Microscopy

Lecture 1: Optical System of the Microscopy I
2012-10-15
Herbert Gross
Lectures:
- Alexander Heisterkamp / IAO
- Rainer Heintzmann / IPHT
- Kai Wicker / IPHT
- Herbert Gross / IAP

Lecture:
- dates: Monday, 14.00 – 15.30
- Location: HS 2, Fröbelstieg 1, Abbeanum

Seminar:
- starts at xxx
- bi-weekly
- location: xxx

Web page on IAP homepage under ‘learning’ provides slides and exercises
<table>
<thead>
<tr>
<th>No</th>
<th>Date</th>
<th>Main subject</th>
<th>Detailed topics</th>
<th>Lecturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.10.</td>
<td>Optical system of a microscope I</td>
<td>overview, general setup, binoculars, objective lenses, performance and types of lenses, tube optics</td>
<td>Gross</td>
</tr>
<tr>
<td>2</td>
<td>22.10.</td>
<td>Optical system of a microscope II</td>
<td>Etendue, pupil, telecentricity, confocal systems, illumination setups, Köhler principle, fluorescence systems and TIRF, adjustment of objective lenses</td>
<td>Gross</td>
</tr>
<tr>
<td>3</td>
<td>29.10.</td>
<td>Physical optics of widefield microscopes</td>
<td>Point spread function, high-NA-effects, apodization, defocussing, index mismatch, coherence, partial coherent imaging</td>
<td>Gross</td>
</tr>
<tr>
<td>4</td>
<td>05.11.</td>
<td>Performance assessment</td>
<td>Wave aberrations and Zernikes, Strehl ratio, point resolution, sine condition, optical transfer function, conoscopic observation, isoplantism, straylight and ghost images, thermal degradation, measuring of system quality</td>
<td>Gross</td>
</tr>
<tr>
<td>5</td>
<td>12.11.</td>
<td>Fourier optical description</td>
<td>basic concepts, 2-point-resolution (Rayleigh, Sparrow), Frequency-based resolution (Abbe), CTF and Born Approximation</td>
<td>Heintzmann</td>
</tr>
<tr>
<td>6</td>
<td>19.11.</td>
<td>Methods, DIC</td>
<td>Rytov approximation, a comment on holography, Ptychography, DIC</td>
<td>Heintzmann</td>
</tr>
<tr>
<td>7</td>
<td>26.11.</td>
<td>Imaging of scatter</td>
<td>Multibeam illumination, Cofocal coherent, Incoherent processes (Fluorescence, Raman), OTF for incoherent light, Missing cone problem, imaging of a fluorescent plane, incoherent confocal OTF/PSF</td>
<td>Heintzmann</td>
</tr>
<tr>
<td>8</td>
<td>03.12.</td>
<td>Incoherent emission to improve resolution</td>
<td>Fluorescence, Structured illumination, Image based identification of experimental parameters, image reconstruction</td>
<td>Heintzmann</td>
</tr>
<tr>
<td>9</td>
<td>10.12.</td>
<td>The quantum world in microscopy</td>
<td>Photons, Poisson distribution, squeezed light, antibunching, Ghost imaging</td>
<td>Wicker</td>
</tr>
<tr>
<td>10</td>
<td>17.12.</td>
<td>Deconvolution</td>
<td>Building a forward model and inverting it based on statistics</td>
<td>Wicker</td>
</tr>
<tr>
<td>11</td>
<td>07.01.</td>
<td>Nonlinear sample response</td>
<td>STED, NLSIM, Rabi the information view</td>
<td>Wicker</td>
</tr>
<tr>
<td>12</td>
<td>14.01.</td>
<td>Nonlinear microscopy</td>
<td>two-photon cross sections, pulsed excitation, propagation of ultrashort pulses, (image formation in 3D), nonlinear scattering, SHG/THG - symmetry properties</td>
<td>Heisterkamp</td>
</tr>
<tr>
<td>13</td>
<td>21.01.</td>
<td>Raman-CARS microscopy</td>
<td>principle, origin of CARS signale, four wave mixing, phase matching conditions, epi/forward CARS, SRS.</td>
<td>Heisterkamp</td>
</tr>
<tr>
<td>14</td>
<td>28.01.</td>
<td>Tissue optics and imaging</td>
<td>Tissue optics, scattering&amp;aberrations, optical clearing,Optical tomography, light-sheet/ultramicroscopy</td>
<td>Heisterkamp</td>
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<tr>
<td>15</td>
<td>04.02.</td>
<td>Optical coherence tomography</td>
<td>principle, interferometry, time-domain, frequency domain.</td>
<td>Heisterkamp</td>
</tr>
</tbody>
</table>
1. Seward, Optical design of microscopes, SPIE Press, 2010
1. Introduction
2. General optical setup of microscopes
3. Binoculars
4. Objective lenses
5. Performance and types of lenses
6. Tube optics
1 Optical System of the Microscopy I

History of Microscopy

- Ernst Abbe: Beginning of scientific microscopy
- Theory of diffraction imaging
- Systematic development and correction of objective lenses
- Major landmark in understanding microscopic resolution
- Ernst Abbe memorial in Jena

Ref: W. Osten
- Scientific calculation of lens systems for microscopes
- Systematic development of new materials together with Otto Schott
- First apochromate
1 Optical System of the Microscopy I
Application Fields of Microscopy

Microscopy

Research
- Biomedical basic research
  - Cell biology
  - biological development
  - toxicology,...
- Material research
  - Micro system technology
  - geology
  - polymer chemistry

Routine applications
- Medical routine
  - Pathology
  - clinical routine
  - forensic,...
- Industrial routine
  - Microscopic surgery
  - ophthalmology

- Pharmacy
  - semiconductor inspection
  - semiconductor manufacturing

Ref: M. Kempe
1 Optical System of the Microscopy I
Image Planes and Pupils

- Principal setup of a classical optical microscope
- Upper row: image planes
- Lower row: pupil planes
- Köhler setup
1 Optical System of the Microscopy I
Microscopic Image Formation

- Basic microscopic system with finite image location
- The objective lens forms an intermediate image, this is observed by the eyepiece
- Magnification of the objective lens

\[ D_{obj} = \frac{D_{ima}}{m_{obj}} \]

- Magnification of the 2-stage imaging setup:

\[ m_{micro} = \frac{t}{f_{obj}} \cdot \frac{250 mm}{f_{eye}} \]
1 Optical System of the Microscopy I
Microscope with Infinite Image Setup

- Basic microscopic system with infinite image location and tube lens
- Magnification of the first stage:
  \[ m_{\text{obj}} = \frac{f_{\text{tube}}}{f_{\text{obj}}} \]

- Magnification of the complete setup
  \[ m_{\text{micro}} = \frac{f_{\text{tube}}}{f_{\text{obj}}} \cdot \frac{250 \, \text{mm}}{f_{\text{eye}}} \]

- Exit pupil size
  \[ D_{\text{Exp}} = 2 \cdot f_{\text{obj}} \cdot NA' = \frac{2 \cdot f_{\text{obj}} \cdot NA}{m_{\text{obj}}} \]
Typically, microscope optical systems are corrected diffraction limited

The resolution therefore follows the Abbe formula

- **Self-luminous object**
  - Pupil is filled

\[
\Delta x = \frac{0.61 \cdot \lambda}{n \cdot \sin u_{obj}}
\]

- **Non-self-luminous object**
  - The relative pupil filling determines
  - the degree of partial coherence and
  - the resolution

\[
\Delta x = \frac{1.22 \cdot \lambda}{n \cdot \sin u_{ill} + n \cdot \sin u_{obj}}
\]

If the magnification exceeds the resolution of the eye of the human observer:
empty resolution

Typical: 500 NA .... 1000 NA
Magnification: objective and eyepiece

\[ m_{\text{micro}} = m_{\text{obj}} \cdot \Gamma_{\text{eye}} \]
1 Optical System of the Microscopy I

Setup of the Microscope

- Standards in microscopic setups
- Terms and normalized lengths
- Differences in the numbers depending on the vendor are possible
1 Optical System of the Microscopy I
Upright-Microscope

- Sub-systems:
  1. Detection / Imaging path
     1.1 objective lens
     1.2 tube with tube lens and binocular beam splitter
     1.3 eyepieces
     1.4 optional equipment for photo-detection
  2. Illumination
     2.1 lamps with collector and filters
     2.2 field aperture
     2.3 condenser with aperture stop
1 Optical System of the Microscopy I
Inverse Microscope

- Additional relay-system in tube
- Applications:
  1. liquid covered probe
  2. sample observed through coverglas
1 Optical System of the Microscopy I
Microscope Stands

Stereo microscopes

Upright microscopes

Inverse microscopes

Routine microscopes

From M. Kempe
- Eyepieces images a finite image of an instrument to infinity
- Viewing with a relaxed eye
- Magnification
- Problem: - Location of the eye pupil inside
  - Pupil of the eyepiece outside: large height of chief ray
- Aperture: usually small

\[ \Gamma = \frac{250 \text{mm}}{f_{\text{eyepiece}}} \]
- Field lens reduces chief ray height
- Eye lens adapts pupil diameter
- Matching of
  1. Field of view
  2. Pupil diameter
  3. Pupil location
- Eye relief:
  - distance between last lens surface and eye cornea
  - required: 15 mm
  - with eyeglasses: 20 mm
- Pupil size: 2-8 mm
1 Optical System of the Microscopy I
Evolution of Eyepiece Designs

- Loupe
- Monocentric
- Von-Hofe
- Plössl
- Erfle
- Erfle type
- Erfle diffractive
- Scidmore
- Bertele
- Wild

- Huygens
- Ramsden
- Kellner
- Kerber
- König
- Nagler 1
- Nagler 2
- Aspheric
- Bertele
- Dilworth
1 Optical System of the Microscopy I
Kellner Eyepiece

- Classical setup
- Corresponds to Ramsden type
- Field lens moved
- Eye lens achromatized
1 Optical System of the Microscopy I
Bertele Eyepiece

- High performance system
- Enlarged eye relief
- Larger field of view: 2 x 33°
- Larger diameter necessary
- More lenses necessary for correction
1 Optical System of the Microscopy I
Microscope Objective Lens

- Focal length as function of magnification for f=165 mm - tubelens
- Large angles in object space
- Colour coding of magnification

<table>
<thead>
<tr>
<th>Colour</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>black</td>
<td>1x</td>
</tr>
<tr>
<td>grey</td>
<td>2x</td>
</tr>
<tr>
<td>red</td>
<td>4x</td>
</tr>
<tr>
<td>yellow</td>
<td>10x</td>
</tr>
<tr>
<td>green</td>
<td>20x</td>
</tr>
<tr>
<td>bright blue</td>
<td>40x</td>
</tr>
<tr>
<td>blau</td>
<td>50x</td>
</tr>
<tr>
<td>dark blue</td>
<td>60x</td>
</tr>
<tr>
<td>white</td>
<td>100x</td>
</tr>
</tbody>
</table>
Legend of data, type and features

- **Type of lens**
- **Special features**
  - (long distance, ...)
- **Magnification**
- **Numerical aperture**
- **Additional data**
  - Immersion
  - Cover glass correction
  - Contrast method
- **Tube length**
- **Thickenss of cover glass**
  - 0 without cover glass
  - Insensitive
- **Mechanical adjustment**
  1. Cover slide
  2. Immersion type
  3. Temperature
  4. Iris diaphragm
- **Contrast**
  - Standard
  - Pol/DIC
  - Ph 0 1 2 3
- **Magnification**
  - 1,0/1.25
  - 2.5
  - 4/5
  - 6.3
  - 10
  - 16/20/25/32
  - 40/50
  - 63
  - 100/150
- **Immersion**
  - Oil
  - Water
  - Glycerin
  - All
1 Optical System of the Microscopy I
Microscope Objective Lens

- Typical ray paths and outfits
1. thread
2. interface plane
3. spring for damage protection
4. -7. middle lens groups
8. correction ring
9. front lens group
10. socket of front lens
### Optical System of the Microscopy I

**Microscope Objective Lens: Performance Classes**

- **Classification:**
  1. performance in colour correction
  2. correction in field flattening
- **Division is rough**
- **Notation of quality classes depends on vendors**
  (Neofluar, achro-plane, semi-apochromate,...)

<table>
<thead>
<tr>
<th><strong>improved field flatness</strong></th>
<th>improved colour correction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Improved</strong></td>
<td>no</td>
</tr>
<tr>
<td><strong>Plan</strong></td>
<td>Plan-achromat</td>
</tr>
<tr>
<td><strong>Plan-Fluorite</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Plan-Apochromat</strong></td>
<td></td>
</tr>
</tbody>
</table>
## Optical System of the Microscopy I

### Microscope Objective Lens: Performance Classes

<table>
<thead>
<tr>
<th>Objective class</th>
<th>Leica</th>
<th>Nikon</th>
<th>Olympus</th>
<th>Zeiss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name</td>
<td>Feature</td>
<td>Name</td>
<td>Feature</td>
</tr>
<tr>
<td>Plan</td>
<td></td>
<td></td>
<td>Plan-</td>
<td>Epi</td>
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<td></td>
<td></td>
<td></td>
<td>LD</td>
<td>Ph</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pol</td>
<td></td>
</tr>
<tr>
<td>Achromat</td>
<td></td>
<td></td>
<td>Achromat</td>
<td>DIC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ph</td>
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<tr>
<td>Plan-Achromat</td>
<td></td>
<td></td>
<td>DIC</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Plan</td>
<td></td>
</tr>
<tr>
<td>Fluorite / Semi-Apochromat</td>
<td>Fluotar</td>
<td></td>
<td>Fluor</td>
<td>UV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wd</td>
</tr>
<tr>
<td>Plan-Fuorit</td>
<td>Plan-</td>
<td>Fluotar</td>
<td>Pol</td>
<td>Epi</td>
</tr>
<tr>
<td></td>
<td>Fluor</td>
<td></td>
<td>DIC</td>
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<td></td>
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<td></td>
<td>Ph</td>
<td></td>
</tr>
<tr>
<td>Apochromat</td>
<td>Apochromat</td>
<td>UV</td>
<td></td>
<td>Wd</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Plan-Apochromat</td>
<td>Plan-</td>
<td>Apochromat</td>
<td>W</td>
<td>UV</td>
</tr>
<tr>
<td></td>
<td>Apochromat</td>
<td>HT</td>
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<td>Ph</td>
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</tbody>
</table>

**Quality classes of the manufacturers**
1 Optical System of the Microscopy I
Microscopic Objective Lens: Performance Classes

- Different color correction types
- Classes of lenses with flattened field:
  Chromatical correction:
  1. Achromate
  2. Fluorite
  3. Apochromate
- Increasing etendue
- Increasing NA / resolution

![Graph showing NA vs magnification for different types of lenses](image-url)
1 Optical System of the Microscopy I
Microscope Objective Lens: Performance Classes

- Quality classes a...d / color and field performance
- Diffraction limited on axis in the green guaranteed
- Usual degradation for outer wavelengths and far off-axis field positions

![Graphs showing W_{rms} vs. rel. field for different wavelengths and field positions.](image)
Three different classes:
1. No effort
2. Semi-flat
3. Completely flat
- Typical parts of lens structure for high NA-objective lenses
- Separation of the lens setup in 3 major sections

**a**
- front part:
  1. spherical aberration: only small
  2. coma: only small
  3. astigmatism: only small
  4. curvature: only small

**b**
- middle part:
  1. spherical aberration: correction
  2. color: correction
  3. coma: correction

**c**
- rear part:
  1. curvature: correction
  2. astigmatism: correction
  3. color: correction
1 Optical System of the Microscopy I
Microscope Objective Lens: Simple Lenses

- Simple type for low aperture and small magnifications
- Improved system for higher apertures by an aplanatic-concentric lens
- Diffraction limited performance up to $NA = 0.65$
Improved design:
- Cemented front lens
- Plane front surface due to practical reasons
- Colour correction significantly better
- Two lenses in the front group
- Colour correction uniformity good
- Field curvature bad:
  best focal plane depends on field height
Optical System of the Microscopy I
Microscope Objective Lens: High NA 100x/0.93

- Point spread function
- Diffraction limit: 80% Strehl ratio
- Typical: performance in the blue critical
Objective lens 100x/0.9
No effort for field flattening
Small range of diffraction limit depending on wavelength and field size
• Seidel surface contributions for 100x/0.90
• No field flattening group
• Lateral color in tube lens corrected
• Distortion plays minor role in bio-med applications in microscopy
Optical System of the Microscopy I

Microscope Objective Lens: Cover glass

- Enhancement of numerical aperture
- Standard data: K5, d=0.17 mm
- Effect on spherical correction for NA > 0.6
- Free working distance decreases with increasing magnification $m$
- Large distance enlarges the lens diameter for large NA

![Diagram showing optical system of microscopy](image)
1 Optical System of the Microscopy I

Microscope Objective Lens Types

- Medium magnification system
  40x0.65

- High NA system 100x0.9
  without field flattening

- High NA system 100x0.9
  with flat field

- Large-working distance
  objective lens 40x0.65
- Floating element to adjust and correct spherical aberration

- Applications:
  1. different thickness values of cover glass
  2. index mismatch at the sample
- Catadioptric lenses:
  1. Schwarzschild design: first large mirror
  2. Newton design: first small mirror

- Advantageous:
  1. Large working distance
  2. Field flattening
  3. Colour correction

- Drawback:
  central obscuration reduces contrast / resolution
- More complicated setups
  1. four surfaces, two refractive
     monolithic
  2. Catadioptric,
     cemented monolithic
Transverse chromatical aberration of microscopic lenses: hard to correct with the other components

Possible Solutions:
1. Compensating ocular, correction in the eyepiece (Abbe)
   Intermediate image not corrected
2. Correcting with the tube lens
   In infinity color corrected (ICS) optics
3. Correcting in the back part of the objective lens
   Using inverted achromates with positive flint lens
- Problem: Lateral colour correction is critical

- Different solutions:
  a) Compensation with eyepiece
  b) Corrector-null-power-component
  c) Compensation with tube lens

- Disadvantage:
  - Intermediate image non corrected
  - no modularity
Optical System of the Microscopy I
Tube Optical System: Tube Lens

- Simple tube lens
- Magnification
  \[ m_{obj} = \frac{f_{tube}}{f_{obj}} \]
- On axis: diffraction limited
- Dominant residual aberration: lateral colour

![Diagram of the tube optical system](image-url)

- Objective exit pupil: \( D_{ExP} \)
- Tube lens: \( y_{TL} \)
- Intermediate image: \( D_{FV} = 25 \text{ mm} \)
- Distance: \( d = 100 \text{ mm} \)
- Tube lens focal length: \( f'_{TL} = 164 \text{ mm} \)
Optical System of the Microscopy I
Tube Optical System: Improved Systems

- Simple tube lens for field position:
  - strong astigmatism
- More complicated tube lenses:
  1. Chromatical correction
  2. Magnification factor modified
  3. Adjust pupil location
- Tube prism systems to generate two bincular channels
- Adjustable pupillary distance required
- Two versions: shift / tilt movement

\[ d_{IPD} = 65 \text{ mm} \]
\[ D = 28 \text{ mm} \]

**a) shift version tube prims set**

**b) tilt version tube prims set**