Microscopy

Lecture 2: Optical System of the Microscopy II

2012-10-22

Herbert Gross

Winter term 2012
# 2 Optical System of the Microscopy II
## Preliminary time schedule

<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>
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<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>
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<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>
2 Optical System of the Microscopy II
Contents of 2nd Lecture

1. Etendue
2. Pupil
3. Telecentricity
4. Confocal systems
5. Illumination setups
6. Köhler principle
7. Fluorescence systems and TIRF
8. Objective adjustment
- No rigid relationship between magnification and aperture
- Product of field size and NA fixes the overall capacity
- Variation of image-sided numerical aperture
Information capacity, etendue, space-bandwidth product, number of PSF's resolved in the field

Non-rigid correlation between magnification and etendue

Largest etendue for medium magnifications

Immersion systems have larger etendue

\[ G = \frac{\pi}{4} \cdot (D_{\text{field}} \cdot NA)^2 \]
Relevance of the system pupil:

- Brightness of the image
  Transfer of energy

- Resolution of details,
  Information transfer, location of Fourier spectrum

- Image quality
  Aberrations due to aperture

- Image perspective
  Perception of depth

- Compound systems:
  matching of pupils is necessary, location and size
2 Optical System of the Microscopy II
Entrance and Exit Pupil

Object point on axis

Upper marginal ray

Lower marginal ray

Upper coma ray

Lower coma ray

Exit pupil

Stop

Entrance pupil

Upper ray

Chief ray

Lower ray

Field point of image

On axis point of image

Outer field point of object
- Object space telecentric
- Real rear stop is not defining the pupil
- Collimated outgoing beam
- Exit pupil usually not accessible
- Diameter of pupil only weak correlated with magnification
- Pupil distortion:

\[ D_{\text{distExp}} = \frac{y_p'}{f' \cdot \sin u} - 1 \]

- Pupil distortion small
  (sine condition corrected)
- Imaging of the pupil is important
- Residual aberrations:
  - sharp edge of aperture is desired
- Real systems: smooth illumination profile

![Diagram of microscope system with lens and pupil stop]

![Graph of intensity I(r) vs radius r]
Example of larger residual aberrations of pupil image:
1. axial shift of pupil with field size
2. no sharp imaging of aperture stop
3. coloured edge of stop boundary
- Vignetting of the pupil:
  1. truncation of the bundle for finite field sizes
  2. chief ray not identical with centroid
  3. perturbation of telecentricity
  4. in microscopic systems mostly at the front lens
- Truncation at front lens
- Pupil vignetting:
  - crescent shape of light cone
- Illumination fall-off towards field boundary
- Truncation of regions with large aberrations as correction method
- Improved performance
- Psf elliptical, anisotropic resolution
- Energy reduced
- Special stop positions:
  1. stop in back focal plane: object sided telecentricity
  2. stop in front focal plane: image sided telecentricity
  3. stop in intermediate focal plane: both-sided telecentricity

- Telecentricity:
  1. pupil in infinity
  2. chief ray parallel to the optical axis
- Design problems with telecentricity: Usual telecentricity only fulfilled for one wavelength

- Telecentricity is related to the centroid ray. Therefore the telecentricity is disturbed by vignetting effects

Graphs and diagrams illustrating the effects of telecentricity and vignetting.
- Laser scan microscope
- Depth resolution (sectioning) with confocal pinhole
- Transverse scan on field of view Digital image
- Only light coming out of the conjugate plane is detected
- Perfect system: scan mirrors conjugate to pupil location
- System needs a good correction of the objective lens, symmetric 3D distribution of intensity
Depth resolved images

Ref.: M. Kempe
- Complete setup: objective / tube lens / scan lens / pinhole lens
- Scanning of illumination / descanning of signal
- Scan mirror conjugate to system pupil plane
- Digital image processing necessary
Scan lens
- Diffraction limited
- Change in pupil location of objective lenses is critical perturbation of telecentricity

![Diagram of optical system](image)

Graphs showing w [°] vs. w rms and y/y_max vs. θ in ° for different wavelengths (480 nm, 546 nm, 644 nm) with plots for paraxial and exact pupil locations.
- Pinhole lens
- Only axial colour is essential
- Usage on axis only due to descanning
- Variable pinhole size not too small: small aperture, retrofocus lens
- Normalized transverse coordinate $v$ 

$$ v = \frac{2\pi}{\lambda} \cdot x' \cdot \sin \alpha $$

- Usual PSF: Airy

- Confocal imaging:
  
  Identical PSF for illumination and observation assumed

$$ I(v) = \left[ \frac{2J_1(v)}{v} \right]^4 $$

Resolution improvement be factor 1.4 for FWhM
- Normalized axial coordinate

- Conventional wide field imaging:
  Intensity on axis
  \[ I(u) = \left( \frac{\sin(u/2)}{u/2} \right)^2 \]
  Axial resolution
  \[ \Delta z_{\text{wide}}^{(\text{approx})} = \frac{0.45 \cdot \lambda}{n'(1 - \cos \theta)} \]

- Confocal imaging:
  Intensity on axis
  \[ I(u) = \left( \frac{\sin(u/2)}{u/2} \right)^4 \]
  Axial resolution improved by factor 1.41 for FWhM
  \[ \Delta z_{\text{confo}} = \frac{0.319 \cdot \lambda}{n'(1 - \cos \theta)} \]
- Large pinhole: geometrical optic
- Small pinhole:
  - Diffraction dominates
  - Scaling by Airy diameter $a = D/D_{\text{Airy}}$
  - Diffraction relevant for pinholes $D < D_{\text{Airy}}$
- Confocal signal:
  Integral over pinhole size
  \[
  S(u) = \int_0^a |U(u, v)|^2 2\pi v \, dv
  \]
- Spherical aberration:
  - PSF broadened
  - PSF no longer symmetrical around image plane during defocus

- Confocal signal:
  - loss in contrast
  - decreased resolution
Depth signal as a function of wavelength
Disturbance by axial colour aberration
- Four possibilities for practical needs
- Epi vs. trans-illumination
- Bright vs. dark field illumination
- Comparison of light cones for imaging and illumination parts
Instrumental realizations

- **a) incident illumination**
  - *bright field*

- **b) incident illumination**
  - *dark field*

- **c) transmitted illumination**
  - *bright field*

- **d) transmitted illumination**
  - *dark field*
• Typical images for different illuminations

- **bright**
  - epi
  - trans

- **dark**
Principle of Köhler illumination:

- Alternating beam paths of field and pupil
- No source structure in image
- Light source conjugated to system pupil
- Differences between ideal and real ray paths
- Types of settings:
  1. Köhler:
     - source into pupil,
     - mostly used
  2. Critical:
     - source into field of view,
     - source structure disturbs image
  3. Projection:
     - source into condenser
  4. Arbitrary
Requirements and aspects:
1. Large collecting solid angle
2. Correction not critical
3. Thermal loading large
4. Mostly shell-structure for high NA
2. Abbe type, achromatic, NA = 0.9 , aplanatic, residual spherical

3. Aplanatic achromatic, NA = 0.85
- Dark-field illumination systems
  1. Trans-illumination
     Cardioid-mirror

  Realizations: approximation of Cardioid curve
2. Epi-illumination
Complicated ring-shaped components around objective lens
- Epi- and trans illumination for TIRF
Fluorescence microscopy is the most frequently employed mode of light microscopy used in biomedical research today.

Setup:

- Necessary components:
  - Dicroitic beam splitter, excitation filter with sharp edge

![Diagram showing the optical system of fluorescence microscopy](image_url)
Total internal reflection microscopy:
Excitation with evanescent field

Advantages:
1. better axial resolution
2. better SNR, no fluorescence background

Problem in optical design:
Extremely small illumination ring-shaped channel around the observation light cone
Adjustment of air gaps to optimize spherical aberration

- Reduced optimization setup

\[ c_j = c_{jo} + \sum_{k=1,4} \Delta c_j \cdot \frac{\partial c_j}{\partial t_k} \], \ j = 2, 4, 6, 8

- Compensates residual aberrations due to tolerances (radii, thicknesses, refractive indices)

<table>
<thead>
<tr>
<th></th>
<th>d₂</th>
<th>d₄</th>
<th>d₆</th>
<th>d₈</th>
<th>c₂₀</th>
<th>c₄₀</th>
<th>c₆₀</th>
<th>c₈₀</th>
<th>W_{rms}</th>
</tr>
</thead>
<tbody>
<tr>
<td>nominal</td>
<td>0.77300</td>
<td>0.17000</td>
<td>3.2200</td>
<td>2.0500</td>
<td>0.00527</td>
<td>-0.0718</td>
<td>0.00232</td>
<td>0.01290</td>
<td>0.0324</td>
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<tr>
<td>d₂ varied</td>
<td>0.77320</td>
<td>0.17000</td>
<td>3.2200</td>
<td>2.0500</td>
<td>0.04144</td>
<td>-0.07586</td>
<td>0.00277</td>
<td>0.12854</td>
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<tr>
<td>d₄ varied</td>
<td>0.77300</td>
<td>0.17050</td>
<td>3.2200</td>
<td>2.0500</td>
<td>0.03003</td>
<td>-0.07461</td>
<td>0.00264</td>
<td>0.01286</td>
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<td>d₆ varied</td>
<td>0.77300</td>
<td>0.17000</td>
<td>3.2250</td>
<td>2.0500</td>
<td>0.00728</td>
<td>-0.07367</td>
<td>0.00275</td>
<td>0.01284</td>
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<tr>
<td>d₈ varied</td>
<td>0.77300</td>
<td>0.17000</td>
<td>3.2200</td>
<td>2.0550</td>
<td>0.005551</td>
<td>-0.0717</td>
<td>0.00235</td>
<td>0.01290</td>
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<tr>
<td>optimized</td>
<td>0.77297</td>
<td>0.16942</td>
<td>3.12670</td>
<td>3.2110</td>
<td>0.000414</td>
<td>0.00046</td>
<td>0.00030</td>
<td>0.01390</td>
<td>0.00468</td>
</tr>
</tbody>
</table>
- Significant improvement for one wavelength on axis
- Possible decreased performance in the field

\[ W_{\text{rms}} \text{ in } \lambda \]

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>480 nm</td>
<td>0.5</td>
</tr>
<tr>
<td>546 nm</td>
<td>1</td>
</tr>
<tr>
<td>644 nm</td>
<td>0</td>
</tr>
</tbody>
</table>

solid lines : nominal

dashed lines : adjusted
Example microscopic lens

Adjusting:
1. Axial shifting lens: focus
2. Clocking: astigmatism
3. Lateral shifting lens: coma

Ideal: Strehl \( D_S = 99.62\% \)
With tolerances: \( D_S = 0.1\% \)
After adjusting: \( D_S = 99.3\% \)

Ref.: M. Peschka
2 Optical System of the Microscopy II
Adjustment and Compensation

- Sucessive steps of improvements

Ref.: M. Peschka