Microscopic Techniques
Outline

1. Optical microscopy
   Conventional light microscopy, Fluorescence microscopy, confocal/multiphoton microscopy and Stimulated emission depletion microscopy

2. Scanning probe microscopy
   Scanning tunneling microscopy (STM), Atomic force microscopy (AFM), Near-field scanning optical microscopy and others

3. Electron microscopy
   Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Scanning transmission electron microscopy (STEM), Focus ion beam microscopy (FIB)
1. Optical Microscopy
Conventional Optical Microscopy

This is an optical instrument containing one or more lenses that produce an *enlarged image* of an object placed in the focal plane of the lens.

Resolution limit: submicron particles approaches the wavelength of visible light (400 to 700nm)

1. Transmission: beam of light *passes* through the sample

   e.g. Polarizing or petrographic microscope

   Samples are usually fine powder or thin slices (transparent)

2. Reflection: beam of light *reflected* off the sample surface

   e.g. Metallurgical or reflected light microscope

   Surface of materials, especially opaque ones
Polarizing Microscope

Polarizer & Analyzer

Only the light component whose vibration direction is parallel to the polarizer is permitted to pass through.

Polarized light microscopy is utilized to distinguish between singly refracting (optically isotropic) and doubly refracting (optically anisotropic) media.
Principle of Polarizing Microscope

The interaction of plane-polarized light with a doubly refracting (birefringent) specimen to produce two individual wave components (ordinary ray and extraordinary ray) that are polarized in mutually perpendicular planes.

Crossed polars:
1. No sample $\rightarrow$ black
2. Isotropic sample $\rightarrow$ black
3. Anisotropic sample $\rightarrow$ color

The interaction of plane-polarized light with a doubly refracting (birefringent) specimen to produce two individual wave components (ordinary ray and extraordinary ray) that are polarized in mutually perpendicular planes.

- Different velocities
- Different propagation direction
Reflected Light Microscope

Half Mirror

Partially reflecting plane glass mirror that deflects light traveling from the horizontal illuminator by 90 degrees into the vertical optical train of imaging components in the microscope.

Objective Lens

- A matching well-corrected condenser properly aligned
- An image-forming objective projecting the image-carrying rays toward the eyepiece
Dark Field vs. Bright Field

Bright field:
- “normal” wide-field illumination method
- bright background
- low contrast

Dark field:
- an opaque disc is placed underneath the condenser lens
- scattered light
- dark background
- high contrast (structural details)

http://www.geog.ucl.ac.uk/~jhope/lab/micro23.stm
Phase Contrast Microscope

- bright-field
- destructive interference patterns in the viewed image (amplitude and phase difference)
- details in the image appear darker/brighter against a background
- colorless and transparent specimen, such as living cells and microorganisms

\[ P = S + D \]

Optical Path Length \( (D) = n \cdot t \)

\[ D = (n_2 - n_1) \cdot t \]

\[ \delta = \frac{2\pi D}{\lambda} \]
Applications of Optical Microscopy

1. Crystal morphology and symmetry

   - Crystal fragments (characteristic shape)
   - Classify isotropic and anisotropic substances
   - Check possible symmetry (parallel extinction)

2. Phase identification, purity and homogeneity

   - Standard optical data (refractive indices and optical axes) for comparison
   - Phase analysis (impurities with separated crystalline/amorphous phase)
   - Single vs. twinned crystal
Applications of Optical Microscopy

3. Crystal defects – grain boundaries and dislocations
   - Defects always present, even in single crystal
   - Chemical etching may preferentially occur at stress sites

4. Refractive index determination

Becke line method:
   - Sample \((n_1)\) is immersed in a liquid \((n_2)\)
   - Out of focus, light is seen to emerge from region of higher \(n\)
Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength.
Fluorescence Microscope

Especially useful in the examination of biological samples:

- **Identify** the particular molecules in complex structure (e.g. cells)
- **Locate** the spatial distribution of particular molecules in the structure
- Biochemical dynamics
- High signal to noise ratio
- Both reflected and fluorescence light

Drawback:
- Chemical labeling
Laser Scanning Confocal Microscope

Scanning a diffraction-limited point of excitation light across the sample

The out of focus light rays are eliminated from the image by the use of a confocal “pinhole”
Laser Scanning Confocal Microscope

Important technique for live cell and tissue imaging, the studies of biochemical dynamics!

Advantages:
• Optical sectioning ability
• 3D reconstruction
• Excellent resolution (0.1-0.2 μm)
• Specific wavelengths of light used
• Very high sensitivity

Drawbacks:
• Expensive
• Complex to operate
• Chemical labeling
• High intensity laser light
Advantages of Confocal Microscope

Conventional microscope

Confocal microscope

Confocal microscope image
Multiphoton Microscope

Advantages:
- Fluorescence only occurs at the focal point
- Able to image deeper into tissue sample

Drawbacks:
- Even more expensive (pulsed laser)
- Localized heating (photobleaching)
Resolution limited by wavelength of light (diffraction)

\[ R = \frac{1.22\lambda}{\text{NA}_{\text{objective}} + \text{NA}_{\text{condenser}}} = \frac{1.22\lambda}{2\text{NA}_{\text{objective}}} \]

NA: numerical aperture
Numerical Aperture

Lens in air:
\[ n \text{ of air: } 1 \]
\[ \sin \theta \leq 1 \]
\[ \text{NA} \leq 1 \]

Lens in oil:
\[ n \text{ of oil } > 1, \text{ similar to coverslip glass (~1.5)} \]
\[ \sin \theta \text{ increase (total internal reflection occur at high } \theta) \]

Overall NA will increase, >1

\[ R = \frac{1.22l}{2 \times \text{NA}_{\text{objective}}} \]
\[ = \frac{1.22 \times (400 \text{nm})}{2 \times (1.4)} \]
\[ = \sim 175 \text{nm} \]
Stimulated Emission Depletion (STED) Microscopy

Prof. Stefan W. Hell (Max Planck Institute for Biophysical Chemistry)

- The excitation spot is ~200 nm by focusing with a lens
- A STED beam (doughnut-shaped and centered over the excitation spot) is used to quench the fluorescent markers before they fluoresce
- Very smaller effective fluorescence spot (~60 nm)
Resolution Enhancement using STED
2. Scanning Probe Microscopy
Scanning Tunneling Microscopy (STM)

1986 Nobel Prize in Physics: Drs. Gerd Binning and Heinrich Rohrer (IBM Zurich)
Invention of the STM

Quantum tunneling:

*In quantum mechanics, an electron has a non-zero probability of tunneling through a potential barrier*
1. When a conducting tip is very close to a conducting/semiconducting surface and a bias voltage is applied, there will be a tunneling current flowing between the tip and the surface.

2. The tunneling current (~pA-nA) is a strong function of the gap between the tip and the surface.
Principle of STM

3. If the tunneling current is monitored and maintained constant by adjusting the gap, the elevation of the surface can be traced.

4. The surface morphology in atomic resolution can be obtained by x-y scan.
Very Sharp Tungsten Tip

- Electrochemical etching method
- Average radius curvature < 50nm

Piezoelectric Scanner

Piezoelectric effect

Inverse piezoelectric effect

Electrical Current Off  Electrical Current On
STM Imaging

HOPG surface (atomically flat)

Atomic resolution (0.1nm)
Scanning Tunneling Spectroscopy (STS)

By ramping the bias voltage, or distance of the tip from surface, the current signal can reveal the local electronic character of the substrate.

Can determine:

- Conductivity
- Bandgap
- Work function
- Density of State

Prof. Øystein Fischer’s research group

http://dpmc.unige.ch/gr_fischer/
Manipulation of Atoms

1. Xenon atom on Ni (110)

2. Closer tip-sample distance

3. Pulling atom with tip

4. Imaging mode

5. Manipulation mode

http://www.almaden.ibm.com
Atomic Force Microscopy (AFM)

**Principle:**

1. The molecular force is a strong function of the separation between two objects.

2. The force can be monitored by the deflection of a cantilever (100-200mm long) which is in turn amplified by the deflection of a laser beam.

3. Constant force is maintained by adjusting the z-position of the surface. A x-y scan will produce the morphology.
Operation Modes of AFM

I. Contact mode

- Tip touching surface
- Interaction force is repulsive ($10^{-8} - 10^{-6}$N)

II. Tapping mode

- >10nm above surface, no contact
- Cantilever set into vibration
- Detect changes in the resonant frequency of cantilever
- Feedback control of height
Applications of AFM

1. Imaging

- Resolution ~nm
- Topology
- Able to image non-conducting materials e.g. polymer and biological samples

Red blood cell

www3.imperial.ac.uk/
2. Force mapping

- To detect the variation of softness, elasticity and stickiness on sample surface
- Useful for composite materials
Applications of AFM

3. Dip-Pen Nanolithography

- Pattern molecules in high resolution
- Functionalize surfaces with patterns of two or more components

Prof. Chad A. Mirkin research group

4. Nanofabrication

Nanoshaving

Nanografting

Richard P. Feynman, 1980

400 nm

60 nm
## Summary of STM and AFM Functions

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<th><strong>STM</strong></th>
<th><strong>AFM</strong></th>
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<tr>
<td><strong>Instrumentation</strong></td>
<td>Tip, scanner, controller</td>
<td>Cantilever, scanner, optics, controller</td>
</tr>
<tr>
<td><strong>Conducting samples</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Non-conducting samples</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Resolution in vacuum</strong></td>
<td>&lt;0.1 Å</td>
<td>~ Å</td>
</tr>
<tr>
<td>In dry air</td>
<td>&lt; 1 Å</td>
<td>~ nm</td>
</tr>
<tr>
<td>In liquid</td>
<td>~ nm</td>
<td>~ 10 nm</td>
</tr>
<tr>
<td><strong>Operation in liquid</strong></td>
<td>Tip coating</td>
<td>No coating needed</td>
</tr>
<tr>
<td><strong>Modes of operation</strong></td>
<td>Constant height</td>
<td>Constant height</td>
</tr>
<tr>
<td></td>
<td>Constant current</td>
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<tr>
<td><strong>Applications</strong></td>
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<td></td>
<td>Tunneling spectroscopy</td>
<td>Force mapping</td>
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<td></td>
<td>Manipulation of atoms/molecules</td>
<td>nanolithography</td>
</tr>
</tbody>
</table>

- STM: Scanning Tunneling Microscopy
- AFM: Atomic Force Microscopy
Near-field Scanning Optical Microscope (NSOM)

Principle of NSOM: Can be simply modeled by the electromagnetic interaction of two very closely positioned nano-objects, which represent a probe and sample.

Aperture-type
- Nanoscale light spot same as aperture size
- Aperture-sample distance is regulated at < 10 nm

Scattering-type
- Sharpened homogeneous metal tip, with enhanced electric field
- Spatial resolution defined by apex diameter

Single Molecule Fluorescence Imaging

- Spatial resolution ~10-30nm
- Single molecule, quantum dot

Images courtesy of Dr. Dan Higgins and Prof. Dr. Paul Barbara
Department of Chemistry, U of Minnesota
Near-field Optical Spectroscopy

NanoRaman Spectroscopy

- Enhanced electric field at the tip
- Resolution as high as 15 nm
3. Electron Microscopy


Resolution and Abbe’s Equation

Abbe’s equation:

\[ R = \frac{0.612 \lambda}{n \sin \alpha} \]

Wavelength of Electron:

\[ \lambda = h (2meV)^{-1/2} \]

Resolution

Numerical aperture

Wavelength of imaging radiation

Electron microscopy:

- Very short wavelength (depends on accelerating voltage, \( \sim 0.04 \text{ Å at } 100 \text{ kV} \))
- Can be deflected by magnetic field (focusing)
**Fundamentals of Electron Microscopy**

*Scanning* electron microscopy (SEM): For studying the texture, topography and surface feature, resolution ~ 10 nm

*Transmission* electron microscopy (TEM): Lattice imaging, resolution < 0.2 nm

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**Resolution (log scale)**

1 Å 10 Å 100 Å 1000 Å 1 μm 10 μm 100 μm 1 mm

0.1 μm 0.1 mm

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**Diagram:**

- SEM
- HREM
- CTEM
- OPTICAL MICROSCOPY
- NAKED EYE
Interaction of Electron with Samples

- SEM or analytical EM
- Conventional TEM & Scanning TEM & Energy analysis

Diagram:

- Incident high-kV beam
- Secondary electrons (SE)
- Characteristic X-rays
- Visible light
- Bremsstrahlung X-rays
- Inelastically scattered electrons
- Direct beam
- Elastically scattered electrons
- Backscattered electrons (BSE)
- Auger electrons
- Electron-hole pairs
- 'Absorbed' electrons
- Specimen
Configuration of SEM
Secondary electrons

- Low energy
- Topographic contrast (surface texture and roughness)
- Resolve surface structure down to 10nm
- Excitation region depends on the accelerating voltage

![Diagram of low atomic number and high atomic number accelerating regions](image)
Backscattered electrons

- High energy
- Both Compositional and Topographic information
- Atomic number contrast
- Lateral resolution is worse than secondary electron image
Characteristic X-ray

- Chemical information of sample
- Energy Disperse X-ray Spectroscopy (EDS)

Detection area is limited by the resolution of SEM (accelerating voltage of electron)
E-beam Lithography

NW

Spin coated PMMA

Exposed to e-beam developing

Lift-off

Metallization

developing

Resolution ~50 to 100nm
In the TEM, we utilize the electrons that go through a very thin specimen (<200nm)

- Scattering electrons (strong interaction between electrons and matter)
- Image, diffraction pattern, x-ray spectrum and electron energy loss spectrum

Non-uniform distribution of electrons contains all the structural & compositional information

\[ 2d \sin \theta = n \lambda \]

When \( d \gg \lambda \), \( \sin \theta \) become very small!
Illumination System

TEM operation using a parallel beam
Function of C2 condenser aperture

*Convergent* beam for (S)TEM

\[ M = \frac{v}{u} \]
Alignment and Adjustment

1. Gun alignment: Electron should follow a straight line through the lens and apertures until it hit the specimen

2. Alignment of C2 aperture

3. Lens aberration
   - Control the minimum possible probe size
   - Aberration corrected TEM

4. Astigmatism
Imaging vs. Diffraction Modes
To select the electrons to form the image by inserting an objective aperture into the back focal plane of the objective lens.
High Resolution Imaging and Diffraction

- Atomic resolution < 0.16 nm
- Lattice spacing, atomic structure
- Interface (different phases, crystal structure)
- Combined with computer simulation
- Crystalline vs. amorphous materials
- Single vs. polycrystalline materials
- Crystal structure and orientation
- Crystal phases, facet
Scanning TEM

- Beam has to scan parallel to the optic axis at all times

STEM signal generated at any point on the specimen is detected, amplified and a proportional signal is displayed at an equivalent point on CRT
Scanning TEM

Dark-field STEM image:

- Annular detector, surrounds the BF detector
- Image contrast is sensitive to the atomic number of imaged materials
- Possible to detect impurities (dopant) using high resolution STEM

Unpublished result, Qian, Li and Lieber
Energy Disperse X-ray Spectroscopy (EDS)

Line scan

Elemental mapping

Highly resolved spatial distribution of elements in specimen
Electron Energy Loss Spectroscopy (EELS)

- Magnetic prism spectrometer
  - Complementary to EDS
  - High energy resolution
  - Atomic composition, chemical bonding, valence and conduction band electronic properties and surface properties
  - Ability to fingerprint different forms of the same element

- Absorption spectroscopy
- Inelastic scattered electrons
## Summary

**Microscopy:**
- Optical microscopy,
- Scanning probe microscopy
- Electron microscopy

**Functions:**
- Imaging (fluorescence, lattice-resolved and topography)
- Chemical analysis
- Structure determination
- Manipulation of atoms and molecules
- Nanolithography, e-beam lithography
- Spectroscopy: surface, electrical and optical properties