Institute of Applied Physics

Friedrich-Schiller-Universität Jena

Metrology and Sensing

Lecture 15: Confocal sensors

2018-02-08

Herbert Gross

Preliminary Schedule

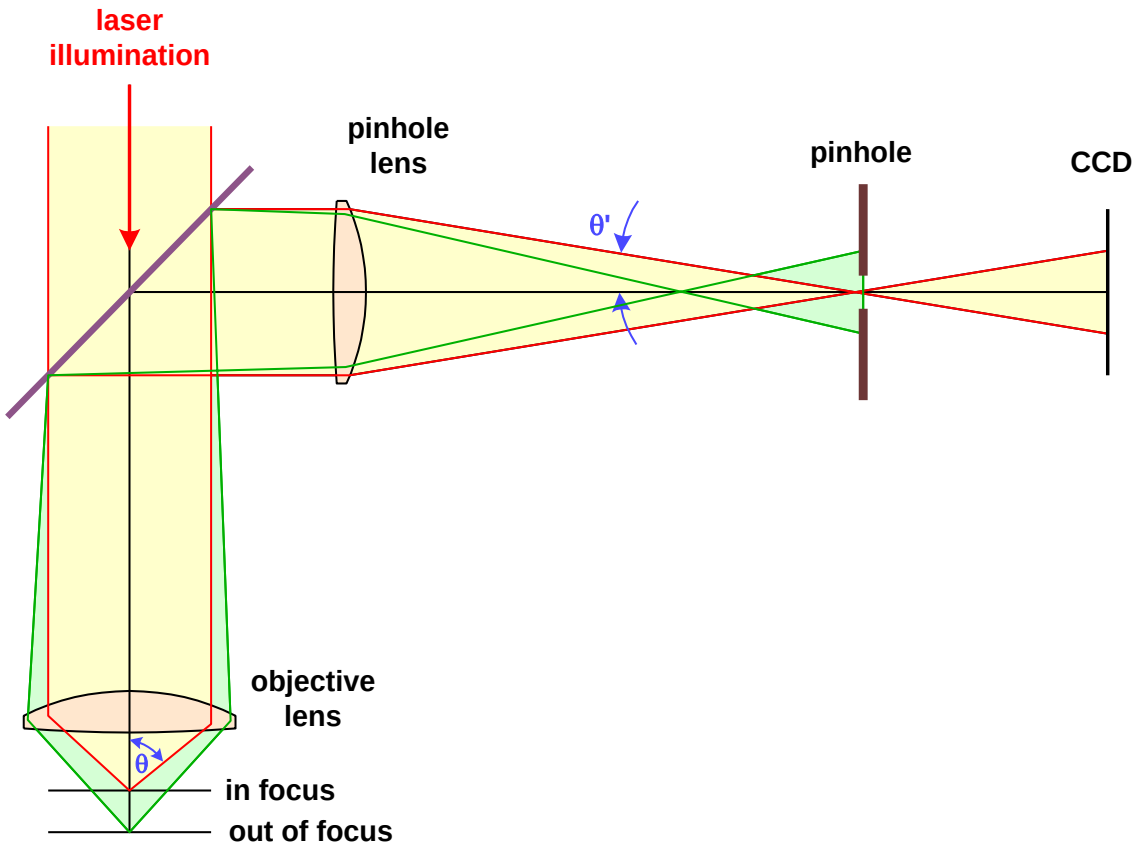
No	Date	Subject	Detailed Content
1	19.10.	Introduction	Introduction, optical measurements, shape measurements, errors, definition of the meter, sampling theorem
2	26.10.	Wave optics	Basics, polarization, wave aberrations, PSF, OTF
3	02.11.	Sensors	Introduction, basic properties, CCDs, filtering, noise
4	09.11.	Fringe projection	Moire principle, illumination coding, fringe projection, deflectometry
5	16.11.	Interferometry I	Introduction, interference, types of interferometers, miscellaneous
6	23.11.	Interferometry II	Examples, interferogram interpretation, fringe evaluation methods
7	30.11.	Wavefront sensors	Hartmann-Shack WFS, Hartmann method, miscellaneous methods
8	07.12.	Geometrical methods	Tactile measurement, photogrammetry, triangulation, time of flight, Scheimpflug setup
9	14.12.	Speckle methods	Spatial and temporal coherence, speckle, properties, speckle metrology
10	21.12.	Holography	Introduction, holographic interferometry, applications, miscellaneous
11	11.01.	Measurement of basic system properties	Bssic properties, knife edge, slit scan, MTF measurement
12	18.01.	Phase retrieval	Introduction, algorithms, practical aspects, accuracy
13	25.01.	Metrology of aspheres and freeforms	Aspheres, null lens tests, CGH method, freeforms, metrology of freeforms
14	01.02.	OCT	Principle of OCT, tissue optics, Fourier domain OCT, miscellaneous
15	08.02.	Confocal sensors	Principle, resolution and PSF, microscopy, chromatical confocal method

Content

- Principle of confocal imaging
- Resolution and PSF
- Pinhole size
- Impact of aberrations
- Scanning
- Examples / applications
- Chromatical confocal method

Confocal Microscope

- 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



Laser Scanning Confocal Microscope

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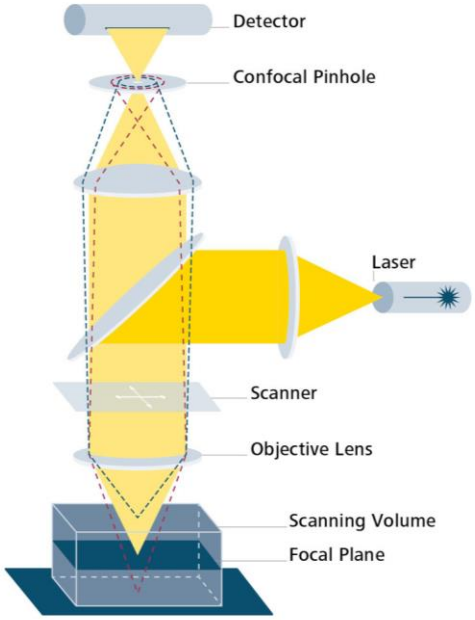
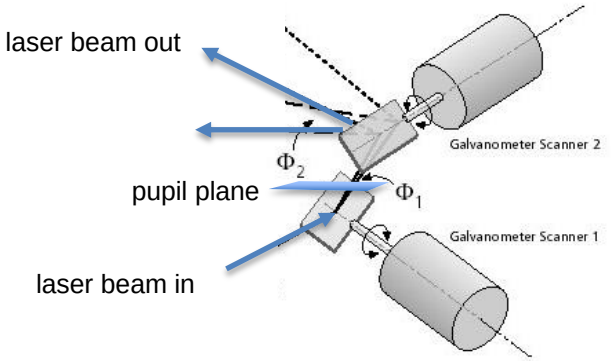
- Depth resolution (sectioning) with confocal pinhole
- Transverse scan over field of view → digital image
- Light from outside of the conjugate plane is rejected at pinhole
- Perfect system: scan mirrors conjugate to pupil location
- System needs a good correction of the objective lens, symmetric 3D distribution of intensity





Ref: M. Kempe

Adapted from: SCANLAB's PC Interface Board RTC3 Manual

Confocal 3D Image Collection

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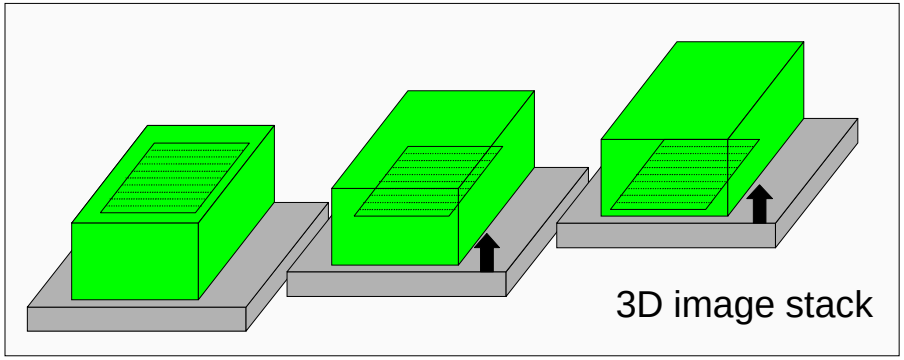
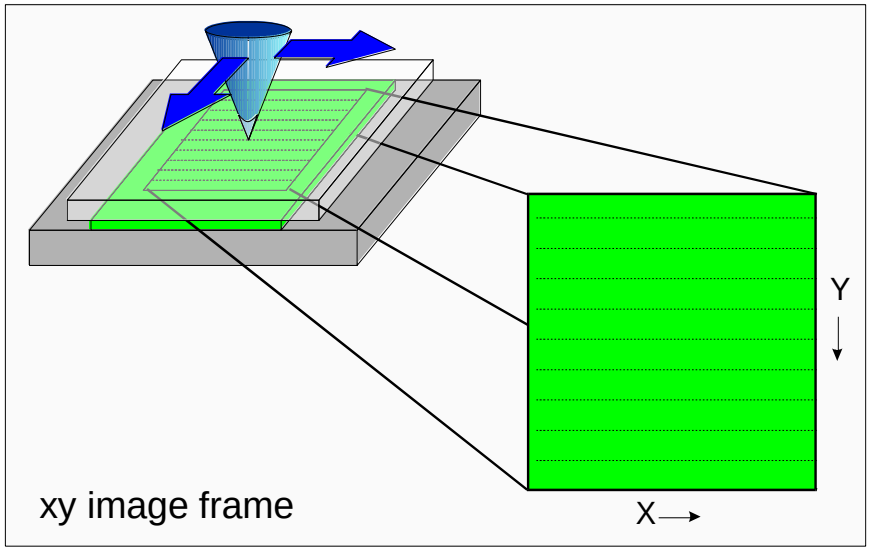
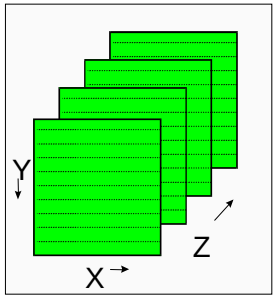
- Contact free optical sectioning
- 3D information collection & reconstruction
- 3D measurement and analysis
- The laser focus is moved over the sample (flying spot method)
- The measured intensity at each spot forms a xy image frame







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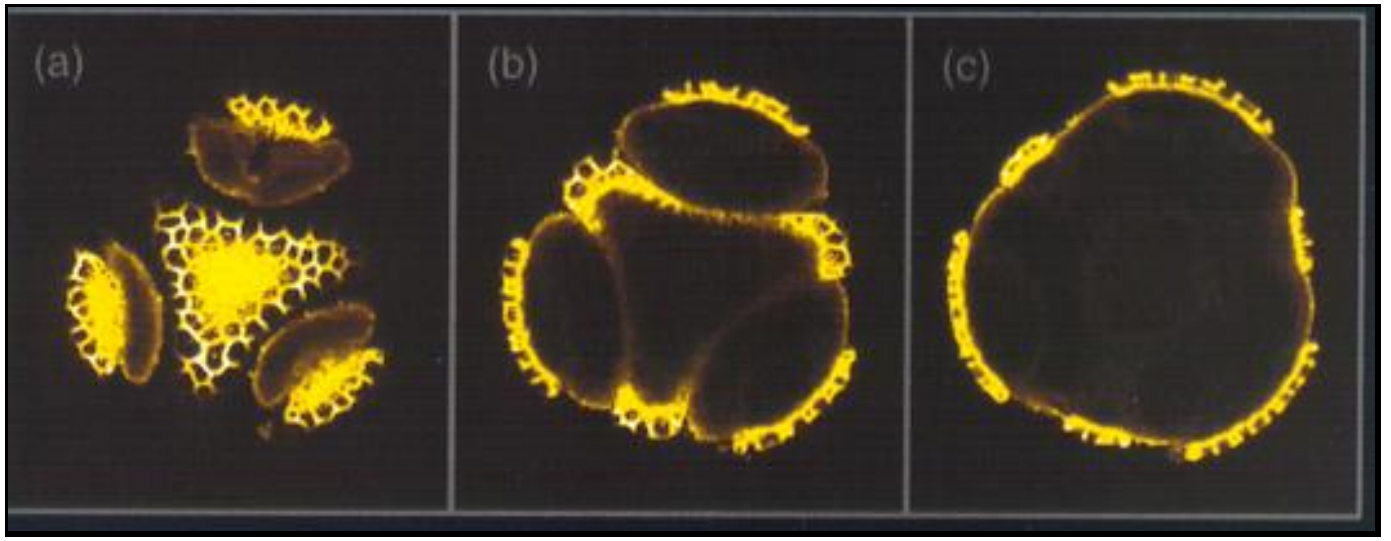
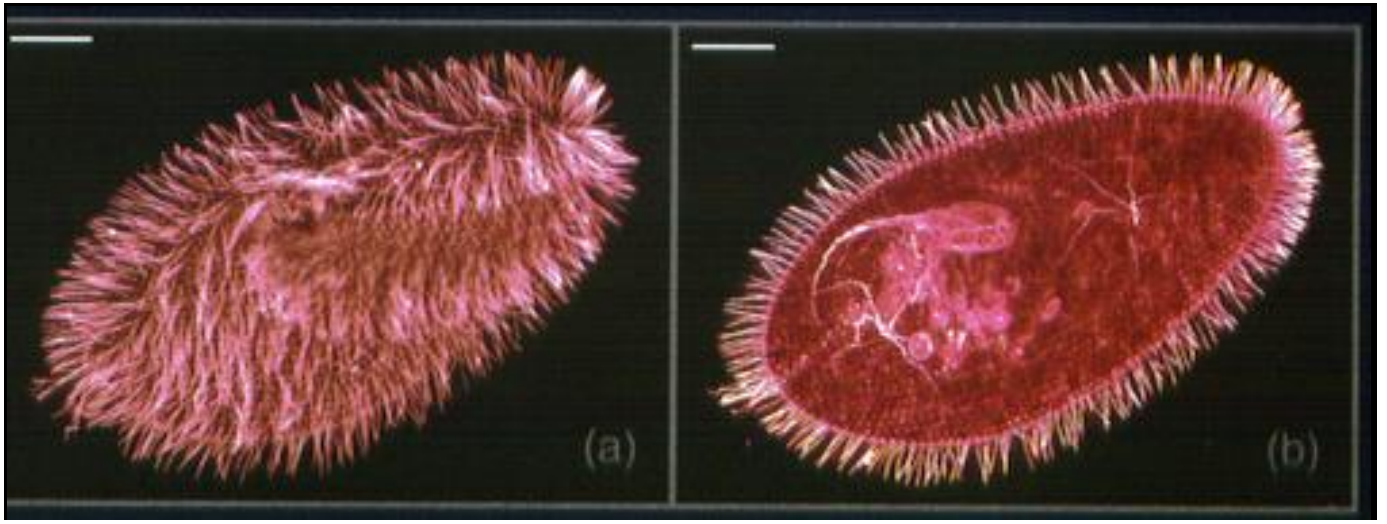
Confocal Images

Depth resolved images





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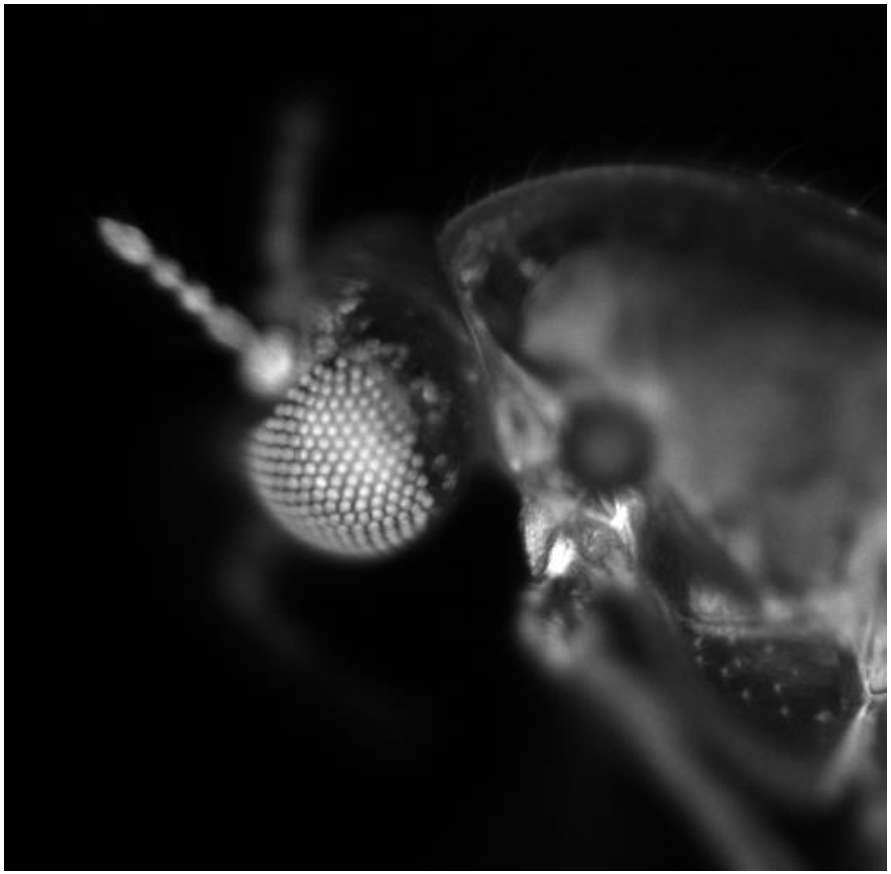
Confocal Microscopy

3-D volume imaging with reconstruction in confocal Laser scan microscope

a) Classical microscopy
depth of object : 300 μm



b) Confocal microscopy
with 3-D reconstruction

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Examples

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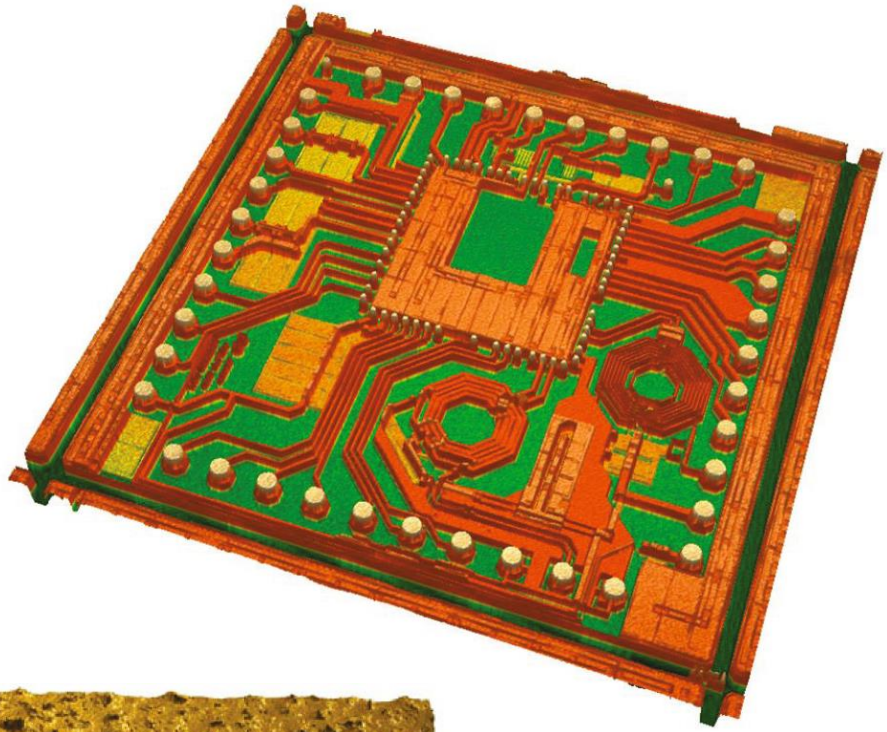
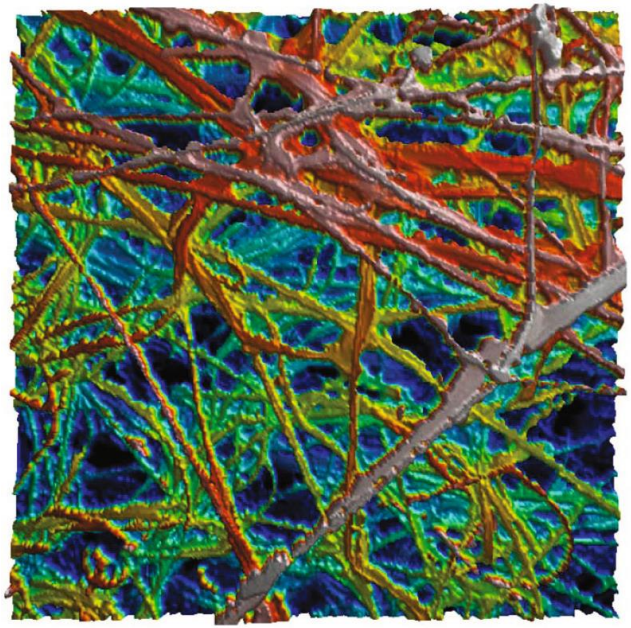
- Microelectronic circuit



- Abbrasive paper

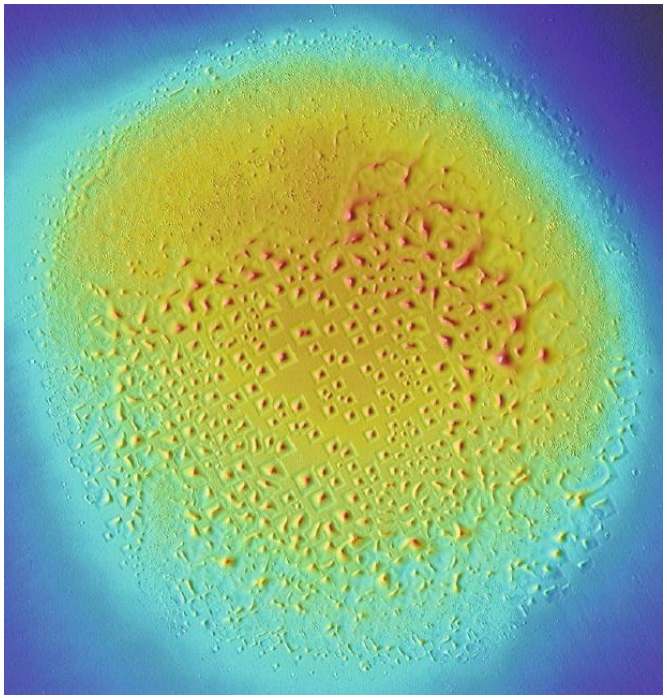


- Smooth paper

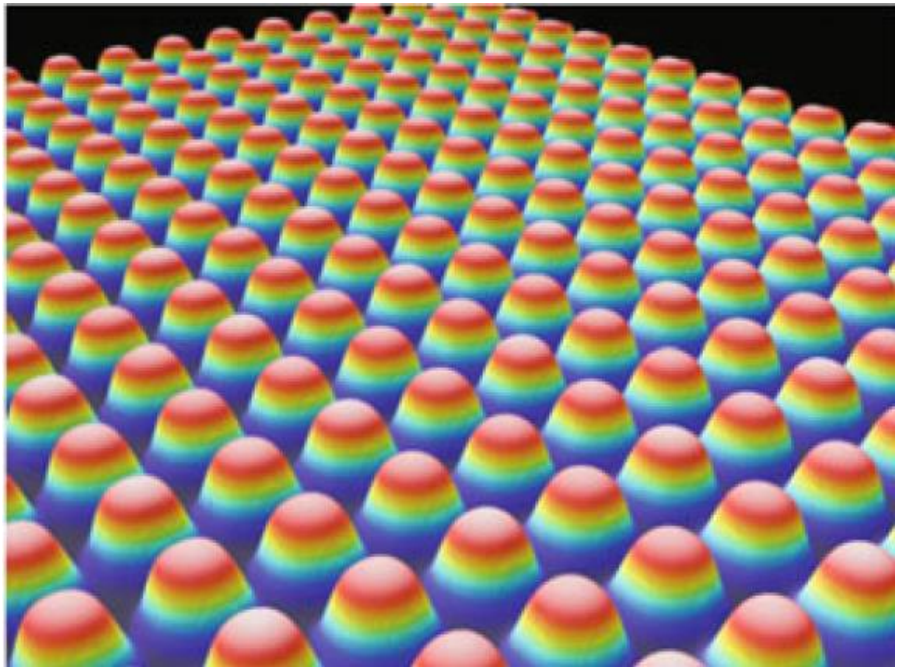
Ref.: R. Leach

Examples

- Silicon surface with stitching



- Microlens array

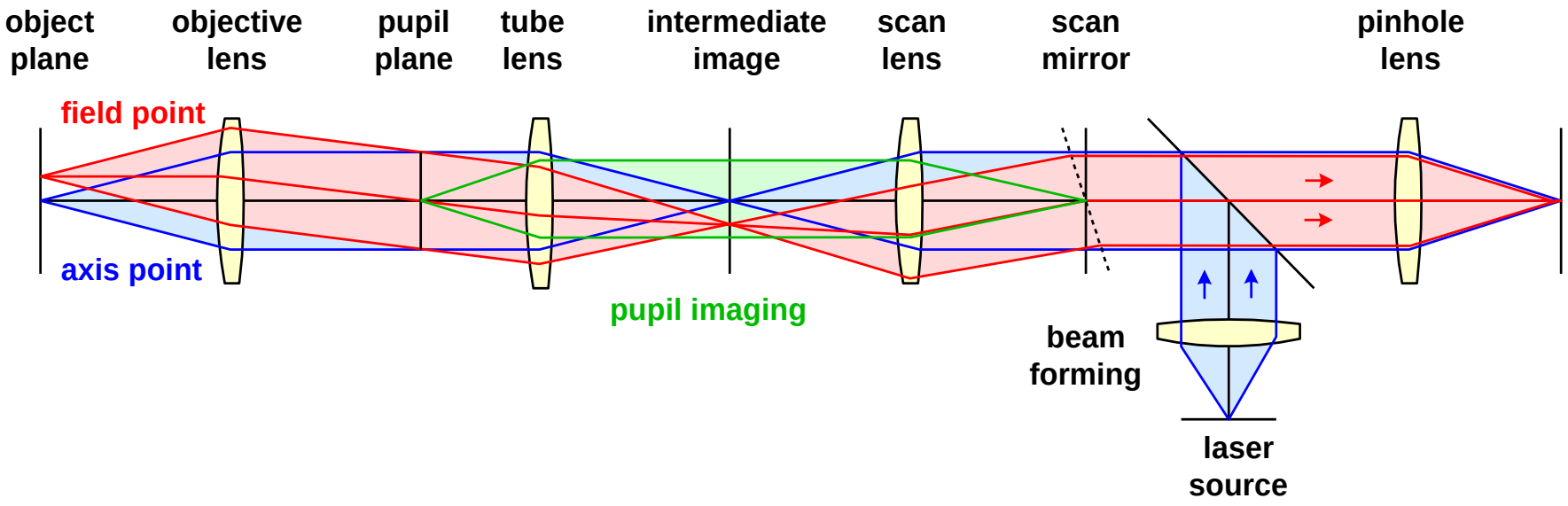


Confocal Microscope – General Aspects

- Laser scan microscope produces only images in combination with software for the image processing
  Realtime image gathering is possible today
- Usually the illumination is a scanning laser beam
- Usually the detection/observation uses the same lens
- The confocal pinhole detection guarantees:
  - a z-sectioning capability
  - a good suppression of straylight out of other planes in the sample
- In scanning systems:
  - the field is generated by transverse scanning with a mirror in a pupil-conjugated plane
  - in case of volume imaging, the z-scan is performed by moving the stage
  - the signal beam is descanned after a beam splitter
  - primary image gathering is monochromatic in a plane-by-plane z-scan
- Due to the very small pinhole, the sensitivity of the microscope is high:
  - strong impact on residual aberrations
  - large environmental sensitivity

Confocal Laser Scan Microscope

- 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



Confocal Laser Scan -Microscope

- Fourier optical model:
  - illumination with point spread function h_{ill}
  - object function plane, t_{obj}, scanned
  - detection with point spread function h_{det}
  - detector function by pinhole size D_{ph}
- General transform of amplitudes

$$U_2 = U_1 * h_{ill}$$

$$U'_2 = U_2 \cdot t_{obj}$$

$$U_3 = U'_2 * h_{det}$$

$$U'_3 = U_3 \cdot D_{ph}$$

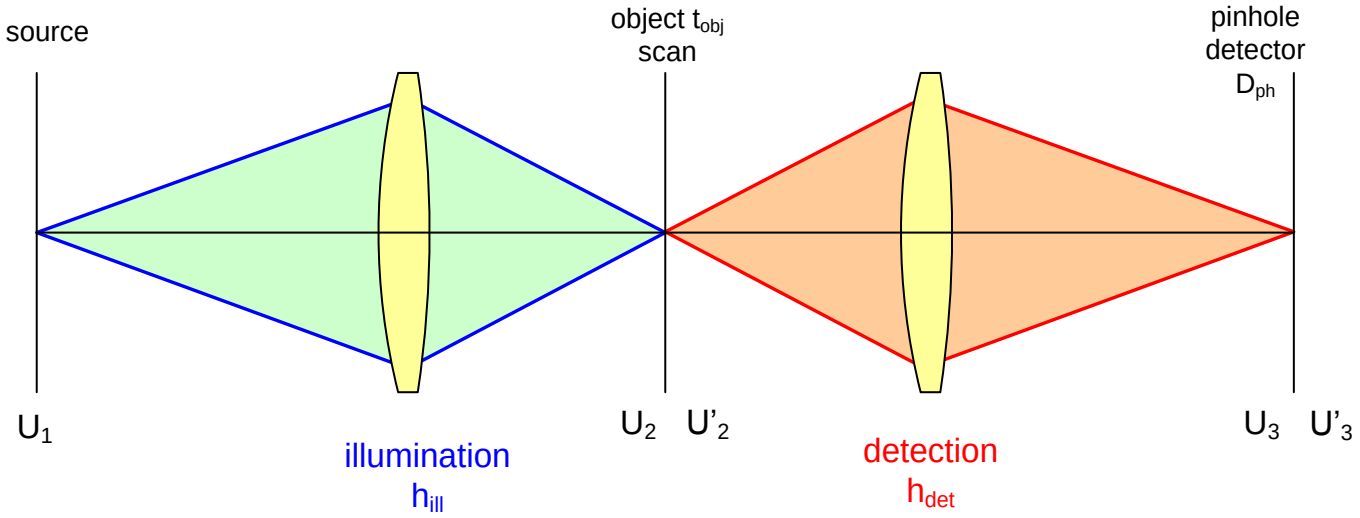


Image in Confocal Laser Scan Microscope

- Amplitude of PSF: h
  Illumination and observation with same lens:
  identical PSF

$$H_{psf} = H_{ill} = H_{obs}$$

- Confocal intensity in image

$$I_{conf} = \left| H_{psf} \right|^4$$

- Real conditions:
  - thick sample, straylight from other z-planes
  - apodization of source due to laser illumination
  - residual aberrations of lenses
  - finite size of the pinhole
  - special shapes of detectors (circle, square, slit,..)
  - Partial coherence of illumination
  - high-NA, vectorial PSF
  - wavelength shift for fluorescence

$$\lambda_1 < \lambda_2$$

- Other/different imaging modes:
  - 2-photon
  - 4π
  - interference
  - structured illumination
  - ...

Ref: M. Wald

Image Formation Confocal LSM

Special cases:

- Brightfield, perfectly small pinhole D=δ(x)δ(y), imaging coherent

$$I_{ima} = \left| \left(h_{ill} \cdot h_{det} \right) \otimes t_{obj} \right|^2$$

- Fluorescence, coherence destroyed perfectly small pinhole

$$I_{ima} = \left| \left(h_{ill} \cdot h_{det} \right) \right|^2 \otimes t_{obj} \qquad \lambda_{ill} < \lambda_{\det}$$

- Point like object $t_{obj} = \delta(x)\ \delta(y)$

$$I_{ima} = \left| h_{ill} \right|^2 \cdot \left[\left| h_{\det} \right|^2 \otimes D_{ph} \right]$$

- Point object and perfectly small pinhole

$$I_{ima} = \left| h_{ill} \right|^2 \cdot \left| h_{det} \right|^2$$

- Plane mirror object t_{obj} = const. perfectly small pinhole

$$I_{ima} = \iint \left| h_{det}(x, y, 2z) \right|^2 dx\, dy$$

$$\lambda_{ill} = \lambda_{\det} \qquad h_{ill} = h_{\det}$$

Ref: M.Wald

Confocal Microscopy Imaging

- Simple model of confocal imaging:
  - illumination with coherent PSF H_{ill}
  - object function T_{obj}
  - observation with coherent PSF H_{obs}

$$I_{conf} = \left| \left(H_{obs} \cdot H_{ill} \right) \otimes T_{object} \right|^2$$

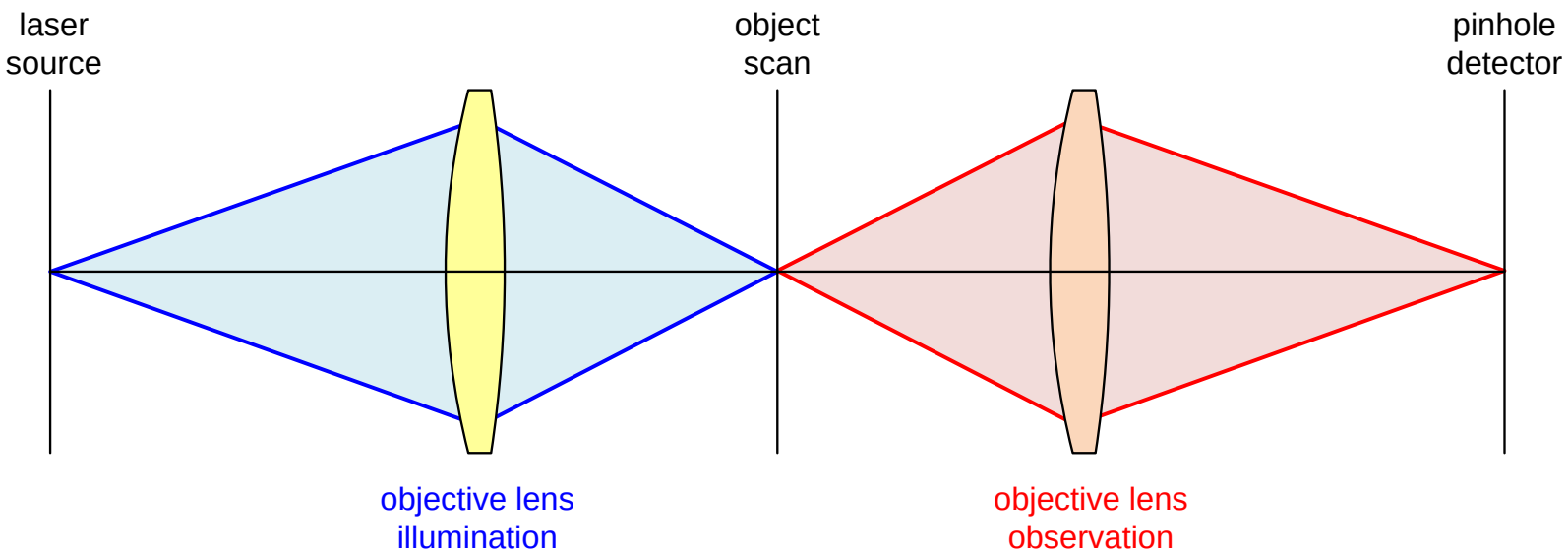
- Rearrangement

  spatial domain

$$I_{conf}(x,y) = \left| \iint T_{obj}(\nu_x,\nu_y) \cdot H_{conf}(\nu_x,\nu_y) \cdot e^{2\pi \cdot i \cdot \left(x\nu_x + y\nu_y \right)} \, d\nu_x \, d\nu_y \right|^2$$

  transfer in frequency domain

$$H_{conf}(\nu_x,\nu_y) = H_{ill}(\nu_x,\nu_y) \otimes H_{obs}(\nu_x,\nu_y)$$



Lateral and Axial Resolution

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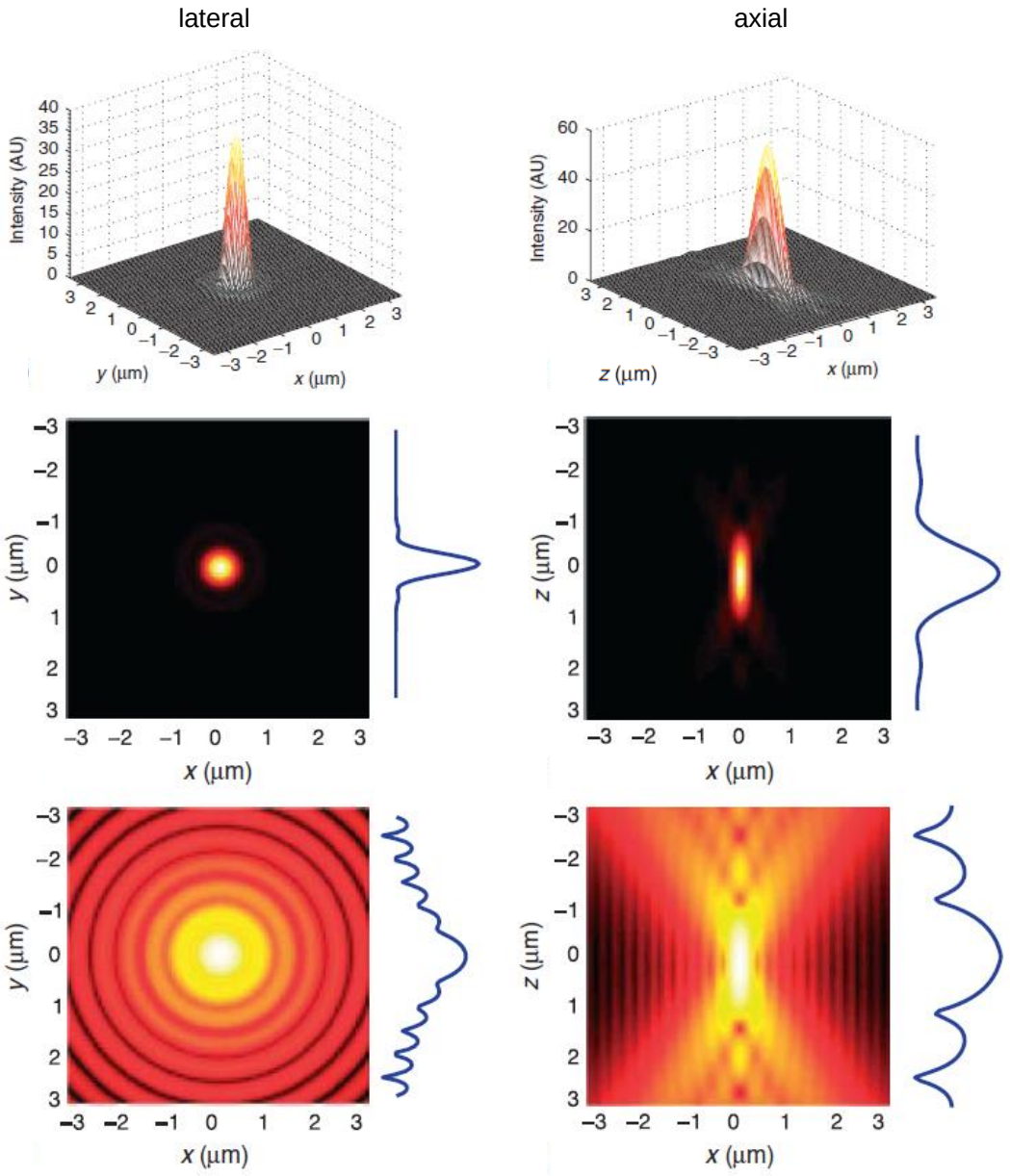
- Intensity distributions



Ref: U. Kubitschek

Confocal PSF

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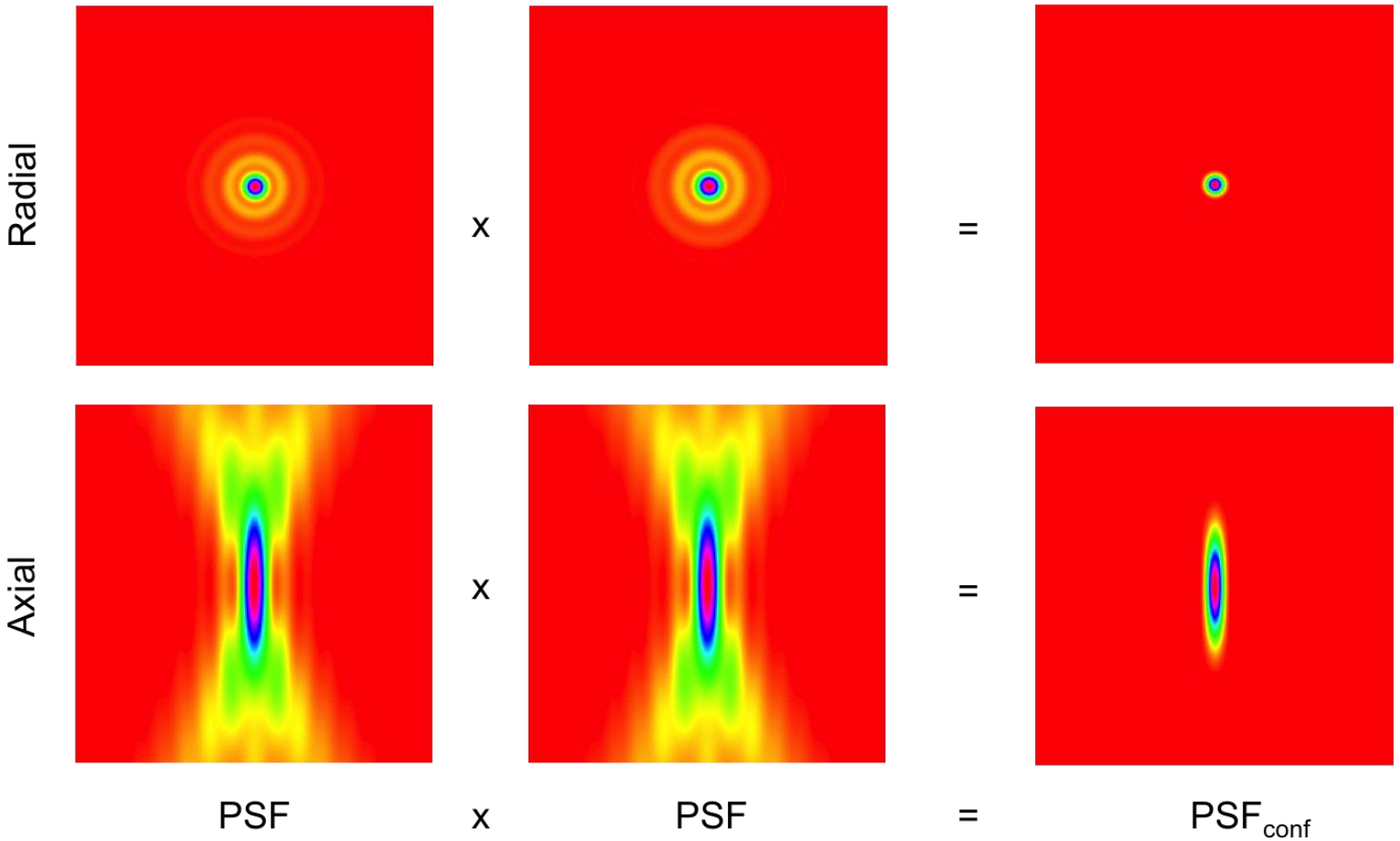
- Change of intensity distributions by confocal mode
  1. lateral
  2. axial



Ref: A. Szameit

Lateral and Axial Resolution

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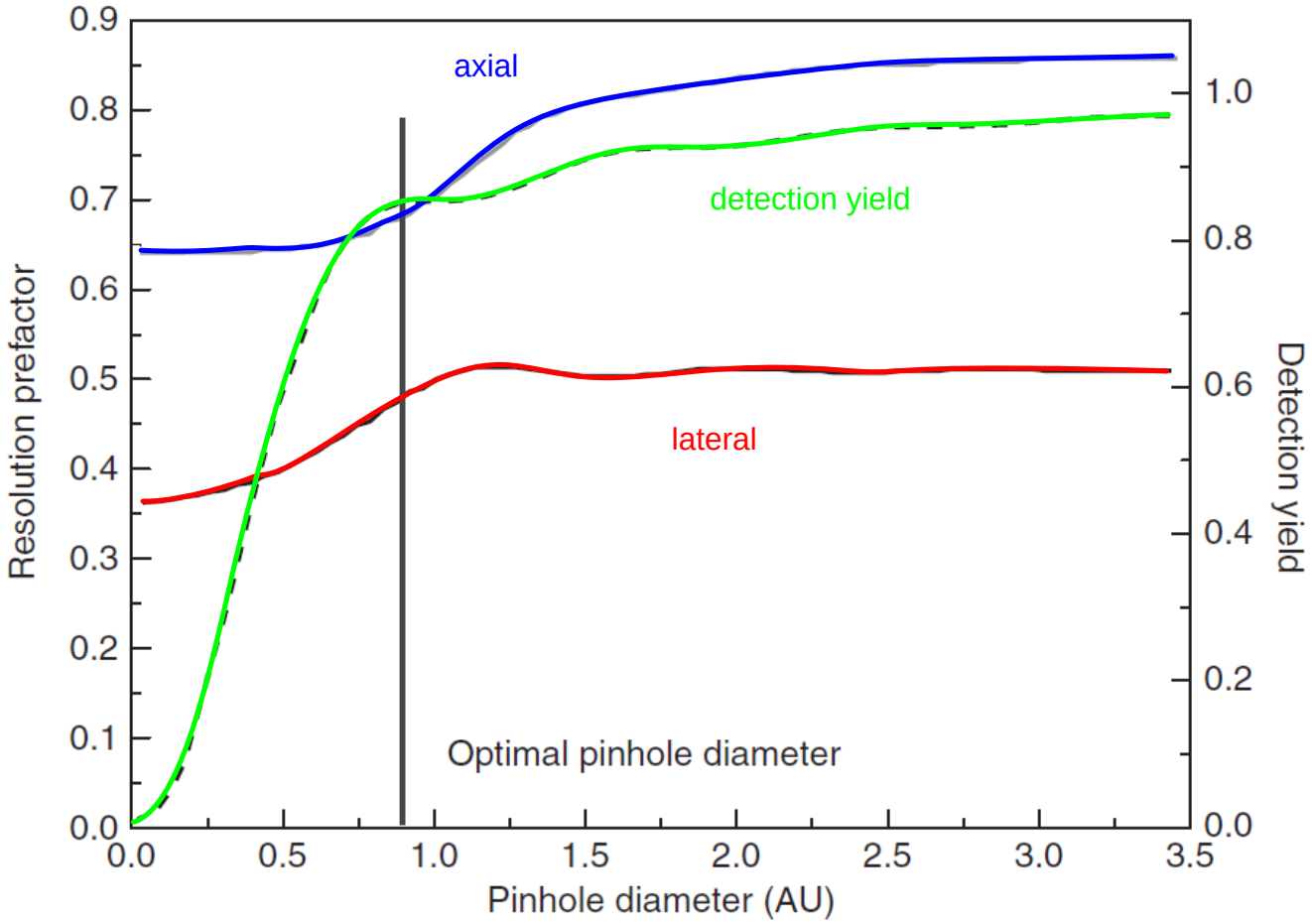
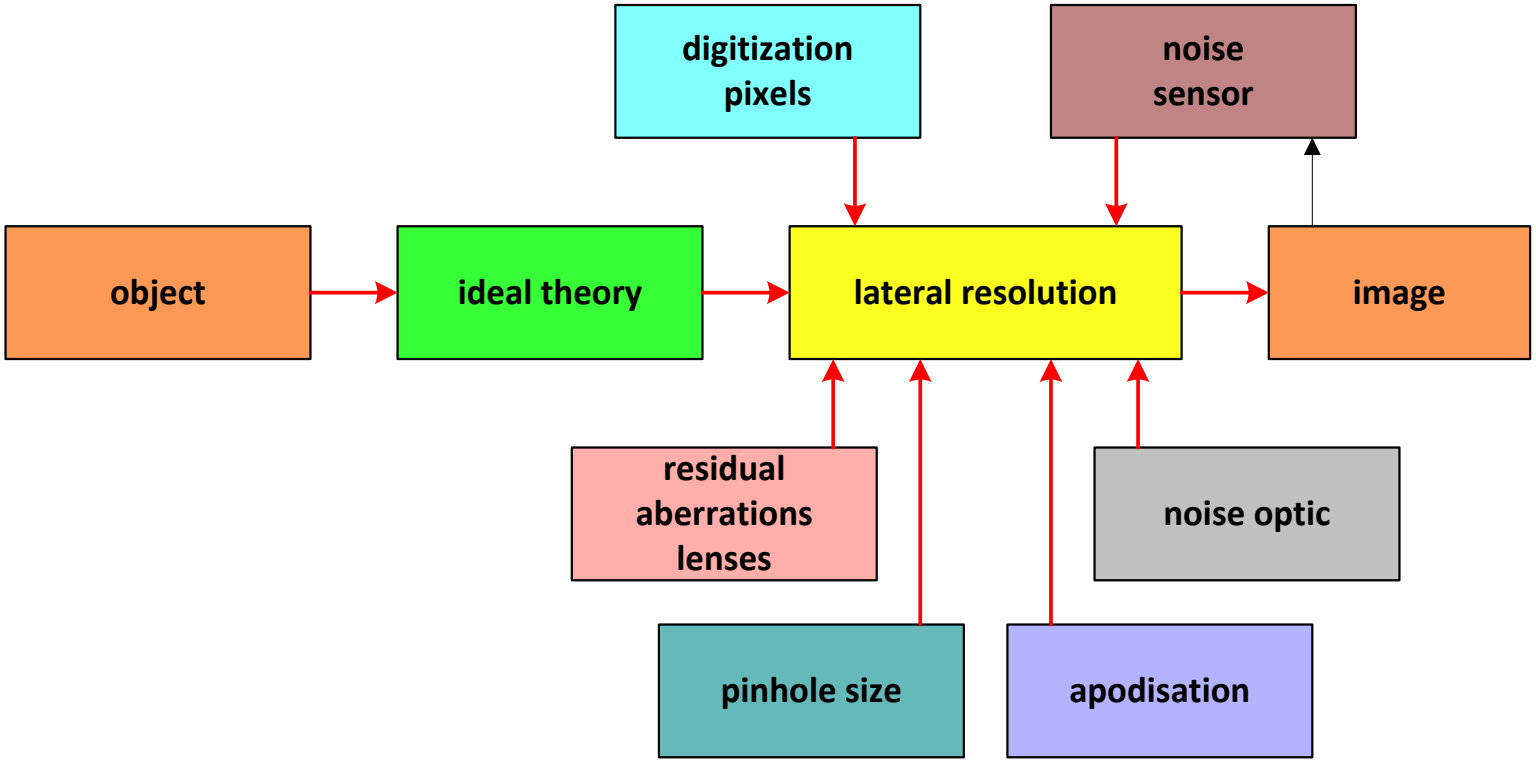
- Tradeoff between:
  1. lateral resolution
  2. axial resolution
  3. signal to noise ratio (detection yield)



Ref: U. Kubitschek

Confocal Resolution

- Confocal microscope:
  - lateral resolution is complicated function
  - not only optical influence functions



Confocal Microscopy: PSF and Lateral Resolution

- Normalized transverse coordinate v

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

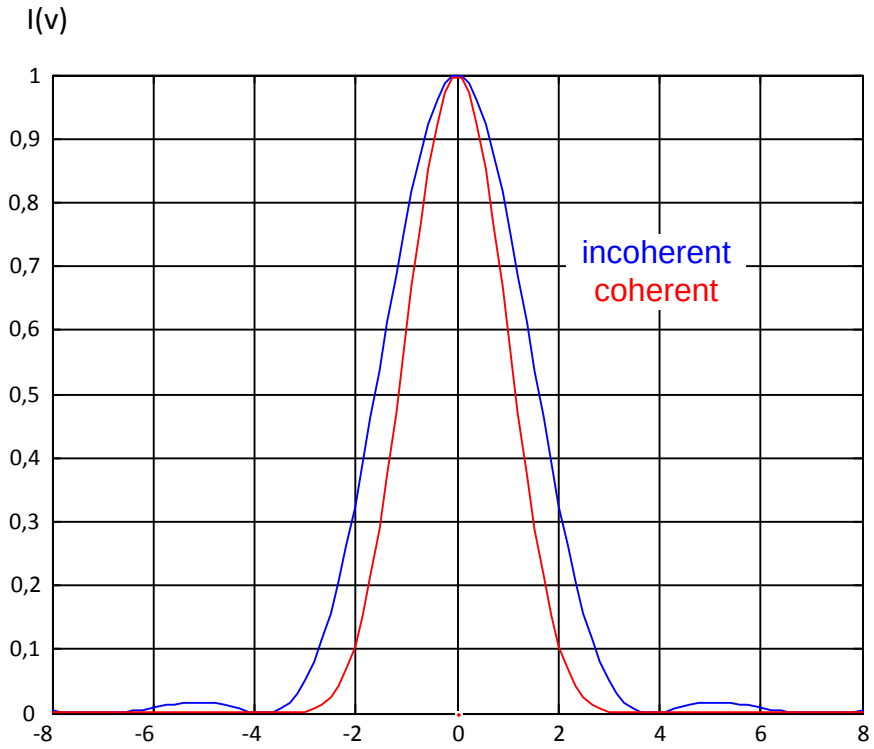
- Usual PSF: Airy

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

- 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



Confocal Microscopy: Axial Sectioning

- 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

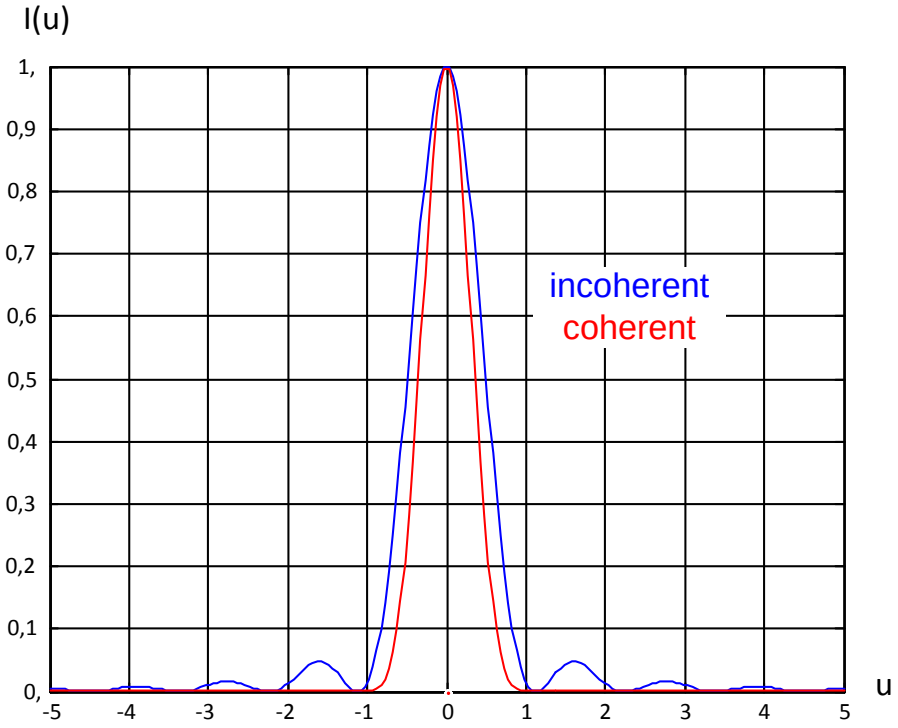
$$\Delta z_{wide}^{(approx)} = \frac{0.45 \cdot \lambda}{n' \cdot (1 - \cos\theta)}$$

- 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

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



Microscopic Resolution

- Signal, lateral and axial resolution depends on imaging mode

Imaging mode	signal	lateral resolution	axial resolution
classical wide field	$S = I_{ill}$	$\Delta x = \frac{0.61 \cdot \lambda}{n \cdot \sin\theta} = 0.5 \cdot D_{airy}$	$\Delta z = \frac{2 \cdot \lambda}{n \cdot \sin^2\theta} = 2 \cdot R_E$
confocal	$S = I_{ill} \cdot I_{obs}$	$\Delta x = \frac{0.40 \cdot \lambda}{n \cdot \sin\theta} = 0.33 \cdot D_{airy}$	$\Delta z = \frac{1.4 \cdot \lambda}{n \cdot \sin^2\theta}$
2 photon	$S = I_{ill}^2$	$\Delta x = \frac{0.70 \cdot \lambda}{n \cdot \sin\theta} = 0.43 \cdot D_{airy}$	$\Delta z = \frac{2.3 \cdot \lambda}{n \cdot \sin^2\theta}$
2 photon confocal	$S = I_{ill}^2 \cdot I_{obs}$		

- Approximation in these formulas: wavelength shift by fluorescence
- Lateral resolution and coherence
  general formula:

$$\Delta x = k \cdot \frac{\lambda}{n \cdot \sin u}$$

factors

	coherent			incoherent		
	Rayleigh	Sparrow	Abbe	Rayleigh	Sparrow	Abbe
Classical	0.82	0.74	1.00	0.61	0.49	0.50
confocal	0.56	0.48	0.50	0.44	0.34	0.25

Confocal Microscopy: Laterale Transfer Function

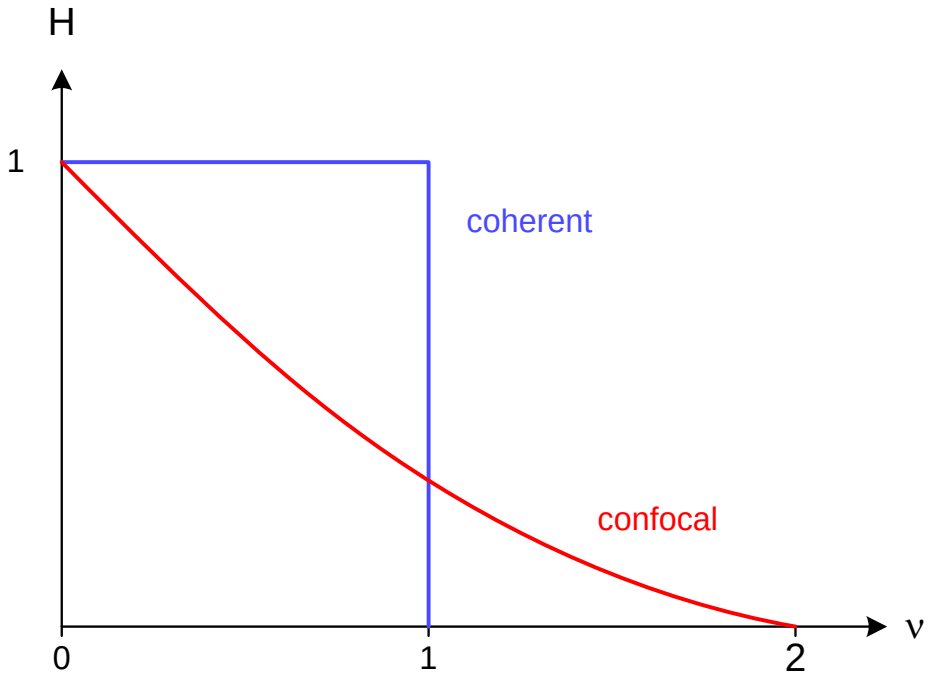
- Ideal coherent transfer function: complex pupil function

$$H_{coh}(\nu) = P\left(\frac{x_p}{\lambda \cdot f}\right)$$

- Confocal transfer function: product in spatial domain, convolution in frequency domain identical to incoherent OTF

$$H_{conf}(\nu) = \frac{2}{\pi} \cdot \left[\arccos\left(\frac{\nu}{2}\right) - \frac{\nu}{2} \cdot \sqrt{1-\left(\frac{\nu}{2}\right)^2}\right]$$

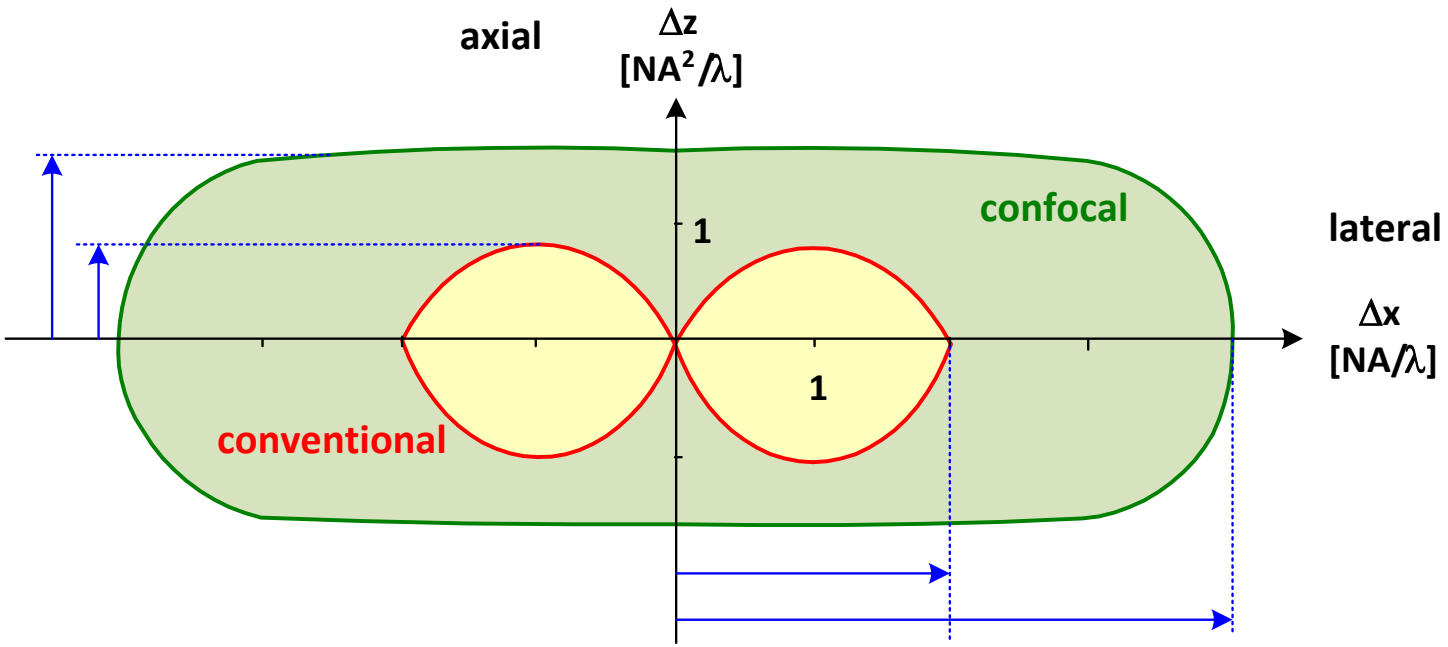
- Confocal system has higher spatial resolution



Resolution Enhancement in Confocal Microscopy

- Increased resolution:
  - axial by factor 2
  - lateral by factor 2
  - no longer missing cone
- In general also improvement of contrast:
  suppression of straylight by pinhole

	lateral	axial
Conventionel	$2\ NA/\lambda$	NA^2/λ
confocal	$4\ NA/\lambda$	$2NA^2/\lambda$



Ref: M. Kempe

CTF in Microscopy

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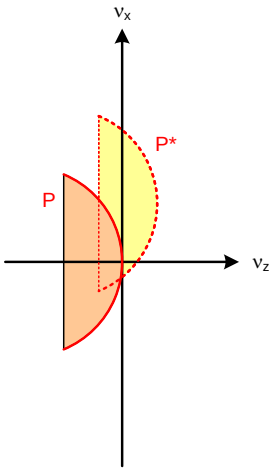
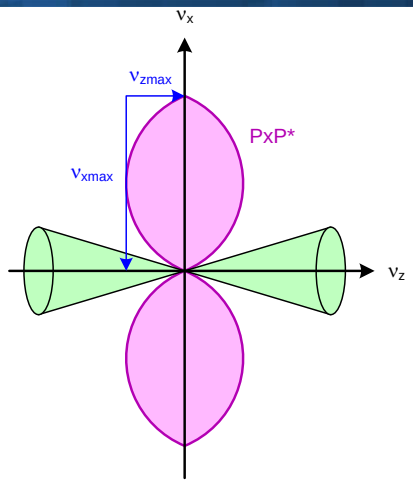
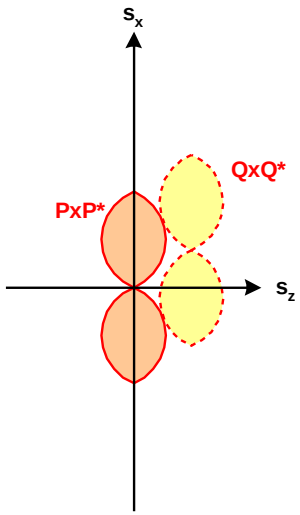
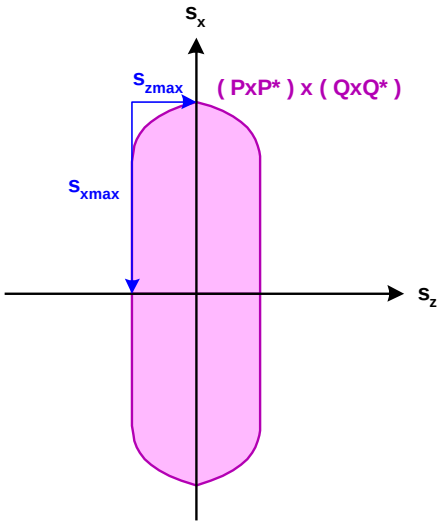
Brightfield



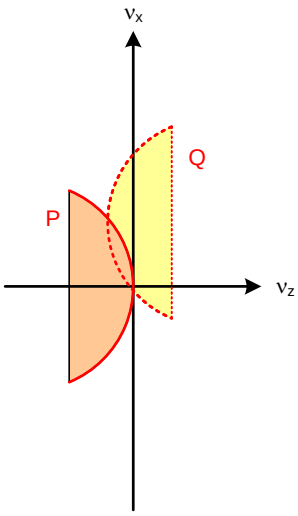


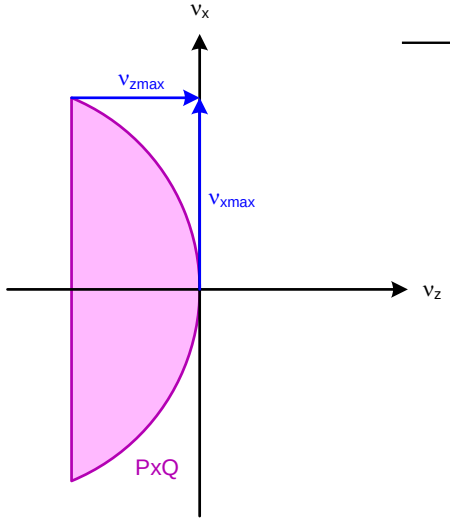
Incoherent laser scan microscope





Coherent laser scan microscope





Lateral Resolution in Confocal Imaging

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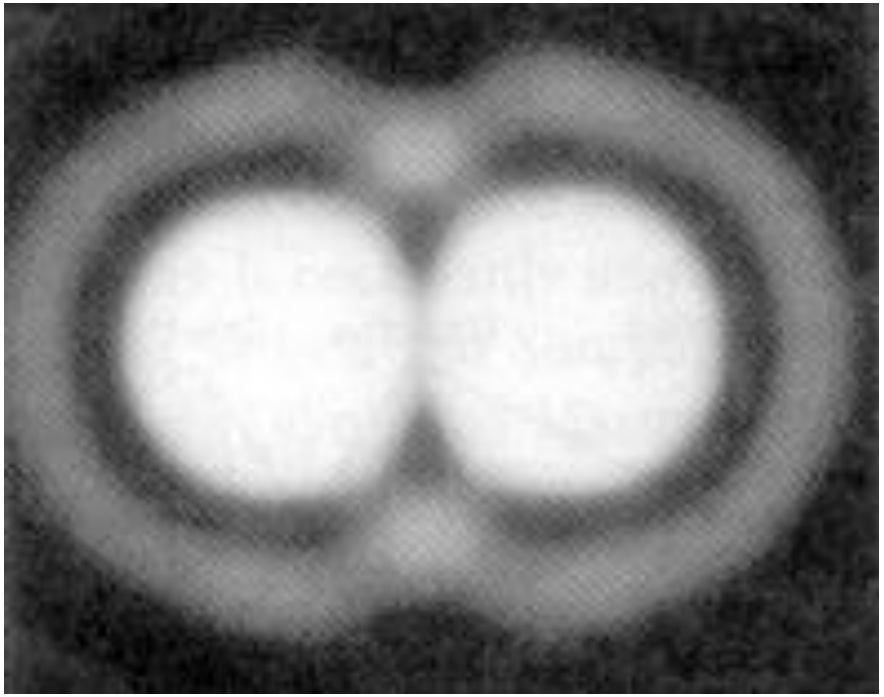
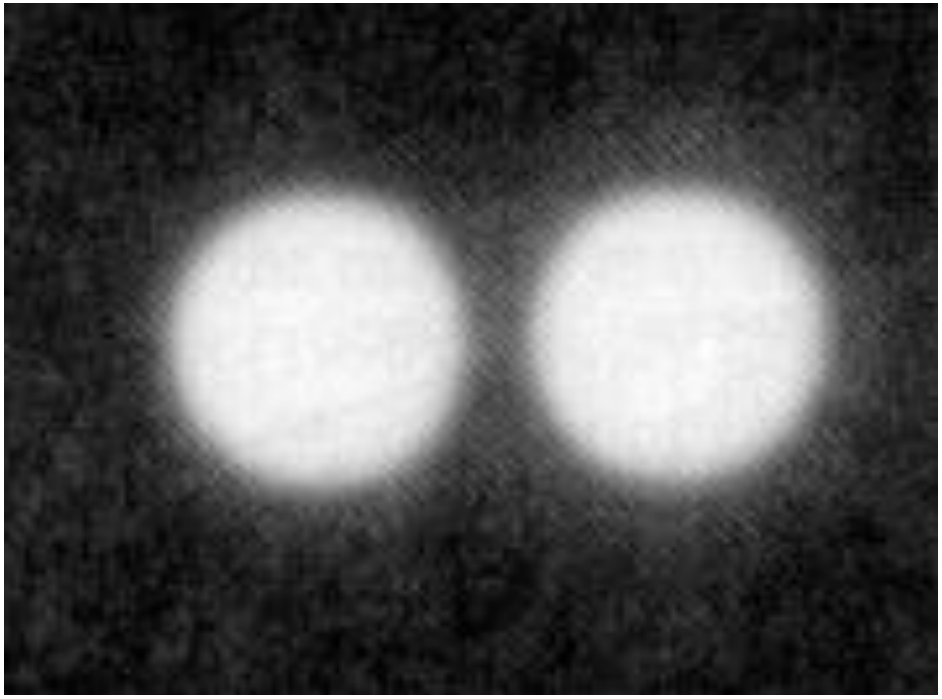
- Comparison of PSF in wide field and confocal imaging
- Improved 2-point resolution in confocal mode

conventional wide field



confocal



Generalized Depth Criterion

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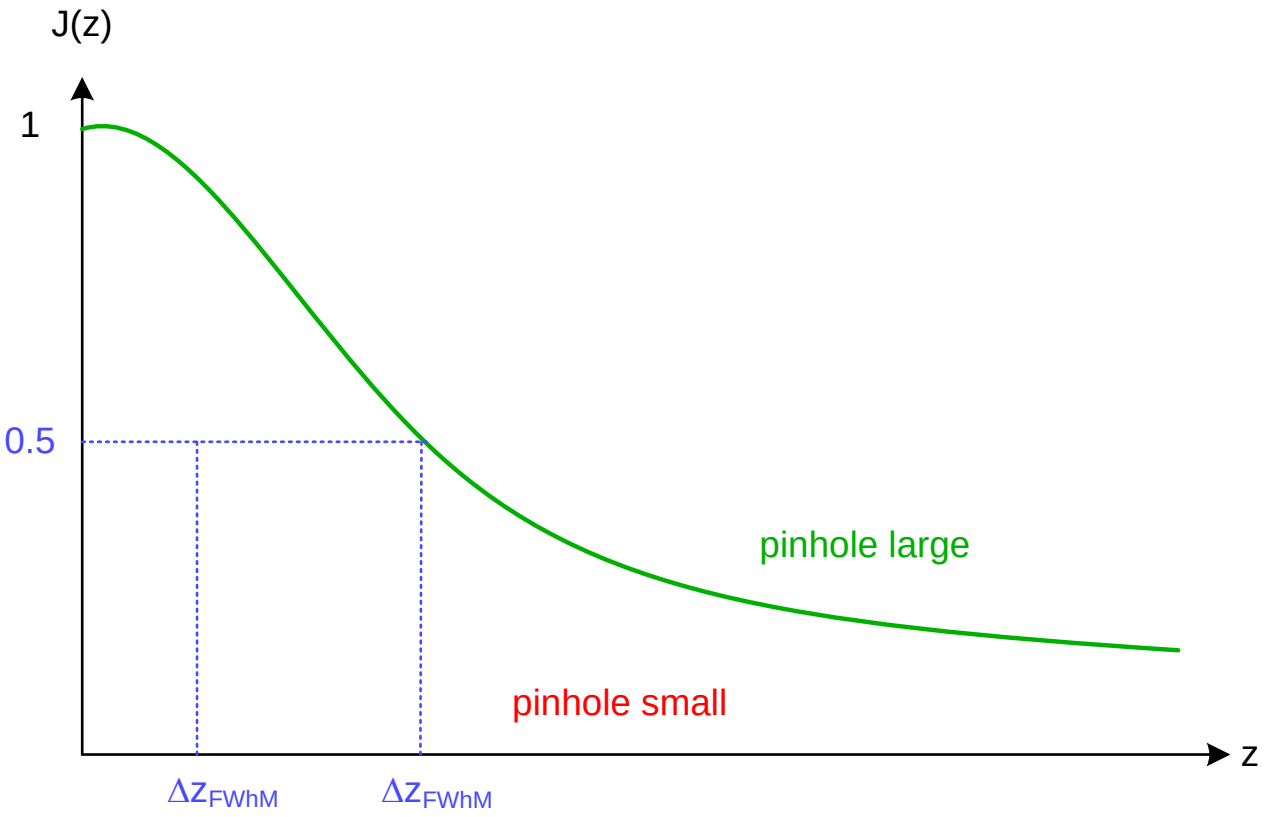
- H_{CTF}: coherent transfer function/PSF
- Integration over spatial frequencies function of the defocussing z
- Depth discrimination:
  FWhM of function J(z) decrease with z

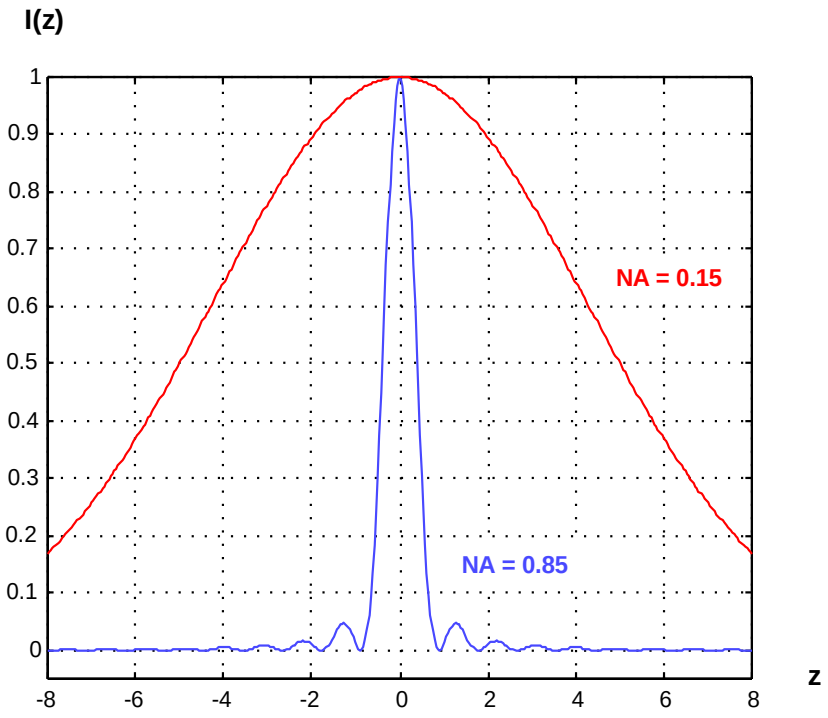
$$J(z) = \int_0^1 \left| H_{CTF}(\nu_x, z) \right|^2 d\nu_x$$

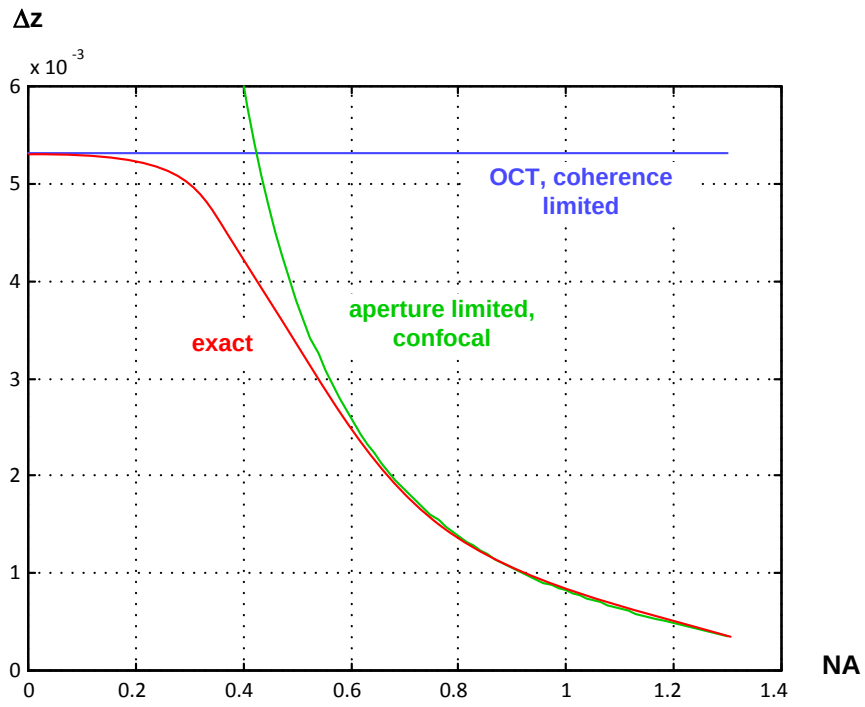
$$\Delta z = \left. \frac{\lambda}{2\pi \cdot n \cdot \sin^2 \theta_o} \right|_{J(z)=1/2}$$



OCT - Microscope

- Large NA:
  confocal, depth discrimination by NA
- Small NA:
  OCT, depth discrimination by axial coherence





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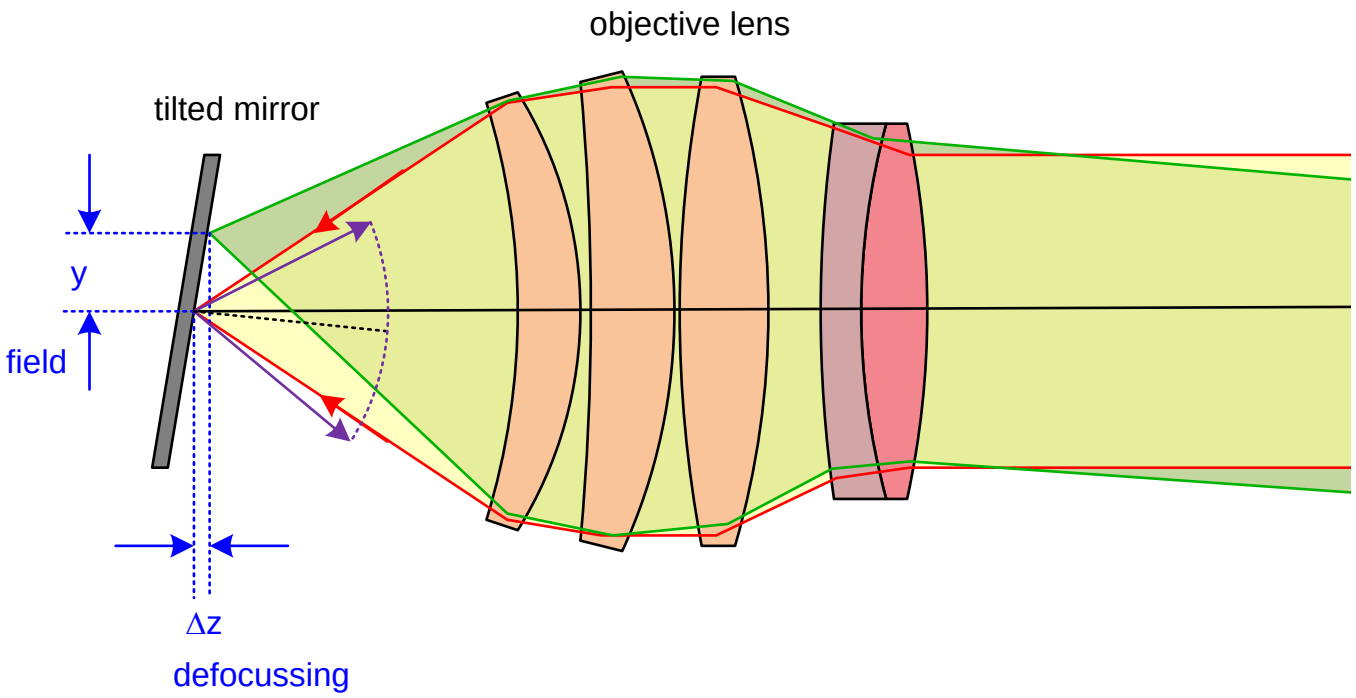
Confocal Depth Signal

- Measurement of the axial confocal signal by using a lateral shifted tilted mirror
- Detection of spherical aberration degradation



Confocal Pinhole Size

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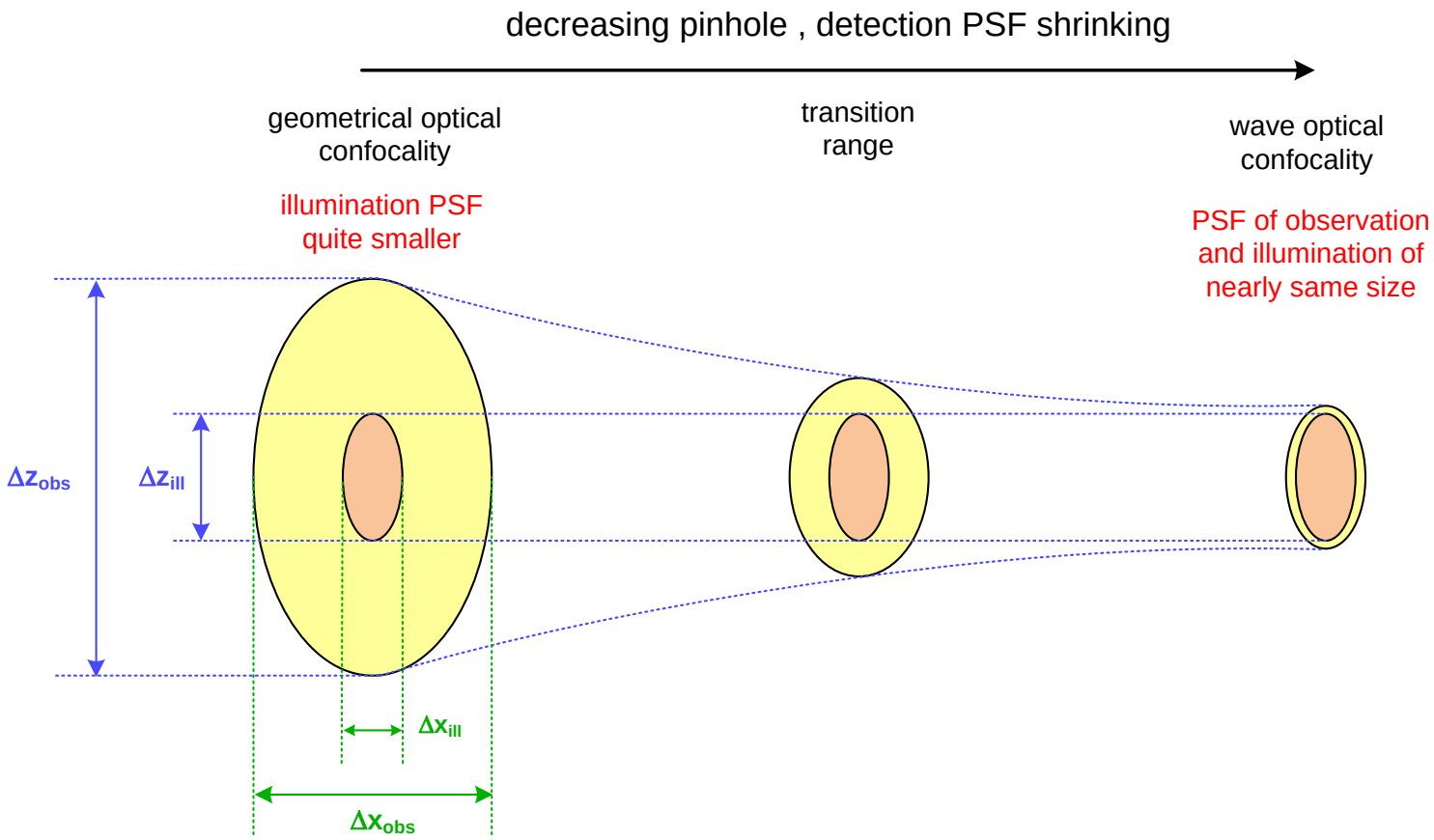
- Change of pinhole size:
  Observation PSF changed
- Changing relative sizes of illumination and observation PSFs



Size of Pinhole and Confocality

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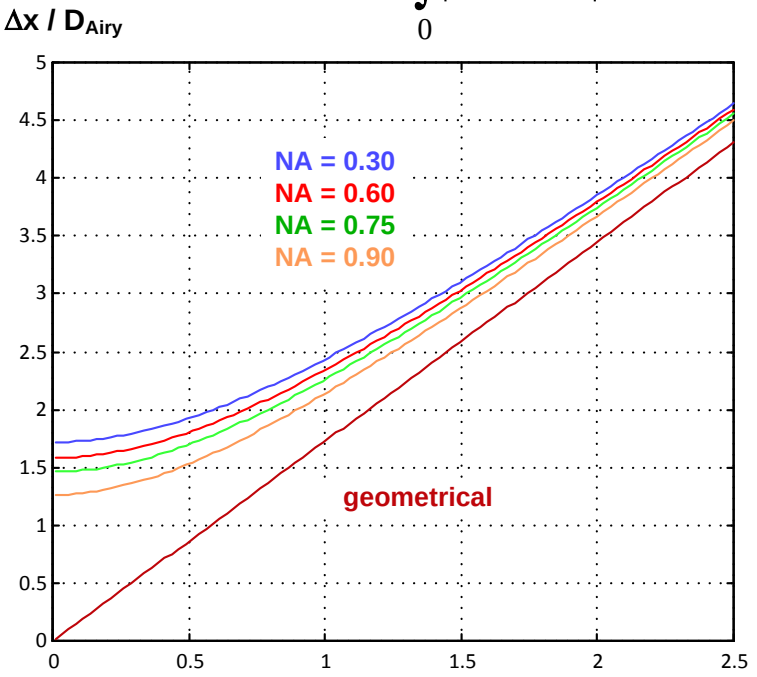
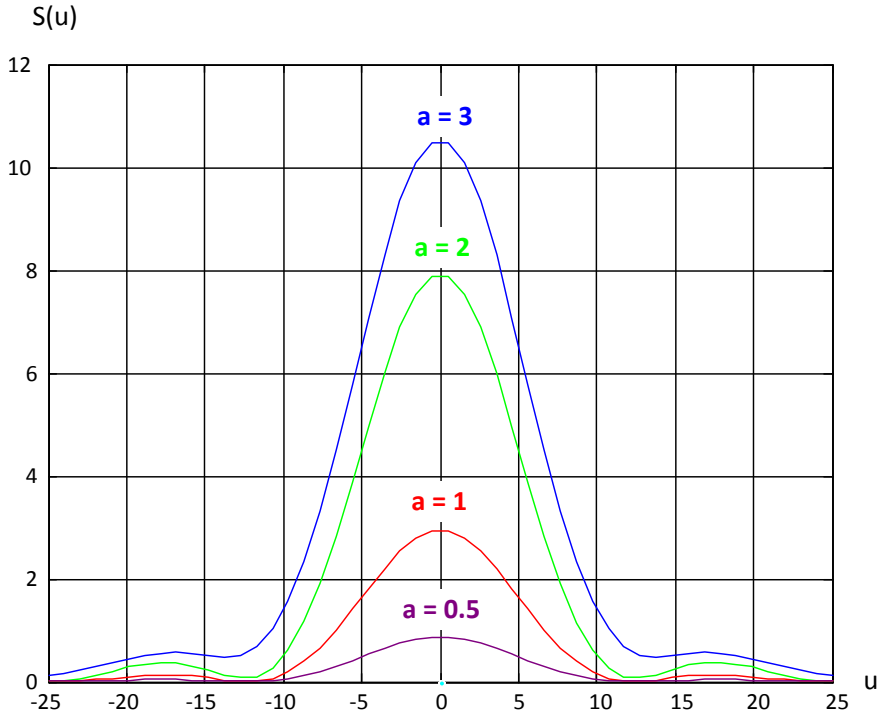
- Large pinhole: geometrical optic
- Small pinhole:
  - Diffraction dominates
  - Scaling by Airy diameter a = D/D_{Airy}
  - diffraction relevant for pinholes
    $D < D_{airy}$
- Confocal signal:
  Integral over pinhole size

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





Confocal Signal for Different Pinhole Sizes

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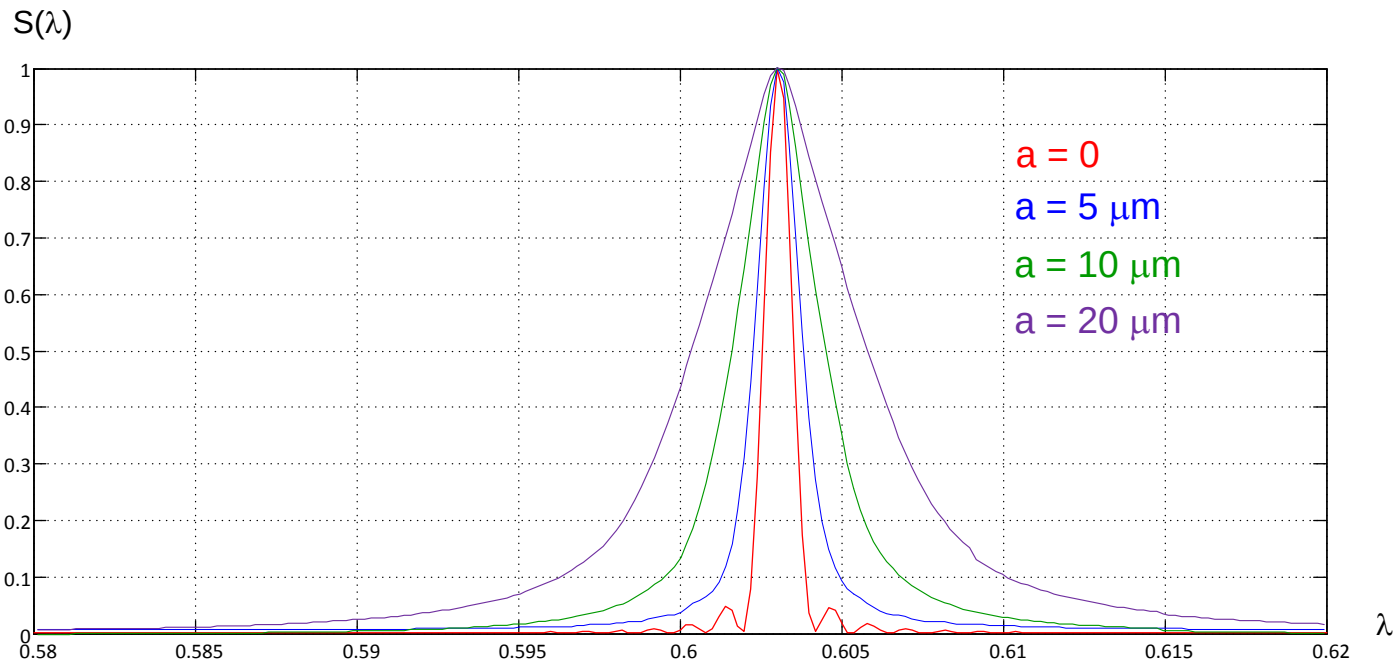
- Numerical result for different sizes a of the fiber radius
- The width increases with the fiber diameter
- The diffraction fine structure disappears with growing a



Confocal Signal and Pinhole Size

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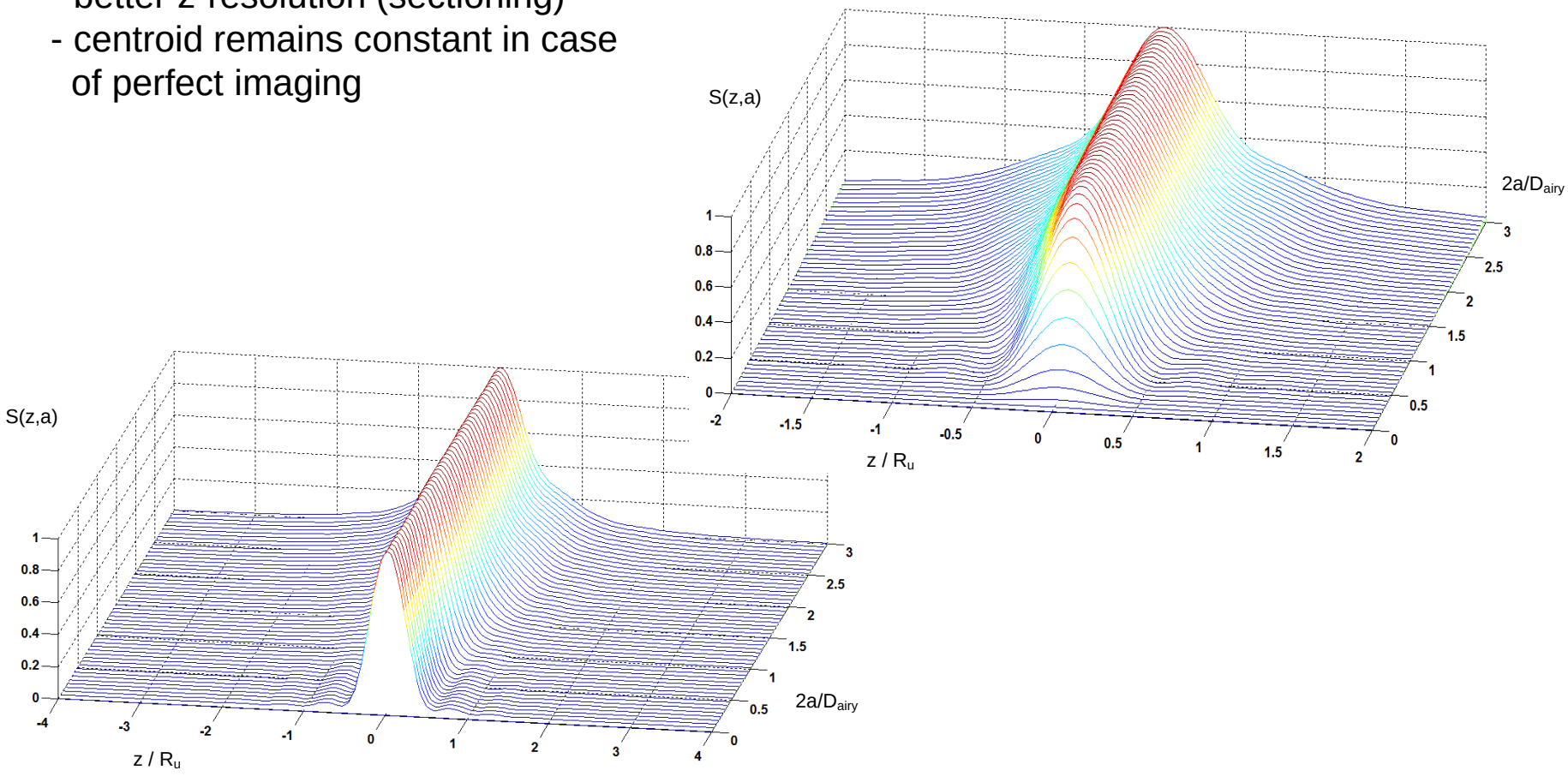
- Confocal signal S(z) without aberrations as a function of the pinhole size a
- Smaller pinhole:
  - low signal (bad SNR)
  - better z-resolution (sectioning)
  - centroid remains constant in case of perfect imaging

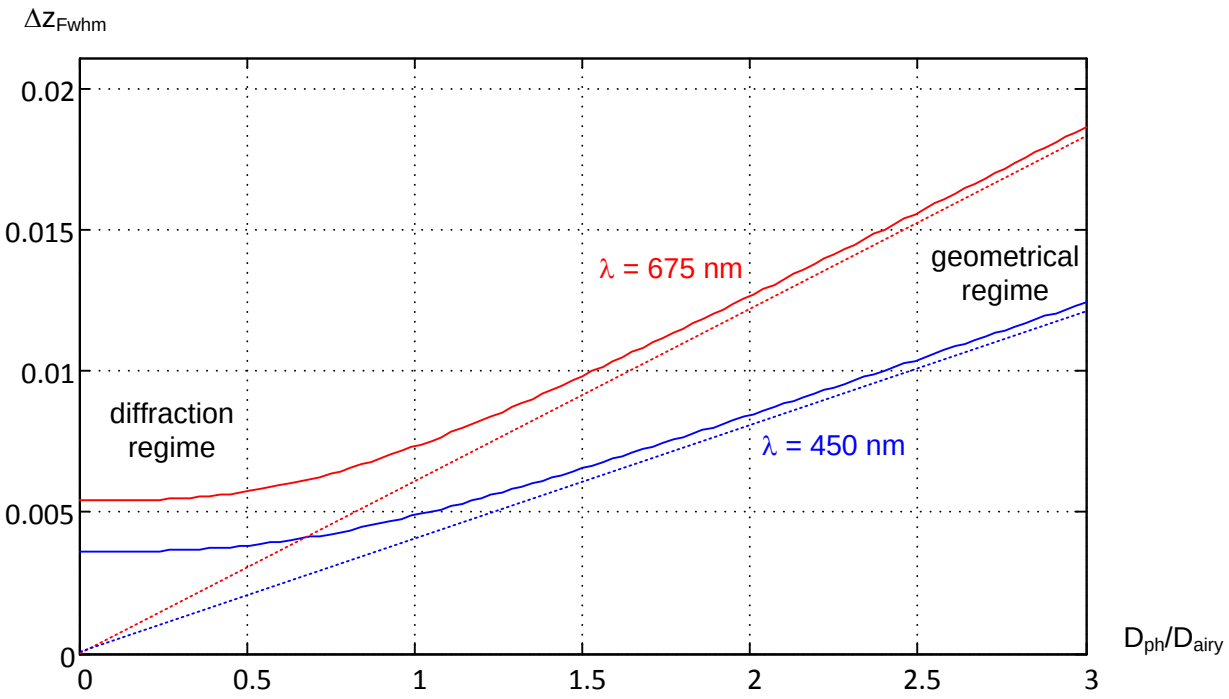


Wilsons Formula

T. Wilson, Jour. of Microsc. 244 (2011) p113, Resolution and optical sectioning in the confocal microscope

Empirical formula for the width of the confocal signal in the case of a finite size pinhole and a fluorescence object
(self luminous, phase information lost)
First factor: diffraction
Second factor: finite size object

$$D_{FWHM} = \frac{0.67 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot \sqrt[3]{1 + 1.47 \cdot \left(\frac{D_{ph}}{D_{airy}} \right)^3}$$



Wilsons Formula: Critical Review

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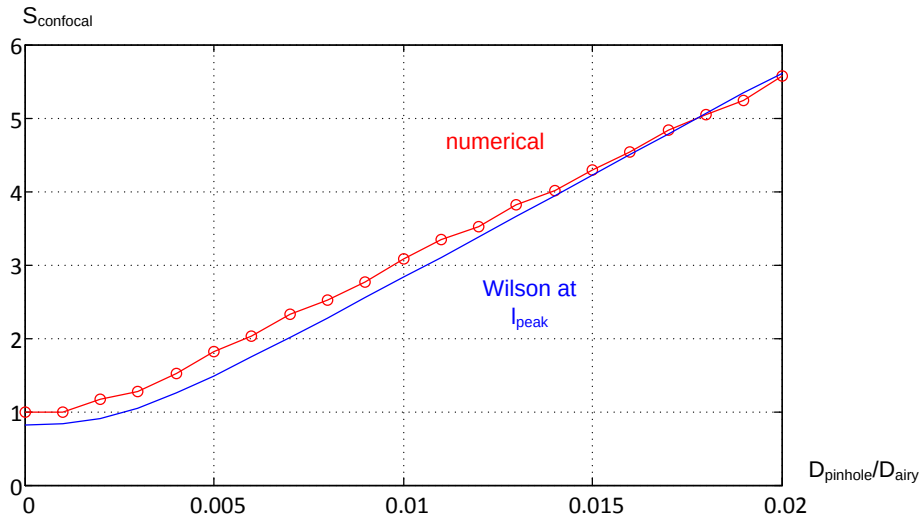
- Formula is valid for:
  1. one wavelength
  2. self luminous object (fluorescence molecule)
  3. perfect corrected spherical aberration
- A different object interaction changes the pre-factor:
  mirror:

$$D_{FWHM} = \frac{0.45 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot \sqrt[3]{1 + 1.47 \cdot \left(\frac{D_{ph}}{D_{airy}} \right)^3}$$

  point reflector:

$$D_{FWHM} = \frac{0.62 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot \sqrt[3]{1 + 1.47 \cdot \left(\frac{D_{ph}}{D_{airy}} \right)^3}$$



  This causes errors of the first factor in the range of 30%
- Incorporation of spherical aberration:
  t.b.d., PCA analysis as approach seems to be promising

Variable Pinhole Diaphragm

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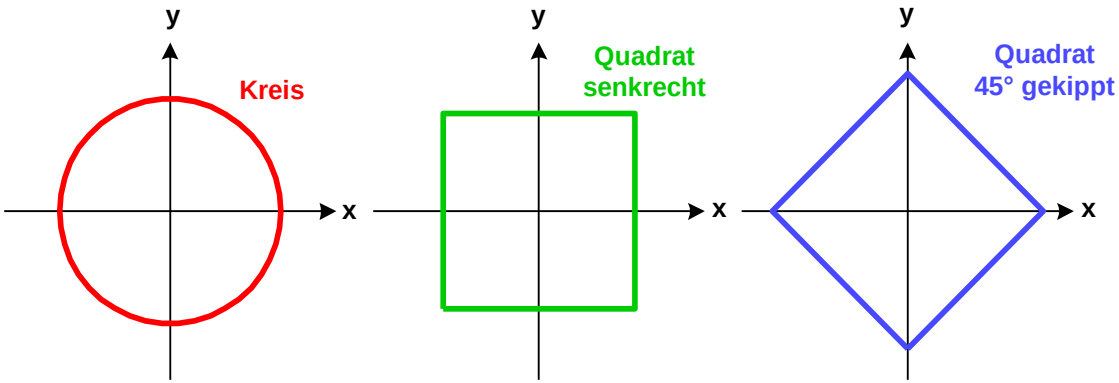
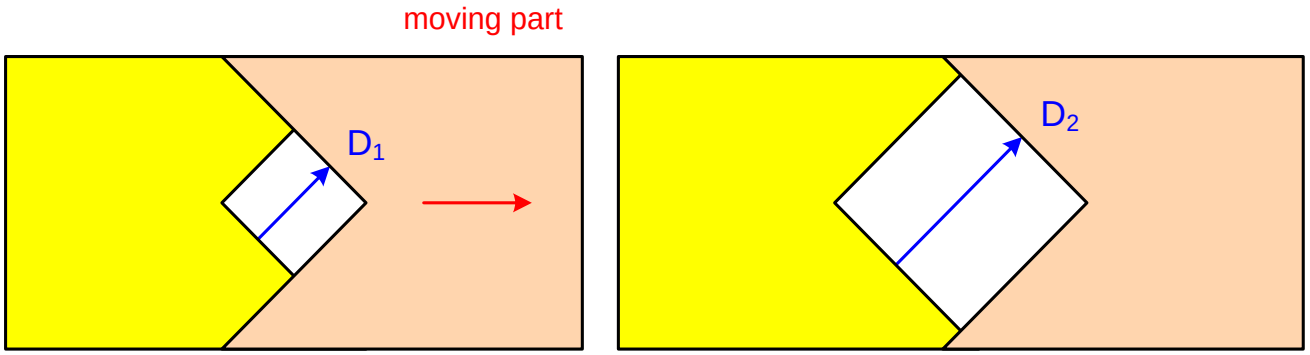


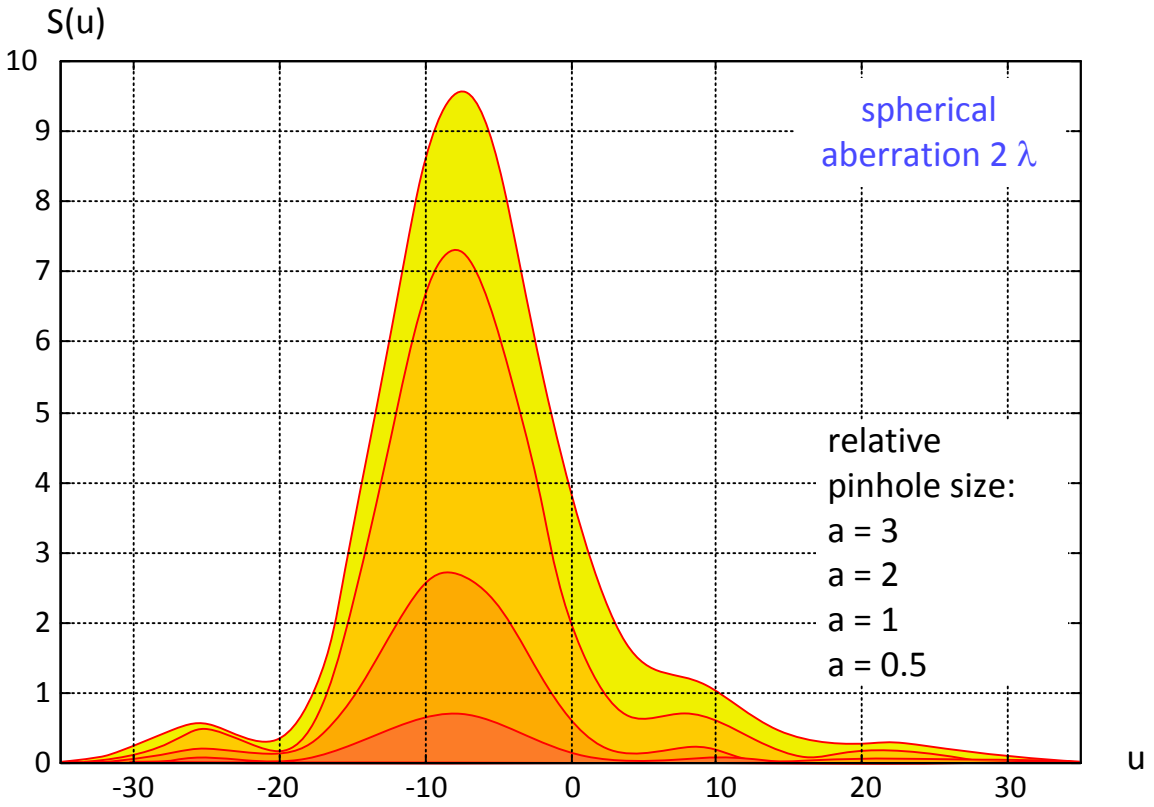
- Real shape of pinhole:
  quadratic or circular
  signal depends on shape
- Variable pinhole easy to realy
  quadratic
- Typical size:
  $D_{pinhole} = 0.5...2\ D_{airy}$
- Easy to fabricate: approx. 30 mm
  very small numerical aperture in pinhole objective lens helps



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



Confocal Signal with Spherical Aberration

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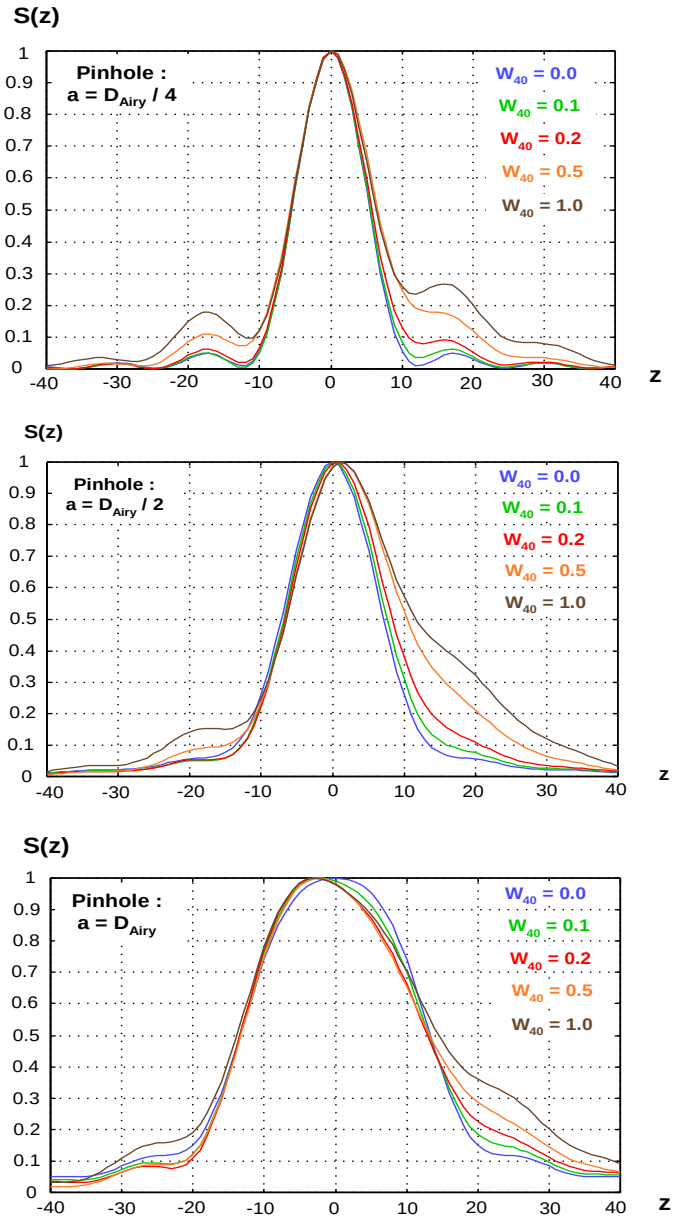
- Spherical aberration with Zernike coefficient W_{40}
- Integration over finite size pinhole with radius a

- Asymmetry and width depends on a and W_{40}
- Large pinhole:
  - depth discrimination decreased
  - fine structure disappears

• Sphärische Aberration mit Koeffizient W_{40}



Confocal Signal with Spherical Aberration

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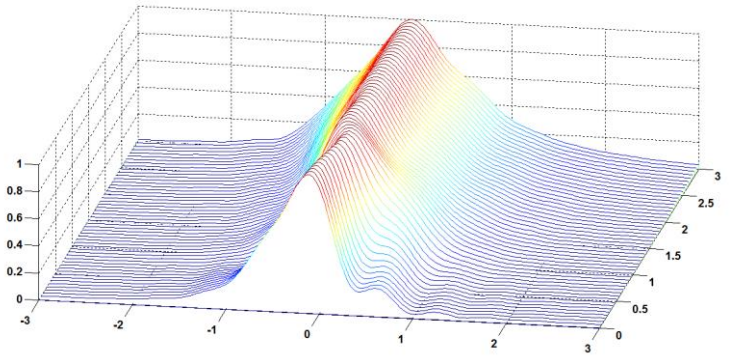
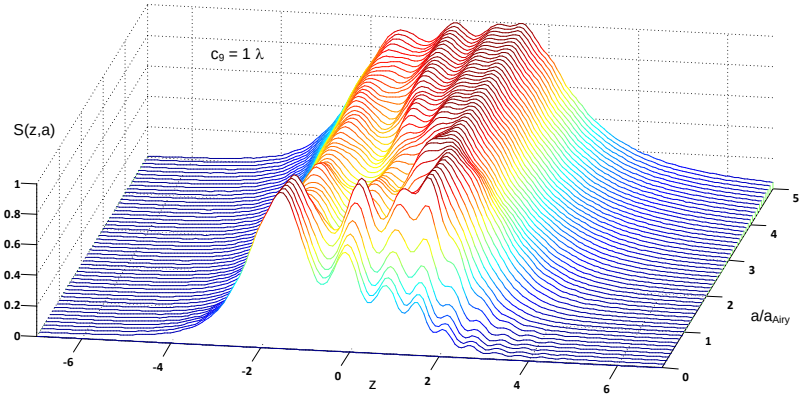
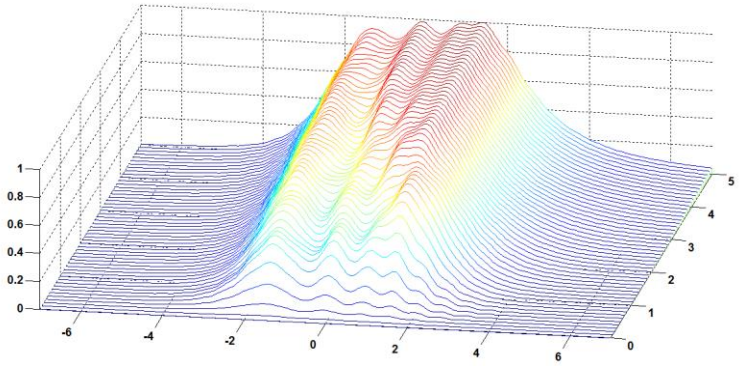
1. $c_9 = 0.3\ \lambda$
   re-normalized



2. $c_9 = 1.0\ \lambda$
   re-normalized



3. $c_9 = 1.0\ \lambda$
   not normalized



Signal Errors due to Spherical Aberration

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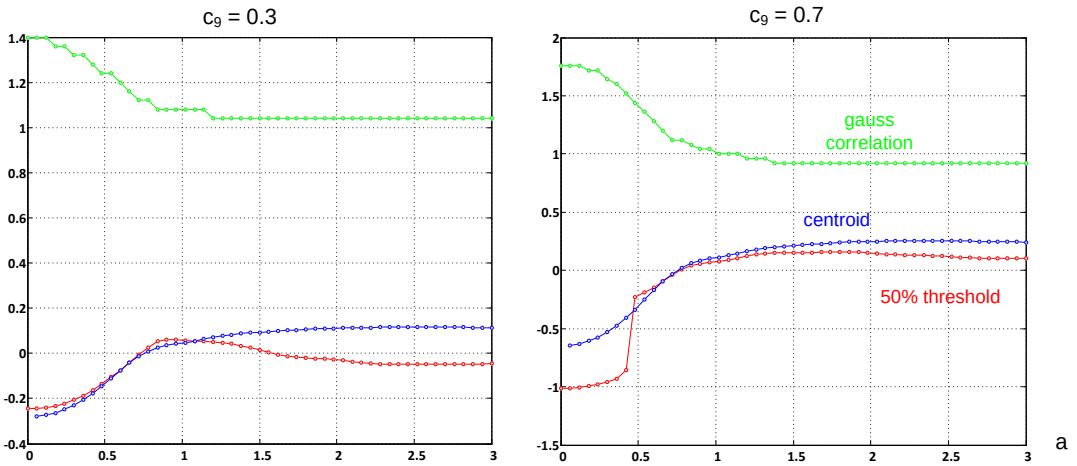
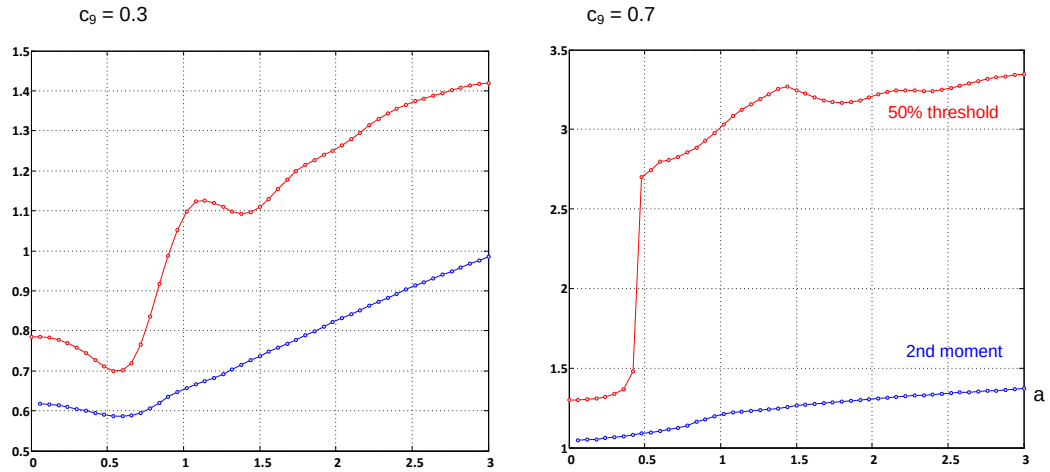
- In the case of spherical aberration the confocal signal curve S(z) is degraded:

  1. in position measurement error
     possible criteria:
     a) centroid
     b) midpoint of 50% threshold



  2. in width
     loss of accuracy
     possible criteria:
     a) 2nd moment
     b) 50% threshold (FWHM)



Numerical Results

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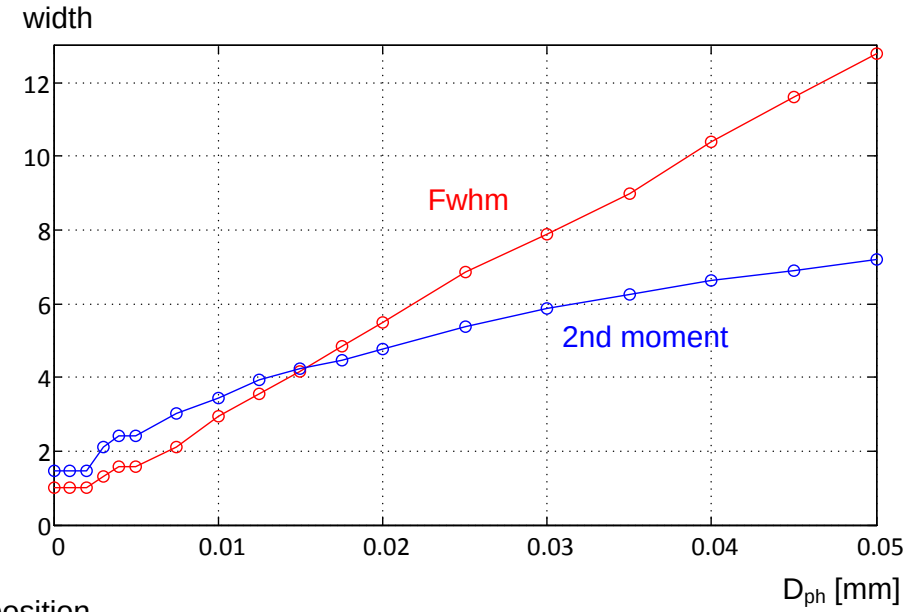
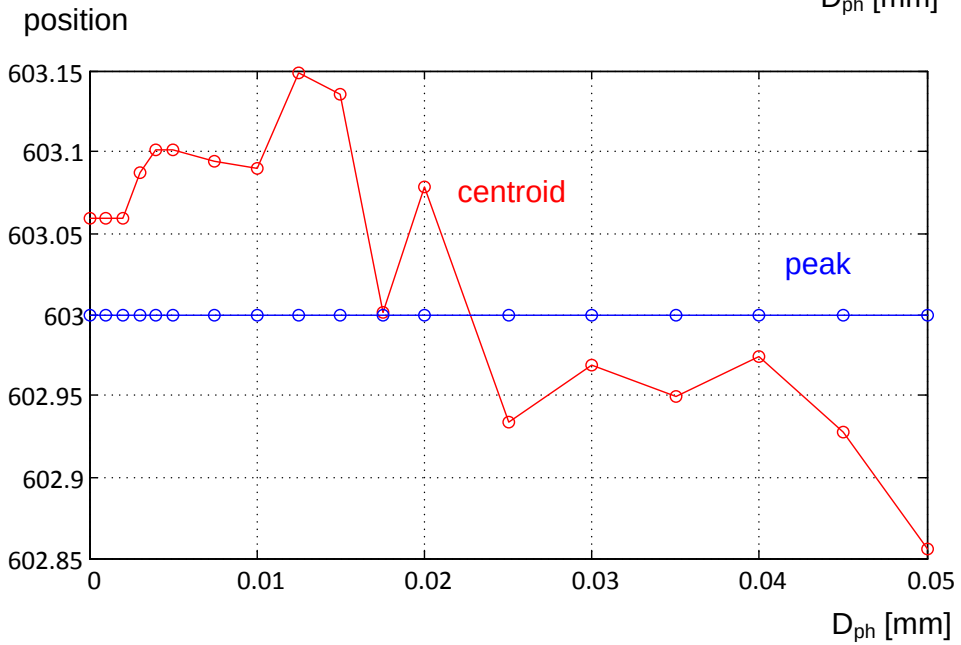
- Width of the confocal signal in the spectral domain

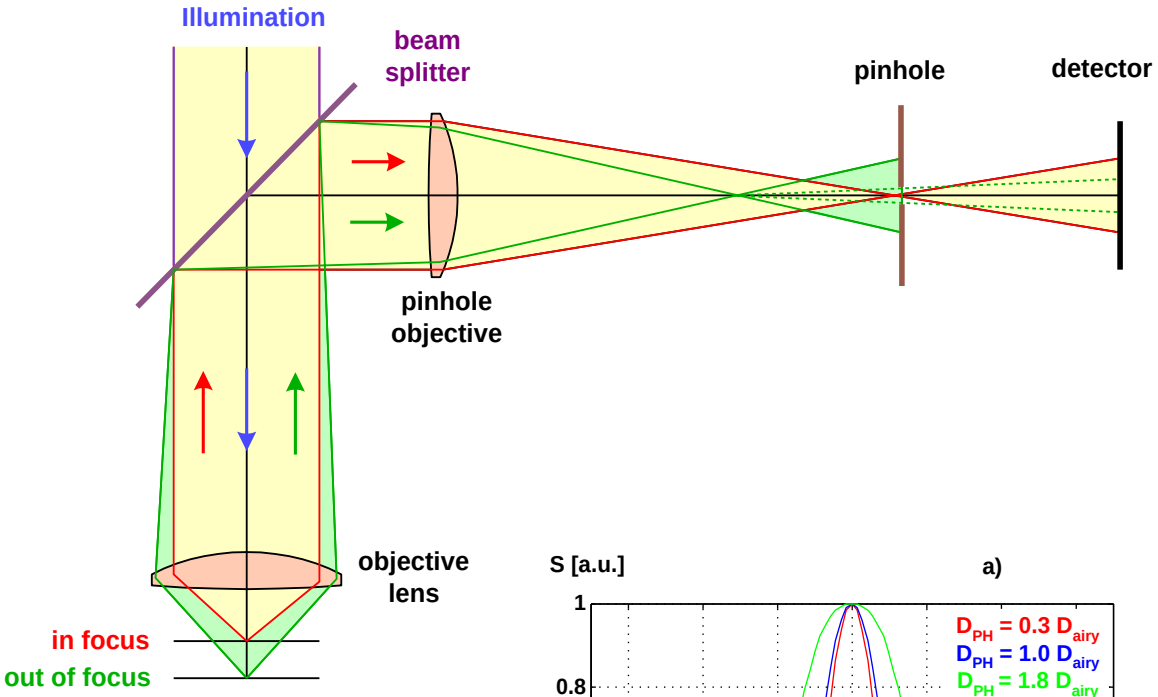


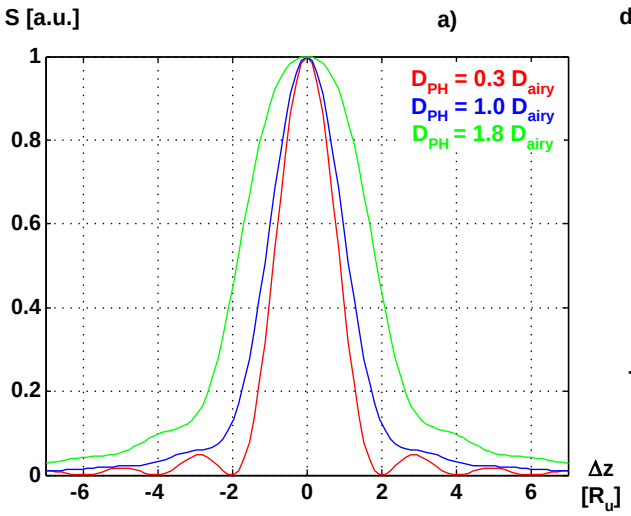
- Location of the sample z position

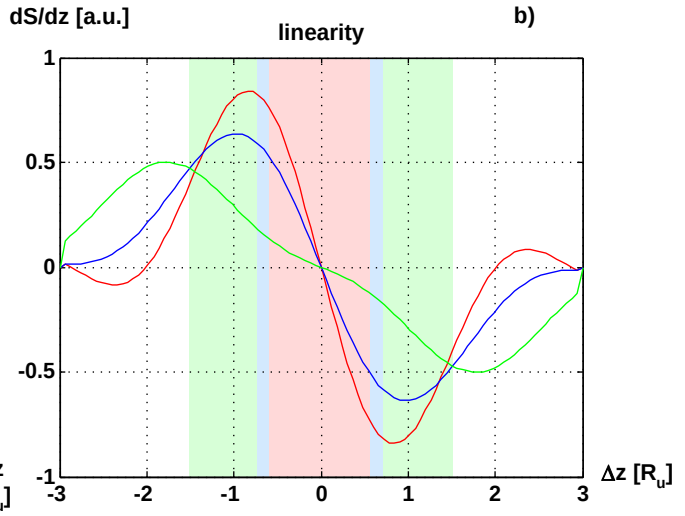


Confocal Distance Sensor

- Principle of the confocal distance sensor





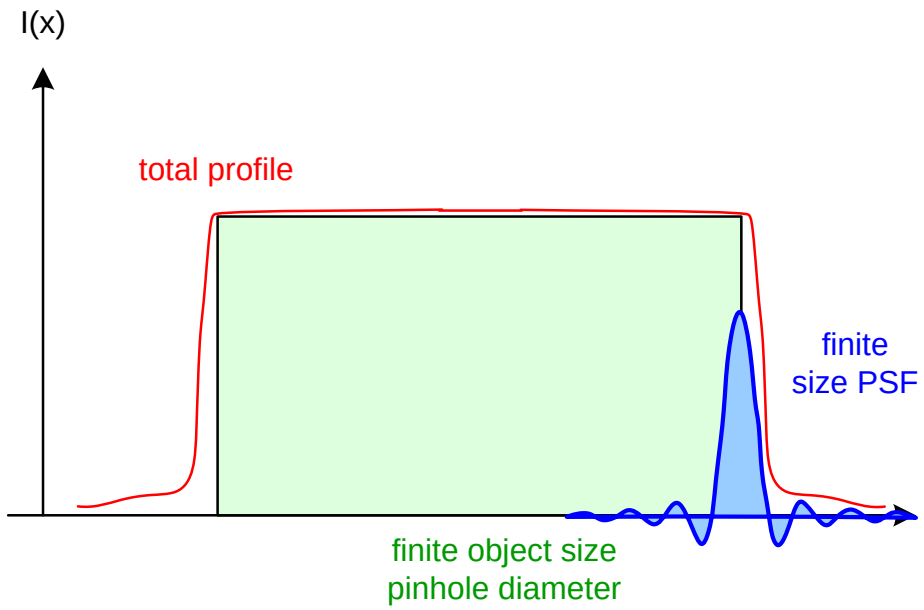


Confocal Depth Measuring System

- The system is described by
  - Zernike c_4, gives the defocus
  - Zernikes c_9, c_{16},... describe the correction of the system
- The point spread function is calculated with the help of the Zernike coefficients as

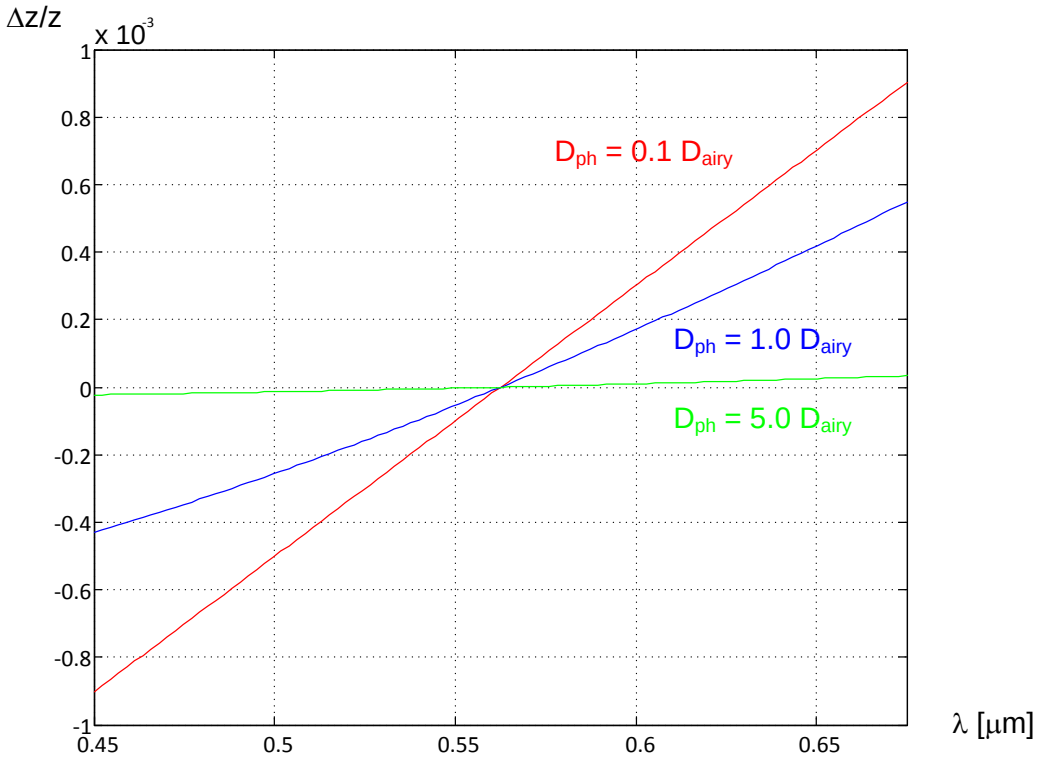
$$h_{psf}(a,\Delta z) = \iint_{r<a} A_o \cdot e^{2\pi i \cdot [c_4 Z_4(x,y) + c_9 Z_9(x,y) + c_{16} Z_{16}(x,y) + \ldots]} dx\, dy$$

- Approximations of the model:
  1. psf considered as shift invariant
  2. perfectly incoherent fiber source
  3. perfectly homogeneous fiber source
  4. in reality, the sample is not a perfect mirror but introduces scattering contributions



Change Over Measuring Range

- Polychromatic illumination
- Airy diameter changes of measuring range
- Measuring accuracy varies over range
- Larger relative influence for small pinholes

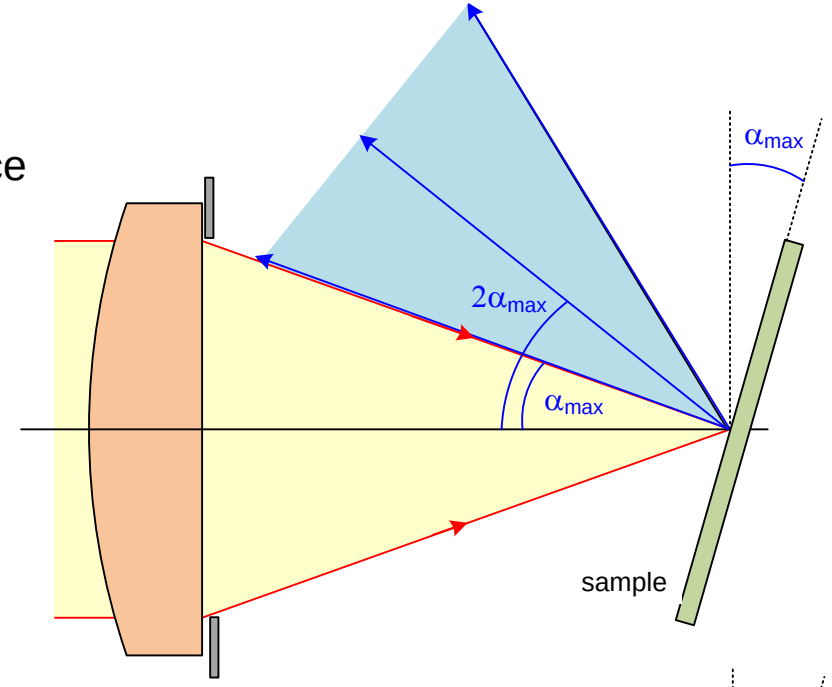


Surface Smoothness

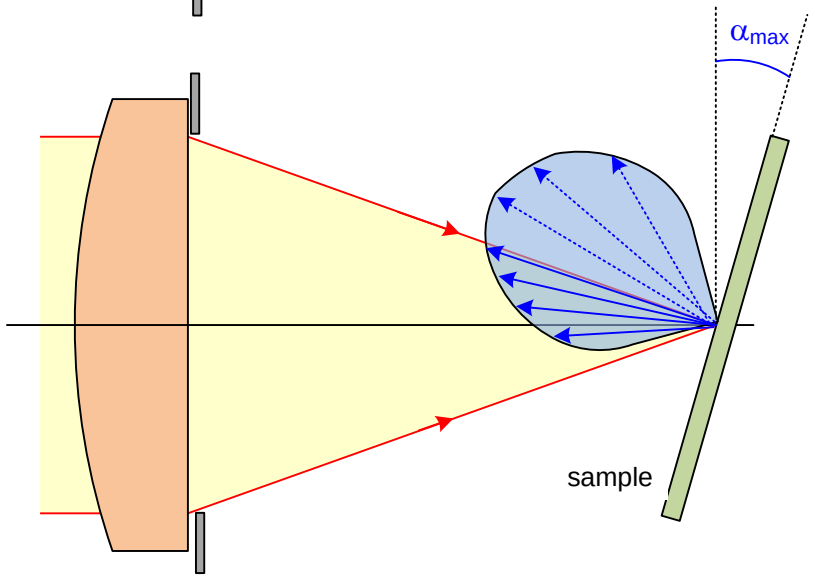
- Smooth / polished surface:
  - only reflected light is measured
  - maximum acceptable slope of the sampe surface

$$\alpha_{\max} = arc\sin(NA)$$

NA	maximum angle α
0.3	18°
0.4	24°
0.5	30°
0.6	37°
0.7	44°

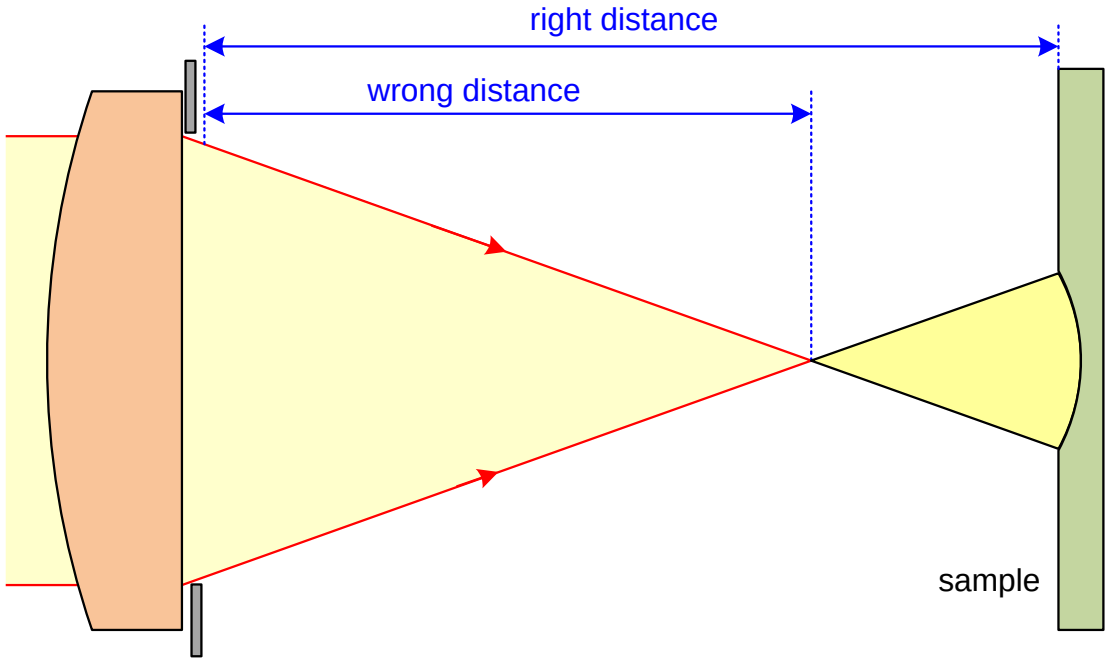


- Diffuse surface:
  - larger slopes can be measured
  - quantitatively the BRDF determines the limit



Ghost Foci

- If parts of a polished sample are spherical in shape:
  - ghost foci with high intensity
  - wrong interpretation of the depth out of the signal



Measurement of Focal Length by Confocal Setup

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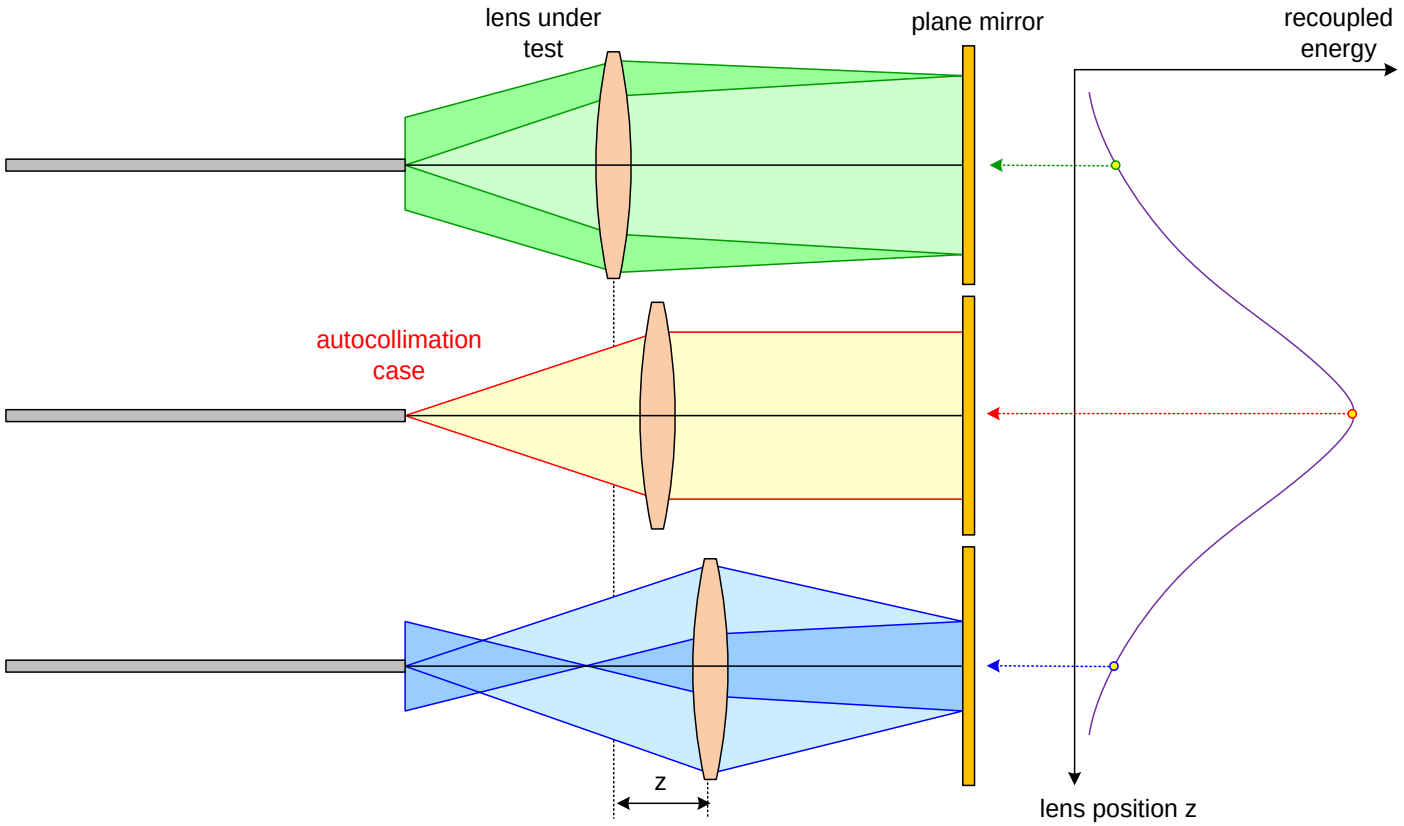
- Setup with fiber and plane mirror for autocollimation
- Change of distance between test lens and fiber
- Analysis of the recoupled power into the fiber (confocal) gives the focal point



Chromatical Confocal Sensor

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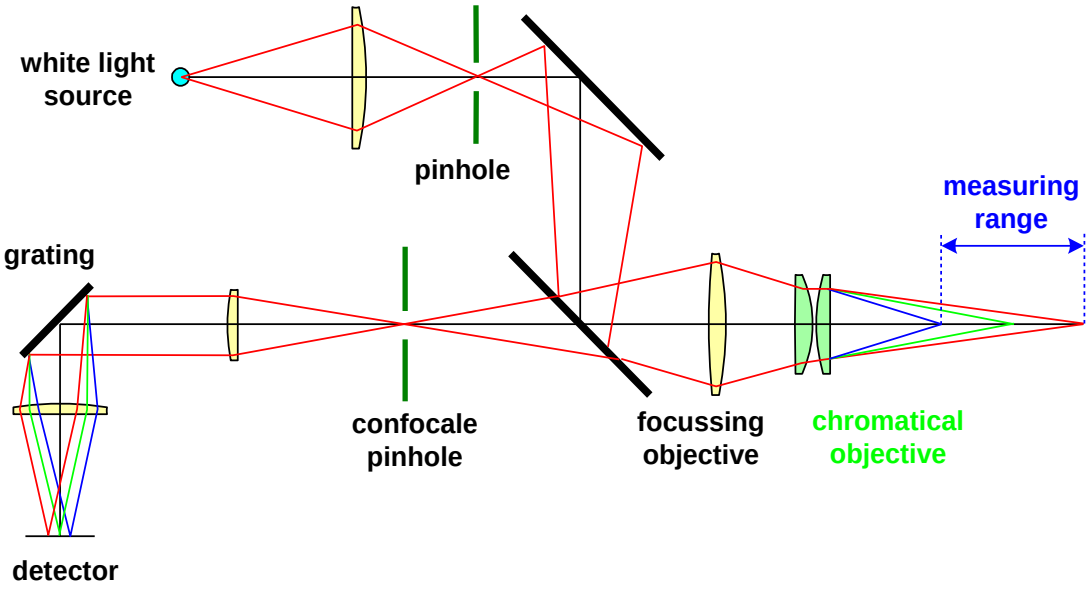
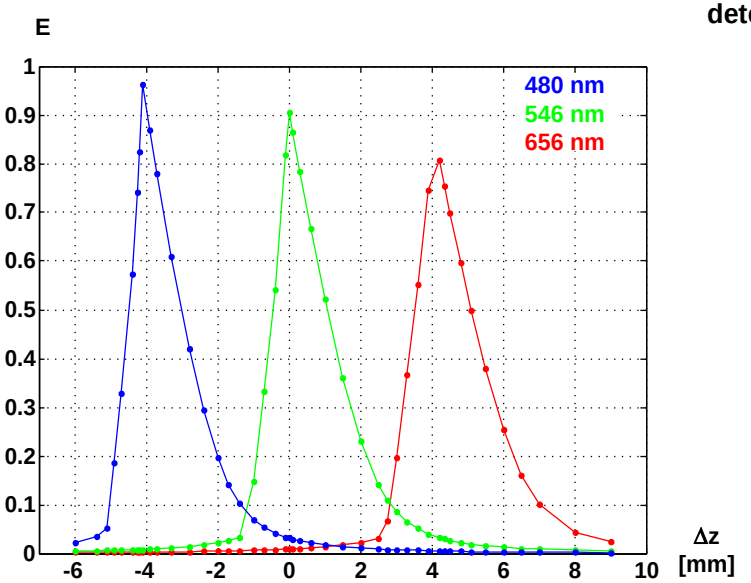
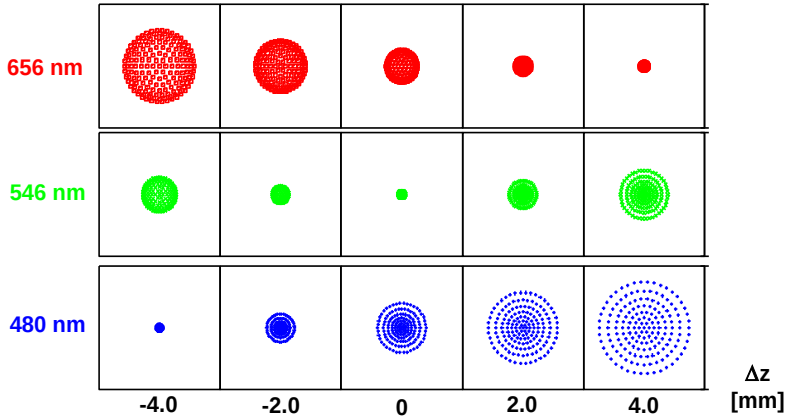
- Spectral sensitive sensor
- Objective lens with large axial chromatical aberration







Confocal Imaging with Hyper Chromate

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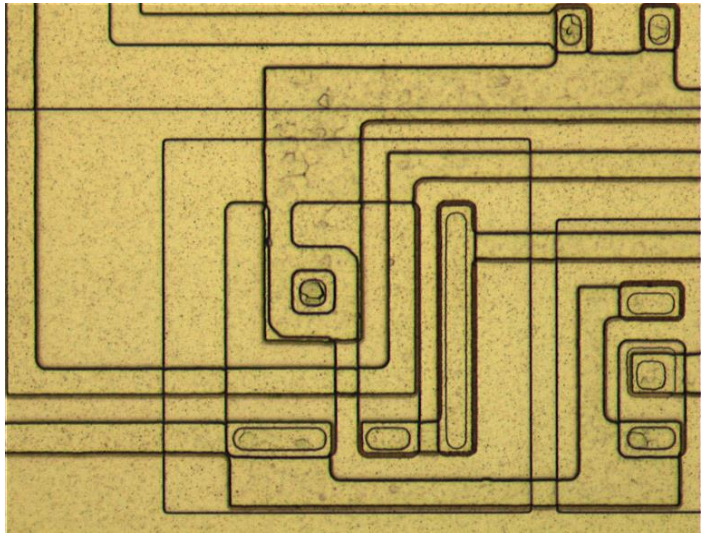
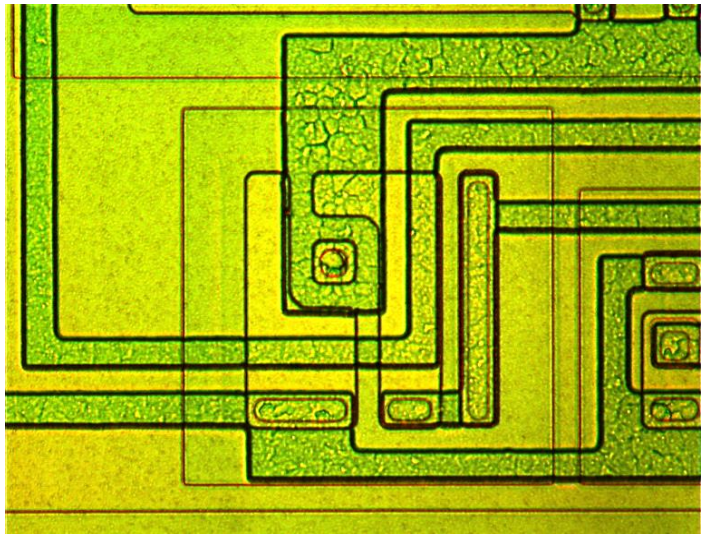
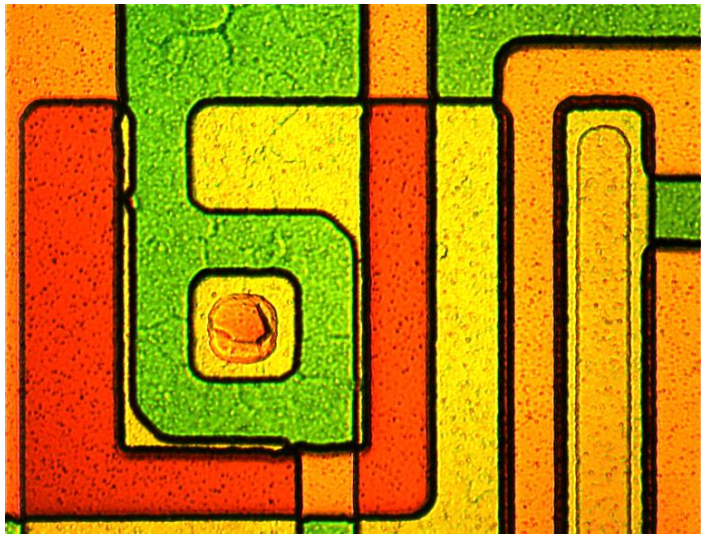
- Wide field 20x0.5
- Confocal with chromate at low aperture 20x0.5
- Confocal with chromate at high aperture 50x0.9







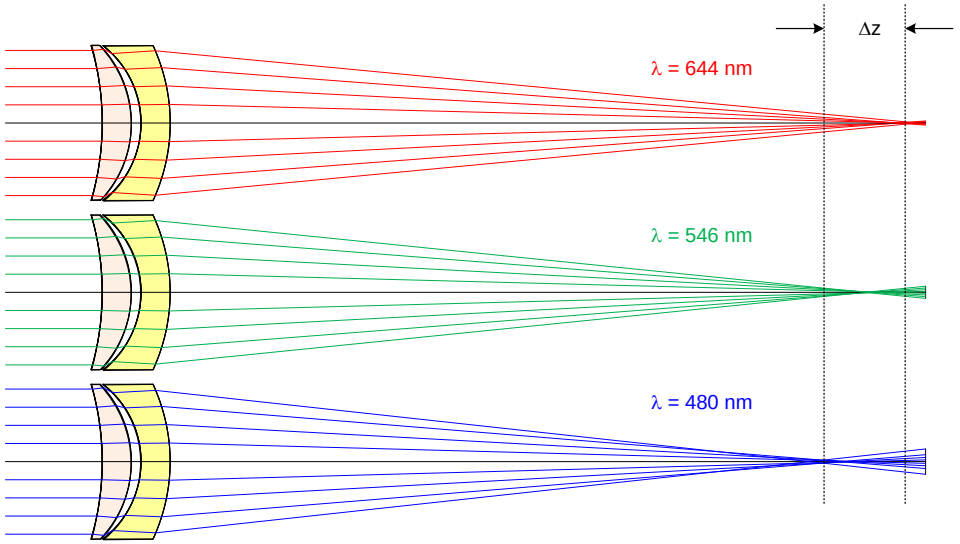
Ref: R. Semmler

Principle

- Goal:
  1. large chromatical spreading (large CHL) Δz
  2. large numerical aperture
  3. corrected spherochromatism

- In the case of a large ratio Δz / f, the numerical aperture shows a considerable change in the measuring interval

- Design approach:
  1. Achromate with positive flint and negative crown
  2. Achromates cascaded
  3. Improved spherochromatism by asphere
  4. monochromatic lens with buried surface adapter



Comparison

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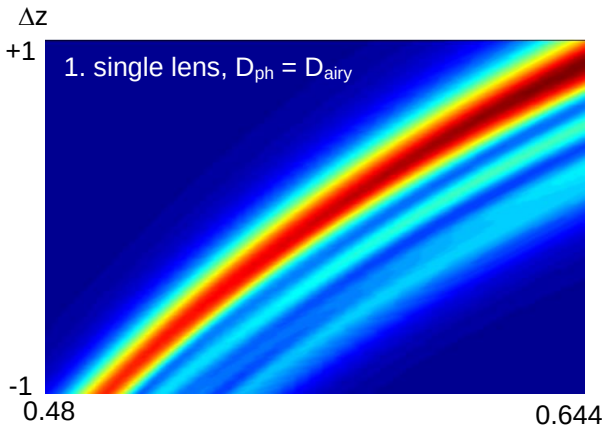
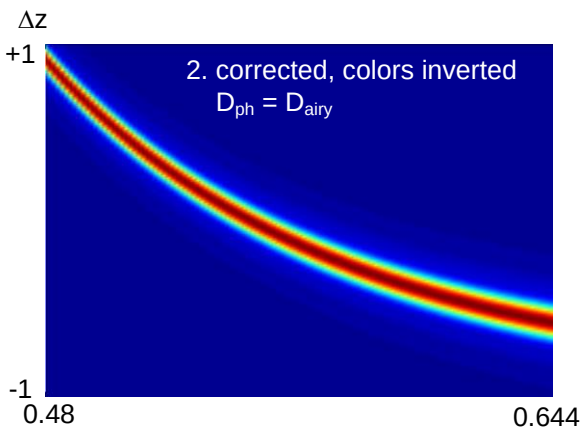
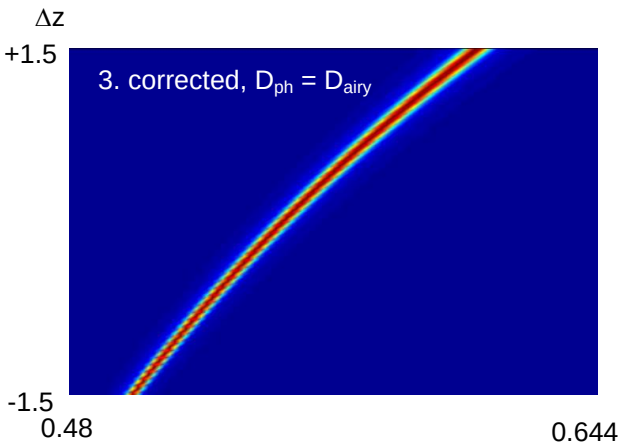
- Confocal signal as a function of distance and wavelength

- Cases:

1. single lens / gauss-aberration corrected
2. pinhole size 1 Airy
3. no quadrature of confocal psf







Optical Design

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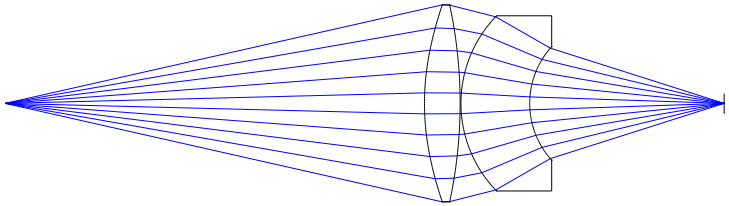
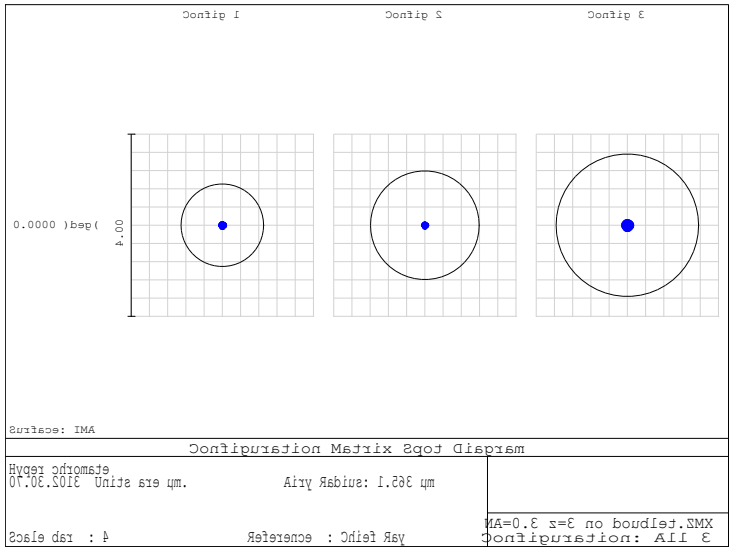
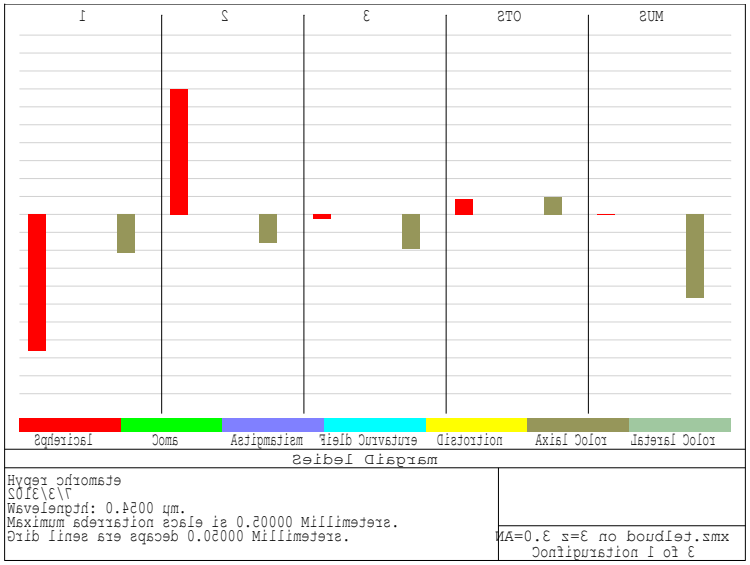
Case 1-1

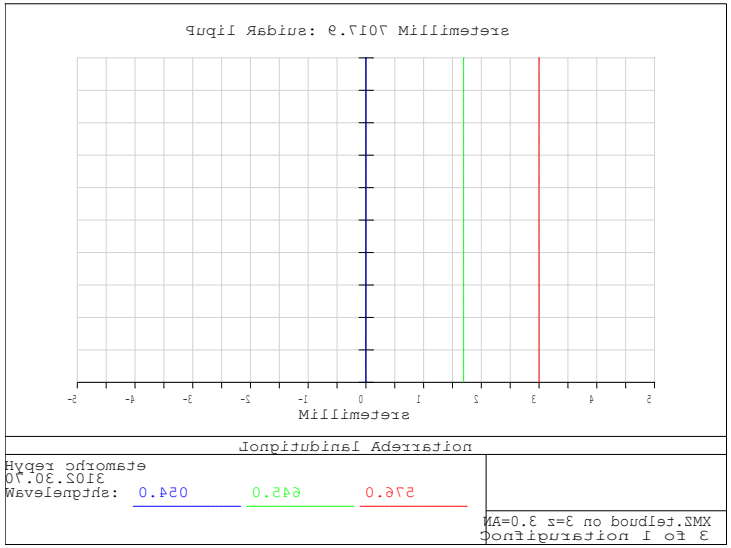
NA_{image} = 0.3, NA_{object} = 0.22
Δz = 3 mm, f = 13 mm
z_{free} = 16.3 mm

1st surface: aspherical









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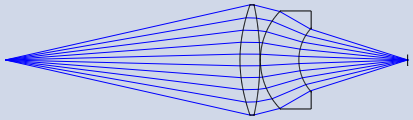
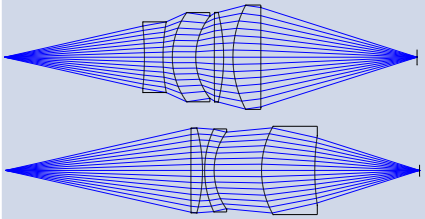
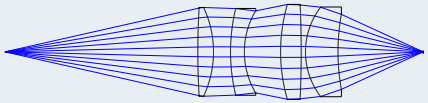
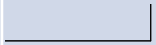
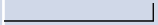
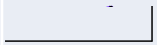
OVERVIEW

Specifications	2 lenses with asphere	Only spherical lenses	Extension in Δz
NA=0.3, Δz=3mm z_{free} = 16 mm			Δz=3.9mm
NA=0.4, Δz=0.4mm z_{free} = 10 mm			
NA=0.4, Δz=1mm z_{free} = 10 mm			Δz=2.5mm
NA=0.7, Δz=0.12mm z_{free} = 3 mm			Δz=0.5mm

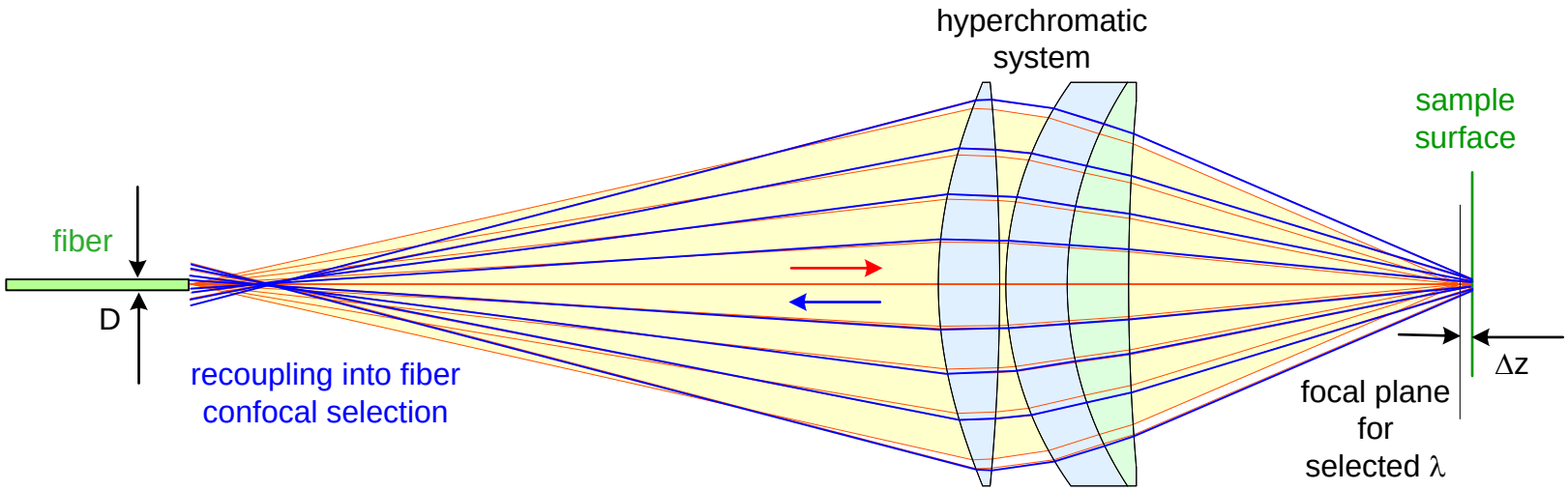
Confocal Depth Measuring System

- Fourier optical model:
  - object/sample to be assumed as a plane mirror
  - fiber source incoherent, diameter D_{fib}, uniformly radiating
  - optical system with point spread function h_{psf}
  - confocal detection by fiber (pinhole) size D_{fib}
- Incoherent imaging model to get the intensity of at the fiber

$$I_{ima}(a,\Delta z) = I_{fib}(a) \otimes \left| h_{psf}(\Delta z) \right|^2$$

- Calculation of the confocal signal by integration over the pinhole

$$S_{conf}(a,\Delta z) = \iint_{r<a} I_{ima}(a,\Delta z)\, dx\, dy$$

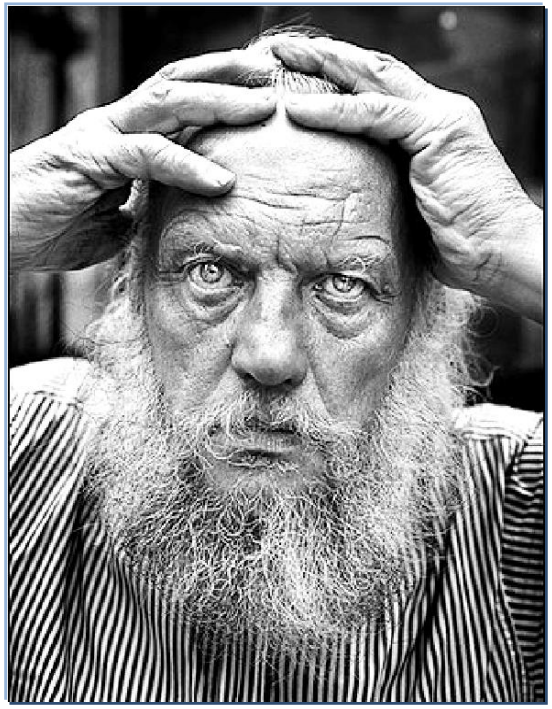


Time is Over

Feedback

nothing clear ?

to complicated ?



to much stuff?

Ref: D. Shafer