Optical microscopy for nanoscale imaging : STED microscopy

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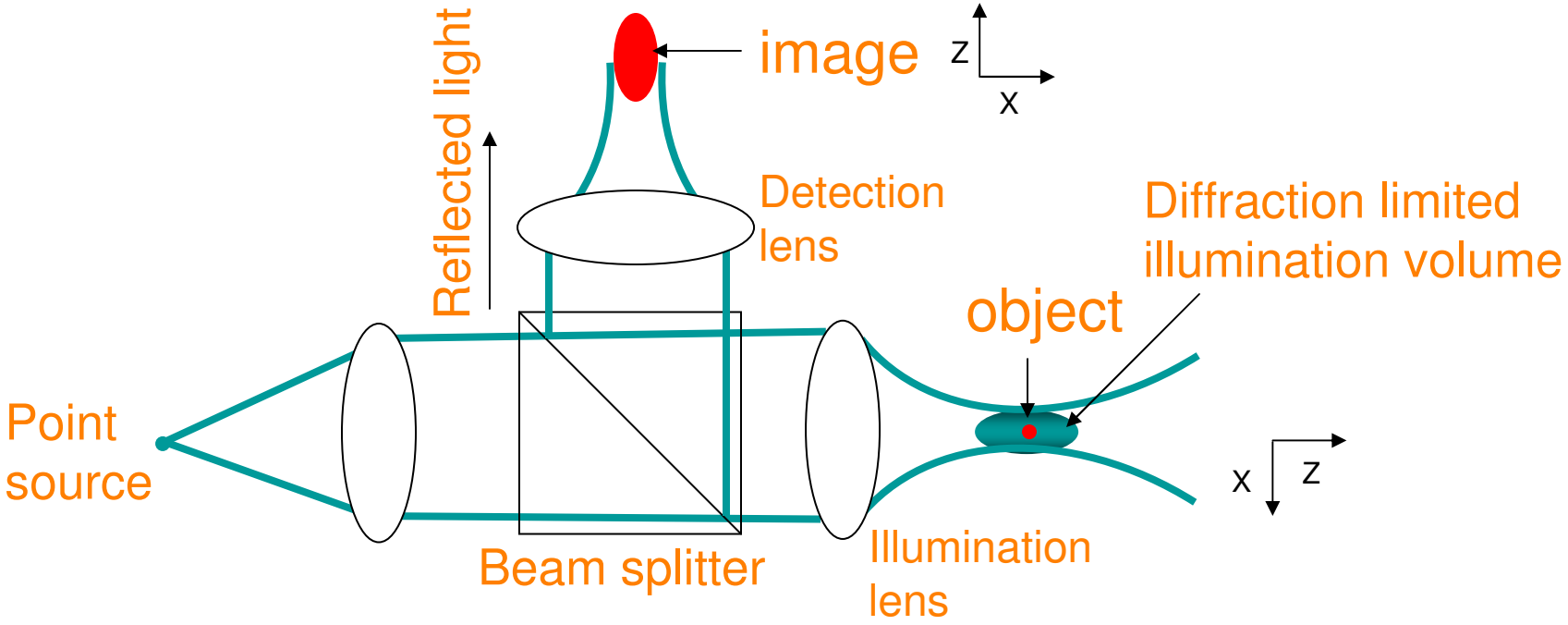
Contents

Far field microscopy

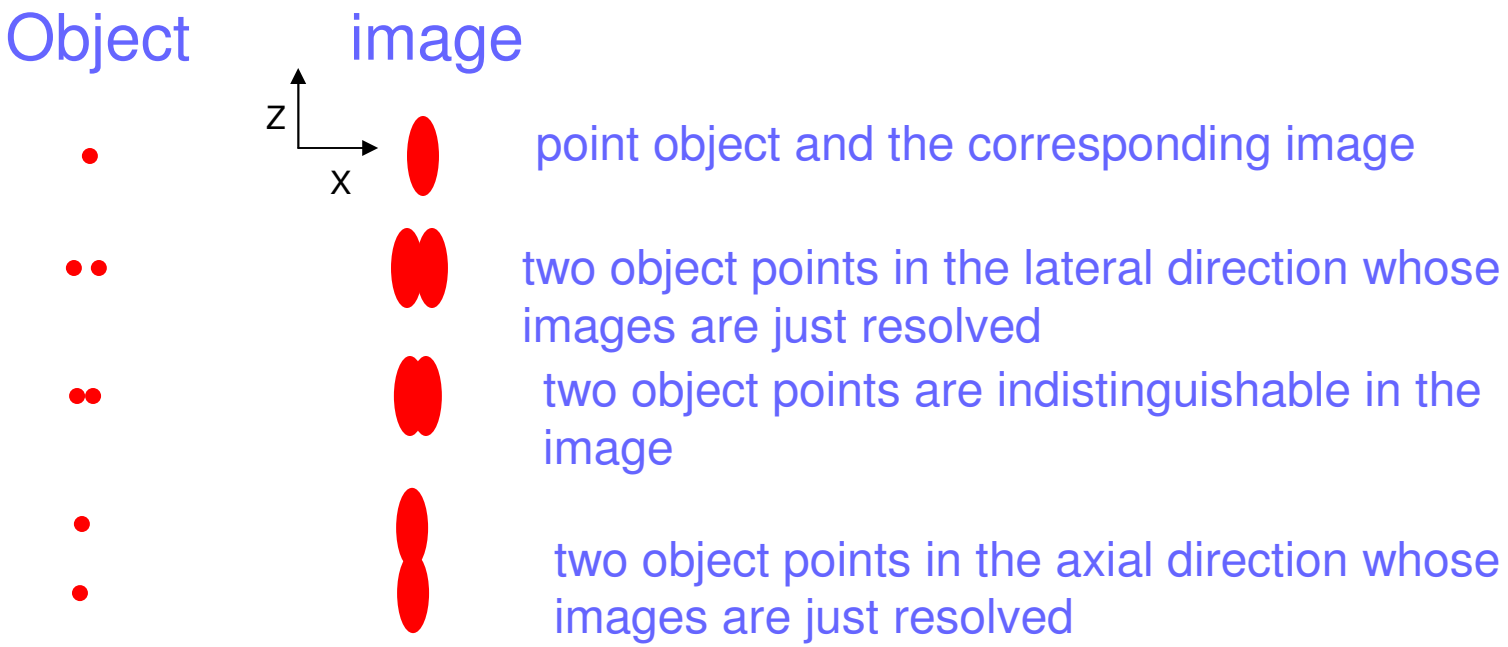
- Nanoscale imaging in near field microscopes
  - For surface study only
  - Vulnerability to artifacts
  - Not suitable for soft materials
- Study of many natural processes requires knowledge about 3D volume of the sample
- Far field microscope can form a 3D image of a 3D object
  - Imaging deep into a biological sample
  - Non-invasive imaging

A simple microscope



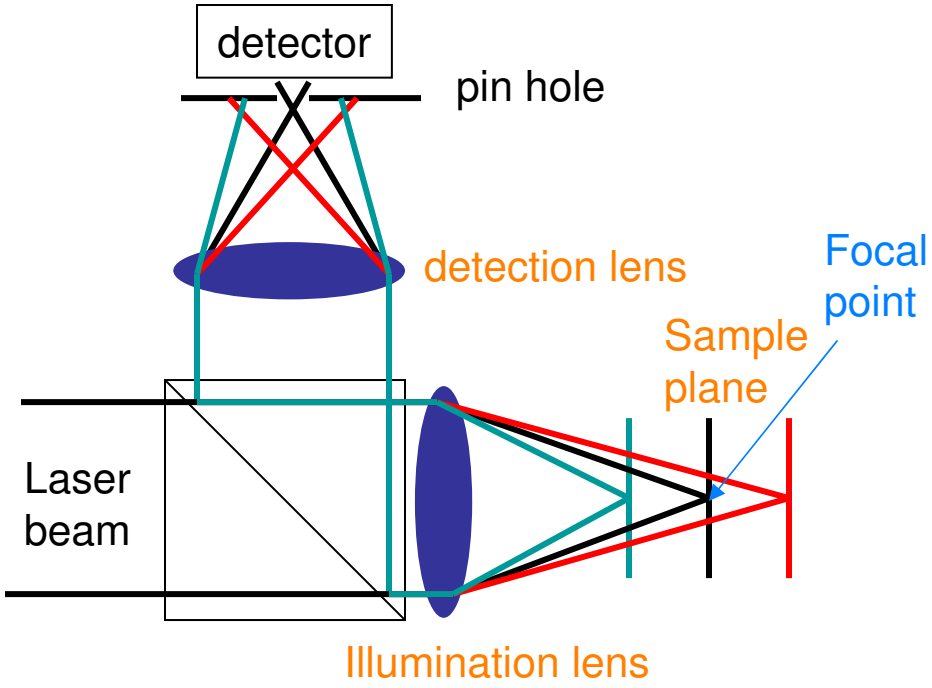
- Collimated beam of wavelength λ is focused by L1 to a diffraction limited volume
- The illumination volume depends on λ, focal length and diameter of the illumination lens
- A point object is imaged into a diffraction limited volume in the image space

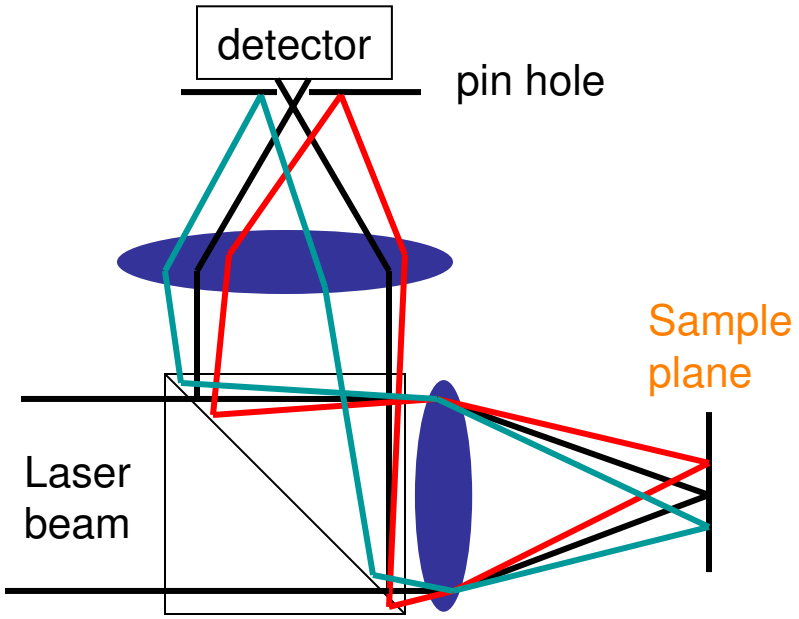
Resolution of a microscope



- Resolution: minimum separation between two point objects whose images are just resolved
  - Contributions from diffractions due to the illumination and detection lenses
- Axial resolution is worse than lateral resolution

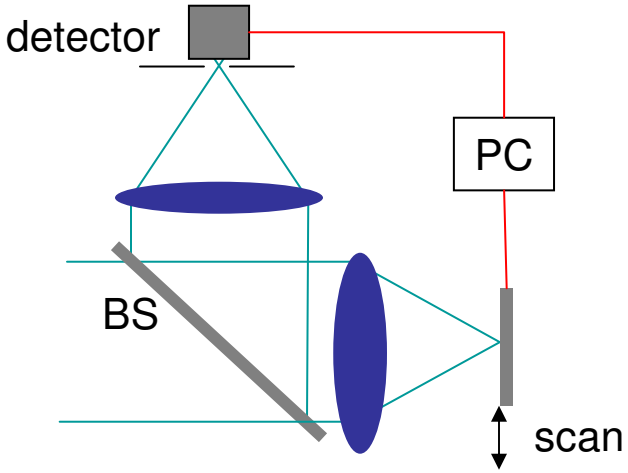
Optical sectioning with a confocal microscope

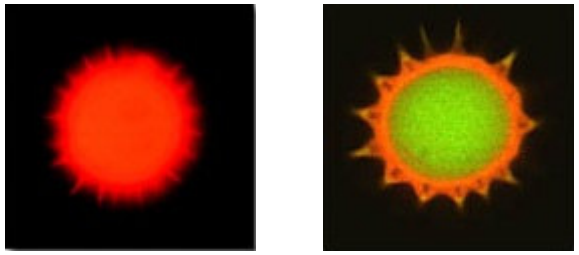




- Confocal arrangement of focal point and pinhole blocks light from out of focus planes or points away from the optic axis
- The detector receives light mostly from the focal point
  - Image, free of out of focus blur, of a point object located at the focal point

Optical sectioning with a confocal microscope



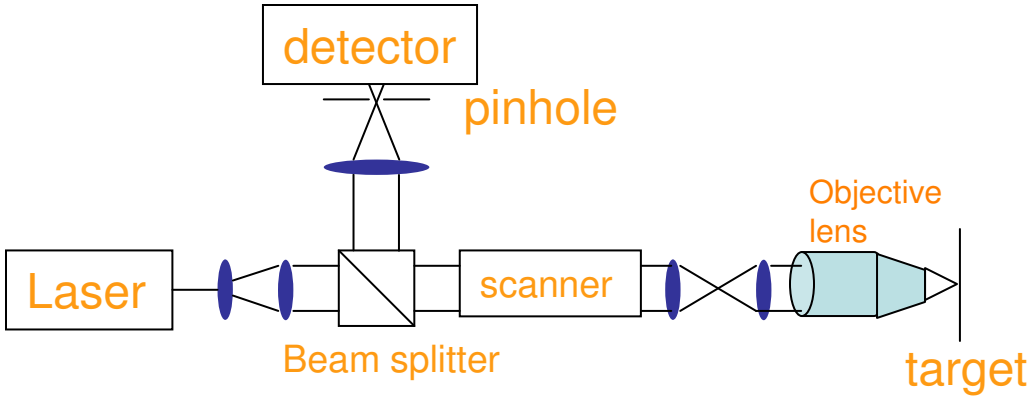


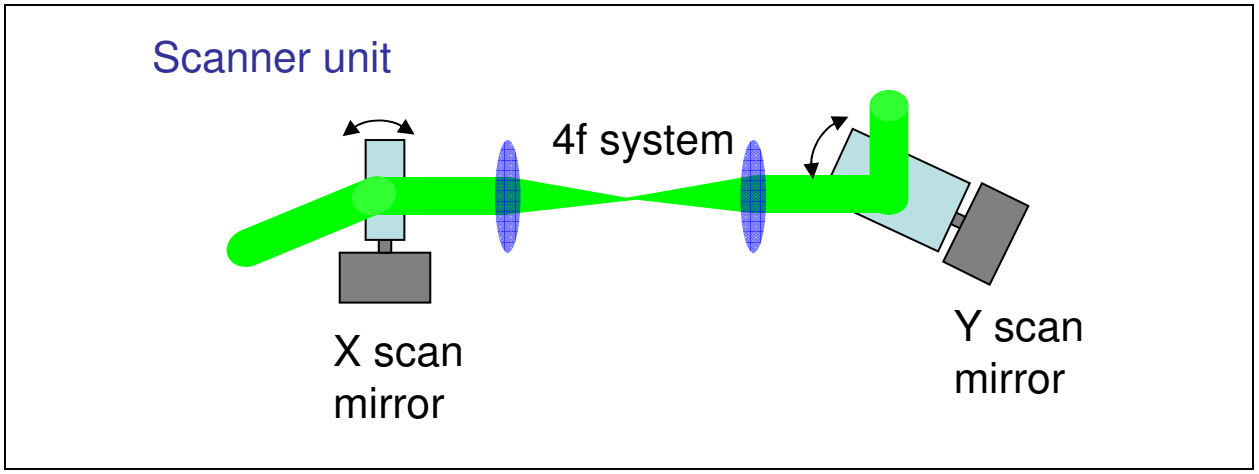
Wide field image Confocal image

(Source :www.olympusfluoview.com)

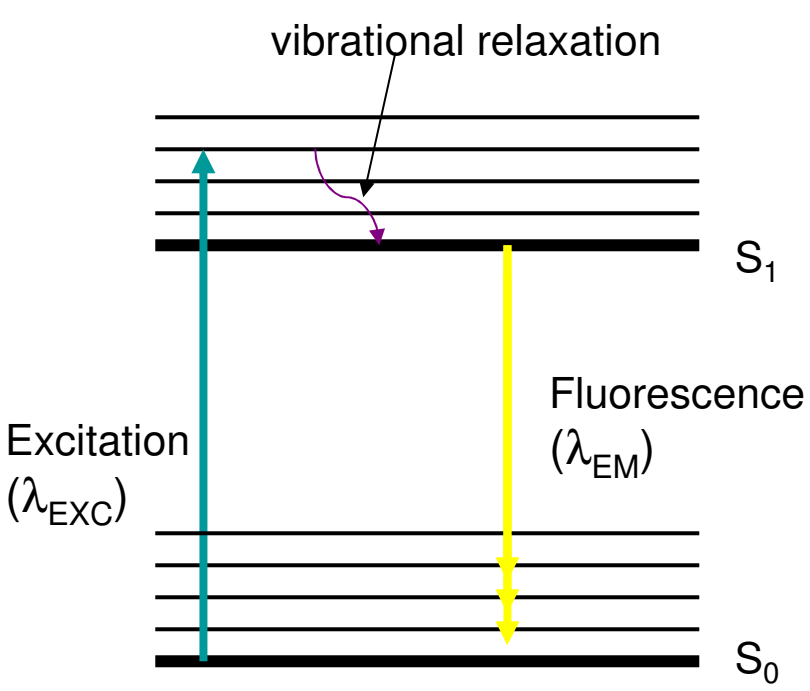
- Either the sample holding stage or the illumination spot is scanned
  - Scanning is controlled by a PC
- For each object point at the illumination spot, the detector signal is stored in the PC
- Results in an optically sectioned image (image corresponds to a sharply defined object plane, devoid of out of focus blur) of the sample
- Much better axial and marginally better lateral resolutions than a conventional (wide field) microscope
- Best resolution: lateral=~$\lambda/2$, axial=~λ

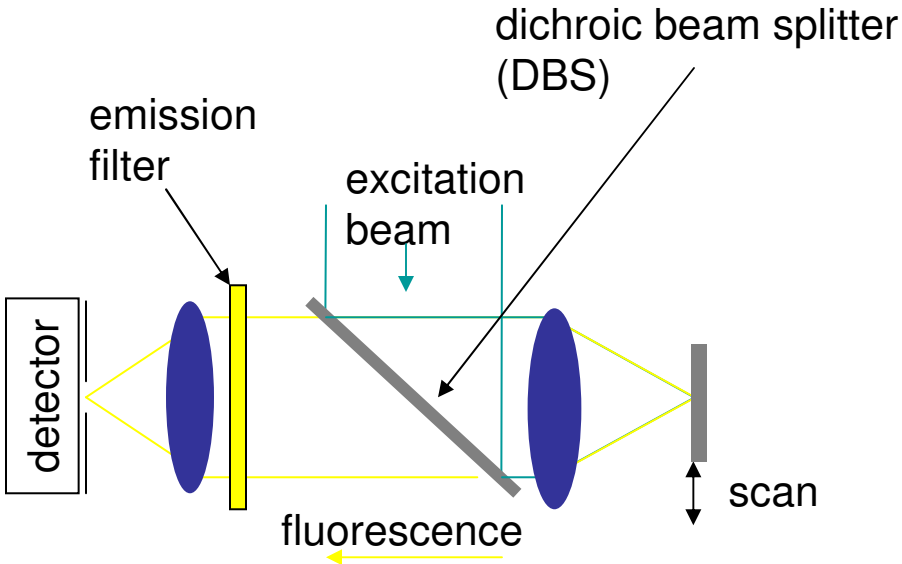
A beam scanning confocal microscope setup





Confocal fluorescence microscope

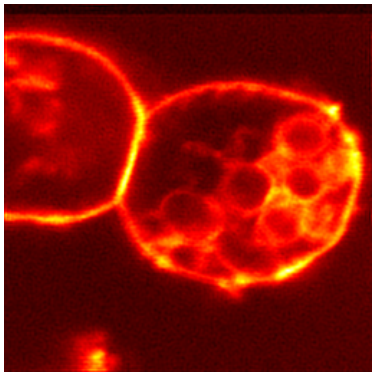




- Molecules (fluorophores) are excited with a laser beam of wavelength (λ_{EXC}), which than undergo a series of spontaneous emissions called fluorescence at the mean wavelength (λ_{EM})
- DBS: reflects λ_{EXC} and transmits λ_{EM}
- Emission filter : blocks reflected light from the sample at λ_{EXC}

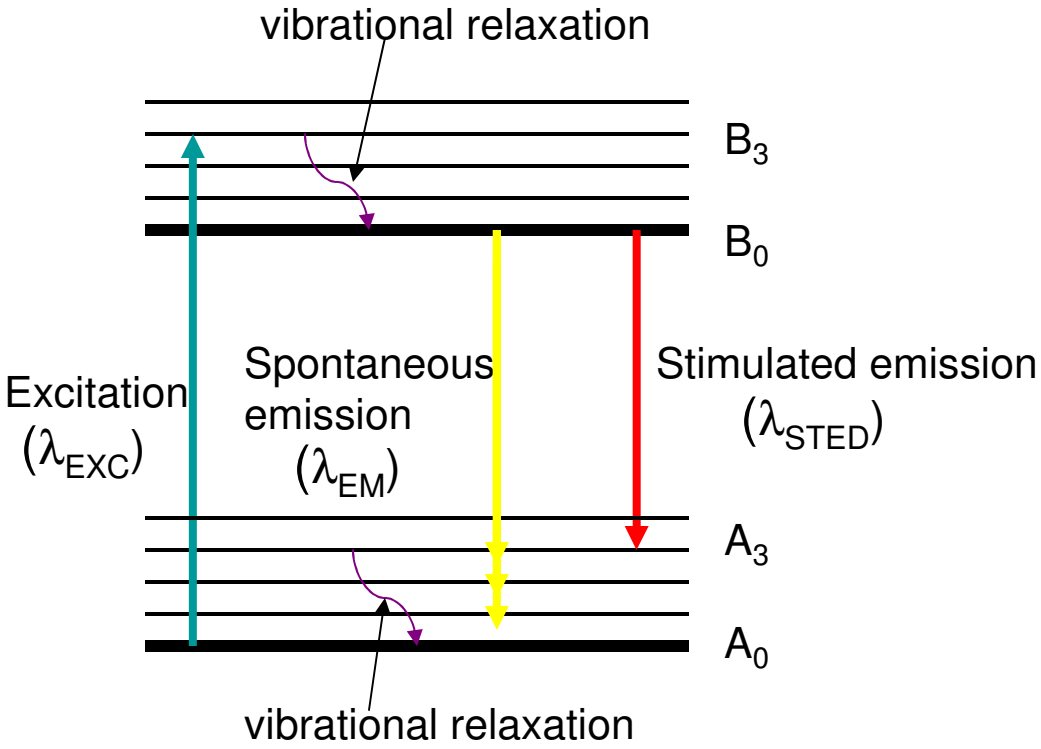
Confocal fluorescence imaging

- The target molecules are tagged with fluorescent probes or fluorophores
- Confocal detection of the fluorescent light in a beam scanning or stage scanning set up
- Fluorescence image provides information about the physical and chemical environment and orientation of the fluorophores and hence of the attached target molecules
- Best resolution working in the UV-visible range (lateral >200 nm, axial >500 nm)
  - Not enough for visualising light-matter interaction at nanoscale



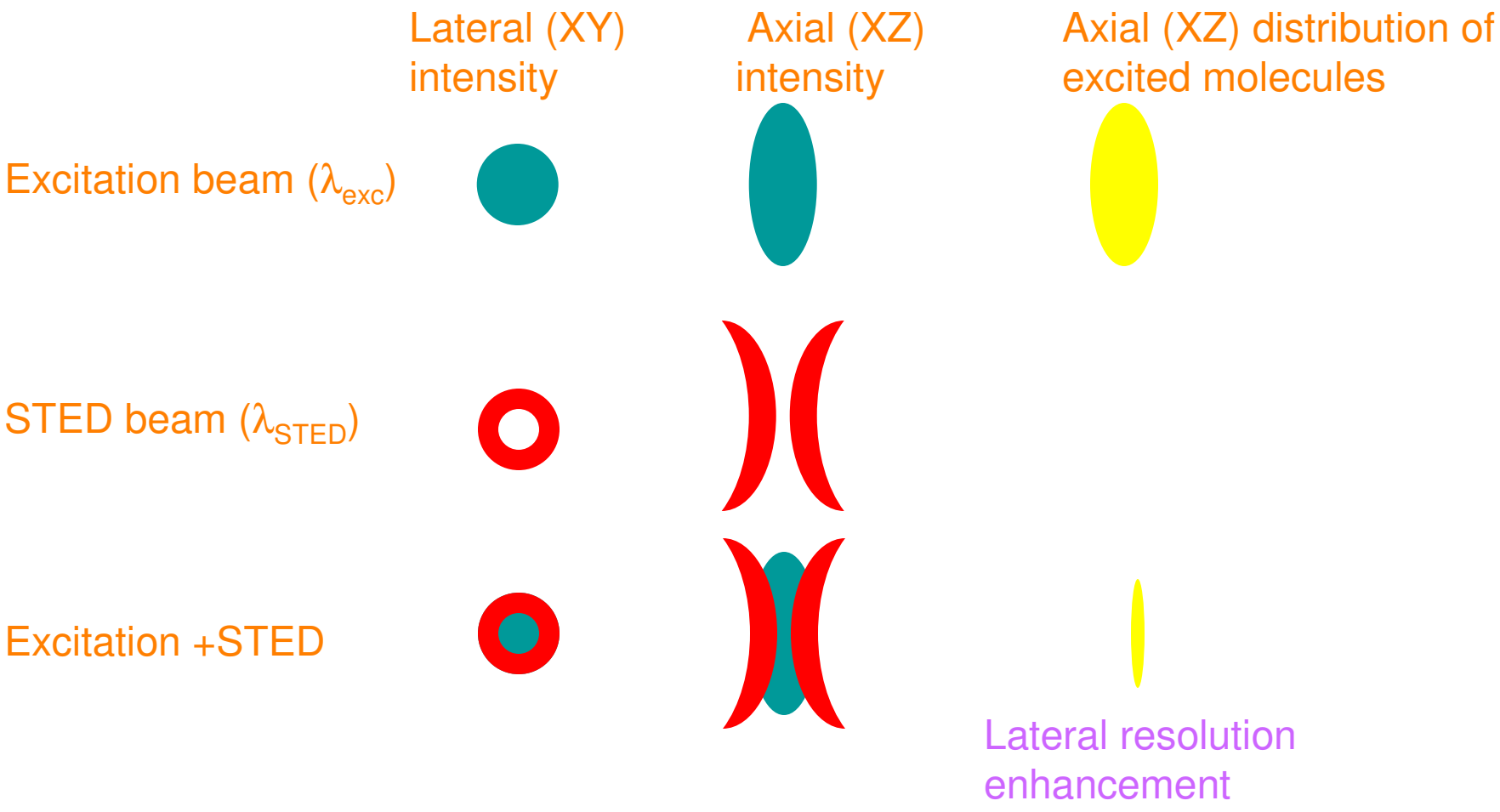
Confocal fluorescence image of human T cells (tagged with di-4-ANEPPDHQ fluorophores)

Stimulated emission depletion (STED)



- Laser beam (λ_{EXC}) excites a molecule to the upper electronic state
- Another laser beam, called STED beam, at (λ_{STED}) shines on the excited molecule
  - Stimulates it to undergo emission at (λ_{STED})
  - No emission at (λ_{EM}) i.e. No fluorescence from the excited molecule

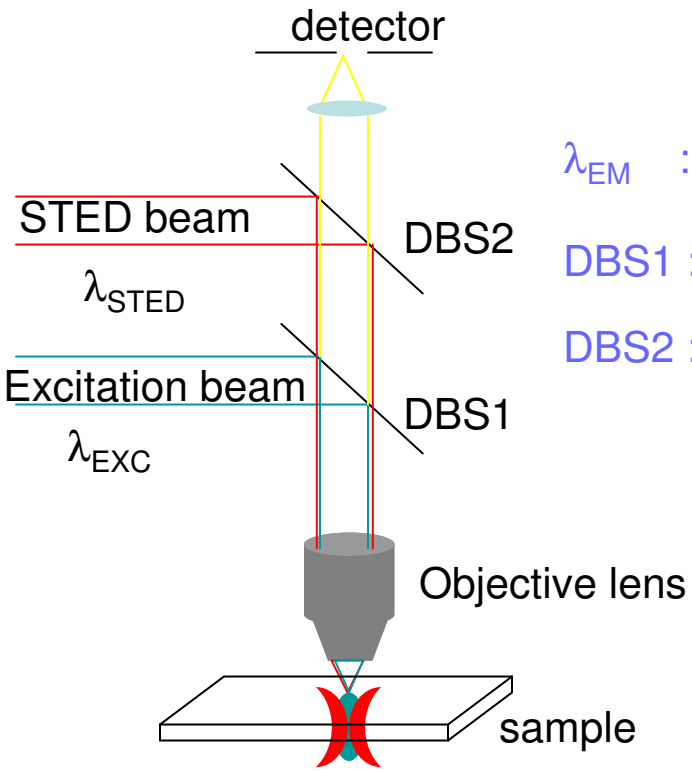
Resolution enhancement with STED



Nanometer resolution with saturated depletion

- For normal STED beam intensity : only marginal improvement in resolution
- Depletion, and hence fluorescence intensity, has a nonlinear dependence on STED beam intensity
- Increasing the power of the STED beam to GW/cm² saturates depletion everywhere except along the optic axis
  - Lateral resolution ~28 nm

STED in a confocal fluorescence microscope



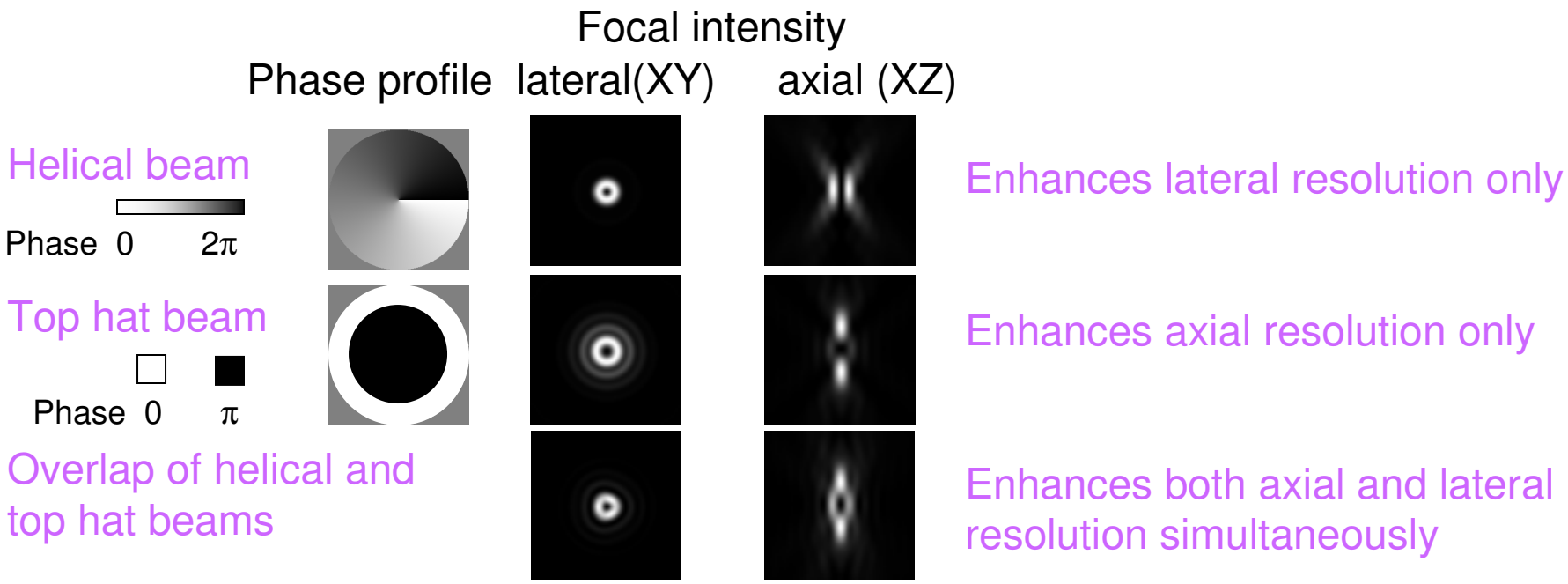
λ_{EM} :mean emission wavelength

DBS1 : reflects λ_{EXC} and transmits wavelengths $>\lambda_{EXC}$

DBS2 : reflects λ_{STED} and transmits wavelengths $<\lambda_{STED}$

- Both excitation and STED beams are pulses following one another, usually derived from the same femto second laser
- Image is formed by scanning the stage or by scanning the beams

Types of STED beams

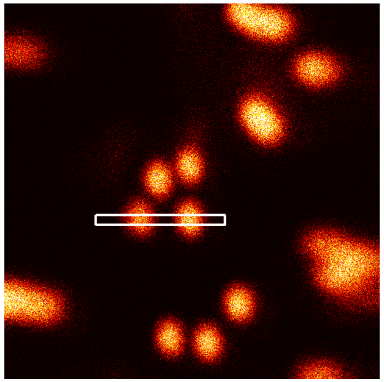


- STED beams are generated using fixed phase plates or programmable diffractive elements such as a spatial light modulator
- Lateral resolution achieved ~28 nm
- Axial resolution achieved ~143 nm

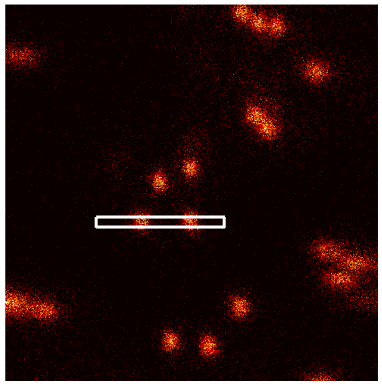
Applications of STED microscopy

Nanoscale imaging of fluorescent beads

Confocal image



STED image



XY plane images of 200 nm fluorescent beads
(source: PhD thesis, B R Boruah, Imperial College London)

Applications of STED microscopy

In biological science

Confocal image STED image

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

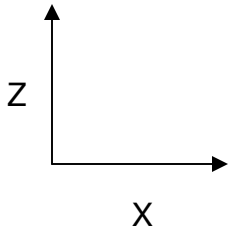
- Reveals nanopattern in the in SNAP-25 protein found in the plasma membrane of mamalian cells (source: Briefings in functional genomics and proteomics, Vol 5, No 4, 289-301)

Applications of STED microscopy

Nanoscale imaging of live cells

Confocal image STED image

Live yeast cells



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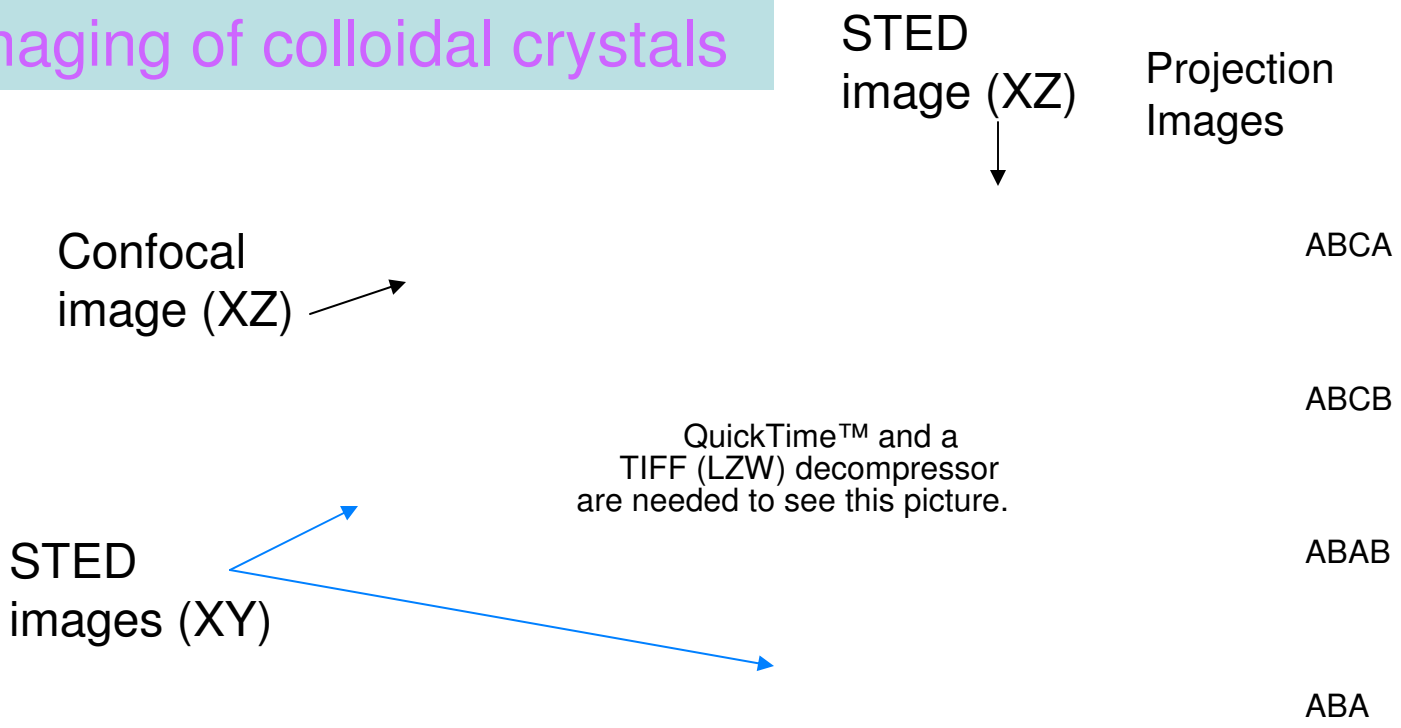
TIFF (LZW) decompressor
are needed to see this picture.

Live E-coli bacteria

(source: PNAS, 97, 15, 2000, 8206-8210)

Applications of STED microscopy

Imaging of colloidal crystals



- Revealing the structure of 3D colloidal crystals (source: Nano Letters, 8, 1309, 2008)

Conclusion

- Near field microscopy can provide nanoscale resolution, however useful for surface study only
- Far field microscopy can be used to image 3D and biological samples
- Laser scanning confocal microscope is a far field microscopy technique that can provide optically sectioned image
  - Can image either with reflected or fluorescence light
  - Useful for 3D visualisation of the sample
  - Resolution is limited by the diffraction effects
- Stimulated emission depletion phenomenon can be used in a confocal fluorescence microscope to get resolution beyond the diffraction barrier
- STED microscope has been used in the study of nano structures in living and non-living samples