NIKON CORPORATION
Instruments Company

Pokročilé mikroskopické techniky

Konfokální mikroskopie

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Super-rezoluční mikroskopie

Ing. Ondřej Sedlák

Nikon CEE GmbH

turning *vision* into information

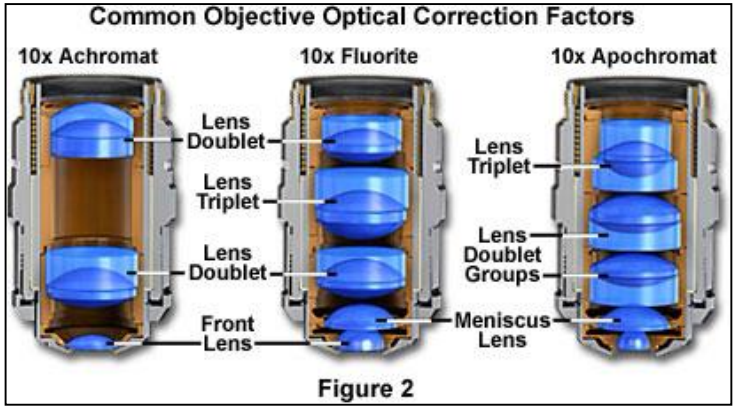

Nikon's First
Microscope
(circa early 1900s)

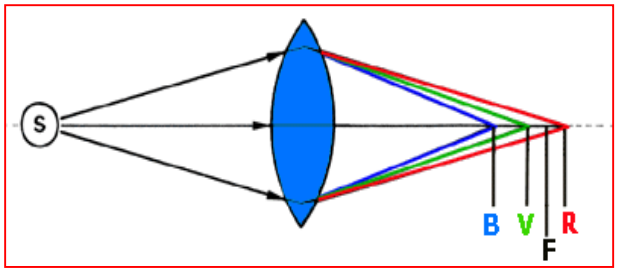

Nikon
Nikon
Nikon Model S
Binocular
Microscope
(circa late 1960s)

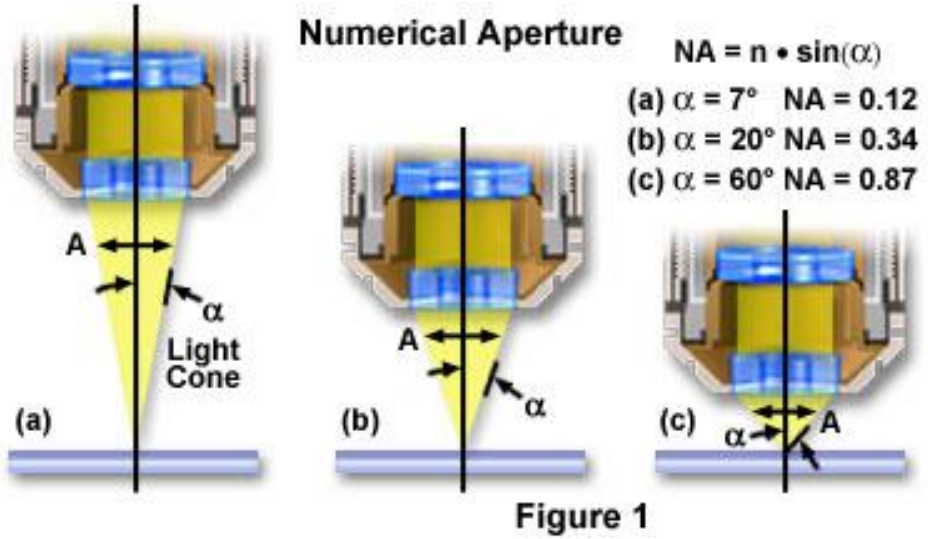

Nikon Eclipse E600
with U-III Film
Camera System
(circa early 1990s)

Plan Apo Lambda series

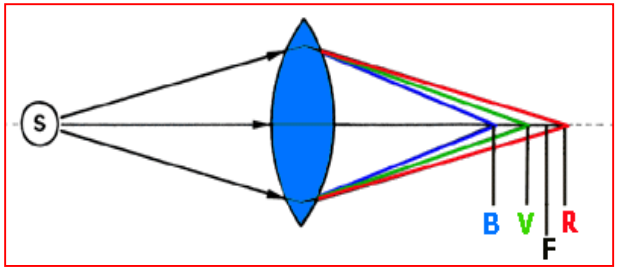

60x Plan Apochromat Objective
Manufacturer
Flat-Field Correction
Lateral Magnification
Specialized Optical Properties
Tube Length
Cover Glass Thickness Range
Cover Glass Adjustment Guage
Nosepiece Mounting Thread
Aberration Correction
Numerical Aperture
Working Distance
Magnification Color Code
Correction Collar
Front Lens Element Assembly
Nikon
Plan Apo
60X / 0.95
DIC M
∞ / 0.11-0.23 WD 0.15
11 14 17 20 23
Figure 1

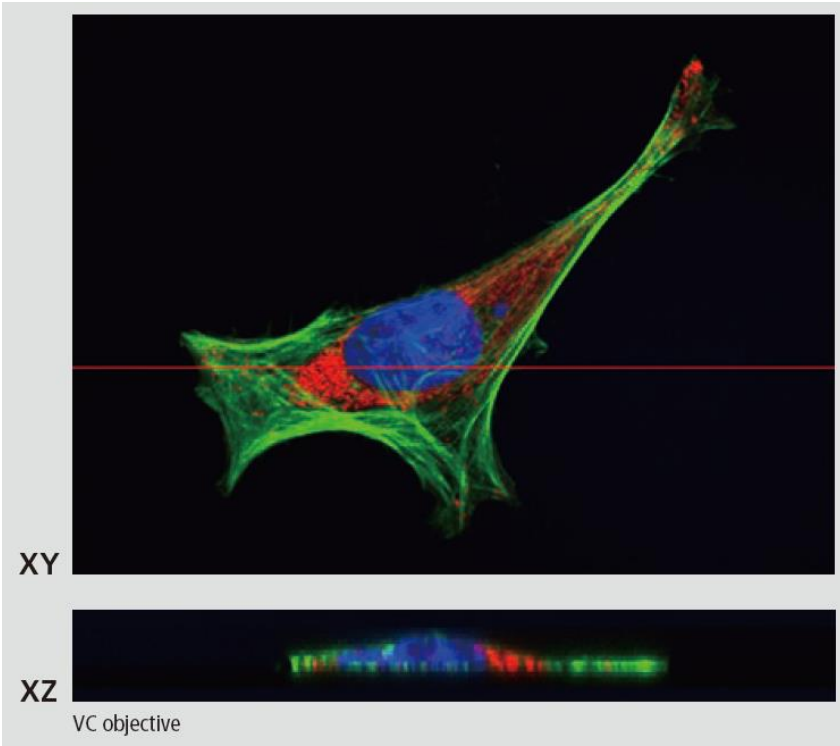

Common Objective Optical Correction Factors
10x Achromat
10x Fluorite
10x Apochromat
Lens Doublet
Lens Triplet
Lens Doublet
Front Lens
Lens Triplet
Lens Doublet Groups
Meniscus Lens
Figure 2


S
B
V
F
R

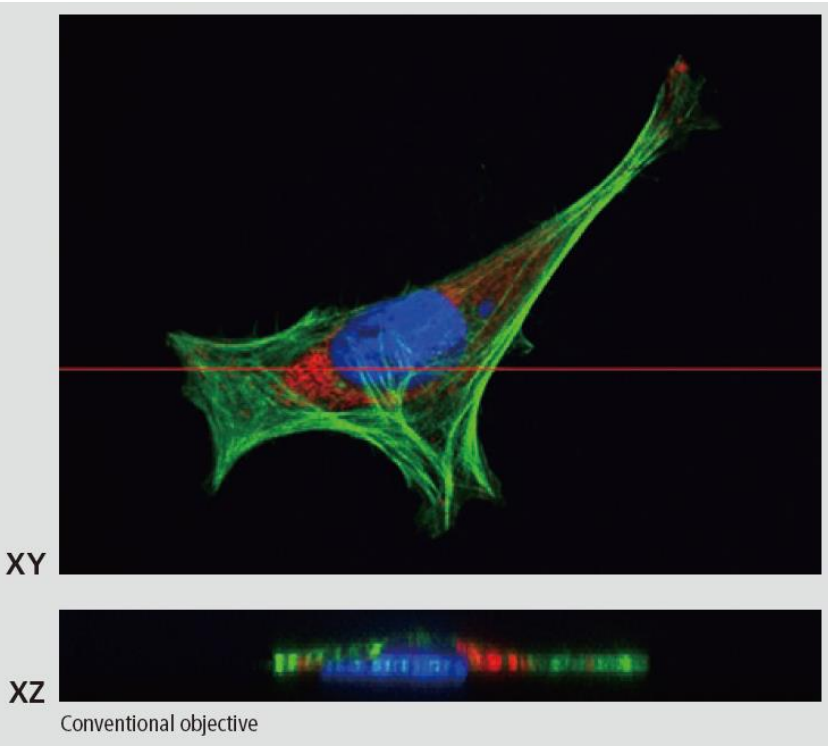

Numerical Aperture
NA = n • sin(α)
(a) α = 7° NA = 0.12
(b) α = 20° NA = 0.34
(c) α = 60° NA = 0.87
A
α
Light Cone
(a)
(b)
(c)
Figure 1

NA = n * sinα



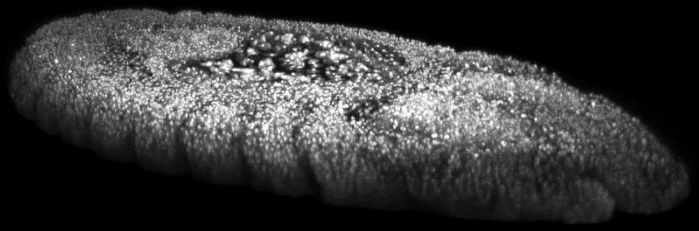


VC objective



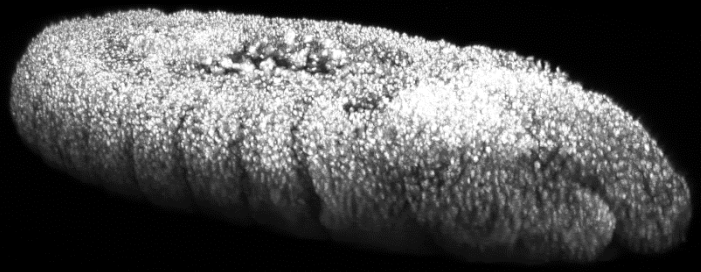
Conventional objective

40x Air PA λ - 0.95 NA, 210 μm WD



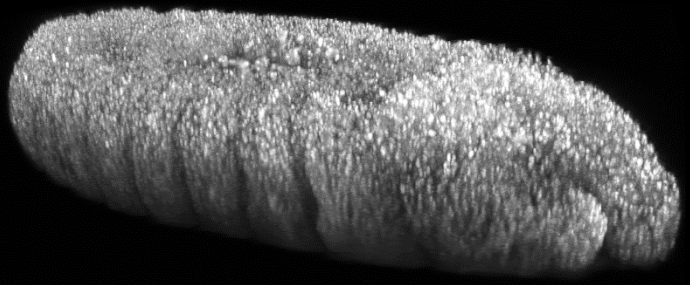
B ✗
D ✗
R ✓
L ✓

40x WI A λS - 1.25 NA, 180 μm WD



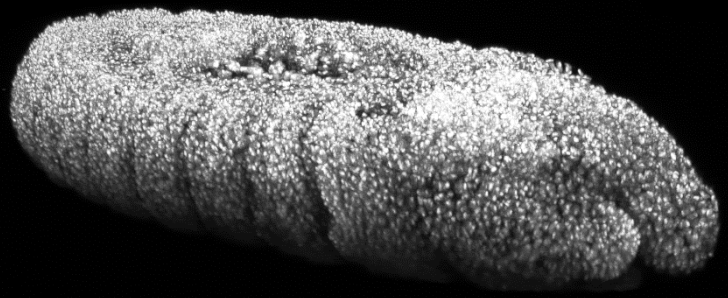
B ✓
D ✓
R ✓
L ✓

40x Oil PF - 1.3 NA, 200 μm WD



B ✓
D ✓
R ✓
L ✗

40x Sil PA λS - 1.25 NA, 300 μm WD



B ✓
D ✓✓
R ✓
L ✓

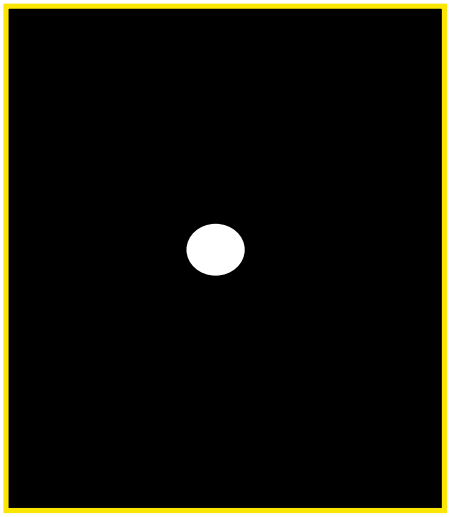
B = Brightness
D = Depth
R = Resolution
L = Live Cell

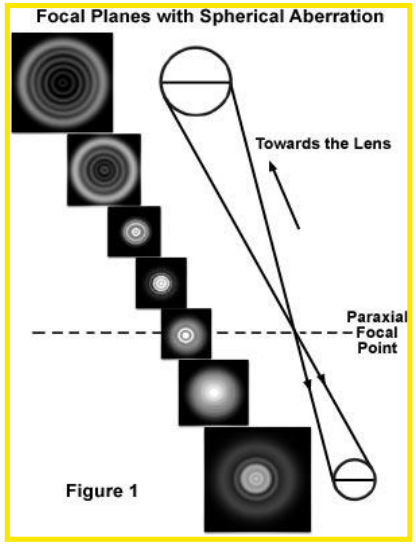
***Drosophila sp.* embryo with DAPI stained nuclei**,
supplied by Dr. Jennifer Sallee, North Central College

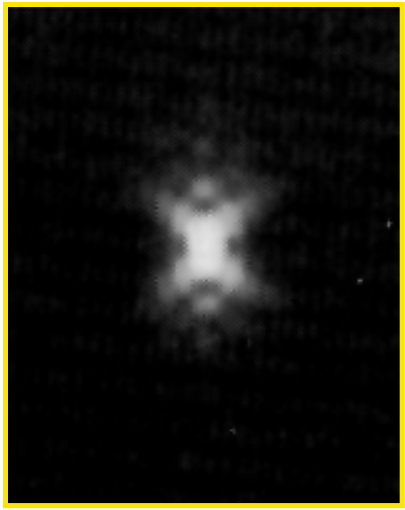
Nikon

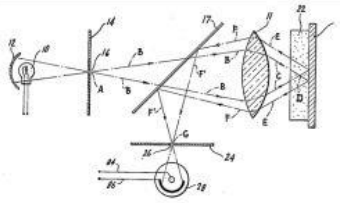
Microscope Image Formation

- An image is a huge array of sub resolution points
- Each point in the image is convolved by the objective to form a “Point Spread Function” (PSF).
- A PSF is unique to a particular objective and microscope configuration









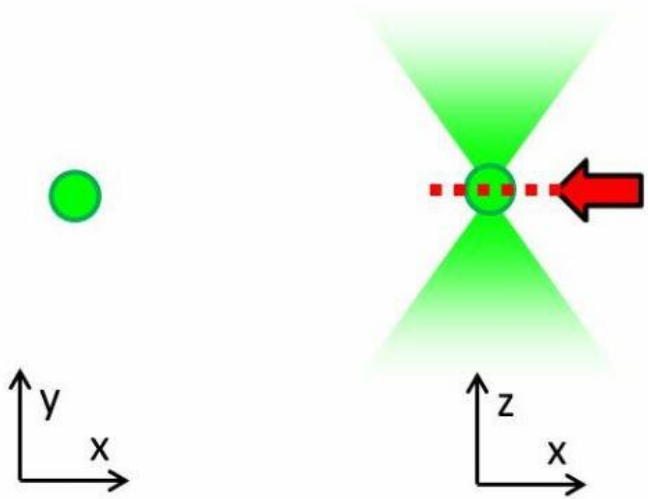
Convolved Point Sources

Point sources of light spread in all direction

Sources exist above and below focal plane

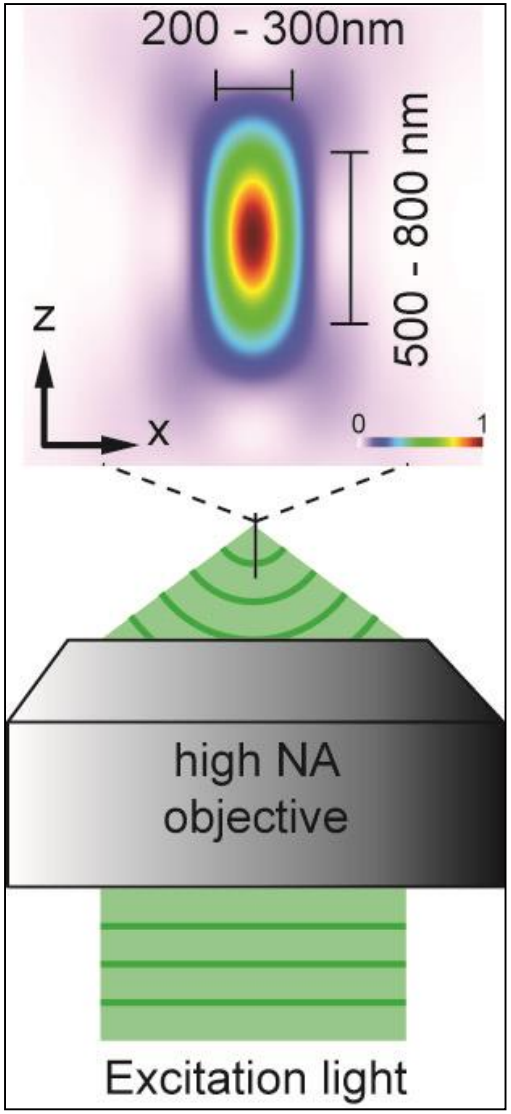
Point Sources

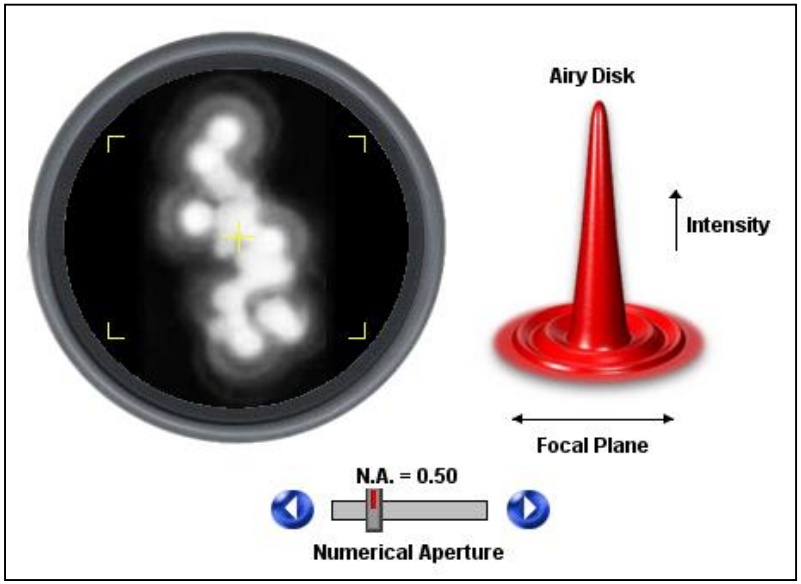
1 2 3

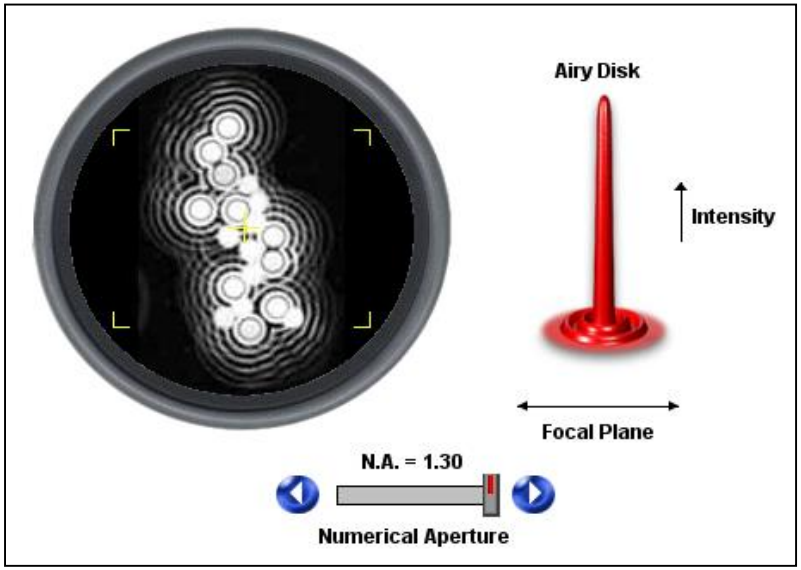


At FOCAL plane

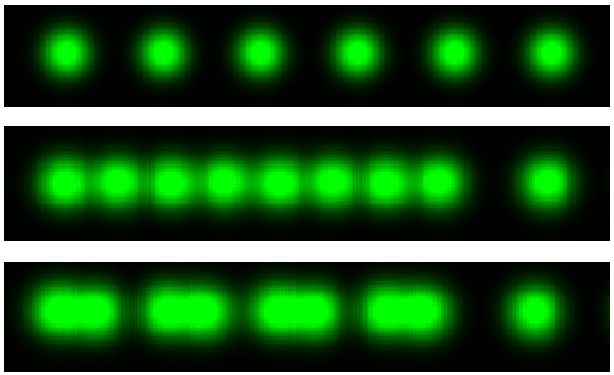
Convolution


200 - 300nm
500 - 800 nm
z
x
0
1
high NA
objective
Excitation light


Airy Disk
Intensity
Focal Plane
N.A. = 0.50
Numerical Aperture


Airy Disk
Intensity
Focal Plane
N.A. = 1.30
Numerical Aperture

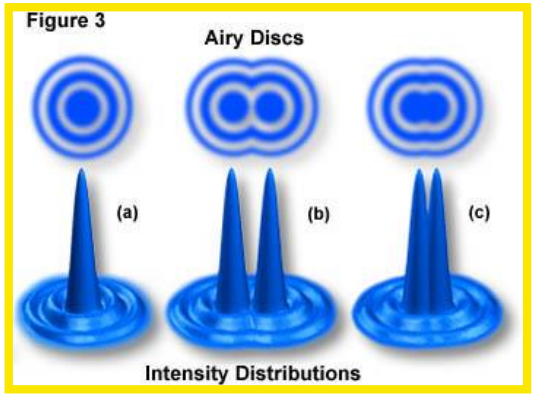
Resolution / Diffraction Limit



Well resolved

Just resolved

Not resolved



Ernst Abbe

Diffraction Limits of Optical Instruments

Ernst Abbe
(1840-1905)

$$d = \frac{\lambda}{2NA}$$

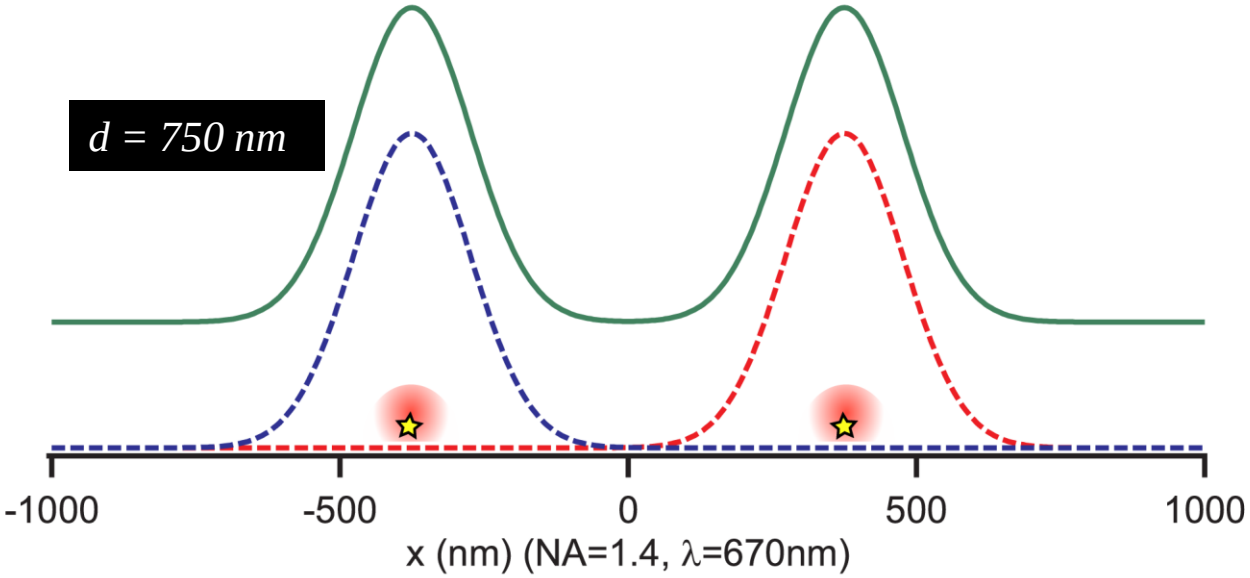
Lord Rayleigh

Resolution Limits of Diffraction Limited Optical Instruments (Epifluorescence)

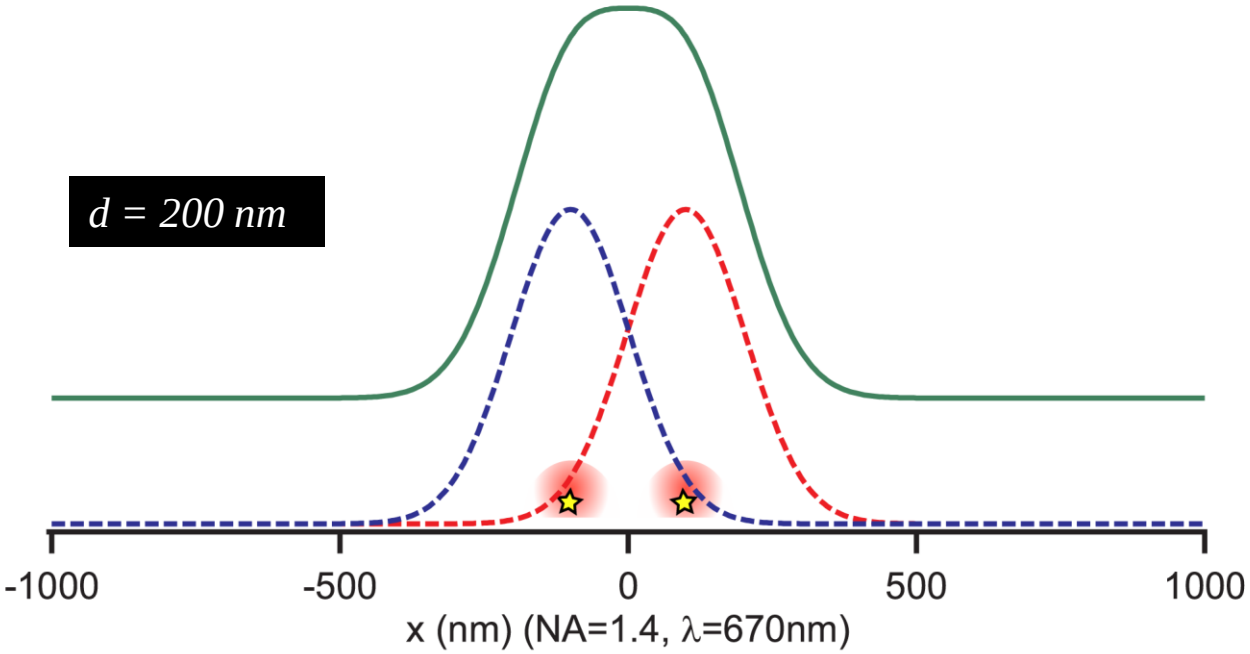
Lord Rayleigh (John Strutt)
(1842-1919)

$$R = \frac{1.22\lambda}{2NA}$$

Resolution Limits



Resolution Limits

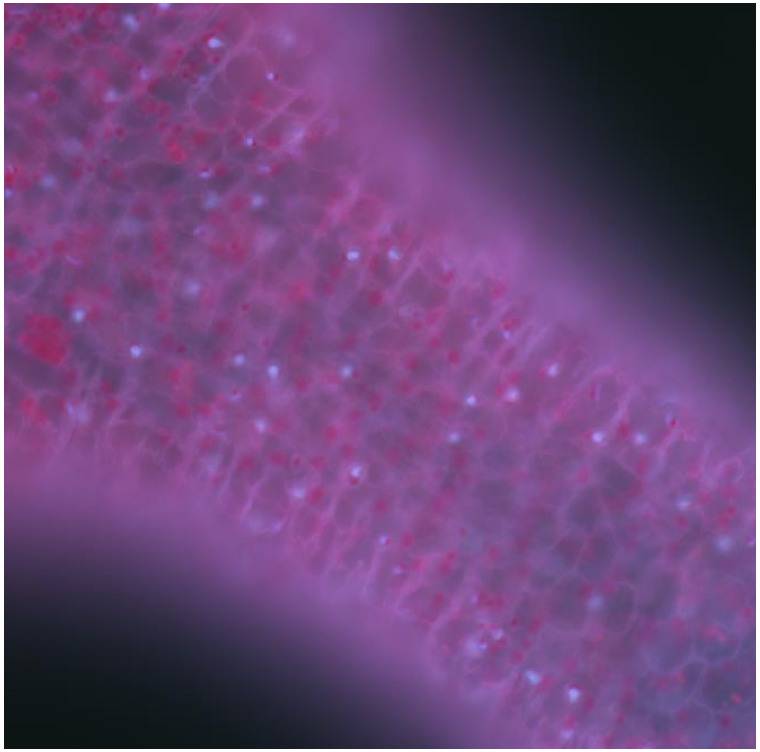


Resolution Limits

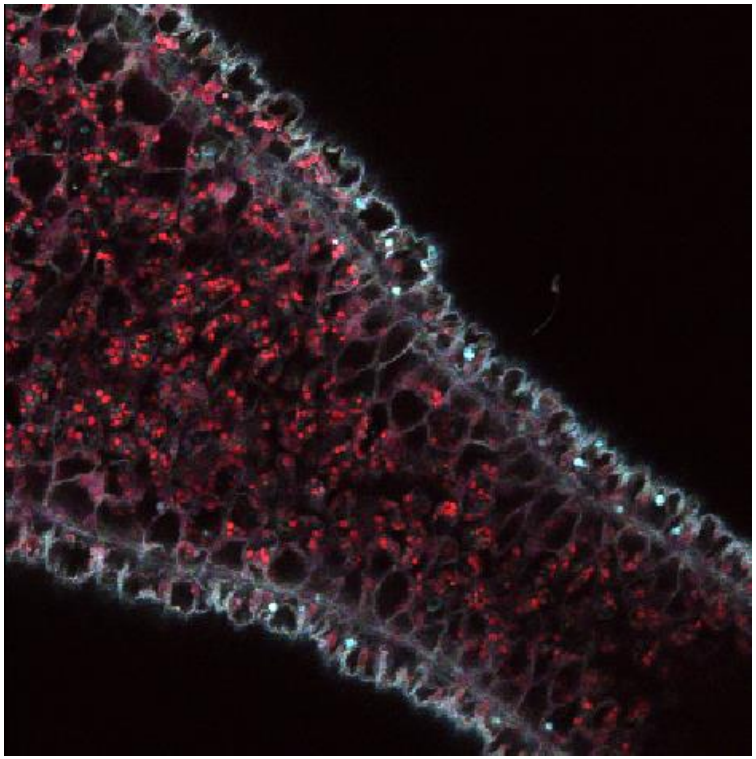
	Approximate resolution
Human eye	**0.05 mm**
Widefield microscopy	**250 nm (X,Y)** **600-850 nm (Z)**
Confocal microscopy	**250 nm (X,Y)** **500 nm (Z)**
Total internal reflection (TIRF)	**50-100 nm (Z)**
SUPER-RESOLUTION	**< 100nm**
Atomic Force Microscopy	1-50 nm
Electron microscopy	< 1 nm

Confocal vs. widefield microscopy

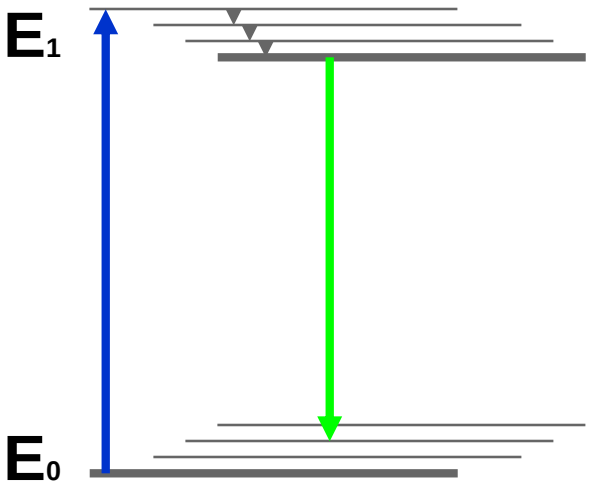
Widefield



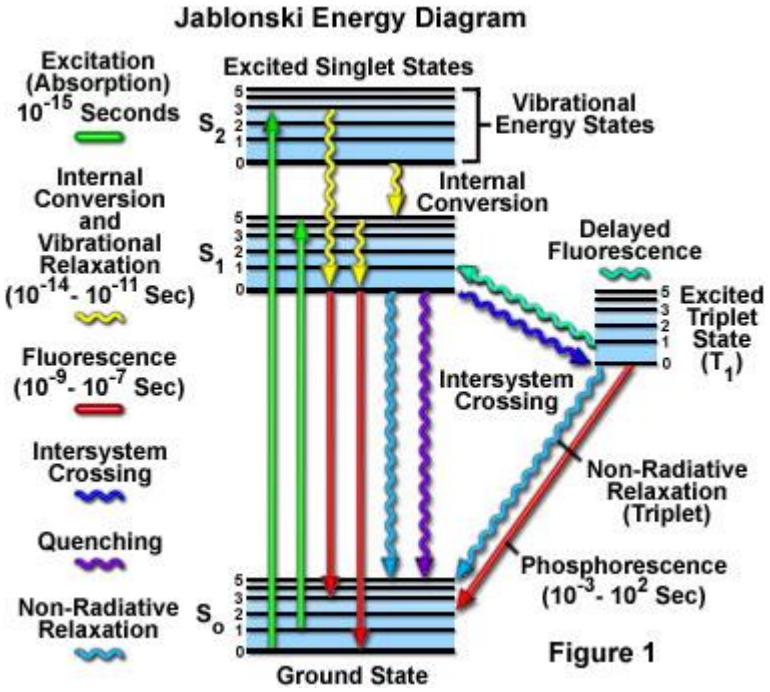
Confocal



Principle of Fluorescence



Jablonski diagram



energy to excite is higher than the emitted energy
this is named the “stokes shift” (a shift to longer wavelengths).

Fluorescence filter block for confocal

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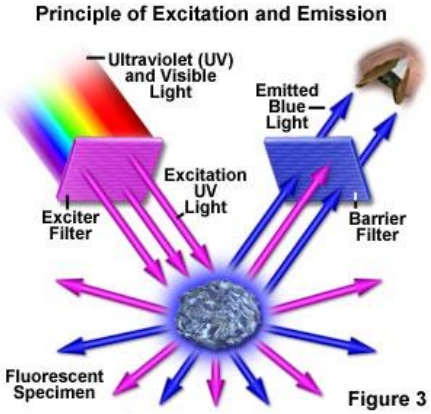
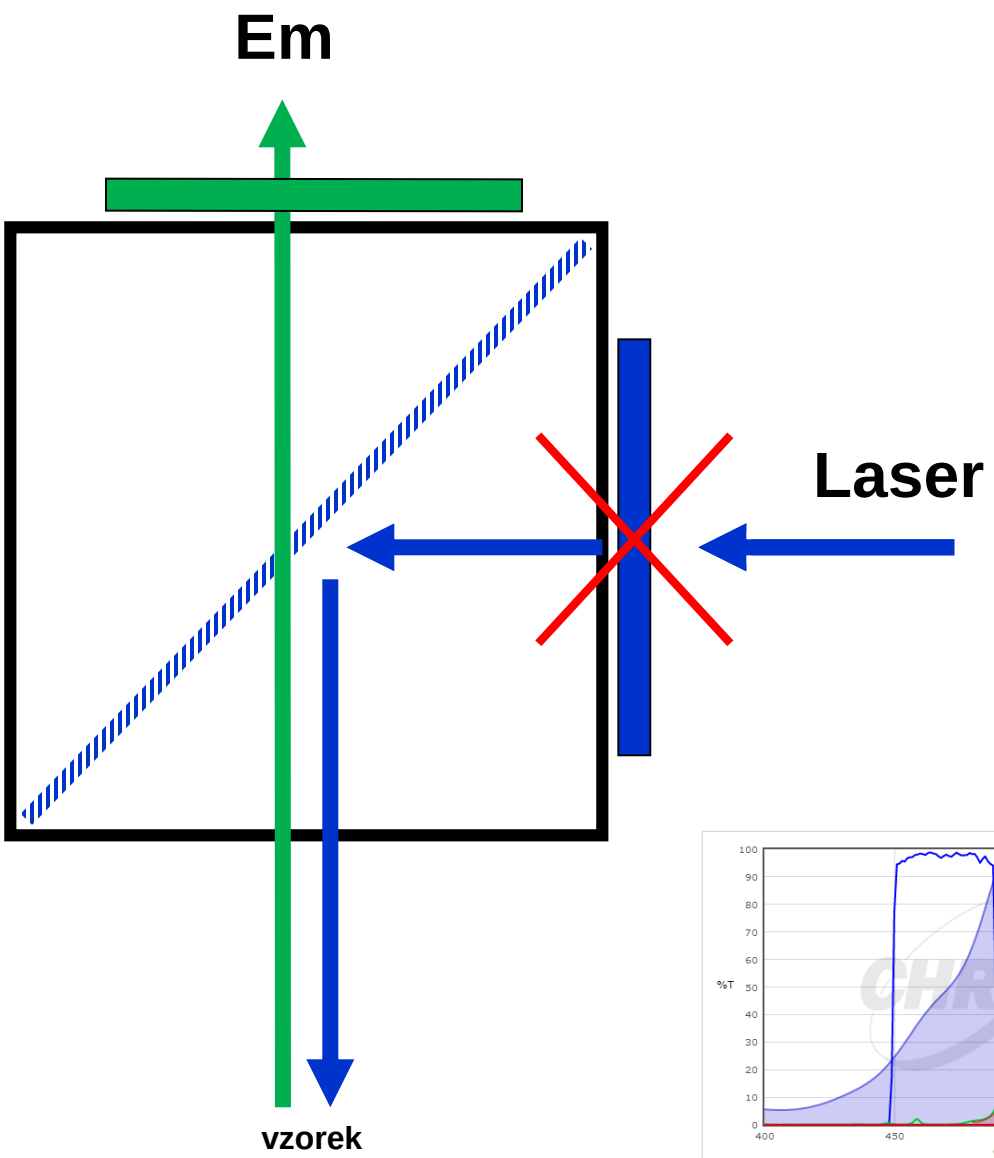
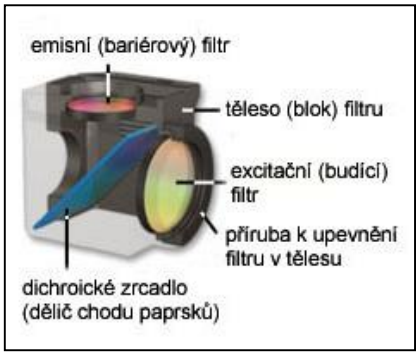
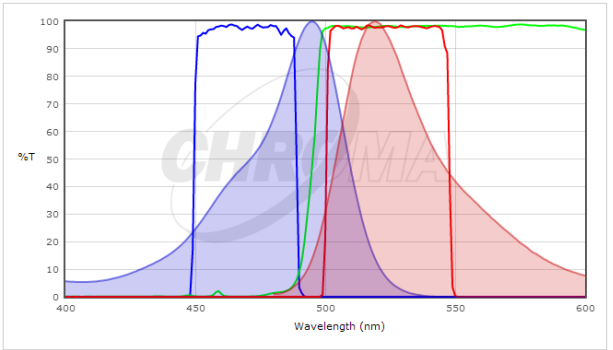




750~1050
638
561
543
514
488
477
457
440
405





Fluorescence filter block for confocal

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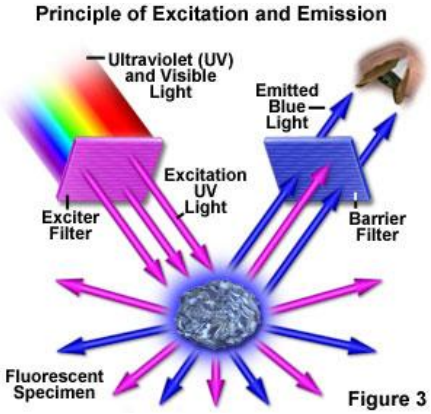

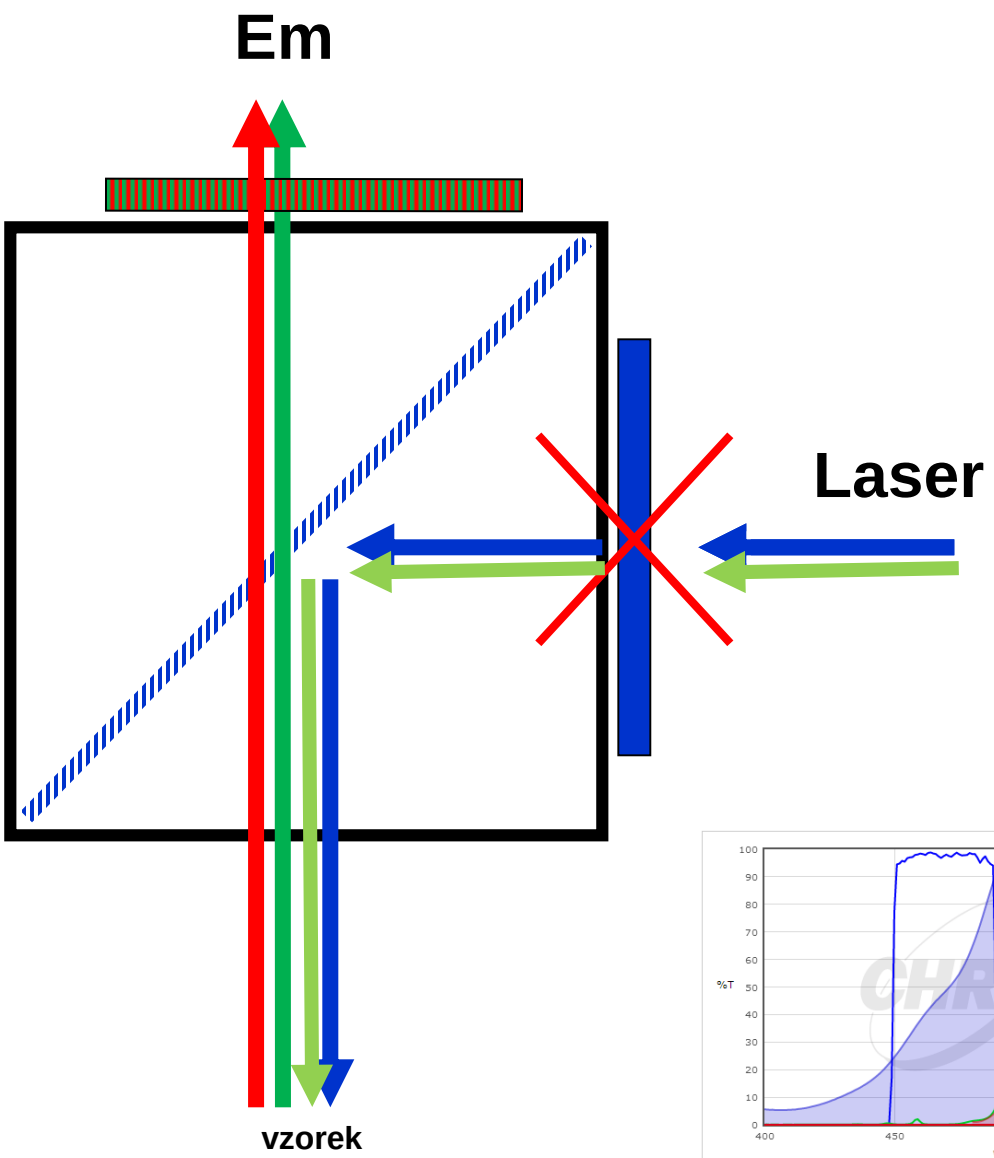

750~1050
638
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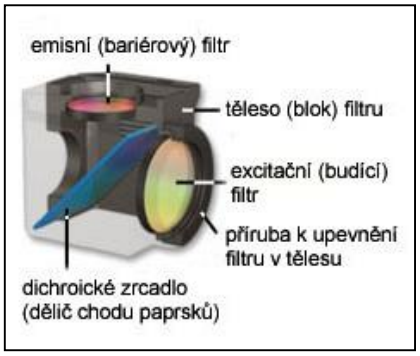
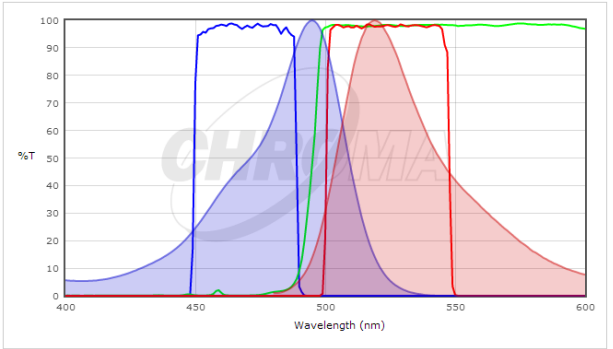
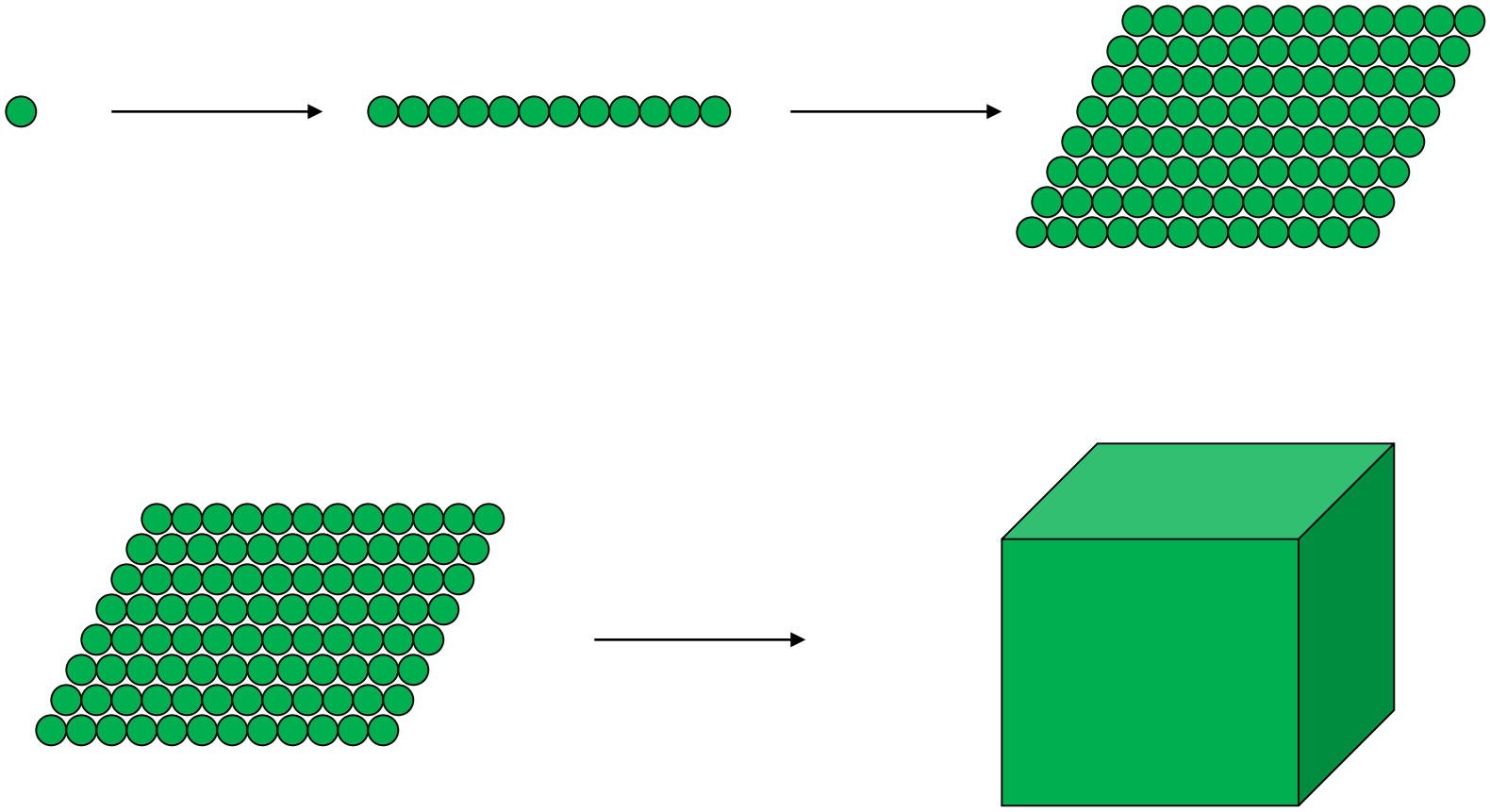
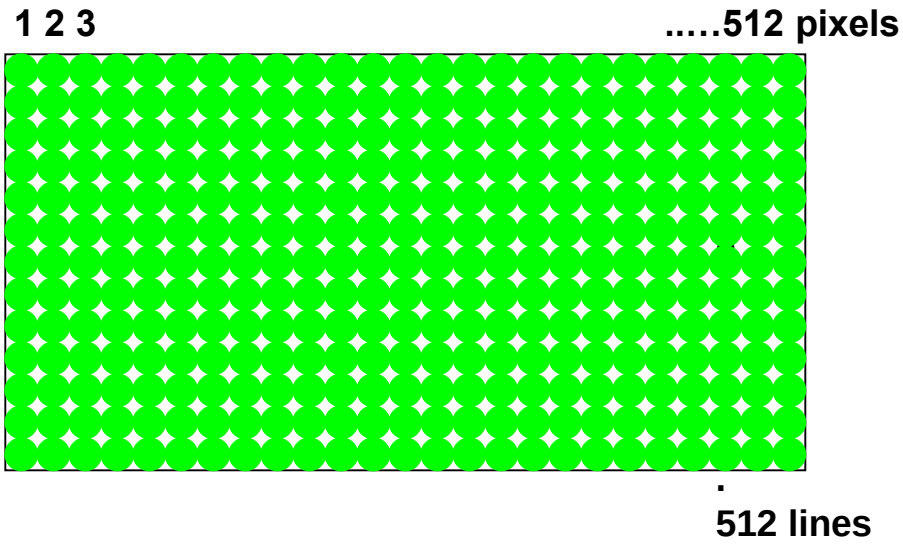




Confocal laser scanning



Confocal laser scanning



Creating f.i. 512x512 image

2 – 30 fps / ~ 420 fps @ 512 x 64 pix.

Confocal v/s widefield microscopy

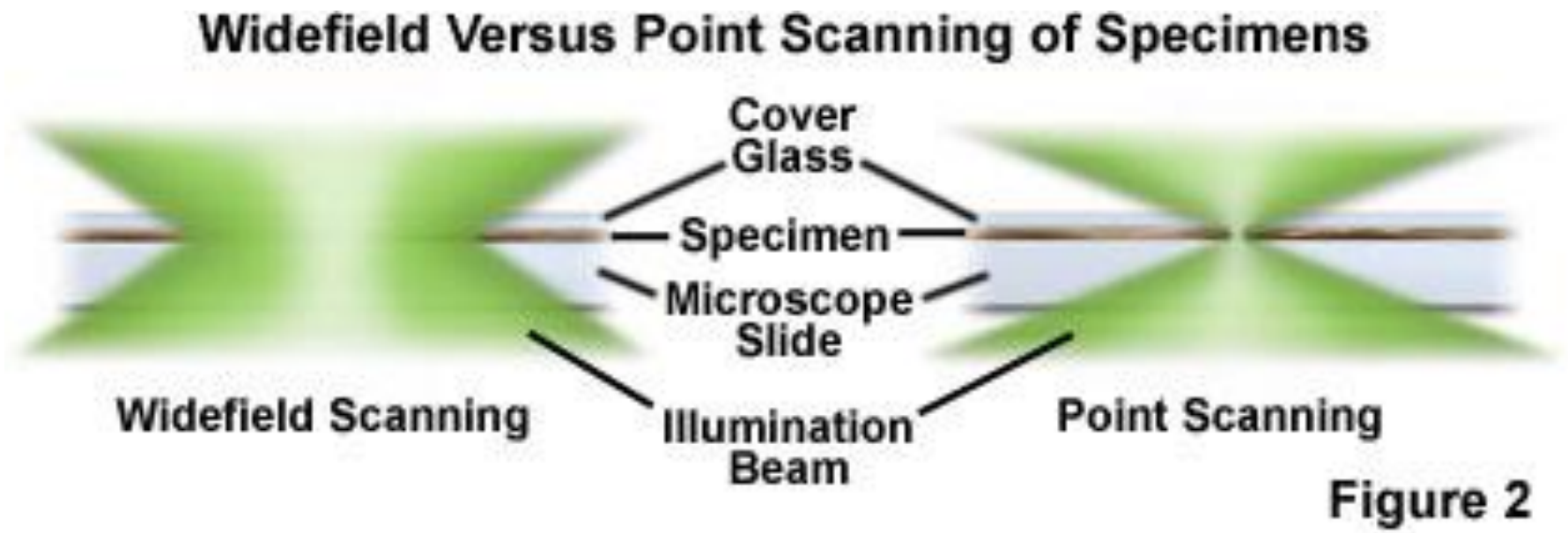
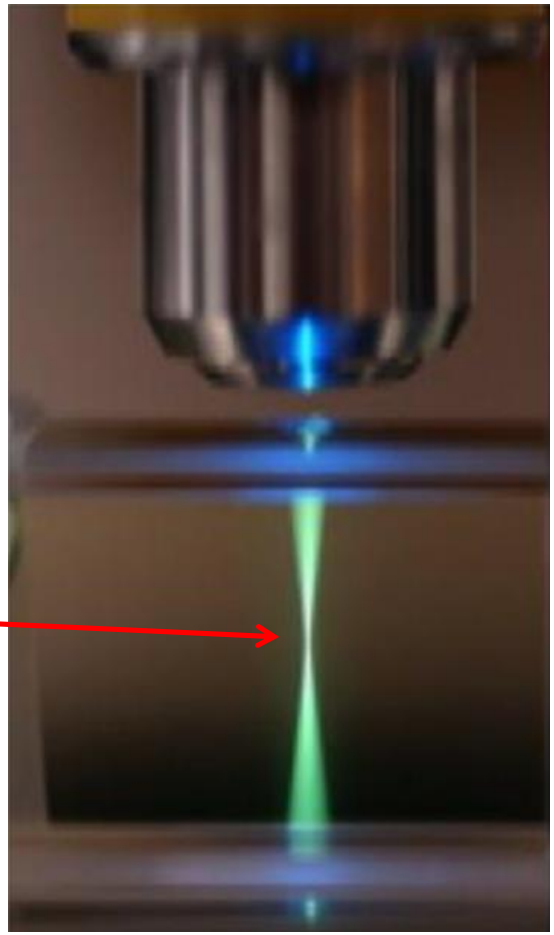


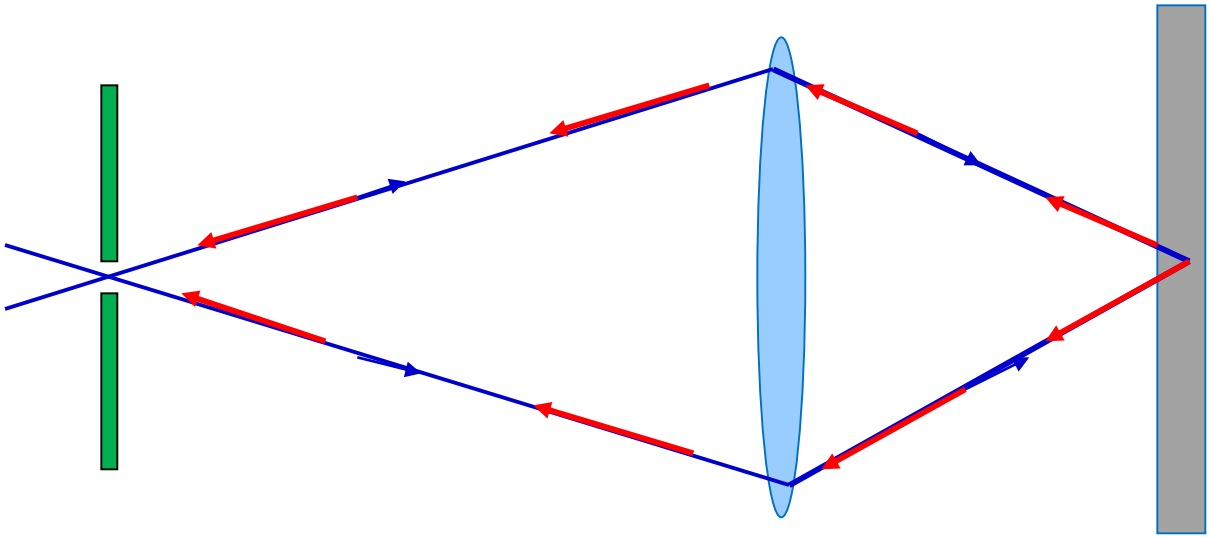
Figure 2

Fluorescent Excitation

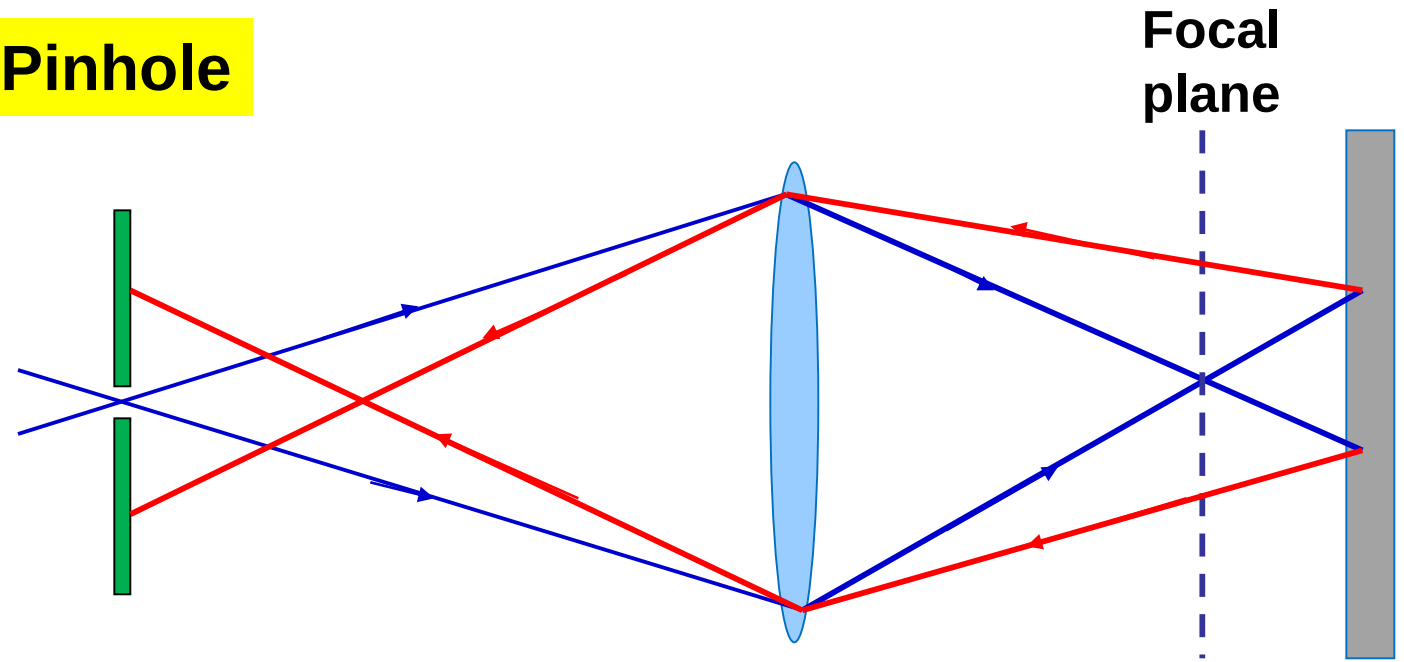


Excitation occurs not only at focus point!

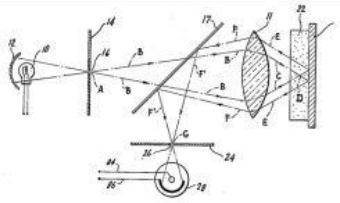
Point scanning principle



Pinhole



Point scanning principle



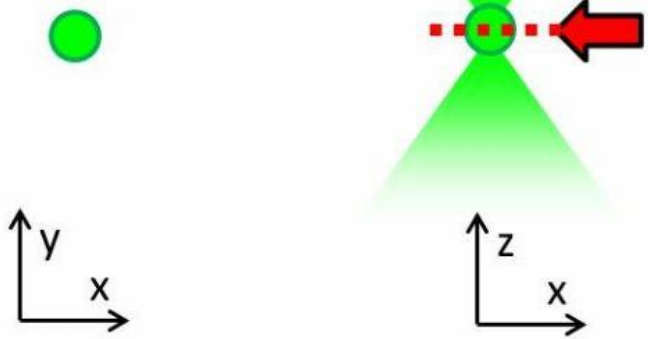
Convolved Point Sources

Point sources of light spread in all direction

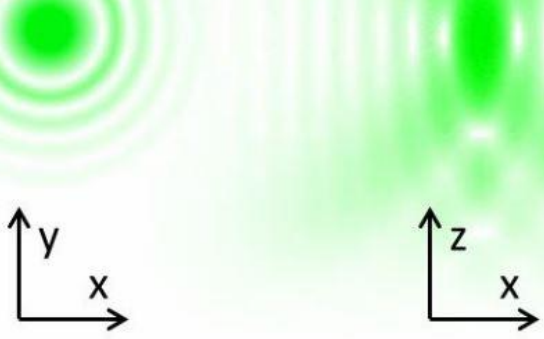
Sources exist above and below focal plane

Point Sources

1 2 3

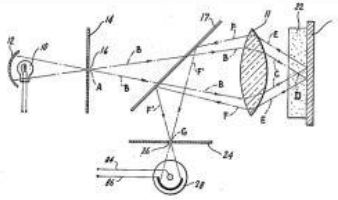


At FOCAL plane



Convolution

Point scanning principle



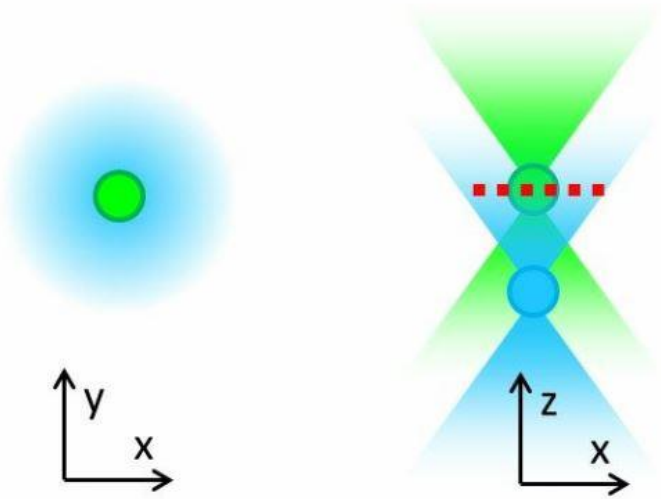
Convolved Point Sources

Point sources of light spread in all direction

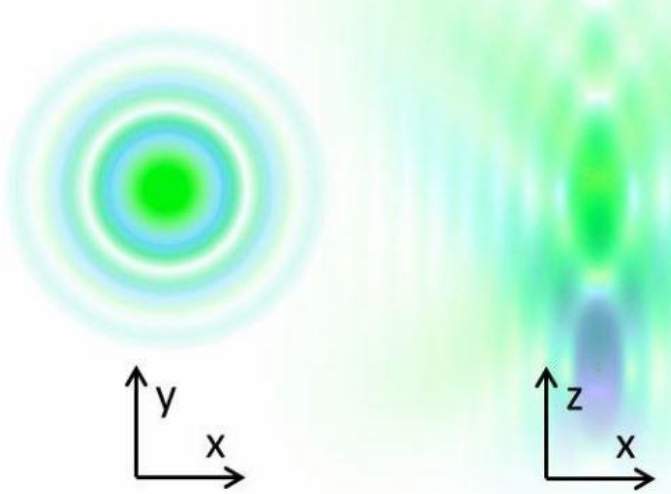
Sources exist above and below focal plane

Point Sources

1 2 3

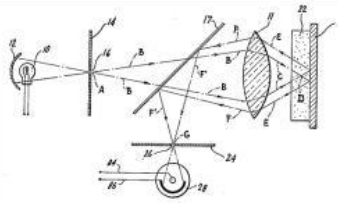


At FOCAL plane



Convolution

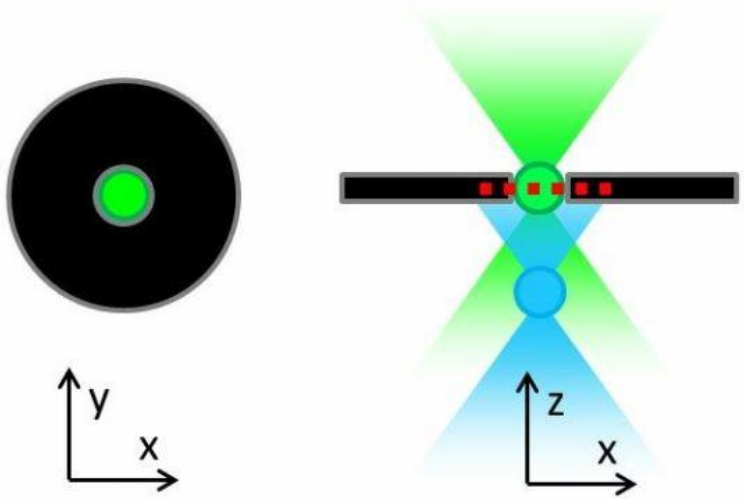
Point scanning principle



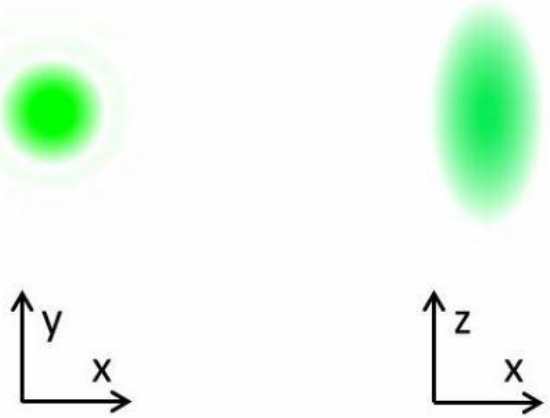
Pinhole Effect

A pinhole in the **con**jugate **focal** plane blocks signal

Pinhole?



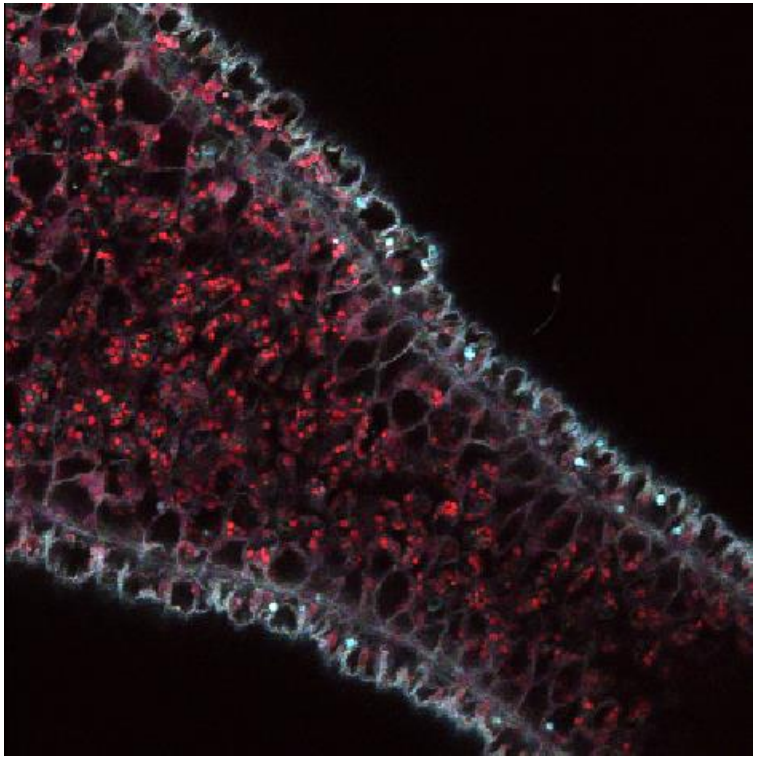
At FOCAL plane



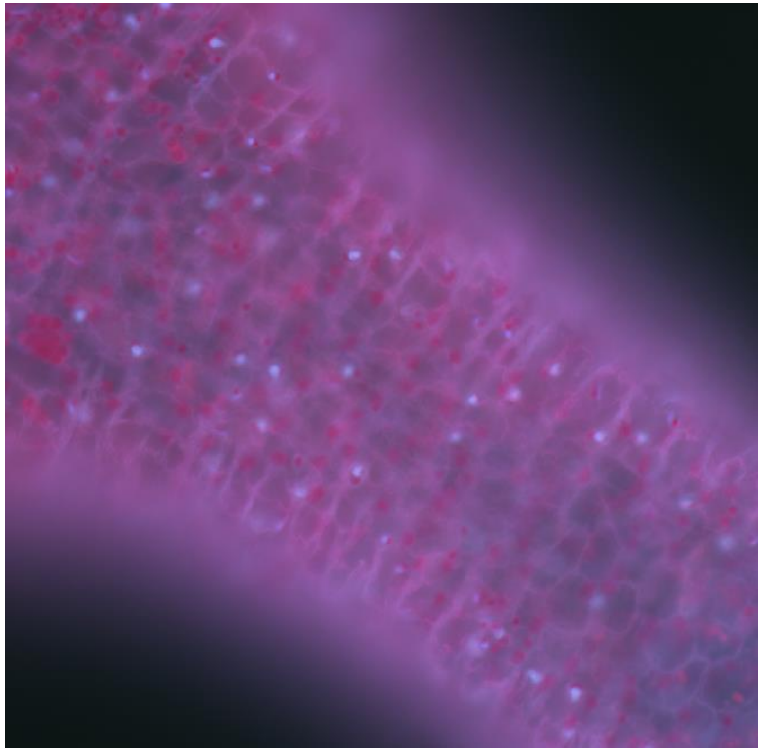
Convolution

Confocal v/s widefield microscopy

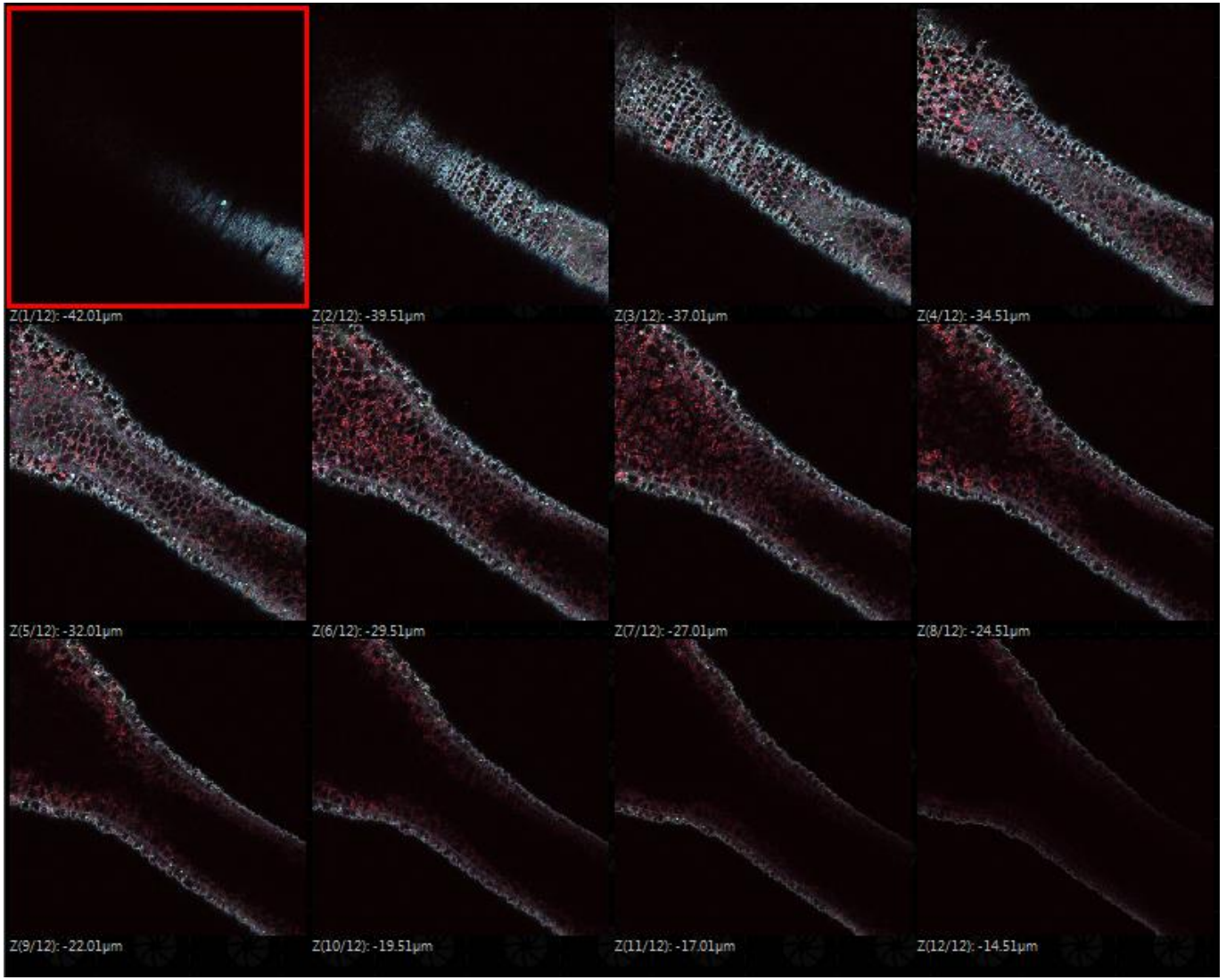
Confocal



Widefield

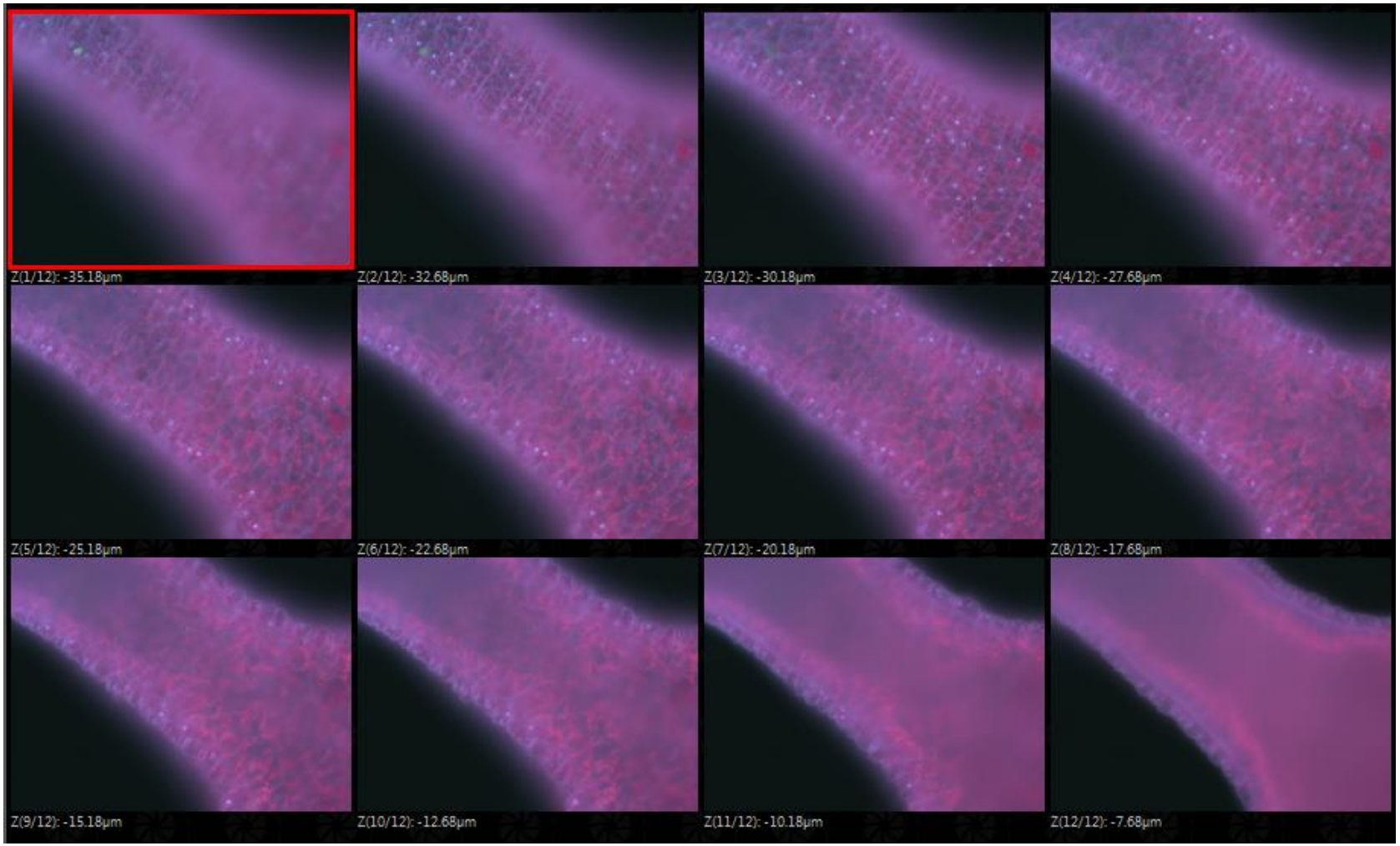


Confocal vs. widefield microscopy



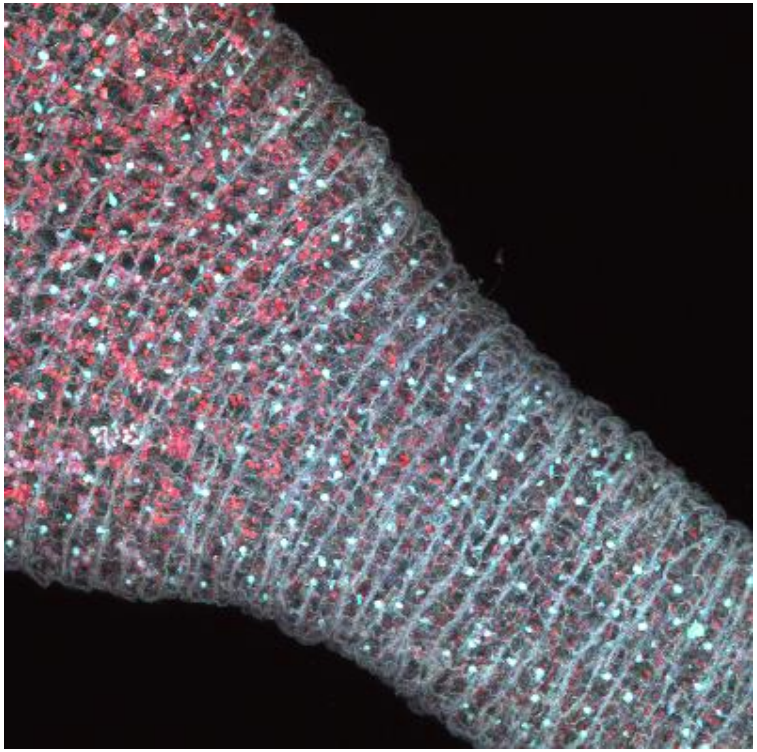
3D data set

Confocal vs. widefield microscopy

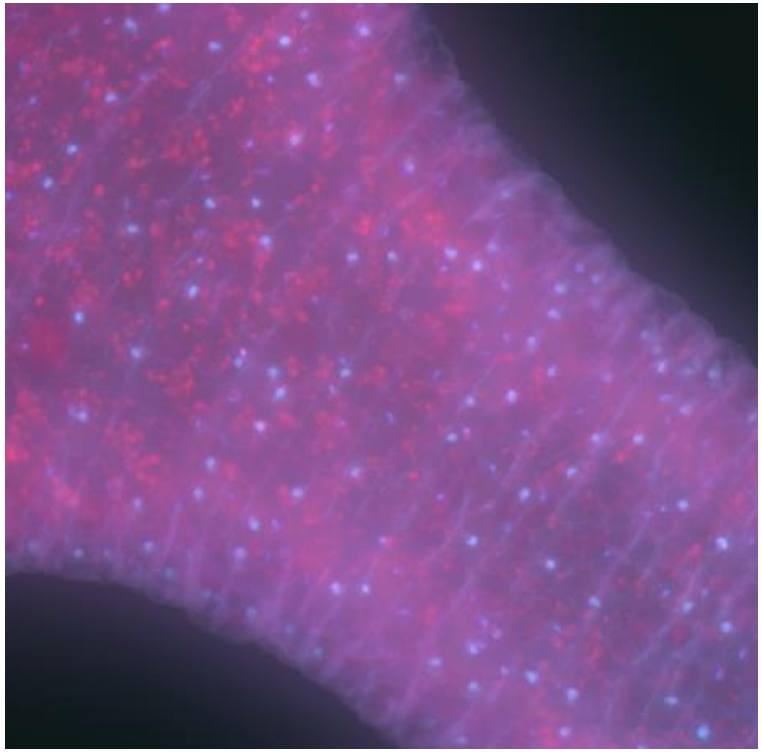


Confocal vs. widefield microscopy

Confocal



Widefield



Maximum Intensity Projection

Confocal vs. widefield microscopy

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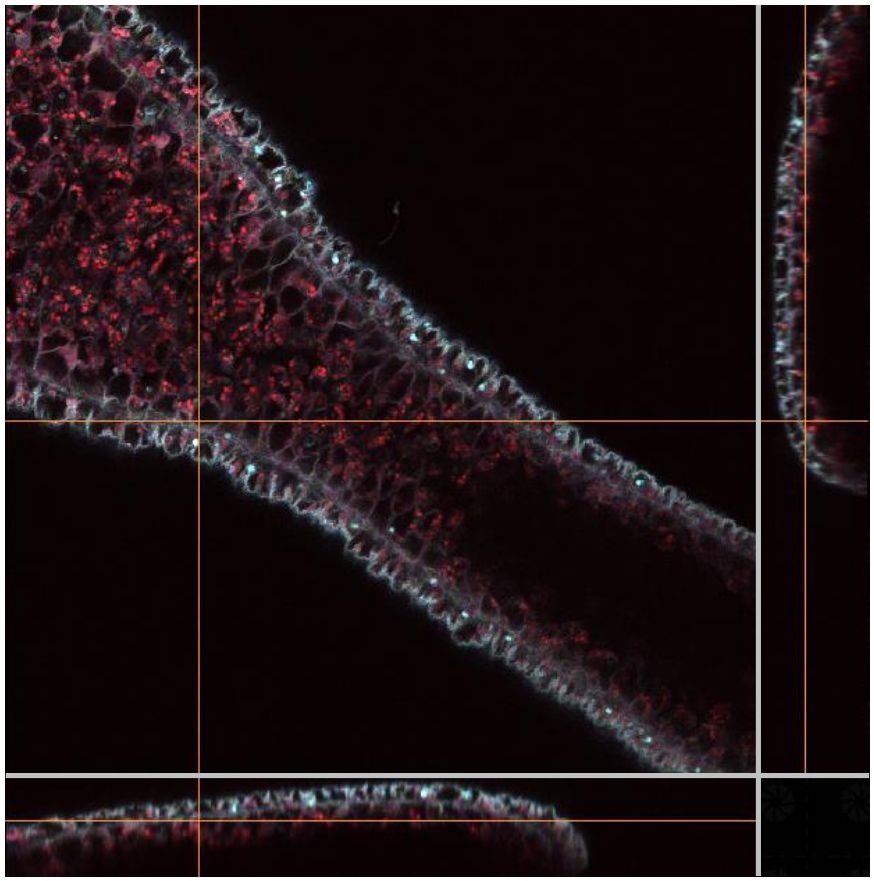
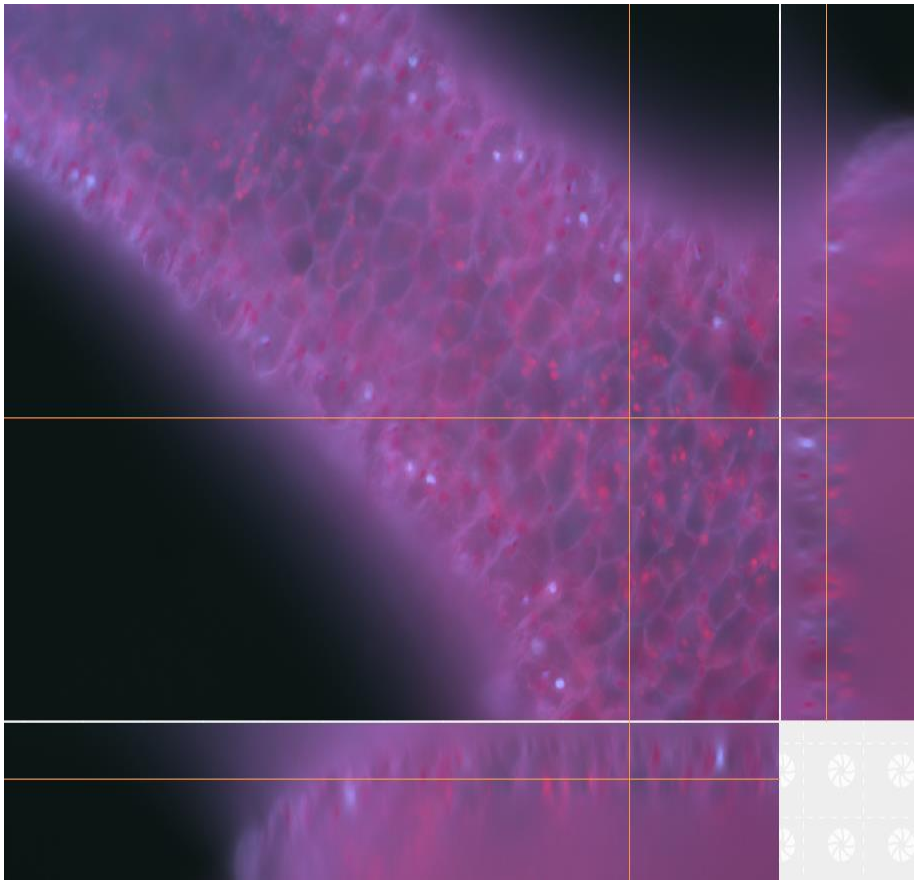
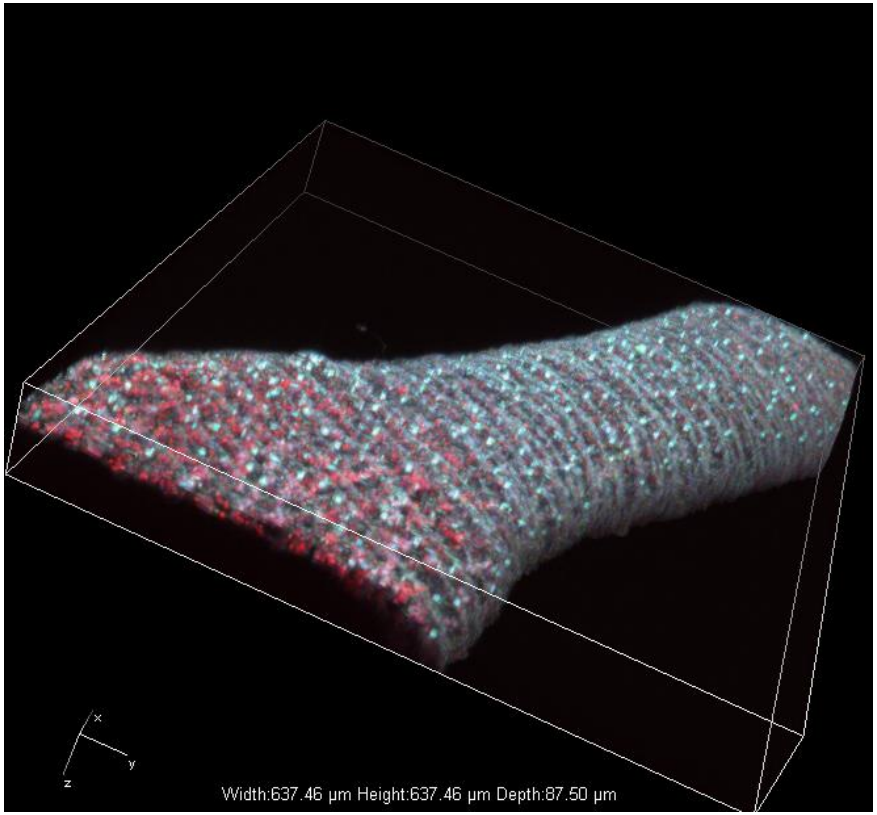
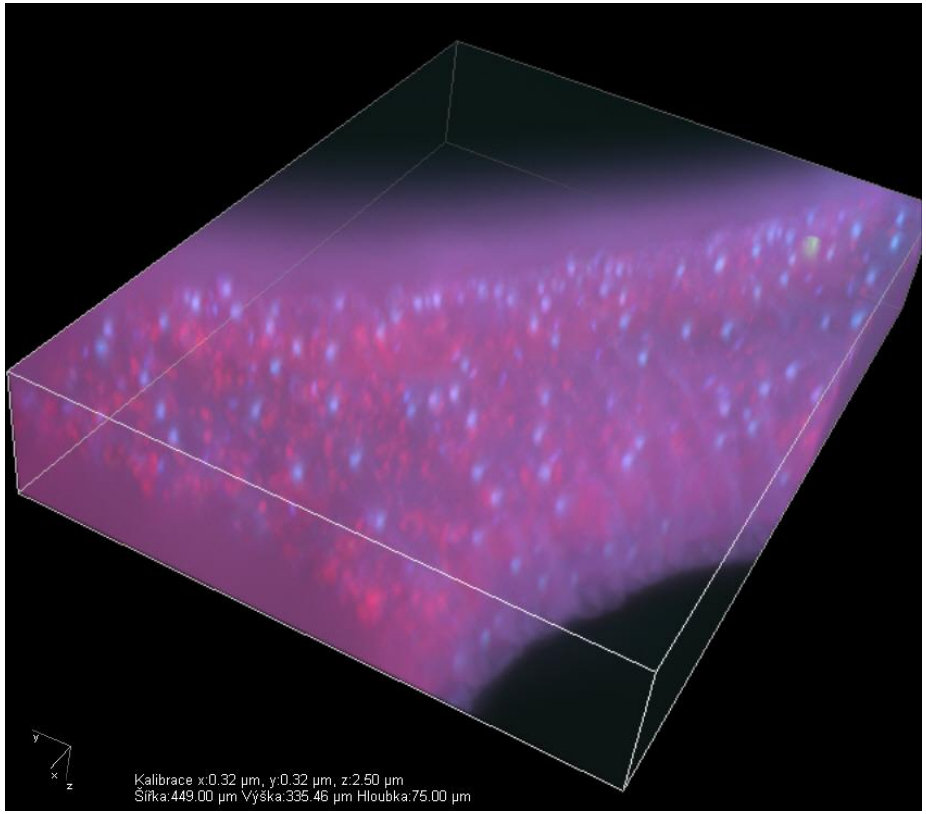




3D data set

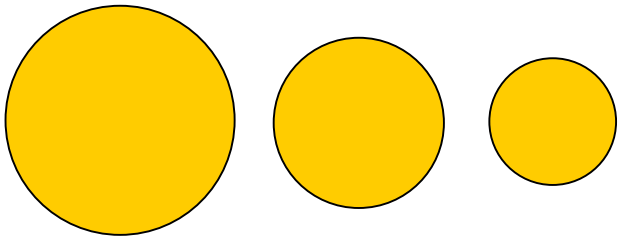
Confocal vs. widefield microscopy

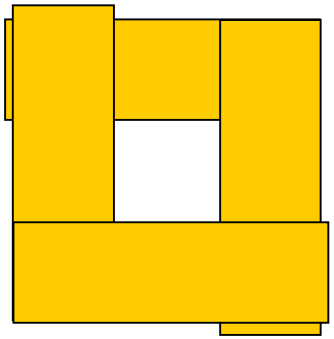


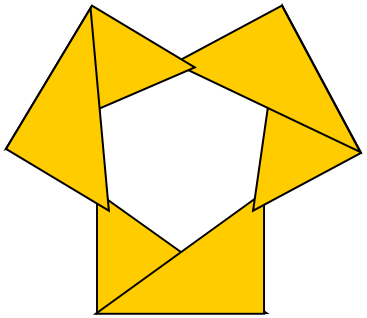


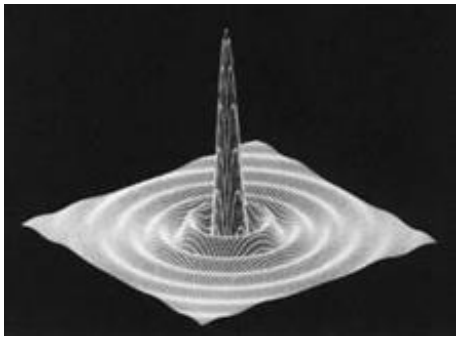
3D data set

Pinhole shape



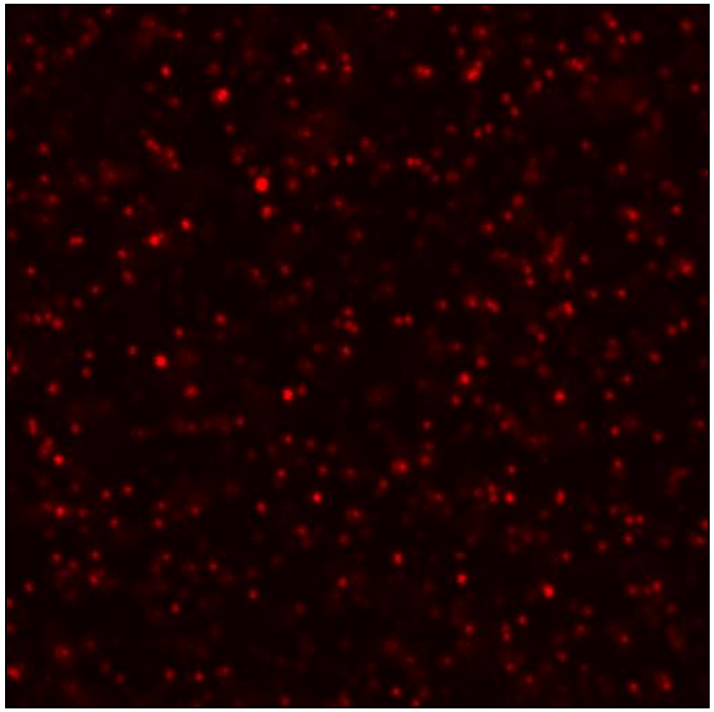




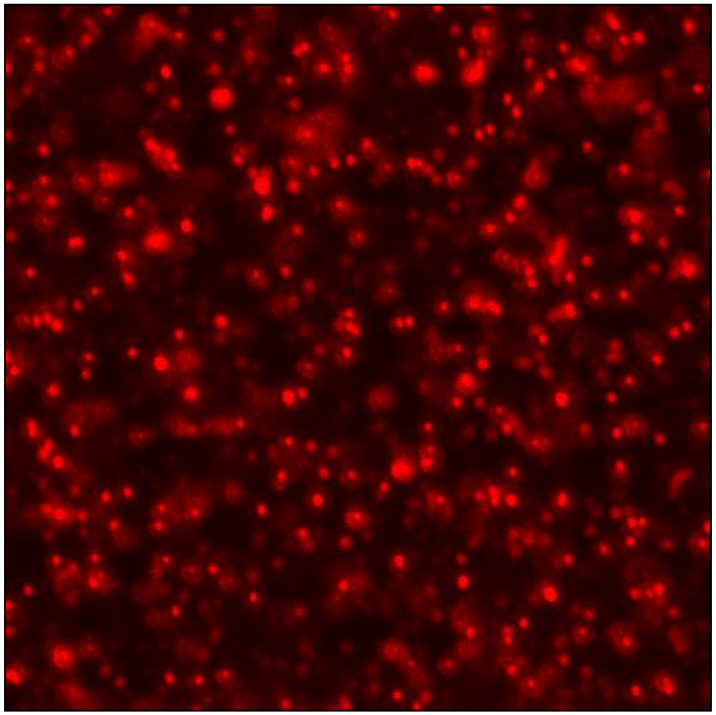


Point spread function

Pinhole size

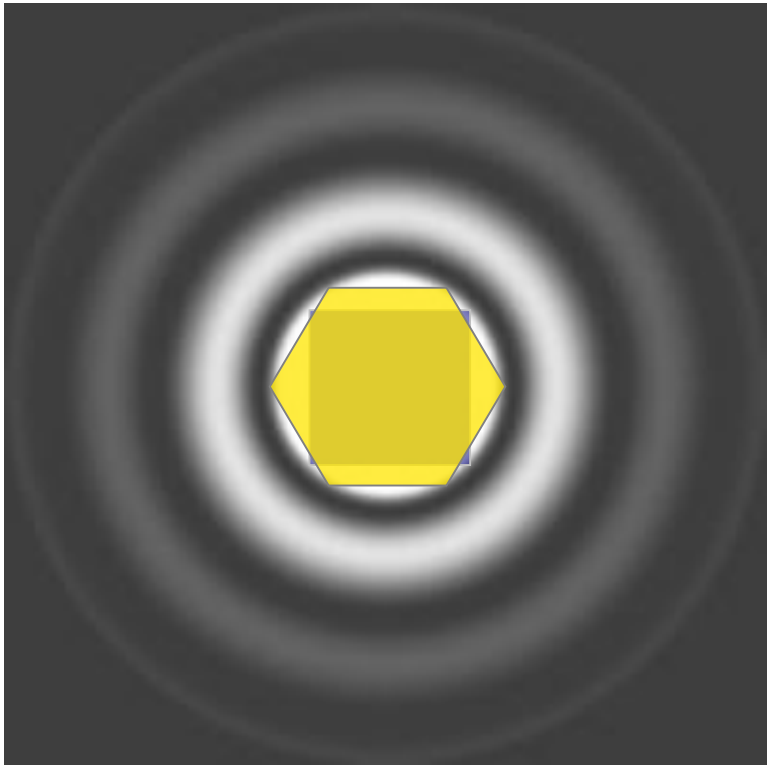


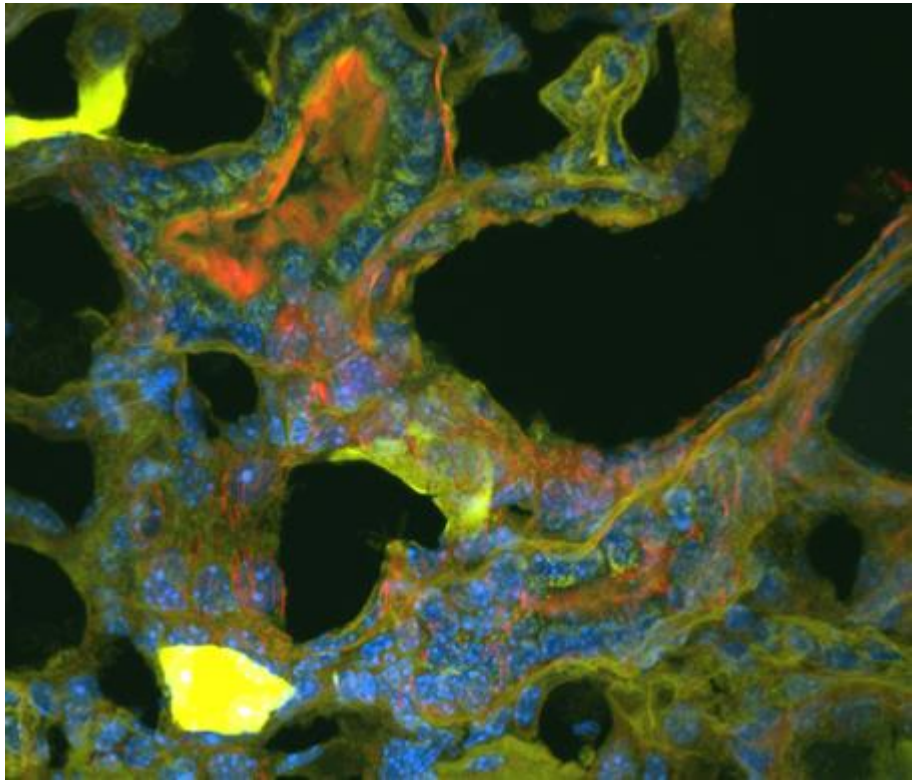
30 um



100 um

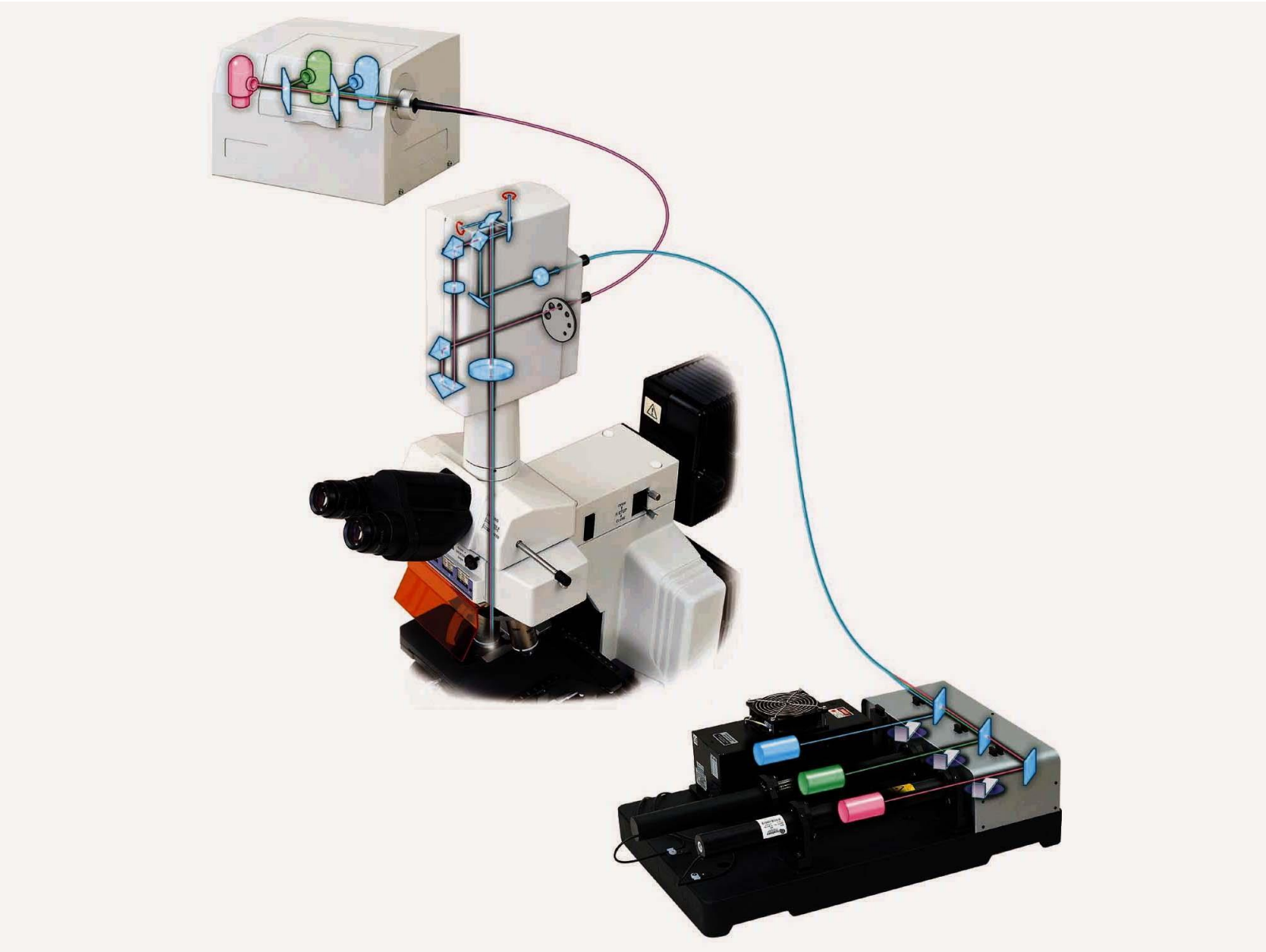
Hexagonal pinhole



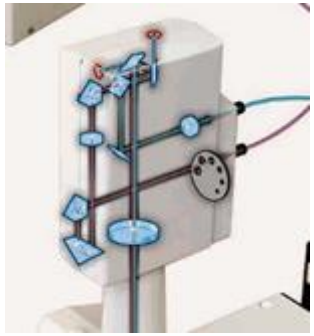


- **30% brighter images**
- **Same optical sectioning performance**

Nikon C2+ optical path

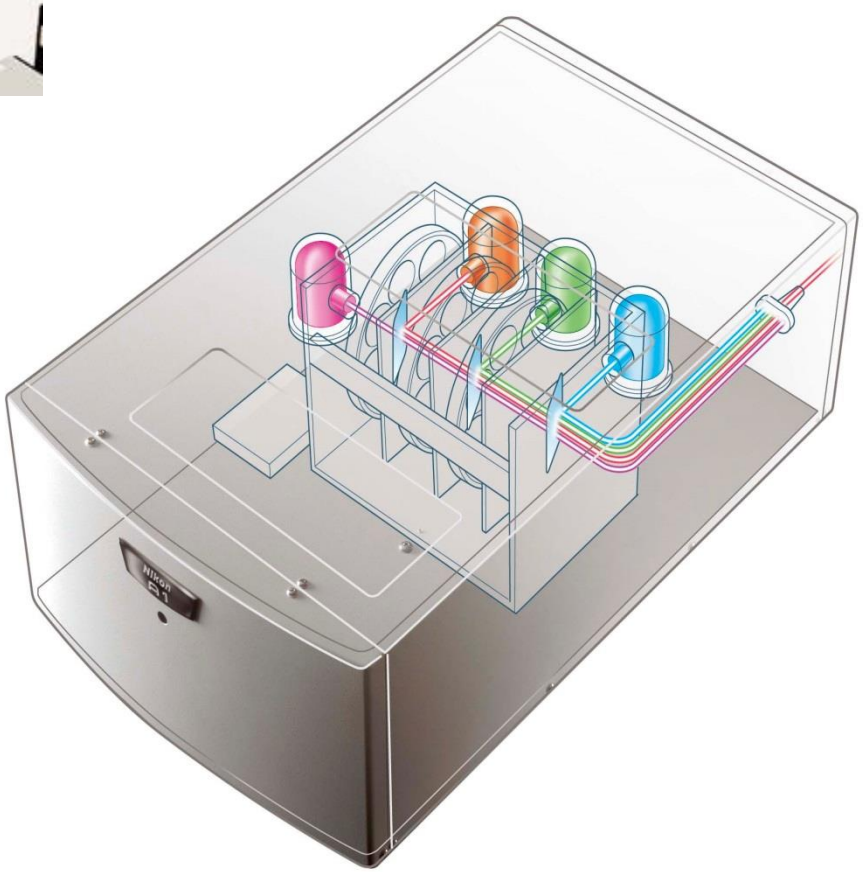


PMT Detector Unit

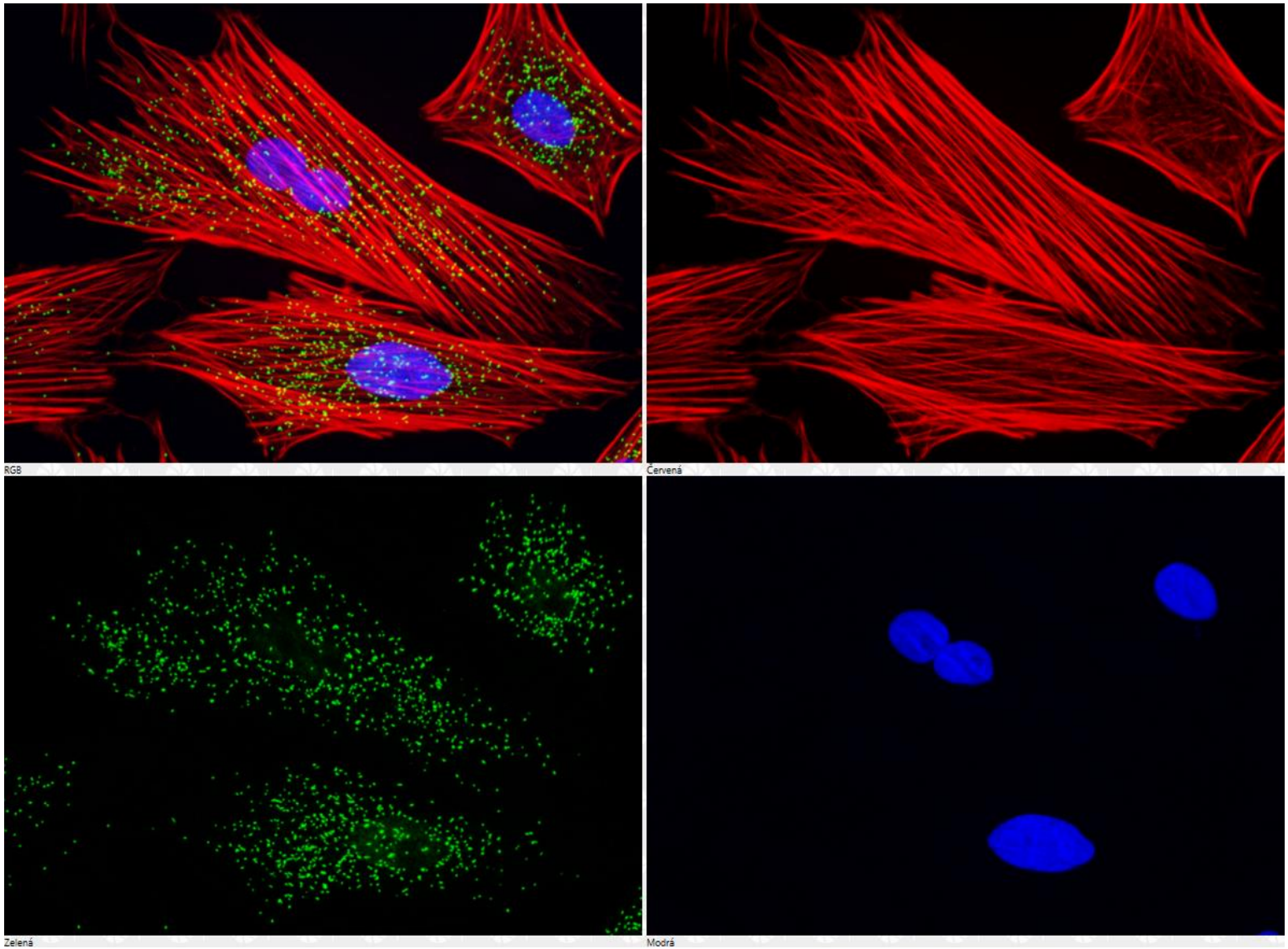


1st DM

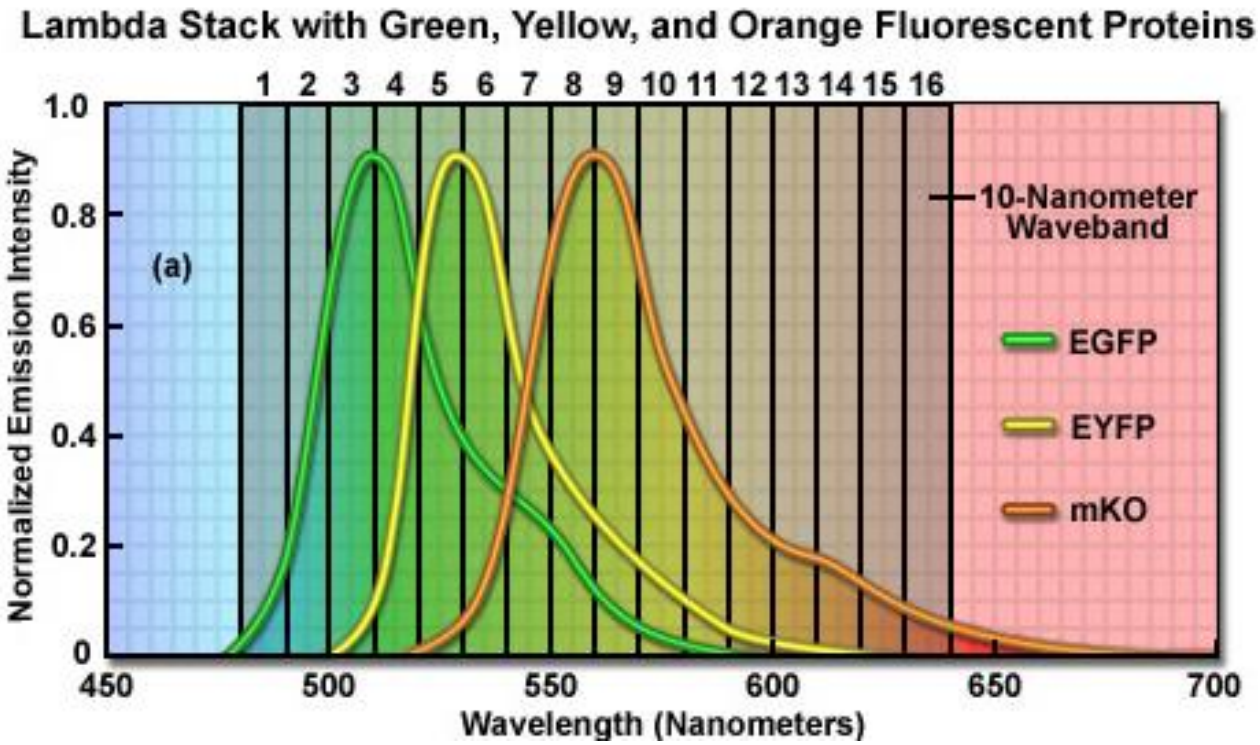
4 PMT detector



2nd DM

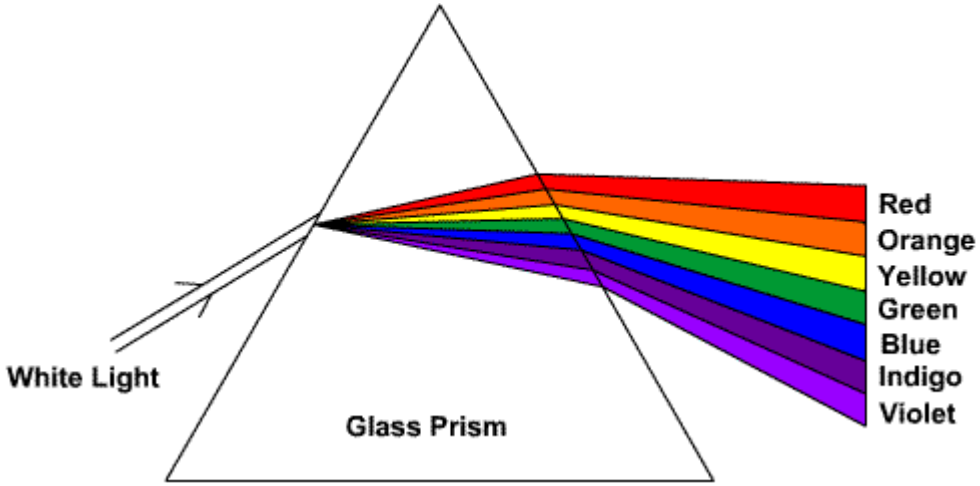

RGB
Červená
Zelená
Modrá

Spectral imaging and Linear Unmixing

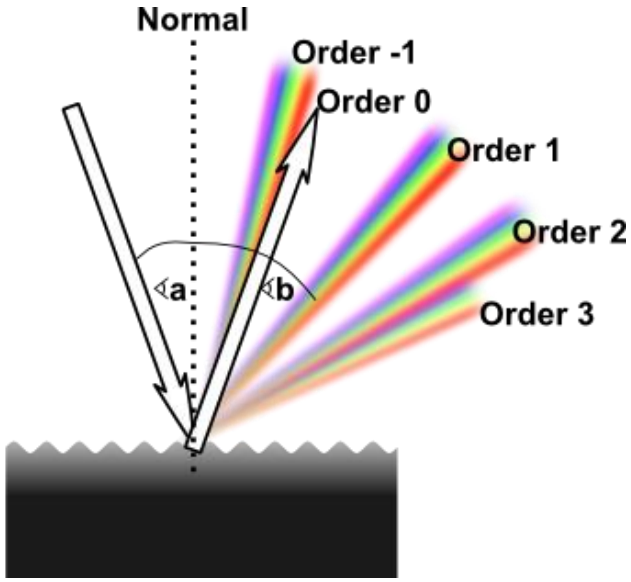


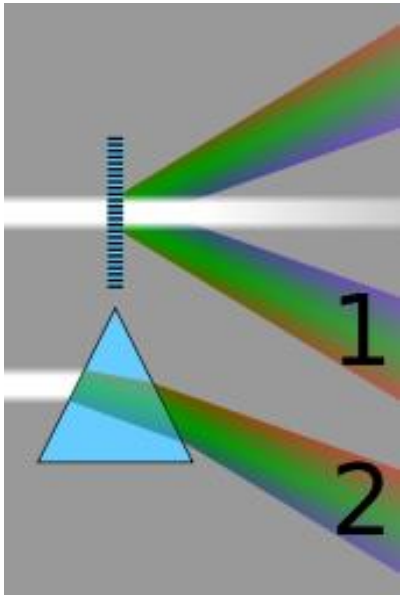
Spectral imaging and Linear Unmixing

- **Two ways of dispersion**
  - **Prism based**



  - **Diffraction Grating based**





Spectral imaging and Linear Unmixing

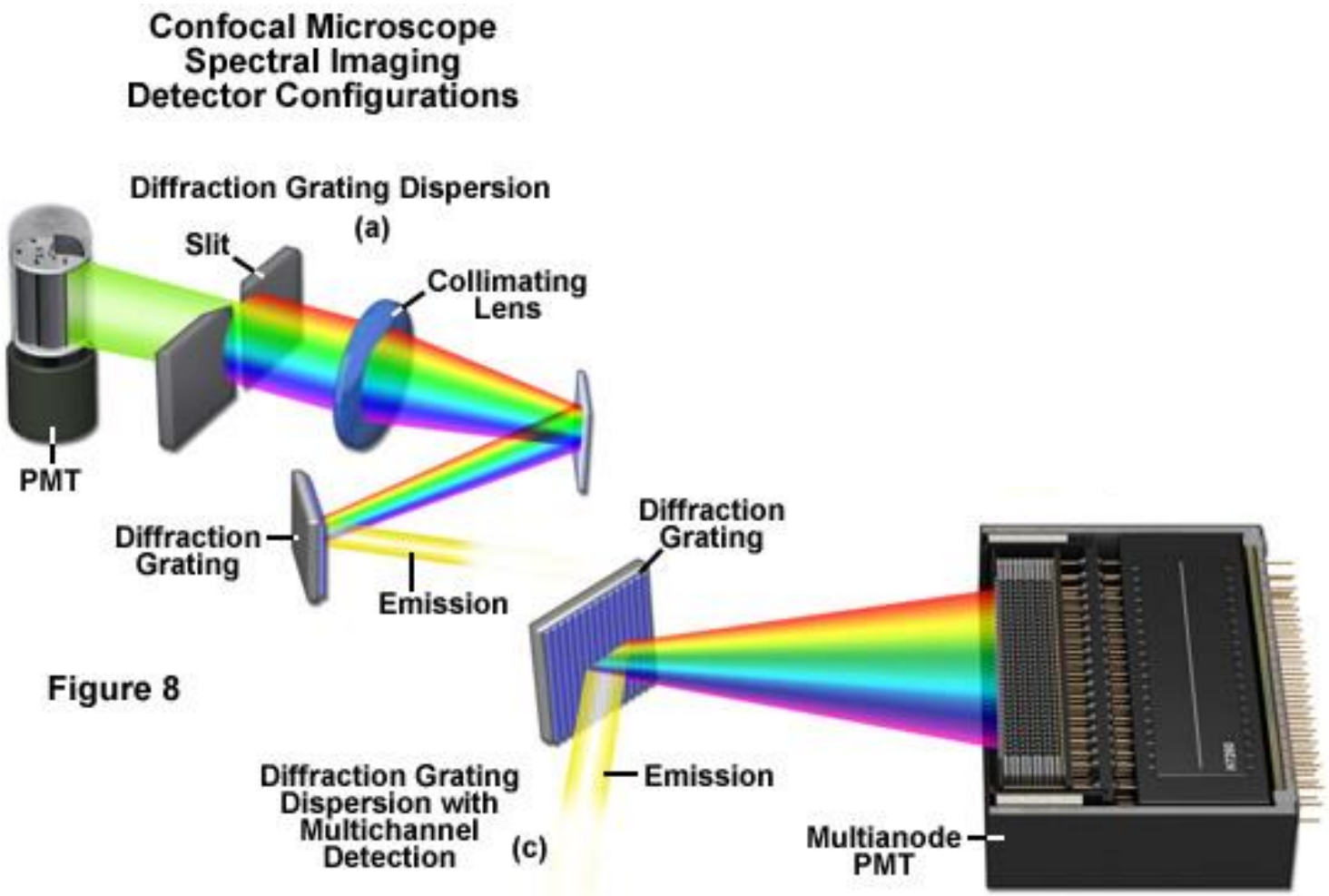


Figure 8

Spectral imaging and Linear Unmixing

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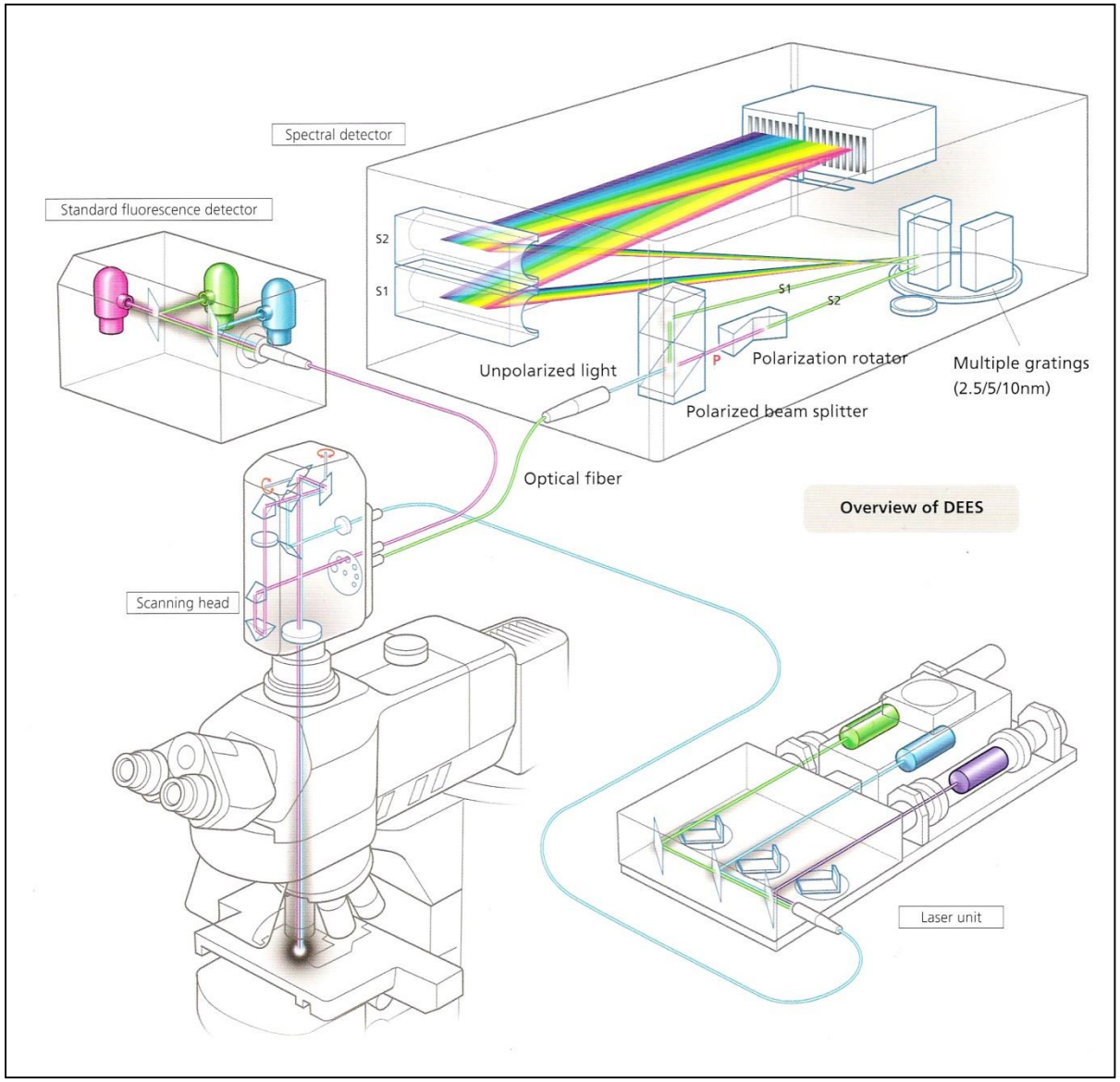


Spectral imaging and Linear Unmixing

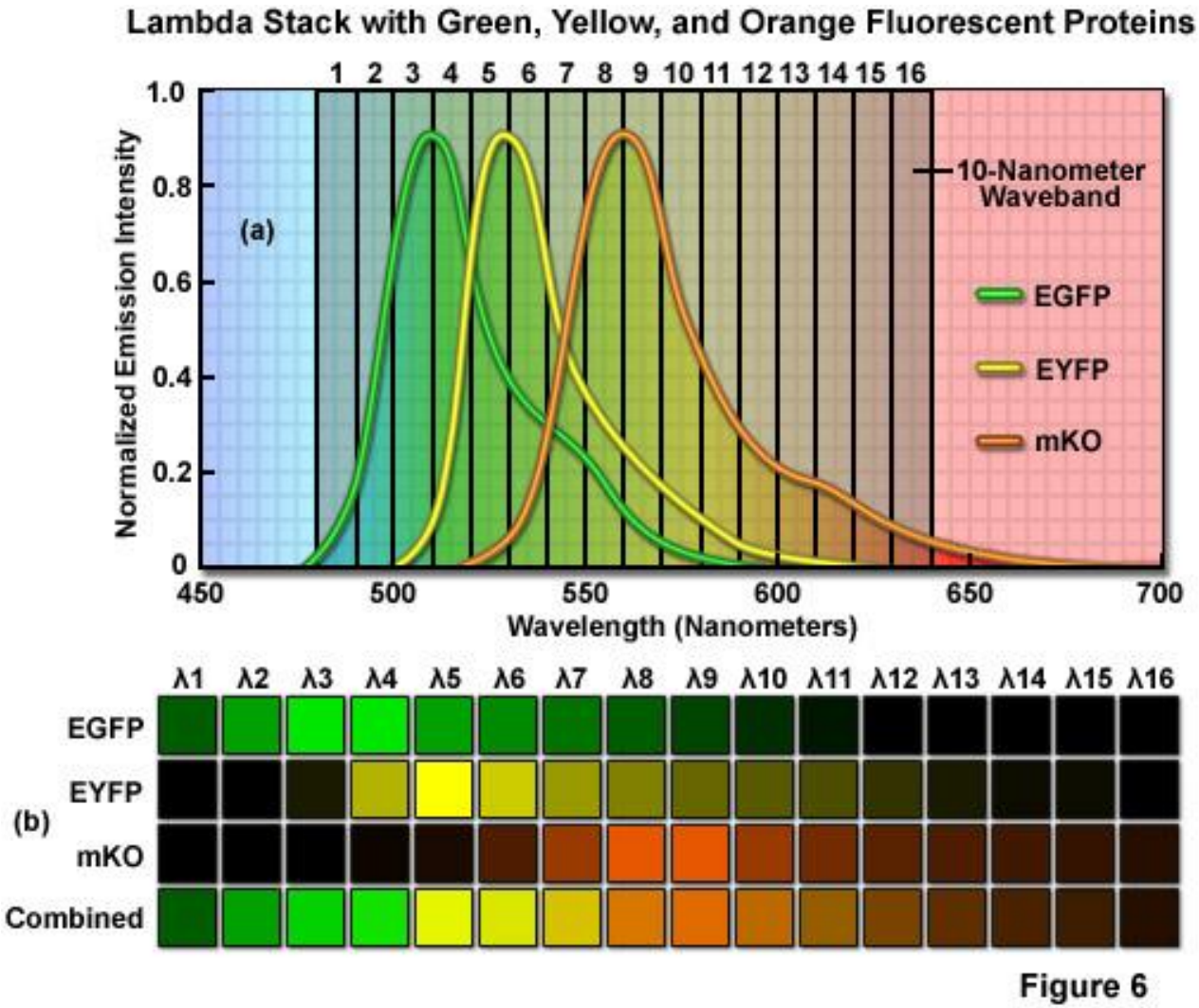
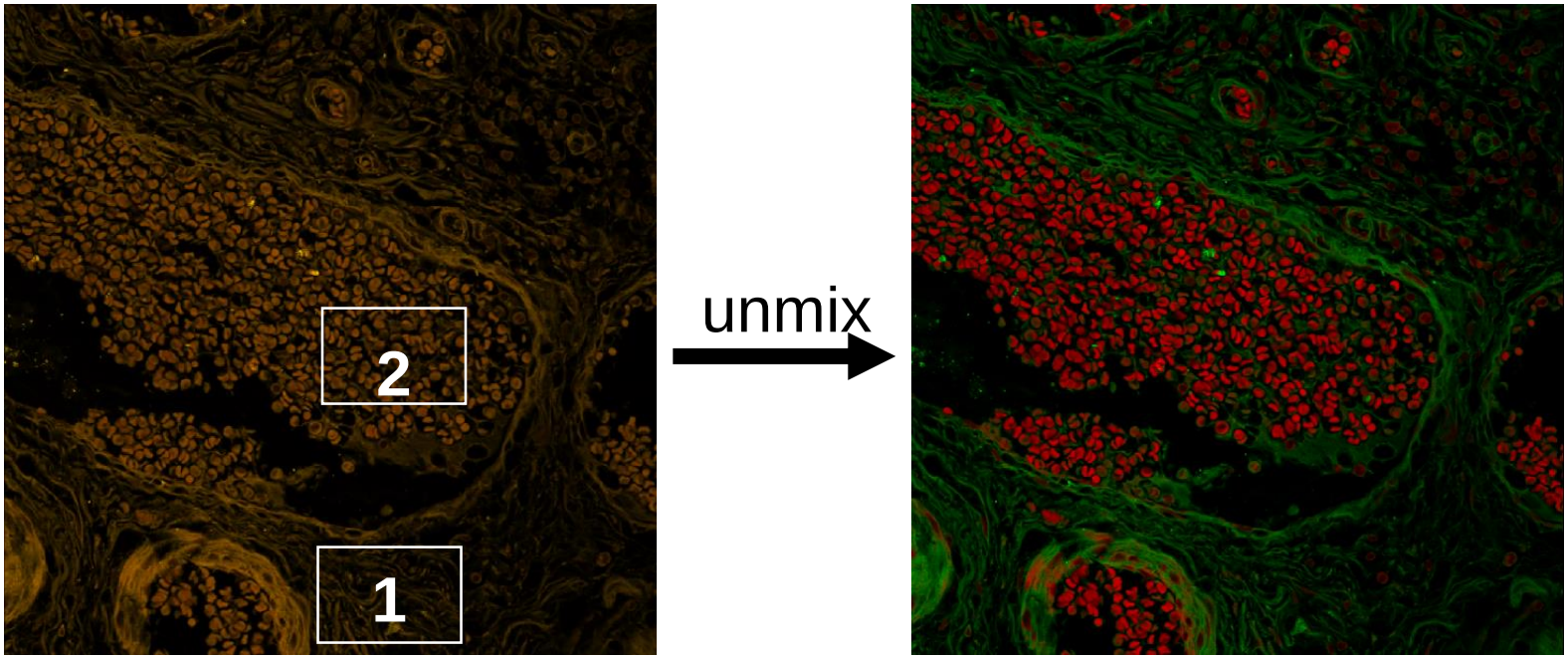
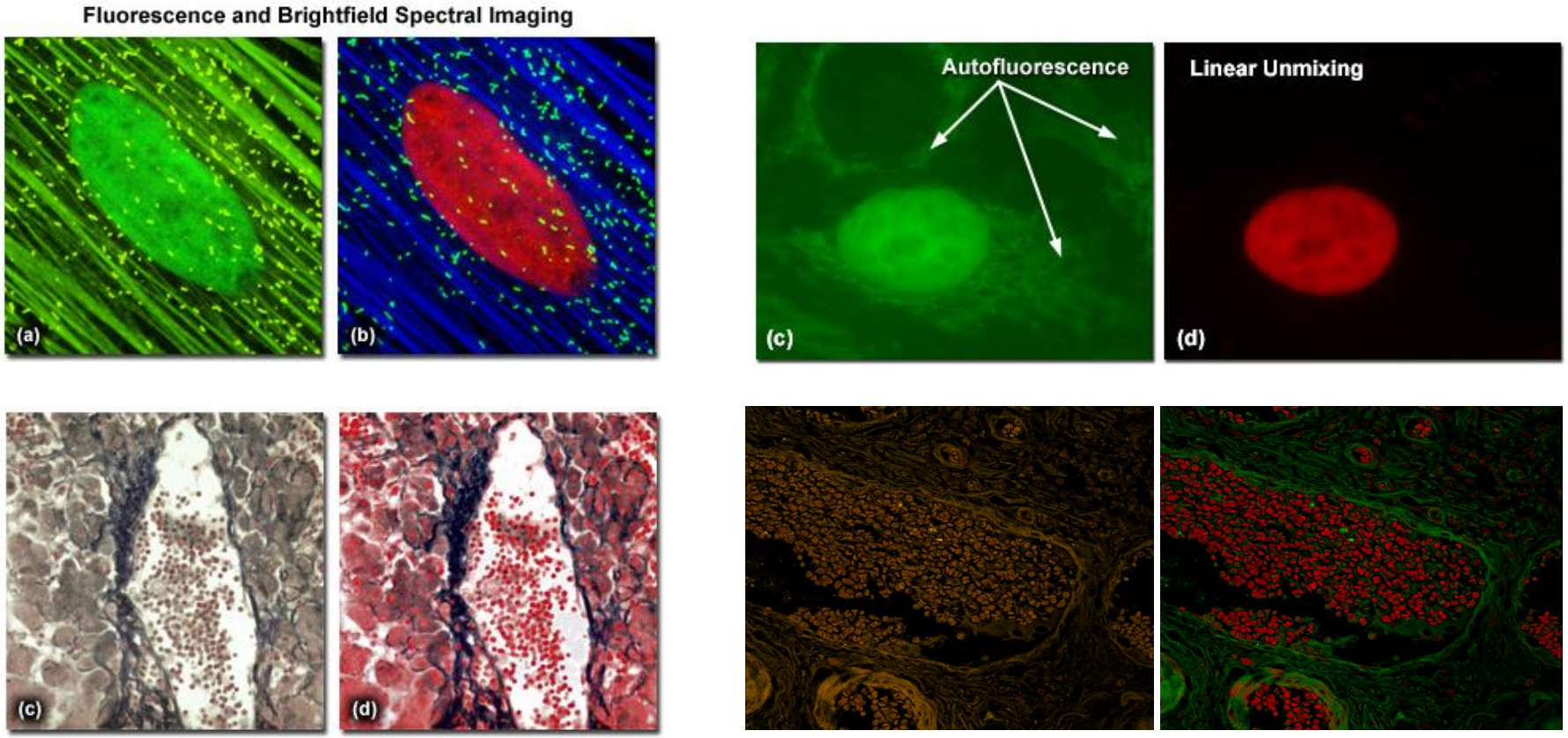


Figure 6

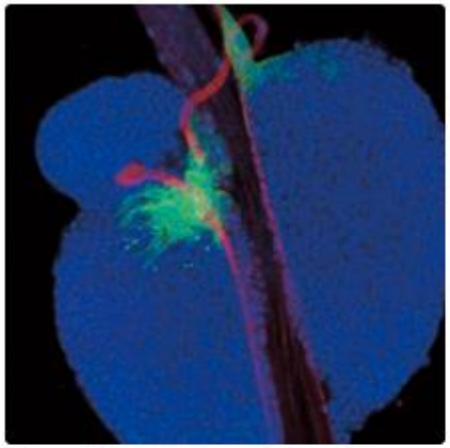
Spectral detection

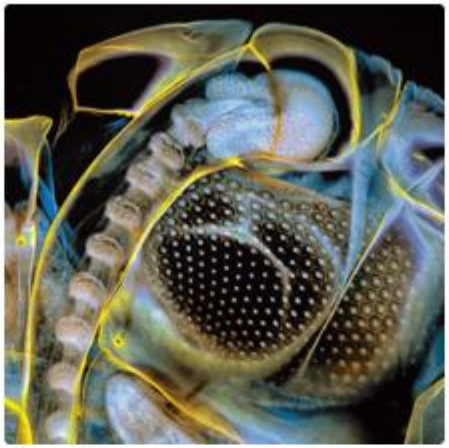


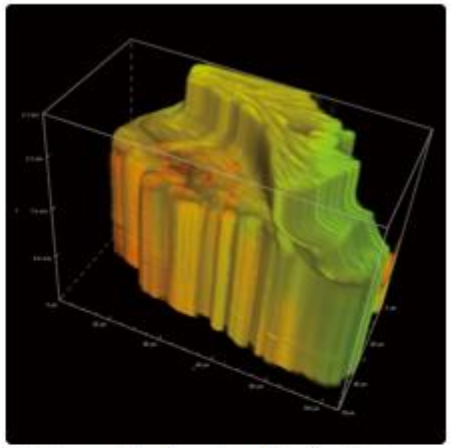
Linear unmixing

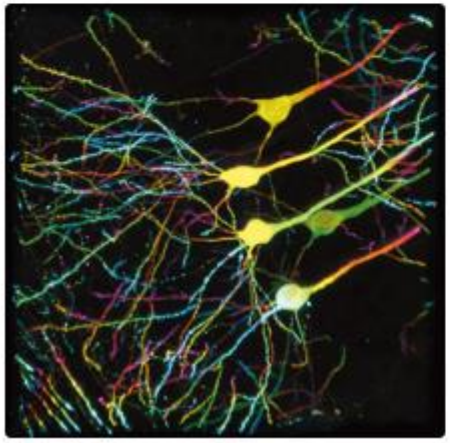


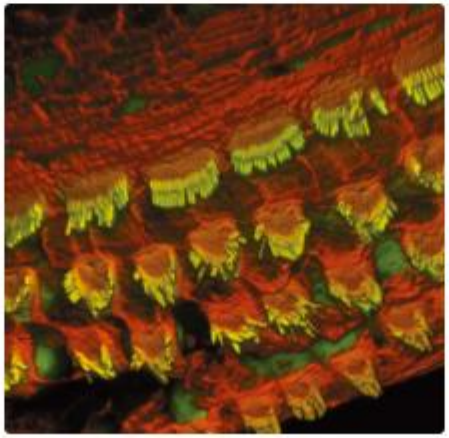
SAMPLE IMAGES

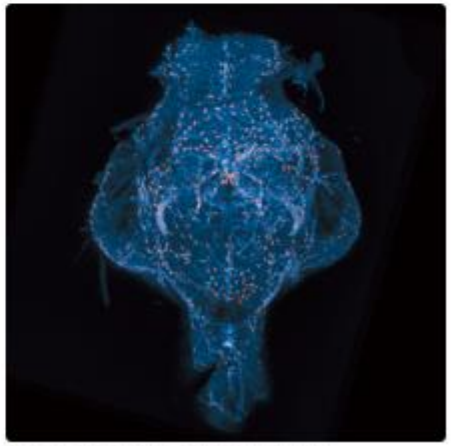

Drosophila sp. Embryonic heart


Mosquito larvae nervous system


XY Time plot of contracting isolated cardiomyocyte

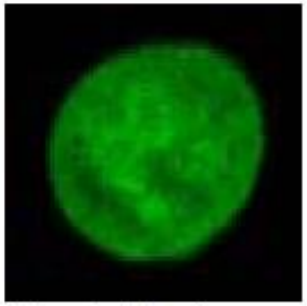

Mouse neurons

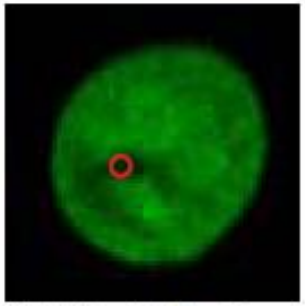

Cochlear cells

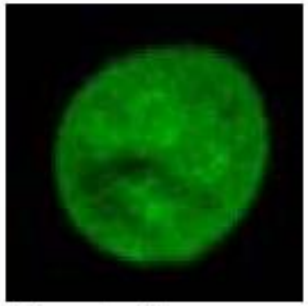

Whole cleared zebrafish head

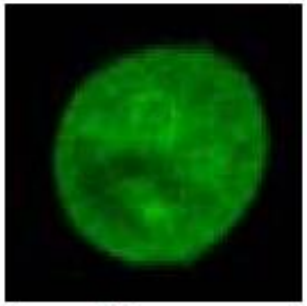
Simultaneous bleaching and imaging

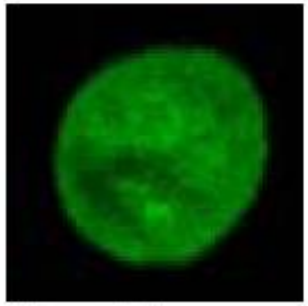
FRAP (Fluorescence Recovery After Photobleaching)

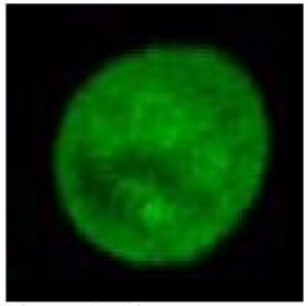

Before photobleaching

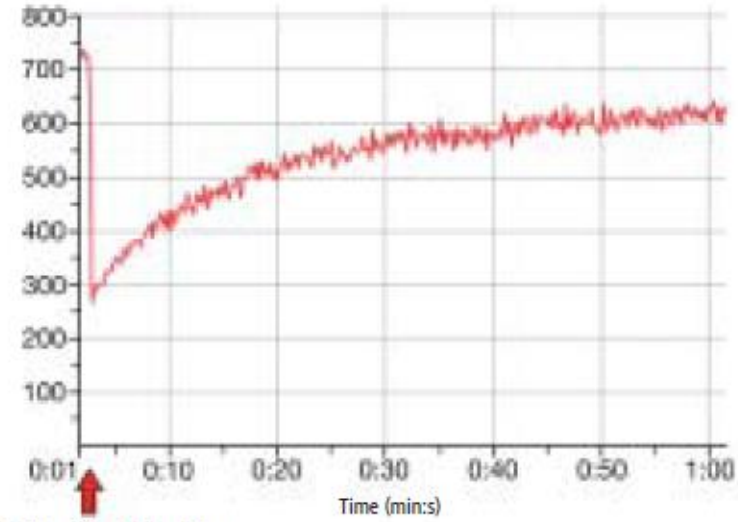

Right after photobleaching


0.3 seconds after photobleaching

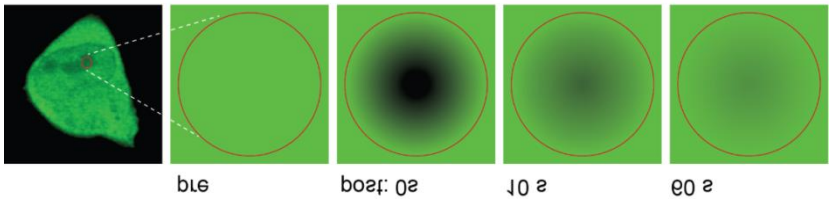

1 second after photobleaching


1.3 seconds after photobleaching


2 seconds after photobleaching

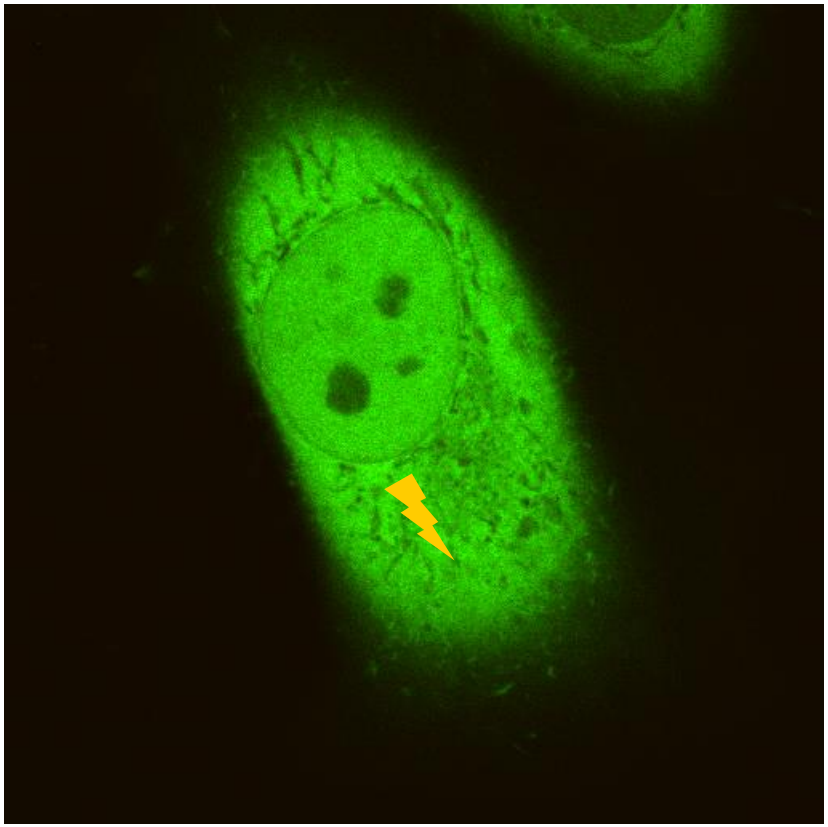


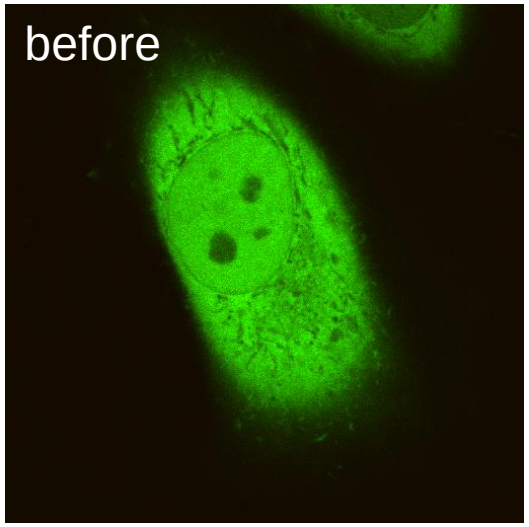
Point of photobleaching

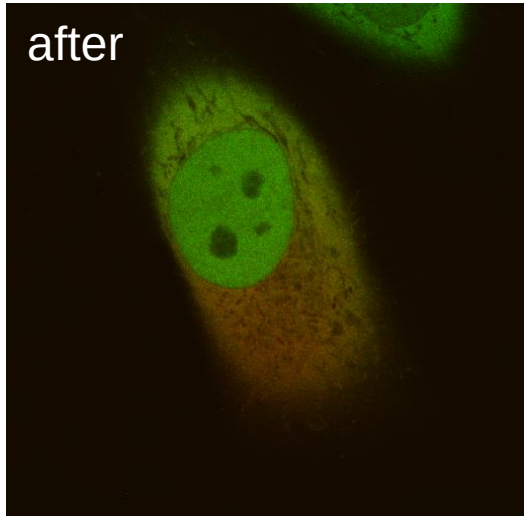


Simult. photostimulation and imaging

Kaede



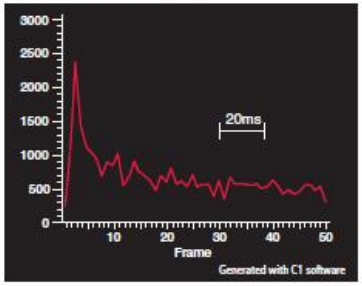




Activation: 405nm
Ex: 488nm / 561nm
Em: 525/50 & 595/50

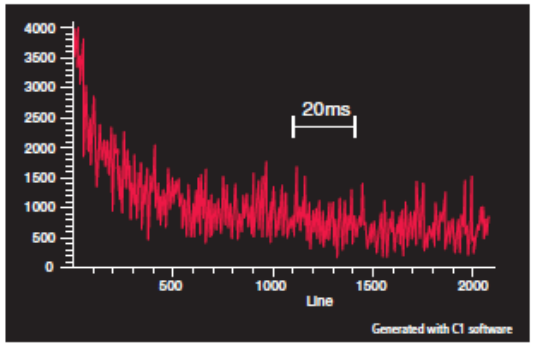
Hi Speed PA-GFP

Observation with band scanning
Imaging at 420 fps (2.4 ms/frame)
Image size: 512 x 32 pixels



420 frames/sec (512/32)

Observation with X-t scanning mode
Imaging with 64 µs time resolution (15,600 lps)



15,600 lines/sec

Multiphoton confocal system

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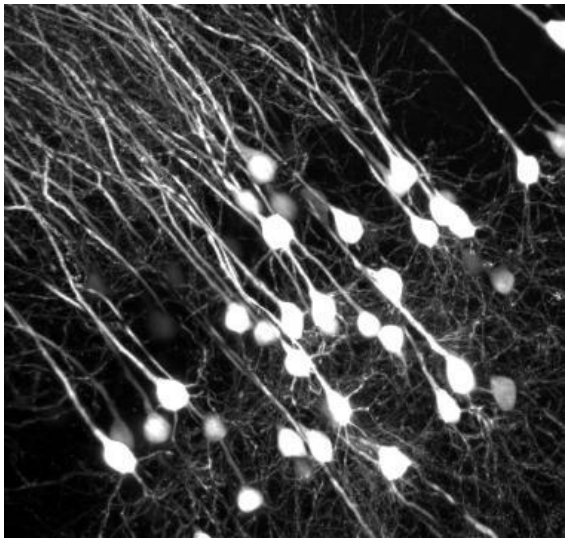
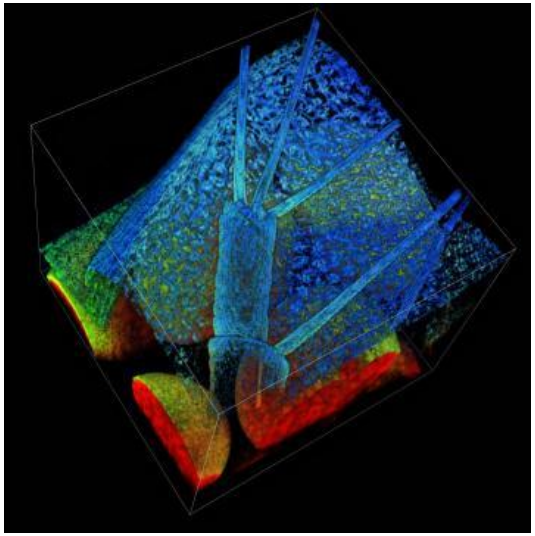
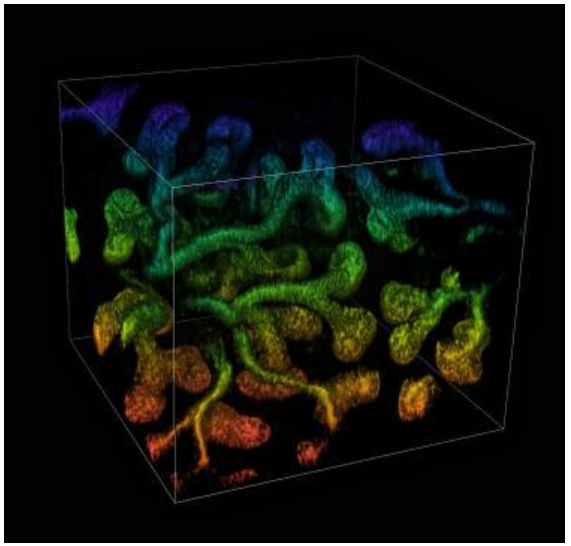






Multiphoton confocal microscopy

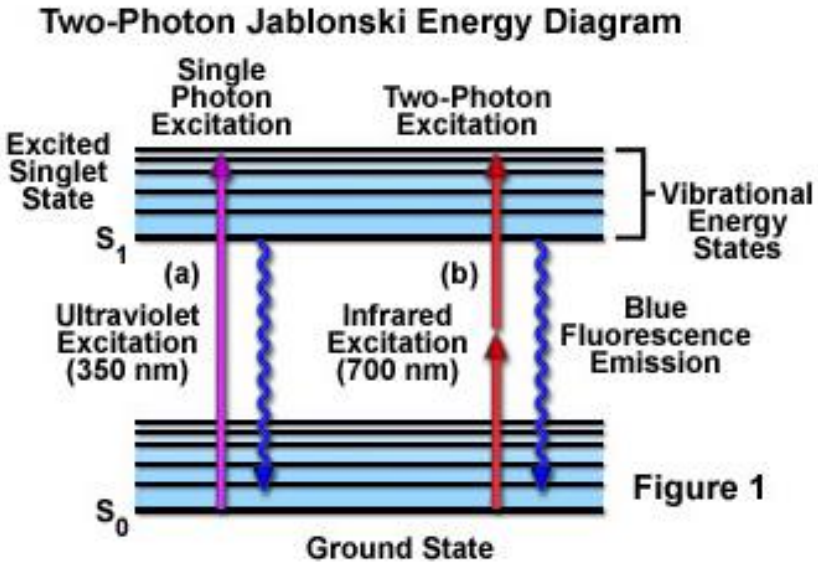


Figure 1

Pulsed lasers pico (E^{-12}) to femto (E^{-15}) second pulses

MP excitation

Fluorophore Excitation in Multiphoton Microscopy

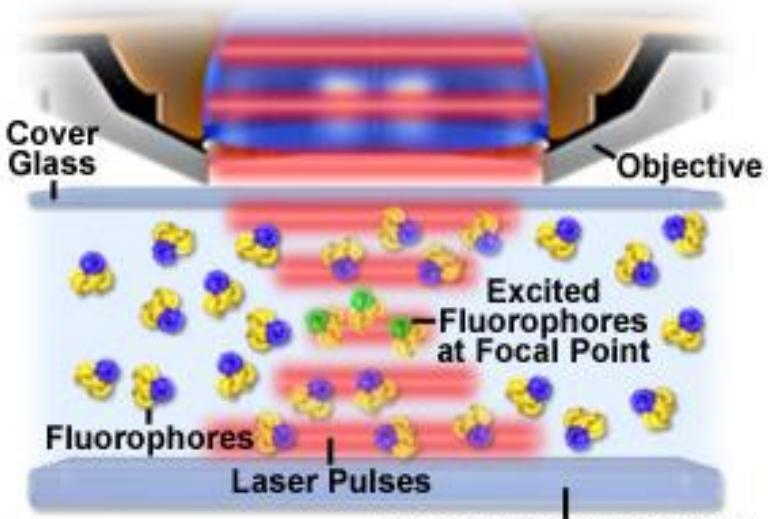
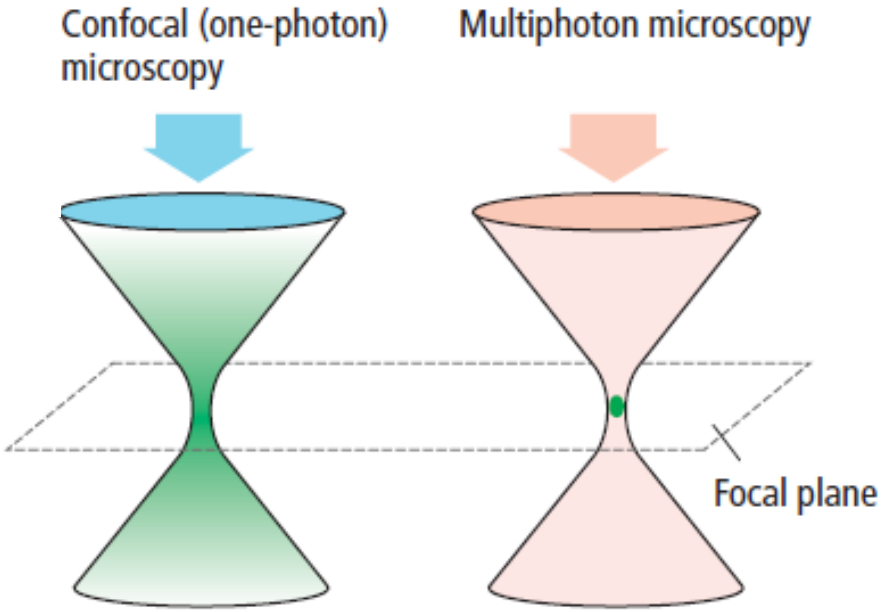


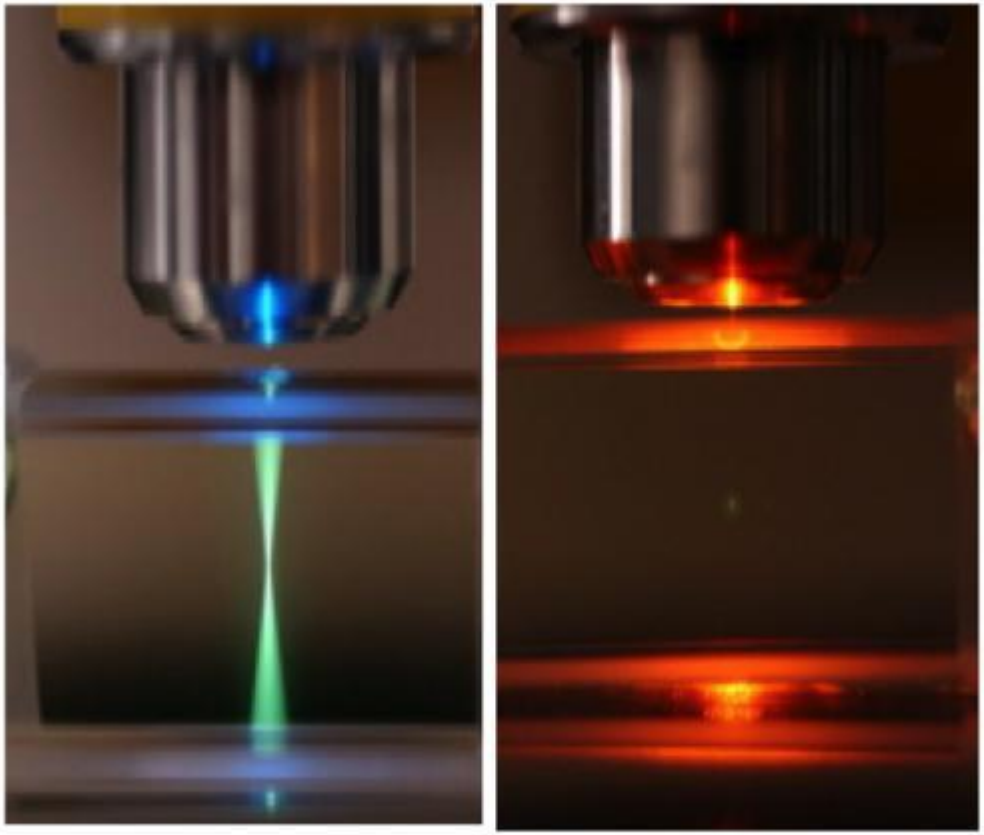
Figure 2



Excitation area in confocal microscopy and multiphoton microscopy

MP excitation

1-photon vs. 2-photon



Photos by Steve Ruzin

Fluorescence from out of focus planes

Fluorescence from focal spot only

MP advantages

Only excitation in focal point

- Much less bleaching
- Much less phototoxicity

Use light in IR range (800-1300nm)

- 2 to 3 times deeper penetration; red light scattered less then blue light
  - 488 light >7 times more
    scattered then 800nm
- No out of focus absorption

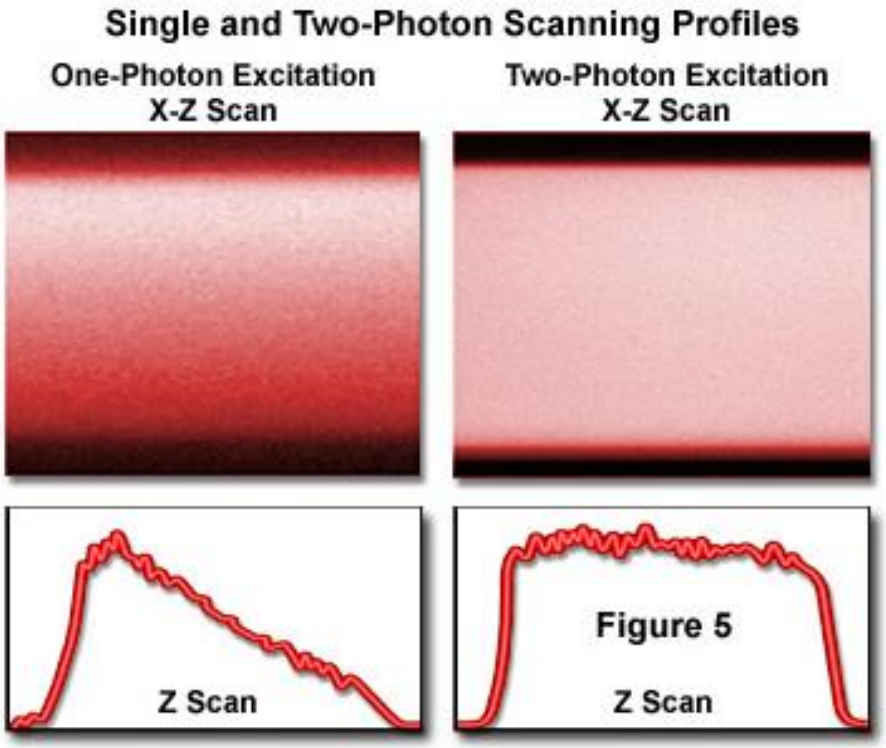
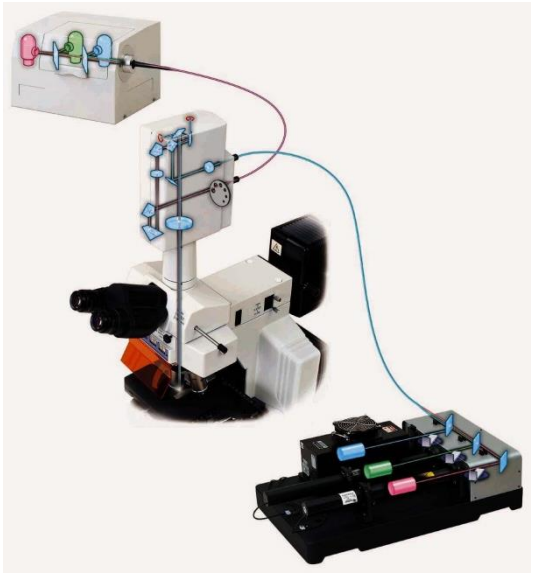


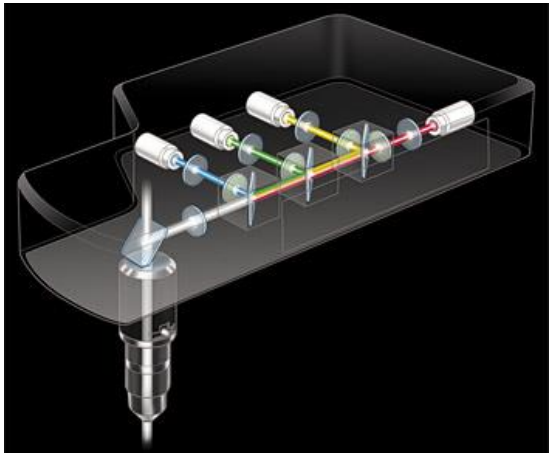
Figure 5

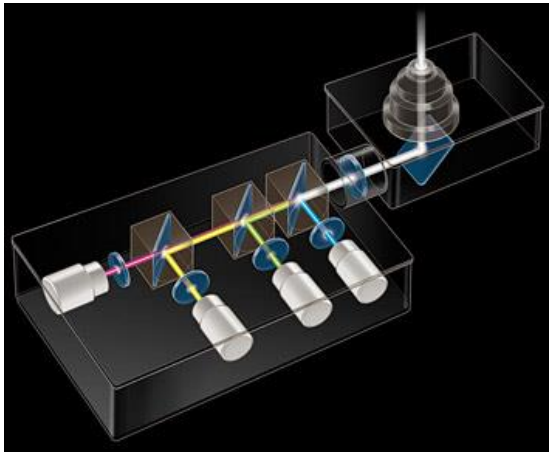
MP advantages

Only excitation in focus point and therefore emission only coming from focus point

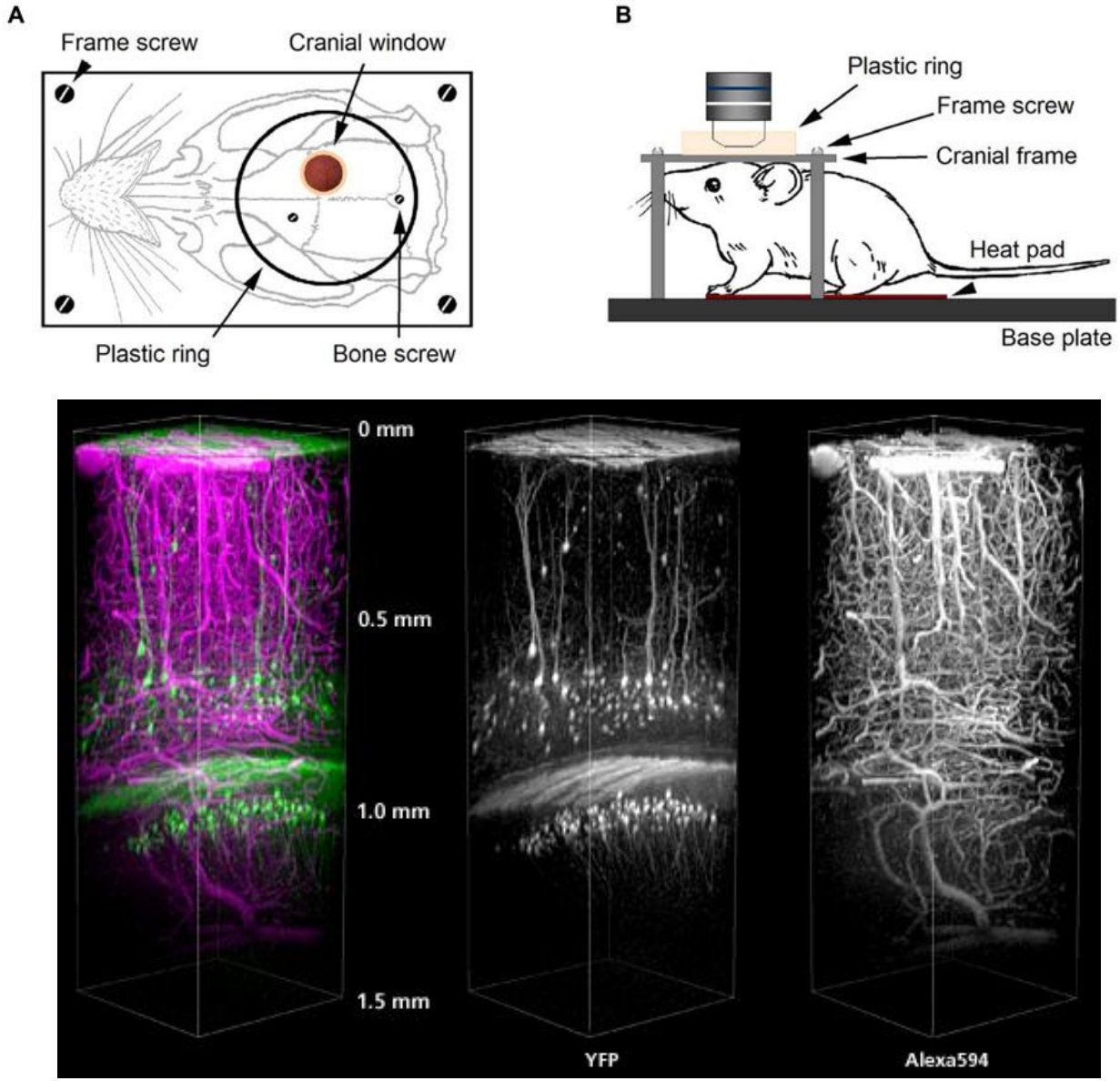
- No pinhole needed
- NDD – Non Descanned Detector







Deep brain imaging in *in vivo* mouse



A1R MP+ GaAsP Epi NDD detector unit

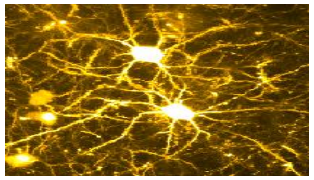
NIKON CORPORATION
Instruments Company

deep brain imaging in *in vivo* mouse

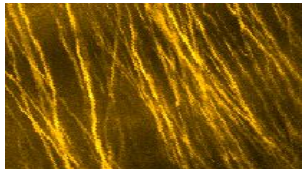
The brain of H-line 4-week-old mouse under anesthetize was studied with the open skull method. The entire shape of dendrites of pyramidal cells in layer V and hippocampal pyramidal cells can be visualized. Surprisingly dendrite of hippocampal pyramidal cells can also be imaged.
Photographed with the cooperation of Dr. Terumasa Hibi,Dr. Ryosuke Kawakami,Dr. Tomomi Nemoto
Research Institute for Electronic Science,Hokkaido University

Objective Lens: **APO LWD 25x 1.10 WD 2.0 mm**
Detector: **EPI NDD GaAsP Detector**

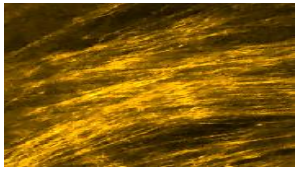
Pyramidal cell in layer V



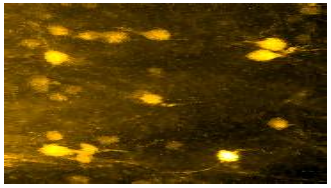
White matter

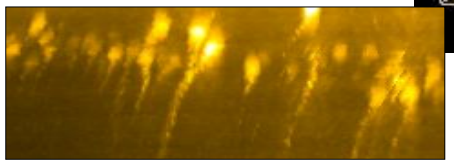


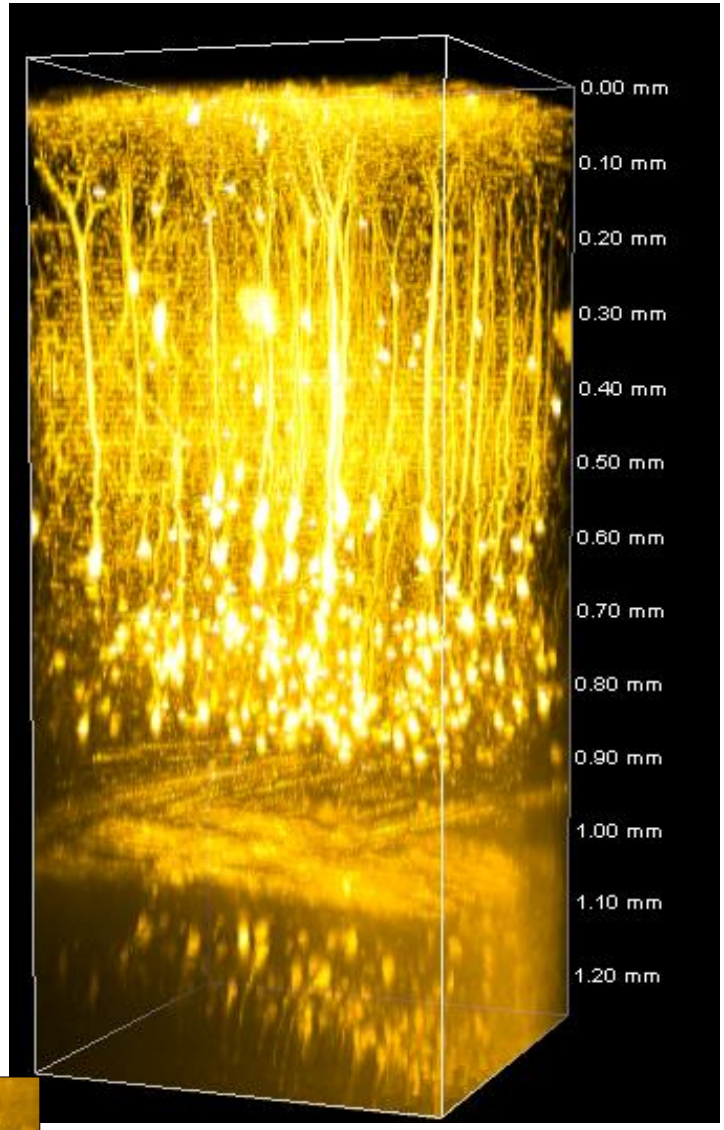
Alveus



Hippocampal pyramidal cell







Hippocampus 3D zoom image

Laser Spinning Disk Systems

NIKON CORPORATION
Instruments Company

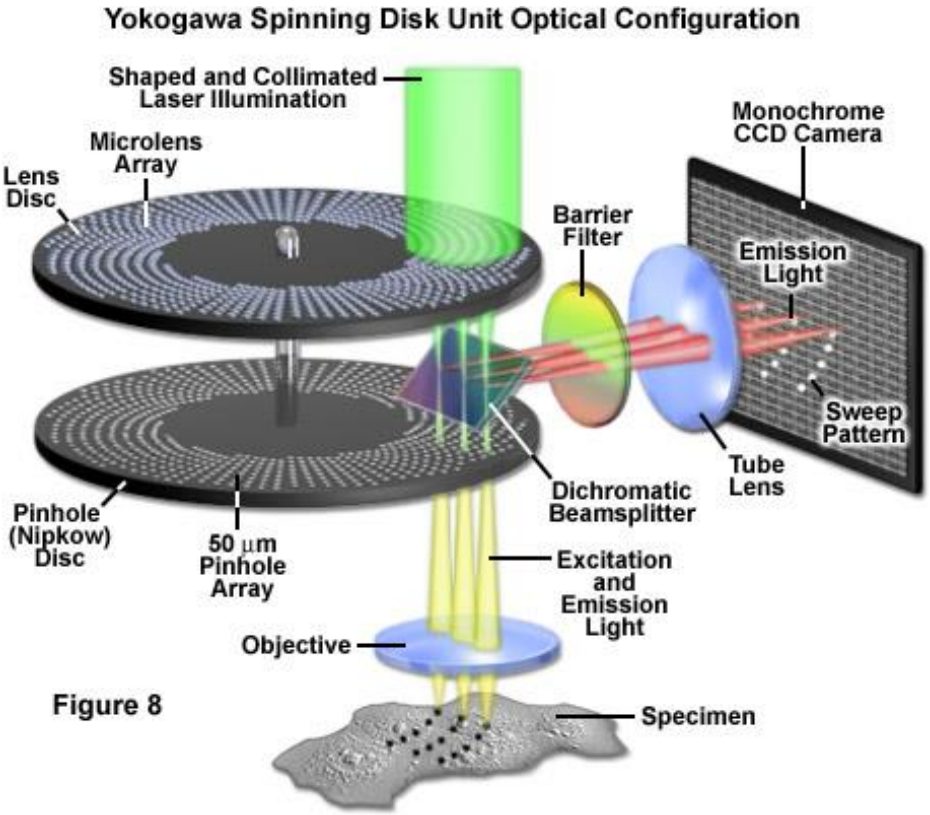


Figure 8

1 kamera = 1 FL kanál

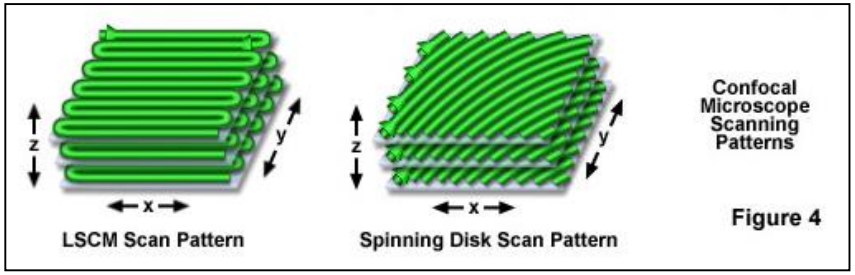
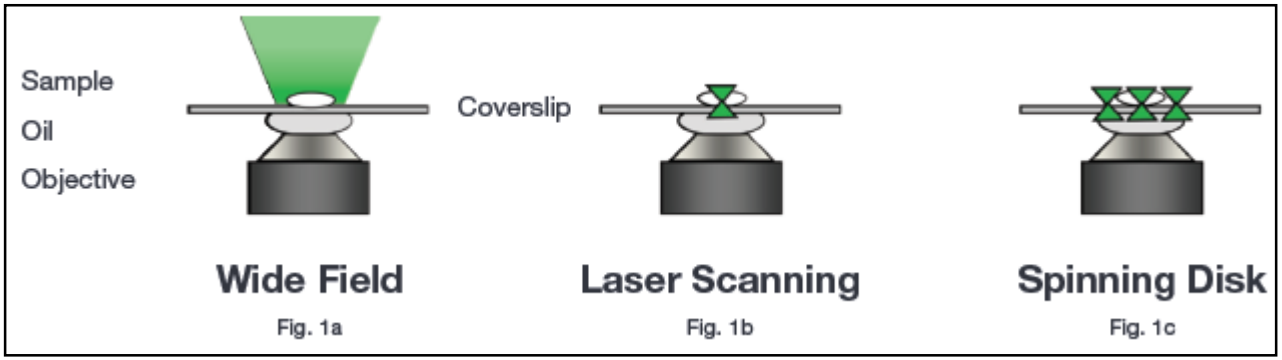
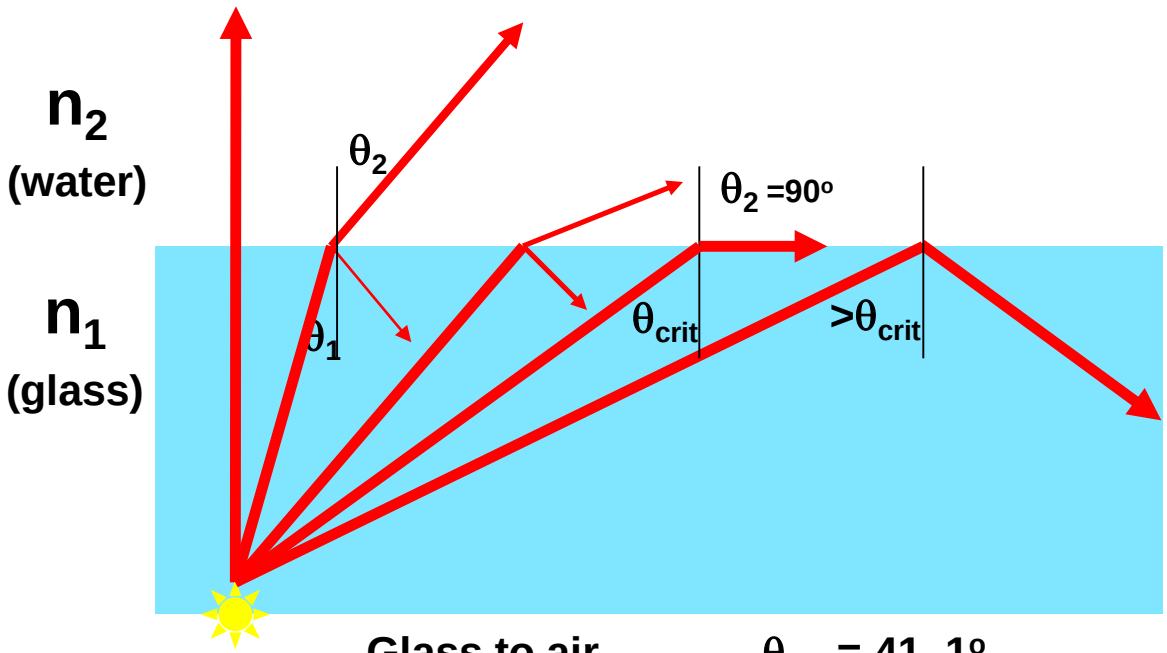


Figure 4



TIRF - Total Internal Reflection Fluorescence



Glass to air	$\theta_{crit} = 41.1^o$
Water to air	$\theta_{crit} = 48.6^o$
Glass to water	$\theta_{crit} = 61.0^o$

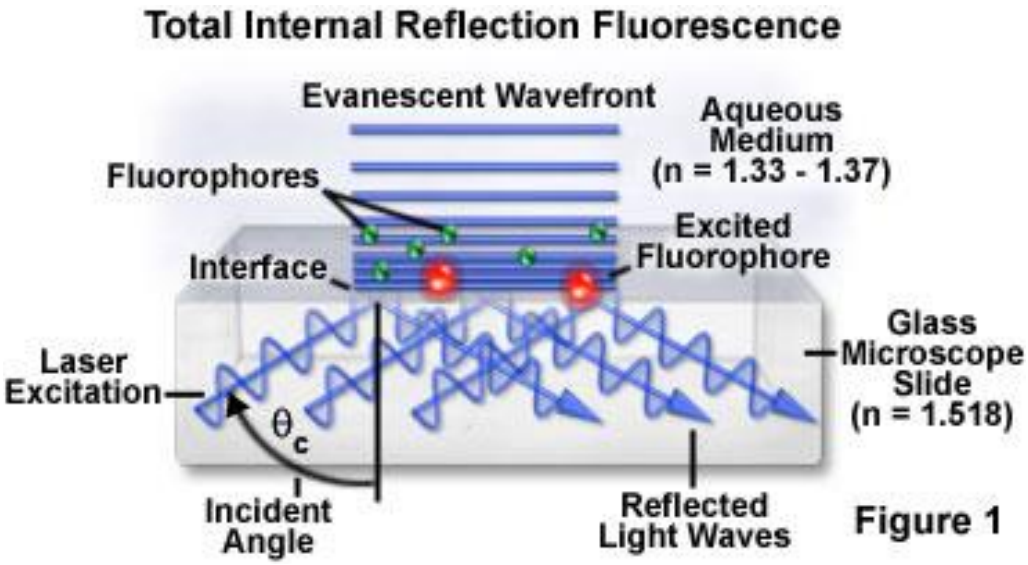
Snell's Law:

$$n_1 \sin\theta_1 = n_2 \sin\theta_2$$

$$\sin\theta_C = n_2 / n_1$$

$$n_{1>} > n_2$$

Evanescent Wave



$$E_z = E_0 \exp(-z/d_p)$$

$$d_p = \frac{\lambda_i}{2\pi n_1 \sqrt{\sin^2\theta_i - (n_2/n_1)^2}}$$

Penetration depth:

- wavelength

- Diff. n1 and n2

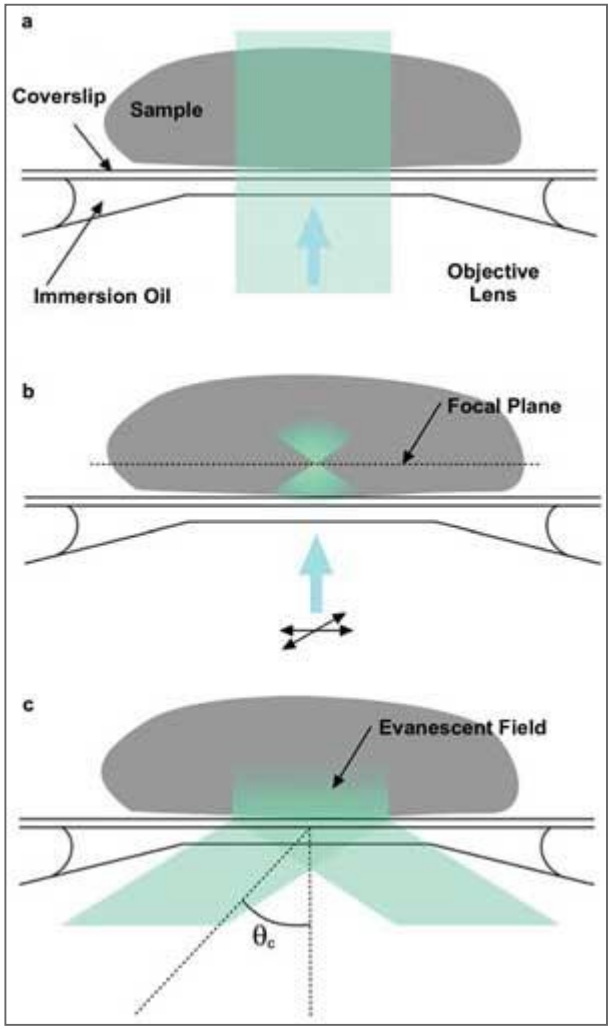
- Incident angle laser beam

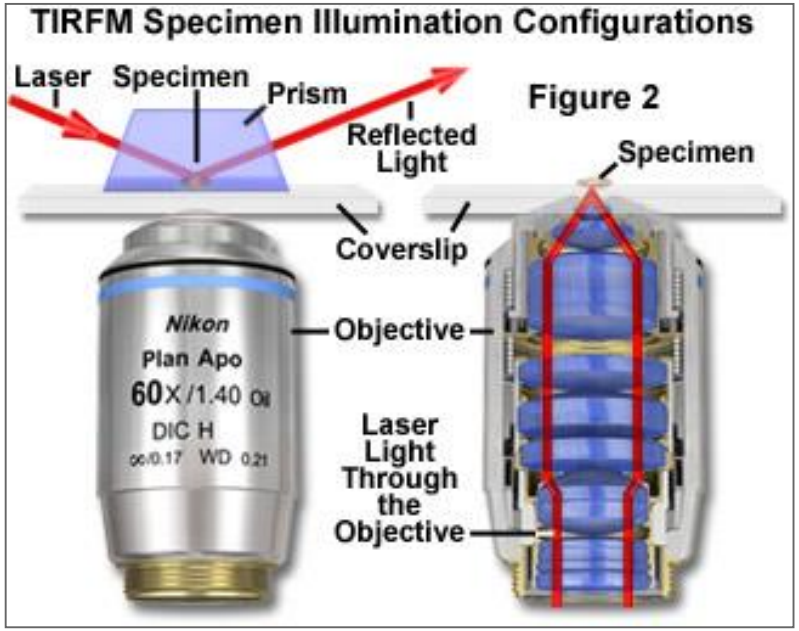
  (> critical angle)

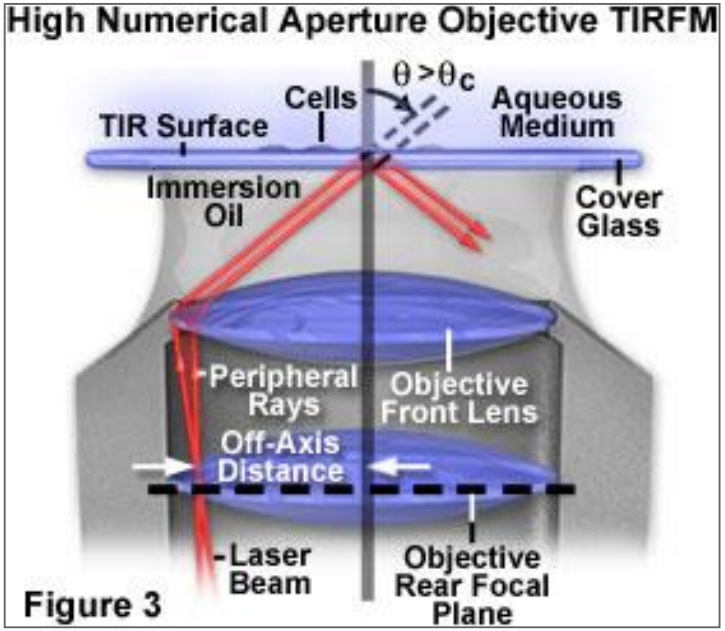
- Typically between 50-200nm

Why is this useful for FL microscopy?

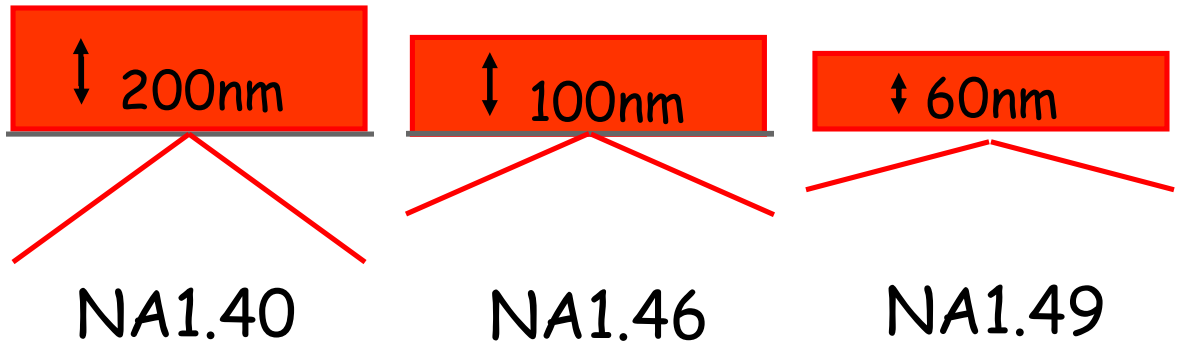
- Excitement of Fluorophores within only 50-200nm of coverslip
- No background Fluorescence from rest of specimen
- Very high increase in S/N
- Measurement single molecule fluorescence possible





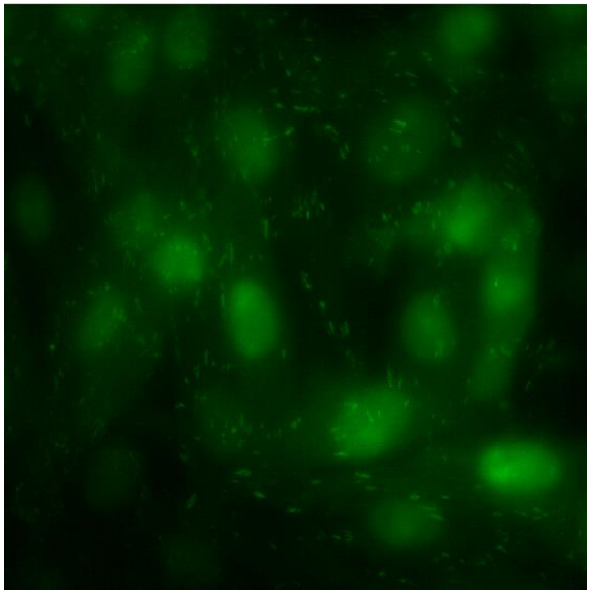


Evanescent wave thickness; axial resolution

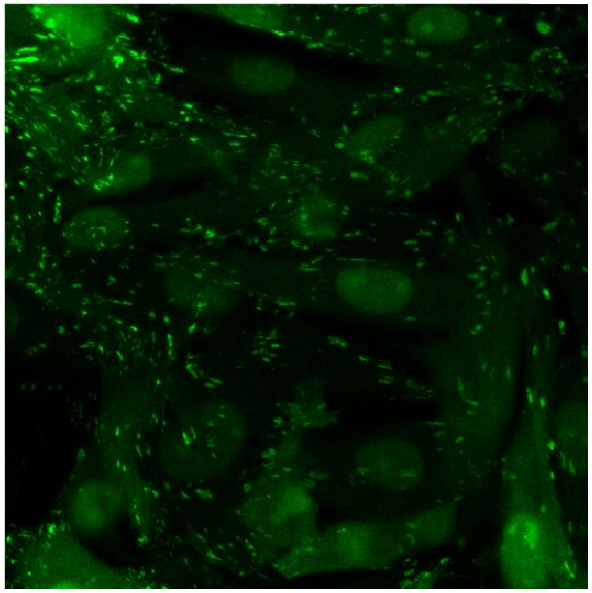


Widefield vs Confocal vs TIRF

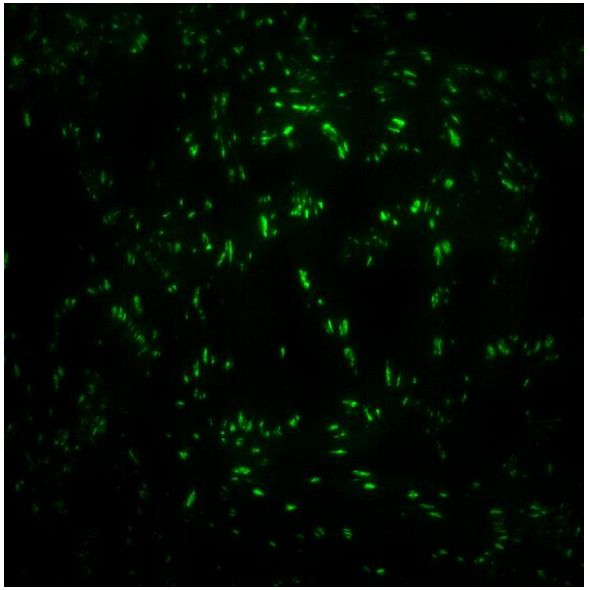
Widefield



Confocal

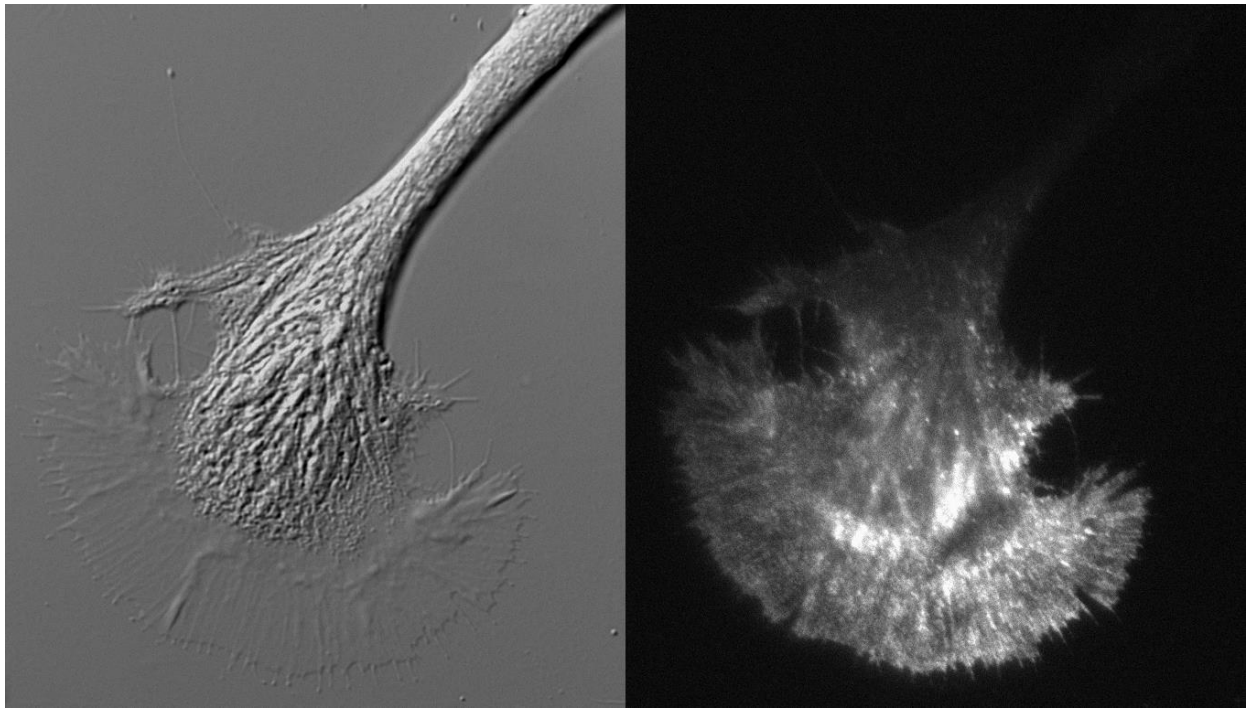


TIRF



S.E. Ledevedec Leiden University (GFP-dSH2)

Neuronal Growth Cone DIC/TIRF



Alexa488 Actin
Imaged by Andy Schaefer, Paul Forscher Lab. , Yale University

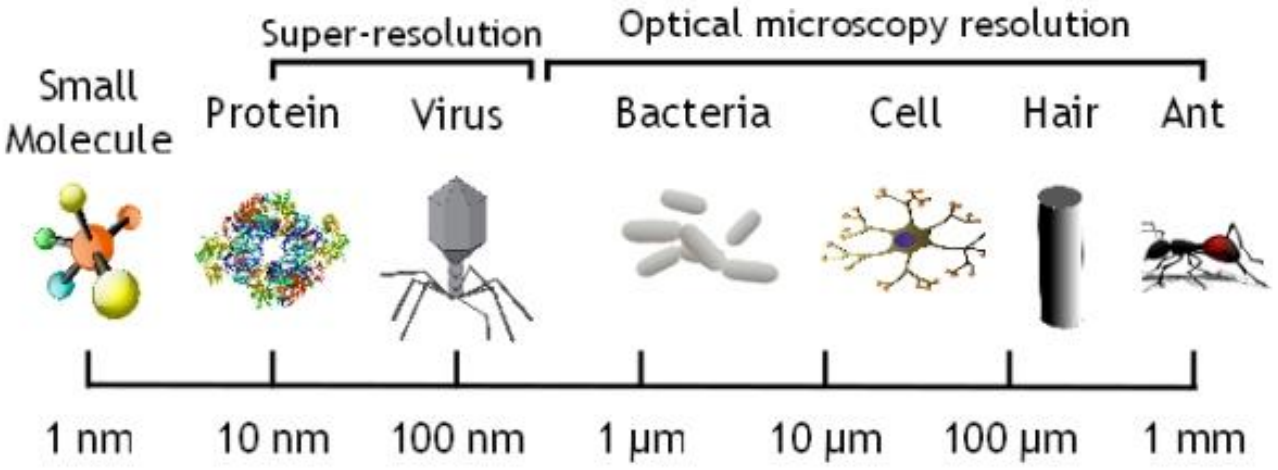
NIKON CORPORATION
Instruments Company

Super-Resolution Microscopy

November 13, 2020

Super-Resolution Microscopy – 2014

Super-Resolution Microscopy




N-STORM

Stochastic Optical Reconstruction Microscopy

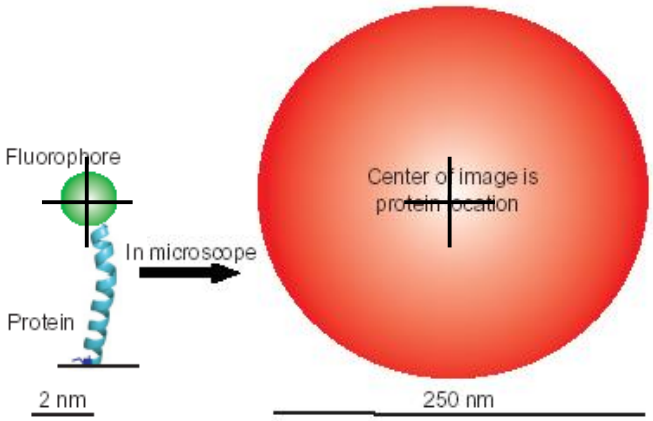
Developed by Dr. Xiaowei Zhuang and colleagues - Howard Hughes Medical Institute, Harvard University

Enhanced resolution that is more than 10 times greater than conventional optical microscopes

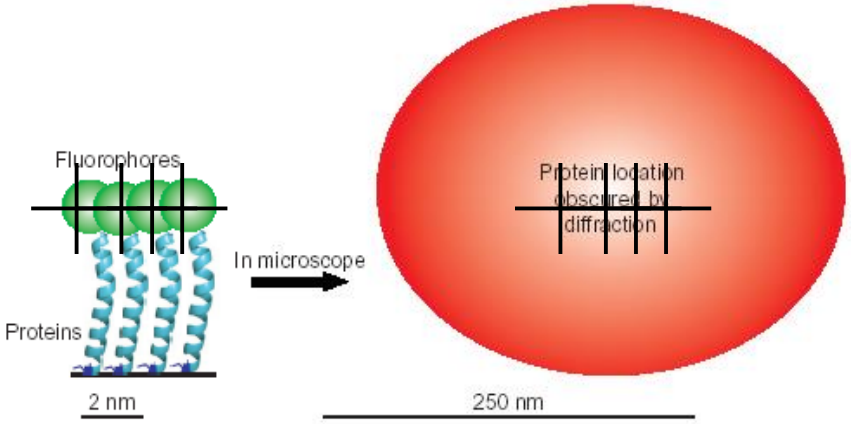
N-STORM reconstructs high resolution fluorescence 2D (20 nm) or 3D (50nm) images from precise localization information of individual fluorophores (SMLM)

N-STORM enables molecular understanding of the specimen

STORM - Principle

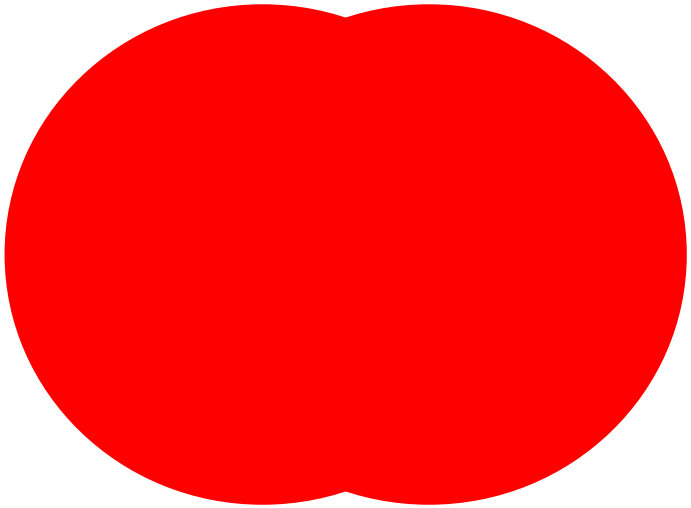


Structures within cells are mostly in the magnitude of 5-100 nm and they are lying close to each other.

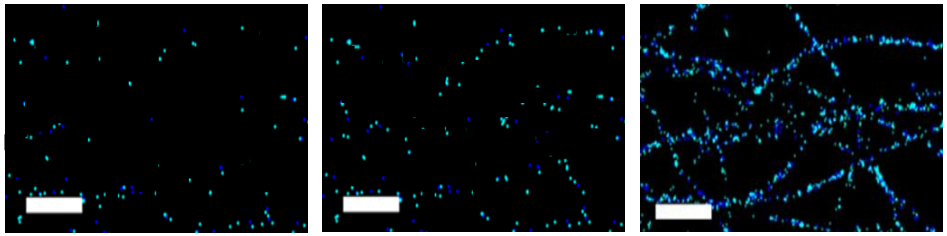


Optical Localisation

Why not switching on the fluorophores individually - one after the other?

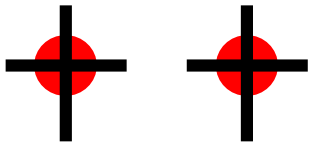


To reconstruct the sample structures one has to repeat this process a few thousand times…

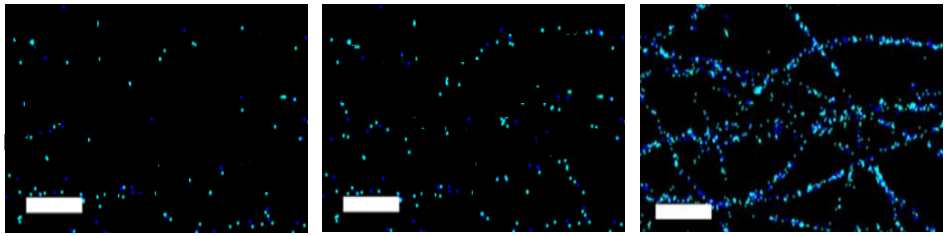


Optical Localisation

Why not switching on the fluorophores individually - one after the other?



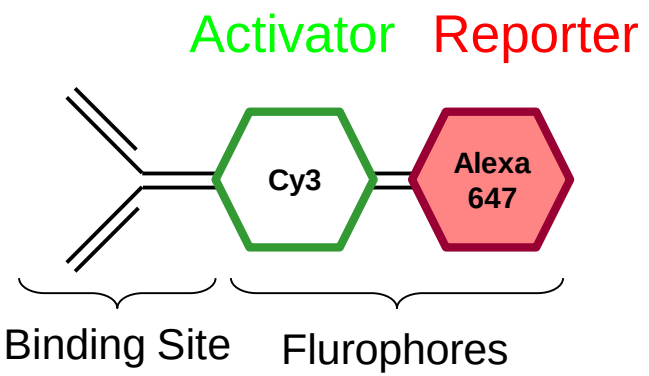
To reconstruct the sample structures one has to repeat this process a few thousand times…



N-STORM with pairs of dyes

The N-STORM works with **photoswitchable** dyes.

These are dye pairs that consist of one shorter wavelenght activator dye and a second dye with longer wavelenght as reporter



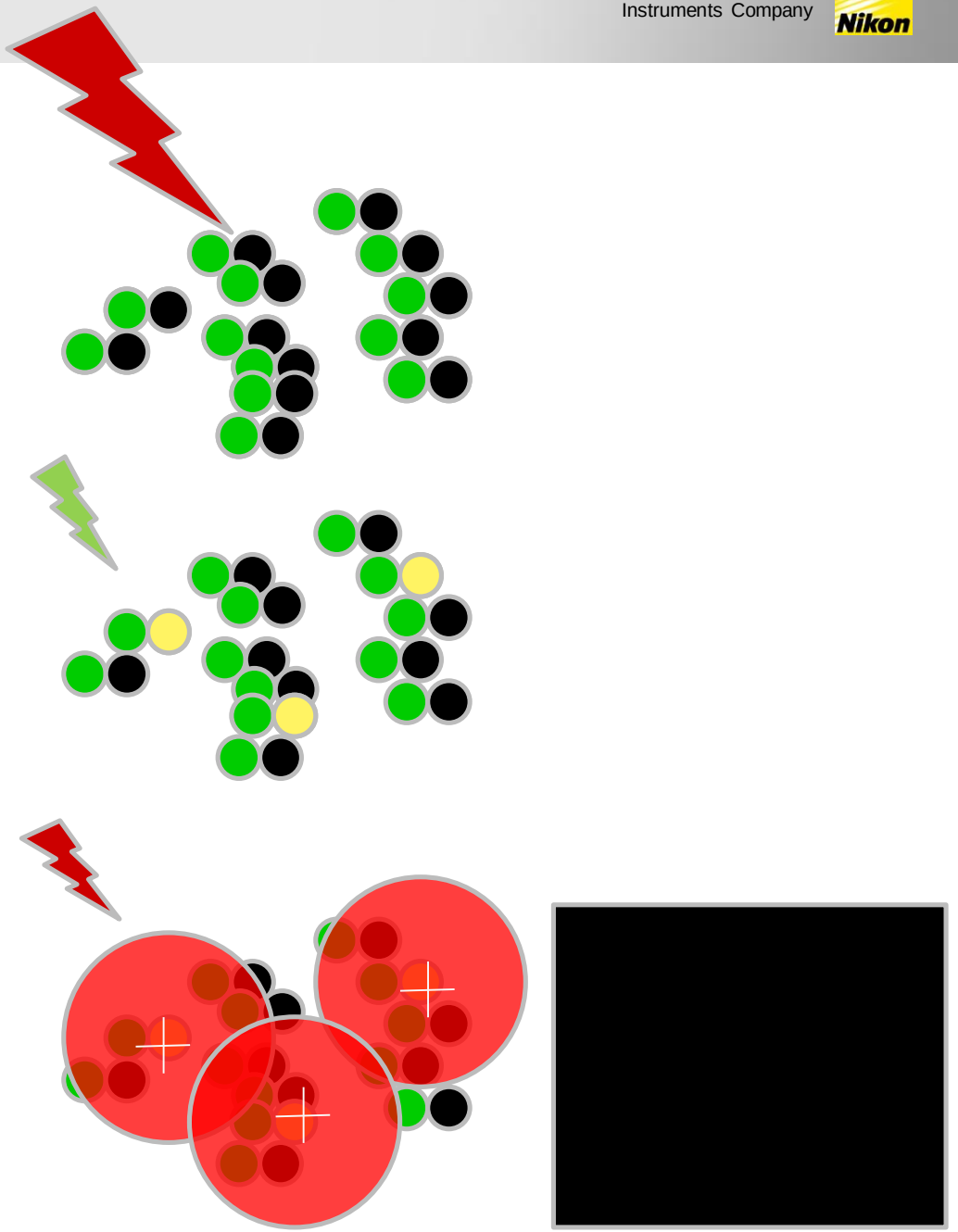
STORM Principle

1) „Switch off“ all reporters with a strong pulse of light at 647 nm.
DARK STATE

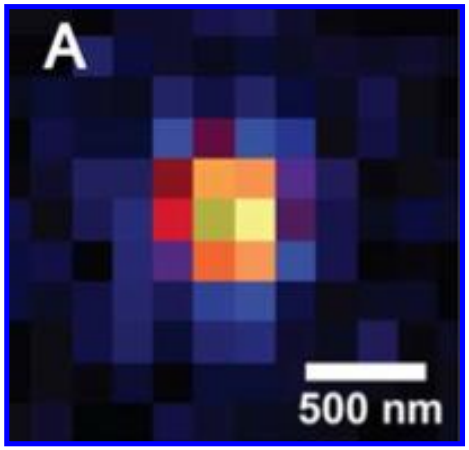
2) Low excitation of the activator with 561nm. The activator **stochastically** enables only some reporters to emit fluorescence.
ACTIVATION

3) Normal Imaging at 647 nm. The positions of the emitting molecules are recorded.
IMAGING & LOCALISATION

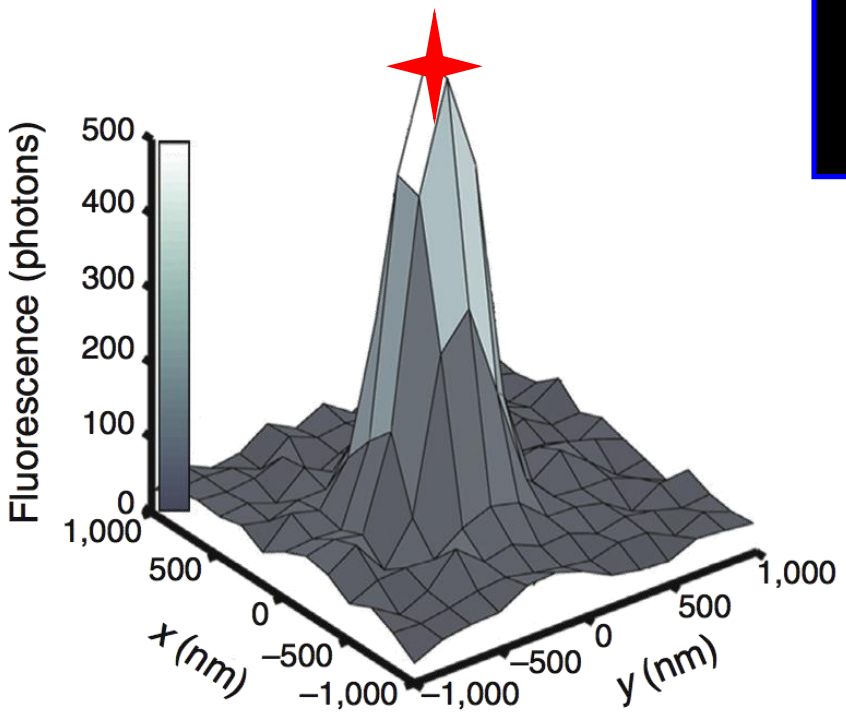
4) Repeat this sequence several 1.000 times.



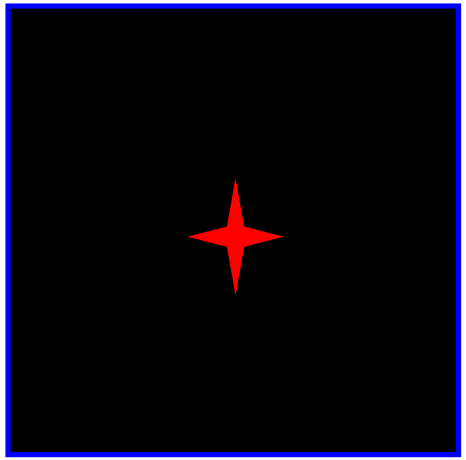
Localizing the PSF Centroid



Diffraction Limited Spot of the emission of a single Reporter on DNA during a single cycle



Point Spread Function (PSF) of the diffraction limited spot



Centroid of the PSF after Gaussian Fit

STORM; super-resolution by localization

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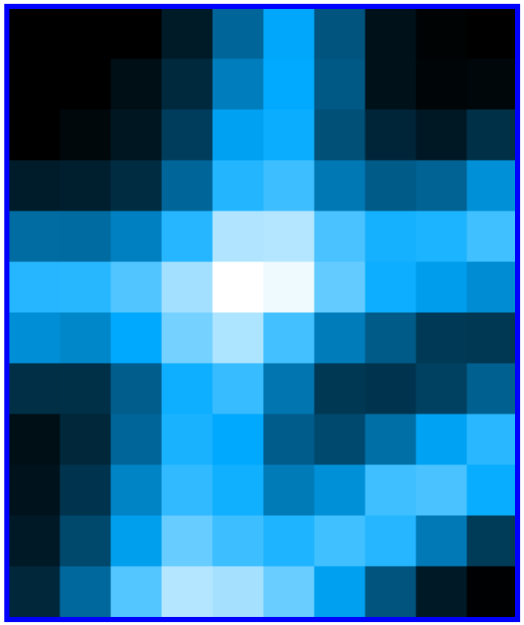
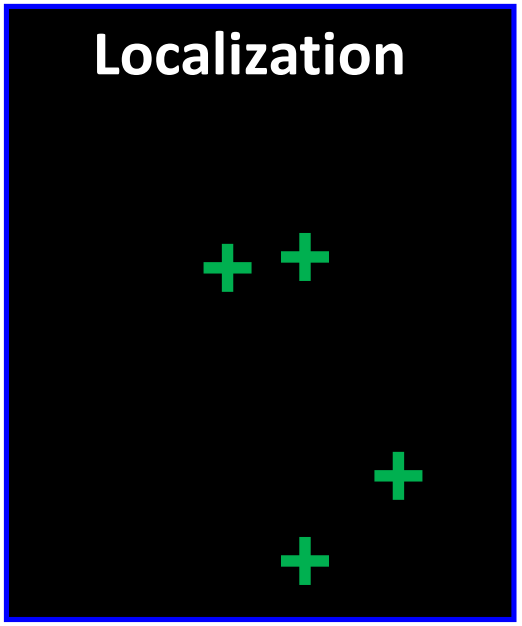
Conventional fluorescence



Raw images

STORM Image

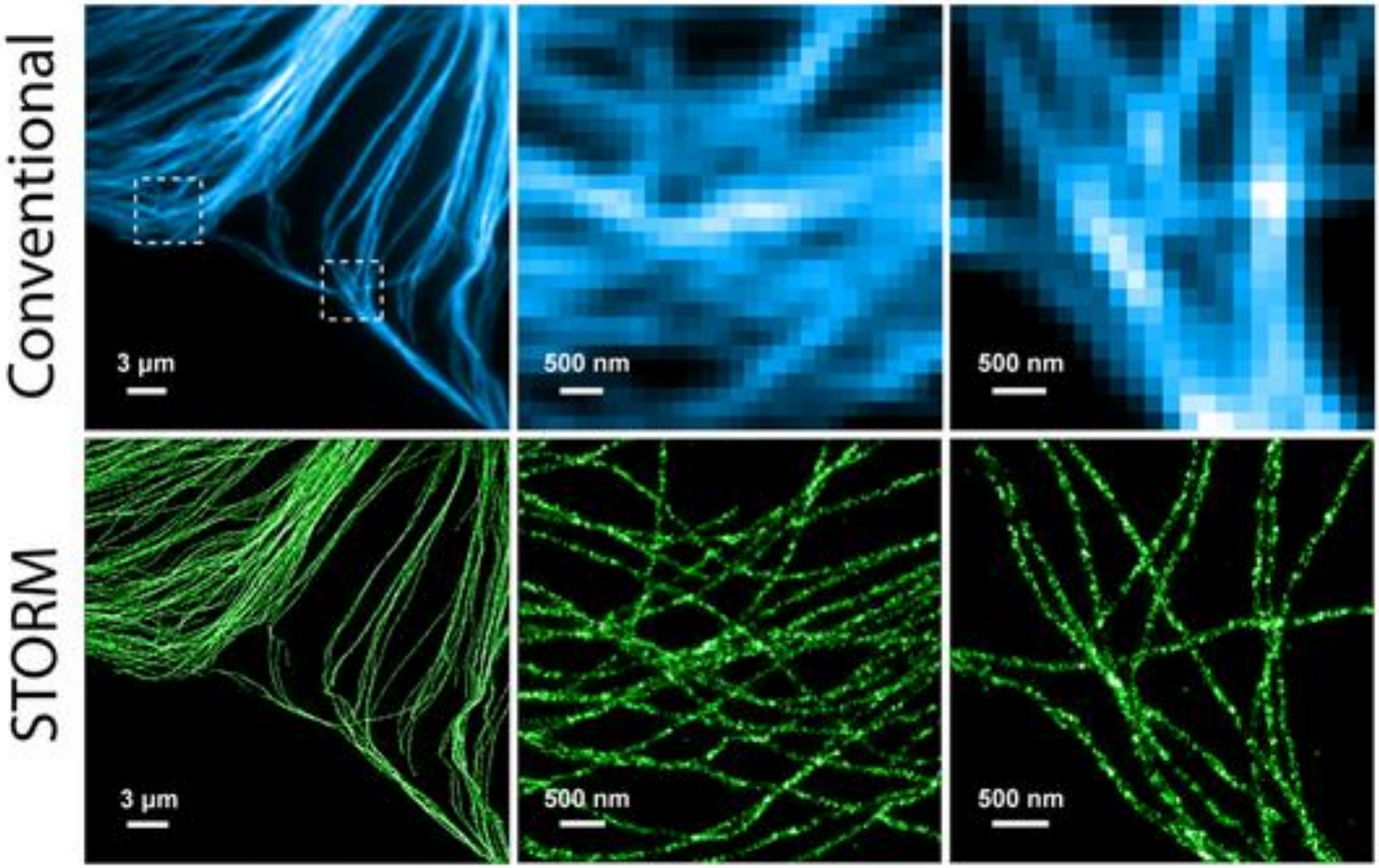


2x real time

Stochastic Optical Reconstruction Microscopy = STORM

Rust, Bates & Zhuang, Nat. Methods, 2006
Bates, Huang, Dempsey & Zhuang, Science, 2007

Conventional vs. STORM

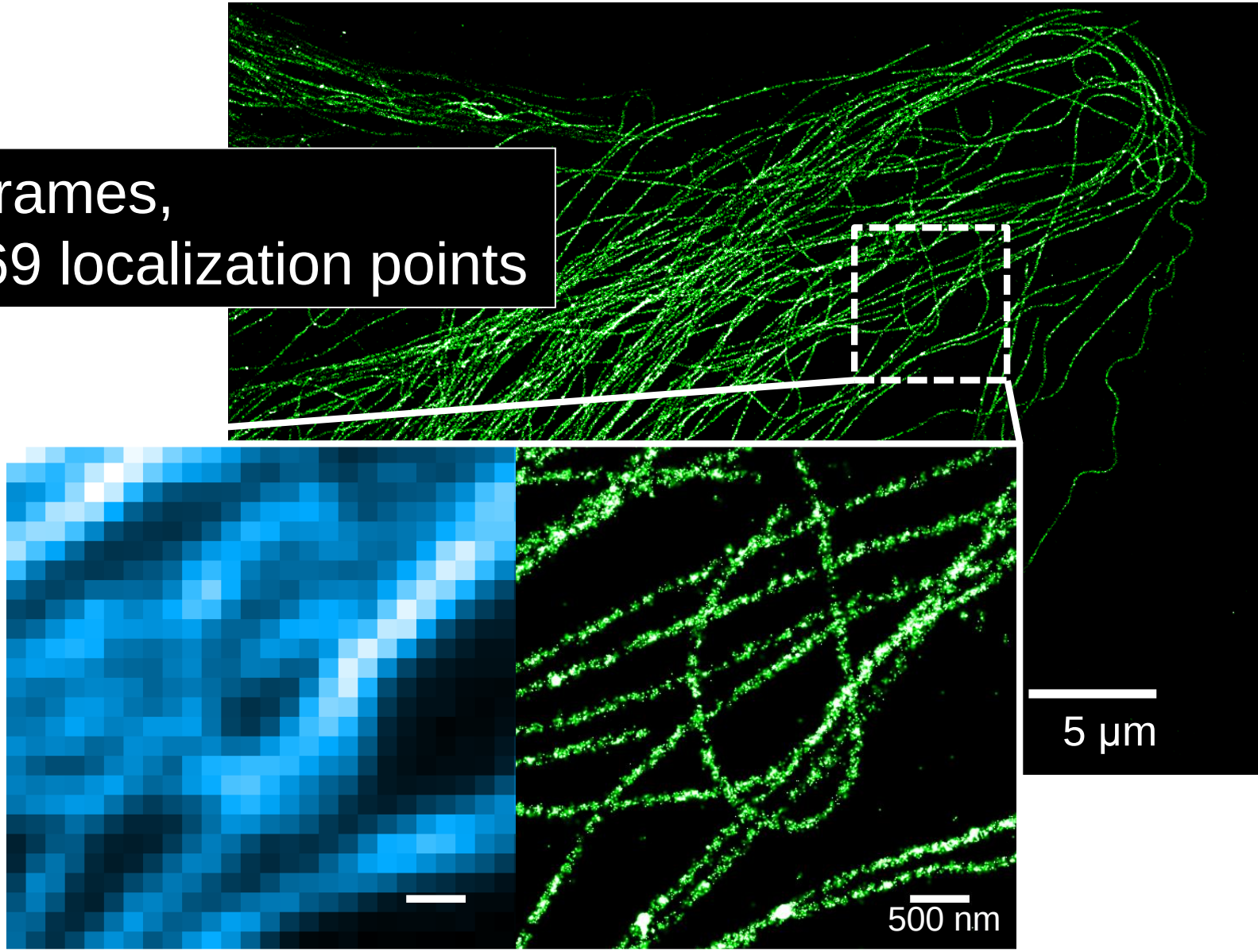


Images of microtubules in a mammalian cell

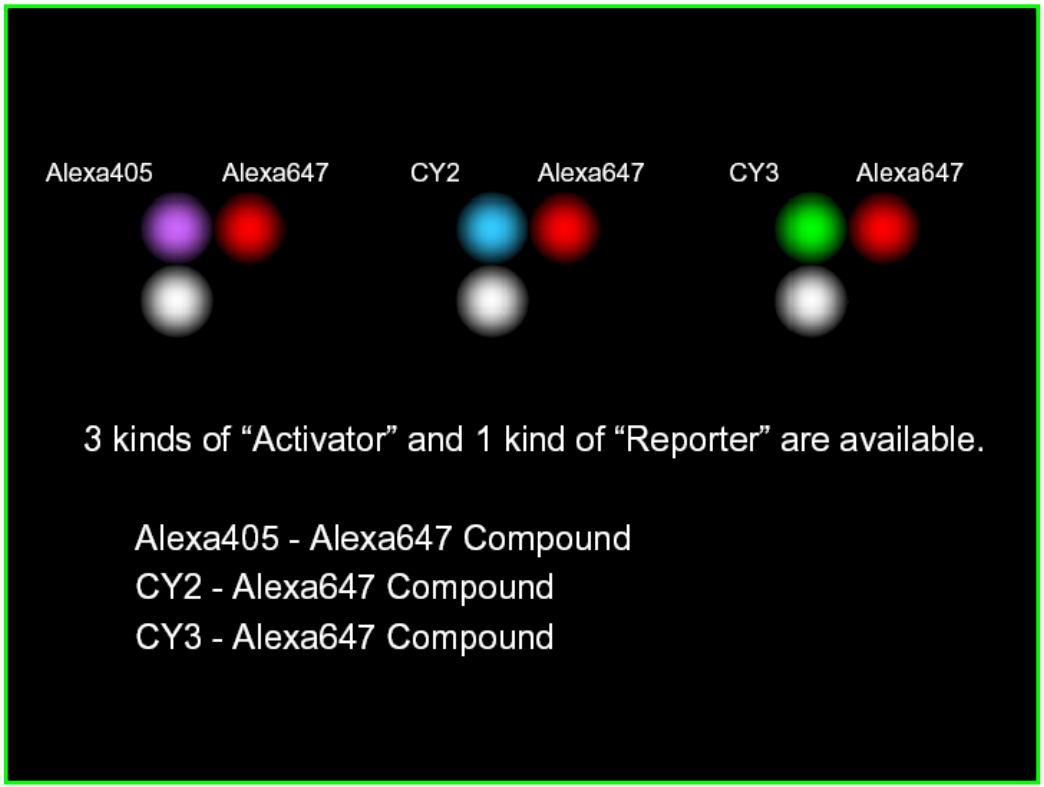
Source: http://zhuang.harvard.edu/storm.html
Images adapted from Science 317, 1749-1753 (2007)

N-STORM - Microtubuli

40,000 frames,
1,502,569 localization points

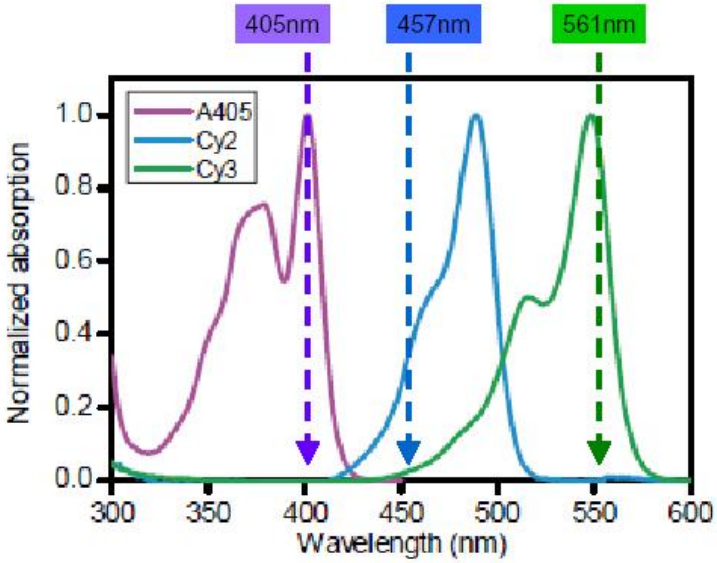


Multicolor N-STORM

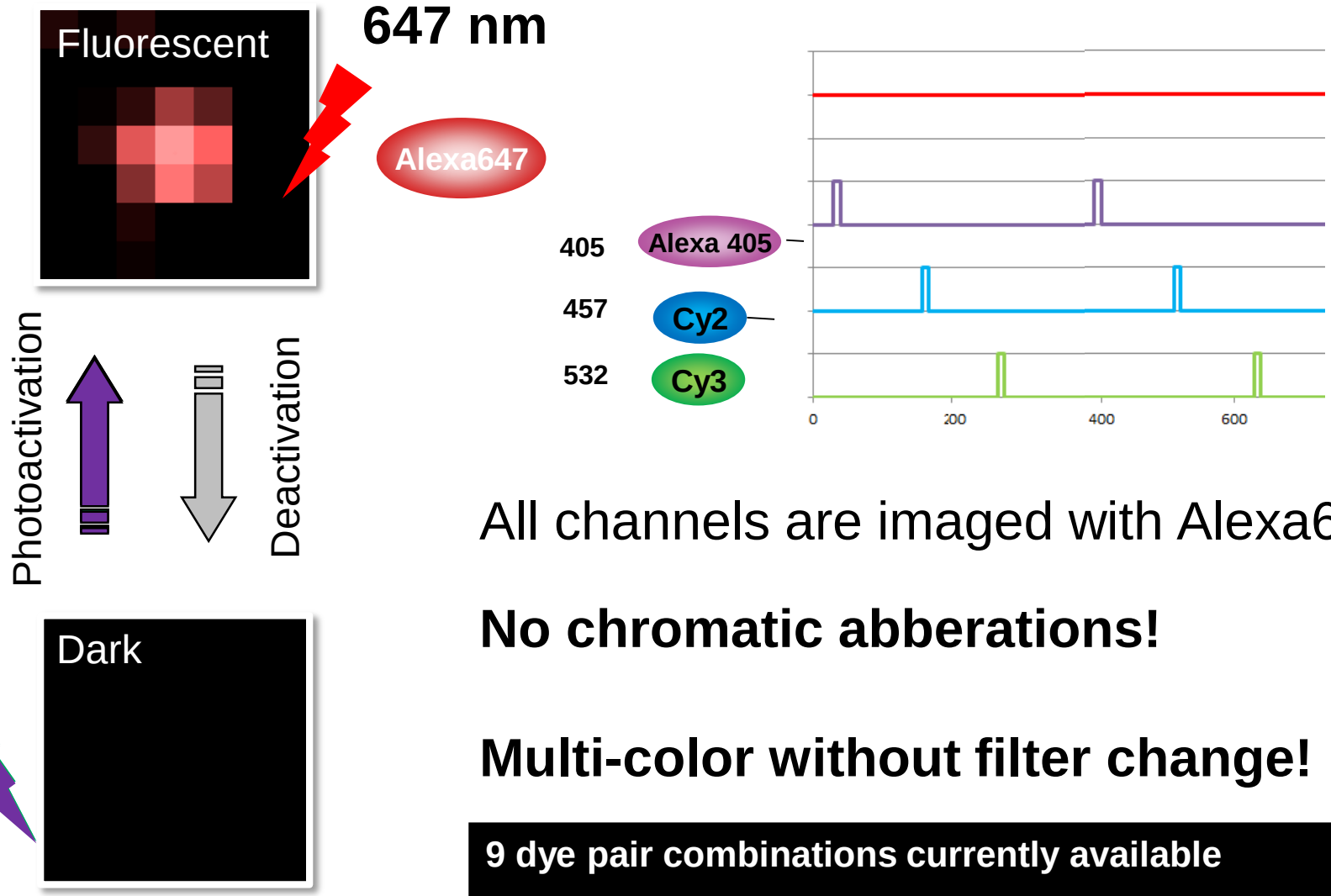


Difference in Excitation

Same Imaging Wavelenght



Sequential activation of Alexa647



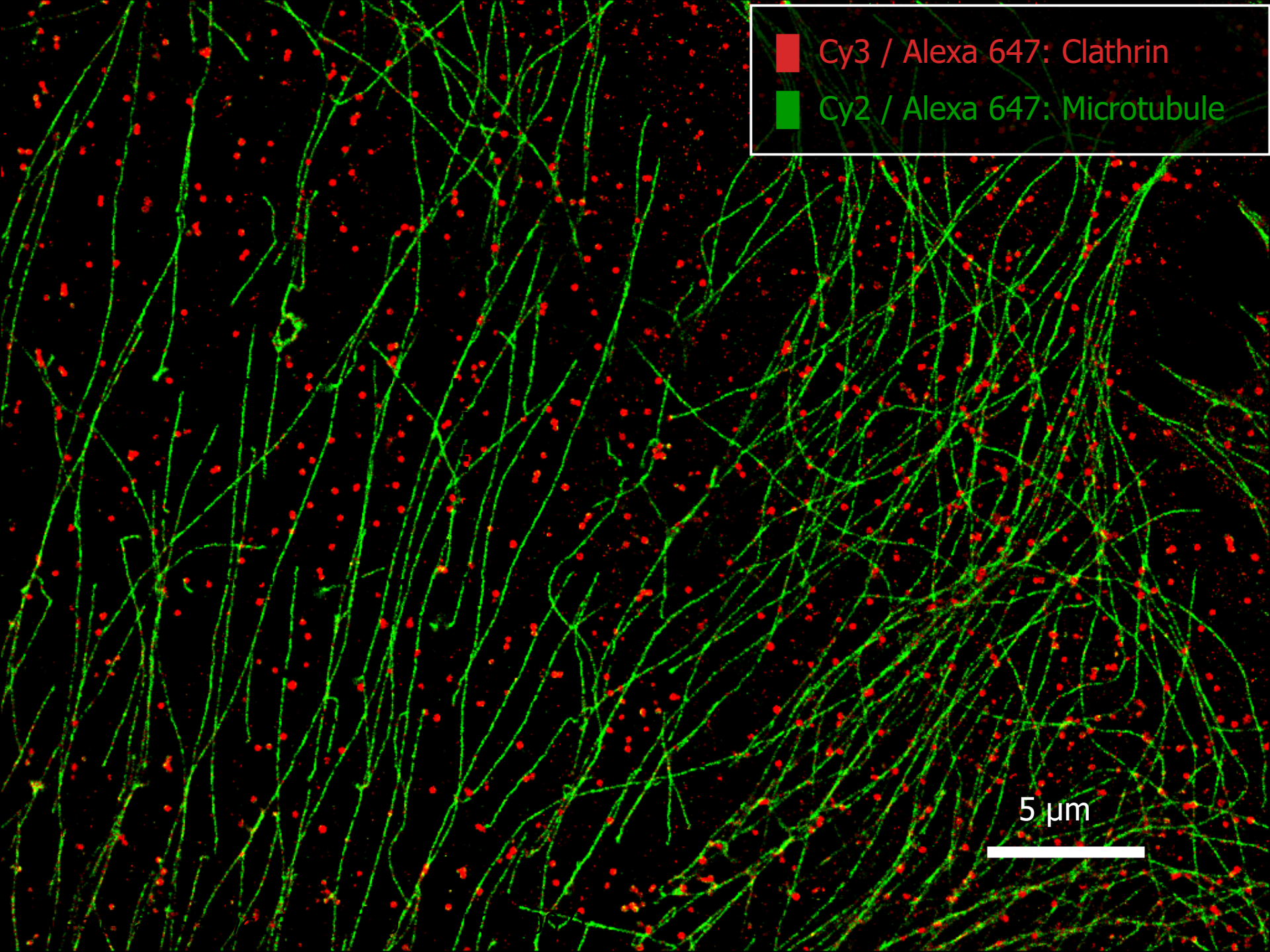
All channels are imaged with Alexa647

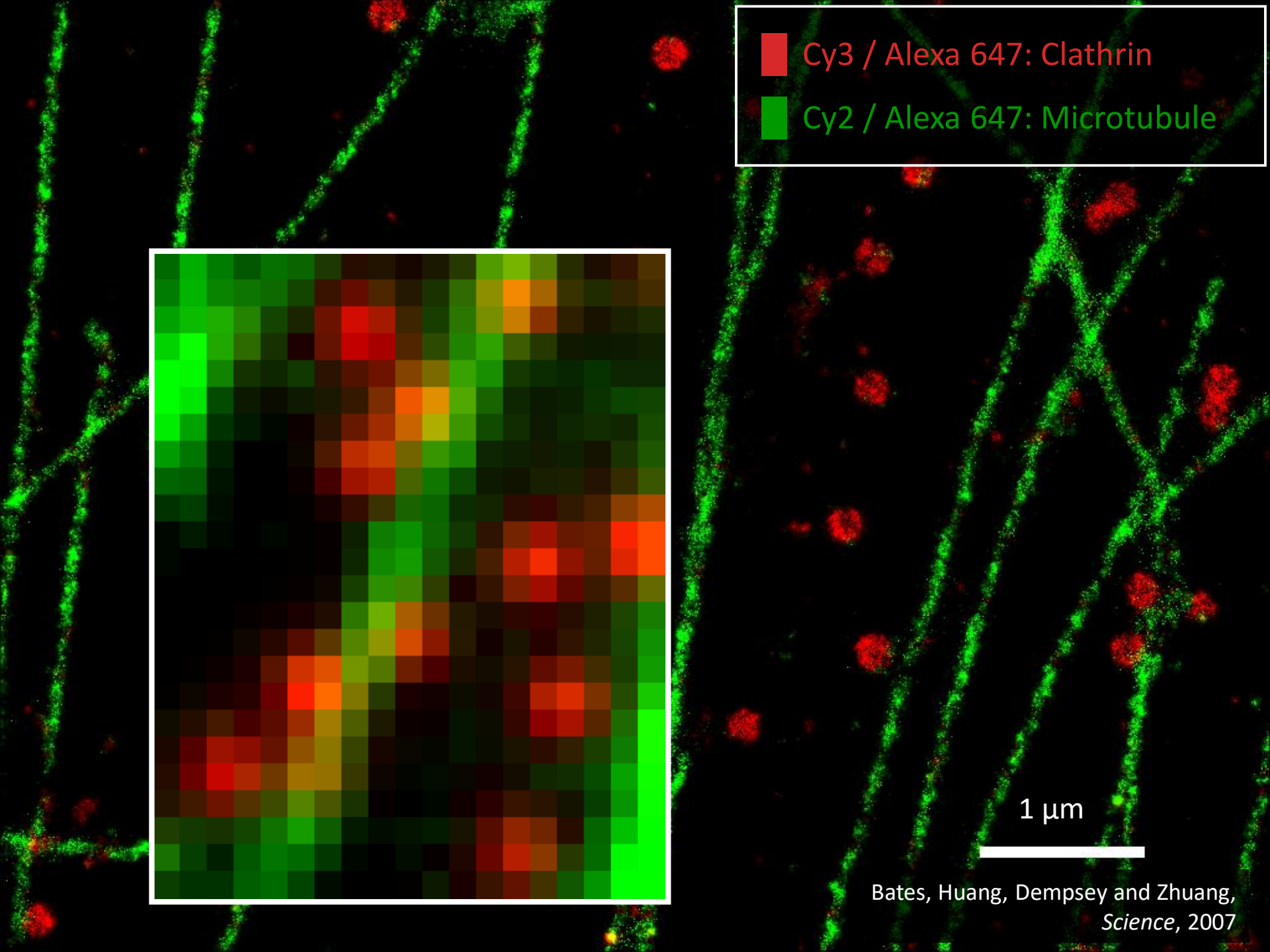
No chromatic abberations!

Multi-color without filter change!

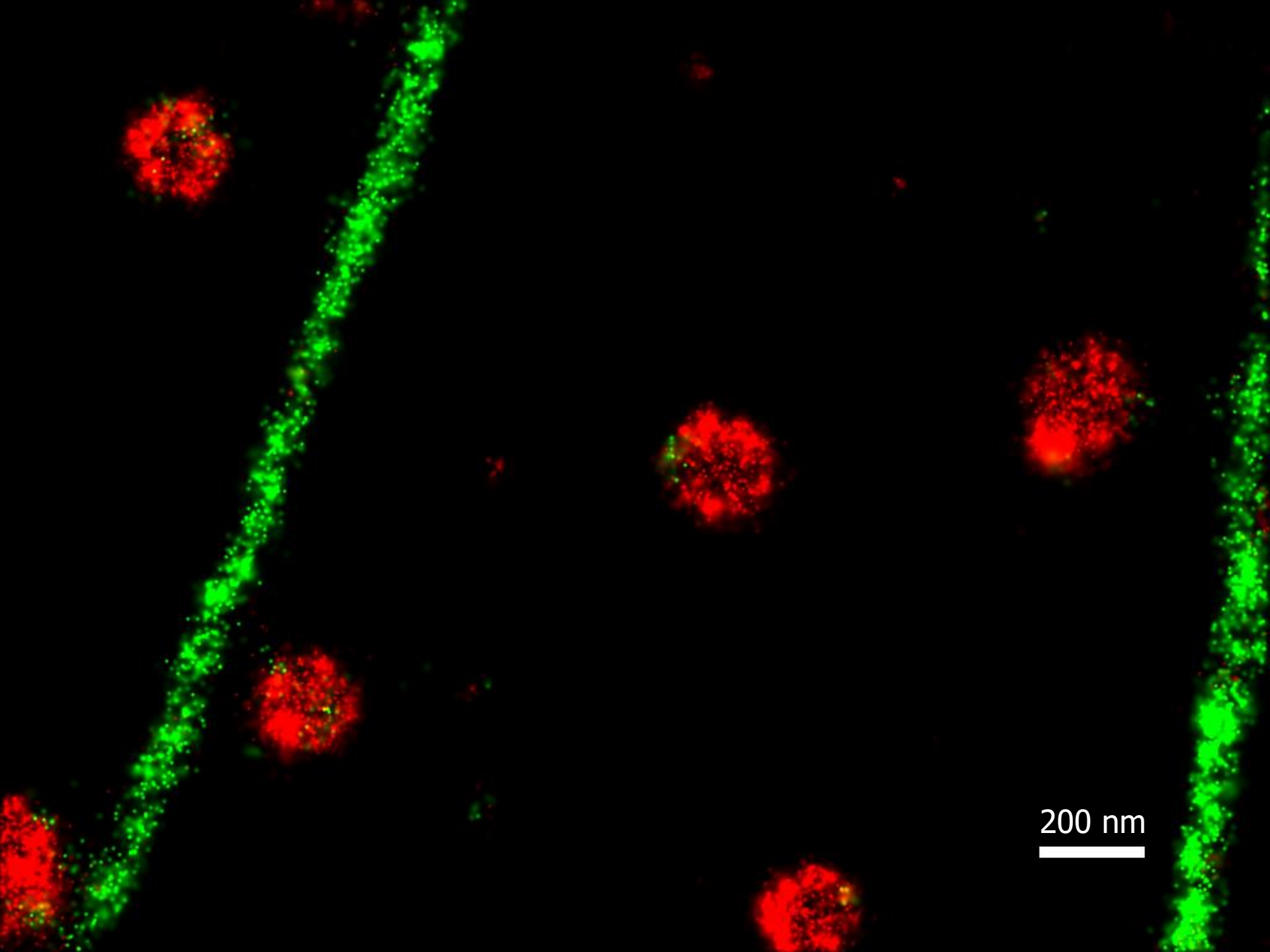
9 dye pair combinations currently available

Max of 3 dye pairs sequentially during acquisition


Cy3 / Alexa 647: Clathrin
Cy2 / Alexa 647: Microtubule
5 µm



Bates, Huang, Dempsey and Zhuang,
Science, 2007


200 nm

N-STORM Applications

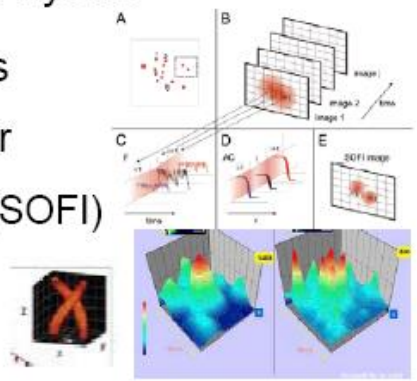
Physics

Pure Physics

Dye behavior

Calculation (SOFI)

3D (SLM)

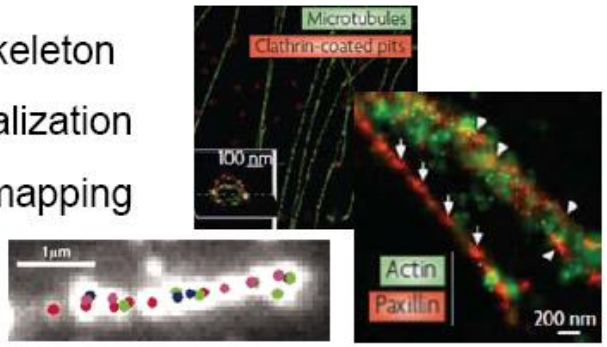


Cell Biology

Cytoskeleton

Colocalization

DNA mapping

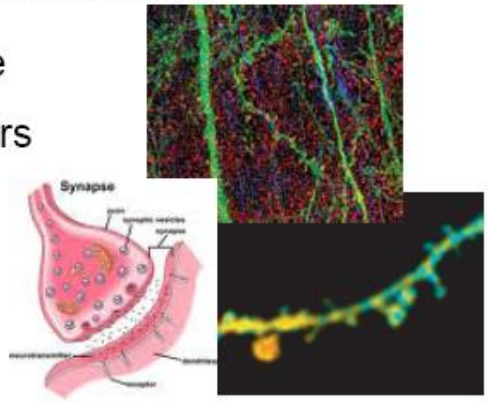


Neuroscience

Synapse

Receptors

Spine

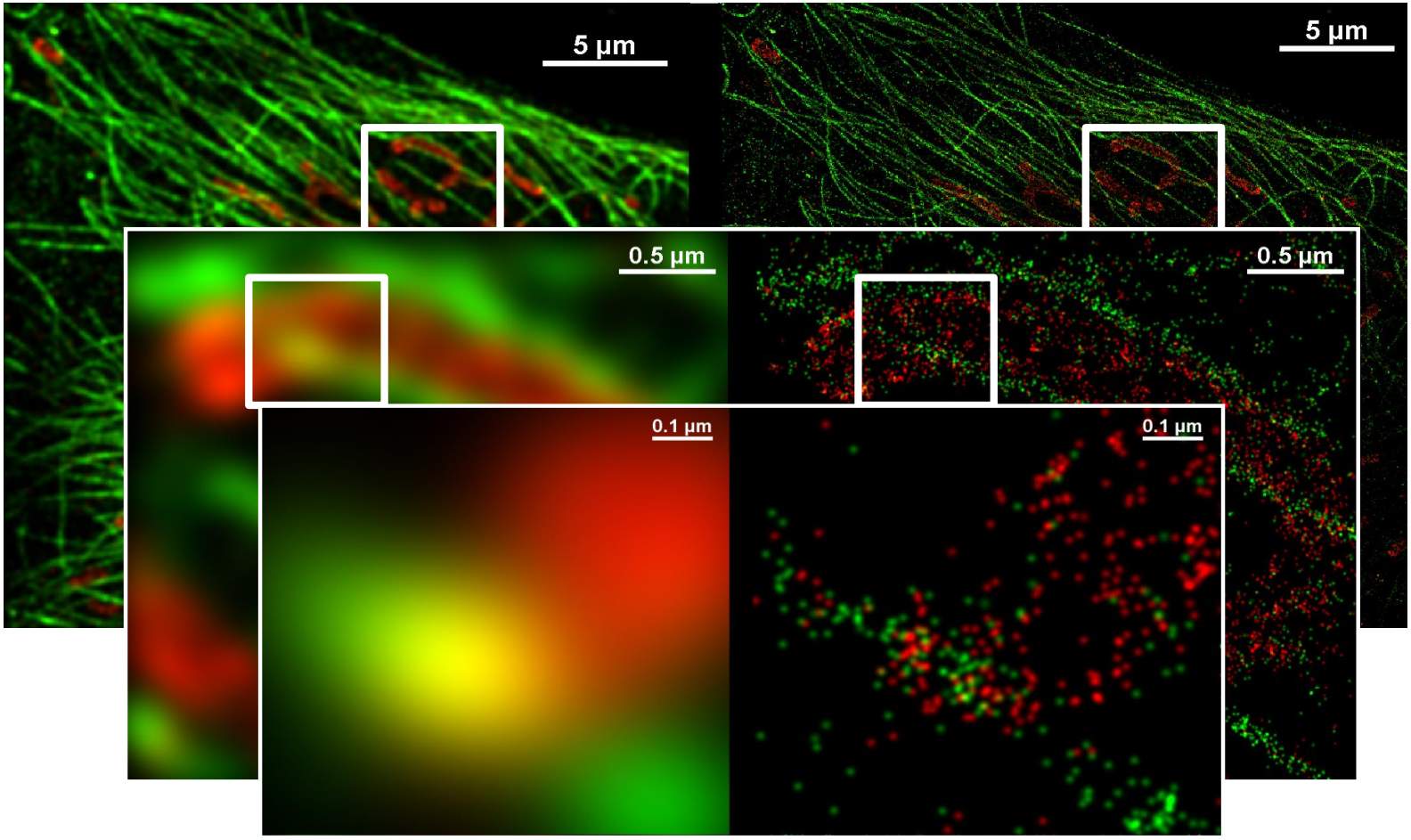


Diagnosis

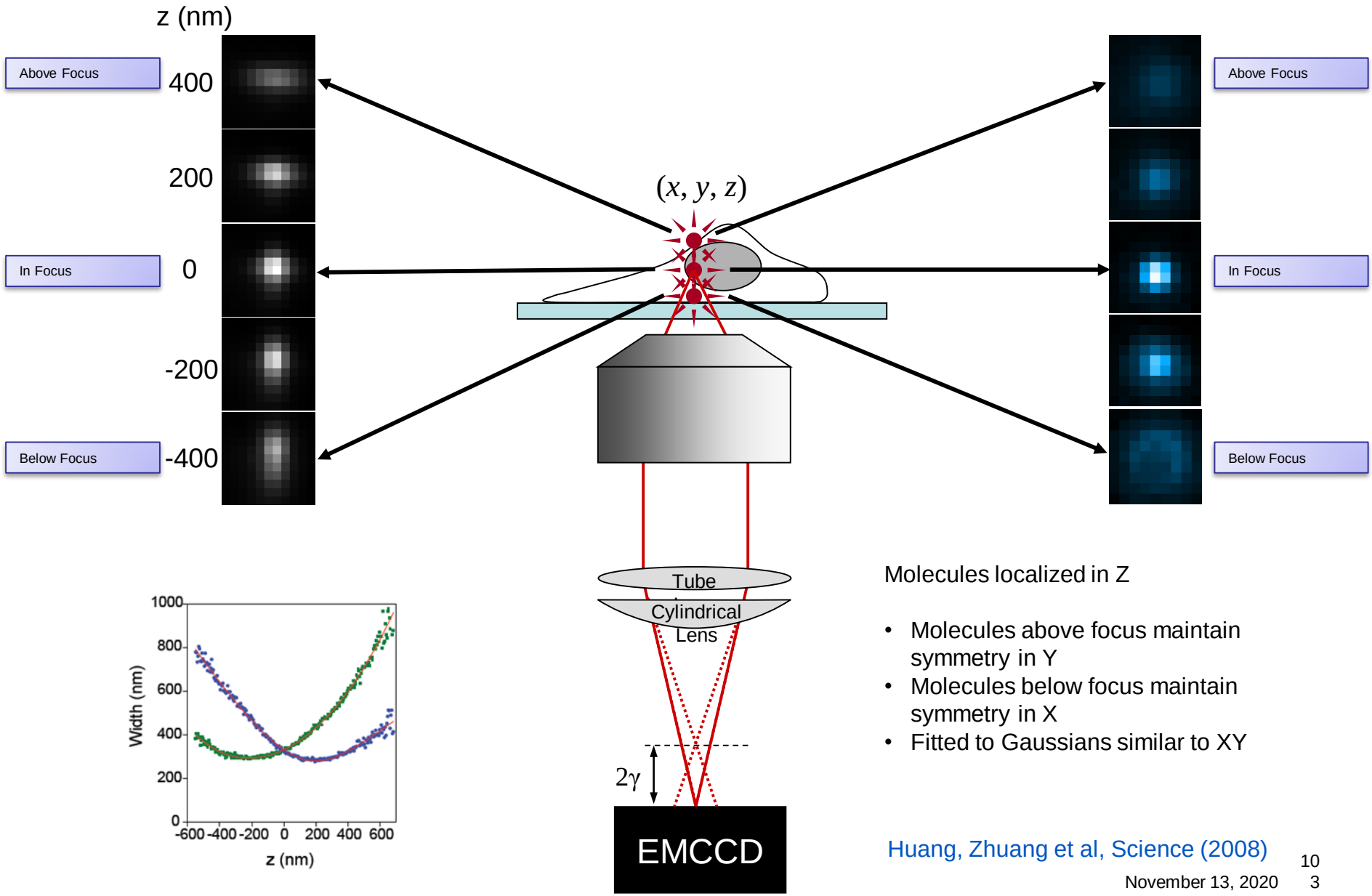
SR FISH

Abnormal Chromosome

N-STORM 10x resolution increase



3D STORM



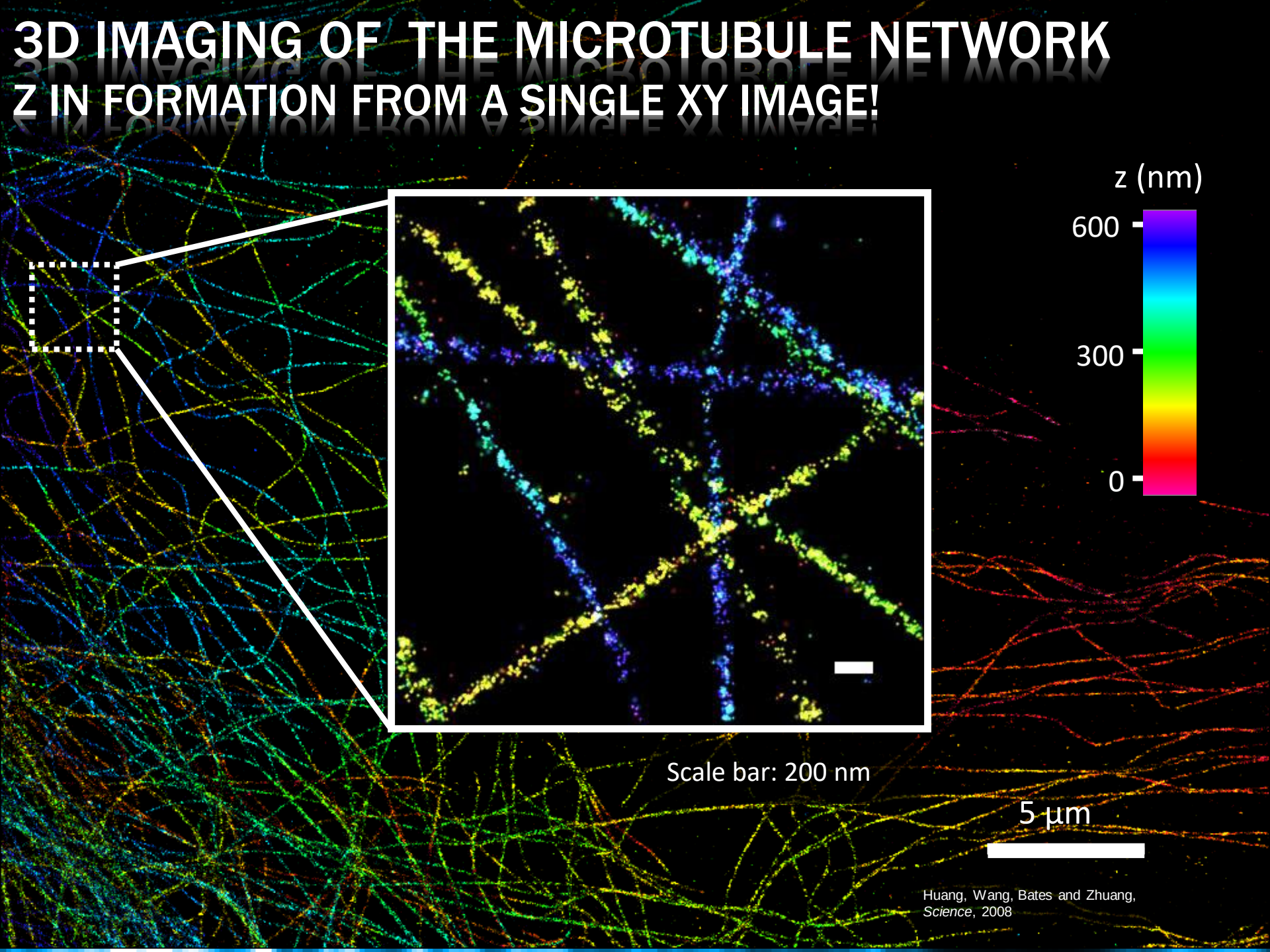
Molecules localized in Z

- Molecules above focus maintain symmetry in Y
- Molecules below focus maintain symmetry in X
- Fitted to Gaussians similar to XY

Huang, Zhuang et al, Science (2008)

3D IMAGING OF THE MICROTUBULE NETWORK

Z IN FORMATION FROM A SINGLE XY IMAGE!



Huang, Wang, Bates and Zhuang, *Science*, 2008

N-STORM Summary

Stochastic Optical Reconstruction Microscopy (2D, 3D)

Photo switchable dyes

Localization of each fluorescent molecule with nanometer precision

Construction of a Super-Resolution image from these points

Activator excitation with 3 lines possible (405, 457, 561)

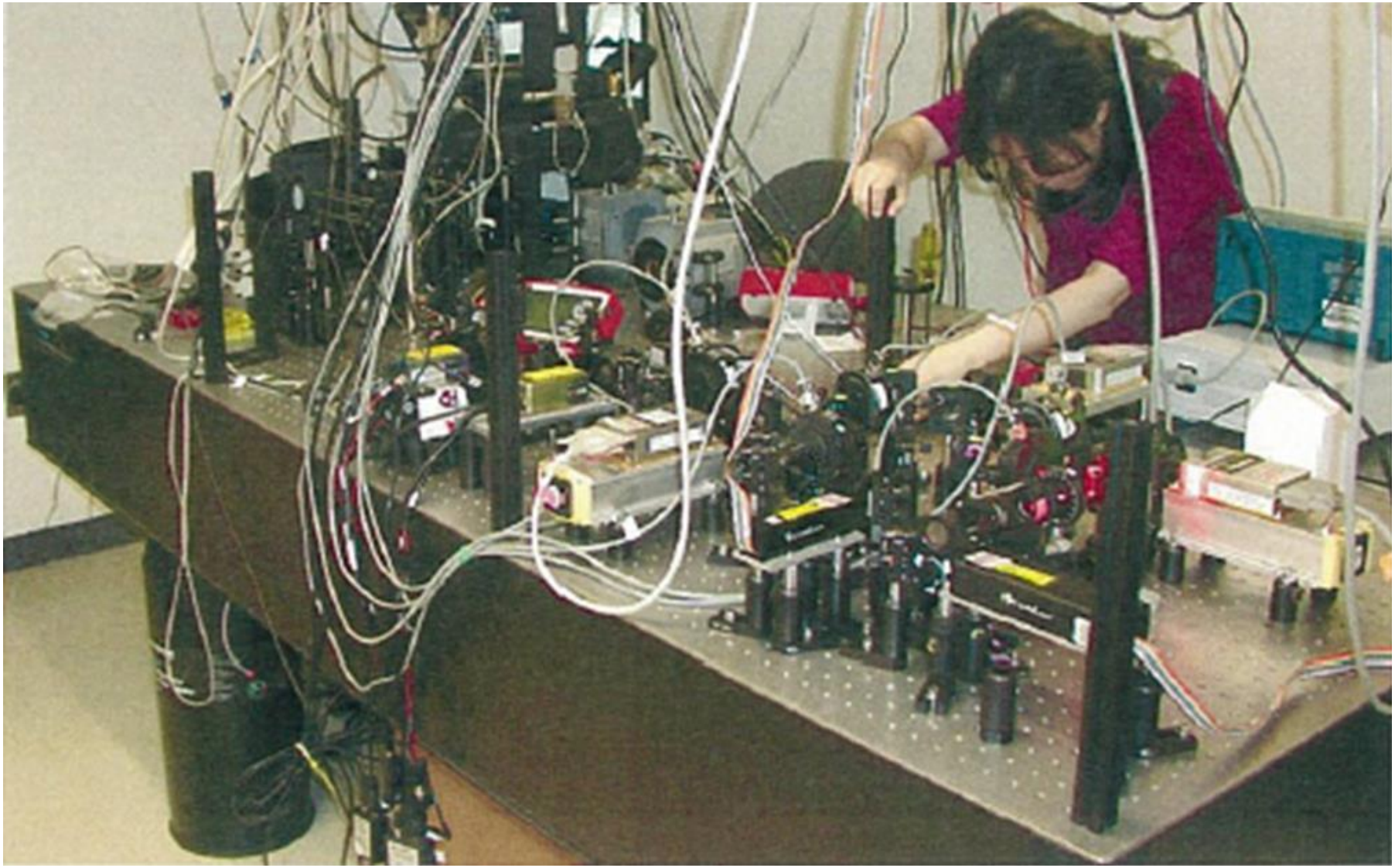
Imaging with high power 647nm laser (300 mW)

3D STORM using astigmatic (cylindrical) lens

10-fold increase in resolution (XY: 20 nm, Z: 50 nm)

Acquisition speed – Minutes

N-STORM Instrumentation



N-STORM Instrumentation

NIKON CORPORATION
Instruments Company


n-SIM

N-SIM

N (**N**ikon) – SIM ("**S**tructured **I**llumination **M**icroscopy")

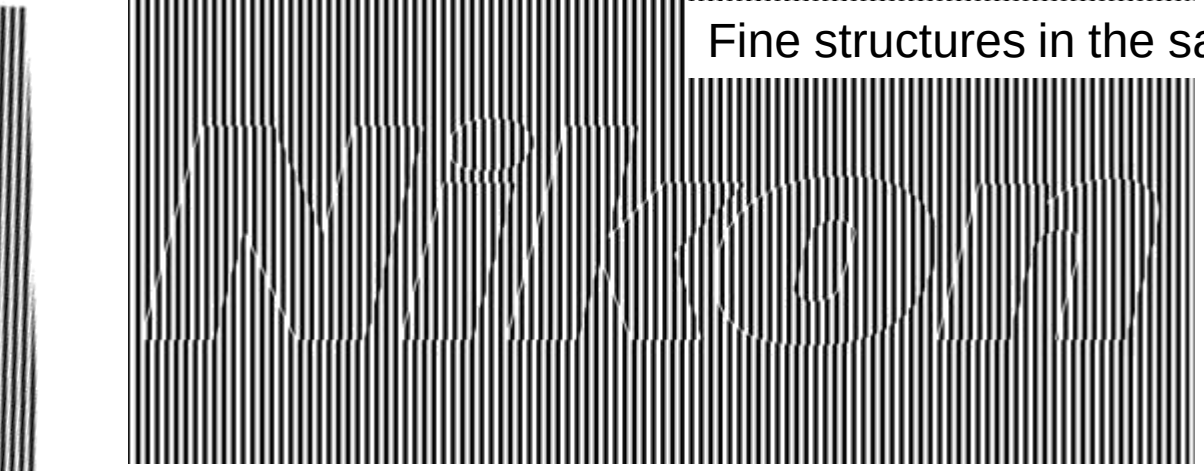
Two times better resolution than diffraction limit: SIM ~ 100 nm / Wide Field ~ 200 nm

Illuminate with diffraction-limited grid pattern - Image reconstruction

Licensed from UCSF

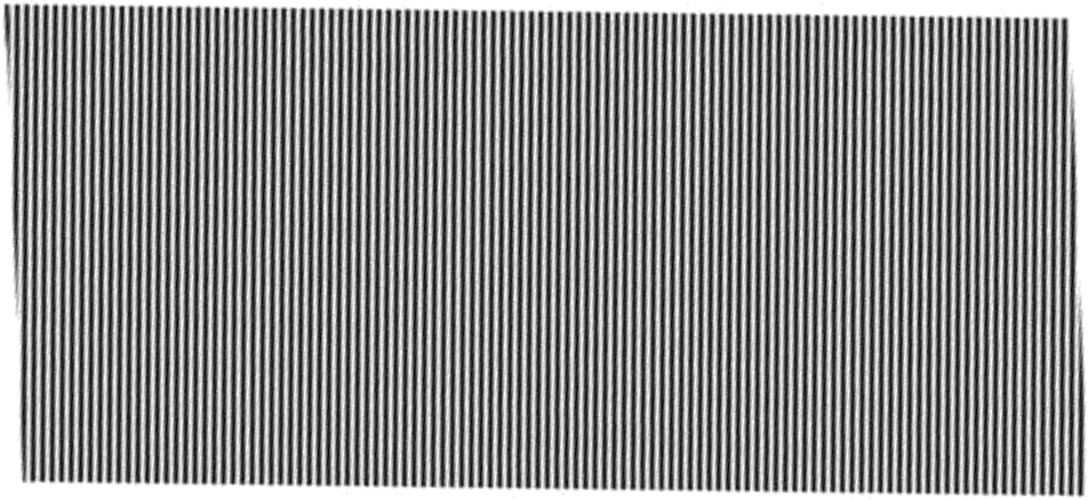
Developed by Dr. Mats G. L. Gustafsson, Dr. John W. Sedat and Dr. David A. Agard of UCSF

N-SIM Principle



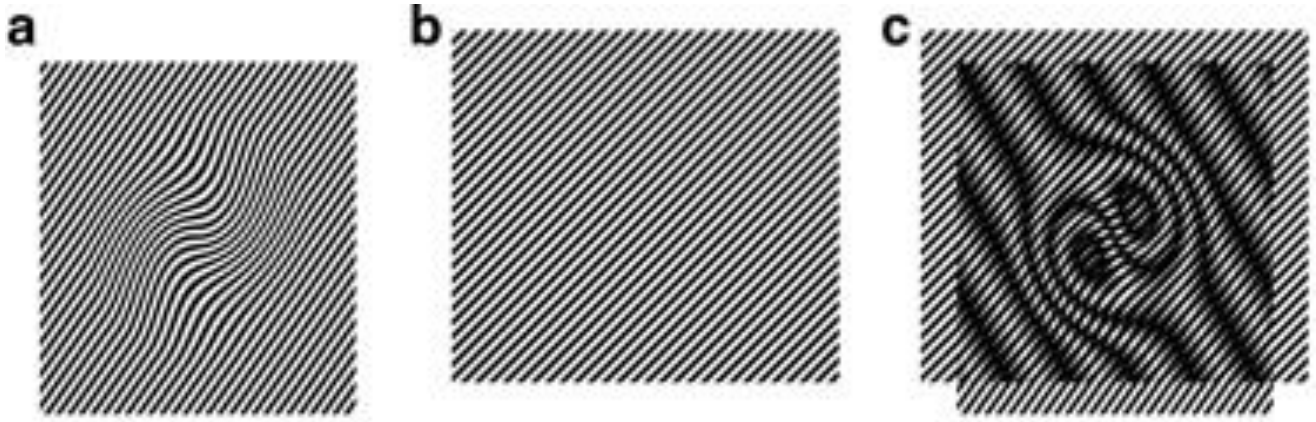
Fine structures in the sample

Moiré pattern



Structured light patterns

N-SIM Principle

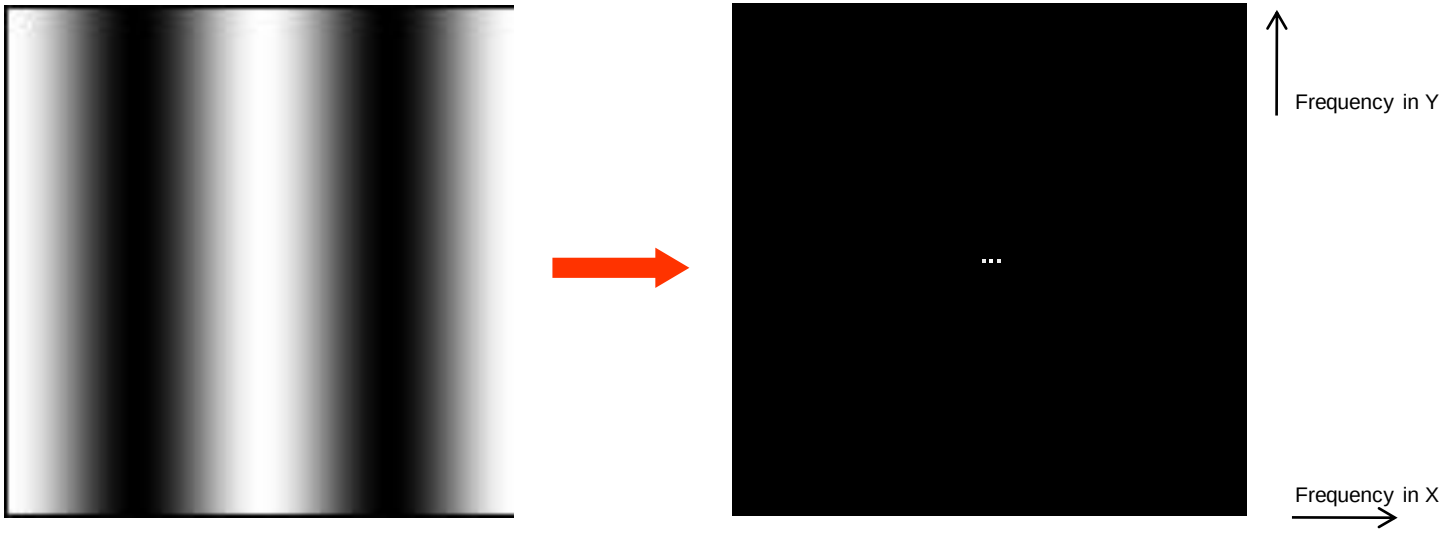


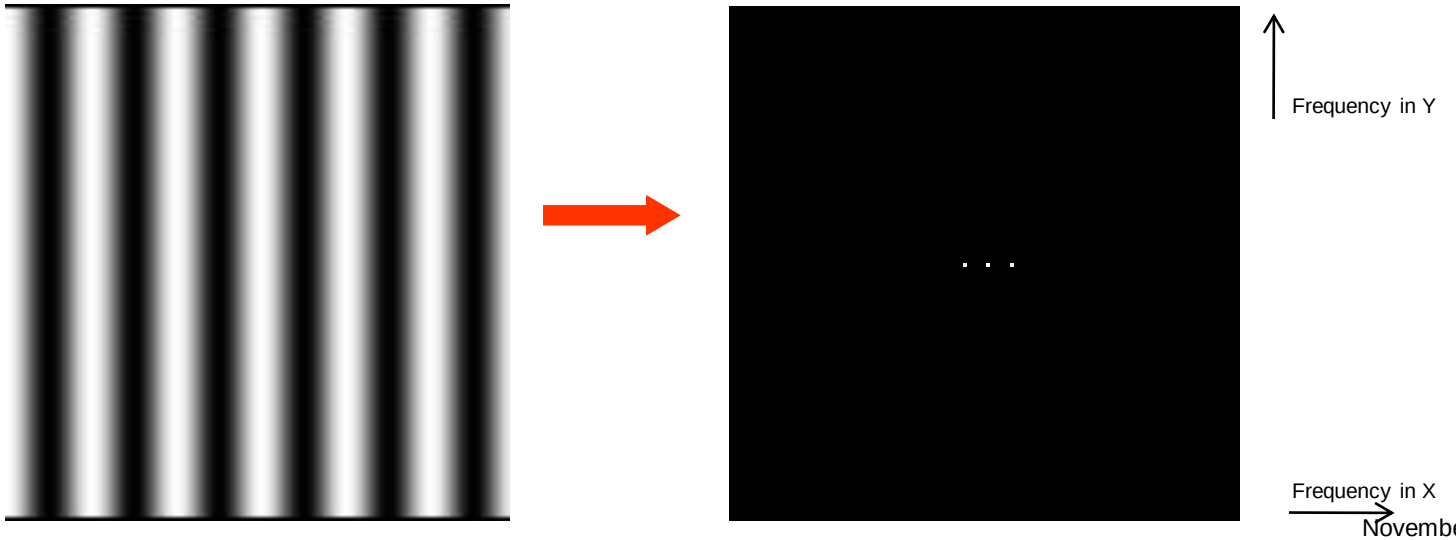
An unknown object (a) is illuminated by a known pattern of light (b) resulting in a moire pattern (c).

From Moire pattern and known pattern it can be concluded to the unkown pattern!

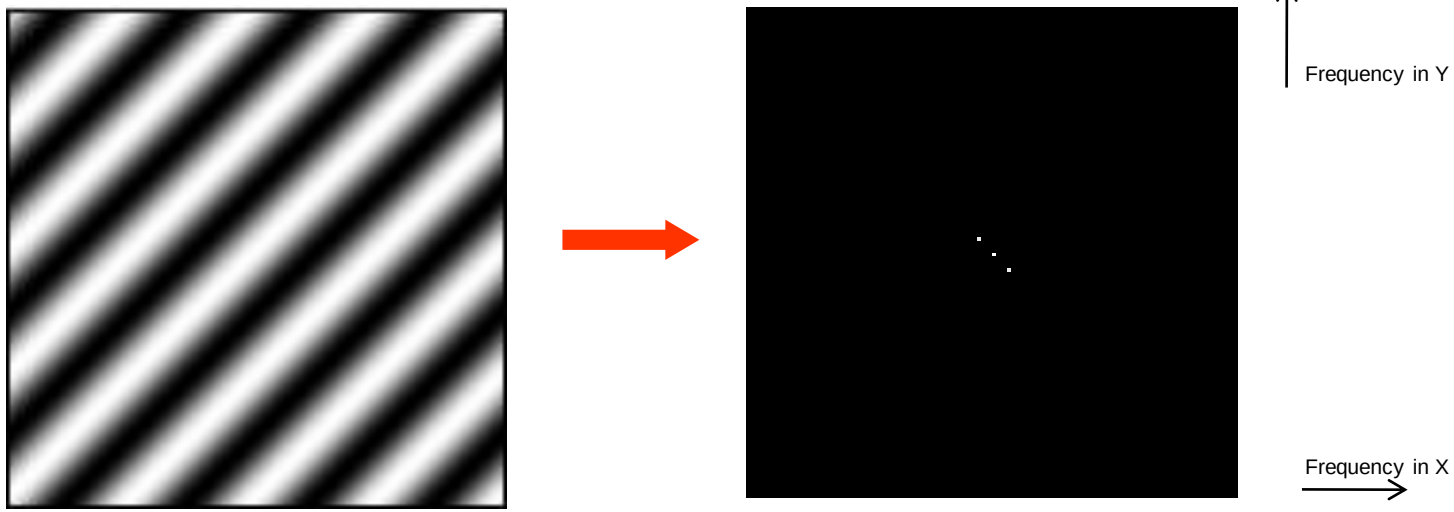
J.B.J. Fourier provides the „key“ to the significant improvement of resolution!

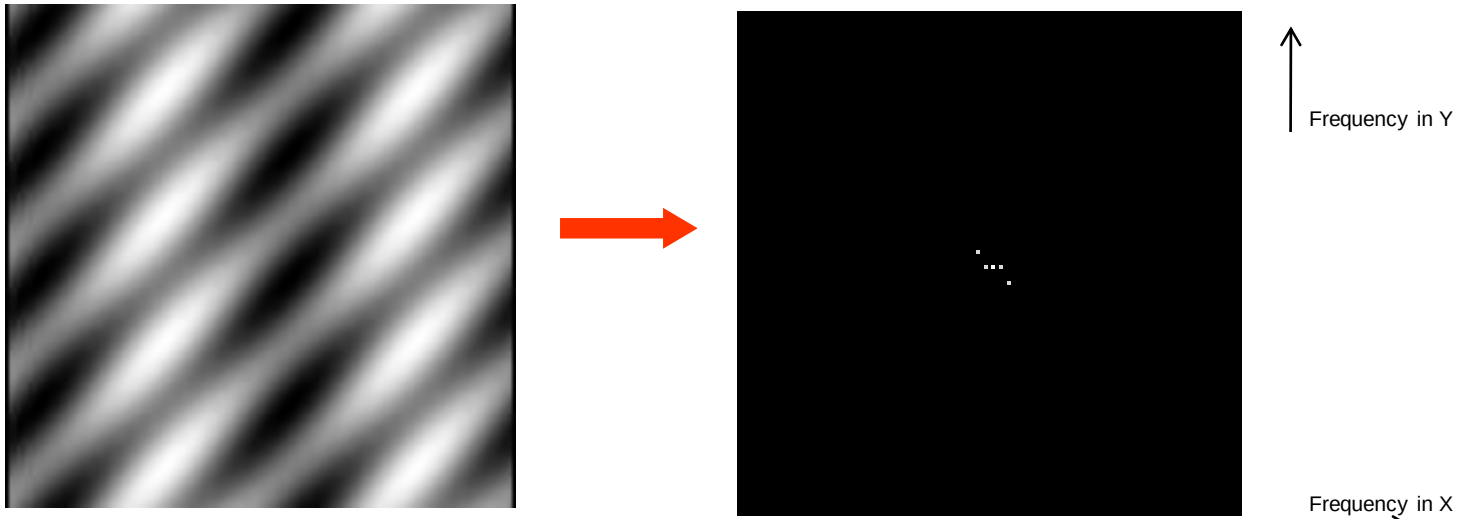
From Real Image to Fourier Image…



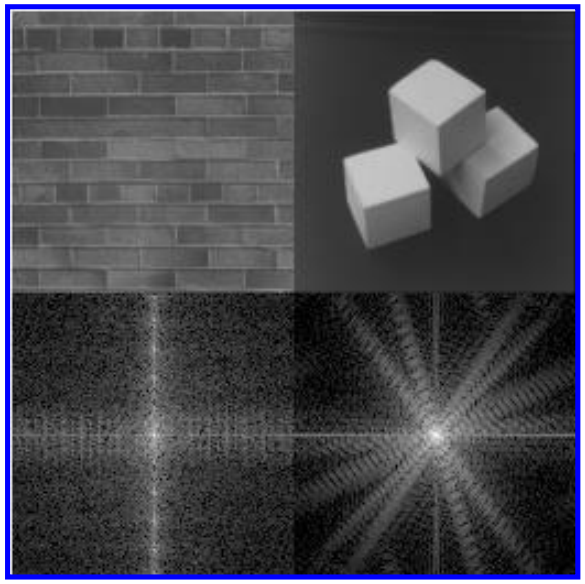


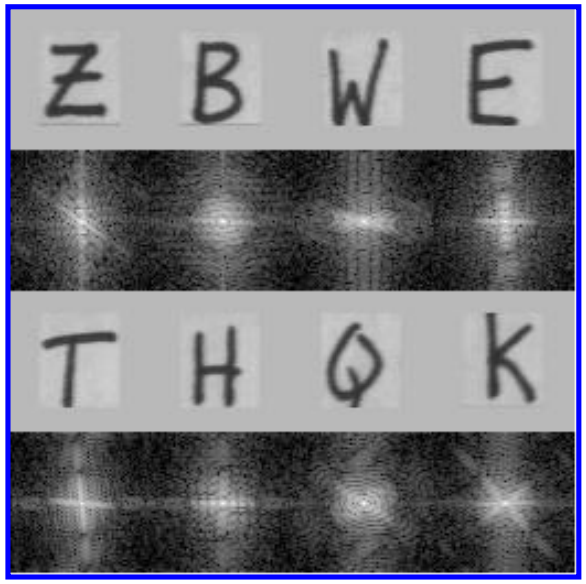
From Real Image to Fourier Image…

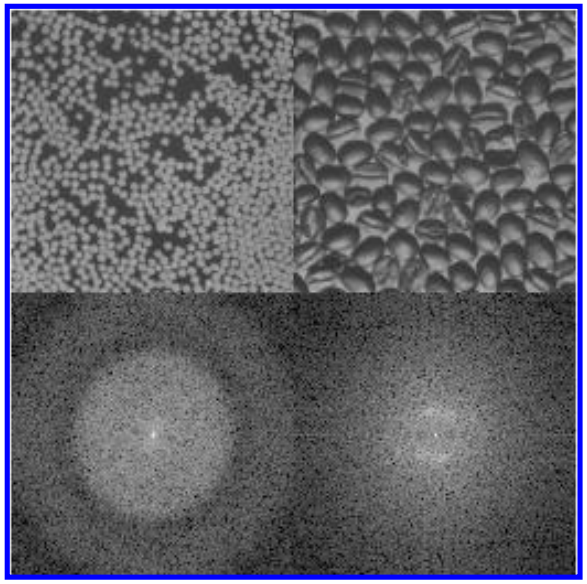




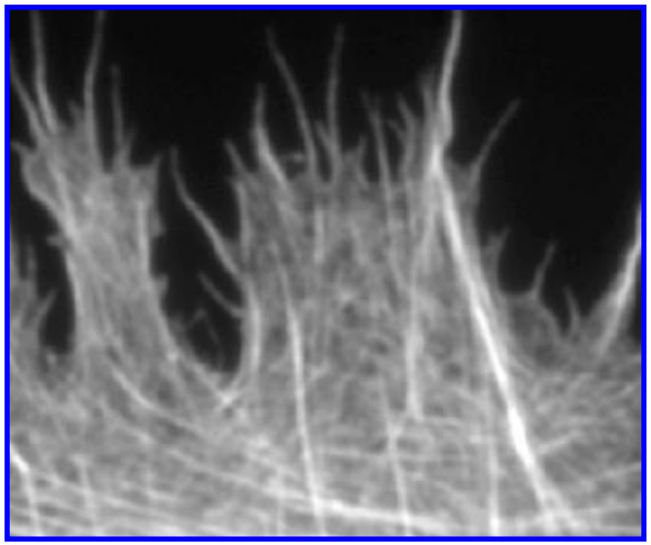
From Real Image to Fourier Image…

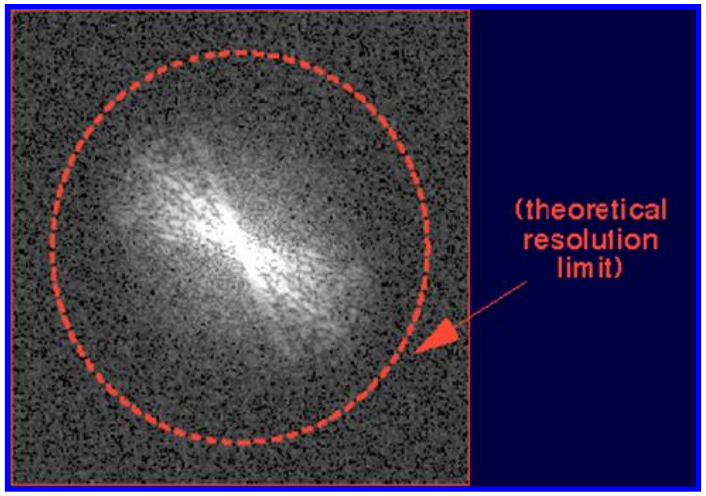






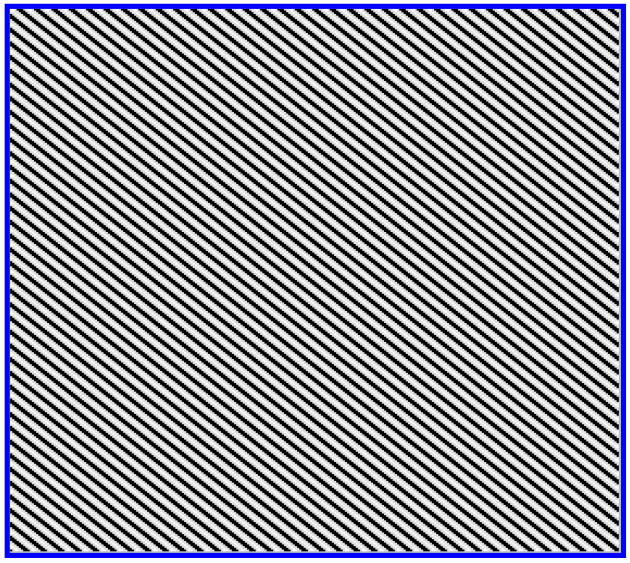
Real space ⇒ Fourier space

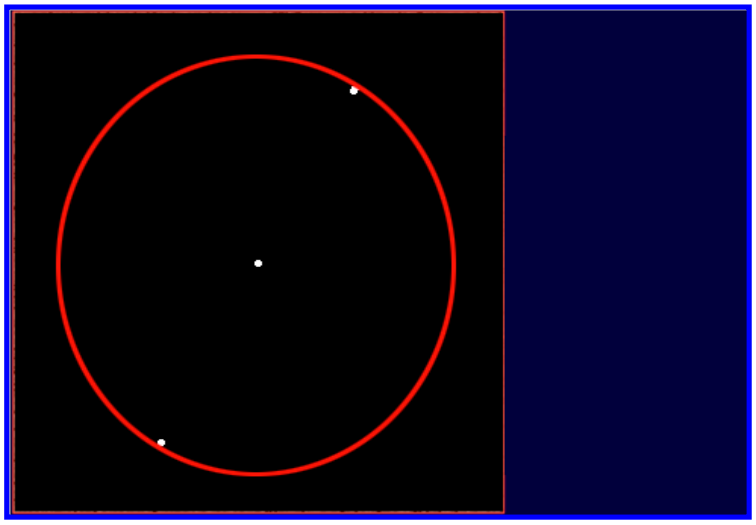




Real space

Fourier space





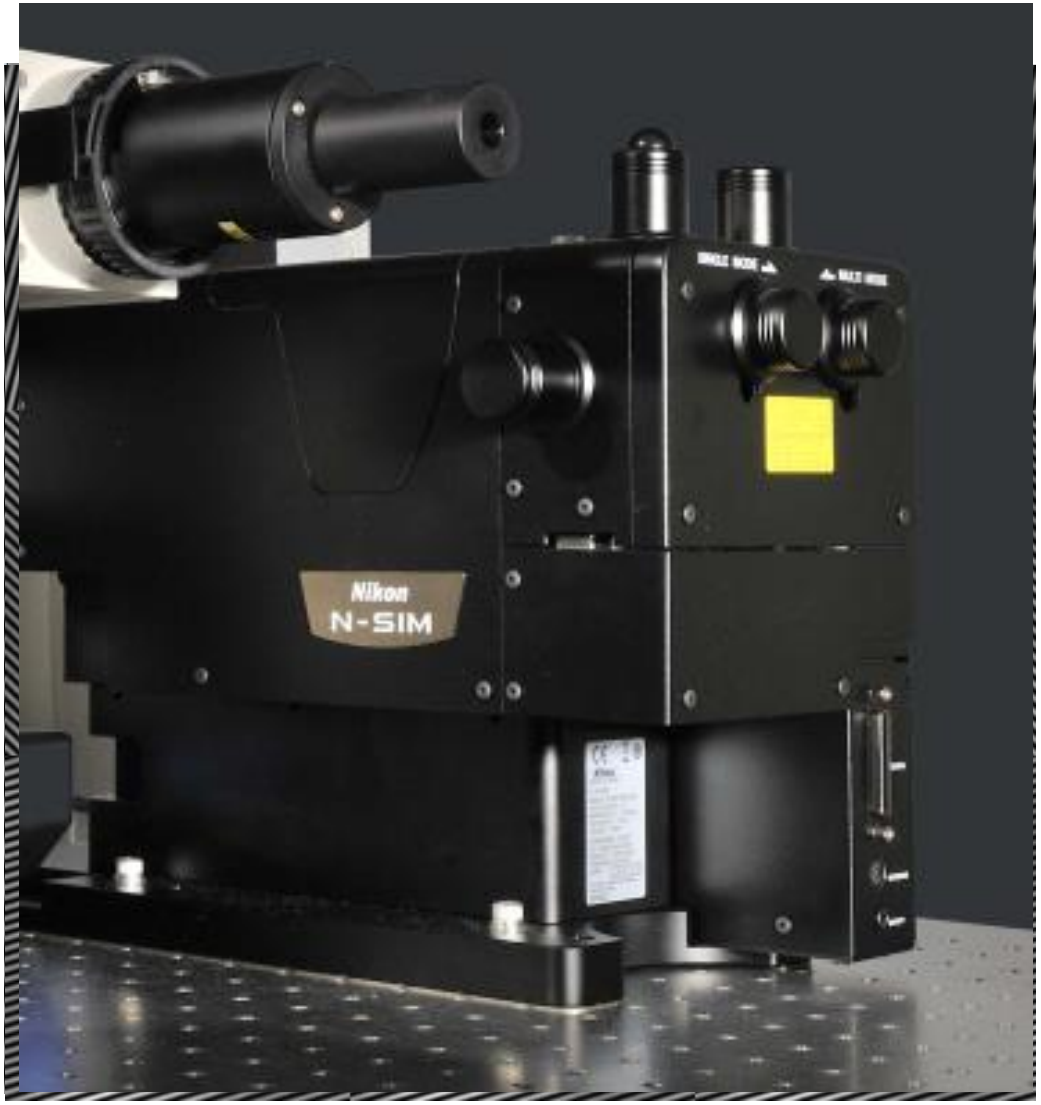
N-SIM Illuminator / SIM Image Set

Diffraction limited grid

Shifted over
3 positions (2D/TIRF-SIM)
5 positions (3D-SIM)

Rotated to 3 orientations

Resulting in 9/15 images
9 (3 shifts x 3 rotations)
15 (5 shifts x 3 rotations)



Reconstruction in reciprocal space

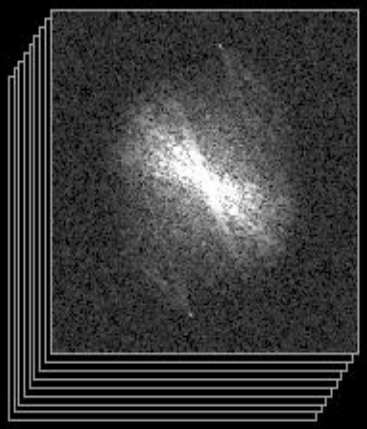
Results from Angle 3 w/3Phases

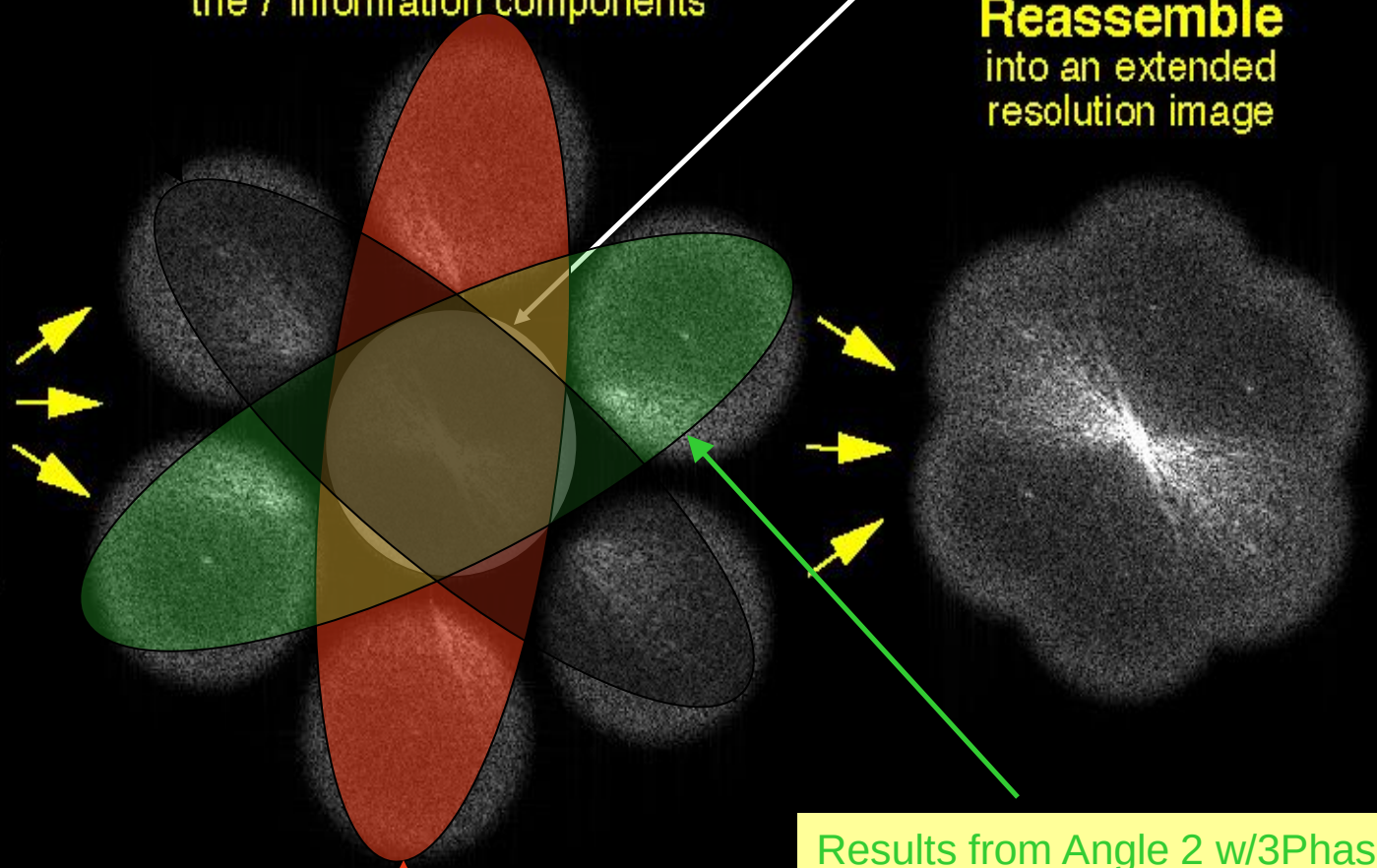
Standard Resolution Info – Same for All Angles and All Phases

Separate
the 7 information components

Acquire
images with
3 pattern angles
x 3 phases.

Reassemble
into an extended
resolution image





Results from Angle 2 w/3Phases

Results from Angle 1 w/3Phases

Mats Gustafsson - UCSF

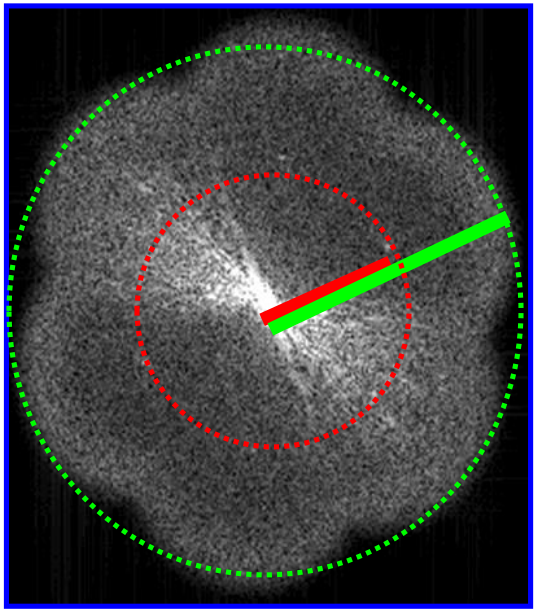
Resolution is extended

Objective aperture radius

Theoretical extended radius after reconstruction processing is 2x original

High frequency (higher resolution) information is further from origin

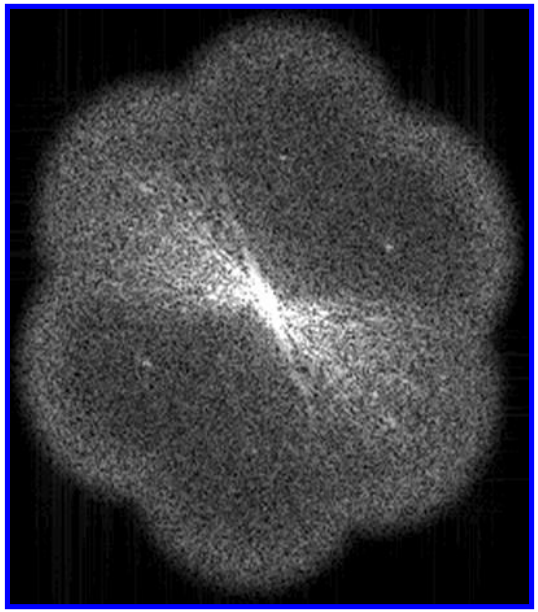
New resolution is 2x original

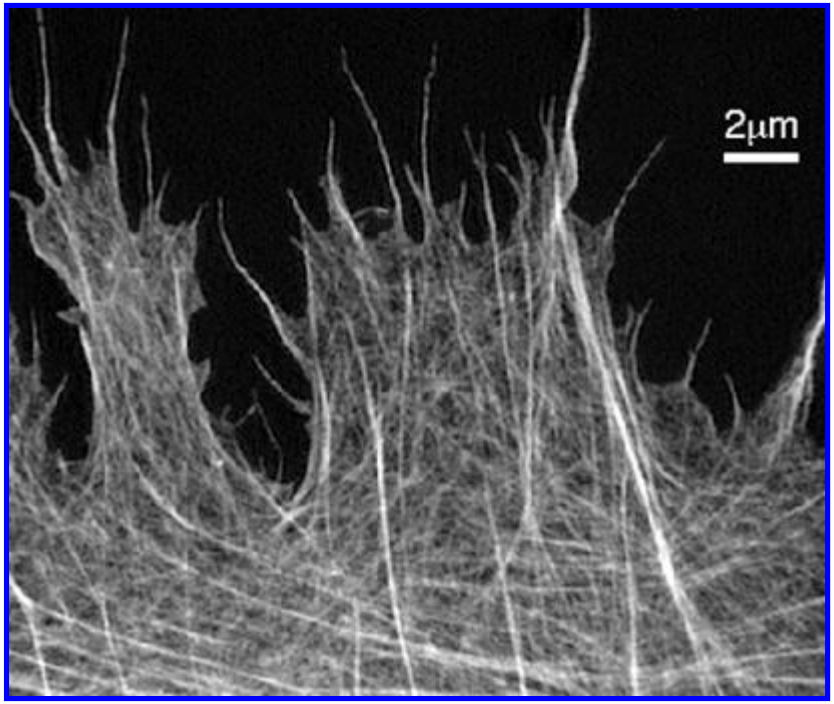


New Resolution Limit using SIM

Resolution is extended

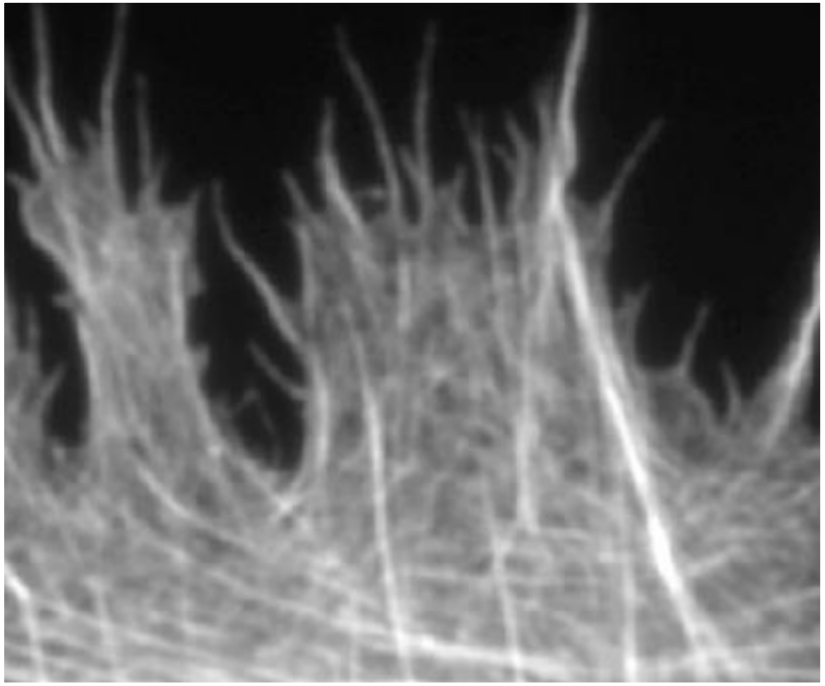
- Final step is to Re-transform extended resolution Fourier image back to real space
- Includes additional processing to compensate for the point spread function of the system.





Conventional vs. SIM

Conventional Epi-FL Microscopy



N-SIM

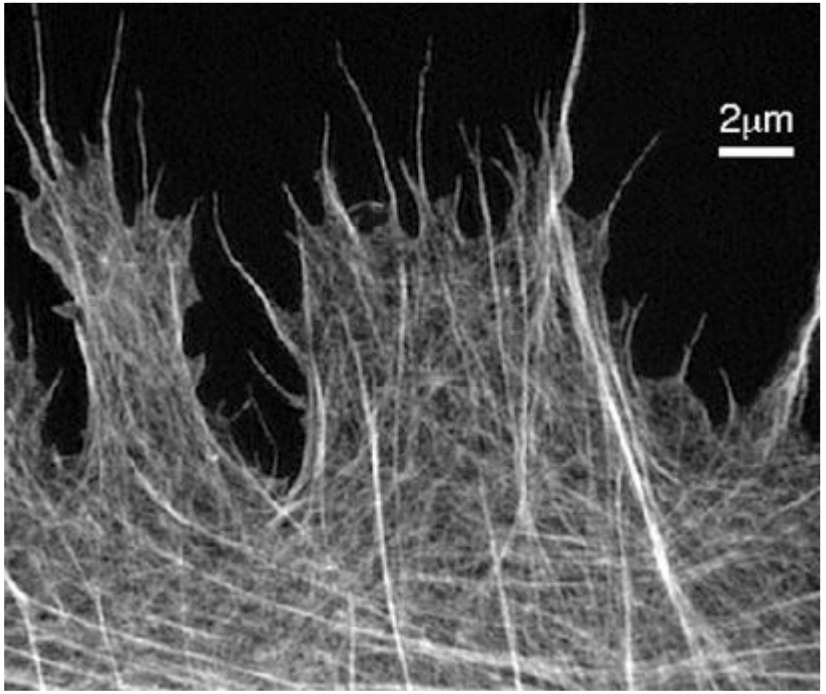
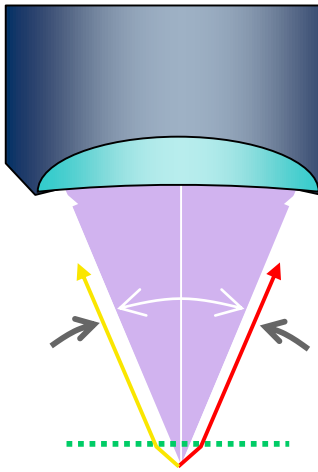


Image From - Mats Gustafsson - UCSF

N-SIM image has twice resolution compared to conventional microscope.

Principle of SIM - Diffraction

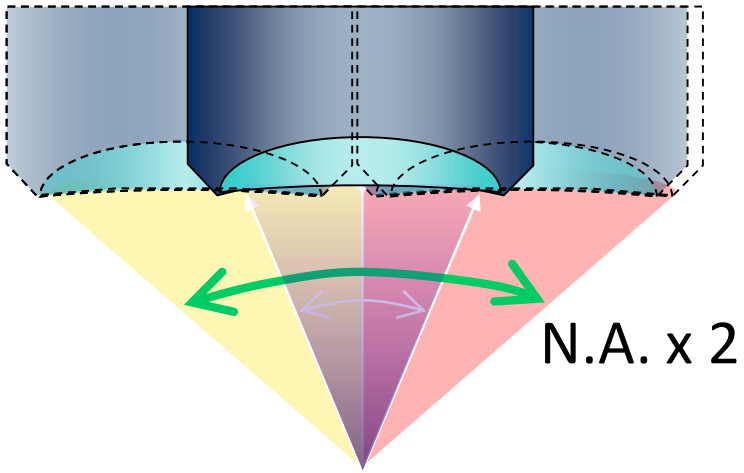


N.A.

Structured
Illumination

Resolution depends on Numerical Aperture (NA).
Diffracted light of the fine structure of sample cannot be captured by objective lens.

However, the fine structure of the sample can be **captured as moire pattern** by illumination of structured light.



N.A. x 2

As a result, we can get images with **double NA**.

N-SIM applications

Flat or sparse samples

Samples features details between 100 and 200 nm

Dynamics ~ seconds or slower (0.6 sec/frame – 2D SIM)
(1.0 sec/frame – 3D SIM)

Actin/Cyto skeleton

Microtubuli

Mitochondria

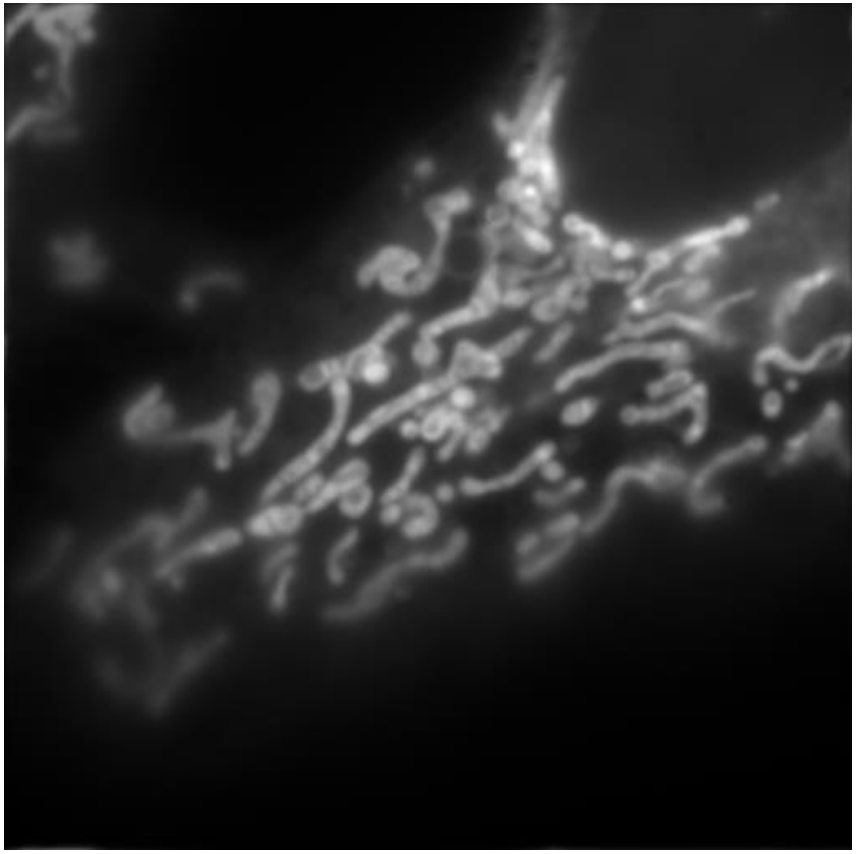
Bacteria

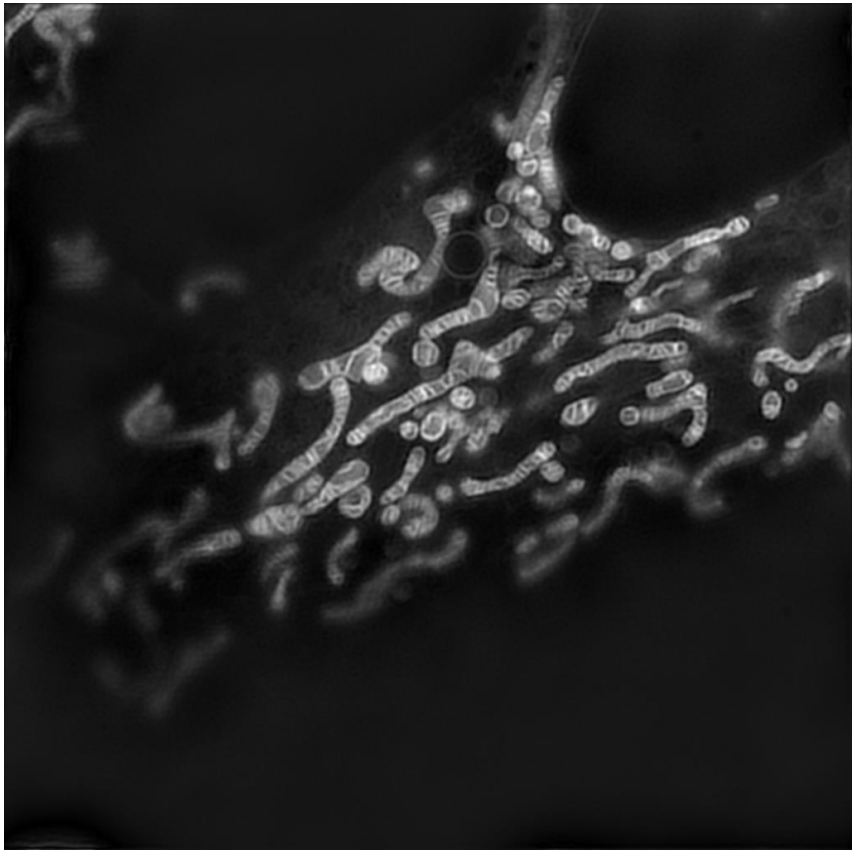
…..

Conventional vs. SIM

Time-Series - 1fps

Conventional vs. SIM

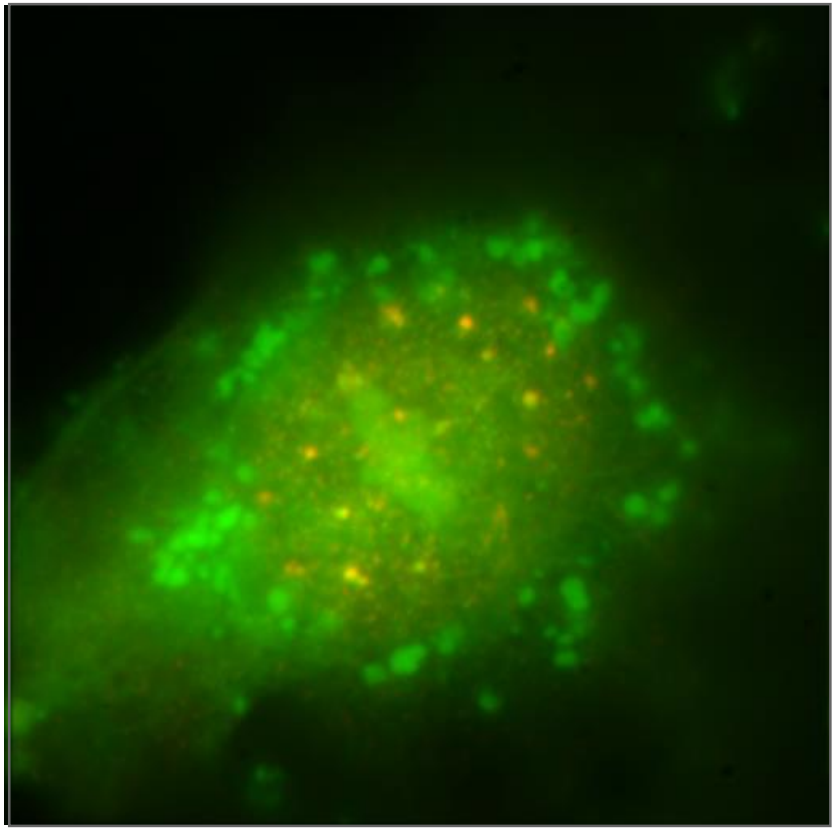




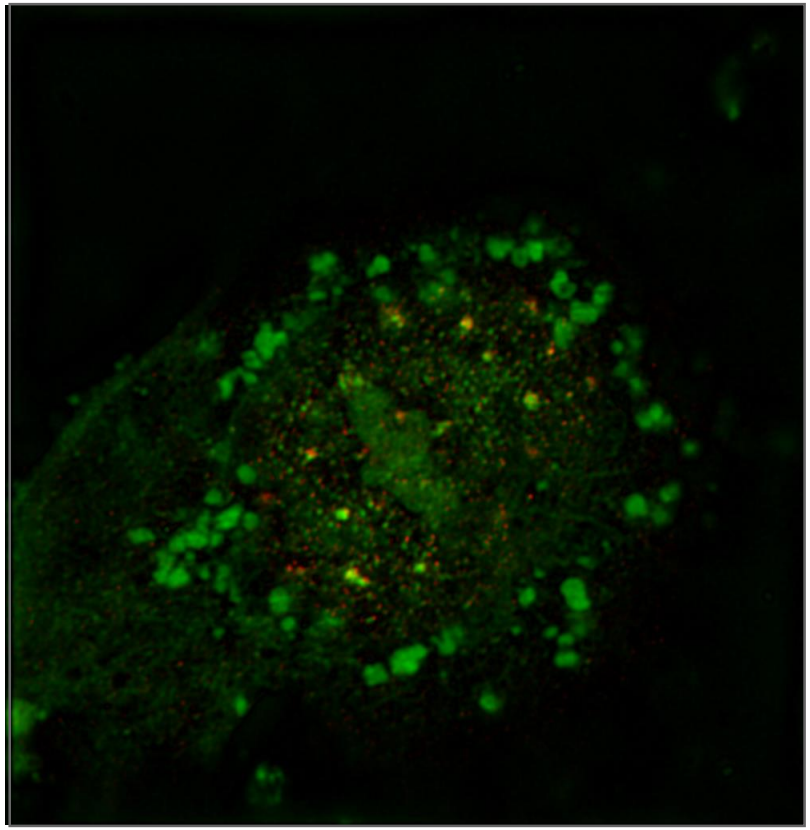
Mitochondria - Acquisition time 1.8sec

Conventional vs. SIM (Multicolor)

Colocalization of VEGF signal receptor (Cy3) and ubiquitin E3 ligase (FITC)

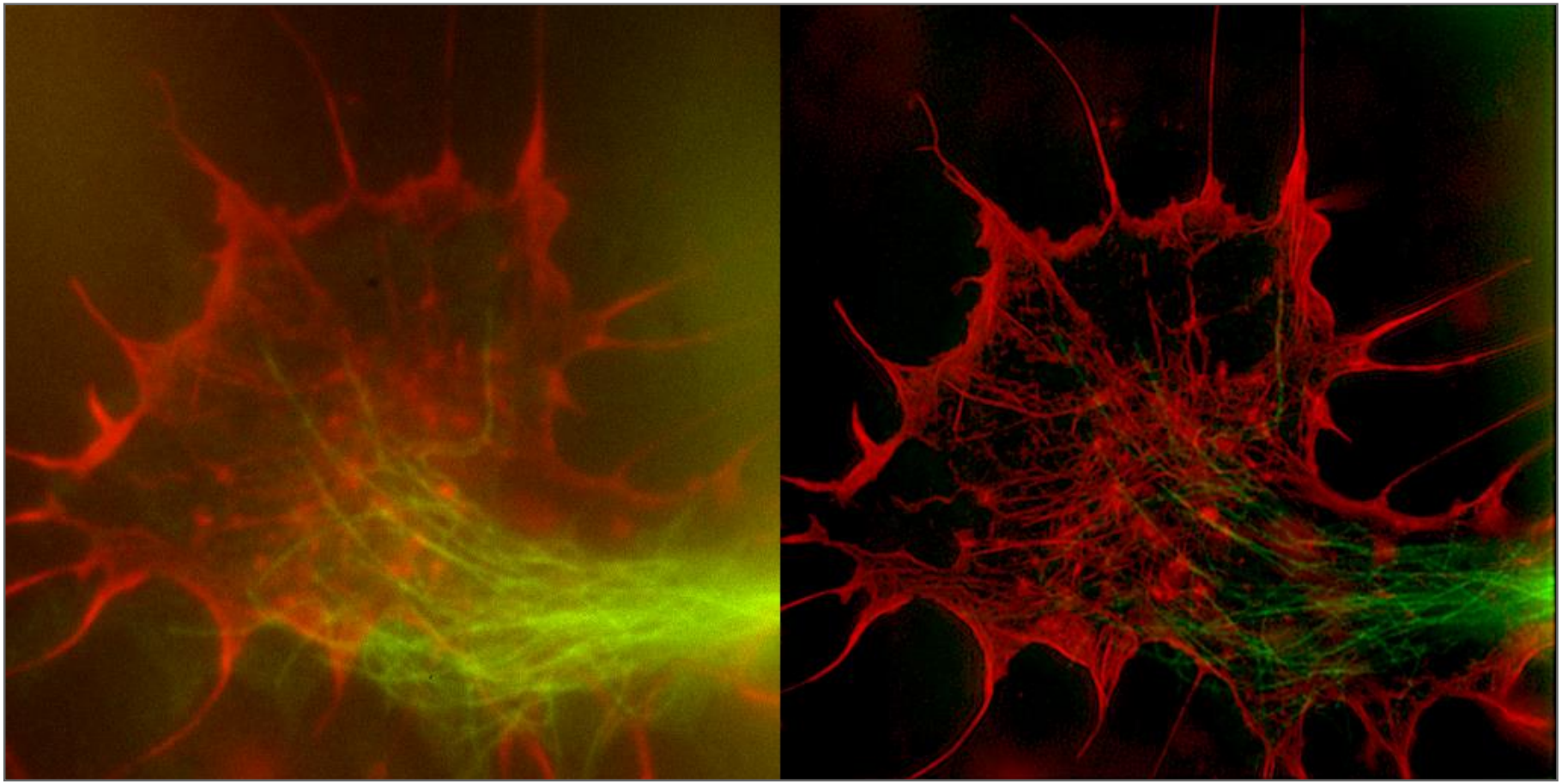


Conventional

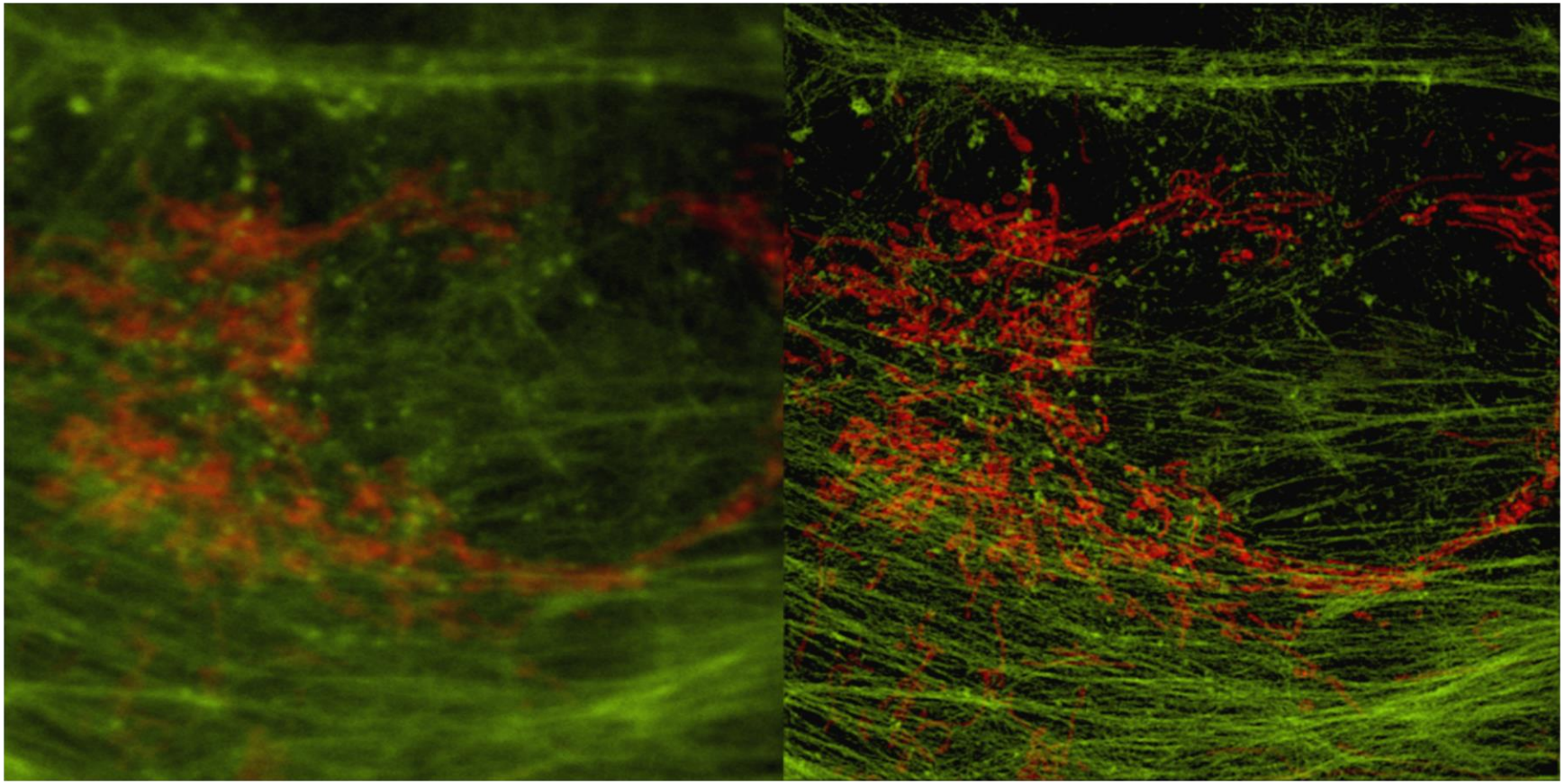


3D-SIM

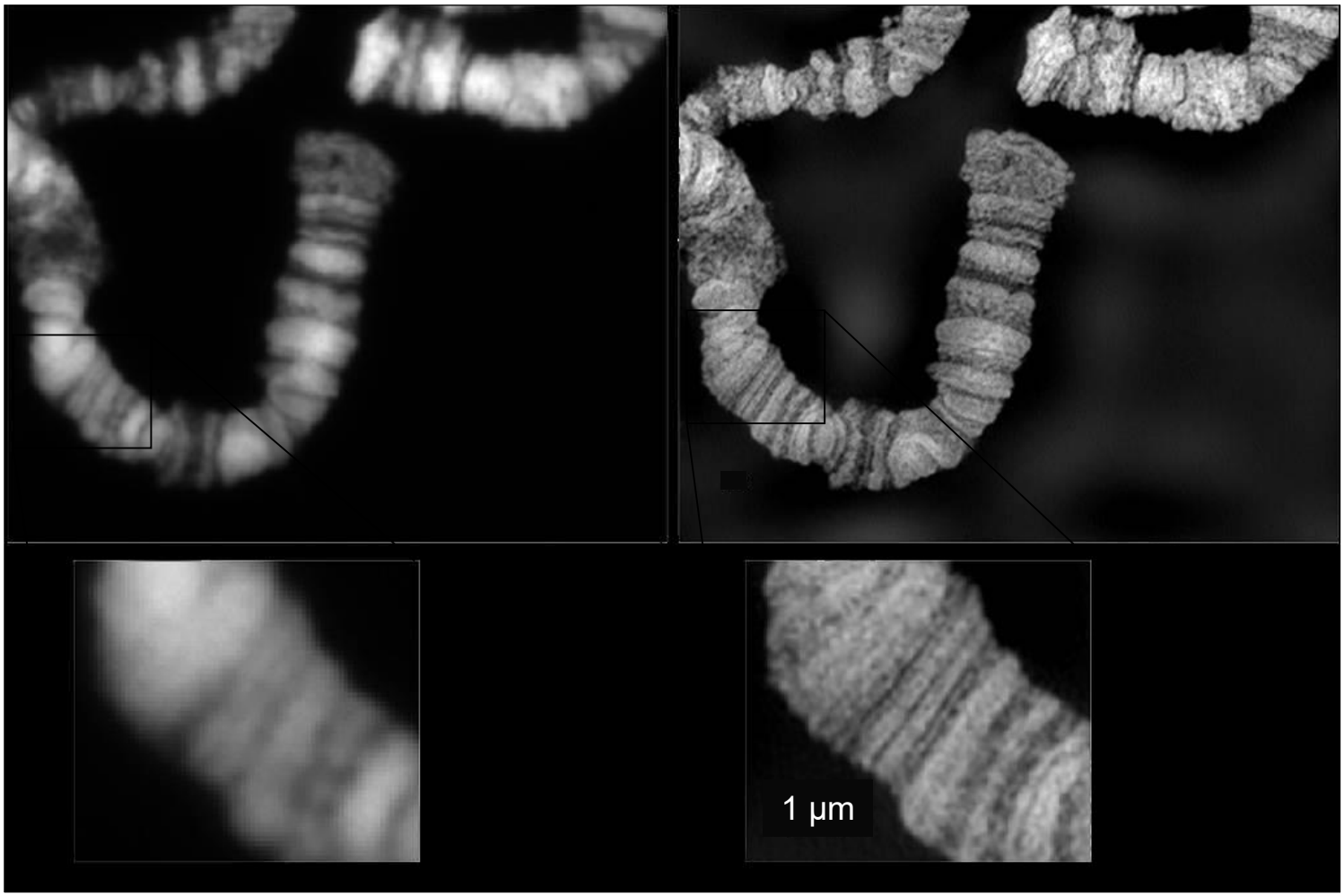
Conventional vs. SIM (Multicolor)



Conventional vs. SIM (Multicolor)



Conventional vs. SIM

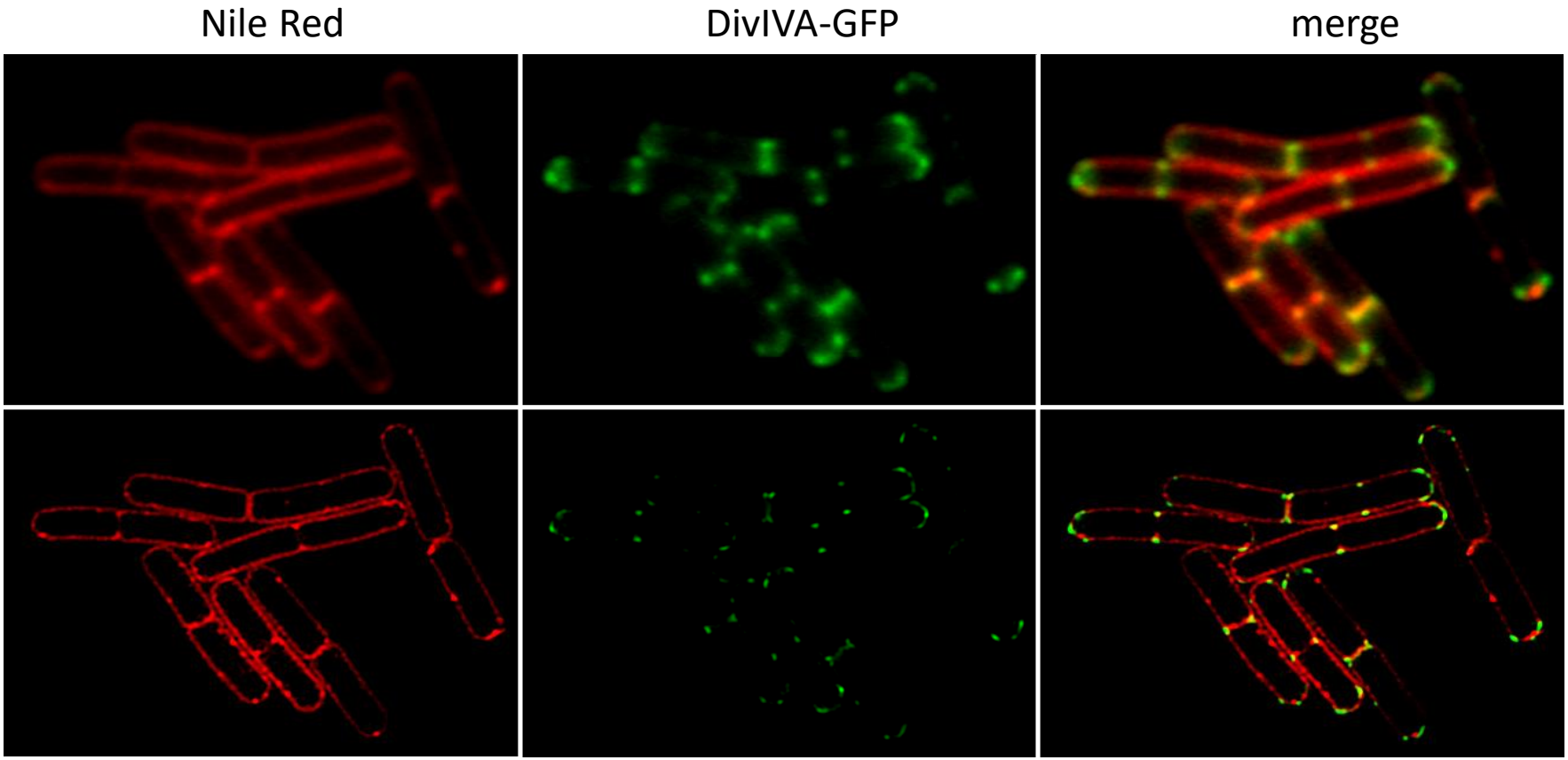


Drosophila polytene chromosome squash

Data by Harry Saumweber

Nikon N-SIM system, 3D-SIM

NIKON CORPORATION
Instruments Company



Bacillus subtilis bacterium stained with membrane dye Nile Red (red), and expressing the cell division protein DivIVA fused to GFP (green).

The superior resolution of N-SIM system allows for accurate localization of the protein during division.

Henrik Strahl & Leendert Hamoen, Centre for Bacterial Cell Biology, Newcastle University

N-SIM Summary

Structured Illumination Microscopy (2D, 3D, TIRF)

Creation of a diffraction pattern on the sample

Interaction of the overlying pattern with fine patterns in the sample creates Moiré effect (= interference pattern)

Based on this information, the original pattern can be determined computationally

Resolution: 2-fold increase in Resolution XY: 100nm, Z: 250 nm (~85 nm with TIRF)

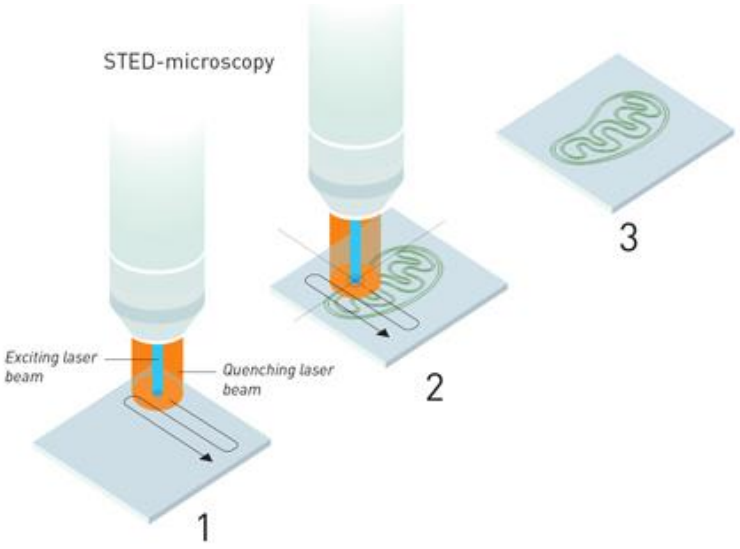
Common staining procedure (standard fluorophores)

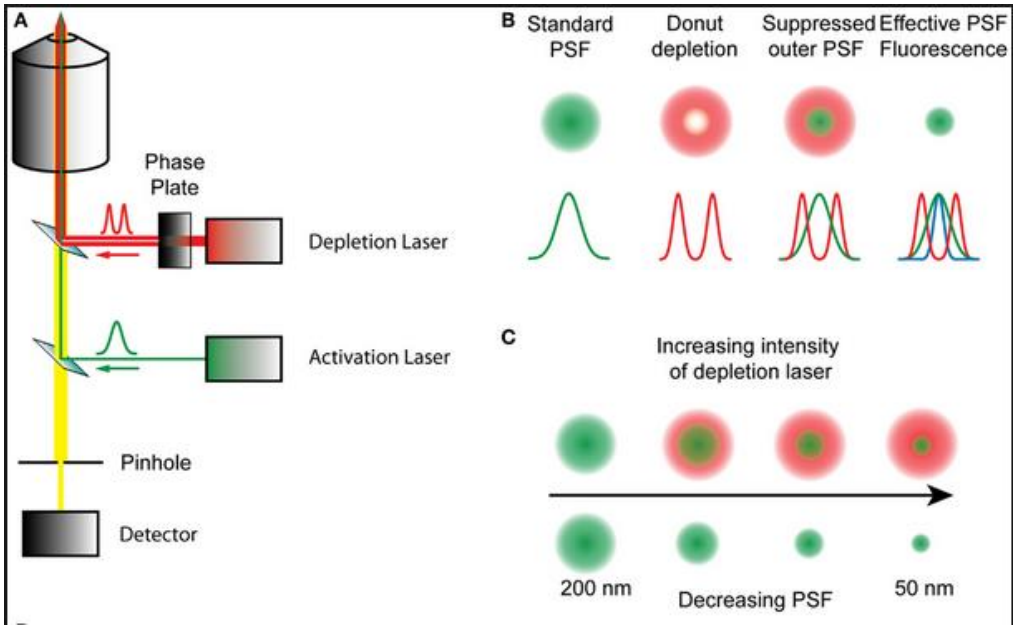
Suitable for live cell imaging

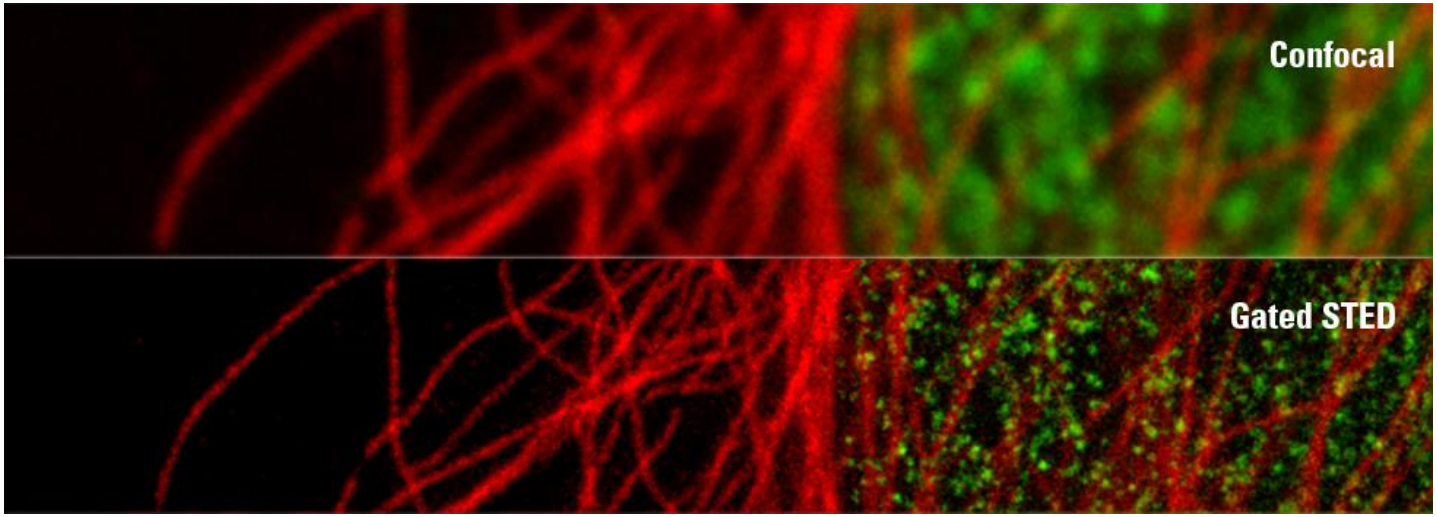
Up to 5 Laser Lines (from: 405/457/488/515/532/561/647)

N-SIM Instrumentation

STED (Stimulated emission depletion)







Děkuji za pozornost