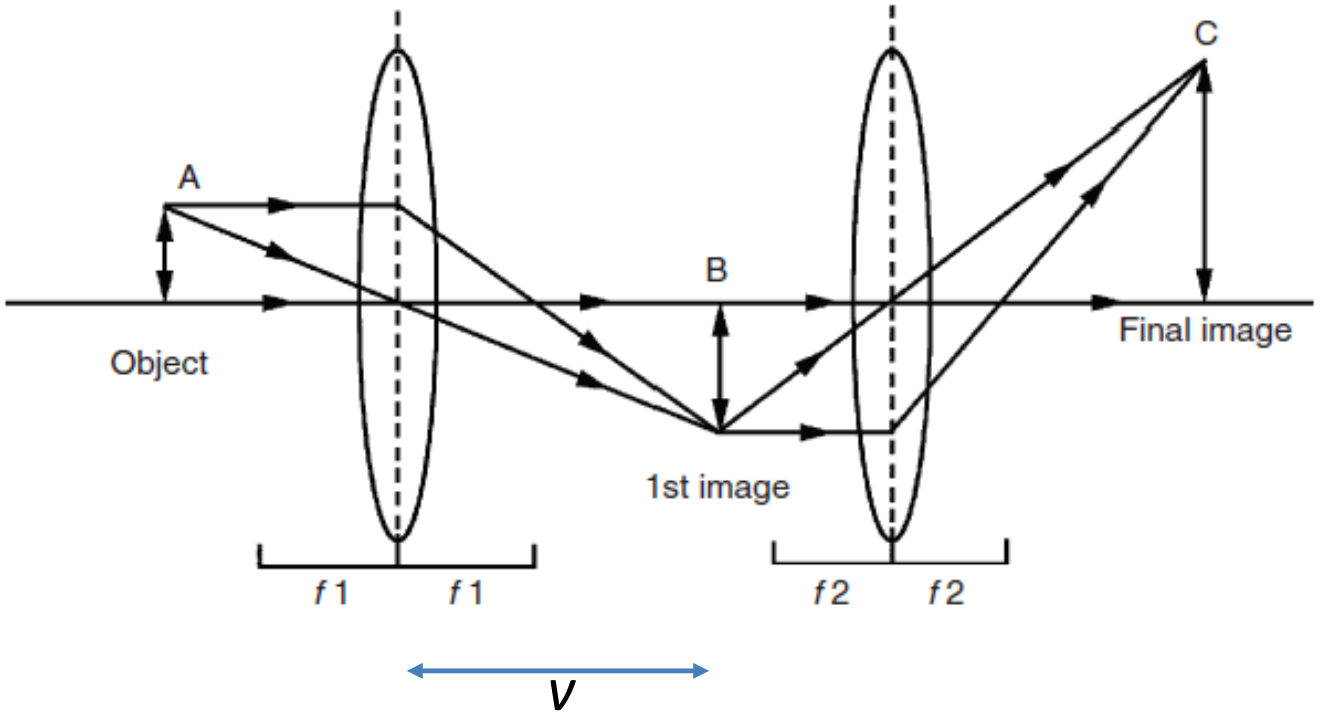
Optical Microscopy: structure and working principles

Lecturer: Kung-Hsuan Lin

TIGP Course: Characterization, Fabrication, and Manipulation at Nanometer Scale

March 26, 2020

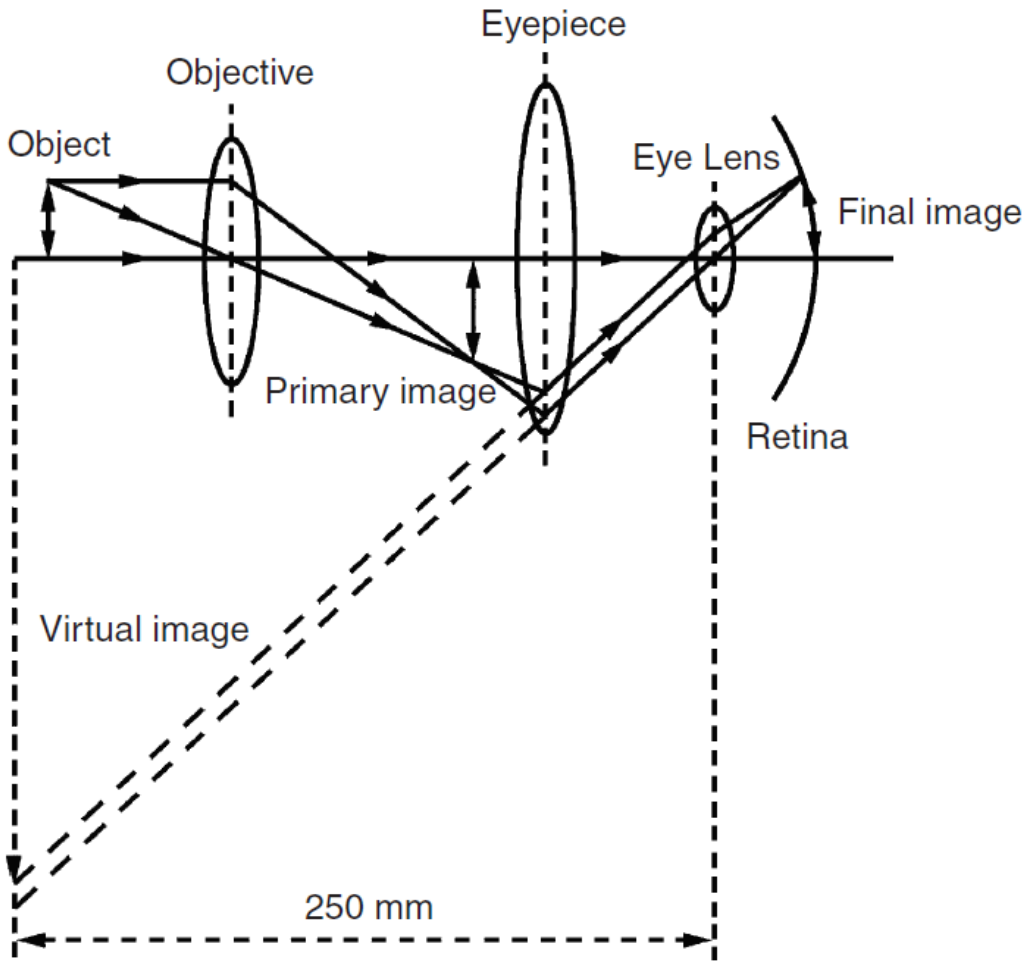
Image Formation



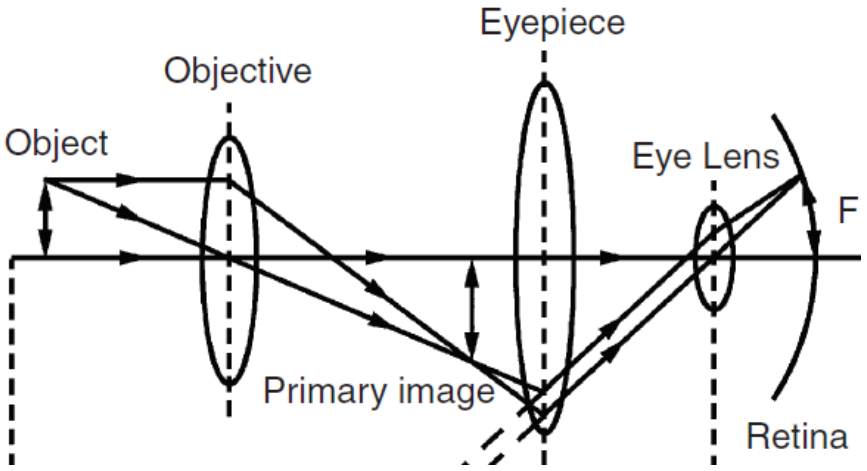
$$M = \frac{v - f}{f}$$

$$M = M_1 M_2 \frac{(v_1 - f_1)(v_2 - f_2)}{f_1 f_2}$$

Image observed by eyes



Tube length & Infinity corrected optics



Tube length:
standardized to the Royal Microscopical Society (RMS) suggestion of 160 mm

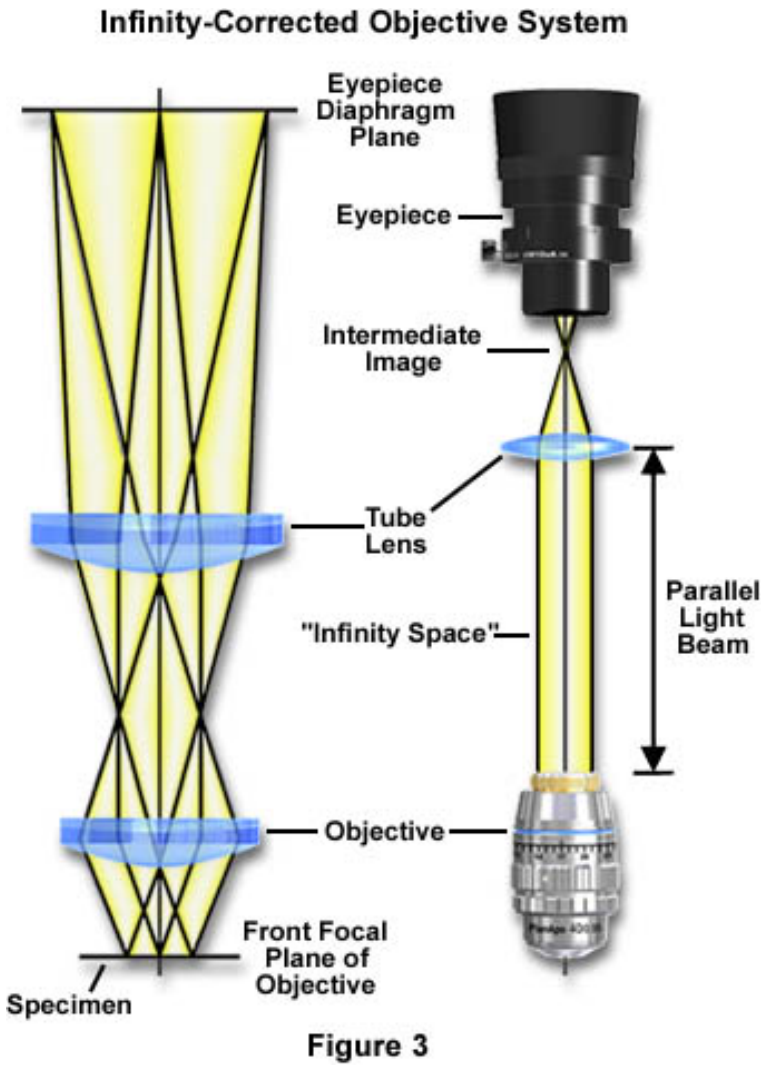
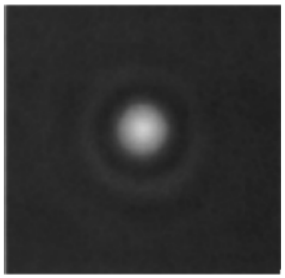
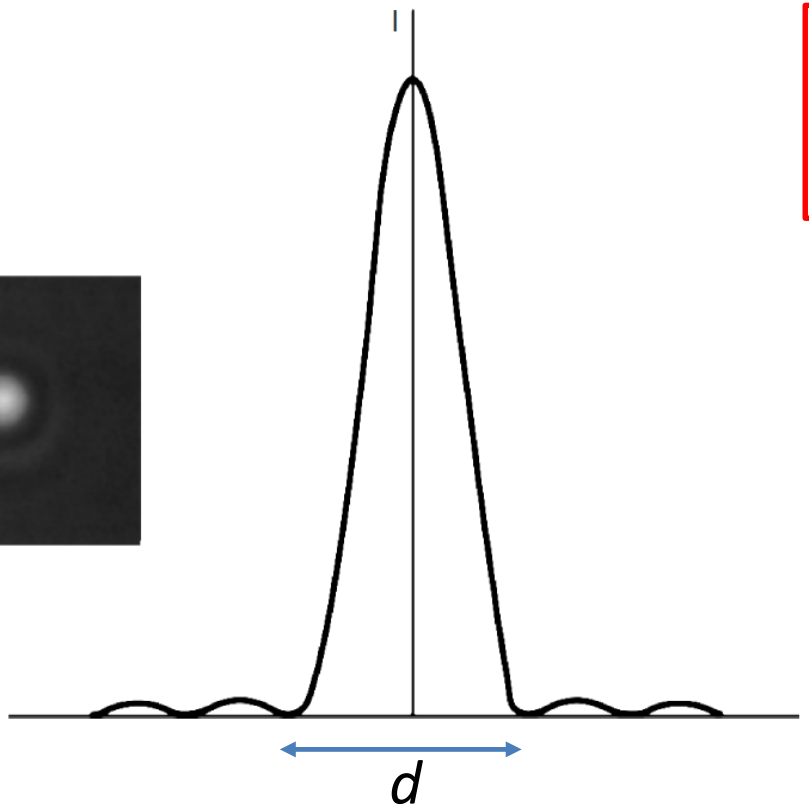


Figure 3

Diffraction limitation

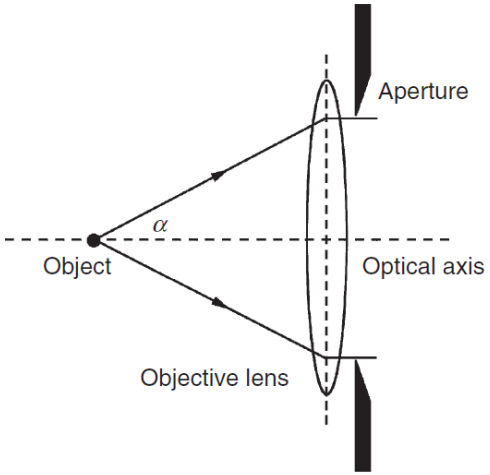
- Due to diffraction, the smallest point to which a can focus a beam of light is the size of the Airy disk. The smallest size is diffraction limited.





$$d = \frac{1.22\lambda}{n\sin\alpha} = \frac{1.22\lambda}{NA}$$

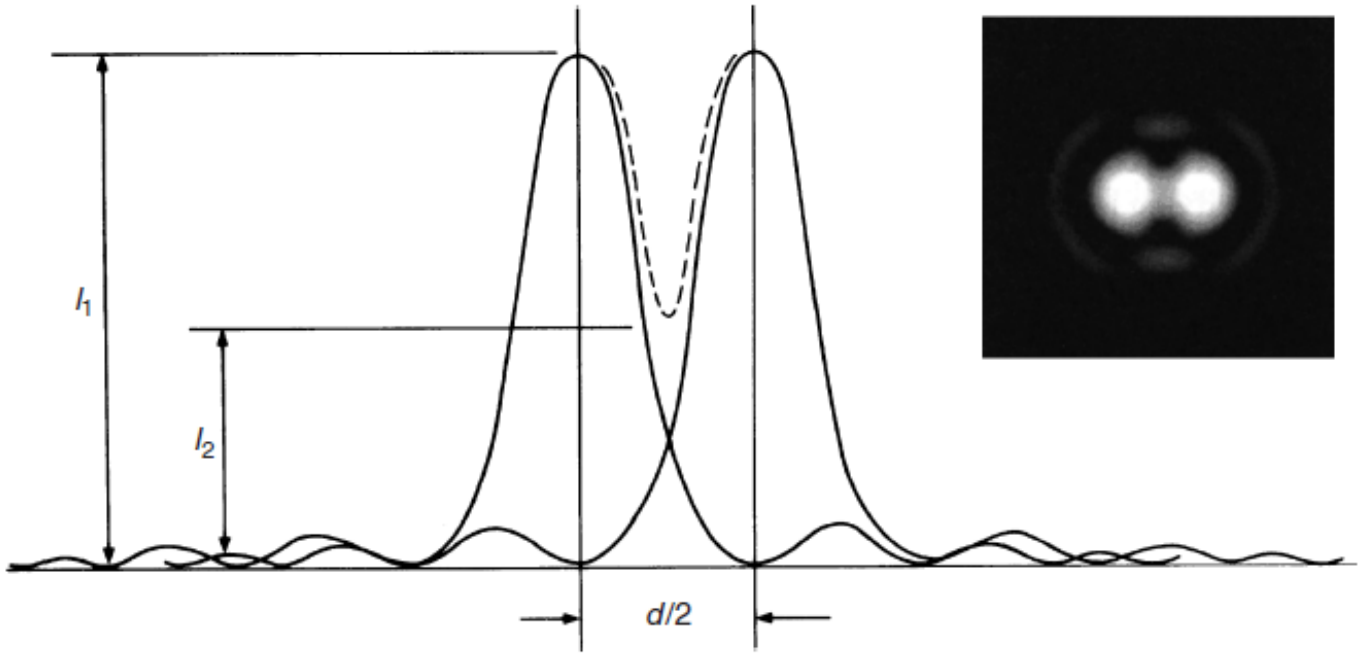
NA: numerical aperture



Optical resolution

- Resolution: the minimum distance to distinguish two point objects.

$$R \equiv d / 2 = \frac{0.61\lambda}{NA}$$



Effective magnification

$$R = d / 2 = \frac{0.61\lambda}{NA}$$

Optical resolution: minimum ~ 200 nm for visible light

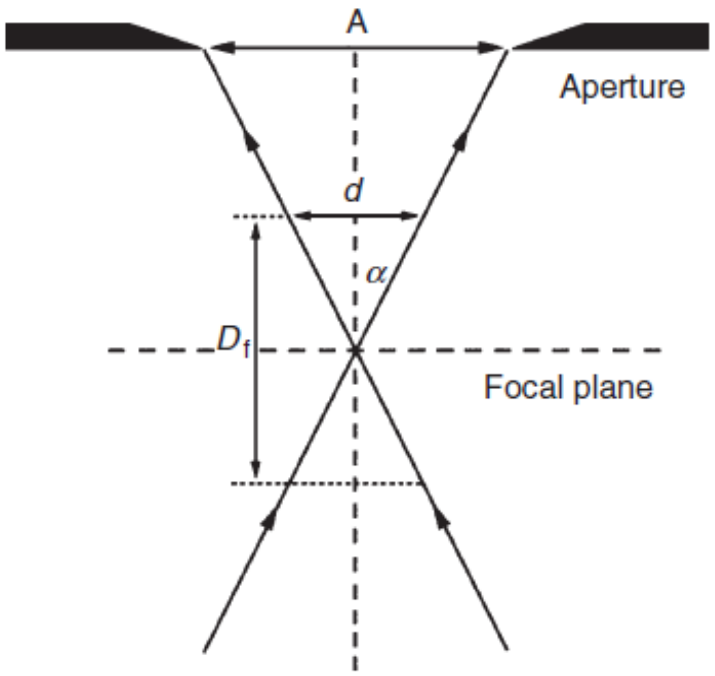
Eye resolution: minimum ~ 0.2 mm
(A microscope should enlarge features to the resolution level of the human eye

X

= 1000

Depth of Field

- The range of position for an object in which image sharpness does not change.



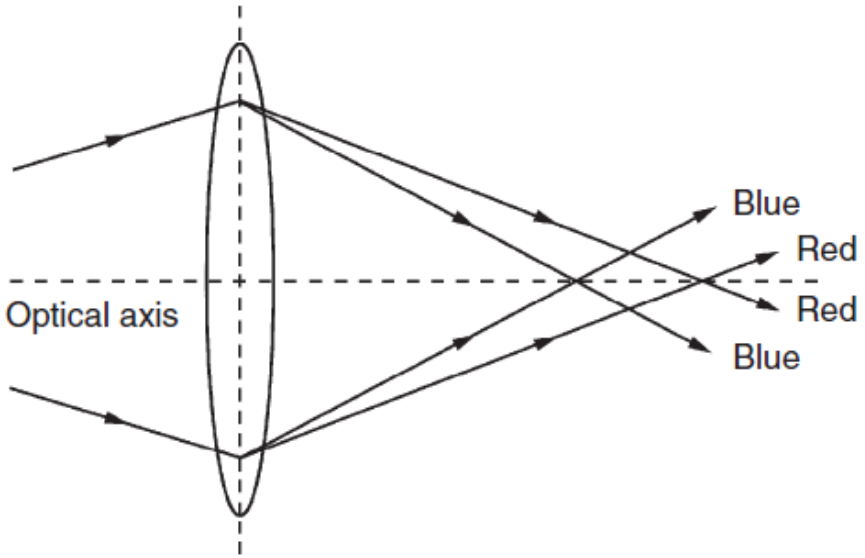
$$D_f \equiv \frac{d}{\tan\alpha}, \qquad d = \frac{1.22\lambda}{NA}$$

Depth of focus

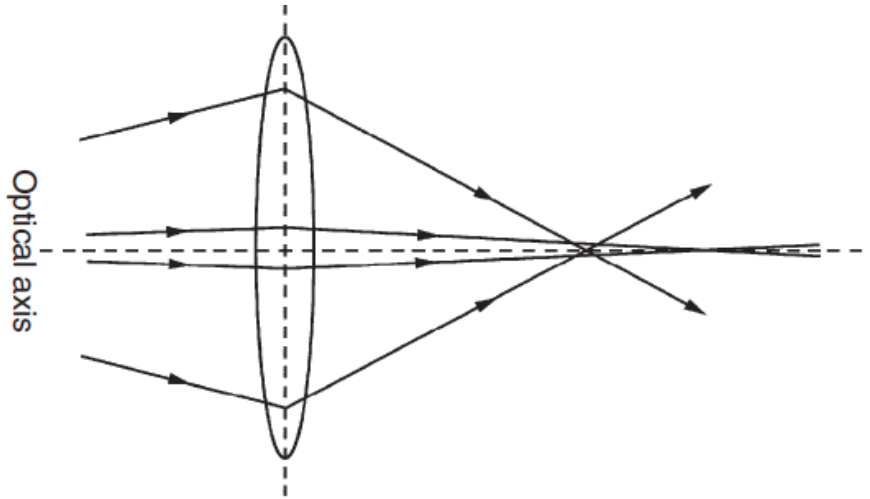
The range of image plane position which is viewed in focus.

Aberration

- Chromatic Aberration

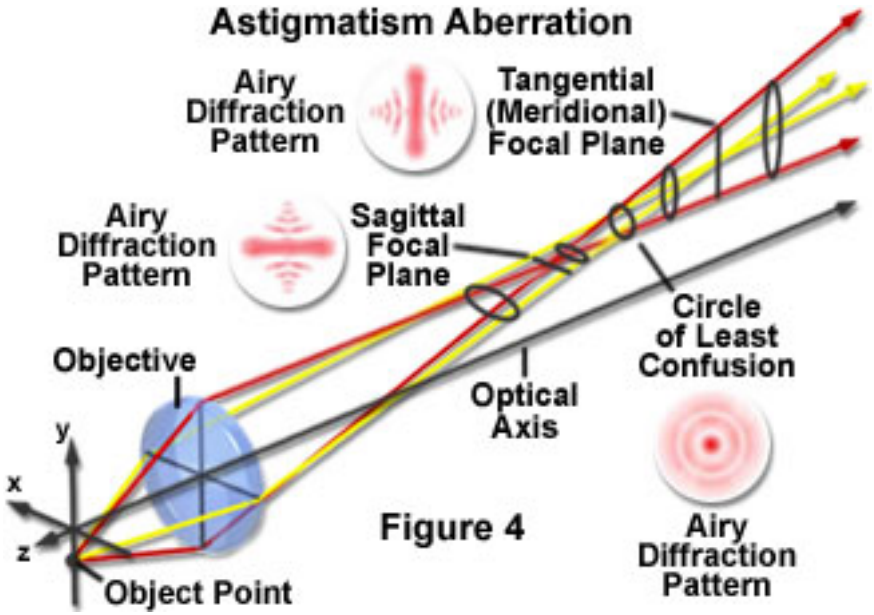


- Spherical Aberration

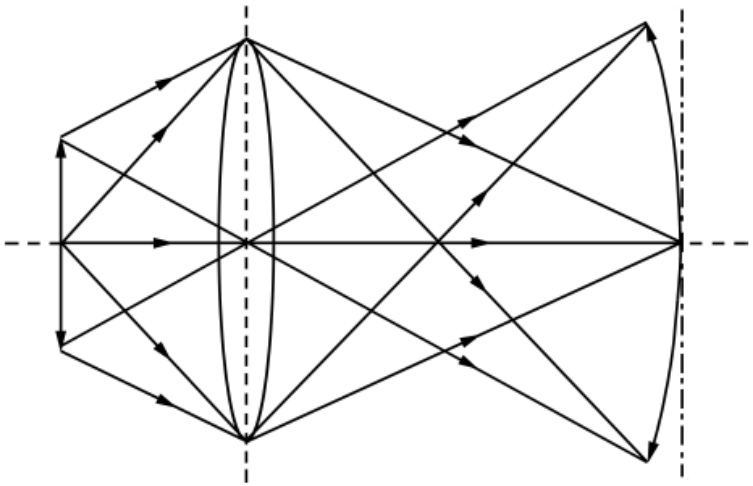


Aberration

- Astigmatism Aberration



- Field Curvature Aberration



Objective lens specification

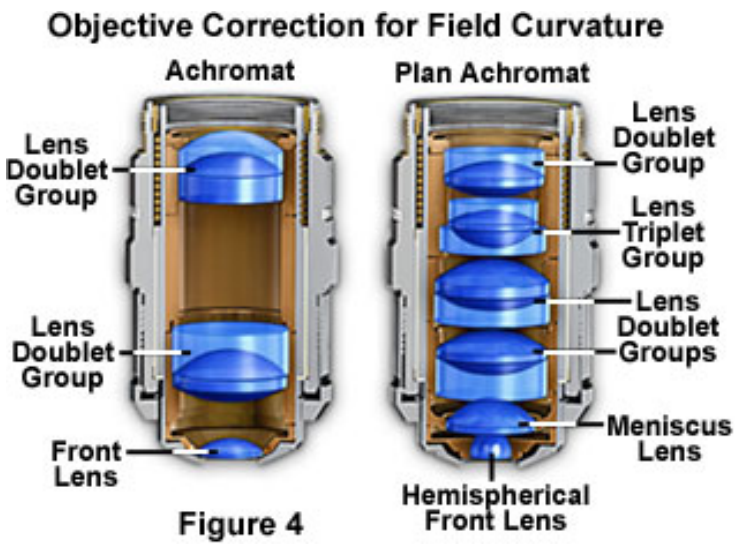
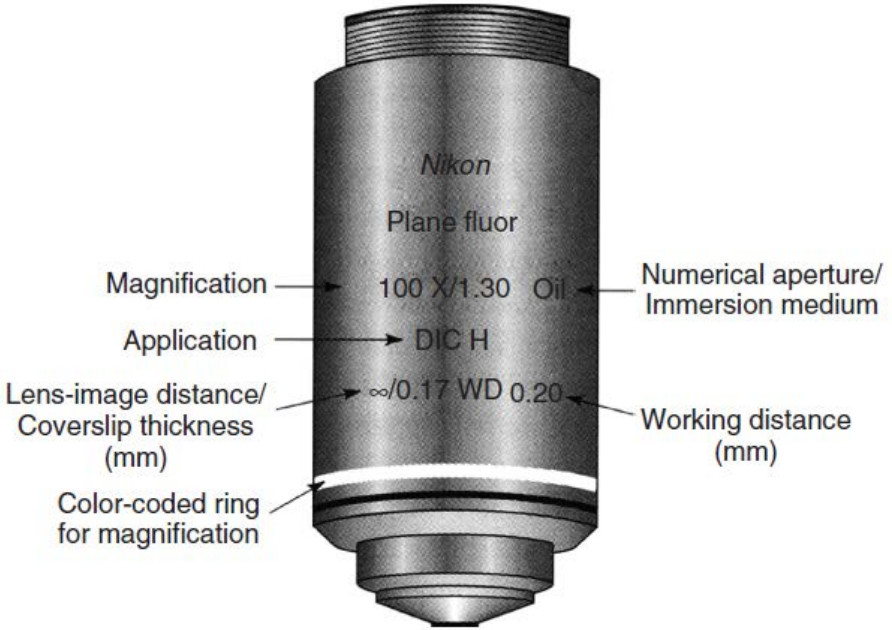
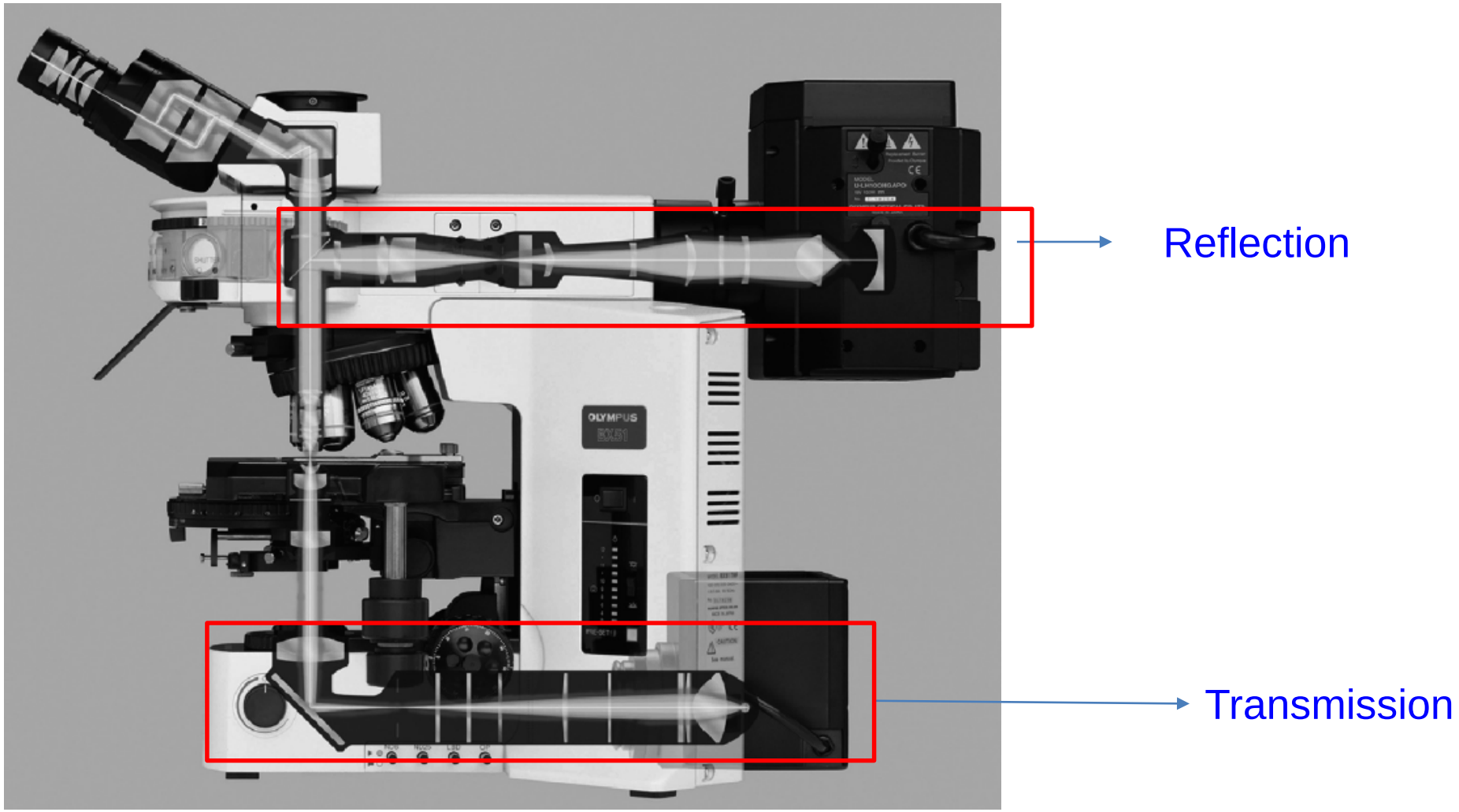


Figure 4



Achromat: correct red and blue
Semiachromat (fluorite): improvement of achromatic aberration
Apochromat: completely eliminate achromatic aberration. And spherical correction of two colors.

Köhler Illumination



Illumination light sources

- Incandescent Lamps

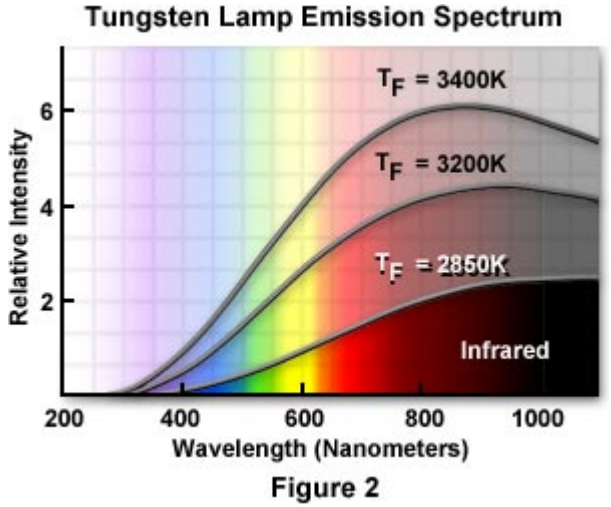


Figure 2

- Arc Lamps

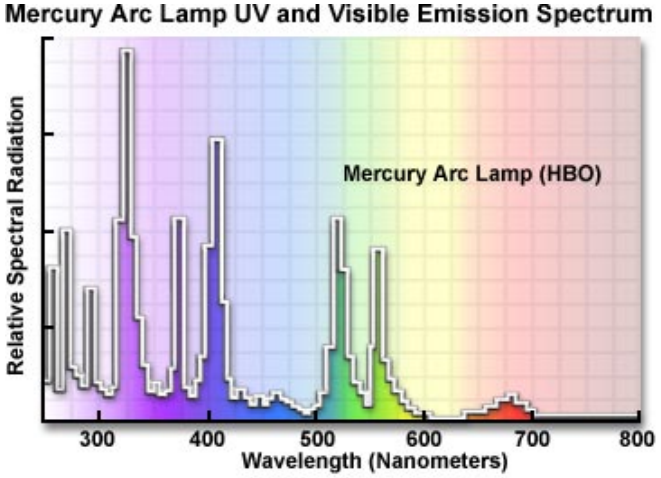
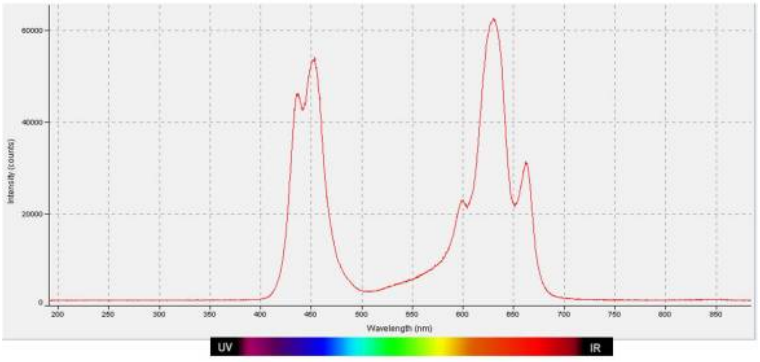


Figure 8

- LED



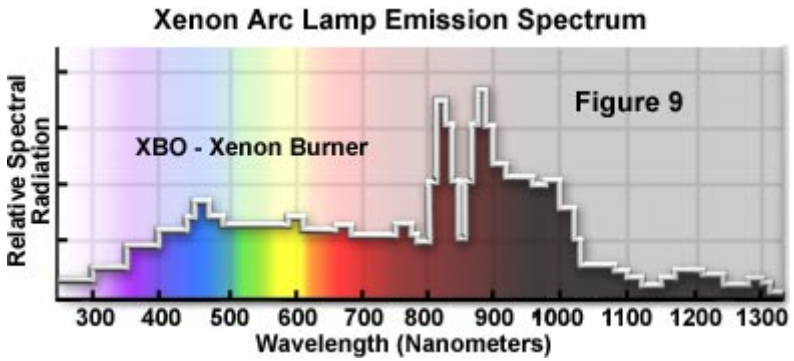
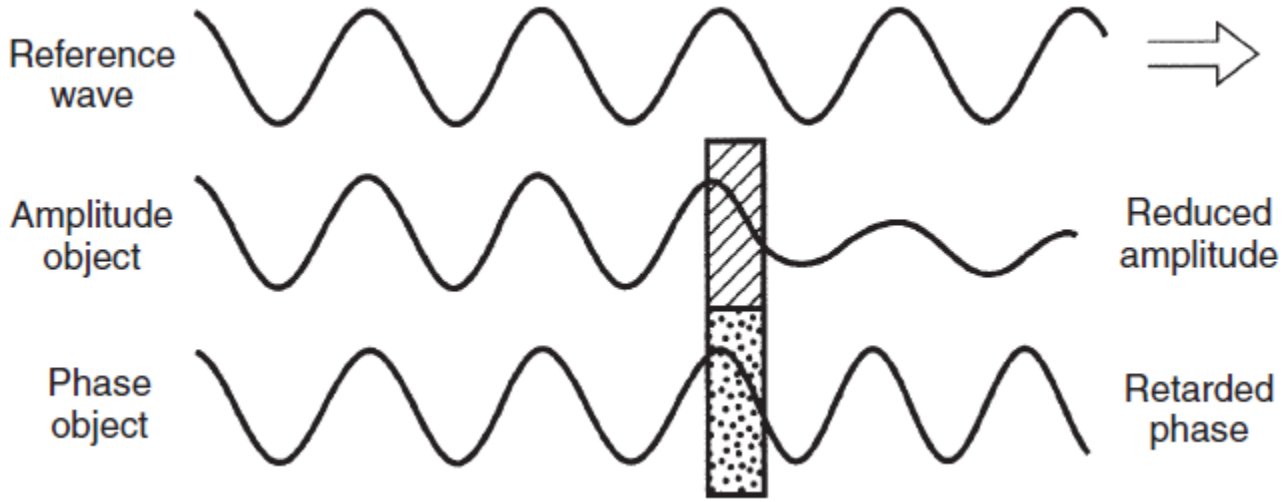


Figure 9

Imaging modes

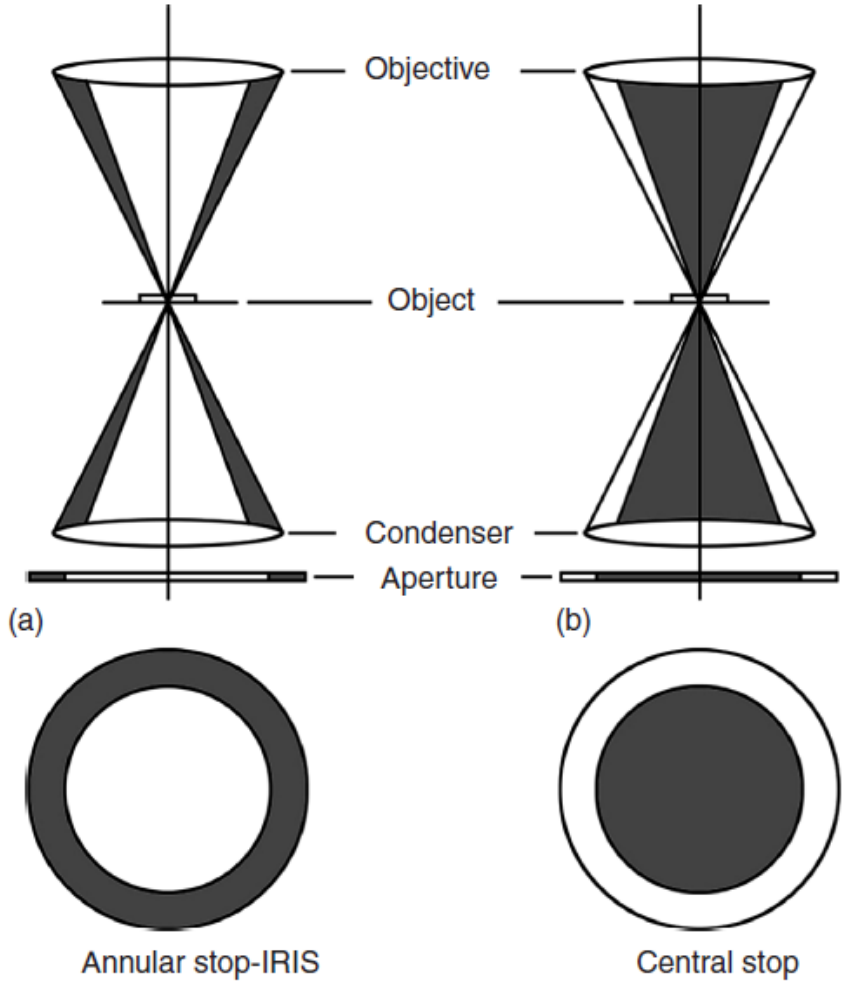


Amplitude object (visible) → Bright-field imaging, Dark-field imaging

Phase object (invisible -> should be converted to intensity) → Phase contrast imaging, Differential interference contrast (DIC) imaging

Fluorescence object (visible) → Fluorescence imaging

Bright-Field and Dark-Field Imaging



Bright-field illumination

Dark-field illumination

Contrast

$$\text{Contrast} = \frac{I_{\text{object}} - I_{\text{background}}}{I_{\text{background}}}$$

Bright-field

Dark-field

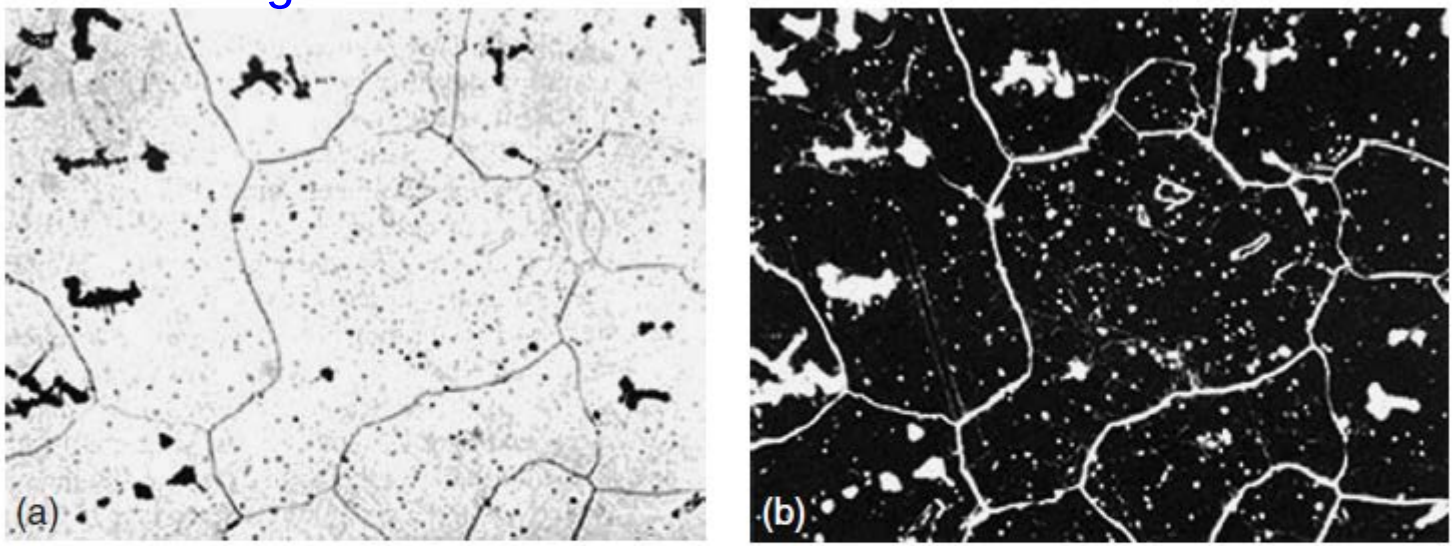
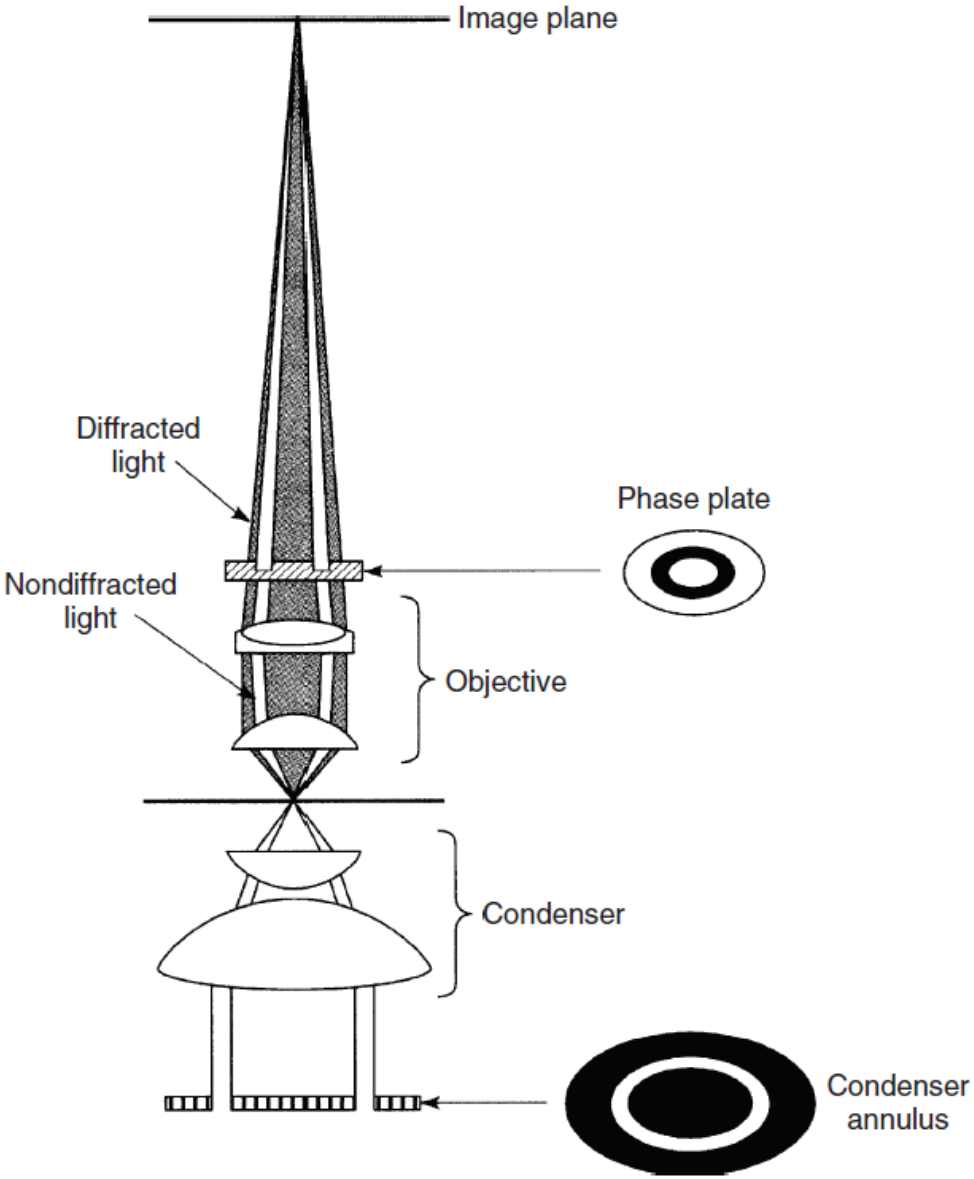
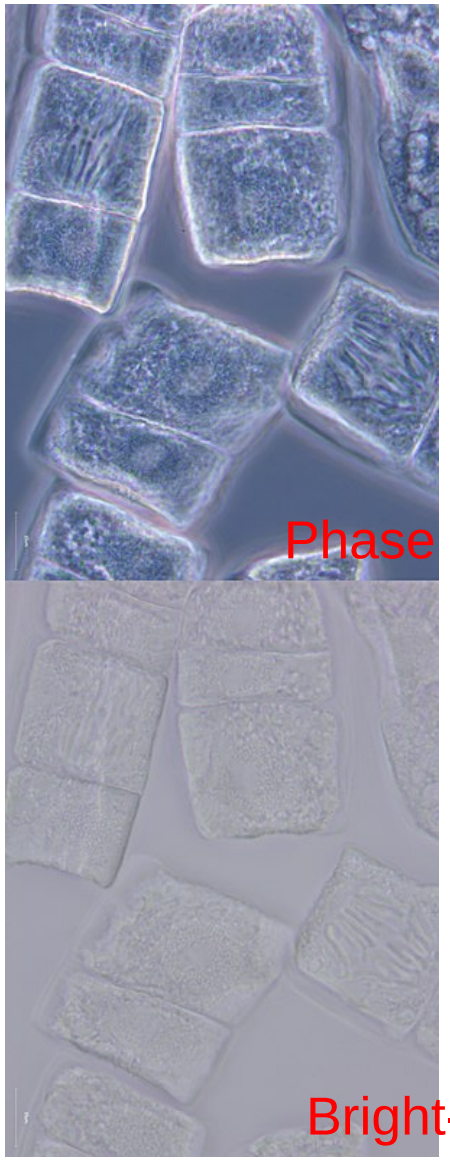


Figure 1.30 Comparison between: (a) bright-field and (b) dark-field images of AISI 1080 high carbon steel. In addition to grain boundaries and oxide particles, annealing twins are revealed in the dark-field image. (Reproduced with permission of ASM International®. All Rights Reserved. *www.asminternational.org*. Ref. [2]. © 1984 ASM International®.)

Phase Contrast Microscopy

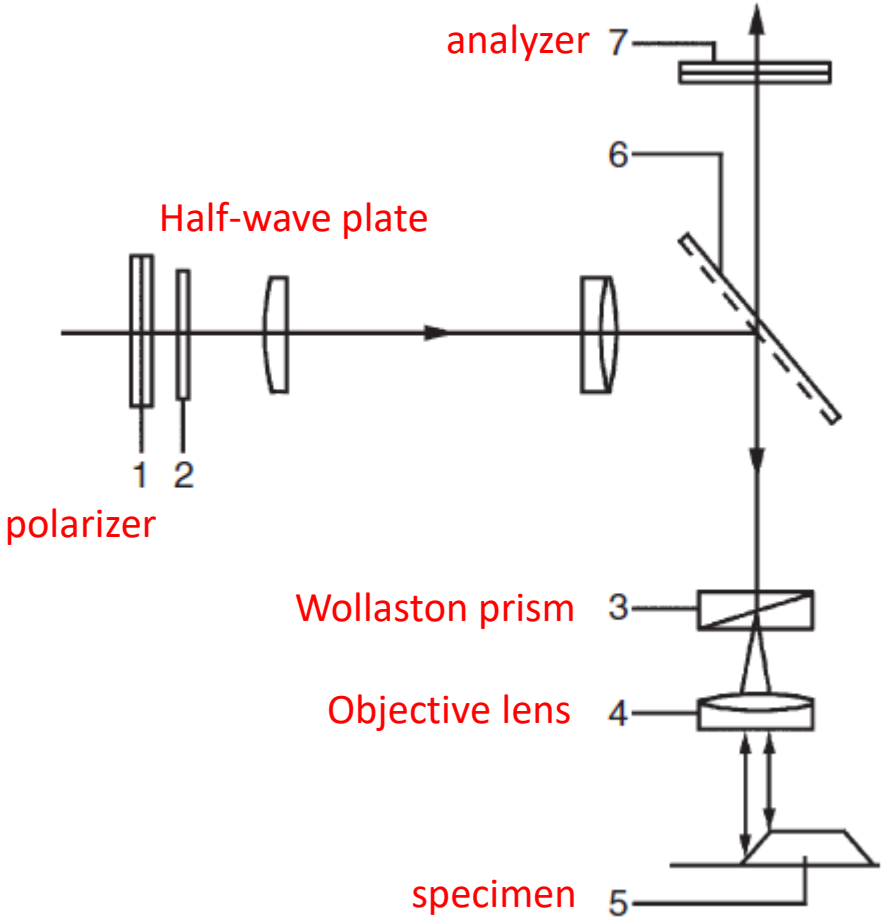


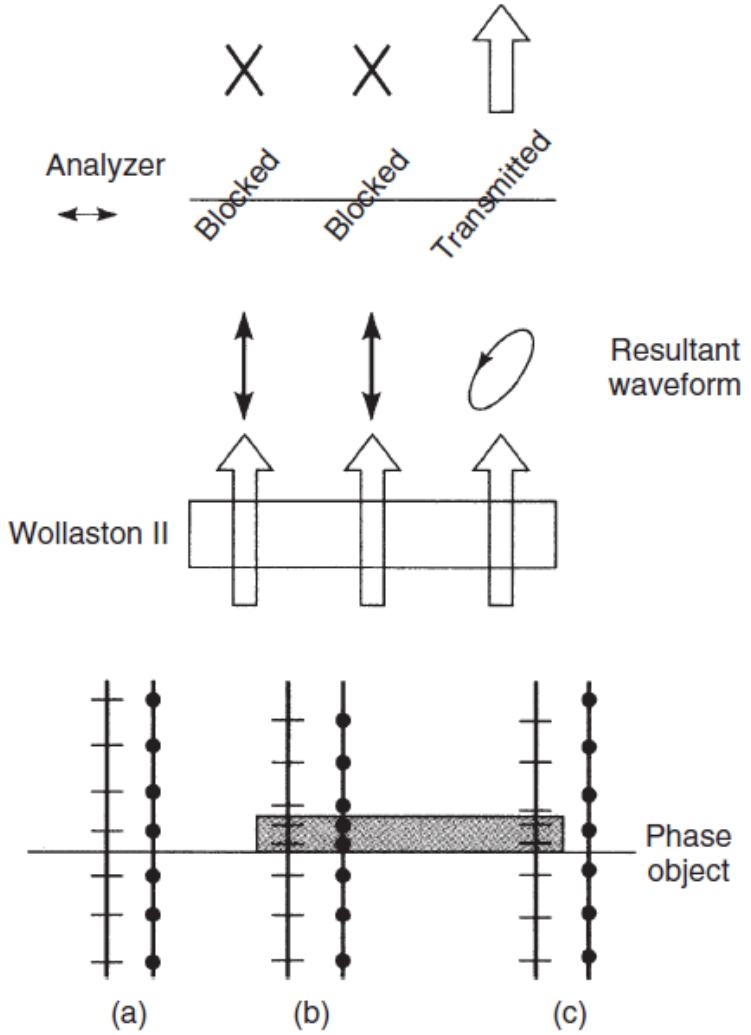


Onion Root Tip Cells

Nomarski Microscopy

Also called Differential Interference Contrast (DIC)

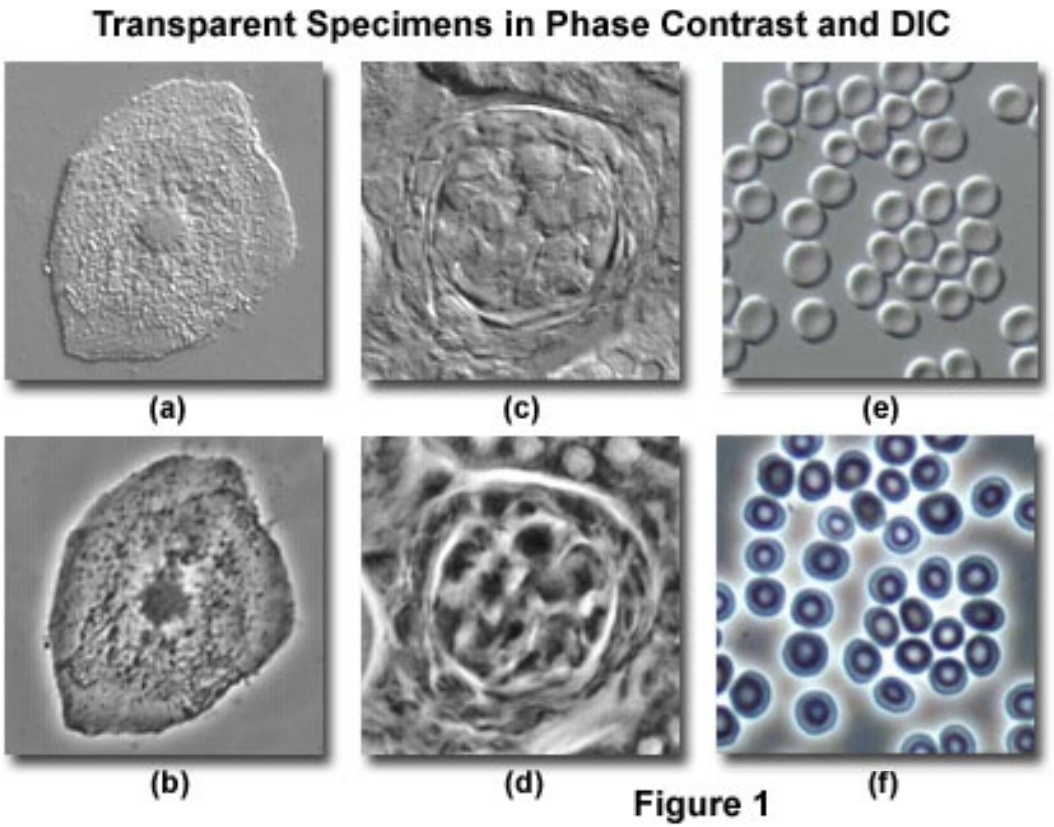




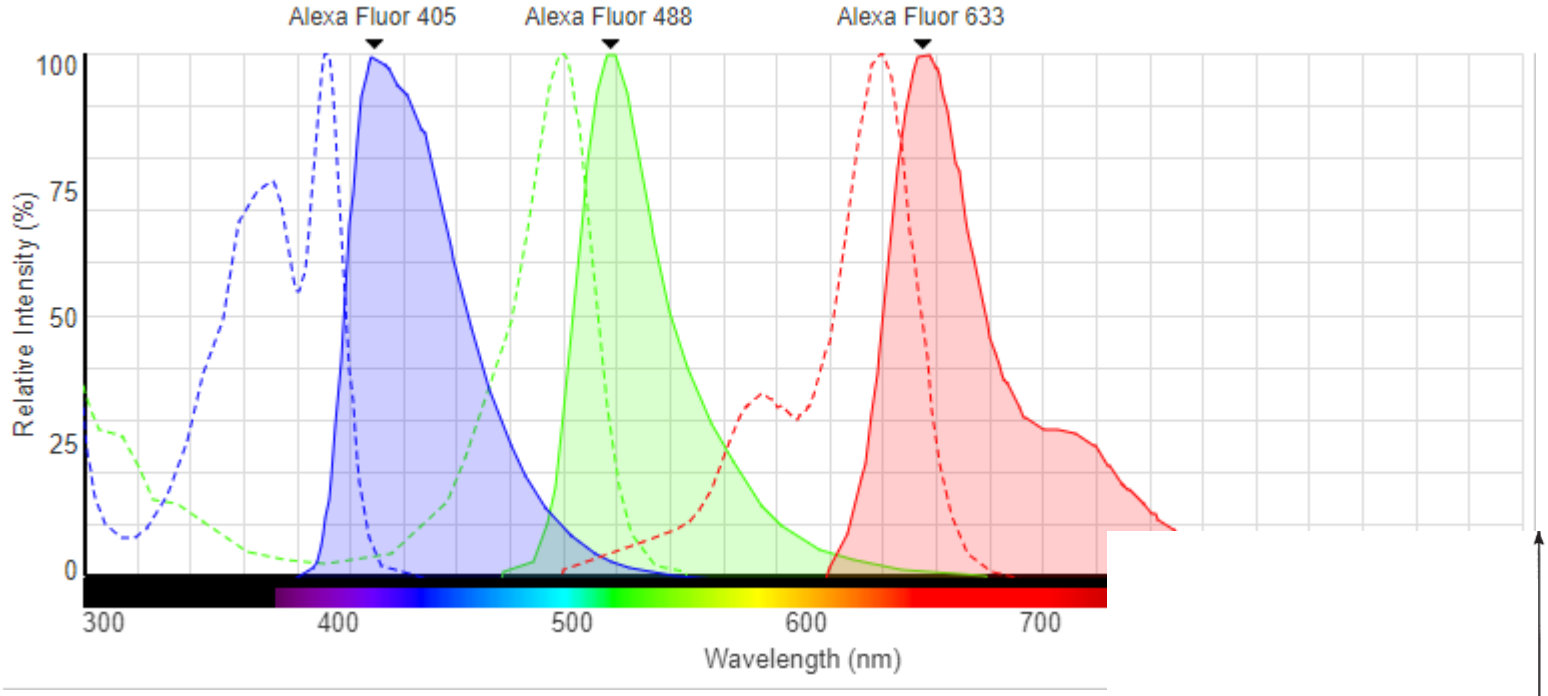
Comparison between phase contrast and DIC

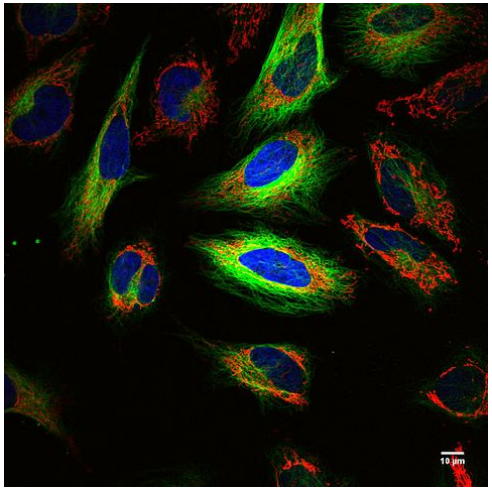
DIC
(more stereo in height)

Phase Contrast
(high contrast, but artifact of halos)

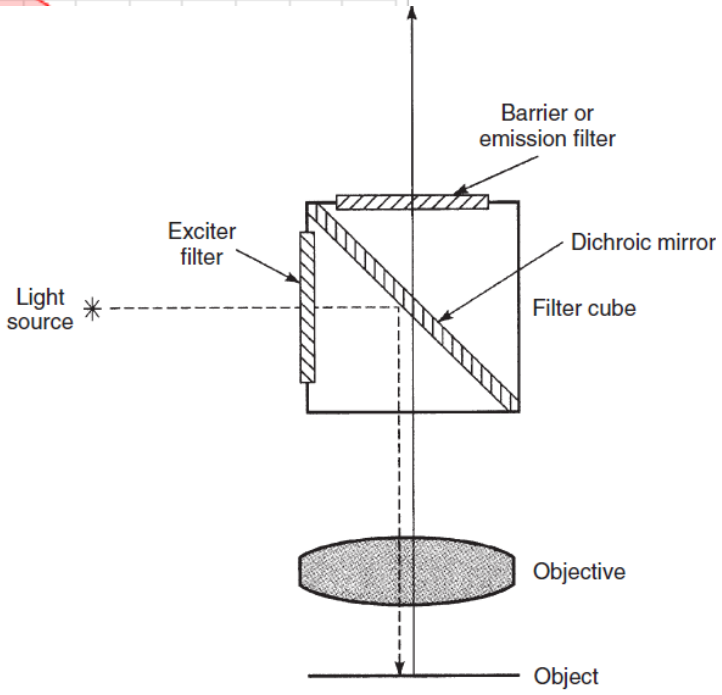


Fluorescence Microscopy

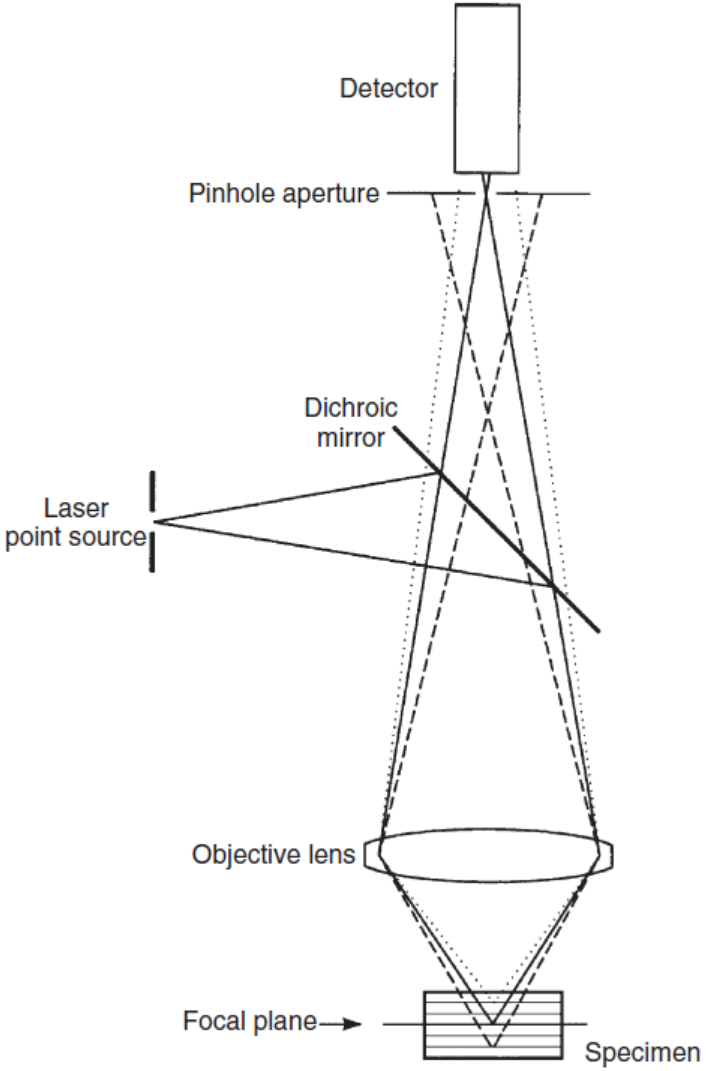




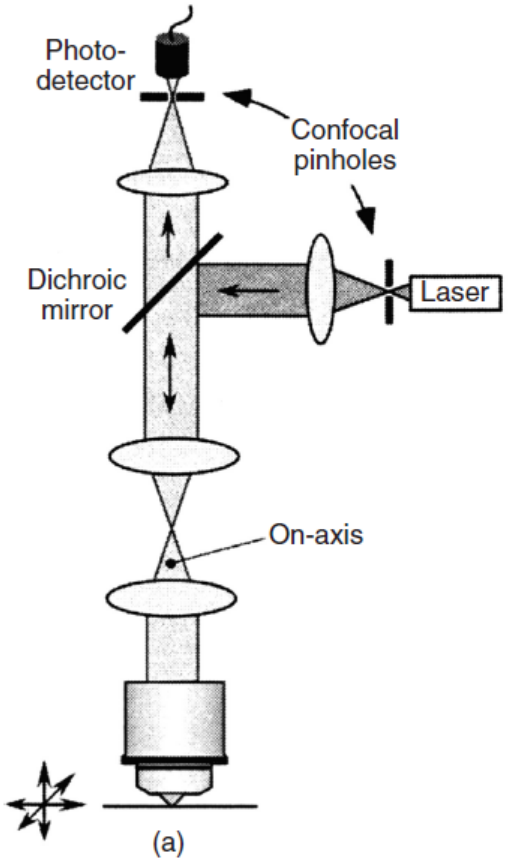
Multicolor fluorescence image of living HeLa cells



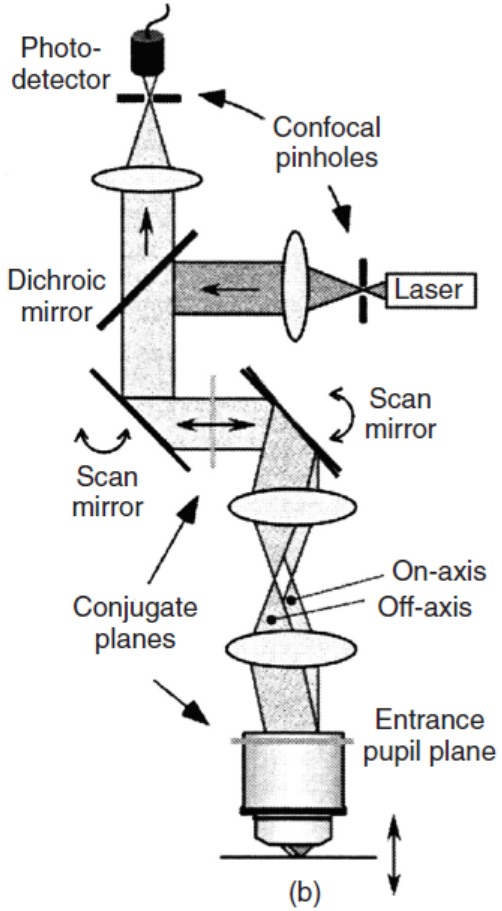
Confocal Microscopy



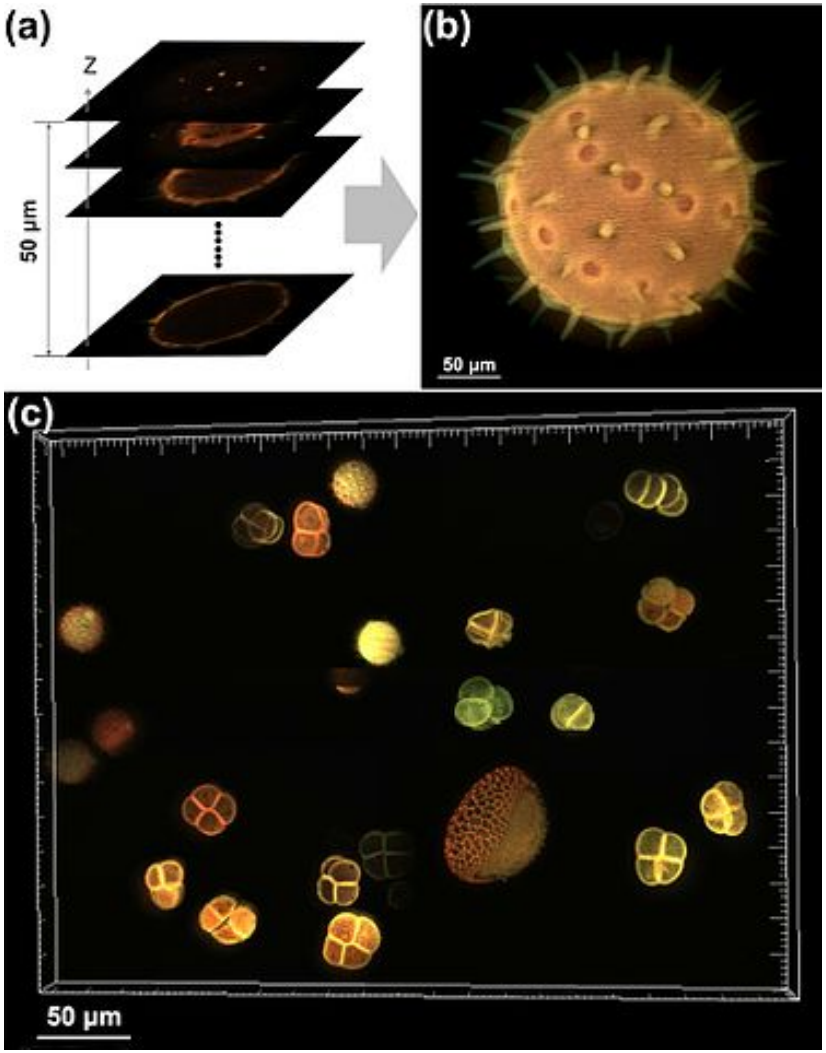
Specimen scanning



Laser scanning



Optical sectioning & 3D image

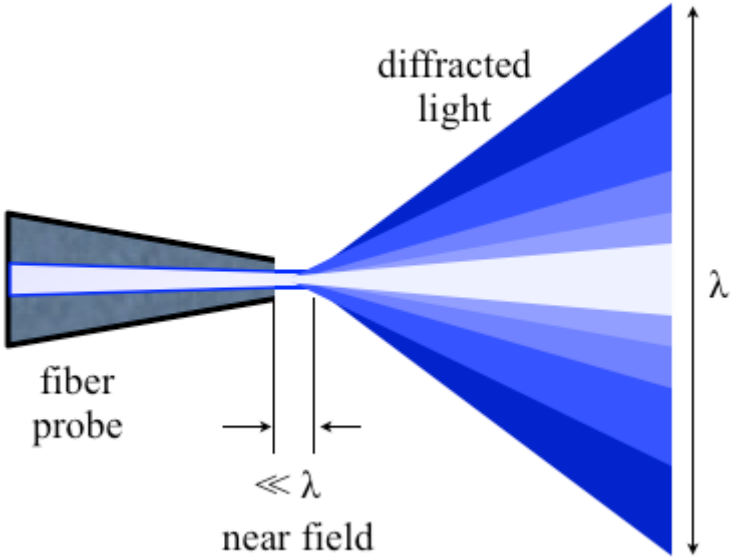


Optical sectioning & 3D fluorescence images of pollens

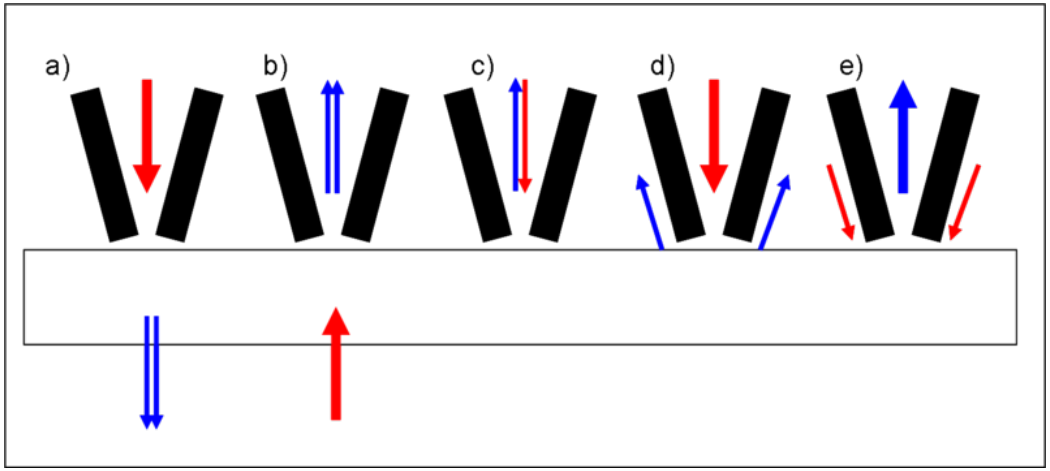
Optical images at nanometer scale

Break the Abbe diffraction limit ~ 200 nm

- Near-field scanning optical microscopy (NSOM), which is a form of scanning probe microscopy (SPM)



Evanescent wave

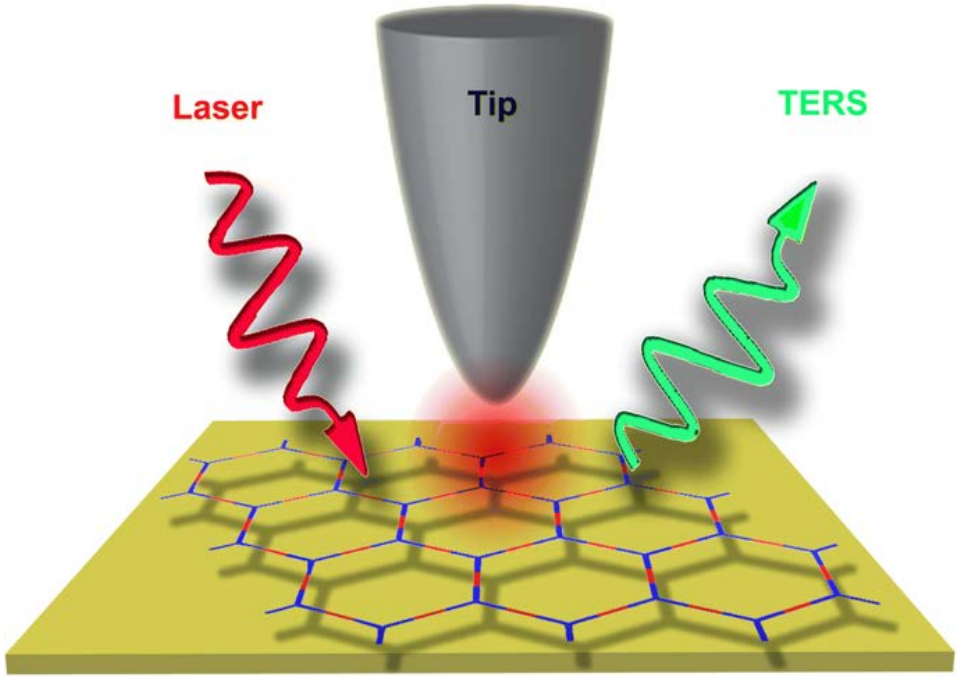


Apertured modes of operation: a) illumination, b) collection, c) illumination collection, d) reflection and e) reflection collection

Optical images at nanometer scale

Break the Abbe diffraction limit ~ 200 nm

- **Apertureless mode**:
  Tip-enhanced Raman Scattering (TERS)



Plasmon Resonance:
Enhanced light field!!

Typical enhancement of Raman Signal: $>10^4$

Far-field optical microscopy with nanometer scale resolution

© Nobel Media AB. Photo: A. Mahmoud

Eric Betzig

Prize share: 1/3

© Nobel Media AB. Photo: A. Mahmoud

Stefan W. Hell

Prize share: 1/3

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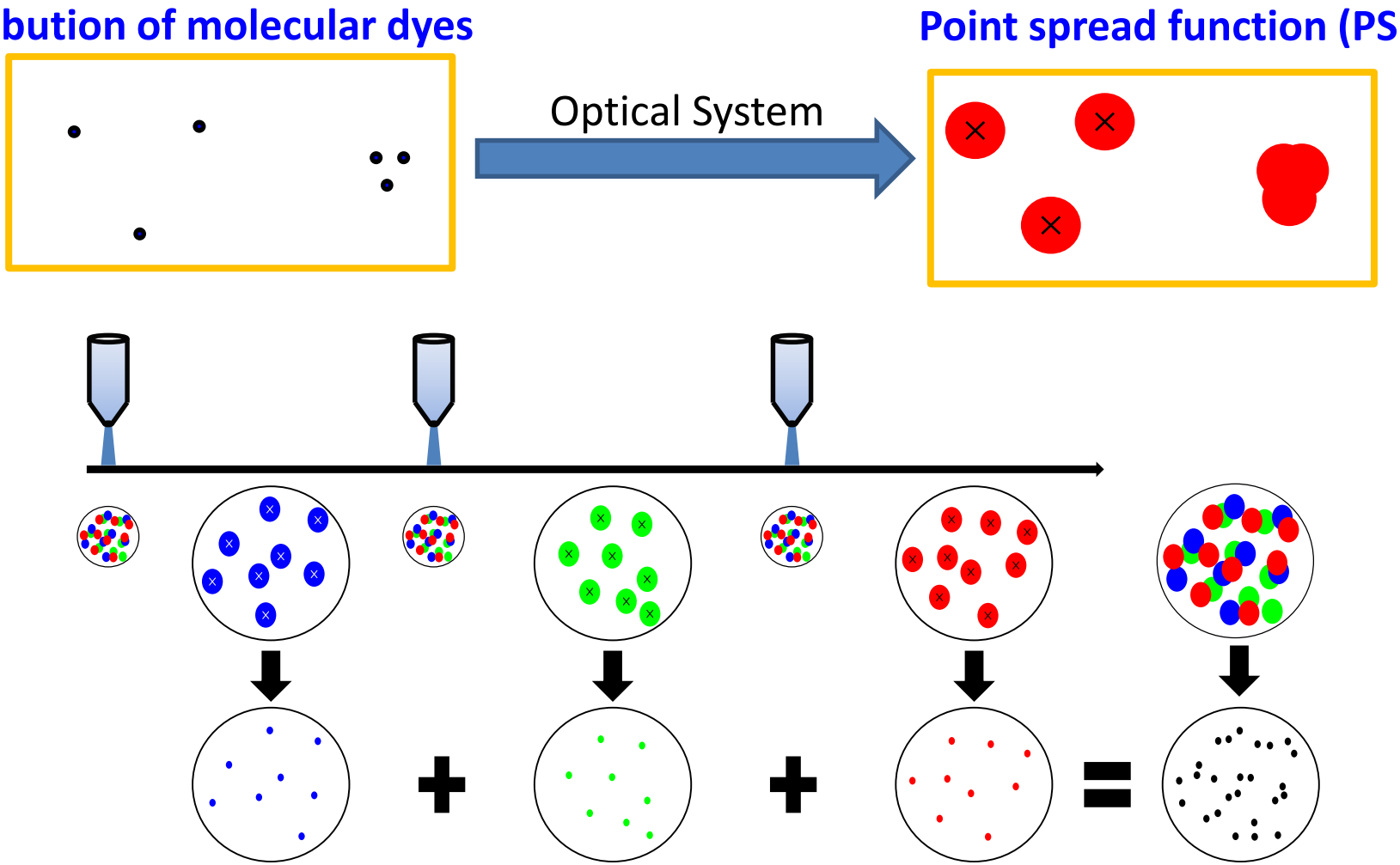
William E. Moerner

Prize share: 1/3

- The Nobel Prize in Chemistry 2014 was awarded for **super-resolved fluorescence microscopy**.

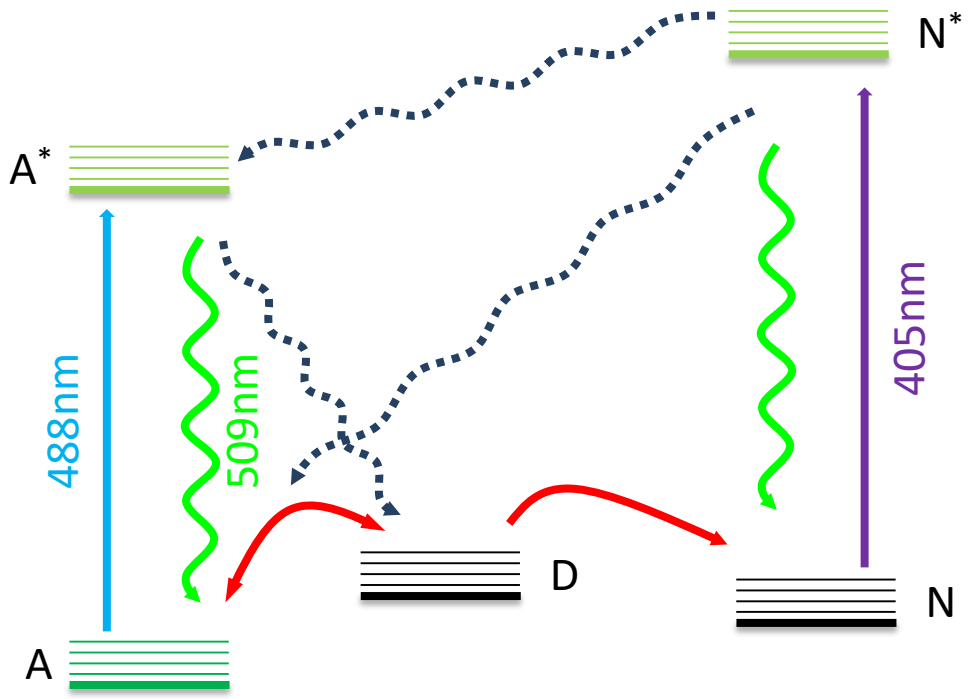
Localization Microscopy



Betzig E (1995) Proposed method for molecular optical imaging. *Opt Lett.*20:237-239.

Switching ON/OFF the fluorescence

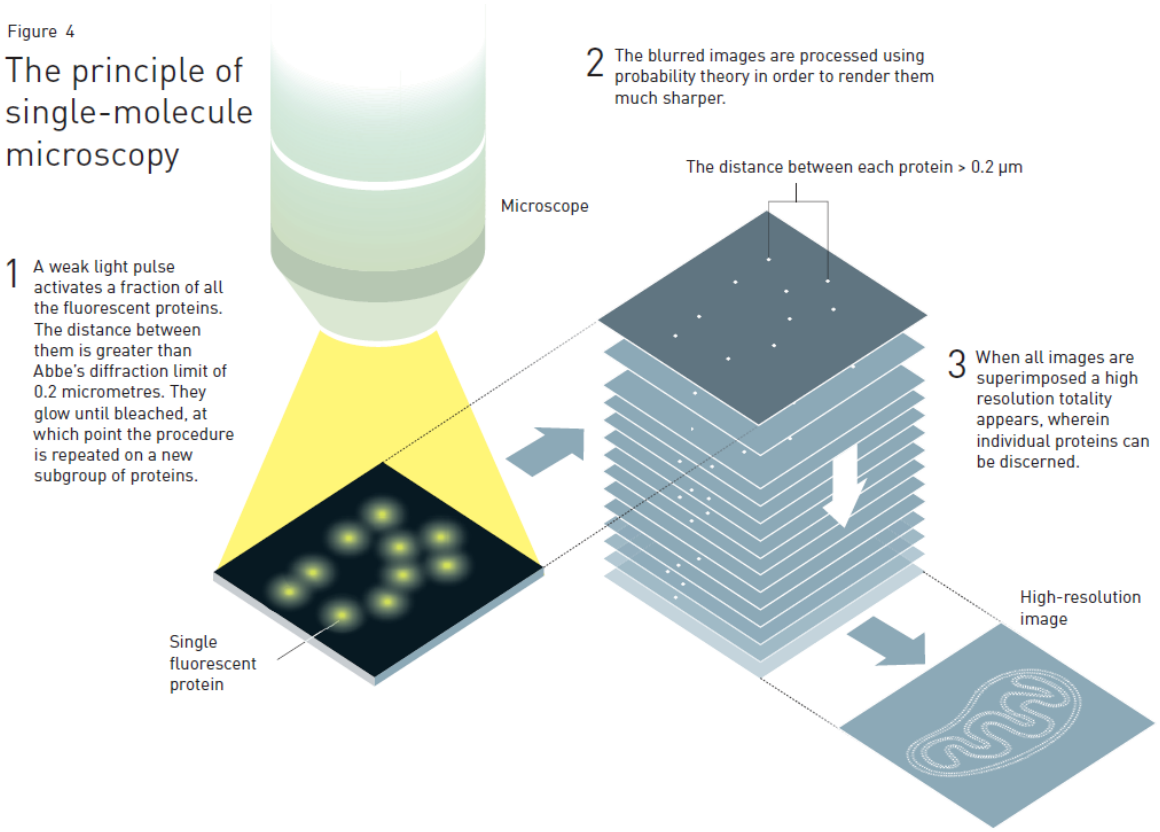
Green fluorescent protein

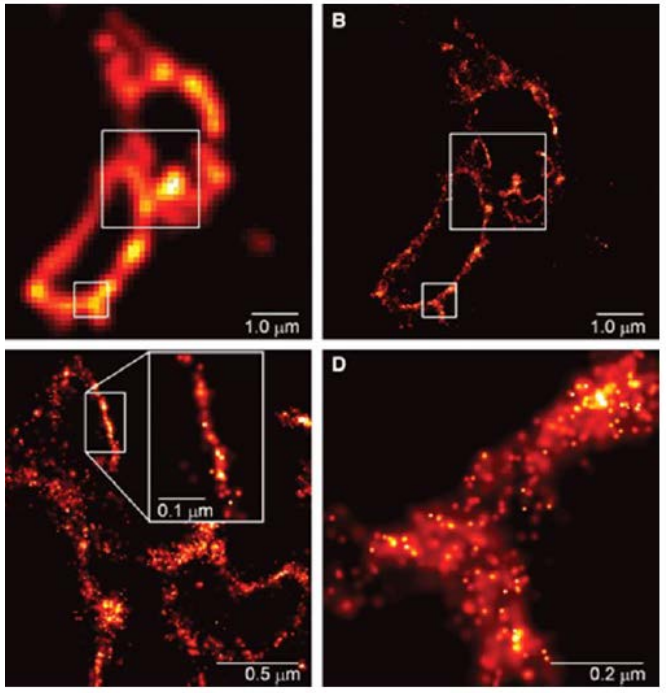


Dickson RM, Cubitt AB, Tsien RY and Moerner WE (1997) On/off blinking and switching behaviour of single molecules of green fluorescent protein. *Nature* 388:355-358.

Photoactivated Localization Microscopy (PALM)

Betzig E, Hess HF et al. (2006) Imaging intracellular fluorescent proteins at nanometer resolution. *Science*313:1642-1645.



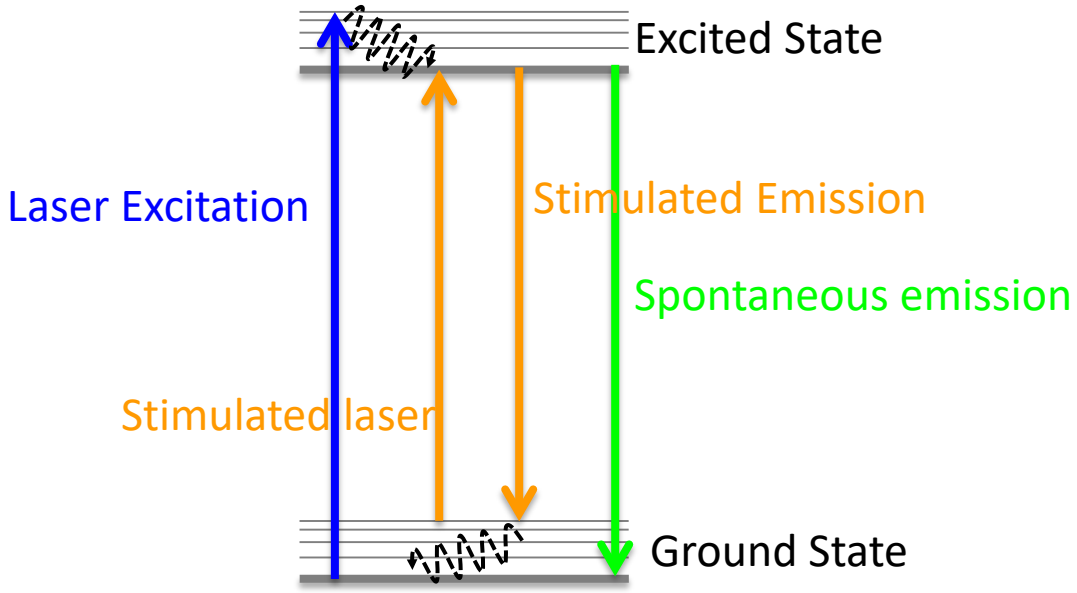


Similar technique: Stochastic optical reconstruction microscopy (STORM)

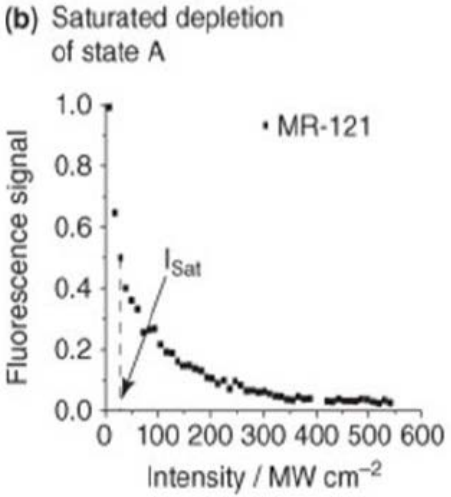
Rust MJ, Bates M and Zhuang X (2006) Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy. (STORM) *Nat Methods* 3:793-795

Switching ON/OFF the fluorescence by Stimulated Emission

Electron energy relaxation



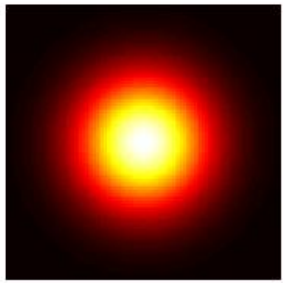
Stimulated emission depletion (STED)



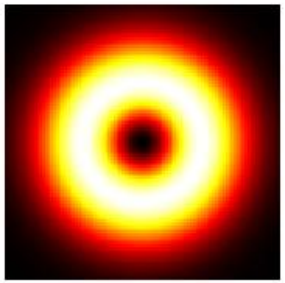
Hell *et al.*, *Current Opinion in Neurobiology* 2004, 14:599–609

Stimulated Emission Depletion (STED) Microscopy

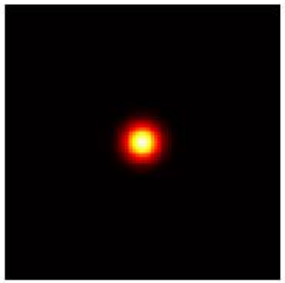
Excitation Laser spot

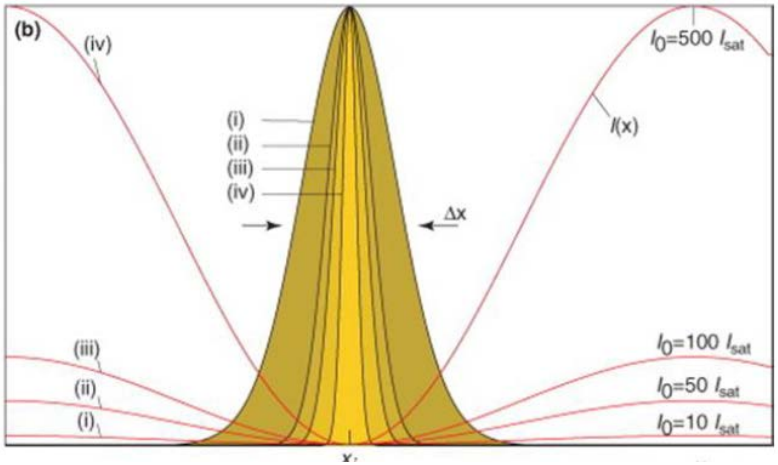


STED laser spot



Fluorescence spot





Excitation

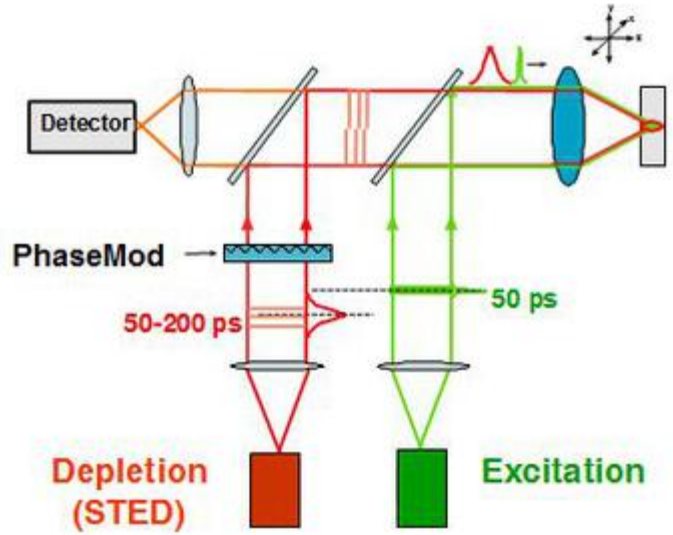
STED spots with donut shape

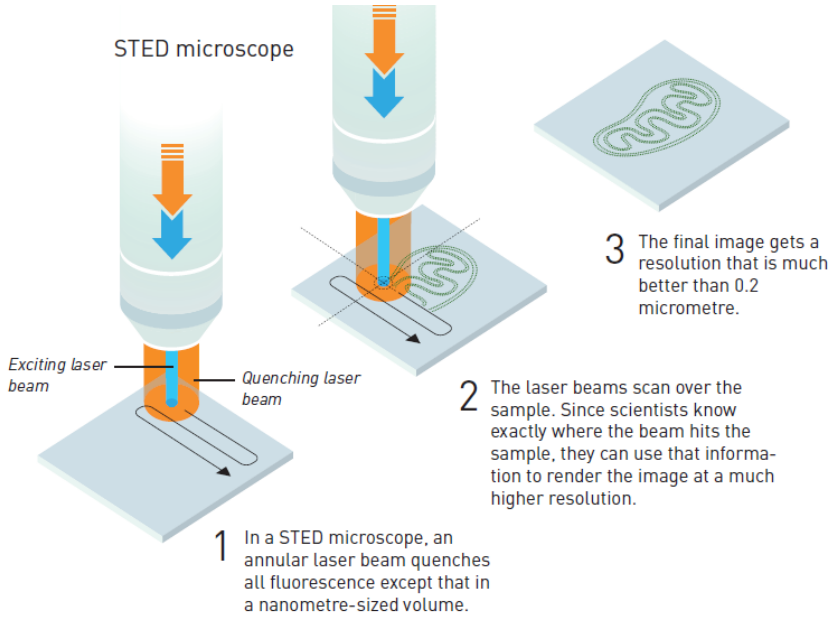
Diffraction limited spot sizes

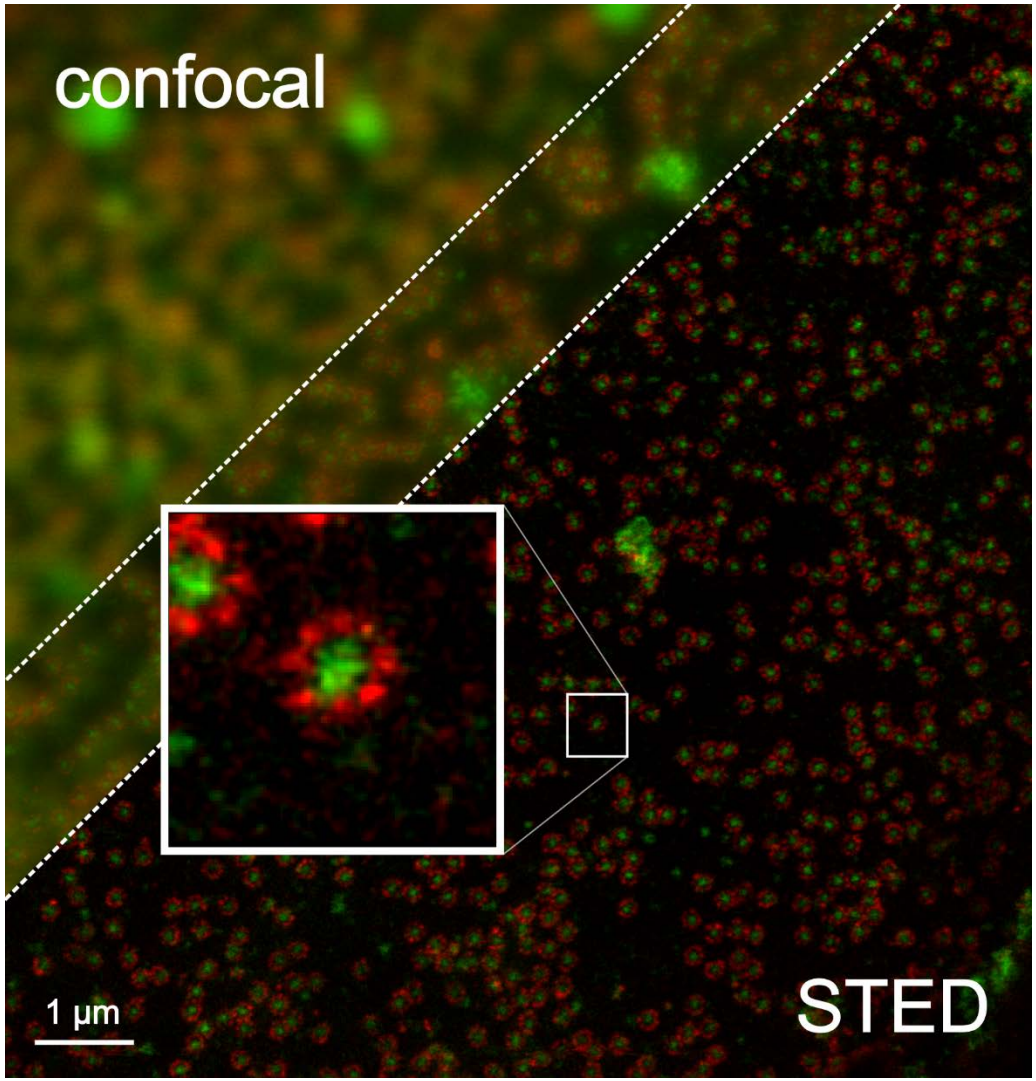
Intensity of STED

Fluorescence

STED Microscopy







Proteins of the nucleus, labelled with fluorescent dyes. Red: gp210 glycoprotein. Green: Several proteins in the central channel

References

- Yang Leng, Materials Characterization: Introduction to Microscopic and Spectroscopic Methods, 2nd Edition, Wiley, Chapter 1

- Olympus Microscopy Resource Center Website (https://www.olympus-lifescience.com/en/microscope-resource/)

- 2014 Chemistry Nobel Prize Website (https://www.nobelprize.org/prizes/chemistry/2014/popular-information/) (https://www.nobelprize.org/prizes/chemistry/2014/advanced-information/)