MICROSCOPY COURSE 2012

INTRODUCTION TO BASIC LIGHT MICROSCOPY
AIM OF THE COURSE

• Teach basic principles of light microscopy
  • Theoretical background
  • Practical aspects
• Faced towards applications of light microscopy in life science
  • Which information can we obtain with light microscopy?
  • What are the limitations?
• Prerequisites
  • Curiosity to learn microscopy
OPTICS HISTORY

- Ancient world
- 1621 W. Snellius Reflection
- 1637 R. Descartes Optical Refraction
- 1657 P. de Fermat Fermat’s principle
- 1665 R. Hooke Light as a wave
  C. Huygens
- 1804 T. Young Interference
- 1818 Fresnel Diffraction/Dispersion
- 1821 Frauenhofer Diffraction Patterns
- 1876 J. C. Maxwell Electromagnetic wave
OPTICS
WHAT’S ALL THE FUZZ ABOUT

• Why do we care about optics?
  • Fundamental working principle of a light microscope
  • Limitations of light microscopy
  • Interaction/communication
    • Scientists (coming from life science) are using microscopes
    • Scientists/engineers (with a strong physics background) are building microscopes
BASICS IN OPTICS

• What is light?
  • How can we describe light?
    • Is the velocity of light finite or infinite?

• Behaviour of light
  • Interaction of light with matter
  • Influence the way light “travels”

• Optical instruments
  • Microscopes
  • Telescopes
Reflection/Refraction
OPTICAL REFRACTION
OPTICAL REFRACTION
SNELL’S LAW

Ibn Sahl of Baghdad: “On burning Mirrors and lenses” (984)

\[
\frac{\sin \theta_1}{\sin \theta_2} = \frac{v_1}{v_2} = \frac{n_2}{n_1}
\]
FERMATS'S PRINCIPLE

• Principle of least time
  • Path taken between two points by a ray of light is the path that can be transversed in the least time
  • Enables a description of optics by means of rays in isotropic media

\[ L(AB) = \int_{AB} nds \]
Characterization of a lens:
Focal length: \( f = 50 \text{ mm} = 0.05 \text{ m} \)
Power: \( 1/f = 20 \text{ m}^{-1} = 20 \text{ dioptr} \)
GEOMETRICAL OPTICS
THIN LENS

Working principle of lenses:
• Refraction
• Curvature
Factors that determine the focal length of a lens

- index of refraction
- index of refraction of the medium
- radius of the front surface
- radius of the back surface

\[
\frac{1}{f} = \left( \frac{n_1}{n_m} - 1 \right) \left( \frac{1}{r_1} - \frac{1}{r_2} \right)
\]

<table>
<thead>
<tr>
<th>Material</th>
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http://hyperphysics.phy-astr.gsu.edu/hbase/tables/indrf.html#c1
SIMPLE LENS TYPES
GEOMETRICAL OPTICS
THIN LENS- IMAGE FORMATION

Principal plane

\[ \frac{1}{f} = \frac{1}{s_0} + \frac{1}{s_1} \]

\begin{align*}
1/f &= 0.26 \text{ cm}^{-1} \\
1/s_0 &= 0.19 \text{ cm}^{-1} \\
1/s_1 &= 0.07 \text{ cm}^{-1} \\
s_0 &= 5.35 \text{ cm} \\
s_1 &= 14.1 \text{ cm} \\
f &= 3.8 \text{ cm}
\end{align*}
GEOMETRICAL OPTICS
THIN LENS- VIRTUAL IMAGE FORMATION

Principal plane

-\( f \)

\( f \)

object

optical axis
GEOMETRICAL OPTICS

TELESCOPE

\[ f_1 = -f_2 \]

\[ d = f_1 + f_2 \]

\[ M = \frac{f_2}{f_1} \]
GEOMETRICAL OPTICS

• Paraxial approximation
  • rays have fixed wavelength
  • rays are almost parallel to the optical axis (small angle)
    $\theta \approx \sin \theta \approx \tan \theta$
  • also known as Gaussian optics

• Ray tracing for “simple” systems
  • powerful tool (simple and intuitive)
GEOMETRICAL OPTICS

• Advanced topics
  • Thick lenses, stops
  • Matrix formalism (ABCD)
    • Straightforward calculation of complicated systems (multiple elements)
    • Convenient for numerical calculations
  • No paraxial approximation
• Disadvantages: No Dispersion
Phase velocity depends on the frequency of the wave.

Rate at which the phase of a wave propagates in space

Example of Dispersion: Rainbow
WAVES
SIMPLE MODEL

\[ V(t) = A \cos(\omega t - \phi) \]

- \( A \): Amplitude
- \( \omega \): Angular frequency
- \( \phi \): Phase shift

\[ T = \frac{2\pi}{\omega} \]
\[ \nu = \frac{1}{T} \] (frequency)
WAVES
INTERFERENCE
**Non-dispersing wave equation**

\[
\frac{\partial^2 f}{\partial x^2} = \frac{1}{v^2} \frac{\partial^2 f}{\partial t^2}
\]

**Wave equation**

\[
f(x,t) = a \exp \left[ 2\pi i \left( \frac{x}{\lambda} - vt \right) \right]
\]

- \(k = \frac{2\pi}{\lambda}\) \text{ wave-number}
- \(\omega = 2\pi v\) \text{ angular frequency}

**Phase velocity**

\[
f(x,t) = a \exp [i(kx - \omega t)]
\]

\(v = \omega / k\)
ATTENUATED HARMONIC WAVE

\[ k \equiv k_1 + ik_2 \]

\[ f = a \exp [i(k_1 + ik_2)x - i\omega t] \]

\[ = a \exp (-k_2x) \exp (i(k_1x - \omega t)) \]
HUYGENS-FRESNEL PRINCIPLE

Refraction

Diffraction
WAVES
PLANE WAVE

\[ \lambda \]

wave fronts

wave vector \( k \)
LIGHT-WAVE THEORY

YOUNG: DOUBLE SLIT EXPERIMENT

Diagram showing light waves passing through two slits (S1 and S2) to create an interference pattern at point F.
LIGHT-WAVE THEORY
FAR FIELD DIFFRACTION

Parallel light

aperture 100 µm

distance R

R > 1.3 m

Observation plane

J. Frauenhofer (1787-1826)
BIREFRINGENCE
DOUBLE REFRACTION

• Discovered by Rasmus Bartholin (1669)
• Unaxial anisotropy of material (e.g. in calcit)
• Unequal indices of refraction ($n_e$ and $n_o$)
  $e$: extraordinary; $o$: ordinary
• Degree of refraction depends on the polarization of light
  (reference: optical axis of material)
ELECTROMAGNETIC WAVES

- Electromagnetic waves were first postulated by J.C. Maxwell
- They were experimentally proven by H. Hertz
WHAT IS LIGHT
PARTICLE/WAVE

1669 Newton
Emanations theory (particle)

1677 Huygens
wave theory

1802 Young
interference

1871 Maxwell
electromagnetic light theory

1886 Hertz
experimental proof of
Maxwells theory
WHAT IS LIGHT
PHOTOELECTRIC EFFECT

Albert Einstein
Nobel price 1921

Experiments by Lennart 1902

- Maximal energy of the photo electrons is independent of the intensity of the light
- Slope is the same for different cathode materials
- Light can be described as particles called photons or light quants. particle-wave duality

Detection of Photons
OPTICS
SUMMARY

• Basic Definitions
  • Reflection
  • Refraction
  • Diffraction
  • Dispersion

• Models to describe optical phenomena
  • Geometrical Optics
  • Wave optics

• Light
  • Particle-wave duality
More about light microscopy/optics

1. Lecture
   Biomicroscopy I + II, Prof. Theo Lasser, EPFL

2. Books
   b) Optics, 4th ed., Eugene Hecht, Addison-Wesley, 2002

3. Internet
   a) http://micro.magnet.fsu.edu
   b) Web sites of microscope manufactures
      Leica
      Nikon
      Olympus
      Zeiss

4. BIOp
   EPFL, SV-AI 0241, Sv-AI 0140
   http://biop.epfl.ch/