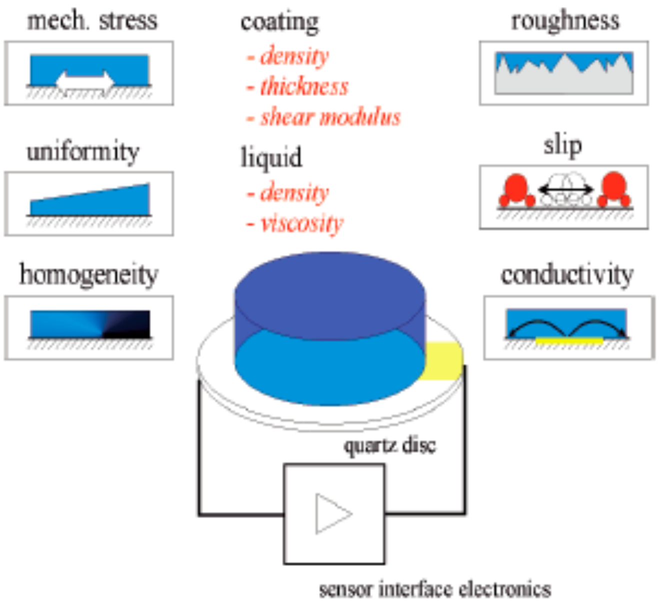
QCM

Quartz crystal microbalance.



Piezoelectric quartz microbalance: A quartz disk ~1 cm in diameter and ~.1 mm thick with circular gold electrodes in the center of each face. When an A.C. voltage is applied across the electrodes the crystal oscillates in a thickness shear mode in which the two faces move parallel to each other and in opposite directions.

Used in this way the quartz becomes a mechanical resonator. Any adsorbed film on the electrodes will make the resonator heavier and so lower it’s resonant frequency. A film thickness measurement is made by monitoring this frequency shift. The crystals used for these experiments were usually driven at their third harmonic ($\mathbf{f}_{o,3} \sim 5.5$ MHz).



Influences on the behaviour of quartz crystals

SPR

(Biacore)

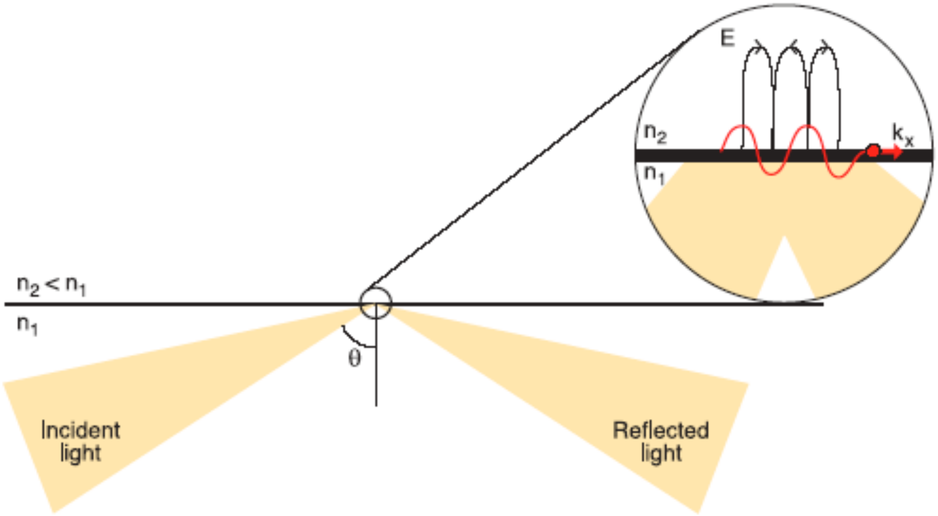
Surface Plasmon Resonance.

- Kinetic Rate Analysis:
- How FAST?
  - k_a, k_d
  - $K_D = k_d/k_a$, $K_A = k_a/k_d$

Concentration Analysis:
How MUCH?
Active Concentration
Solution Equilibrium
Inhibition

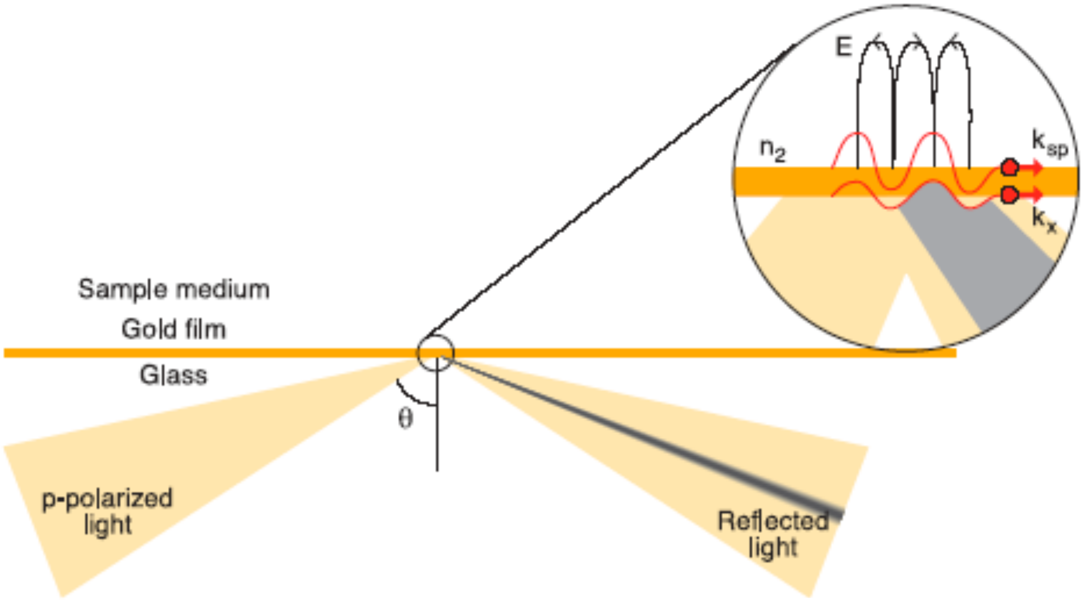
Affinity Analysis: HOW STRONG?
K_D, K_A
Relative Ranking

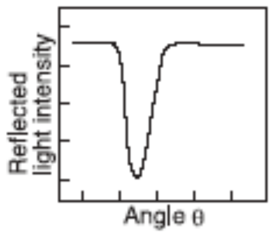
- Yes/No Data
  - Ligand Fishing

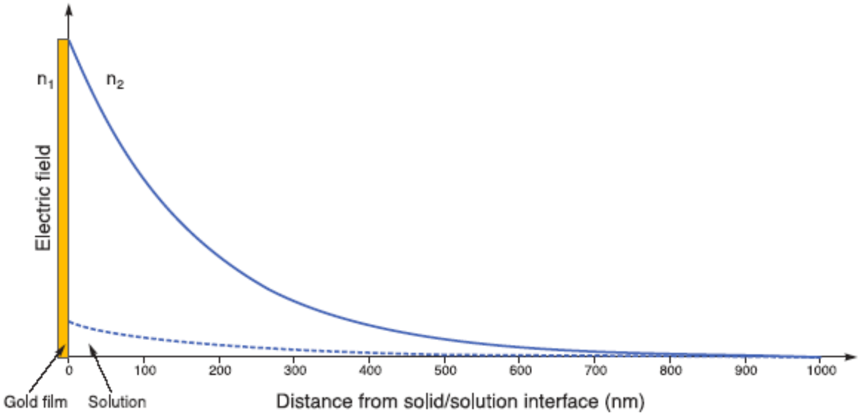


Total internal reflectance (TIR) at interface of differing refractive indices. Angle of incidence above a critical value get TIR. Evanescent field (E) is setup.

TIR / SPR


E
n_2
k_{sp}
k_x
Sample medium
Gold film
Glass
θ
p-polarized light
Reflected light


Reflected light intensity
Angle θ


Electric field
n_1
n_2
0
100
200
300
400
500
600
700
800
900
1000
Gold film
Solution
Distance from solid/solution interface (nm)

Biacore's proprietary SPR technology

- Non-label
- Real-time
- Unique, high quality data on molecular interactions
- Simple assay design
- Robust and reproducible
- Walk-away automation
- Small amount of sample required

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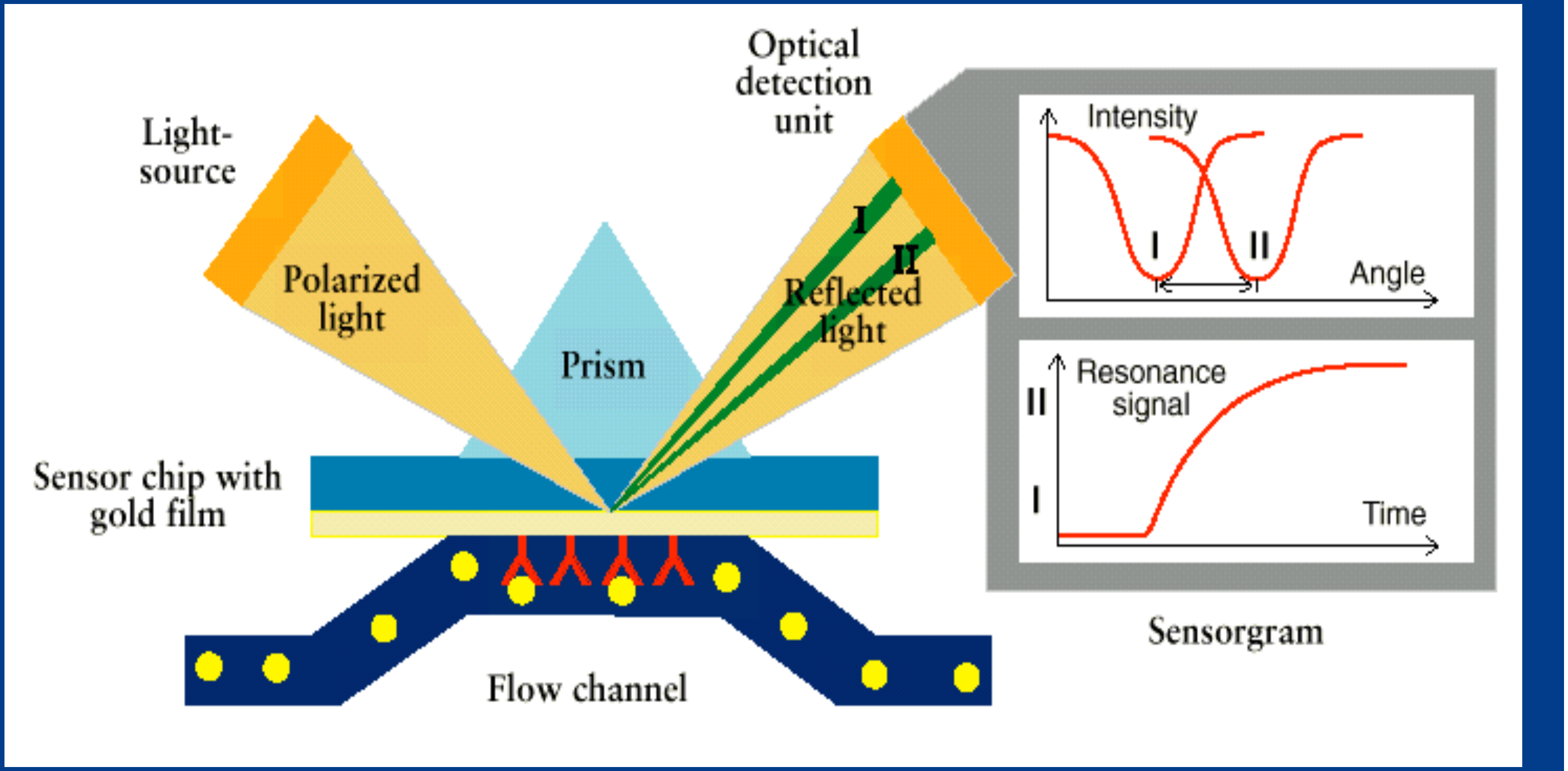
Biomolecular Binding in Real Time

Principle of Detection



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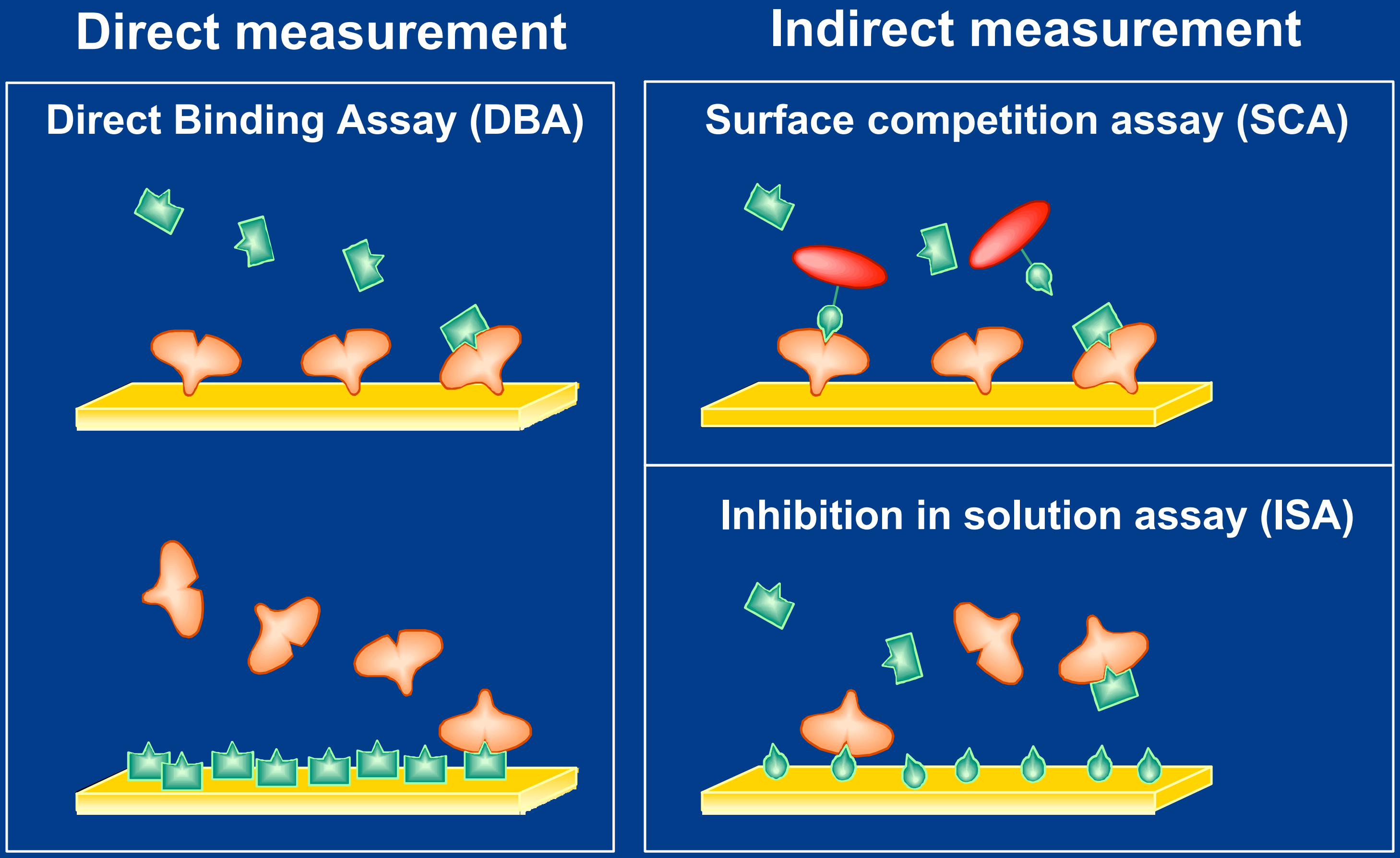
Flexibility in Assay Design

- Multiple assay formats providing complementary data



Direct measurement

Direct Binding Assay (DBA)

Indirect measurement

Surface competition assay (SCA)

Inhibition in solution assay (ISA)

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SERS:
Surface Enhanced Raman Spectroscopy

- **The Raman Effect.** When light is scattered from a molecule most photons are elastically scattered. The scattered photons have the same energy (frequency) and wavelength as the incident photons. However, a small fraction of light (approximately 1 in 10^7 photons) is scattered at optical frequencies different from, and usually lower than, the frequency of the incident photons. The process leading to this inelastic scatter is the termed the Raman effect. Raman scattering can occur with a change in vibrational, rotational or electronic energy of a molecule. Chemists are concerned primarily with the vibrational Raman effect. The difference in energy between the incident photon and the Raman scattered photon is equal to the energy of a vibration of the scattering molecule. A plot of intensity of scattered light versus energy difference is a Raman spectrum.
- **Resonance-Enhanced Raman Scattering.** Raman spectroscopy is conventionally performed with green, red or near-infrared lasers. The wavelengths are below the first electronic transitions of most molecules, as assumed by scattering theory. The situation changes if the wavelength of the exciting laser within the electronic spectrum of a molecule. In that case the intensity of some Raman-active vibrations increases by a factor of 10^2-10^4. This resonance enhancement or resonance Raman effect can be quite useful.
- **Surface-Enhanced Raman Scattering.** The Raman scattering from a molecule adsorbed on or even within a few Angstroms of a structured metal surface can be 10^3-10^6X greater than in solution. This surface-enhanced Raman scattering is strongest on silver, but is observable on gold and copper. SERS arises from
  - 1) enhanced electromagnetic field at the surface of the metal (wavelength of the incident light is close to the plasma wavelength of the metal, conduction electrons in the metal surface are excited into an extended surface electronic excited state called a surface plasmon resonance). Molecules adsorbed or in close proximity to the surface experience an exceptionally large electromagnetic field. Vibrational modes normal to the surface are most strongly enhanced.
  - 2) formation of a charge-transfer complex between the surface and analyte molecule. The electronic transitions of many charge transfer complexes are in the visible, so that resonance enhancement occurs. Molecules with lone pair electrons or pi clouds show the strongest SERS.