Superresolution Imaging for Neuroscience

Jan Tonnesen, U. Valentin Nägerl

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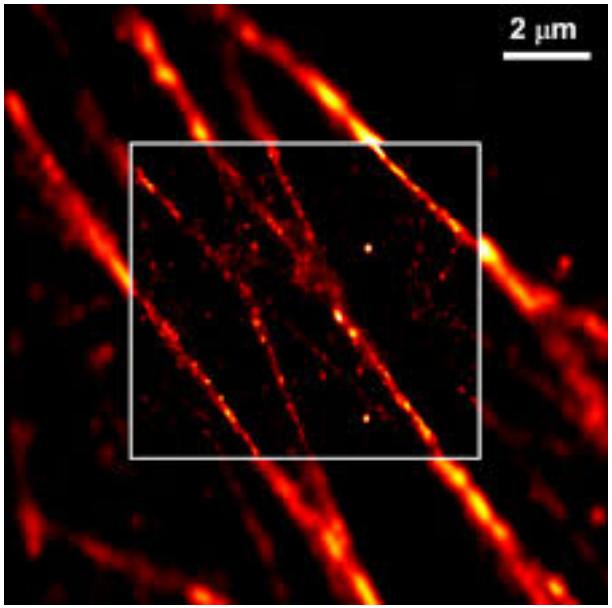


Lola Bautista

MORPHEME

MORPHologie Et IMagEs

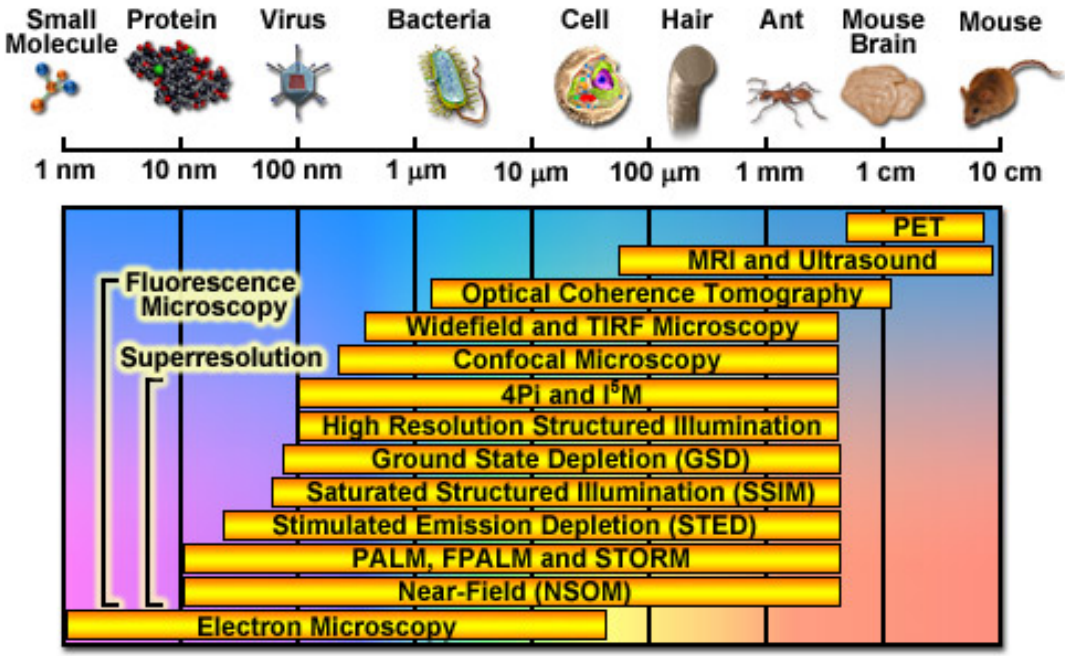
June 4, 2014

Agenda

- Introduction
- Fluorescence Microscopy
- Superresolution
- STED Microscopy
- PALM/STORM Microscopy
- SIM Microscopy
- Discussion
- References

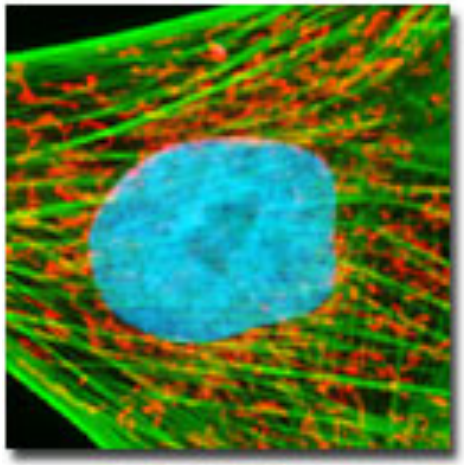
Introduction

- Several techniques can generate images of animal and human subjects at resolutions between 10 cm and 10μm.
- Fluorescence microscopy techniques can readily resolve a variety of features in isolated cells and tissues



http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html

Fluorescence Microscopy



- **Fluorescence** is a phenomenon whereby light is first absorbed by a crystal or molecule and rapidly re-emitted at a slightly longer wavelength (lower energy).
- The microscope irradiates the specimen with a desired and specific band of wavelengths, and then separate the much weaker emitted fluorescence from the excitation light.
- The techniques of fluorescence microscopy can be applied to organic material, formerly living (biological) material, or to living material or to inorganic material.
- Spatial resolution $>$ 250 nm

Superresolution

It refers to various methods for improving the resolution of an optical imaging system beyond its diffraction limit.

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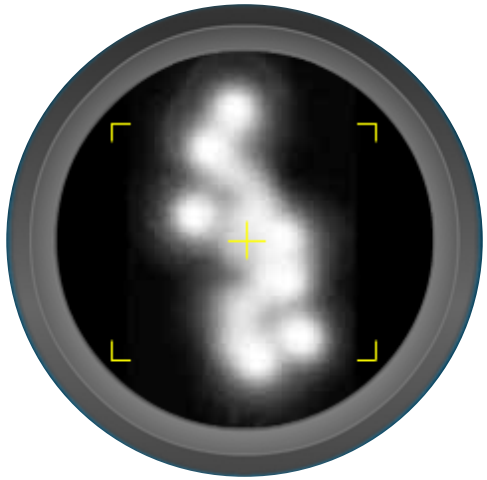
- **Postprocessing or computational superresolution:** aims at recovering the object spectrum beyond the cutoff frequency of the optical system by using some prior information about the object.
- **Information theory approach:** it makes transparent the fundamental trade-off of the signal-to-noise ratio and the field of view.

Resolution: physical facts

- **Resolution:** the smallest distance between two points on a specimen that can still be distinguished as two separate entities.



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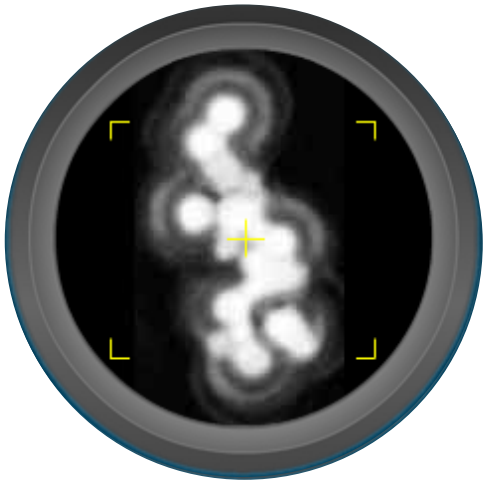

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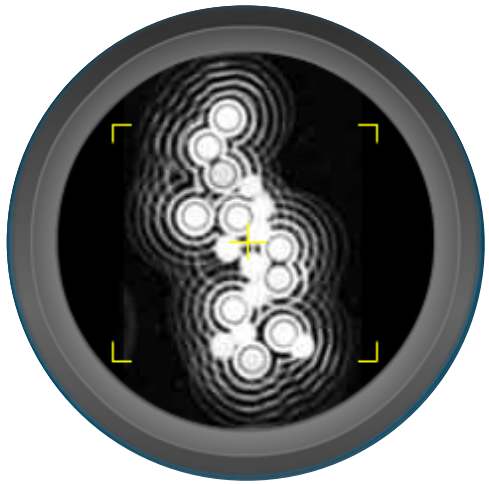
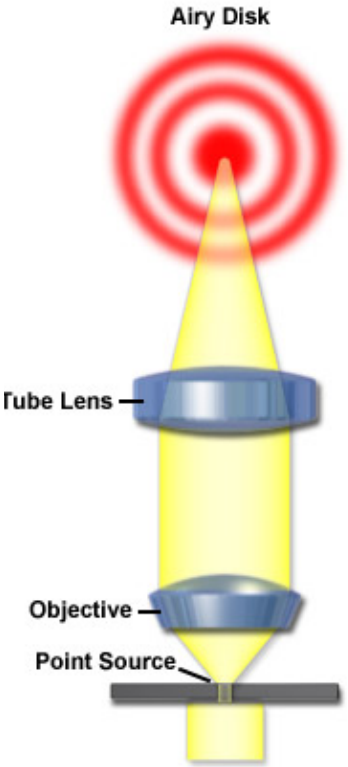


- Due to the wave nature of light and the diffraction associated with these phenomena, **the resolution of a microscope objective is determined by the angle of light waves that are able to enter the front lens** and the instrument is therefore said to be **diffraction limited**.

Resolution: physical facts



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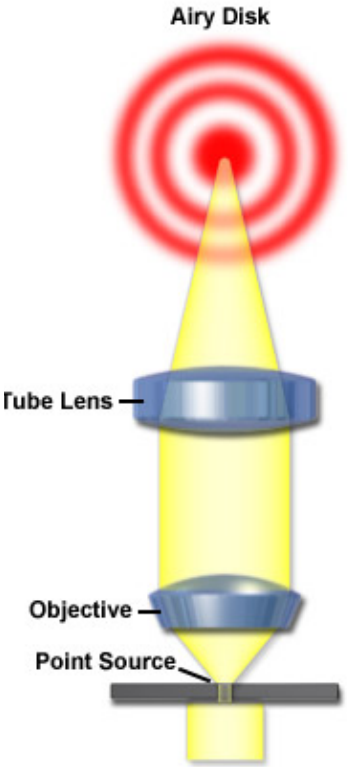

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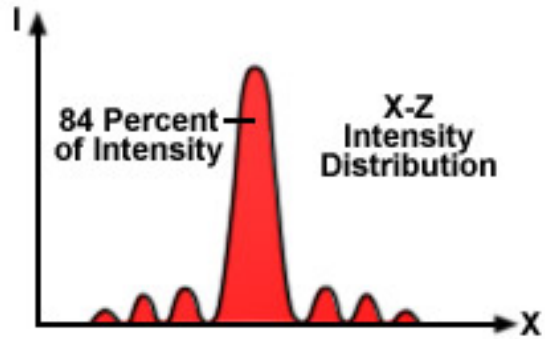

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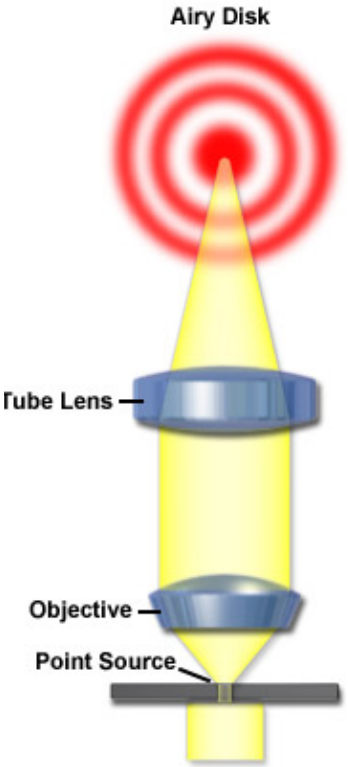

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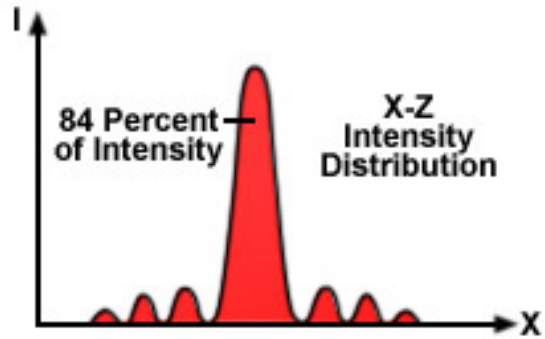
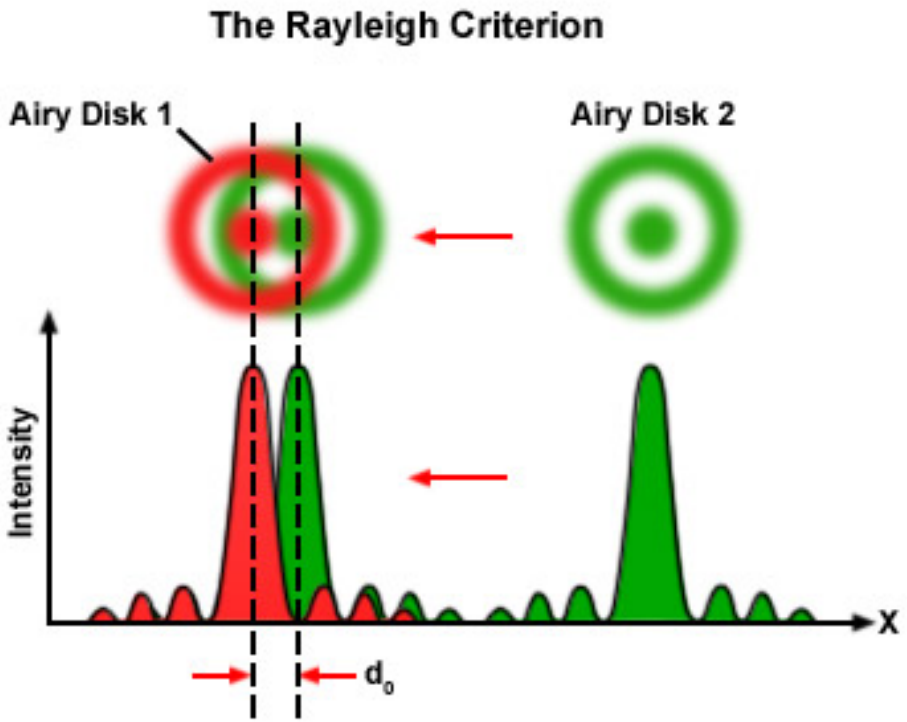


Resolution: physical facts

- **The Rayleigh Criterion**

  Two adjacent object points are defined as being resolved when the central diffraction spot of one point coincides with the first diffraction minimum of the other point in the image plane.



STED:STimulated Emission Depletion Microscopy

- Creates sub-diffraction limit features by altering the effective PSF of the excitation beam using a second laser that suppresses fluorescence emission from fluorophores located away from the center of excitation.

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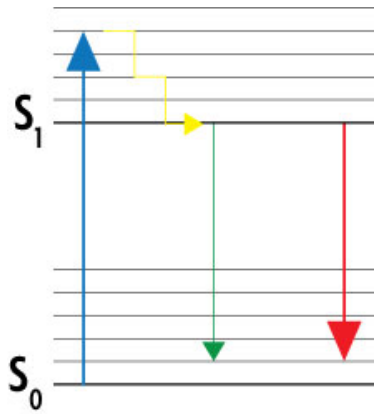

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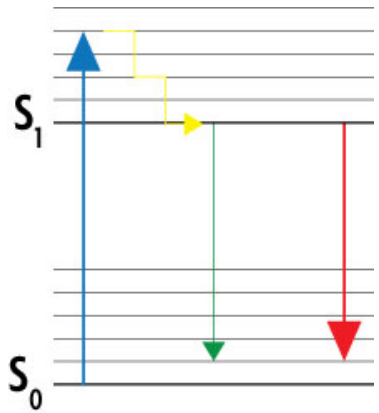

- The suppression of fluorescence is achieved through stimulated emission that occurs when an excited-state fluorophore encounters a photon that matches the energy difference between the ground and excited state.

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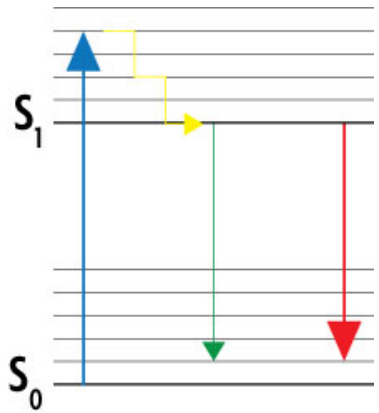

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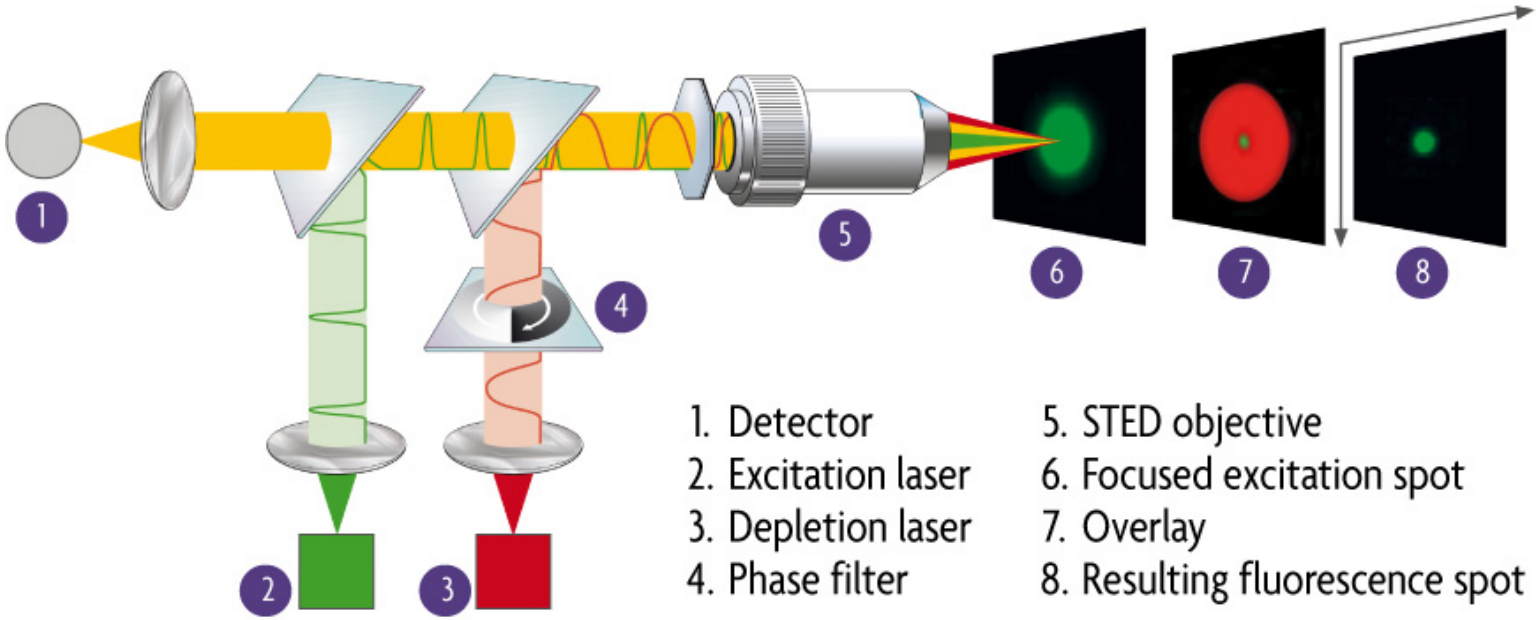
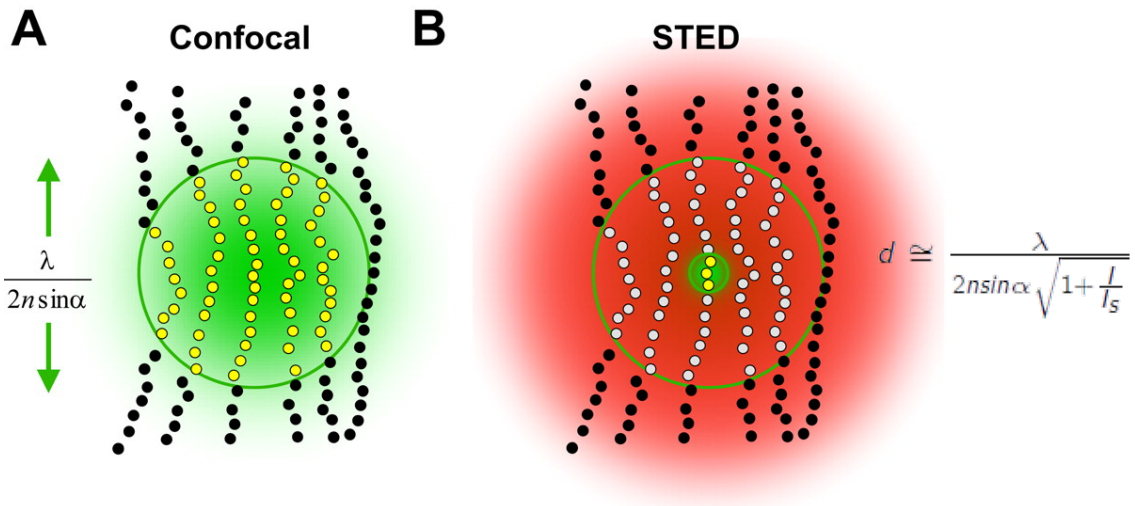
STED Microscopy

- The process effectively depletes selected regions near the focal point of excited fluorophores that are capable of emitting fluorescence.



http://www.activemotif.com/catalog/627/sted-microscopy-products

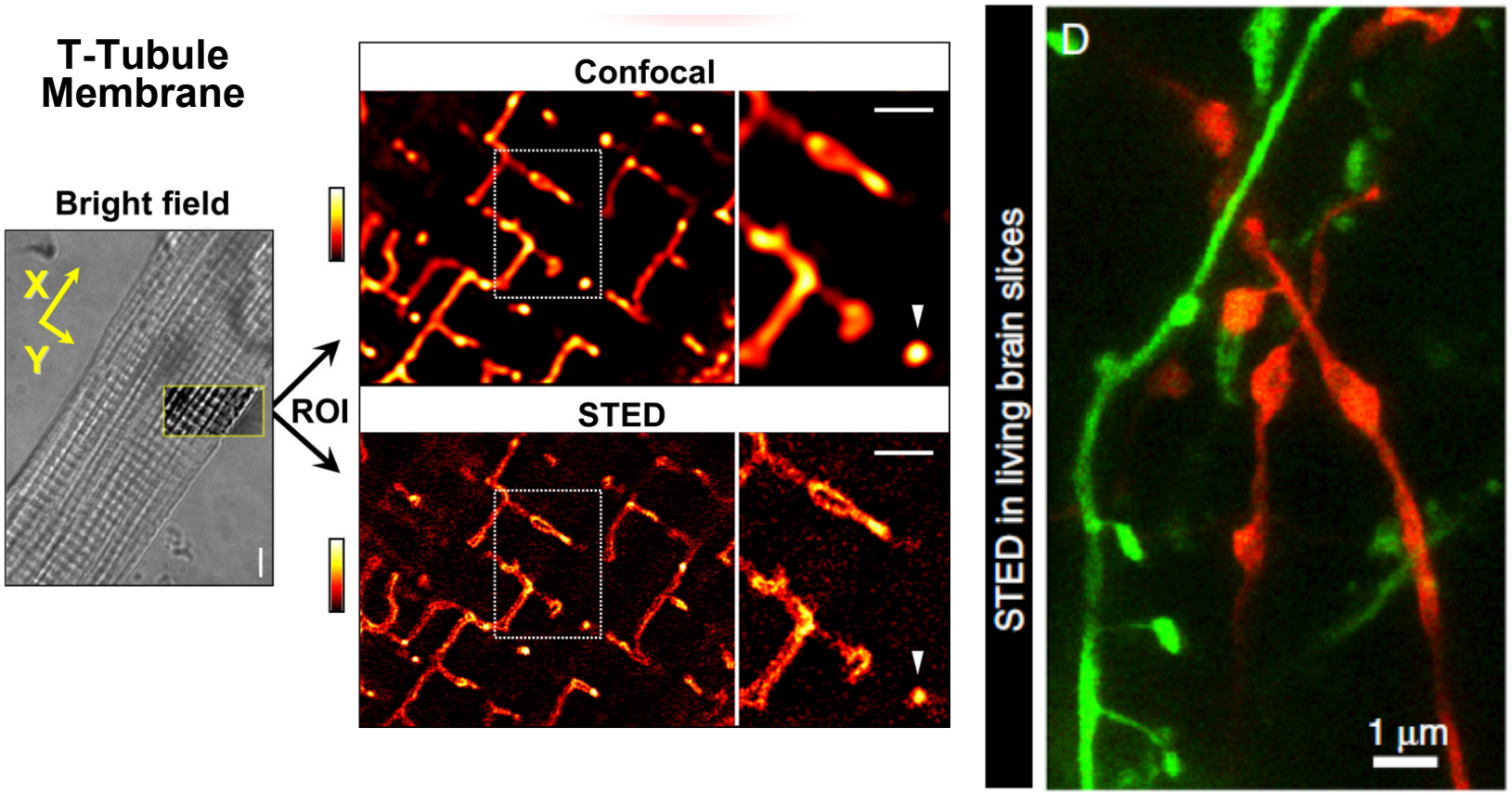
STED Microscopy



http://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/stedfundamentals/indexflash.html

The lateral resolution is typically between 30 and 80 nm. Axial resolution, on the order of 100 nm have been demonstrated.

STED Microscopy

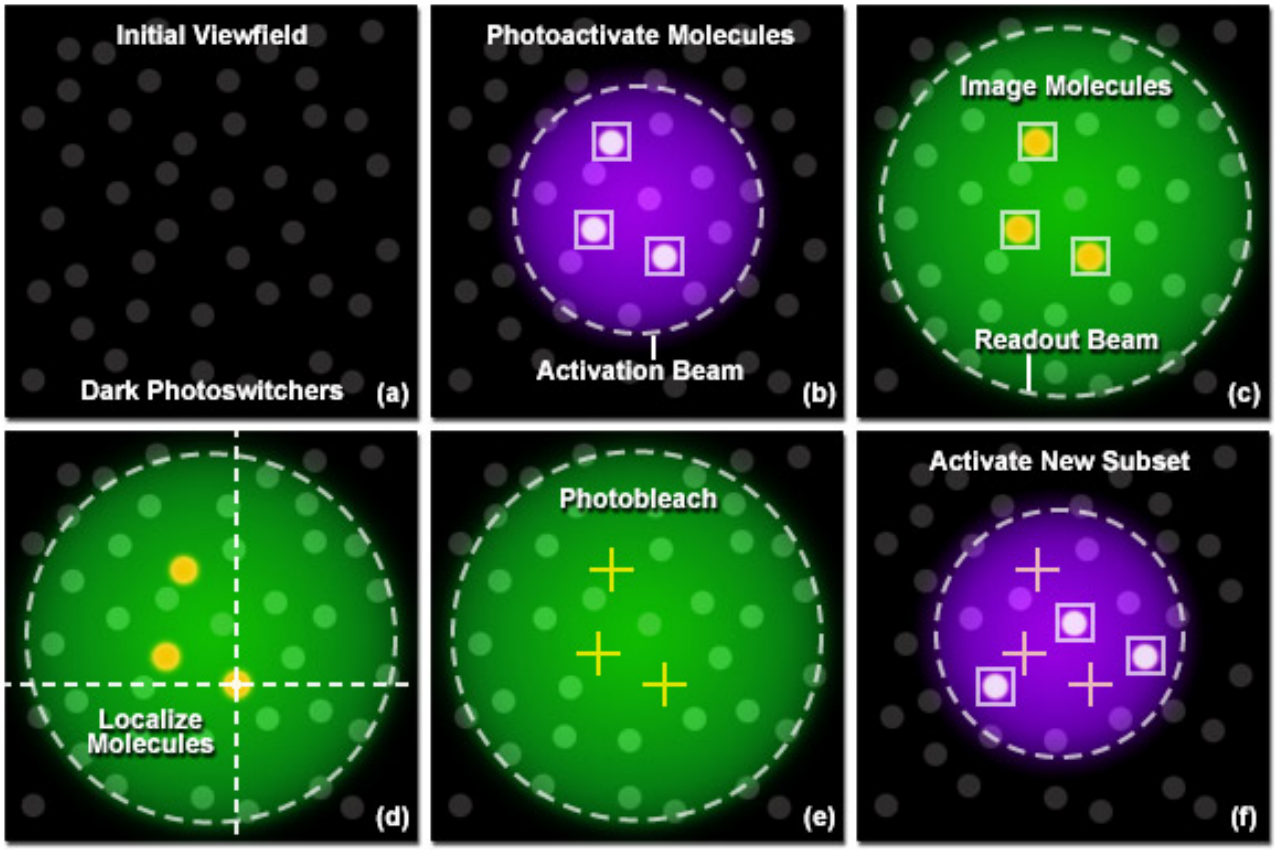


PALM: Photo Activated Localization Microscopy
STORM: STochastic Optical Reconstruction Microscopy

- Methods that are based on stochastic on/off switching of single fluorescent molecules and their computational localization in wide-field illumination.
- PALM was initially developed using fluorescent proteins
- STORM was developed using organic dyes such as cyanine

PALM/STORM

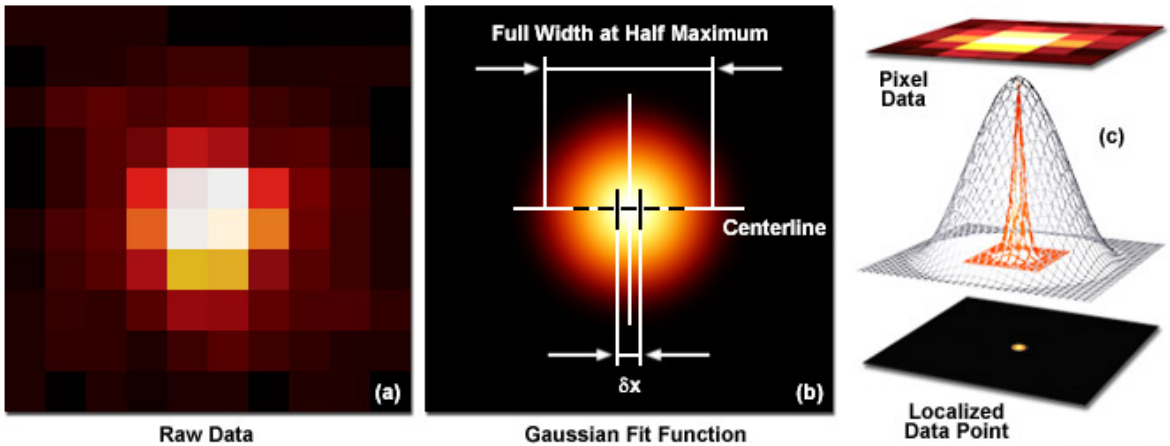
Both methods use the principle of single-molecule localization microscopy



http://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/practicalaspects.html

PALM/STORM

- If the emission from the two neighbouring fluorescent molecules is made distinguishable, then it is possible to overcome the diffraction limit.
- Once a set of photons from a specific molecule is collected, it forms a diffraction limited spot in the image plane of the microscope.
- The center of the spot can be found by fitting the observed emission profile to a Gaussian function in two dimensions.



PALM/STORM

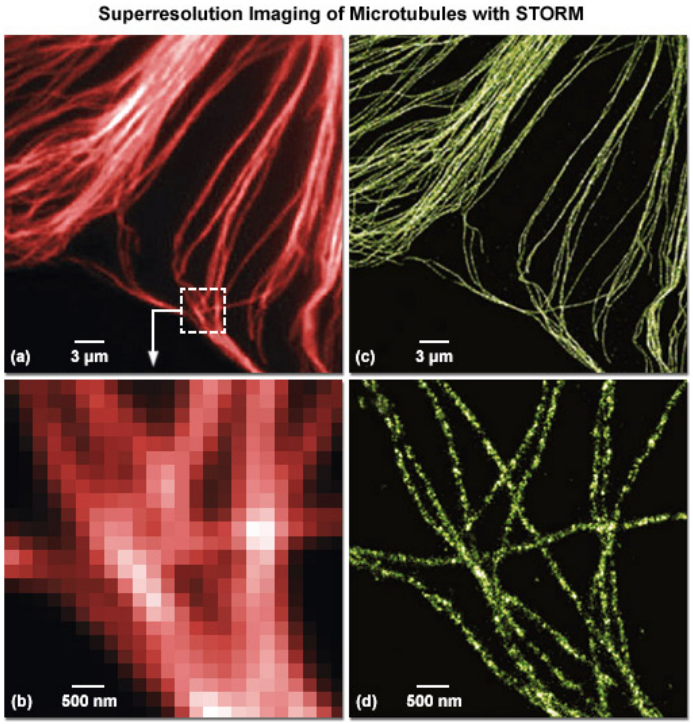
- A superresolved image is constructed out of a large number of conventional wide-field images, each containing the positional information of different subsets of dispersed single fluorescent molecules.

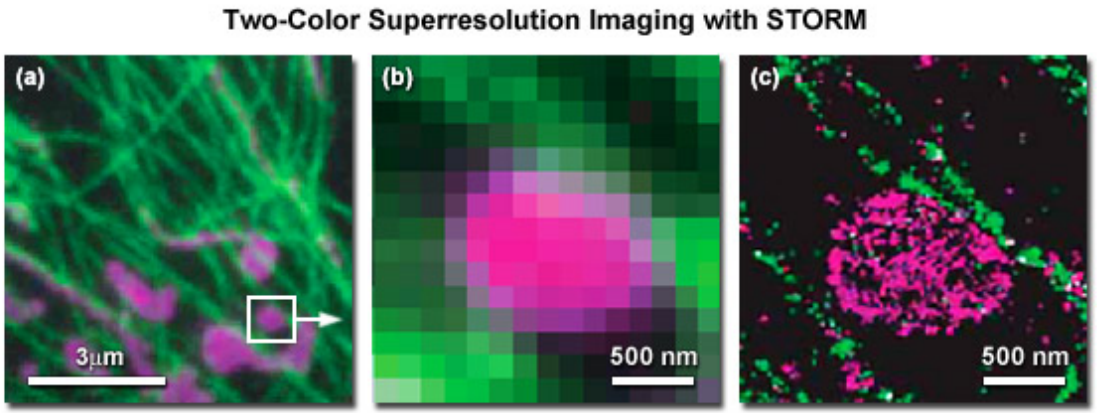
- The resulting information of the position of the centers of all the localized molecules is used to build up the super-resolution image.

$$\sigma = \sqrt{\left(\frac{s_i^2 + \frac{a^2}{12}}{N}\right) \cdot \left(\frac{16}{9} + \frac{8\pi s_i^2 b^2}{a^2 N}\right)}$$

- **N** the number of collected photons
- **a** the pixel size of the imaging detector
- b^2 the average background signal
- s_i the standard deviation of the Point Spread Function

PALM/STORM



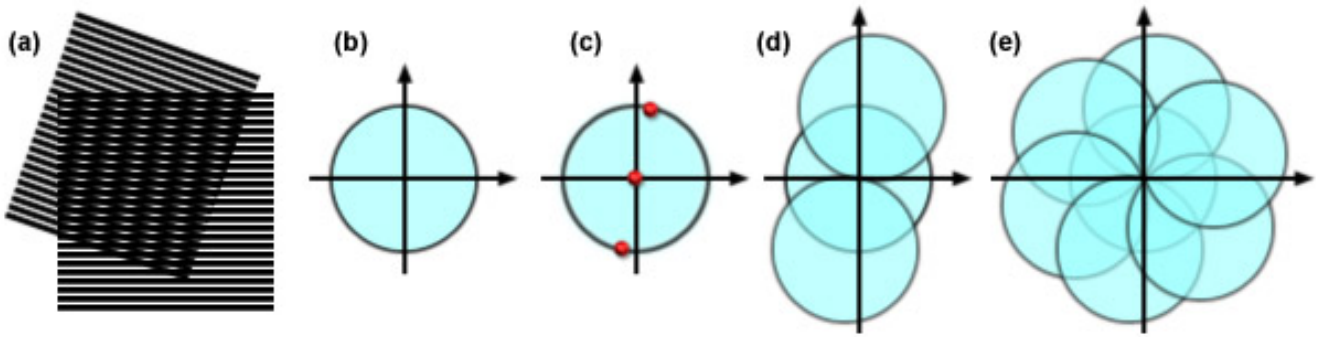


http://www.microscopyu.com/articles/superresolution/stormintro.html

http://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/palmbasics/indexflash.html

SIM: Structured Illumination Microscopy

- Illuminates a sample with a series of sinusoidal striped patterns of high spatial frequency (by passing light through a movable optical grating and projected via the objective onto the specimen).
- Coarse interference patterns (moiré fringes) arise, which are transferred to the image plane.



http://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/hrsim/indexflash.html

SIM

- The high frequencies containing information on fine details of the sample structure.
  - The higher spatial frequencies normally get filtered out by the microscope objective. However, when the specimen is illuminated by spatially varying (patterned or structured) excitation light, these spatial frequencies are effectively shifted to lower ones that can be resolved by the imaging system.

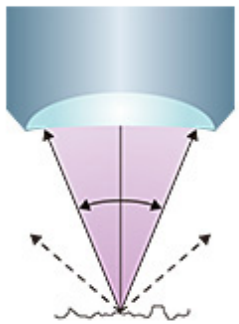


Fig. A: Resolution is limited by the NA of the objective

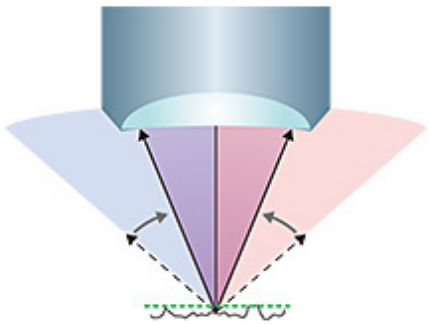


Fig. B: The product of Structured Illumination and normally un-resolvable specimen structure produce recordable moiré fringes containing the specimen information at double the conventional resolution limit.

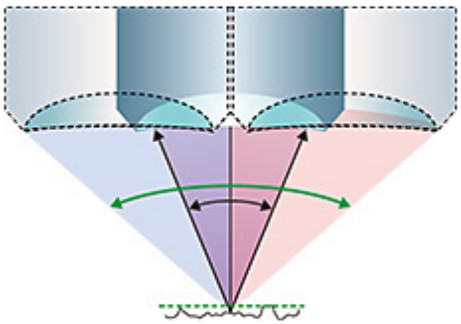
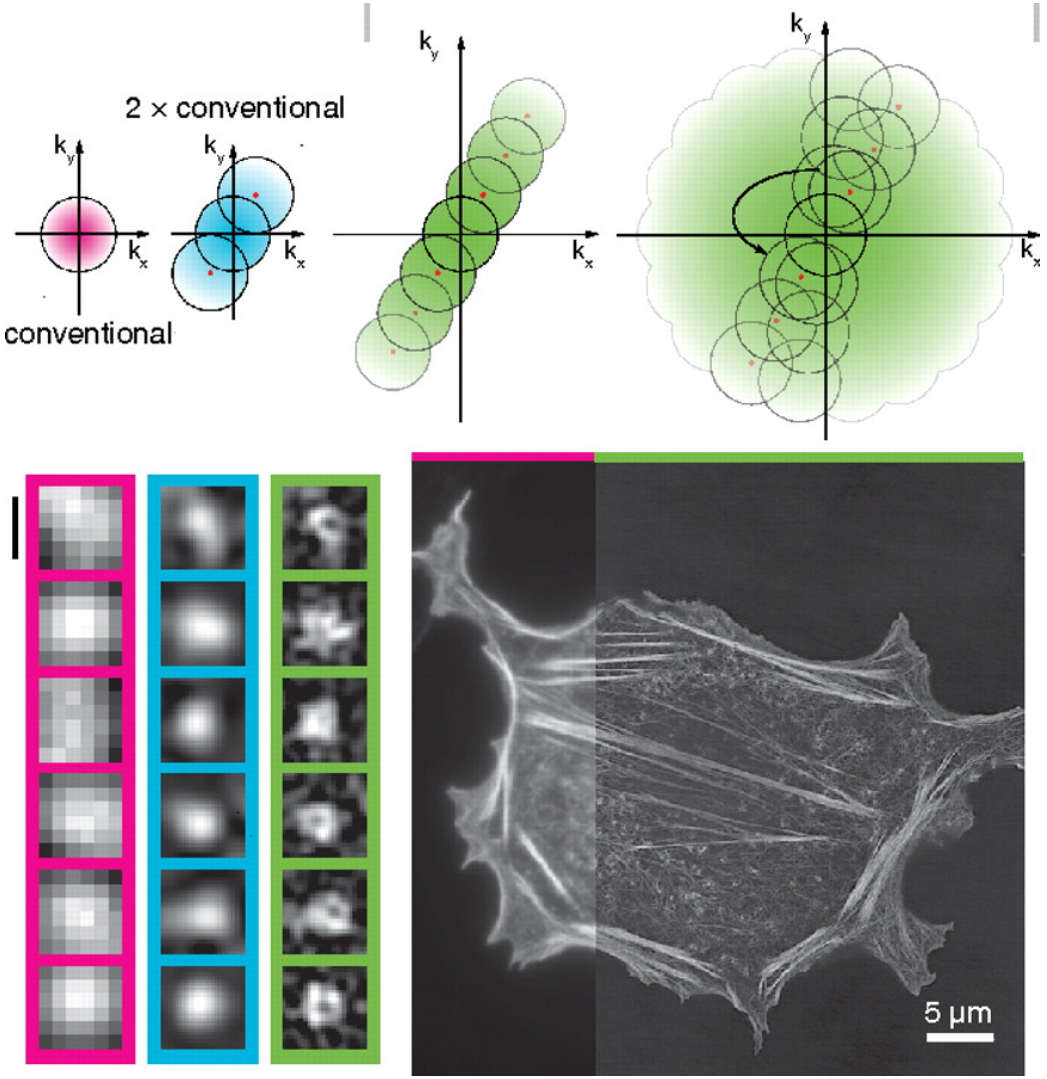


Fig. C: Images with resolutions equivalent to those captured with objective lenses with approximately double the NA are achieved.

SIM



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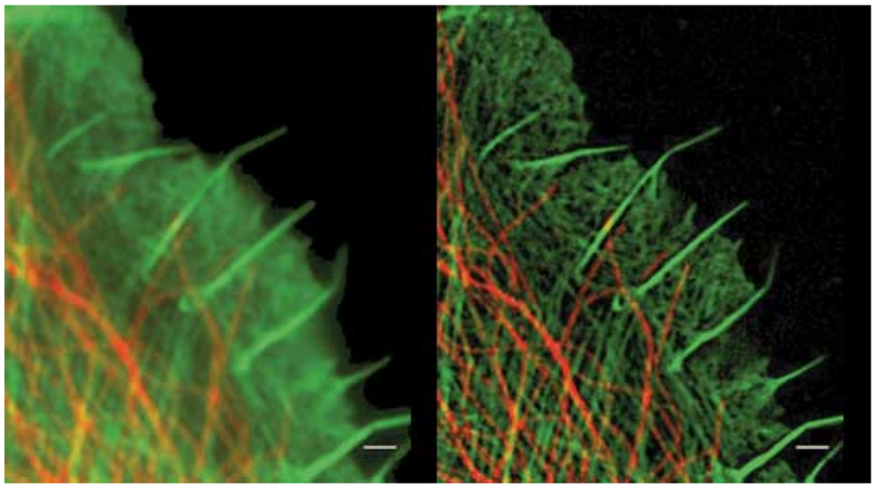

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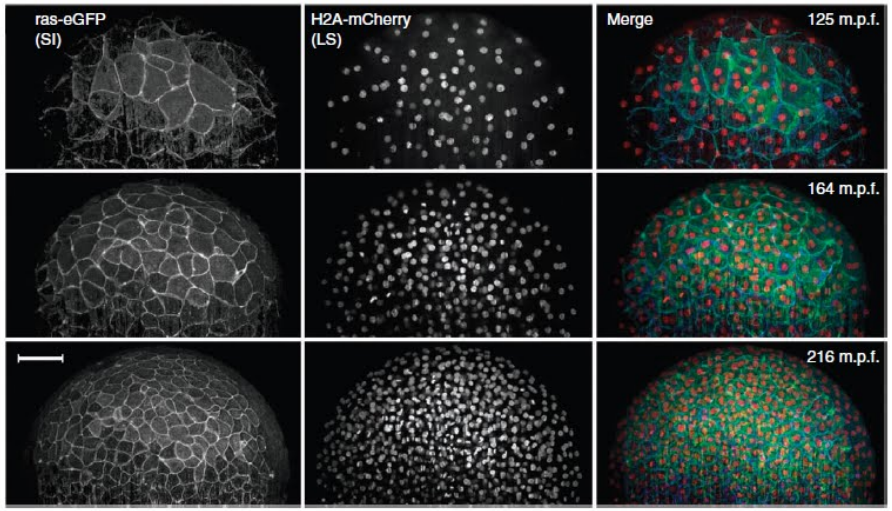
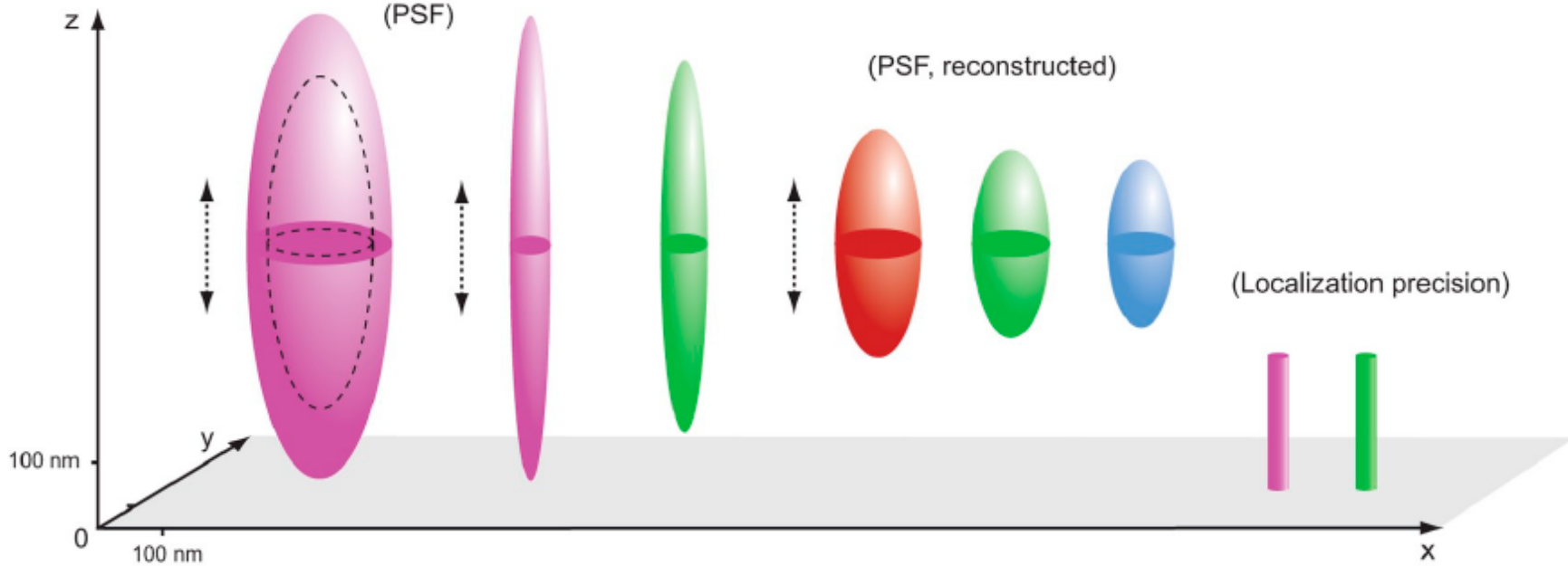


http://biophotonicsreview.blogspot.fr/2010/07/combining-digital-scanned-laser-light.html

Discussion

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [nm]	460-670	670	520	620	520	460	670	520



~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} µm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

Discussion (1)

- One promising area is the investigation of plasma membrane proteins and membrane microdomains.
- Photoactivation-facilitated high-density single-particle tracking provides a powerful approach to study dynamic phenomena.
- Superresolution allows the visualization of fine structures within membrane organelles.
- It is expected they can expand the current understanding of nucleus structures (highly condensed DNA packaging).
- To provide understanding of the interactions between nucleic acids and proteins.
- In microbiology offers an opportunity for the bacterial imaging problem (organization of chromosomes and proteins).

Discussion (2)

- In neurobiology, for the study of subneuronal structures (synapses), neurotransmissors and neuroreceptors.
- Determining the complete neuronal wiring diagram may require even higher resolution.

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