Institute of
Applied Physics
Friedrich-Schiller-Universität Jena

# Microscopy

Lecture 2: Optical System of the Microscopy II

2012-10-22

Herbert Gross

# 2 Optical System of the Microscopy II
# Preliminary time schedule



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

# 2 Optical System of the Microscopy II
# Contents of 2nd Lecture

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1. Etendue
2. Pupil
3. Telecentricity
4. Confocal systems
5. Illumination setups
6. Köhler principle
7. Fluorescence systems and TIRF
8. Objective adjustment

# 2 Optical System of the Microscopy II
# Microscope Objective Lens

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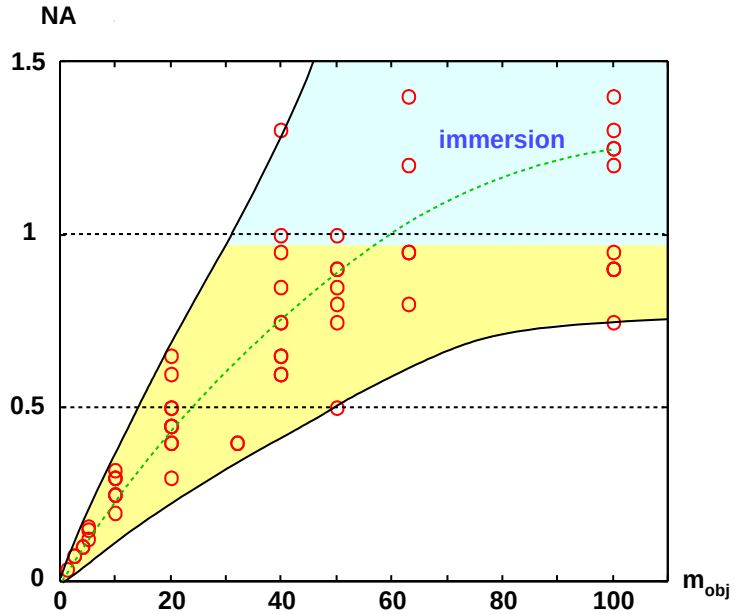
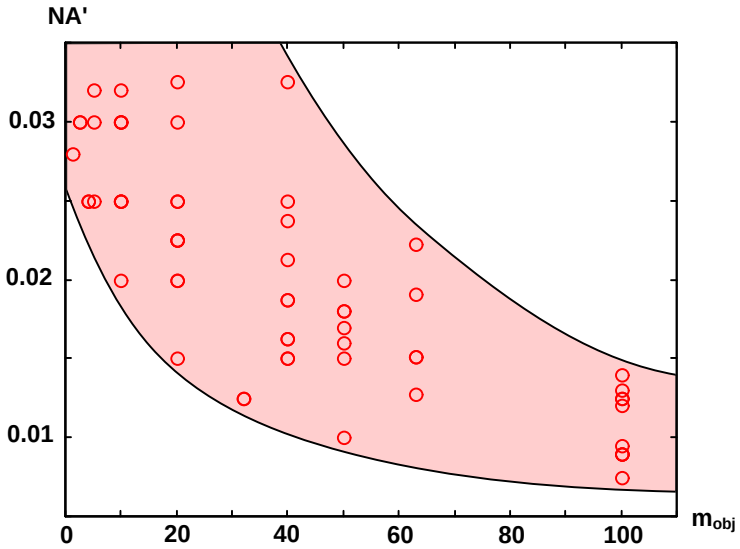
- No rigid relationship between magnification and aperture
- Product of field size and NA fixes the overall capacity
- Variation of image-sided numerical aperture



# 2 Optical System of the Microscopy II
# Etendue of Microscope Objective Lenses

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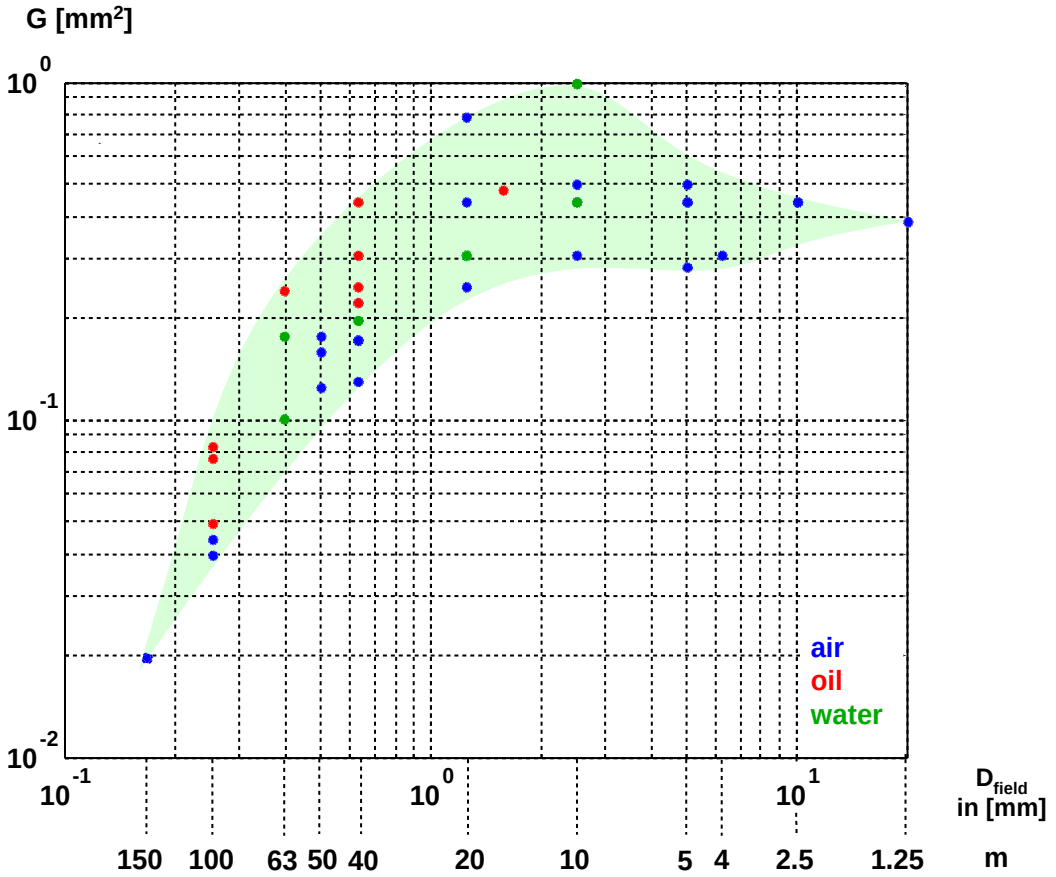
- Information capacity, etendue, space-bandwidth product, number of PSF‘s resolved in the field

$$G = \frac{\pi}{4} \cdot \left( D_{field} \cdot NA \right)^2$$

- Non-rigid correlation between magnification and etendue
- Largest etendue for medium magnifications
- Immersion systems have larger etendue



# 2 Optical System of the Microscopy II
# Properties of the Pupil



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

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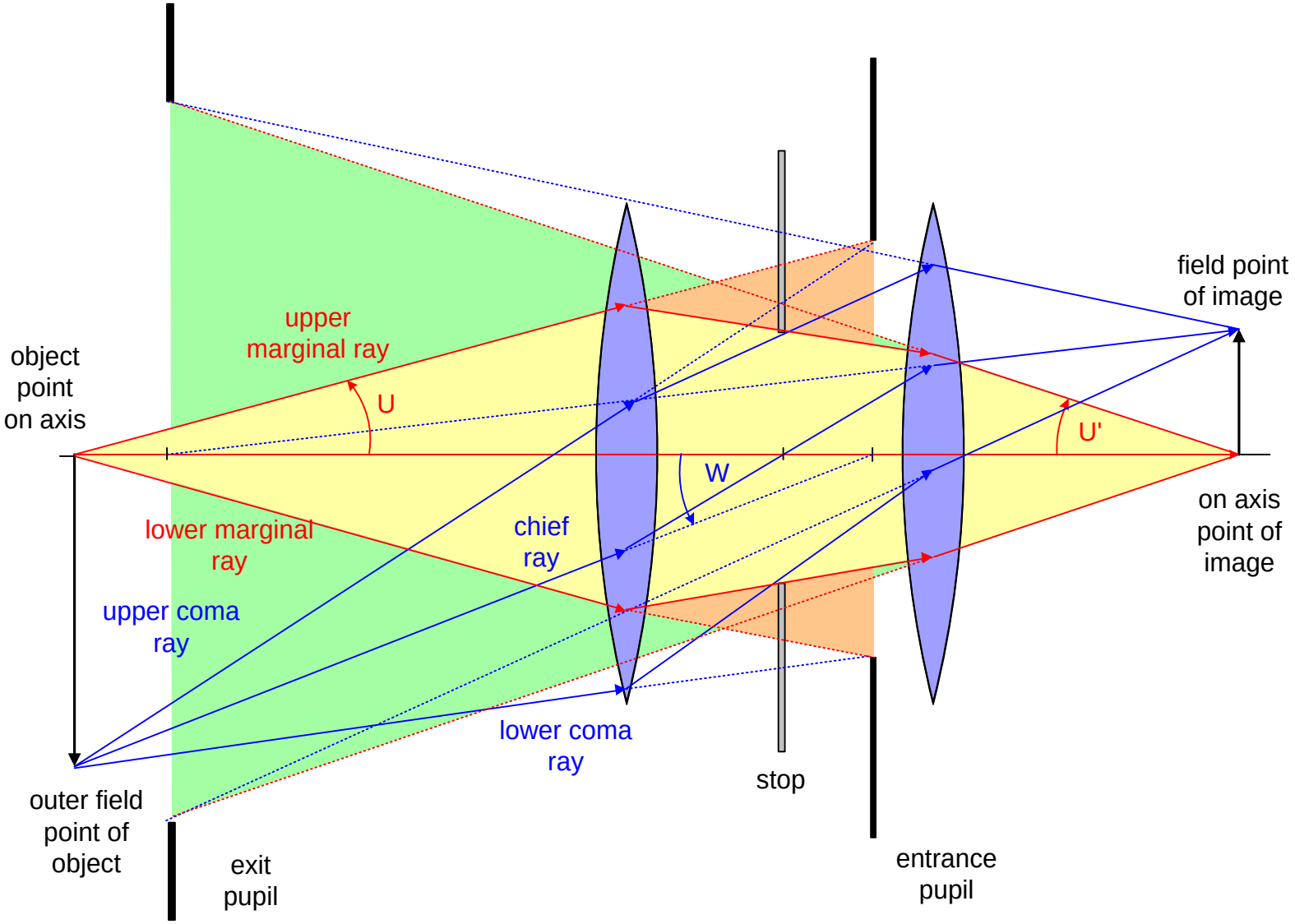

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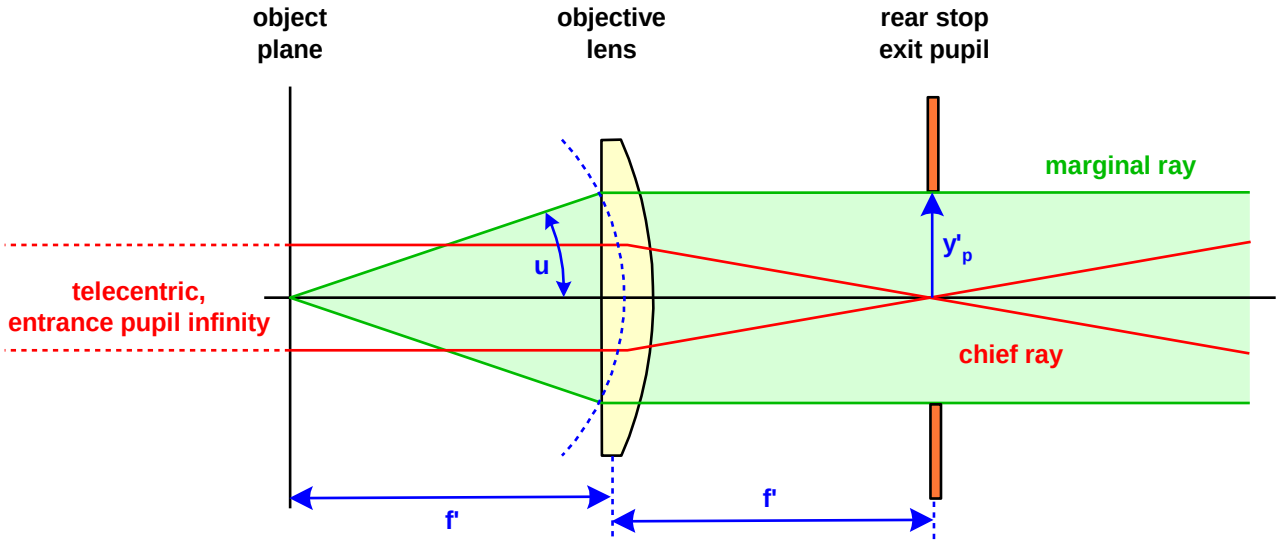
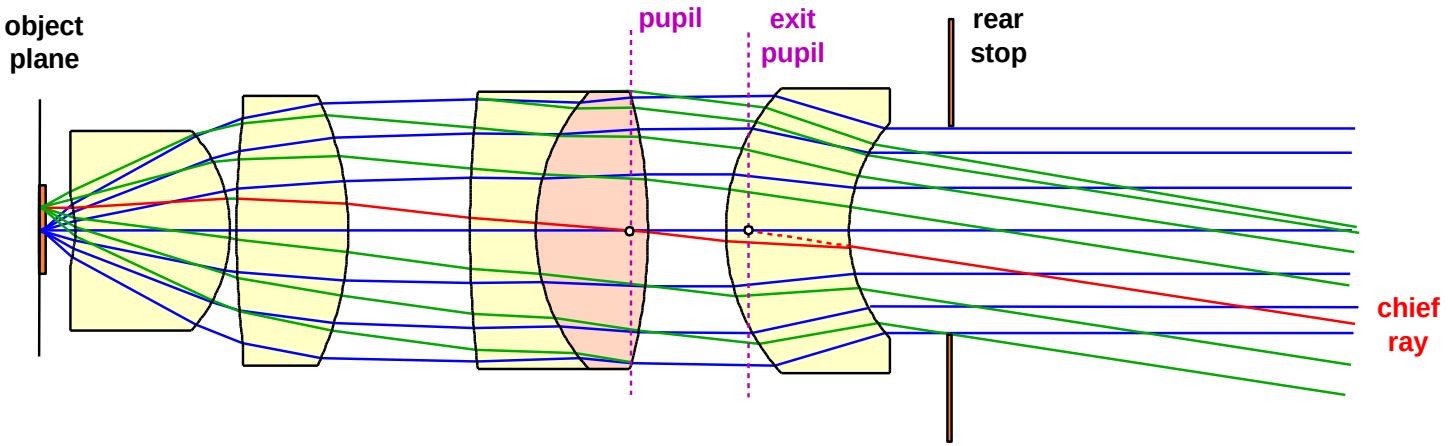
- Object space telecentric
- Real rear stop is not defining the pupil
- Collimated outgoing beam
- Exit pupil usually not accessible



# 2 Optical System of the Microscopy II
# Microscope Objective Lens: Pupil

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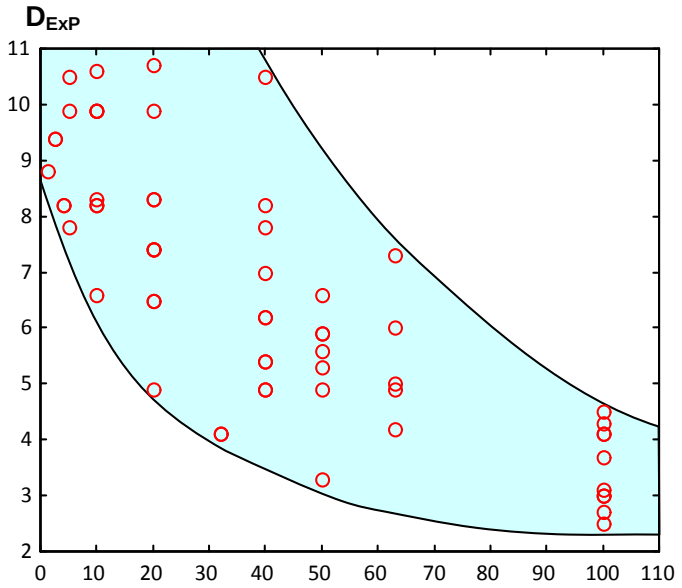
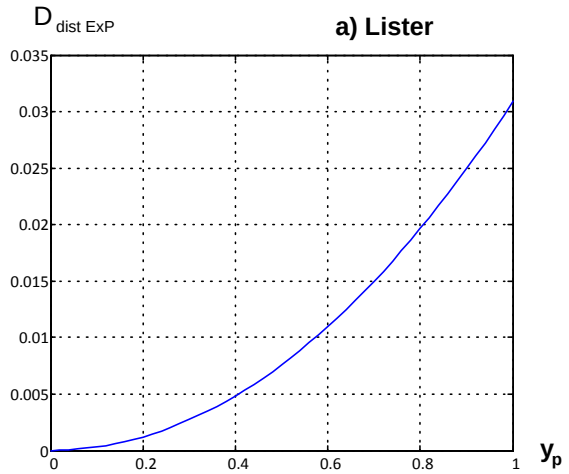
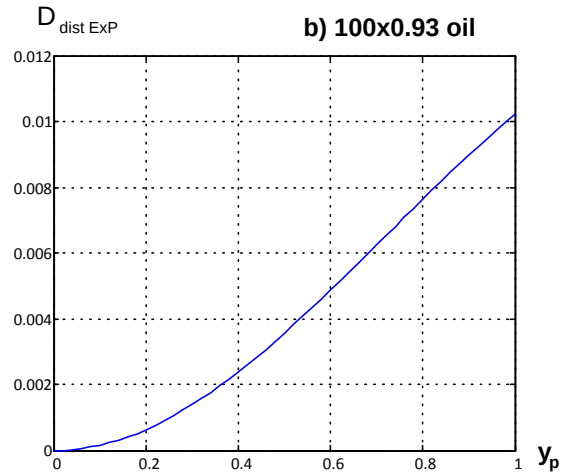
- Diameter of pupil only weak correlated with magnification
- Pupil distorsion:

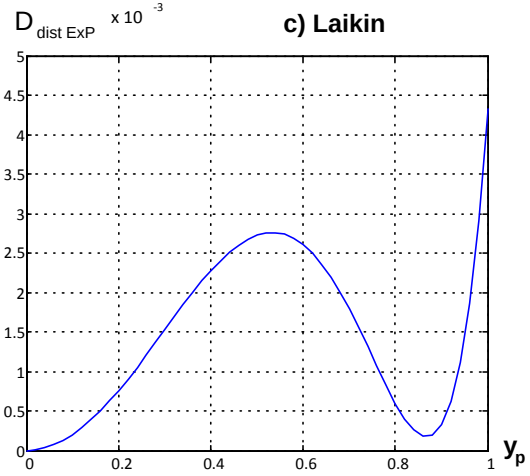
$$D_{distExP} = \frac{y'_p}{f' \cdot \sin u} - 1$$

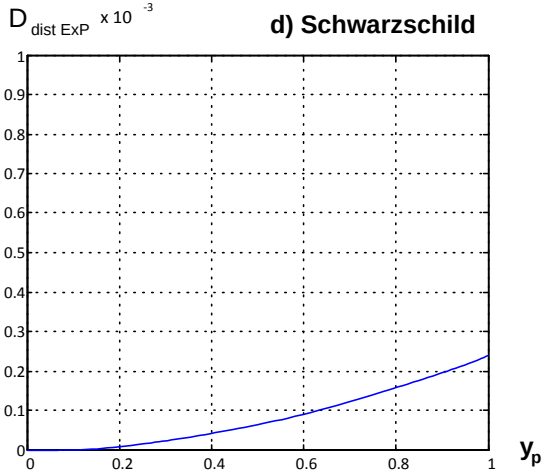



- Pupil distortion small
  (sine condition corrected)



# 2 Optical System of the Microscopy II
# Microscope Objective Lens: Pupil

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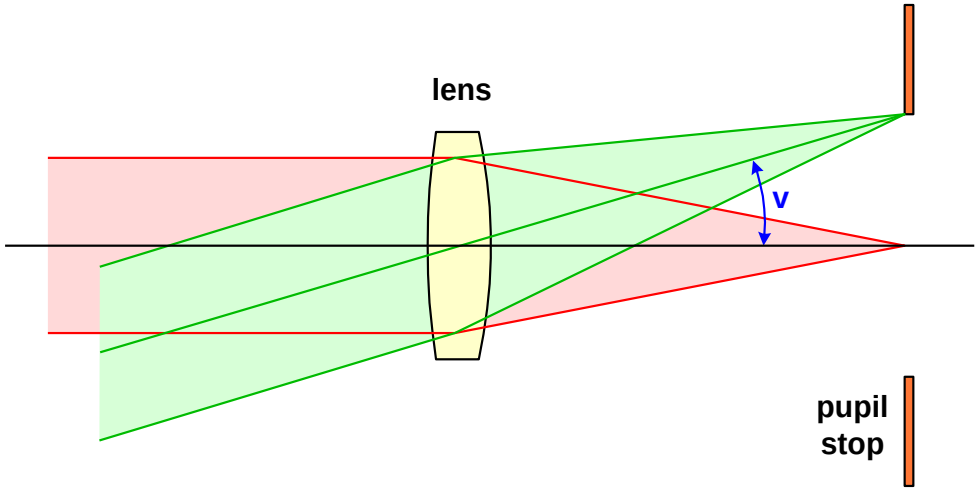
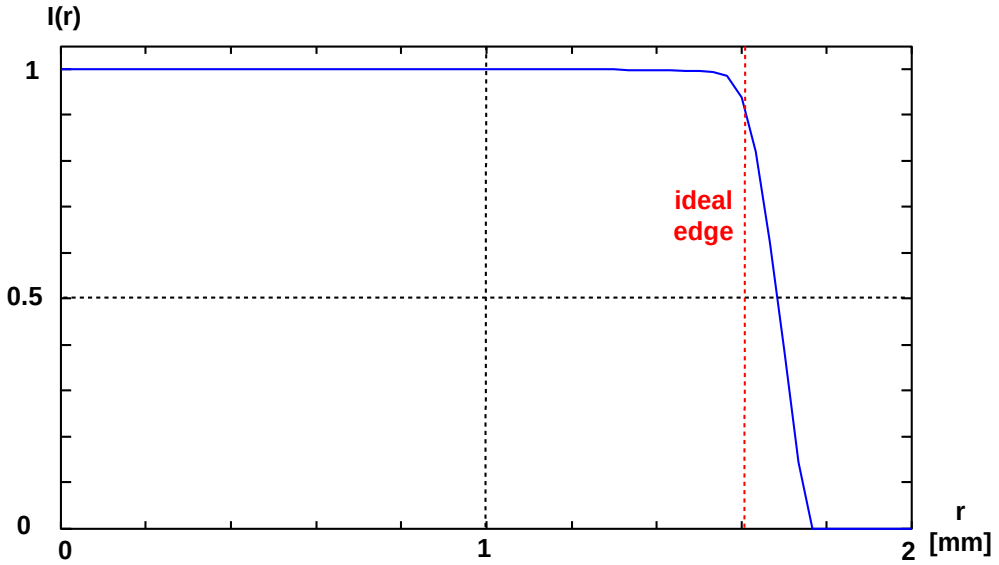
- Imaging of the pupil is important
- Residual aberrations :
  sharp edge of aperture is desired
- Real systems : smooth illumination profile



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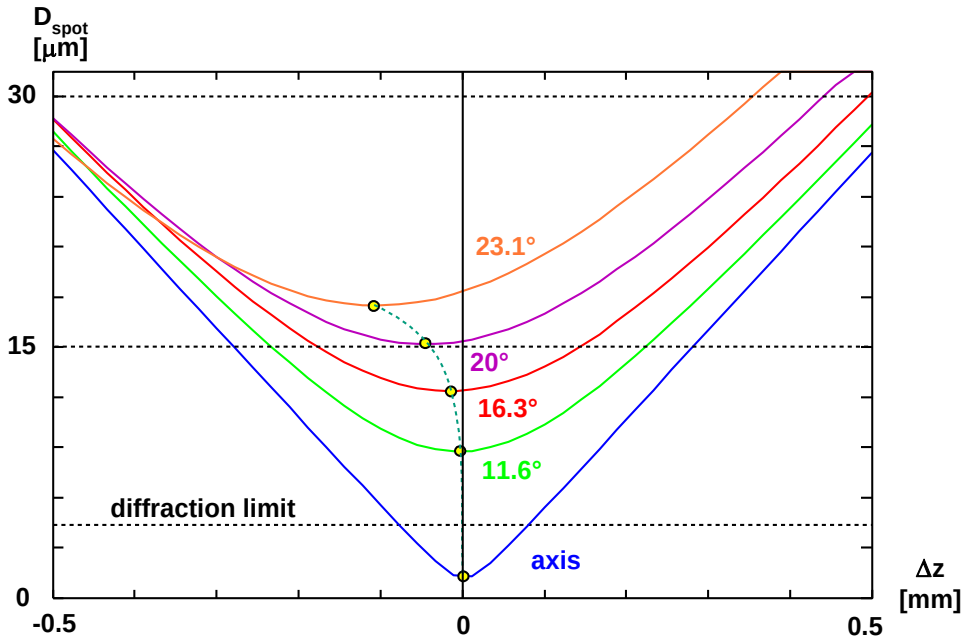
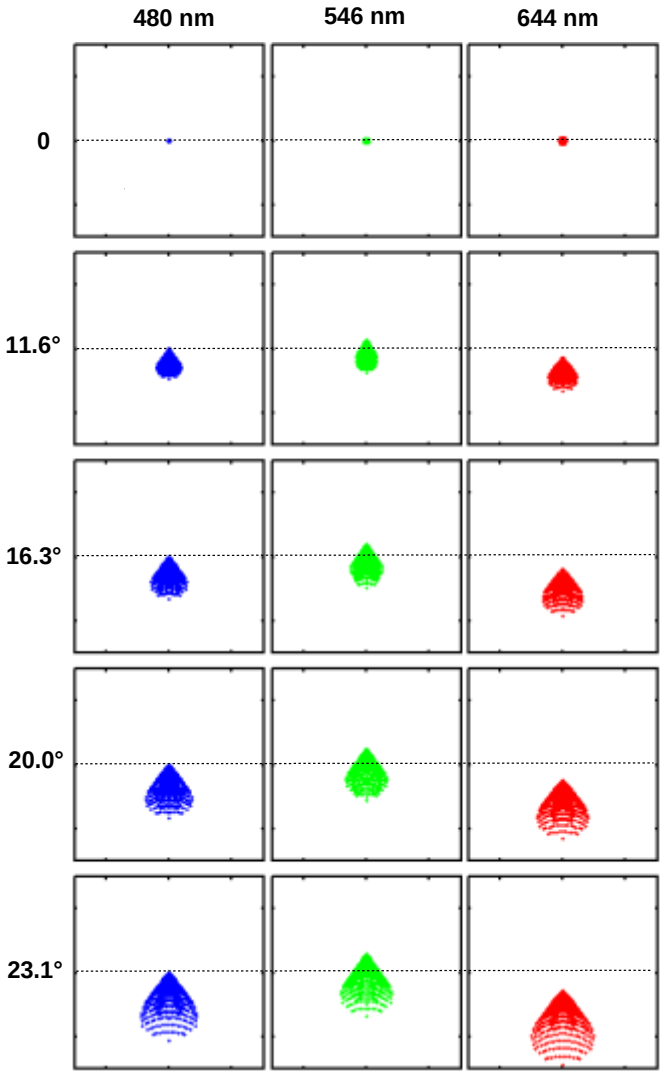
- Example of larger residual aberrations of pupil image :
  1. axial shift of pupil with field size
  2. no sharp imaging of aperture stop
  3. coloured edge of stop boundary



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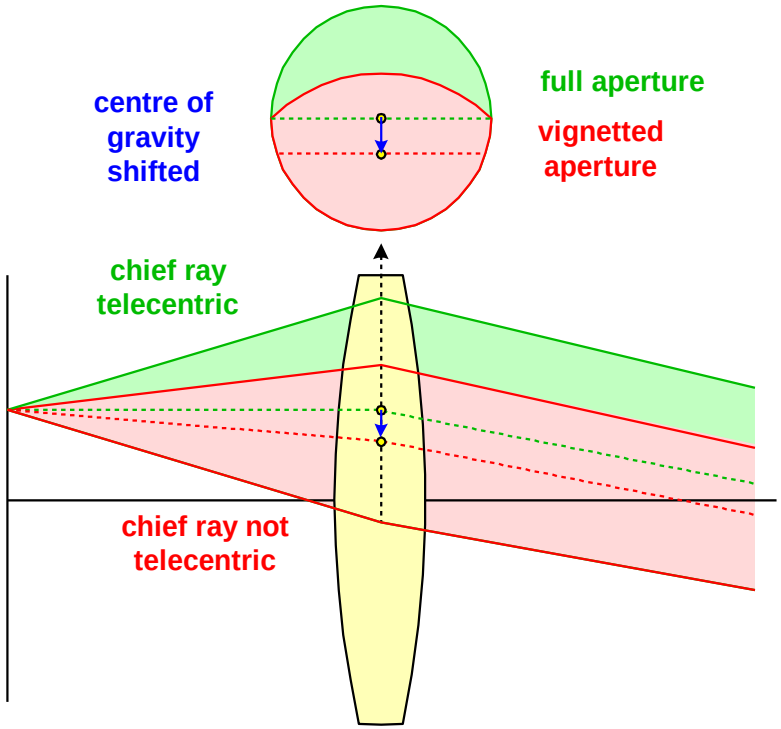
- Vignetting of the pupil :
  1. truncation of the bundle for finite field sizes
  2. chief ray not identical with centroid
  3. perturbation of telecentricity
  4. in microscopic systems mostly at the front lens



# 2 Optical System of the Microscopy II
# Vignetting

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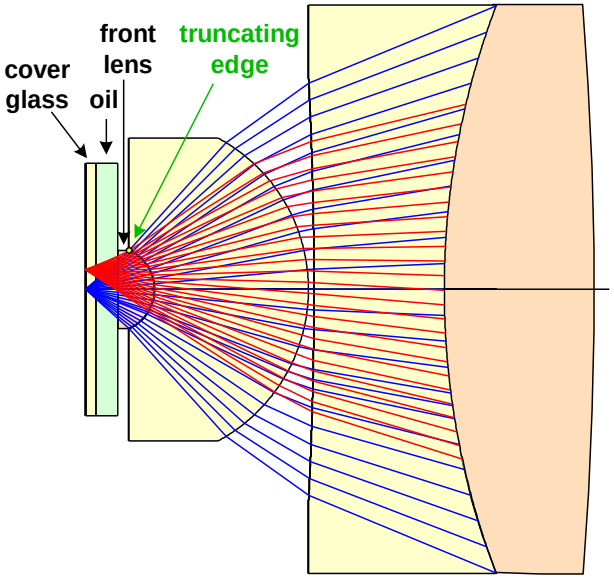
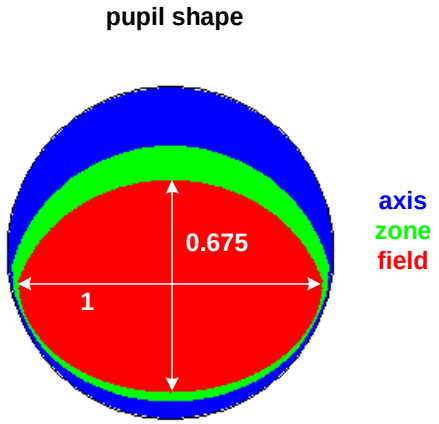
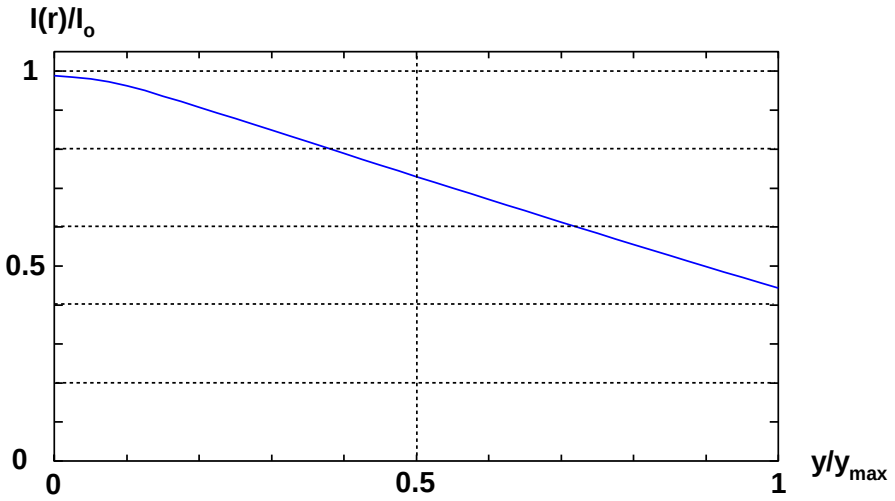
- Truncation at front lens
- Pupil vignetting:
  crescent shape of
  light cone
- Illumination fall-off towards
  field boundary



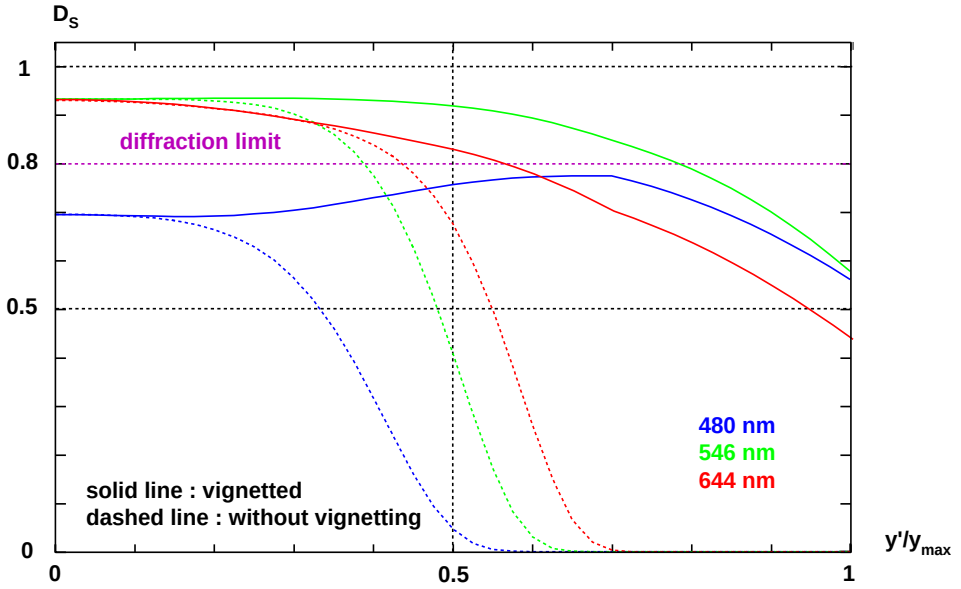



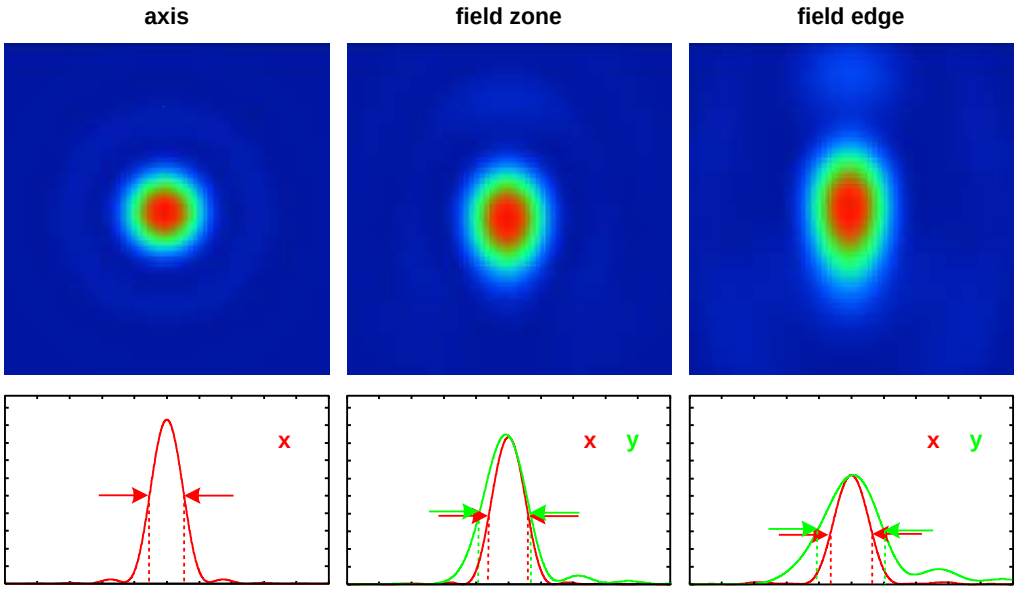



# 2 Optical System of the Microscopy II
# Vignetting



- Truncation of regions with large aberrations as correction method
- Improved performance
- Psf elliptical, anisotropiuc resolution
- Energy reduced



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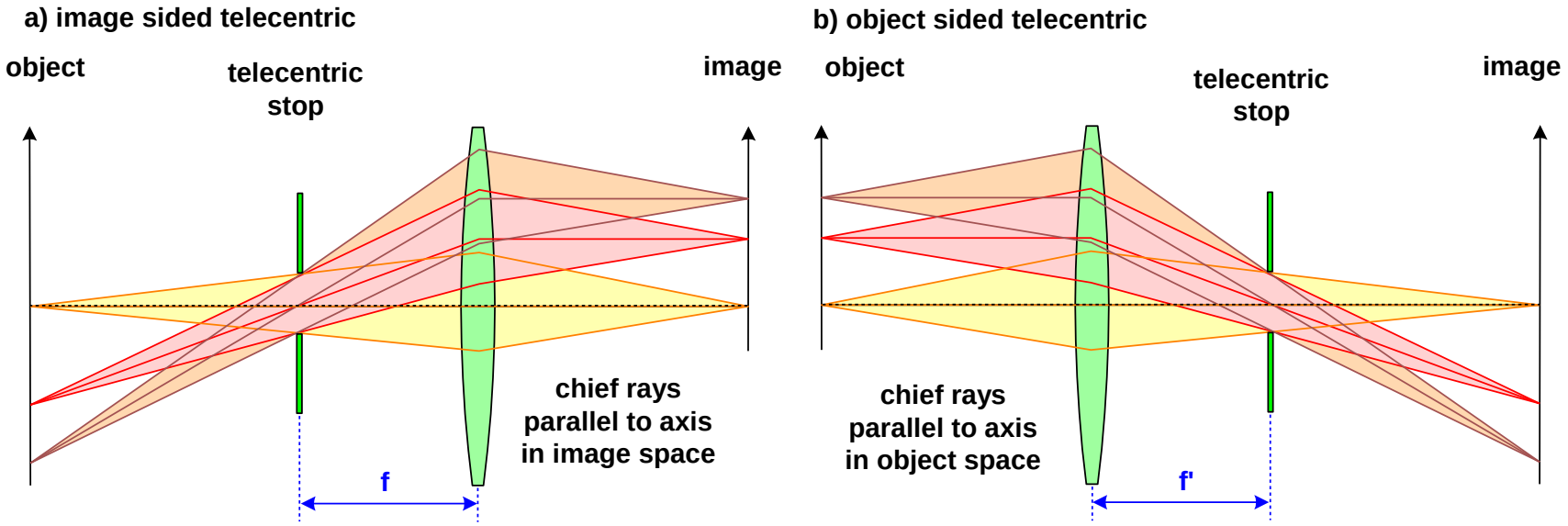
- Special stop positions:
  1. stop in back focal plane: object sided telecentricity
  2. stop in front focal plane: image sided telecentricity
  3. stop in intermediate focal plane: both-sided telecentricity

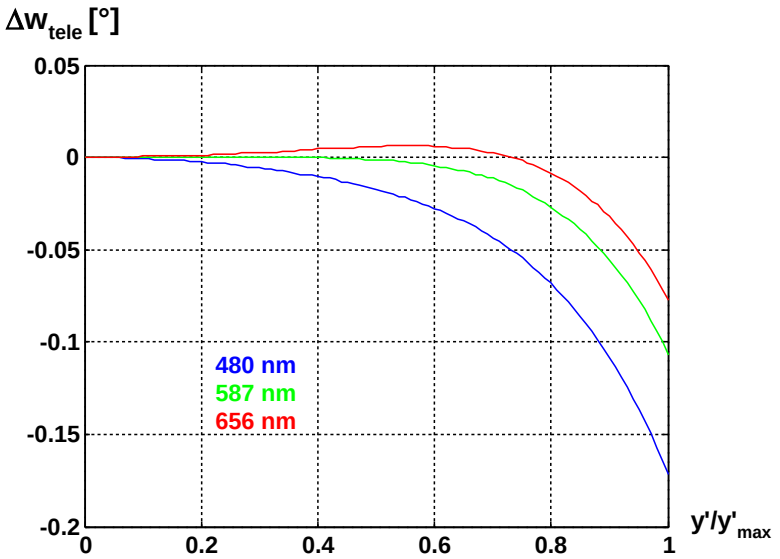
- Telecentricity:
  1. pupil in infinity
  2. chief ray parallel to the optical axis



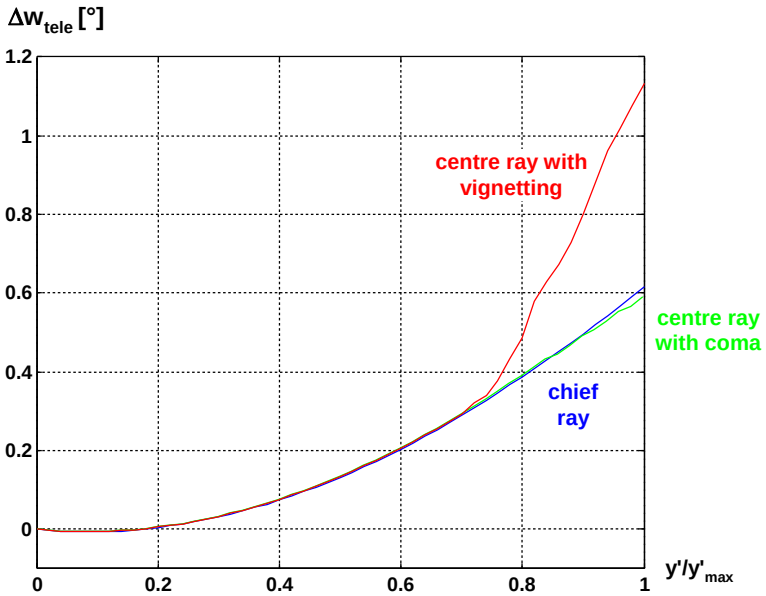
# 2 Optical System of the Microscopy II
# Telecentricity

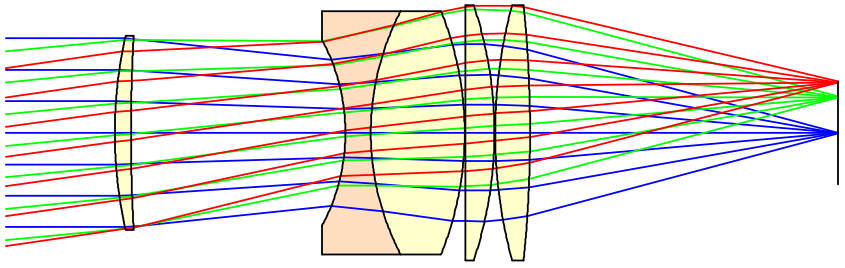


- Design problems with telecentricity:
  Usual telecentricity only fulfilled for one wavelength




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



# 2 Optical System of the Microscopy II
# Confocal Microscope

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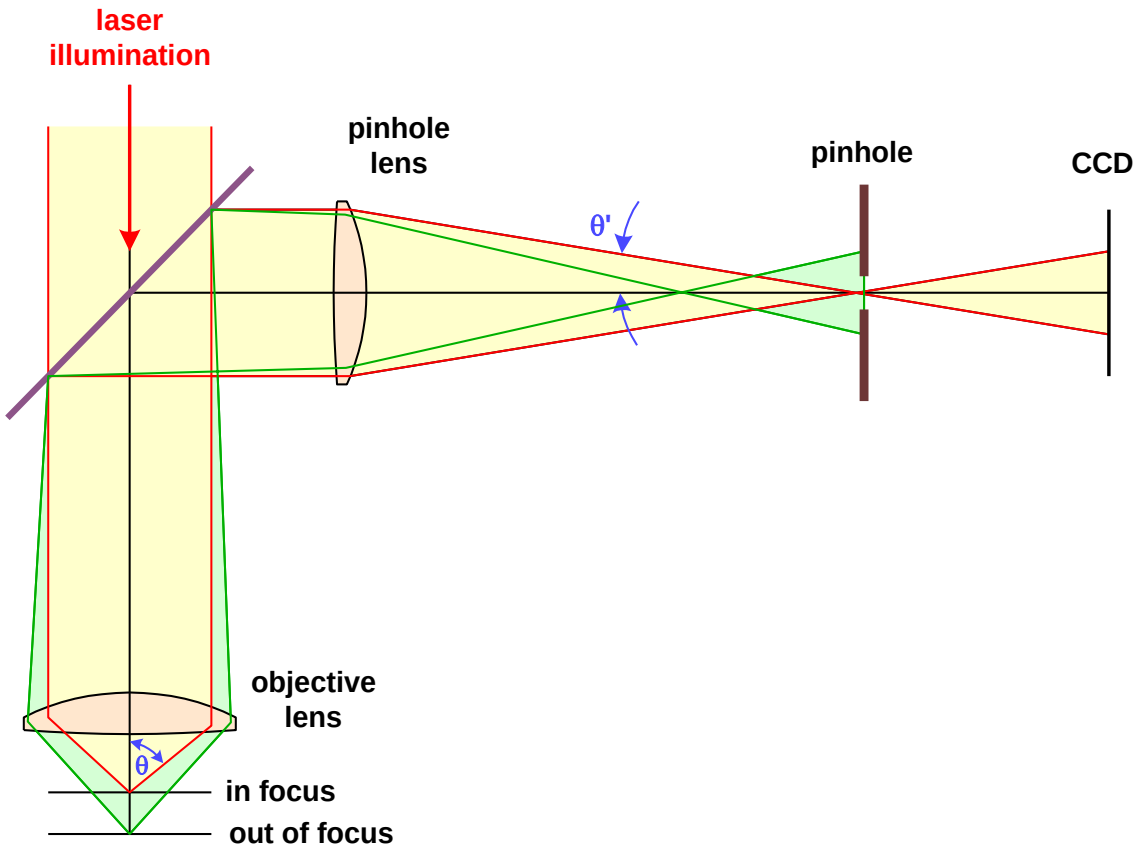
- Laser scan microscope
- Depth resolution (sectioning) with confocal pinhole
- Transverse scan on field of view Digital image
- Only light comming out of the conjugate plane is detected
- Perfect system: scan mirrors conjugate to pupil location
- System needs a good correction of the objective lens, symmetric 3D distribution of intensity



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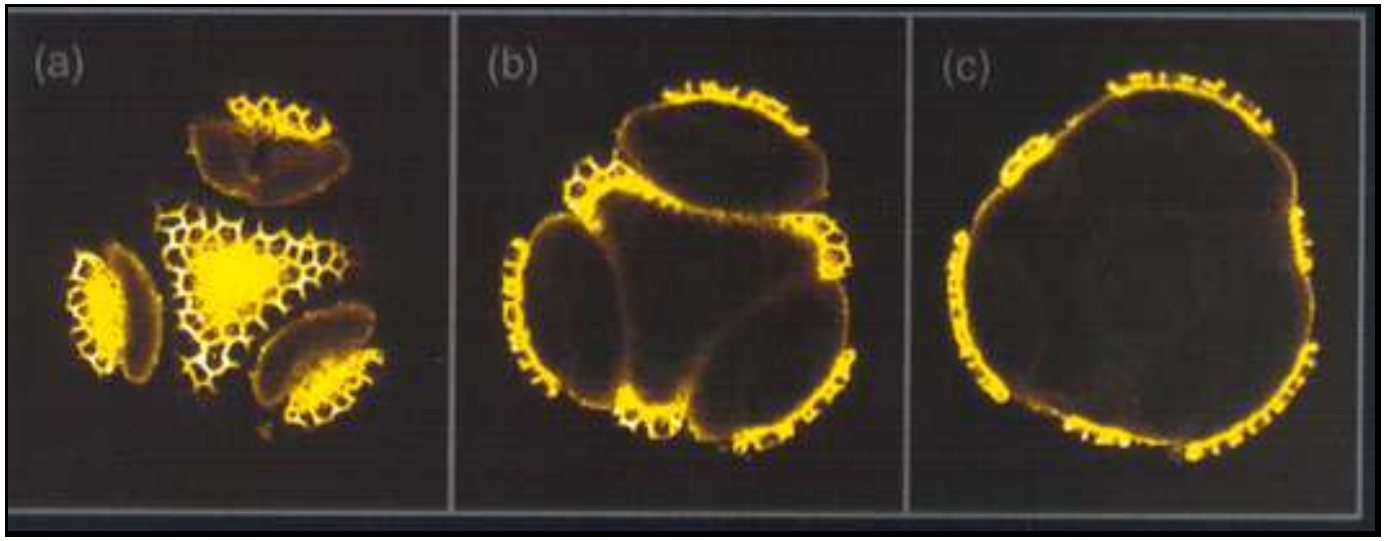
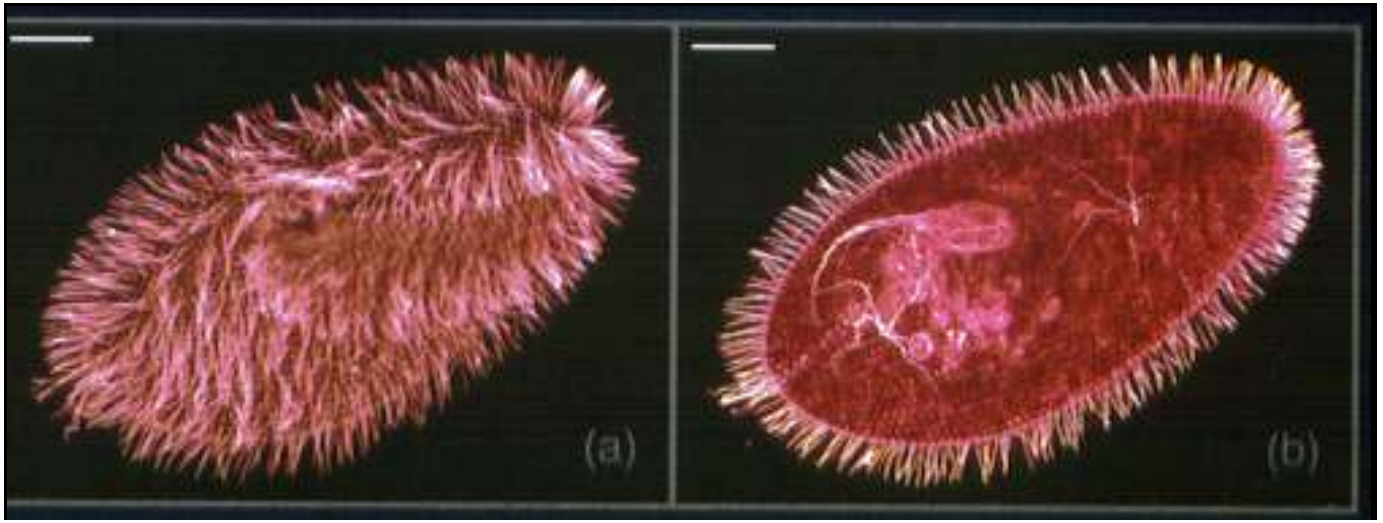
Depth resolved images




Ref.: M. Kempe

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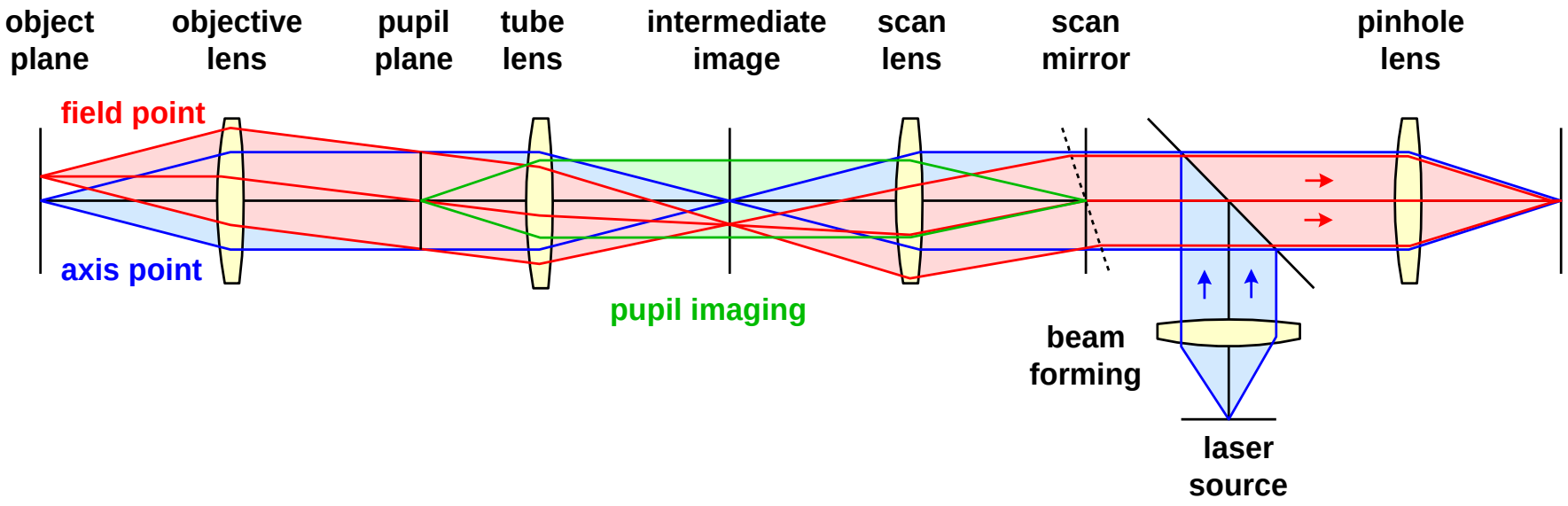
- Complete setup: objective / tube lens / scan lens / pinhole lens
- Scanning of illumination / descanning of signal
- Scan mirror conjugate to system pupil plane
- Digital image processing necessary



# 2 Optical System of the Microscopy II
# Confocal Laser Scan Microscope

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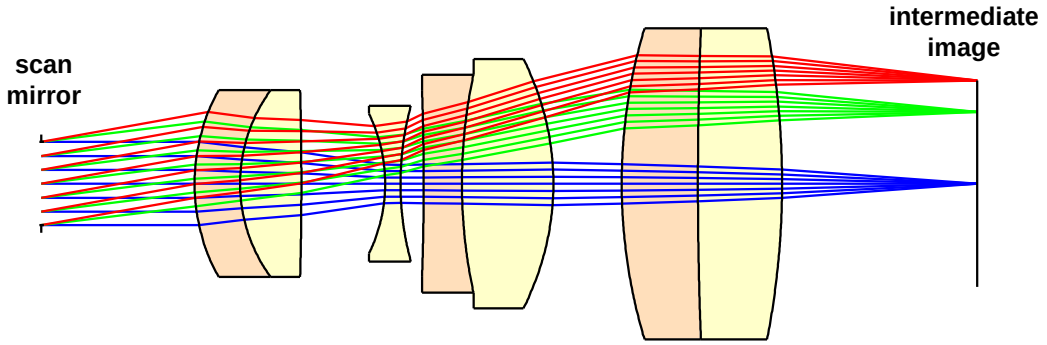
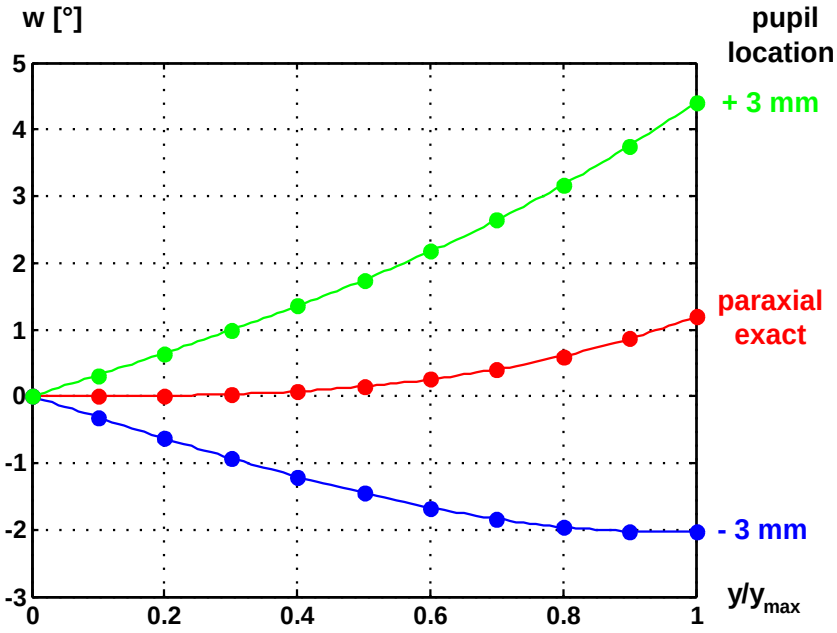
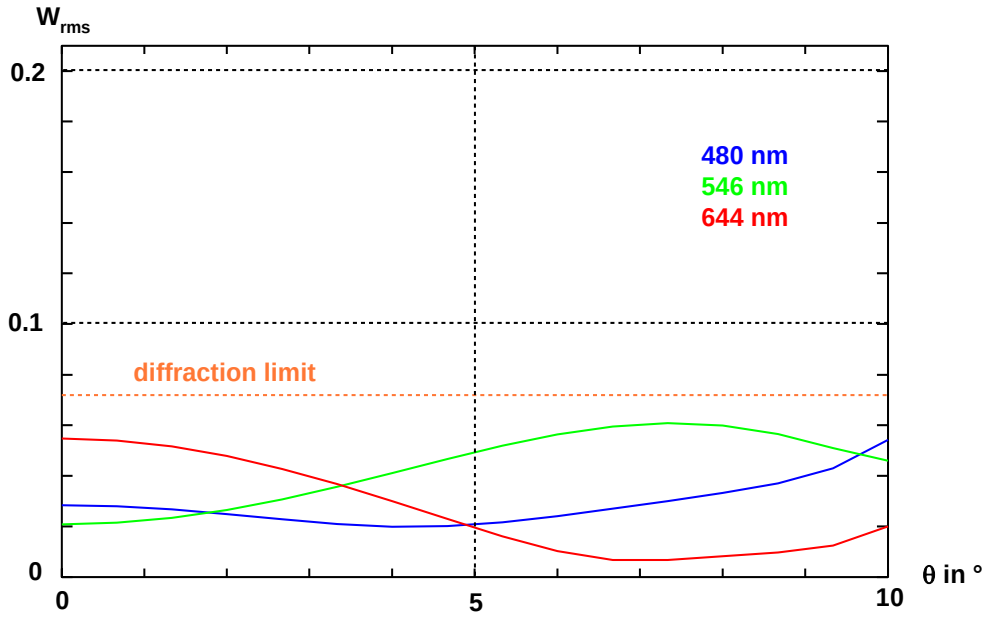
- Scan lens
- Diffraction limited
- Change in pupil location of objective lenses is critical perturbation of telecentricity



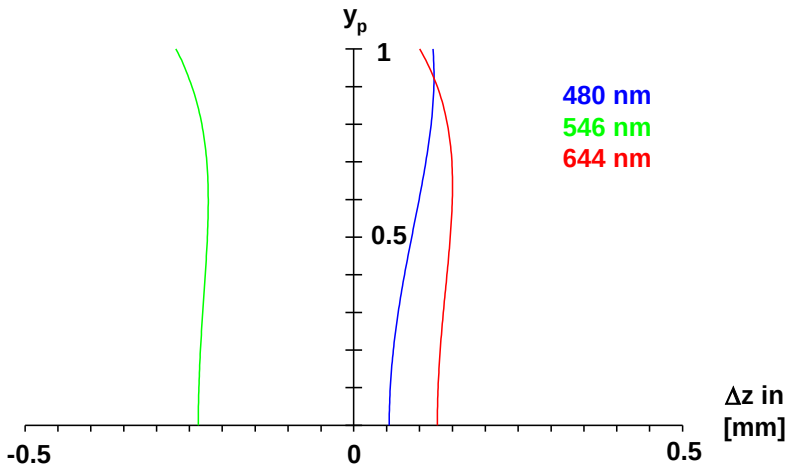



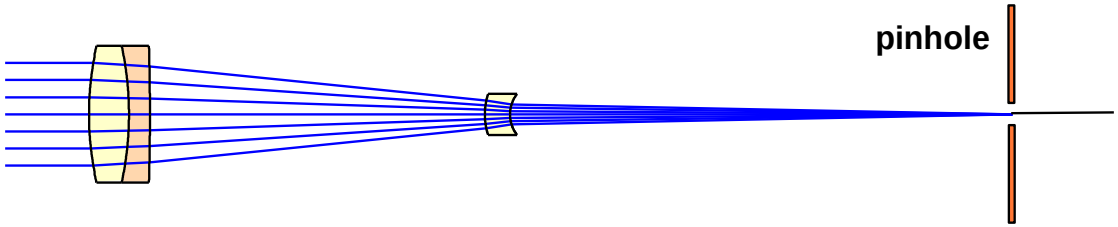



# 2 Optical System of the Microscopy II
# Confocal Laser Scan Microscope



- Pinhole lens
- Only axial colour is essential
- Usage on axis only due to descanning
- Variable pinhole size not too small: small aperture, retrofocus lens



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- Normalized transverse coordinate v

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

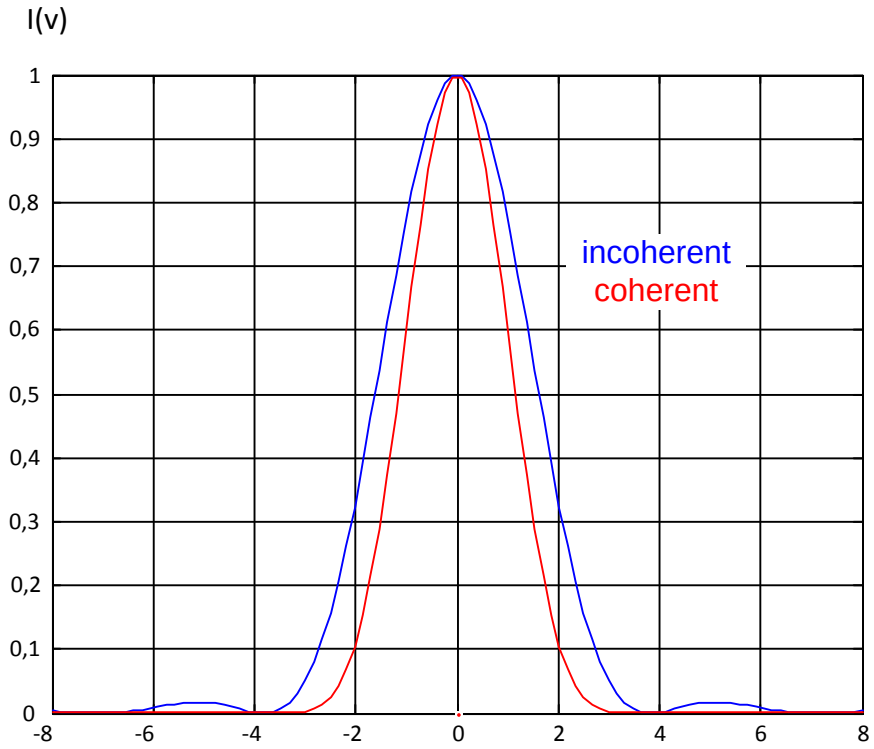
- Usual PSF: Airy

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

- 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



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- Normalized axial coordinate

$$u = \frac{8\pi}{\lambda} \cdot z \cdot \sin^2(\alpha/2)$$

- Conventional wide field imaging:
  Intensity on axis

$$I(u) = \left[\frac{\sin(u/2)}{u/2}\right]^2$$

  Axial resolution

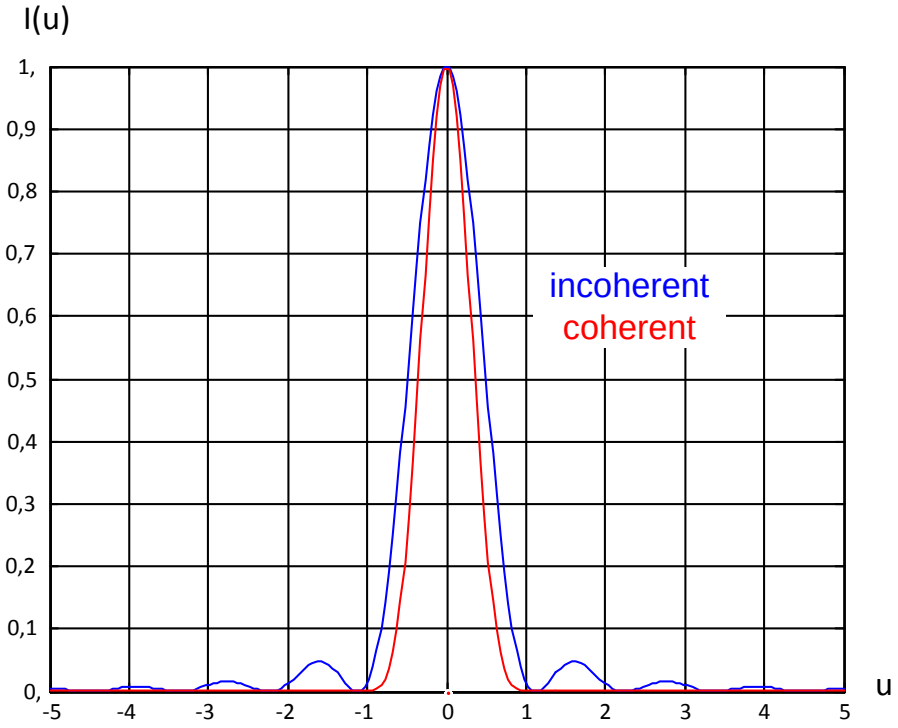
$$\Delta z_{wide}^{(approx)} = \frac{0.45 \cdot \lambda}{n' \cdot (1 - \cos\theta)}$$

- 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

$$\Delta z_{confo} = \frac{0.319 \cdot \lambda}{n' \cdot (1 - \cos\theta)}$$



# 2 Optical System of the Microscopy II
# Size of Pinhole and Cnfocality

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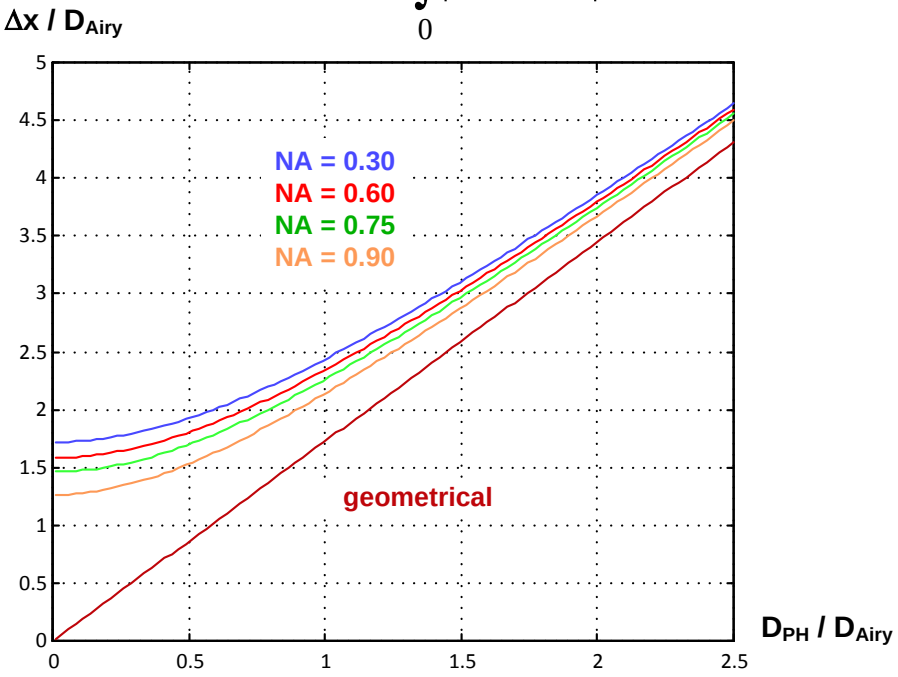
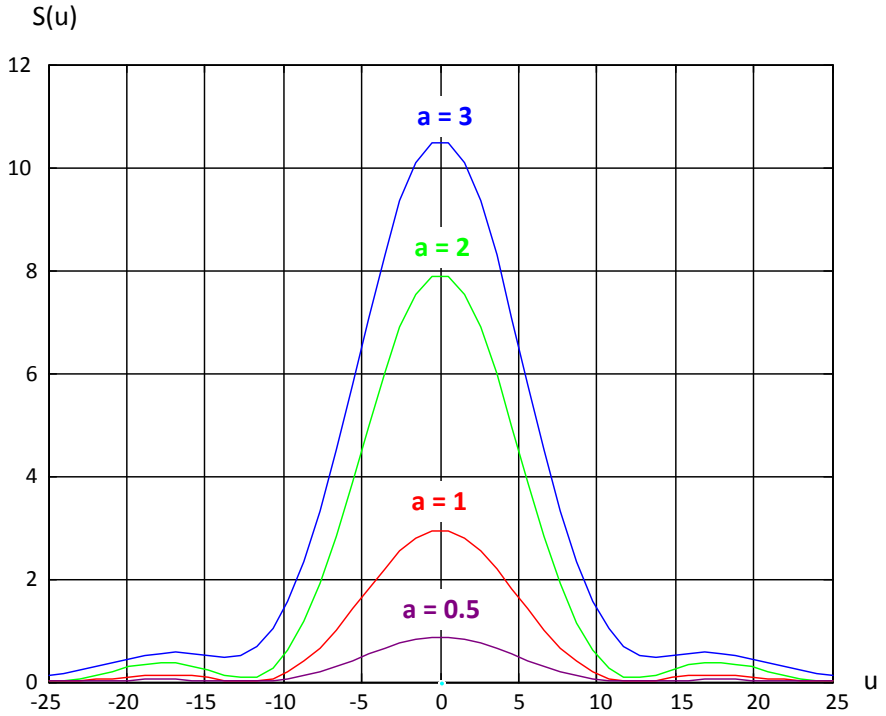
- Large pinhole: geometrical optic
- Small pinhole:
  - Diffraction dominates
  - Scaling by Airy diameter a = $D/D_{Airy}$
  - diffraction relevant for pinholes
    $D < D_{airy}$
- Confocal signal:
  Integral over pinhole size

$$S(u) = \int_0^a |U(u,v)|^2 \, 2\pi v \, dv$$



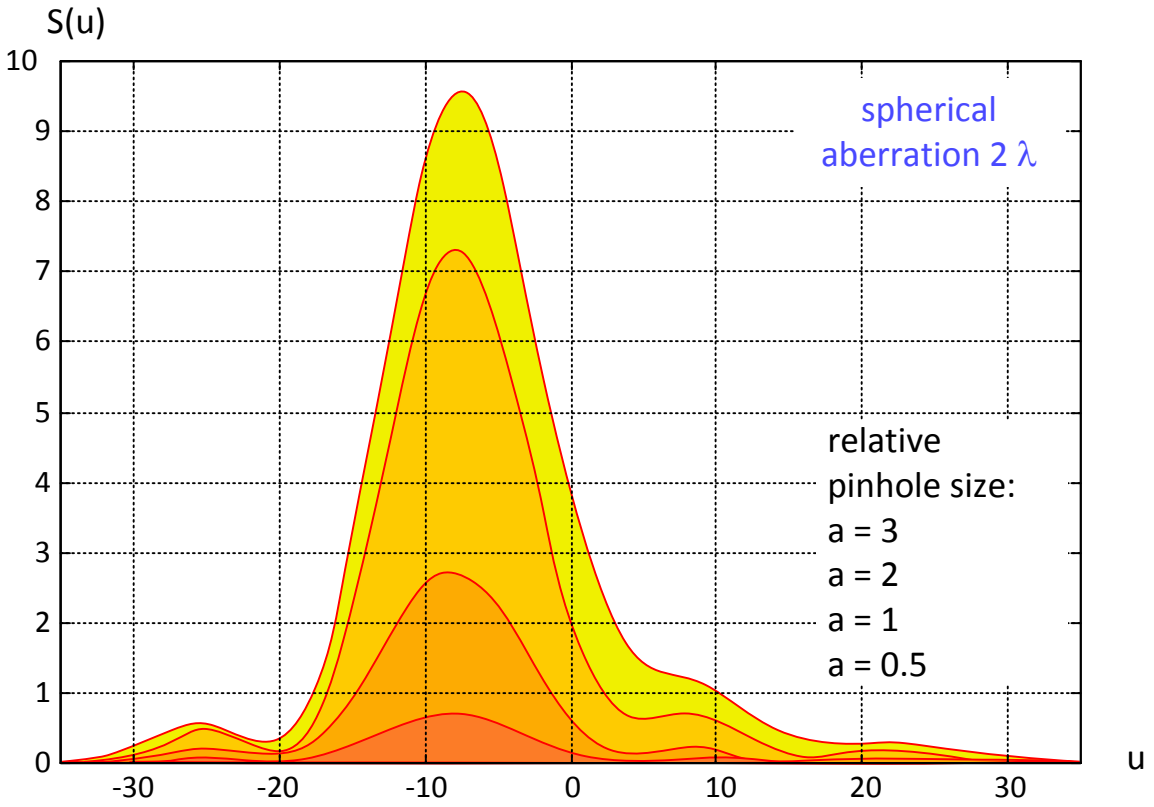



# 2 Optical System of the Microscopy II
# Confocal Signal with Spherical Aberration



- Spherical aberration:
  - PSF broadened
  - PSF no longer symmetrical around image plane during defocus

- Confocal signal:
  - loss in contrast
  - decreased resolution



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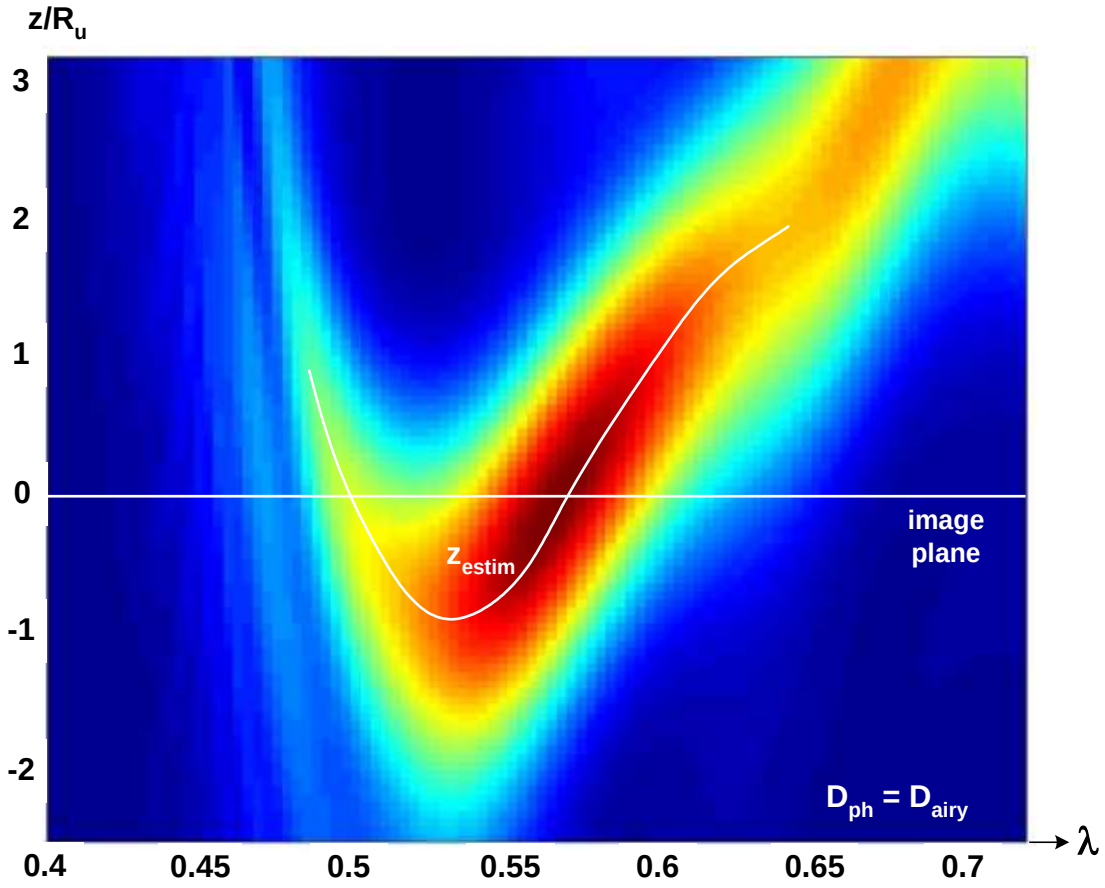
- Depth signal as a function of wavelength
- Disturbance by axial colour aberration



# 2 Optical System of the Microscopy II
# Illumination Optics: Overview

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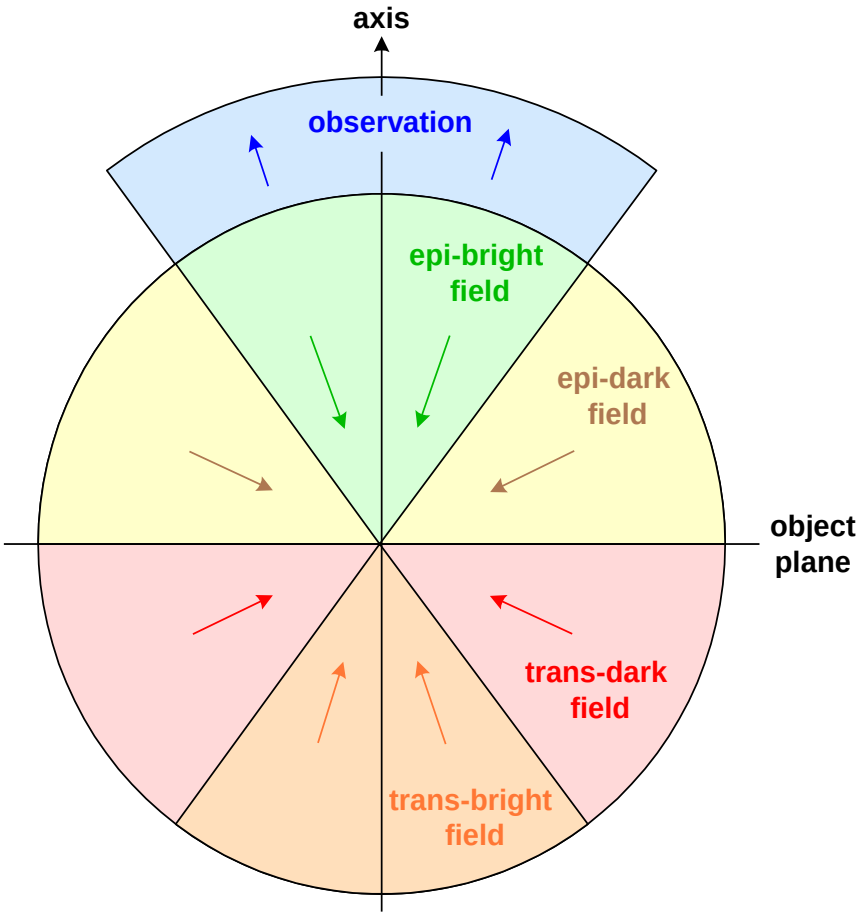
- Four possibilities for practical needs
- Epi vs. trans-illumination
- Bright vs. dark field illumination
- Comparison of light cones for imaging and illumination parts



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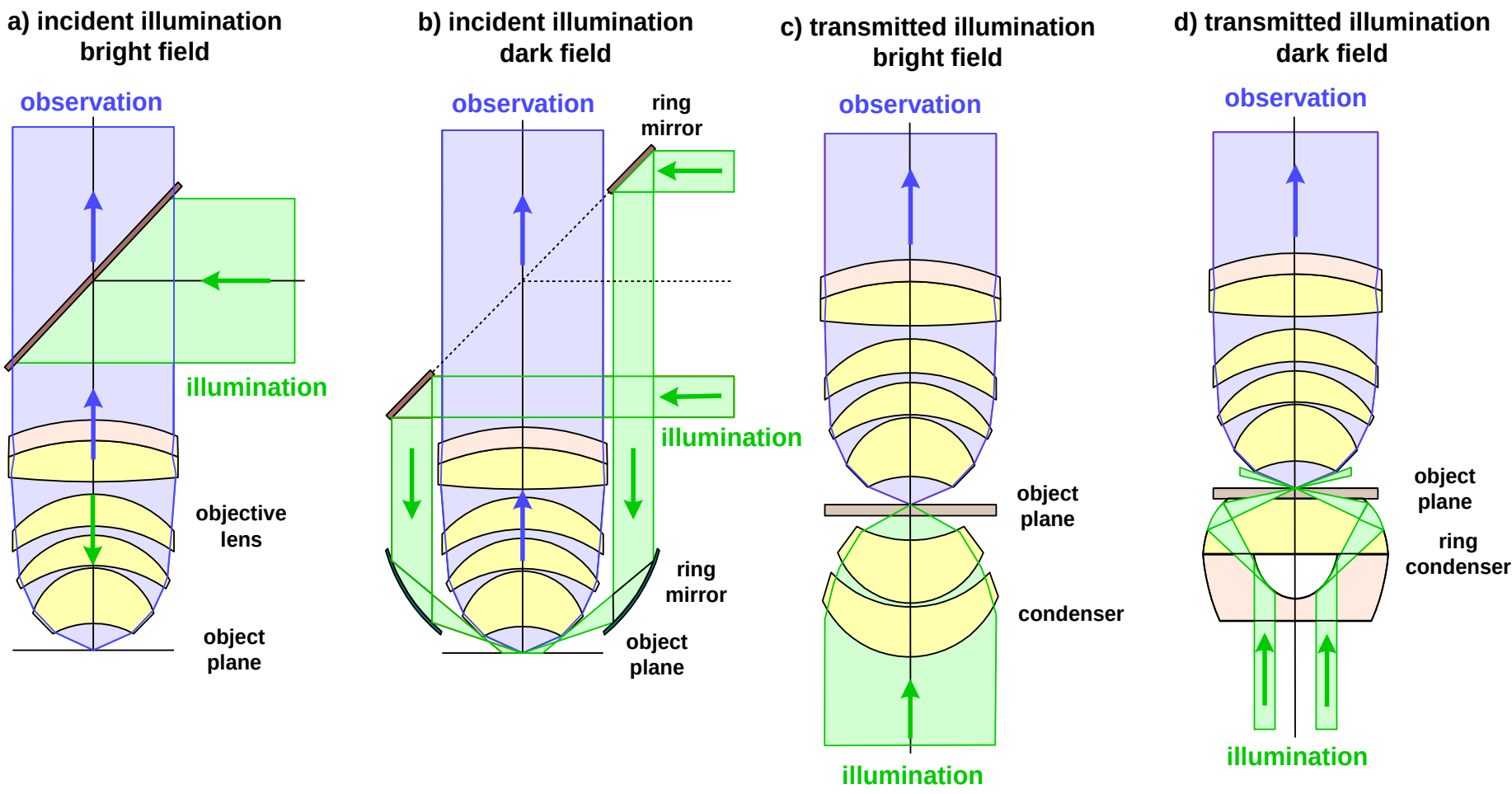
- Instrumental realizations



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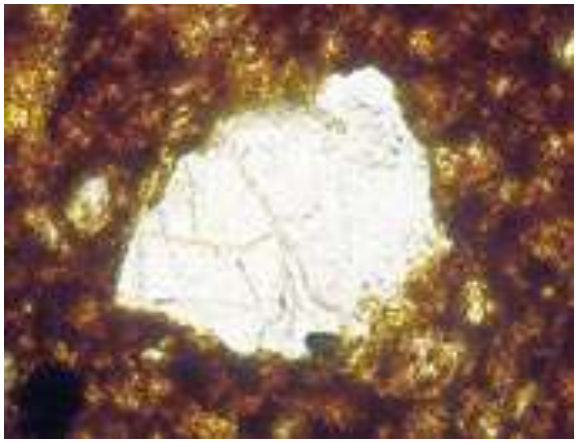
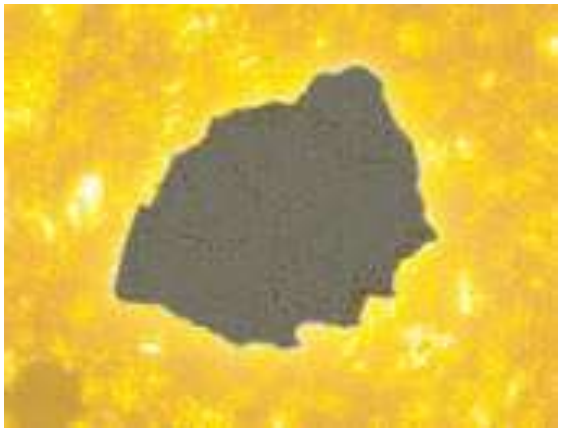
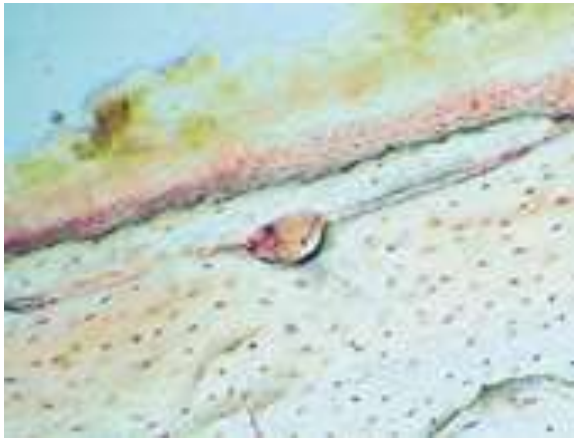
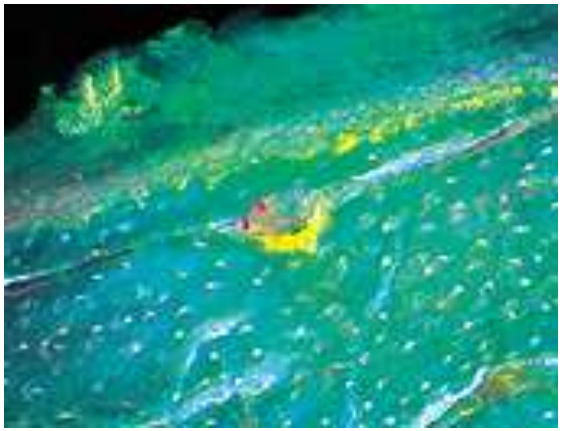
- Typical images for different illuminations

| | epi | trans |
|---|---|---|
| **bright** |  |  |
| **dark** |  |  |

# 2 Optical System of the Microscopy II
# Köhler Illumination Principle

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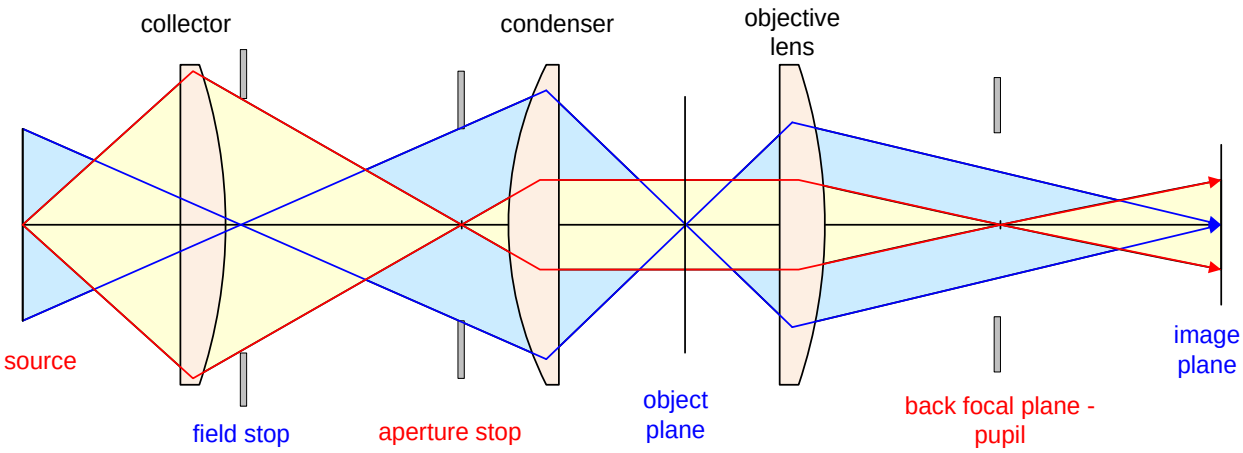
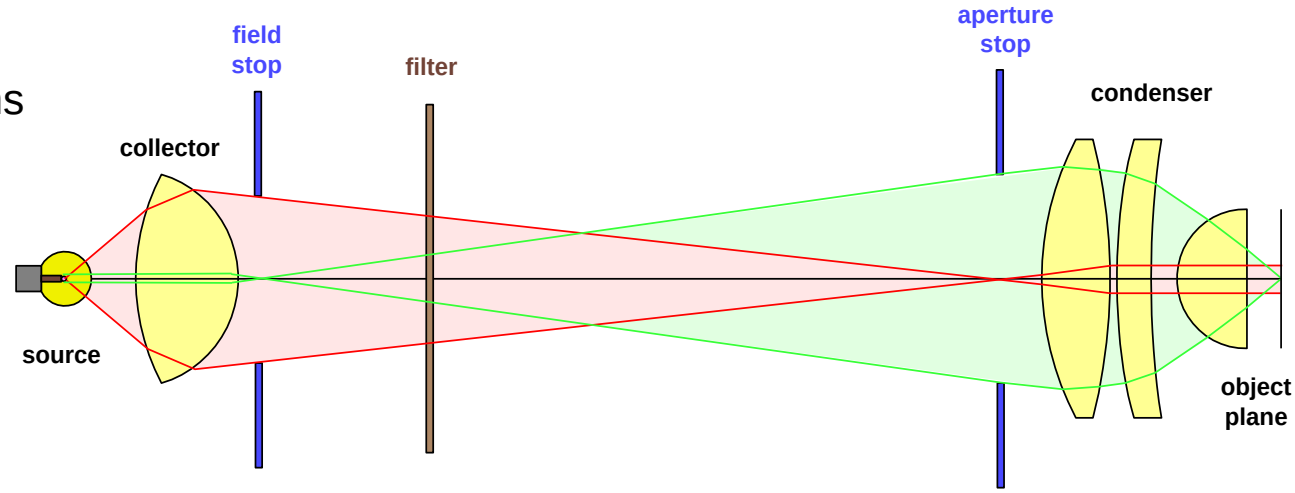
Principle of Köhler illumination:

- 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



# 2 Optical System of the Microscopy II
# Illumination Optics: Overview

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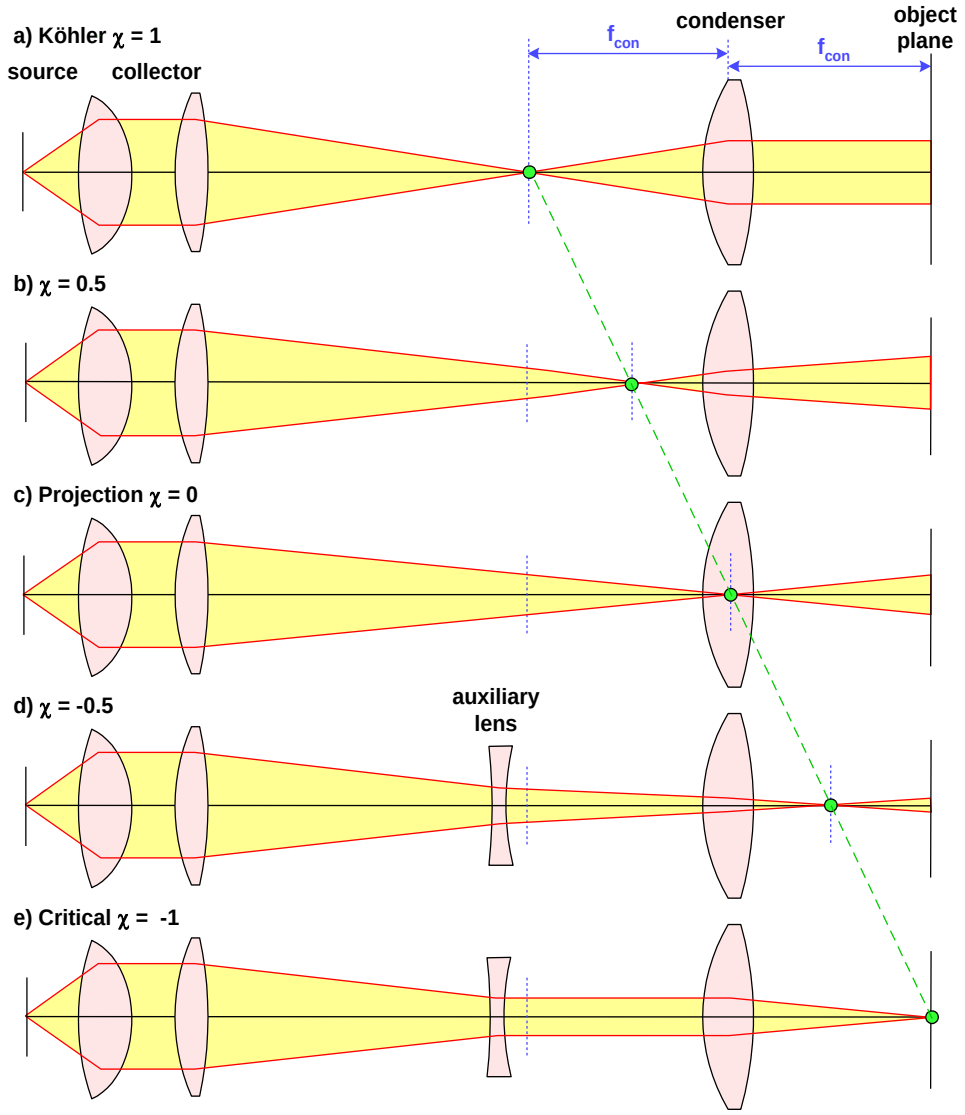
- Types of settings :
  1. Köhler :
     source into pupil,
     mostly used
  2. Critical :
     source into field of view,
     source structure disturbs image
  3. Projection :
     source into condenser
  4. Arbitrary



# 2 Optical System of the Microscopy II
# Illumination Optics: Collector

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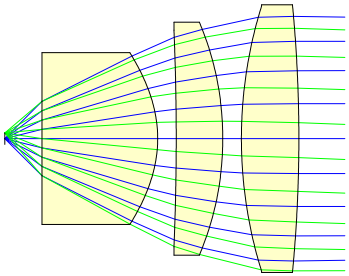
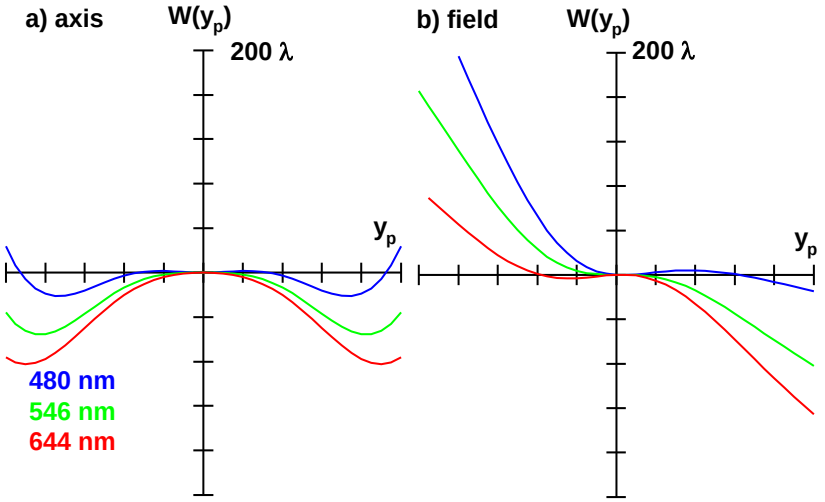
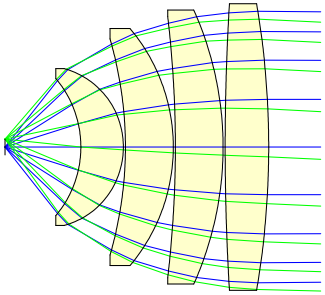
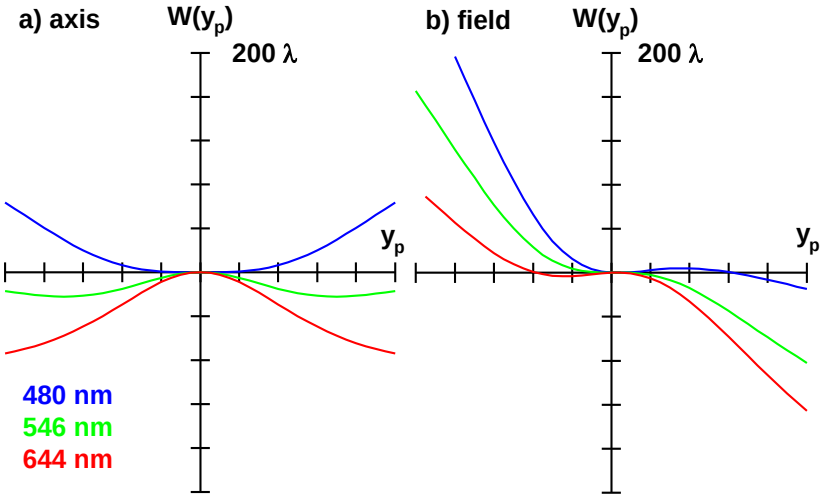
- Requirements and aspects:

1. Large collecting solid angle
2. Correction not critical
3. Thermal loading
   large
4. Mostly shell-structure
   for high NA







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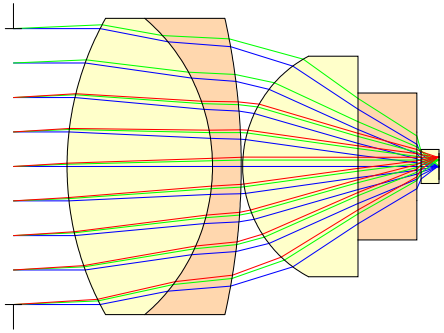
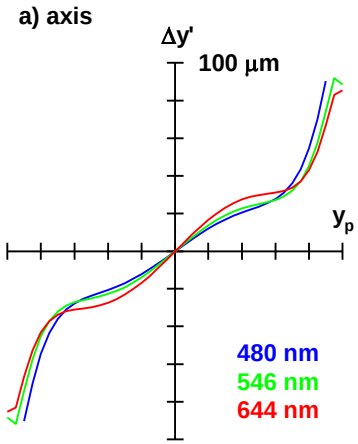
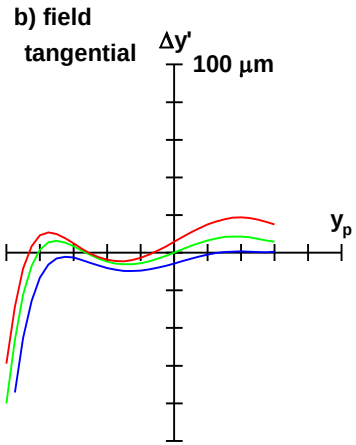
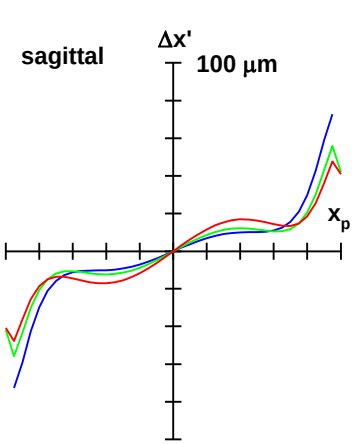
2. Abbe type, achromatic, NA = 0.9 , aplanatic, residual spherical

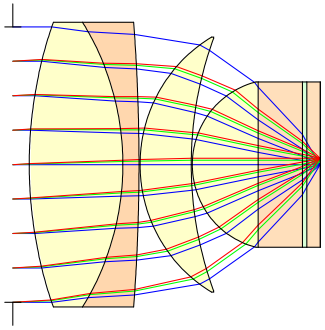


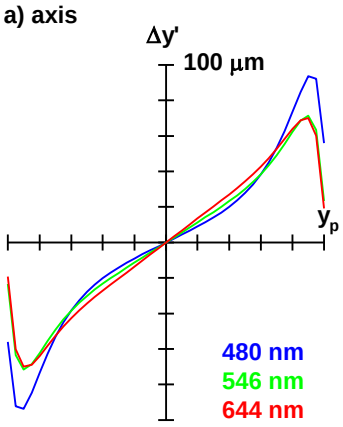


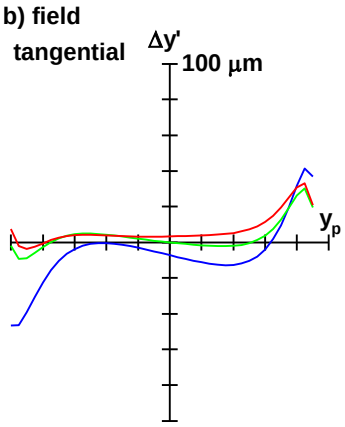



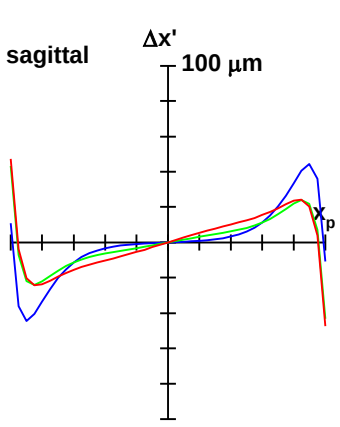




3. Aplanatic achromatic, NA = 0.85





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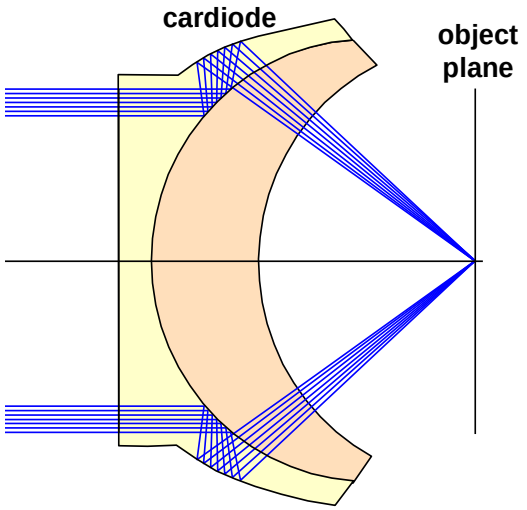
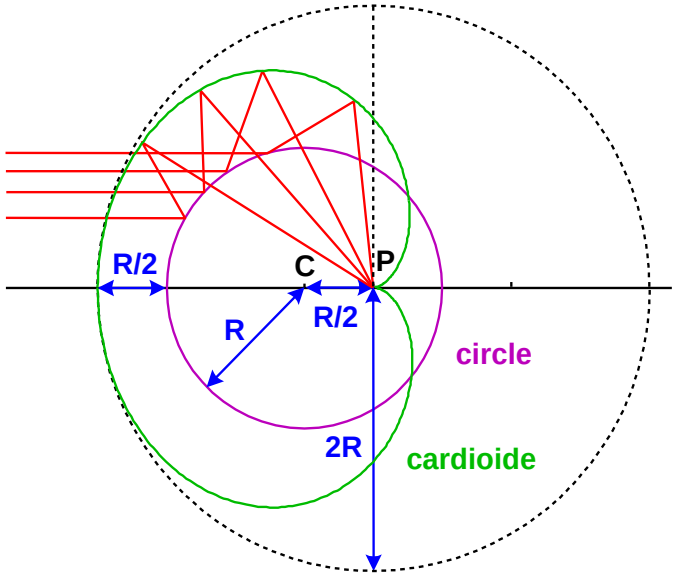
- Dark-field illumination systems

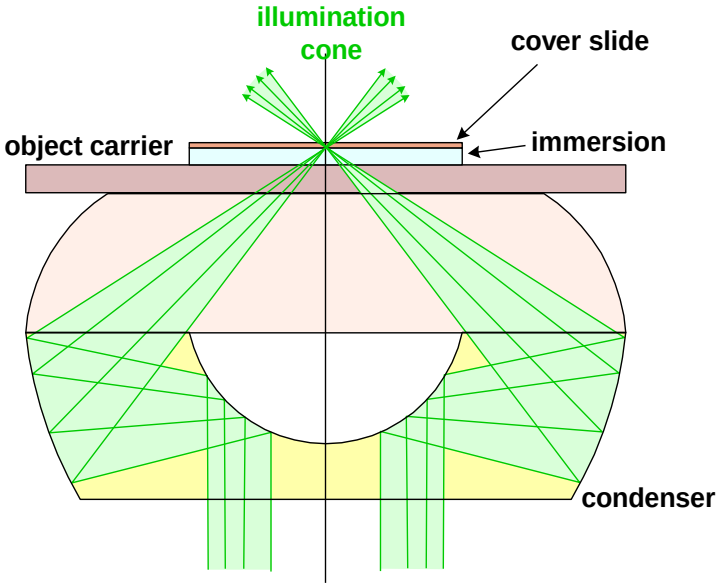
  1. Trans-illumination

     Cardioid-mirror




Realizations: approximation of Cardioid curve



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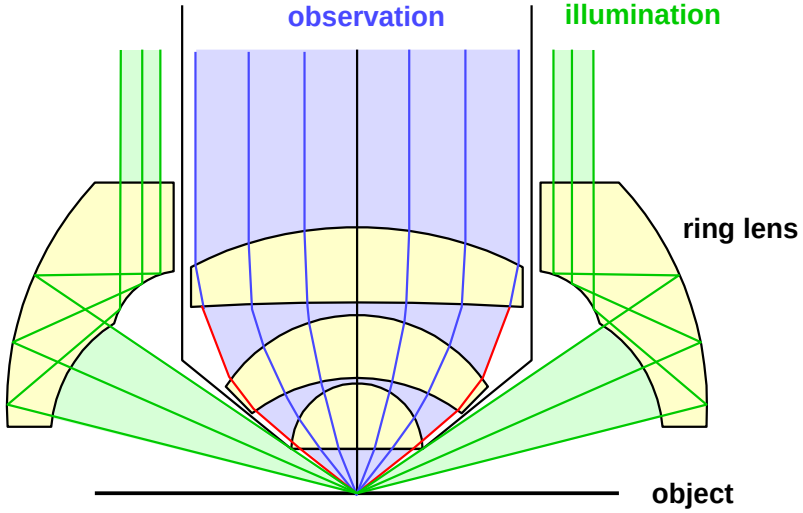
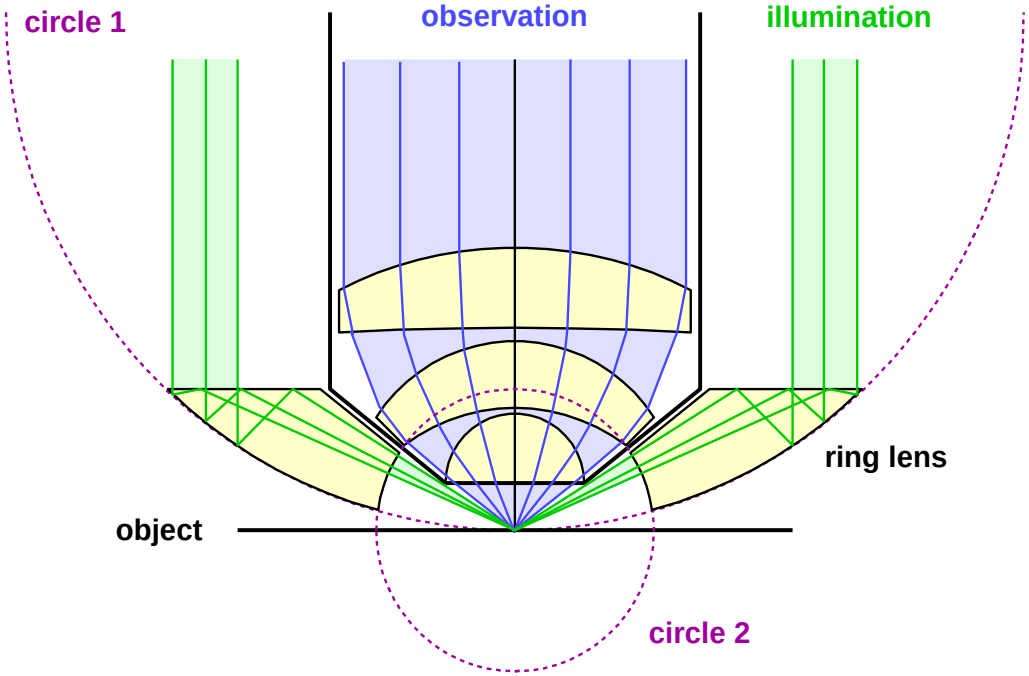
2. Epi-illumination

Complicated ring-shaped components around objective lens



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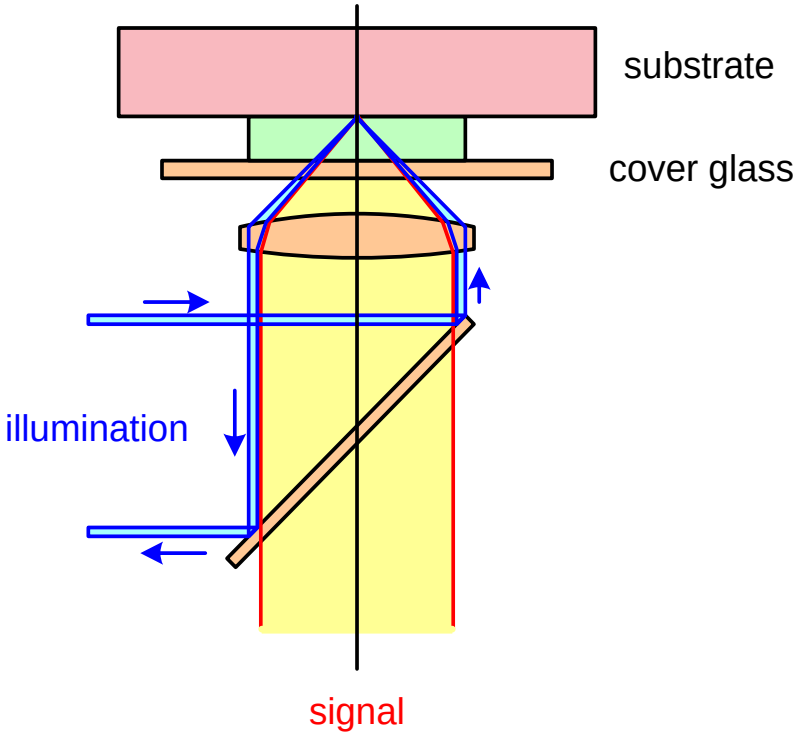
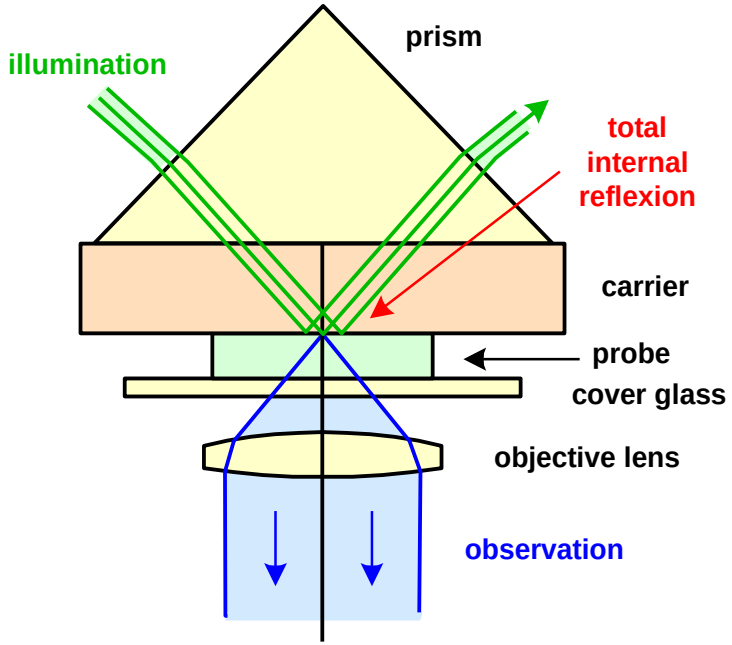
- Epi- and trans illumination for TIRF



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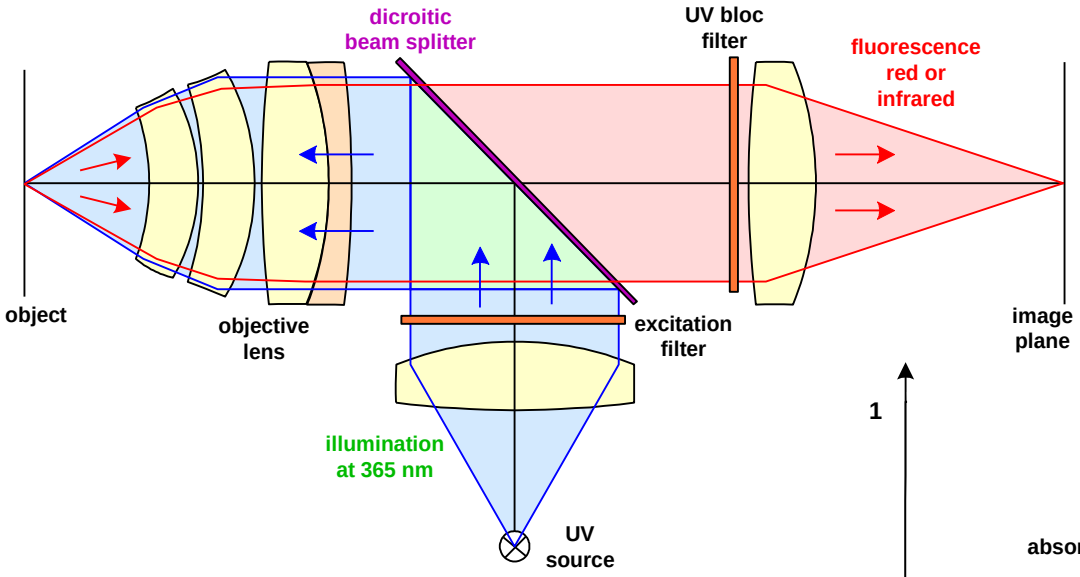
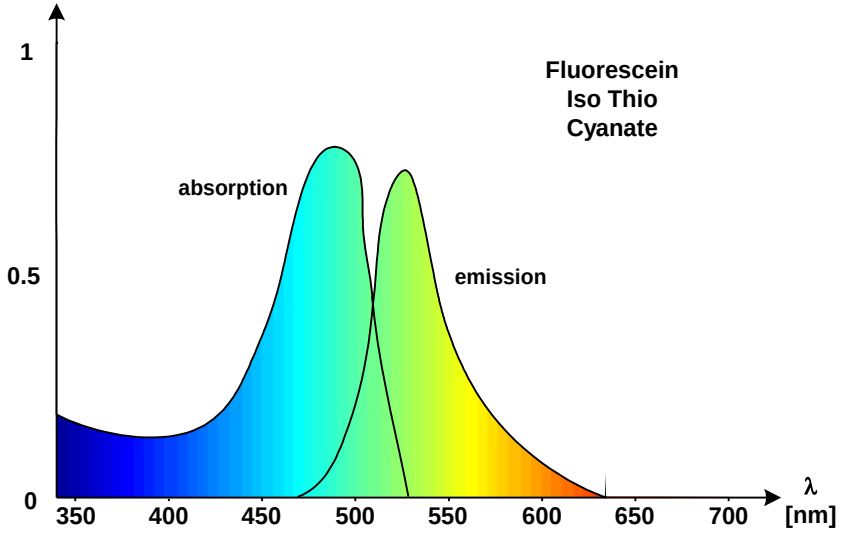
- Fluorescence microscopy is the most frequently employed mode of light microscopy used in biomedical reserach today
- Setup:




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

# 2 Optical System of the Microscopy II
# TIRF Microscopy

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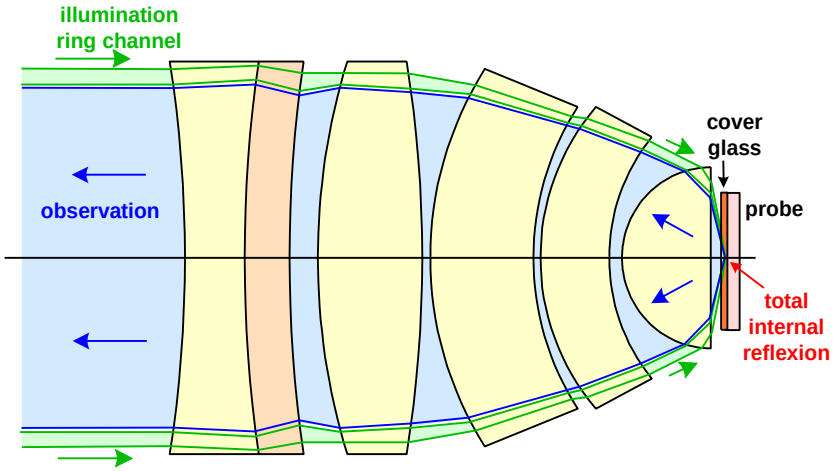
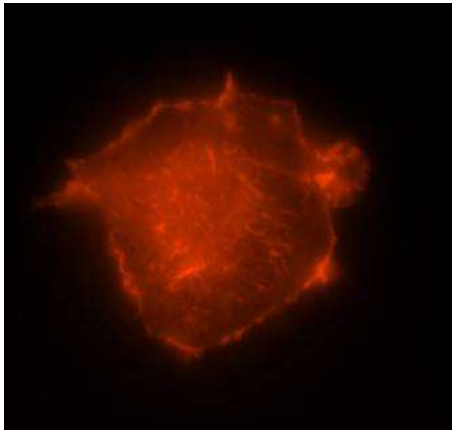
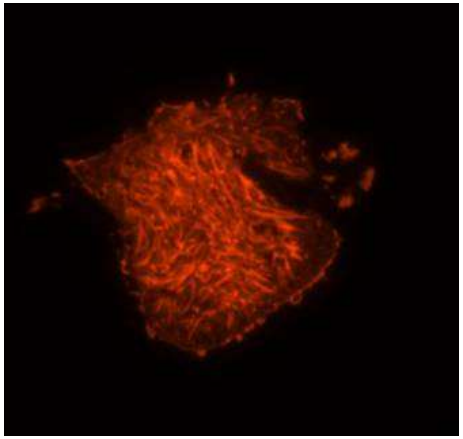
- Total internal reflection microscopy:
  Excitation with evanescent field
- Advantages:
  1. better axial resolution
  2. better SNR, no fluorescence background
- Problem in optical design:
  Extremly small illumination ring-shaped channel around the observation light cone

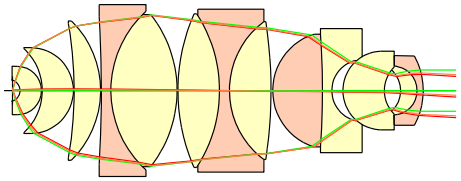



Epi-Fluorescence



TIRF






# 2 Optical System of the Microscopy II
# Adjustment of Objective Lenses

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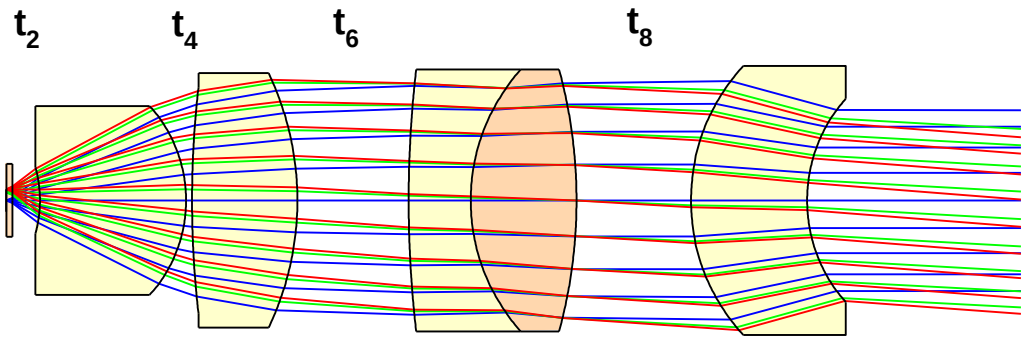
- Adjustment of air gaps to optimize spherical aberration
- Reduced optimization setup




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

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

| | $d_2$ | $d_4$ | $d_6$ | $d_8$ | $c_{20}$ | $c_{40}$ | $c_{60}$ | $c_{80}$ | $W_{rms}$ |
|---|---|---|---|---|---|---|---|---|---|
| **nominal** | **0.77300** | **0.17000** | **3.2200** | **2.0500** | **0.00527** | **-0.0718** | **0.00232** | **0.01290** | **0.0324** |
| $d_2$ varied | 0.77320 | 0.17000 | 3.2200 | 2.0500 | 0.04144 | -0.07586 | 0.00277 | 0.12854 | |
| $d_4$ varied | 0.77300 | 0.17050 | 3.2200 | 2.0500 | 0.03003 | -0.07461 | 0.00264 | 0.01286 | |
| $d_6$ varied | 0.77300 | 0.17000 | 3.2250 | 2.0500 | 0.00728 | -0.07367 | 0.00275 | 0.01284 | |
| $d_8$ varied | 0.77300 | 0.17000 | 3.2200 | 2.0550 | 0.005551 | -0.0717 | 0.00235 | 0.01290 | |
| **optimized** | **0.77297** | **0.16942** | **3.12670** | **3.2110** | **0.000414** | **0.00046** | **0.00030** | **0.01390** | **0.00468** |

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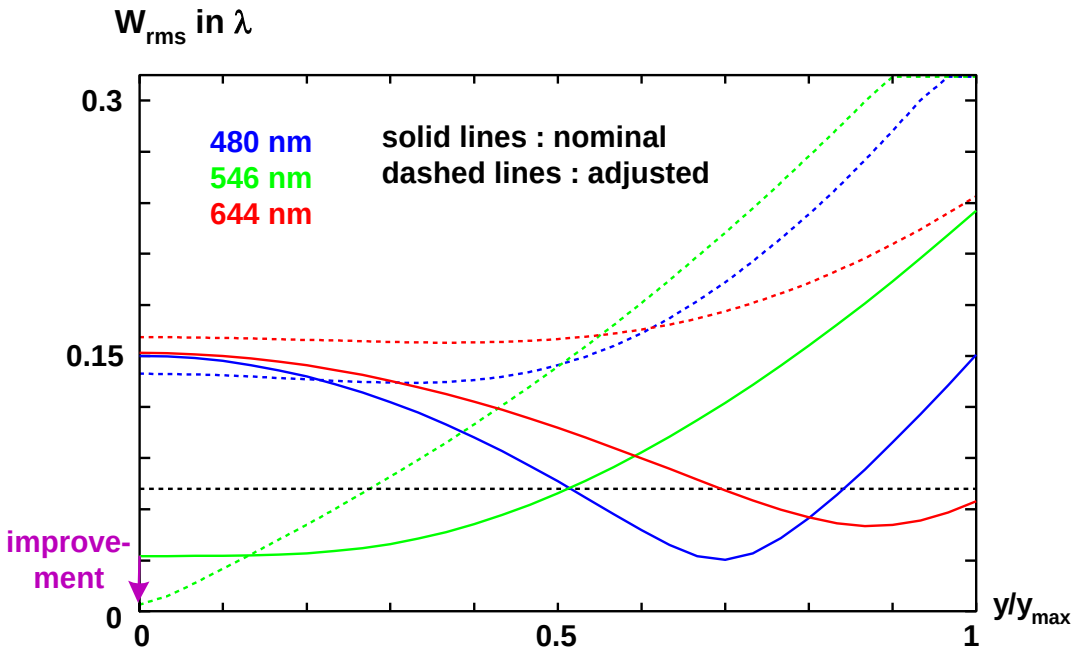
- Significant improvement for one wavelength on axis
- Possible decreased performance in the field



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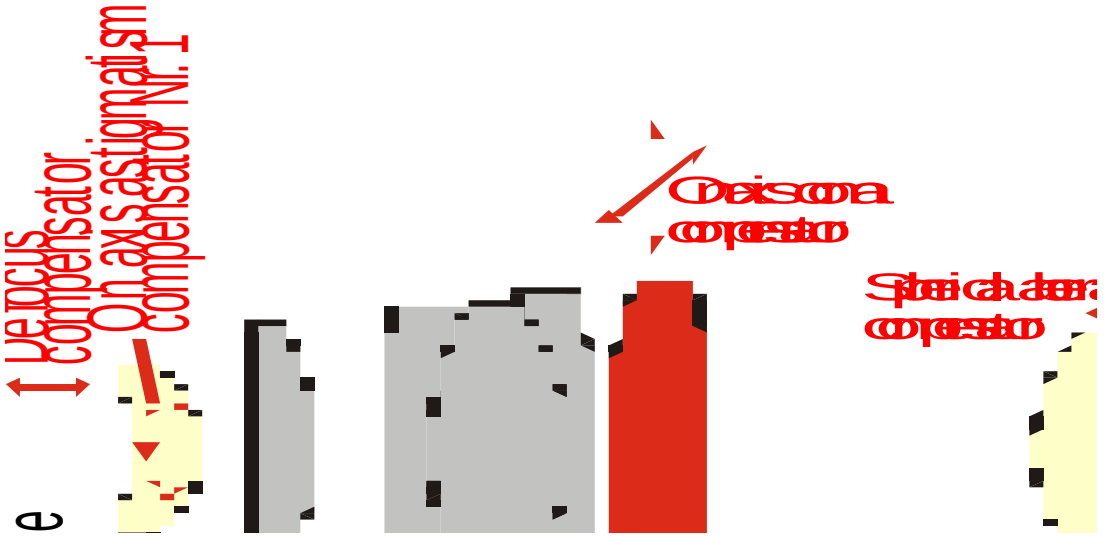
- Example microscopic lens

- 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

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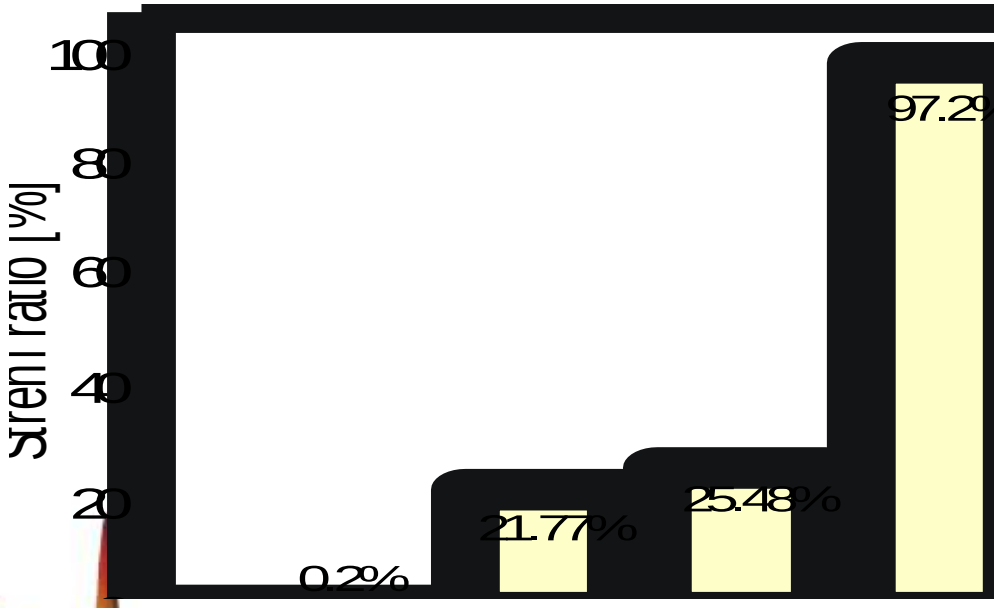
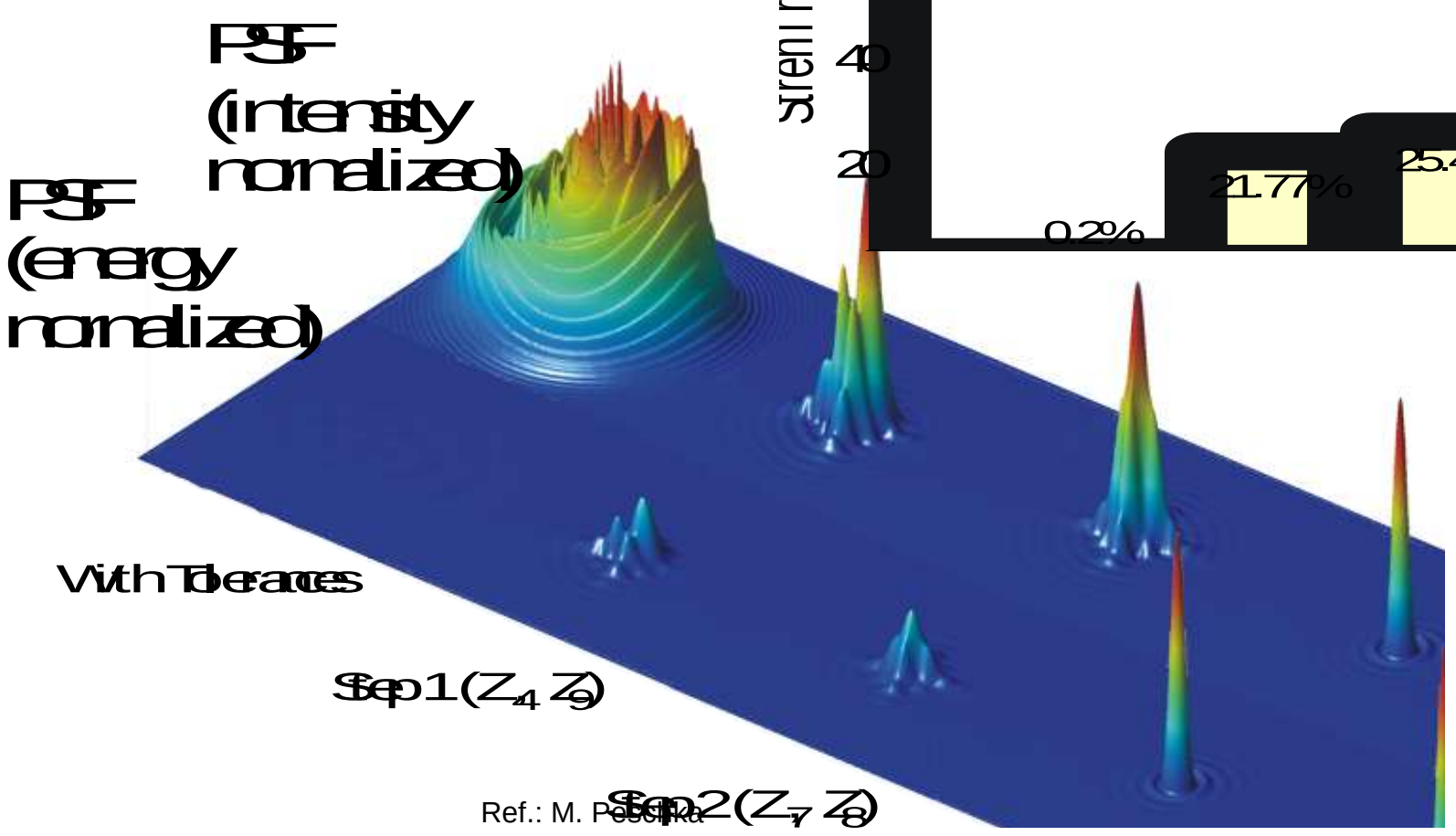
- Sucessive steps of improvements

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