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Friedrich-Schiller-Universität Jena

Metrology and Sensing

Lecture 13-1: Confocal sensors

2020-02-09

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Content

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- Principle of confocal imaging
- PSF
- Examples

Confocal Microscope

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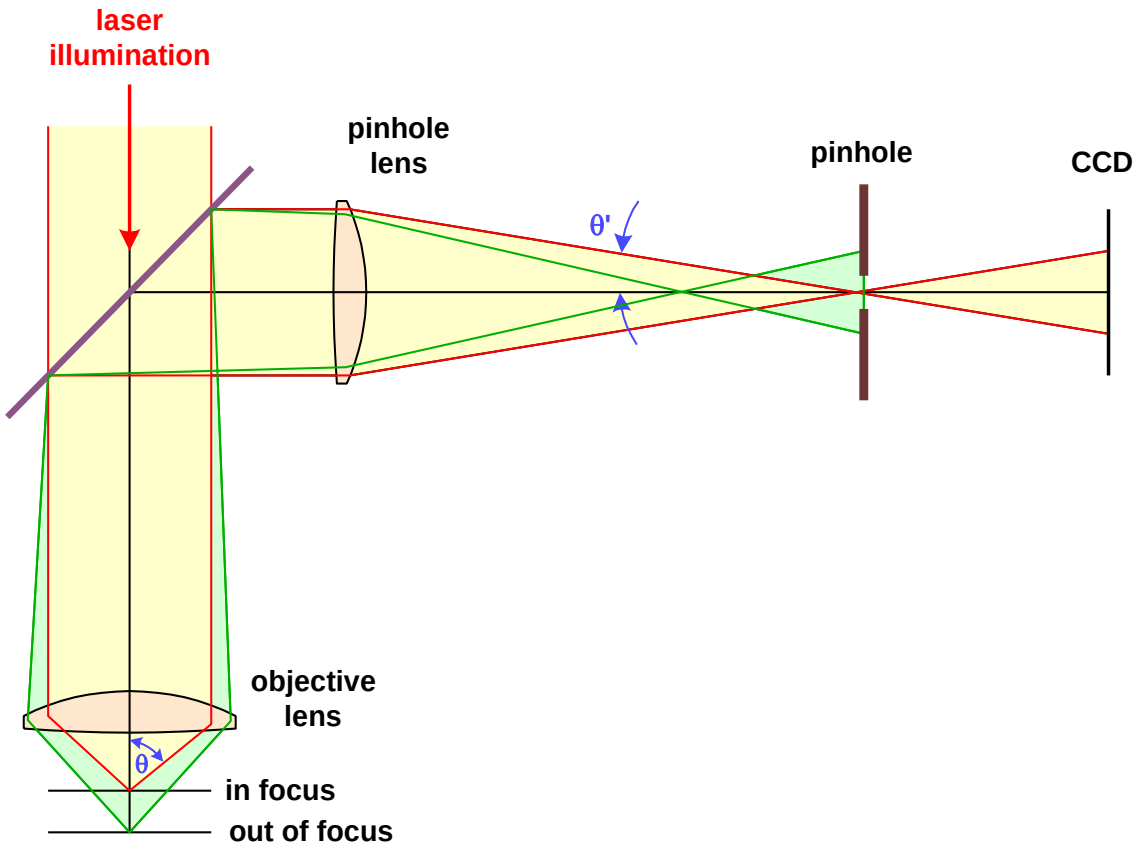
- Laser scan microscope
- Depth resolution (sectioning) with confocal pinhole
- Transverse scan on field of view Digital image
- Only light comming out of the conjugate plane is detected
- Perfect system: scan mirrors conjugate to pupil location
- System needs a good correction of the objective lens,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



Confocal 3D Image Collection

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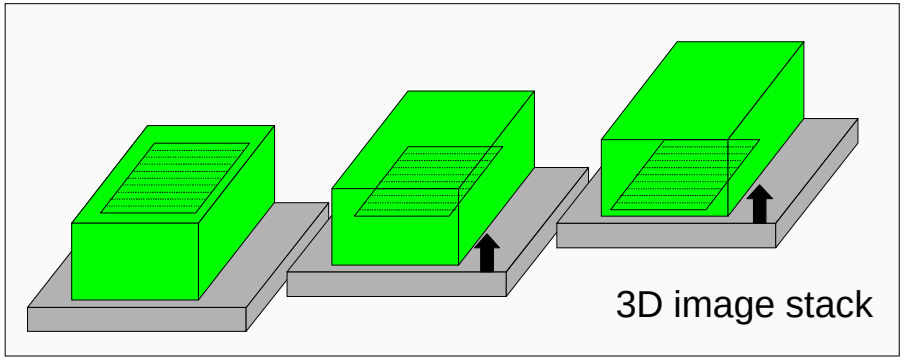
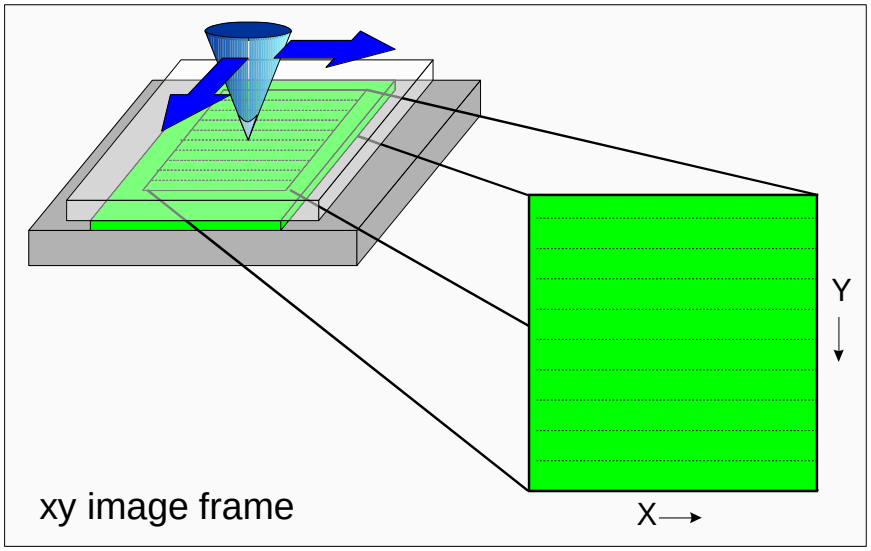
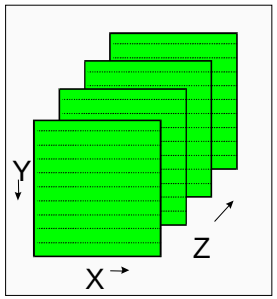
- Contact free optical sectioning
- 3D information collection & reconstruction
- 3D measurement and analysis
- The laser focus is moved over the sample (flying spot method)
- The measured intensity at each spot forms a xy image frame



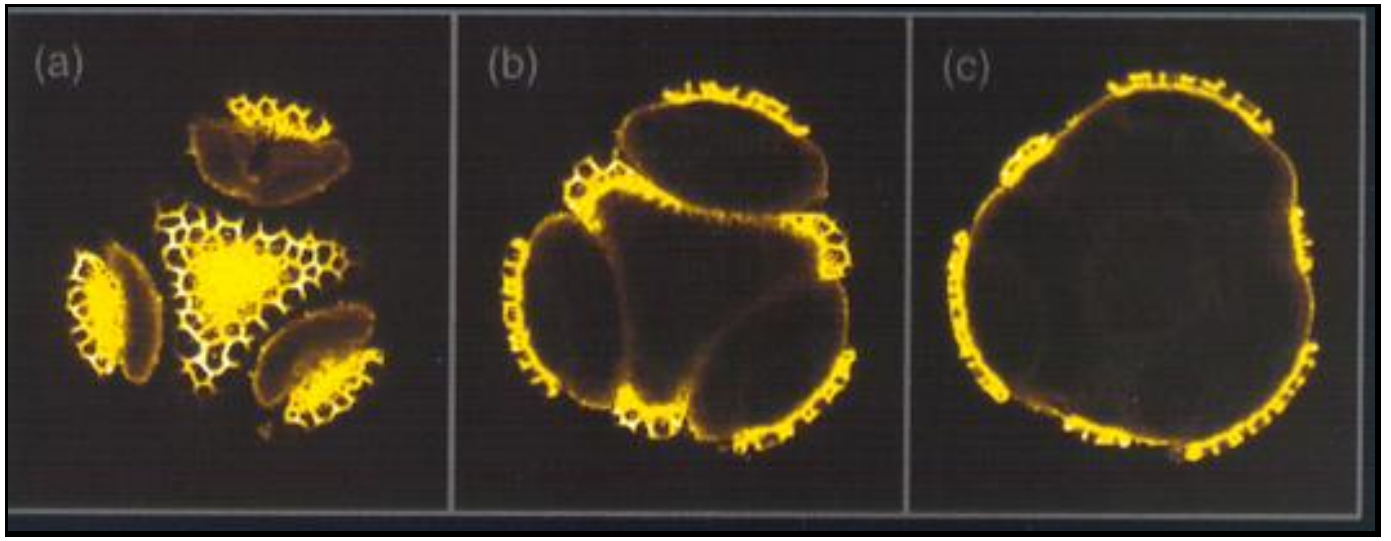




Ref: M. Kempe

Confocal Images

Depth resolved images



Ref.: M. Kempe

Confocal Microscopy

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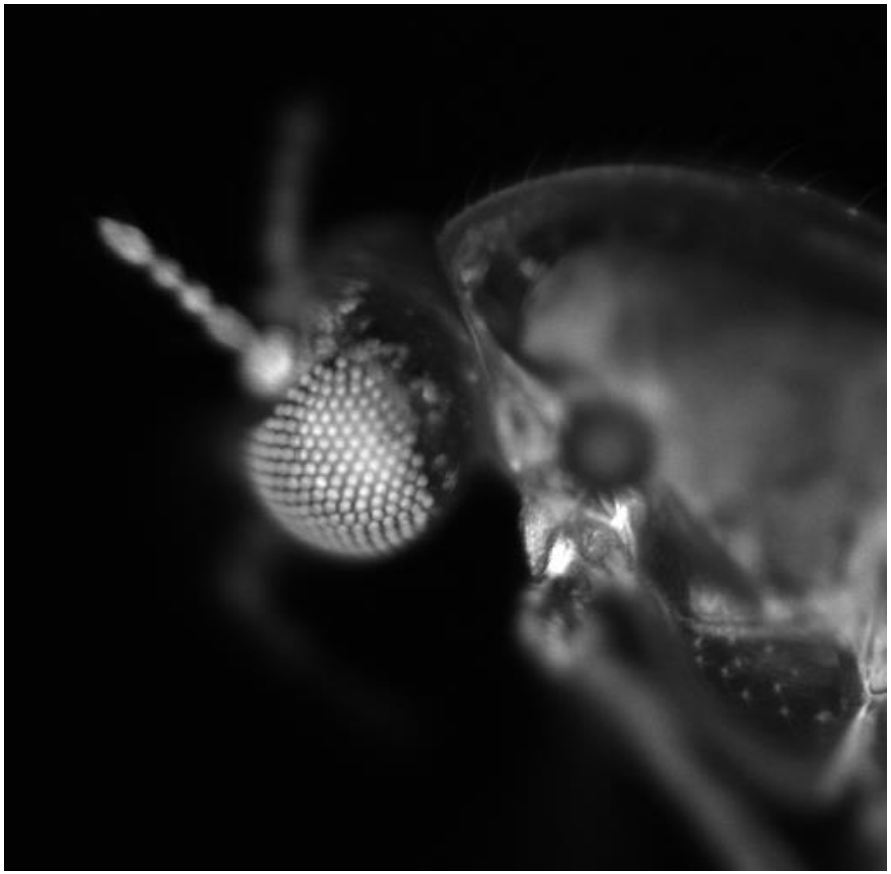
3-D volume imaging with reconstruction in confocal Laser scan microscope

a) Classical microscopy
depth of object : 300 μm

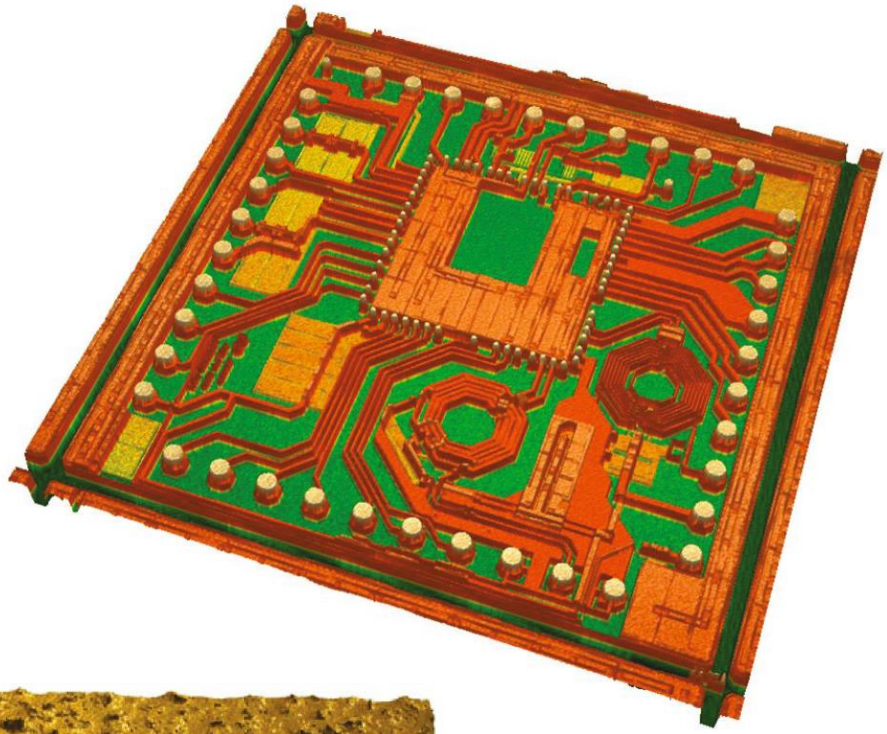


b) Confocal microscopy
with 3-D reconstruction

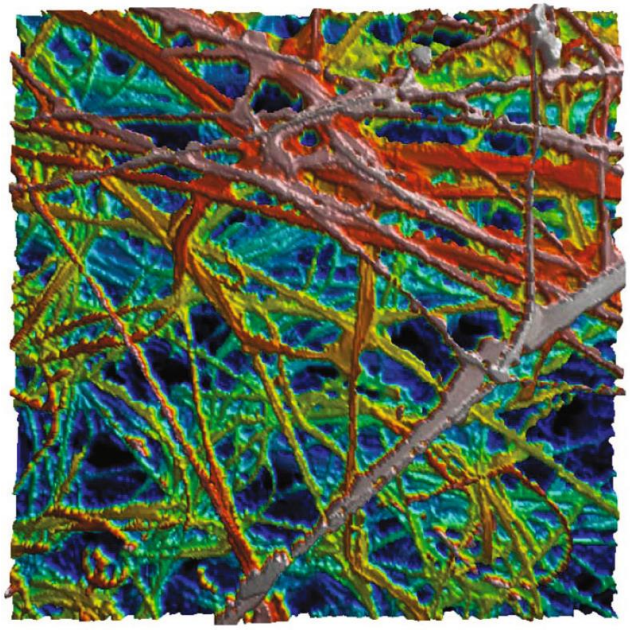
Ref: M. Kempe

Examples

- Microelectronic circuit



- Abbrasive paper



- Smooth paper

Ref.: R. Leach

Examples

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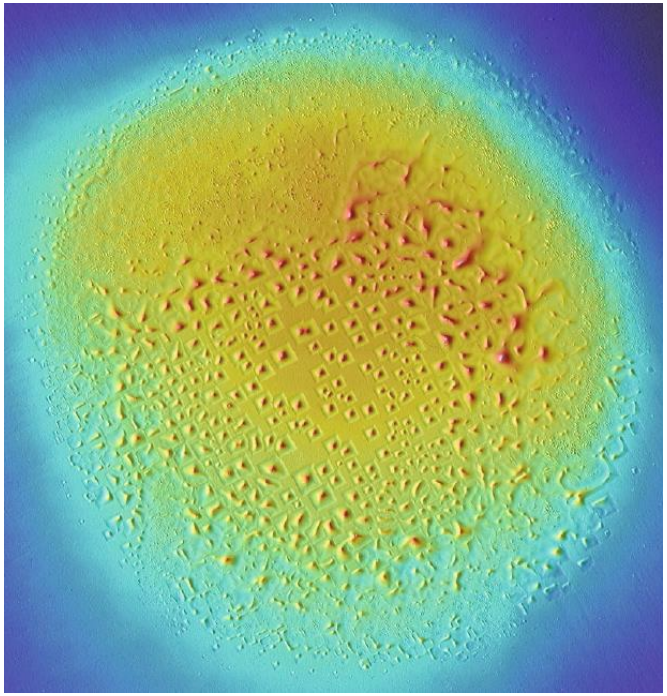
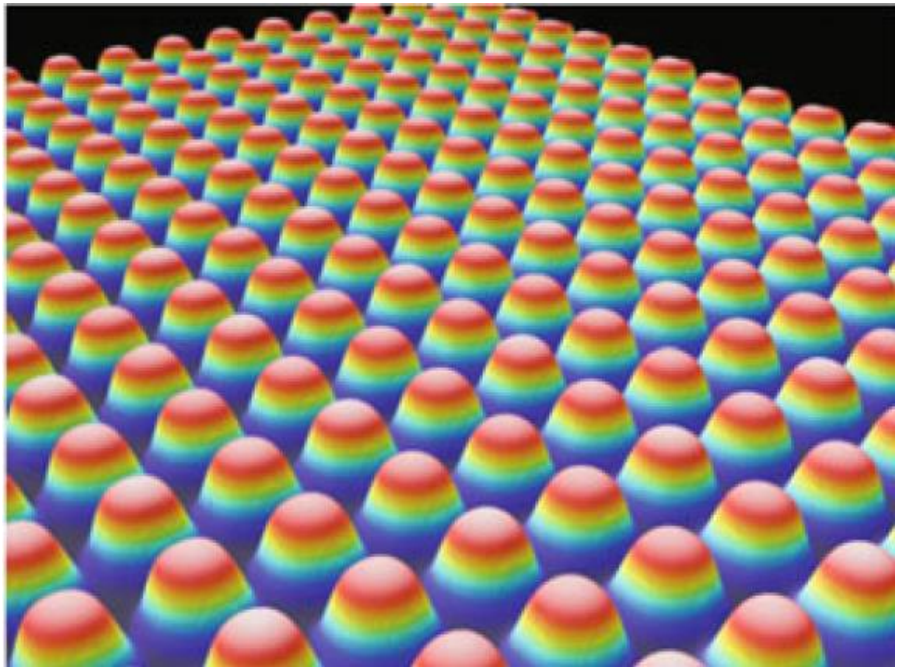
- Silicon surface with stitching



- Microlens array



Confocal Microscope – General Aspects

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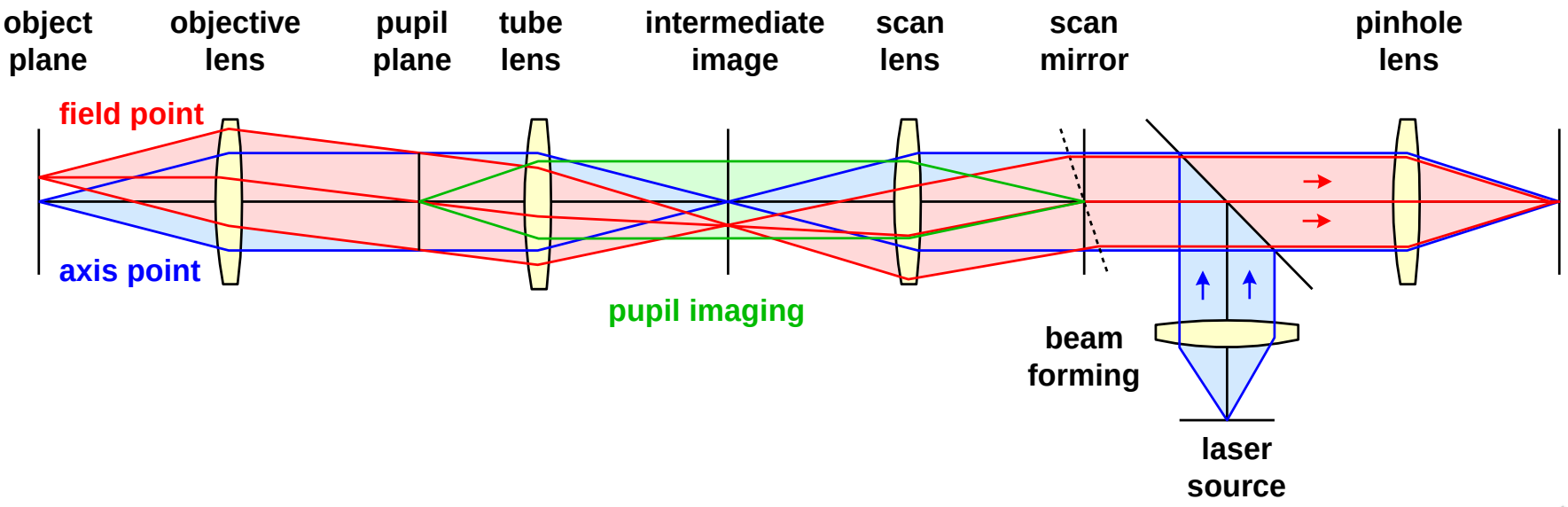
- Laser scan microscope produces only images in combination with software for the image processing
  Realtime image gathering is possible today
- Usually the illumination is a scanning laser beam
- Usually the detection/observation uses the same lens
- The confocal pinhole detection guarantees:
  - a 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



Lateral and Axial Resolution

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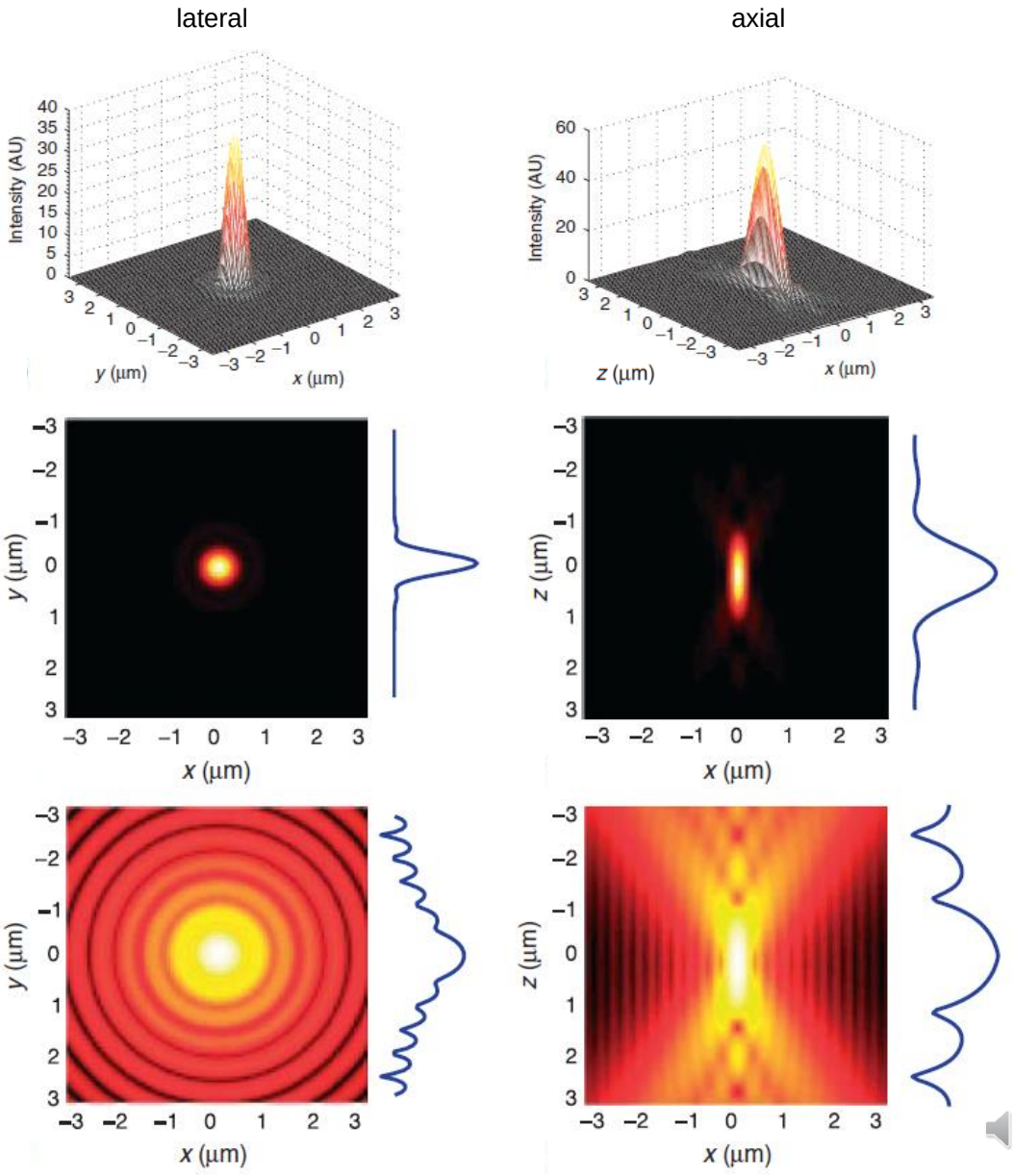
- Intensity distributions



Ref: U. Kubitschek

Confocal PSF

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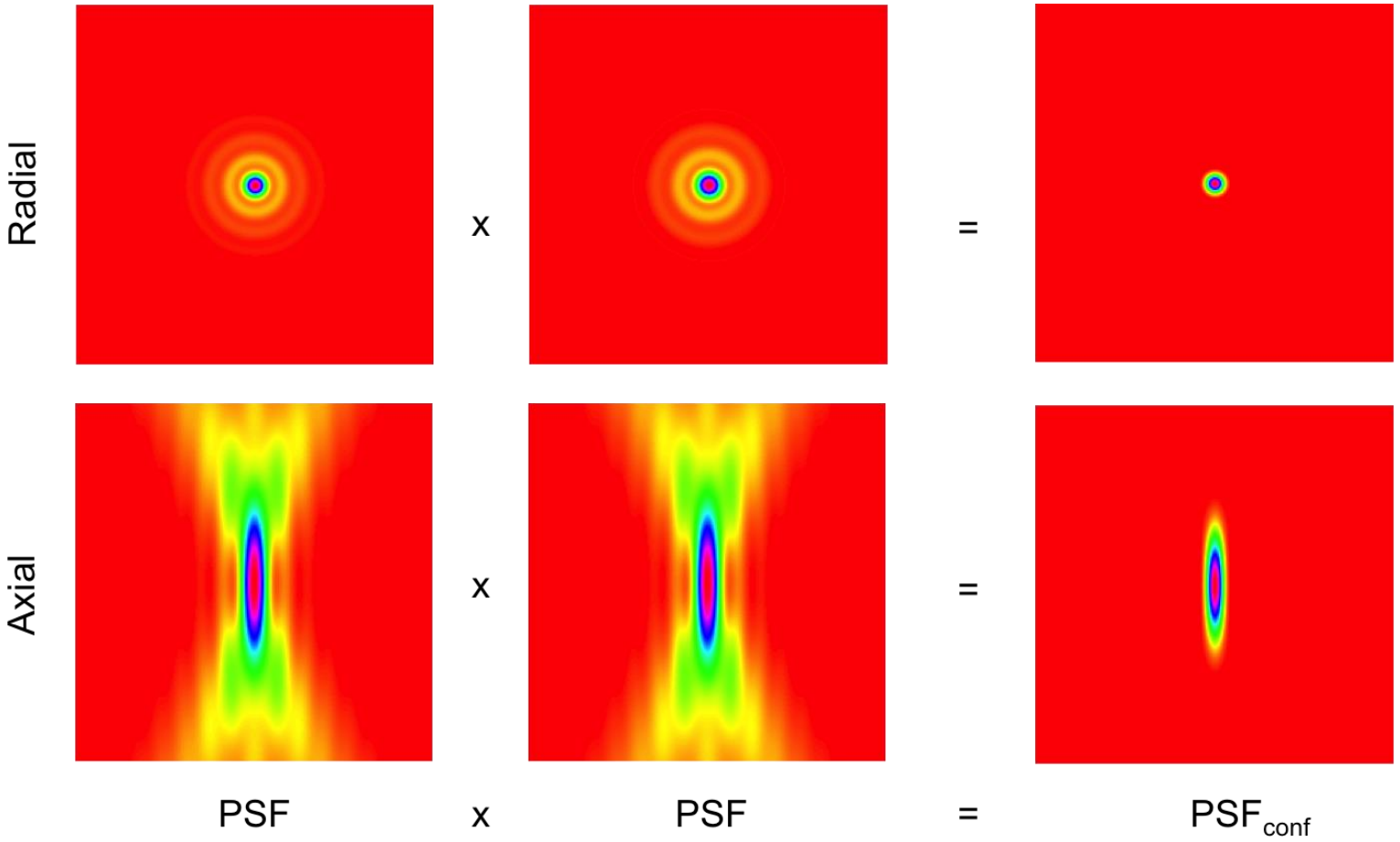
- Change of intensity distributions by confocal mode
  1. lateral
  2. axial



Ref: A. Szameit

Lateral Resolution in Confocal Imaging

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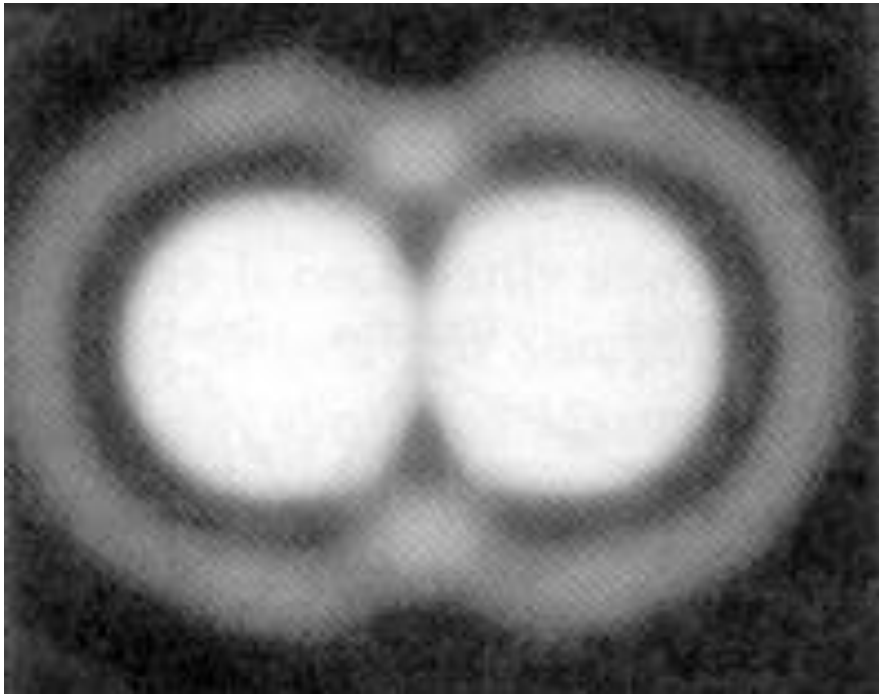
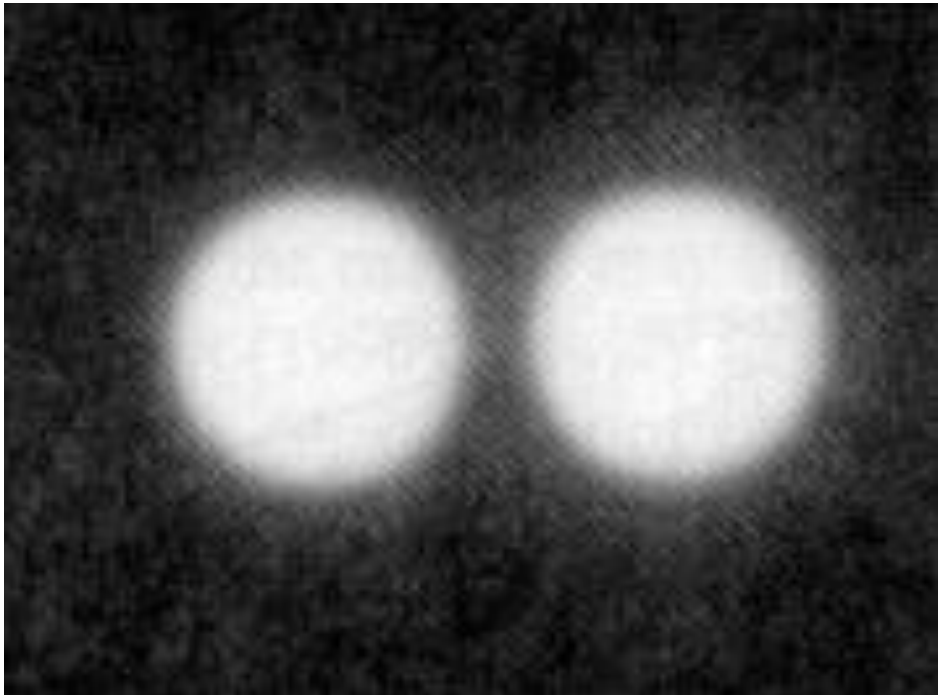
- Comparison of PSF in wide field and confocal imaging
- Improved 2-point resolution in confocal mode

conventional wide field



confocal



Microscopic Resolution

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- 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