MICRO-562

Biomicroscopy II

Instructors:
Hatice Altug and Arne Seitz

TA:
Daniil Riabov

Biomicroscopy-II Syllabus – (TENTATIVE)

Lecture 1	Course	Brief Review, Dark-Field
Lecture 2	Course	Phase Contrast & Polarization, Birefringence
Lecture 3	Course	DIC & Fluorescence Microscopy Techniques (FRET & TIRFM)
Lecture 4	Course	FRAP & Introduction to Confocal Microscopy
Lecture 5	Course	Advanced Microscopy-I
Lecture 6	Course	Advanced Microscopy-II
Lecture 7	TP	Practical: Intro to Wide-Field Microscopy
Lecture 8	TP	Practical: Transmission Techniques
Lecture 9	TP	Practical: Intro to Fluorescence
Lecture X	**EASTER**	
Lecture 10	TP	Practical: Intro to Confocal
Lecture 11	TP	Practical: Fluo/Conf
Lecture 12	TP	Practical: Fluo/Conf
Lecture 13	**EXAM**	
Lecture X	**HOLIDAY**	

Optical Microscopy

- **Wide-field microscopy methods:**
  - **Bright-field**
  - **Dark-field**
  - **Phase Contrast**
  - **Differential Interference Contrast (DIC or aka Nomarski)**
  - **Polarization**
  - **Fluorescence**

- **Large varieties of fluorescence microscopy methods exist:**

  Conventional fluorescence microscope *(i.e. epi-fluorescence)*
  FRET: Förster resonance energy transfer
  TIRF: Total Internal Reflection Fluorescence Microscopy
  FRAP: Fluorescence Recovery After Bleaching Microscopy
  Confocal Microscopy
  Super-resolution microscopy: STED, STORM, PALM…
  Multiphoton (i.e. two-photon) or Nonlinear Microscopy
  4-Pi Microscopy
  SIM: Structured illumination microscopy ……..

  ….. *and many more*

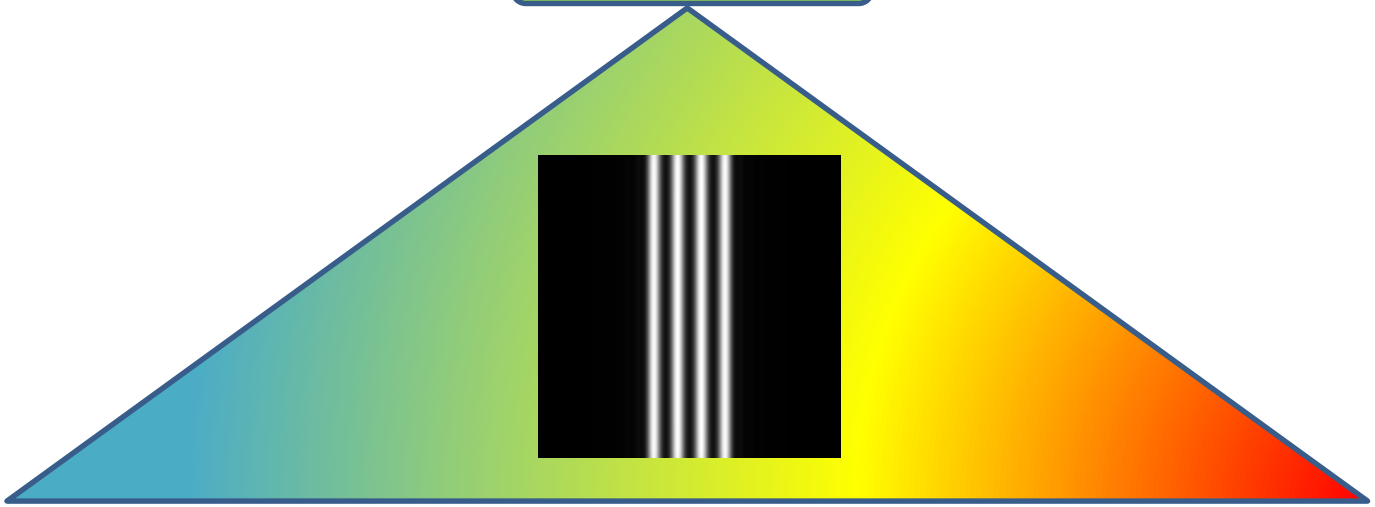
Reminder:Important aspects in microscopy:

… the ability to see stuff

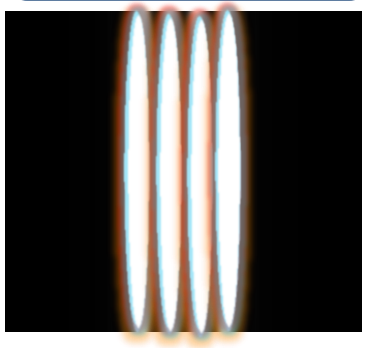
- Magnification
- Image quality – aberrations, illumination, alignment etc
- Contrast
- **Instrument Resolution**

Reminder:Important aspects for microscopy

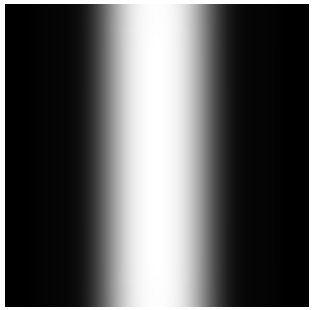
Magnification



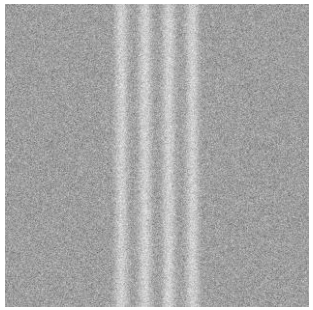
Geometrical aberrations



Resolution

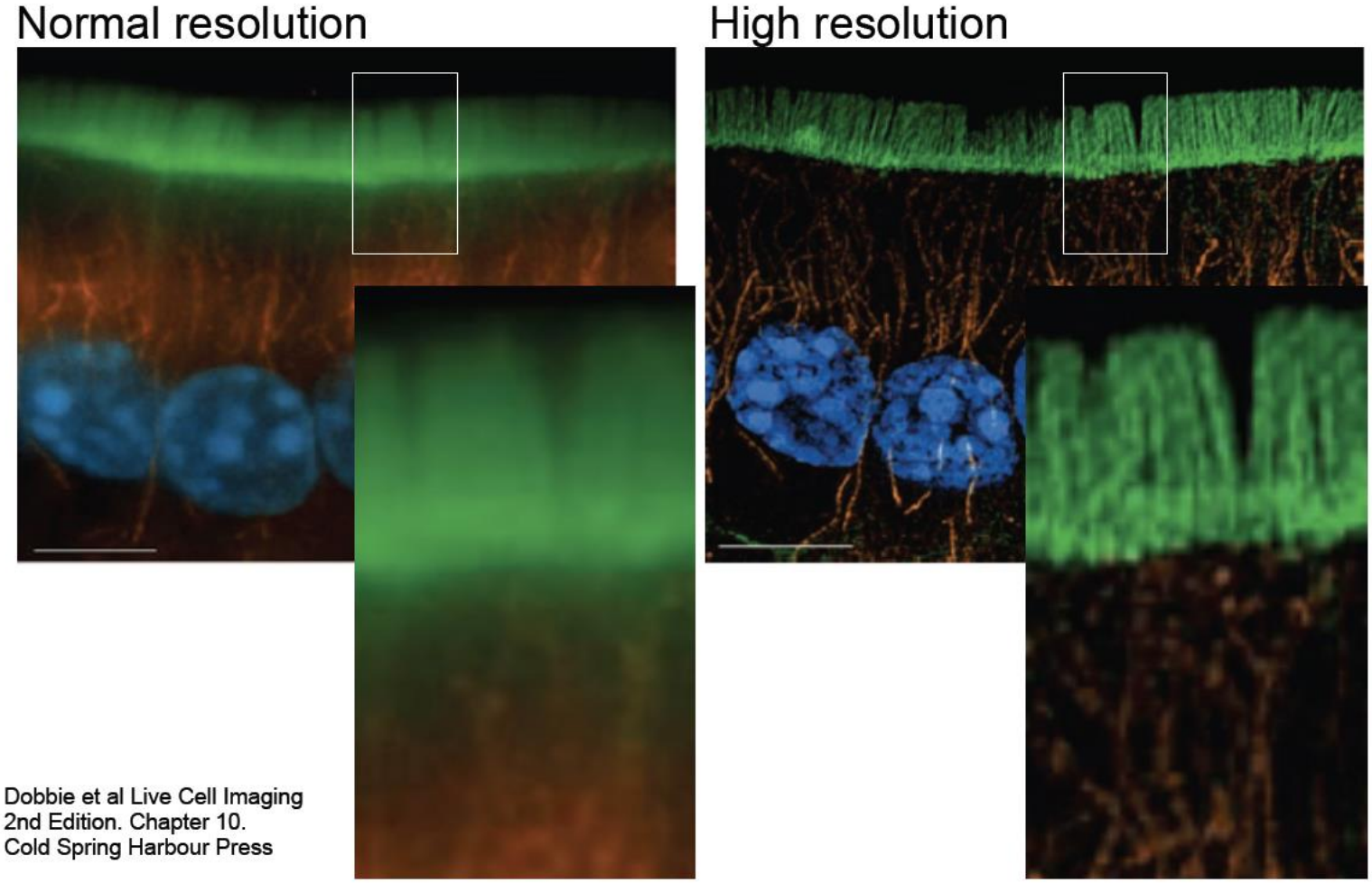


Contrast



Reminder: Resolution in microscopy

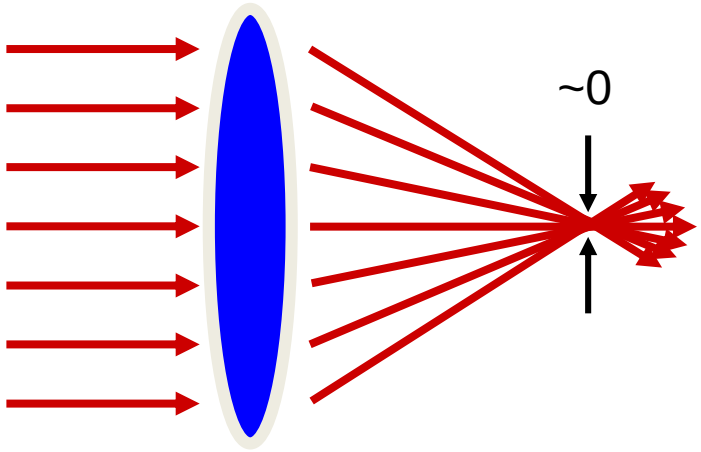
… the ability to see stuff



Dobbie et al Live Cell Imaging
2nd Edition. Chapter 10.
Cold Spring Harbour Press

Reminder: Resolution Limit

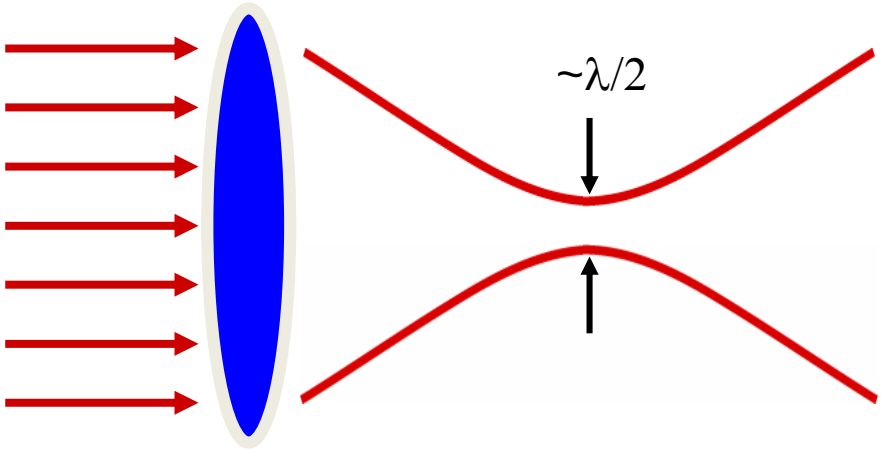
Ray pictures seem to imply that we could focus a beam to a point with zero dimension.



"Ideal" focusing is not taking place
→ due to diffraction

The possible focal spot size is about a wavelength.

This spot size also gives the best resolution one can achieve with a microscope/imaging system.

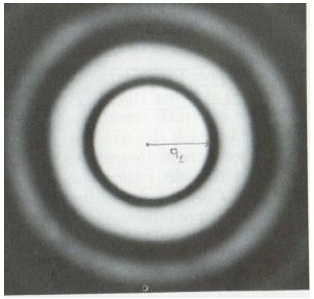


Reminder: Microscopy resolution by 2 methods

1. **Rayleigh criteria**

→ Diffraction of a circular aperture & Airy disk

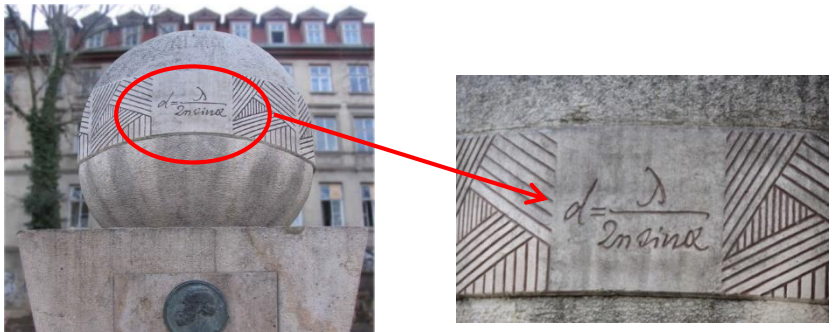
$$d_{min} \approx 0.61 \frac{\lambda_0}{NA} \approx 0.61 \frac{\lambda_0}{nsin(\theta)}$$



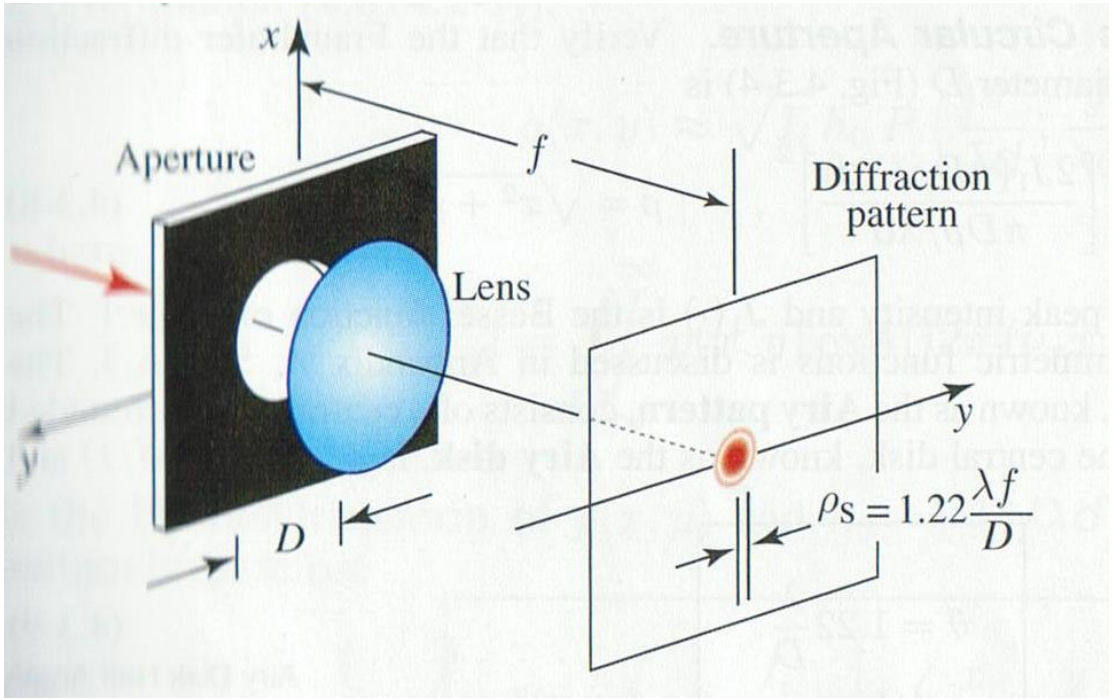
2. **Abbe's theory for image formation**

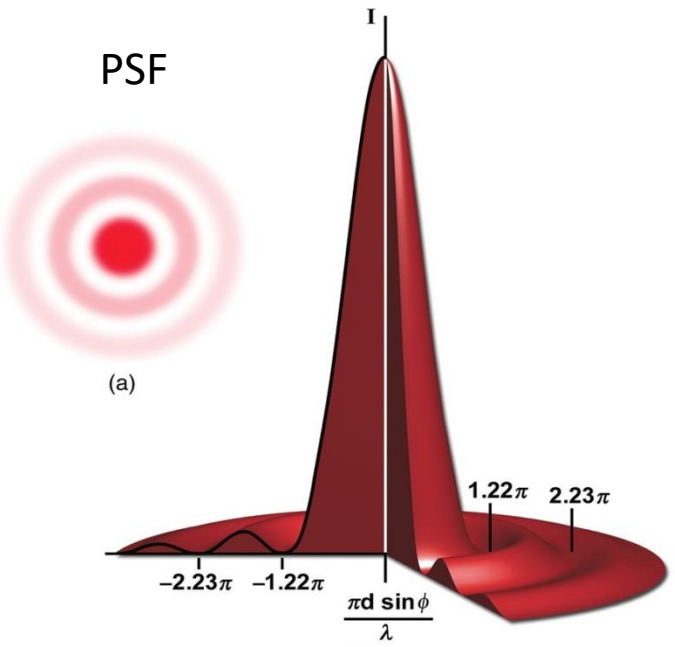
→Collecting more diffraction orders for higher resolution

$$d_{min} \approx 0.5 \frac{\lambda_0}{NA} \approx 0.5 \frac{\lambda_0}{nsin(\theta)}$$



Reminder: Diffraction Limited Spot Size

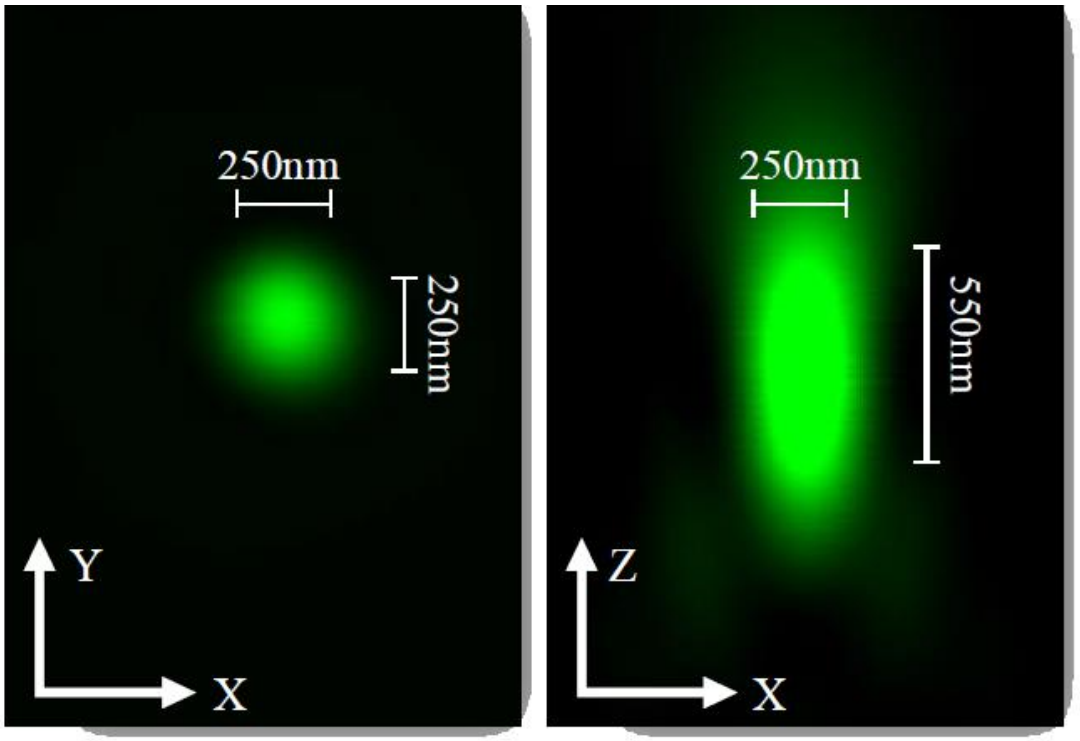




$$\rho_s = 1.22\,\lambda f/D$$

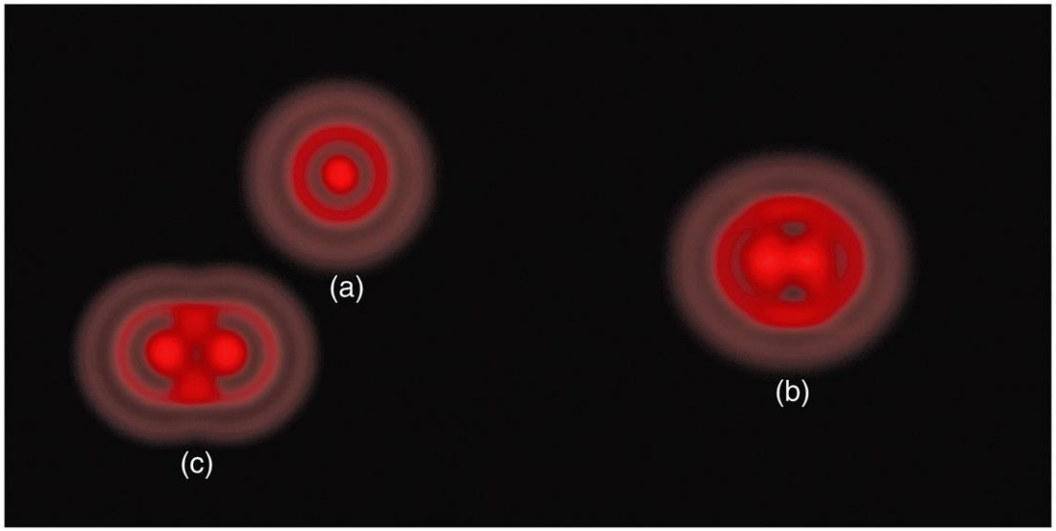
- Central spot of the PSF is called Airy disk with radius, ρ_s
- Contains 84% of power

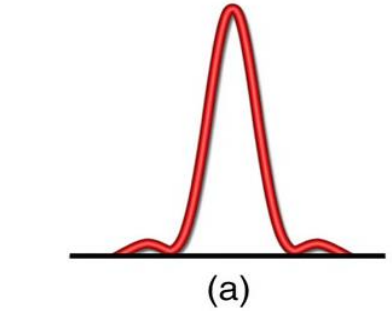
Imaging PSF - Diffraction Limited Spot

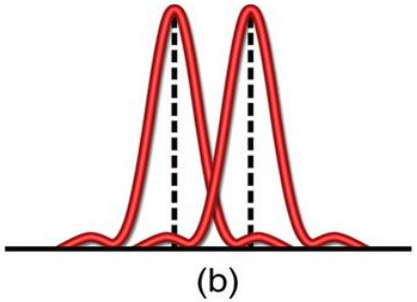


- PSF image obtained with a conventional confocal microscope.
- Laser light with 640 nm wavelength was mapped out by an 80 nm gold bead positioned inside the focal spot and imaged with a 100x oil 1.4 NA objective.

Reminder: Rayleigh Criterion for Resolution







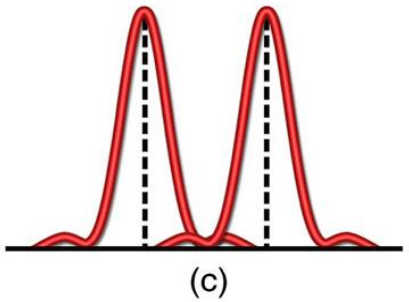
The resolution of a microscope is the shortest distance two points can be separated and still be observed as 2 points.

At the image plane:

a) Profile of a single diffraction pattern

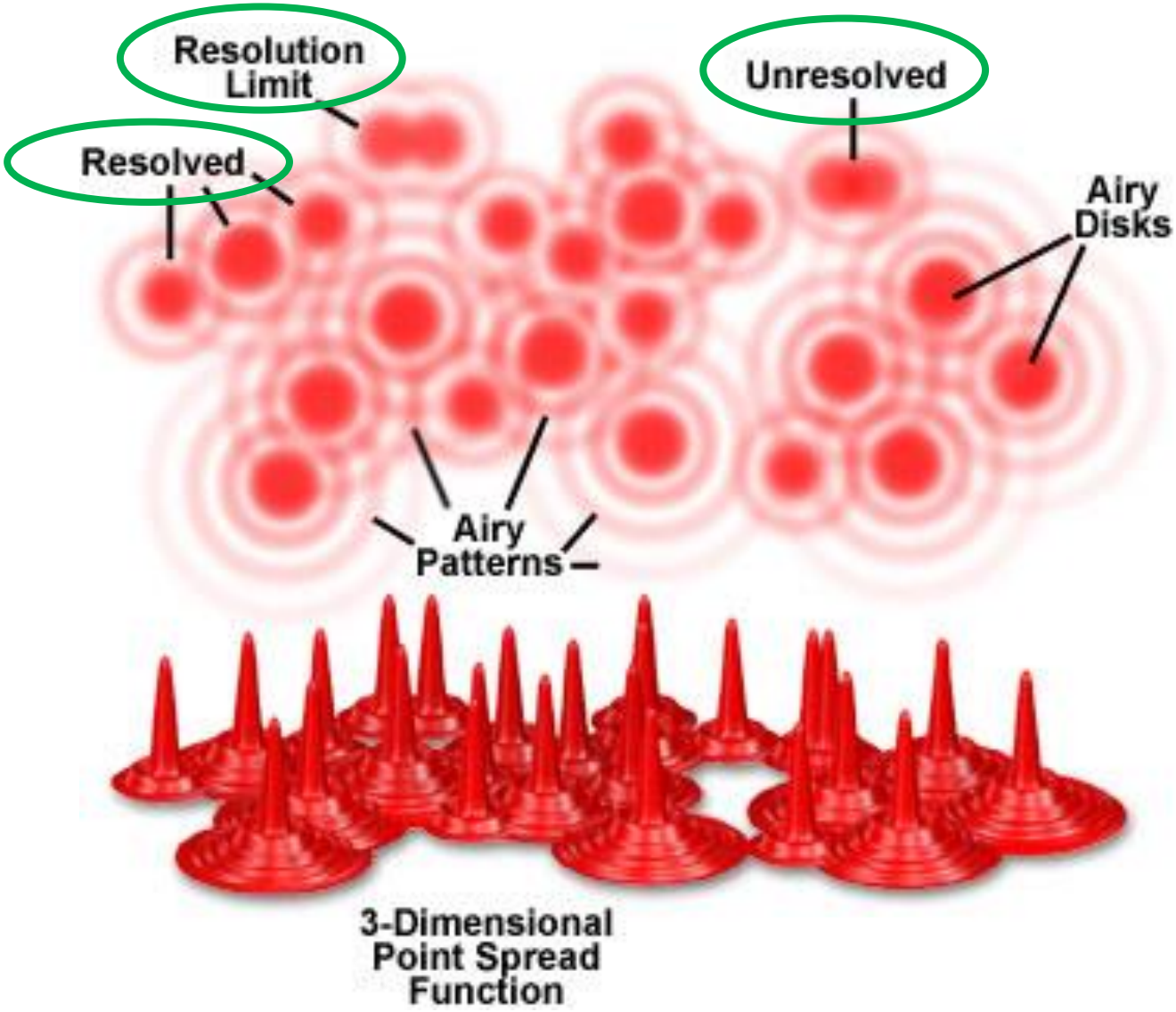
b) Profile of two disks separated at the Rayleigh limit: maximum of one disk overlaps the first minimum of the other disk. These two points are barely resolve.
➔ **Minimum resolvable feature size is ρ_s**

c) Profile of two disks at a separation such that the maximum of each disk overlaps the 2nd minimum of the other disk. These two points are clearly resolvable

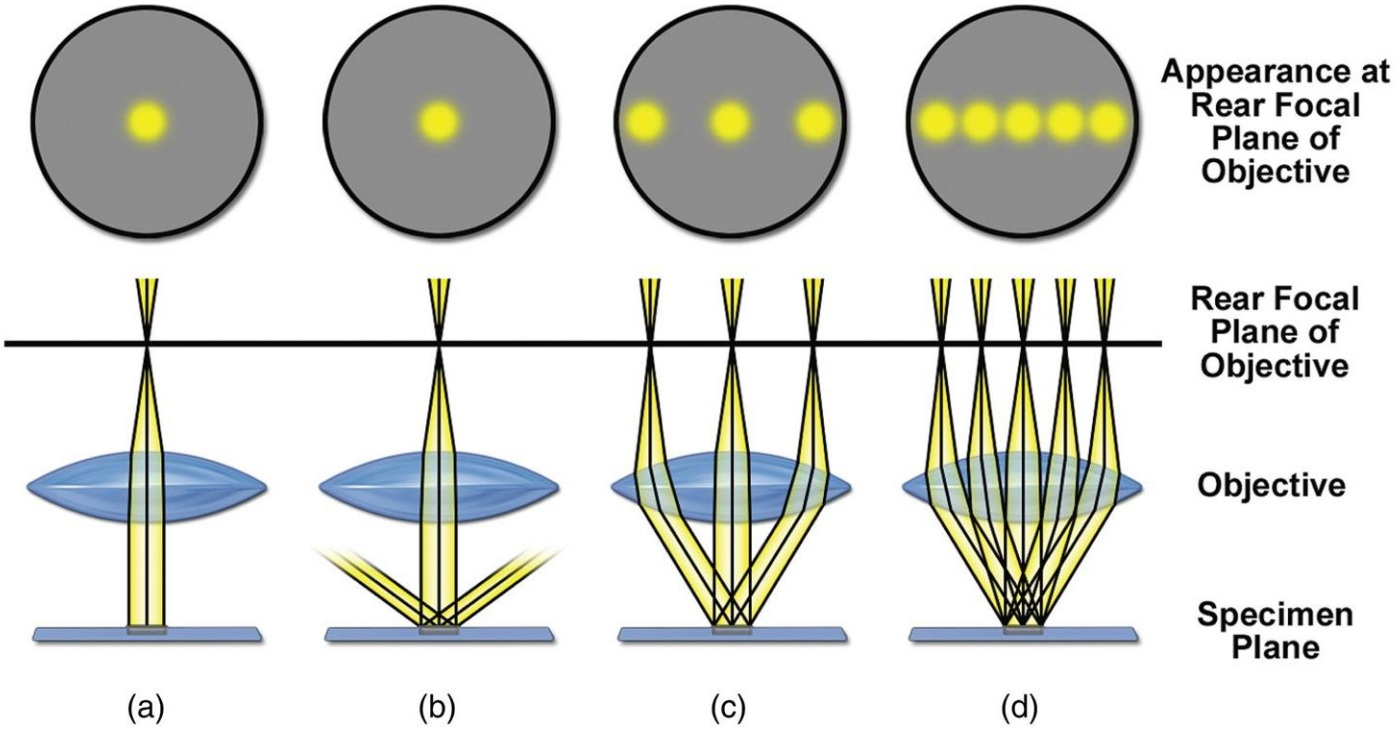


Reminder: Rayleigh Criterion for Resolution Limit

Minimum resolvable distance requires that the two PSF's (i.e. Airy disks) don't overlap



Reminder: Abbe's theory



a) No diffracting object in the specimen plane: no image
b) Grating as specimen. Collect no diffraction other than 0^{th} order ➔ no image
c) Grating as specimen. Collect 0^{th} & 1^{st} orders diffraction ➔ Image with minimum resolution
d) Grating as specimen. Collect multiple higher orders ➔ Image with higher resolution

Abbe showed that we need at least TWO adjacent orders
Ex: 0^{th} & 1^{st}

2014 Nobel Prize in Chemistry: Super Resolution Microscopy

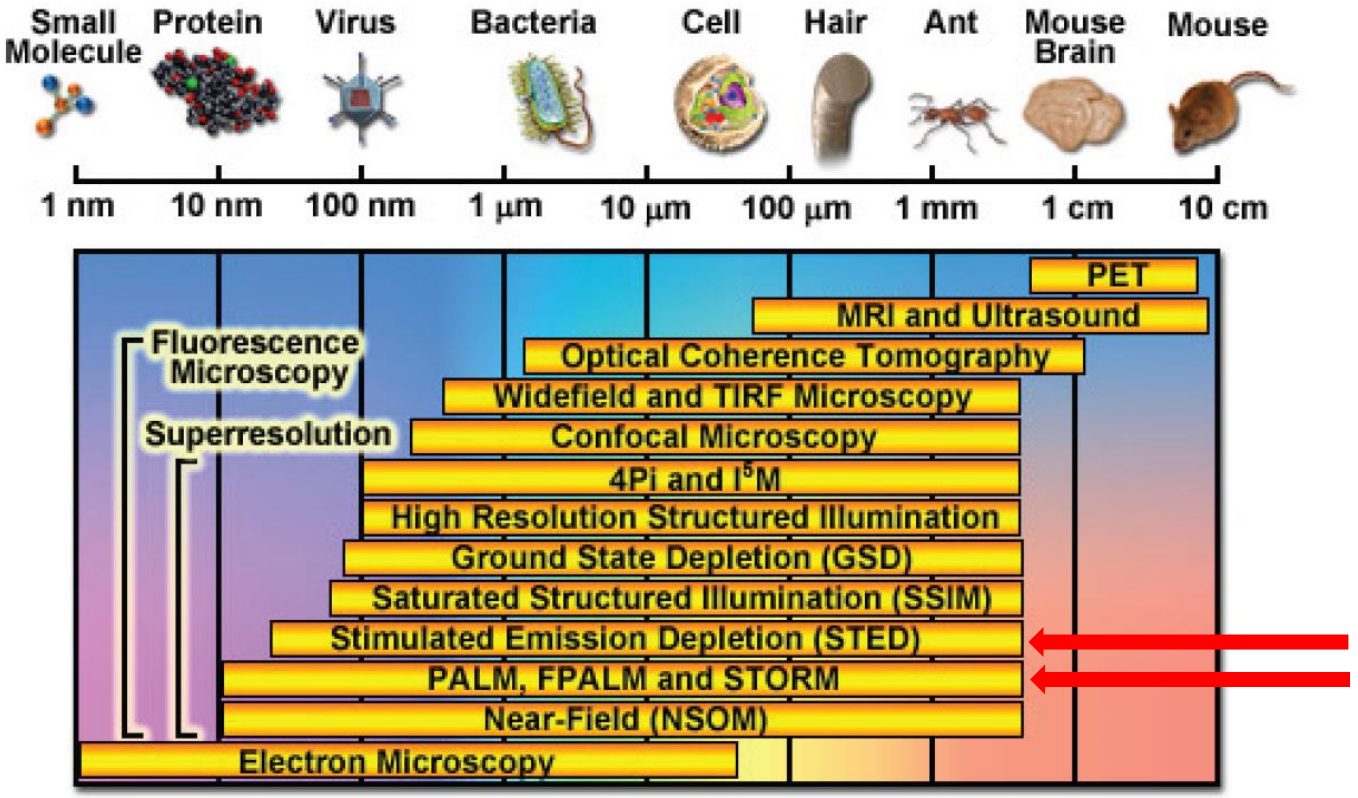
Eric Betzig
Howard Hughes Medical Institute
VA, USA

Stefan W. Hell
Max Planck Institute for
Physical Chemistry and
Cancer Research Center
Germany

William E. Moerner
Stanford University
CA, USA

***"for the development of super-resolved fluorescence microscopy"*.**
-- opening a window into the nanoworld

Super Resolution Microscopy



Two "<u>far-field</u> <u>super-resolution</u>" methods exploit the following mechanisms:

- **<u>Non-linear process to sharpen the PSF</u>**, as in stimulated emission depletion (STED), which is pioneered by Stephan Hell.
- **<u>Localization of individual molecules,</u>** as in photo-activated localization microscopy (PALM), which is pioneered by Eric Betzig.

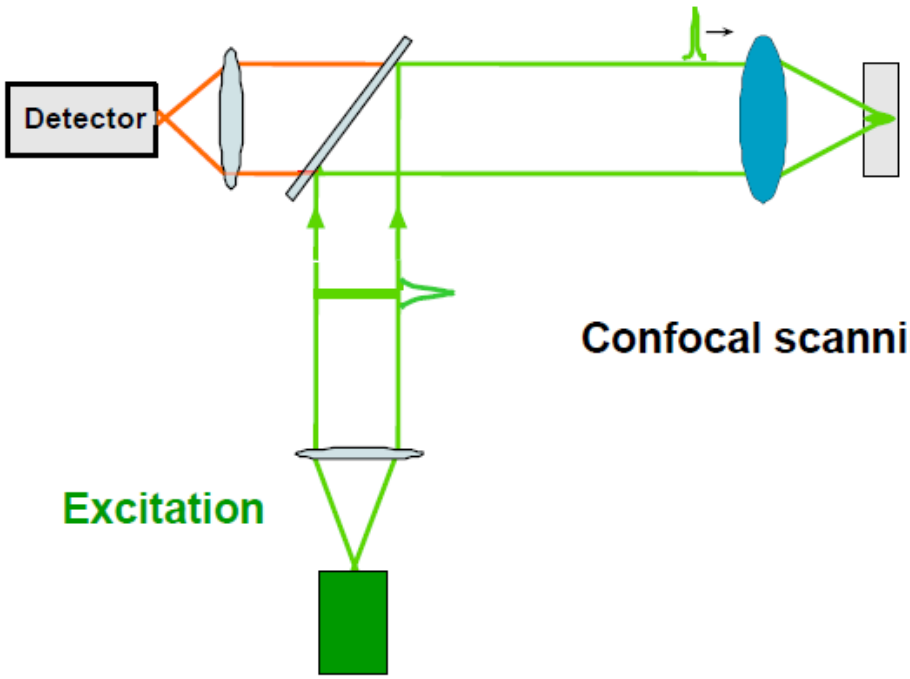
2014 Nobel Prize in Chemistry: Super Resolution Microscopy

Stimulated Emission Depletion (STED) Microscopy

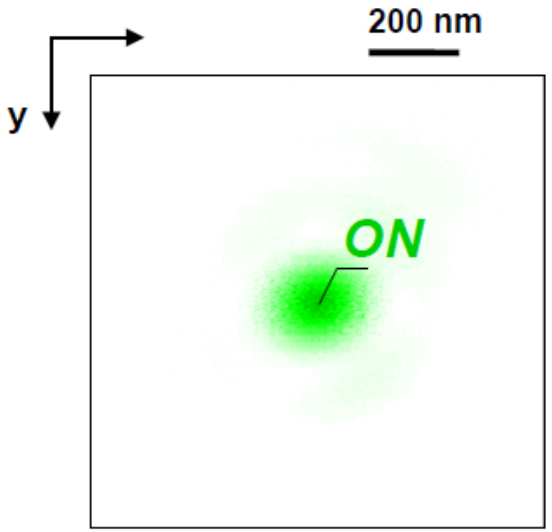
Stefan W. Hell
Director of Max Planck Institute for
Physical Chemistry and Cancer Research Center
Germany

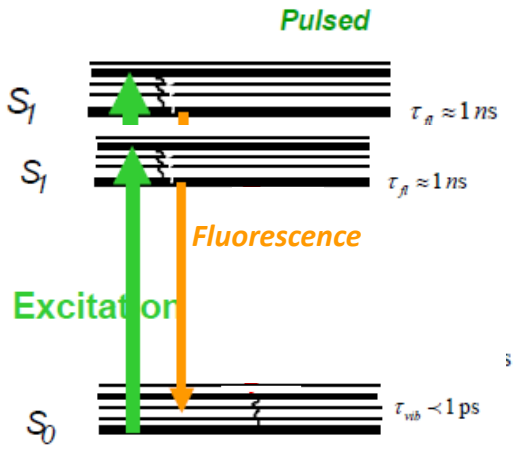
- It uses two overlapping synchronized laser beams, which arrive at the sample position consecutively.
- The first one (excitation laser) excites and the second one (depletion or STED laser) “de-excites” (or depletes) the excitation within the sample.

STED: The base is a confocal fluorescence microscopy

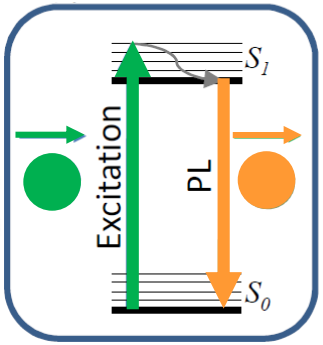


Confocal scanning microscope

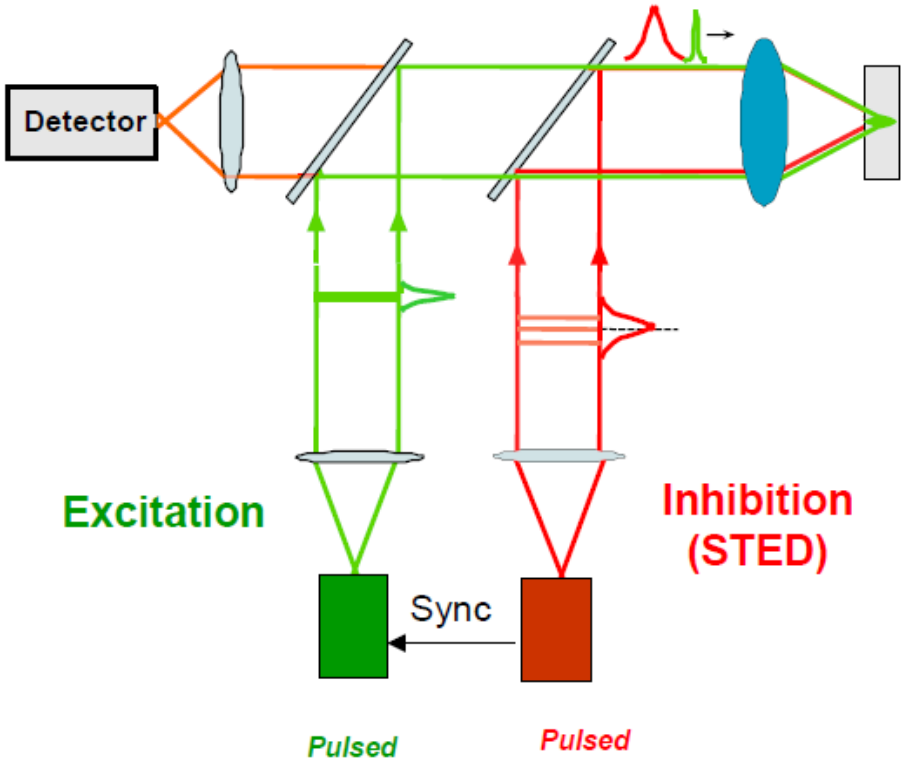


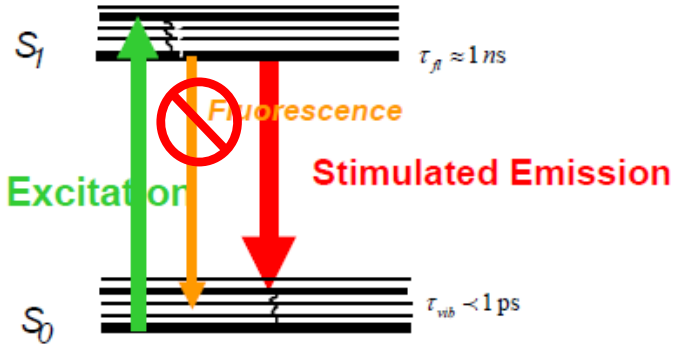


- Fluorescence occurs when a dye is excited to a higher energy state and **spontaneous relaxation** of the electron to the ground state by generating a new photon, based on the dye's characteristic energy levels.
- Due to vibrational relaxations, fluorescence or so called photo-luminescence (PL) is always *red-shifted*.

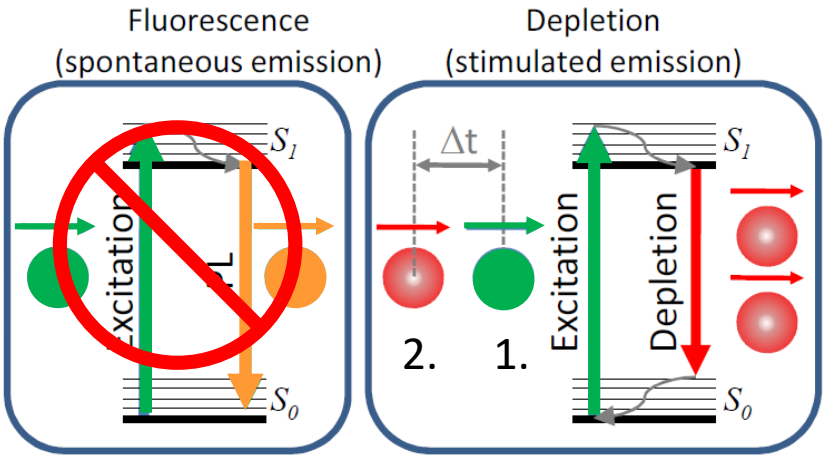


STED Microscopy: Stimulated Emission Depletion

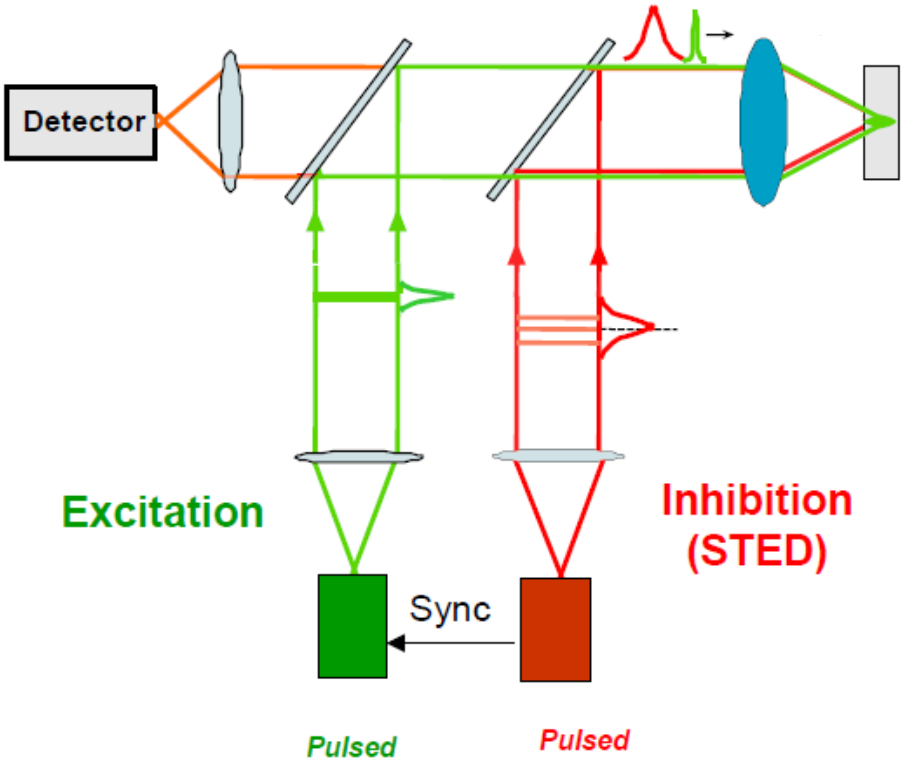




- If the sample is illuminated with a second laser beam **while still being in the excited state**, the second laser (aka inhabitation or STED laser) kicks the electron back to the ground state without fluorescence (spontaneous) emission.
- Instead of spontaneous emission, a process known as the **stimulated emission** takes place, and the second photon copies itself. In other words the energy difference between the ground and the excited state is used to generate a photon which is exactly the same as the incoming depletion photon in wavelength, polarization and more importantly, direction of propagation.
- As a result, the dye returns to the ground state without generating fluorescence emission.

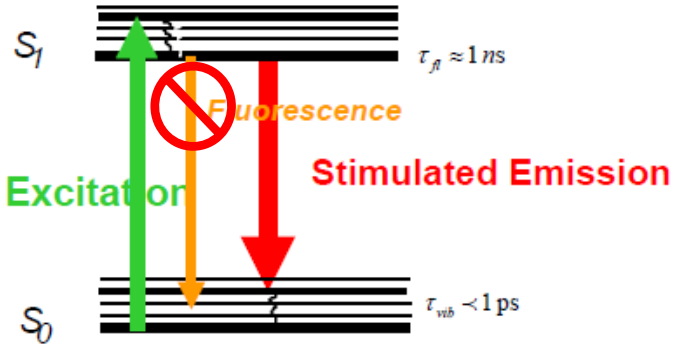


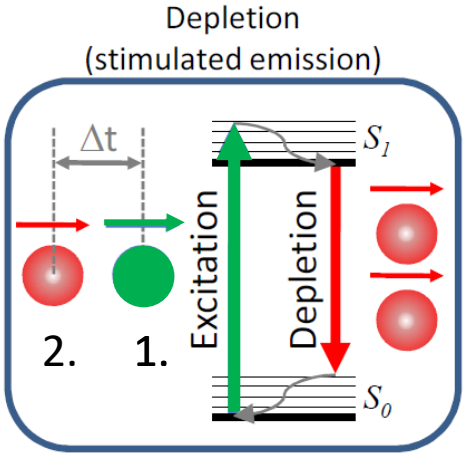
STED Microscopy Parameters – 1 (Temporal Control)



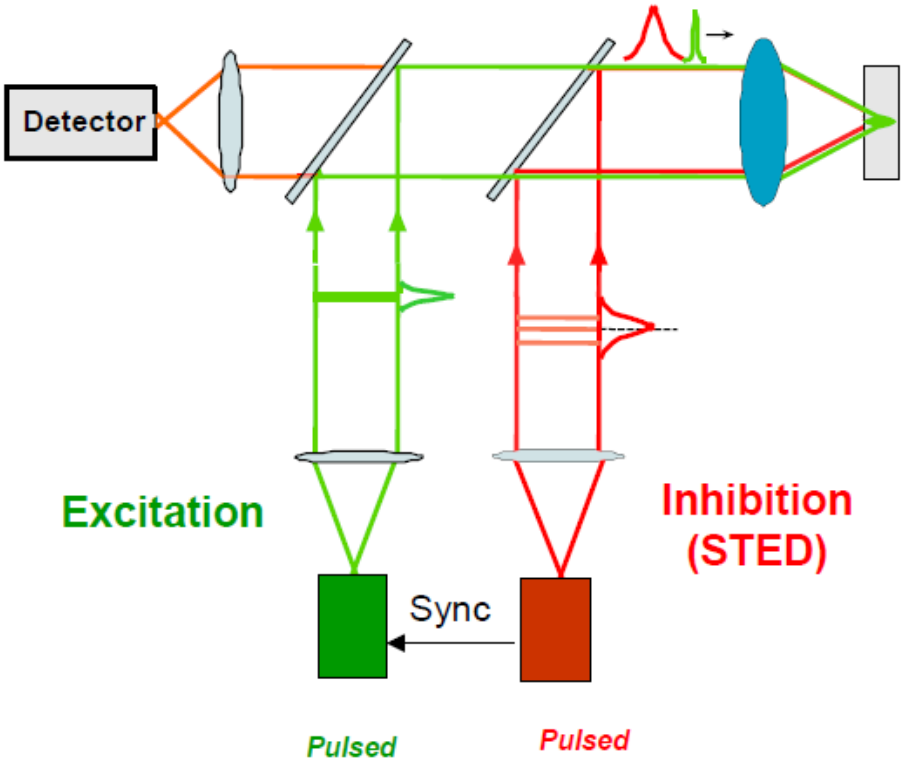
An important optimization parameter is the <u>time-delay (Δt) between excitation laser and STED laser</u>.

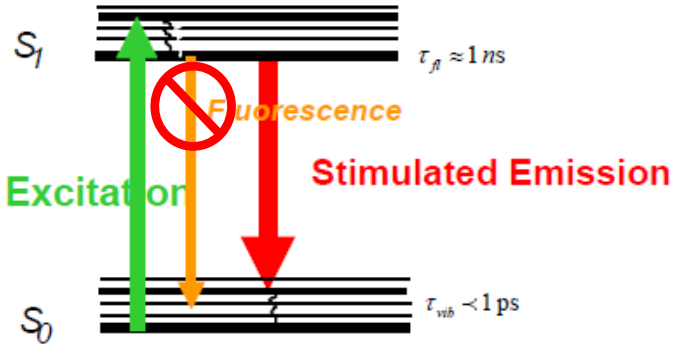
- STED laser beam should arrive at the sample when the electron is still in the excited state.
- Pulse width of the STED laser cannot be longer than the excited state lifetime of the molecule.
- Typical pulse width is 200-300 ps.





STED Microscopy Parameters – 2 (Spectral Control)

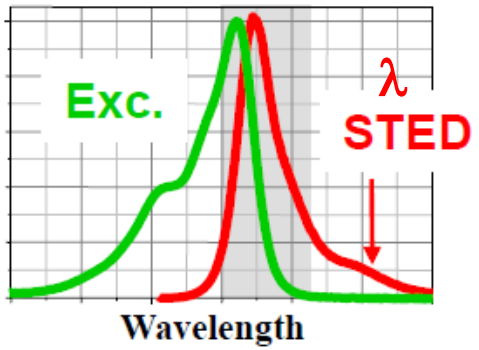




Another important optimization parameter is the <u>wavelength (λ)</u> of the STED laser beam.

- It should not overlap with the absorption spectrum of the dye.
- But, it should be in the range of the emission wavelength of the dye.

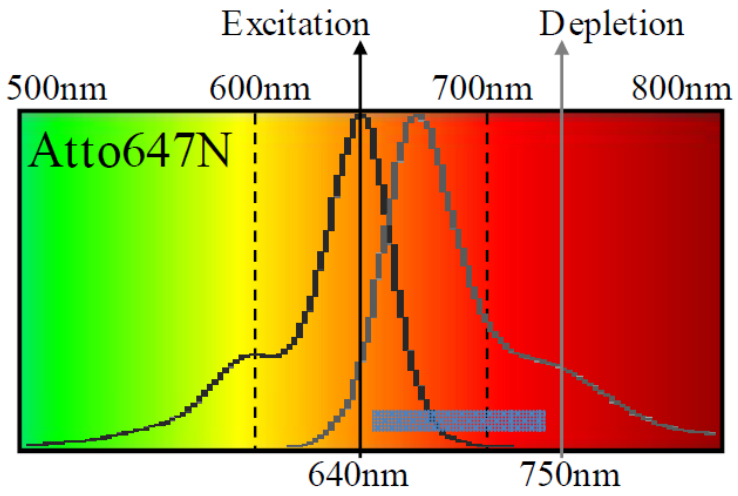
- Therefore, the wavelength of the STED laser beam is **selected to be in the tail of the emission** spectrum of the dye **without overlapping with the absorption spectrum to avoid re-absorption**.

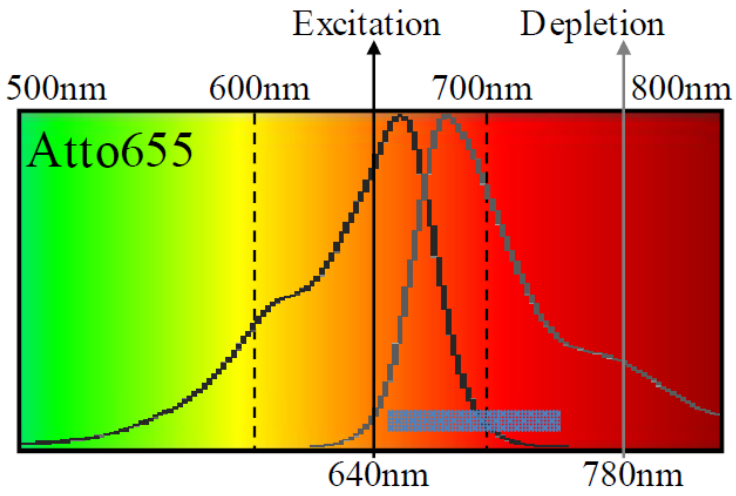


Example: Lasers & Dyes for Stimulated Emission Depletion

Absorption (i.e. excitation) & emission characteristics of two dyes suitable for STED imaging:

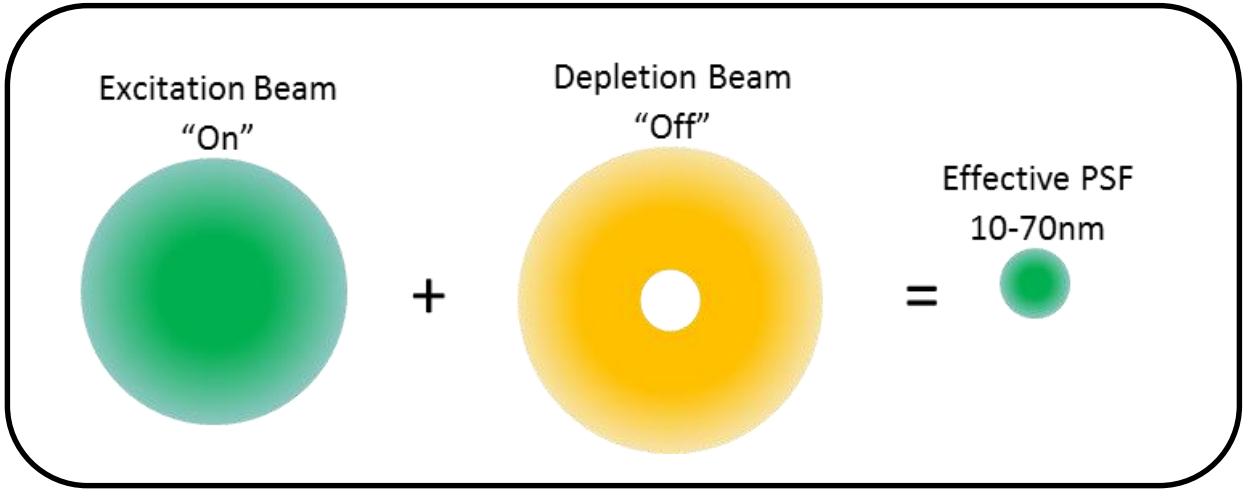
- Arrows show the ideal wavelength for excitation laser and depletion (STED) laser.
- Solid horizontal gray bars show the emission range collected by the detector.





STED Microscopy Parameters – 3 (Spatial Control)

Stimulated Emission Depletion should take place in a "**Selected Region"**

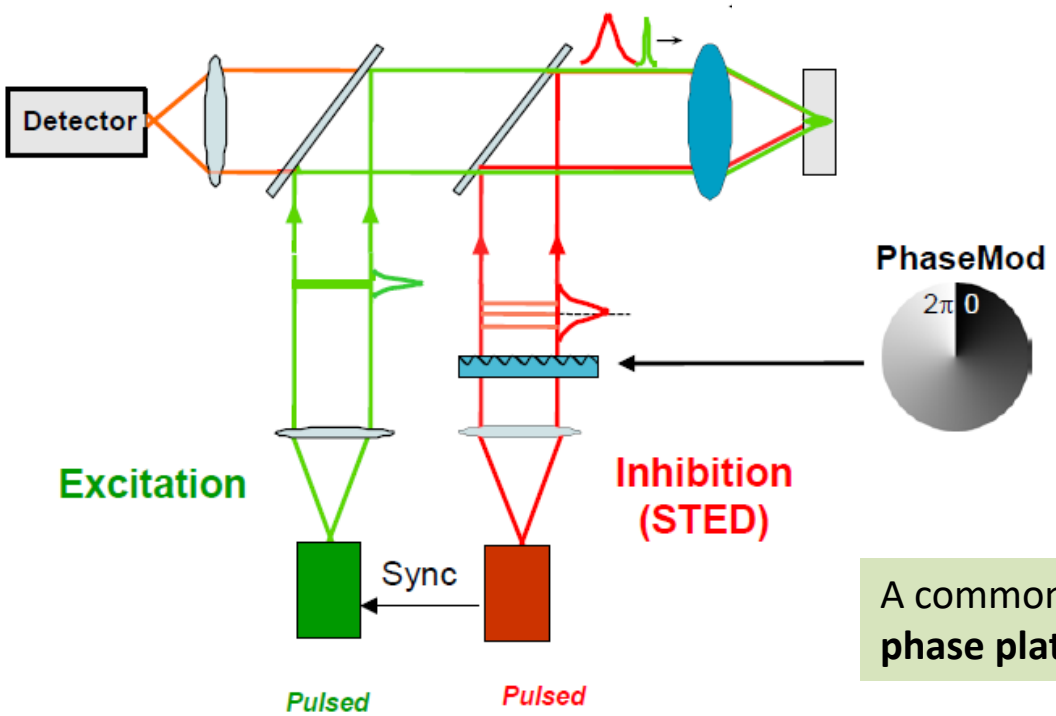


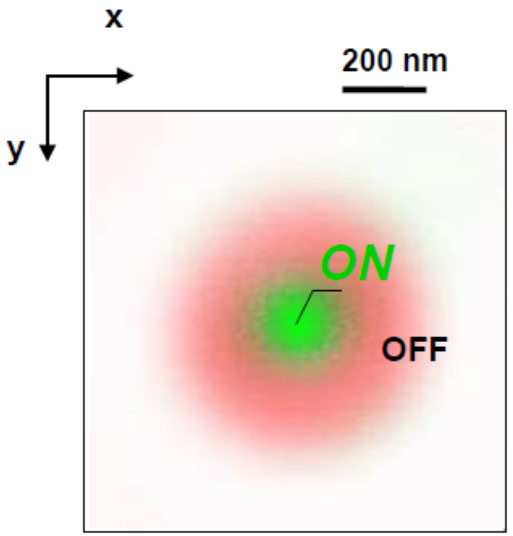
The essence of STED microscopy is to overlap spatially two beams (which act as excitation laser and depletion laser, consecutively) in selected regions as illustrated above:

- In this configuration, the STED laser (shown in yellow) possess zero-fields in the center and maximum in the periphery, which exhibits a doughnut-shaped phase pattern.
- The quality of the doughnut phase pattern of the depletion beam, which overlaps with the excitation beam determines the effective PSF (thus ultimate resolution) of the setup.
- Therefore, control of spatial overlap is an additional important optimization parameter in STED microscopy.

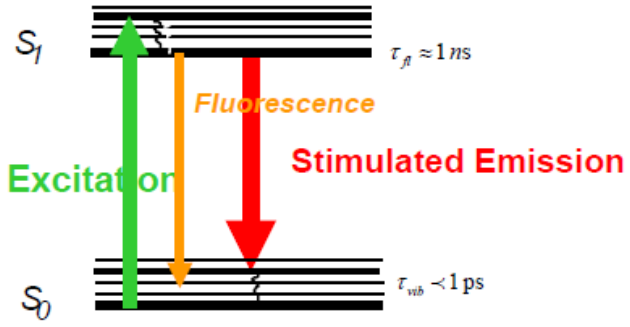
There are several methods for doughnut generation based on different techniques in wavefront engineering based on **phase-mask**.

Stimulated Emission Depletion Takes Place in a Selected Region: **Phase Mask**



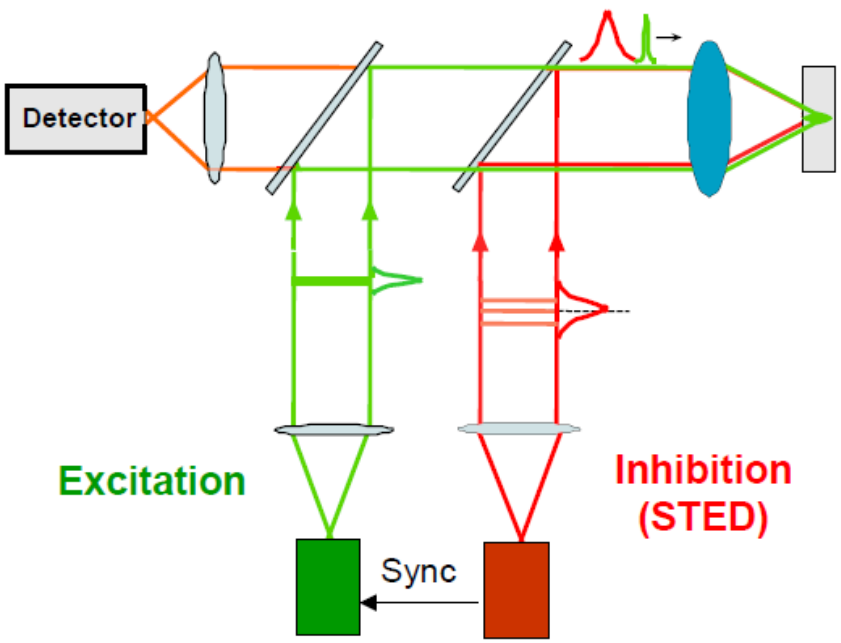


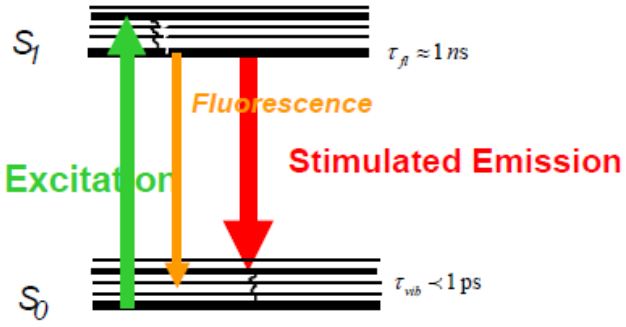
A commonly used phase mask is with "**Vortex" phase plates**.



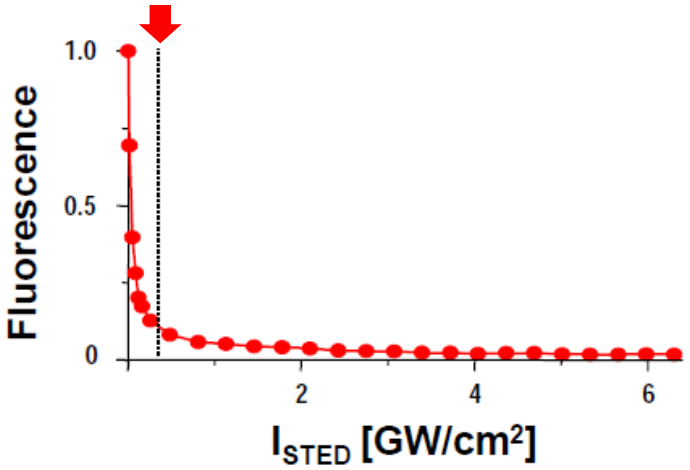
- STED laser beam, after being tuned in polarization by a half-waveplate, passes through a **vortex plate, which imprints a helical phase ramp of 2π onto the wavefront.**
- Next, it passes through a quarter-waveplate before entering the objective lens, which creates a circular polarization.
- This leads to destructive interference of all vectorial light fields at the focal point and creates a minimum field at the center of the focus ➔ thus resulting a **doughnut pattern**.

STED Microscopy Parameter- 4 (STED Laser Intensity Control)





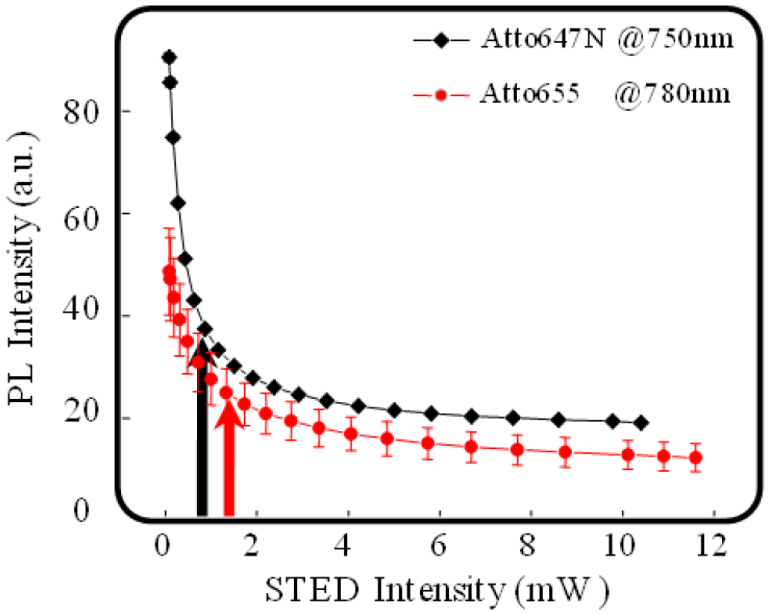
- The key in super-resolution imaging based on STED method is the **non-linear dependence** of the depleted population of the excited electrons on STED laser beam intensity.
- **If the STED laser intensity is more than a certain threshold, spontaneous emission is dominated by the stimulated emission.**
- This leads to strong suppression of the fluorescence or what is known as *depletion*.
- Thus, STED laser intensity is another parameter in STED microscopy.

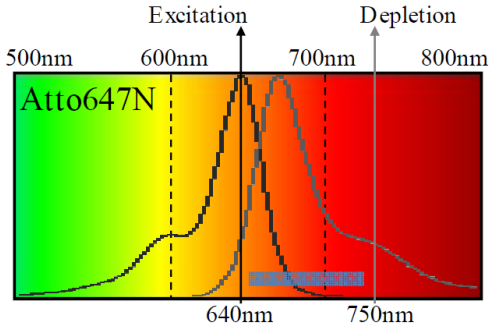


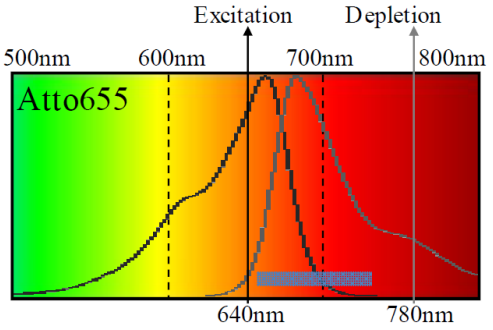
Saturation Intensity & STED Laser

- Fluorescence depletion of two common dyes in STED microscopy, Atto647N (black, diamonds) and Atto655 (red, circles), as a function of the depletion laser intensity.

- Stimulated emission becomes the dominant process after increasing the depletion laser intensity more than a critical depletion threshold, which is defined as the intensity of the laser at 50% depletion of the photo-luminescence (PL) (marked by arrows for both dyes).

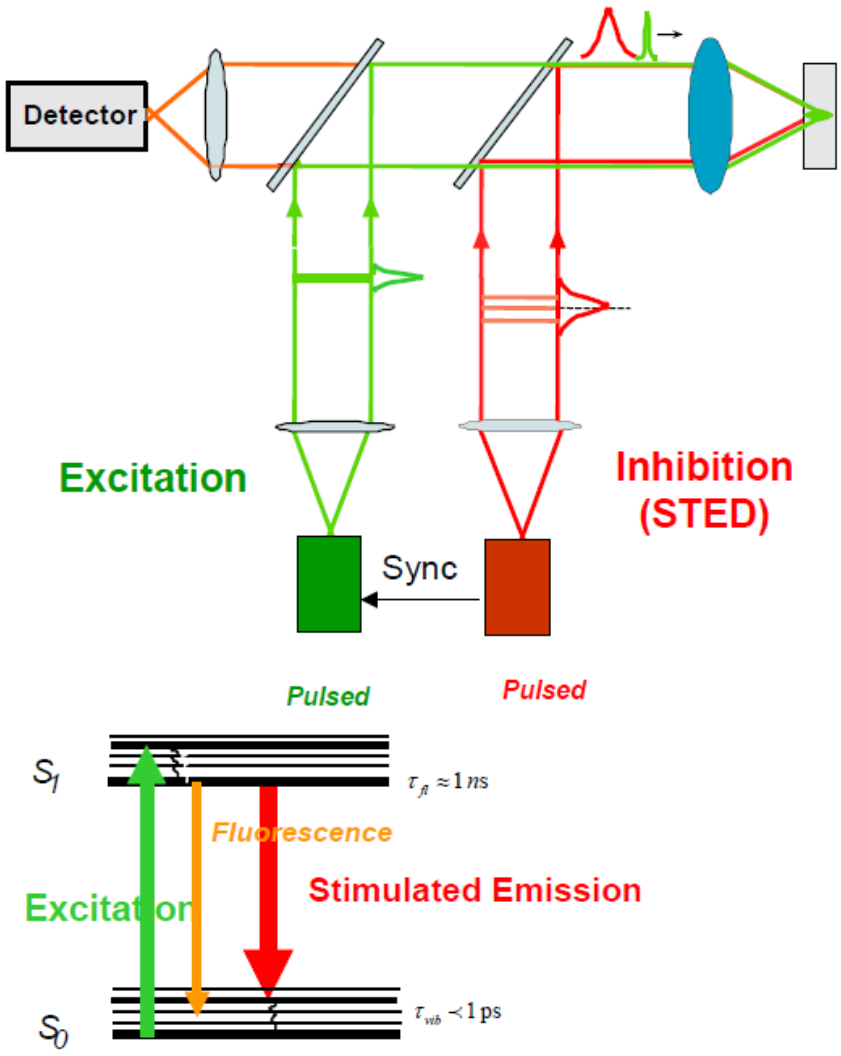






- Depletion process is characterized by placing a layer of dye on the coverslip and then subjecting this to the excitation and depletion laser beams and finally detecting the fluorescence signal using an avalanche photodiode.
- Excitation wavelength is 640 nm for both dyes. Wavelength of the depletion laser is tuned to be on the tail of the emission spectrum with no overlap with the absorption spectrum: 750 nm (Atto647N) and 780 nm (Atto655).

Resolution in Stimulated Emission Depletion



Two factors determine the resolution of STED microscopy:

- STED laser intensity (I or I_{STED})
- Saturation intensity of the fluorophore (I_{sat}).

Extended version of the optical resolution limit for STED is defined by Westphal and Hell as:

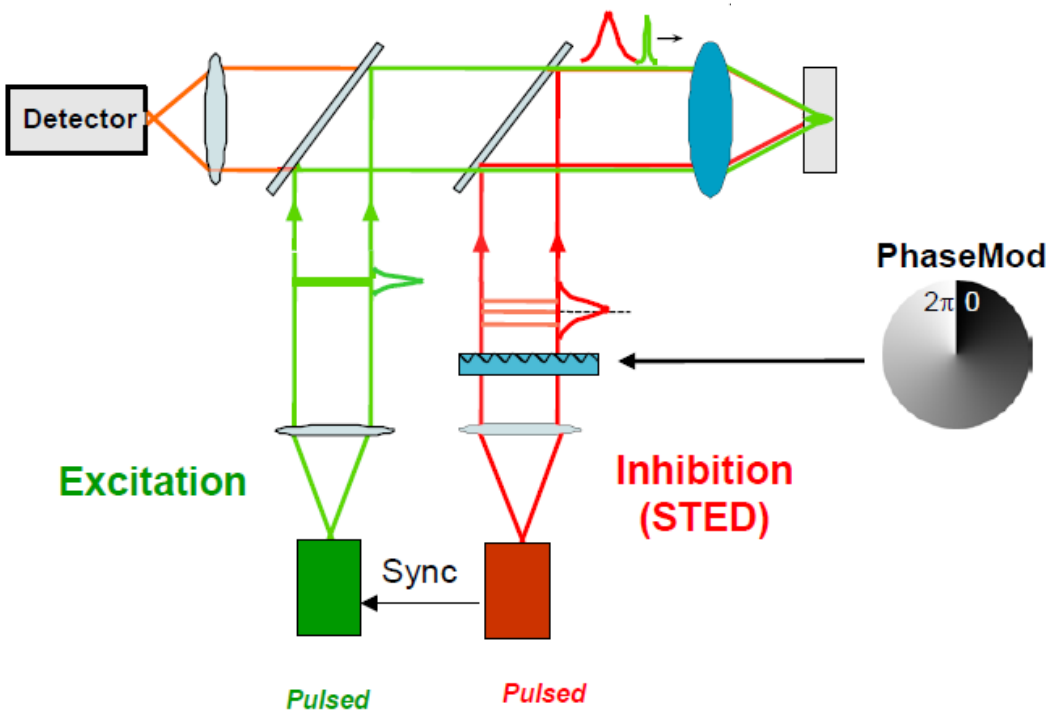
$$d_{min}(I) \approx \frac{1}{2} \frac{\lambda}{nsin(\theta)\sqrt{1 + \frac{I}{I_{sat}}}}$$

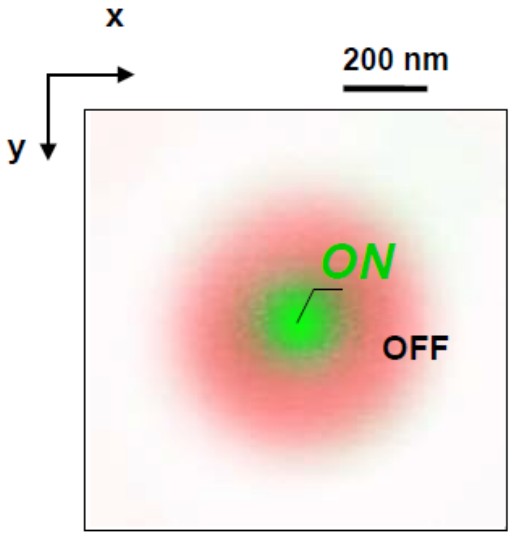
- λ is the wavelength of the excitation laser
- *n.sin*θ is the numerical aperture of the objective with
  - *n* as the refractive index of the medium
  - θ as half the focal angle
- *I* is the intensity of the STED laser
- I_{sat} is the saturation intensity of the fluorophore

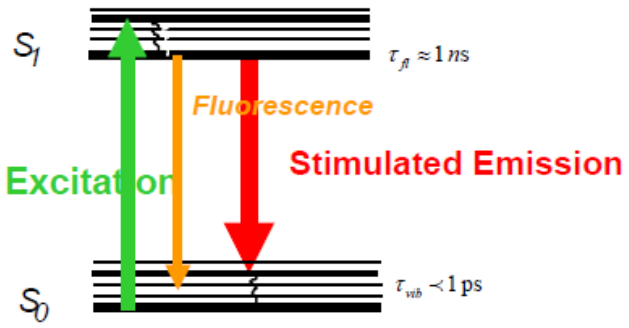
$$I_{sat} \approx \frac{1}{\sigma\tau} \frac{hc}{\lambda}$$

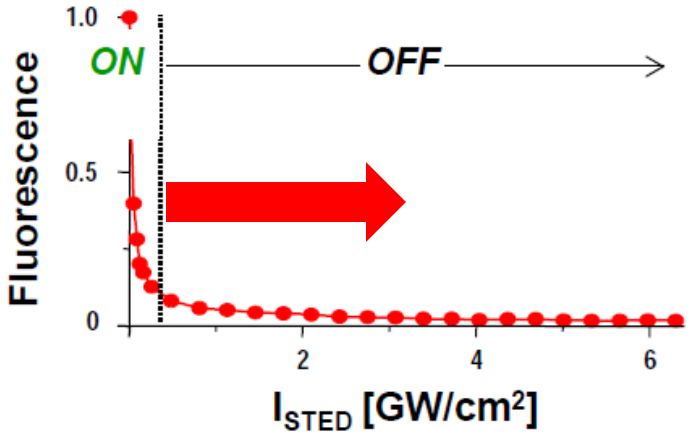
- σ is the absorption cross section of the dye (cm^2)
- τ is the fluorescence lifetime (1/s)
- hc/λ is the energy of the photon (Joule)

Intensity of STED Laser & Resolution

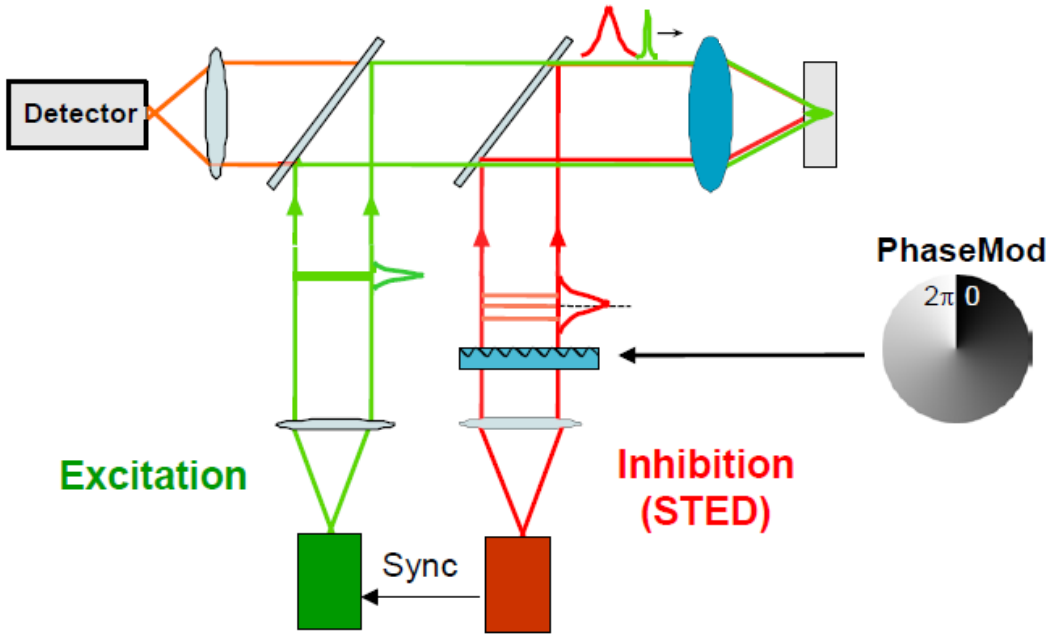


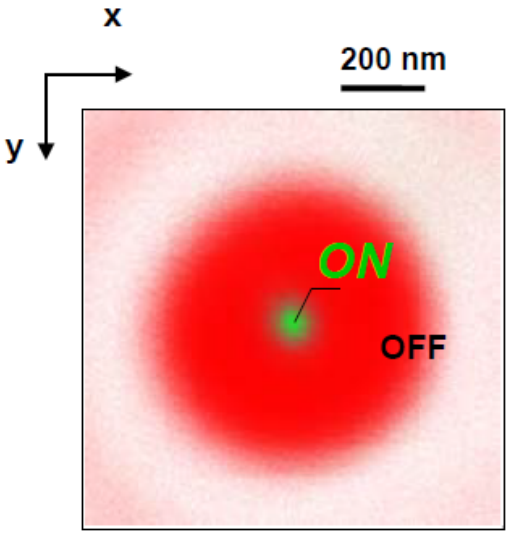


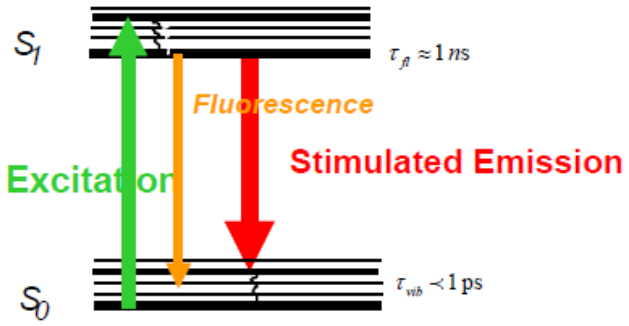


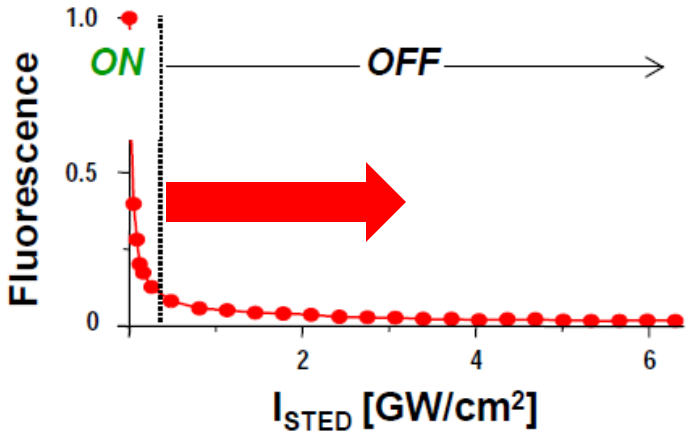


Intensity of STED Laser & Resolution





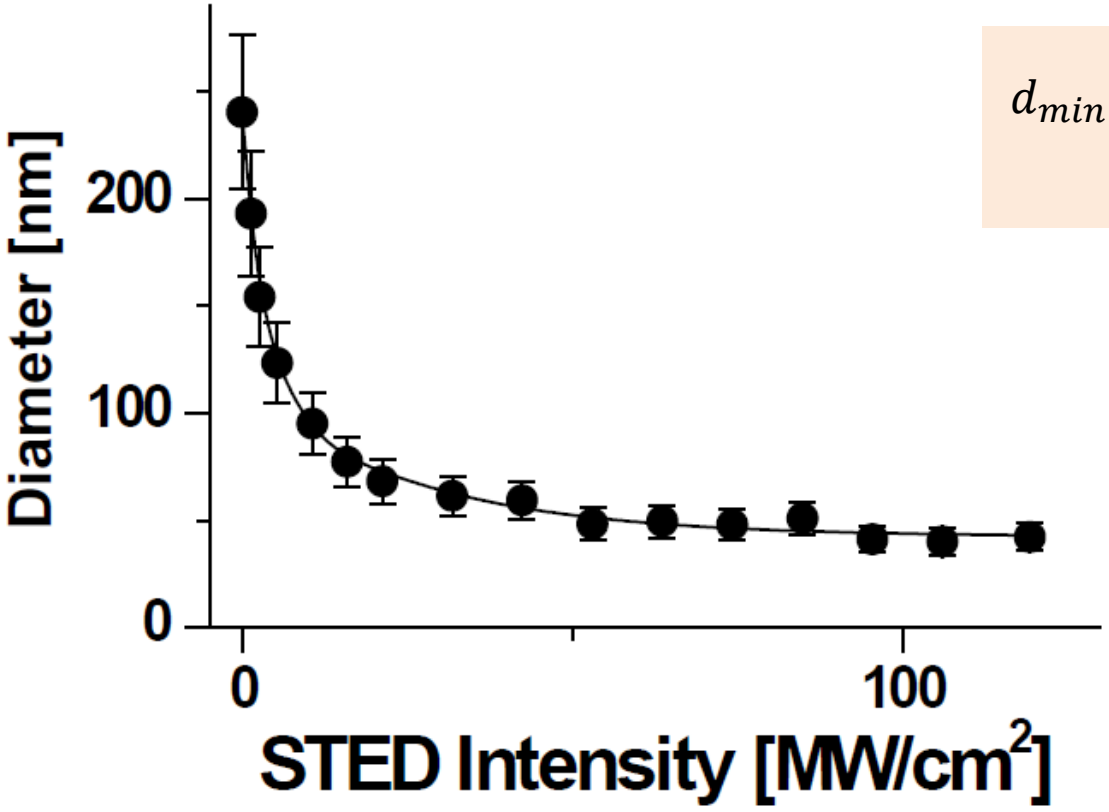




STED Microscopy

Dynamical control of resolution

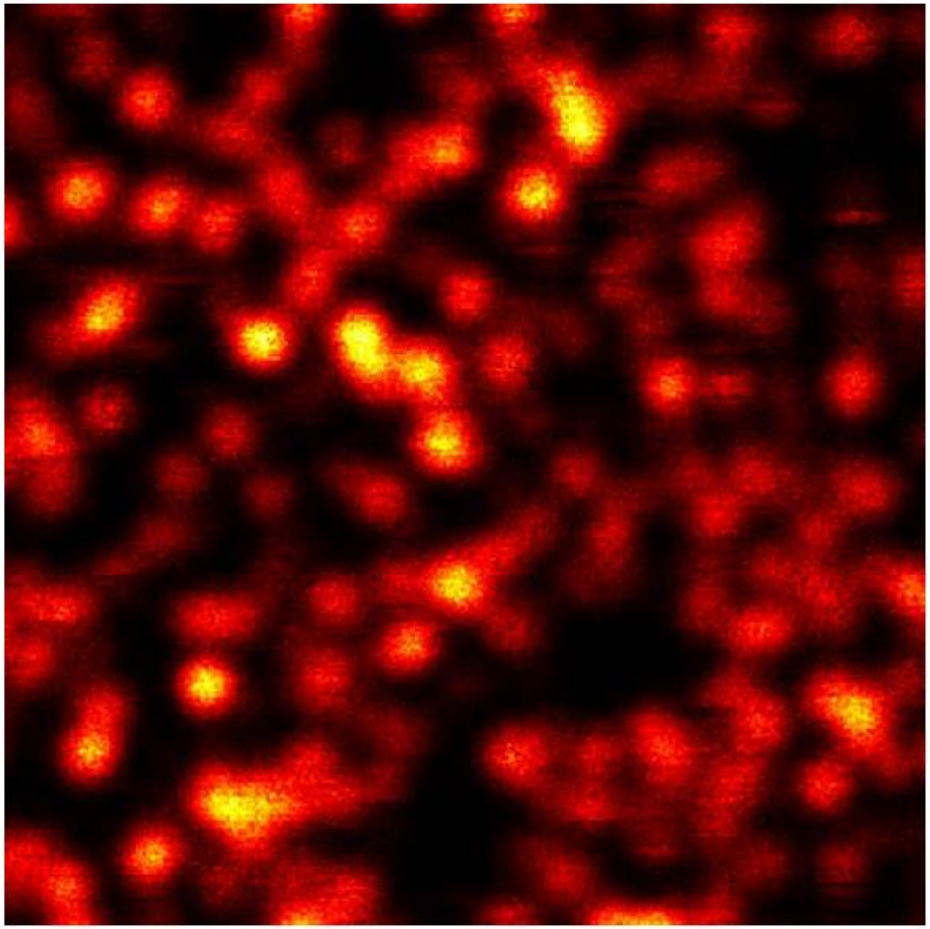
Nanoscale observation areas: CONTINUOUS TUNING of spatial resolution

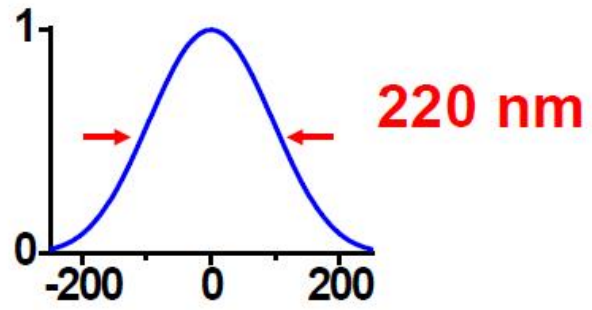


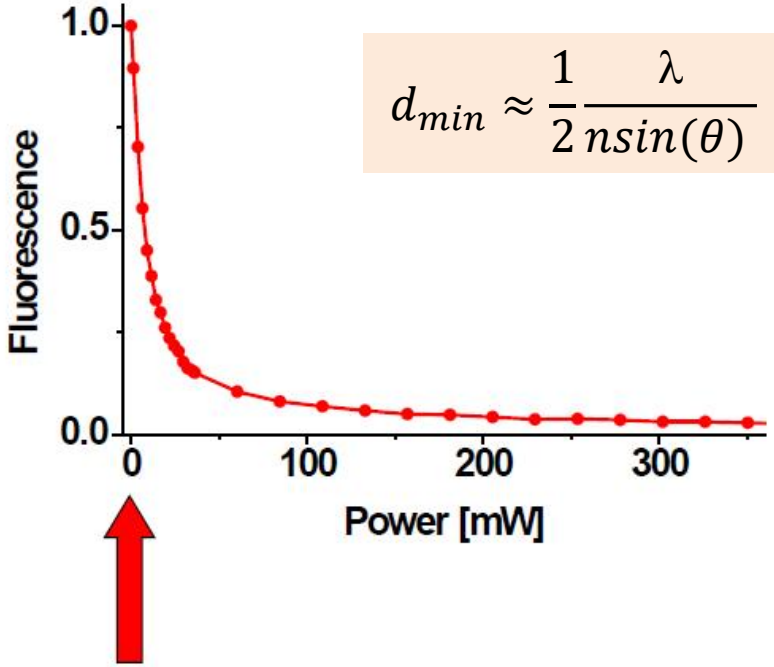
$$d_{min}(I) \approx \frac{1}{2} \frac{\lambda}{n sin(\theta) \sqrt{1 + \frac{I}{I_{sat}}}}$$

STED Microscopy

Sub-Diffraction Imaging





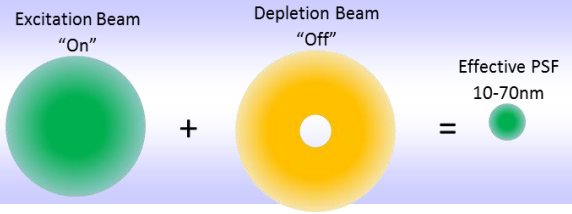


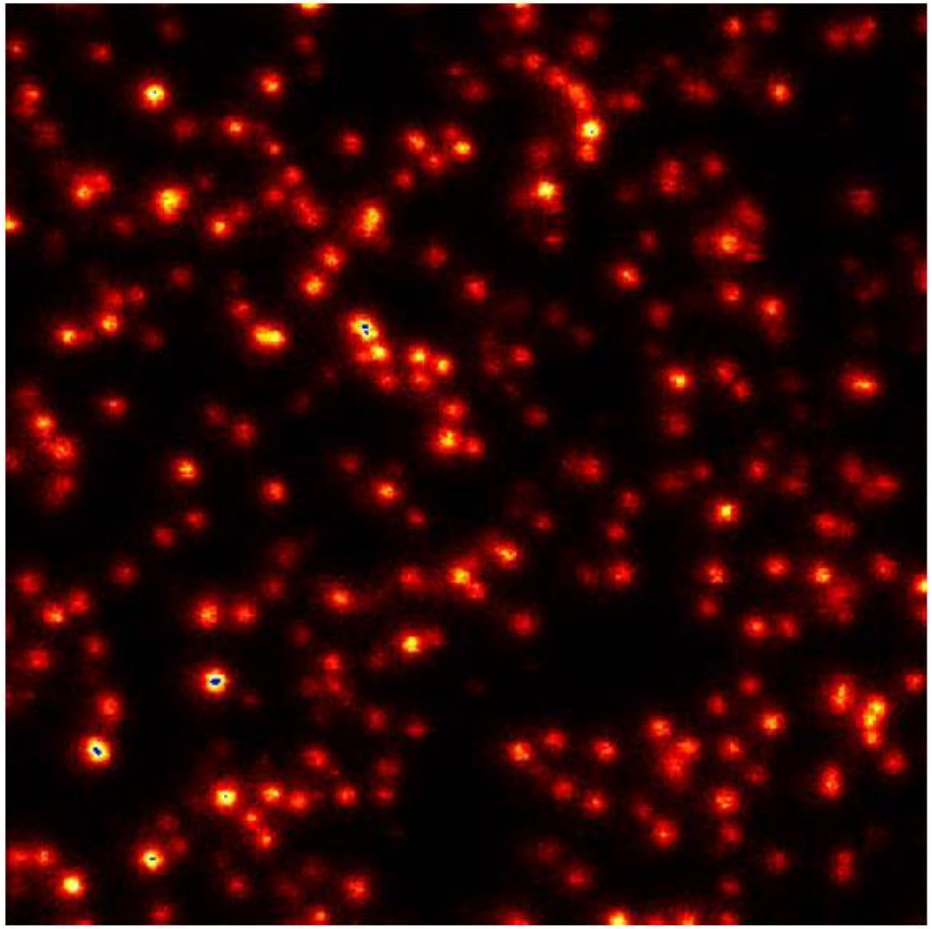
$$d_{min} \approx \frac{1}{2}\frac{\lambda}{nsin(\theta)}$$

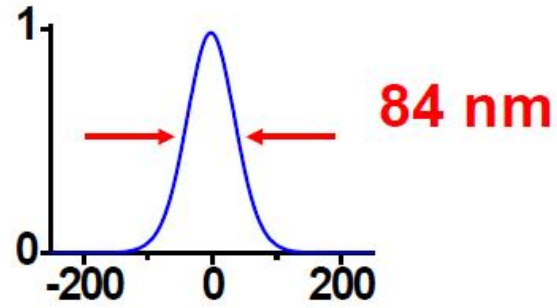
20nm Crimson beads
633nm exc, 90ps, 30kW/cm²
785nm STED 200ps, 76MHz

STED Microscopy

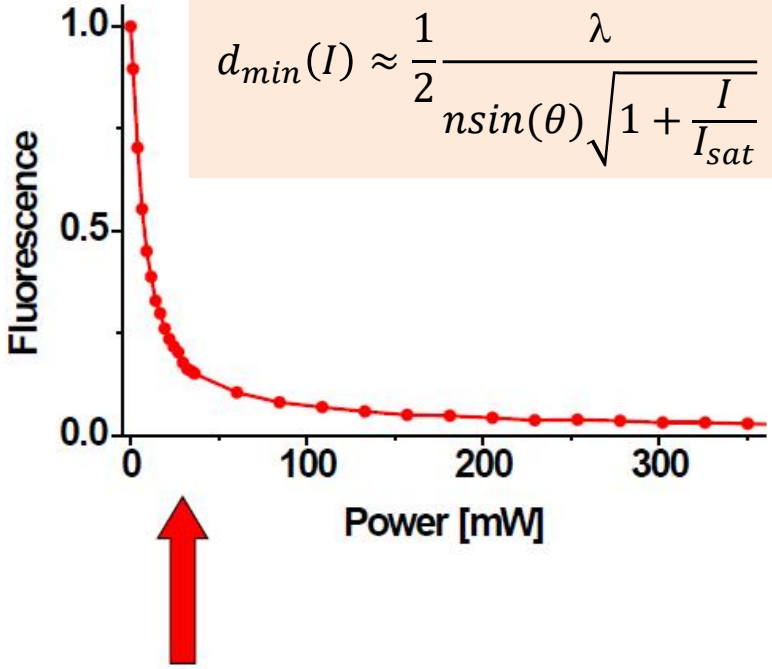
Sub-Diffraction Imaging







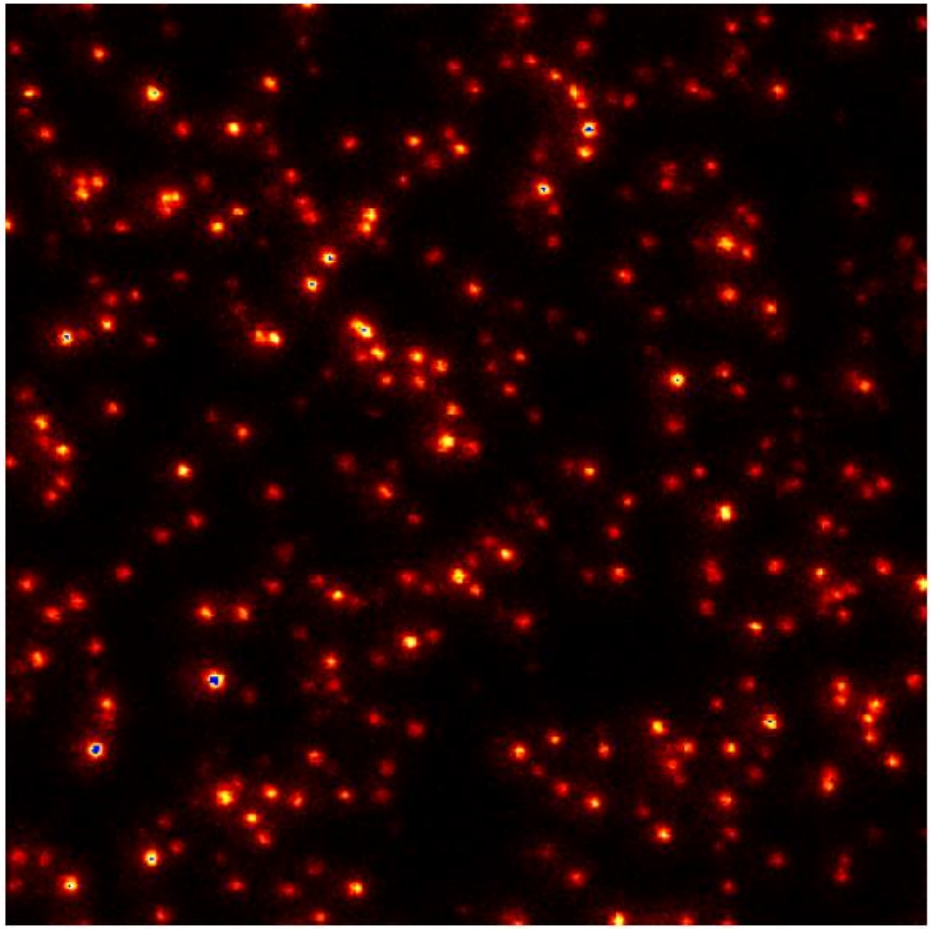
$$d_{min}(I) \approx \frac{1}{2}\frac{\lambda}{nsin(\theta)\sqrt{1+\frac{I}{I_{sat}}}}$$

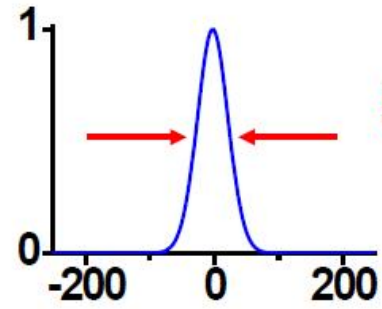


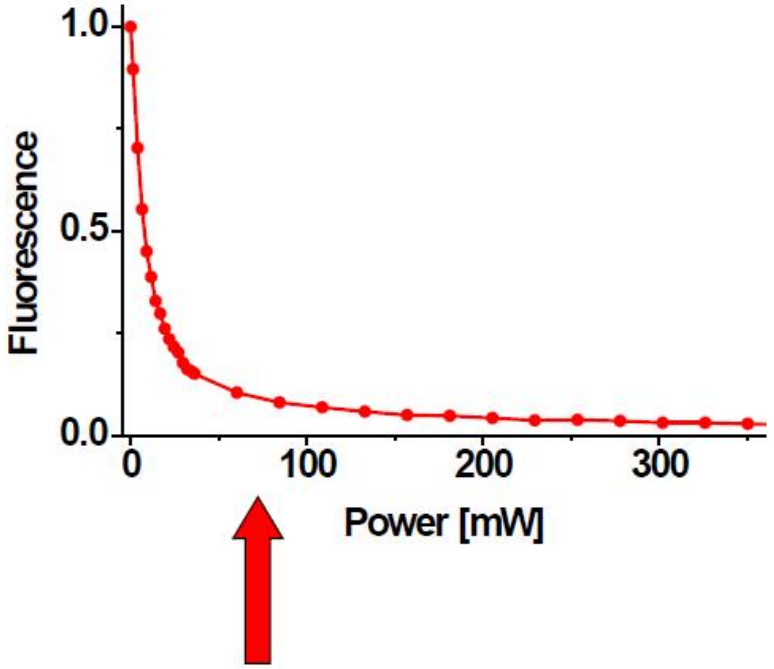
20nm Crimson beads
633nm exc, 90ps, 30kW/cm²
785nm STED 200ps, 76MHz

STED Microscopy

Sub-Diffraction Imaging







20nm Crimson beads
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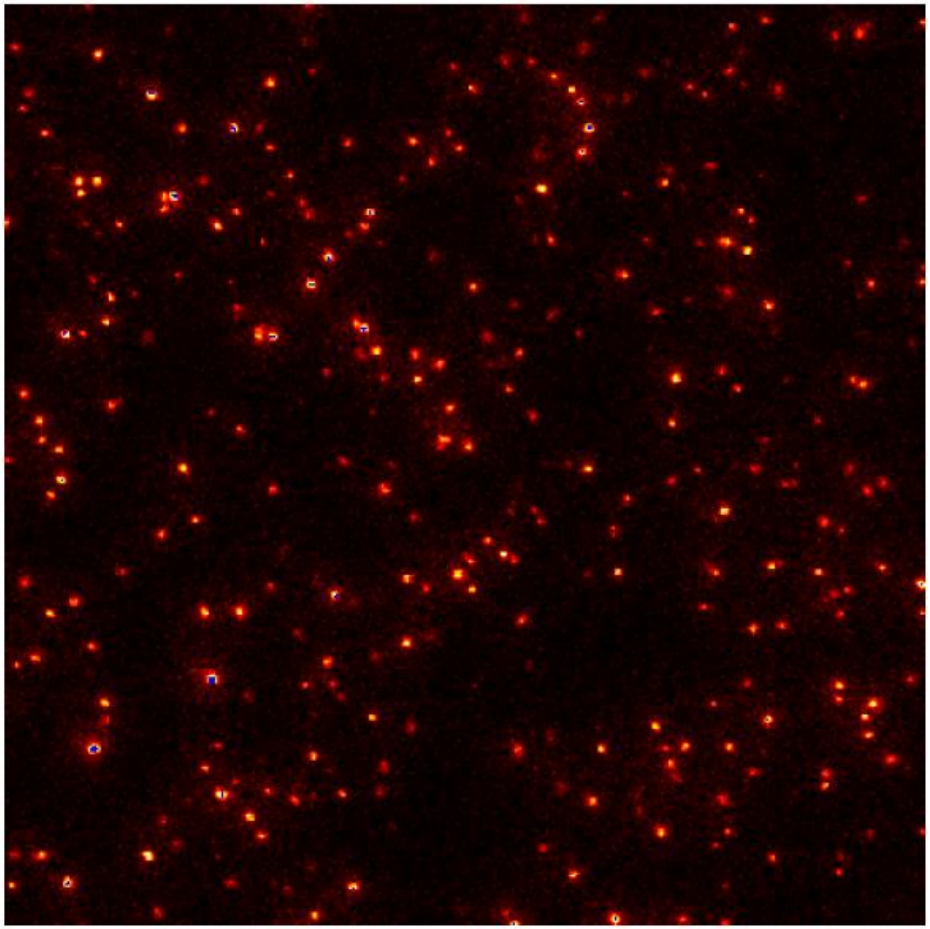
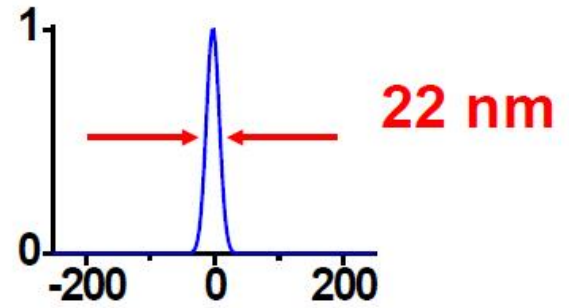
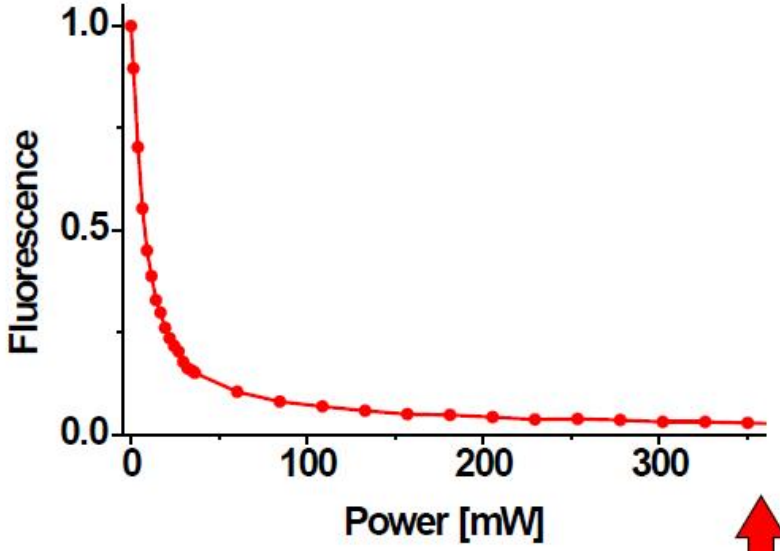
STED Microscopy

Sub-Diffraction Imaging



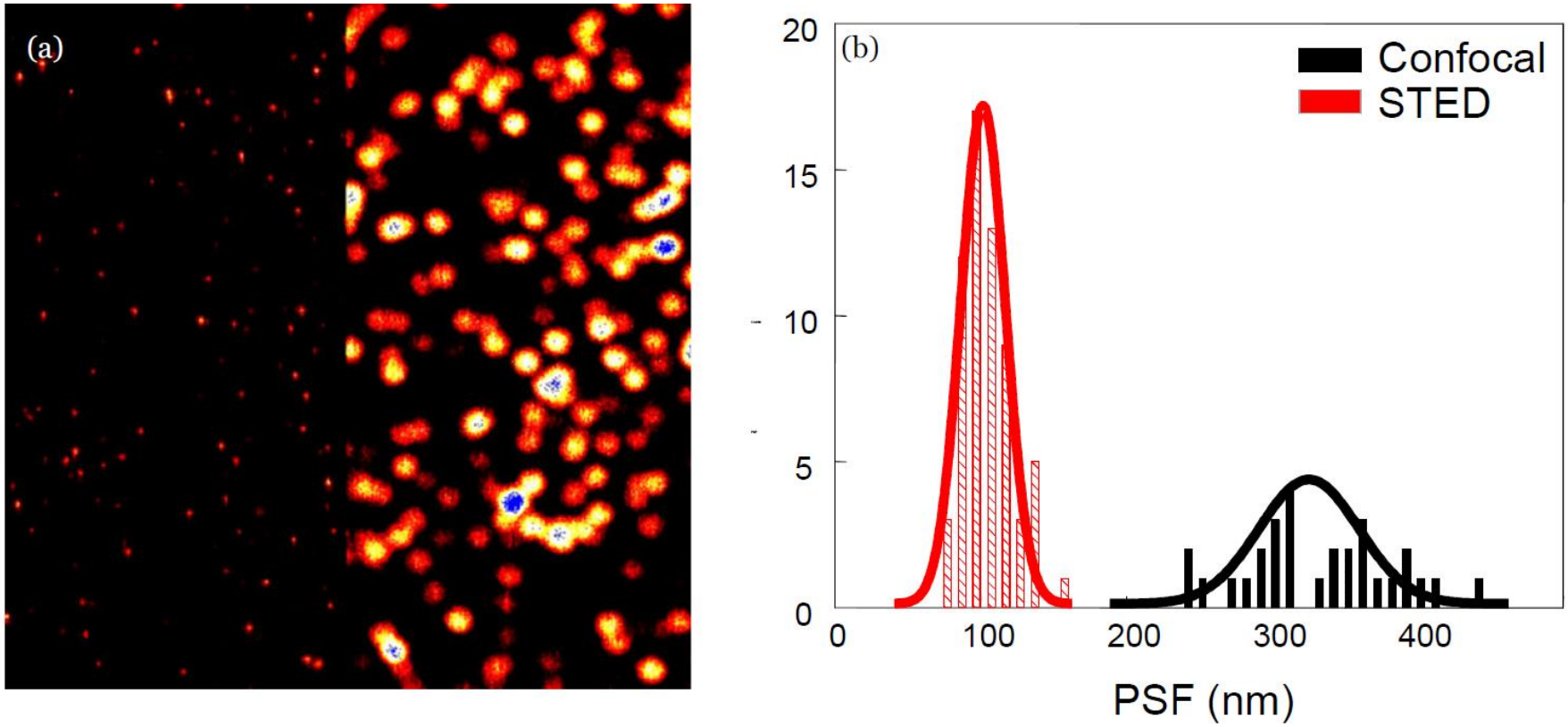




20nm Crimson beads
633nm exc, 90ps, 30kW/cm²
785nm STED 200ps, 76MHz

STED Microscopy

Sub-Diffraction Imaging

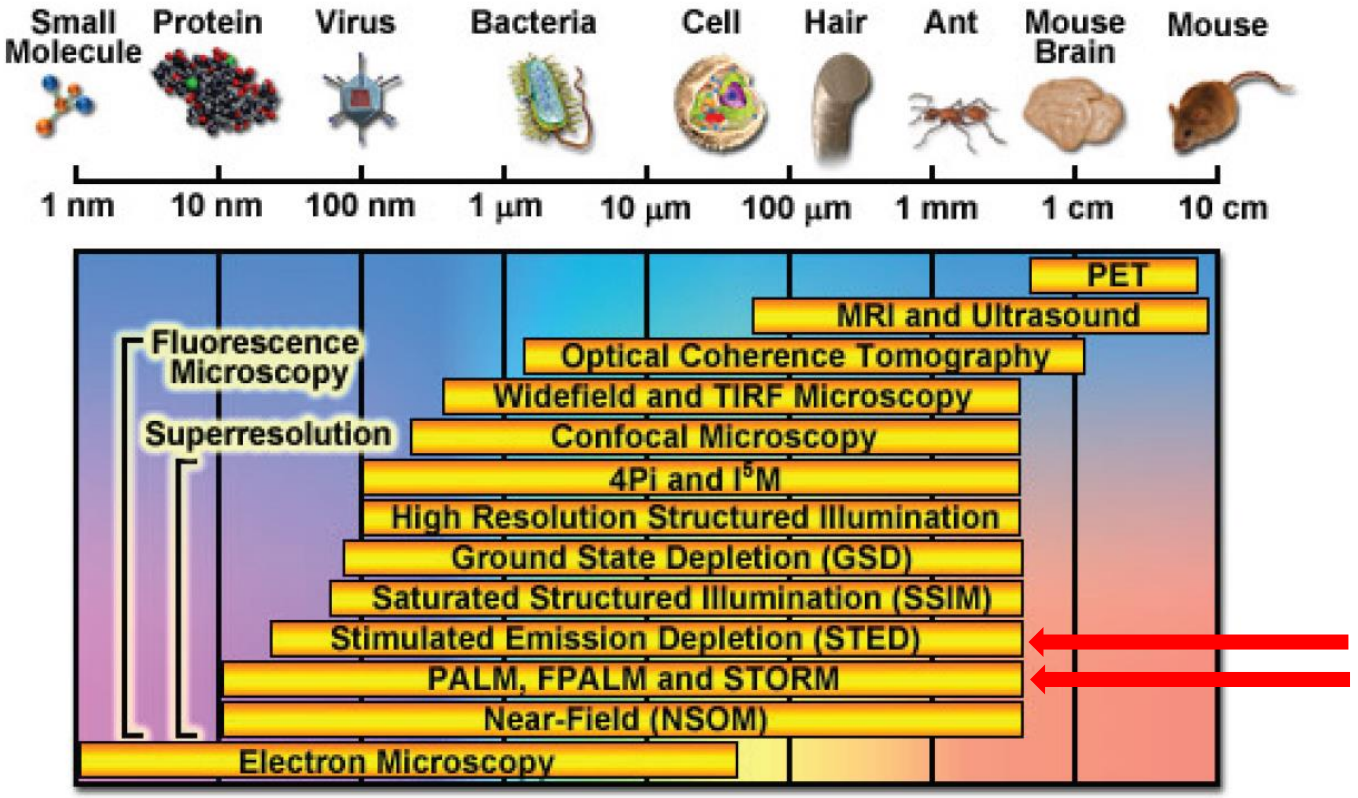


Determination of the STED microscope's resolution:

(a) STED (left hand) and confocal (right hand) images of crimson beads filled with Atto647N. The improved resolution on the STED image is clearly visible by the naked eye. Image size is 8.7 μm

(b) Resolution has been quantified in (b). Two dimensional Gaussian fit for more than 90 beads imaged with STED and confocal to determine and histogram the resolution of the microscope. These histograms are represented in (b) which show the resolution of STED to be around 90-100 nm with a relatively smaller distribution compared to confocal which is centered around 300 nm.

Super Resolution Microscopy



Two "<u>far-field</u> <u>super-resolution</u>" methods exploit the following mechanisms:

- **<u>Non-linear process to sharpen the PSF</u>**, as in stimulated emission depletion (STED), which is pioneered by Stephan Hell.
- **<u>Localization of individual molecules,</u>** as in photo-activated localization microscopy (PALM), which is pioneered by Eric Betzig.

2014 Nobel Prize in Chemistry: Super Resolution Microscopy

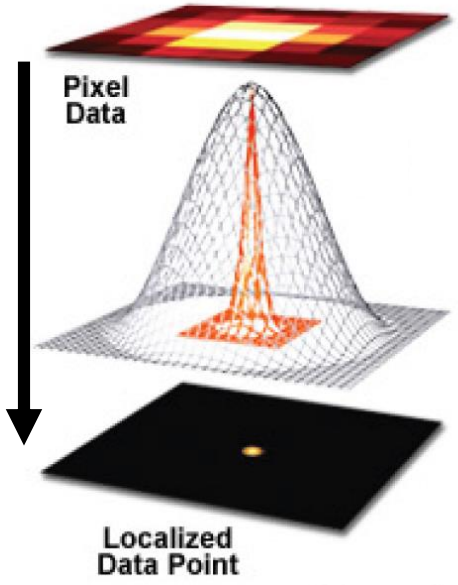
Eric Betzig
Howard Hughes Medical Institute, VA, USA

PhotoActivated Localization Microscopy (PALM)
Also called FPALM, STORM

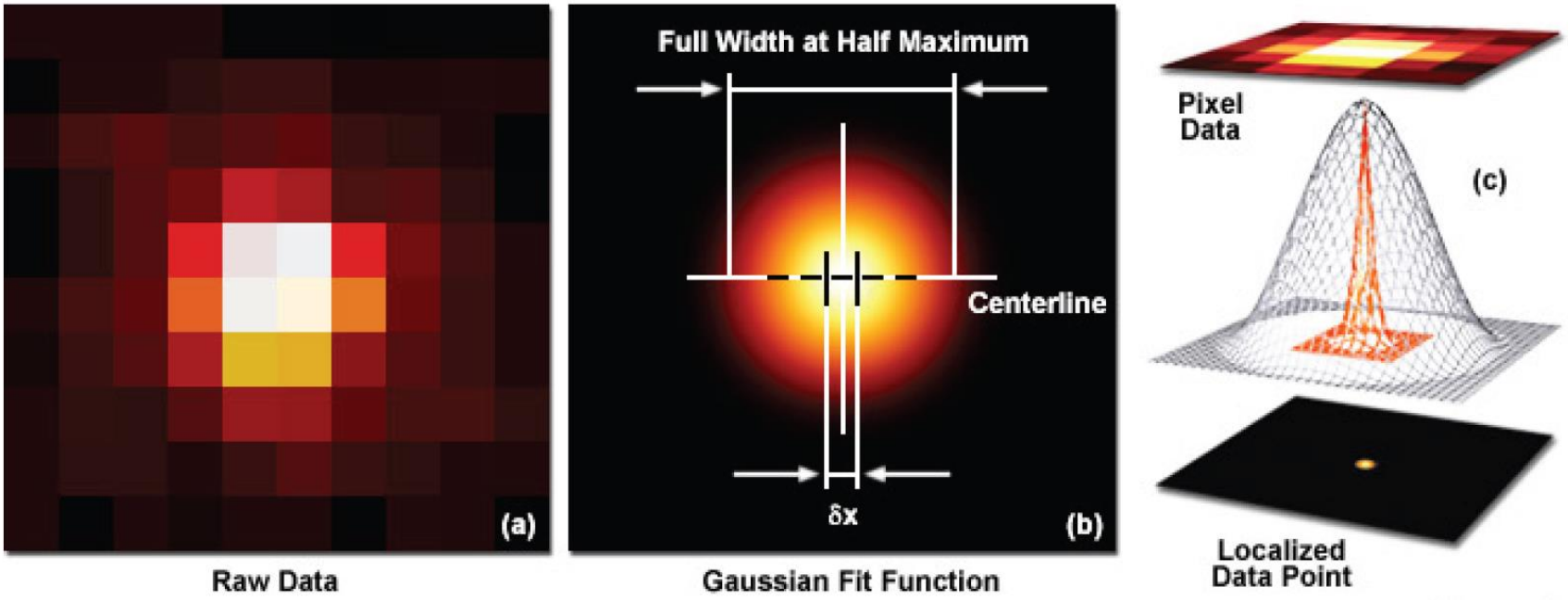
It is based on single molecule localization microscopy.

In a lateral specimen field containing single molecule emitters:

- Central portion of each diffraction-limited spot recorded on a digital camera image plane corresponds to the position of a molecule can be localized with nanometric precision by analyzing the distribution of its photons on the pixels of a CCD detector.



Fitting Single-Molecule Pixel Data to a Gaussian Function



(a) A molecule imaged with N photons will appear noisy and spread with a width approaching the diffraction limit.

(b)-(c) To localize the centroid of the diffraction limited spot, image is typically analyzed by applying a least squares fit to a Gaussian approximation of the PSF.

➔ **In this way, the effective localization precision can be at nm accuracy!!**

2014 Nobel Prize in Chemistry: Super Resolution Microscopy

Eric Betzig
Howard Hughes Medical Institute, VA, USA

PhotoActivated Localization Microscopy (PALM)
Also called FPALM, STORM

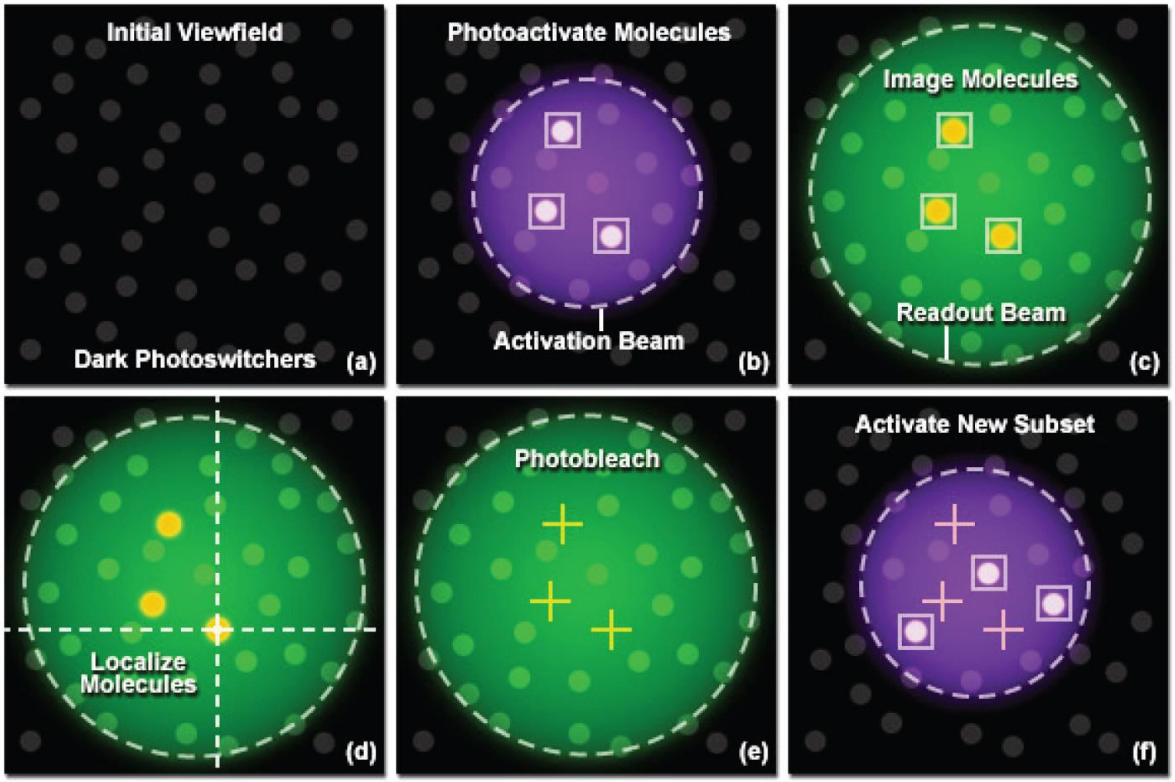
It is based on single molecule localization microscopy.

- The concept of identifying and localizing individual molecules was first described by Werner Heisenberg in the 1930s and was mathematically re-established during the 1980s and 1990s.

➔ One can localize molecules with an accuracy of several nanometers and resolve details down to 10 nm or less.

➔ In practice, it is typically 20 –50 nm, which is still many times better than Abbé's diffraction limit (~200 nm) that can be obtained using standard wide field microscopy.

Principle of Single-Molecule Localization Microscopy



(a) Specimen is labeled with photoswitchers initially residing in inactive (or "dark") state.

(b) UV light (purple activation beam) is used to "photoactivate" a small number of molecules in the specimen (those surrounded by boxes). This number is maintained at a very low level by ensuring a sufficiently weak laser intensity at the focal plane. Photoactivation of the molecules occurs stochastically and the probability of activation is proportional to location and the intensity of the activation laser.

(c) Activated molecules are imaged (orange boxed circles) with a readout laser (green beam) and an EMCCD camera.

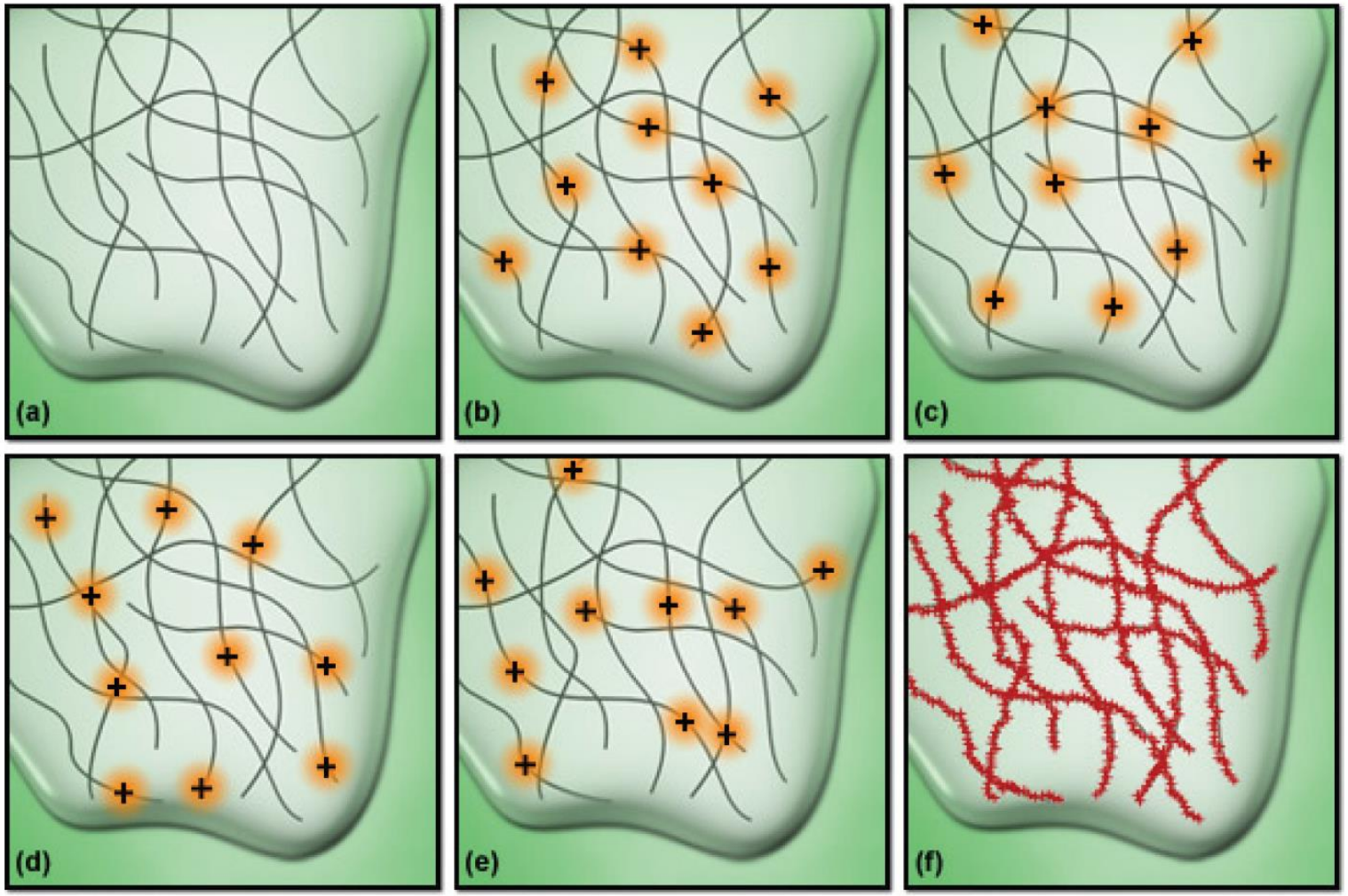
(d) Enough photons are collected from each photoactivated molecule to **localize the PSF with "high" precision**.

(e) During readout, photoactivated molecules spontaneously photobleach, thus reducing the number of active molecules.

(f) A new set of molecules is photoactivated to repeat the sequence, which is reiterated until all of the molecules in the specimen have been exhausted.

➔ **The final super-resolution image is constructed by plotting the measured positions of the localized fluorescent probes.**

Basic Principle of Image Construction



The final image is composed of the sum of tens of thousands of individual points

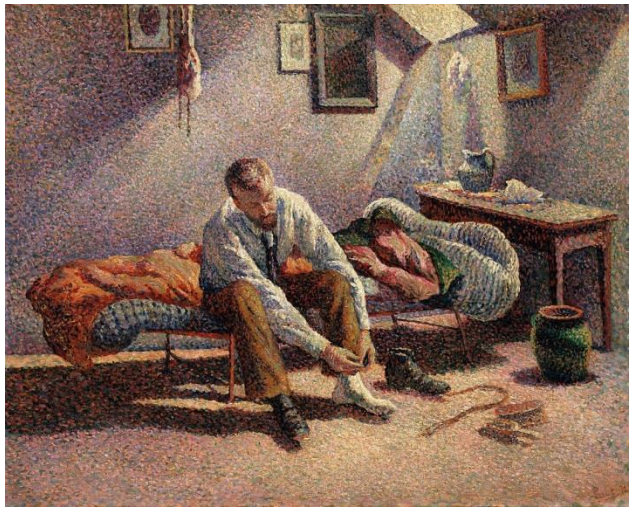
https://www.youtube.com/watch?v=RE70GuMCzww

PALM ←→ Pointillism

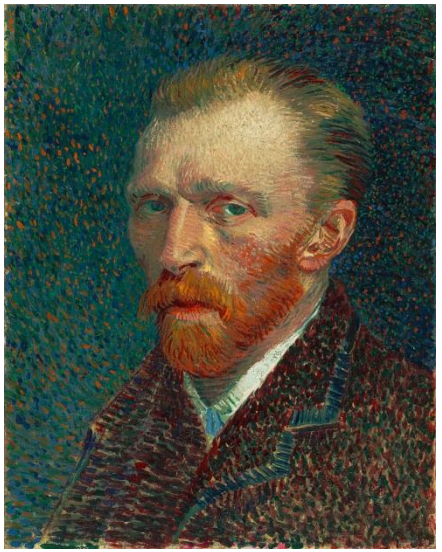
The final image is composed of the sum of tens of thousands of individual points
→ This has been compared with the painting technique called *pointillism*.

Pointillism /ˈpɔɪntɬlɪzəm/ is a painting technique in which small, distinct dots of color are applied in patterns to form an image. Georges Seurat and Paul Signac developed the technique in 1886, branching from Impressionism.

Detail from Seurat's *La Parade de Cirque* (1889),
showing the contrasting dots of paint used in Pointillism

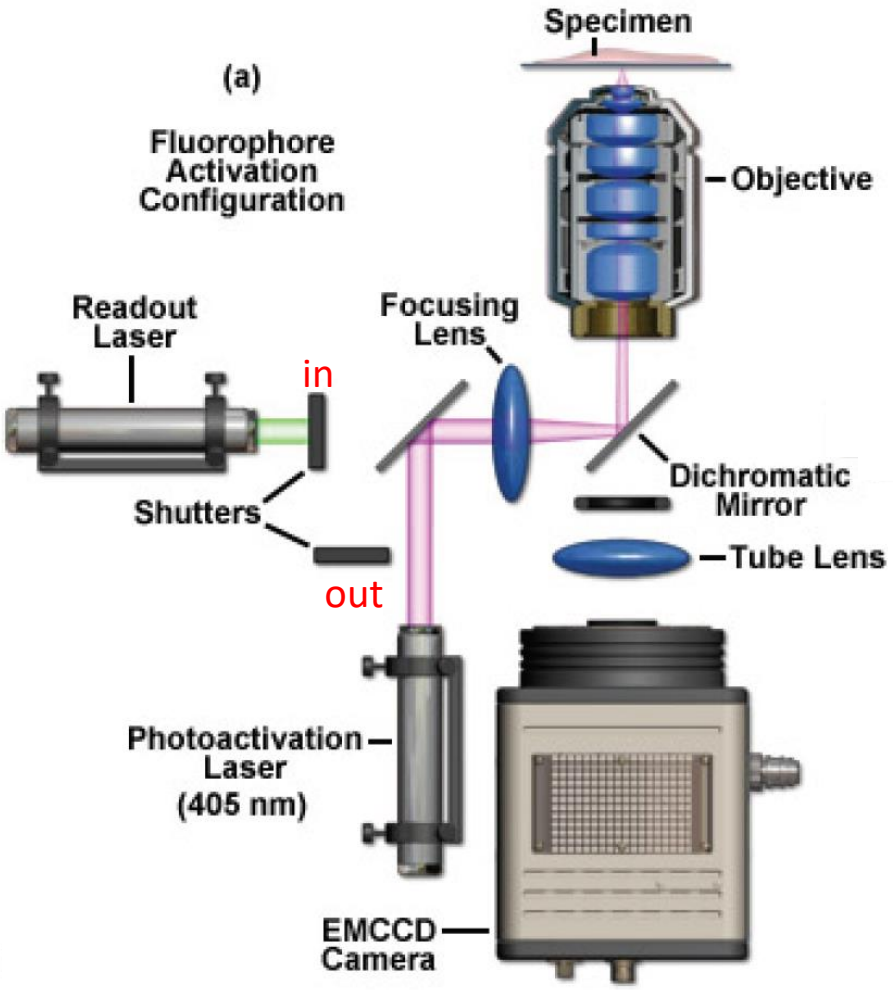


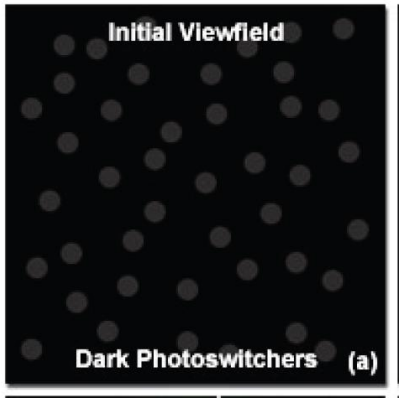
Maximilien Luce,
Morning, Interior, 1890
using pointillist technique.

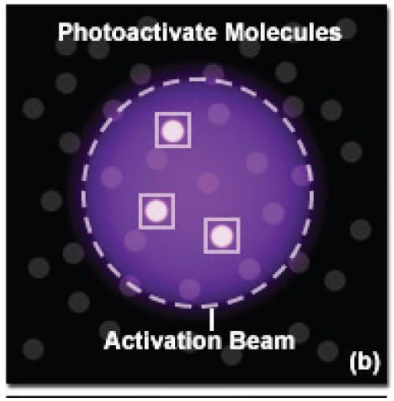


Van Gogh, *Self Portrait*, 1887
using pointillist technique

Optical Path of PALM





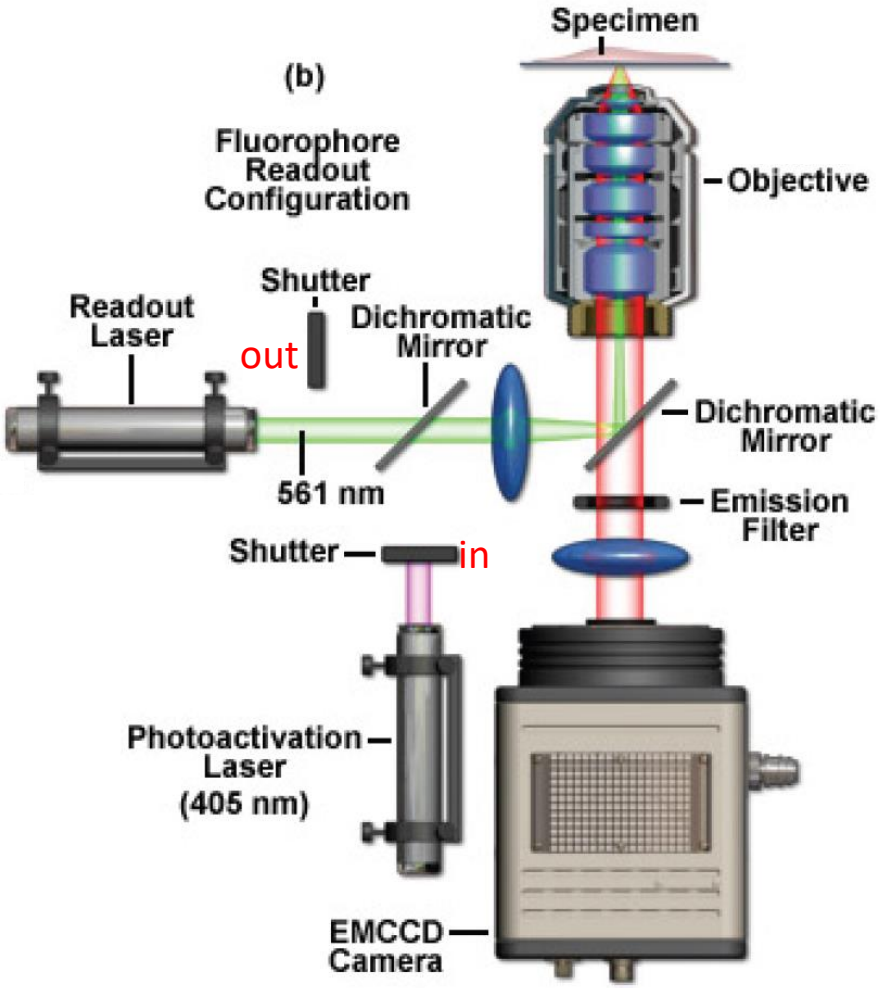


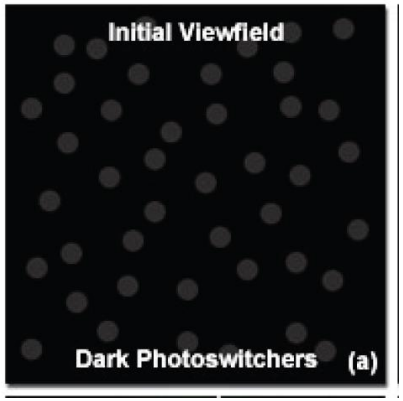
During the photo-activation step:

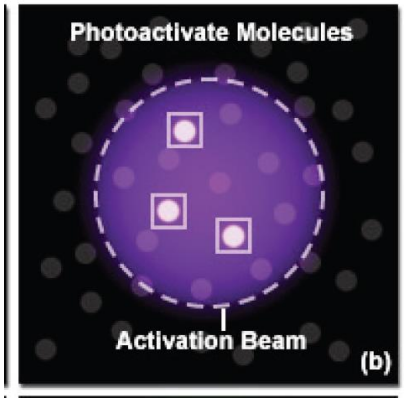
Shutter in front of the photoactivation laser is “out of the way”

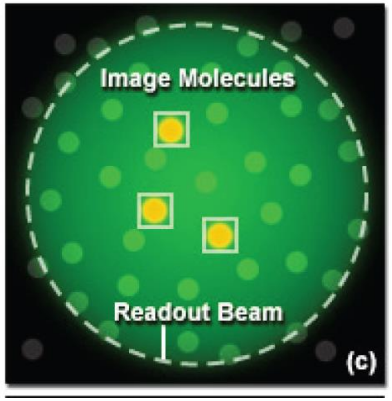
Shutter in front of the readout laser is “in of the way”

Optical Path of PALM







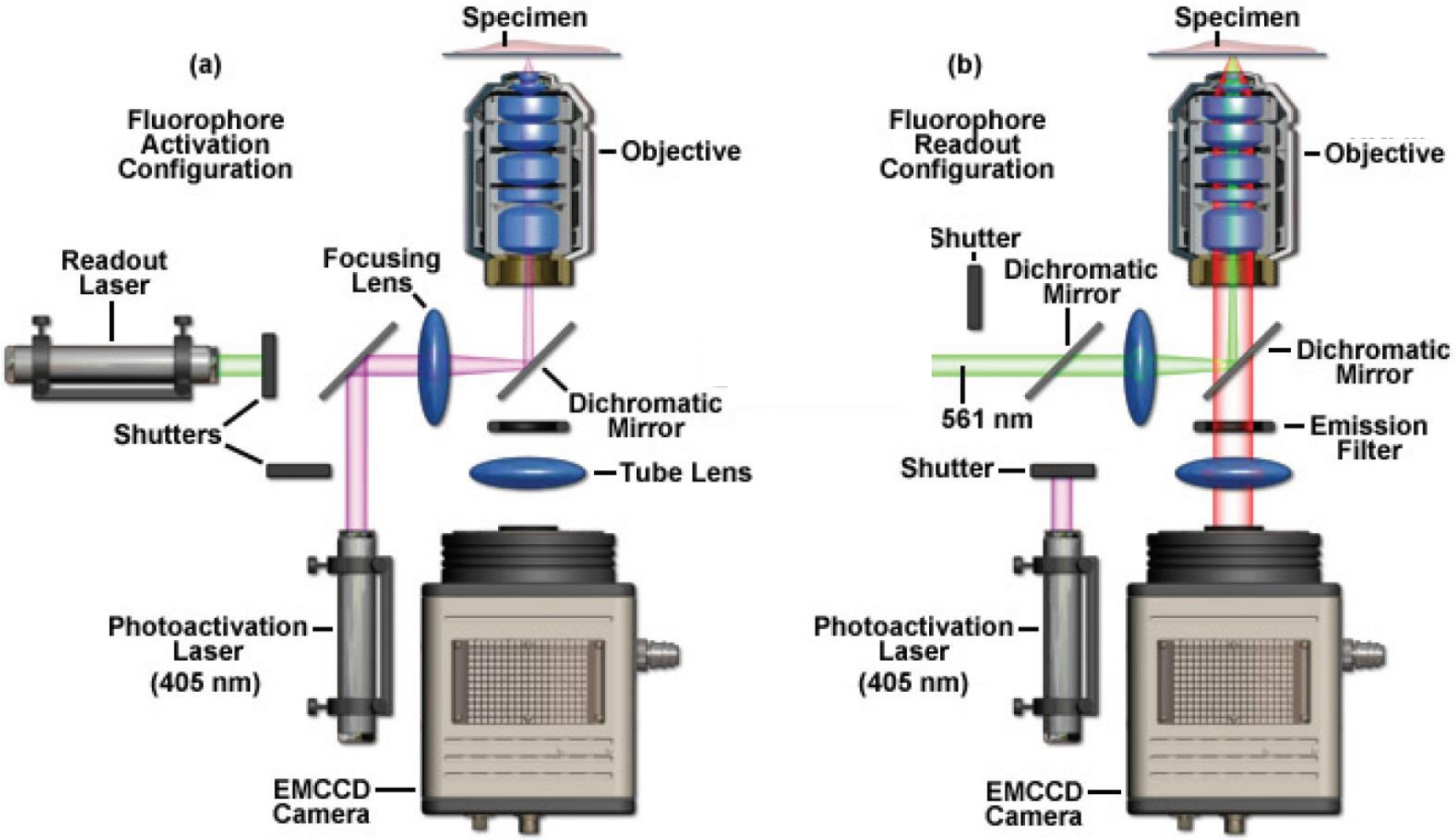


During the read-out step:

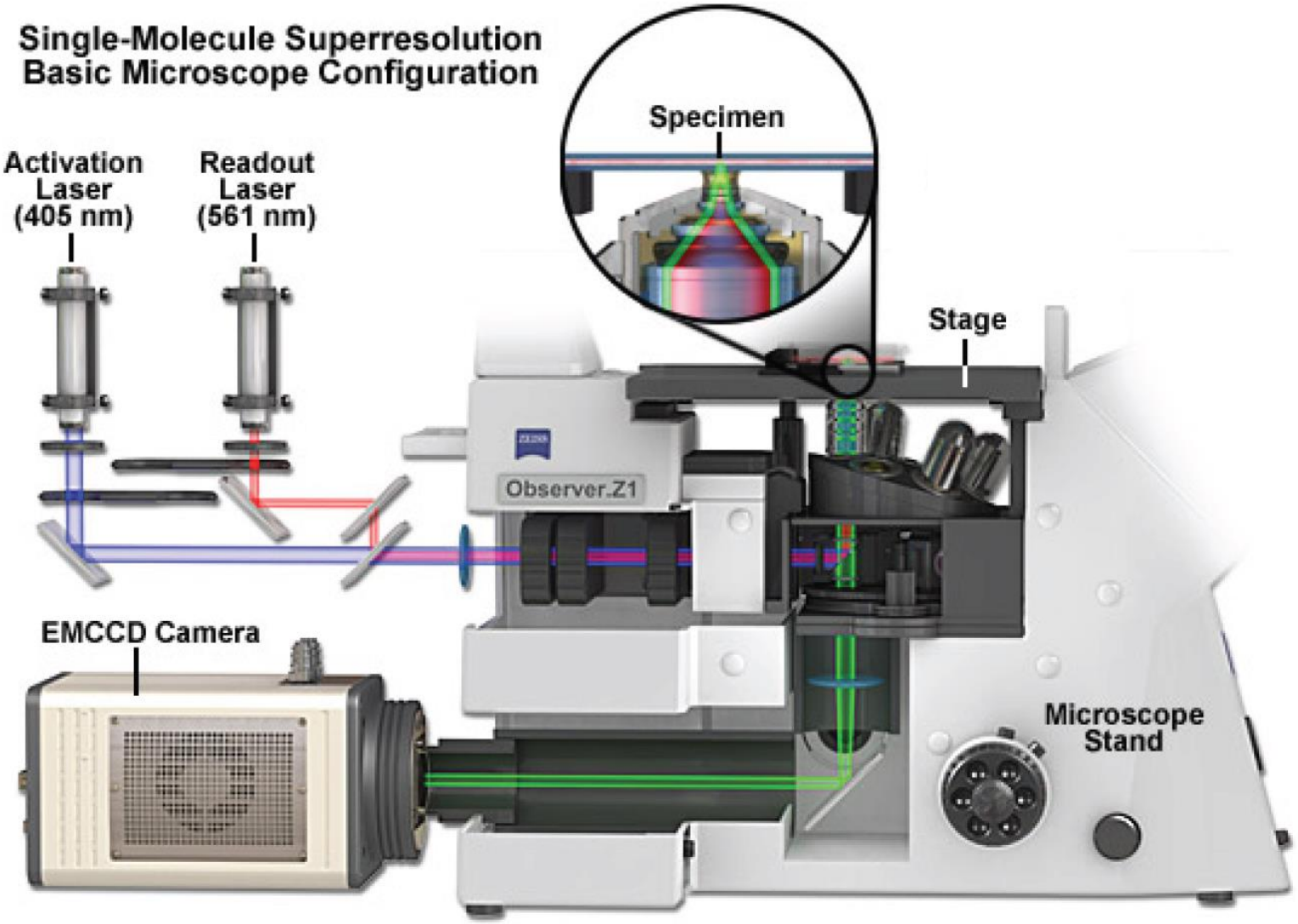
Shutter in front of the photoactivation laser is “in the way”

Shutter in front of the readout laser is “out of the way”

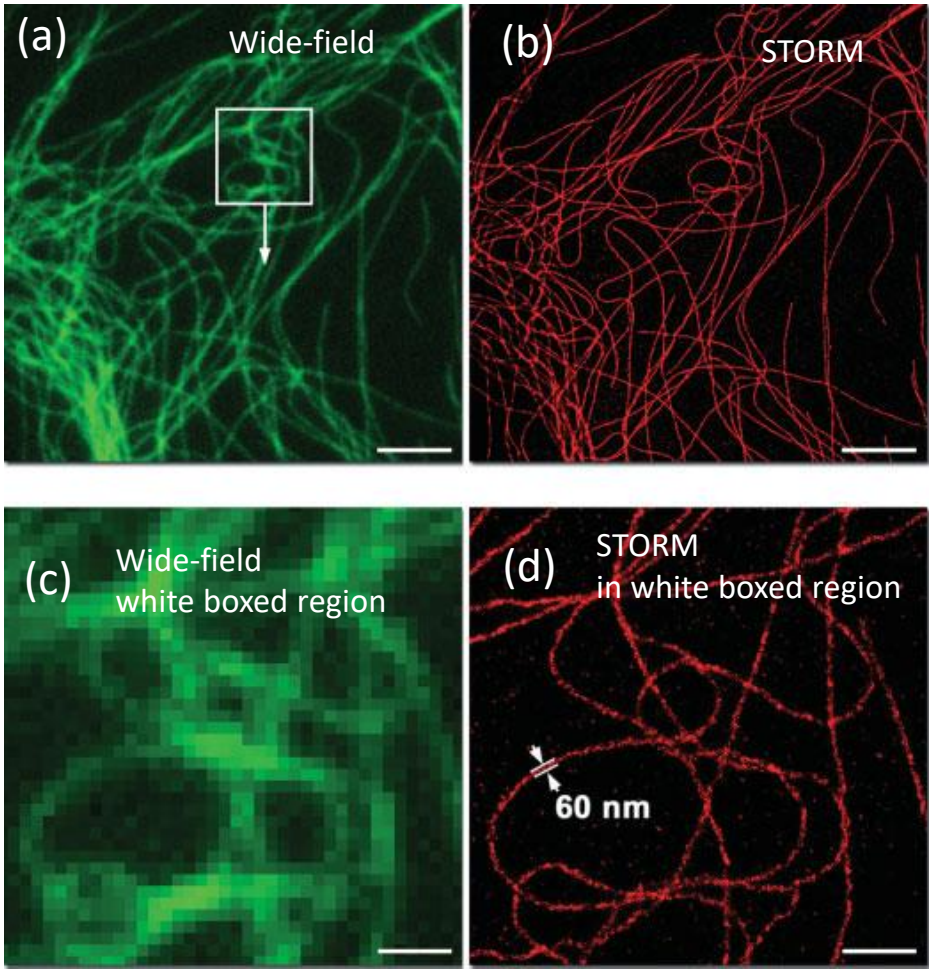
Optical Path of PALM



Implementation of PALM in an Inverted Microscope



Bio-Imaging with Super-Resolution Microscopy



(a, b) scale bar = 5 µm

(c, d) scale bar = 1 µm

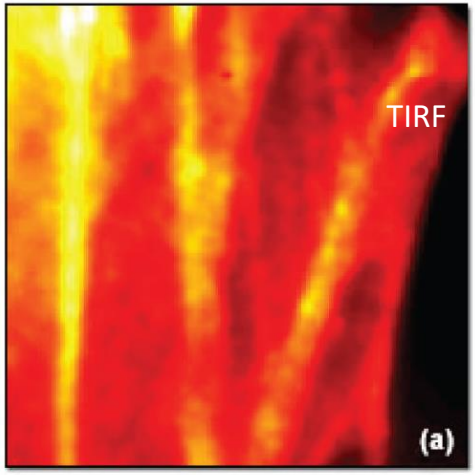
Superresolution imaging of microtubules with STORM (Stochastic Optical Reconstruction Microscopy).

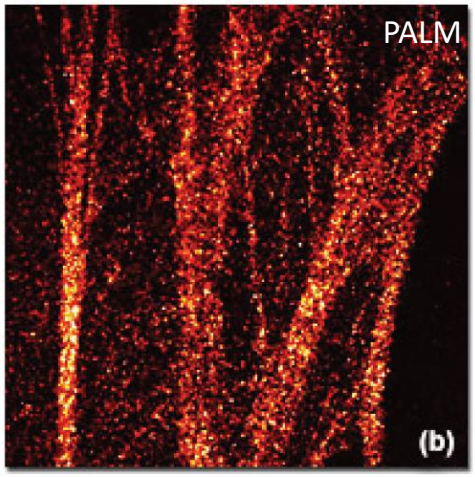
- Microtubules in rat-kangaroo kidney cells were labeled with antitubulin primary antibodies and secondary Fab antibody fragments conjugated to Alexa Fluor 405 and Alexa Fluor 647.
- STORM images were generated using a pulsed 405 nm activation laser and 657 nm imaging laser.

(a) Conventional wide-field fluorescence image.
(b) STORM image of the field viewed in panel a.
(c) Enlarged wide-field image of the boxed area in panel a.
(d) STORM image of the area shown in panel c with a single microtubule diameter (60 nm) indicated by the white arrows.

Bio-Imaging with Super-Resolution Microscopy

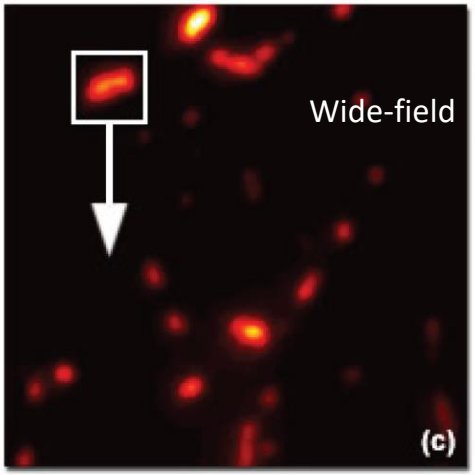
Filamentous actin cytoskeleton network

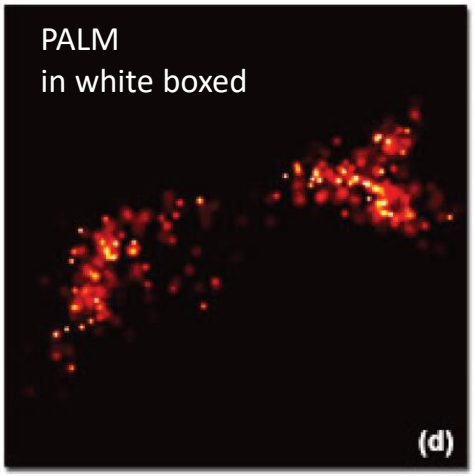




Label: tandem Eos fluorescent protein (fused with human beta-actin)

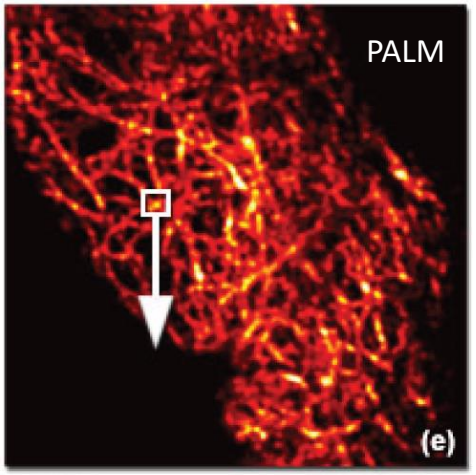
Single mitochodrion (white box)

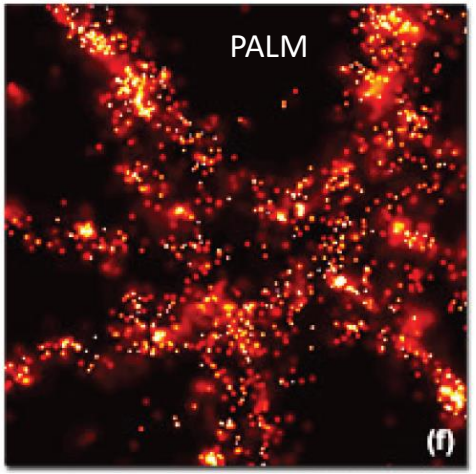




Label: dimeric Eos (fused to the mitochondrial targeting sequence)

Filament network in HeLa cell

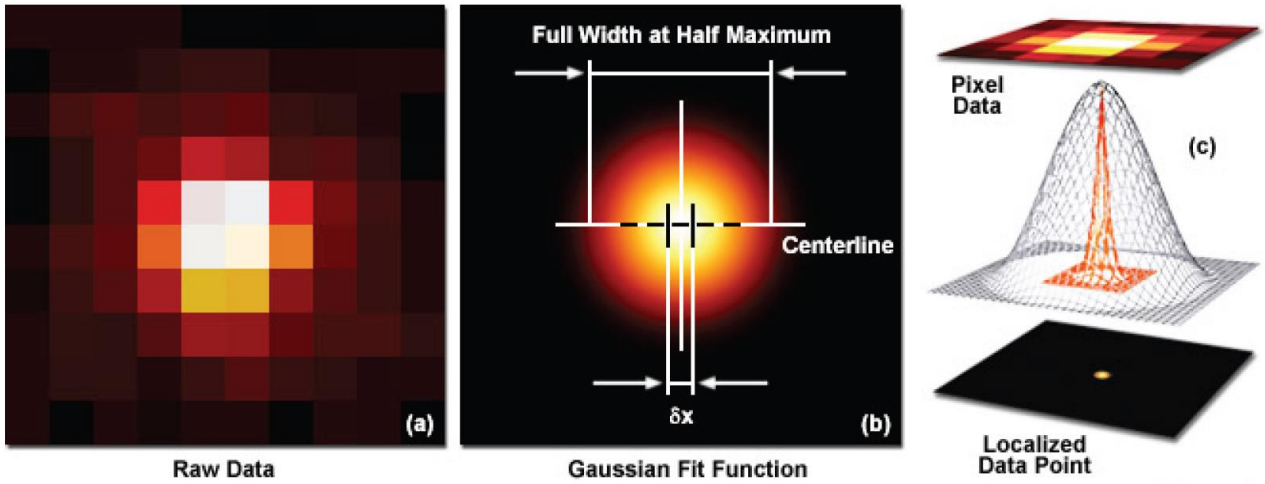




Label: monomeric version of Eos fluorescent protein (fused to cytokeratin)

Photo-activation conducted by 405nm diode laser & read-out using 561 nm diode pumped solid state laser

Localization Precision in Single-Molecule Imaging



- The central portion of each diffraction limited spot (a.k.a. PSF) corresponds to the probable position of a molecule and can be localized with nanometric precision by gathering a sufficient number of photons.
- Determining the center of localization is **based on a statistical curve-fitting** of the measured photon distribution to a Gaussian function.
- The uncertainty in determining the center or mean value of the photon distribution ($\mu = x_0, y_0$) is given by :

$$\sigma_{\mu_i} = \sqrt{\left(\frac{s_i^2}{N}\right) + \left(\frac{a^2/12}{N}\right) + \left(\frac{8\pi s_i^4 b^2}{a^2 N^2}\right)}$$

This is also called localization precision

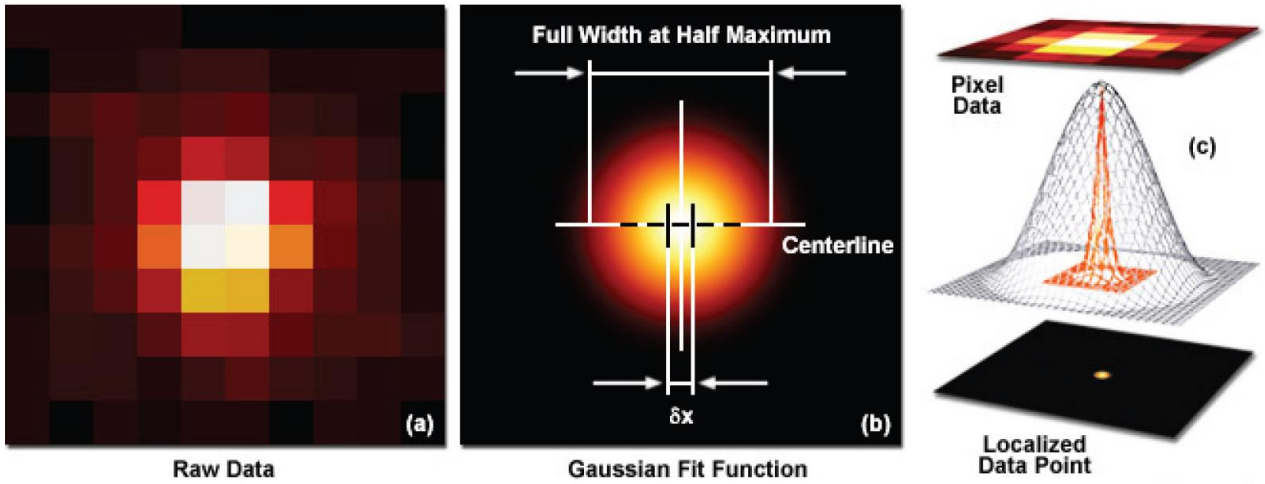
- s_i is the PSF in direction i (index i refers to x or y direction).
- N is the number of photons gathered
- a is the pixel size of the imaging CCD detector
- b is the standard deviation of the background, which includes background fluorescence emission combined with detector noise.

1st term refers to the photon (shot) noise

2nd terms takes into account that pixel size of the detector is finite.

3rd term takes into account the effect of the background noise

Fitting Single-Molecule Pixel Data to a Gaussian Function



$$\sigma_{\mu_i} = \sqrt{\left(\frac{s_i^2}{N}\right) + \left(\frac{a^2/12}{N}\right) + \left(\frac{8\pi s_i^4 b^2}{a^2 N^2}\right)}$$

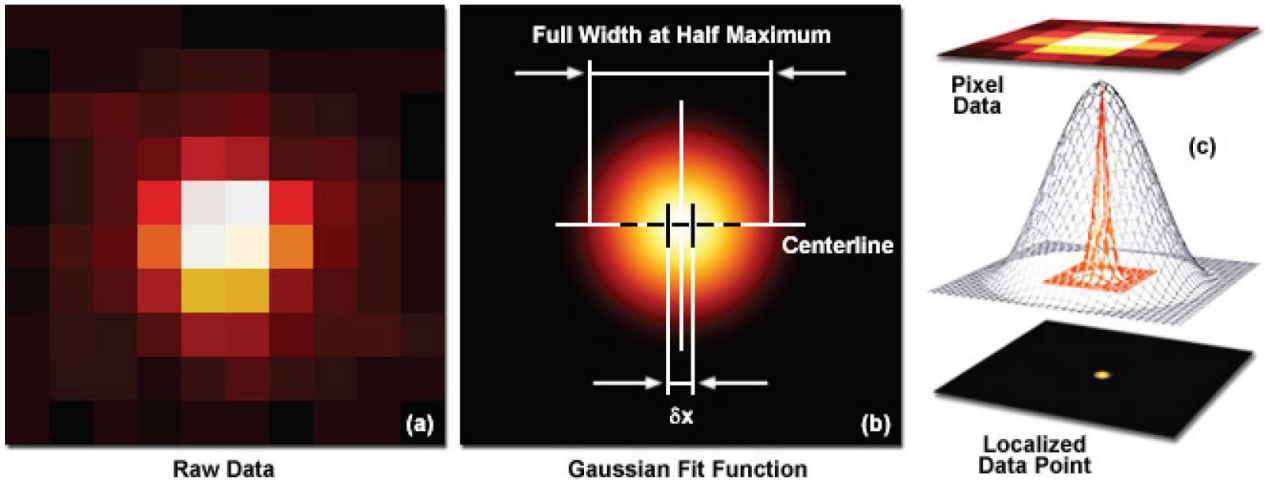
- Background can arise from autofluorescence, residual fluorescence of surrounding probes that have entered the dark state…

- The key to have high precision for molecular localization is to minimize the background noise and maximize photon output from the fluorescent probe.

- With high end cameras having small pixel size and negligible background noise, localization can be approximated as:

$$\sigma_{x,y}^2 \approx \frac{s_{x,y}^2}{N}$$

Fitting Single-Molecule Pixel Data to a Gaussian Function



$$\sigma_{x,y}^2 \approx \frac{s_{x,y}^2}{N}$$

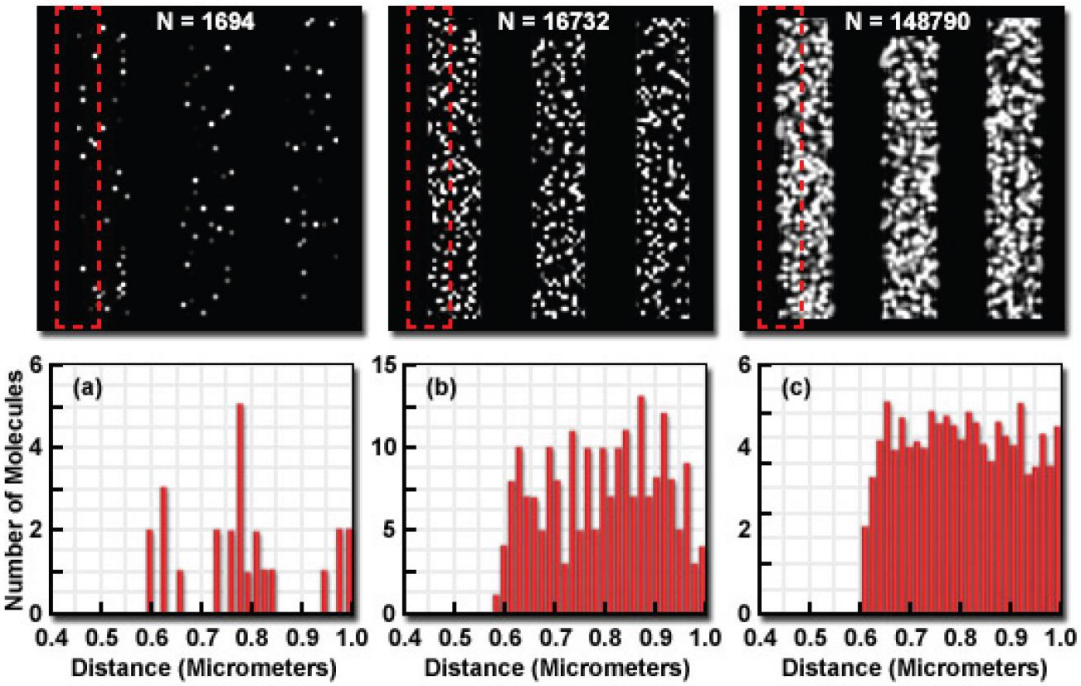
Let's assume to have **~250 nm PSF** and operate in the best case scenario of shot noise limit (i.e. absence of other noises such as background).

- If **10000 photons are collected** , the center of localization can be determined with **an accuracy of ~2 nm**.
- In contrast, if **only 400 photons are collected**, then the localization **accuracy drops to ~20 nm** or worse.

Density of Labelled Molecules in Localization Microscopy

➔ **The other key determinant of resolution is the density of labeled molecules in the specimen.**

- According to the **Nyquist-Shannon sampling theorem**, the sampling interval (mean distance between neighboring localized molecules) must be at least twice as fine as the desired resolution, or two data points per resolution unit → Otherwise, the feature of interest will be under-sampled and not resolved.
- **Example:**
- To achieve 10 nm lateral resolution, molecules must be spaced a minimum of 5 nm apart in each dimension.
- This means a minimum density of 40,000 molecules/μm^2 (or ~2,000 molecules in a diffraction limited region of 250 nm in diameter).



Illustrative example:

- The pattern in each simulation is a line structure with stripes of 1μm width and increasing density (a) to (c).
- The red dashed boxes in the upper panel correspond to the zoomed regions that are shown in the lower histogram graphs, which plot the number of localized molecules vs distance.
- **Even though the localization precision is the same in all three cases, when a limited number of molecules are localized as in (a), the ability to discern the edge is limited.** Whereas at high molecular density as in (c), lateral profile of the edge is clearly visible.

An introductory video about super-resolution microscopy for biology

https://www.youtube.com/watch?v=UCJ6oQSdxN0

A video lecture about developing PALM microscopy by Eric Betzig and Harald Hess

https://www.youtube.com/watch?v=GcQ24khZzvU

A video lecture on STED by Stephan Hell:
https://www.youtube.com/watch?v=YyBGiZZSslY