BIOSCIENCE IMAGING FACILITY

Bringing new perspectives to life

Microscopy | Live Animal Imaging

A collaborative environment that provides the knowledge, instruments, and expertise needed to visualize life at scales ranging from single molecules to entire animals.

- Project specific instrument training & advice.
- Consultation on sample preparation, image processing, and data analysis.
- Charge-back rates; Collaborations are encouraged.

Microscopy

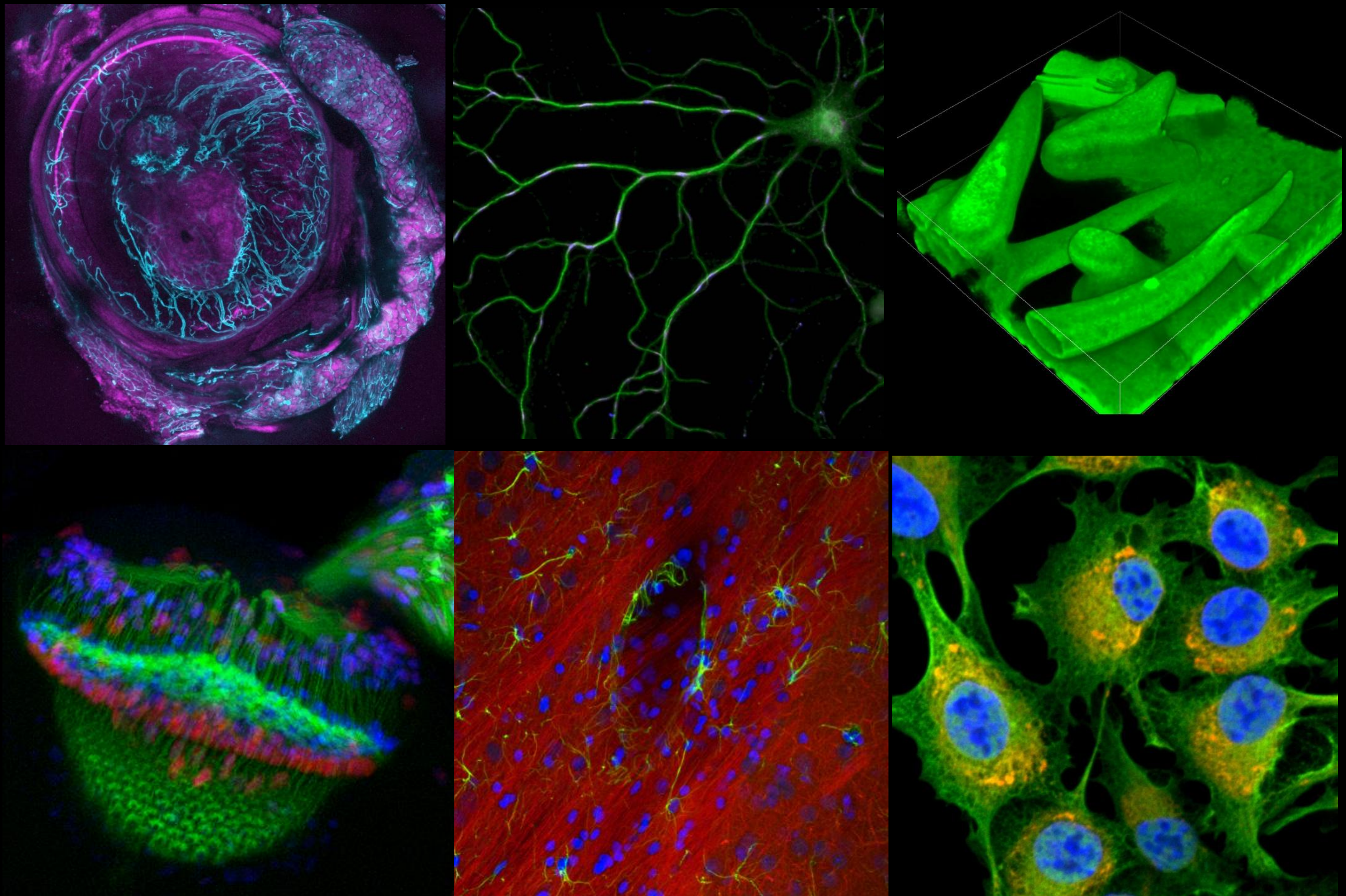
Nikon A1Rsi Confocal

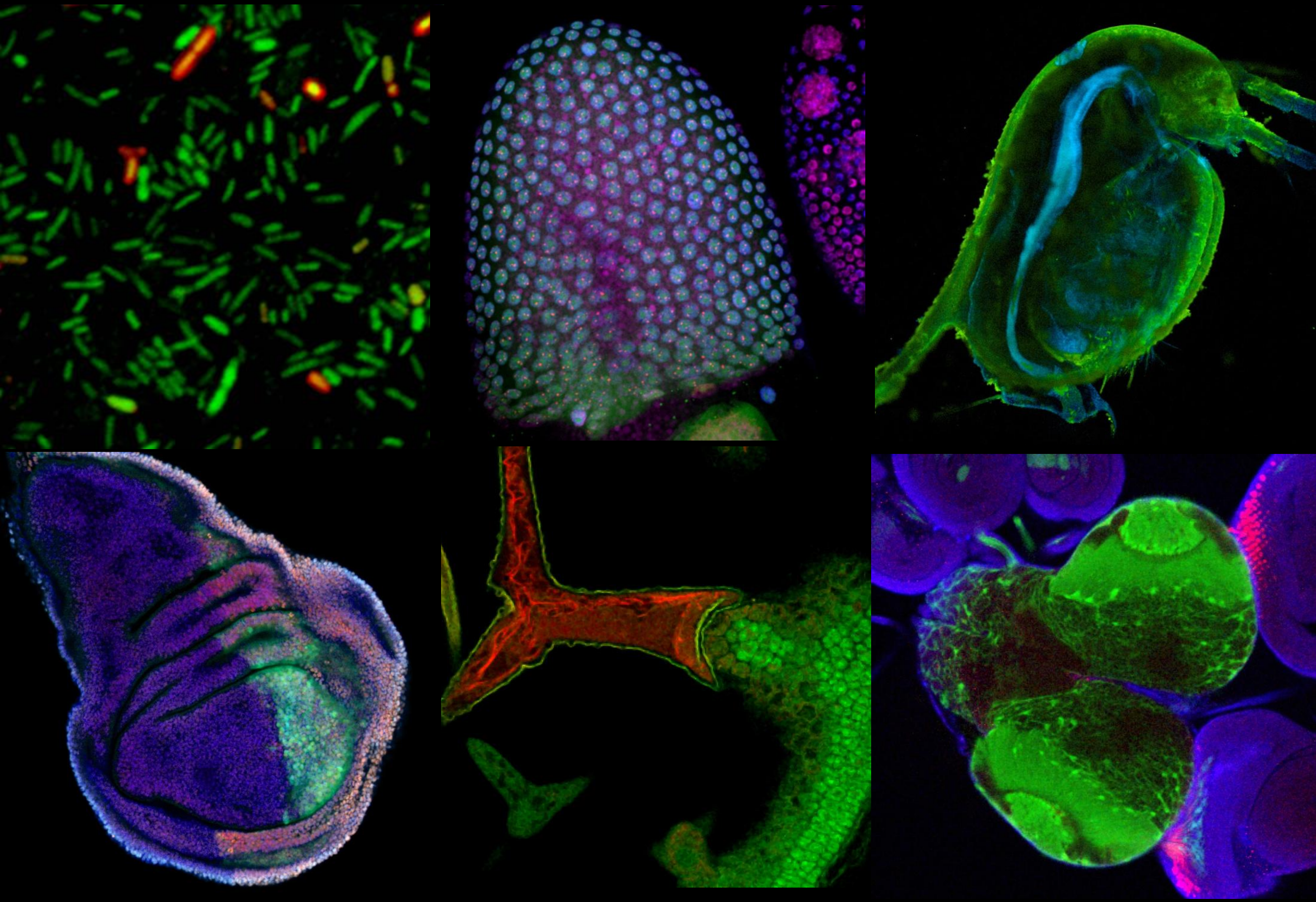
Nikon STORM/SIM

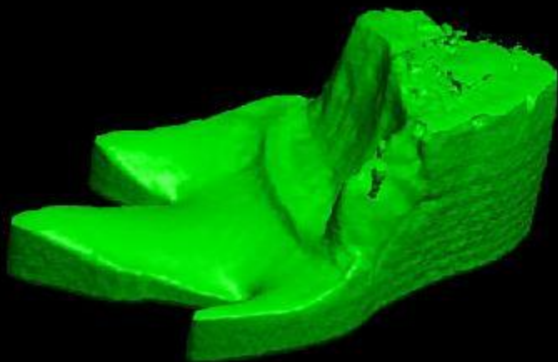
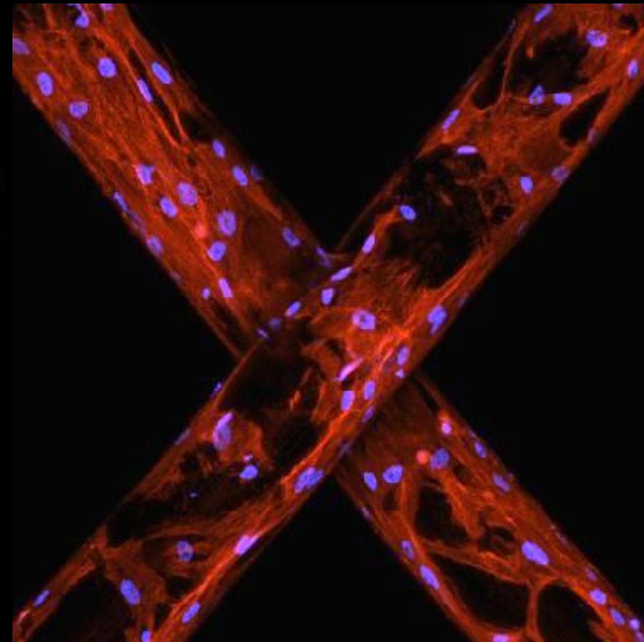
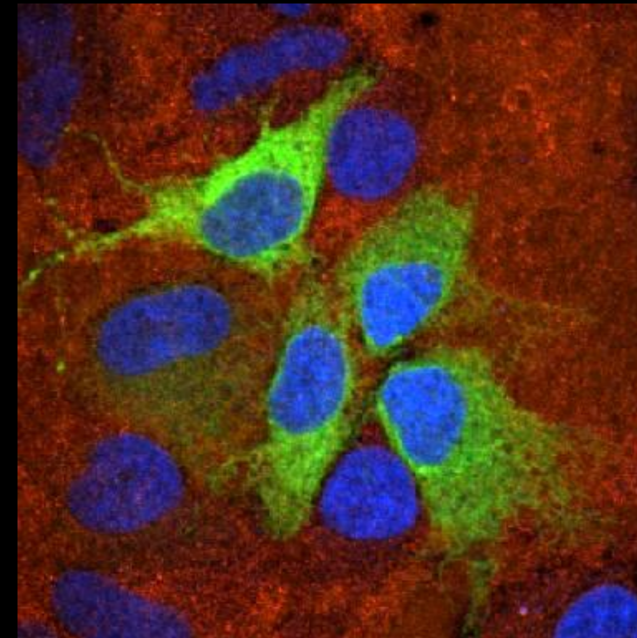
Nikon A1R Multi-photon

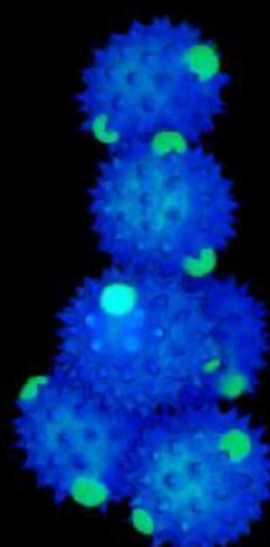
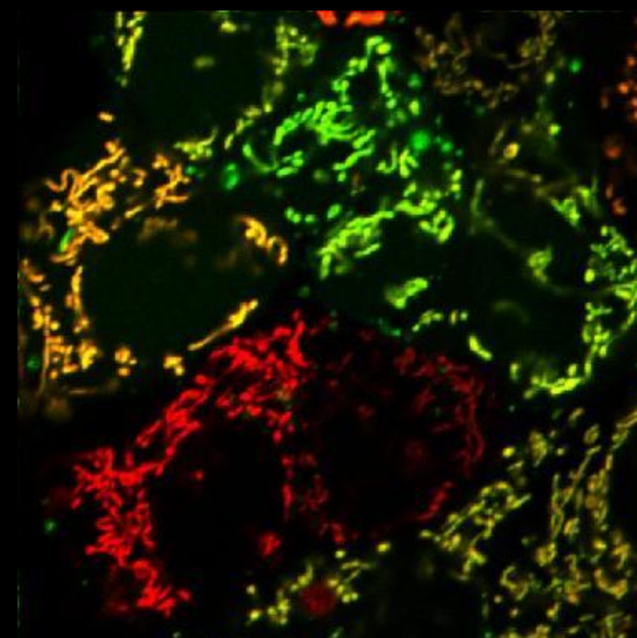
Zeiss 710 Confocal

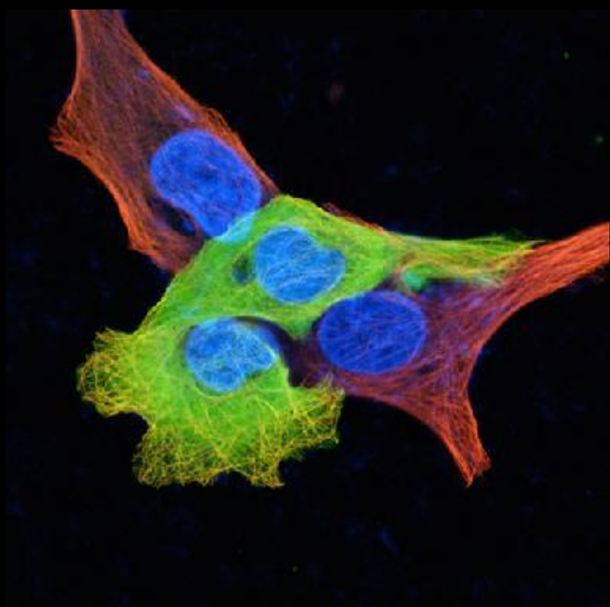
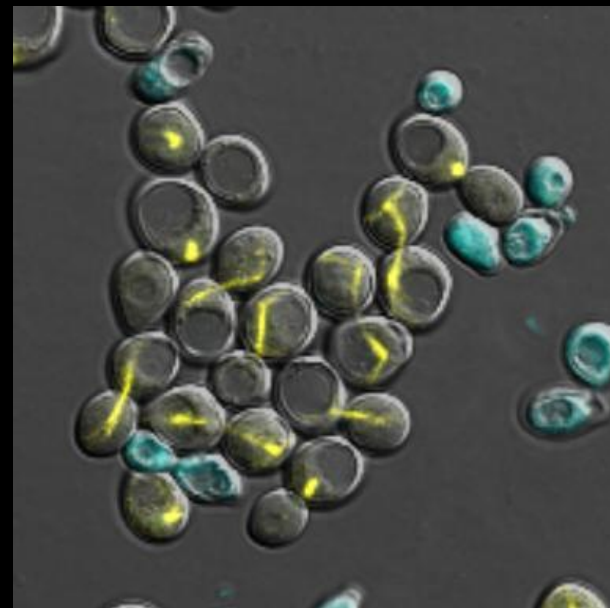
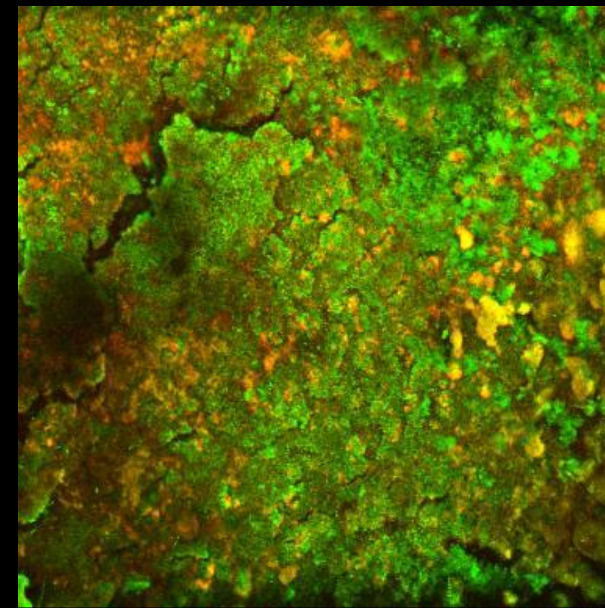
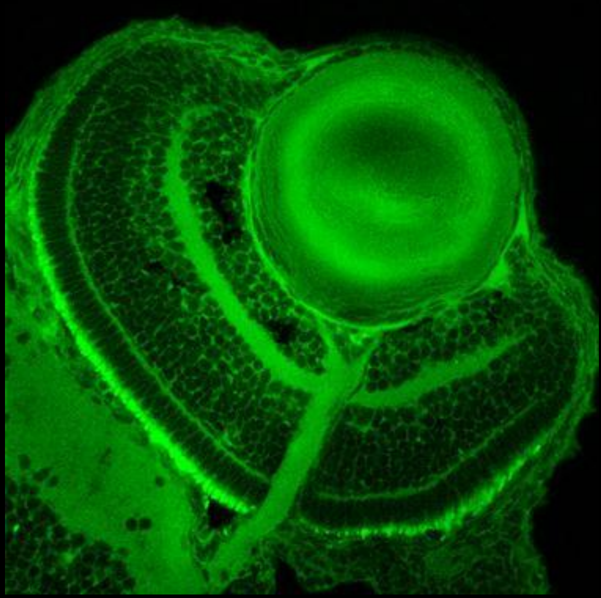
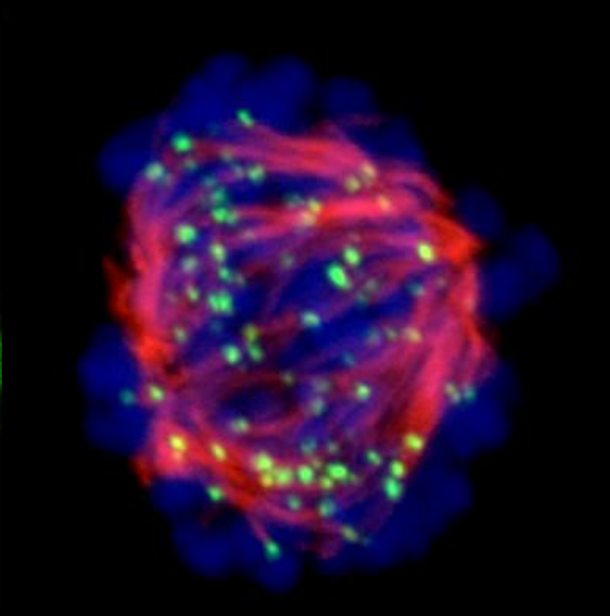
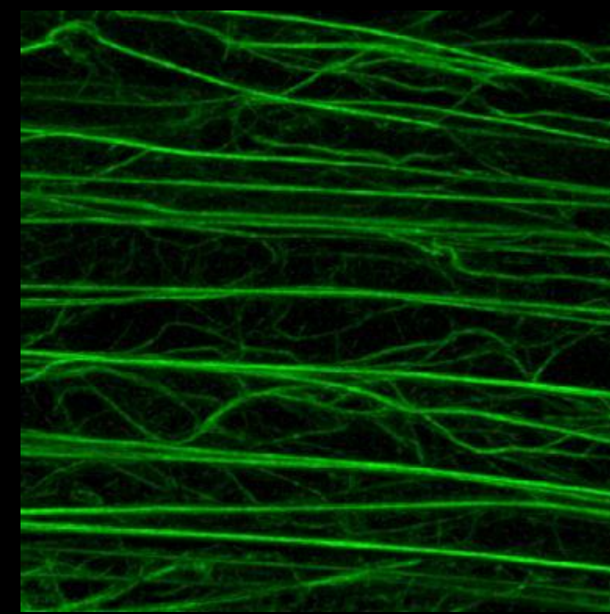
<u>Also</u>: Nikon 90i Widefield, Nikon Ti-S Phase, Nikon SMZ1000



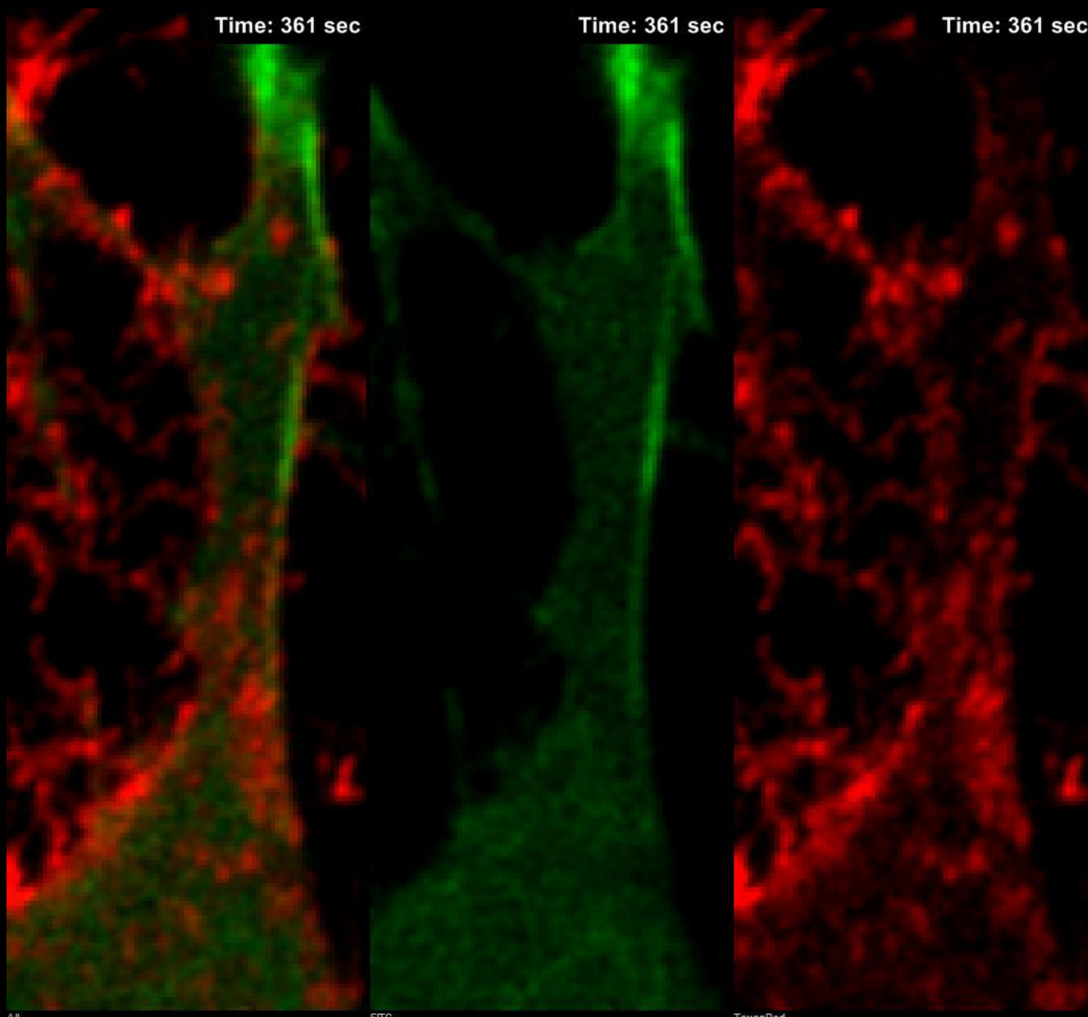


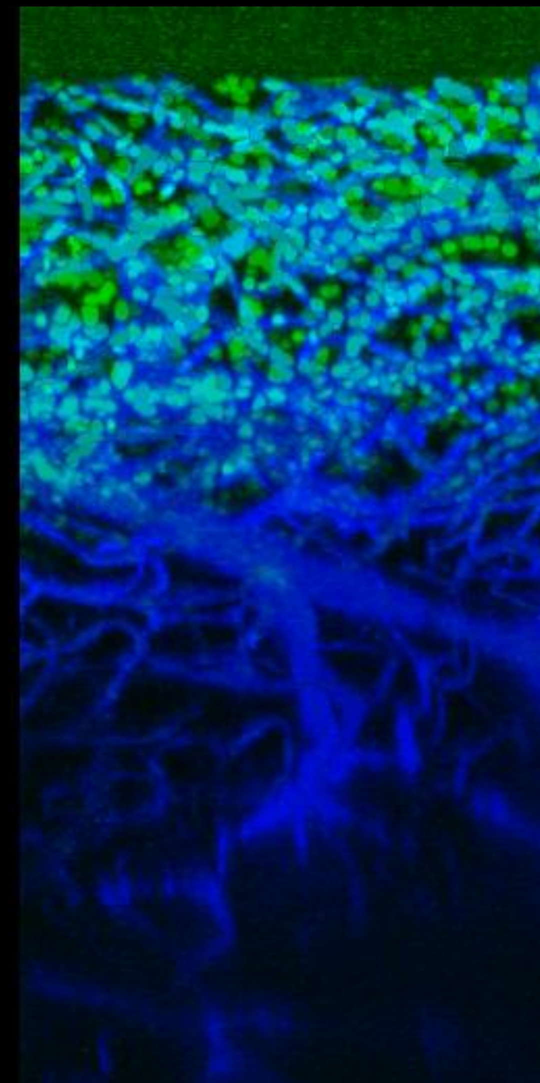




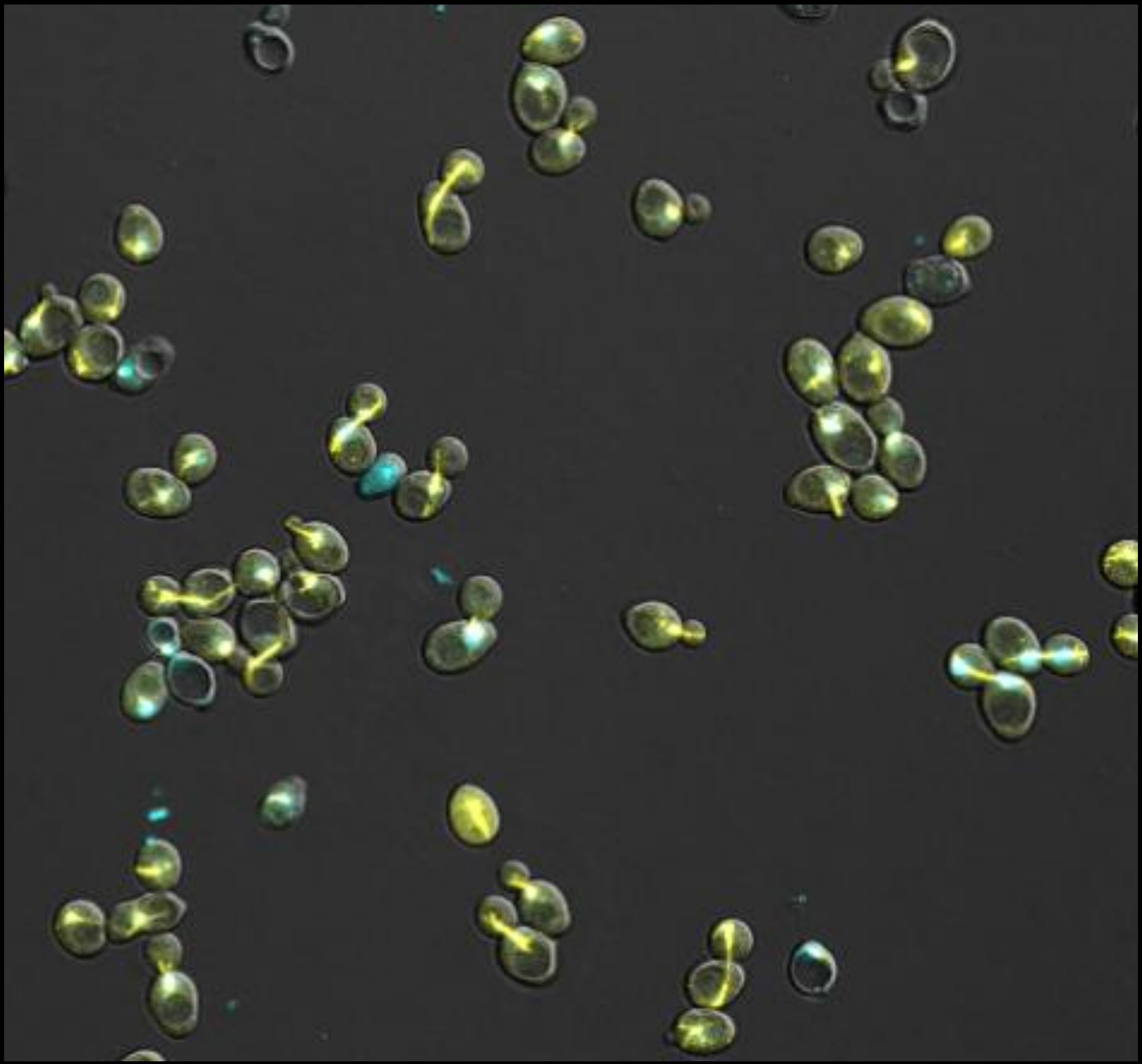







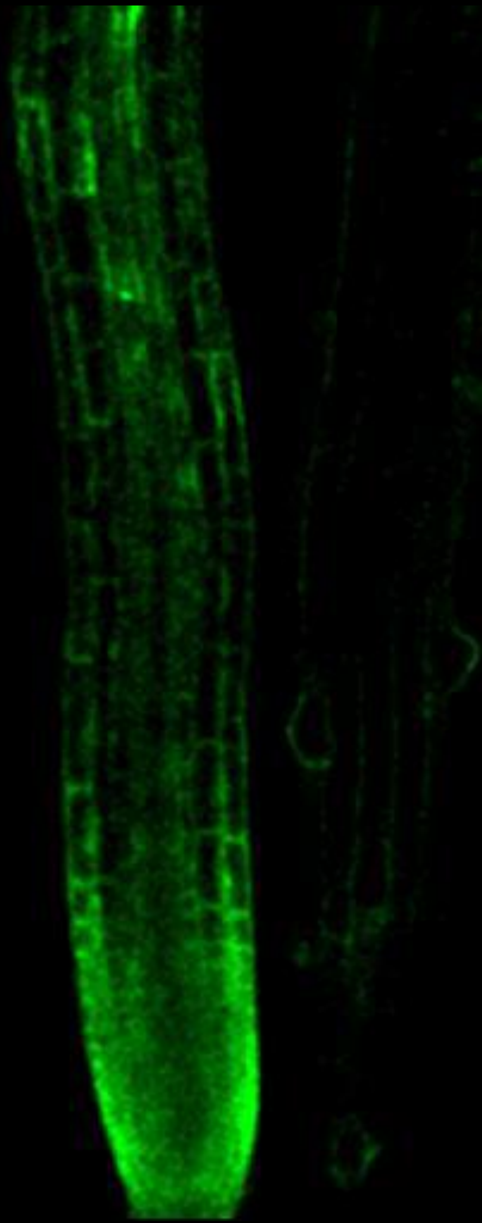
Fast Time-lapse and 3D Imaging



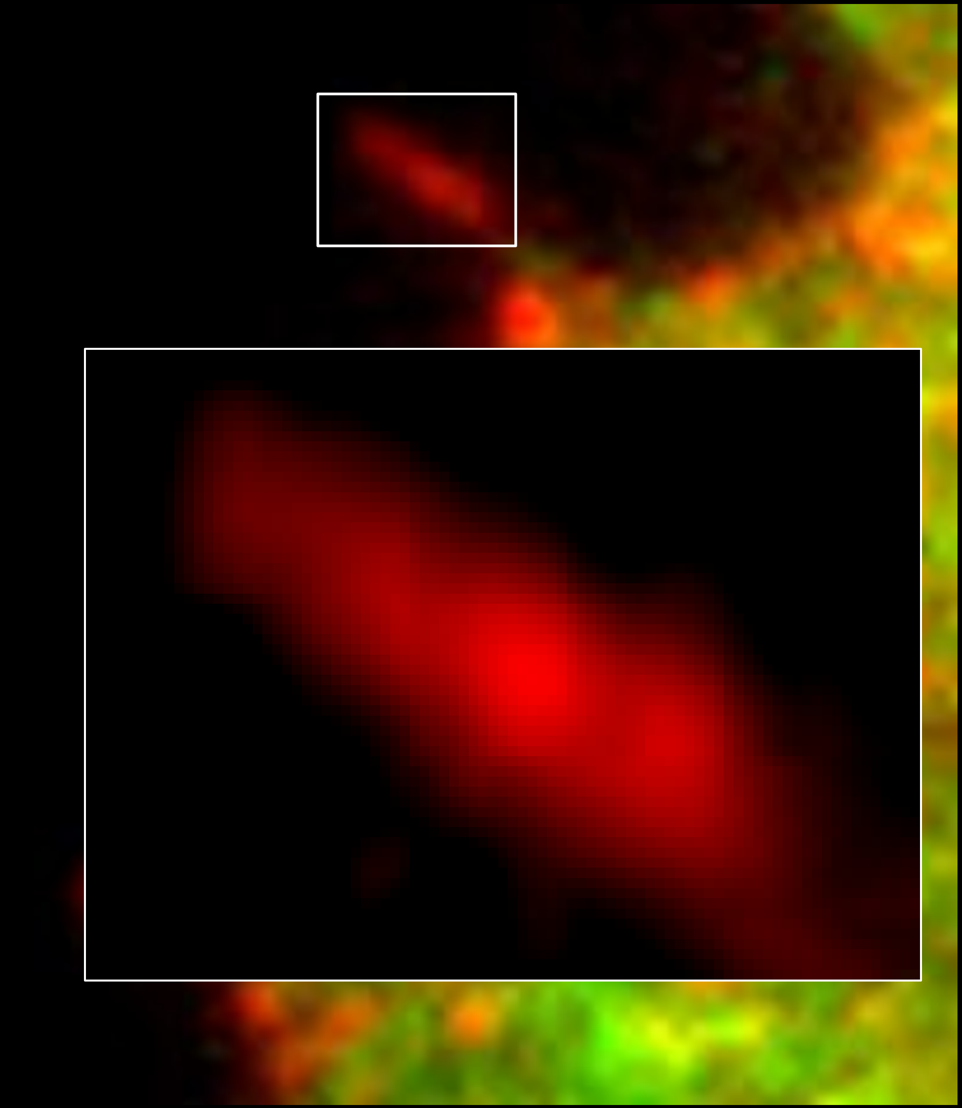


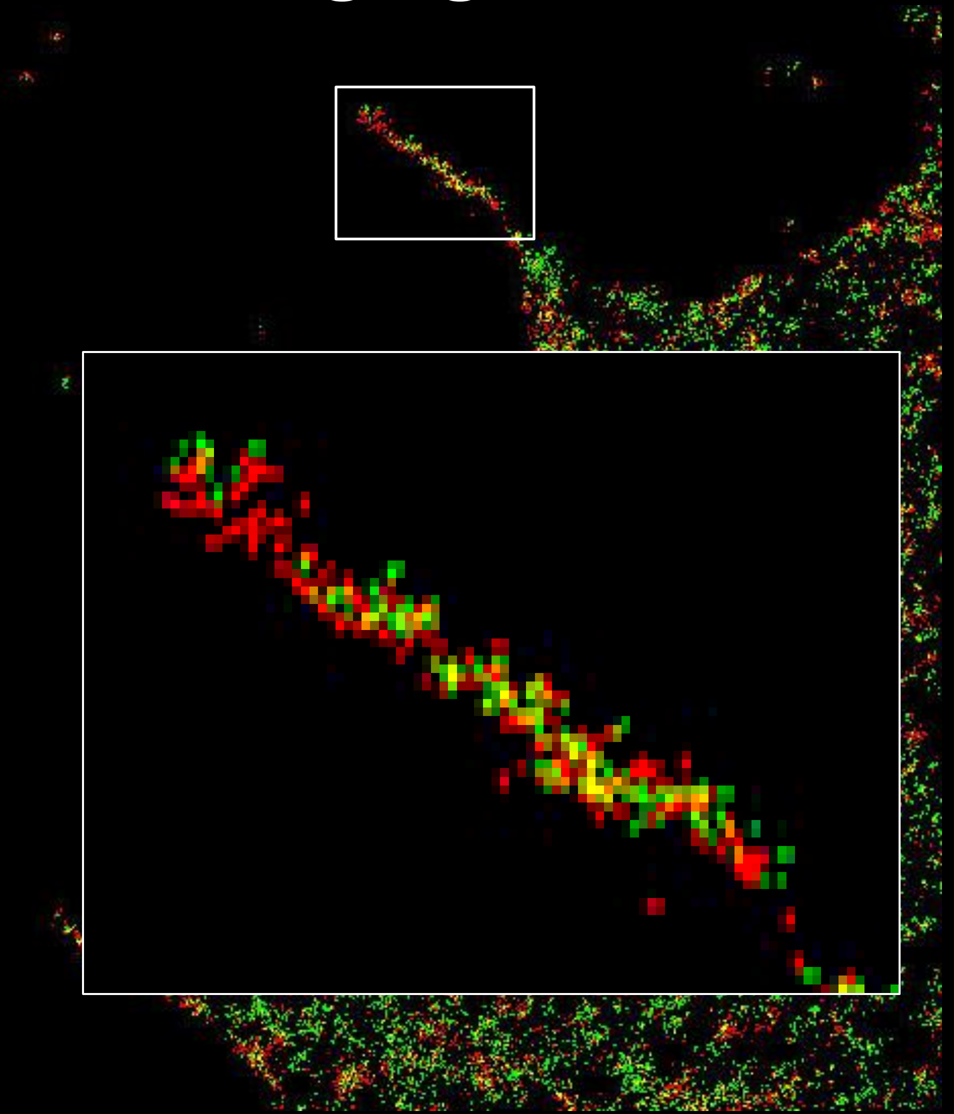
Fast Time-lapse and 3D Imaging





Super Resolution Imaging



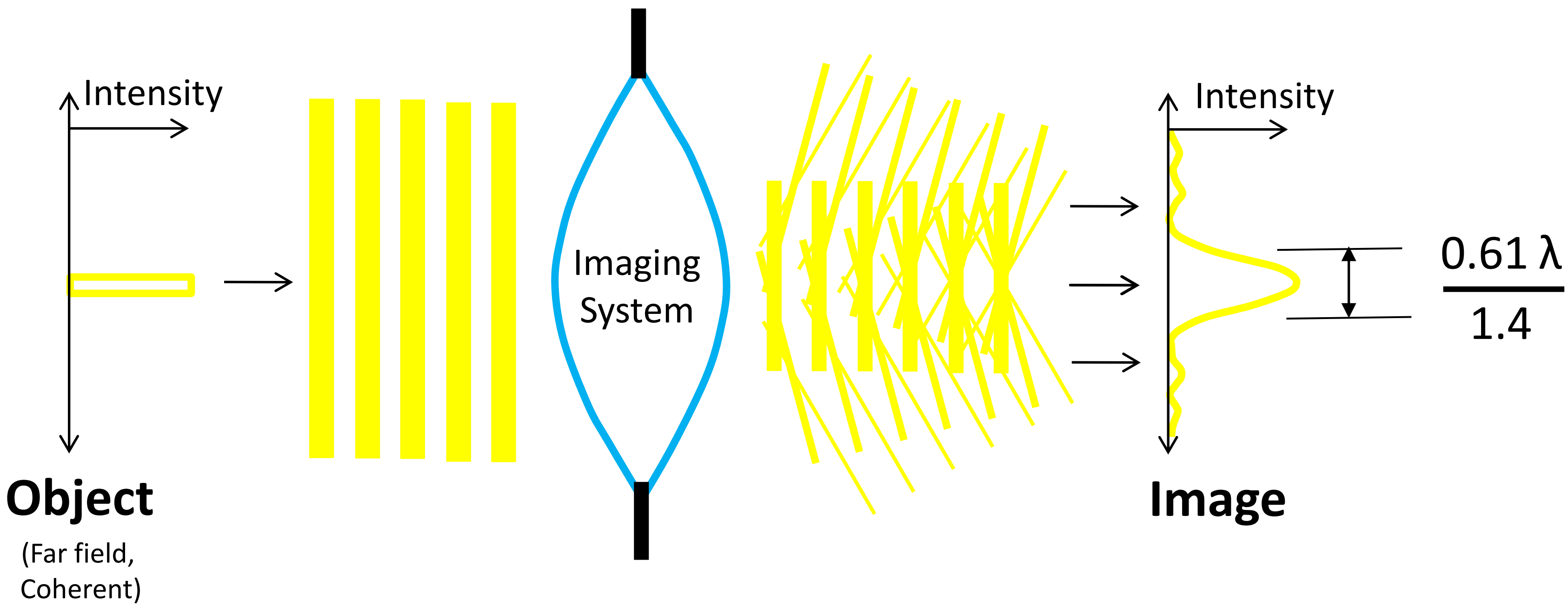


Resolution in Light Microscopy

Resolution is the distance that must separate two points in order for them to be distinguishable.

What is your resolution? E E E E E E E E

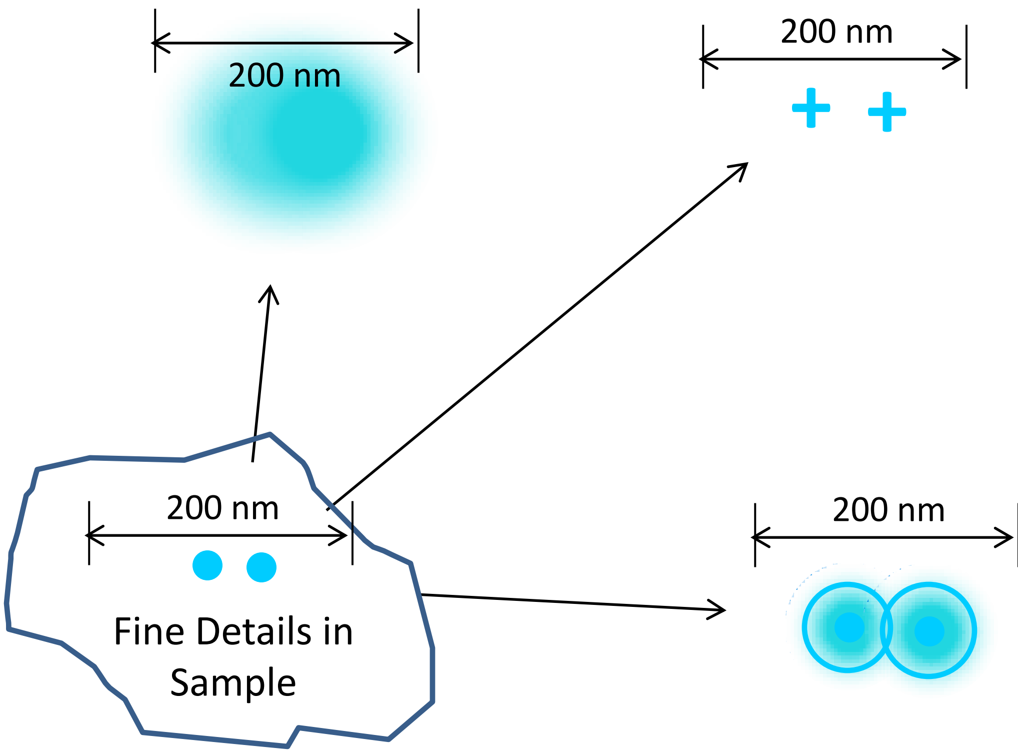
Due to a physical process called **diffraction**, the resolution of even a perfect microscope is no better than about 200 nm.



Super Resolution Light Microscopy

Super resolution microscopy is any technique that offers better than 200 nm resolution.

Wide-field or Confocal
(details lost)



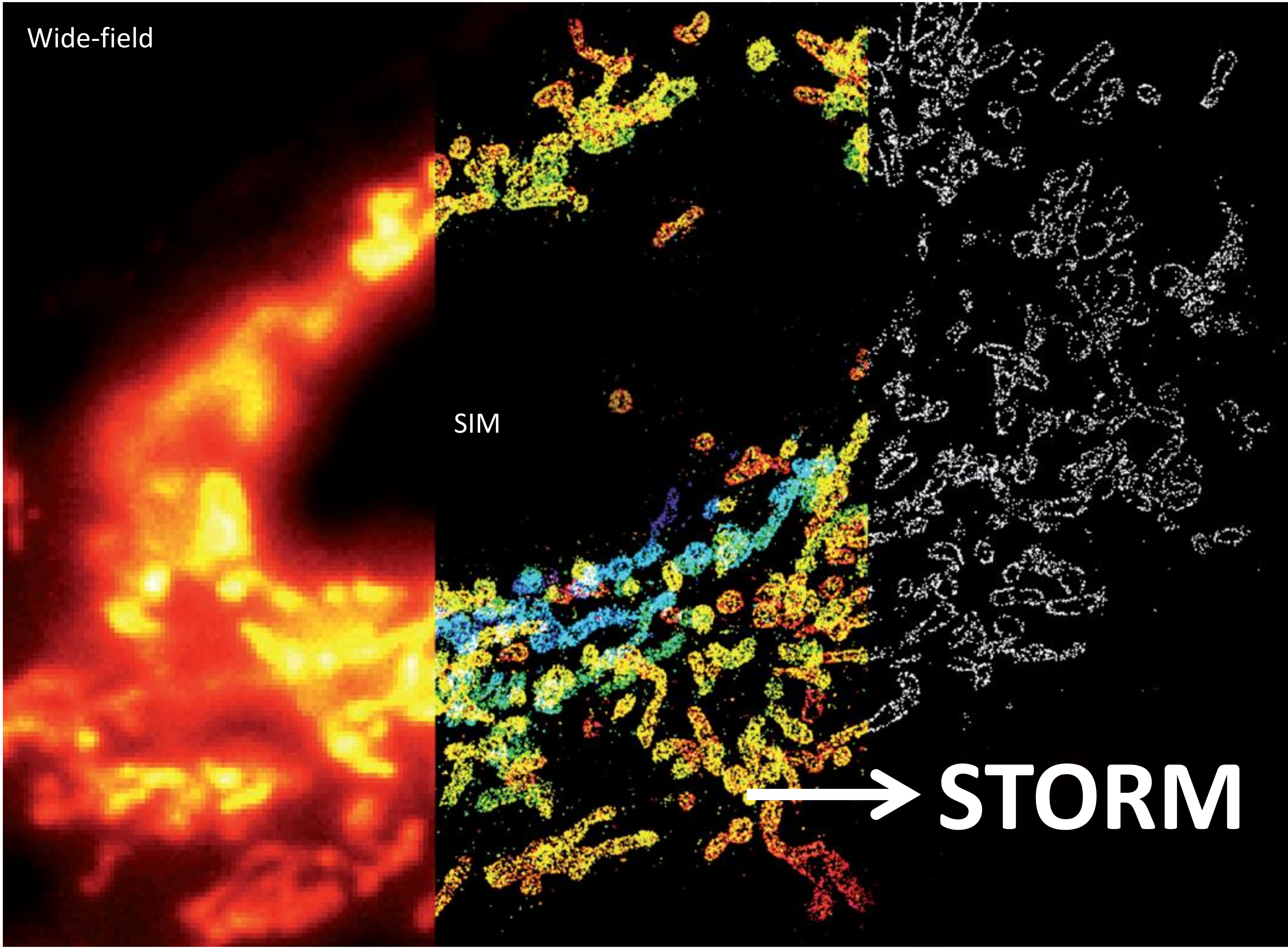
Stochastic Optical Reconstruction Microscopy (STORM)

Locates individually the peak of each fluorophore's image. >20 nm resolution.

(aka Photoactivated Localization Microscopy, or PALM)

Structured Illumination Microscopy (SIM)

Uses multiplication to shift high sample frequencies to lower frequencies that can be resolved and then uses lots of math to recover the originals. >100 nm resolution.



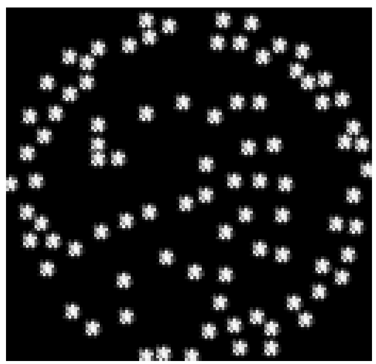
X. Zhuang, Nature, 2011

How STORM breaks the diffraction-limit

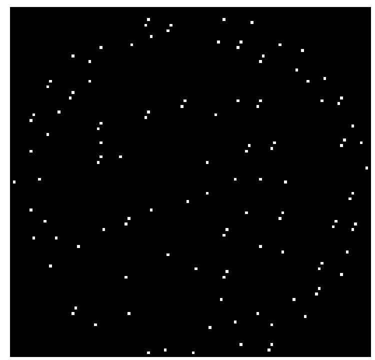
Using special dyes and optics, it is possible to image only a few dye molecules per frame. Then, a computer can localize the peak of each molecule's image and after thousands of frames a localization plot (the 'image') can be constructed.

Object

Raw Data



Localizations



Many, many Localizations →

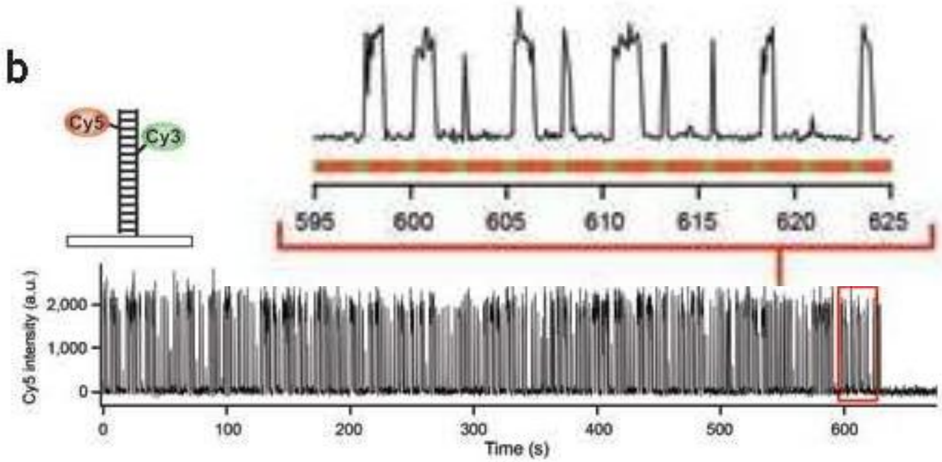
'Localization Plot'

Wide-field:

STORM dyes must be sparsely activated

If all of the fluorophores fluoresced at once, their images would overlap and the peaks could not be localized (e.g. epifluorescence).

Photoswitchable dye pairs are best



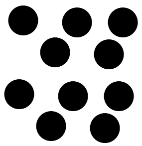
Rust, Nat Methods, 2006

New dyes and strategies are emerging.

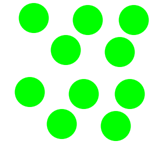
Photoactivatable dyes can work

(aka PALM)

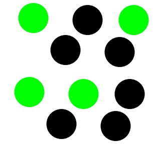
1. Activate with UV laser



2. Bleach with excitation laser



3. Collect image after substantial photobleaching

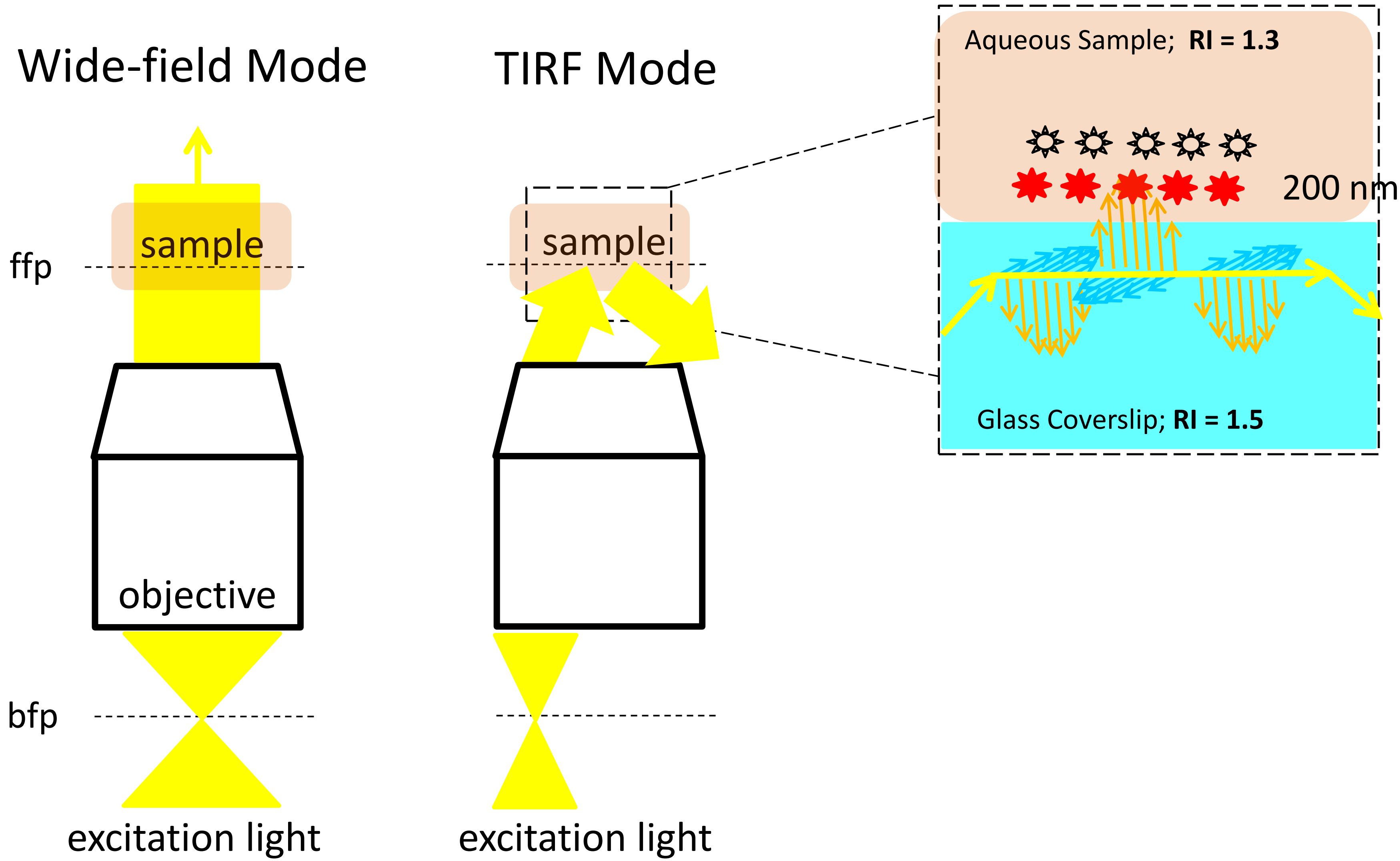


4. Repeat …

e.g. Betzig, Science, 2006

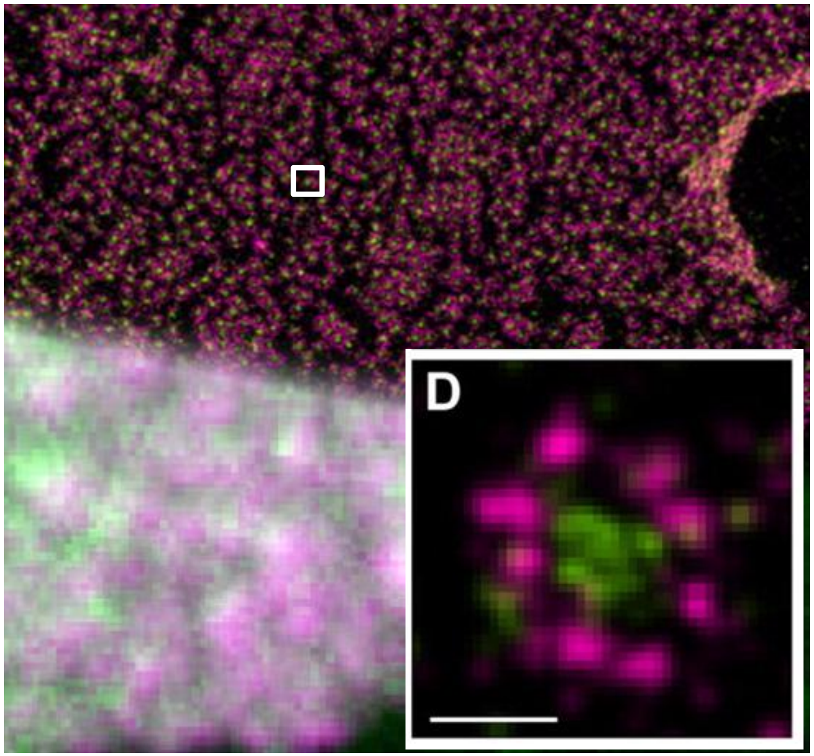
STORM is usually performed in TIRF mode

Single molecule imaging is not possible when out-of-focus light is present.



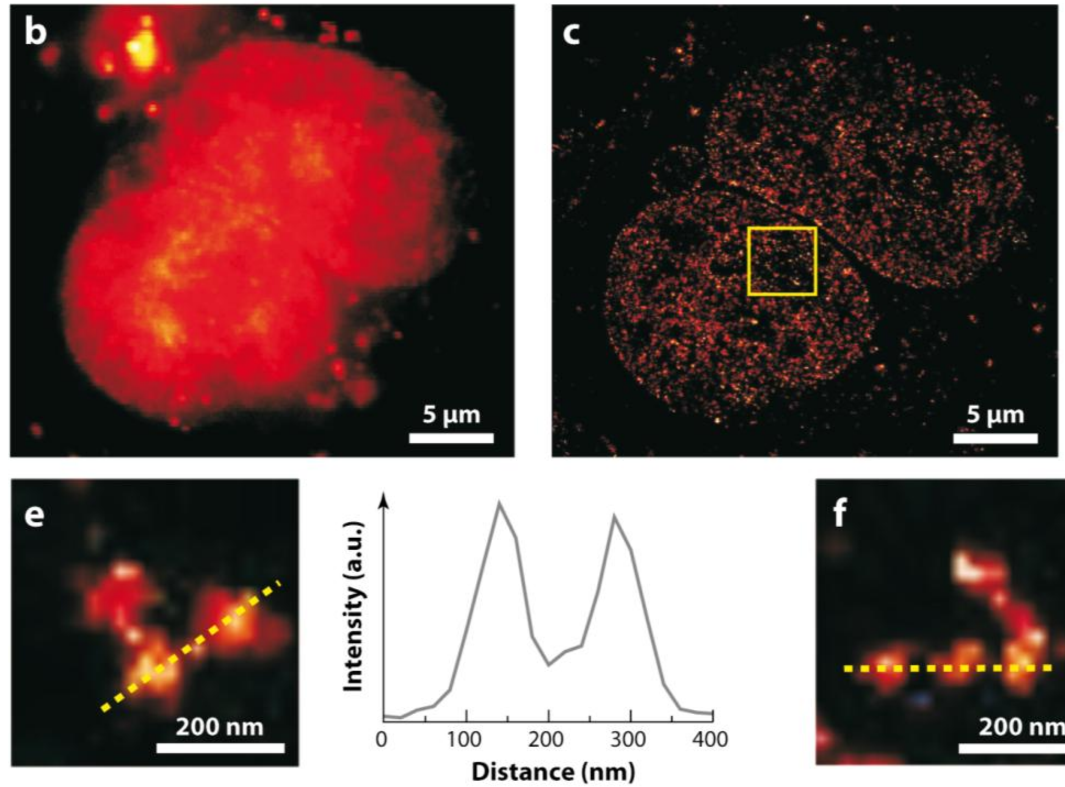
STORM application examples

Nuclear Pore Architecture

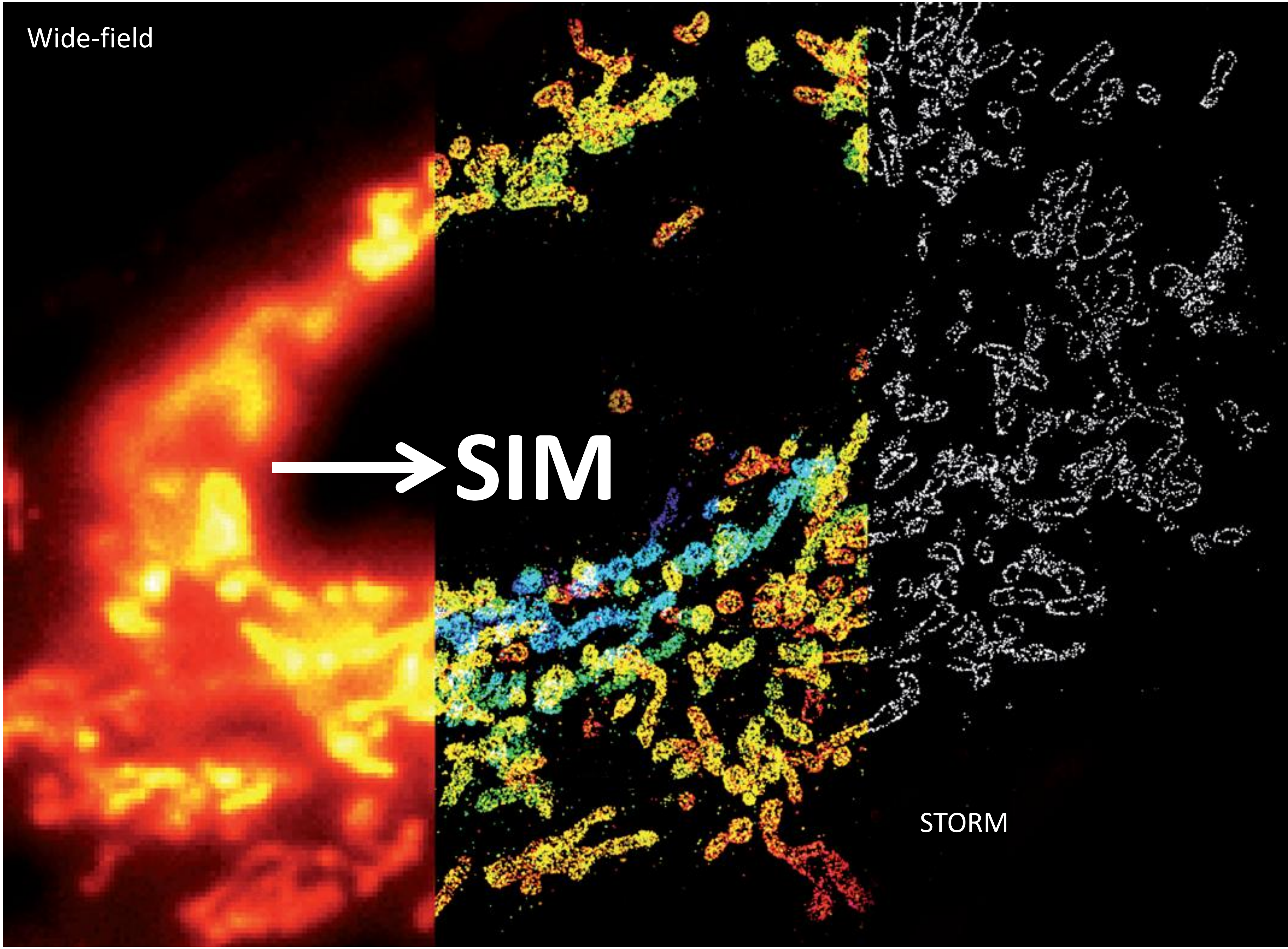


Löschberger A et al. J Cell Sci 2012

Histones as ‘beads on a string’



Van de Linde, Ann Rev Phys Chem, 2012



X. Zhuang, Nature, 2011

How SIM breaks the diffraction-limit (simplified!)

Sinusoidal patterns of excitation light are shown onto a fluorescent sample, which multiplies high sample frequencies by the excitation pattern. Thus, the high frequencies are converted to lower frequencies that can pass through the objective. The original frequencies are then (approximately) recovered using advanced mathematics.

High Sample Freq: $\sin(f_1 x + p_1)$ ⟷

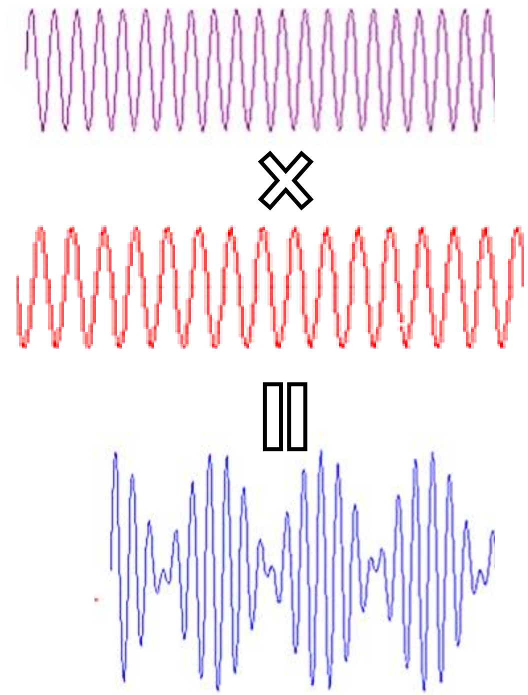
$\times$

Excitation Freq: $\sin(f_2 x + p_2)$ ⟷

$=$

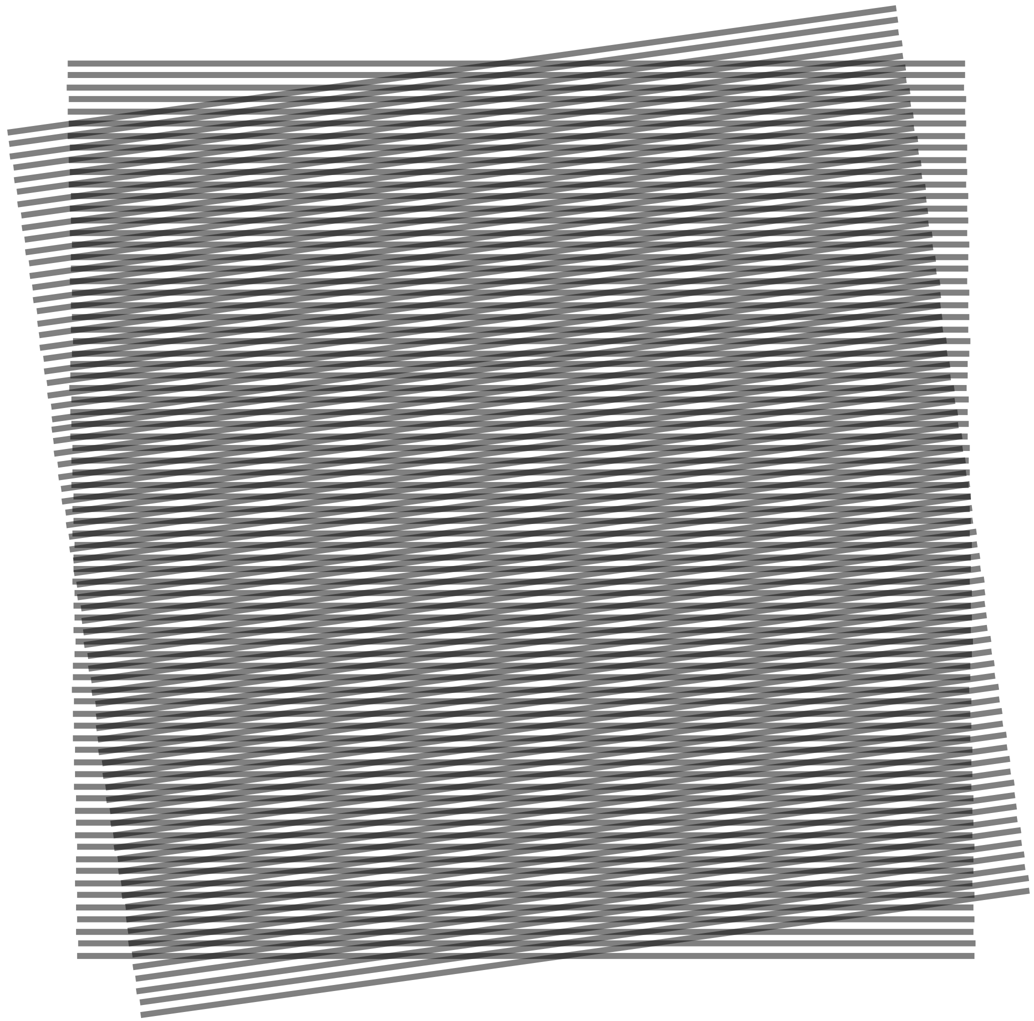
Em: $\frac{1}{2}\left[\cos((f_1 - f_2)x + P) - \cos((f_1 + f_2)x - P)\right]$ ⟷

(Where $P = P(p_1, p_2)$)



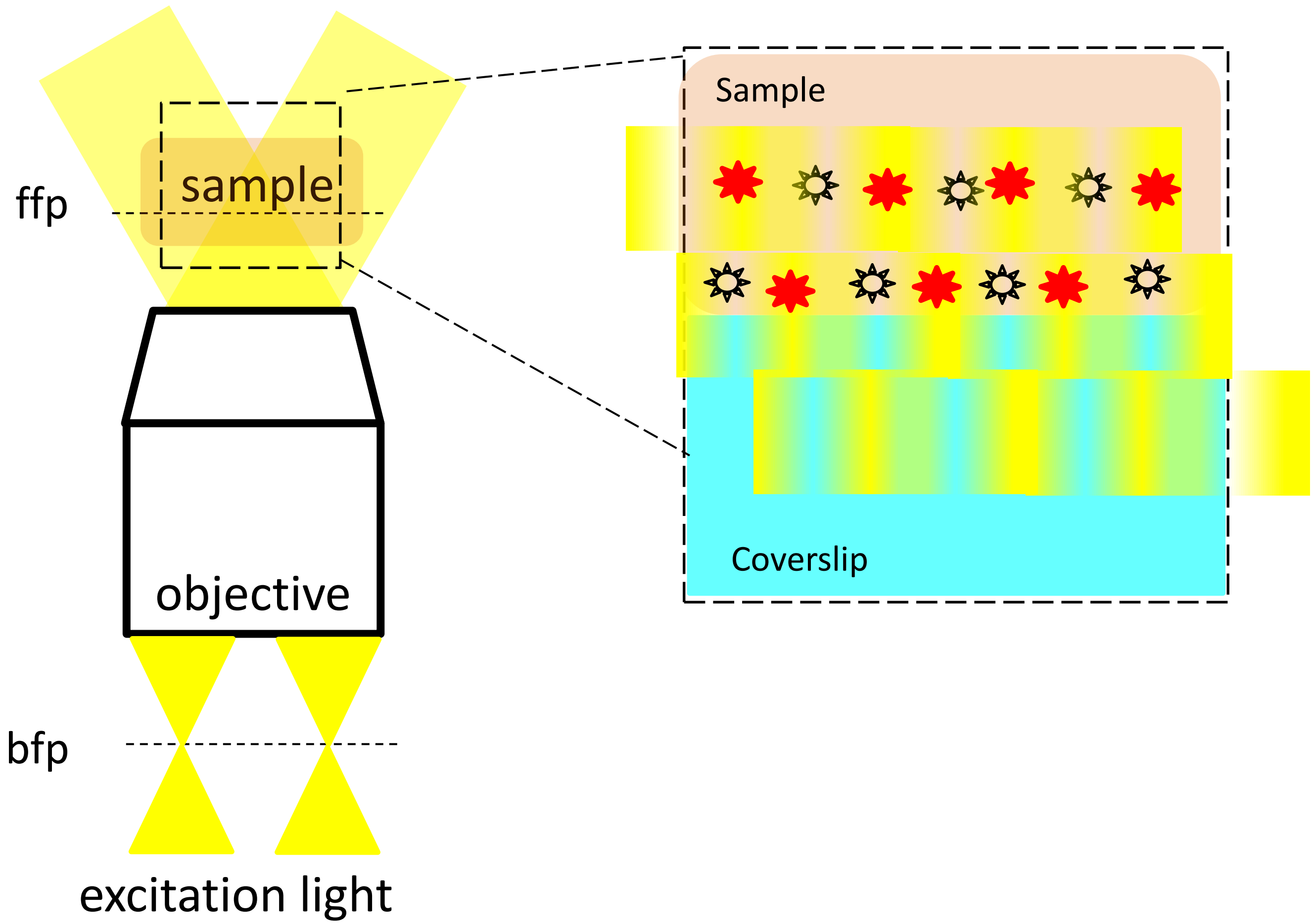
Since $(f_1 - f_2) <= f_2$, $f_1 <= 2f_2$. Thus, SIM resolution is at best 2x traditional resolution.

Moire Fringes: A 2D SIM analogy



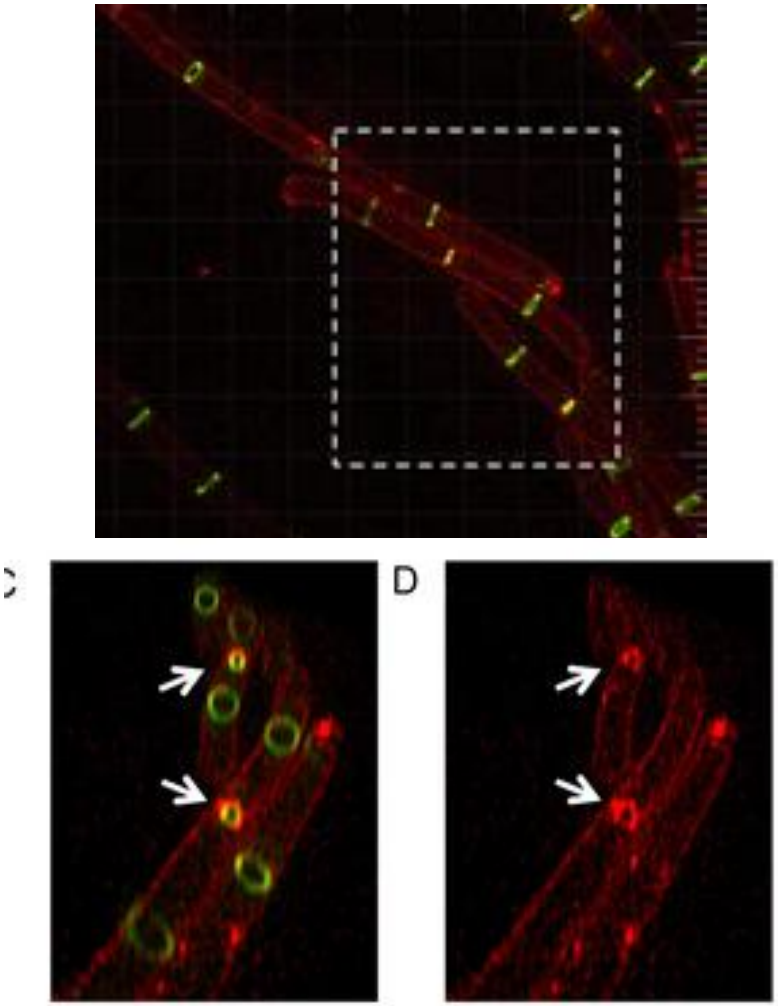
SIM operates in wide-field mode

Two (or three) points of excitation light are placed at a high NA position within the back-aperture. Thus, a sinusoidal interference pattern is produced in the front focal plane.



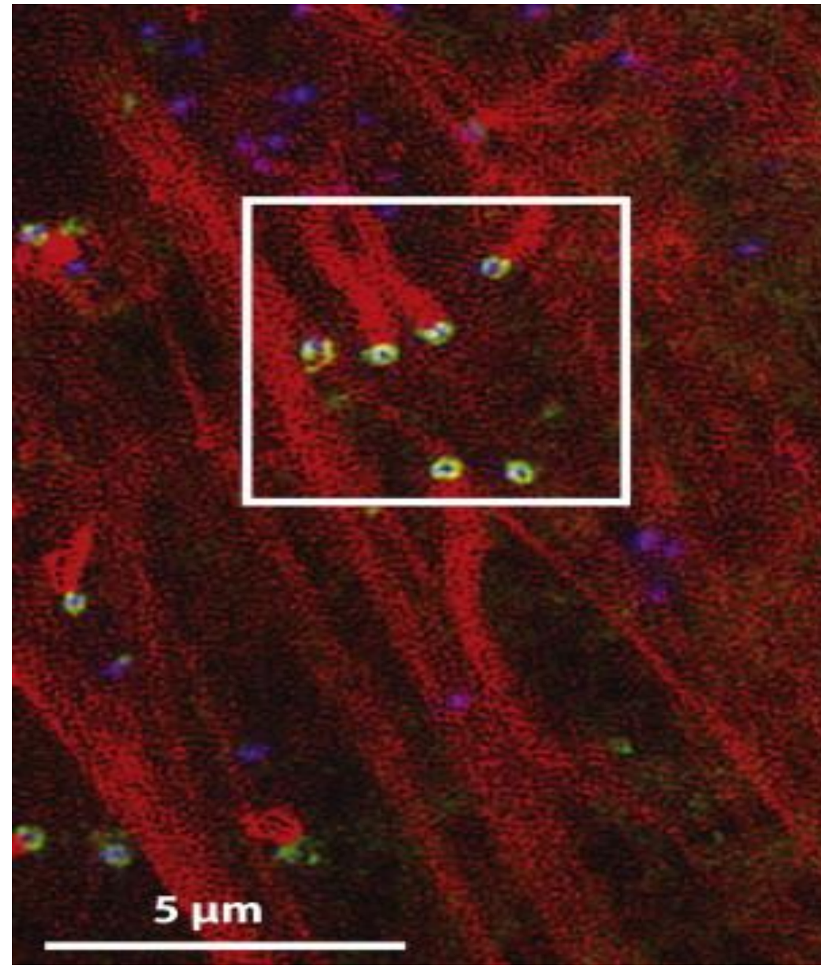
SIM application examples

Bacterial Septum



Strauss MP, et al., PLoS Biol, 2012.

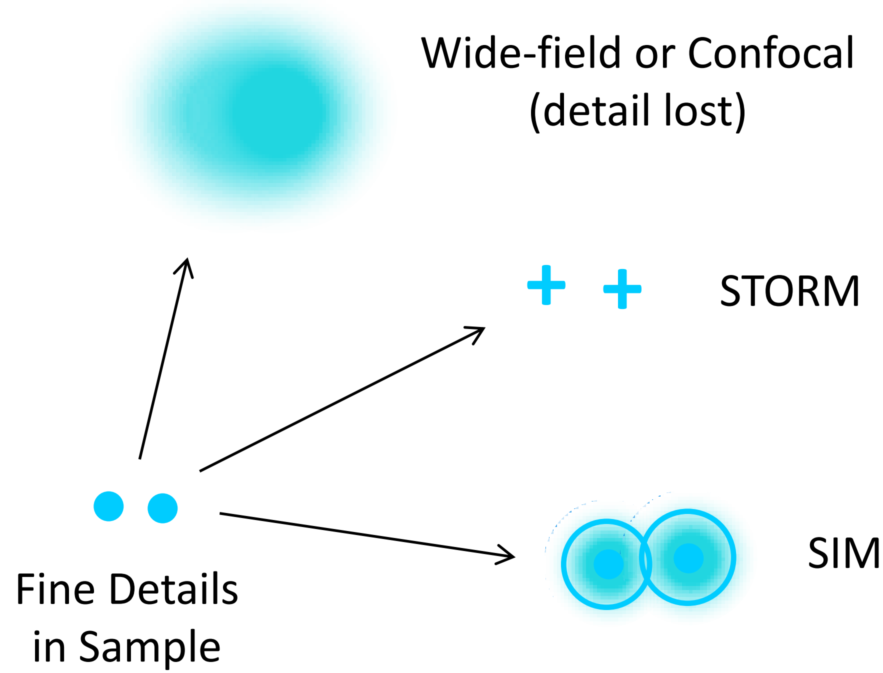
Virus on actin filaments



Horsington J, et al. J Virological Methods. 2012.

Summary

STORM and **SIM** are new fluorescent imaging technologies that exceed diffraction-limited resolution.



	STORM	**SIM**
XYZ resolution:	20-50 nm	100-200 nm
Special dyes needed?	Yes	No
Quantitative?	Likely	No
Artifact prone?	No	Yes
Extra sample preparation?	A little – fixed only	No – live possible

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Thank You!

Come and try our microscopes!

For more information…

Email: abtaylor@purdue.edu

Web: Google “Bindley Imaging”