Optical Characterization Methods

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amc.mrl.Illinois.edu
Optical characterization

- Light properties
- Light-matter interactions
- Instrumentation and methods
- Applications
- Strengths and limitations
- Complementary techniques
Light properties

- Direction of propagation
- Electric field direction or polarization
- Photon energy or wavelength
- Intensity
- Speed (constant in vacuum = 299,792,458 m/s = 670,616,632 mph)
Light interactions

- Transmission
- Reflection
- Absorption
- Emission
- Scattering
- Refraction

Non-linear effects
- SFG
- SHG
- DFG
- Multi-photon absorption
Light – matter interaction

**Size**

**Lattice structure, dopants**

**Temperature**

**Stress**

**Microstructure**

**Concentration**

**Thickness**

**Composition**

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The interaction of radiation with matter
Spectroscopy

Graphs showing transmission and absorbance spectra.
Transmission, Reflection, Absorption

What is measured:
The transmitted and reflected light intensity as a function of the incident photon energy, which depends on the material’s electronic, atomic, chemical and morphological structure.
Spectrophotometry (UV-VIS-NIR)

Instrumentation:

- Sample
- Light source
- Detector
- Transmission
Spectrophotometry (UV-VIS-NIR)

Instrumentation:

Detector → Sample → Specular reflectance

Light source
Spectrophotometry (UV-VIS-NIR)

Instrumentation:

Light source

Detector

Sample

Diffuse transmission
Spectrophotometry (UV-VIS-NIR)

Instrumentation:

Light source

Detector

Sample

Diffuse reflectance
Spectrophotometry (UV-VIS-NIR)

Instrumentation:
Optical band gap determination of CdS thin films as a function of growth substrate temperatures

Tauc’s relation:

\[ \alpha h \nu = A (h \nu - E_g)^m \]

\( m = 0.5 \) for direct and 2 for indirect allowed transitions.

Using absorbance to determine Au/Hg concentration in water solutions

Spectrophotometry (UV-VIS-NIR)

Beer-Lambert Law

\[ \text{Abs} = K \ell c = a \ell \]

\[ \text{Abs} = \log \left( \frac{1}{T} \right) \]
Using transmission interference fringes to determine thickness

\[ \lambda_m = \frac{T_m}{\nu} = 2n \sin \theta \]
Excitations in materials
  • Plasmons

Plasmons are quanta of collective motion of charge-carryers in a gas with respect of an oppositely charged background. They play a significant role on transmission and reflection of light.

Spectrophotometry (UV-VIS-NIR)

Phys. Today, 64, 39 (2011)
Plasmonic crystal Brillouin zone from the transmission spectra measured for many different angles of incidence.
Spectrophotometry (UV-VIS-NIR)

Optical Materials Express, 332858 (2018)

Applied Materials Today 8, 68 (2017)
Spectrophotometry (UV-VIS-NIR)

ACS Nano 12, 10880 (2018)
Fourier Transform IR spectroscopy (FTIR)

Normal vibrational modes in molecules:

- CO$_2$ (4 modes)
  - $v_1 = 1388$ cm$^{-1}$
  - $v_2 = 667$ cm$^{-1}$
  - $v_3 = 2349$ cm$^{-1}$

- H$_2$O (3 modes)
  - $v_1$ (3657 cm$^{-1}$)
  - $v_2$ (1595 cm$^{-1}$)
  - $v_3$ (3756 cm$^{-1}$)

- PO$_4$ (9 modes)

Number of modes:
- 3N-6 for non-linear molecules
- 3N-5 for linear molecules
Fourier Transform IR spectroscopy (FTIR)

Normal vibrational modes in solids:

Sb/GaAs(110)  GaN  SWCNT

http://www.phonon.fc.pl

T. A. Beu and A. Farcaș 2016 *EPL* 113 37004
Fourier Transform IR spectroscopy (FTIR)

IR active vibrations

The intensity of a vibrational absorption depends on the strength of the transition dipole momentum, so a vibration mode j will be “IR active” only when \( \left( \frac{\partial \mu}{\partial Q_j} \right)_0 \neq 0 \).

\[
\begin{align*}
\text{Cl}_2 & \quad \nu_1 = 558 \text{ cm}^{-1} \\
\text{CO}_2 & \quad \nu_1 = 1388 \text{ cm}^{-1} \\
\text{HCl} & \quad \nu_1 = 1443 \text{ cm}^{-1} \\
& \quad \nu_2 = 667 \text{ cm}^{-1} \\
& \quad \nu_3 = 2349 \text{ cm}^{-1}
\end{align*}
\]

IR Inactive

\[
\left( \frac{\partial \mu}{\partial Q_j} \right)_0 = 0
\]

IR Active

\[
\left( \frac{\partial \mu}{\partial Q_j} \right)_0 \neq 0
\]
Fourier Transform IR spectroscopy (FTIR)

The Nobel Prize in Physics 1907
Albert A. Michelson

"for his optical precision instruments and the spectroscopic and metrological investigations carried out with their aid"

Instrumentation:
The FTIR uses a Michelson interferometer with a moving mirror, in place of a diffraction grating or prism.

\[ \Delta L = n\lambda \Rightarrow \text{constructive interference} \]
\[ \Delta L = (n+1/2)\lambda \Rightarrow \text{destructive interference} \]

Beam splitter
fixed mirror
moving mirror
sample
detector

Detector voltage

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Fourier Transform IR spectroscopy (FTIR)

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The Nobel Foundation

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\[ \Delta L = (n+1/2) \lambda \Rightarrow \text{destructive interference} \]

Detector voltage vs. Time

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Fourier Transform IR spectroscopy (FTIR)

\[ I(\nu) = \int_{-\infty}^{\infty} S(t) e^{2i\pi \nu t} \, dt \]
Fourier Transform IR spectroscopy (FTIR)

Polystyrene

Transmission (%) vs. Wavenumber (cm⁻¹)

3 mil
1.5 mil

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Fourier Transform IR spectroscopy (FTIR)

FTIR can be used to identify components in a mixture by comparison with reference spectra.

*Discovery of beeswax as binding agent on a 6th-century BC Chinese turquoise-inlaid bronze sword*

Wugan Luo, Tao Li, Changsui Wang, Fengchun Huang

*J. of Archaeological Sci. 39 (2012), 1227*
Fingerprinting:

FTIR can be used to identify components in a mixture by comparison with reference spectra.

Complementary characterization techniques, like XRD can provide conclusive evidence for the identification.

J. of Archaeological Sci. 39 (2012), 1227
Fourier Transform IR spectroscopy (FTIR)

Images from Wikipedia and eurotalc.com
Spectra from *Developments in Clay Science*, Vol. 8, Ch. 5

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Spectrophotometry (UV-VIS-NIR)

Images from Wikipedia and eurotalc.com
Spectra from *Developments in Clay Science*, Vol. 8, Ch. 5

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Fourier Transform IR spectroscopy (FTIR)
Fourier Transform IR spectroscopy (FTIR)
Strengths:

• Very little or simple sample preparation.
• Simplicity of use and data interpretation.
• Short acquisition time, for most cases.
• Non destructive.
• Broad range of photon energies.
• High sensitivity (~ 0.1 wt% typical for FTIR).

Limitations:

• Reference sample is often needed for quantitative analysis.
• Many contributions to the spectrum are small and can be buried in the background.
• Usually, unambiguous chemical identification requires the use of complementary techniques.
• Limited spatial resolution.

Complementary techniques:

Raman, Electron Energy Loss Spectroscopy (EELS), Extended X-ray Absorption Fine Structure (EXAFS), XPS, Auger, SIMS, XRD, SFG.
Light scattering
Light scattering

Sir Chandrasekhara Venkata Raman

The Nobel Prize in Physics 1930 was awarded to Sir Venkata Raman "for his work on the scattering of light and for the discovery of the effect named after him".
What is measured:
The light inelastically scattered by the material.

Basic principle:
The impinging light couples with the lattice vibrations (phonons) of the material, and a small portion of it is inelastically scattered. The difference between the energy of the scattered light and the incident beam is the energy absorbed or released by the phonons.
Raman spectroscopy

Impinging light couples with vibration modes of the material:

- Phonons
- Molecular vibrations

atomic structure
chemical composition
physical state
temperature.

http://exciting-code.org

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Raman spectroscopy

Inelastic scattering:

The dependence of the polarizability tensor $\tilde{\alpha}$ on the normal coordinate $Q$ associated with a normal vibrational mode of a material, for small amplitude oscillations near the equilibrium can be written:

$$\alpha = \alpha_0 + \left( \frac{\partial \alpha}{\partial Q} \right) Q = \alpha_0 + \alpha' Q$$

For a harmonic oscillation ($Q = Q_0 \cos \omega t$) and $E = E_0 \cos \omega_0 t$, the time dependence of the induced dipole momentum $\mu'$ will be:

$$\mu' = \alpha_0 E_0 \cos \omega_0 t + \frac{1}{2} \alpha' Q_0 E_0 [\cos(\omega_0 - \omega) t + \cos(\omega_0 + \omega) t]$$

meaning that the dipole oscillates with three frequencies simultaneously, corresponding to the three possible scattering modes (Rayleigh, Stokes Raman and anti-Stokes Raman)
Fourier Transform IR spectroscopy (FTIR)

**IR active vibrations**

The intensity of a vibrational absorption depends on the strength of the transition dipole momentum, so a vibration mode j will be “IR active” only when \( \left( \frac{\partial \mu}{\partial Q_j} \right)_0 \neq 0 \).

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
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<td>CO(_2)</td>
<td>HCl</td>
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<tr>
<td>( \nu_1 ) = 558 cm(^{-1})</td>
<td>( \nu_1 ) = 1388 cm(^{-1})</td>
<td>( \nu_1 ) = 1443 cm(^{-1})</td>
</tr>
<tr>
<td>IR Inactive</td>
<td>( \left( \frac{\partial \mu}{\partial Q_j} \right)_0 = 0 )</td>
<td>( \left( \frac{\partial \mu}{\partial Q_j} \right)_0 \neq 0 )</td>
</tr>
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<td></td>
<td>( \nu_2 ) = 667 cm(^{-1})</td>
<td>( \nu_3 ) = 2349 cm(^{-1})</td>
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</table>

Z. Naturforsch. 58a, 36 (2003); IOP Concise Physics - Molecular Photophysics and Spectroscopy, David L Andrews

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Raman spectroscopy

Raman active vibrations

The intensity of the Raman scattering linked to a vibrational state depends on the change in the polarizability tensor \( \left( \frac{\partial \alpha}{\partial Q_j} \right)_0 \neq 0 \)

Z. Naturforsch. 58a, 36 (2003); IOP Concise Physics - Molecular Photophysics and Spectroscopy, David L Andrews
FTIR and Raman:
The two techniques are complementary (different selection rules).
Molecular and crystalline structure characterization

Raman spectroscopy
Presence of N vacancies yields poor crystallinity

Substitutional C fills N vacancies improving the crystallinity

C incorporates interstitially causing a degradation of the crystal lattice

Raman spectroscopy

Molecular and crystalline structure characterization

PHYSICAL REVIEW B 68, 155204 (2003)
Raman spectroscopy

Crystalline structure defect characterization

Graphene

Raman spectroscopy

Crystalline structure defect characterization

Graphene

Raman spectroscopy

Crystalline structure defect characterization

The inset shows the linear dependence between the two parameters at low defect concentration.
Raman spectroscopy

Type of single wall carbon nanotubes (SWNT)

$G' = 2D$

$G'' = 2D'$

Advances in Physics 60, 413 (2011)

Raman spectroscopy

(a) Intensity (a.u.) vs. Raman shift (cm\(^{-1}\)) for different samples: Gr/flat SiO\(_2\), Gr/50 nm NSs, and Gr/20 nm NSs.

(b) Color map showing \(\omega_G\) and \(\omega_{2D}\) for Gr/20 nm NSs and Gr/flat SiO\(_2\).

(c) Graph showing the relationship between \(\omega_G\) and \(\omega_{2D}\) for different nanosphere diameters: 200 nm, 100 nm, 50 nm, and flat.

(d) Graph showing the average strain (%) vs. nanosphere diameter (nm) for different samples.
Raman spectroscopy

Phase transitions

(a) Different Raman Modes

<table>
<thead>
<tr>
<th></th>
<th>Si-I (cm$^{-1}$)$^\dagger$</th>
<th>Si-III (cm$^{-1}$)$^\dagger$</th>
<th>Si-XII (cm$^{-1}$)$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>300, 520</td>
<td>166, 171</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>a-Si (cm$^{-1}$)$^\dagger$</td>
<td>384</td>
<td>352</td>
<td></td>
</tr>
<tr>
<td>475, 510</td>
<td>432, 463</td>
<td>397</td>
<td></td>
</tr>
</tbody>
</table>

$^\dagger$ Ref 2,3

J. Raman Spectroscopy 41, 334 (2010)
Raman spectroscopy

Chemical composition
Component identification
Components distribution

Raman spectroscopy

Primary Strengths:
- Very little sample preparation.
- Structural characterization.
- Non destructive technique.
- Chemical information.
- Complementary to FTIR.

Primary Limitations:
- Expensive apparatus (for high spectral/spatial resolution and sensitivity).
- Weak signal, compared to fluorescence.
- Limited spatial resolution (diffraction limited).

Complementary techniques:
FTIR, EELS, Mass spectroscopy, EXAFS, XPS, AES, SIMS, XRD, SFG.
Excitations in materials
•Plasmons

Plasmons are quanta of collective motion of charge-carriers in a gas with respect of an oppositely charged background.

They can be driven by photons at resonance to build large standing wave electric fields.

That leads to a strong enhancement of Raman scattering, proportional to fourth power of the E field strength.
Surface Enhanced Raman Spectroscopy (SERS)

Typically achieved with corrugated gold/silver surface or gold/silver nanoparticles with molecules of interest attached.

Capable of boosting Raman signal up to 14 Orders of Magnitude or more! *Science* **275**, 1102 (1997)

Surface Enhanced Raman Spectroscopy (SERS)

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*Science* 275, 1102 (1997)

Anal. Methods, 6, 9547 (2014)
Raman spectroscopy

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Confocal Raman Microscopy

Confocal Raman Image
Carbon Nanotubes

This also works with a single metalized sharp tip, such as an STM or AFM tip!

Not only do you get the electric field enhancement, but now the source of the Raman signal is extremely localized.
Tip Enhanced Raman Spectroscopy (TERS)

Confocal Raman Image

Tip Enhanced Raman Image

Carbon Nanotubes

Near-field scanning optical nanospectroscopy

- Fiber Laser
- DFG
- BS
- NeaSNOM
- AFM cantilever
- detector
- d
- RM
Near-field scanning optical nanospectroscopy

Nano-FTIR

Nature Communications 4, 2890
Near-field scanning optical nanospectroscopy

Nano-FTIR

Nature 000, 1-4 (2012) doi:10.1038/nature11253

https://www.youtube.com/watch?v=mcom2uN1TR4
Near-field scanning optical nanospectroscopy

Nano-FTIR

Science 344, 1369
Near-field scanning optical nanospectroscopy

**Strengths:**
- No sample preparation.
- Non destructive technique.
- Sub diffraction limit resolution (20 nm).

**Requirements and limitations:**
- Slow data acquisition.
- Limited to fairly flat samples (AFM-like).
- Interaction between tip and sample may make analysis difficult.

**Complementary techniques:**
AFM, SEM, TEM, Confocal microscopy.
Luminescence

Lifetime: Phosphorescence, fluorescence

Mechanism: Photoluminescence, bioluminescence, chemoluminescence, thermoluminescence, piezoluminescence, etc.
What is measured:
The emission spectra of materials due to radiative recombination following photo-excitation.

Basic principle:

[Diagram showing the concept of direct and indirect band gaps in photoluminescence]
What is measured:
The emission spectra of materials due to radiative recombination following photo-excitation.

Basic principle:
Photoluminescence

Number of layers in 2D materials

(a) PL spectra for mono- and bilayer MoS$_2$.
   Inset: PL QY of thin layers for N = 1–6.
(b) Normalized PL spectra by the intensity of peak A of thin layers of MoS$_2$ for N = 1–6. Feature I for N = 4–6 is magnified for clarity.
(c) Band-gap energy of thin layers of MoS$_2$, inferred from the energy of the PL feature I for N = 2–6 and from the energy of the PL peak A for N = 1. The dashed line represents the (indirect) band-gap energy of bulk MoS$_2$.

Defects in 2D materials

(a) Defect induced PL emission. (a) Schematic diagram of electron beam irradiation on monolayer WSe$_2$ sample during the EBL process.
(b) PL spectrum of pristine monolayer WSe$_2$ and monolayer WSe$_2$ after EBL. The inset shows optical image of WSe$_2$ with PMMA patterned by EBL, scale bar is 5 $\mu$m.
(c) PL spectra of a pristine WSe$_2$ under different e-beam irradiation density.
Photoluminescence

Carrier concentration

Photoluminescence spectra of InN layers with different carrier concentrations.

1 - $n = 6 \times 10^{18}$ cm$^{-3}$ (MOCVD);
2 - $n = 9 \times 10^{18}$ cm$^{-3}$ (MOMBE);
3 - $n = 1.1 \times 10^{19}$ cm$^{-3}$ (MOMBE);
4 - $n = 4.2 \times 10^{19}$ cm$^{-3}$ (PAMBE).

Photoluminescence

In$_x$Ga$_{1-x}$N alloys. Luminescence peak positions of catodoluminescence and photoluminescence spectra vs. concentration $x$.

The plots of luminescence peak positions can be fitted to the curve $E_g(x)=3.48 - 2.70x - bx(1-x)$ with a bowing parameter of $b=2.3$ eV.

Photoluminescence

Electronic band structure

Conduction band

Valence band

Width and quality of semiconductor quantum wells.

<table>
<thead>
<tr>
<th>Depth (nm)</th>
<th>Material</th>
<th>Layer Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>GaAs</td>
<td>cap</td>
</tr>
<tr>
<td>3</td>
<td>InGaAsN</td>
<td>QW</td>
</tr>
<tr>
<td>35</td>
<td>GaAs</td>
<td>barrier</td>
</tr>
<tr>
<td>5</td>
<td>InGaAsN</td>
<td>QW</td>
</tr>
<tr>
<td>35</td>
<td>GaAs</td>
<td>barrier</td>
</tr>
<tr>
<td>9</td>
<td>InGaAsN</td>
<td>QW</td>
</tr>
<tr>
<td>100</td>
<td>GaAs</td>
<td>buffer</td>
</tr>
<tr>
<td>GaAs (001) SUB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Photoluminescence

**Photoluminescence**

**Strengths:**
- Very little to none sample preparation.
- Non-destructive technique.
- Very informative spectrum.

**Limitations:**
- Often requires low temperature.
- Data analysis may be complex.
- Many materials luminescence weakly.

**Complementary techniques:**
- Ellipsometry, Modulation spectroscopies, Spectrophotometry, Raman.

www.glofish.com
Polarization

Guimond and Elmore - Oemagazine May 2004

http://www.photophysics.com/
Polarization

Light Waves Vibrating Perpendicular to the Highway

Light Waves Vibrating Parallel to the Highway

Incident ray (unpolarised)

Reflected ray (polarised)

θ

Reflected ray (slightly polarised)

www.bobatkins.com

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What is measured:
The changes in the polarization state of light upon reflection from a mirror like surface.
Ellipsometry

Basic principle:
The reflected light emerges from the surface elliptically polarized, i.e. its p and s polarization components are generally different in phase and amplitude.

\[
\tan(\Psi') e^{i\Delta} = \frac{\tilde{R}_p}{\tilde{R}_s}
\]
Ellipsometry

\[ \tilde{n} = \mathbf{n} + ik \]

\[ \tilde{n}_1 \sin \phi_1 = \tilde{n}_2 \sin \phi_2 \]

\[ \tilde{r}_{12}^{p,s} = \frac{\tilde{n}_{2,1} \cos \phi_1 - \tilde{n}_{1,2} \cos \phi_2}{\tilde{n}_{2,1} \cos \phi_1 + \tilde{n}_{1,2} \cos \phi_2} \]

\[ \tilde{R}_{p,s} = \frac{\tilde{r}_{ab}^{p,s} + \tilde{r}_{bc}^{p,s} e^{-2i\beta}}{1 + \tilde{r}_{ab}^{p,s} \tilde{r}_{bc}^{p,s} e^{-2i\beta}} \]

\[ \beta = \frac{2\pi d}{\lambda} \tilde{n}_b \cos \phi_b \]

\[ \tan(\Psi) e^{i\Delta} = \frac{\tilde{R}_p}{\tilde{R}_s} \Rightarrow \]

\[ \tan(\Psi) = \frac{\tilde{R}_p}{\tilde{R}_s} \]

\[ \Delta = \delta^r - \delta^i \]
Ellipsometry

Applications

➢ Film thickness

| SiO₂ | 21.1 Å |
| Si   |       |

SiO₂ thickness 21.1 ± 0.5 Å

χ² = 4.31
Ellipsometry

Applications

➢ Film thickness

$\text{SiO}_2 \quad 18.7 \pm 0.2 \ \text{Å}$

$\text{SiO}_2 \quad 4923.1 \pm 0.2 \ \text{Å}$
Ellipsometry

Applications

- Composition
- Surface roughness
- Film thickness

Ellipsometric $\Psi(\lambda)$ and $\Delta(\lambda)$ spectra of Cd$_{1-x}$Zn$_x$S thin films deposited under the different concentration of ammonia: 0.19, 0.38, 0.56, and 0.75 M.

<table>
<thead>
<tr>
<th>[NH4OH] (M)</th>
<th>Thickness (nm)</th>
<th>Roughness (nm)</th>
<th>ZnS (%)</th>
<th>Band-gap (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19</td>
<td>42.12</td>
<td>23.77</td>
<td>99.7</td>
<td>3.49</td>
</tr>
<tr>
<td>0.38</td>
<td>73.79</td>
<td>7.15</td>
<td>45.5</td>
<td>2.52</td>
</tr>
<tr>
<td>0.56</td>
<td>50.89</td>
<td>5.94</td>
<td>32.3</td>
<td>2.45</td>
</tr>
<tr>
<td>0.75</td>
<td>18.59</td>
<td>4.54</td>
<td>5.2</td>
<td>2.43</td>
</tr>
</tbody>
</table>

Ellipsometry

Applications

- Composition
- Surface roughness
- Film thickness
- Band gap energy
- Optical constants (dielectric function)

Ellipsometry

**Strengths:**
- Fast.
- Measures a ratio of two intensity values and a phase.
  - Highly accurate (even in low light levels).
  - No reference sample necessary.
  - Not susceptible to scatter, lamp or purge fluctuations.
  - Increased sensitivity, especially to ultrathin films (<10nm).
- Can be used in-situ.

**Limitations:**
- Flat and parallel surface and interfaces with measurable reflectivity.
- A realistic physical model of the sample is required to obtain most useful information.

**Complementary techniques:**
Optical microscopy

Figure 6
Optical microscopy

"Conventional" Optical Microscopy
Optical microscopy

- Bright field
- Phase contrast
- Dark field
- Polarizing
Optical microscopy
# Optical microscopy

## Contrast-Enhancing Techniques for Optical Microscopy

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Imaging Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmitted Light</strong></td>
<td></td>
</tr>
<tr>
<td>Transparent Specimens</td>
<td>Phase Contrast</td>
</tr>
<tr>
<td>Phase Objects</td>
<td>Differential Interference Contrast (DIC)</td>
</tr>
<tr>
<td>Bacteria, Spermatozoa, Cells in Glass Containers, Protozoa, Mites, Fibers, etc.</td>
<td>Hoffman Modulation Contrast</td>
</tr>
<tr>
<td></td>
<td>Oblique Illumination</td>
</tr>
<tr>
<td>Light Scattering Objects</td>
<td>Rheinberg Illumination</td>
</tr>
<tr>
<td>Diatoms, Fibers, Hairs, Fresh Water Microorganisms, Radiolarians, etc.</td>
<td>Darkfield Illumination</td>
</tr>
<tr>
<td>Light Refracting Specimens</td>
<td>Phase Contrast and DIC</td>
</tr>
<tr>
<td>Colloidal Suspensions powders and minerals Liquids</td>
<td></td>
</tr>
<tr>
<td>Amplitude Specimens</td>
<td>Brightfield Illumination</td>
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<tr>
<td>Stained Tissue</td>
<td></td>
</tr>
<tr>
<td>Naturally Colored Specimens</td>
<td></td>
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<tr>
<td>Hair and Fibers</td>
<td></td>
</tr>
<tr>
<td>Insects and Marine Algae</td>
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<tr>
<td>Fluorescent Specimens</td>
<td>Fluorescence Illumination</td>
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<tr>
<td>Cells in Tissue Culture</td>
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<td>Fluorochrome-Stained Sections</td>
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<tr>
<td>Smears and Spreads</td>
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<td>Liquid Crystals</td>
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<td>Melted and Recrystallized Chemicals</td>
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<tr>
<td>Hairs and Fibers</td>
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<tr>
<td>Bones and Feathers</td>
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</tbody>
</table>

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## Optical microscopy

### Contrast-Enhancing Techniques for Optical Microscopy

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<th>Specimen Type</th>
<th>Imaging Technique</th>
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<td><strong>Reflected Light</strong></td>
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<td>Specular (Reflecting) Surface</td>
<td>Brightfield Illumination, Phase Contrast, DIC, Darkfield Illumination</td>
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<td>Thin Films, Mirrors</td>
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<tr>
<td>Diffuse (Non-Reflecting) Surface</td>
<td>Brightfield Illumination, Phase Contrast, DIC, Darkfield Illumination</td>
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<td>Thin and Thick Films</td>
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<td>Rocks and Minerals</td>
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<td>Hairs, Fibers, and Bone Insects</td>
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<tr>
<td>Amplitude Surface Features</td>
<td>Brightfield Illumination, Darkfield Illumination</td>
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<td>Dyed Fibers</td>
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<td>Mineral Thin Sections</td>
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<td>Bones and Feathers</td>
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<td>Single Crystals</td>
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<td>Oriented Films</td>
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Optical microscopy
Resolution

$\frac{d}{\approx \frac{0.61 \lambda}{NA}}$ Rayleigh criterion

$\frac{d}{\approx \frac{\lambda}{NA_{col} + NA_{obj}}} \approx \frac{\lambda}{2 \cdot NA}$ Abbé criterion
Optical microscopy

\[ \text{NA} = (n) \sin(\mu) \]

(a) \( \mu = 7^\circ \) \( \text{NA} = 0.12 \)

(b) \( \mu = 20^\circ \) \( \text{NA} = 0.34 \)

(c) \( \mu = 60^\circ \) \( \text{NA} = 0.87 \)
Confocal microscopy

- Increased contrast => 200:1.
- Slightly increased in plane resolution (1.5 x)
- Significantly increased resolution along the optical axis.
- Scanning image formation.
**Confocal microscopy**

The relation of the first ring maximum amplitude to the amplitude in the center is 2% in case of conventional point spreading function (PSF) in a focal plane, while in case of a confocal microscope this relation is 0.04%.

\[
r_{\text{res}} = 0.61 \frac{\lambda}{n \sin \theta} = 1.22 \frac{\lambda}{D} F
\]

\[
r_{\text{conf}} = 0.44 \frac{\lambda}{n \sin \theta} = 0.88 \frac{\lambda}{D} F
\]
Confocal microscopy

Laser Scanning Confocal Microscopy

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Confocal microscopy

- **<fibroblasts network on epoxy>** data/image courtesy of Joselle McCracken, Nuzzo Group
- **<cells bridge the gap>** data/image courtesy of Joselle McCracken, Nuzzo Group
Confocal microscopy

Scratch on glass
Confocal microscopy

Laser barrier curtain
Confocal microscopy

Mermaid butterfly wing
Confocal microscopy

Brass sphere

<table>
<thead>
<tr>
<th>Area</th>
<th>Ra (µm)</th>
<th>Rq (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area1</td>
<td>8.159</td>
<td>10.616</td>
</tr>
<tr>
<td>Area2</td>
<td>12.537</td>
<td>15.763</td>
</tr>
</tbody>
</table>

\[
Ra = \frac{1}{n} \sum_{i=1}^{n} |y_i|
\]

\[
Rq = \sqrt{\frac{1}{n} \sum_{i=1}^{n} y_i^2}
\]
Confocal microscopy

50 nm calibration standard
Confocal microscopy

Strengths:
- Optical sectioning.
  - Three-dimensional images.
  - Software localization of signal can bring z resolution to 20 nm.
- Improved contrast (200:1).
- Better resolution lateral (1.5x).
- Field of view defined by the scanning range.

Limitations:
- Image is scanned, resulting in slower data acquisition.
- High intensity laser radiation can damage some samples.
- Cost (typically 5x more than a comparable wide-field system).
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September 5th, 2019

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