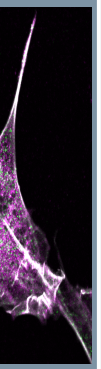
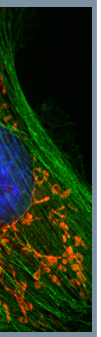

Super Resolution Microscopy

SIM
AIRYSCAN
PALM/STORM

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Biological Physics and Cell Signaling Group
IFGW/IB
athomaz@ifi.unicamp.br

Instituto de Física Gleb Wataghin

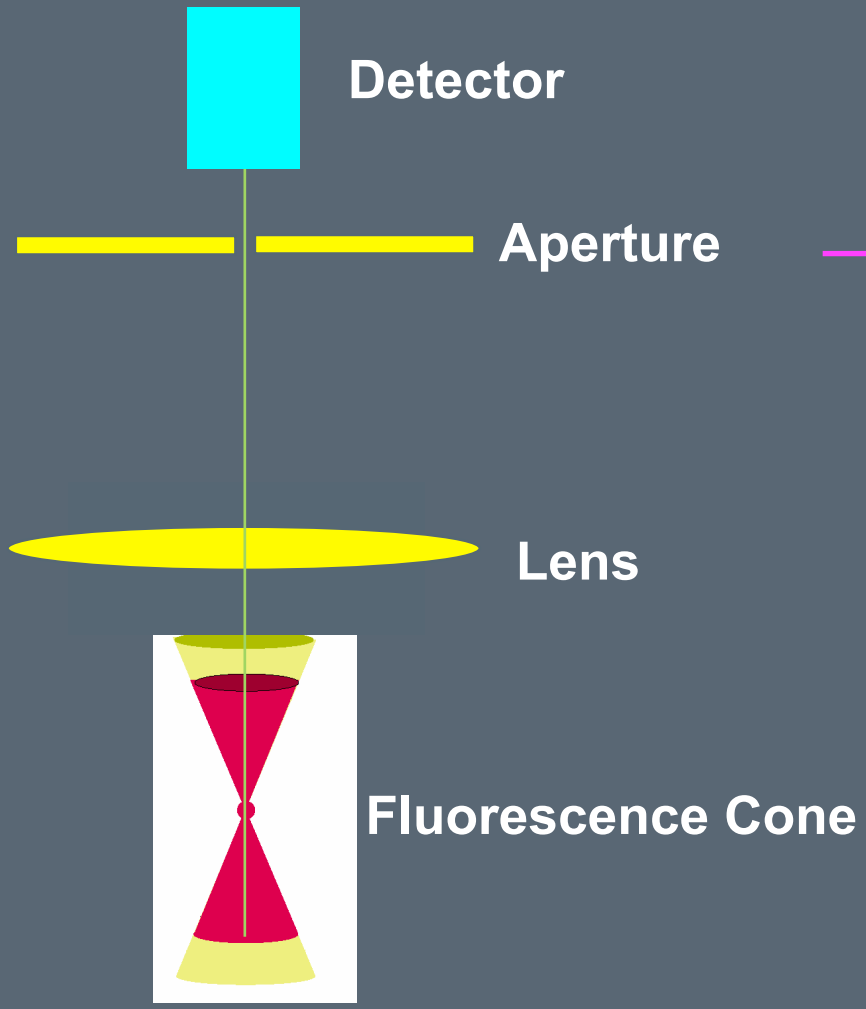
Instituto de Biologia

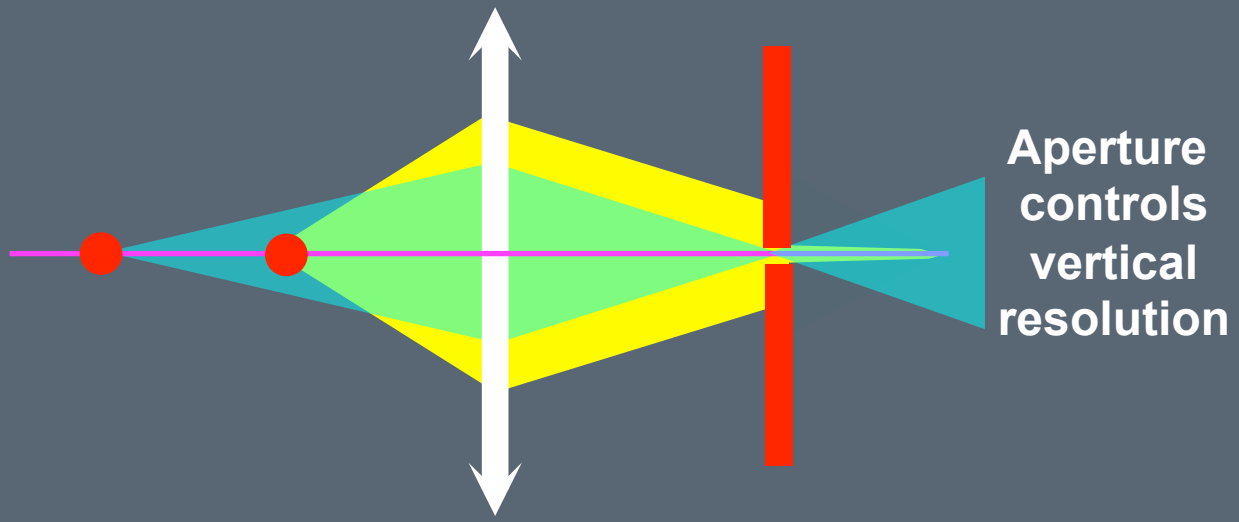
INFABIC

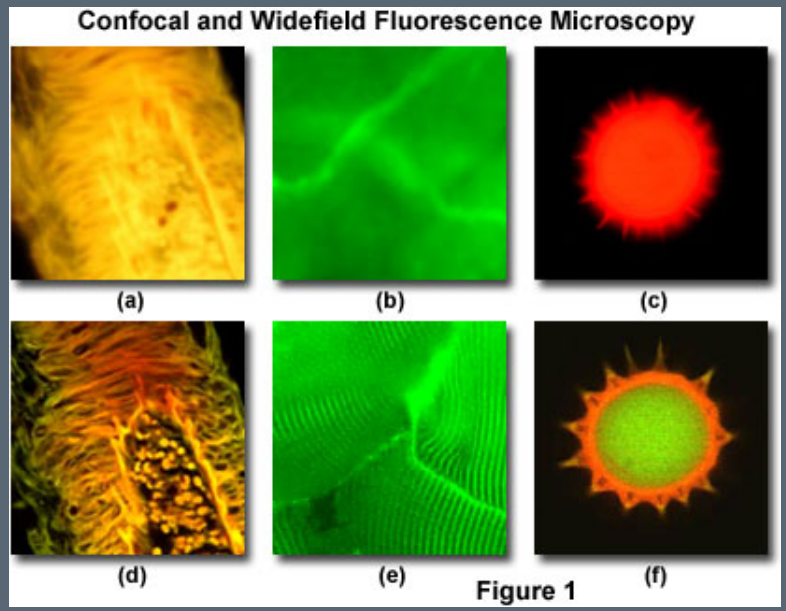
Resolution In Microscopy

How to achieve super resolution?

Confocal Microscopy: 3D images



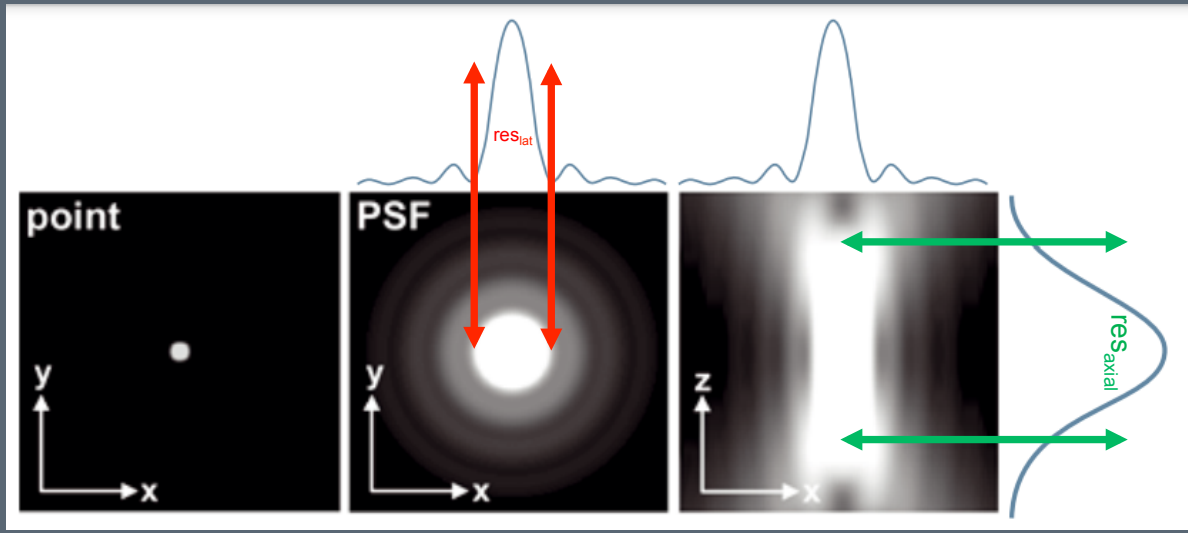




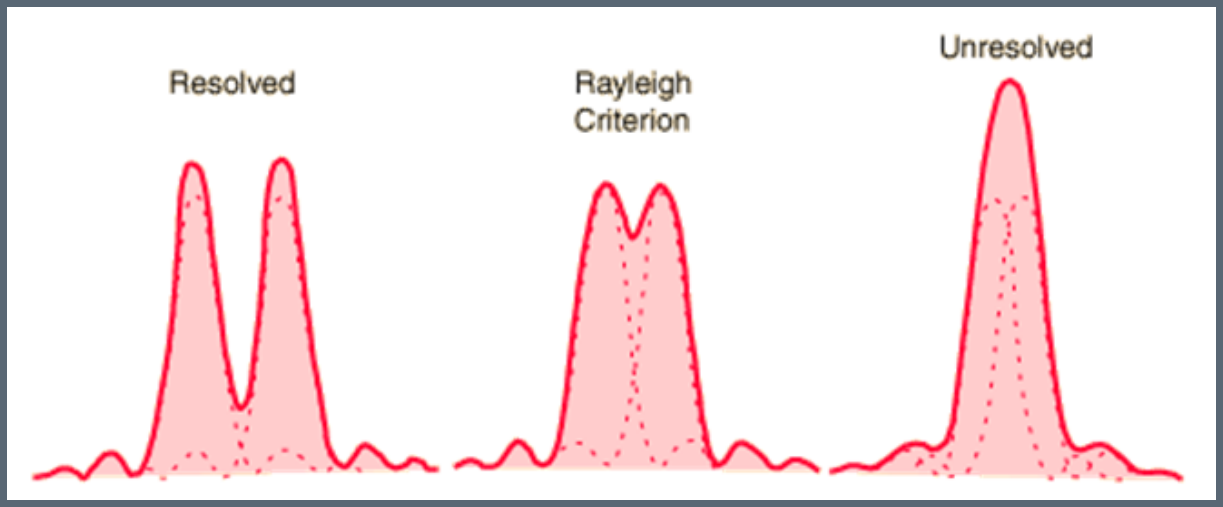
RESOLUTION – DIFFRACTION LIMITED

Point Spread Function PSF

Point like source has a size on the detector



$res \downarrow lat$ $\lambda / NA \uparrow$ $n\lambda / NA \uparrow$



Hayleigh criterion

Super resolution Microscopy

We need to deal with the diffraction limit!

Multiple techniques:

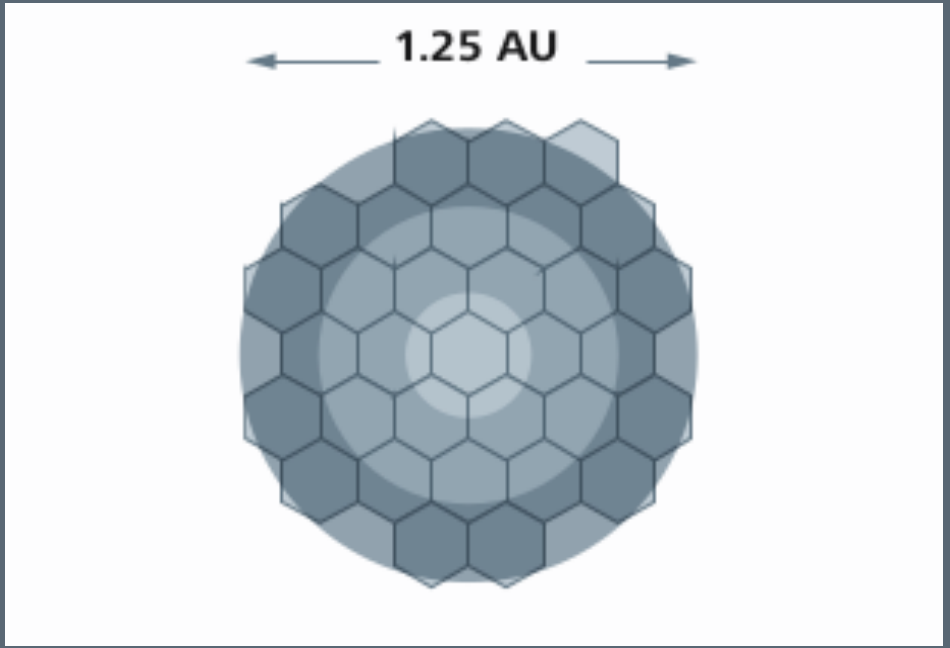
- SIM (Structured Illumination Microscopy) ~ 100nm
- Airy Scan ~ 100nm
- STED (Stimulated Emission Depletion Microscopy) ~ 60nm
- PALM (Photoactivated Localization Microscopy) ~ 10nm
- STORM (Stochastic Optical Reconstruction Microscopy) ~ 10nm

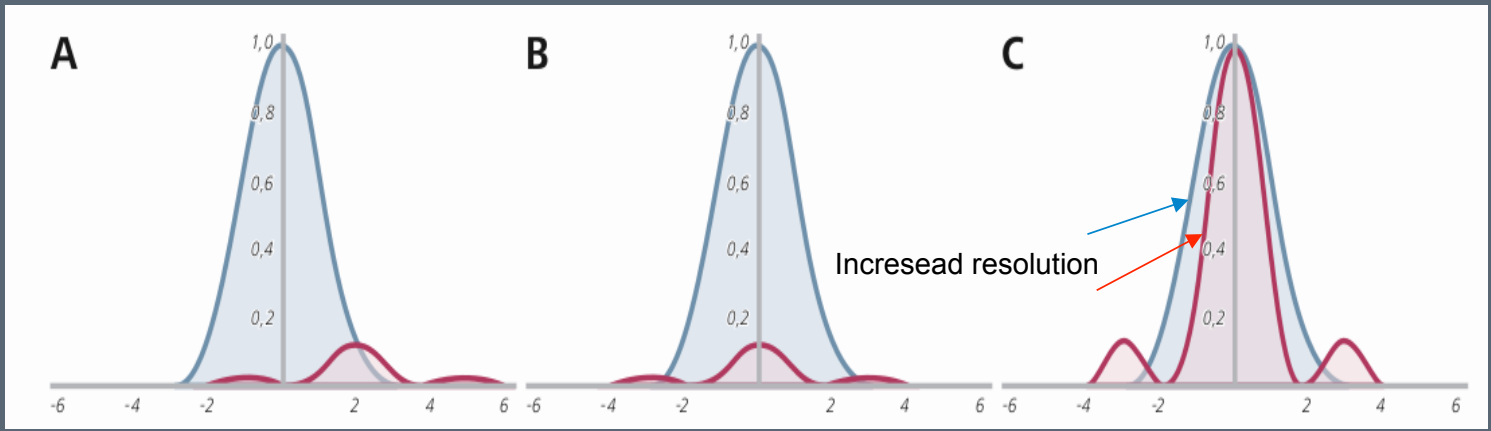
Airy Scan Microscopy

AIRY SCAN MICROSCOPY

32 GaAsP detectors concentric with microscope optical axis

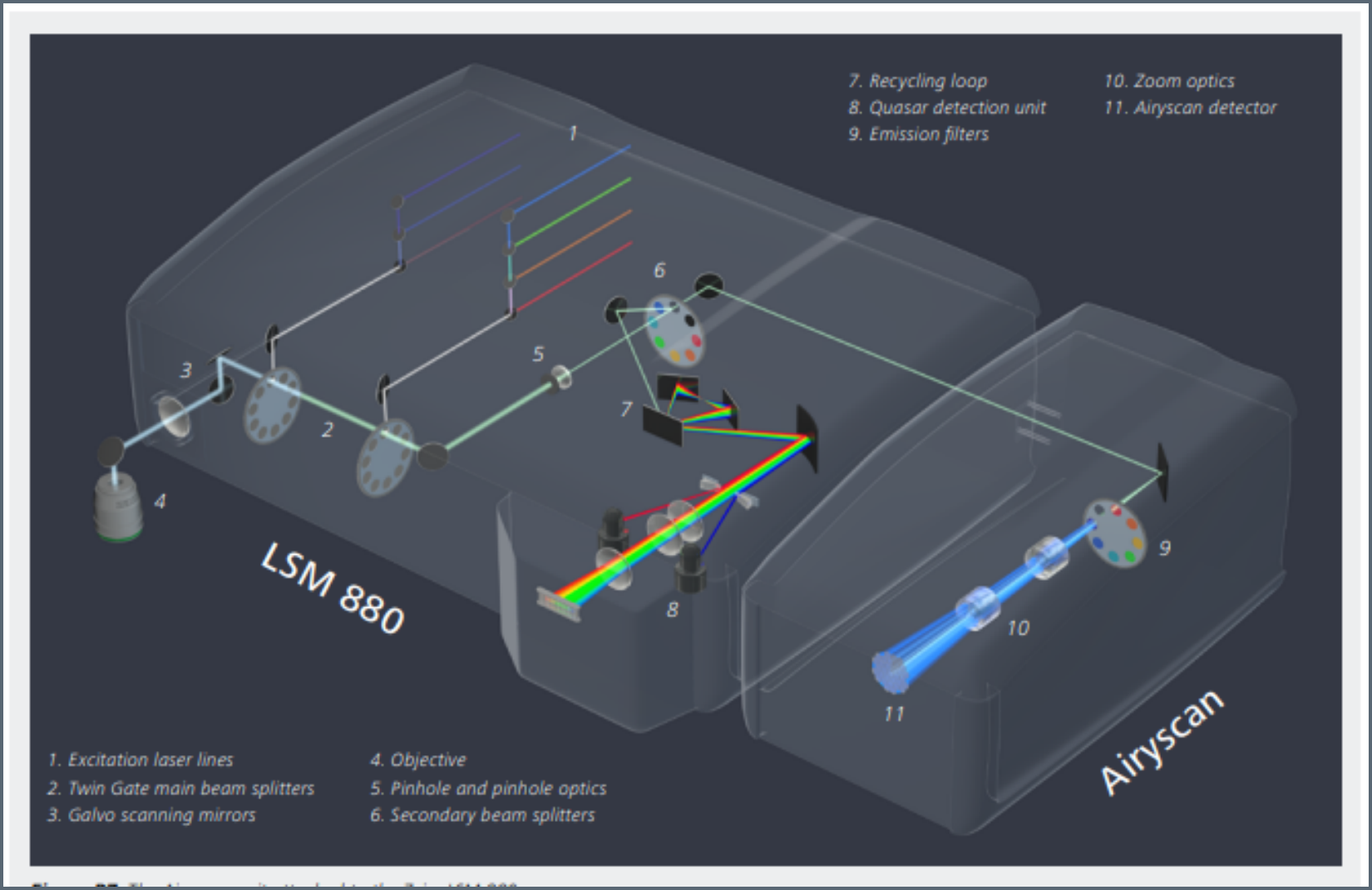
It is like 32 pinholes!





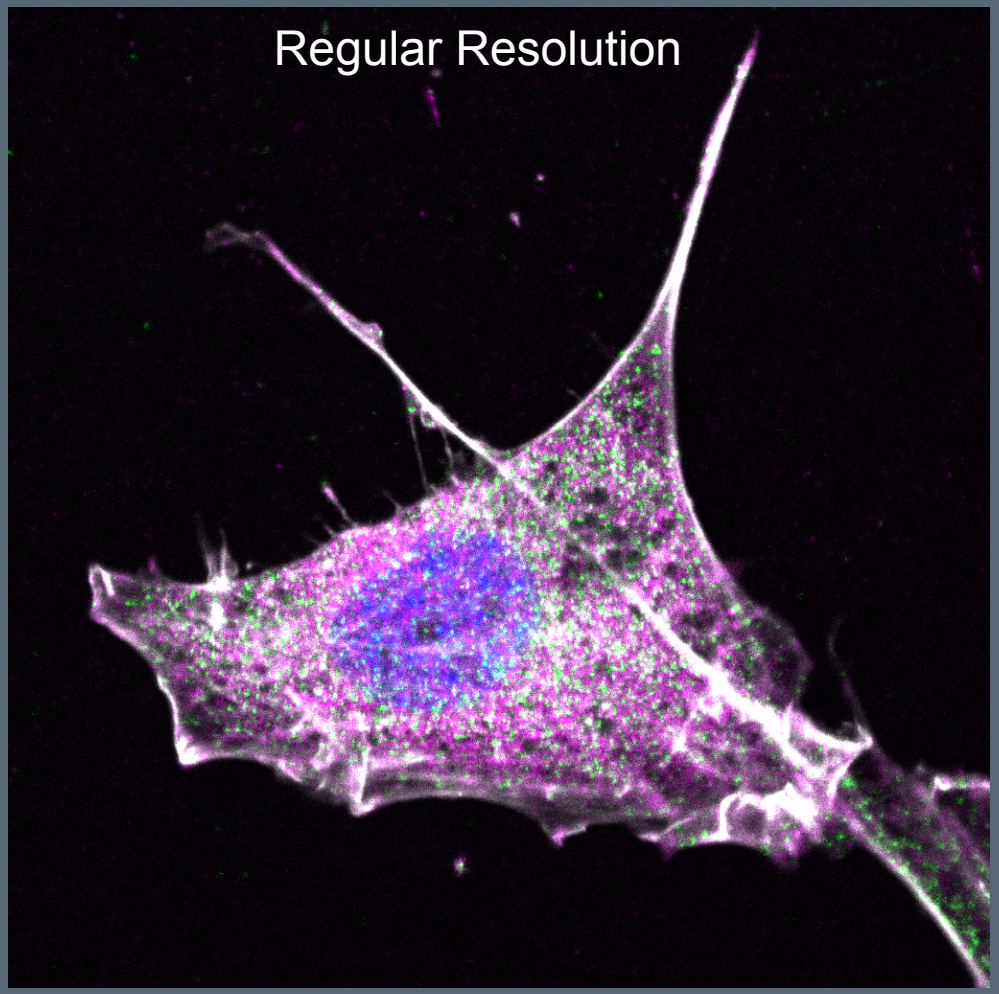
Each dislocated PSF is calculated back to the optical axis with better resolution

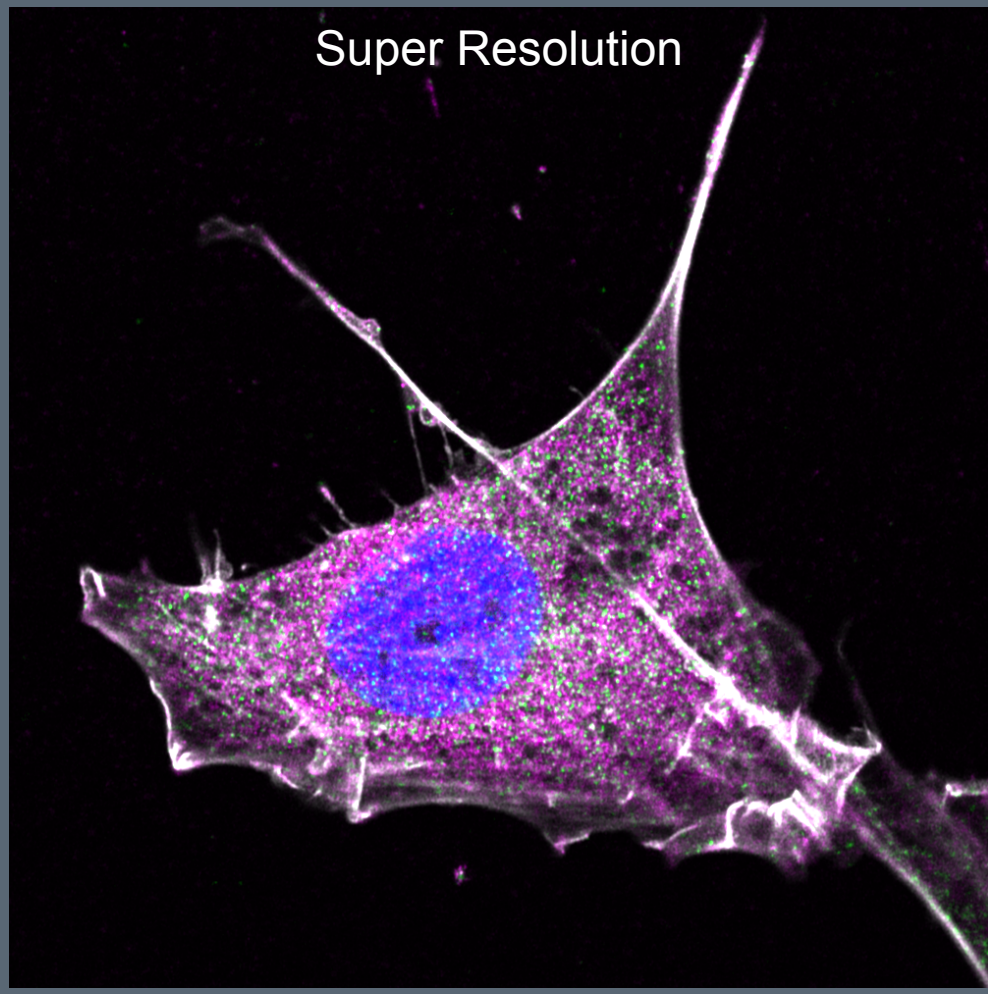
AIRY SCAN MICROSCOPY



AIRY SCAN MICROSCOPY

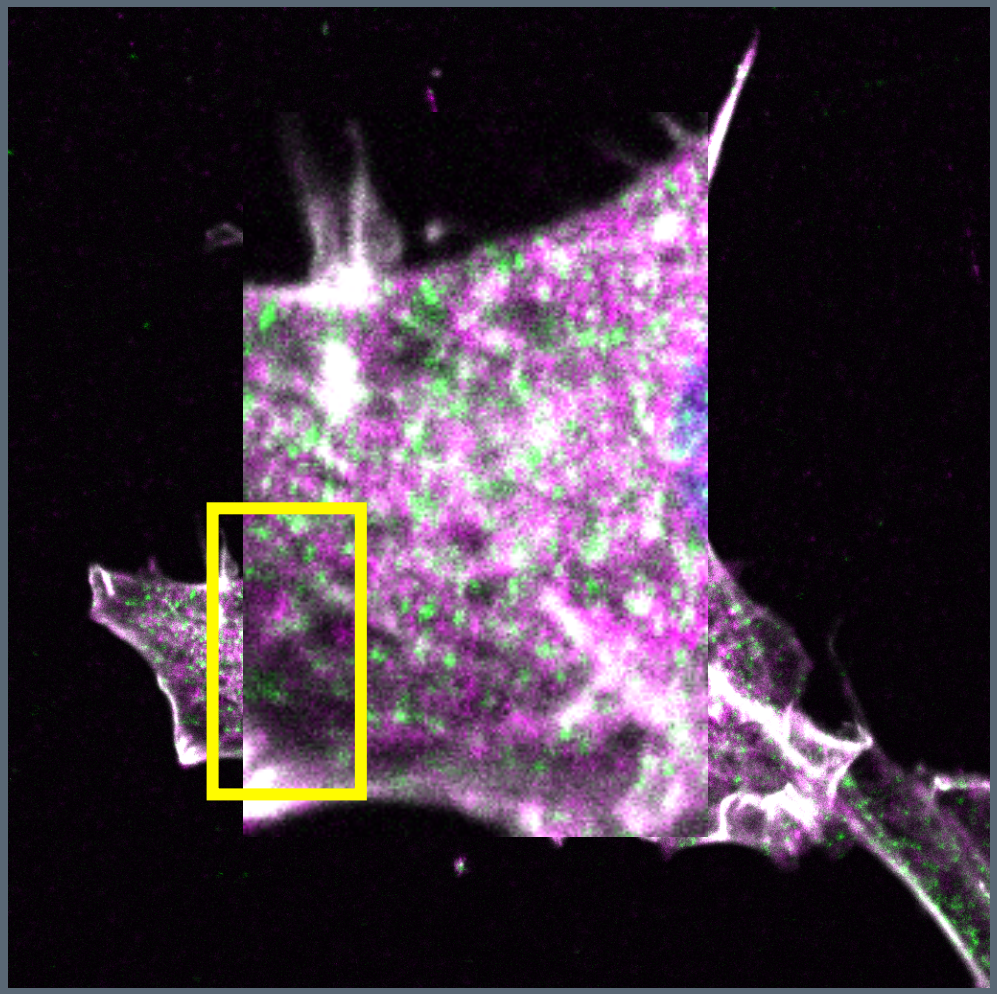
AIRY SCAN MICROSCOPY

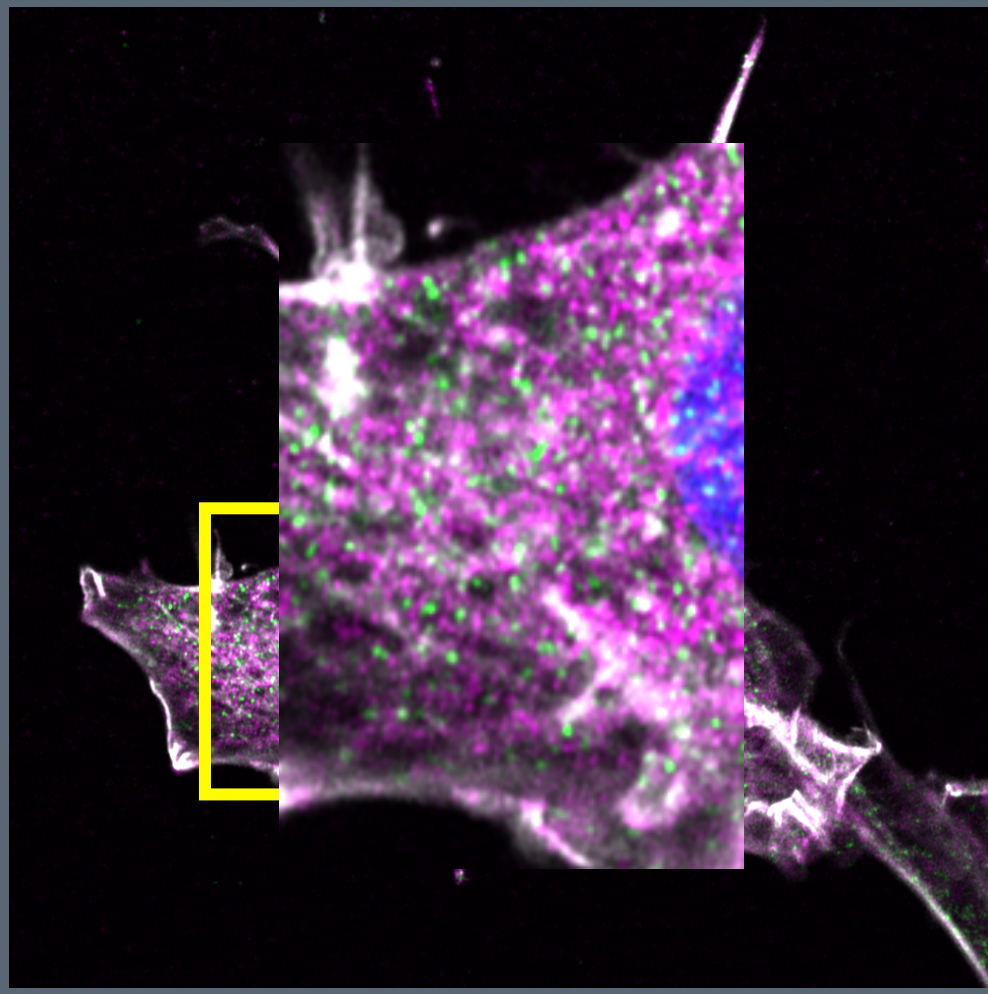




NHI-3T3 Fibroblasts in culture
Gray – Actin
Magenta – PTK2
Green – Myosin Va
Blue - Nucleus

AIRY SCAN MICROSCOPY





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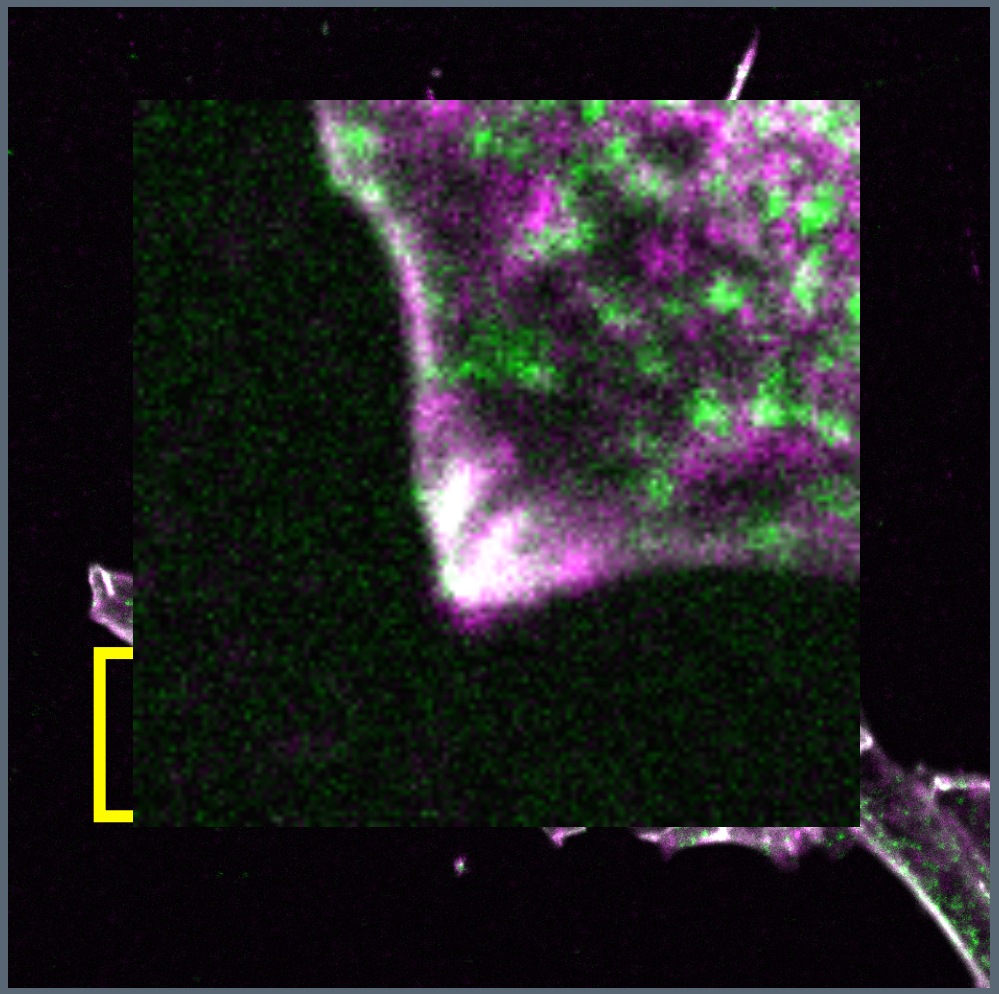
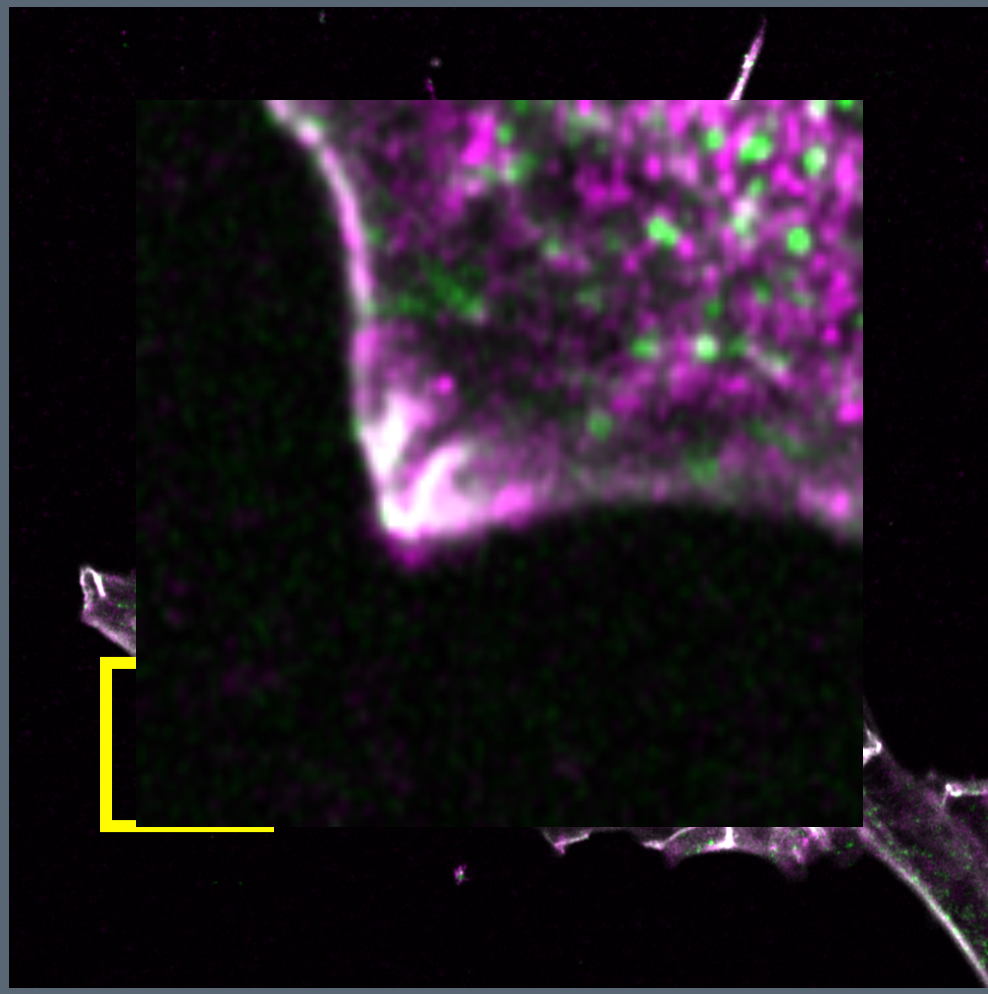
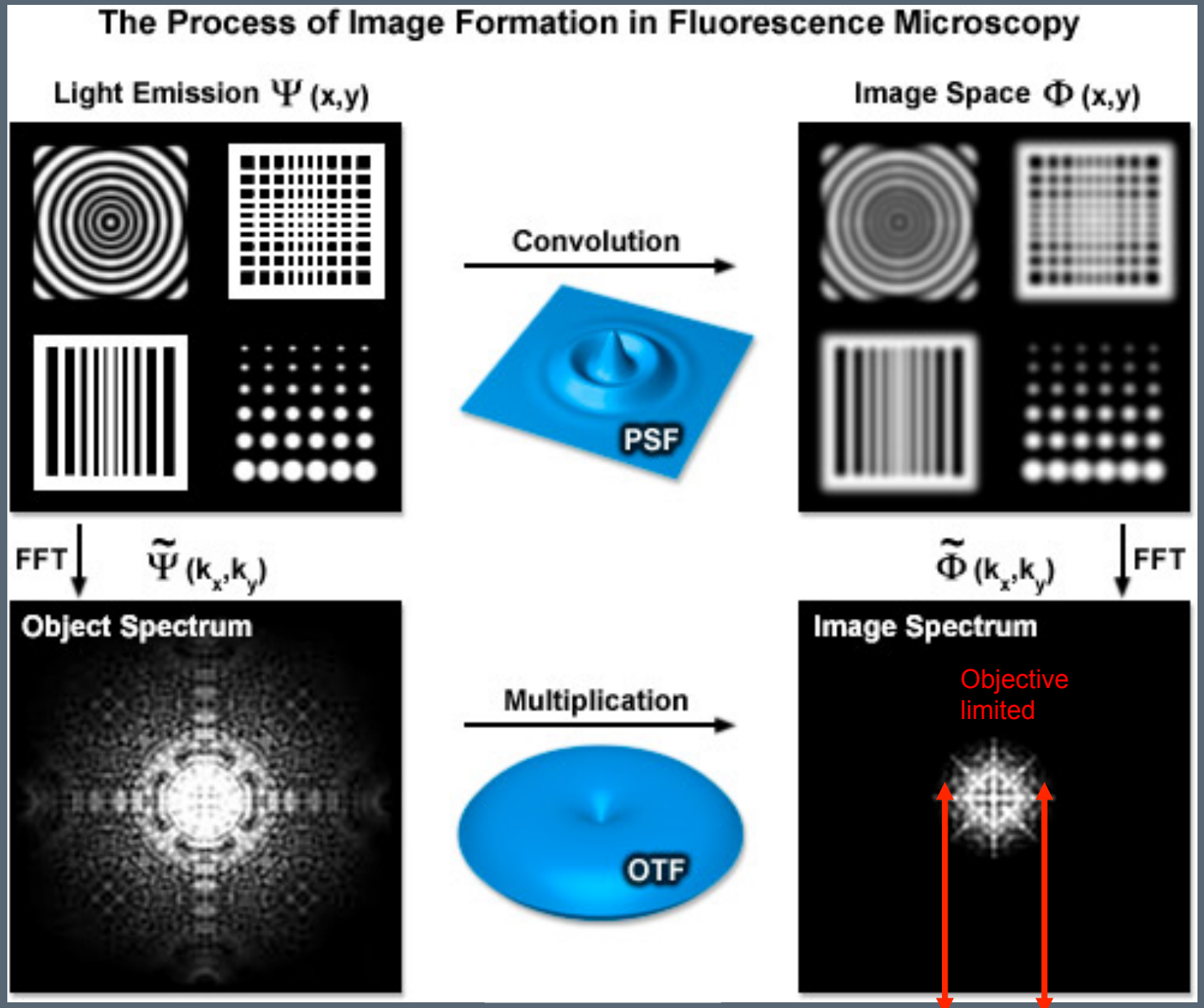




NHI-3T3 Fibroblasts in culture
Gray – Actin
Magenta – PTK2
Green – Myosin Va
Blue - Nucleus

SIM Microscopy

SIM (Structured Illumination Microscopy)



Effect of diffraction:
Blurs the final image!

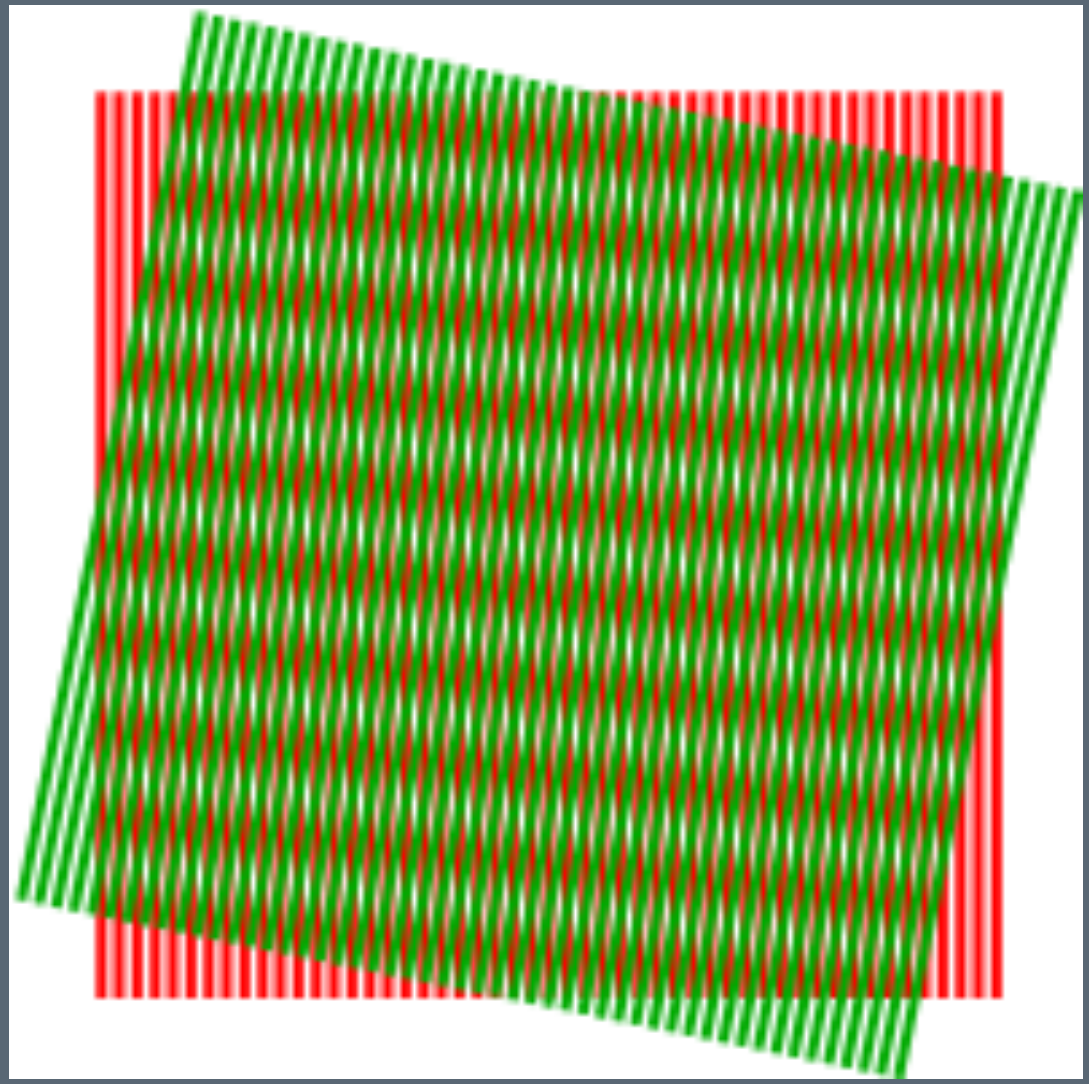
↓

Fast Fourier Transfor

↓

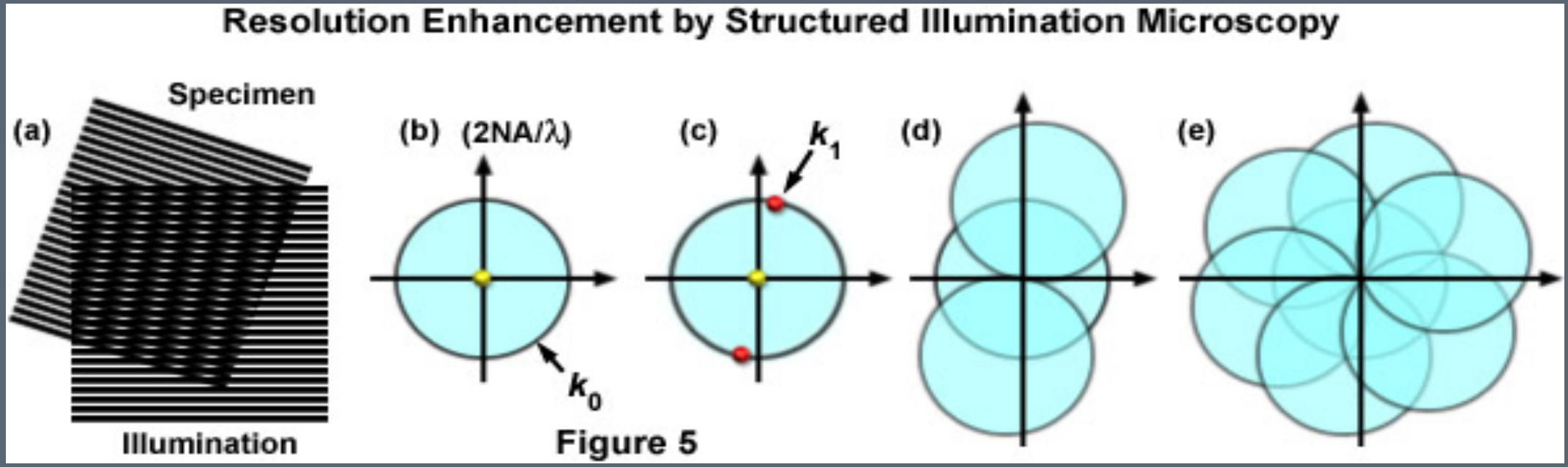
Effect of diffraction:
Clips the frequencies

MOIRE FRINGES



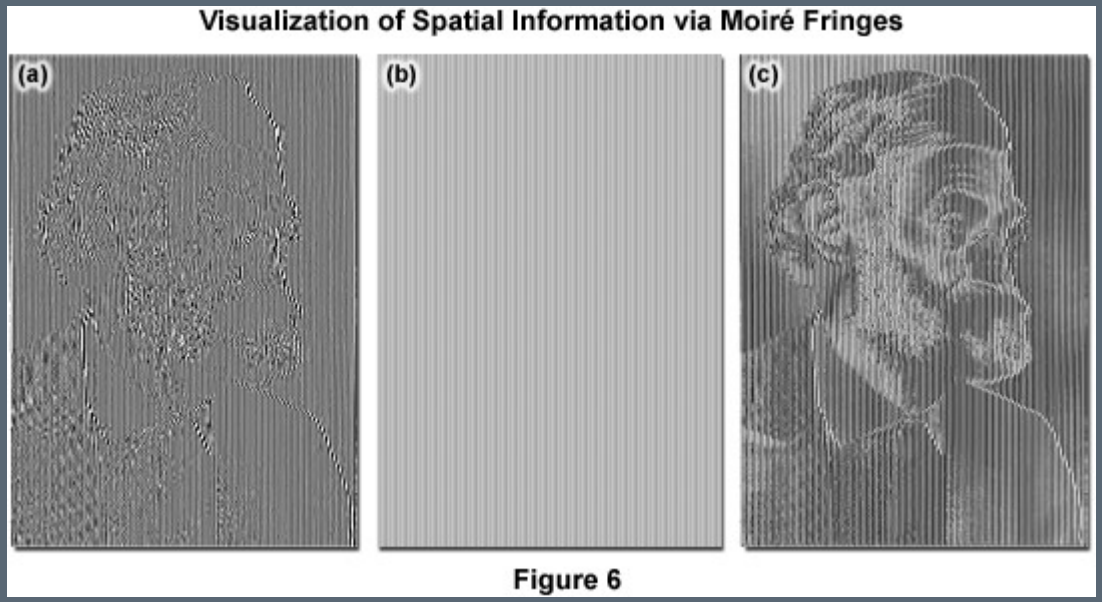
Pattern created by interference between waves with close frequencies

SIM (Structured Illumination Microscopy)



By illuminating a structure on the sample we can get information from higher frequencies!

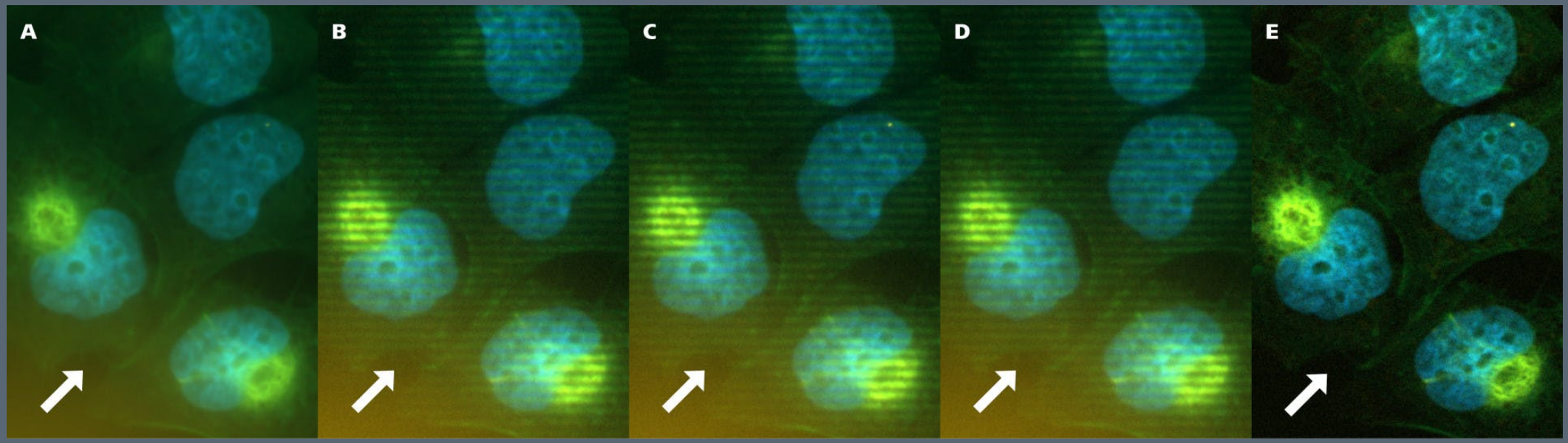
SIM (Structured Illumination Microscopy)



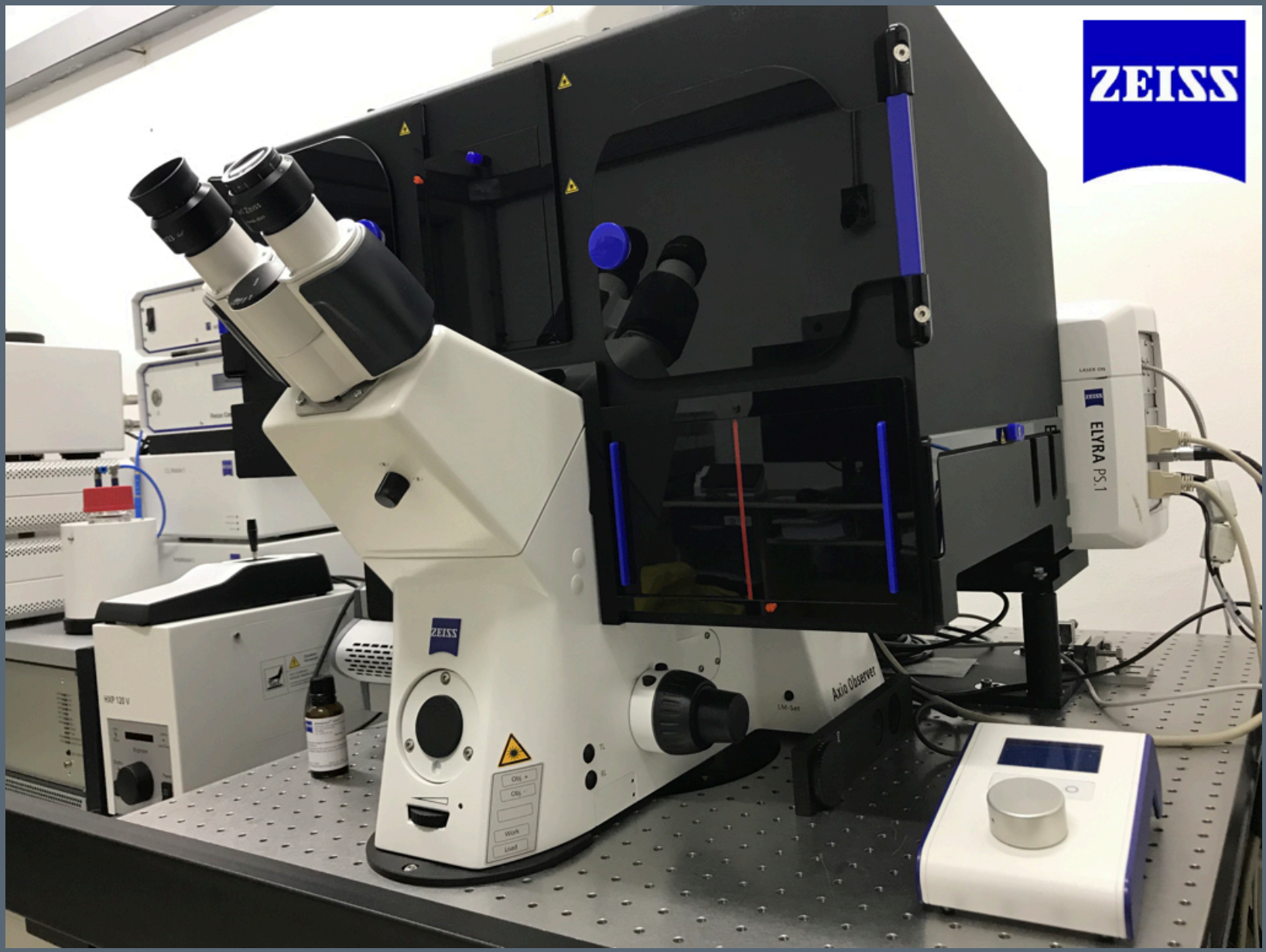
By illuminating a structure on the sample we can get information from higher frequencies!

APOTOME

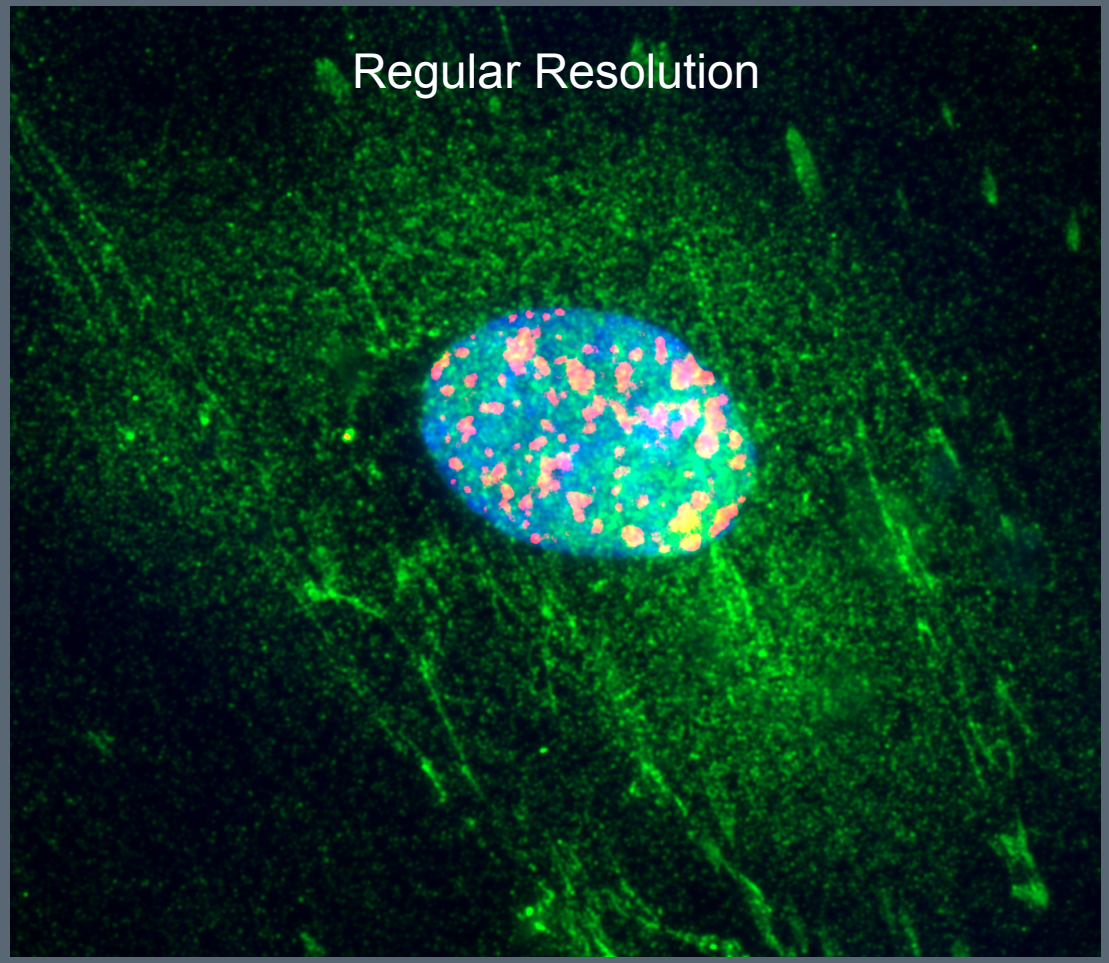
http://zeiss-campus.magnet.fsu.edu/tutorials/opticalsectioning/apotome/indexflash.html

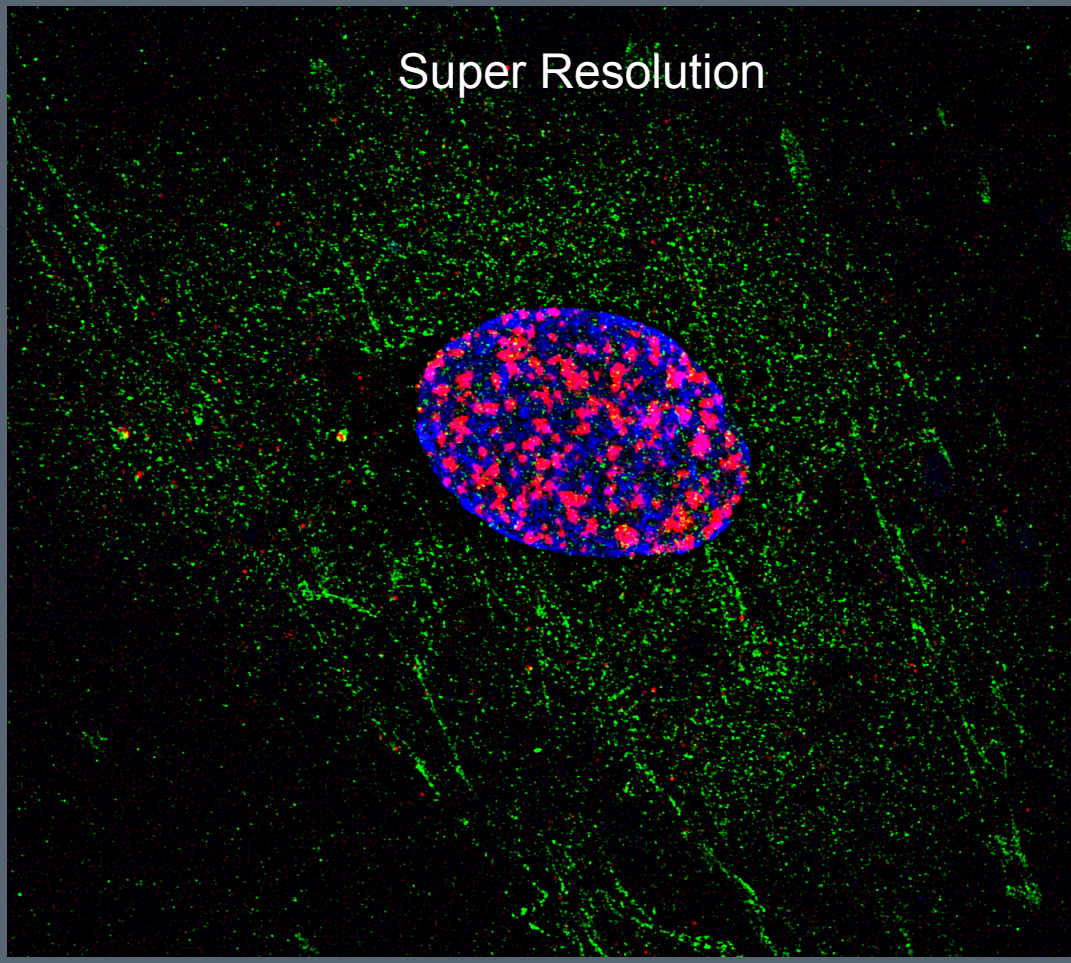


SIM



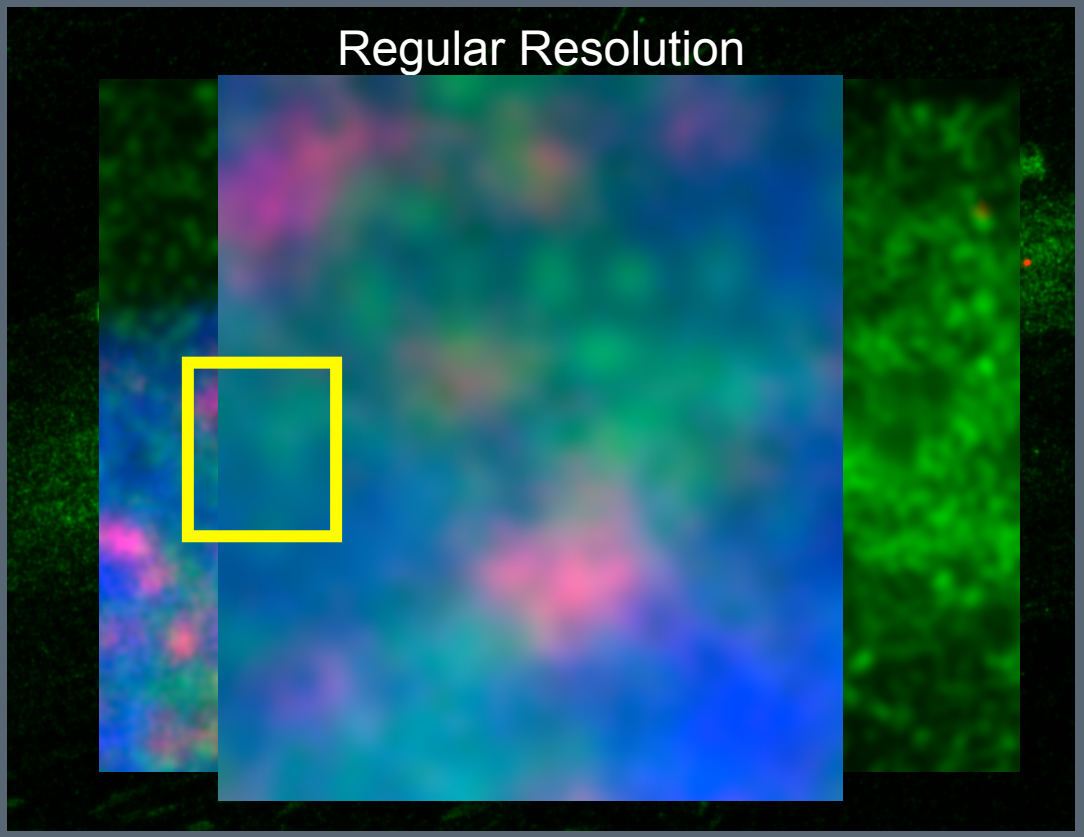
SIM

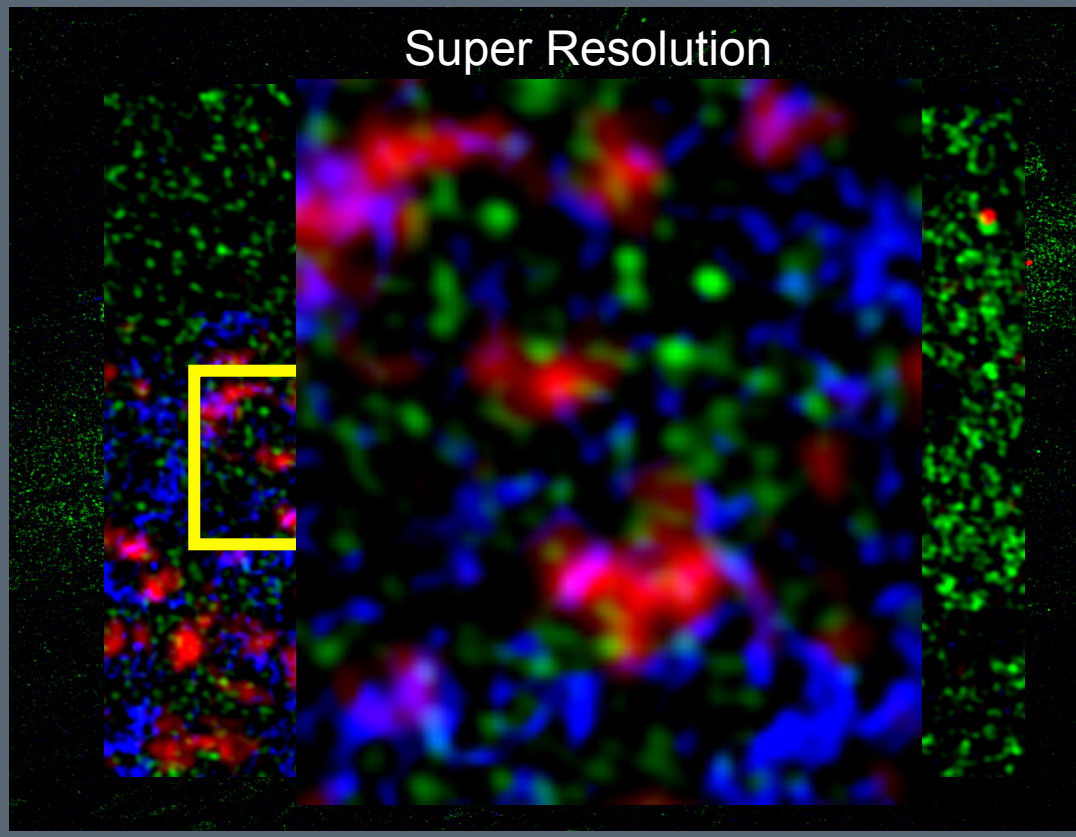




Cells treated with DOXO – used in cancer treatment
H9C2 Myocytes in culture
Green – PTK2
Red – γH2AX
Blue - Nucleus

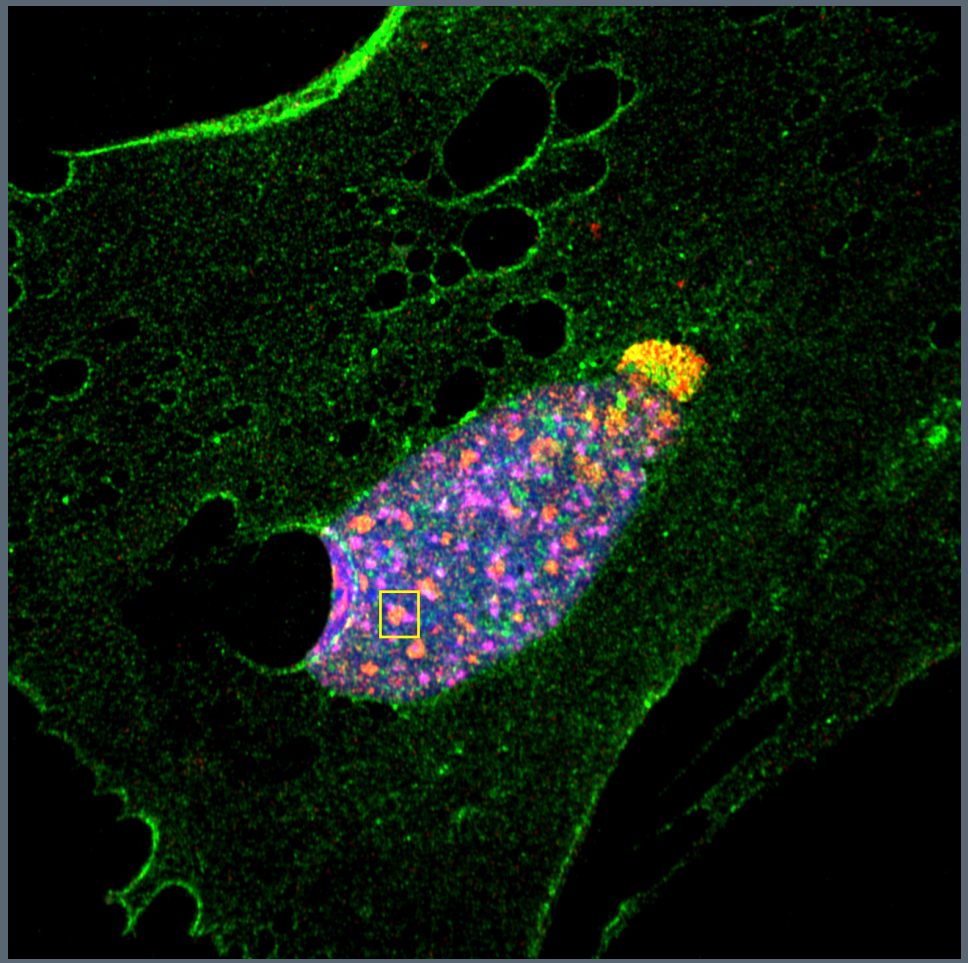
SIM

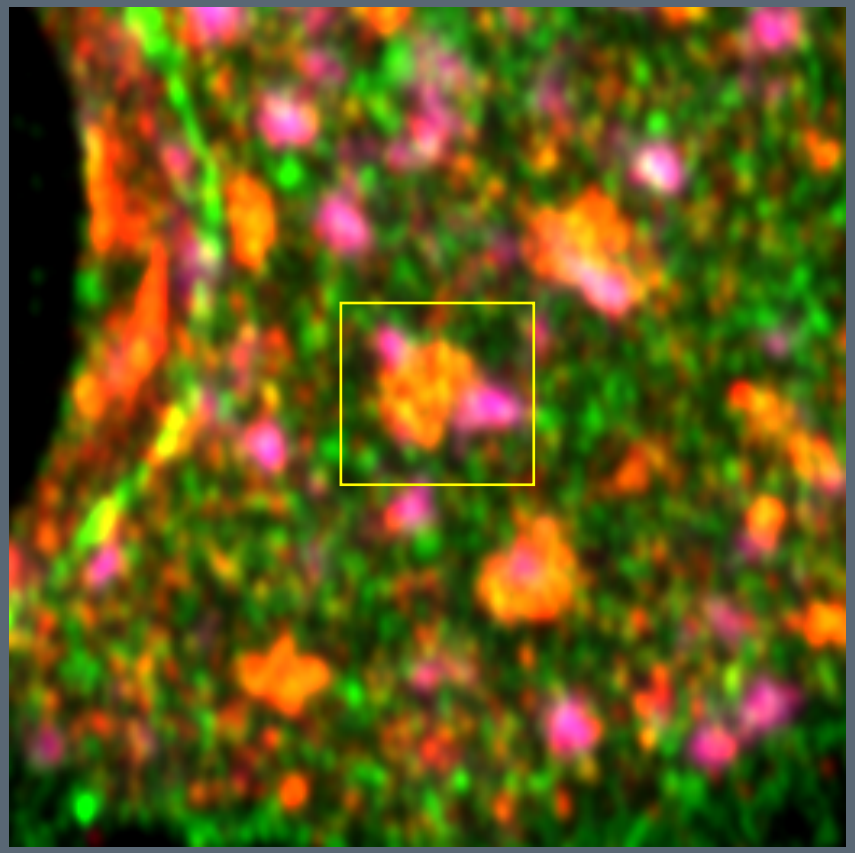




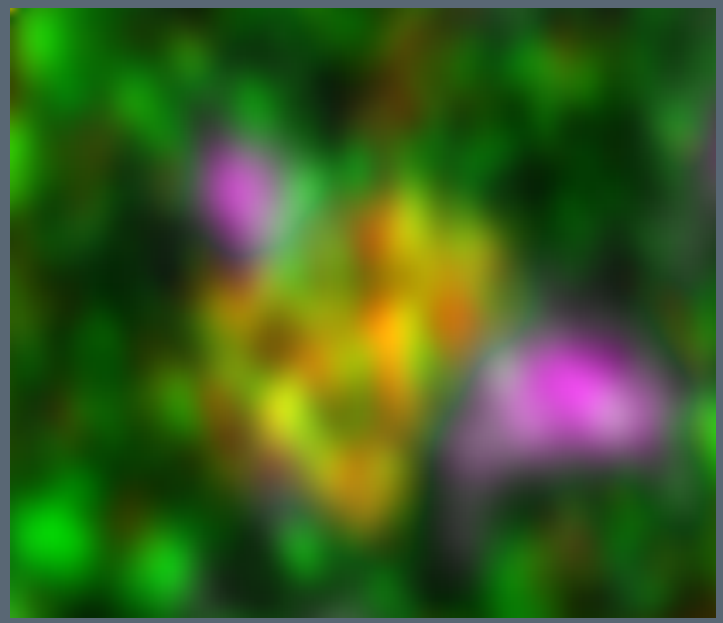
Cells treated with DOXO – used in cancer treatment
H9C2 Myocytes in culture
Green – PTK2
Red – γH2AX
Blue - Nucleus

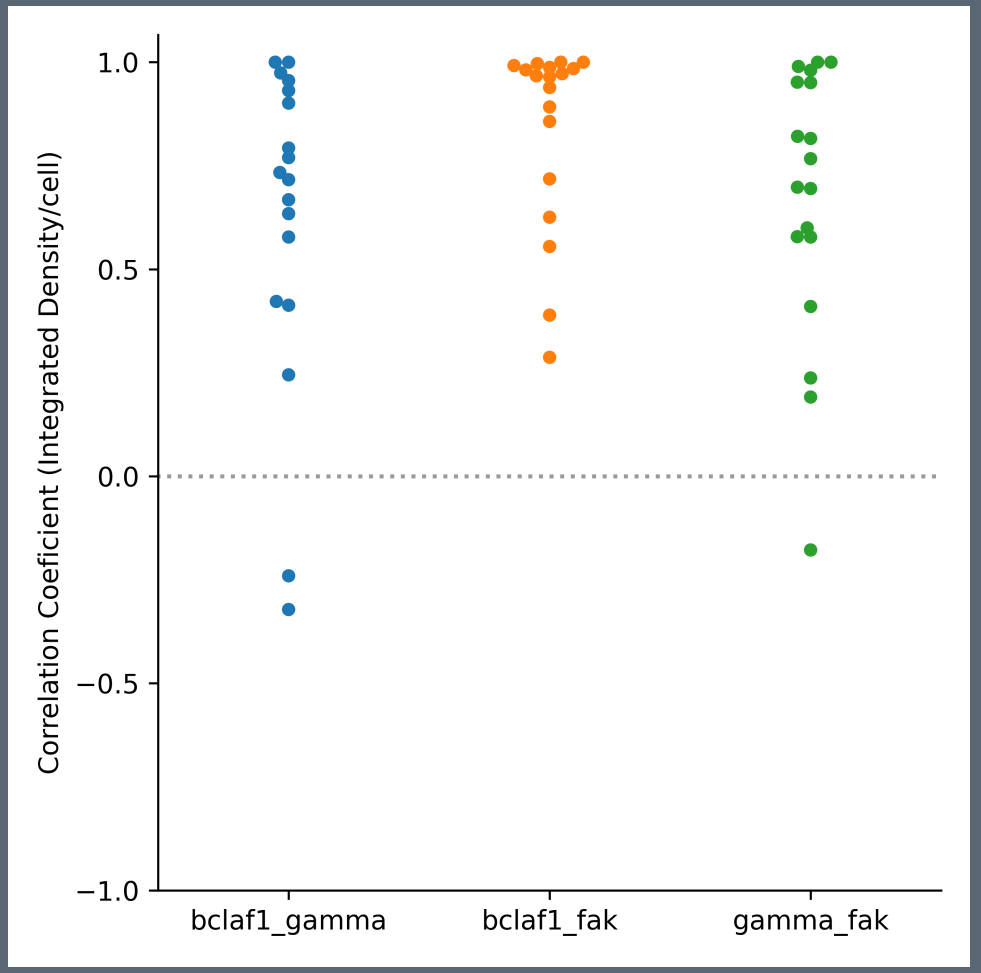
PTK2 and BCLAF1 are near γ-H2AX sites

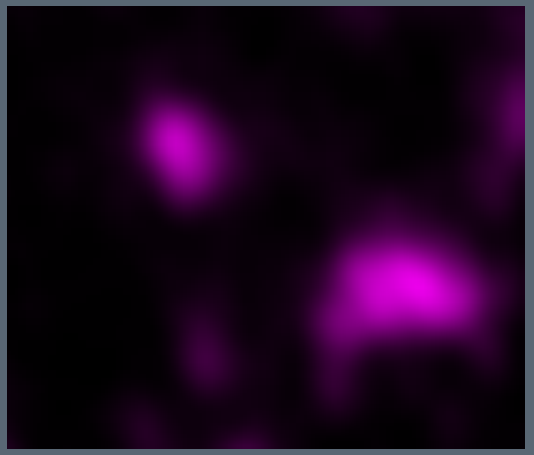


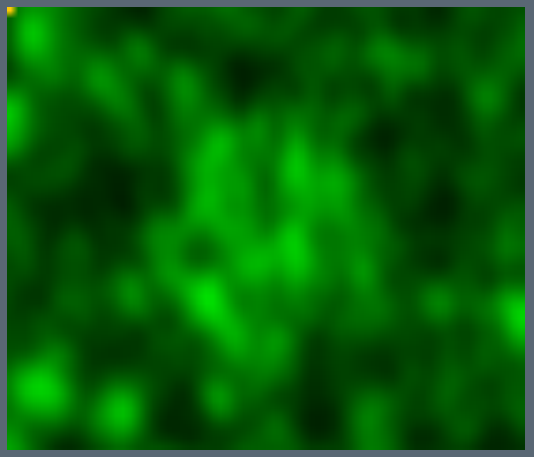


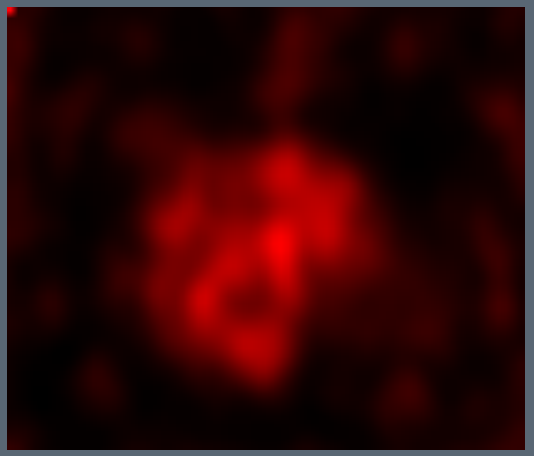
PTK2
BCLA
γ-H2a
DAPI

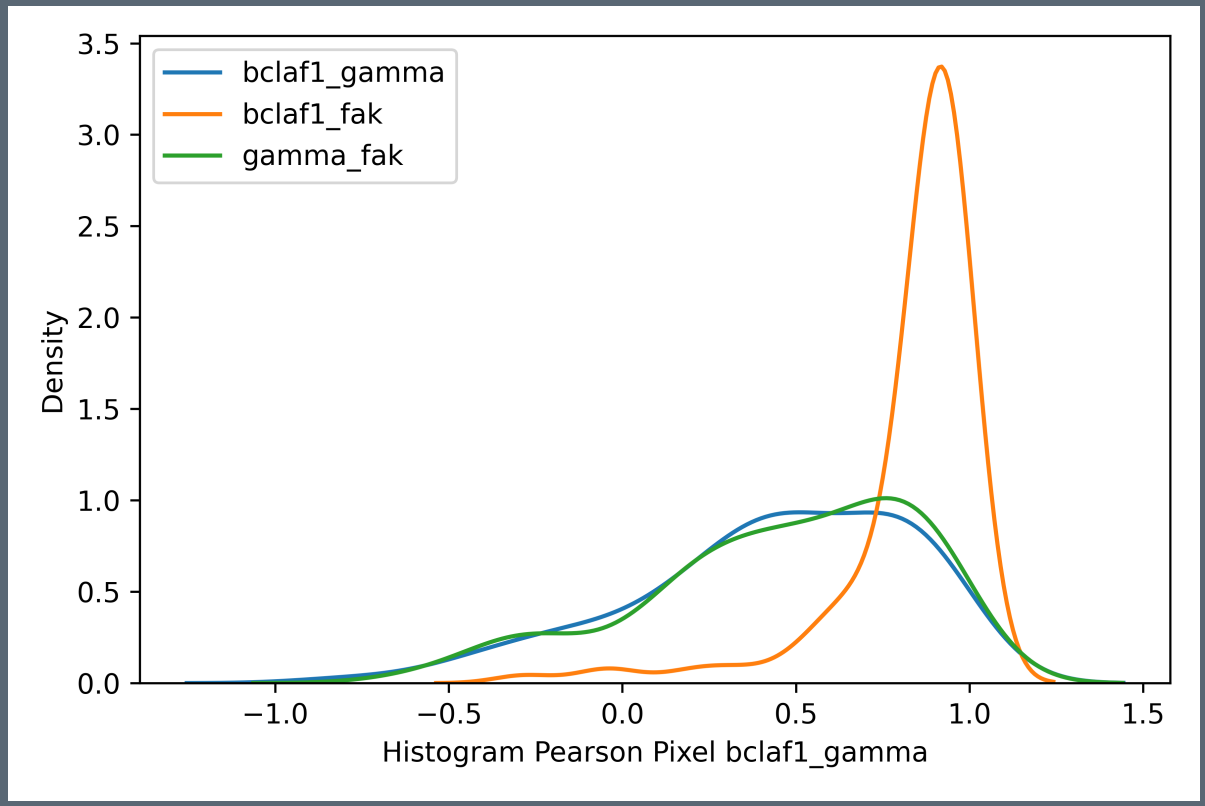



Correlation Coeficient (Integrated Density/cell)
1.0
0.5
0.0
−0.5
−1.0
bclaf1_gamma
bclaf1_fak
gamma_fak









Pixel position correlation

$r \;\; cov(p\downarrow \;\; p\downarrow \;\;)/\sqrt{\square}var(\;\; p\downarrow \;\;)var \; p\downarrow$

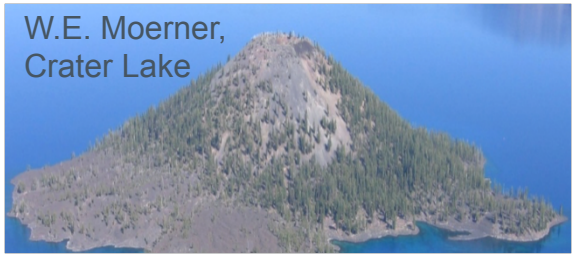
STORM
PALM

Super-Accuracy:
Nanometer Distances w Single Molecules

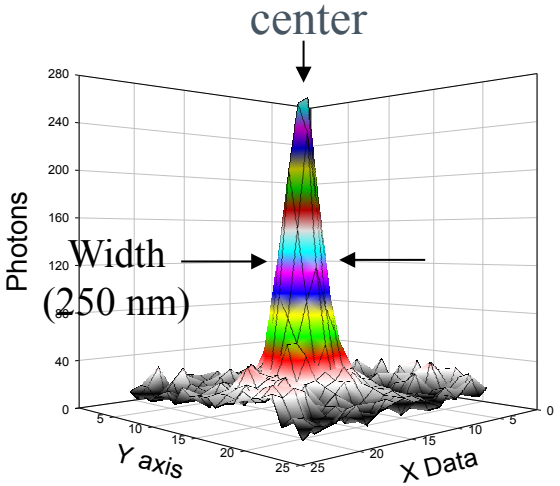
FIONA

Fluorescence **I**maging with
One **N**anometer **A**ccuracy

1.5 nm accuracy
1-500 msec



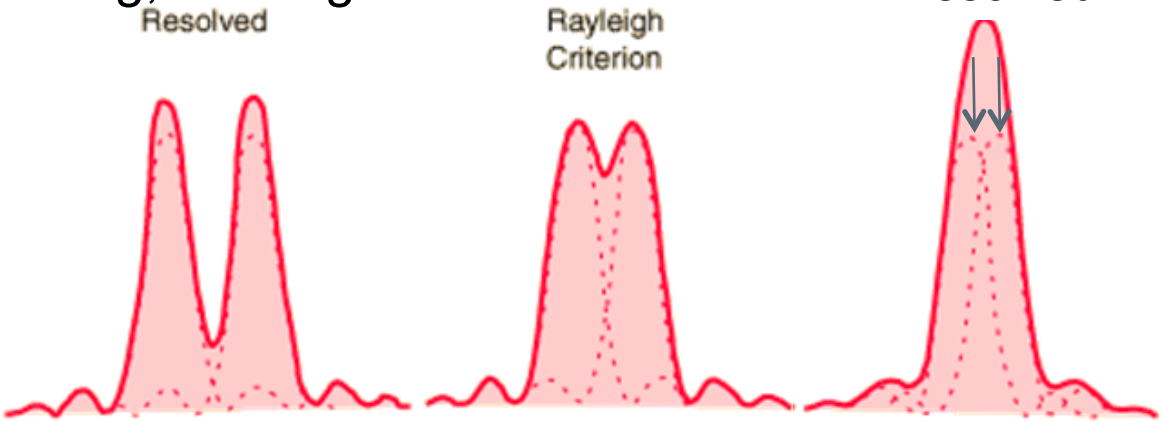
Center can be found much more accurately than width



$\Delta x_{center} = width/\sqrt{N}$
$\approx 250/\sqrt{10k} = 1.3$ nm

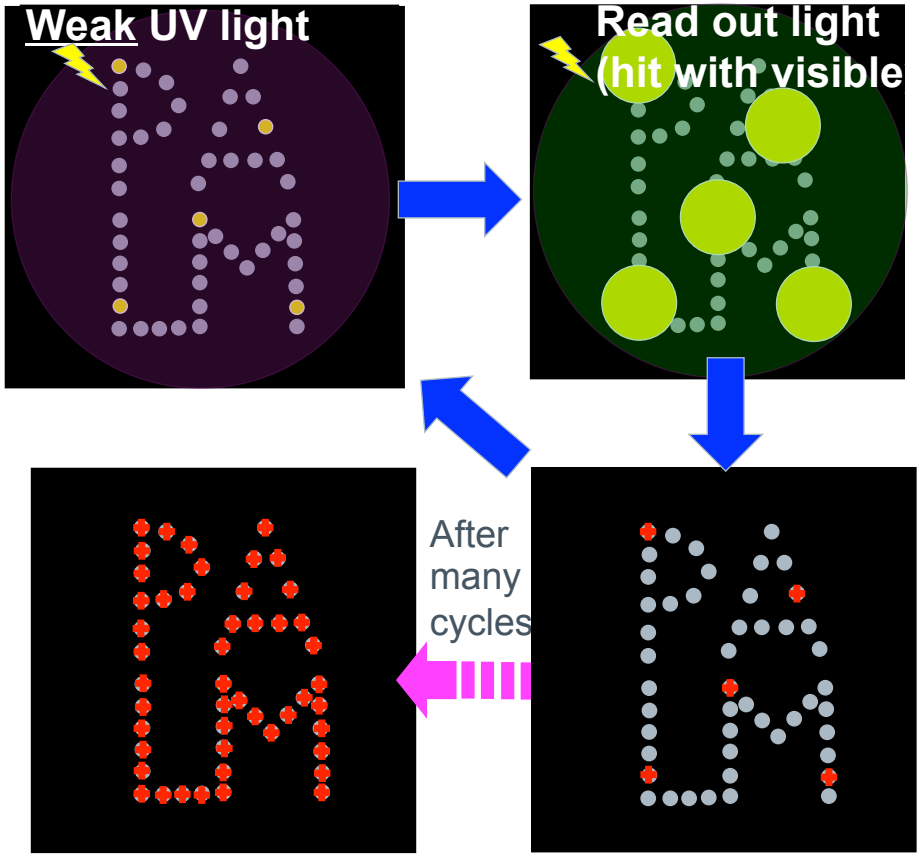
Super-Resolution: PALM/STORM. between (activatable) molecules

Betzig, Zhuang



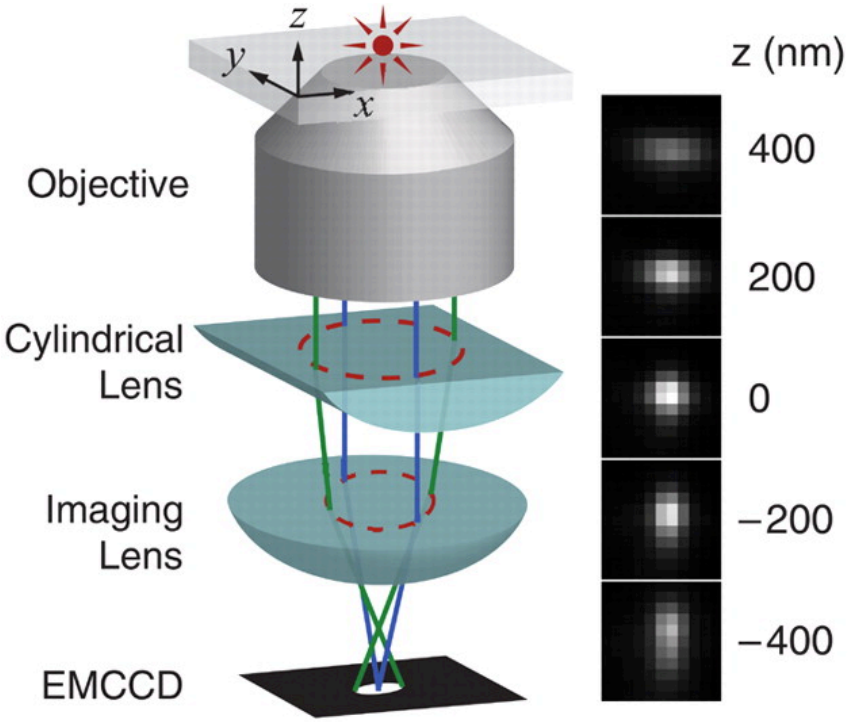
Yildiz et al, Science, 2003

PALM - Photo-activated localization super-resolution microscopy



The PALM cycle

Betzig et al. Science 2006



3D super-resolution

Huang et al. Science 2008

PALM imaging with 10 ~ 20 nm resolution (localization precision).

Nobel Prize in Chemistry 2014

for the development of super-resolved fluorescence microscopy

Photo: A. Mahmoud

Eric Betzig

Prize share: 1/3

PALM

Photo: A. Mahmoud

Stefan W. Hell

Prize share: 1/3

STED

Photo: A. Mahmoud

William E. Moerner

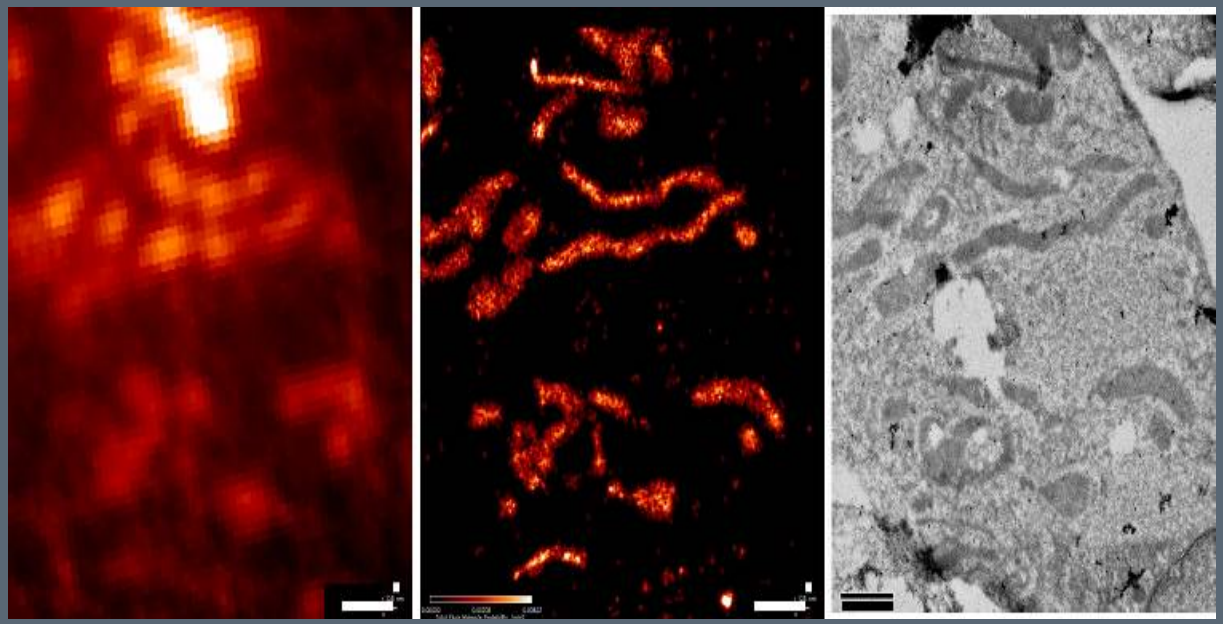
Prize share: 1/3

First single molecule measurement

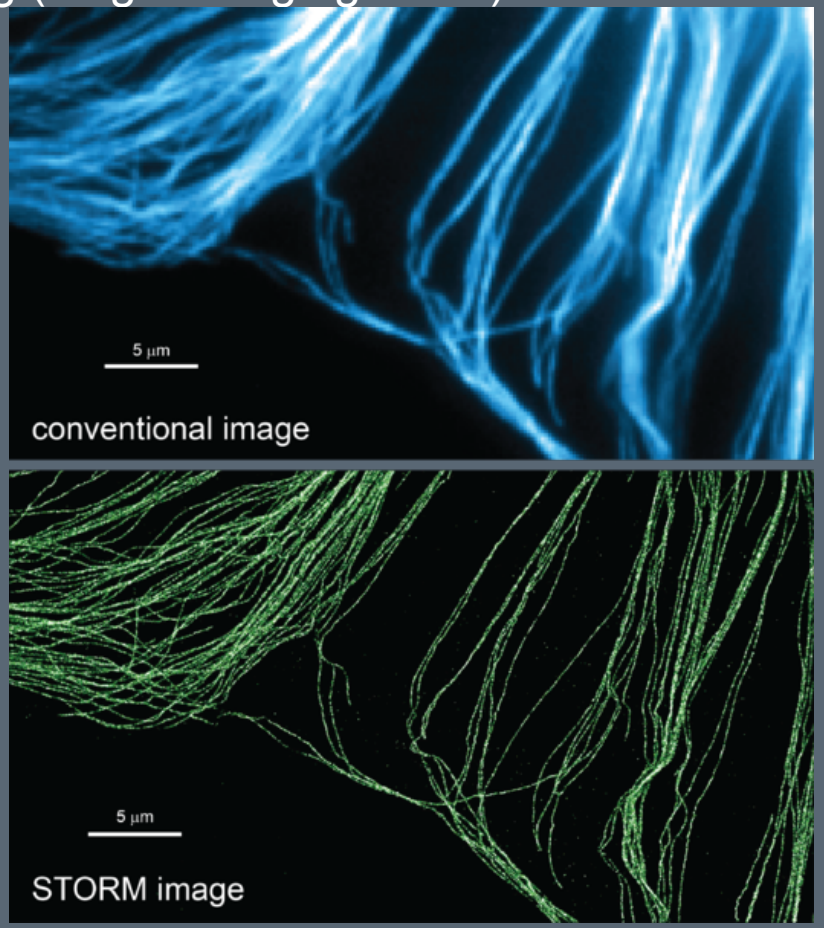
PALM X STORM

The main difference between PALM and STORM is the fluorophores used for the experiment:

PALM: photo switchable/convertible fluorescent proteins (live cell imaging)
STORM: uses organic dyes as fluorescent probes for imaging (longer imaging times)

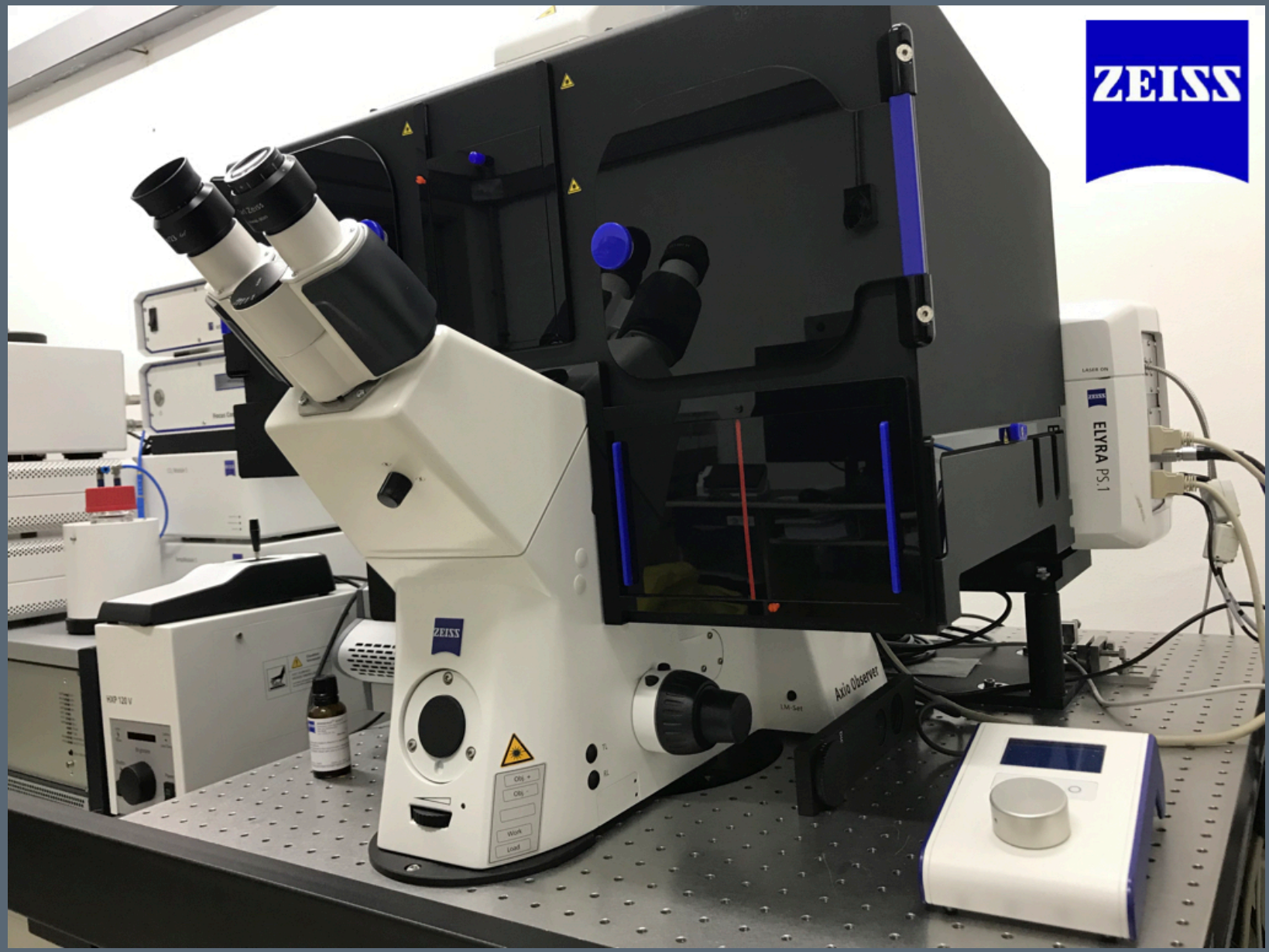


E. Betzig *et al.*, "Imaging intracellular fluorescent proteins at nanometer resolution," (in English), *Science,* Article vol. 313, no. 5793, pp. 1642-1645, Sep 2006, doi: 10.1126/science.1127344

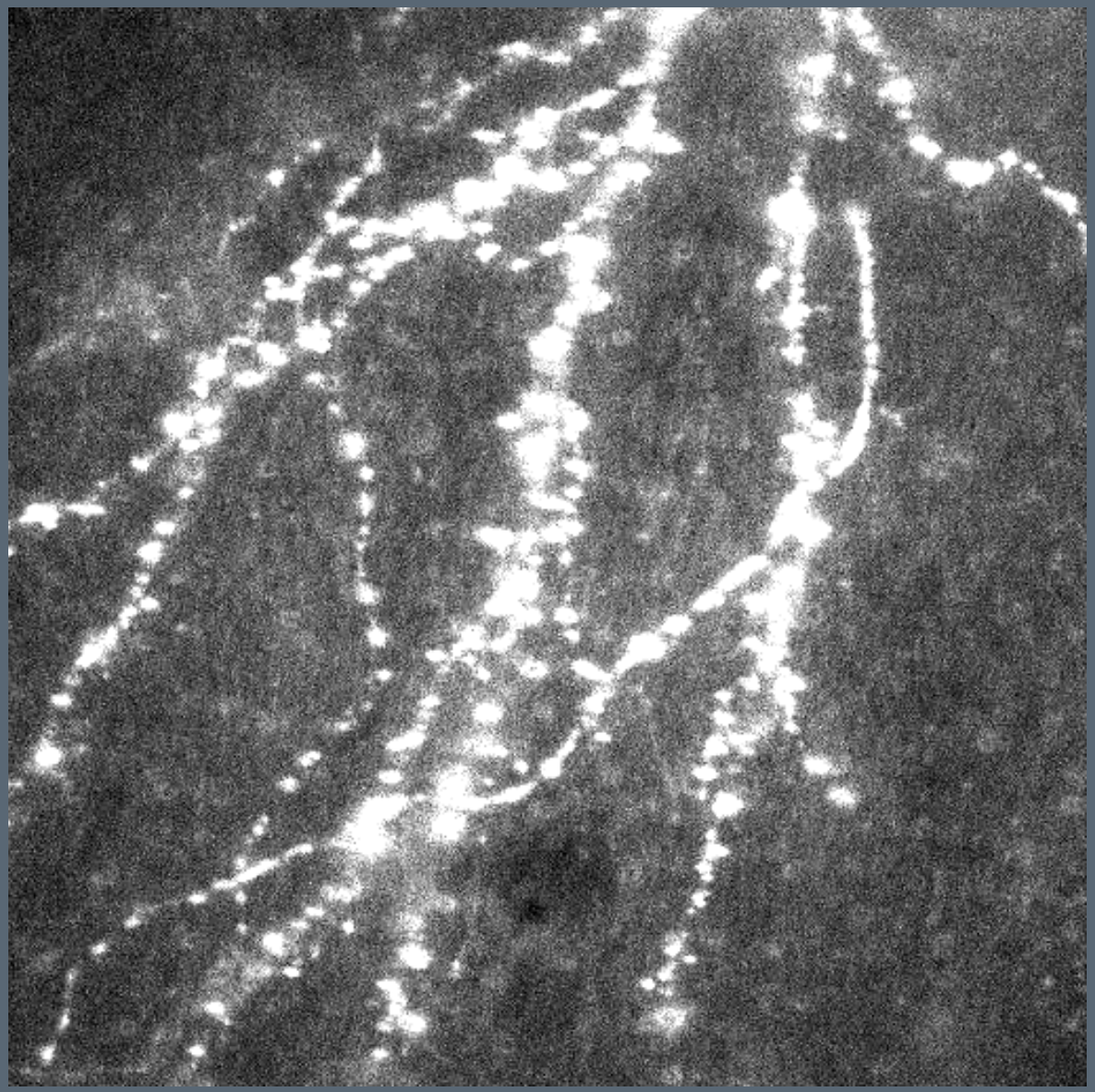


Multicolor Super-Resolution Imaging with Photo-Switchable Fluorescent Probes
M. Bates, B. Huang, G. T. Dempsey, X. Zhuang
Science *317* 1749-1753 (2007)

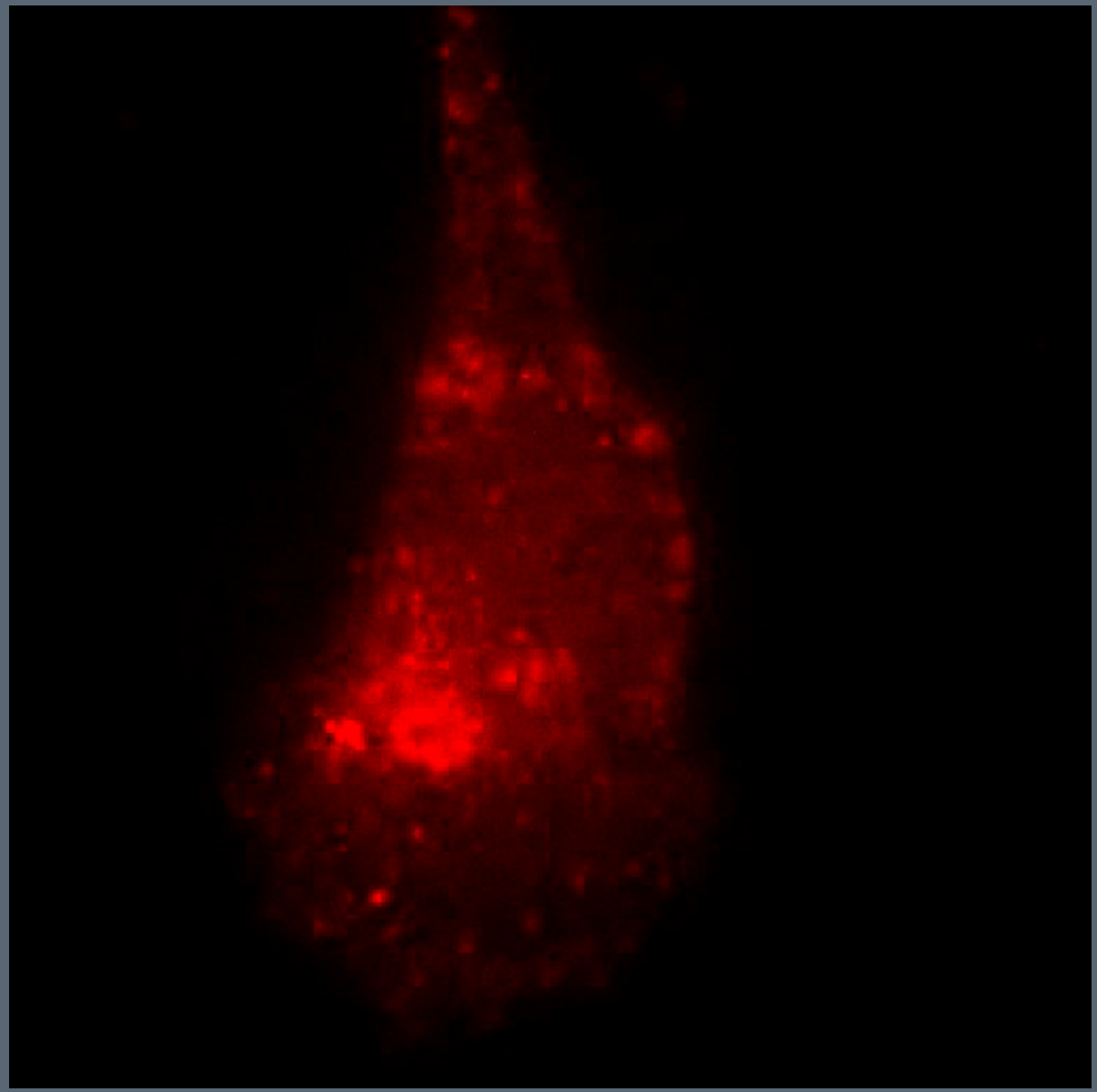
PALM / STORM



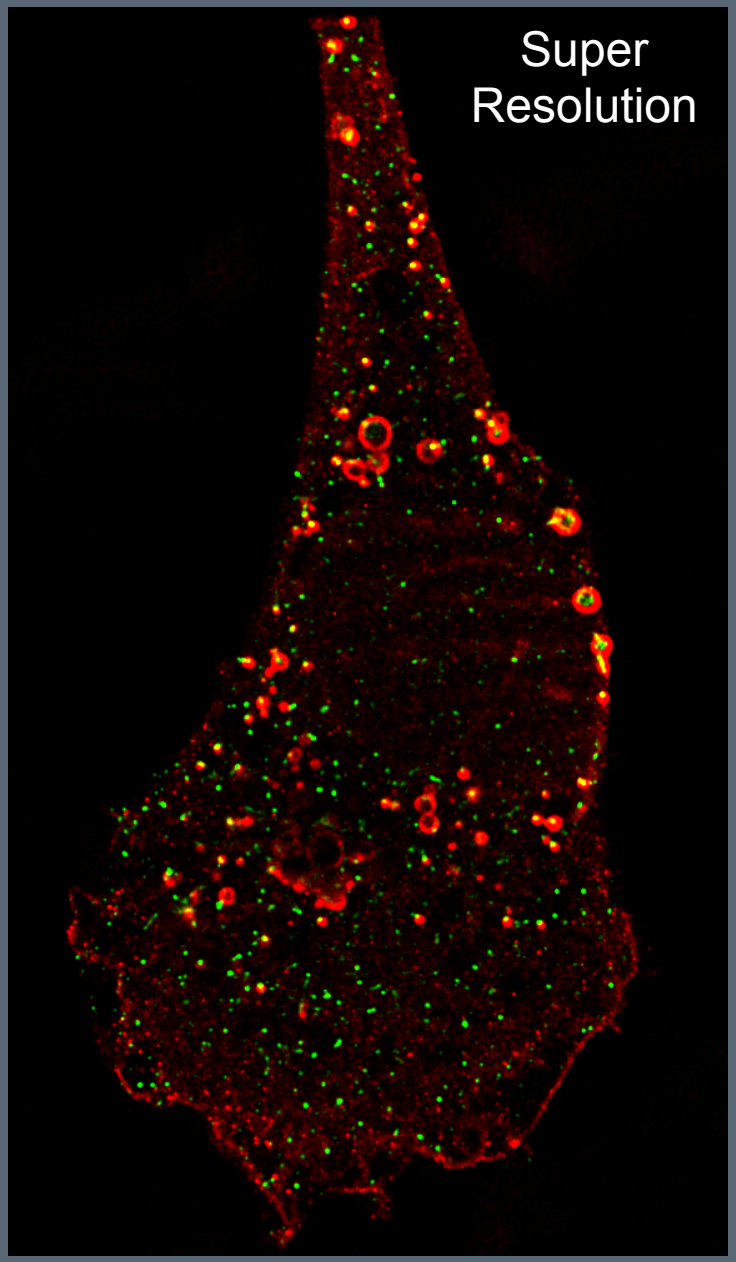
PALM



STORM



STORM



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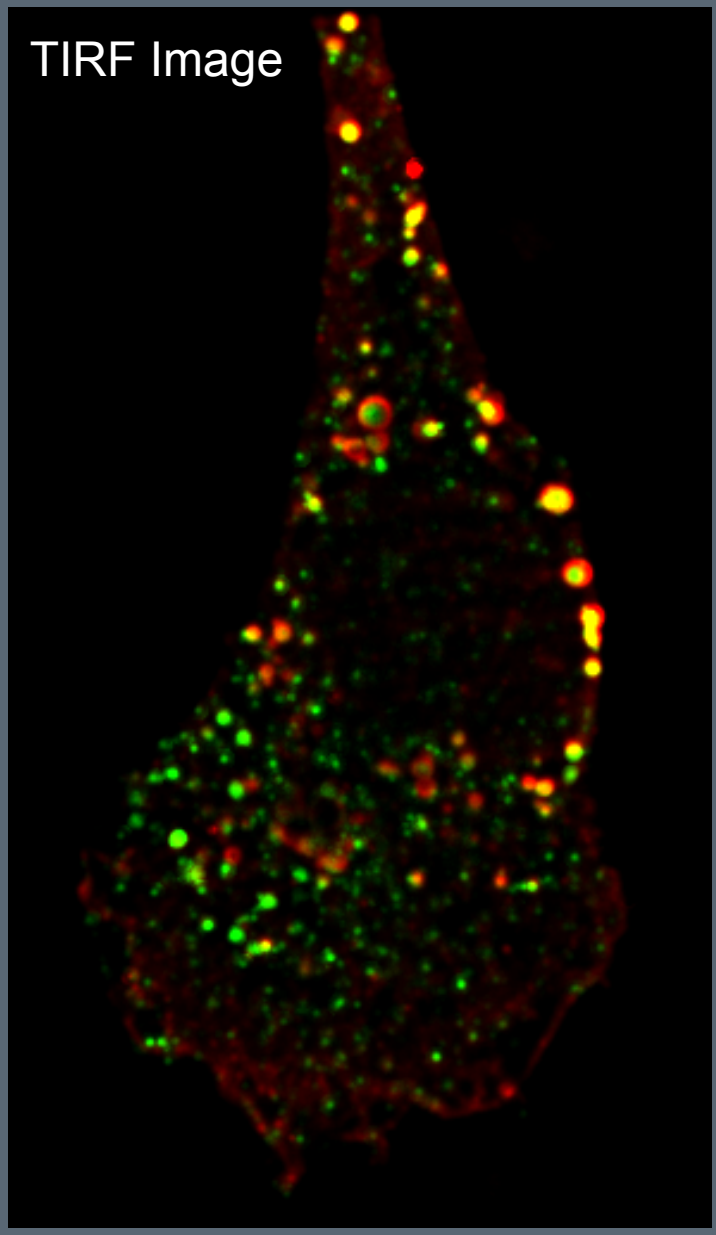
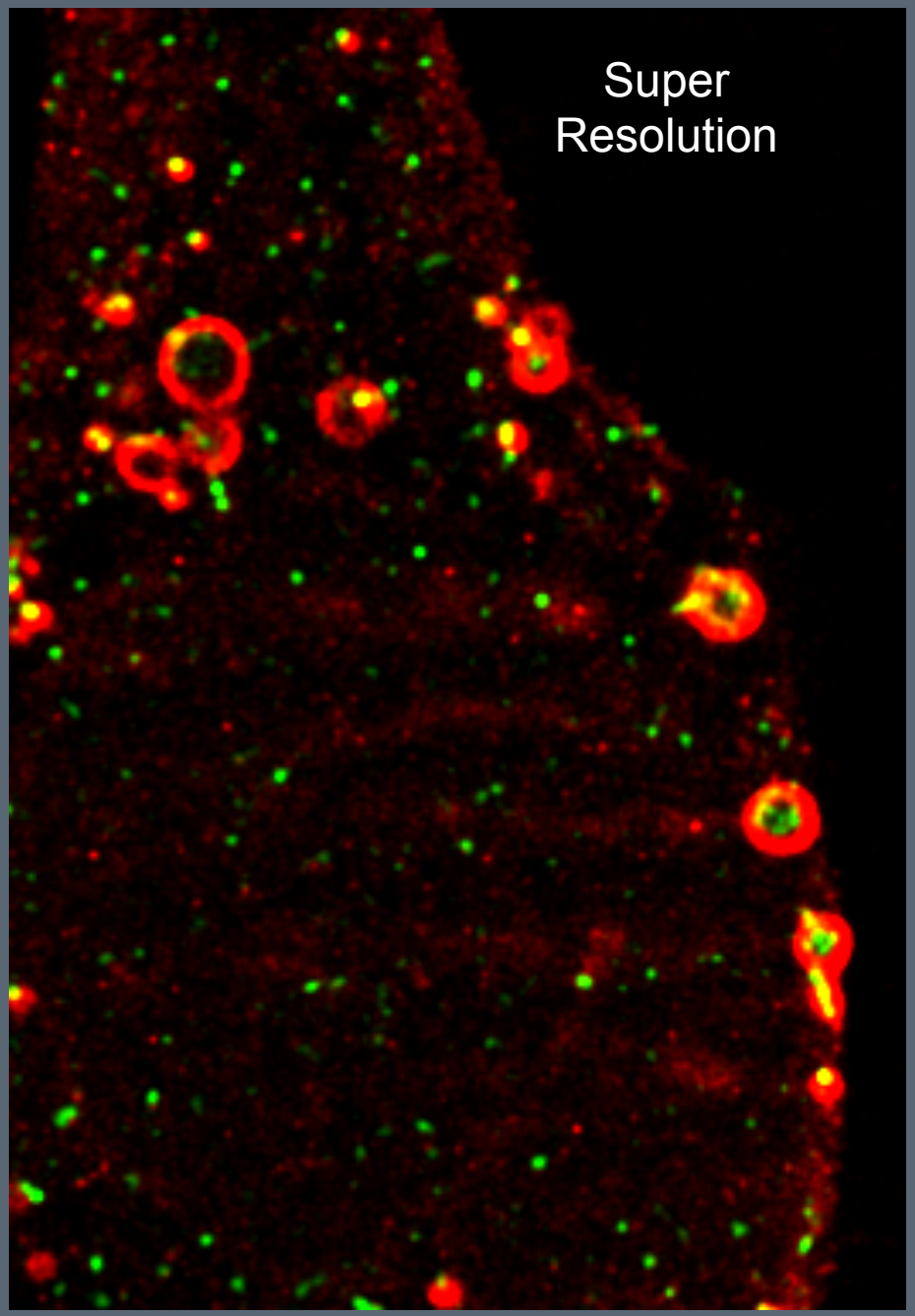
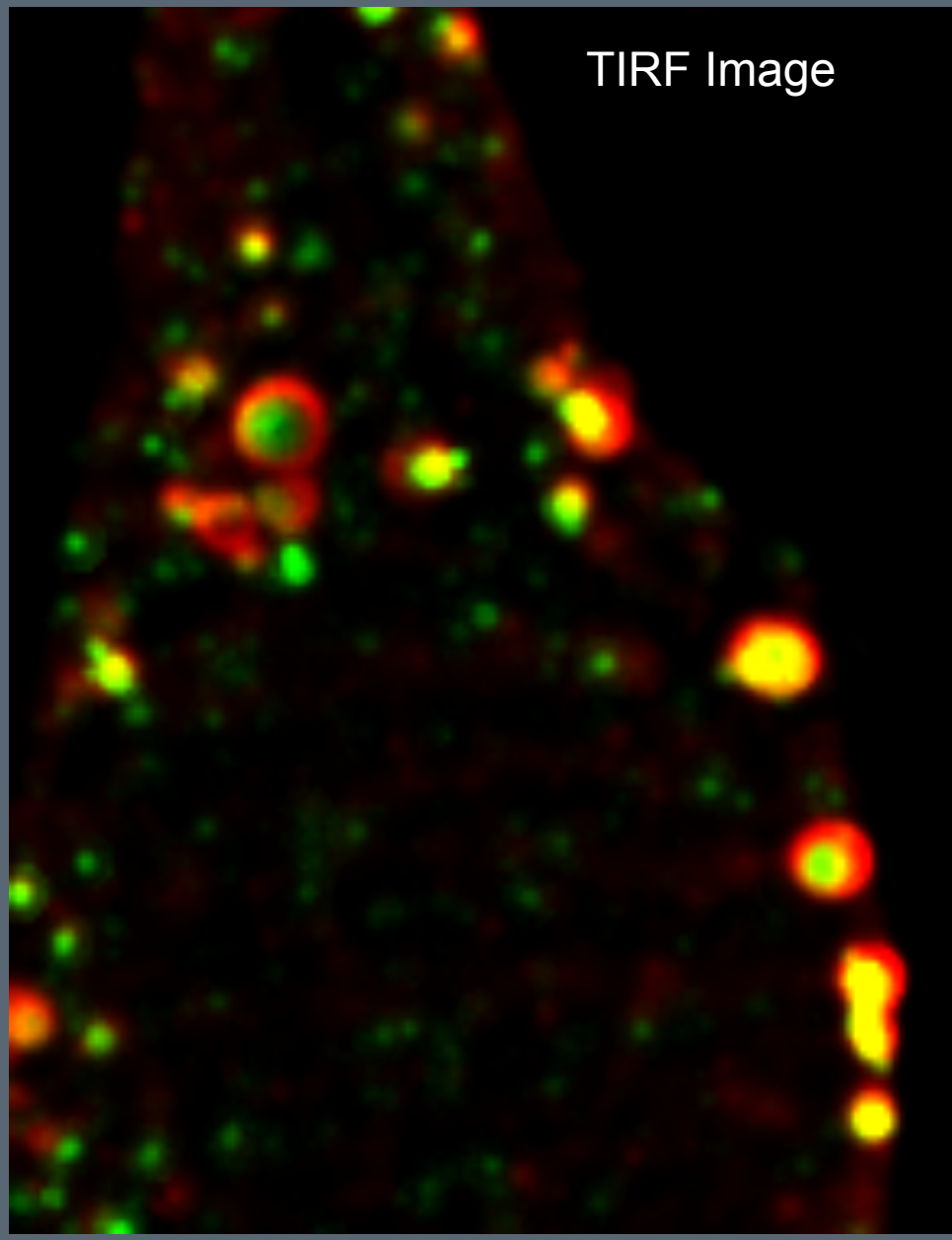


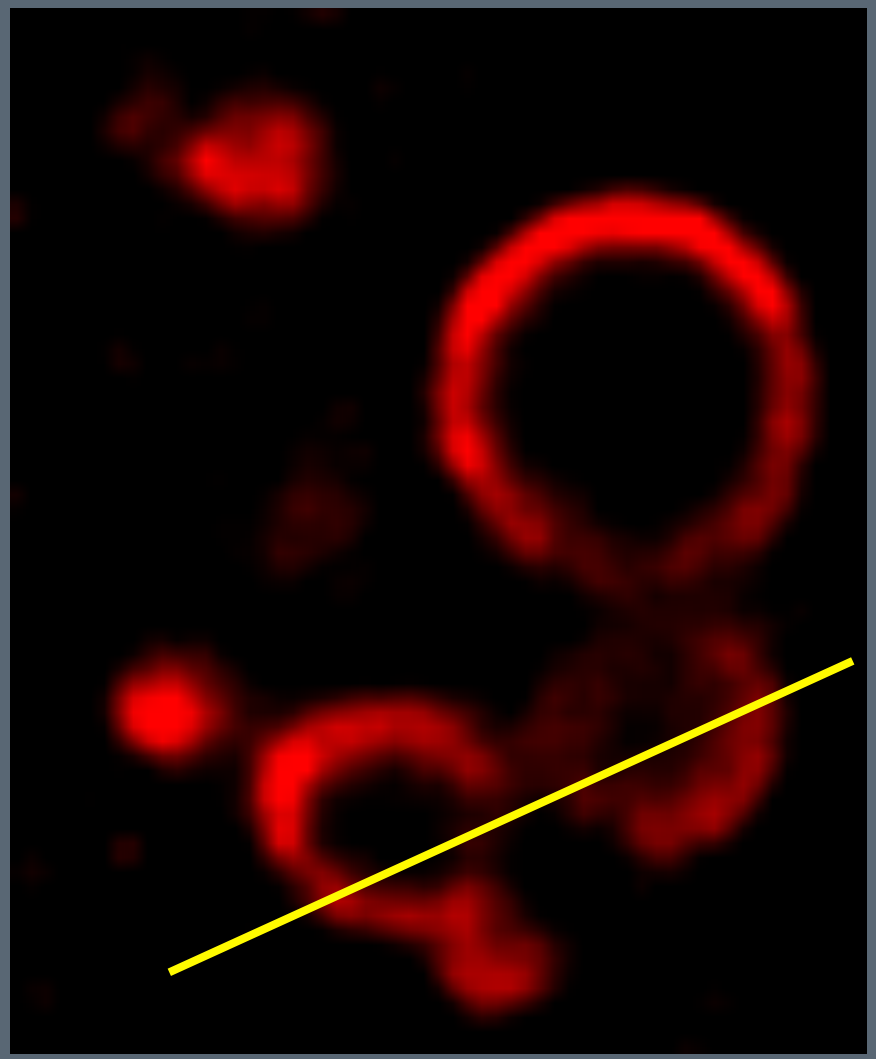
STORM



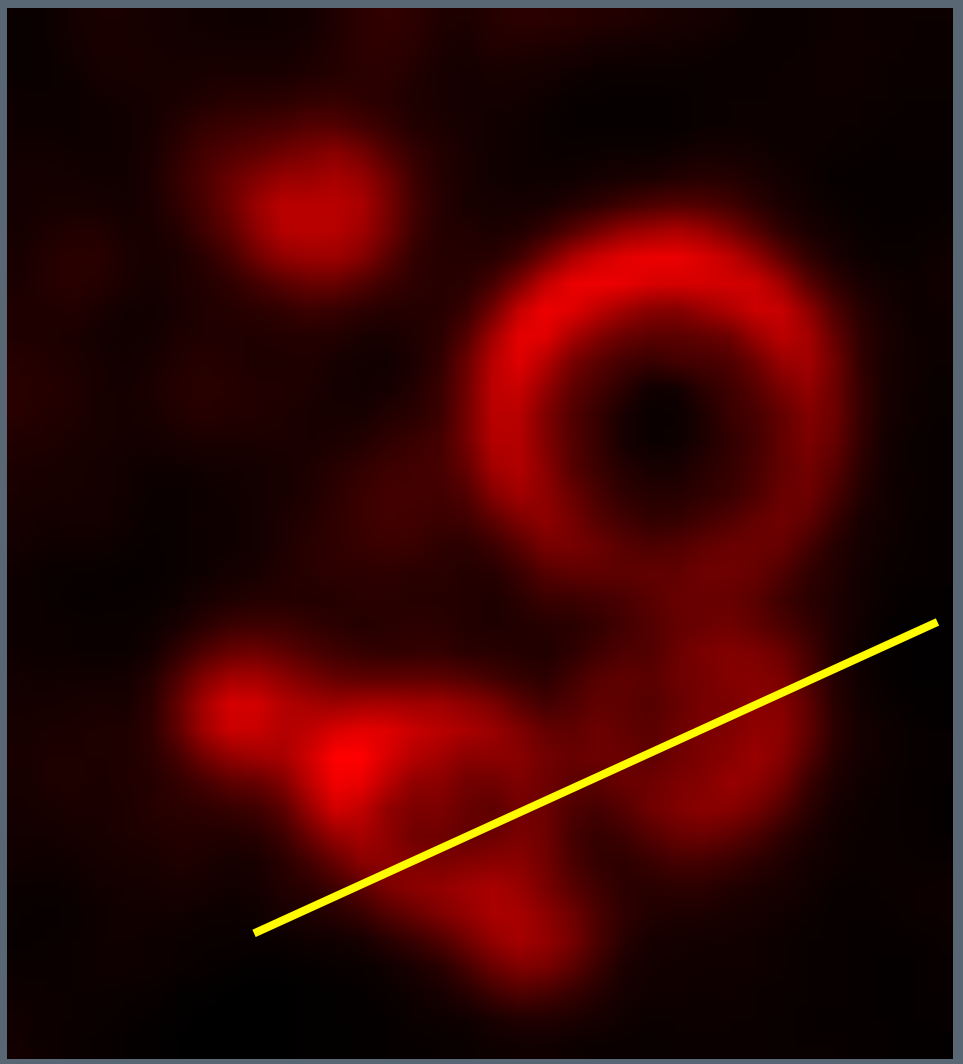


STORM

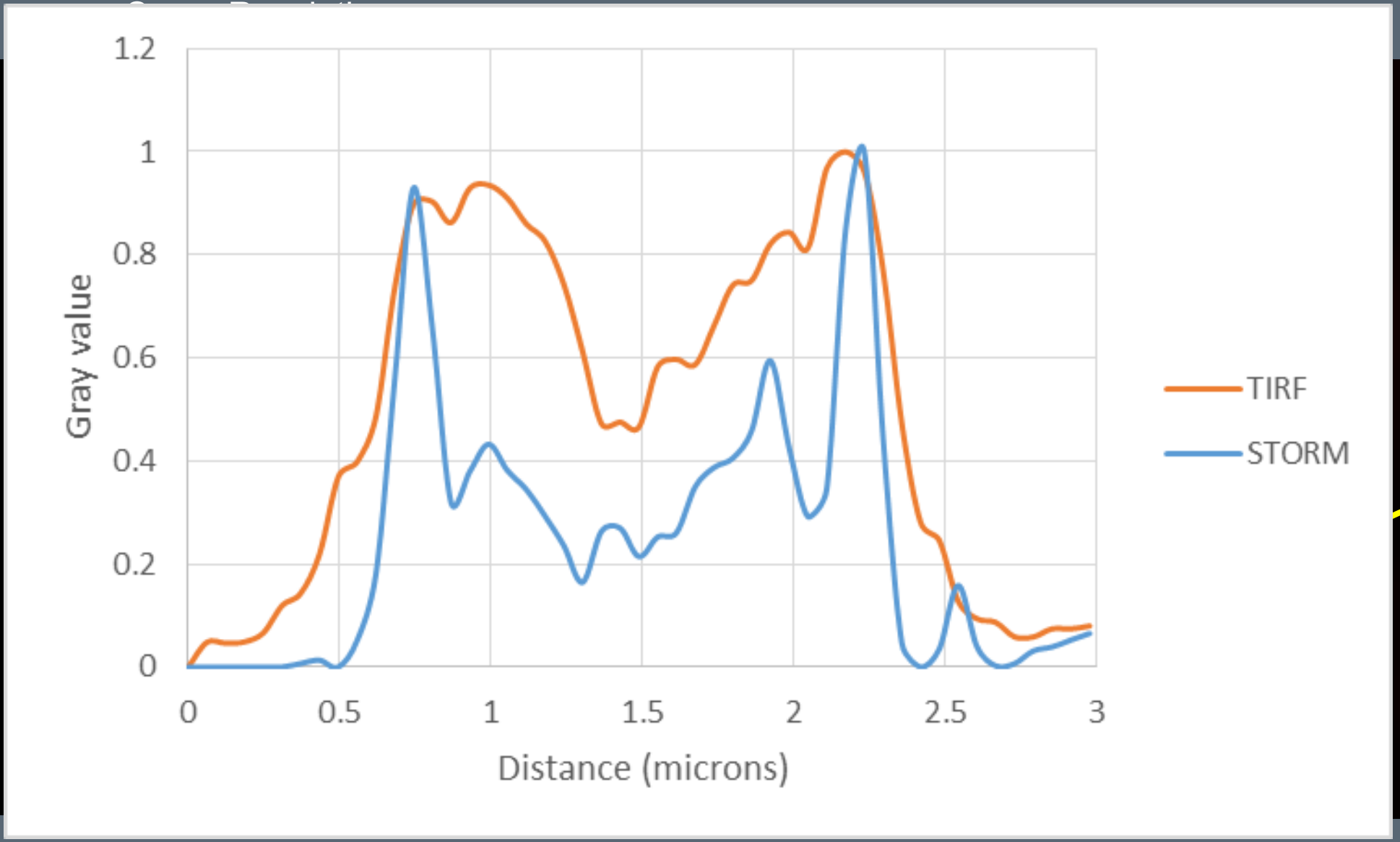
Super Resolution



TIRF Image

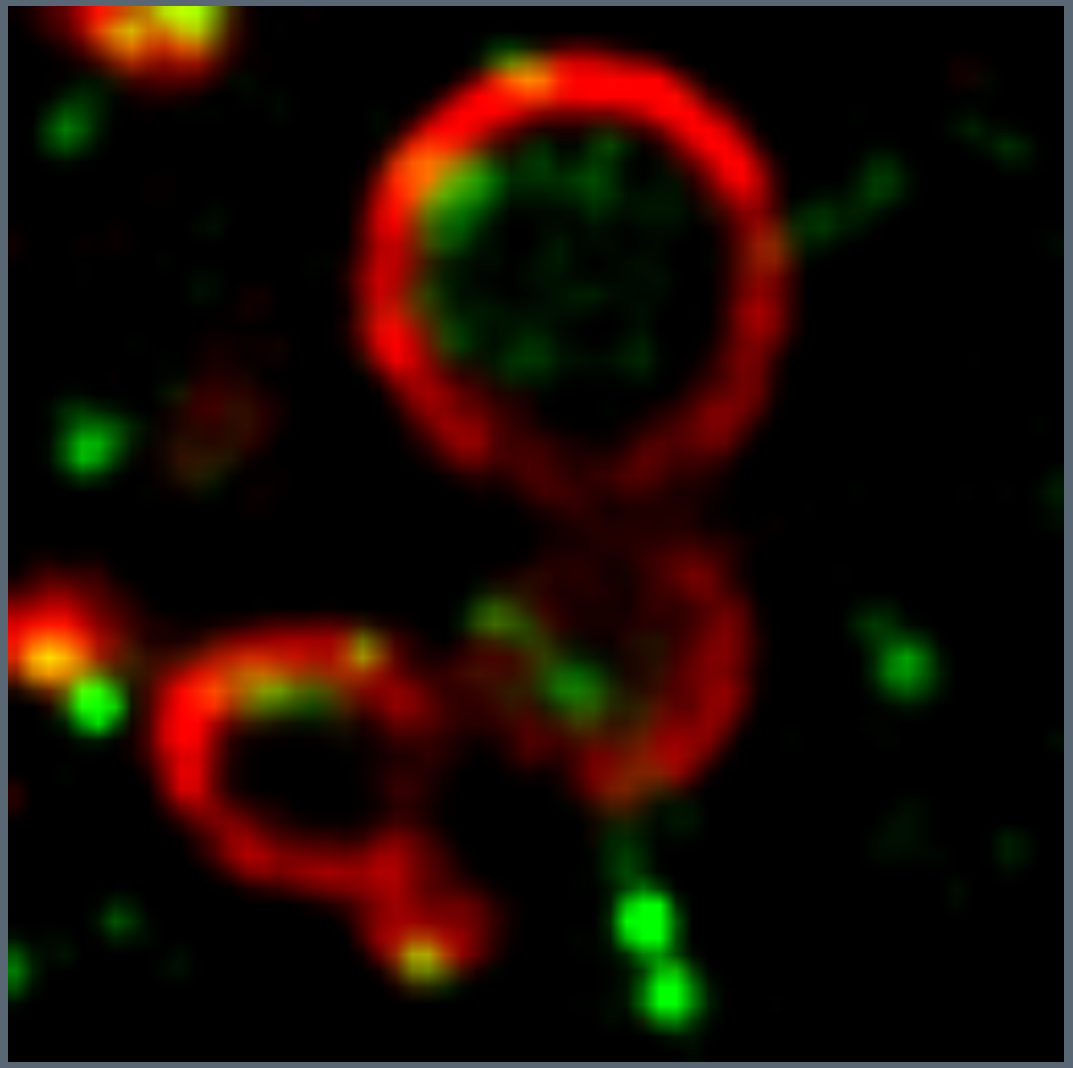


STORM

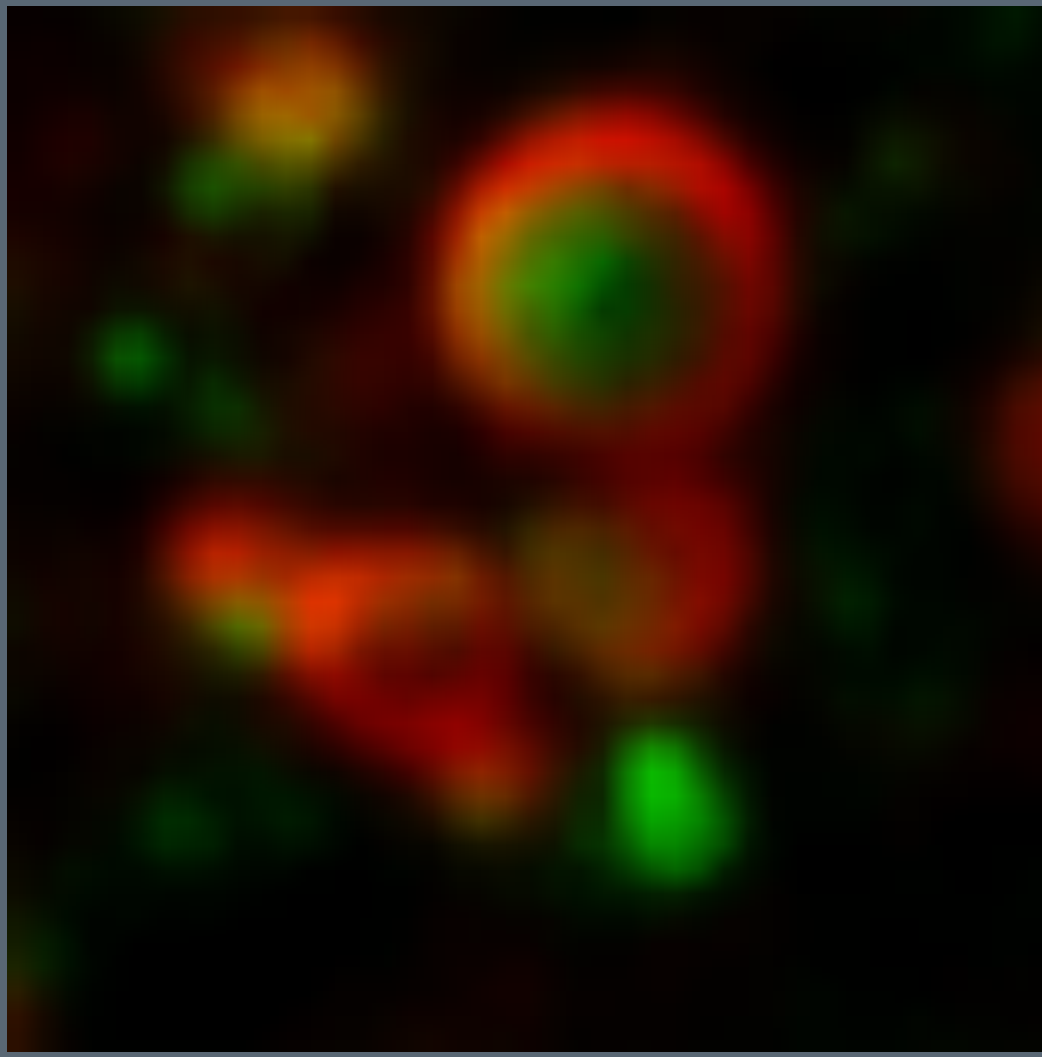

1.2
1
0.8
0.6
0.4
0.2
0
Gray value
0
0.5
1
1.5
2
2.5
3
Distance (microns)
TIRF
STORM

STORM

Super Resolution

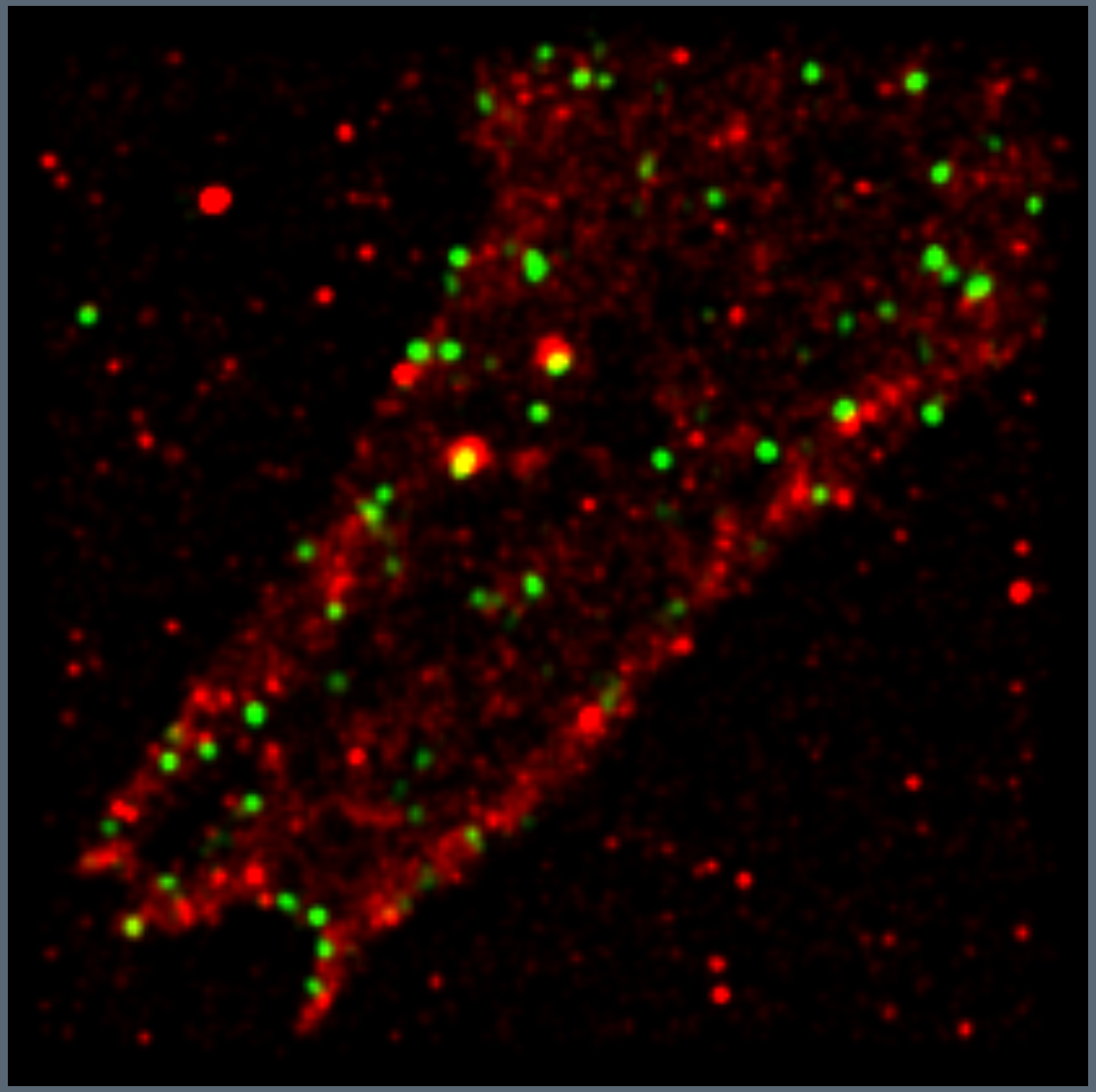


TIRF Image

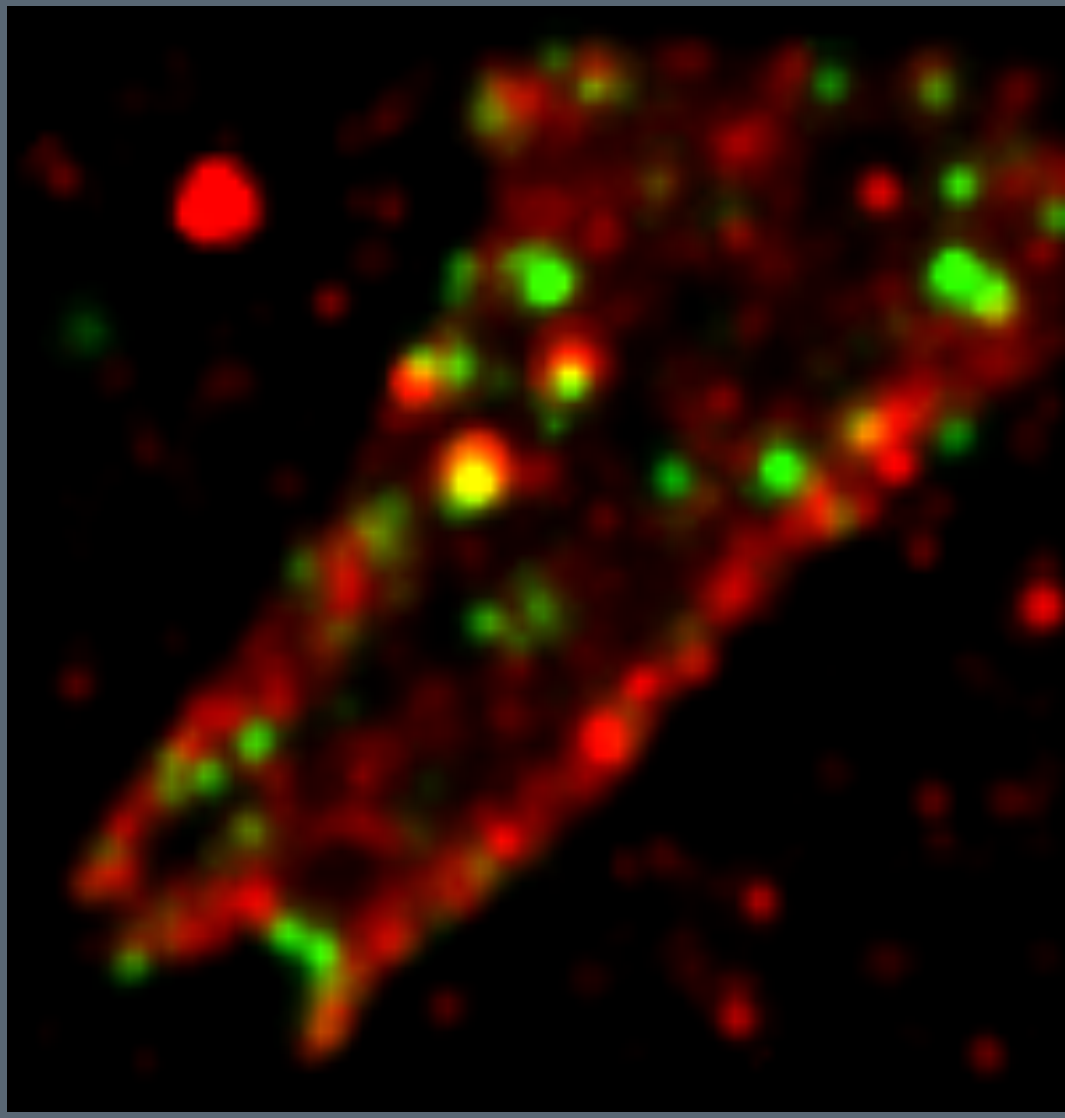


STORM

Super Resolution

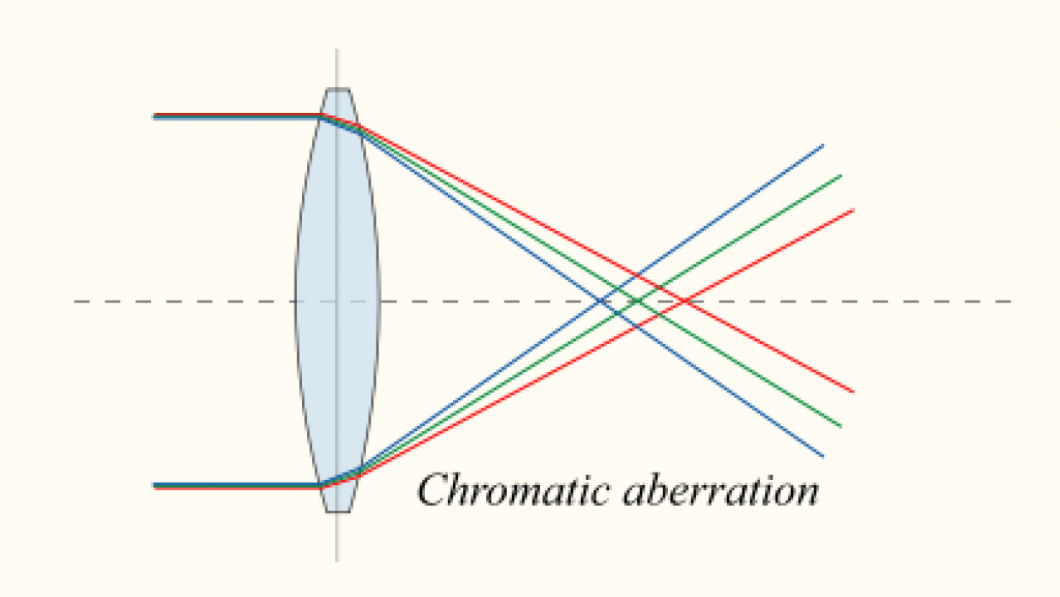


TIRF Image



Consequences of Super Resolution

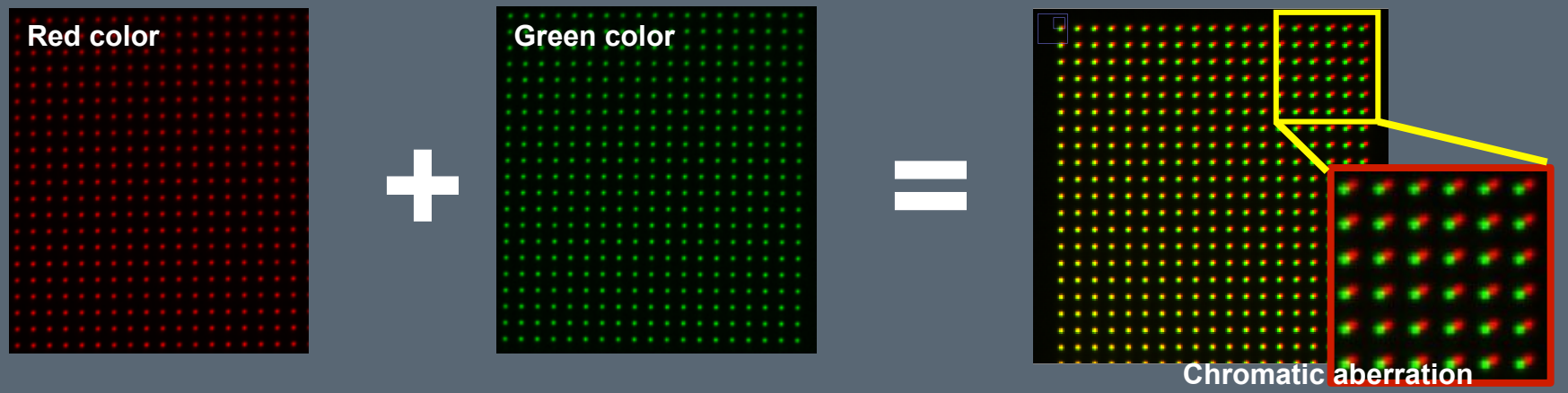
Chromatic Aberration in Microscopy



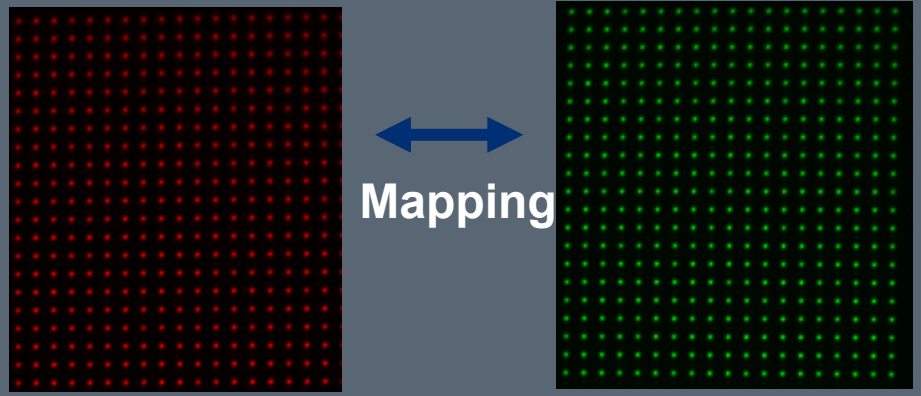
Each color focuses on a different position because of small differences in the refractive index

Lateral chromatic aberration is greater for objectives of short focal length and can range from 1.1 to 1.9 percent of the radial distance from the optic axis

Nanoholes to correct chromatic aberration



100 nm size nanoholes on silver coated coverslip
Nanoholes place every 1.5 μm



We obtained a **mapping function** that is taken by comparing individual dots between red and green channel

Mapping - red and green channel
(1 ~ 5 nm error)

SAMPLE DRIFT

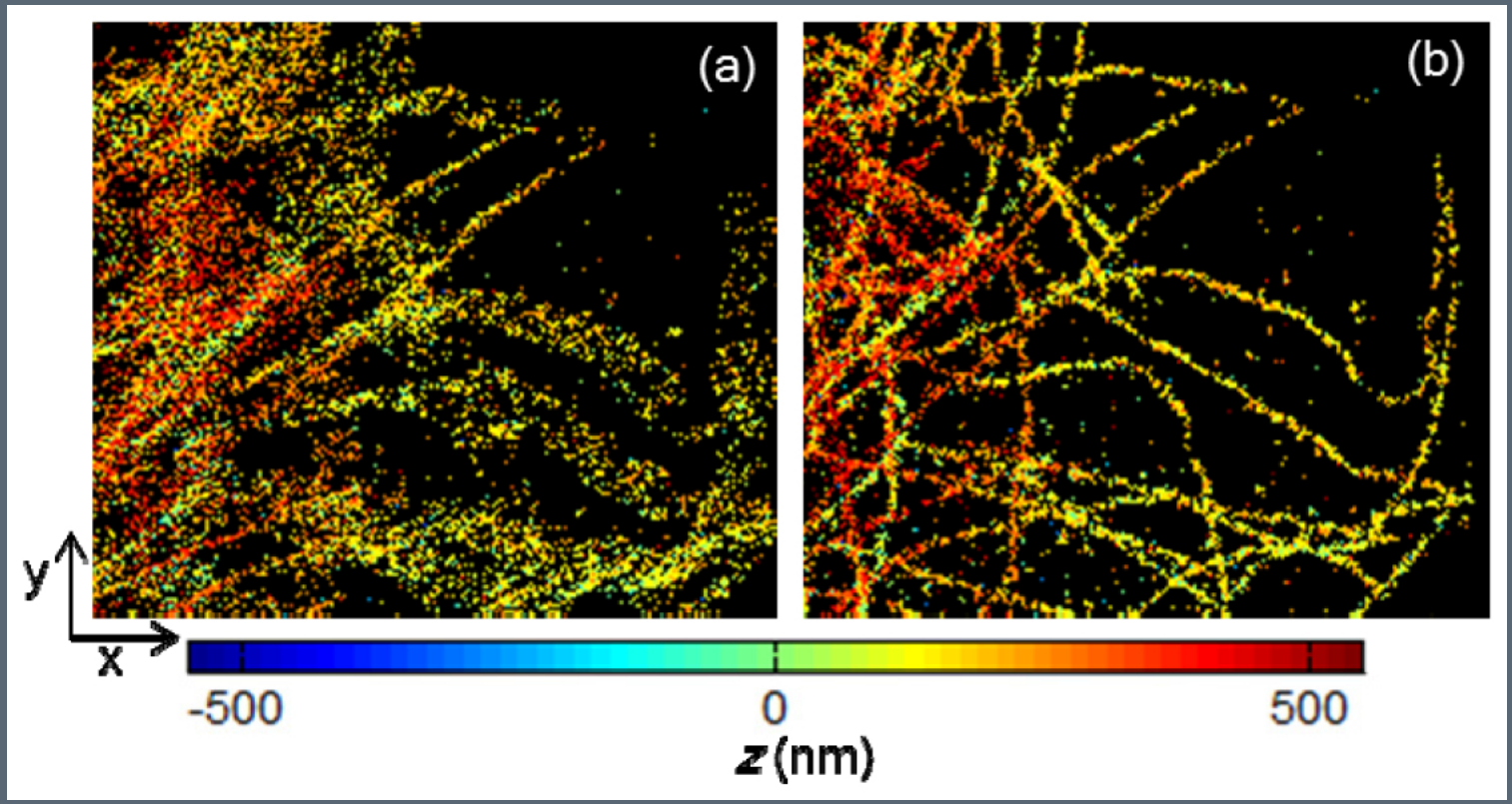
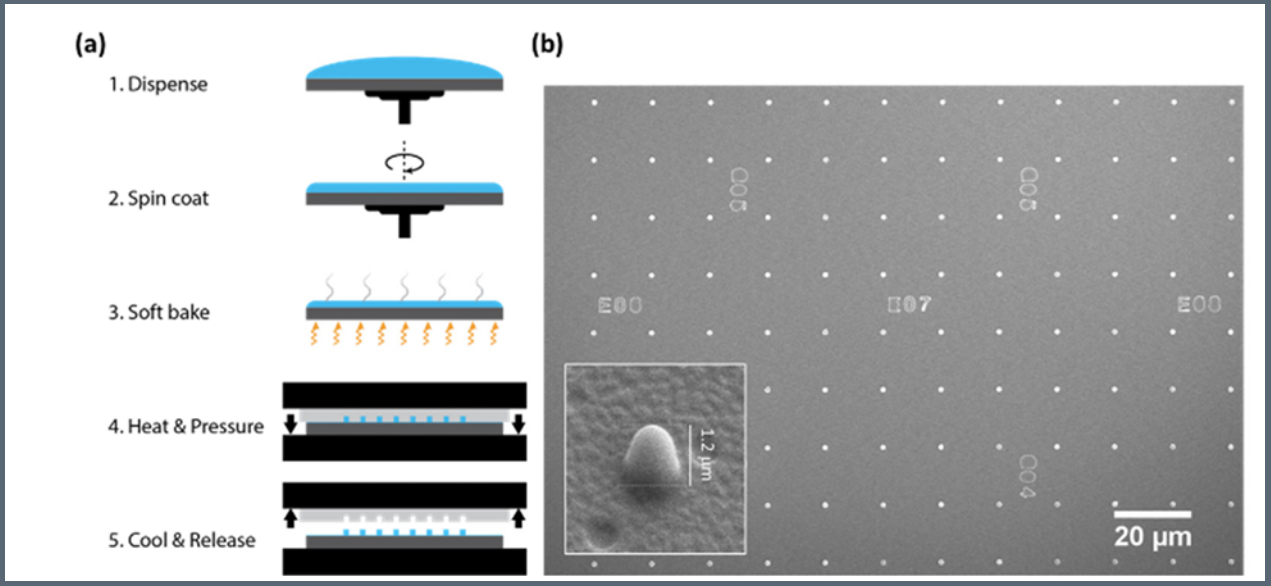
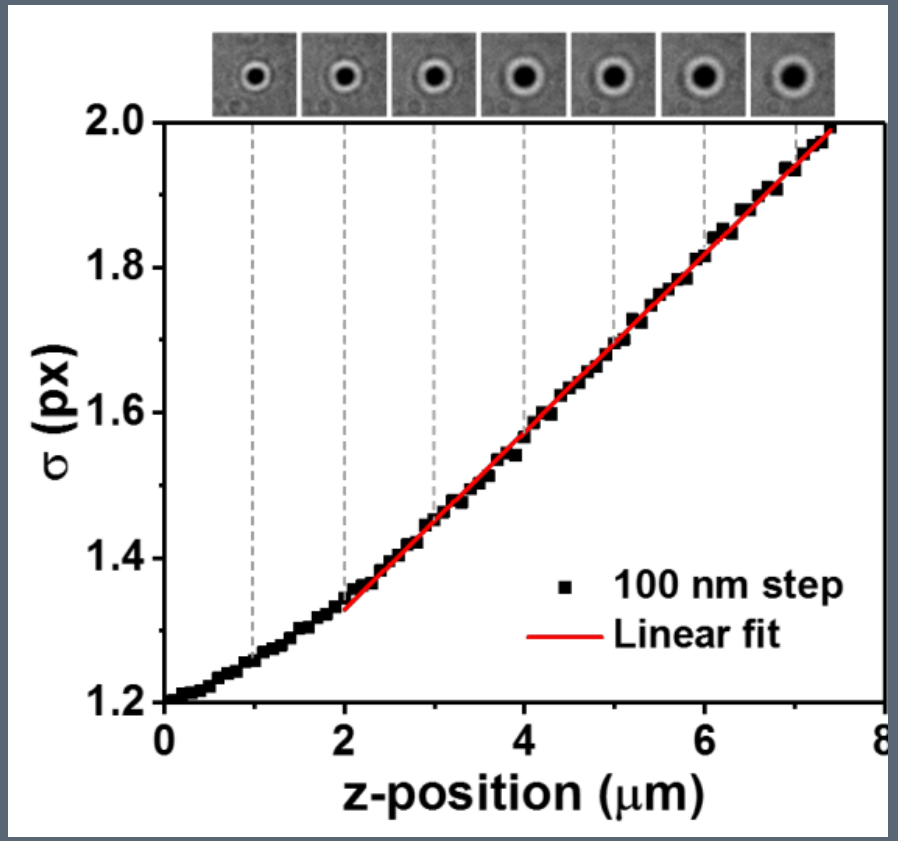


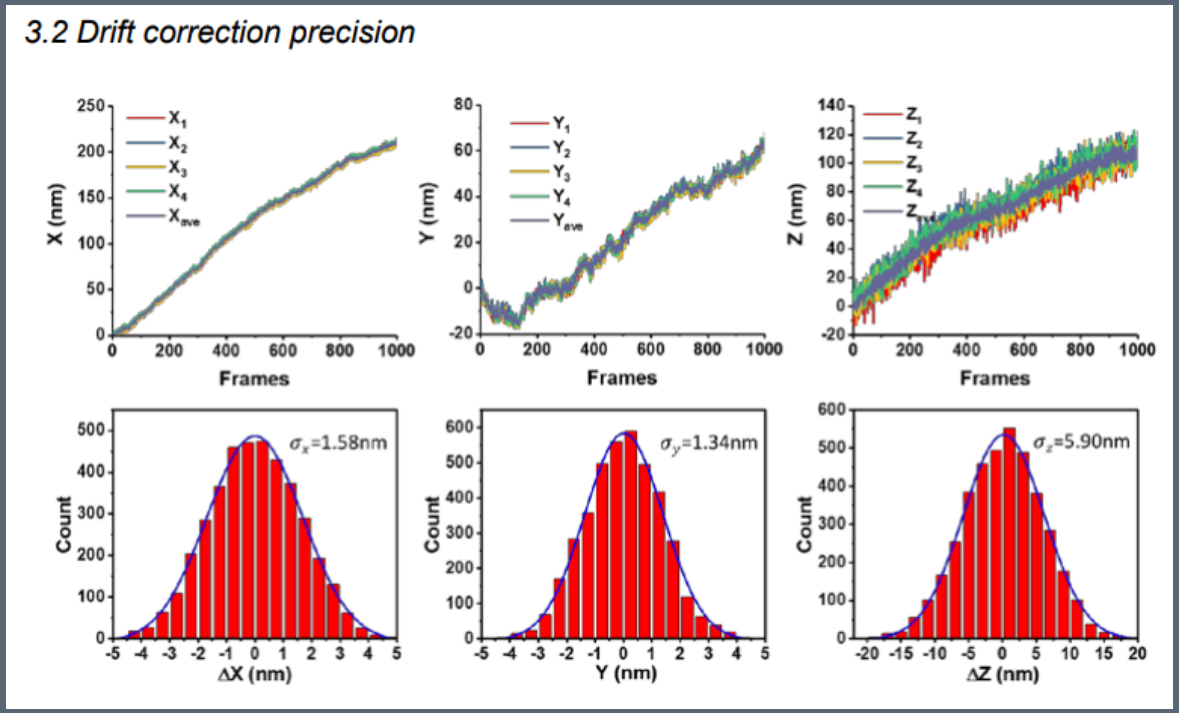
Fig. 1

Citation
Ginni Grover, Wyatt Mohrman, Rafael Piestun, "Real-time adaptive drift correction for super-resolution localization microscopy," Opt. Express **23,** 23887-23898 (2015);
https://www.osapublishing.org/oe/abstract.cfm?uri=oe-23-18-23887

SAMPLE DRIFT - CORRECTION

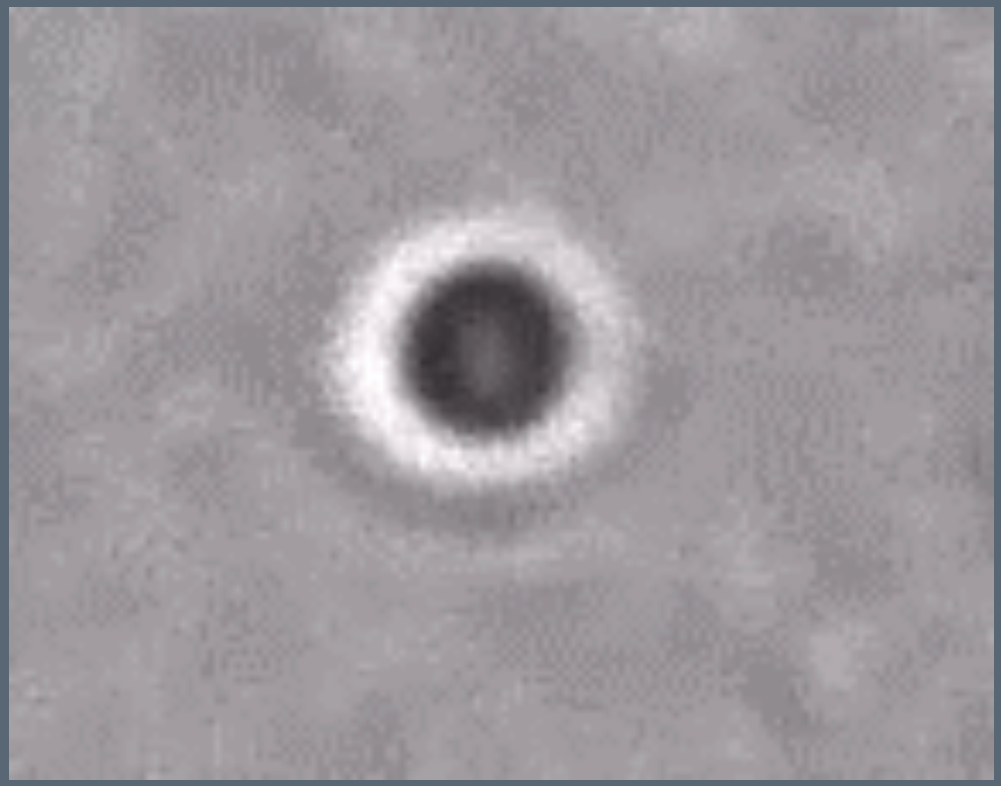




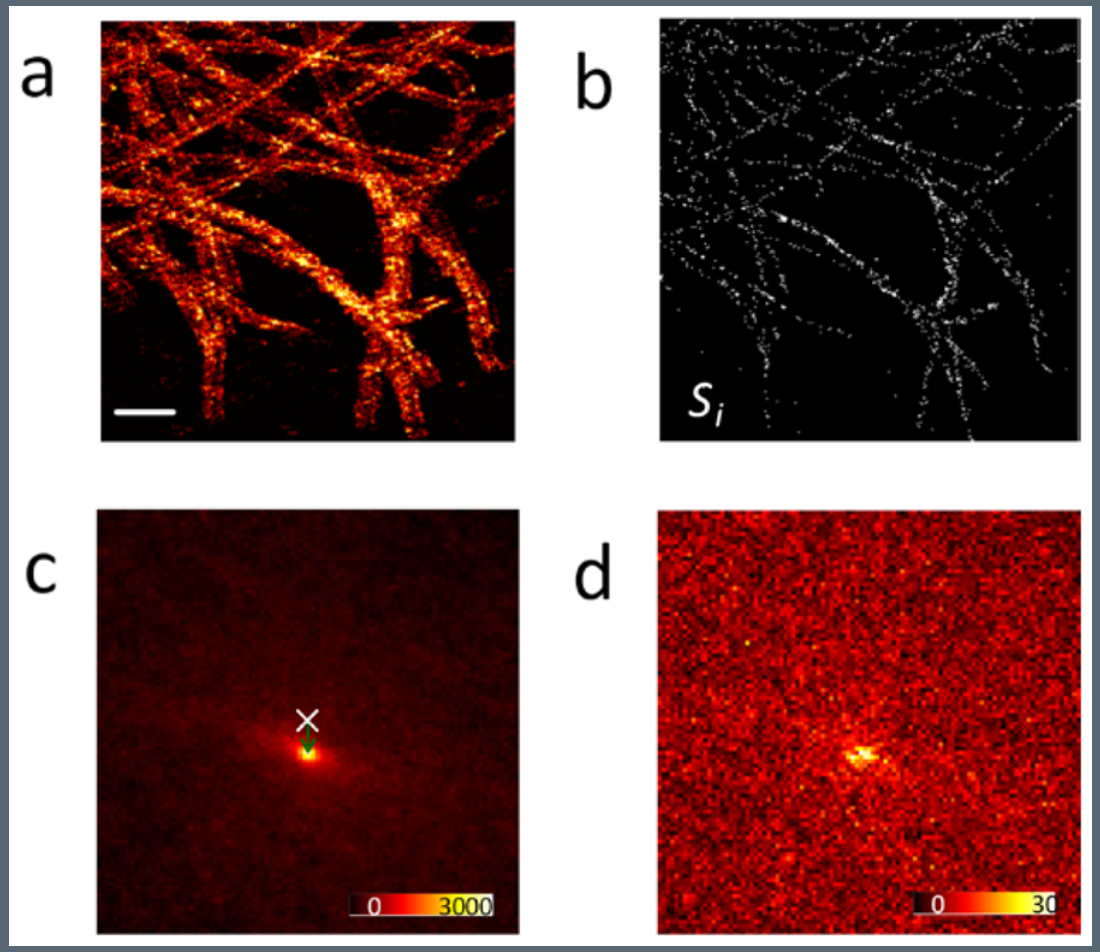


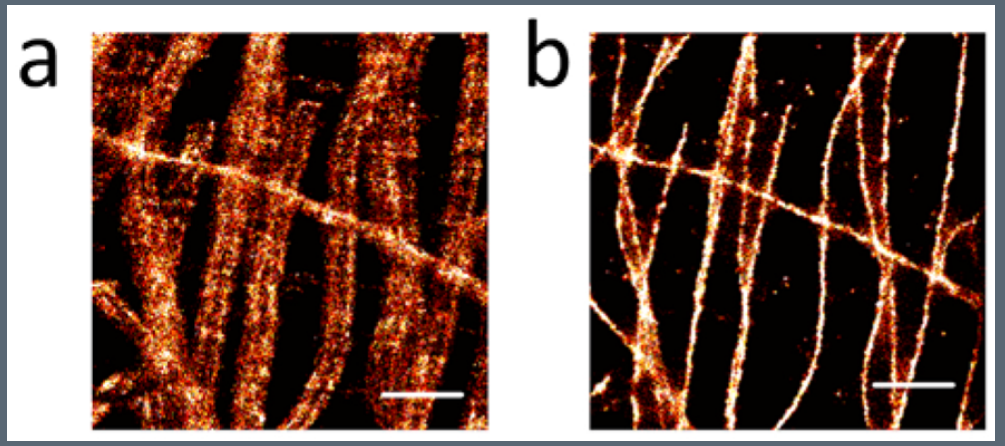
Yeoan Youn, Yuji Ishitsuka, Chaoyi Jin, Paul R. Selvin, "Thermal nanoimprint lithography for dr
correction in super-resolution fluorescence microscopy," Opt. Express **26,** 1670-1680 (2018)
https://www.osapublishing.org/oe/abstract.cfm?uri=oe-26-2-1670

SAMPLE DRIFT - CORRECTION



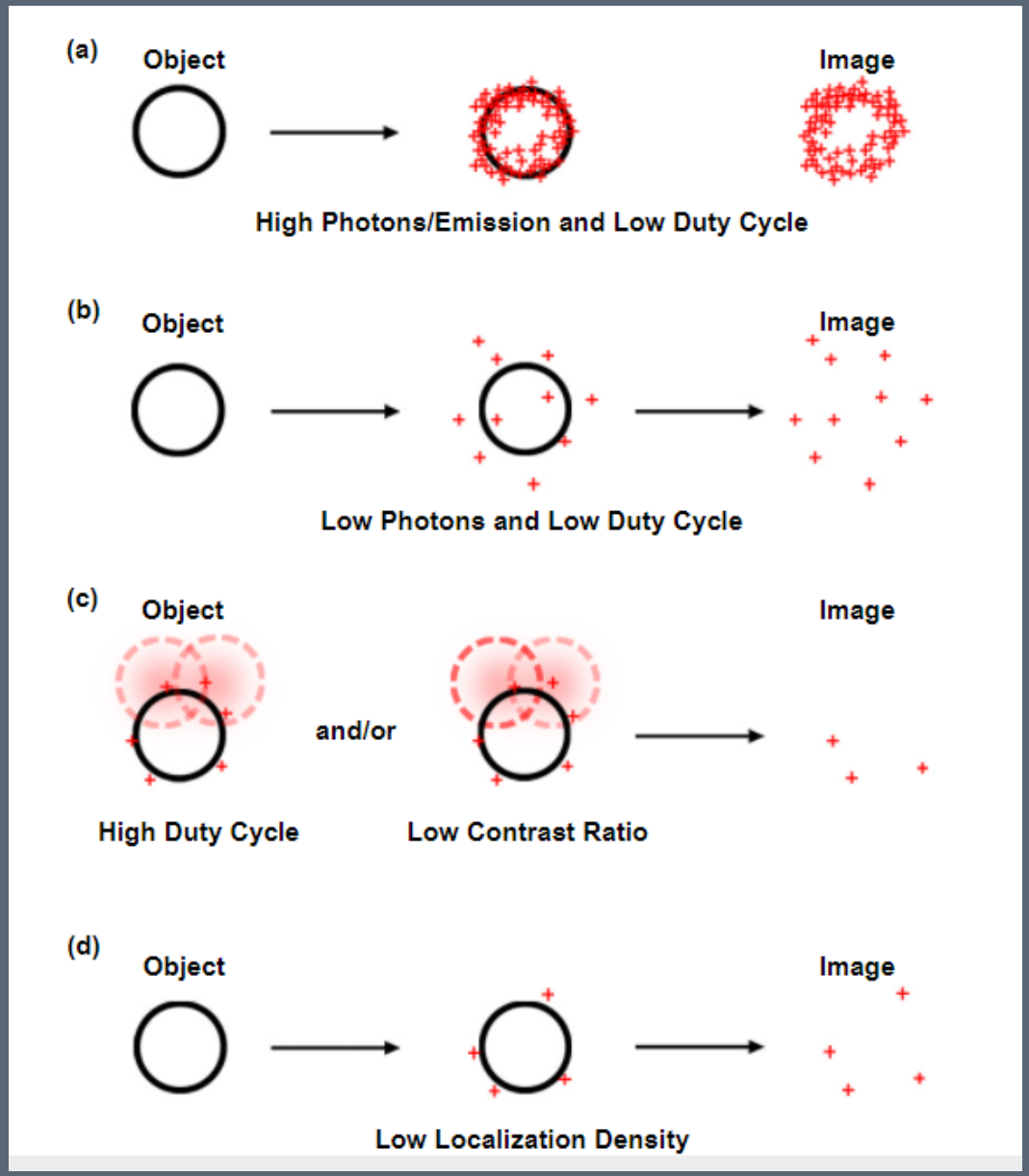
SAMPLE DRIFT - CORRECTION





Yina Wang, Joerg Schnitzbauer, Zhe Hu, Xueming Li, Yifan Cheng, Zhen-Li Huang, Bo Huang, "Localization events-based sample drift correction for localization microscopy with redundant cross-correlation algorithm," Opt. Express **22,** 15982-15991 (2014);
https://www.osapublishing.org/oe/abstract.cfm?uri=oe-22-13-15982

DYES FOR STORM



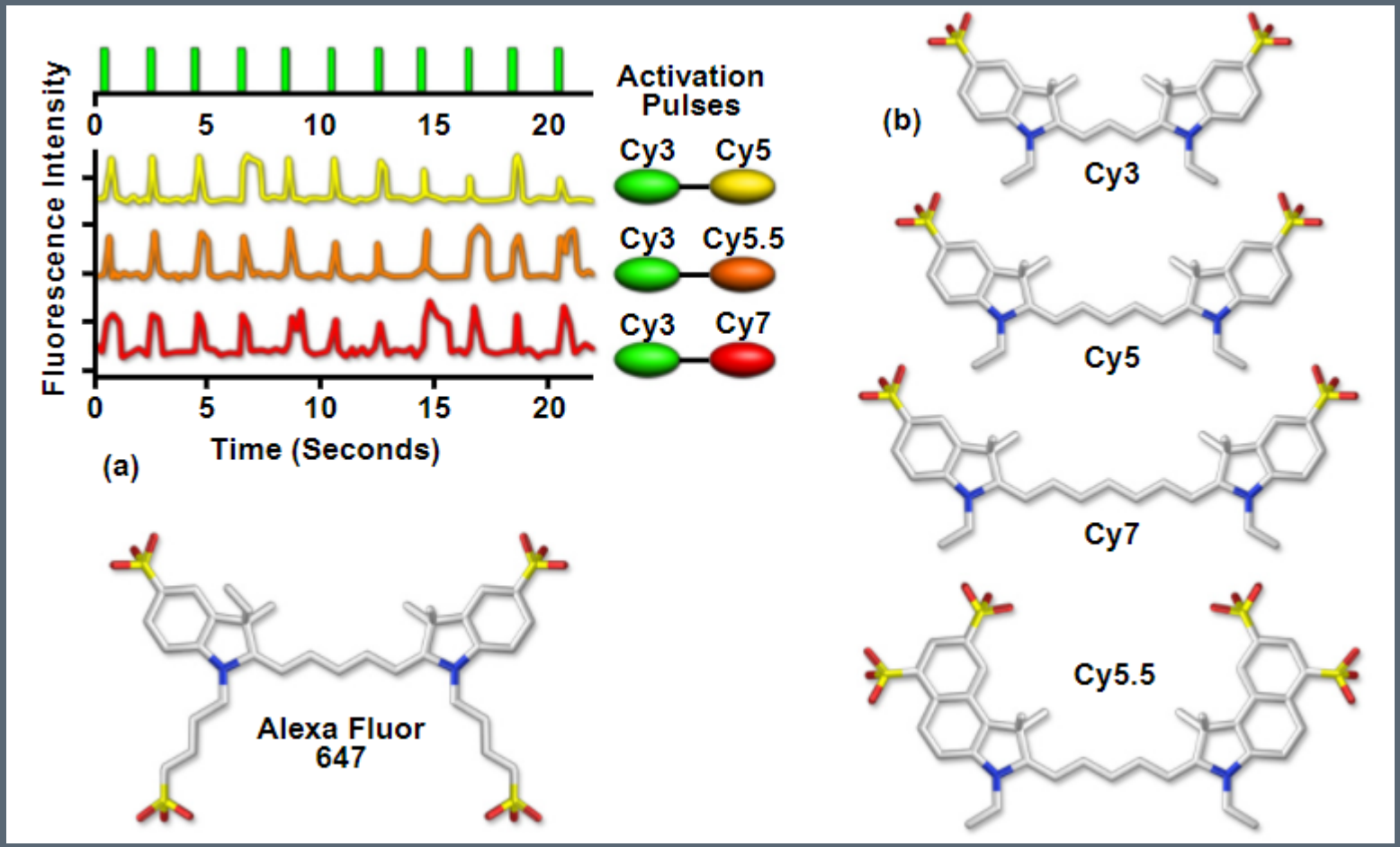
Precision ~ *width/* $\sqrt{\square N}$

Duty cycle

Switching cycles

https://www.microscopyu.com/techniques/super-resolution/single-molecule-super-resolution-imaging

DYES FOR STORM



"Classic" STORM

1 dye activator

1 dye reporter

https://www.microscopyu.com/techniques/super-resolution/single-molecule-super-resolution-imaging

DYES FOR STORM

dSTORM—Alexa Fluor® 488–568 | dSTORM—Alexa Fluor® 594–750 | nSTORM

	Alexa Fluor® 488	Alexa Fluor® 532	Alexa Fluor® 555	Alexa Fluor® 568
Target	Label/conjugate	Label/conjugate	Label/conjugate	Label/conjugate
STORM buffer	MEA	MEA	MEA	MEA
Bibliography	Citations	Citations	Citations	Citations
Laser line (nm)	488	488	488	561
Standard filter set	FITC	TRITC	TRITC	RFP
Ex/Em (nm)	495/519	532/554	555/580	578/603

dSTORM—Alexa Fluor® 488–568 | dSTORM—Alexa Fluor® 594–750 | nSTORM

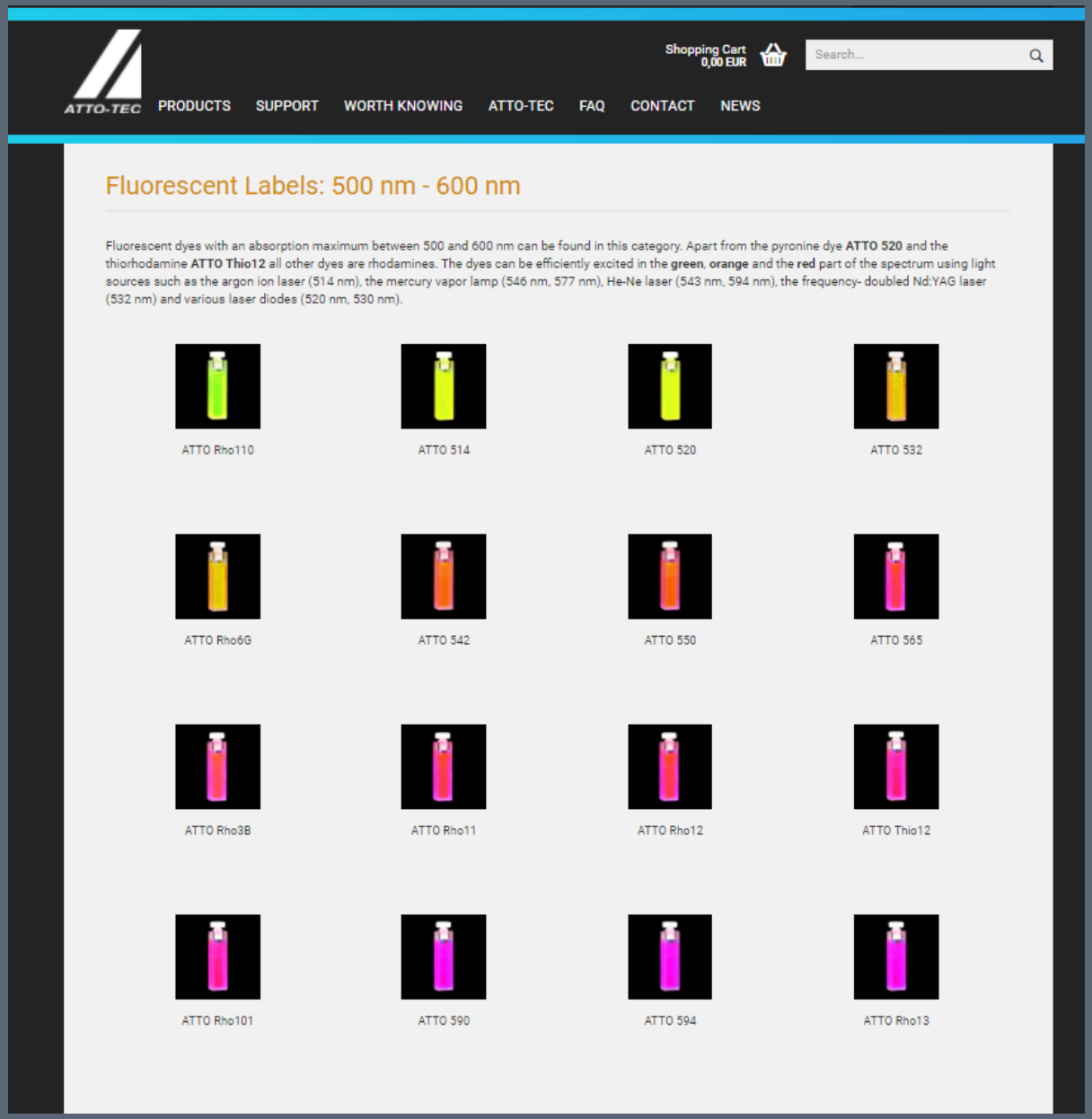
	Alexa Fluor® 594	Alexa Fluor® 647	Alexa Fluor® 680	Alexa Fluor® 750
Target	Label/conjugate	Label/conjugate	Label/conjugate	Label/conjugate
STORM buffer	MEA	BME	BME	TCEP
Bibliography	Citations	Citations	Citations	Citations
Laser line (nm)	594	594/633	633	633
Standard filter set	Texas Red® dye	Cy®5	Cy®5.5	Cy®7
Ex/Em (nm)	590/617	650/665	679/702	749/775

ThermoFisher SCIENTIFIC

dSTORM

Only 1 dye activated by buffer

DYES FOR STORM



dSTORM

Only 1 dye activated by buffer

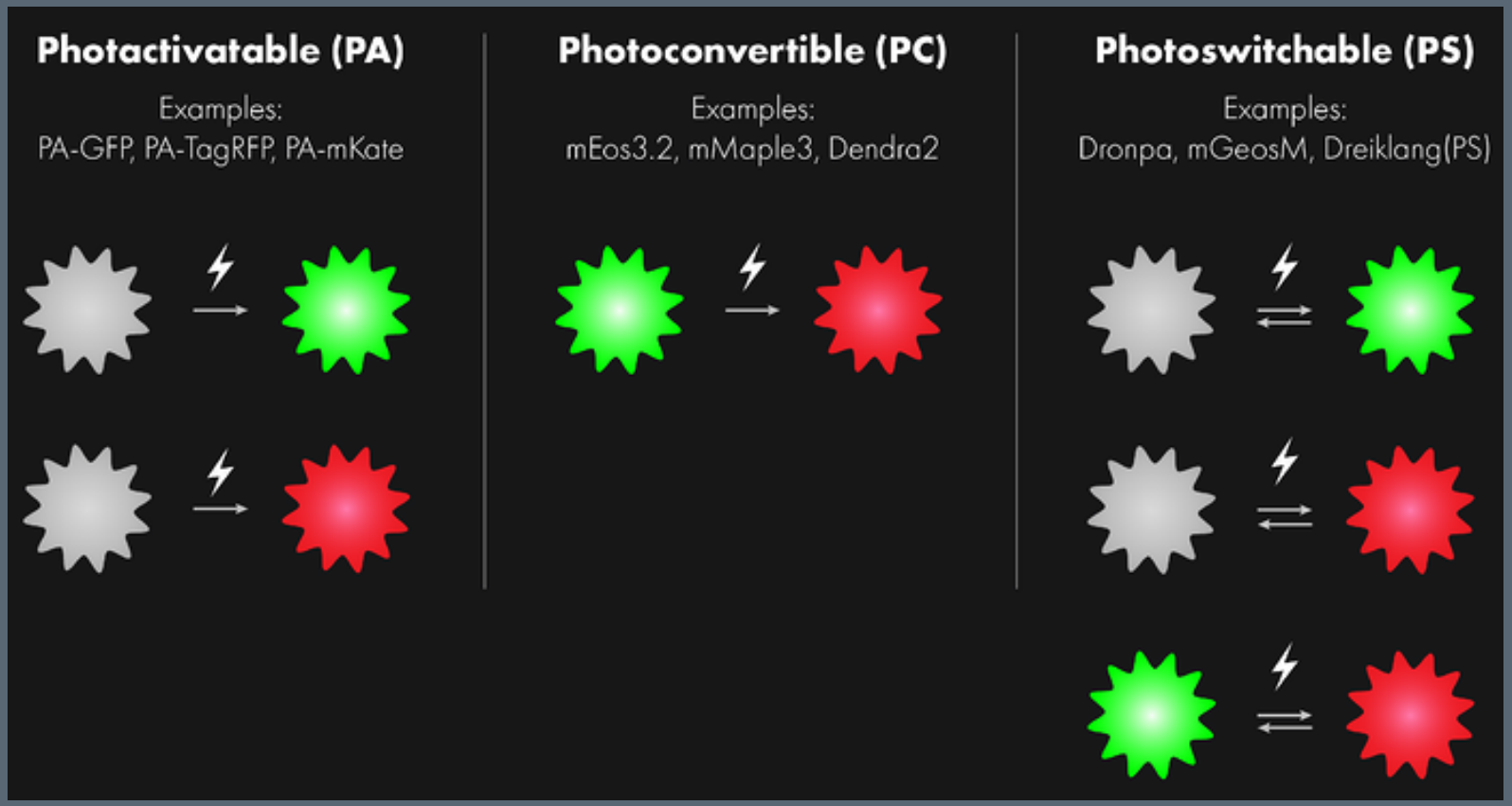
STORM BUFFERS

Basic imaging buffer	Dye-specific buffers		
	MEA	BME	TCEP
• 50 mM TRIS, 10 mM NaCl (to pH 8) • GLOX (0.5 mg/mL glucose oxidase, 40 µg/mL catalase, 10% glucose)	+ MEA to10 mM	+ BME to 140 mM	+ TCEP to 10–100 mM (need 1 mM ascorbic acid and methyl viologen)
Dempsey et al. *Nat Methods* 8:1027–36	Dempsey et al. *Nat Methods* 8:1027–36	Dempsey et al. *Nat Methods* 8:1027–36	Vaughan et al. 2013 *J Am Chem Soc* 135(4):1197–200

In order to image native AMPARs, we labeled AMPARs using Anti-GluA2-Alexa647 after fixation. For STORM imaging, we added imaging buffer consisting of 5 mM MEA (Sigma: 30070, St. Louis, MO) solution (~pH 8.0) and additionally added 40 mM Sodium D/L-lactate (Sigma: 71720, St. Louis, MO) and EC-Oxyrase (Sigma: SAE0010, St. Louis, MO) in PBS in order to improve the photo-stability.

https://elifesciences.org/articles/27744#s4

PALM PHOTOACTIVABLE PROTEINS

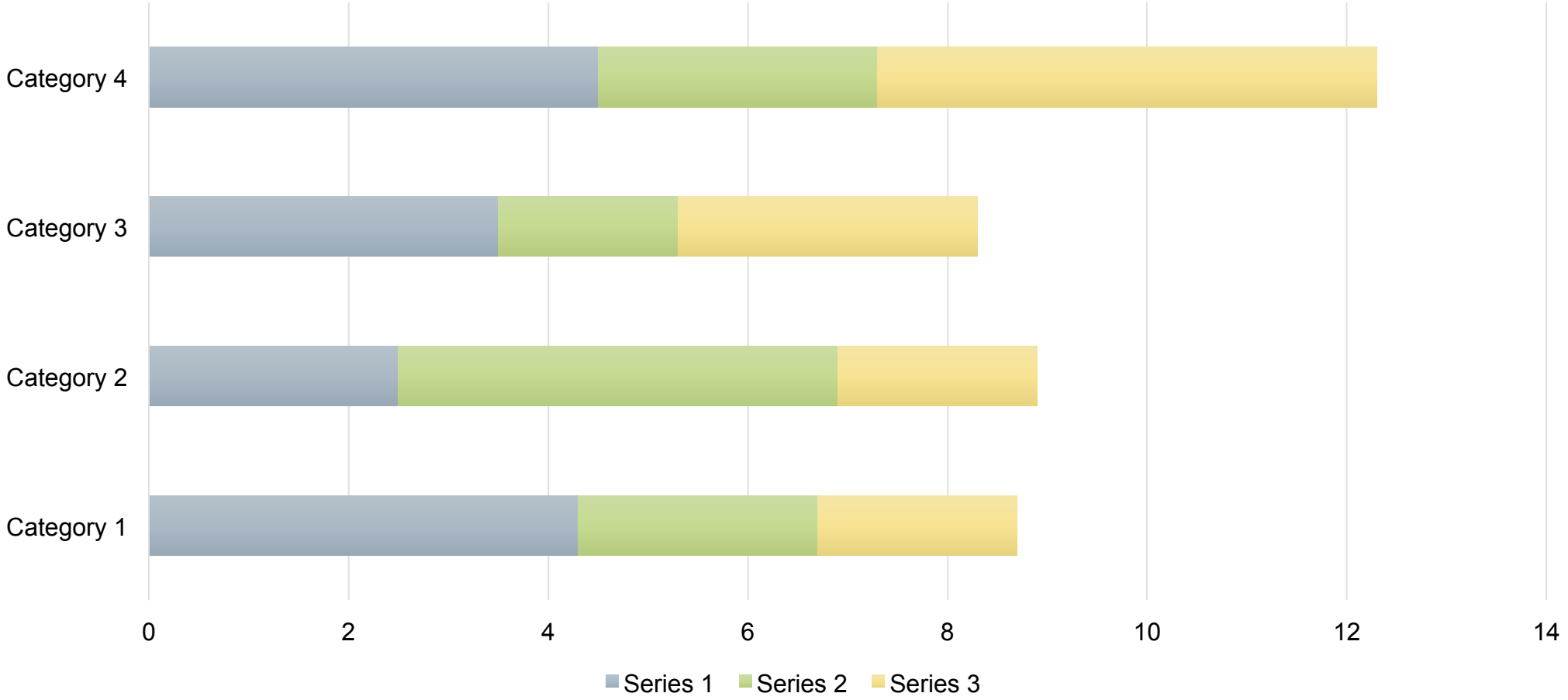


PA – Emits after activation
PC – Changes emission after activation
PS – Reversible between stats

Acknowledgement

Laboratório de Física Biológica e Sinalização Celular

Title and Content Layout with Chart

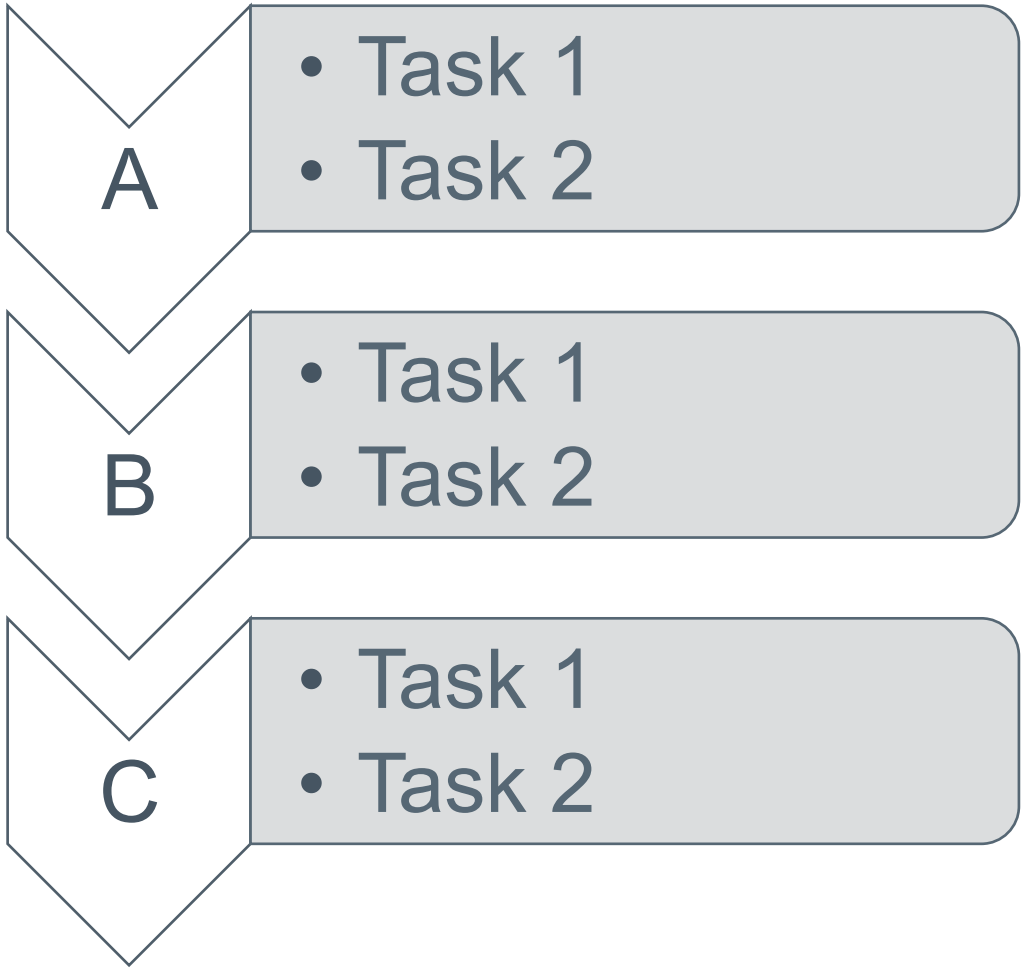


Two Content Layout with Table

Class	Group A	Group B
Class 1	82	95
Class 2	76	88
Class 3	84	90

- First bullet point here
- Second bullet point here
- Third bullet point here

Two Content Layout with SmartArt



› First bullet point here

› Second bullet point here

› Third bullet point here

Add a Slide Title - 1

Add a Slide Title - 2

Add a Slide Title - 3

ADD A SLIDE TITLE - 4

ADD A SLIDE TITLE - 5