Radiation based Microscopy

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Outline

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

• 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)

object:

• medicine, biology (cells, bacteria, virus, colloidal particles, …)

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

- **magnification** (linear): \( A \rightarrow B = MxA \)

- **resolution** (resolving power): capability to separately identify 2 different points (objects)
  (human eye: 100-200 \( \mu \)m)

- **enhancing techniques**: Image Analysis & Processing
Diffraction and interference

**Huygens principle:**
- Hit scatterers work as equal, independent sources
- \( \rightarrow \) each wavefront = \( \sum \) array of wavelets

\[ \Delta s = n \Delta l \]

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

Airy function profile

\[ \delta = 0.61 \frac{\lambda}{\mu \sin \alpha} \]

Abbe eq.
Rayleigh resolution limit

**diffraction:**

\[ d_{\text{min}} = 0.61 \frac{\lambda}{NA} \], \hspace{1cm} NA = n \sin \alpha

N.B.,
resolution :
- undefined for an isolated object
- ≠ magnification \( M \)
Resolution limit: wave nature of light

Nyquist sampling theorem:

\[ \sim \text{need at least 2 data points} \]
\[ \text{(i.e. 1 max & 1 min) per 1 period (wavelength)} \]
\[ \text{to map an oscillating signal (light wave)} \]
\[ \text{without aliasing} \]

(based on Fourier transform theory)

look out: \textit{aliasing} gives \textit{pseudo-resolution}!
Resolution limit: particle nature of light

Heisenberg principle:
\[ \Delta x \Delta k_x, \Delta y \Delta k_y, \Delta z \Delta k_z > \frac{\hbar}{2} \]

if \( \Delta x, \Delta y < \lambda \), \( \Delta k_x, \Delta k_y \) large

invariance of \( k^2 = k_x^2 + k_y^2 + k_z^2 \)  \[ \Rightarrow k_z^2 < 0, \quad k_z = i |k_z| \]

\[ e^{ik_z z} \rightarrow \text{Evanescent field:} \]

*spatial frequencies* \( >1/\lambda \)

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...

down to “molecular detection”!

van Hulst
\[ \text{JCP 18, 7799 '00} \]

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: $\lambda = \frac{h}{p}$
- 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)*
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?

\[ \lambda_{\text{DeBroglie}} = \frac{h}{p} \]

Planck constant: 
\[ h = 6.63 \times 10^{-34} \text{ Js} \]

- tennis ball: \( M \approx 100 \text{ g}, v \approx 100 \text{ km/h} \) \( \rightarrow \lambda_M \approx 2 \times 10^{-24} \text{ Å} \)
- continuous (non-oscillating) wave
- electrons: \( m_e \approx 9 \times 10^{-31} \text{ kg}, v \approx 1/3 c \) \( \rightarrow \lambda_e \approx 0.02 \text{ Å} \approx 10^{22} \lambda_M \), "observable"

<table>
<thead>
<tr>
<th>( \lambda(E) ) [nm, eV]</th>
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<tbody>
<tr>
<td>( \gamma )</td>
</tr>
<tr>
<td>( e )</td>
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\[ E_\gamma = h\nu = \frac{hc}{\lambda_\gamma} \]
\[ E_e = \frac{p_e^2}{2m_e} = \frac{h^2}{2m_e}\lambda_e^2 \]

i.e. electrons – vs – photons
\( \rightarrow \) “softer” diffraction limit

- possible radiation damage
  but:
  - controlled potential (i.e. conductive) samples required
  - UHV environment required
examples of **HR-TEM**:  

\[ E \geq 100 \text{ kV} \]

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**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.

**Figure 4.55** Lattice image of a SiC particle located within an alumina grain. The alumina lies along a low-index plane axis, and is the source of the lattice image. A more 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

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)

… ESEM

• 1988 ElectroScan (USA)
  → Philips → FEI
Scan-enhanced optical microscopy

**CSOM** (Marvin Minsky patent, 1957)
Confocal Scanning Optical Microscope

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

**CLSM (or LSCM)**
Confocal Laser Scanning Microscopy

- for fluorescence detection

**TPE**
Two Photon Excitation

**Infrared laser (Mode-locked)**
Scanning mirror

Dichroic

Beam Splitter

Green PTM

Red Filter

Green Filter

Objective

Fluorophores

Focal Plane
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 high 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:

\[ J \propto T^2 \exp\left(\frac{-e\phi}{kT}\right) \]

- filament life (hrs)
  - \(W\): ~100, ~50
  - \(\text{LaB}_6\): ~1000, ~1000
  - FEG: ~2000, ~4000

- “FEG”:
  - operated at RT
  - e tunnel out of the cold filament
  - very sharp and clean
**electron-solid interactions**

\[ 0 = I_{\text{tot}} = -I_b + \delta I_b + \eta I_b + I_{sc} \]

\[ I_{sc} = (1 - \delta - \eta) I_b \]

= 0 (for insulating samples) at \( E = E_2 \)

<table>
<thead>
<tr>
<th>material</th>
<th>( E_2 ) (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>resist</td>
<td>0.6</td>
</tr>
<tr>
<td>amorphous C</td>
<td>0.8</td>
</tr>
<tr>
<td>teflon</td>
<td>1.9</td>
</tr>
<tr>
<td>quartz</td>
<td>3.0</td>
</tr>
<tr>
<td>alumina</td>
<td>3.5</td>
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BSE yield \( \eta = n_{\text{BSE}} / n_{\text{incid}} = 0.2 - 0.4 \)

SE yield \( \delta = n_{\text{SE}} / n_{\text{incid}} = 0.1 \) at 30 keV

\( < 1 \) at 1-2 keV
Interaction zone

- at the boundary $E \sim kT$
- as the path length in the sample increases, $<E>$ decreases and $E$ spread $\Delta E$ increases

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

envelope of inelastically scattered $e^-$ from the incident beam

1 nm - elettroni Auger
5 - 50 nm - elettroni secondari
Elettroni riflesi
Raggi X caratteristici
Raggi X del continuo
Raggi X di fluorescenza secondaria

Superficie del campione

$Z$

$E$
Source performance parameters

size

e source brightness: $\beta = J / \Omega$ (A / m² sterad) $\beta \propto V_0$

energy spread

W $\rightarrow$ LaB₆ $\rightarrow$ W FEG

$\beta$: 3÷10x 10÷100x

s: 20÷50 μm 10 nm

d = s M₁ M₂

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

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

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

limits to resolution: sensitivity, aberration
“Aberrations”

→ effective probe size:  \( d^2 \rightarrow d'^2 = d^2 + \sum d_i^2 \)

**diffraction:**  
(source)

Airy disc diameter  
\( d_{\text{differ}} = 1.22 \frac{\lambda}{\alpha} \)

**spherical aberration:**  
(lenses)

\( d_{\text{spher}} = \frac{C_{\text{spher}} \alpha^3}{2} \)

**chromatic aberration:**  
(source)

\( d_{\text{chrom}} = \frac{C_{\text{chrom}} \alpha \Delta E_{\text{tot}}}{E_0} \)

**astigmatism:**  
(somewhat controlled)

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

*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.
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 $\alpha$ exists

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

i.e. need very low aperture (in optics: $\alpha$ up to 1!)

$$\alpha \approx \tan \alpha = \frac{p}{D_{\text{focus}}} \rightarrow D_{\text{focus}} = \frac{p}{\alpha} = 100 - 1000 \text{ p} = 1-10 \text{ mm if 1 cm } \leftrightarrow 1000 \text{ p}$$

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

SE weak $\Rightarrow$ easy to collect at low detector bias

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

- hi inner bias accelerate 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
\[ I_{opt}(0) = I_{opt}(\theta) \cos \theta \]

\[ I_{SE}(\theta) = I_{SE}(0) \sec \theta \]

voltage contrast

magnetic contrast
  surface domains
  at free surface
  \( F = -e/c \ vXB \)
  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
\( \eta \sim -0.025 + 0.016 \, Z \)

\( Z \quad \eta \)
C : 6 0.05
Au: 79 0.5

Electron Channeling Pattern

for large single crystals

Bragg condition
\( \sin\theta = \lambda/2d_{ij} \)

topographic “contrast”

magnetic contrast
e.g.: SEM of EC-STM tips

**standard insulating coatings** \( [A_{lree} < (10 \, \mu m)^2] \):

- poliacrylics
- epoxy
- Apiezon wax

Tip coating device assembly

Apiezon wax coated Au tip. The very tip (several \( \mu m \)) remains uncoated.
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

\[ E_R = 2 \left[ \frac{p \cos \theta}{R^3} \right] (1 + i k R) e^{i k R} \]
\[ E_\theta = \left[ \frac{p \sin \theta}{R^3} \right] [1 + i k R - (k R)^2] e^{i k R} \]
\[ H_\phi = \left[ \frac{p \sin \theta}{R^3} \right] [i k R - (k R)^2] e^{i k R} \]

→ NF (kR<1):
- changes more rapidly than FF
- stronger where FF vanishes

(light wavevector, k=ω/c=2π/λ)
Photon tunneling

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

→ Near Field Optical Microscopy :

Fresnel formulas for refraction at the interface between 2 media
Fiber tip formation by Heat and Pull

+ very smooth taper surface
- very low throughput (~10^-7)
due to long taper (i.e. small angle \(2\alpha \sim 10^\circ\))
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

After 30 min ...

... 2 h ...

H$_2$SO$_4$ > 5 h ...

apex radius ~ 50 nm, cone angle ~ 26°
NFO Microscopy setups 1/2

according to the “geometry”…

- position of the components
- direction of light

... plus many others …
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:**
  \[ \Phi \leq 50 \text{ nm} \]

- **Controlled All Solid State Electrolysis:**
  \[ \text{AgPO}_3: \text{AgI} \]
Constant Distance Mode, but:

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

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

    - STM "crown tip" of metal coating

(longitudinal, TE illumination)
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