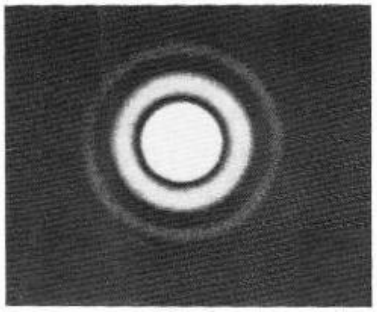
Charles Chevalier's Horizontal Microscope (circa 1834)

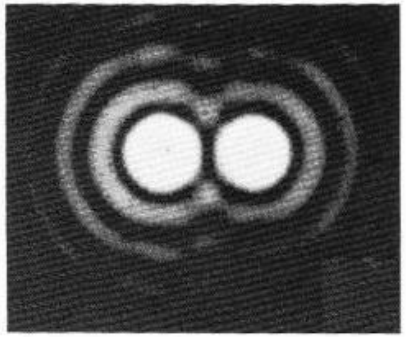
neuro technology studio

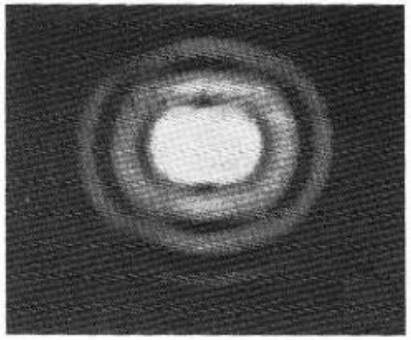
Visual Microscopy Workshop Series

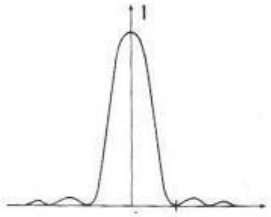
Super-Resolution
A case study on STED

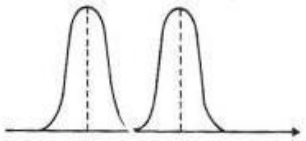
Lai Ding
BWH NeuroTechnology Studio



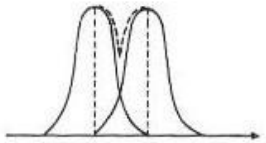








resolved



not resolved

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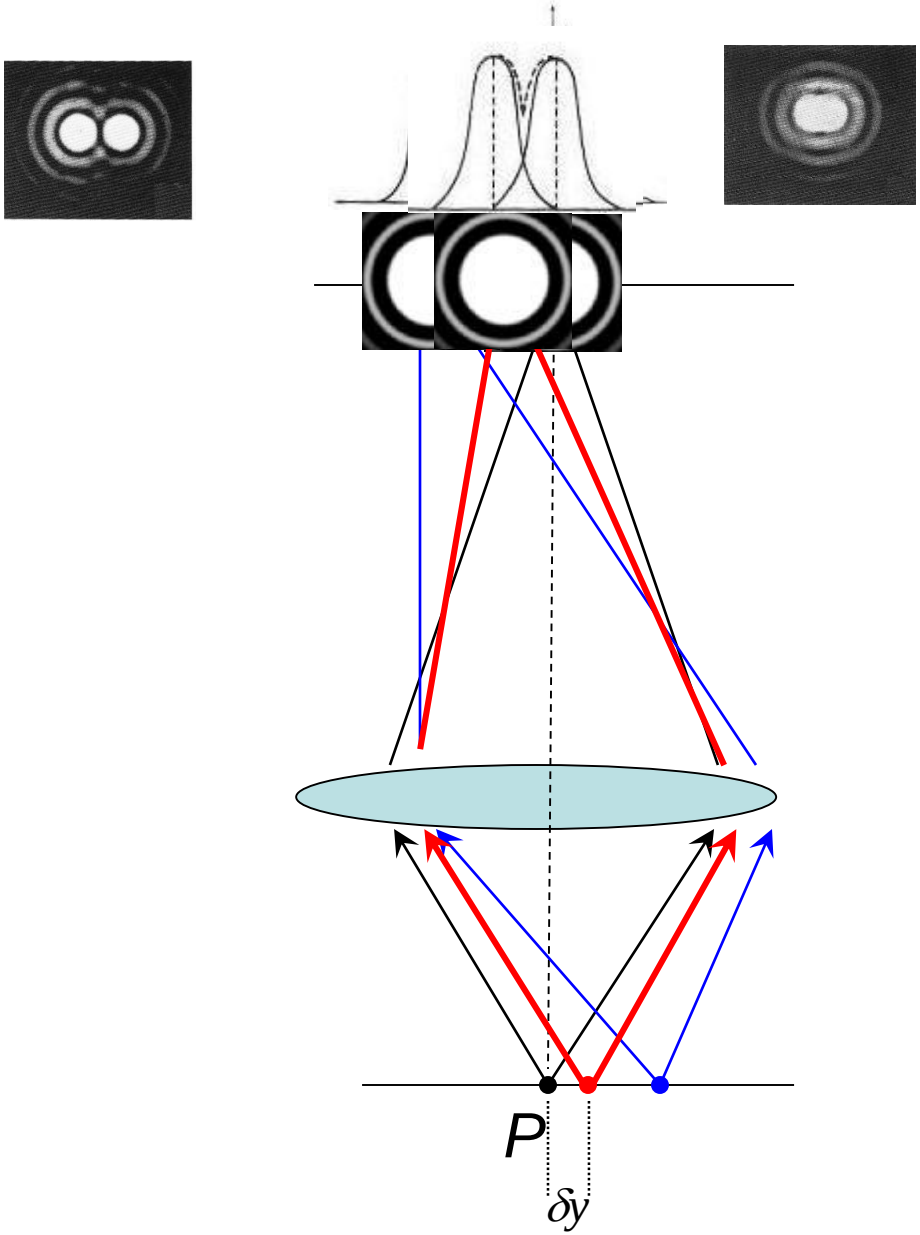
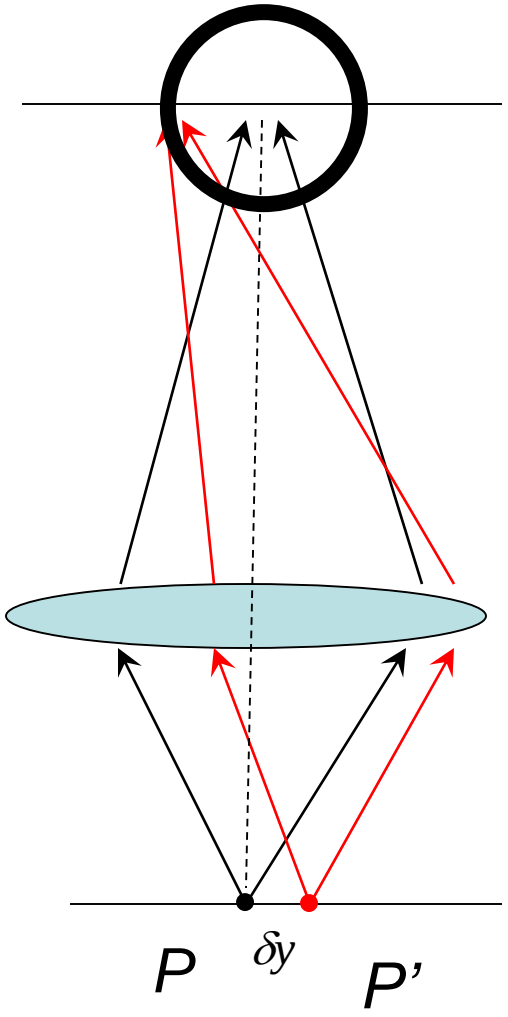
Resolution limit

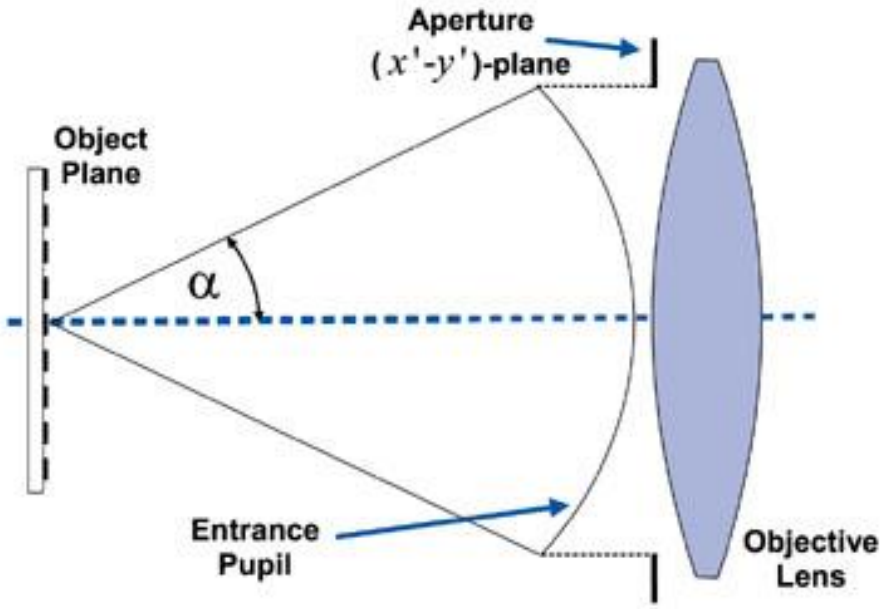
The distance between two points (on object side), which we can barely resolve them as two distinguished disks on image side


P
δy



$$\delta y = 0.61\ \lambda / \text{N.A.}$$

Definition of N.A. (Numerical Aperture)



N.A. = n * sin(α)

Optical Imaging

Glass lens n 1.6

Visible light λ 400nm -700nm

δy = 0.61 λ / N.A. sin(α) < 1

Best resolution you can get (lateral direction) ~ 150nm

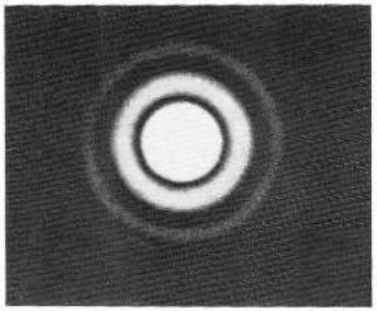
The resolution you can expect ~ 200nm (high mag oil objective)

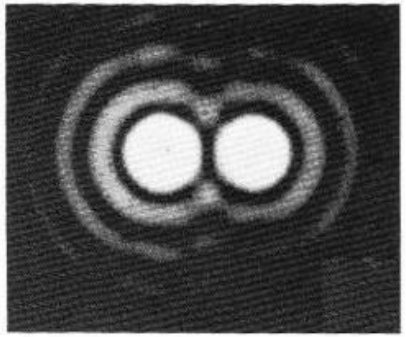
The resolution you actually get : ????

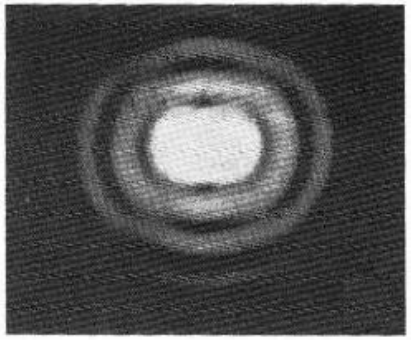
Want to achieve higher resolution?

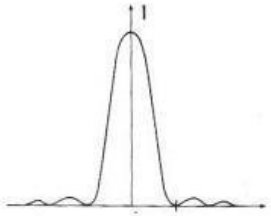
Resolution (r) = 0.61 λ /N.A.

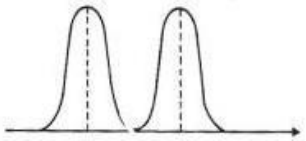
use lower λ, UV, EM, X-ray …



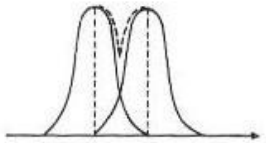








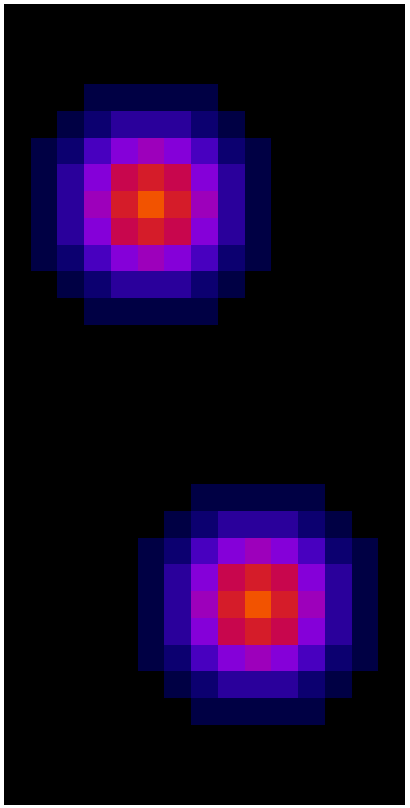
resolved

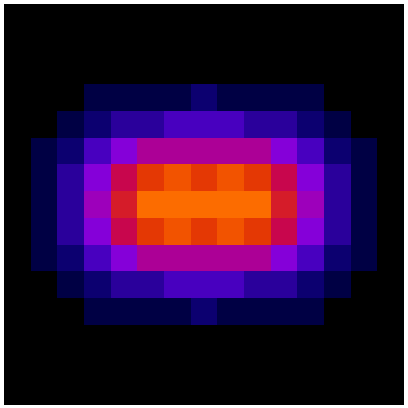


not resolved

• • • ••

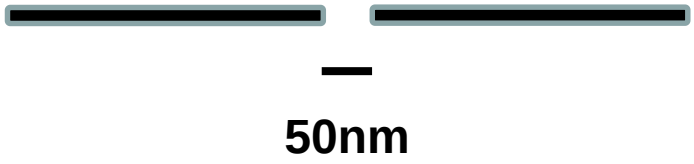
We have this problem, because those two points are shining at the same time



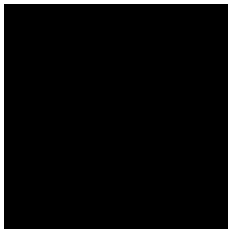


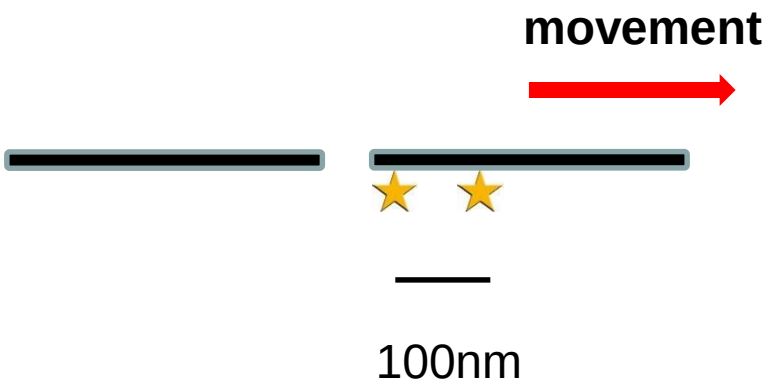
Not resolved

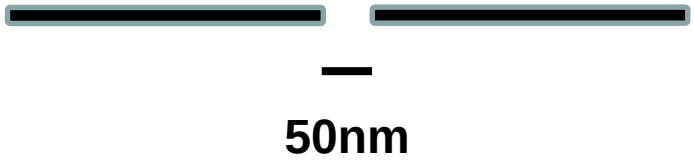
100nm



pos 1

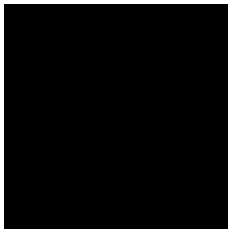




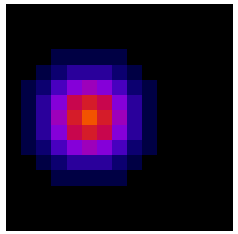


50nm

pos 1

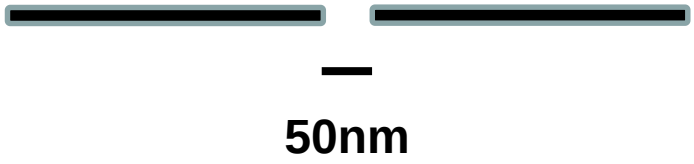


pos 2

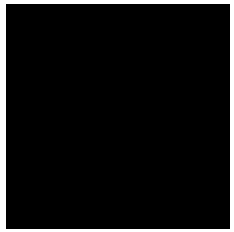


movement

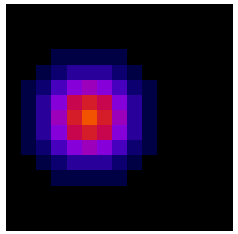
100nm



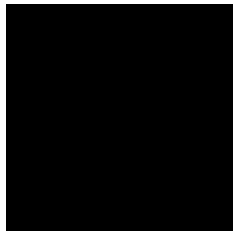
pos 1

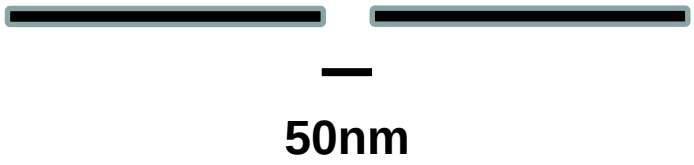


pos 2

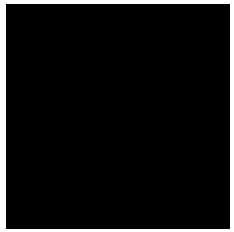


pos 3





pos 1



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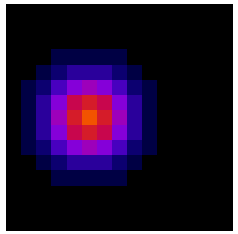

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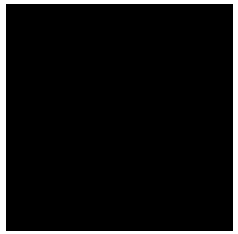
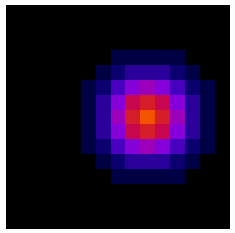
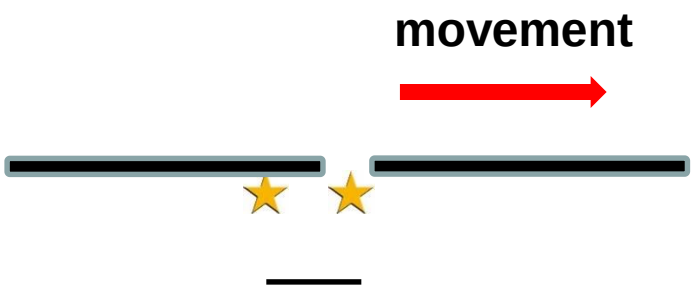
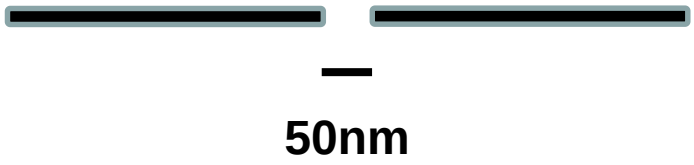
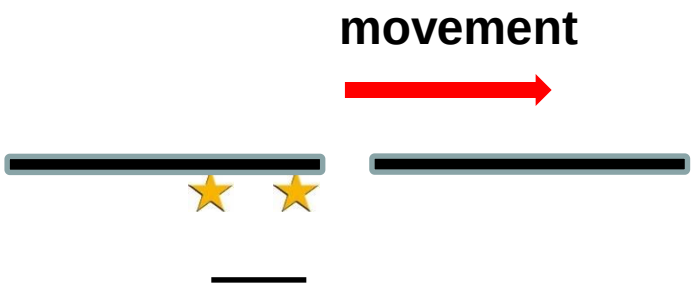


pos 4

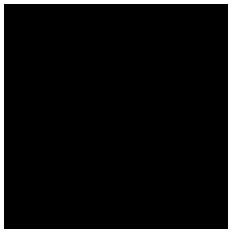








pos 1



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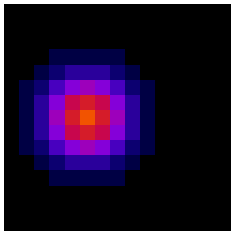
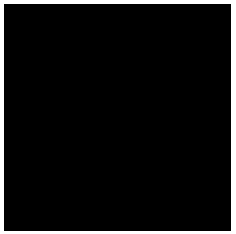
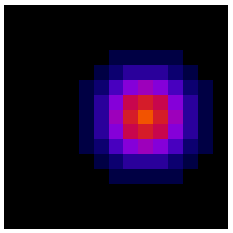


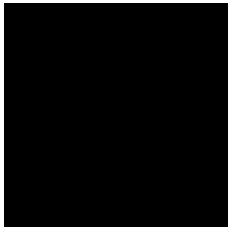
pos 3

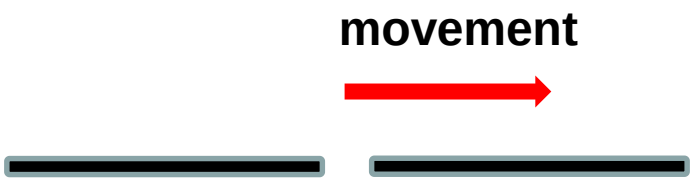


pos 4



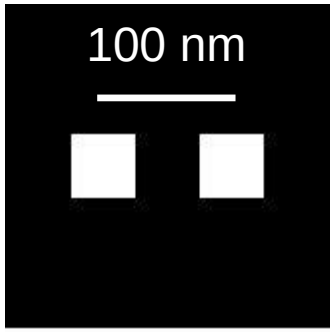
pos 5

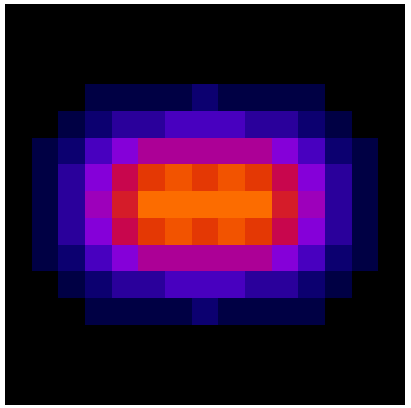


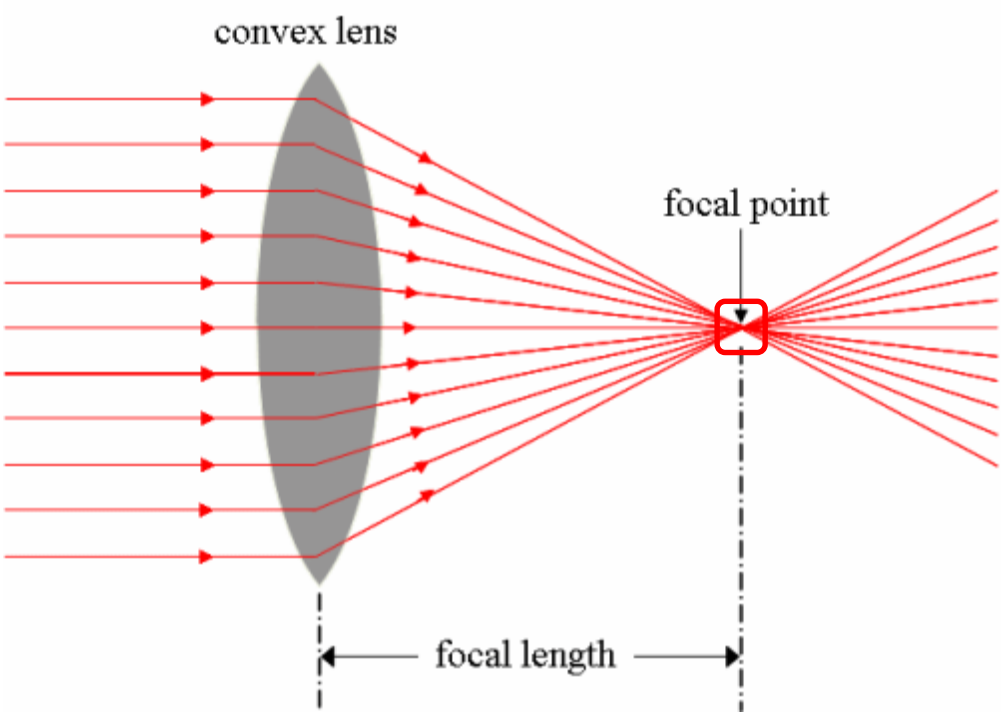


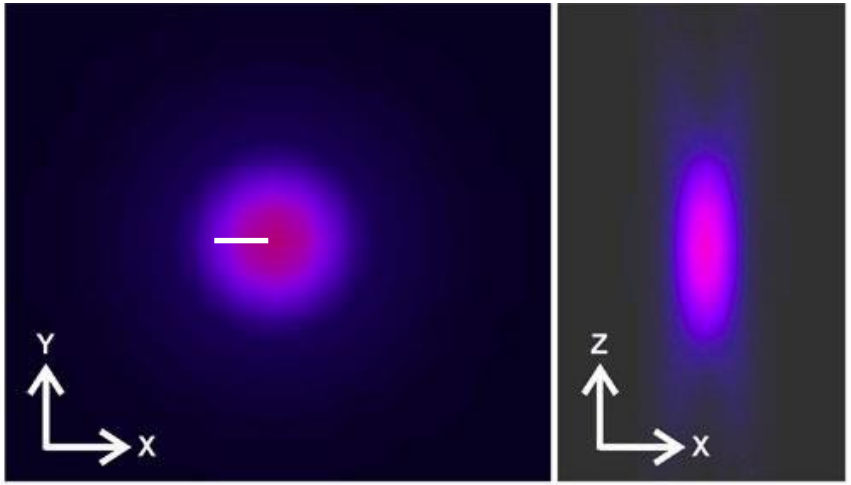
50nm step

each position as a pixel

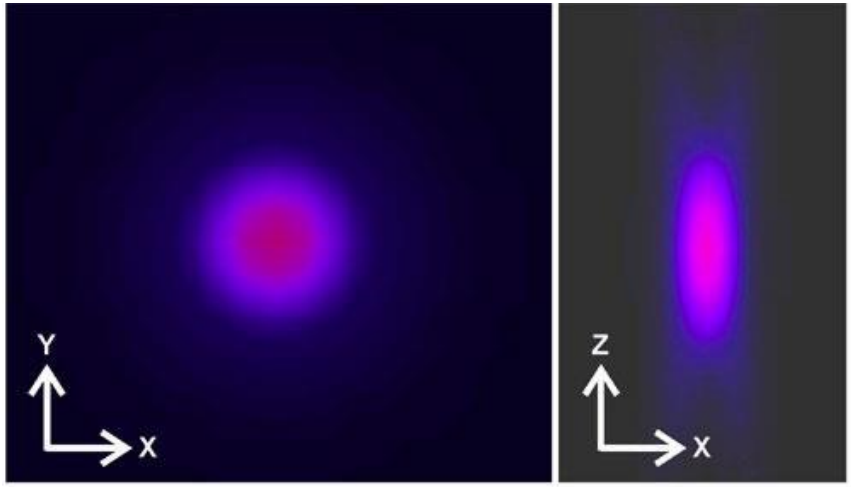








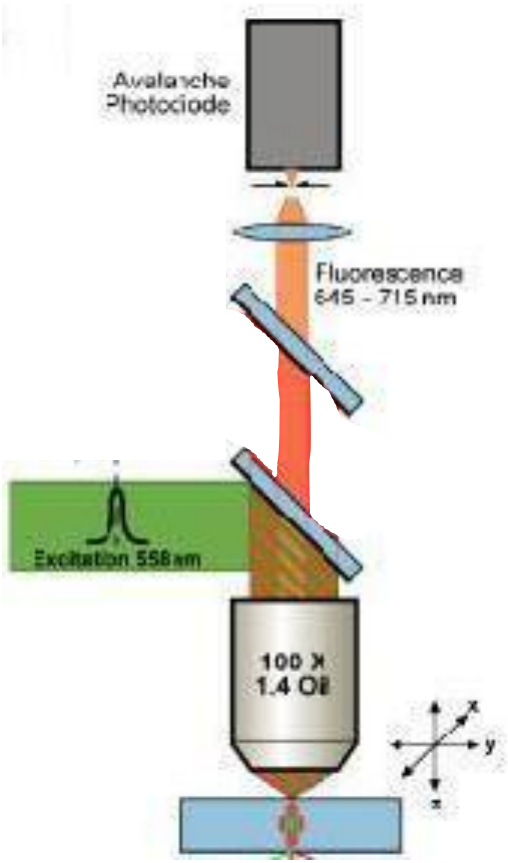
The excitation spot on sample is at the same level of the resolution level ~200nm (100X/1.4)

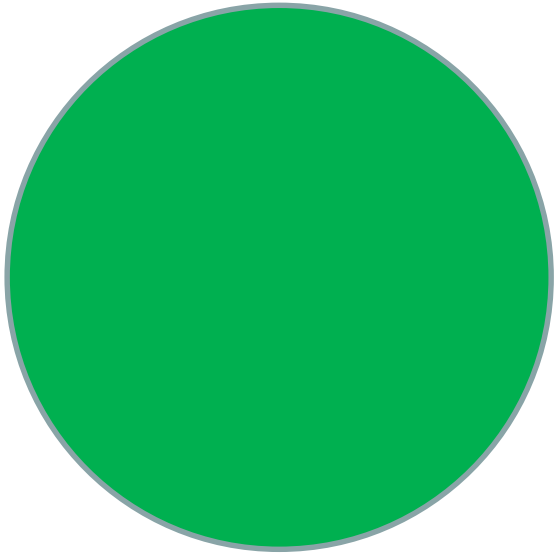


PSF of the excitation light basically controls the resolution limit of the point scan imaging system

So, how to make it smaller?

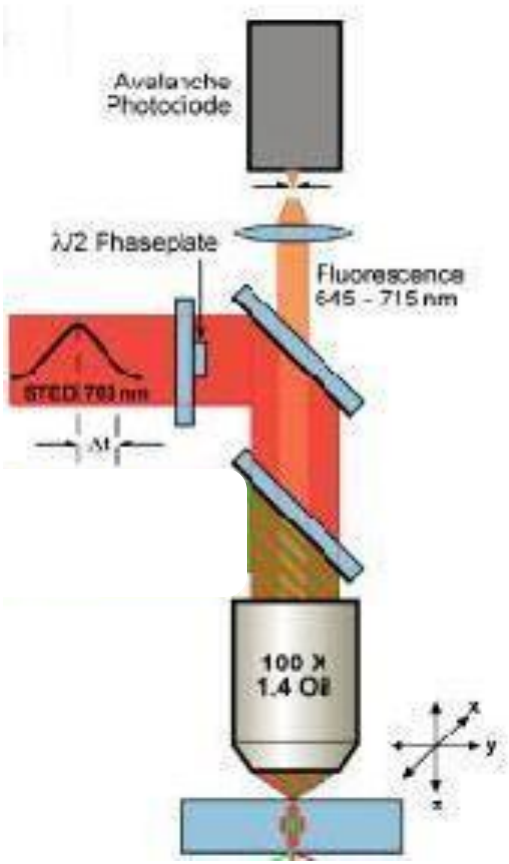
Stimulated Emission Depletion (STED-) microscope

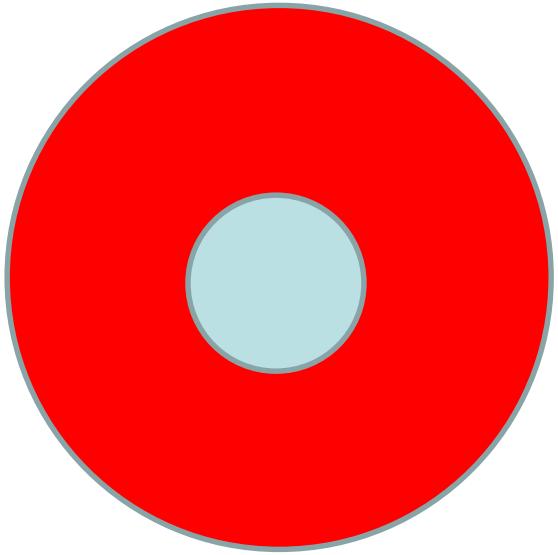




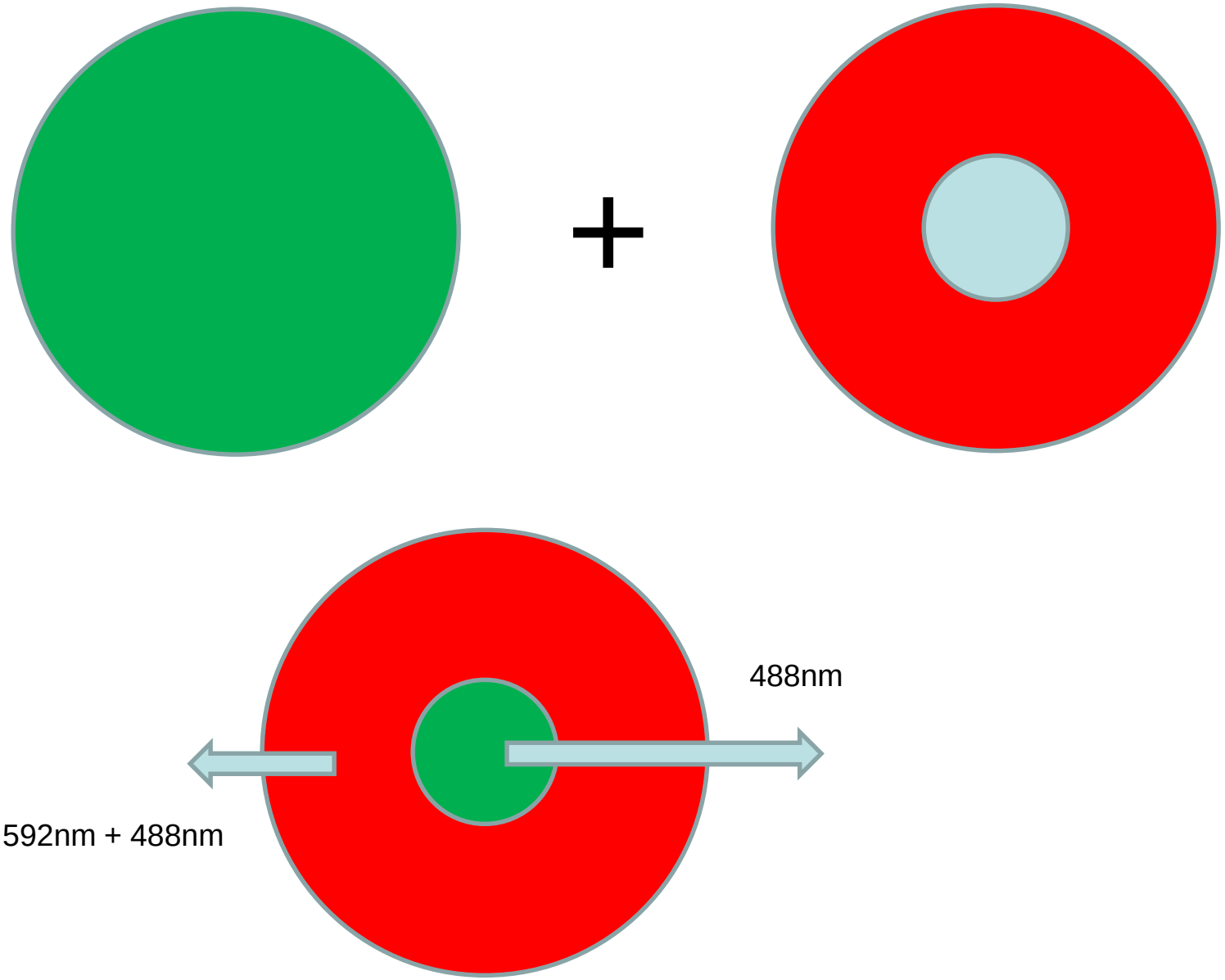
400nm

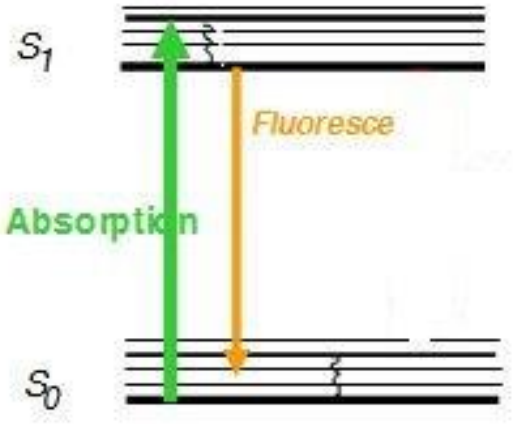
488nm excitation


Avalanche
Photodiode
λ/2 Phaseplate
Fluorescence
645 - 715 nm
STED 760 nm
100 x
1.4 Oil
x
y
z

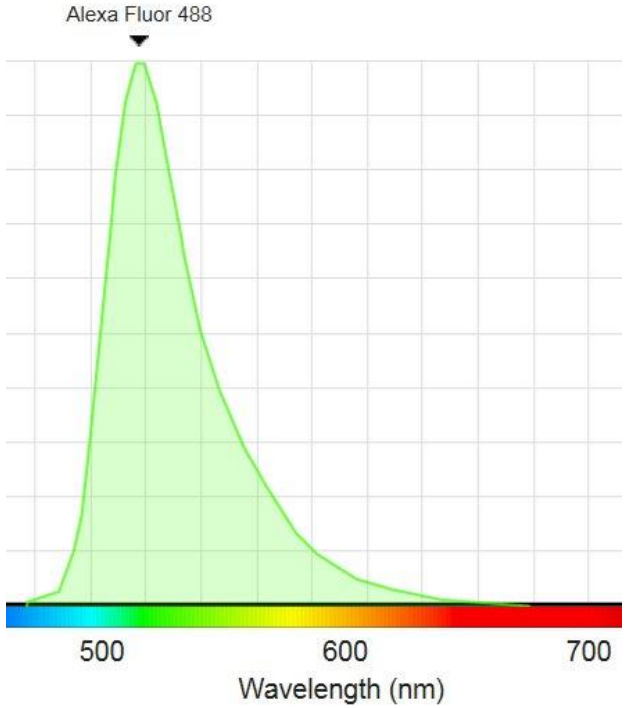


592nm

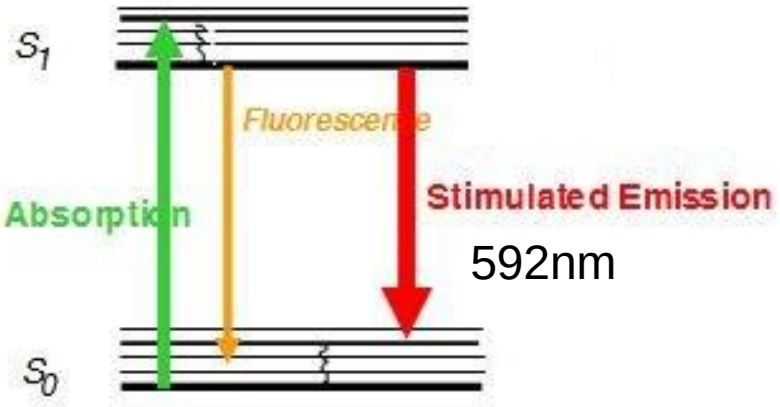

488nm
592nm + 488nm

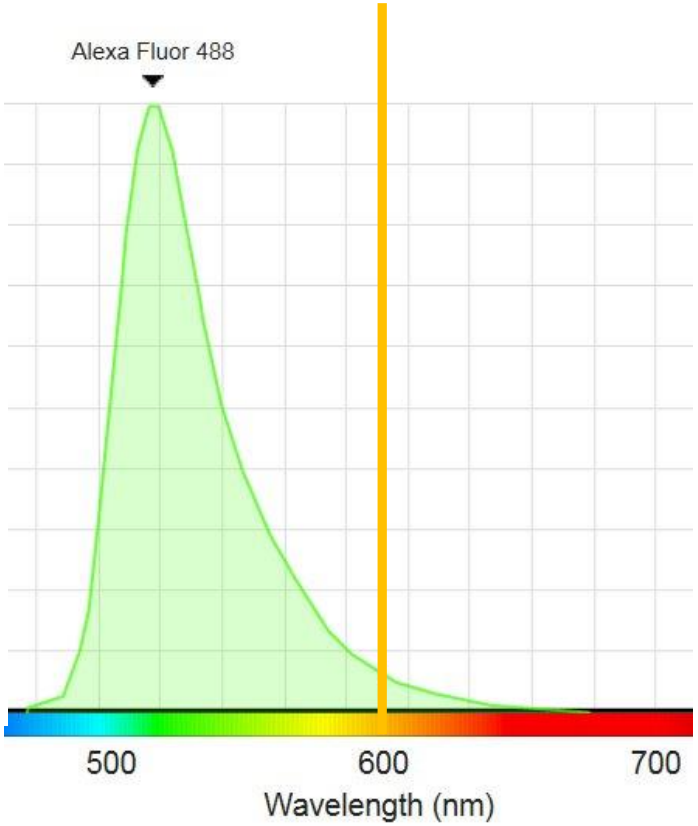


488nm excitation

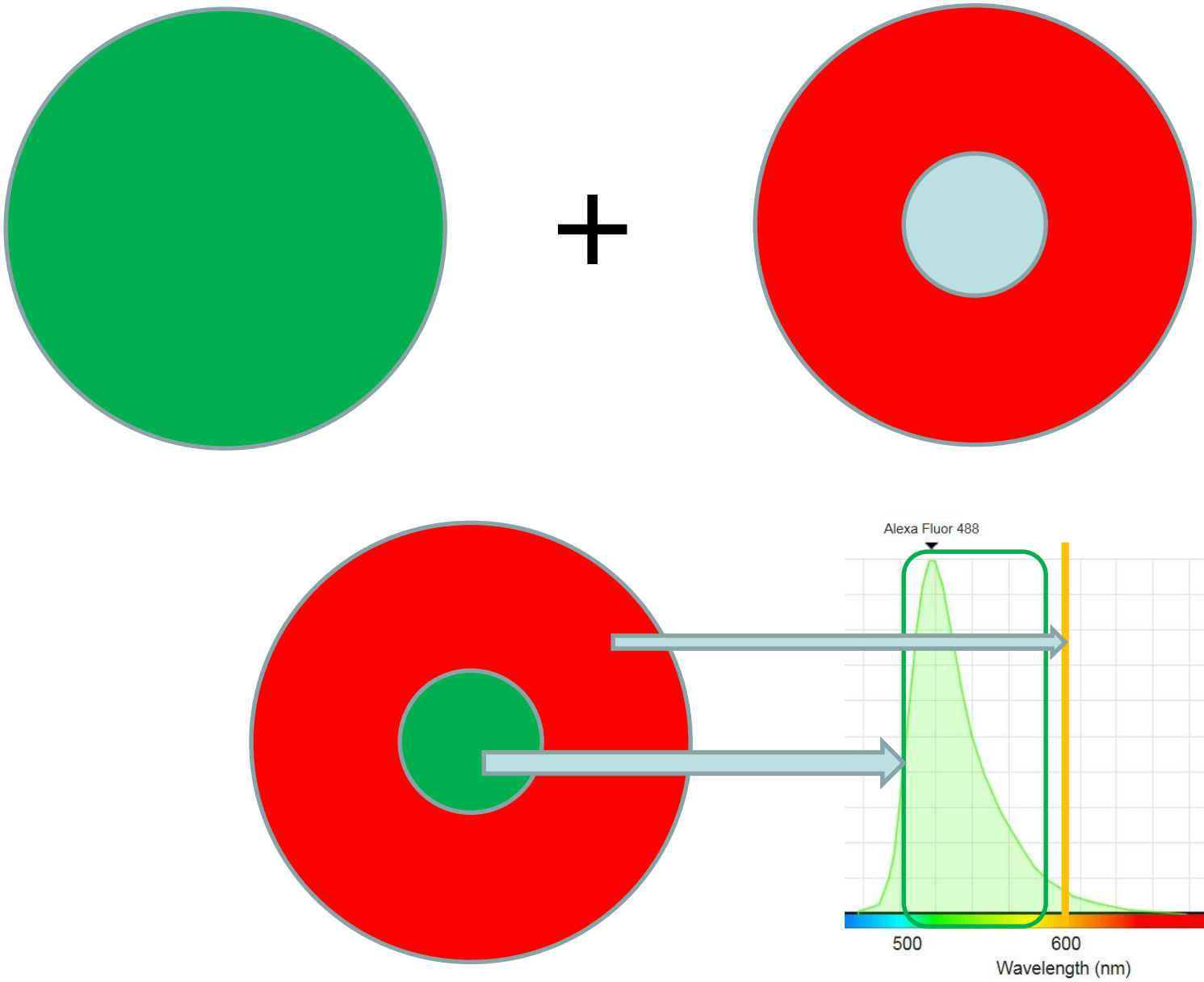


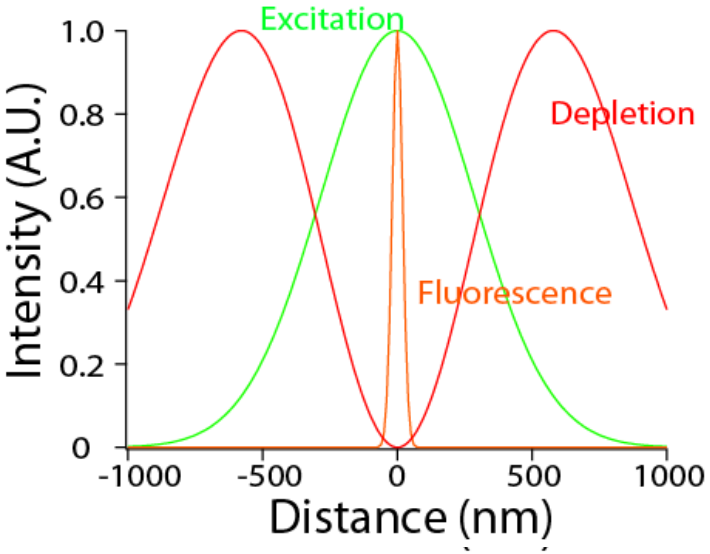
592nm

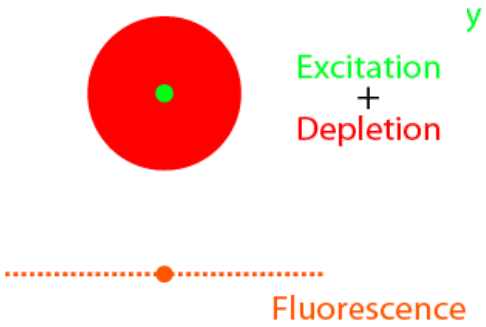




Majority of the fluorescence molecules will emit at wavelength of 592nm


Alexa Fluor 488
500
600
700
Wavelength (nm)

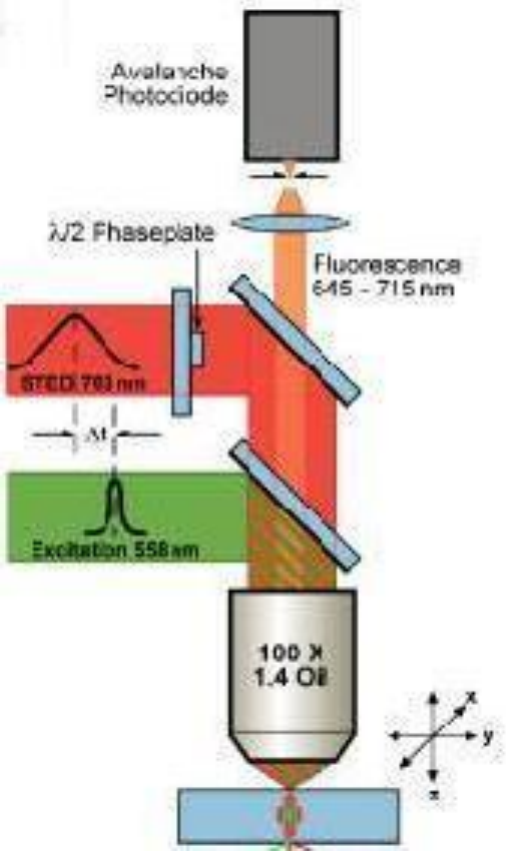


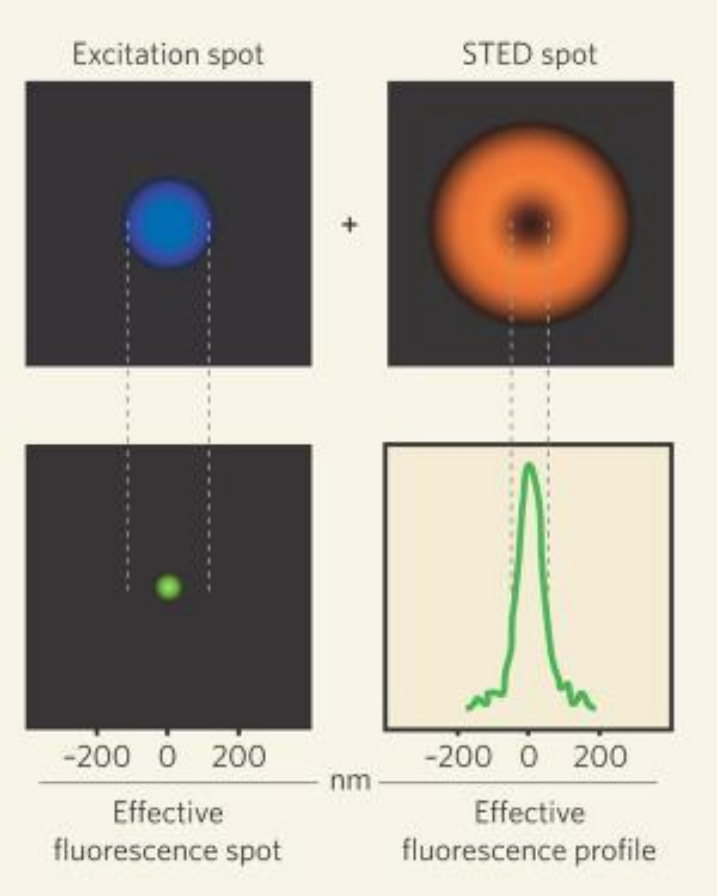


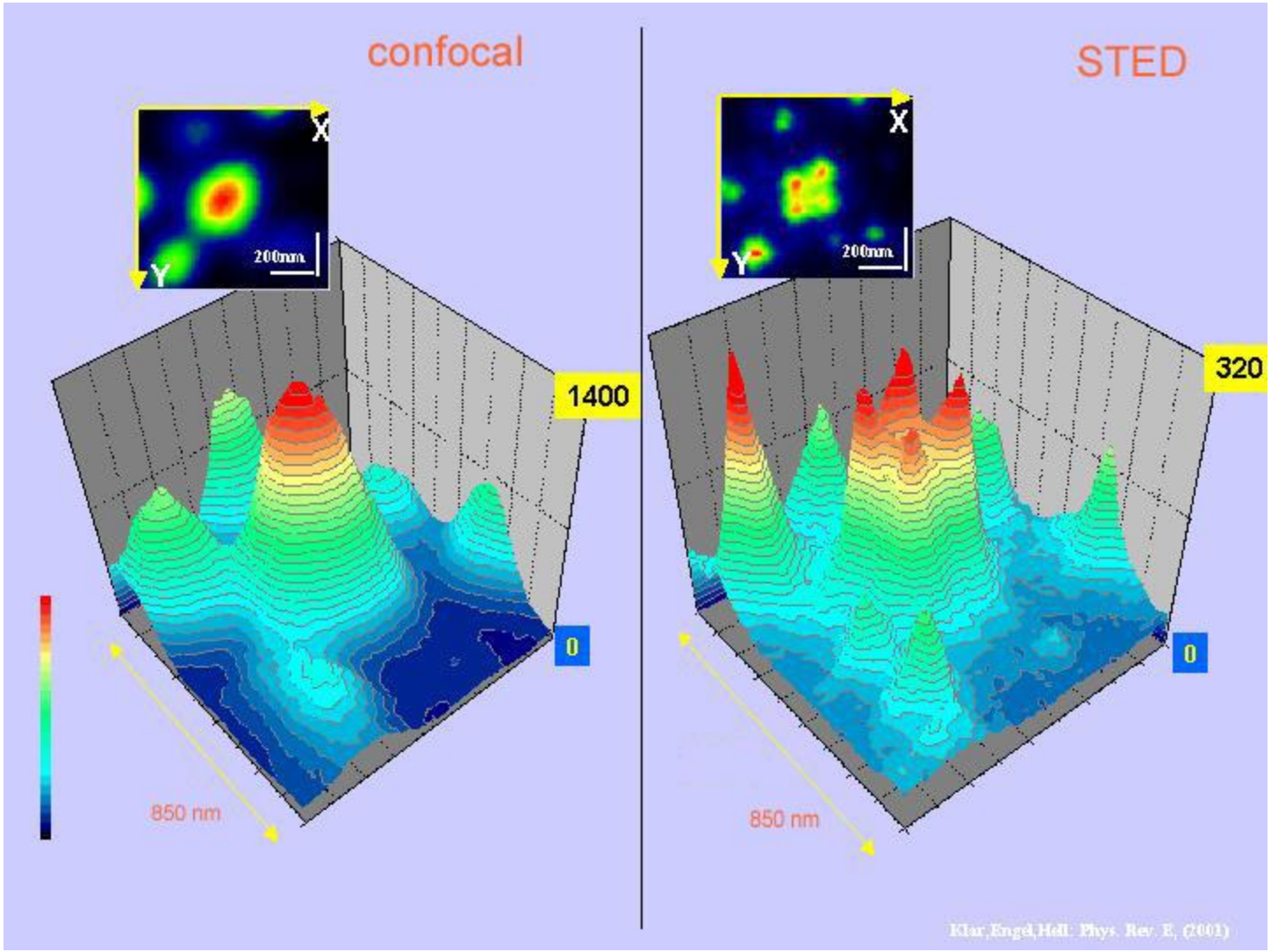
$$\Delta r \approx \frac{\lambda}{2NA\sqrt{1+Imax/Isat}}$$

"Unlimited" resolution results from inhibiting fluorescence **around** the central peak of excitation

Jun Ding Stanford U

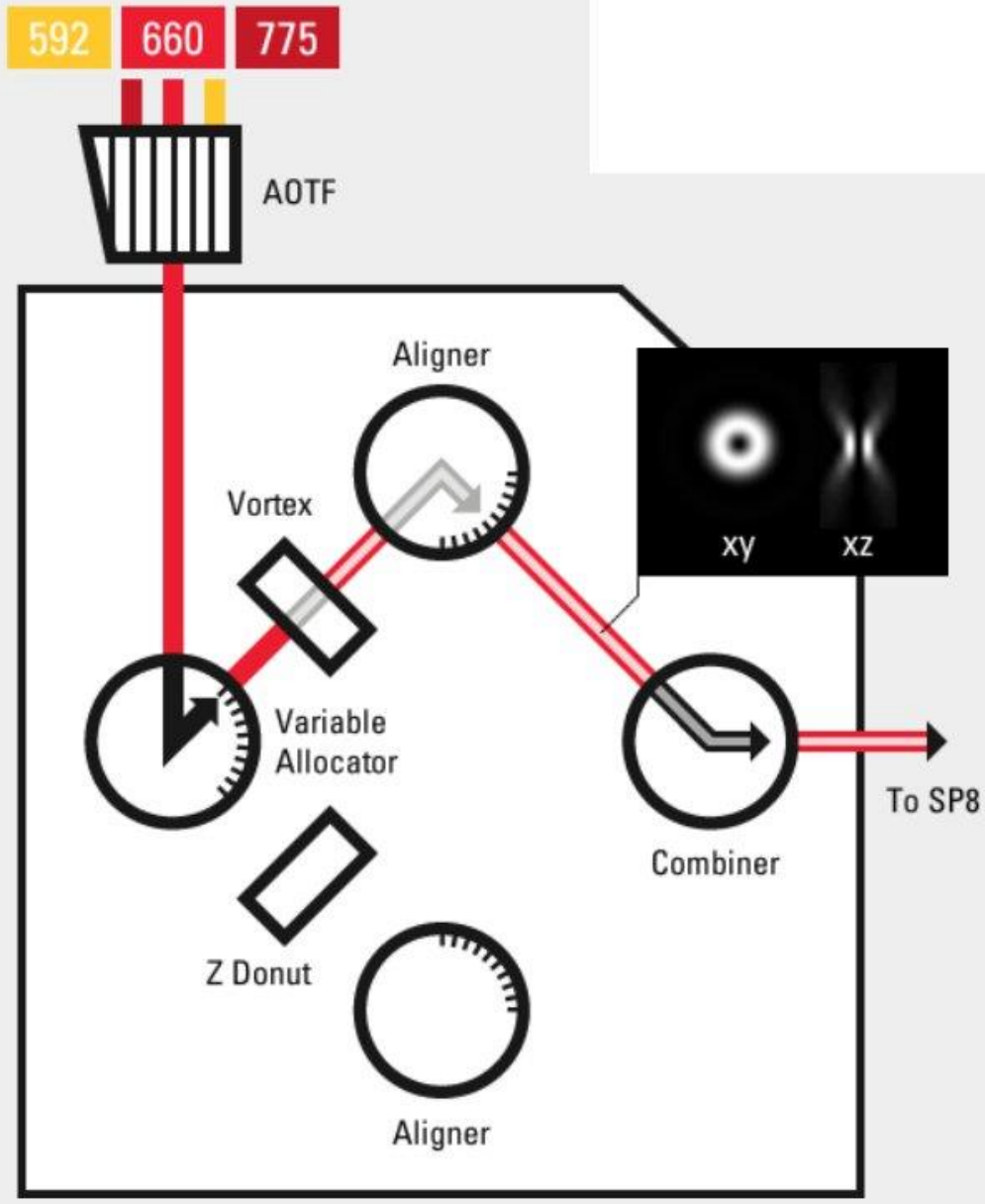

Avalanche Photodiode
λ/2 Phaseplate
Fluorescence 645 - 715 nm
STED 703 nm
Excitation 558 nm
100 x 1.4 Oil
y
z

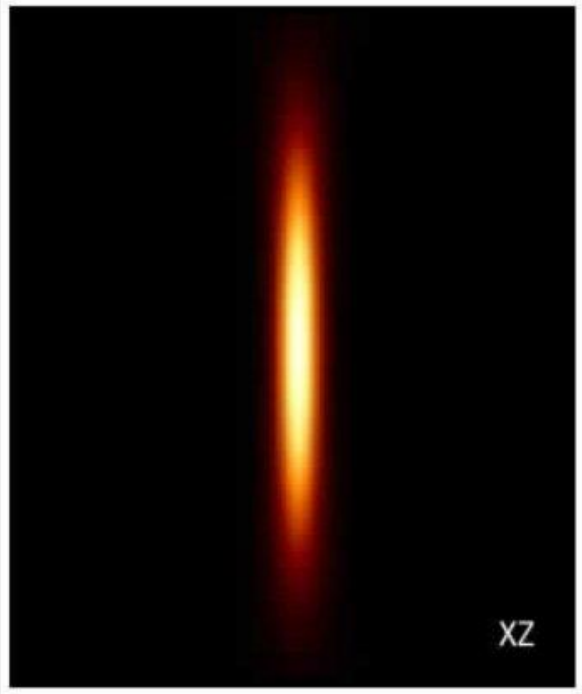

Excitation spot
STED spot
+
-200 0 200
nm
-200 0 200
Effective fluorescence spot
Effective fluorescence profile



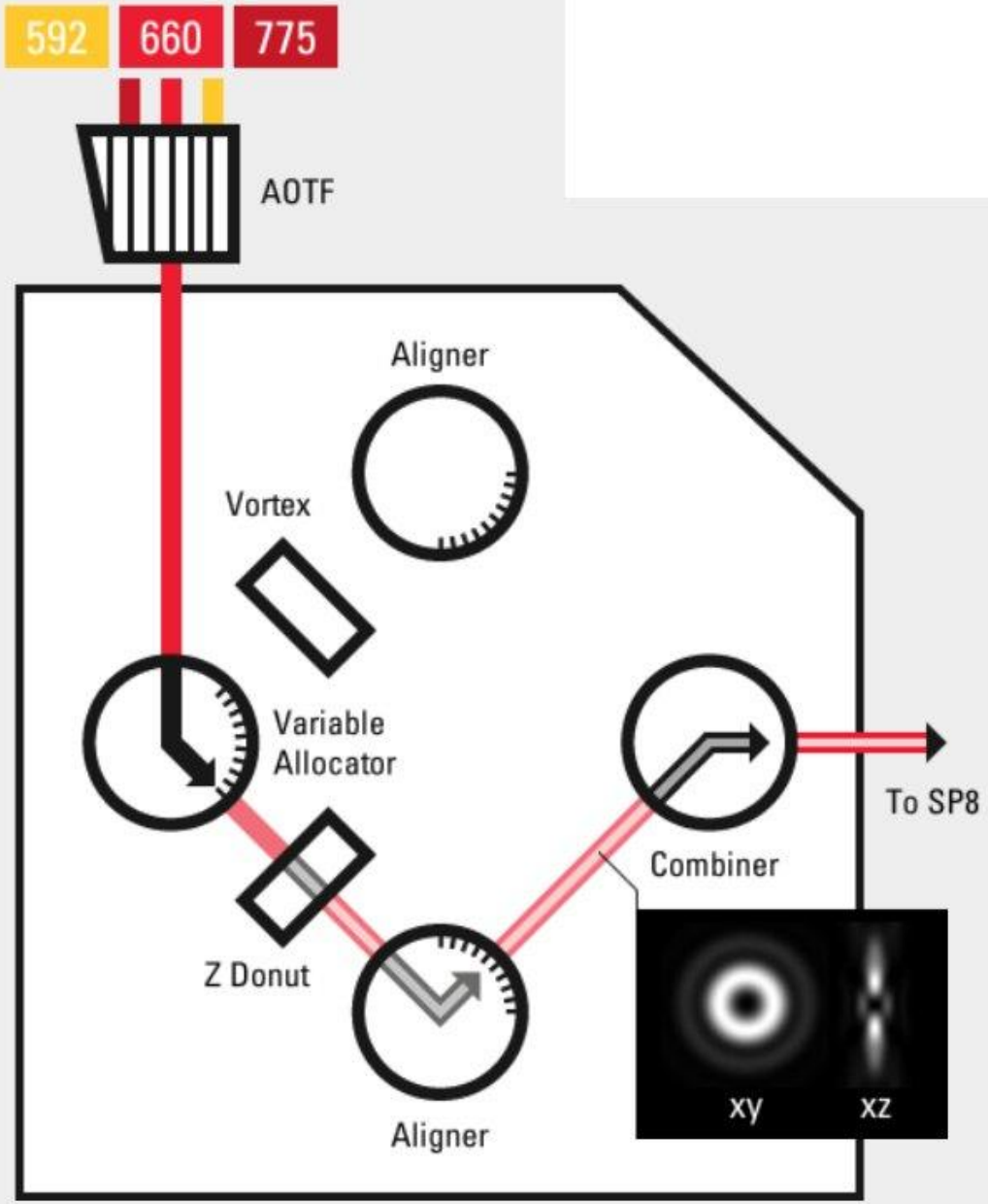
lateral resolution : ~ 40 nm

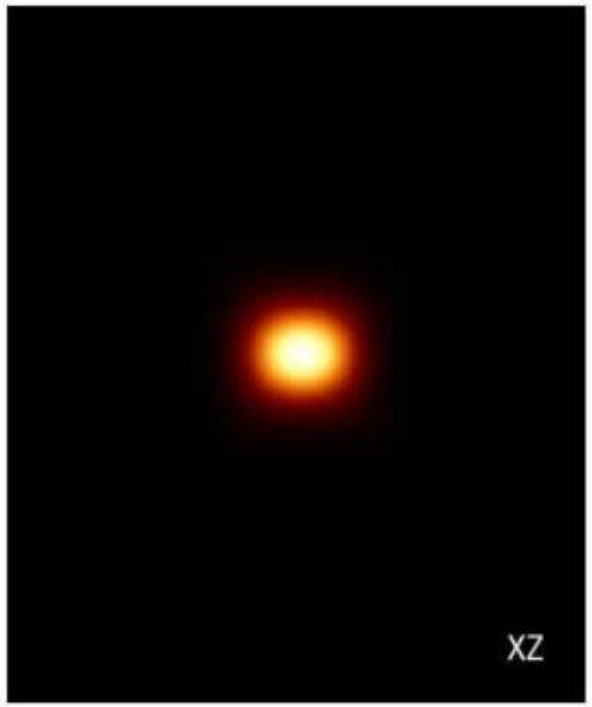
Leica STED 3X



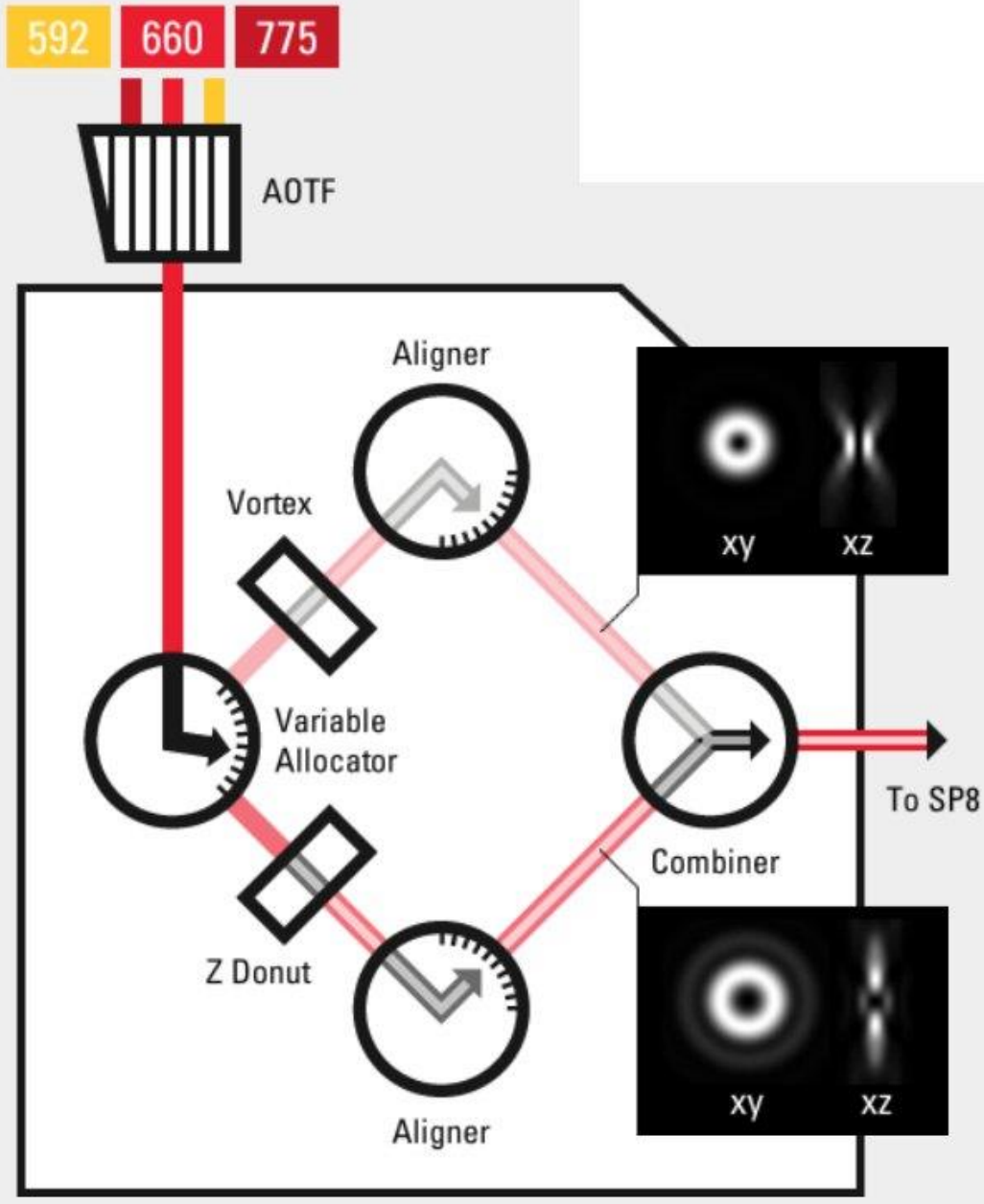


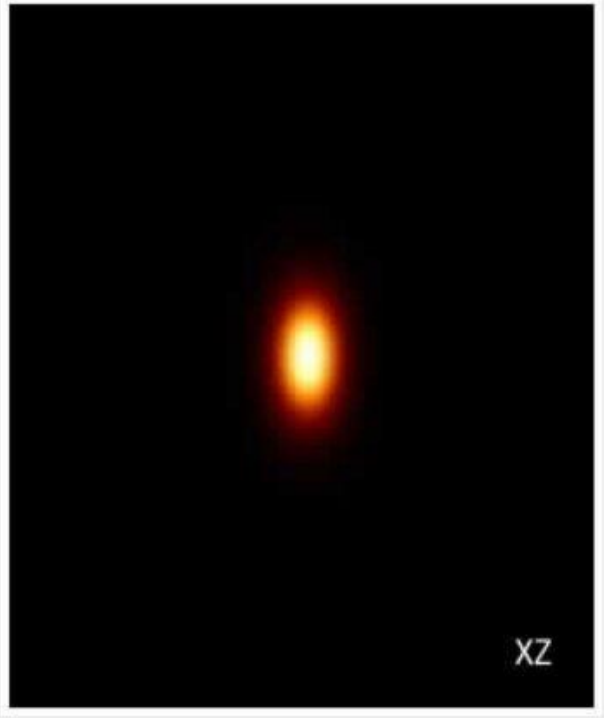
Effective Focus



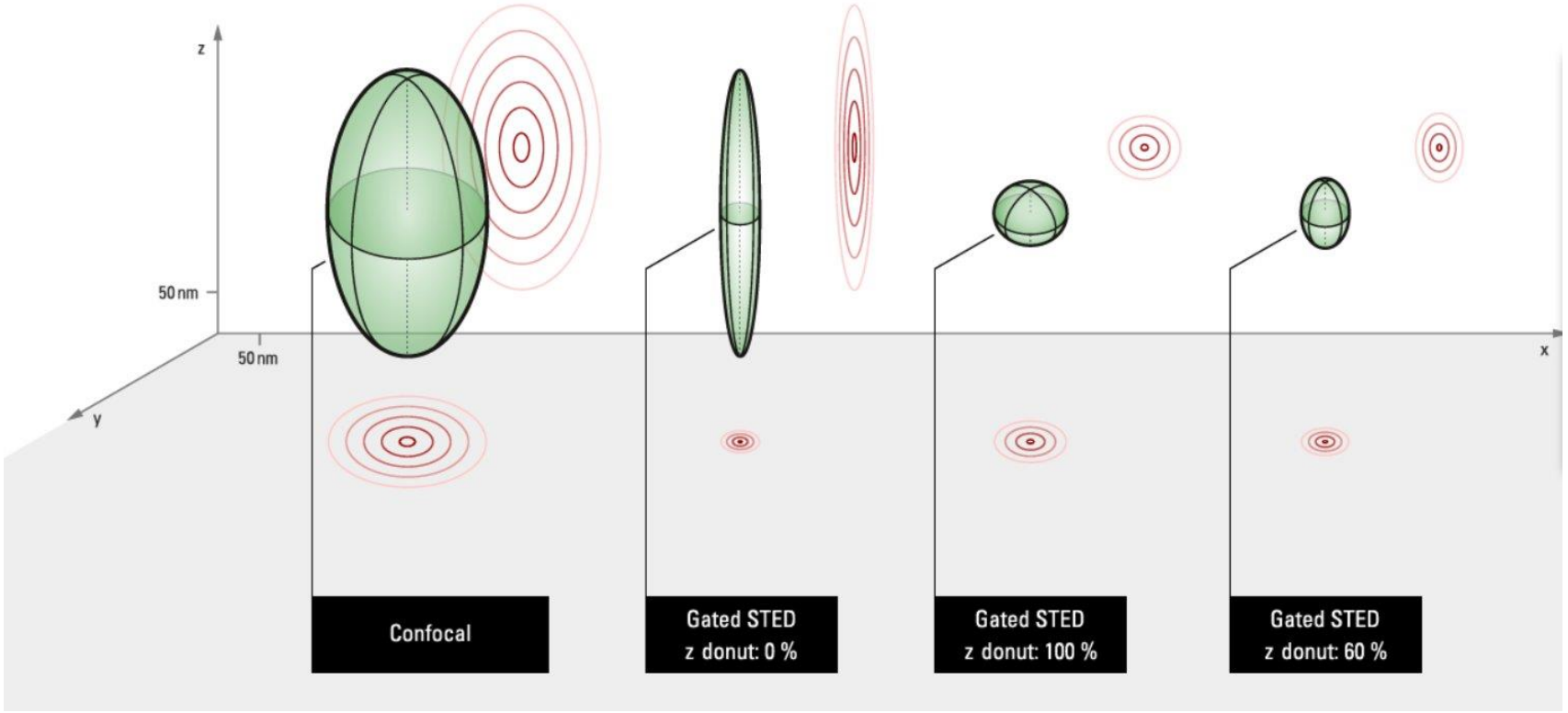


Effective Focus





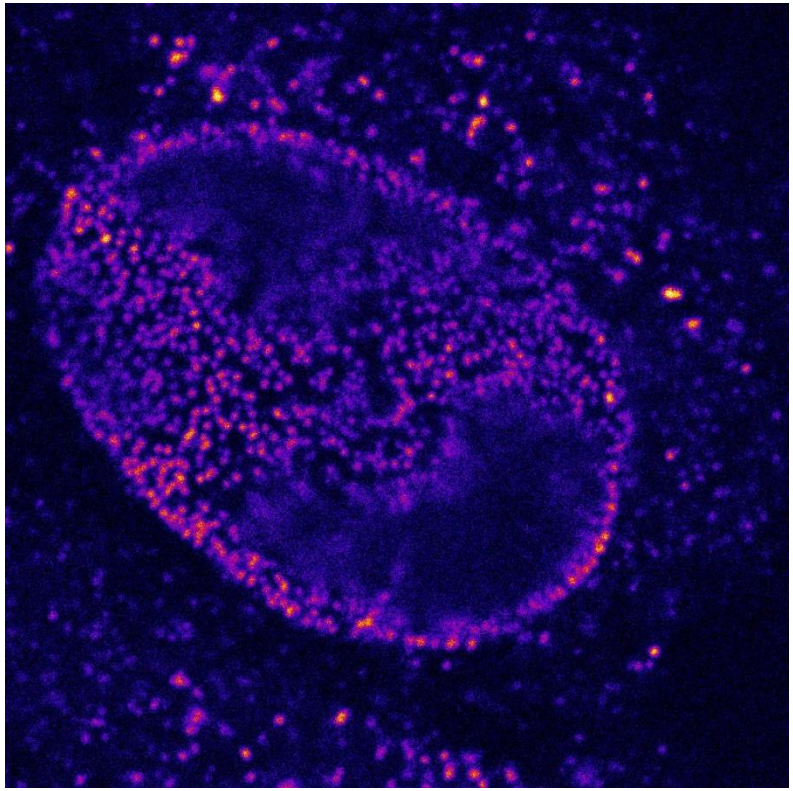
Effective Focus

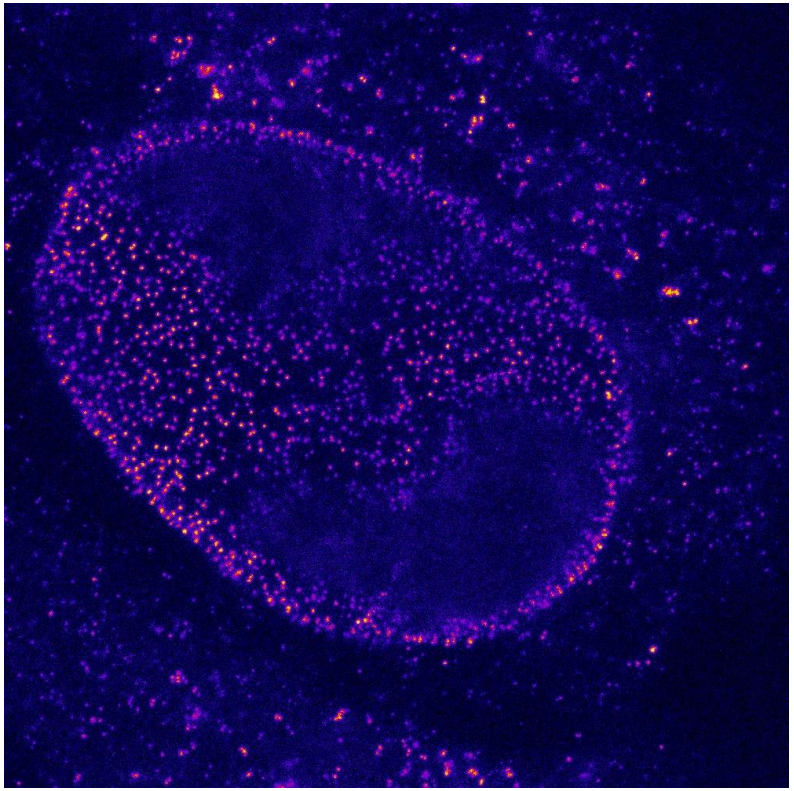

z
50 nm
50 nm
y
x
Confocal
Gated STED
z donut: 0 %
Gated STED
z donut: 100 %
Gated STED
z donut: 60 %

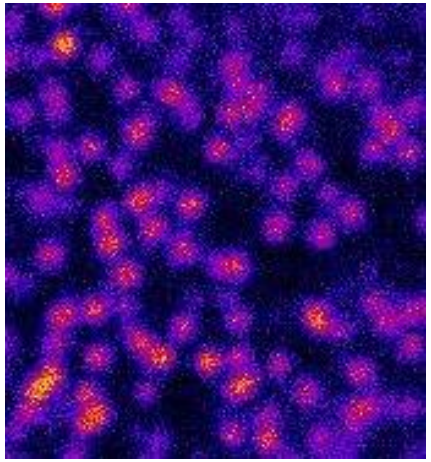
Live Demo

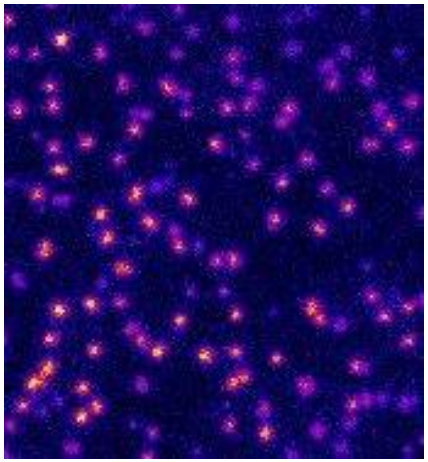
confocal

STED









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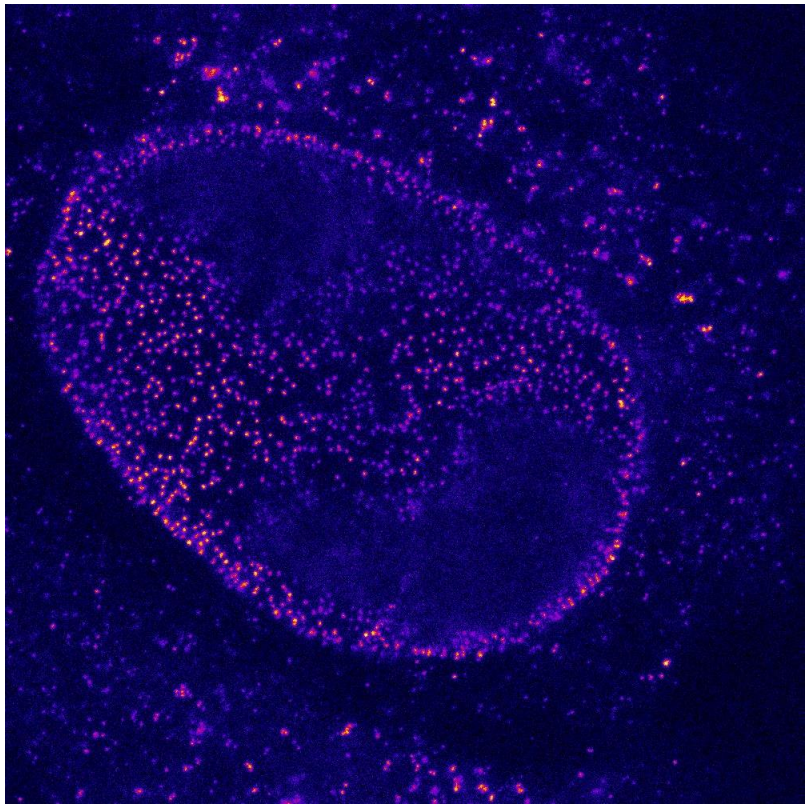
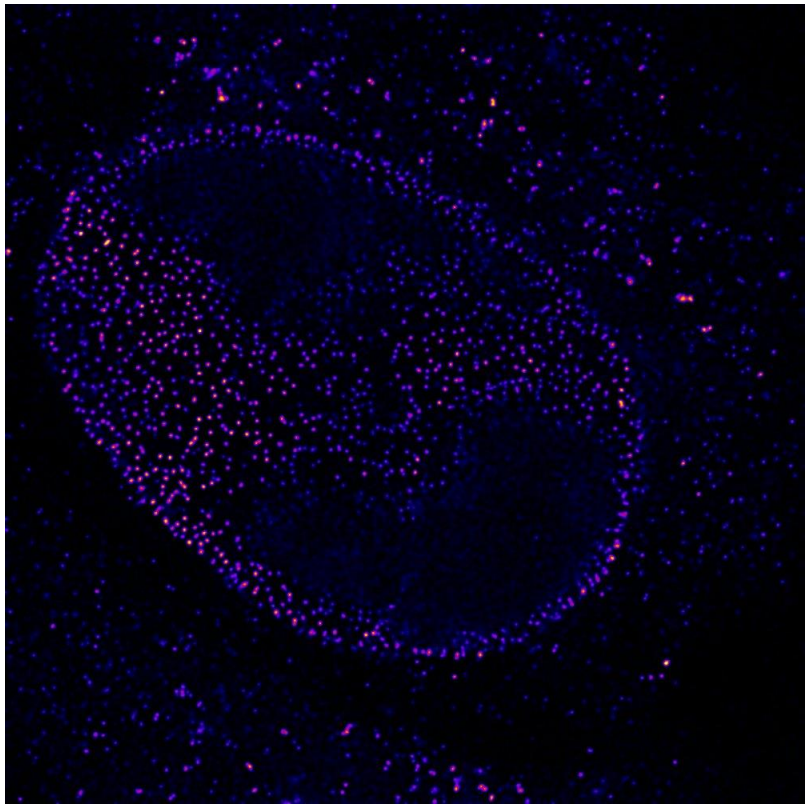
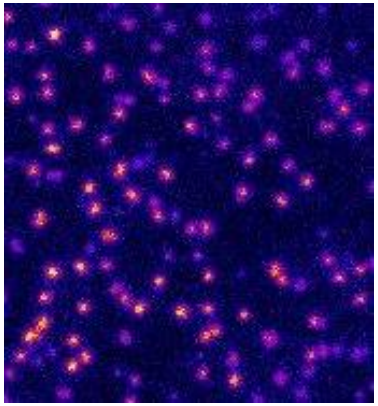
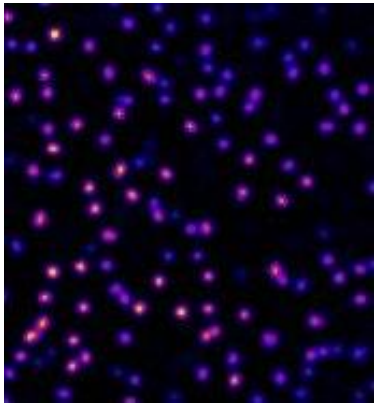
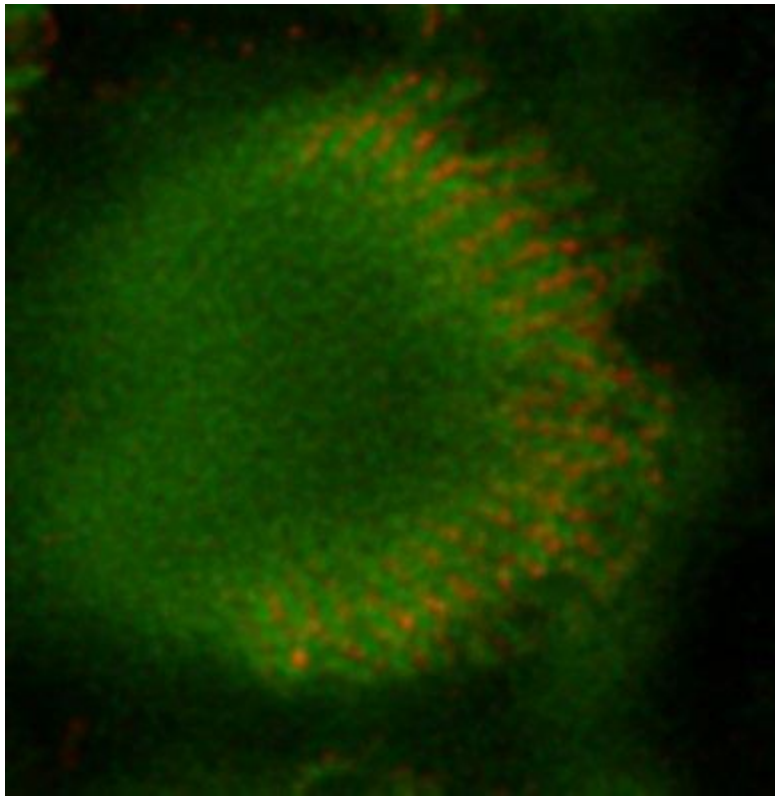


STED Deconvolution









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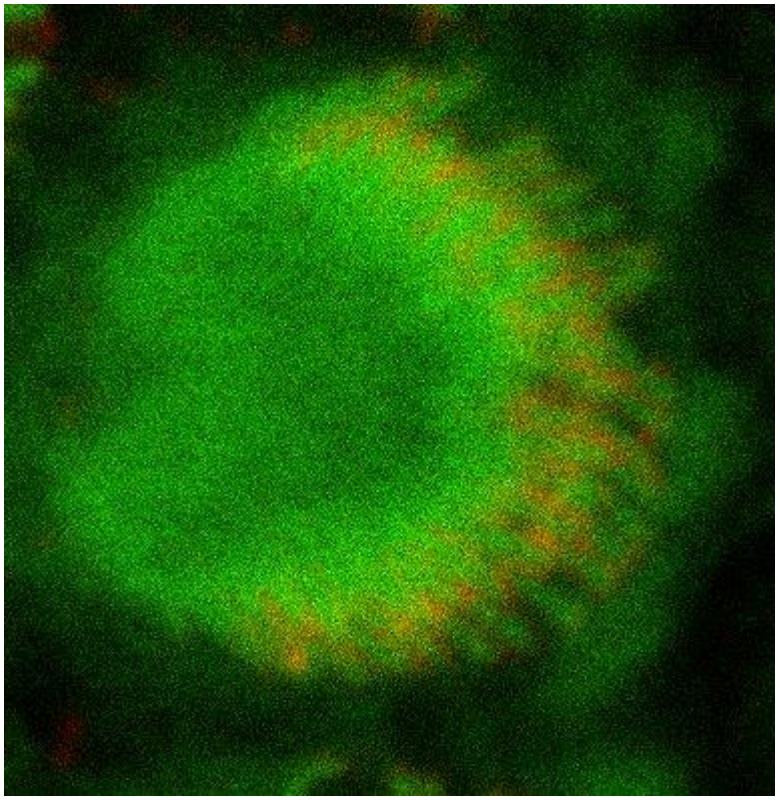


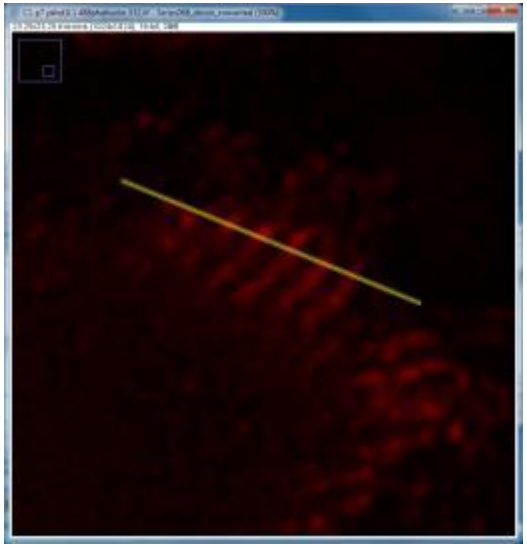
Confocal

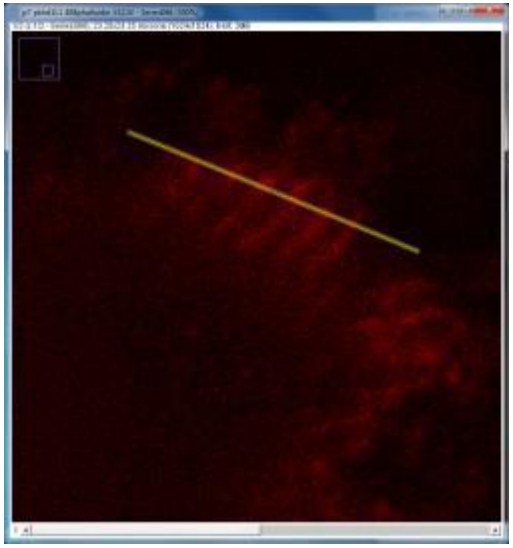
stereocilia of inner ear receptor cells

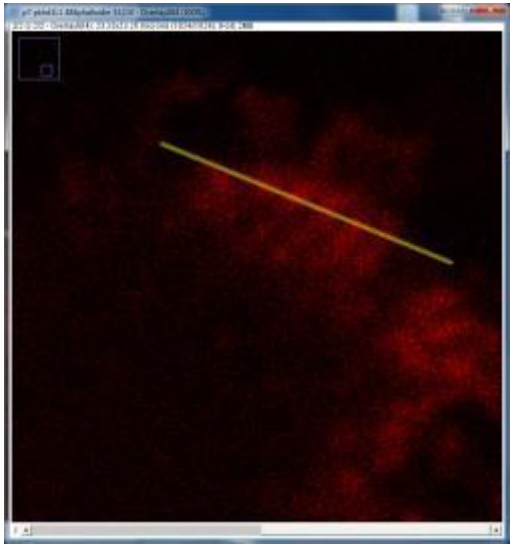
STED +Decon

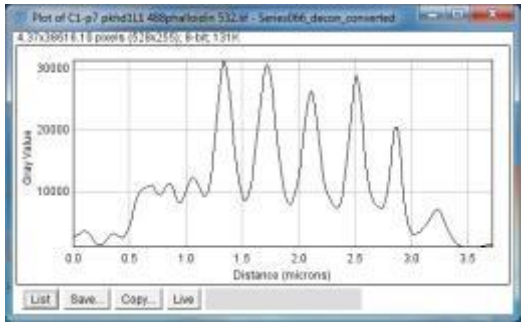
STED

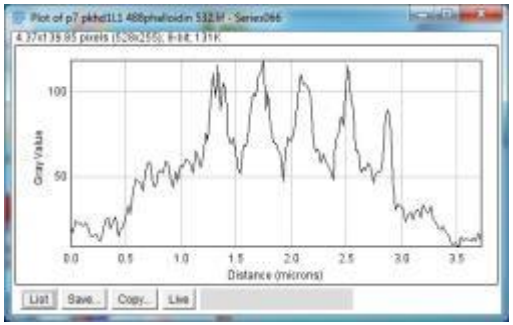
Confocal

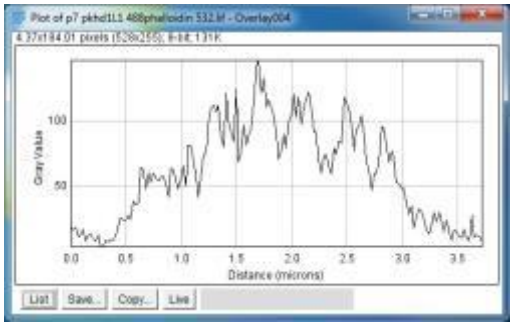


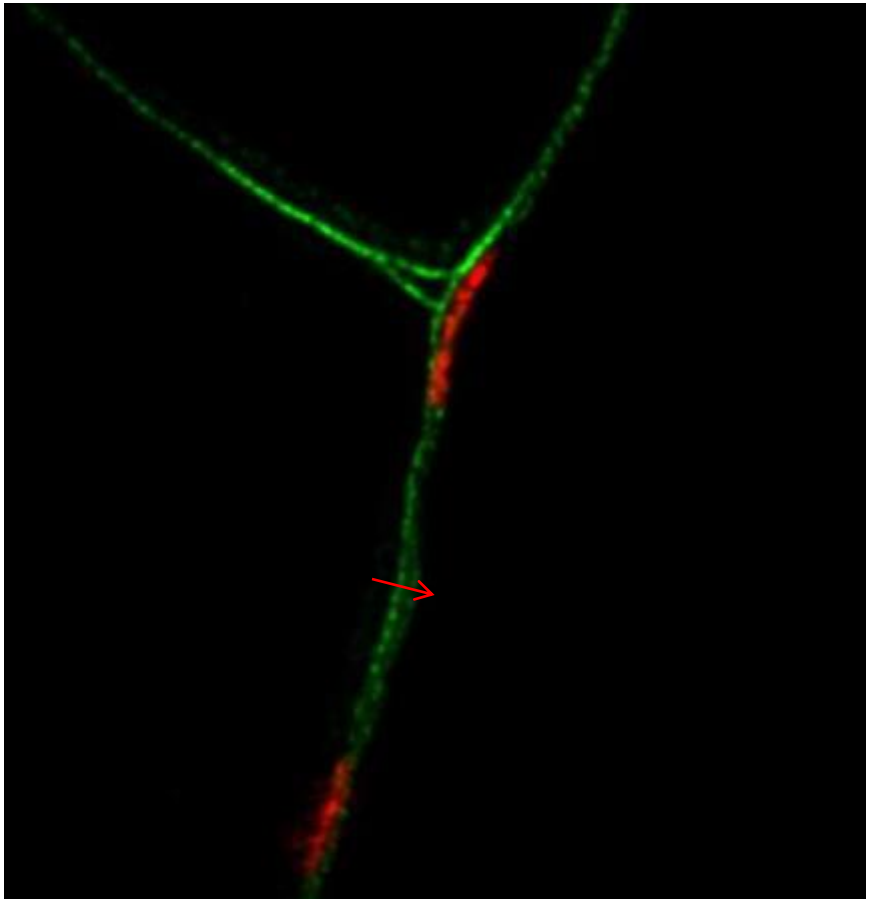


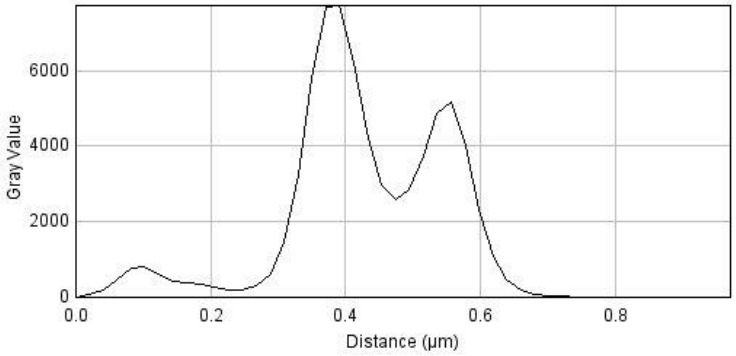












Mitochondrial (red) moves along axon (green)

Limitations:

Sample must be less than 15um thick.

Sample should be treated carefully, use coverslip #1.5

Depletion laser can bleach the sample very quickly.

Slow acquisition speed.

DAPI is NOT recommended

Most importantly : Do you NEED it?

“Super-Resolution Microscopy”

Nature Method of the year 2008

http://www.nature.com/nmeth/video/moy2008/index.html

Chemistry Nobel awarded for super-resolution microscopy

Oct 8, 2014 5 comments

Pushing the limit: Eric Betzig, Stefan Hell and William Moerner

The 2014 Nobel Prize for Chemistry has gone to Eric Betzig, Stefan Hell and William Moerner for developing super-resolution microscopy techniques based on the fluorescence of molecules. The prize is worth SEK 8m (£690,000) and will be shared by the three winners, who will receive their medals at a ceremony in Stockholm on 10 December.

Betzig is a US citizen and works at the Howard Hughes Medical Institute, Hell is a German citizen and is at the Max Planck Institute for Biophysical Chemistry in Göttingen, and Moerner is a US citizen based at Stanford University.

Breaking Abbe's diffraction resolution limit in fluorescence microscopy with stimulated emission depletion beams of various shapes

Thomas A. Klar, Egbert Engel, and Stefan W. Hell*
High Resolution Optical Microscopy Group, Max-Planck-Institute for Biophysical Chemistry,
37070 Göttingen, Germany

Physical Review E.

Nanoscope?

Jena, Germany