Radiation based Microscopy

Marco Salerno

Outline

- conventional optical microscopy
- electron microscopy
- Near Field optical microscopy
- comparison with other “indirect” microscopies

Microscopy

object:

• visual description of nature

"At a microscope the surface of objects is seen, magnified and made clearer, yet do not think that you're seeing their intimate essence",

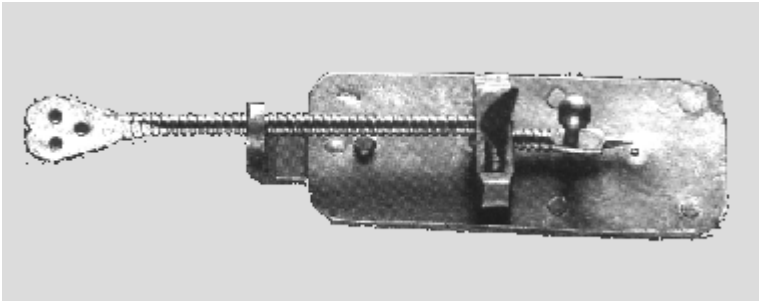
Feng-shen Yin-Te (1771-1810)

applications:

- medicine, biology (cells, bacteria, virus, colloidal particles, …)
- geology (crystals, …)
- material science (phases, particles,
- chemistry, physics (molecules, atoms, …)

after the telescope (Galileo, early 1600), Anton van Leeuwenhoek (~mid 1600):

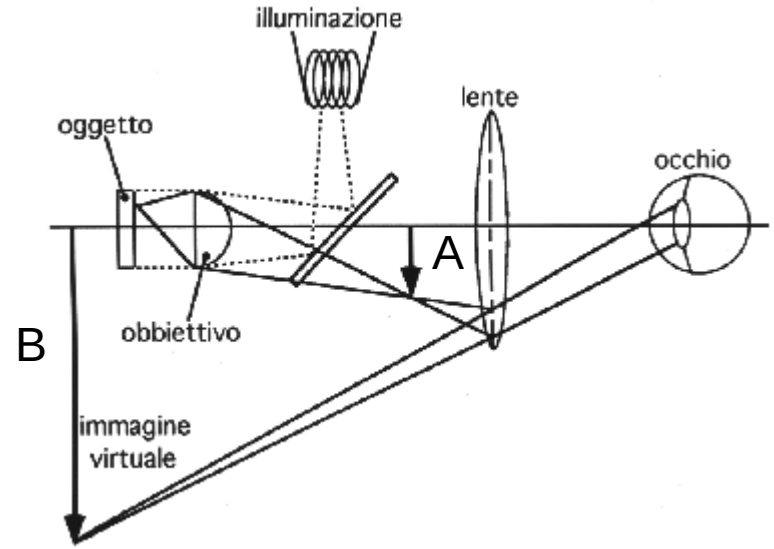
- protozoa
- spermatozoa
- bacteria
- Red blood cells



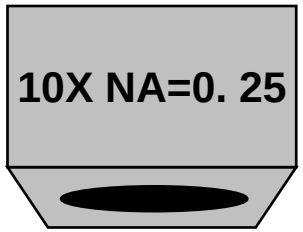
optical "microscope"

“Power” of a microscope

Conventional
Optical
Microscope



obiettivo:



- **magnification** (linear): A → B = MxA
- **resolution** (resolving power): capability to separately identify 2 different points (objects)

  (human eye: 100-200 μm)
- **enhancing techniques: I**mage **A**nalysis **& P**rocessing

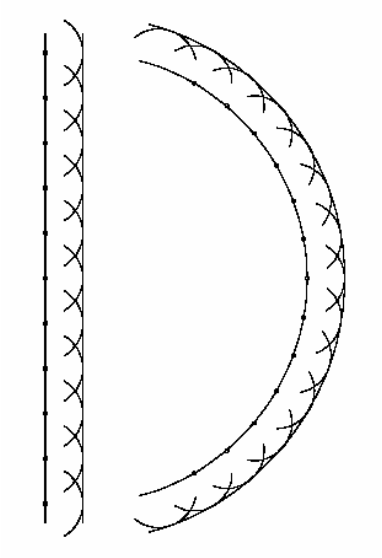
Diffraction and interference

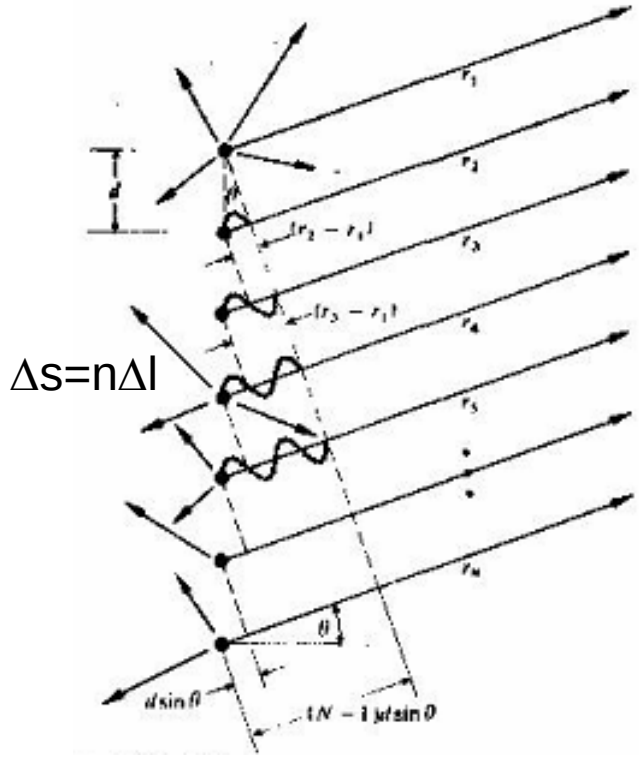
Huygens principle:

hit scatterers

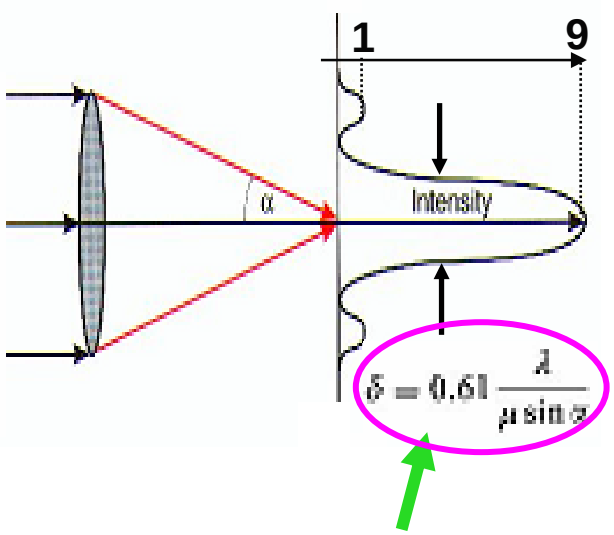
work as equal, independent sources

→ each wavefront = Σ array of wavelets





Airy function profile

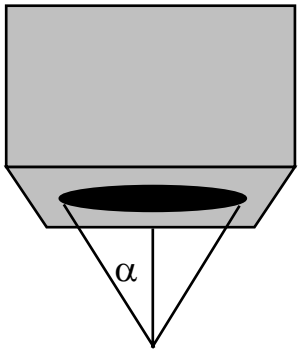


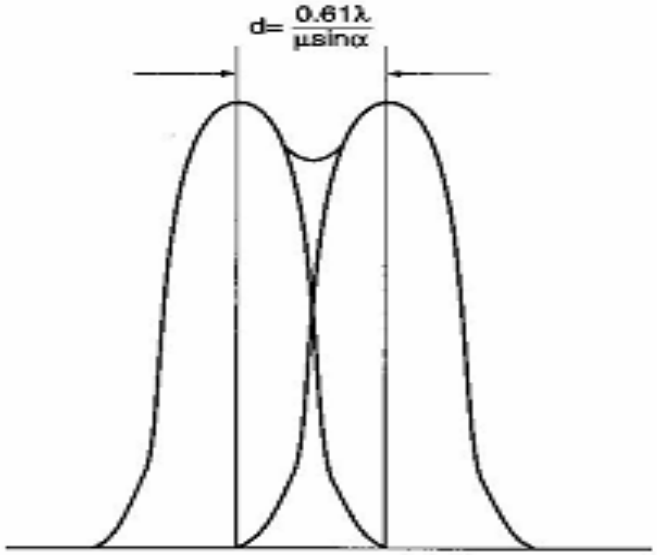
Abbe eq.

i.e. identical in-phase coherent oscillators (radiating antennas)

Rayleigh resolution limit

diffraction: $d_{min} = 0.61\frac{\lambda}{NA}$, $NA = n \sin\alpha$





N.B.,

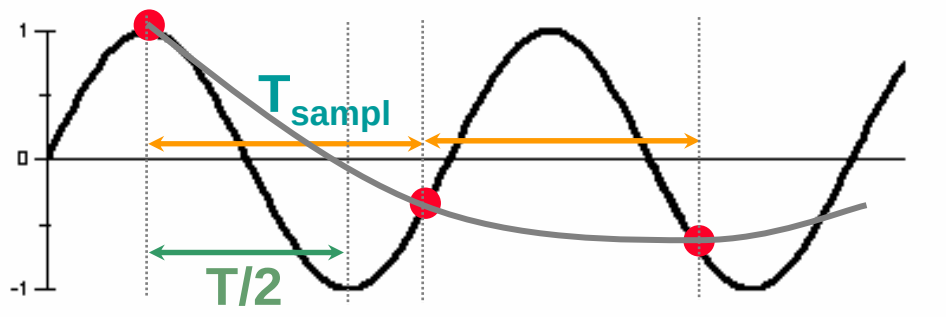
resolution :

- ***undefined for an isolated object***
- ***≠ magnification M***

Resolution limit: wave nature of light

Nyquist sampling theorem:

~ need at least 2 data points
(i.e.1 max & 1 min) per 1 period (wavelength)
to map an oscillating signal (light wave)
without aliasing

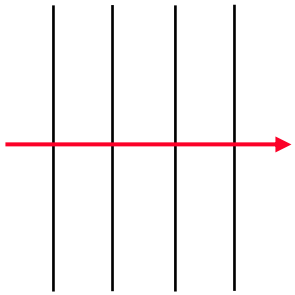


(based on Fourier transform theory)

look out : ***aliasing*** gives ***pseudo-resolution*** !

Resolution limit: particle nature of light

$e^{i k_z z}$



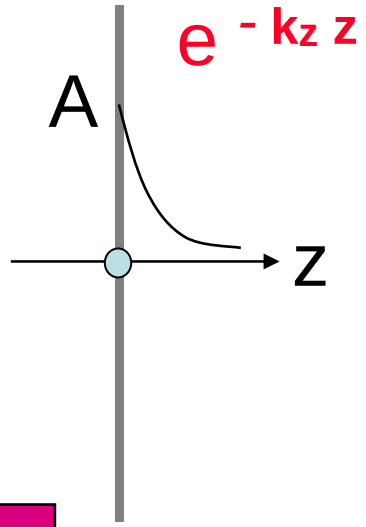
Heisenberg principle :

$\Delta x \, \Delta k_x, \ \Delta y \, \Delta k_y, \Delta z \, \Delta k_z > \hbar$

if $\Delta x, \Delta y < \lambda$, $\Delta k_x , \Delta k_y$ large

invariance of $k^2 = k_x^2 + k_y^2 + k_z^2$

$\Rightarrow \ k_z^2 < 0 \ , \ k_z = i \, | k_z |$



$e^{- k_z z}$

→ Evanescent field :

spatial frequencies >1/λ

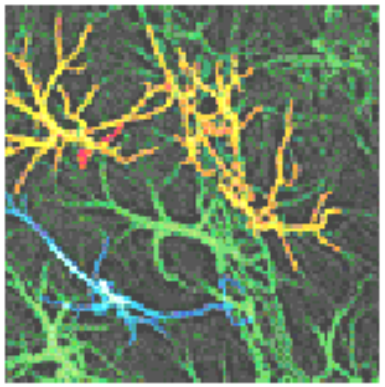
decay exponentially with distance

Sensitivity of a microscope

- necessary for resolution
- not sufficient !

e.g.: **fluorescence microscopy :**

fluorescent molecule dyes
(F or Cr based *fluorochromes*)
chemically stick
(due to pH, Ca^{2+}, Mg^{2+},
hydrophobic/philic…)
to objects of interest
and label them…

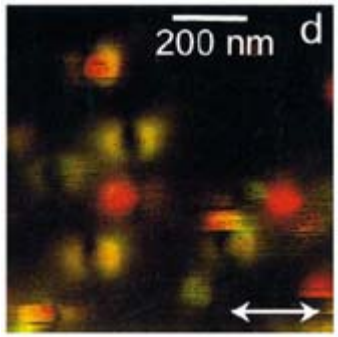


rabbit nerve cells

down to **“molecular detection”**! …

van Hulst

JCP 18, 7799 ‘00



diC18 molecule spots
(with polarized light)

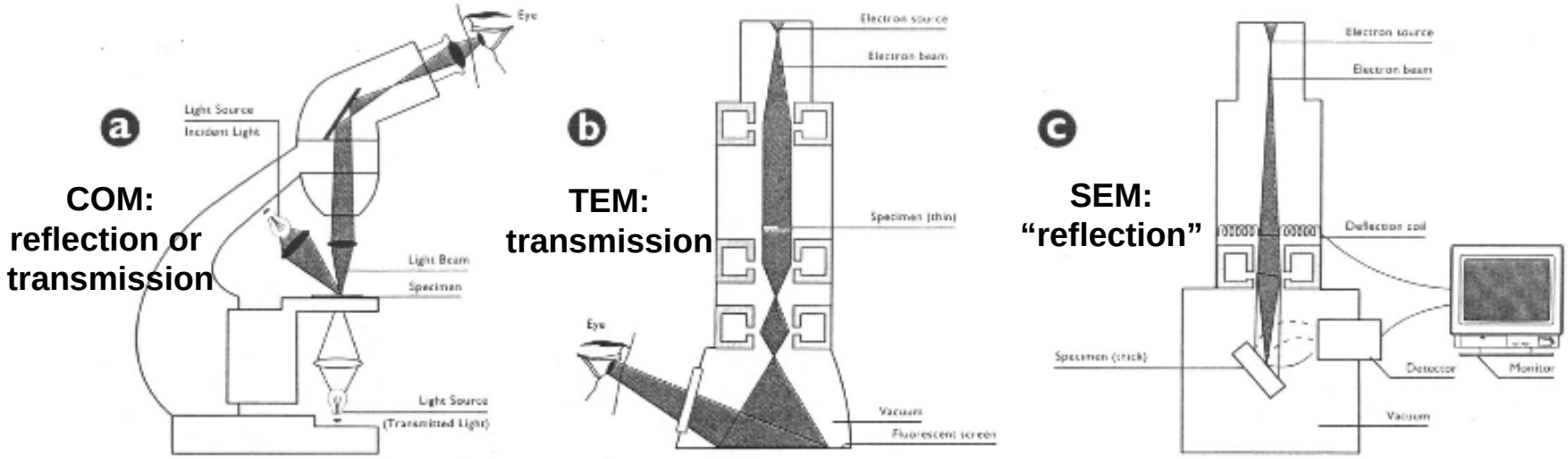
…. but this does not mean **“molecular resolution”**

birth of Electron Microscopy

- Thomson 1897: discovery of the e^-
- De Broglie 1924: $\lambda = h/p$
- Bush 1926: **E**, **B** axial fields: slow for charged particles

- Ernst Ruska 1934: TEM
  (commercial: 1939, Siemens & Halske)
- Knoll-Von Ardenne 1935-38: TEM + scan coils

- Zworykin-Hillier-Snyder ’40s SEM
- Oatley ’50s-’60s
  (commercial: 1965, Cambridge Instruments)



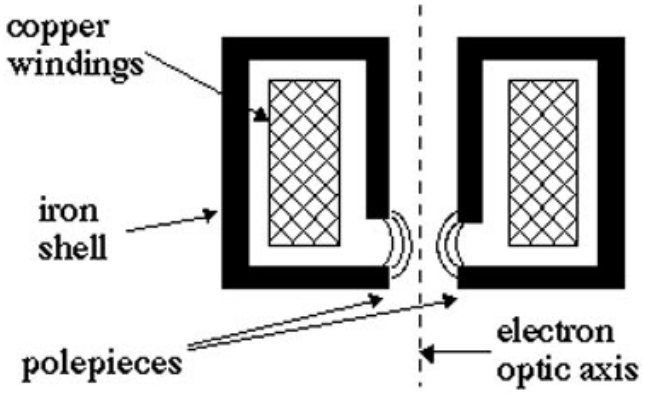
COM: reflection or transmission

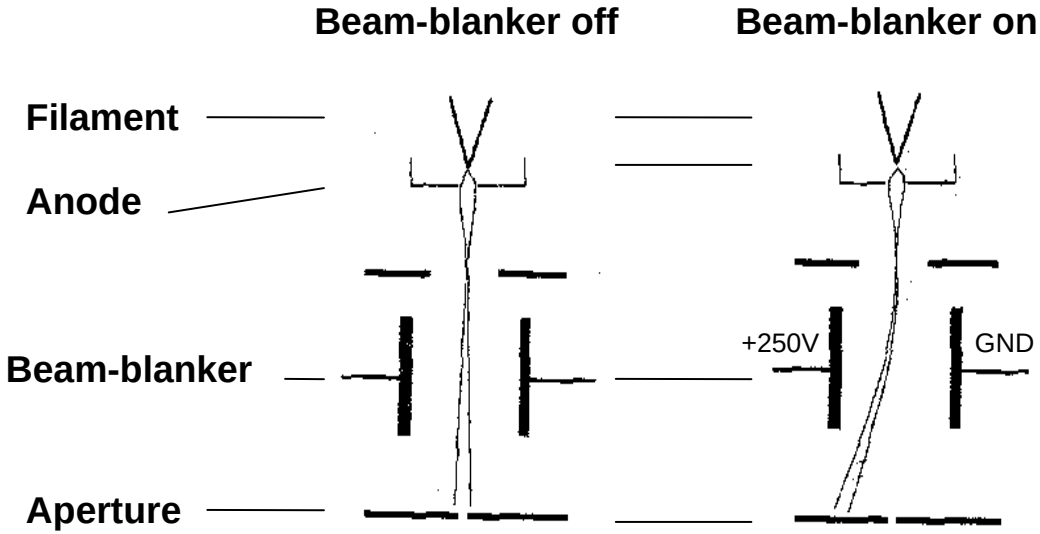
TEM: transmission

SEM: “reflection”

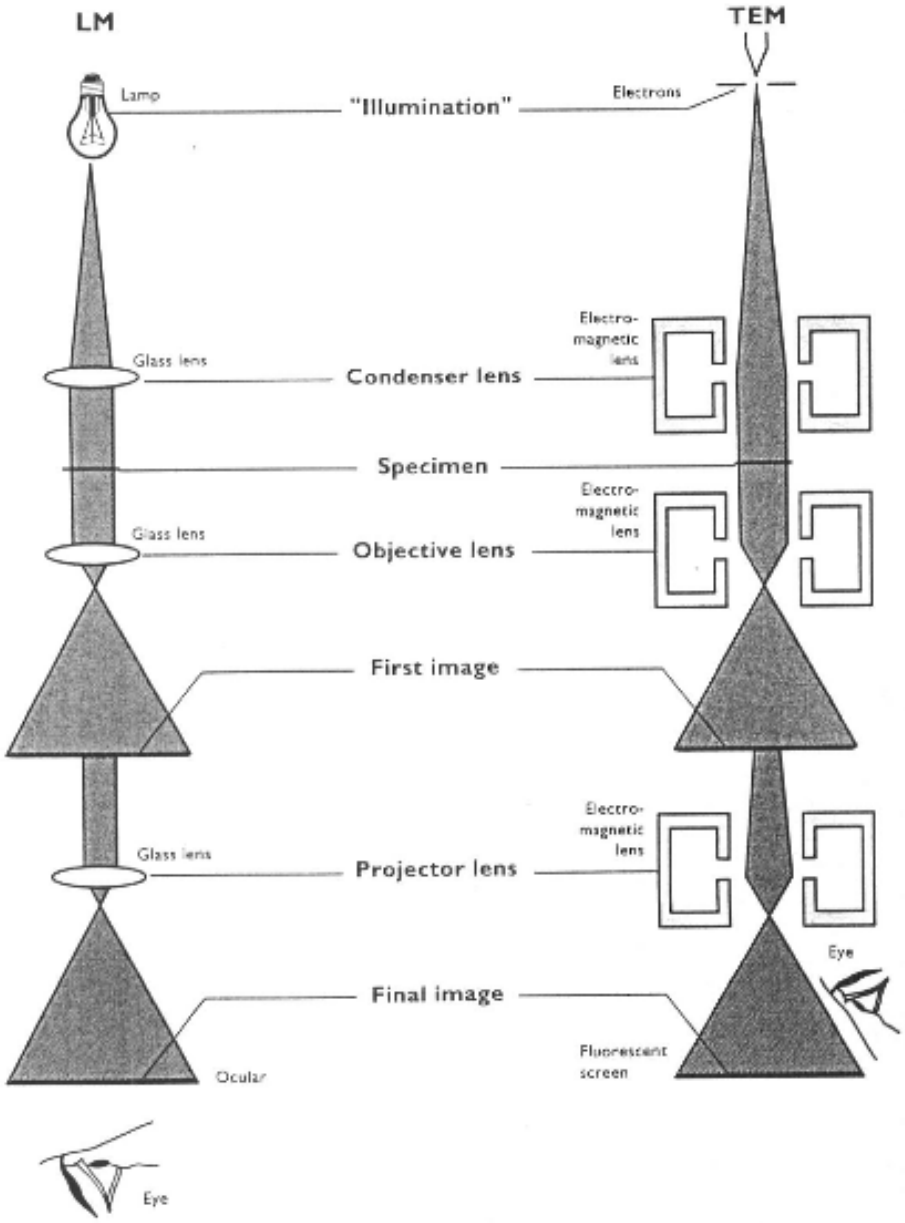
intermediate column components

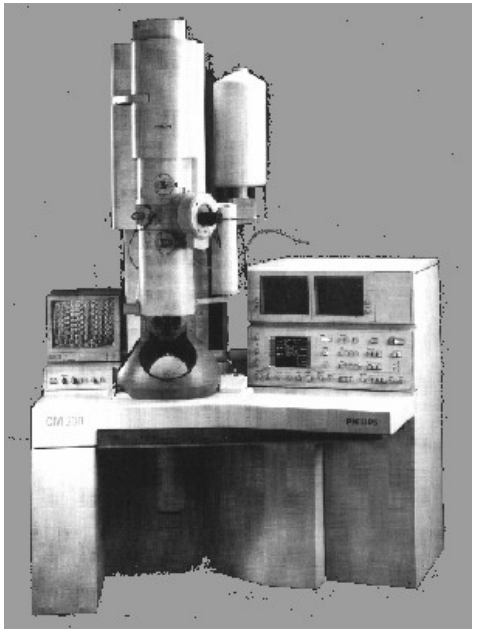
- *(e source)*
- 2 or more **em lenses** (focusing)
- beam **blanker** (deflection: on/off stopper)
- stigmator
- apertures (alignment)
- *(detector)*

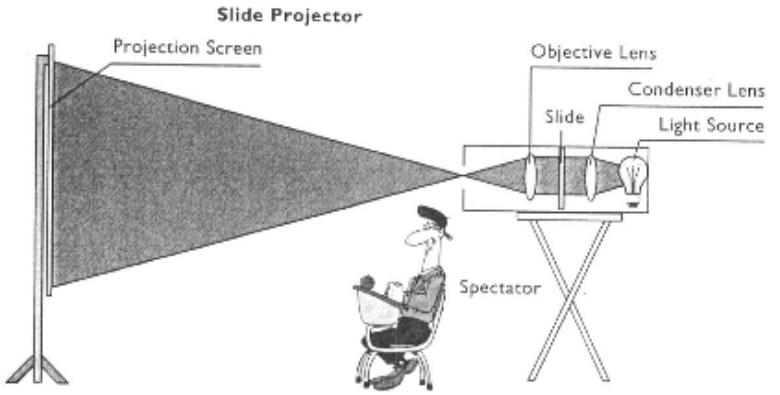


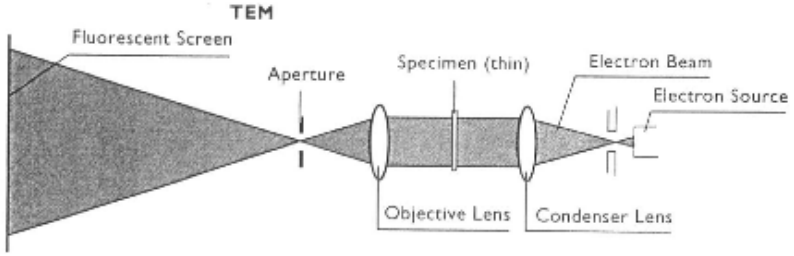


TEM

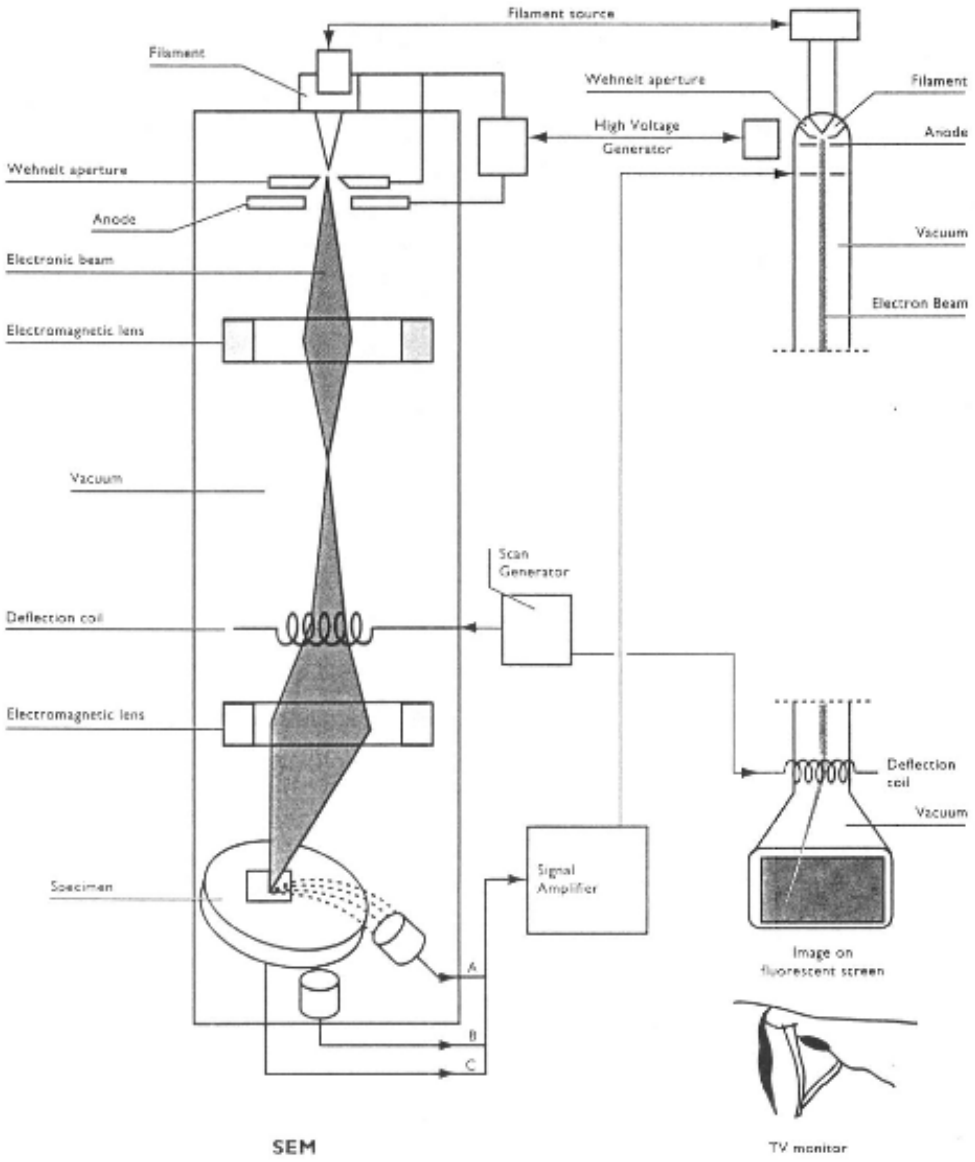

LM
TEM
Lamp
"Illumination"
Electrons
Glass lens
Condenser lens
Electro-magnetic lens
Specimen
Glass lens
Objective lens
Electro-magnetic lens
First image
Glass lens
Projector lens
Electro-magnetic lens
Eye
Final image
Ocular
Fluorescent screen
Eye




Slide Projector
Projection Screen
Objective Lens
Condenser Lens
Slide
Light Source
Spectator


TEM
Fluorescent Screen
Aperture
Specimen (thin)
Electron Beam
Electron Source
Objective Lens
Condenser Lens

SEM



+ easy to use/understand (optical image quality)
+ no thin specimens
+ high resolution, high field depth, analytical capability ~ TEM, at lower price: 200-500 k€
→ > 20'000 SEM installed worldwide, +1 / day
- slow

Why higher resolution?

$$\lambda_{DeBroglie} = h / p$$

Planck constant:
$h=6.63\ 10^{-34}$ Js

- tennis ball: M~100 g, v~100 km/h → λ_M~2 10^{-24} Å
  ~ continuous (non-oscillating) wave
- e^-: m_e~9 10^{-31} kg, v~1/3 *c* → λ_e~0.02 Å ~10^{22} λ_M, "observable"

$E_\gamma = h\nu = hc/\lambda_\gamma$ →

$E_e = p_e^2/2m_e = h^2/2m_e\lambda_e^2$ →

	λ(E) [nm, eV]
γ	**1240 / E**
e	**1.23 / √E**

i.e. electrons – vs – photons
→ "softer" diffraction limit

but:
- possible radiation damage
- controlled potential (i.e. conductive) samples required
- UHV environment required

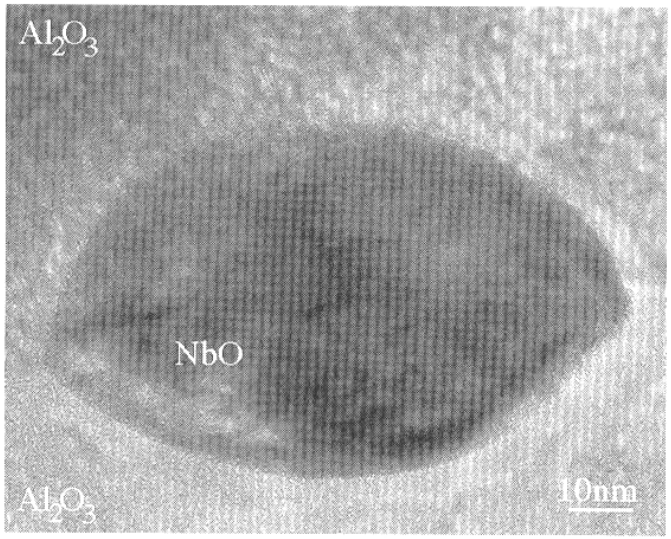


Figure 4.53 Transmission electron micrograph of a NbO particle located at a grain boundary in polycrystalline alumina. Phase contrast (lattice fringes) and mass–thickness contrast vary from the alumina grain to the NbO grain

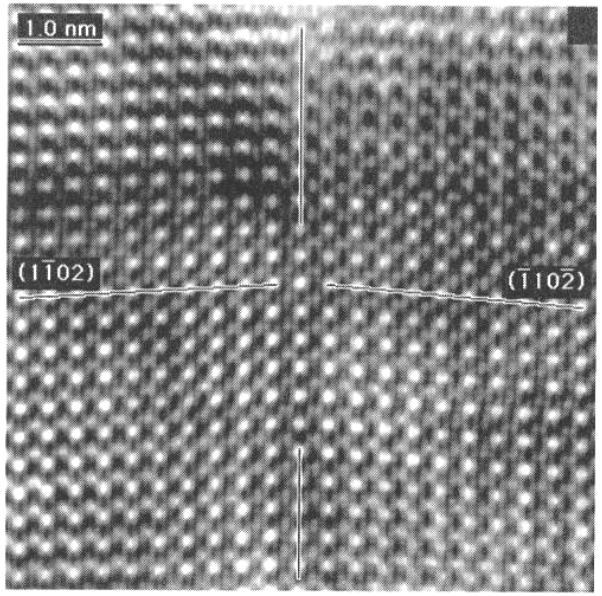


Figure 4.54 Lattice image of a rhombohedral twin in alumina

examples of **HR-TEM**:

E>=100 kV

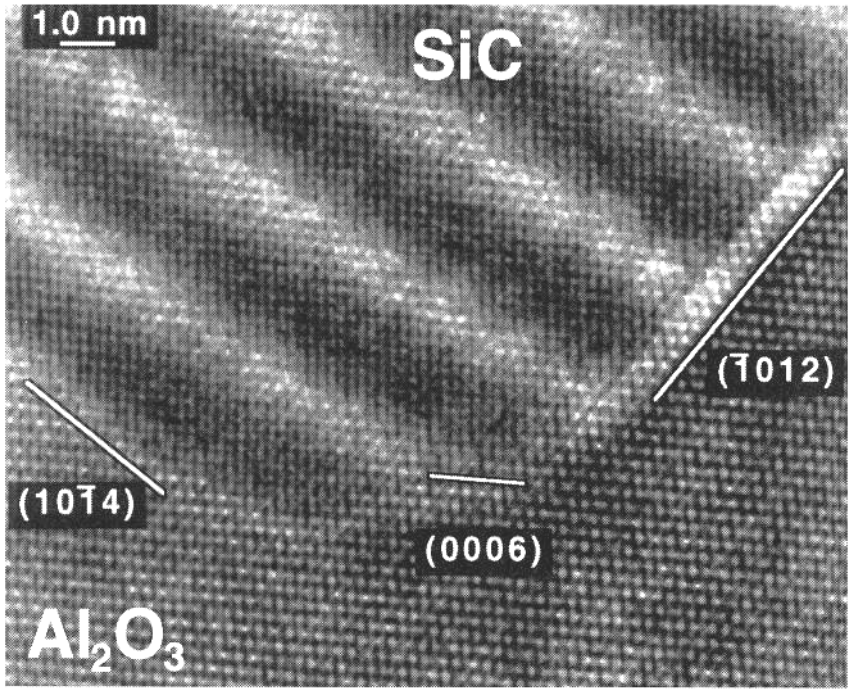


Figure 4.55 Lattice image of a SiC particle located within an alumina grain. The alumina lies along a low-index-zone axis, and is the source of the lattice image. A moiré pattern appears within the SiC particle due to overlap between the alumina and SiC (in the direction of the electron beam)

extremely detailed informations about thin crystalline samples (after interpretation of data and for flat sample slices only)

More electron microscopes…

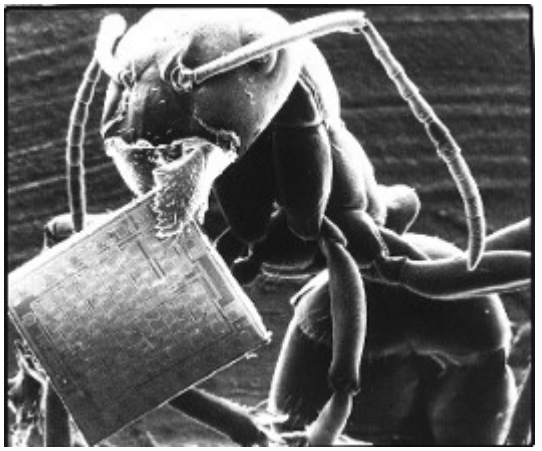
STEM …

- in 1991 true atomic resolution: 1.4 Angstroem
- today almost all TEM are STEM

… ESEM

- 1988 ElectroScan (USA) → Philips → FEI

T=0 °C, P=6.5-13 mbar, RH up to 100%

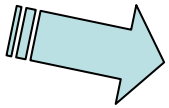


samples can be: - biologic (i.e. soft, dirty, degassing)
- insulators (water vapor ions drain charge away)

Scan-enhanced optical microscopy

CSOM *(Marvin Minsky patent, 1957)*

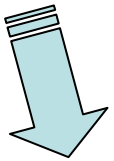
Confocal Scanning Optical Microscope



CLSM (or LSCM)

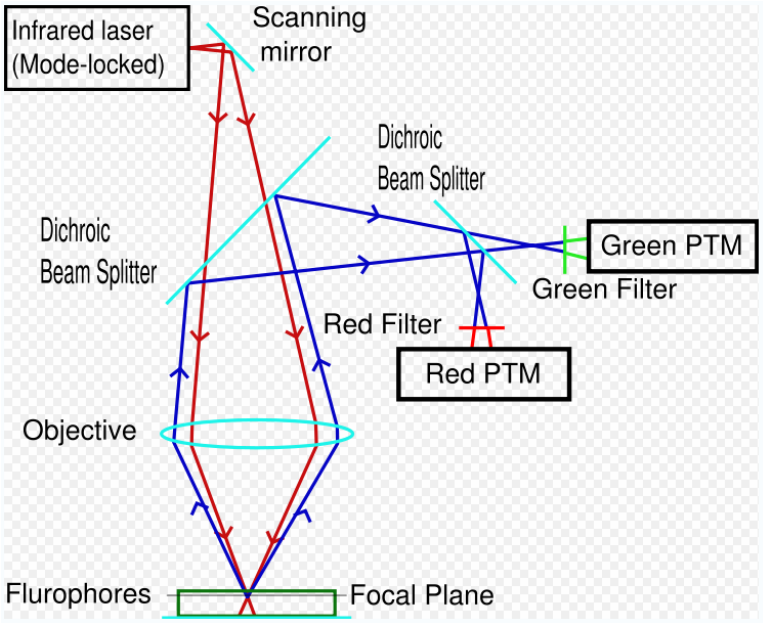
Confocal Laser Scanning Microscopy

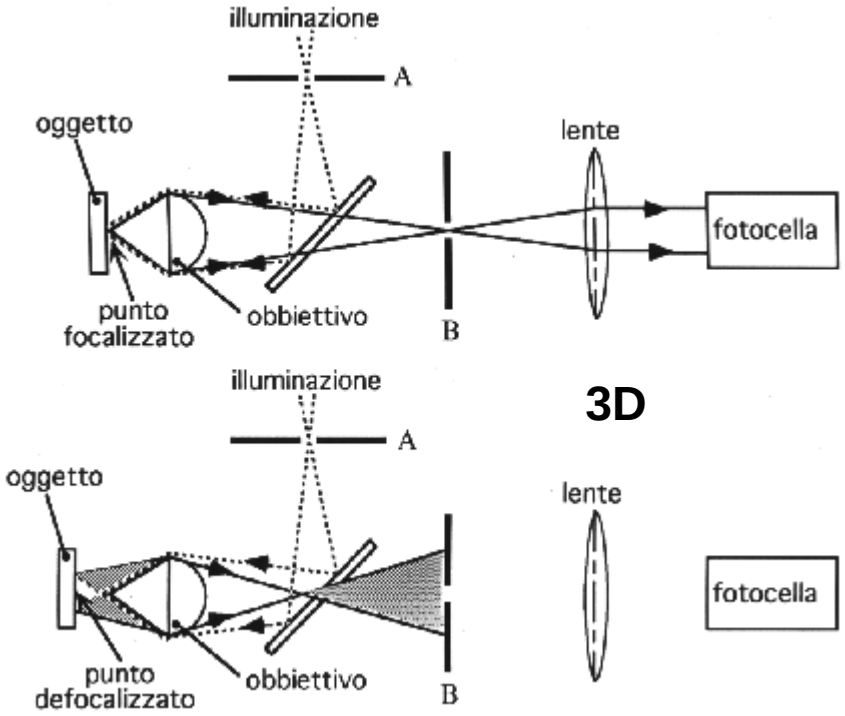
• for fluorescence detection



TPE

Two Photon Excitation



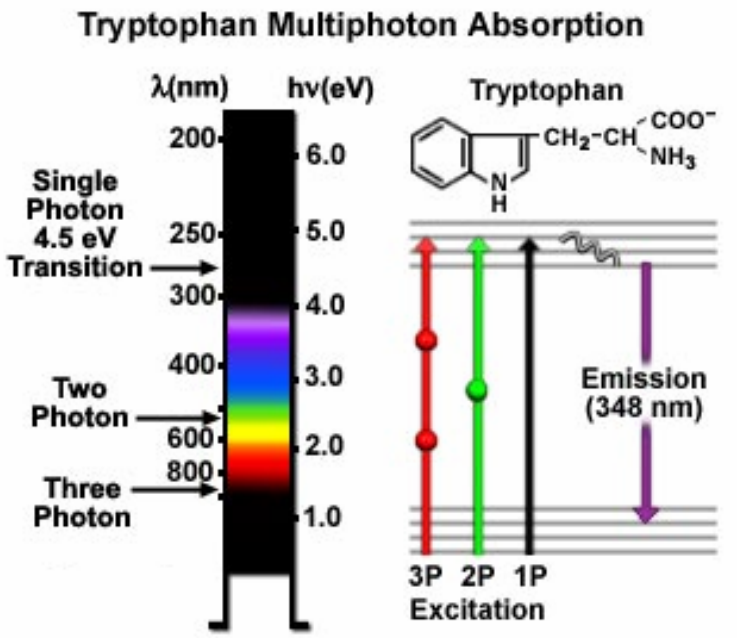


3D

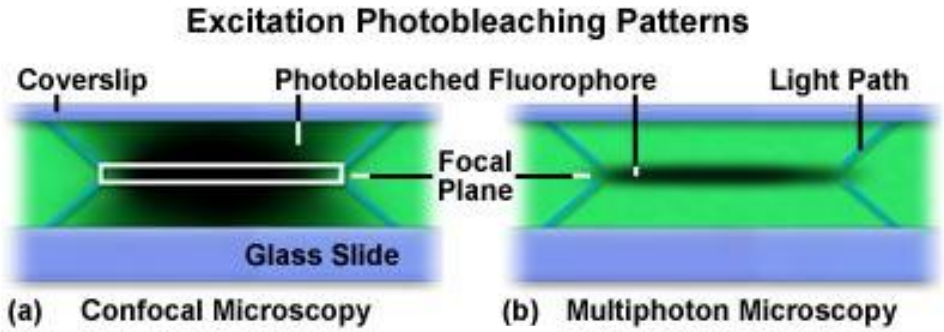
• artificial image reconstruction
• simultaneous focalization required through 2 pinholes
• optical resolution improved, particularly axial
• SPM: not true 3-D (+ perturbative)

Two (or Multi) Photon Excitation

+ not a real confocal (no pinhole) yet intrinsic confocal-like effect →reduced photobleaching
+ infrared exciting photons: lower damage to biological sample



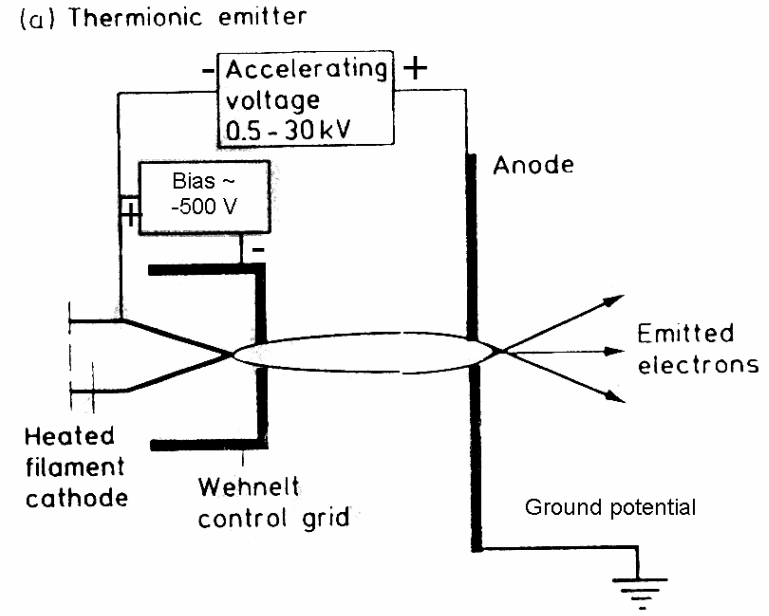
- needs hi excitation photon flux for near-simultaneous 2-photon emission → expensive pulsed laser e.g. fs Ti:sapphire

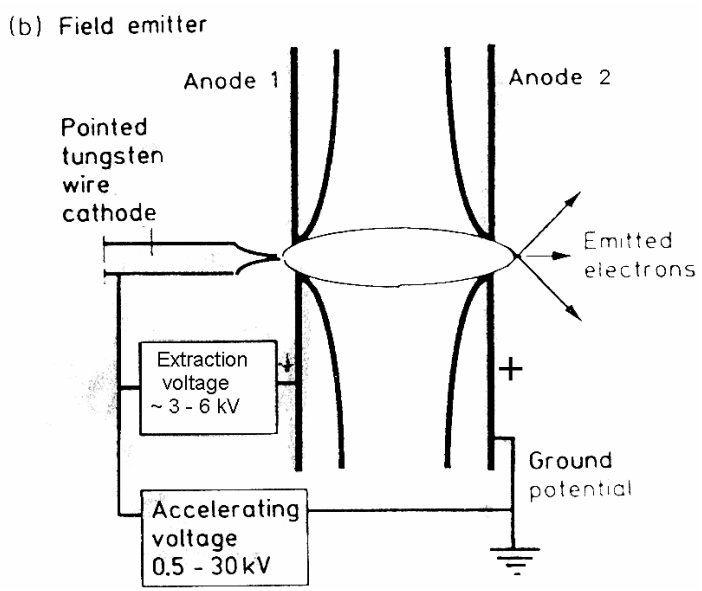


SEM electron source

- column → **gun (e source)** + e path “cylinder”
- console (control hw – sw)

~10^{-7} mbar **gun types** ~10^{-9} mbar





Richardson law:

$J \propto T^2 \exp(-e\phi / kT)$

$\phi_W = 4.5$ V

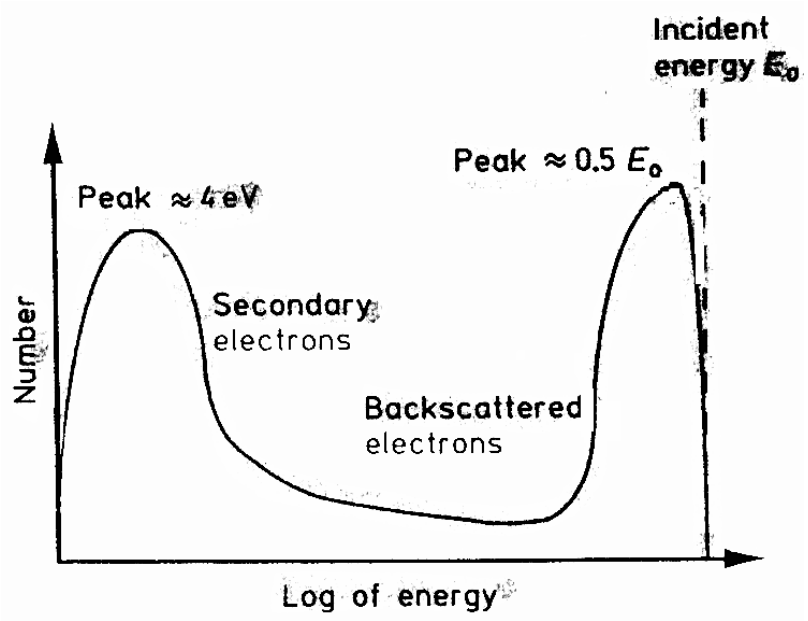
$\phi_{LaB6} = 2.6$ V

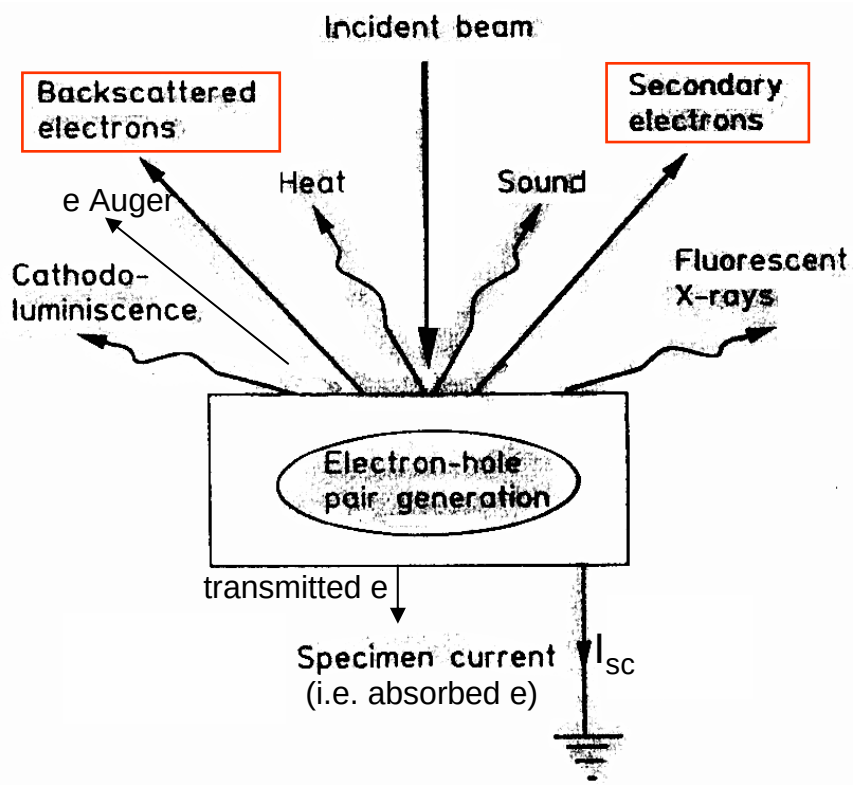
filament	life (hrs)	cost(€)
W	~ 100	~ 50
LaB_6	~1000	~ 1000
FEG	>~2000	~ 4000

“FEG”:

- operated at RT
- e tunnel out of the cold filament
- very sharp and clean

electron-solid interactions





$0=I_{tot}=-I_b+\delta I_b+\eta I_b+I_{sc}$

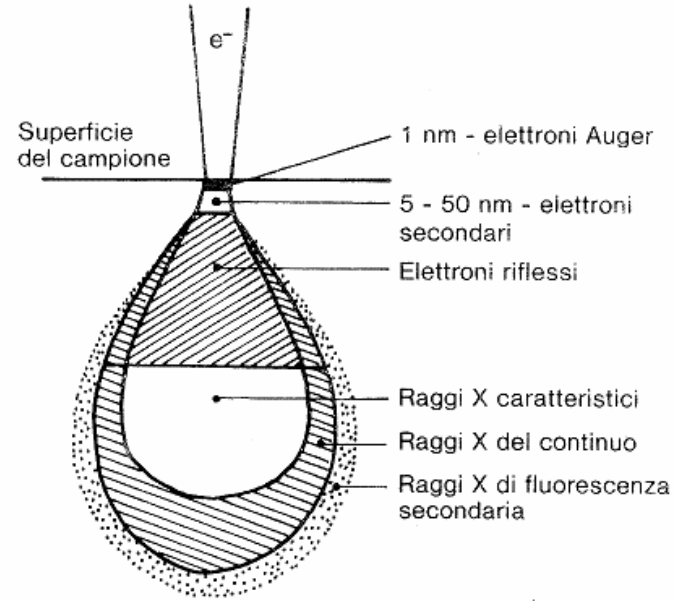
→ $I_{sc}=(1-\delta-\eta)I_b$
=0 (for insulating samples) at $E=E_2$

material	*E_2 (keV)*
resist	0.6
amorphous C	0.8
teflon	1.9
quartz	3.0
alumina	3.5

BSE yield $\eta=n_{BSE}/n_{incid}$=0.2-0.4

SE yield $\delta=n_{SE}/n_{incid}$ = 0.1 at 30 keV
<~1 at 1-2 keV

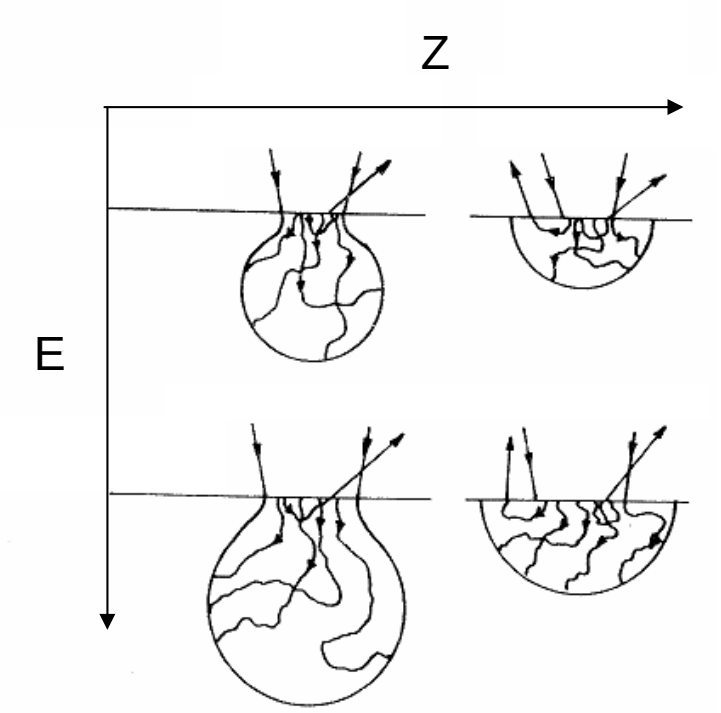
Interaction zone



envelope of
inelastically scattered
e^- from the incident beam

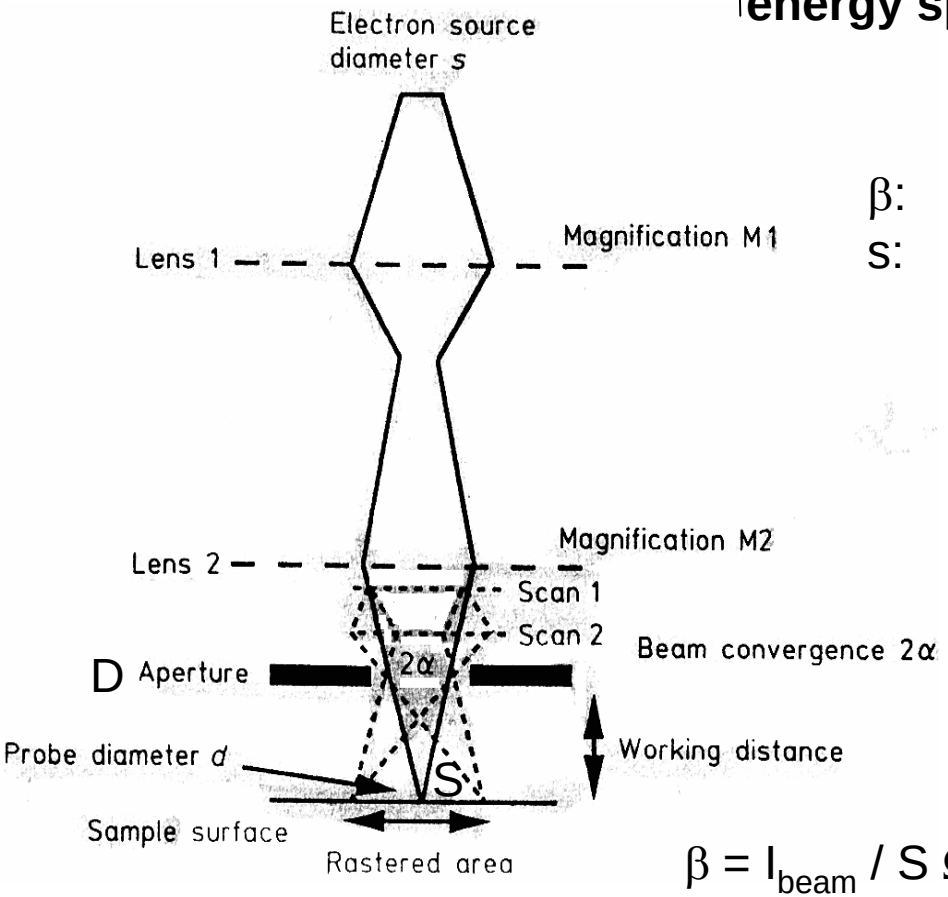
- at the boundary E~kT
- as the path length in the sample increases, <E> decreases and E spread ΔE increases

BSE :
- from deeper regions than SE
  → lower resolution
- low collection efficiency (<5%)
- strong direction memory
  → shadows



Source performance parameters

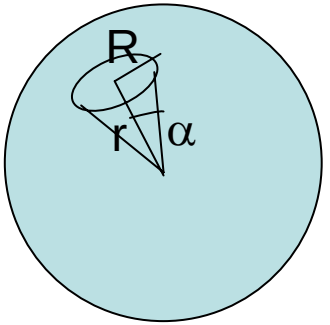
e source | **size**
brightness: $\beta = J / \Omega$ $(A / m^2\ \text{sterad})$ $\beta \propto V_0$
energy spread



	W	→	LaB_6	→	W FEG
β:		3÷10x		10÷100x	
s:	20÷50 μ				10 nm

$d = s\ M_1\ M_2$

$\alpha \sim \tan\alpha \sim (D/2) / WD$



$\Omega = 4\pi R^2/r^2$,
$R/r = \tan\alpha \sim \alpha$ →

$\beta = I_{beam} / S\ \Omega = I_{beam} / \pi\ (d/2)^2\ \ 4\pi\alpha^2$ → $I_{beam} = \pi^2\ \beta\ \alpha^2\ d^2$

limits to resolution:
- sensitivity
- aberration

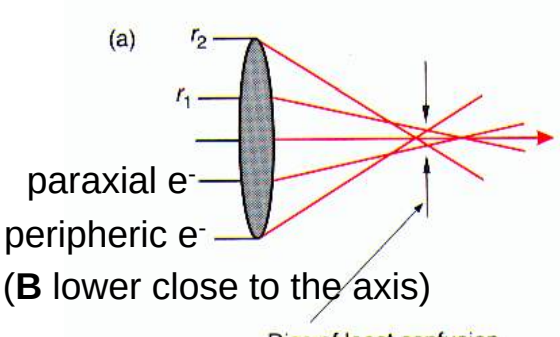
“Aberrations”

→ effective probe size: $d^2 \rightarrow d'^2 = d^2 + \sum_i d_i^2$

diffraction:
(source)

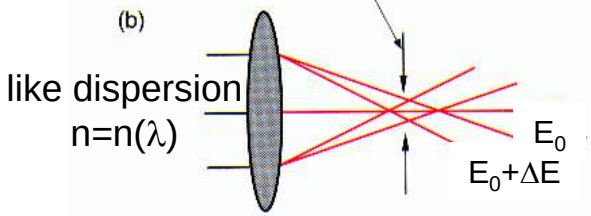
Airy disc diameter $d_{diffr}=1.22\ \lambda/\alpha$

spherical aberration:
(lenses)



$$d_{spher} = \frac{C_{spher}\alpha^3}{2}$$

chromatic aberration:
(source)



$$d_{chrom} = C_{chrom}\alpha\frac{\Delta E_{tot}}{E_0}$$

Figure 4.4 Spherical (a) and chromatic aberration (b) prevent a parallel beam from being brought to a point focus. Instead, a disc of least confusion is formed in the focal plane

(**astigmatism:**
somewhat controlled)

Brandon-Kaplan
Microstruct. Charact. of Materials
Wiley (1999)

consequences on imaging properties

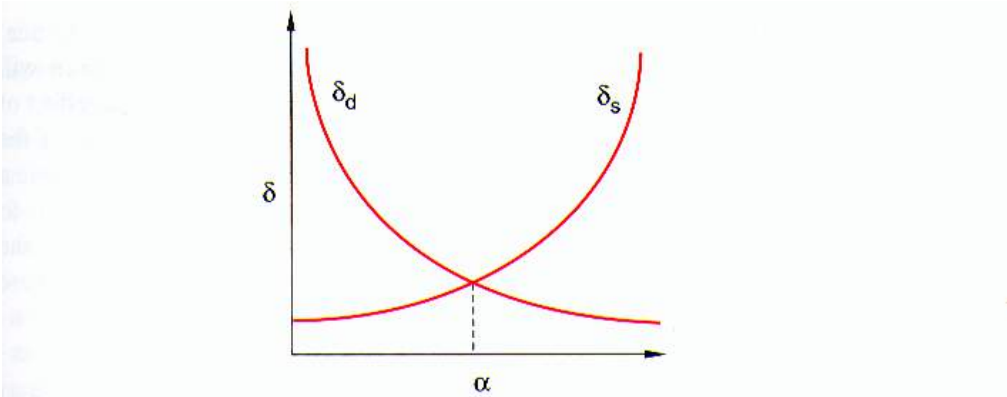
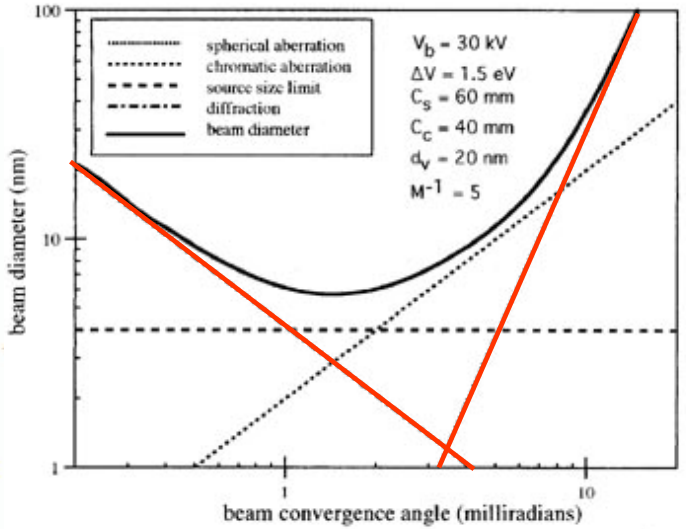
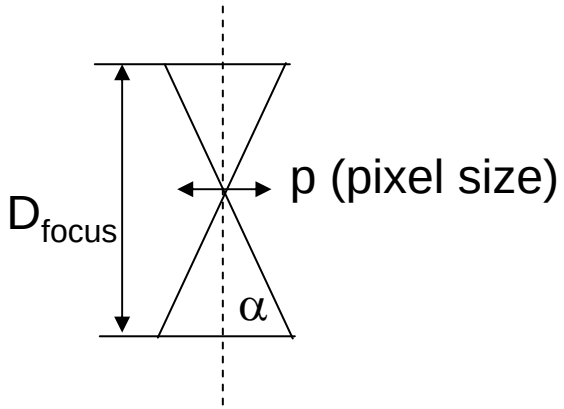


Figure 4.5 The diffraction and the spherical aberration limits on resolution have an opposite dependence on the angular aperture of the objective, so that an optimum value of α exists



$$0 = \partial(d'^2) \rightarrow d'^2 \text{ is min for } \alpha = \left(\frac{d}{C_{spher}}\right)^{1/3} = 1-10 \ \ mrad$$

i.e. **need very low aperture** (in optics: α up to 1!)



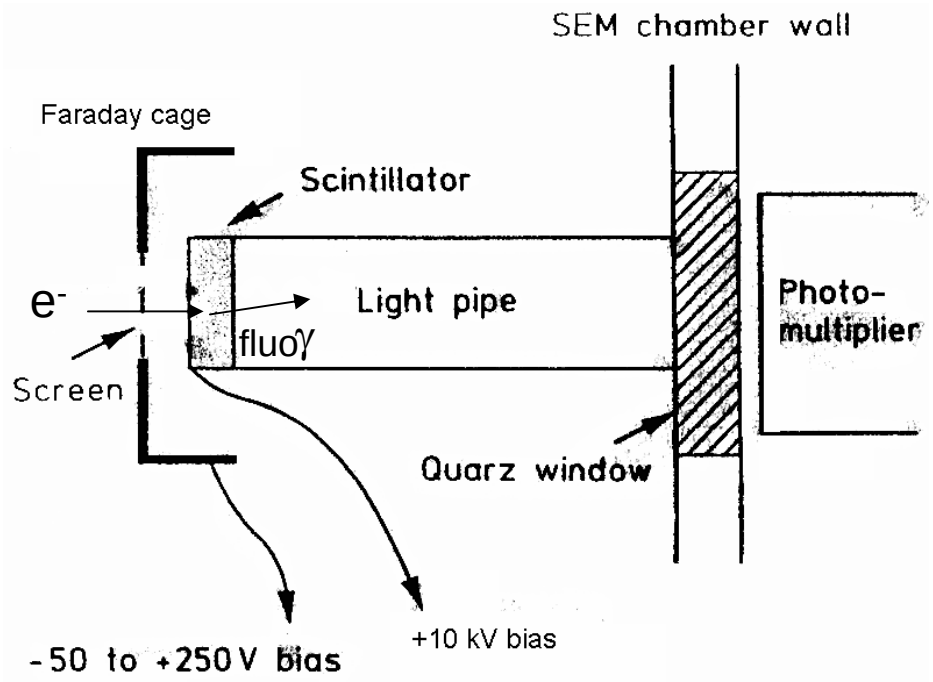
$$\alpha \approx \tan\alpha = \frac{p}{D_{focus}} \rightarrow D_{focus} = \frac{p}{\alpha} = 100-1000 \ \ p =$$

$$= 1-10 \ \ mm \quad \text{if 1 cm} \leftrightarrow 1000 \text{ p}$$

i.e. **get very hi focus depth (3D effect)**

SE detection

SE weak → easy to collect at low detector bias



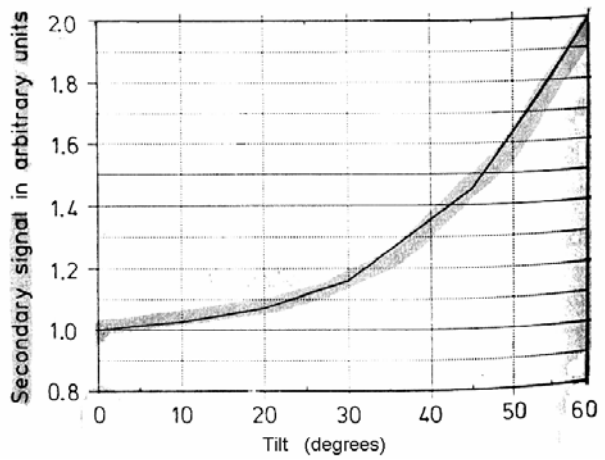
dynodes …

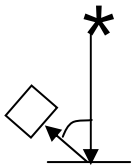
annular ring (in-lens) **Everhart-Thornley** detector

- hi inner bias accelate e- after entrance
- Faraday cage screens the beam

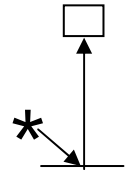
SE imaging

topographic contrast
dominant one
~90% micrographs



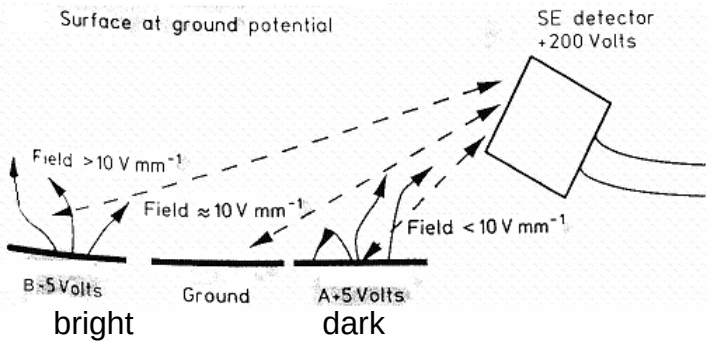


$I_{opt}(\theta) = I_{opt}(0) \cos\theta$
Lambert law

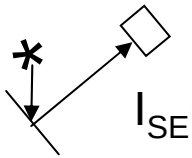


reciprocity principle
$I_{opt}(0) = I_{opt}(\theta) \cos\theta$

voltage contrast



bright dark



$I_{SE}(\theta) = I_{SE}(0) \sec\theta$

magnetic contrast

surface domains → F = -e/c vXB
at free surface inward (darker)
or backward (brighter)

BSE imaging

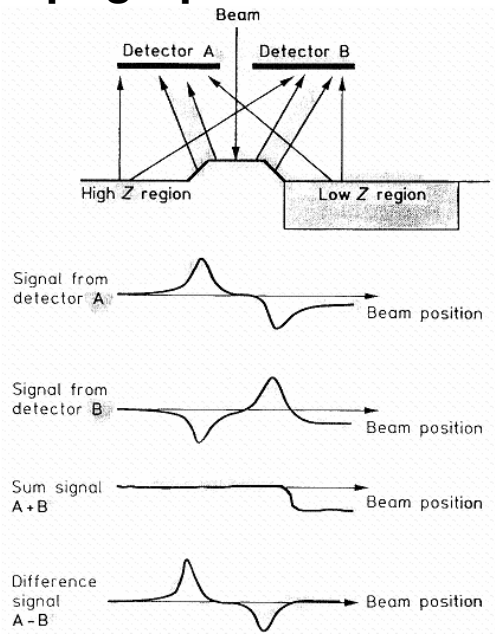
Z contrast
dominant one

escape depth $R_{BS} \propto (A/Z^{0.9})\,(E^{1.7}/\rho)$

for E>5 keV $\eta \sim -0.025 + 0.016\,Z$

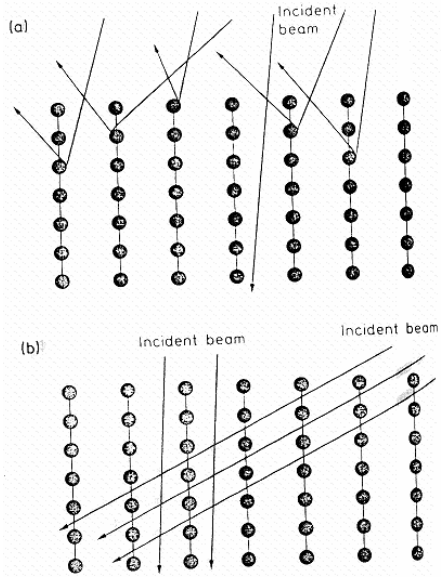
	Z	η
C :	6	0.05
Au:	79	0.5

topographic "contrast"



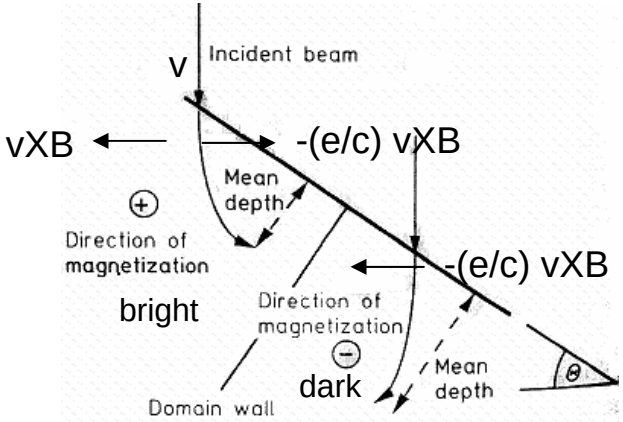
Electron Channeling Pattern

for large single crystals



Bragg condition $\sin\theta = \lambda/2d_{ijk}$

magnetic contrast

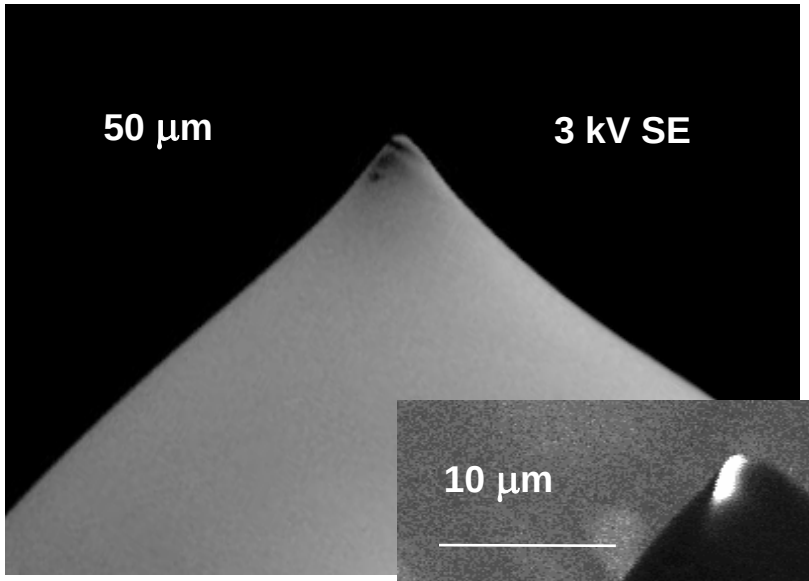


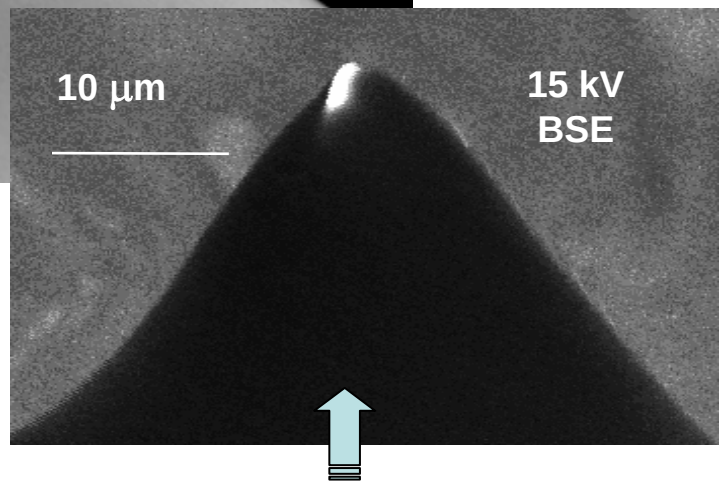
e.g.: SEM of EC-STM tips

standard insulating coatings ***[A_{free} < (10 μm)²] :***

- **poliacrylics**
- **epoxy**
- **Apiezon wax**

Tip coating device assembly





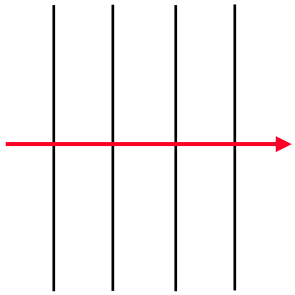
Apiezon wax coated Au tip. The very tip (several μm) remains uncoated.

Hole at the bottom of Optics

and below λ_{vis}, e.g. transmission of a 150 nm Ø pinhole ?

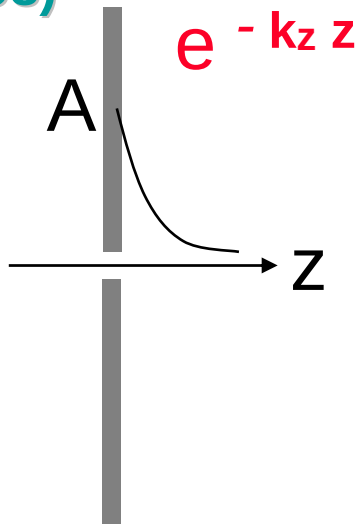
EXTRAORDINARY! **Ebbesen *et al*,**
Nature 391, 667 (1998)

$e^{i k_z z}$



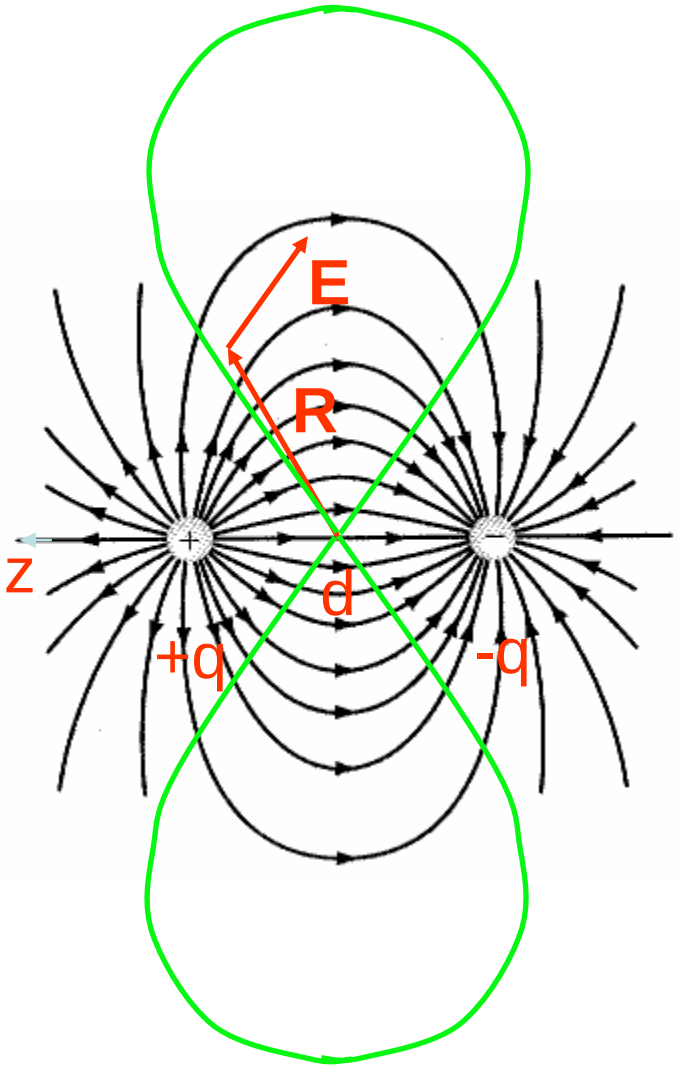
→ Near Field Optics :

"... involving the passage of light to, from, through, or near an element with ***subwavelength*** *sized* ***features****, and coupling to another element at* ***subwavelength distance****."*



NFO laws can be quite different from the FFO ones

NF - vs - FF example: electric dipole



FF radiation pattern profile (~cos²θ)

$p = qd$

$$E_R = 2\left[\frac{p\cos\theta}{R^3}\right](1 + i\,k\,R)\; e^{i\,k\,R}$$

$$E_\theta = \left[\frac{p\sin\theta}{R^3}\right][1 + i\,k\,R - (k\,R)^2]\; e^{i\,k\,R}$$

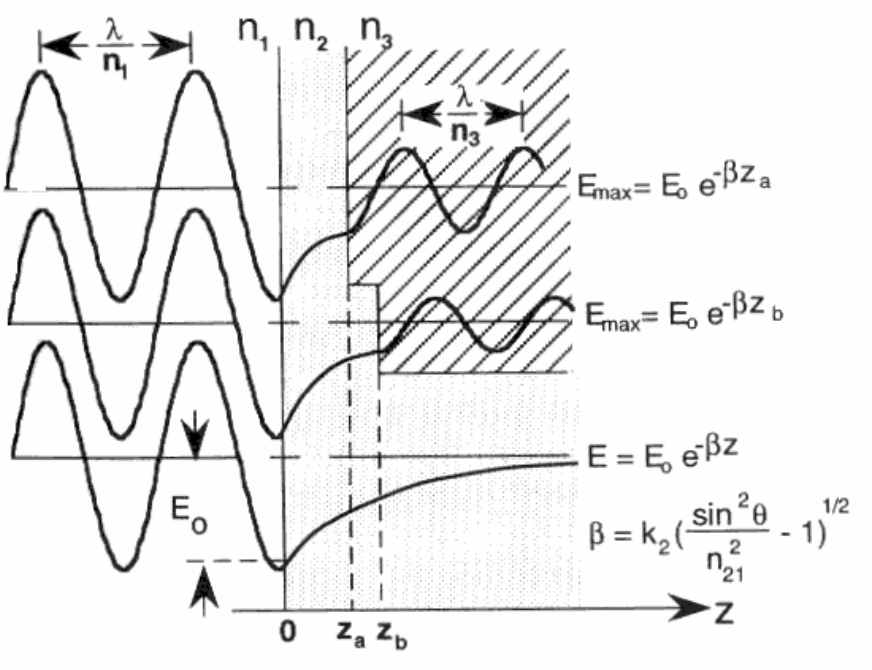
$$H_\phi = \left[\frac{p\sin\theta}{R^3}\right][\; i\,k\,R - (k\,R)^2]\; e^{i\,k\,R}$$

→ NF (kR<1) :

- *changes more rapidly than FF*
- *stronger where FF vanishes*

($\vec{k}$ light wavevector, $k=\omega/c=2\pi/\lambda$)

Photon tunneling

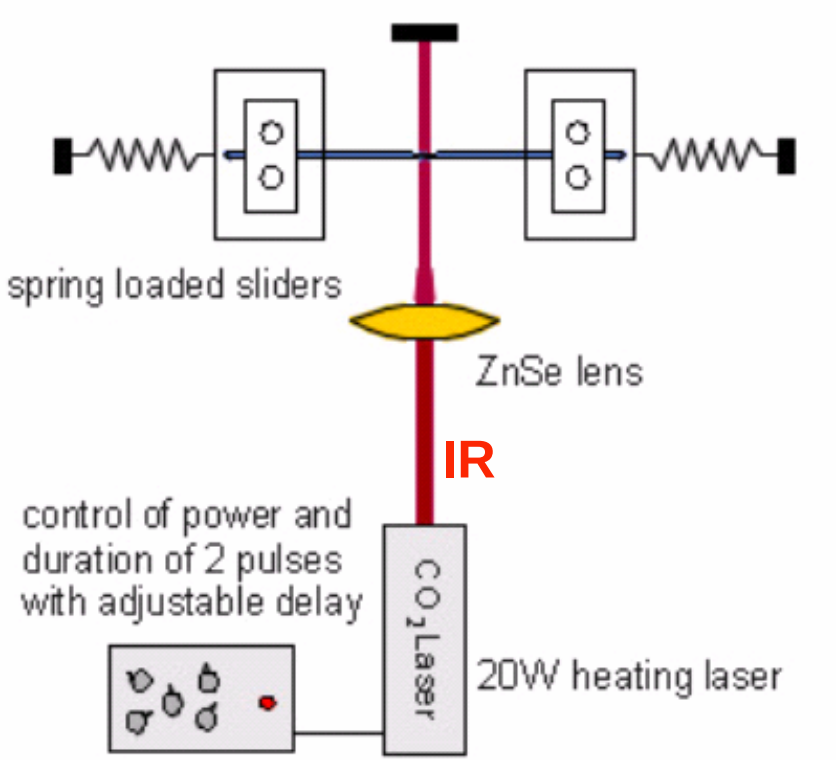


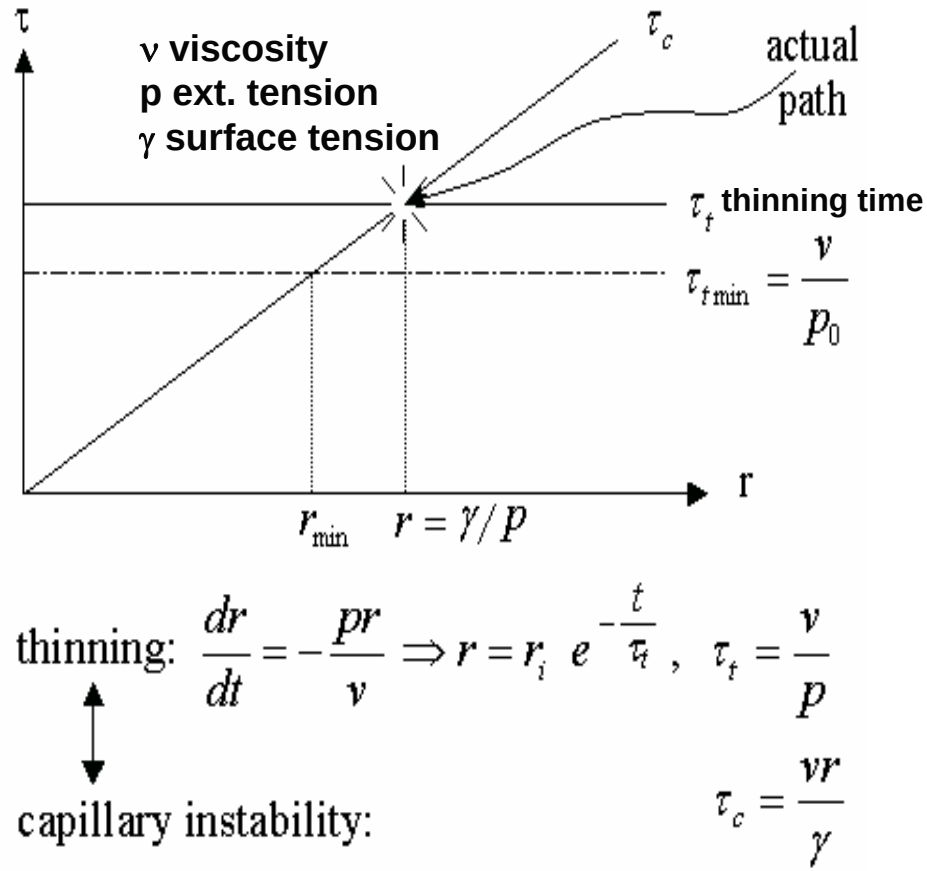
Fresnel formulas
for refraction at the interface between 2 media

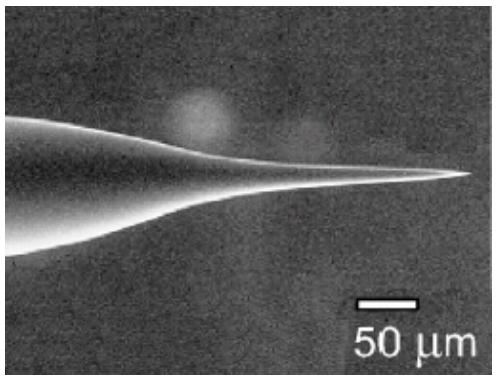
→ Near Field Optical Microscopy :

1928 : Synge's idea (lacking tech for tiny holes and scan)
1972 : first μ-wave experiments by Ash and Nichols
1984 : birth of SNOM with first visible imaging
at Dieter Pohl lab in Zurich

Fiber tip formation by Heat and Pull

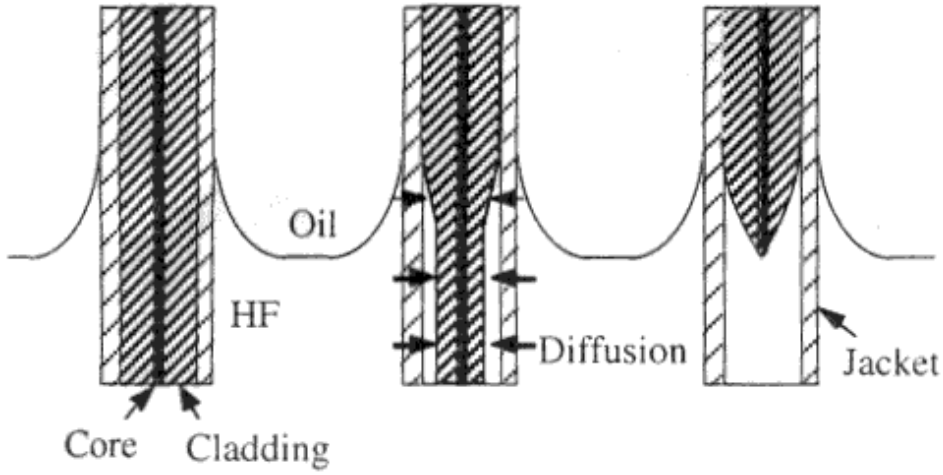




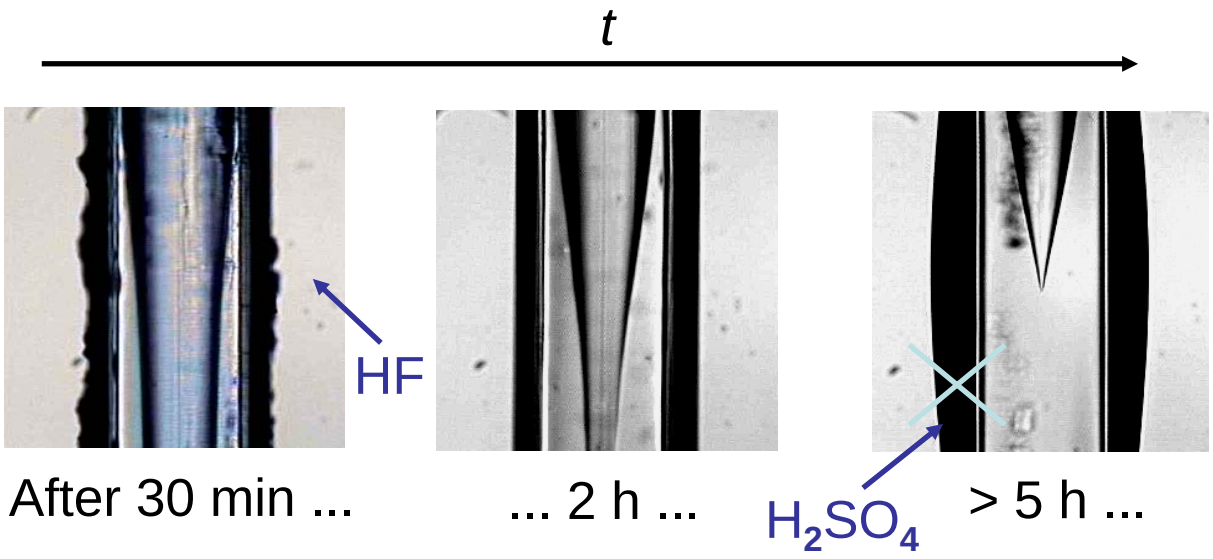


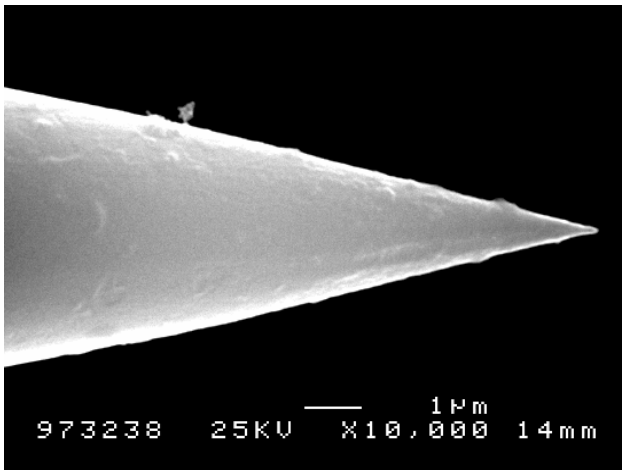
+ very smooth taper surface
- very low throughput (~10^{-7})
due to long taper (i.e. small angle 2α~10°)

Fiber tip formation by Tube Etching



+ ease of manufacturing
+ typical etching large cone angle
+ smooth taper surface compared to standard etching
+ self-limiting process
- off-center (off core?) apex
- HF vapor attacks fiber holder above



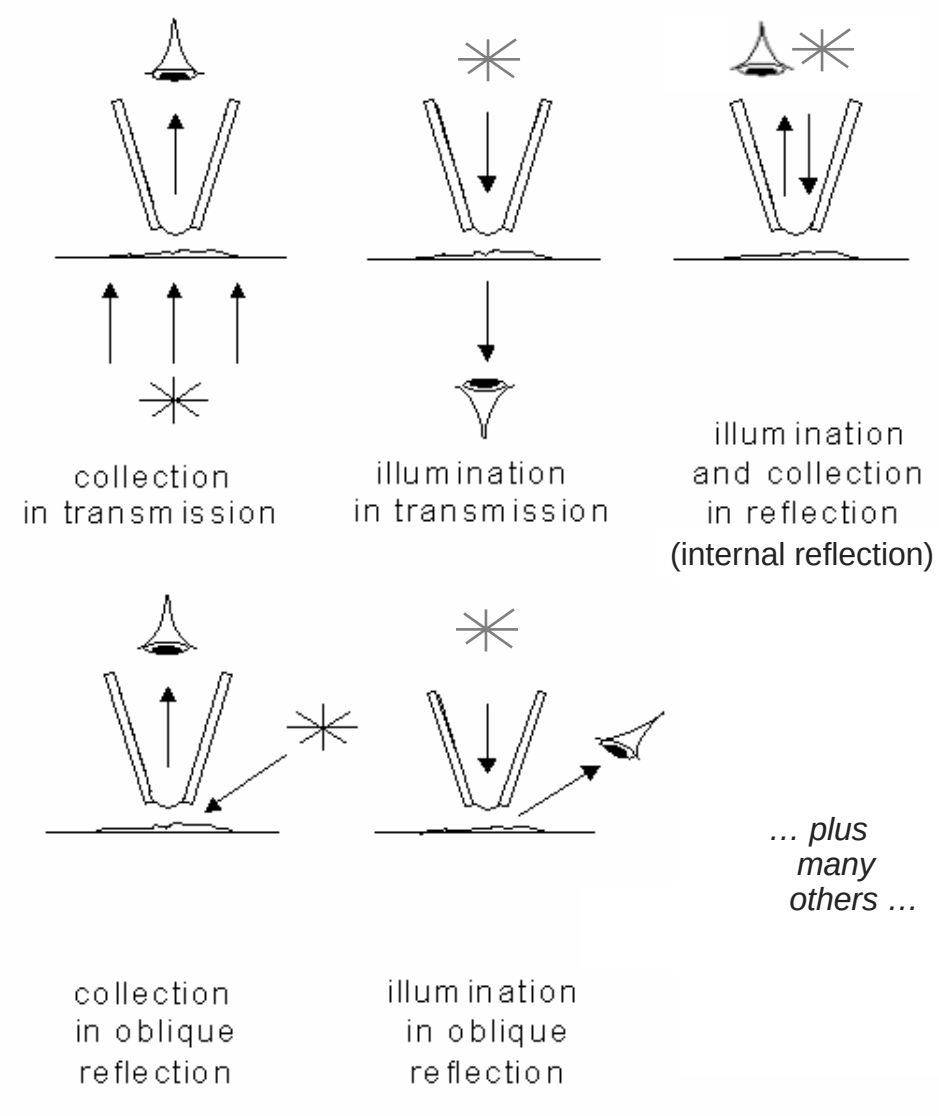


apex radius ~ 50 nm,
cone angle ~ 26°

NFO Microscopy setups 1/2

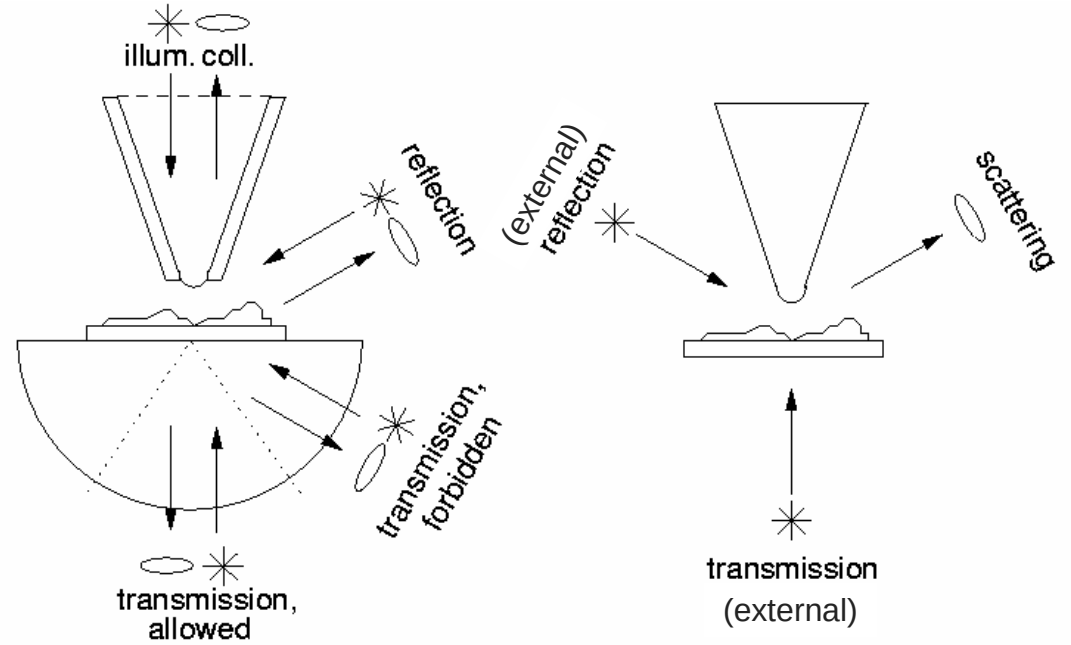
according to the "geometry"…

- position of the components
- direction of light

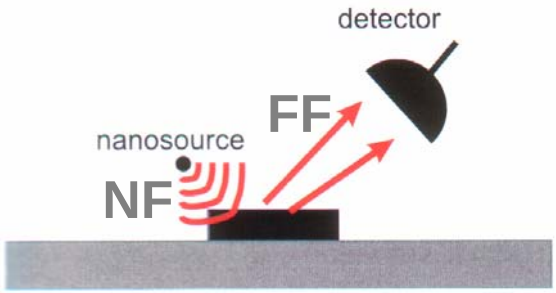


NFO Microscopy setups 2/2

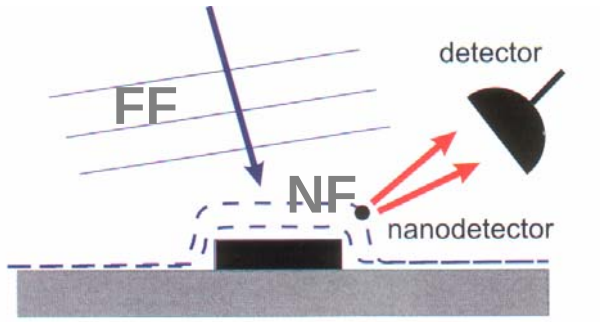
…according to the "physics"



- Aperture type (SNOM or a-SNOM)



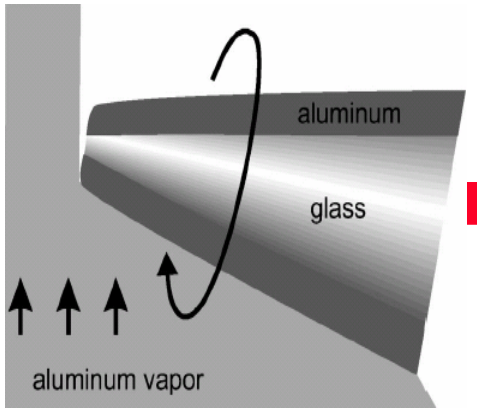
- Apertureless type (scattering or al-SNOM)

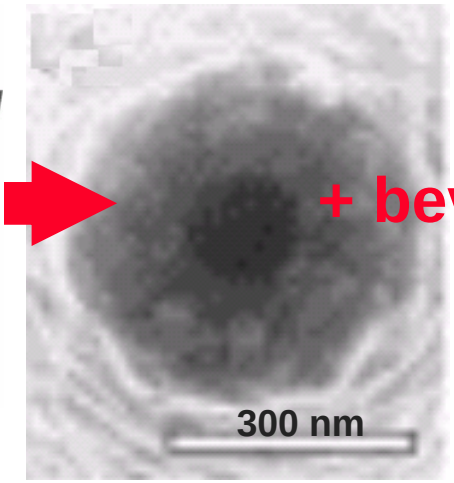


NFO probes: aperture formation

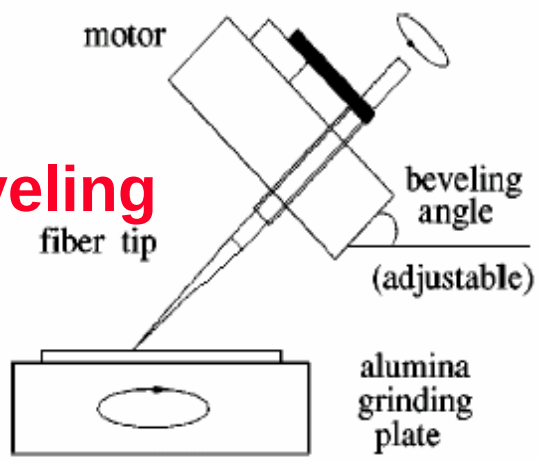
- **shadowed evaporation:**

Ø<=50 nm

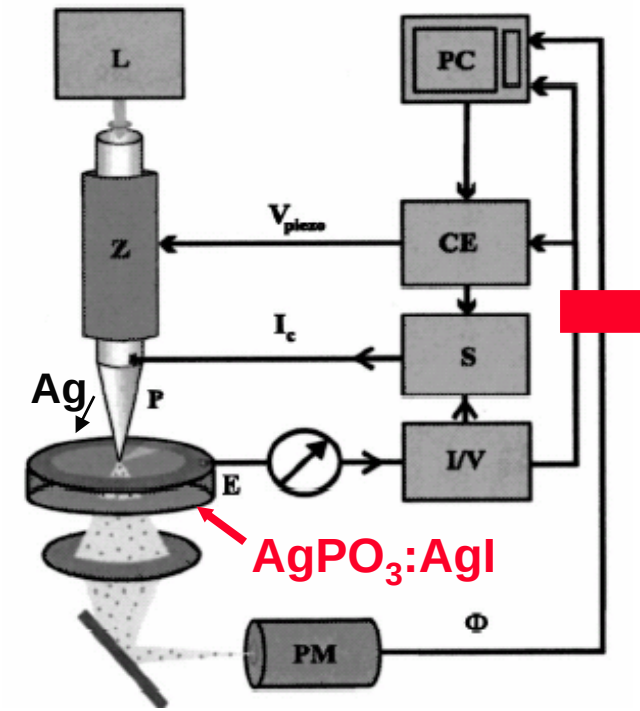


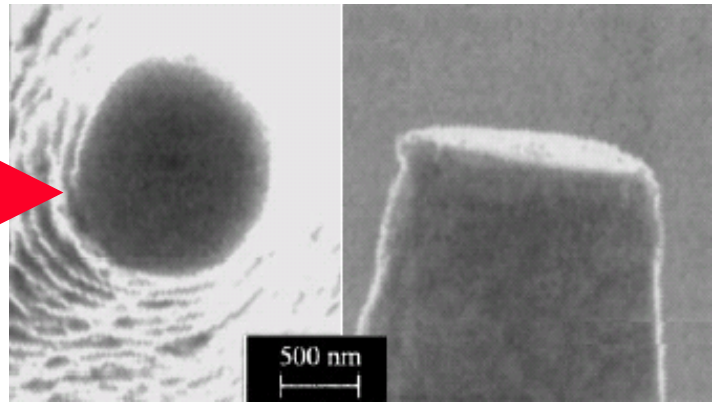


+ beveling



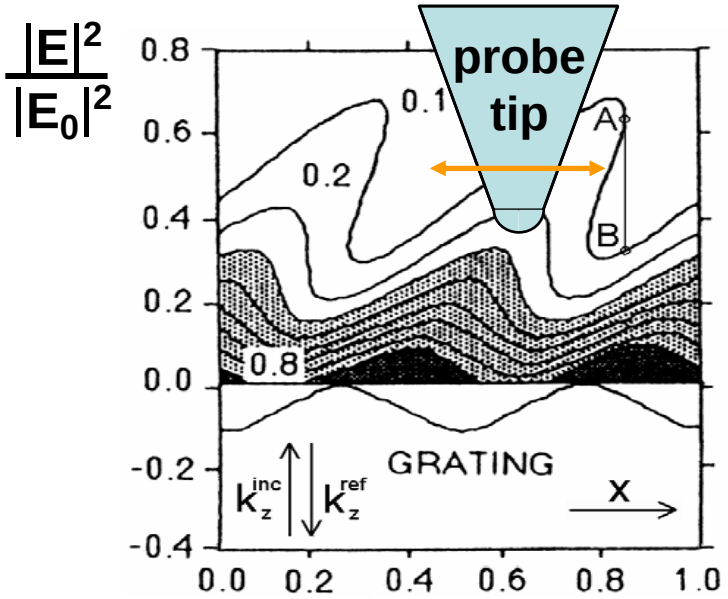
- **Controlled All Solid State Electrolysis :**





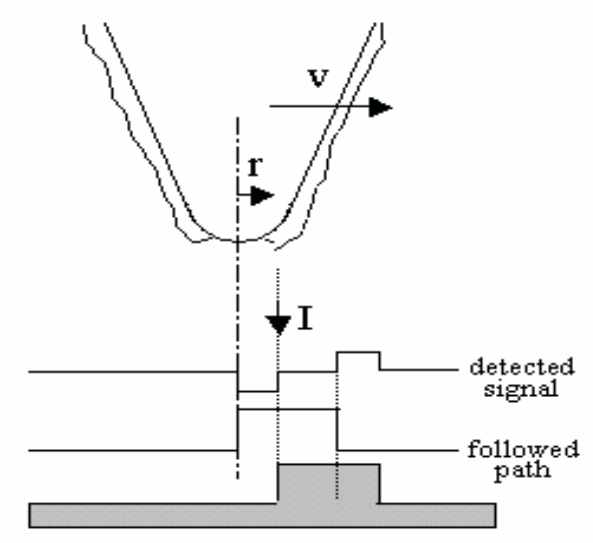
Constant Distance Mode, but :

- NFO signal isointensity profiles not a function, e.g.:



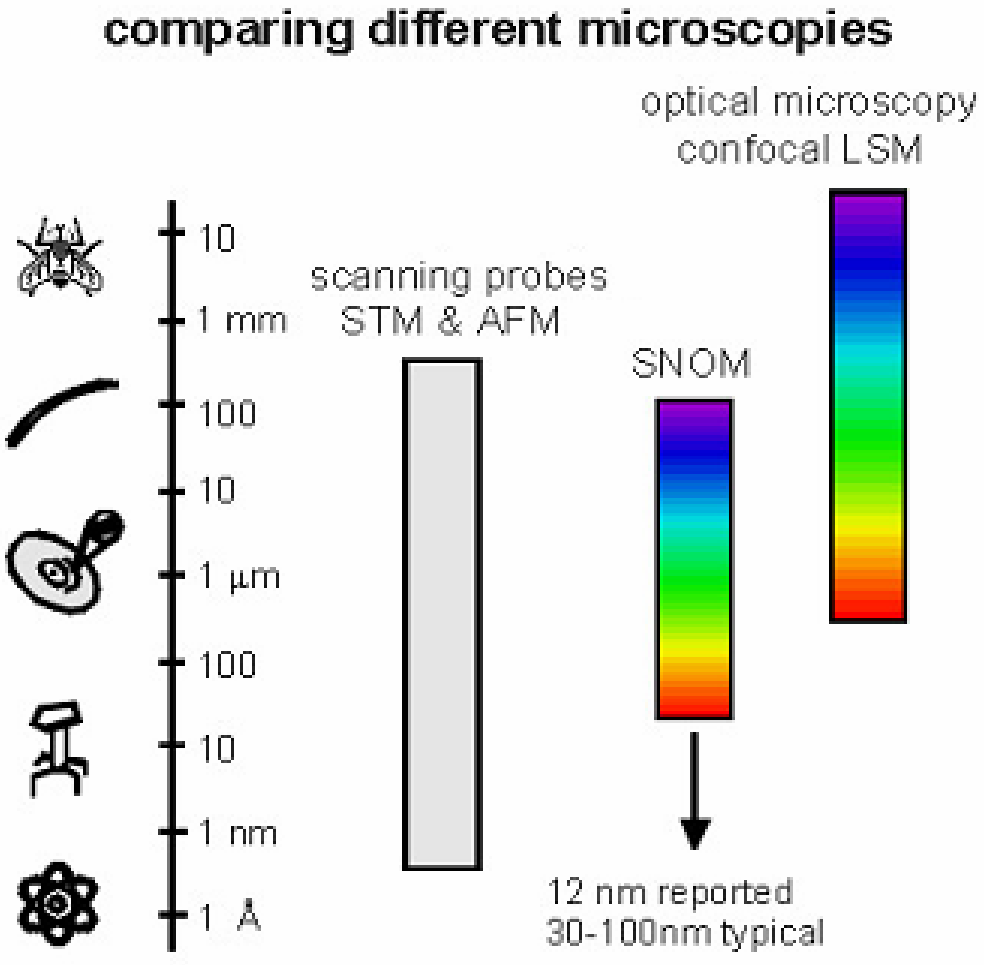
(longitudinal, TE illumination)

- most independent distance signals have off-centered tip, e.g.:



STM "crown tip" of metal coating

NSOM: the power of light at high resolution



contrast from:

- **absorption**
- **reflection**
- **emission**
- **polarization**

e.g. *fluorescence*:

- **fluorochrome dye labeling, based on**
  - **pH or Mg^{2+} Ca^{2+}**
  - **hydrophobic/philic interactions**
  - **specific bonding to prot. or nucleic acids**
- **Stokes shift**
- **$\tau \leq 10$ ns**

+ multi-labeling - photo-bleaching

Conclusions

- a number of microscopic techniques available today:
  - conventional optical microscopy:
    user friendly but diffraction-limited (res.~200 nm)
  - electron microscopy: very high (~5 nm, SEM)
    or even atomic (TEM) resolution
  - Near Field optical microscopy:
    ~30 nm res., and spectroscopic capabilities

- our choice will depend on what we want to “see”
  - for “force” sensing, viscoelastic properties etc.:
    → go AFM (next seminar !)

- always be aware of artifacts:
  “the map is not the territory”, Alford Korzybski