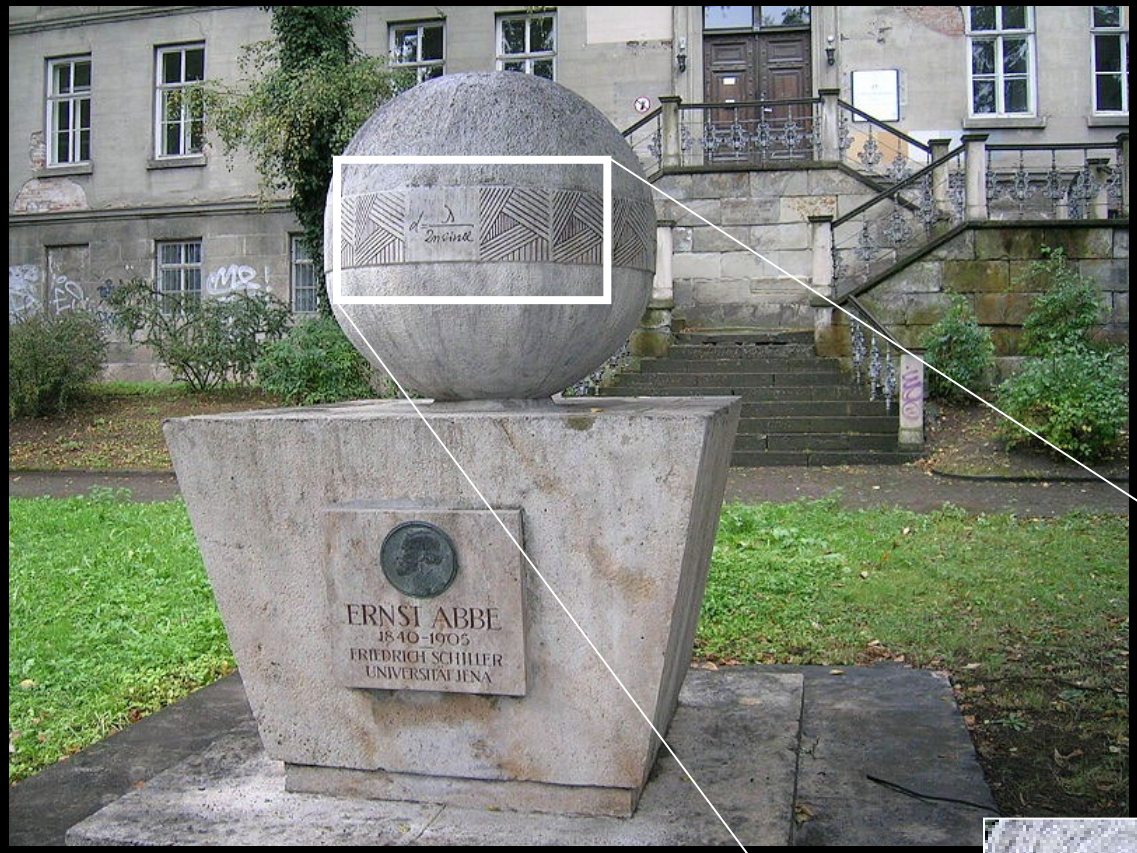
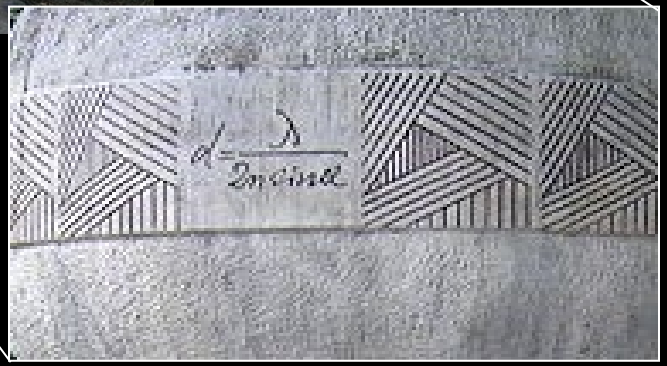
Super-Resolution Microscopy

Johan Slotman

11-11-2026

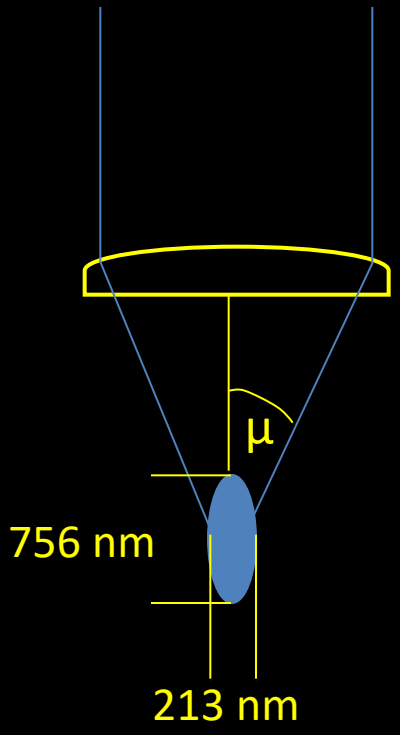




Abbe's law

Abbe, E. (1873) Beitrage zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. Arkiv. Mikroskop. Anat. 9, 413-468.

Lateral (XY) and Axial (Z) resolution



Lateral (XY) resolution:
XY_{min}= 0.61*wavelength/Numerical aperture
XY_{min}= 0.61*488/1.4 = 213 nm

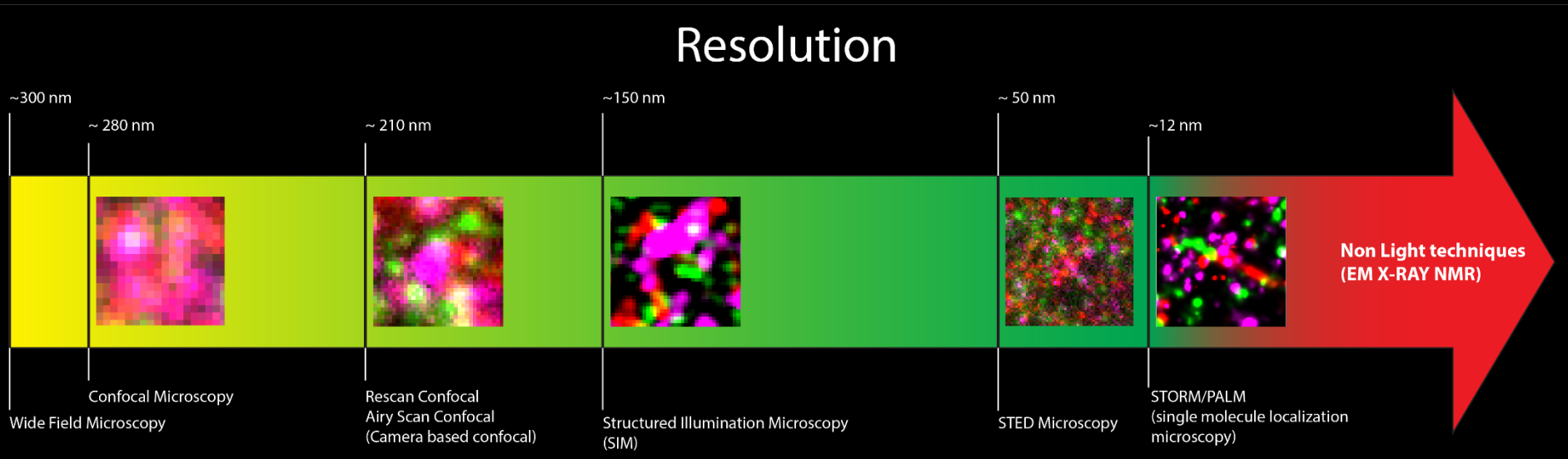
Numerical aperture = refractive index * sin (μ)

Axial (Z) resolution:
Z_{min}=2*wavelength*refractive index/(Numerical aperture)^2
Z_{min}=2*488*1.518/$(1.4)^2$=756 nm

Super-resolution microscopy beyond the abbe-limit

Super-resolution microcopy: light microscopy techniques that are able to capture images with a higher resolution than the diffraction limit

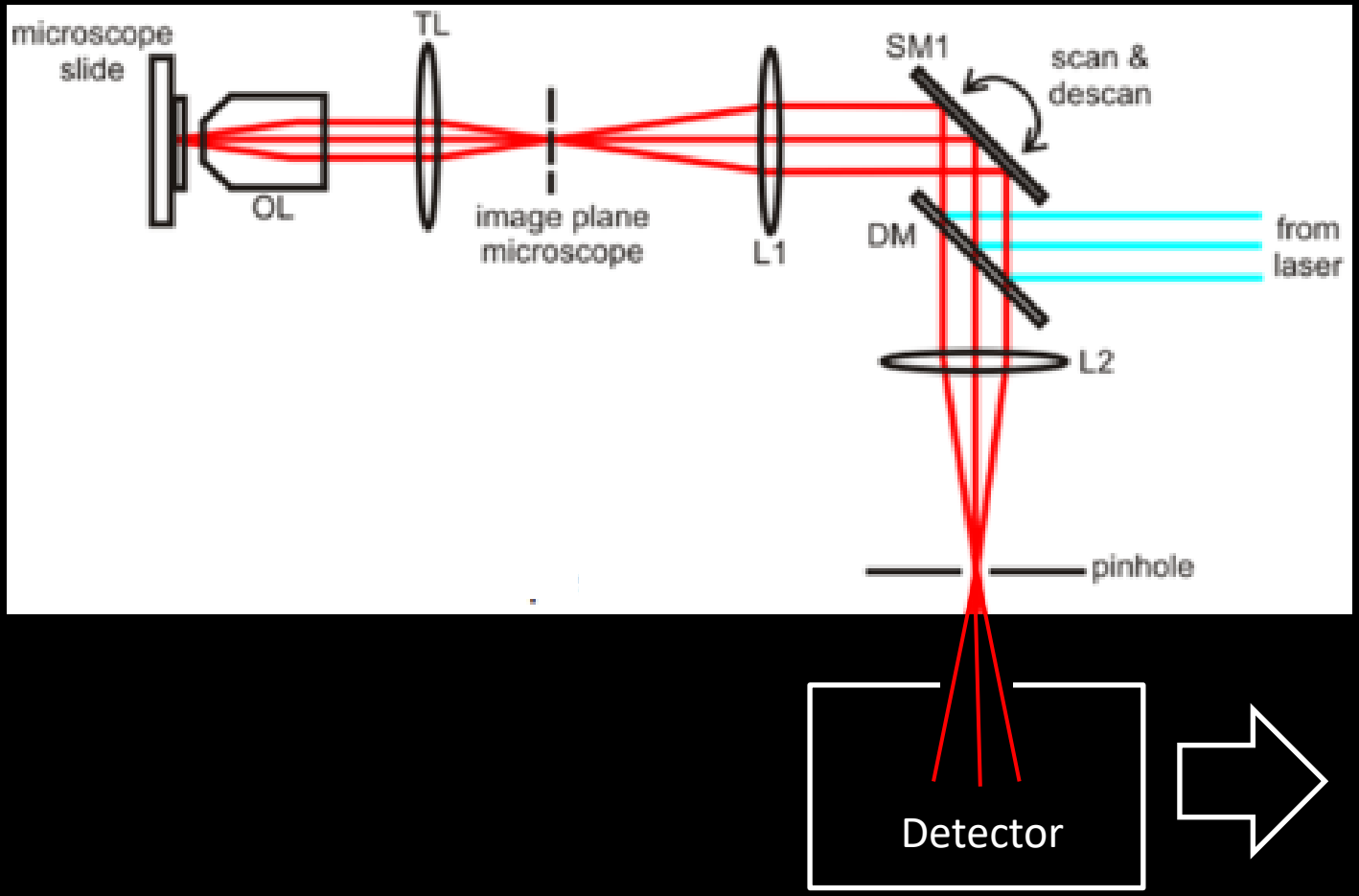
The many flavors of super-resolution microscopy



A mechanical magnification

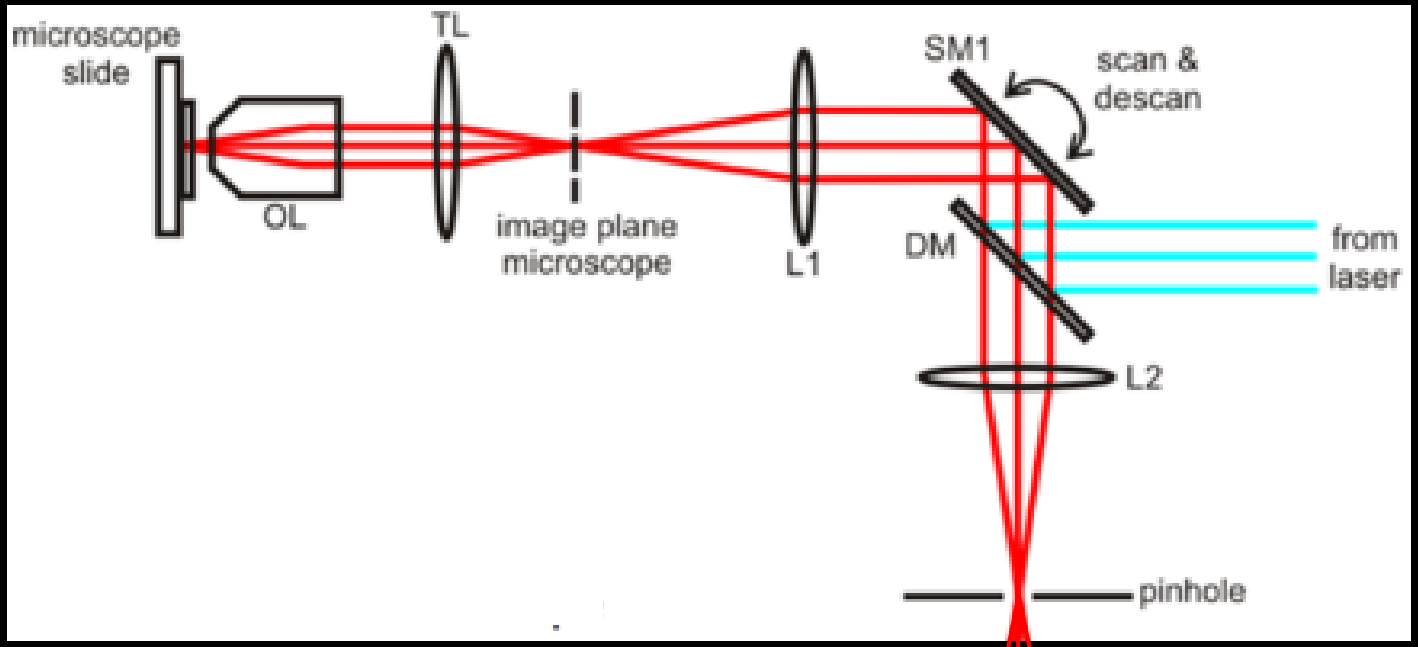
RE-SCAN CONFOCAL

Confocal imaging using a detector

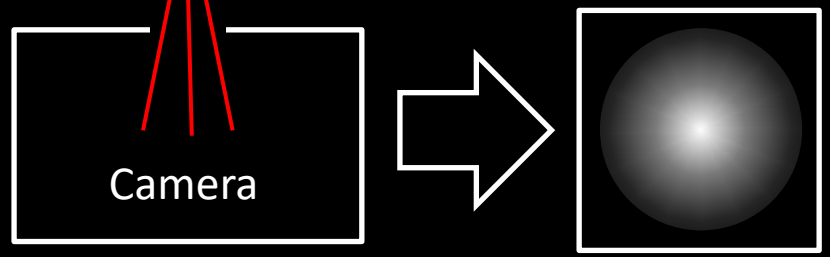


8

Confocal imaging using a camera

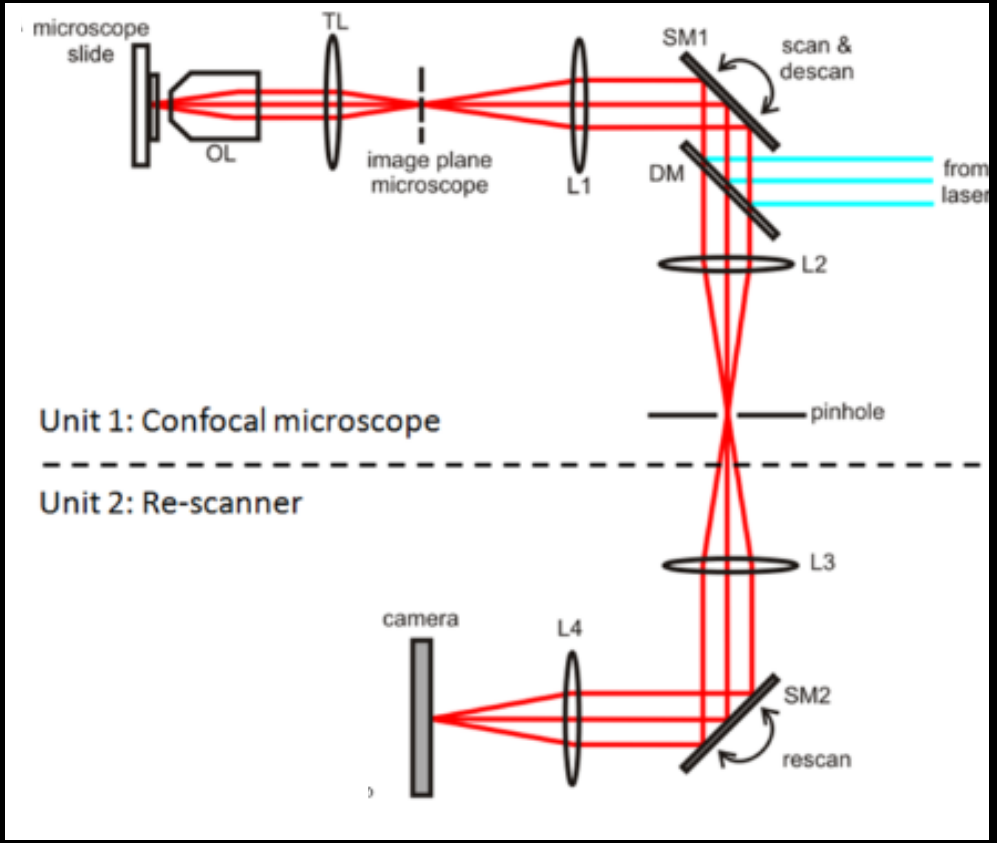


ISM
Airyscan (32 detectors
i.e. small camera)



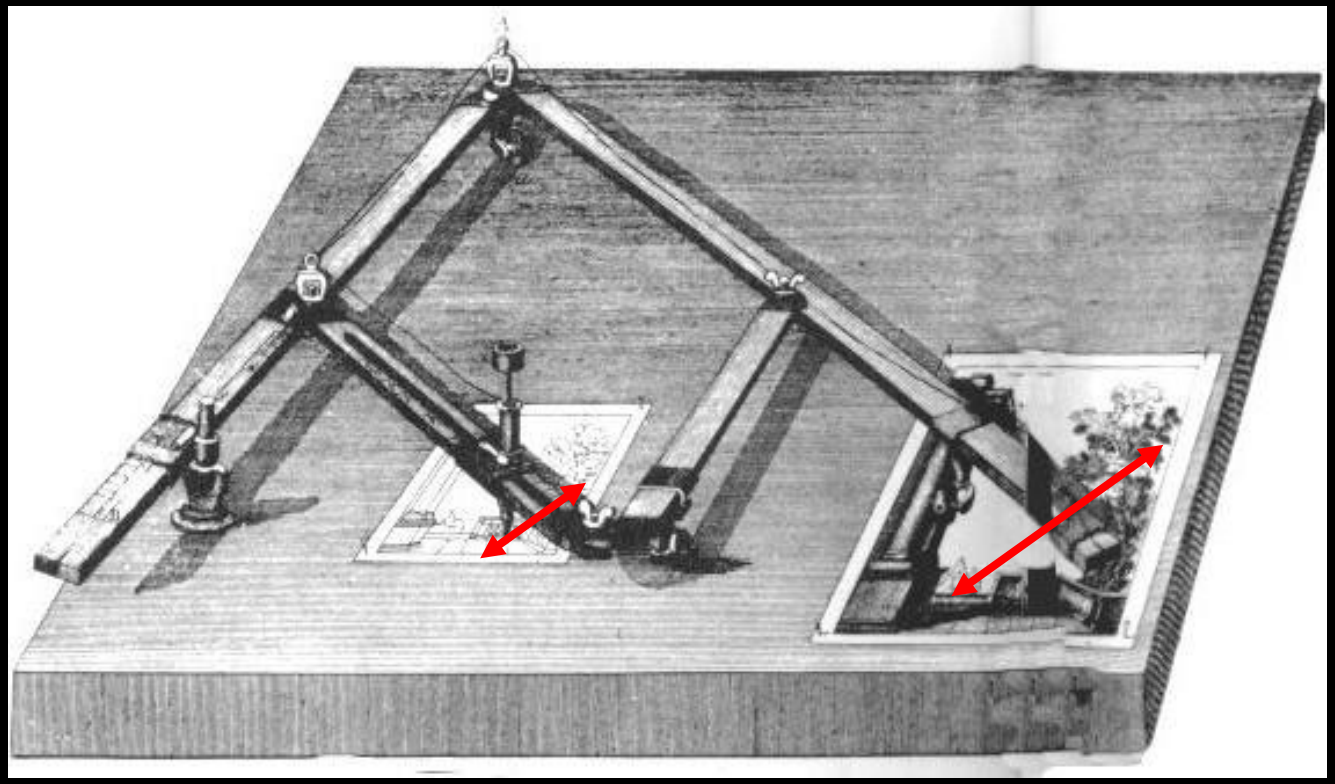
Rescanning

- Writing the image back on the camera
- Exposing the camera for the duration of one image scan



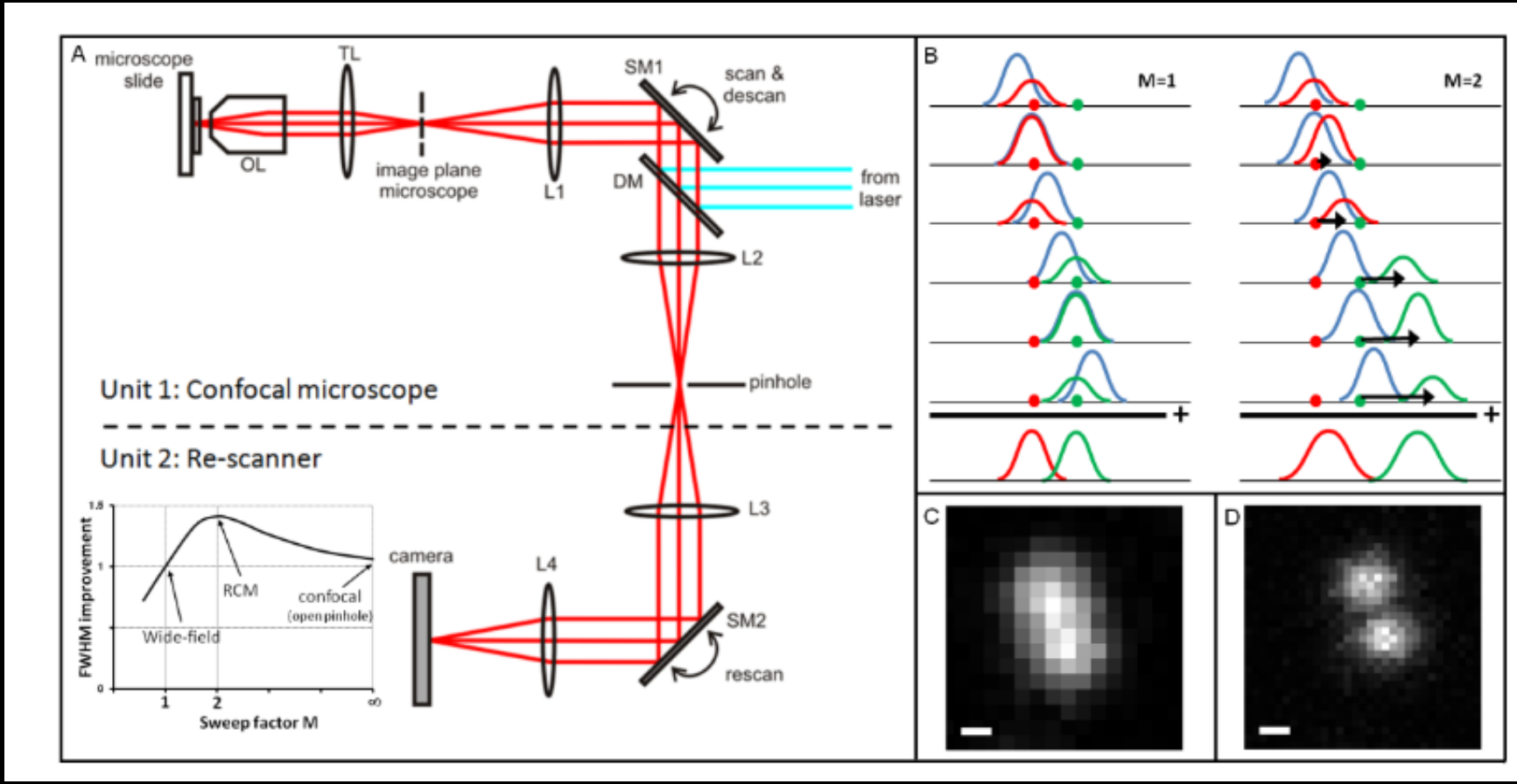
De Luca et. Al. Biomed Opt Express. 2013 Oct 25;4(11):2644-56.

Gaining resolution by rescanning



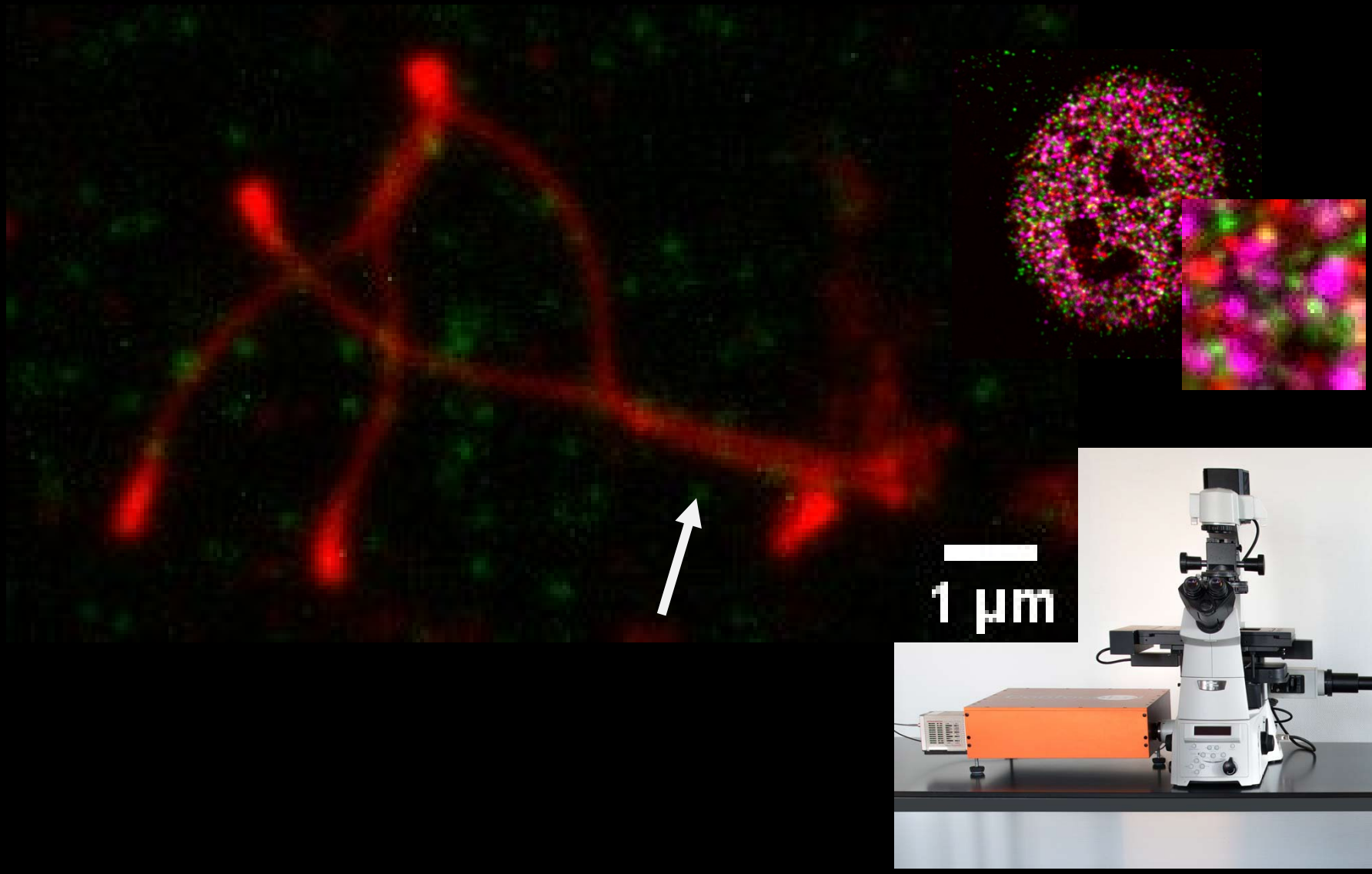
Mechanical magnification by increasing the sweep

Gaining resolution by rescanning with different sweep factors

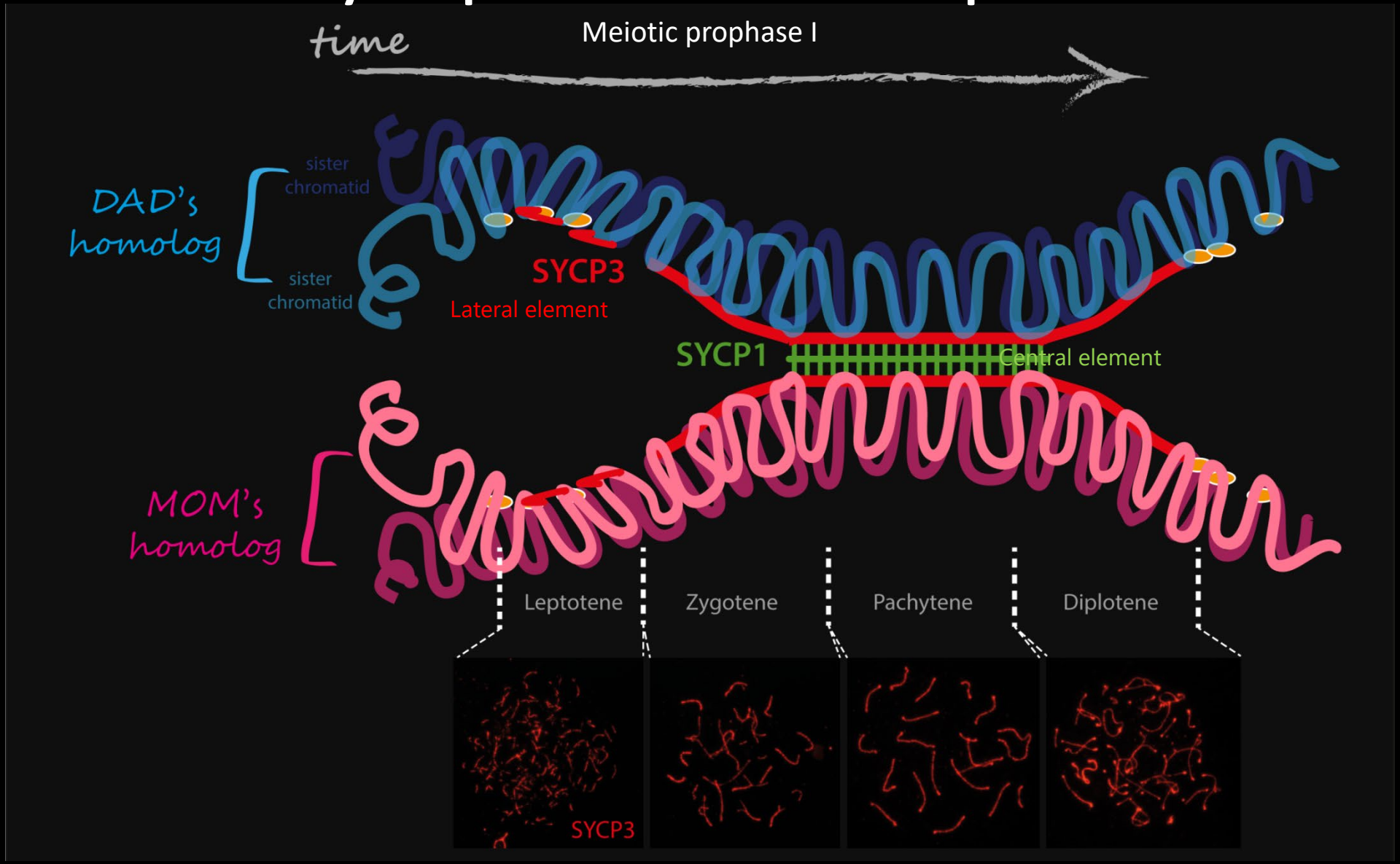


Maximum resolution at sweep factor 2
(increase of $\sqrt{2}$ times over widefield)

Examples of RCM microscopy



Synaptonemal complex



Summary Re-Scan Confocal

- Multi color
- Very suitable for live cell imaging
- Confocal technique: Optical sectioning
- Small field of view (specific to our system)
- Fixed field of view
- $\sqrt{2}$ times resolution increase

Putting a structure in the illumination light

SIM MICROSCOPY

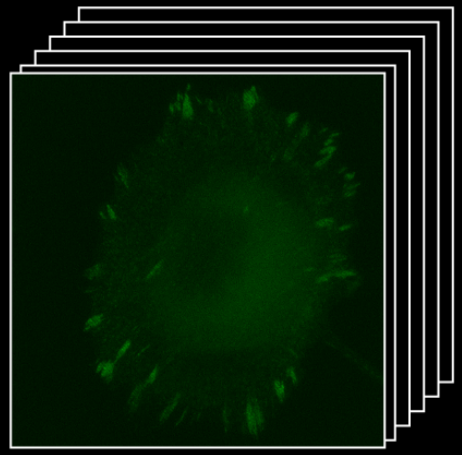
Structured Illumination Microscopy

- Illumination of a sample with a light pattern
- Two time resolution increase compared to Wide Field microscopy



In short

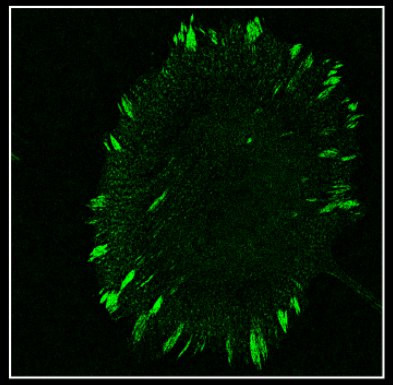
Acquisition



Reconstruction

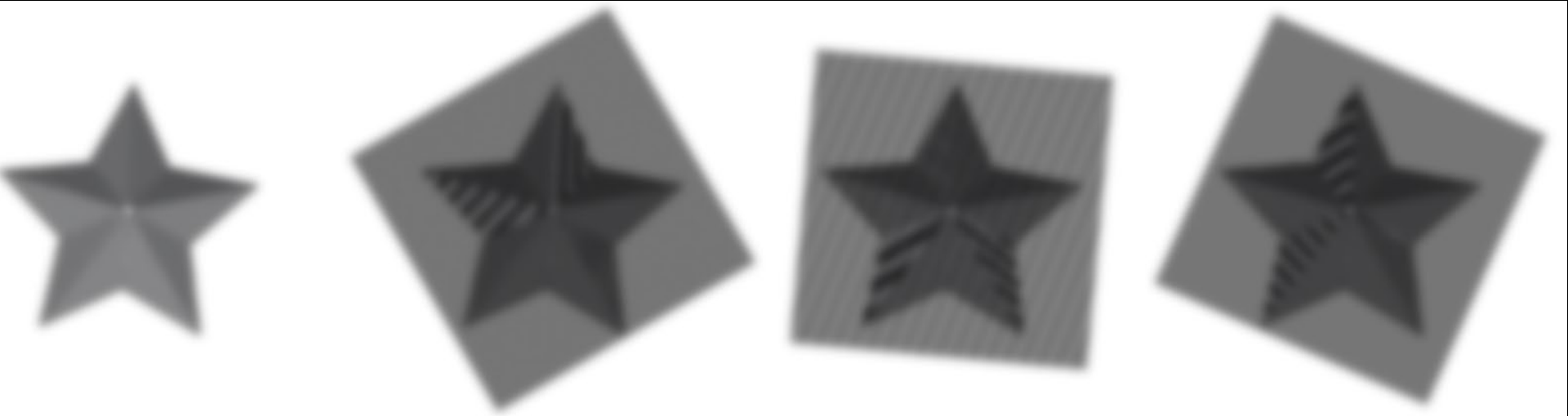


Analysis



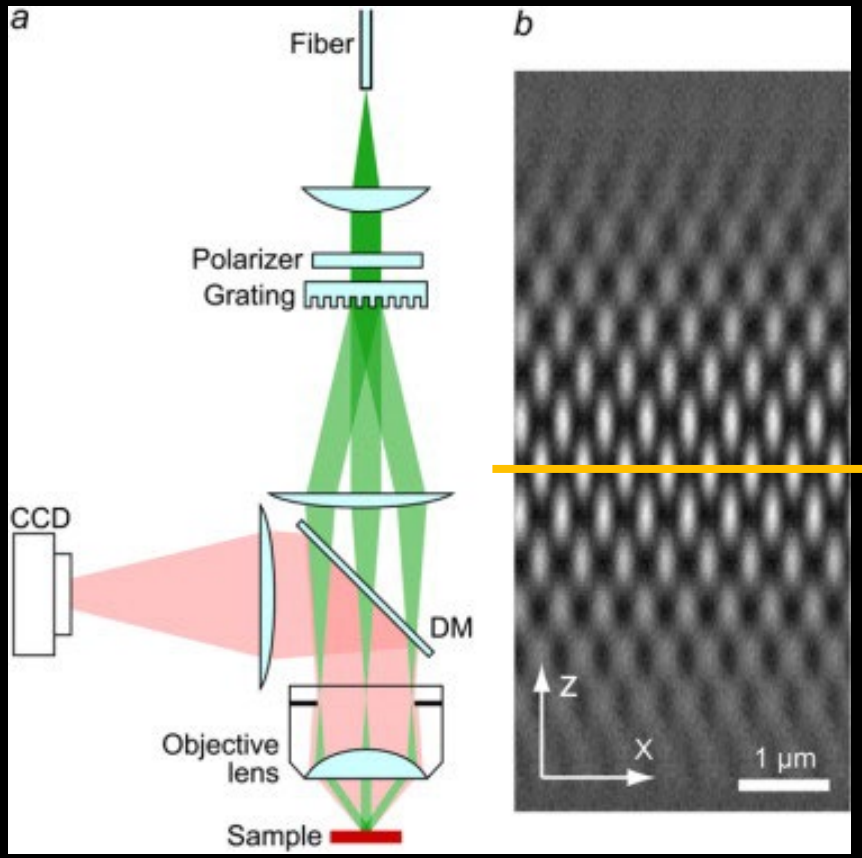
How?

- Moiré patterns
- Emission from a sample illuminated by a pattern results in a image that contains emission data that is shifted in “frequency space”

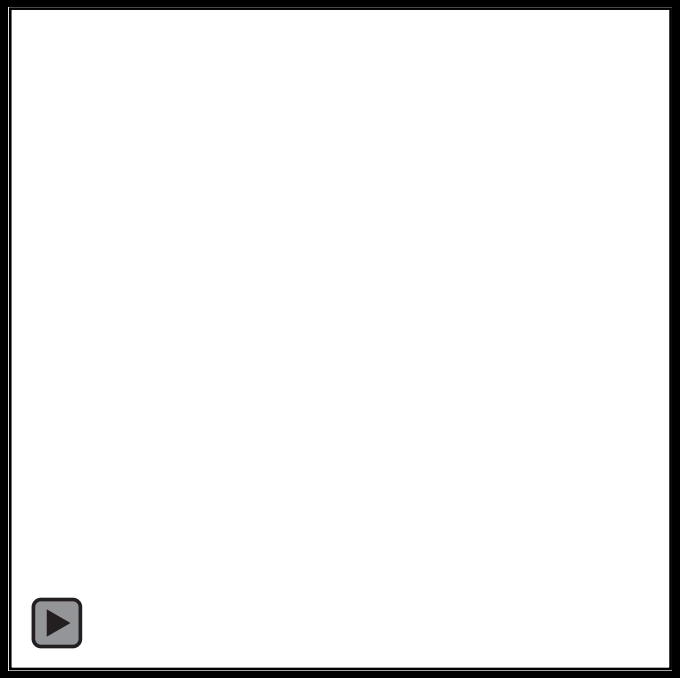


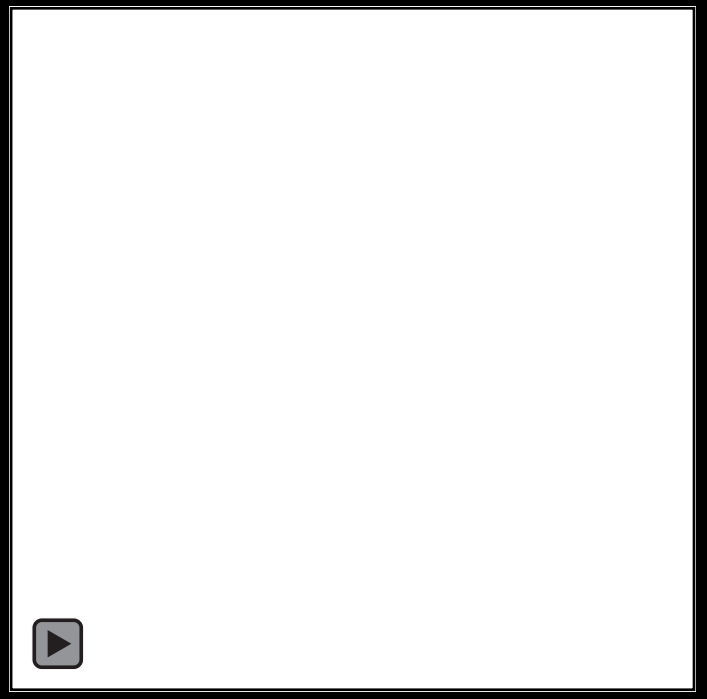
Acquisition

- An interference pattern is created using a laser and a grating
- The object is illuminated with the 3d pattern
- Images are acquired for 5 or 3 pattern rotations and 5 phases for each rotation
- The 15 or 25 images are reconstructed into one high resolution image



Projected grid is rotated and shifted





Why multiple phases?

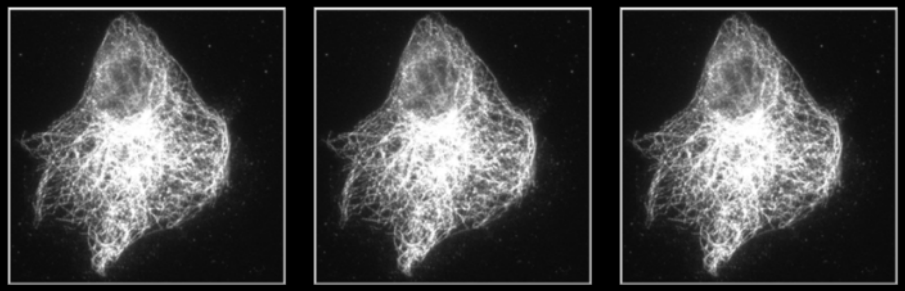
- Each image contains high-resolution information of only a portion of imaged object
- Several phases are needed to get a full image



Why Multiple rotations?

- A set of phases at one rotation angle contains high-resolution information in one direction
- Information is shifted in one angle in “frequency space” to lower frequency information

rotation 1 2 3

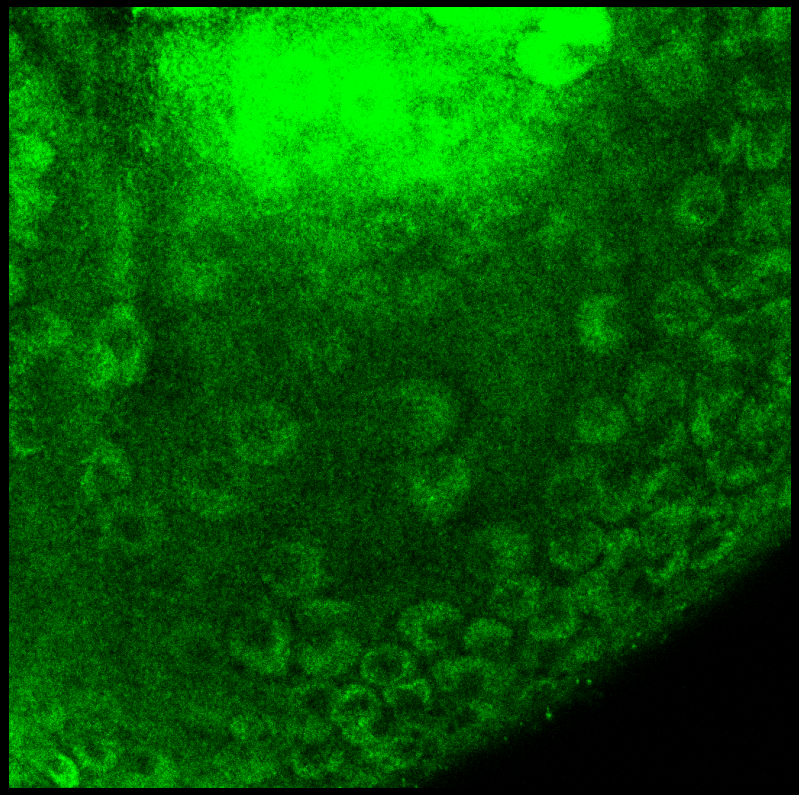


Information in image

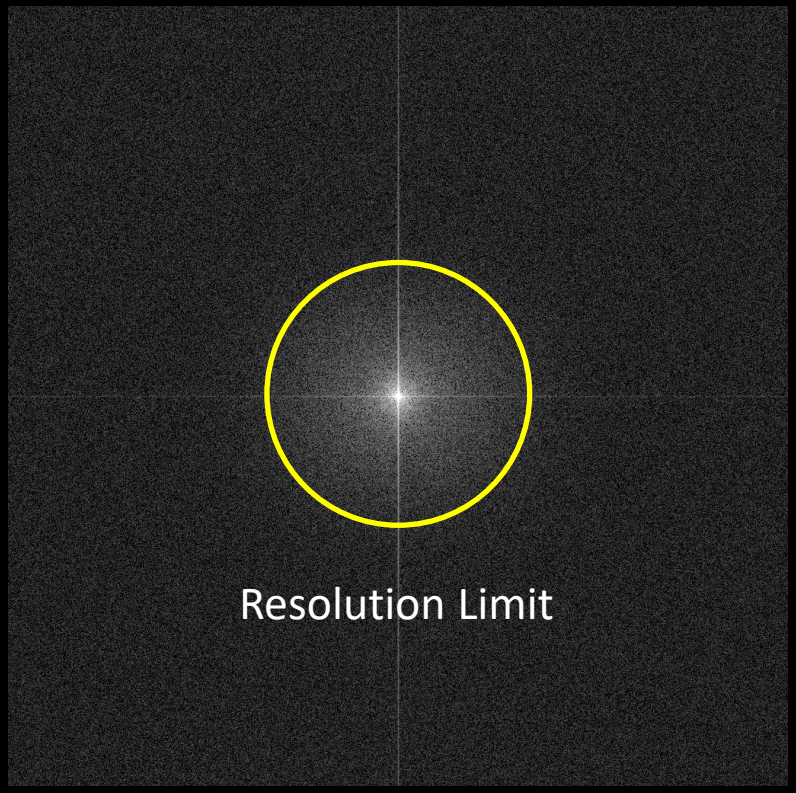
Widefield image
+ high frequency data from the rotation

Fourier Transform of an image

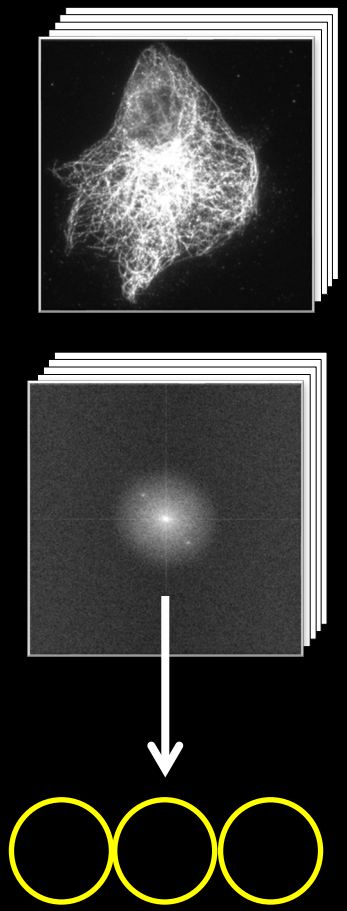
"Real space"

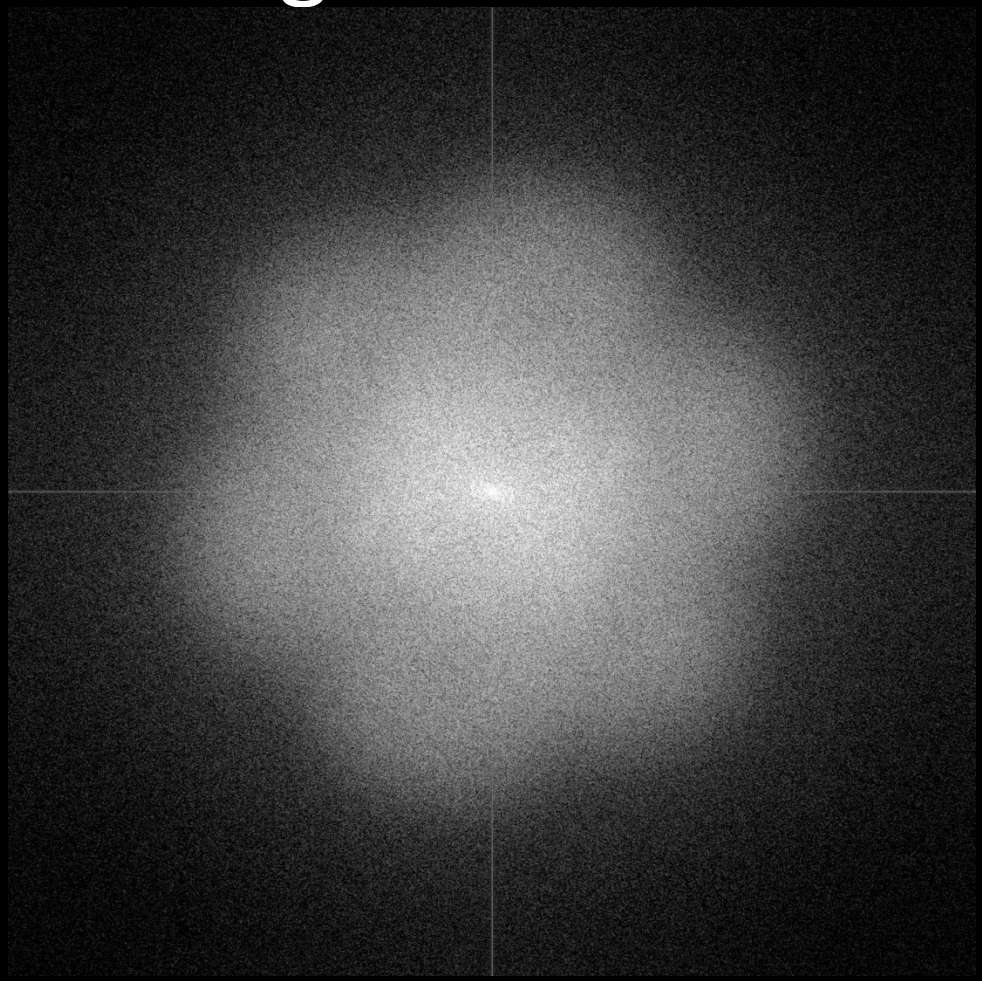


"Frequency space"

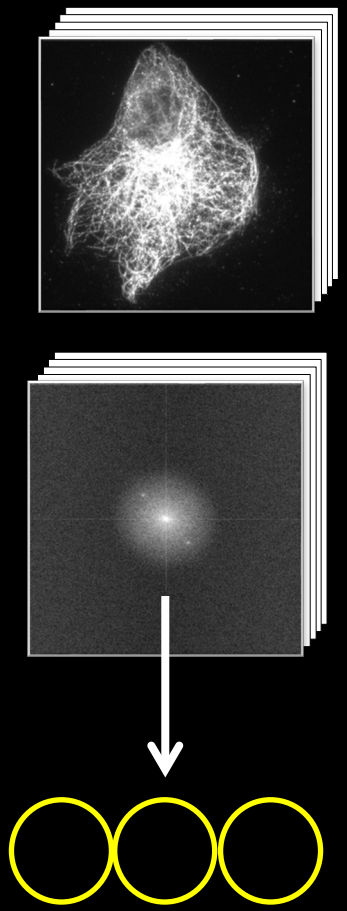


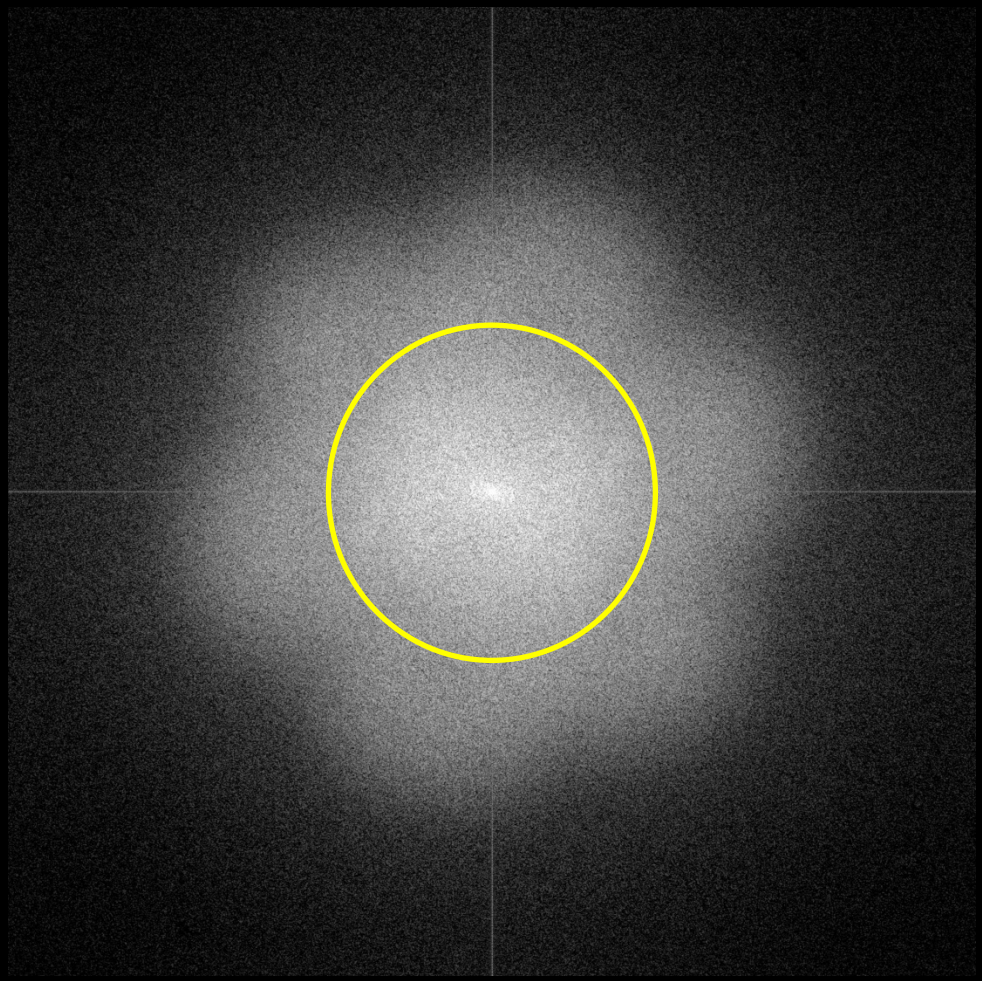
Fourier Transform of reconstructed image





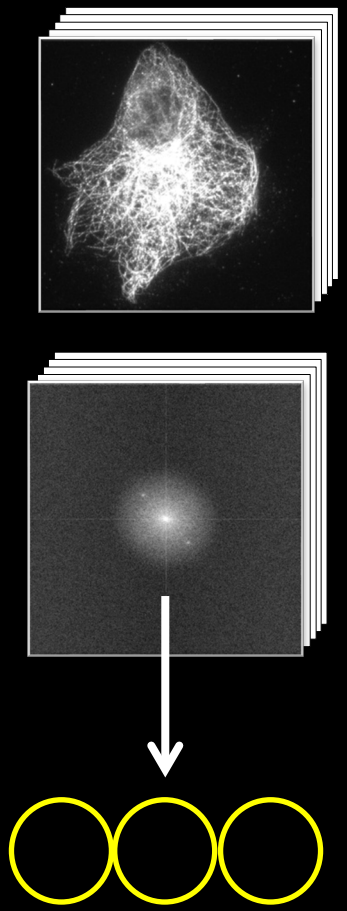
Reconstructed Image

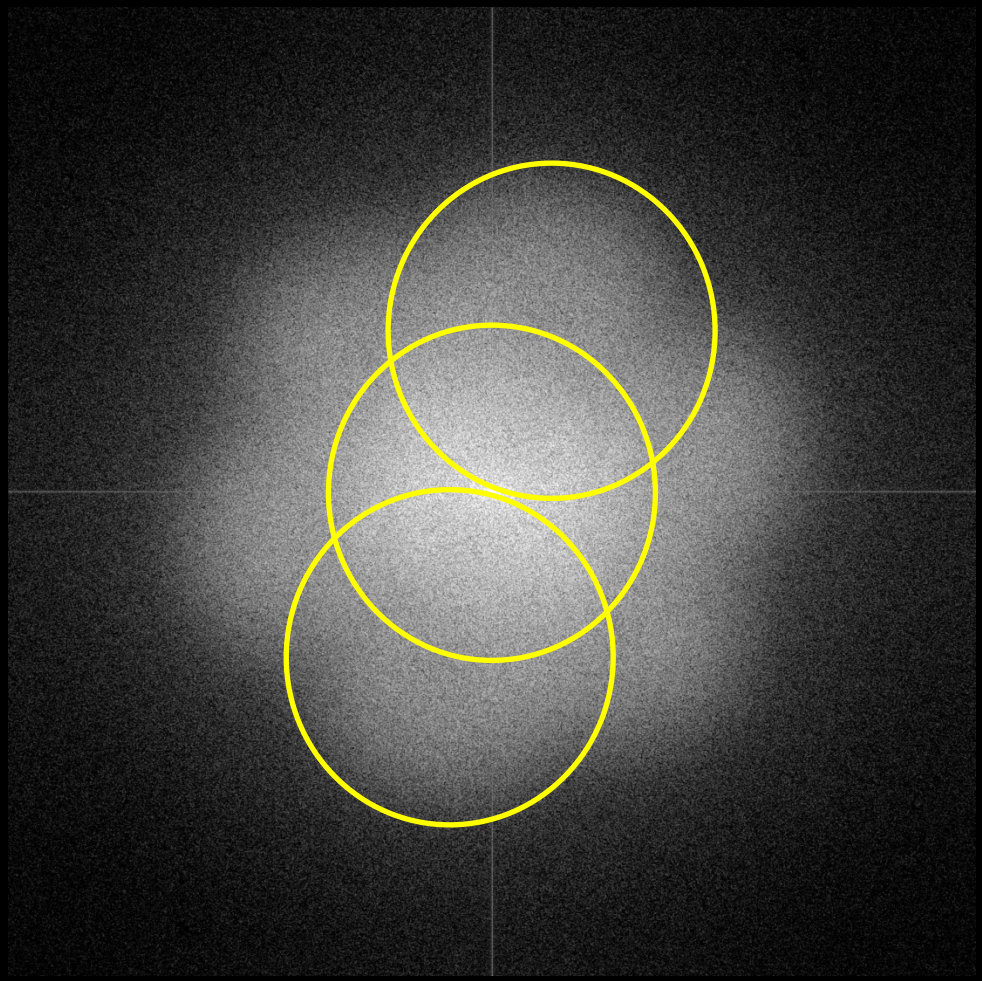




Each grating rotation contains the center information

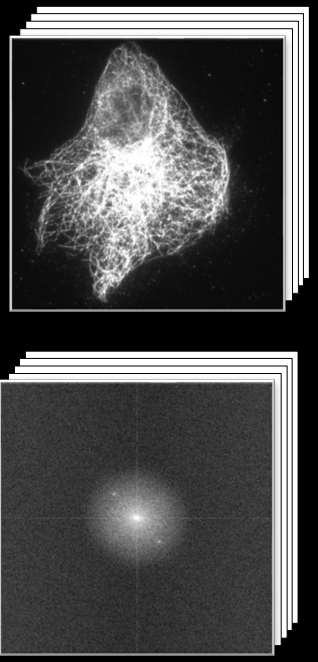
Reconstructed Image

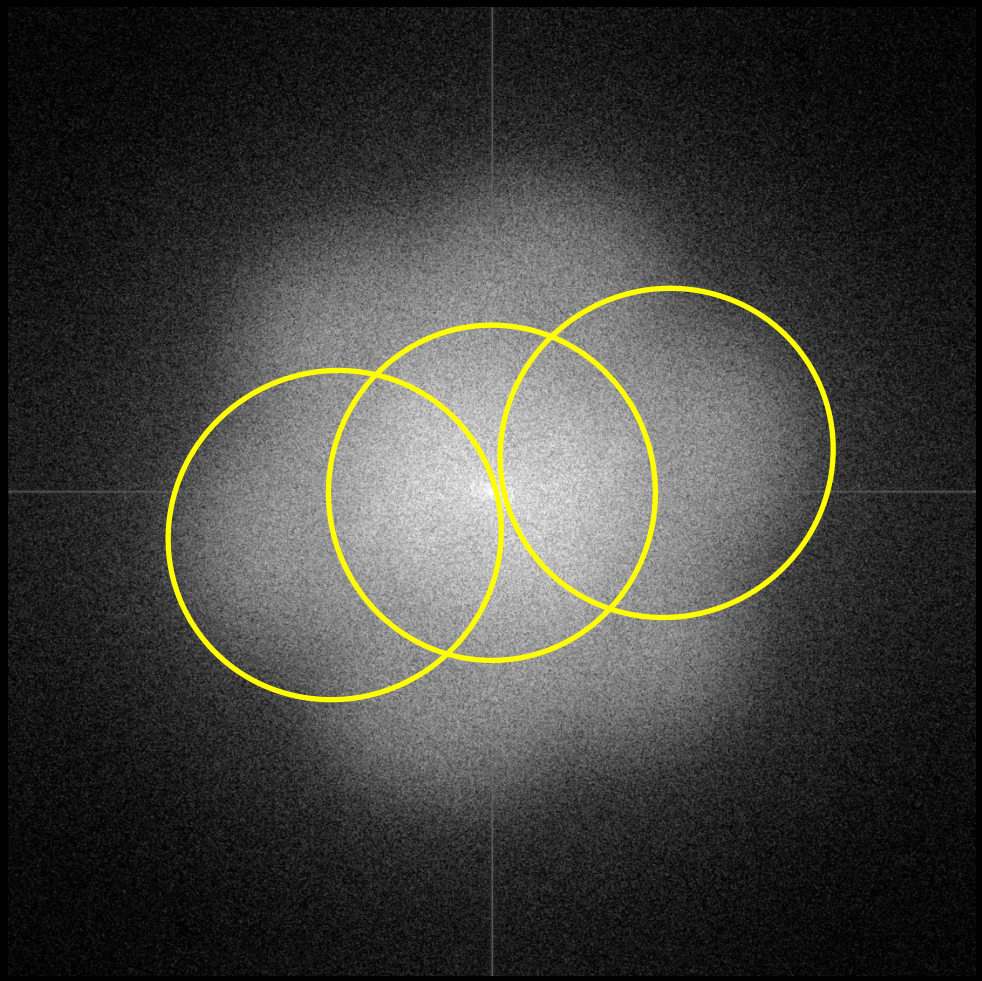




And additional information about higher frequency domains

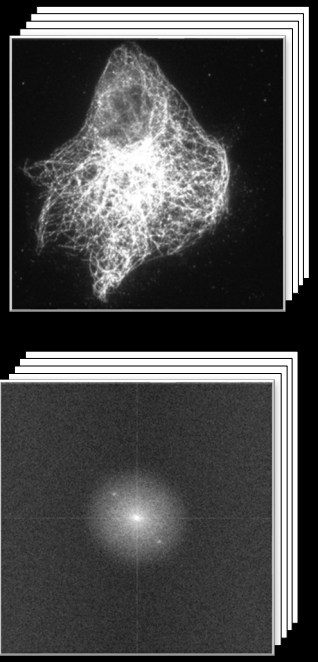
Reconstructed Image

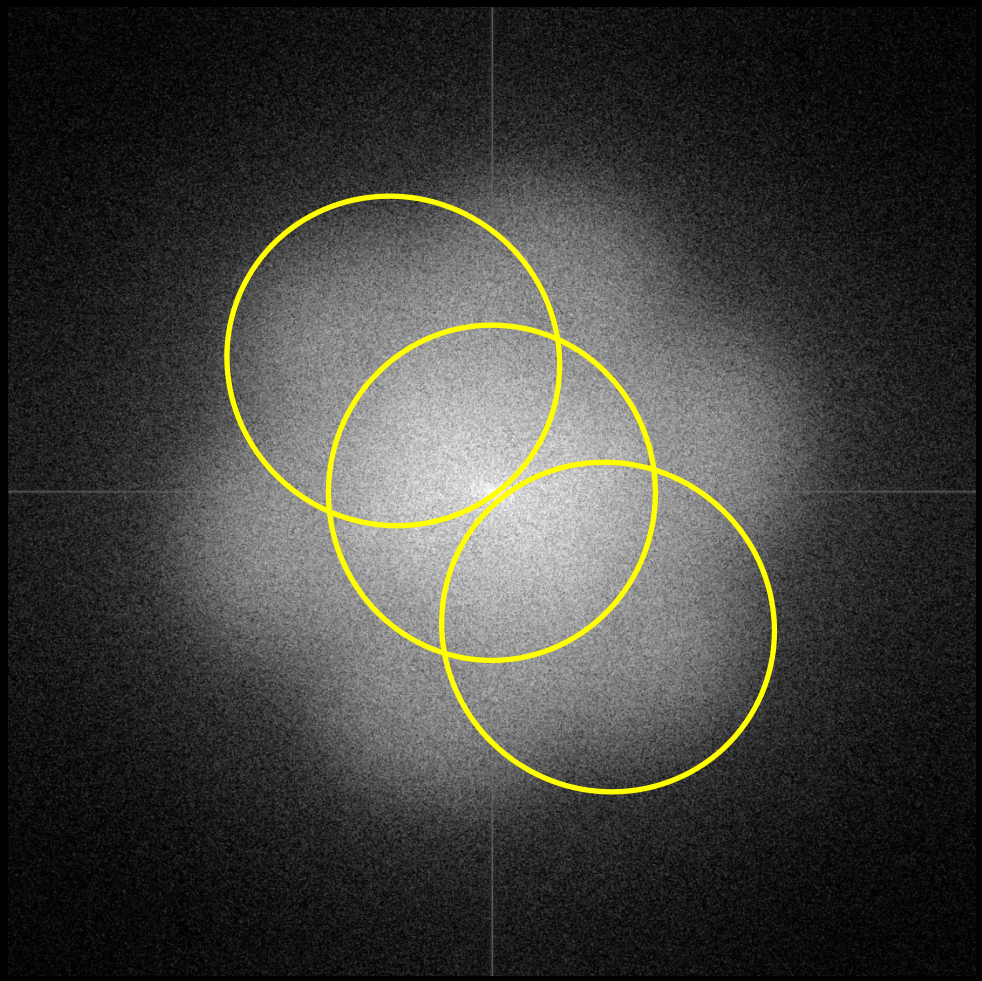




And additional information about smaller frequency domains

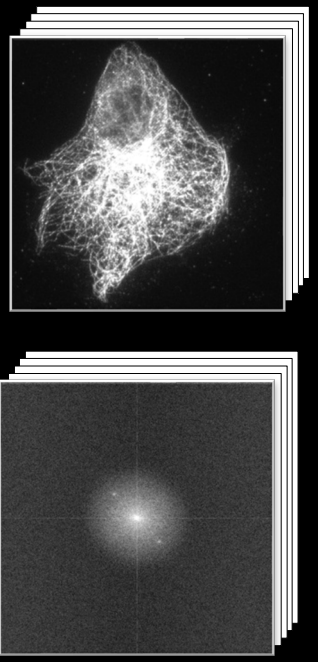
Reconstructed Image

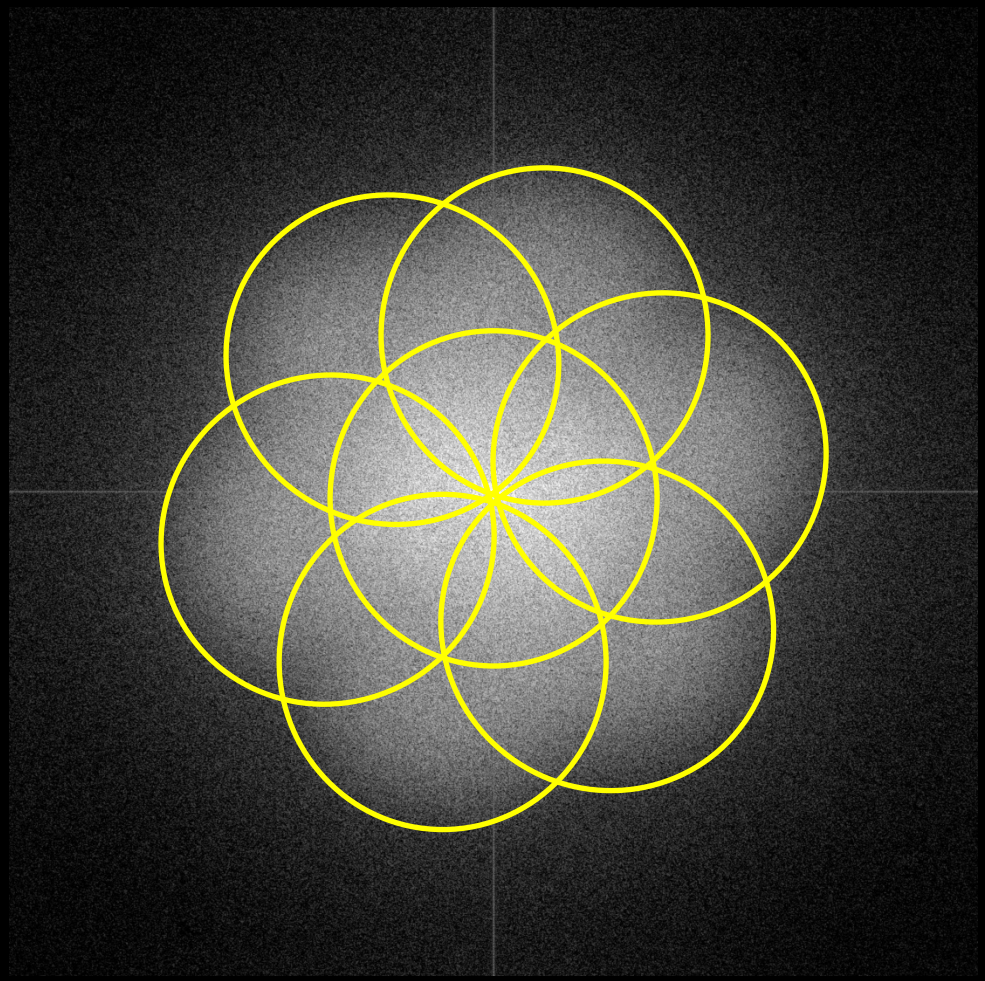




And additional information about smaller frequency domains

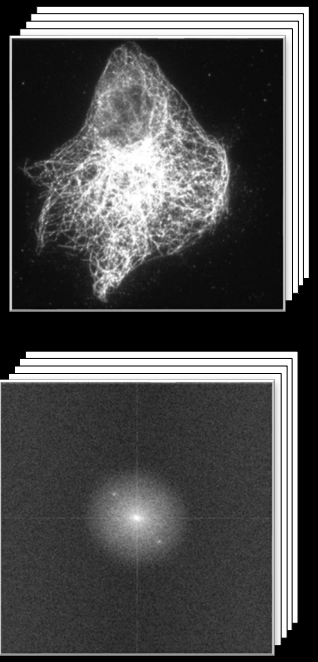
Reconstruced Image

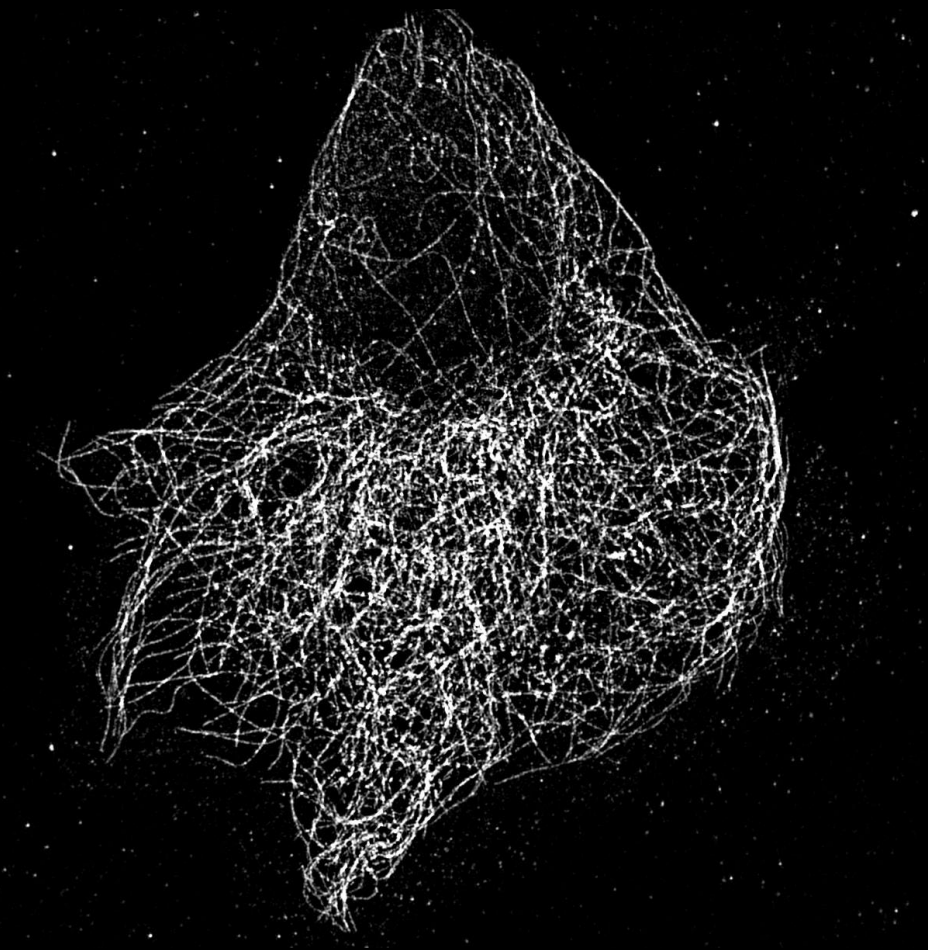




Final image is reconstructed in a weighted fashion

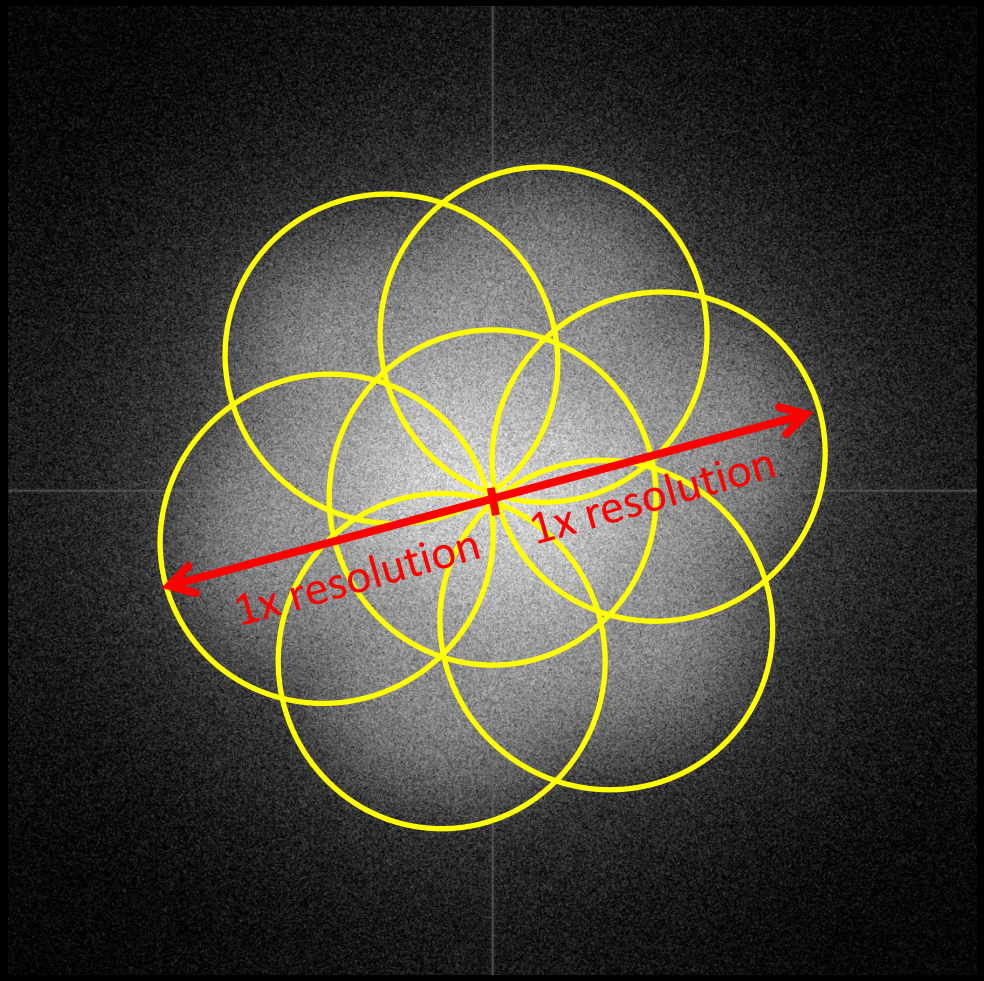
Reconstruction of Image



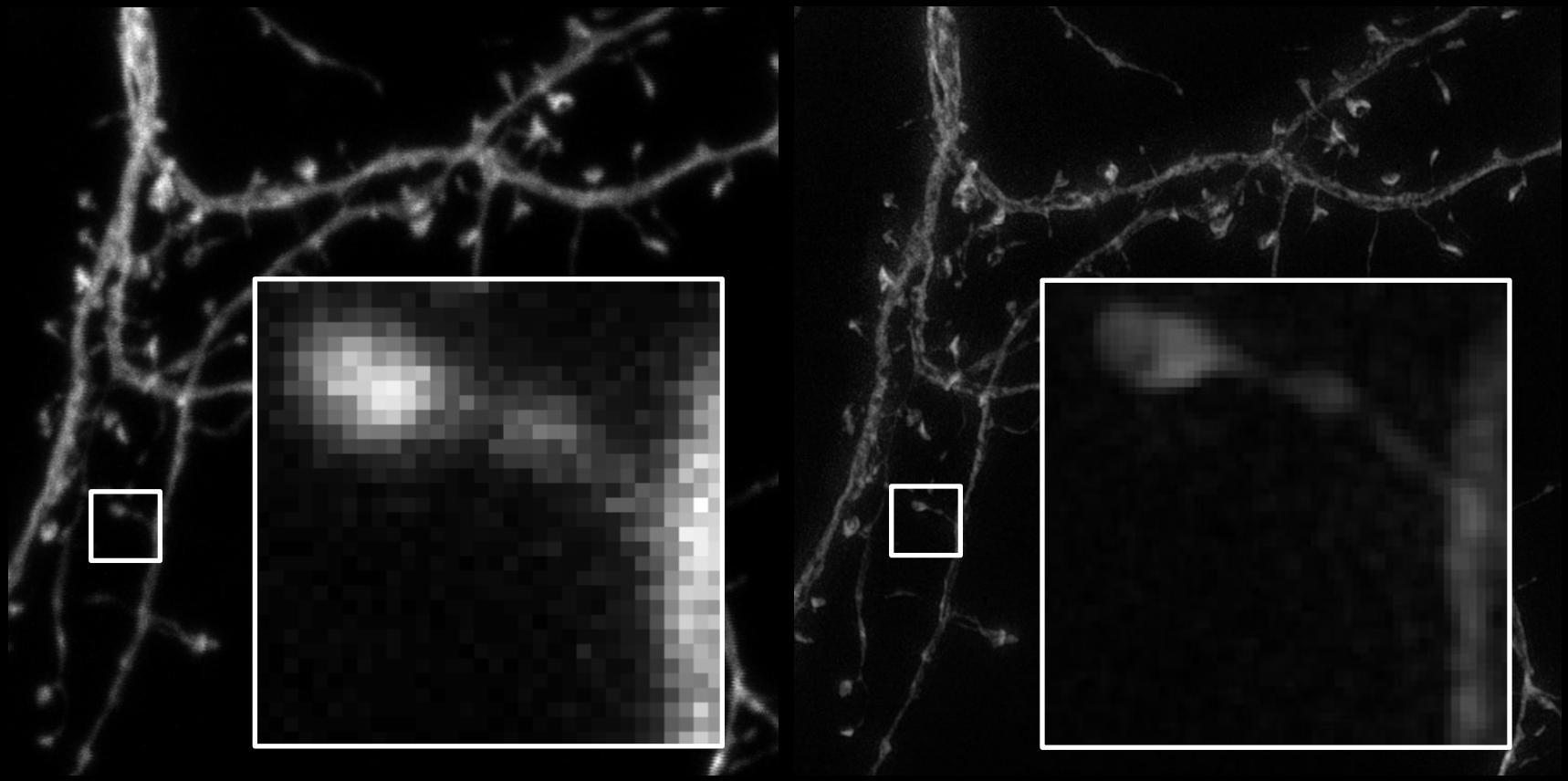


What does the FT of the reconstruction tell us

- Rotations are needed to get a full circle
- The maximum resolution increase is 2 times



What does resolution increase mean for biological samples

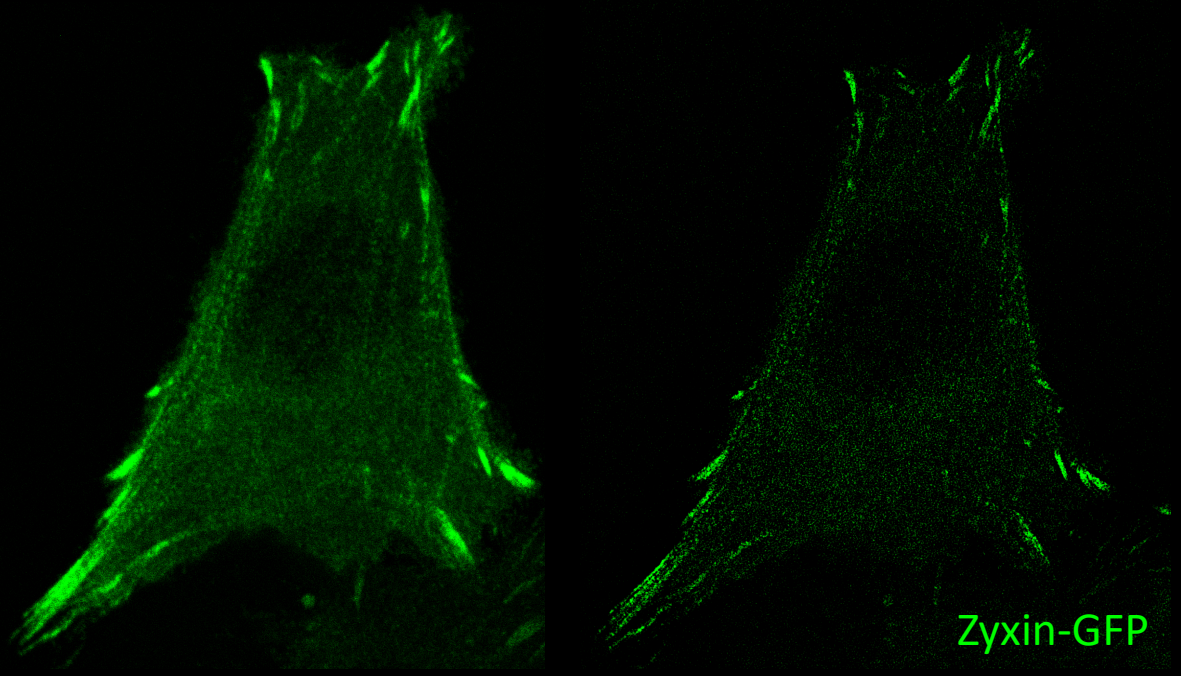


Confocal

SIM

What does resolution increase mean for biological samples

- Gain of structural information within structures



Confocal SIM

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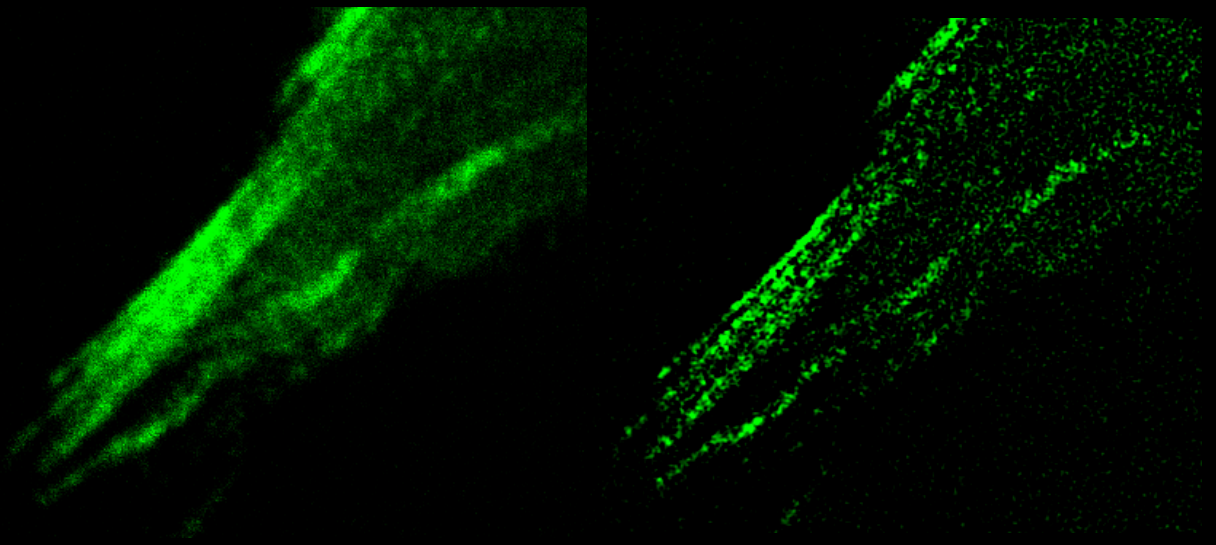
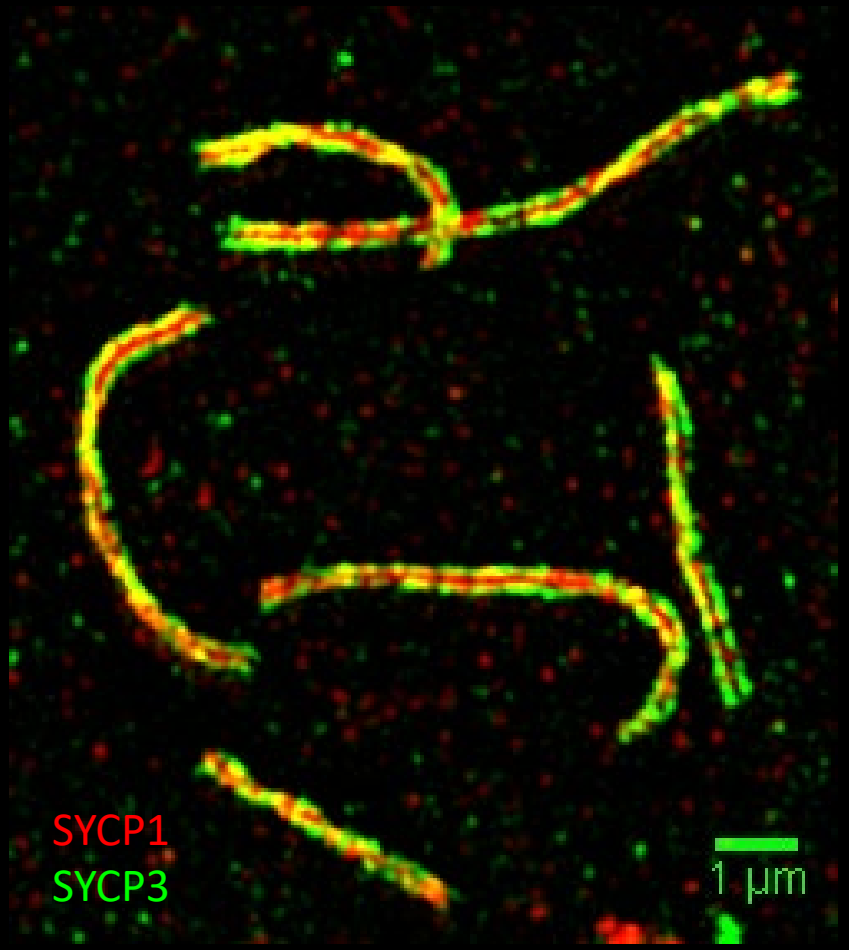


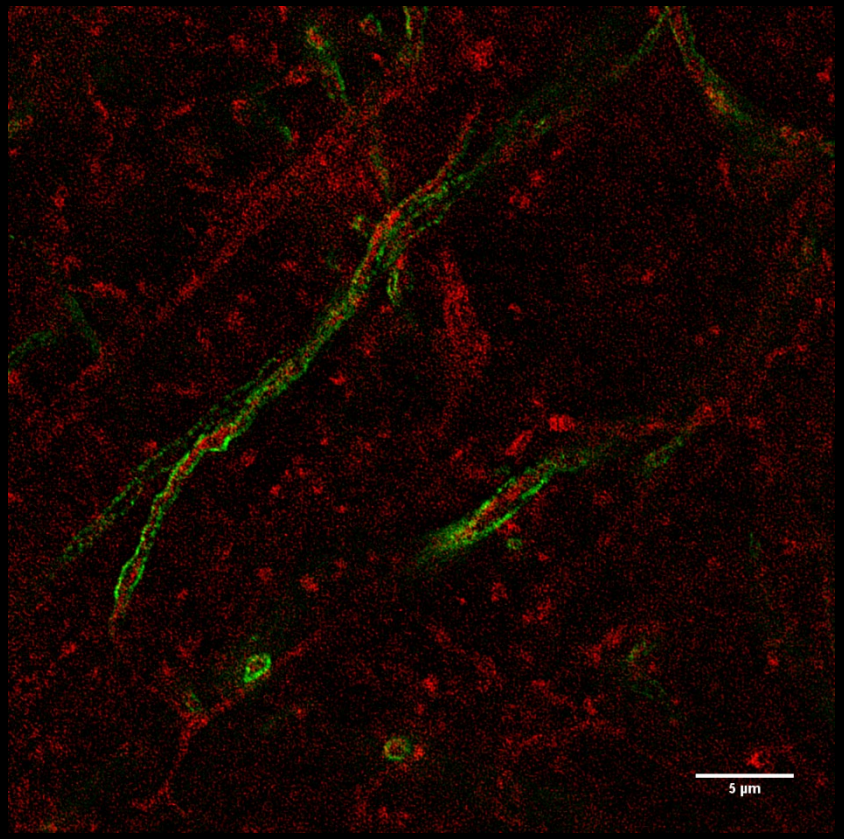
What does resolution increase mean for biological samples

- Gain of structural information within structures
- Separation of structures



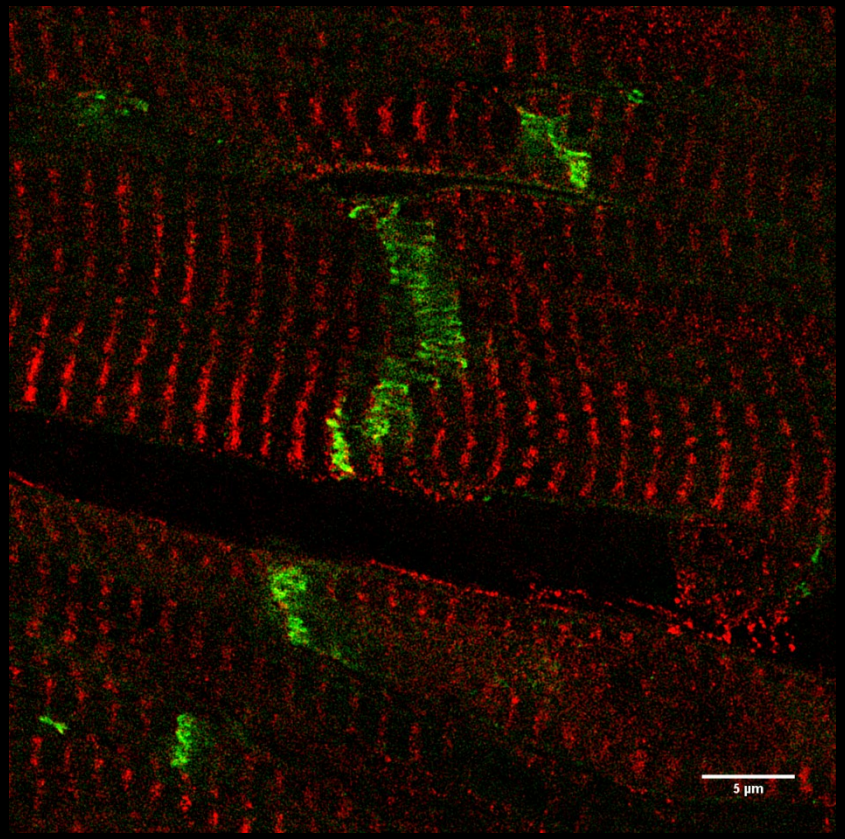
Federica Federici

Structured Illumination in tissue slices



Myeline sheets in mouse brain tissue

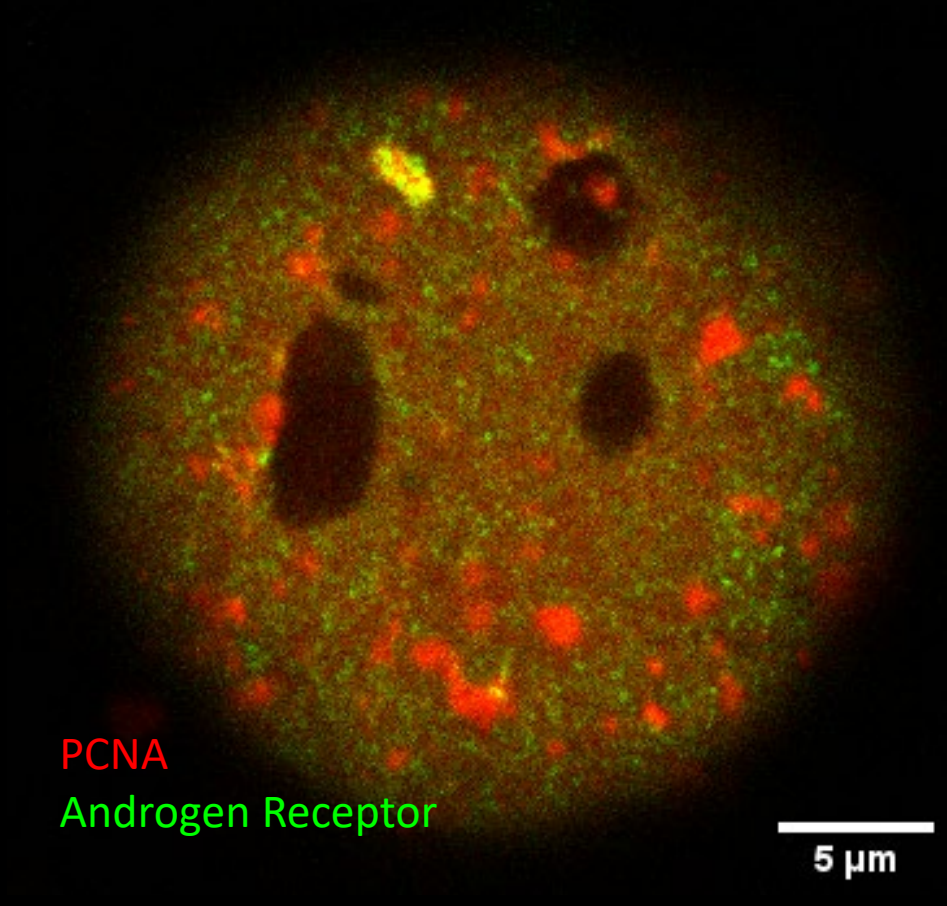
Jeffrey Stedehouder / Steven Kushner



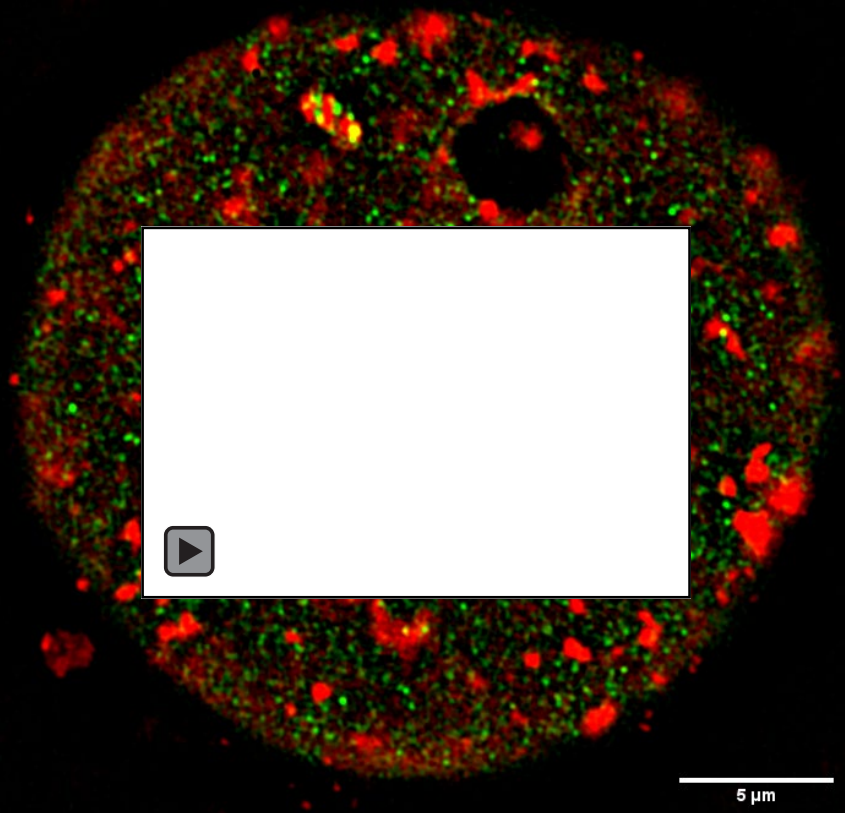
Intercalated discs in mouse hart tissue

Nuo Yu / Niels Galjart

What does resolution increase mean for biological samples



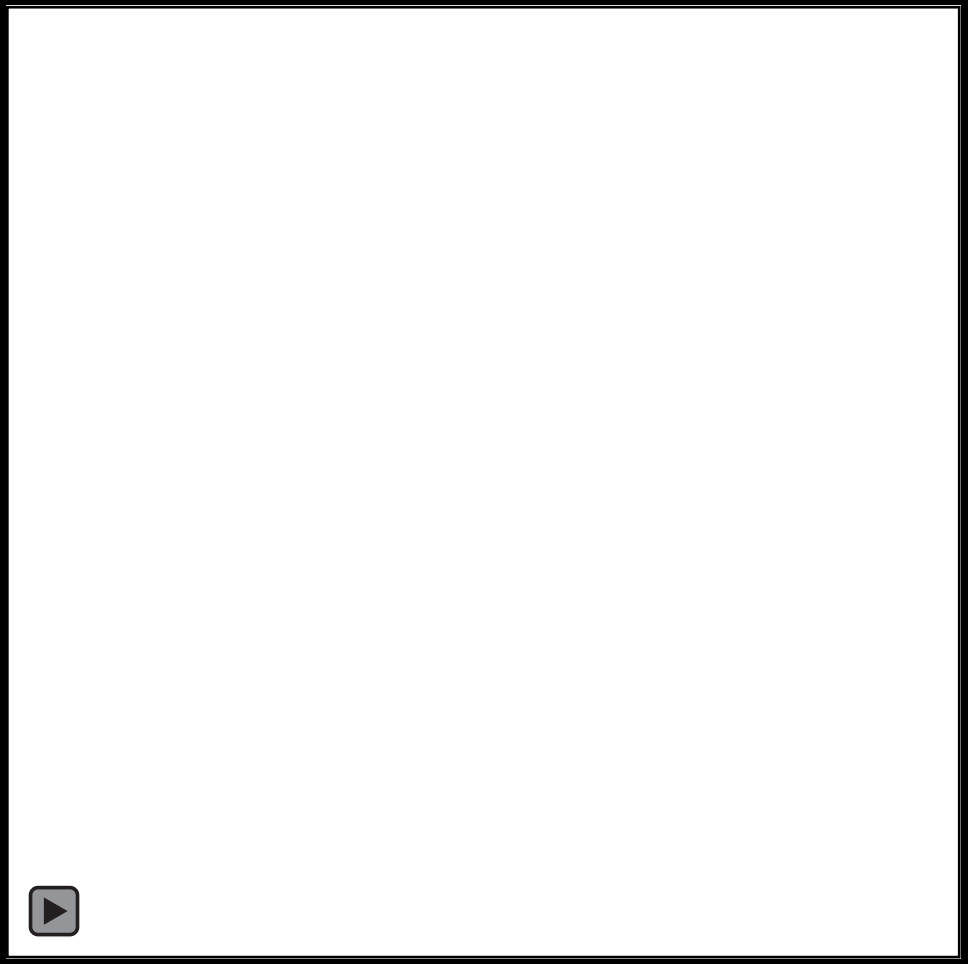
Confocal



SIM

What does resolution increase mean for biological samples

- Separation of structures
- Gain of structural information within structures
- Structural information in time



Interval = 2min

Average projection of 20 z-slices

Summary SIM

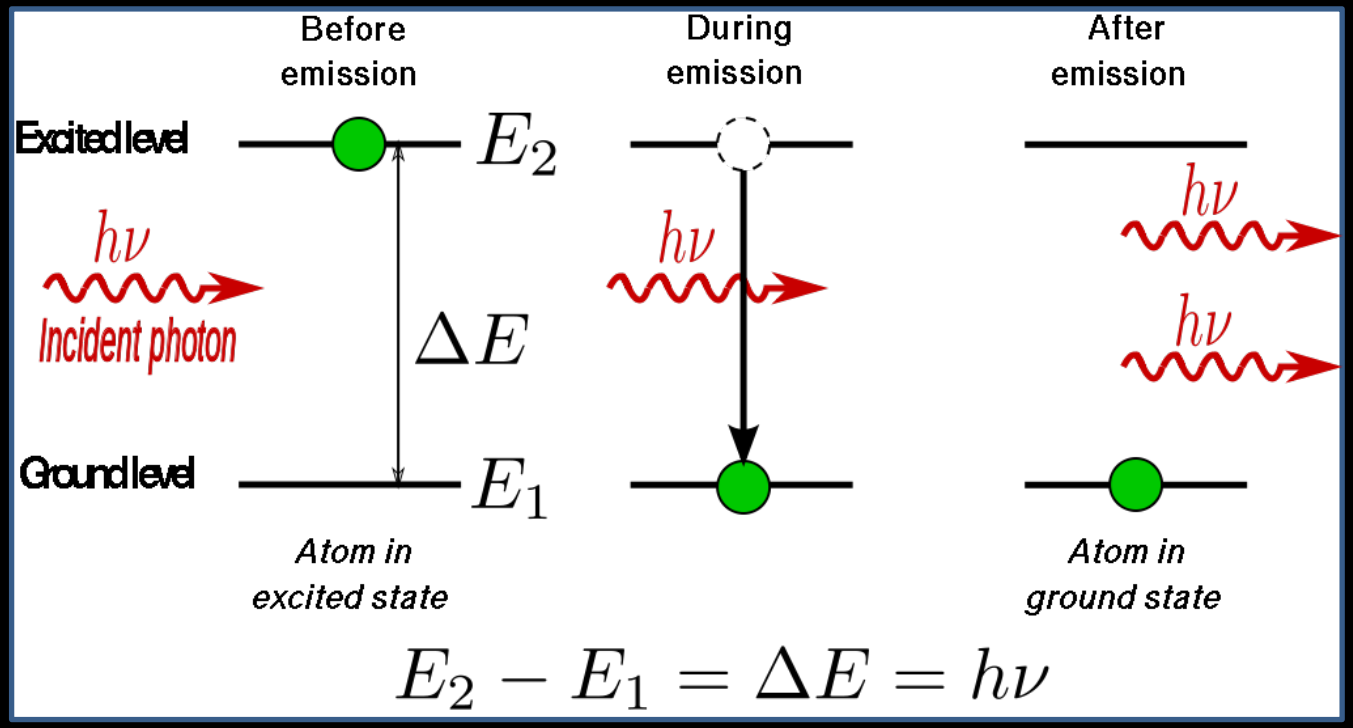
- Increased resolution in X, Y and Z up to 2 times widefield resolution
- Applicable to life cell imaging
- Multiple colors possible
- Multiple images per plane necessary
- Post-Processing

Imaging through the hole of donut

STED MICROSCOPY

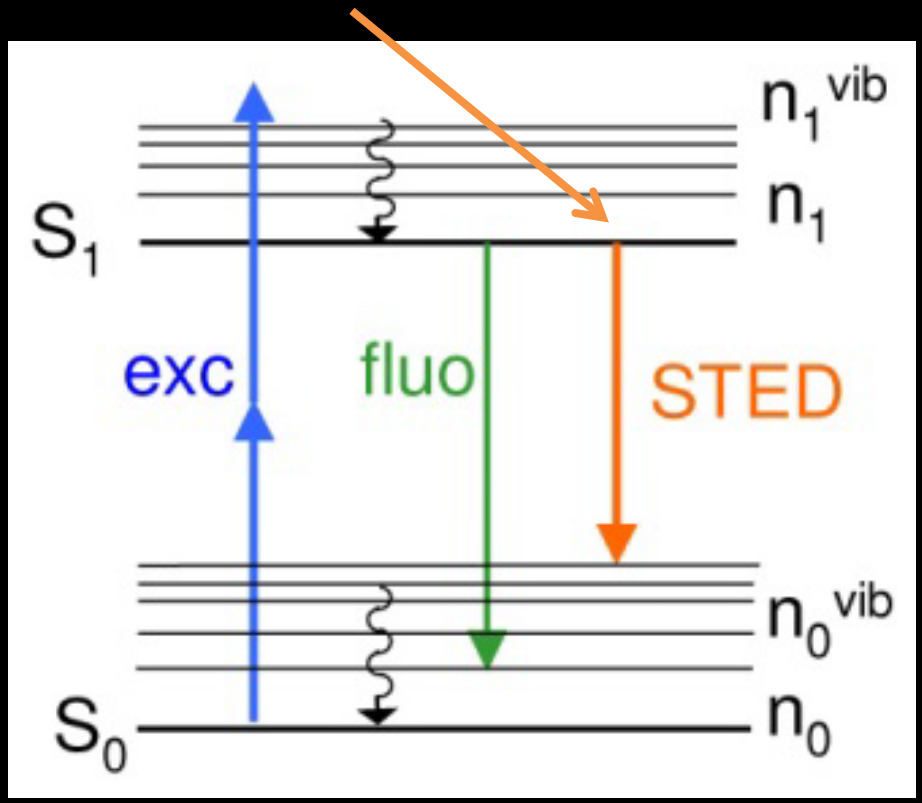
Stimulated Emmision Depletion Microscopy

- Stimulated Emmision

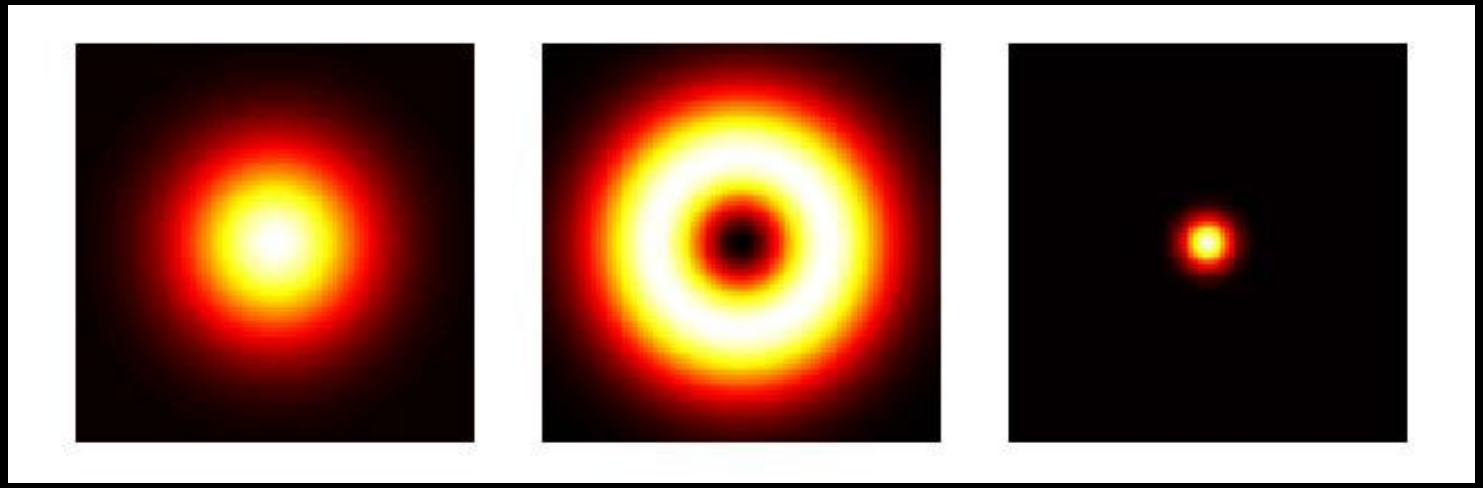


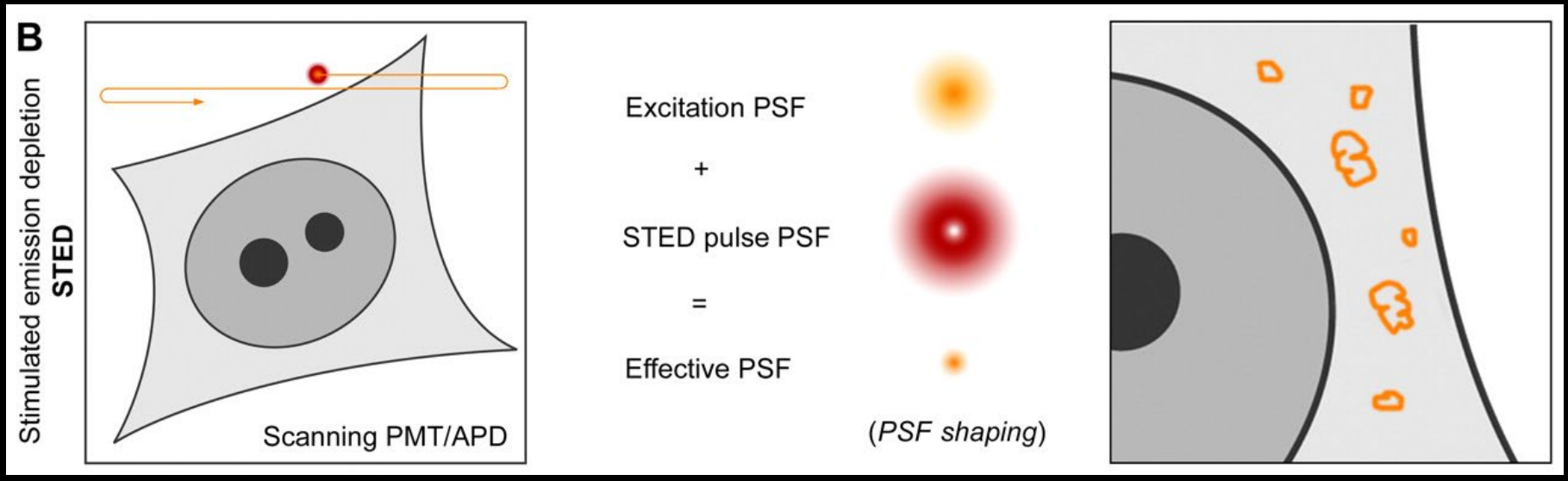
Stimulated Emmision Depletion Microscopy

- Stimulated Emmision

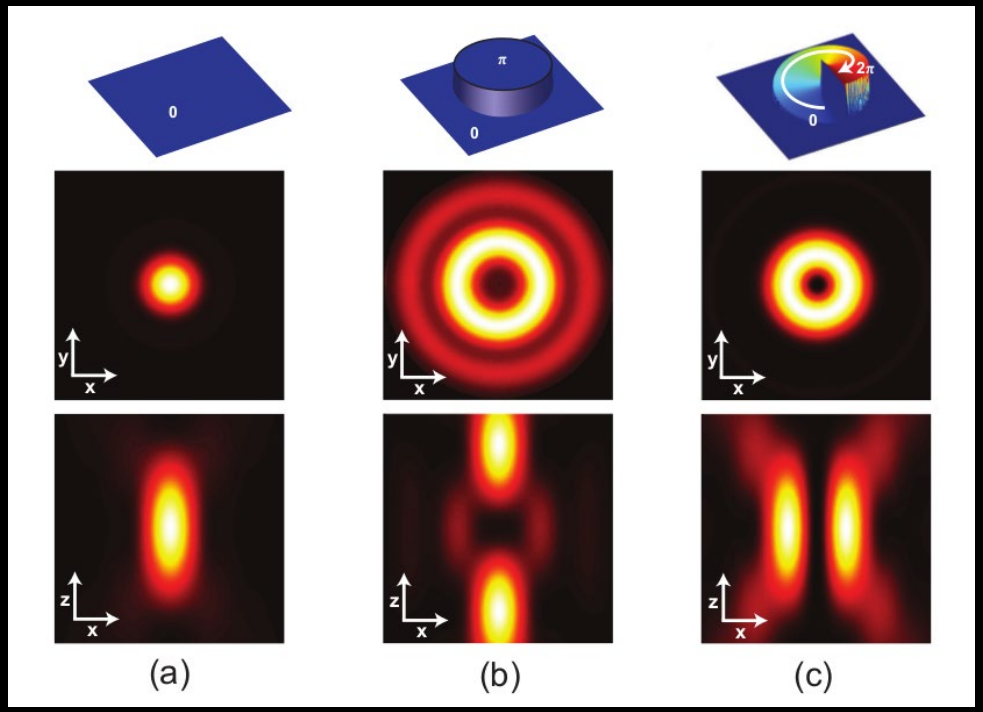


Stimulated Emmision Depletion Microscopy

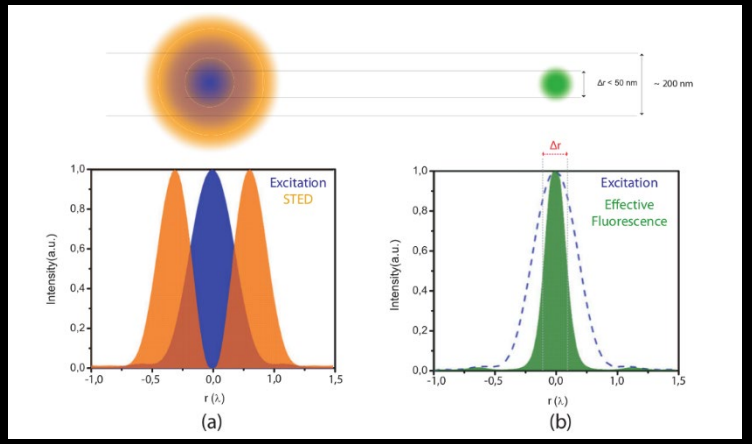




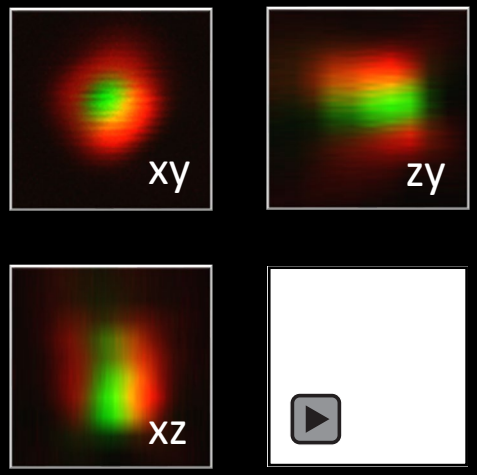
- STED is nonlinear
- The STED Beam can be shaped in different ways
- Donut (c) gives the highest xy-resolution but low z-resolution
- ‘Z-STED’ gives the highest z-resolution but lower xy-resolution
- Mixed mode gives intermediate x,y,z-resolutions



$$d = \frac{0.45\lambda}{NA\sqrt{1 + \frac{I}{I_{SAT}}}}$$

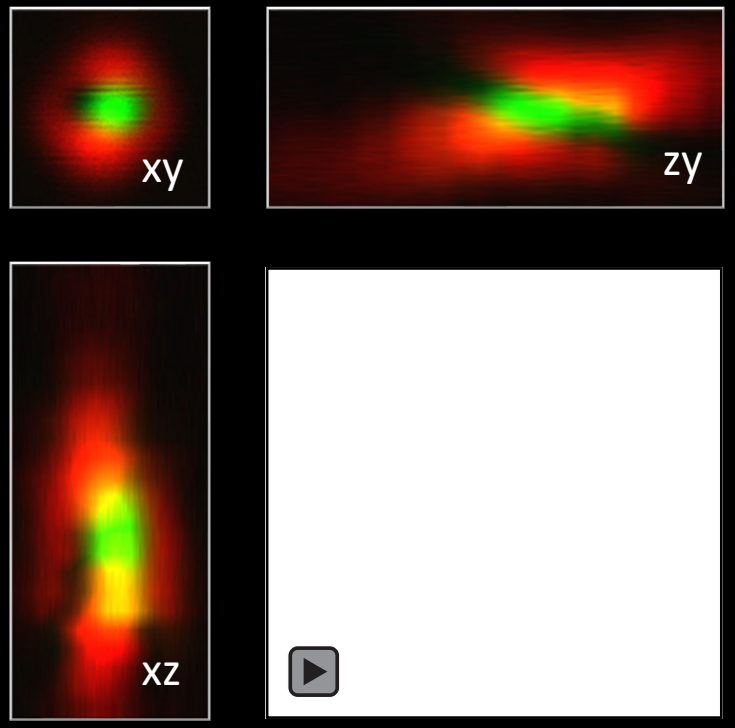


STED PSF



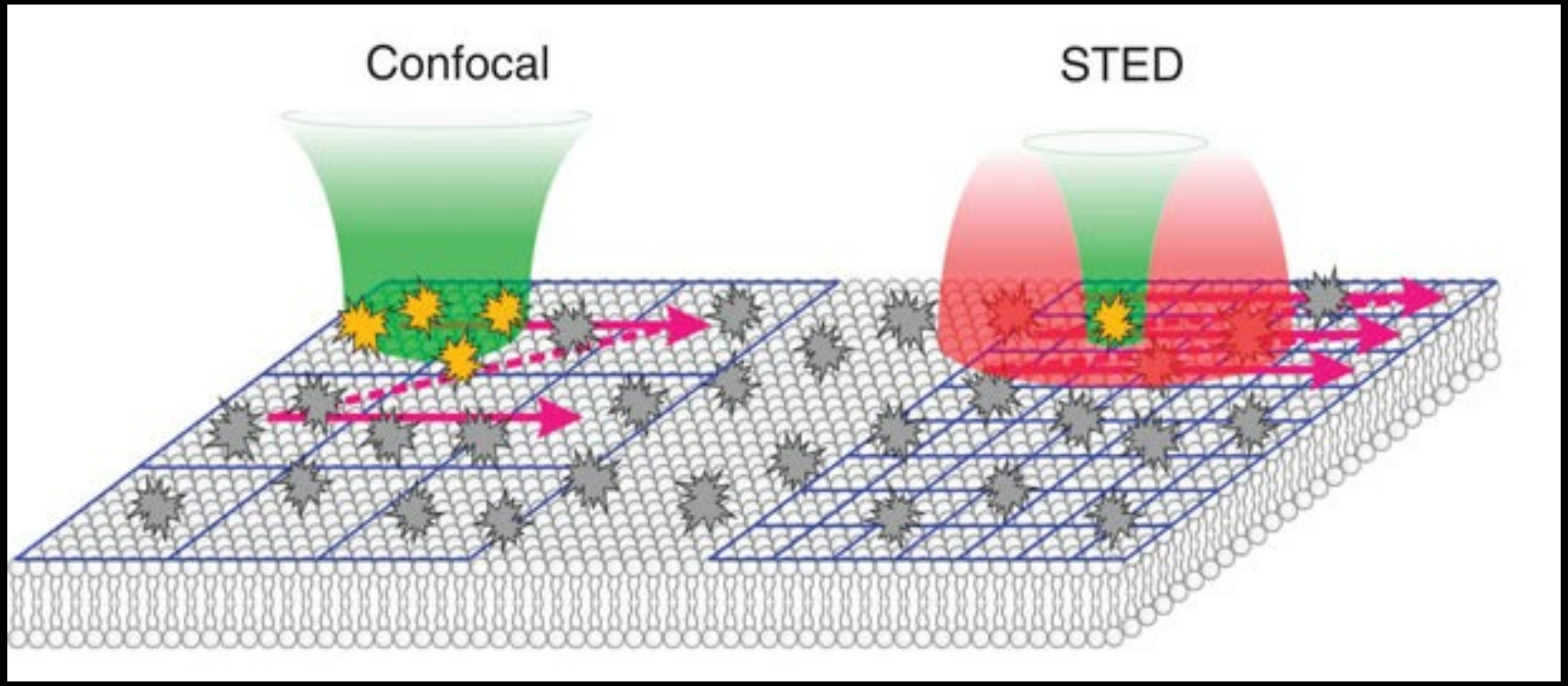
Depletion PSF
Excitation PSF

3D STED PSF



Depletion PSF
Excitation PSF

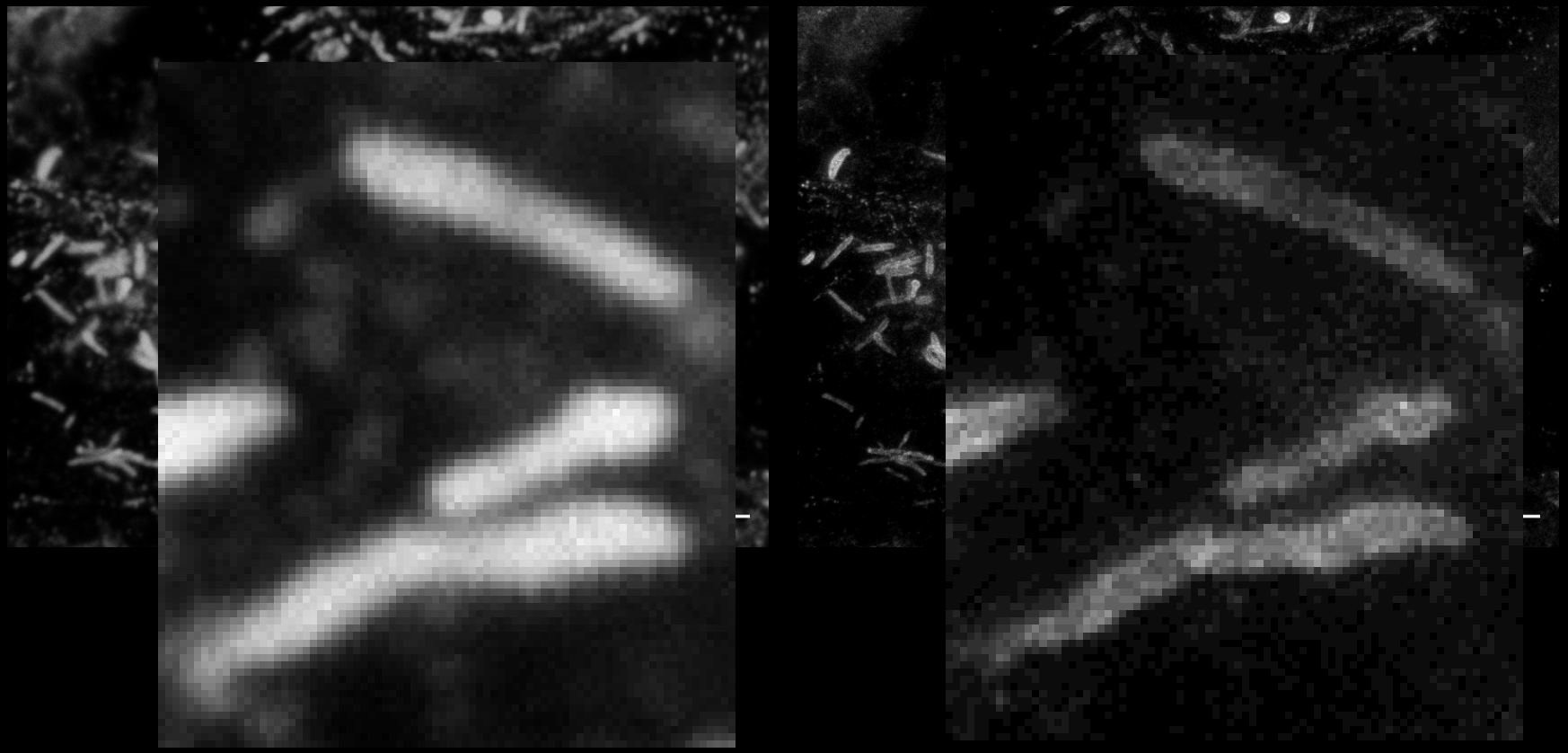
The STED beam depletes specific exitation of fluophores



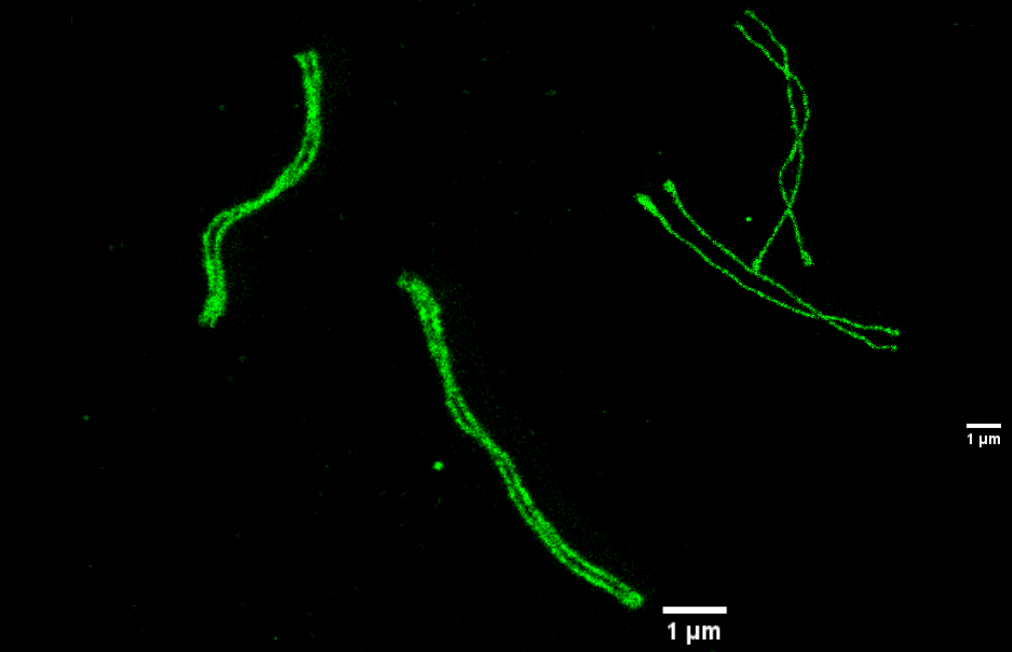
STED microscopy of weibel palade bodies

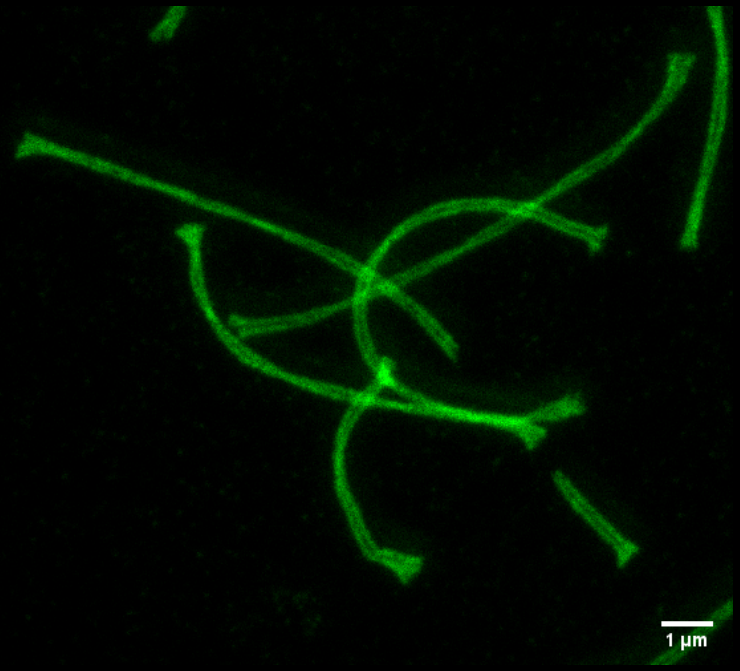
Confocal

STED



Single Color STED, SYCP3 (Alexa Fluor 555)

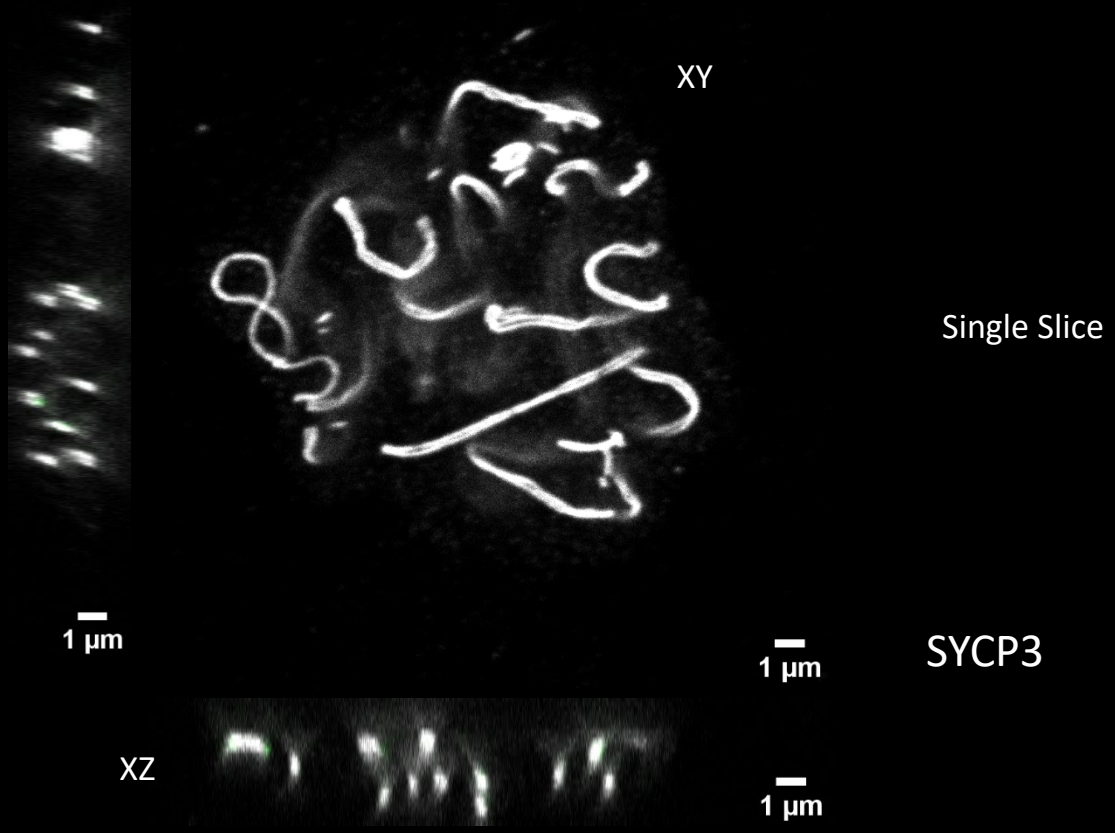




Lieke Koornneef

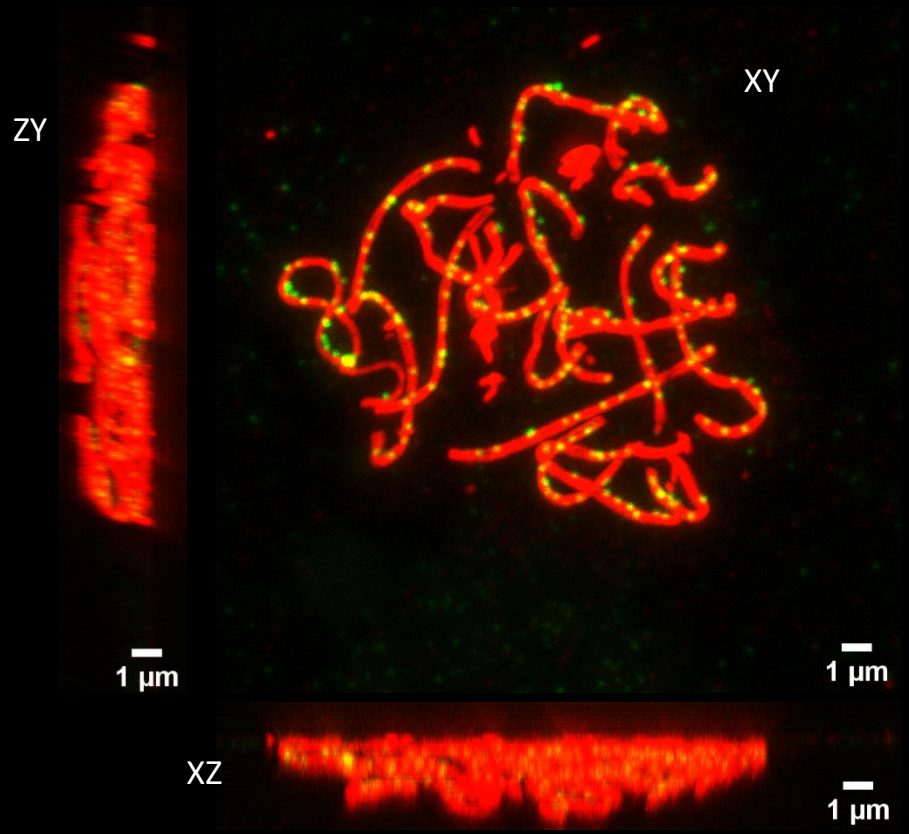
SYCP3

3D STED Synaptonemal Complex



Aditya Mhaskar

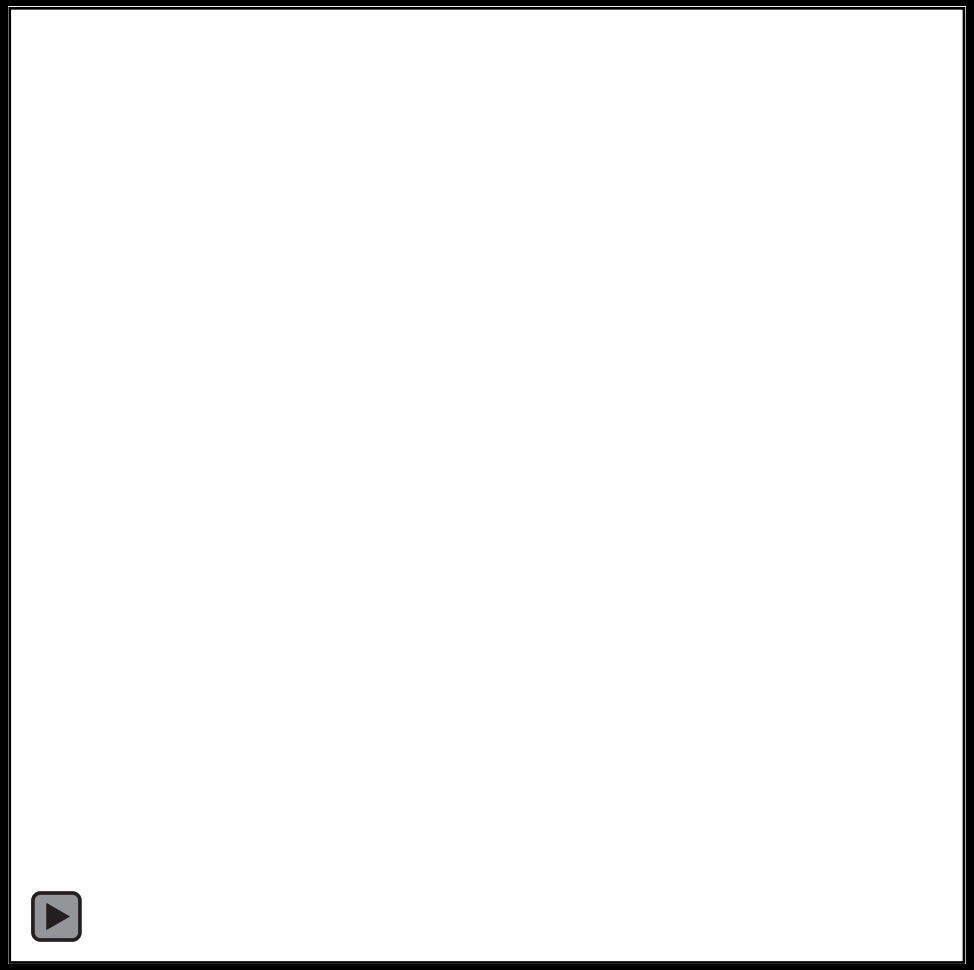
3D STED Synaptonemal Complex



MAX Projection

SYCP3
RAD51

Aditya Mhaskar



SYCP3
RAD51

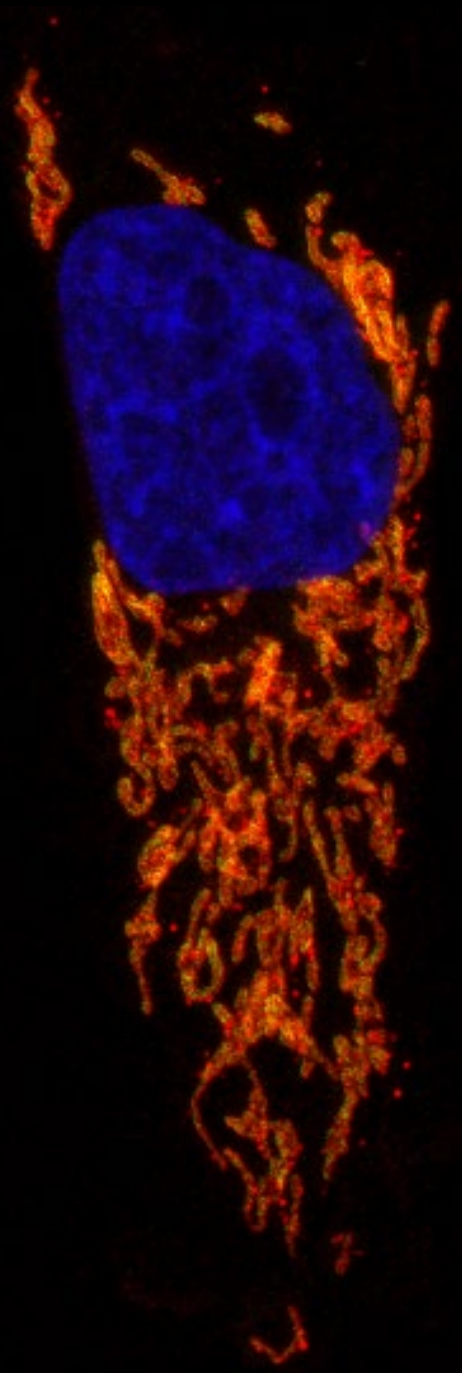
Aditya Mhaskar

Summary STED

- 30 ~ 80 nm resolution
- No post-processing
- Resolution increase in x,y and z

- Limited number of fluorophores
- High-laser powers
- Low excitation signals

Thank You for
your attention



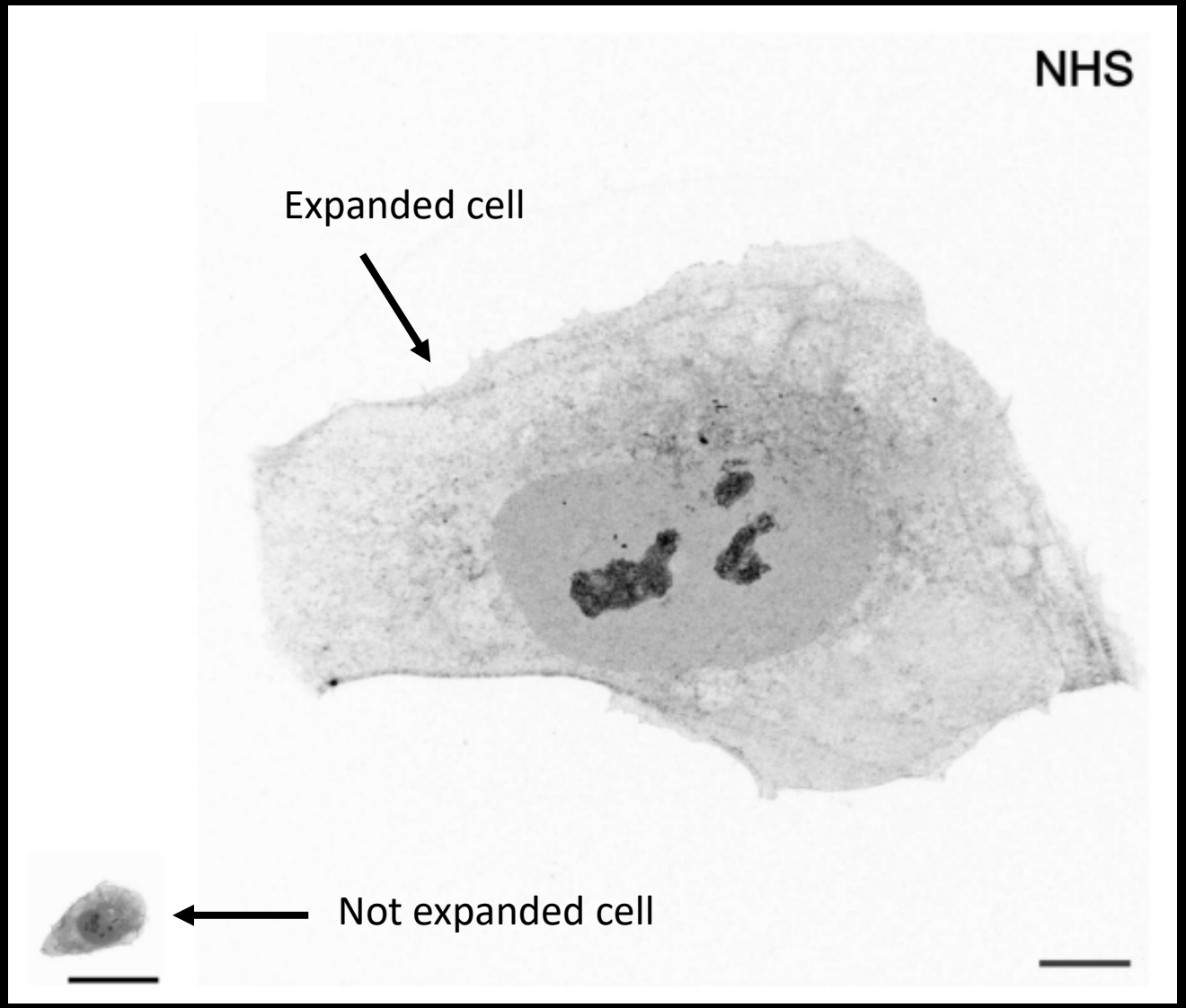
Getting “super-resolution” without a super-resolution microscope

EXPANSION MICROSCOPY

Changing the sample: Expansion Microscopy

- Two different cells not expanded or expanded in a gel
- All proteins stained by a NHS ester coupled fluorophore
- Imaged on regular confocal microscope

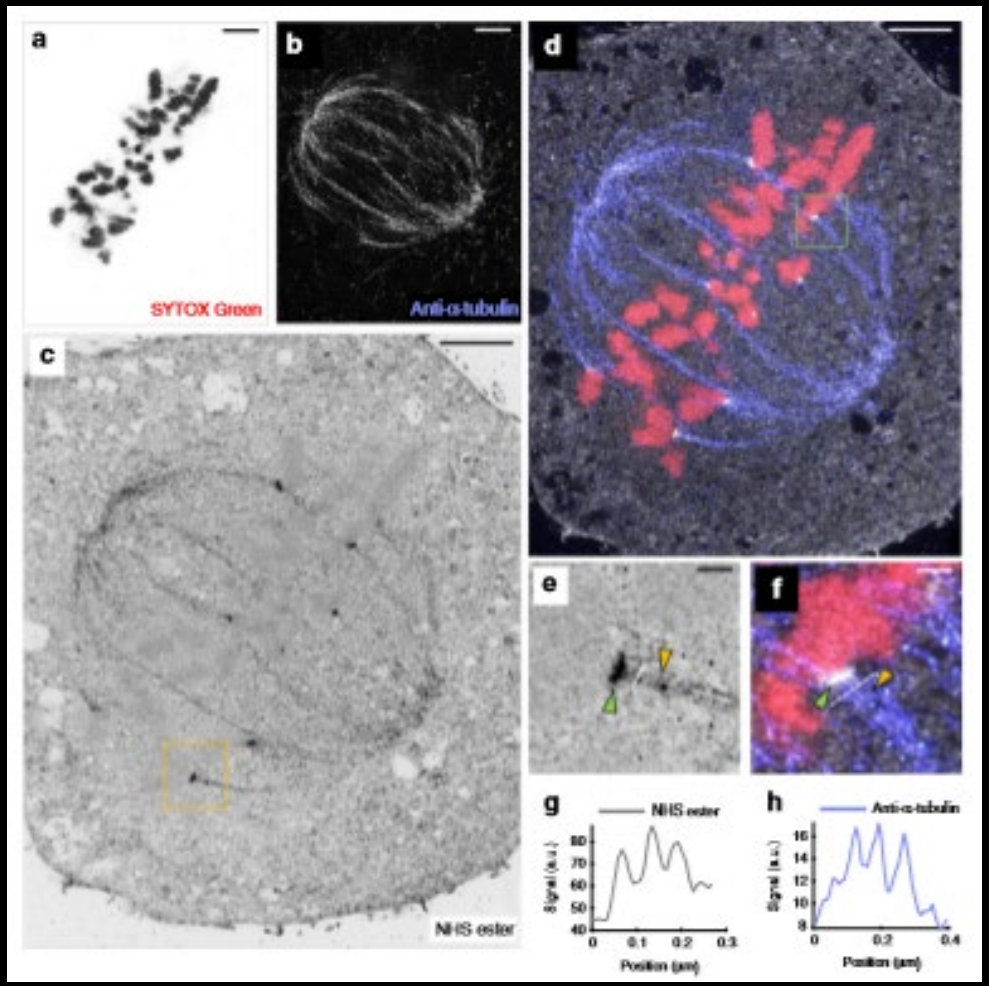
- Resolution increase by physically increasing the size of the sample



Lisa Baltus

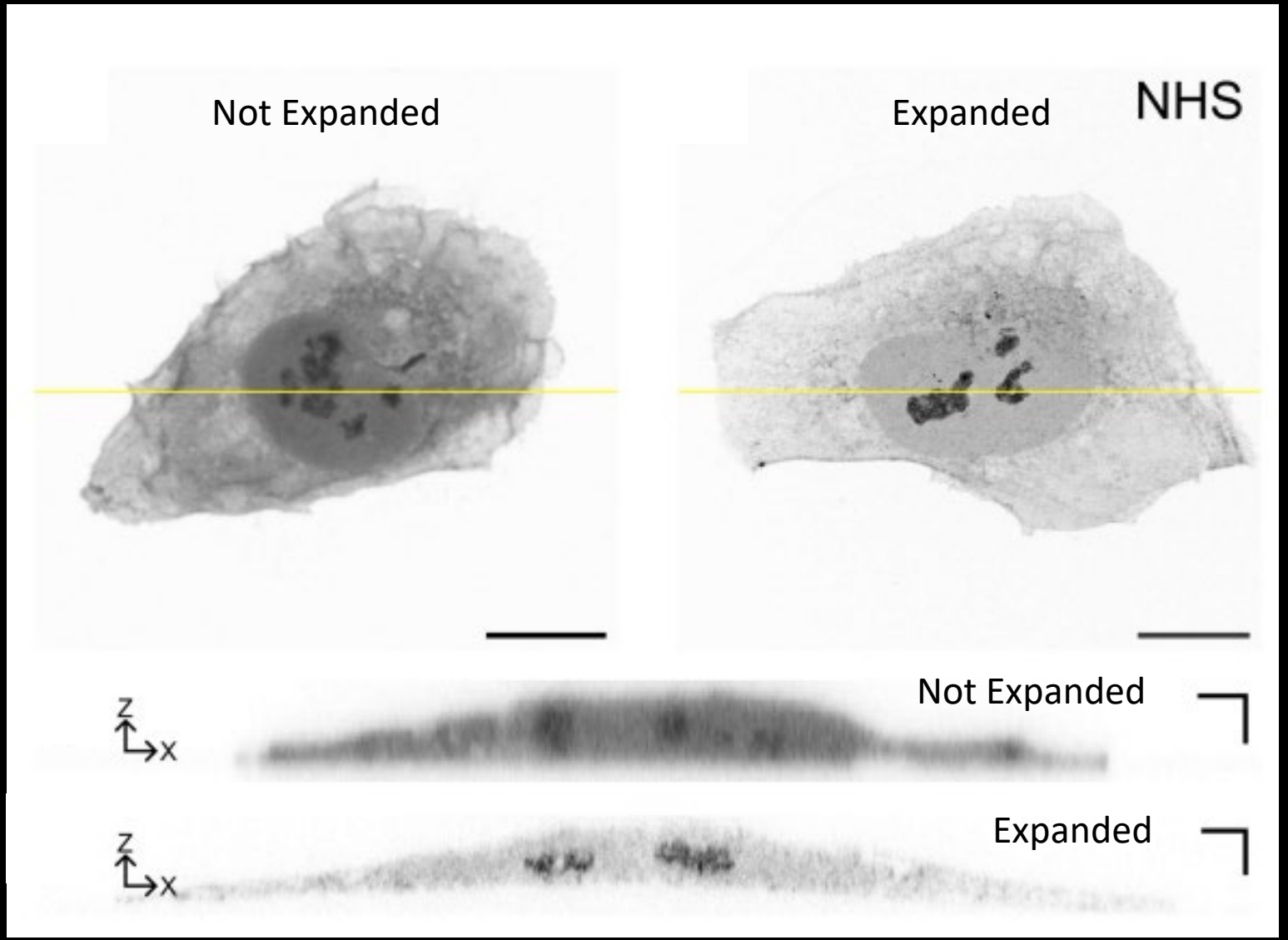
Counterstaining of proteins and DNA

Getting toward EM resolutions with the benefits of fluorescence microscopy



M'Saad & Brewersdorf Nature comm. 2020

3D imaging

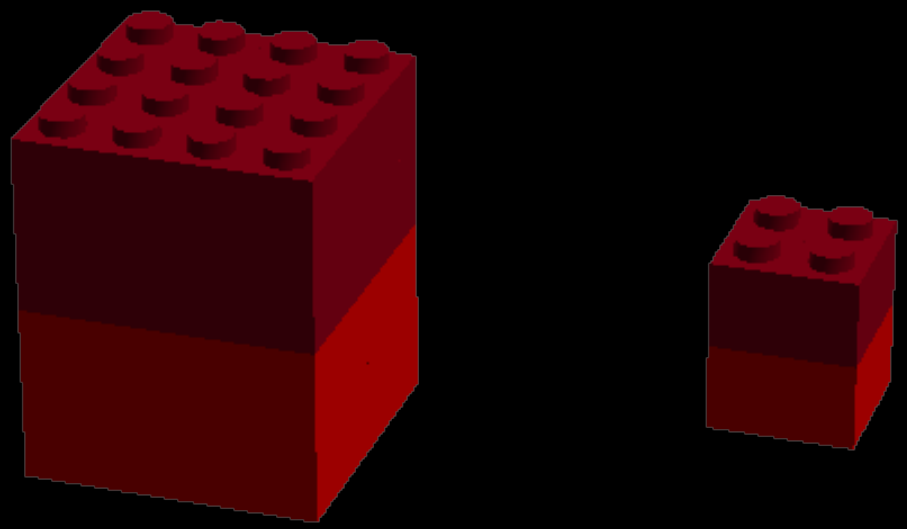


Lisa Baltus

Summary Expansion Microscopy

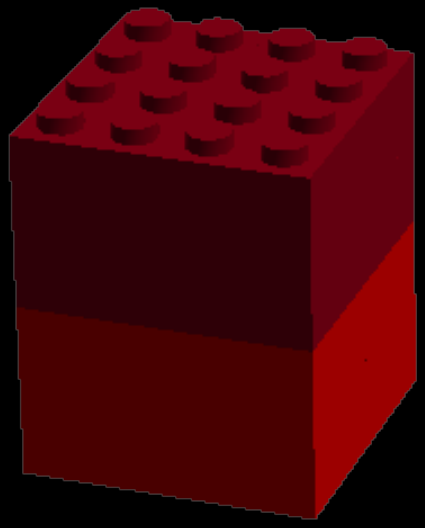
- Sample preparation technique
- Fixed samples
- Multi color
- 3D
- Can be combined with super resolution microscopy

- Difficult to relate back to original sample in measurements
- Limited working distances of lenses, samples get really big!

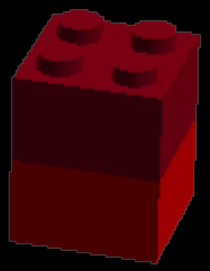


Resolution increase

- A two fold resolution increase
- Objects appear 8 times smaller (2^3)



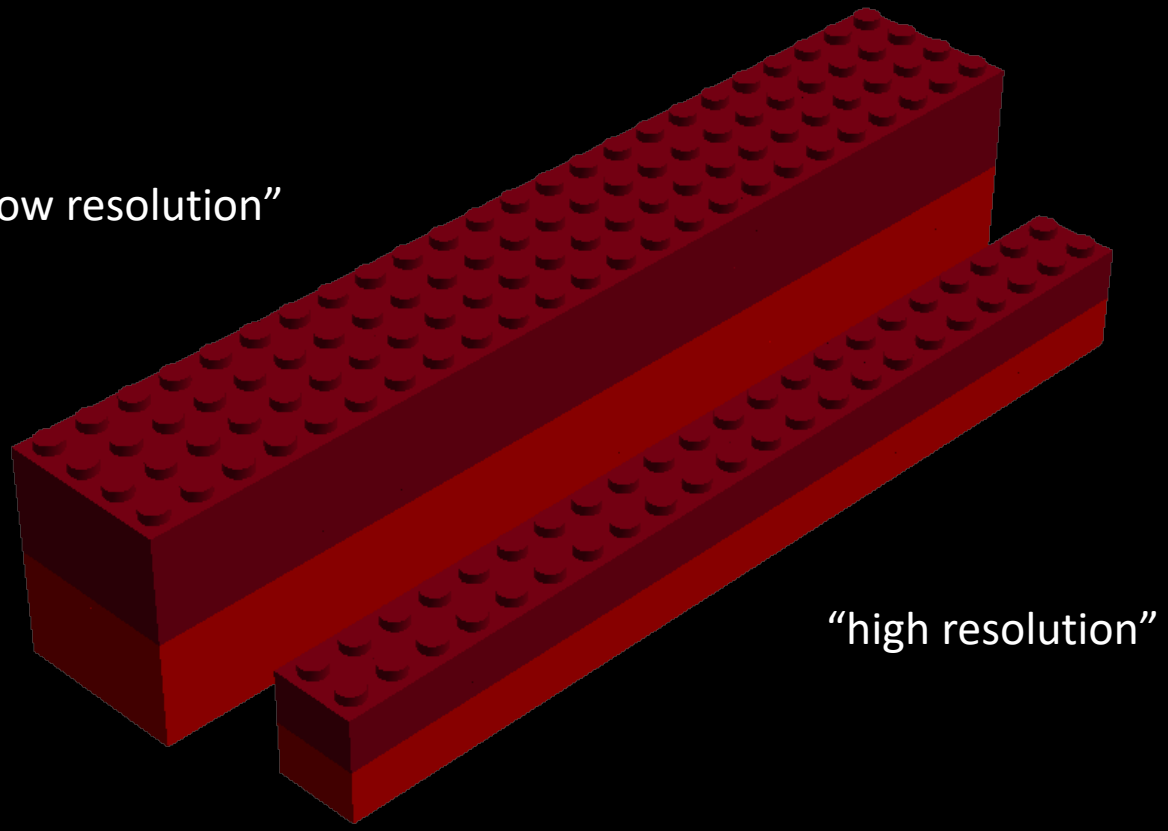
Resolution of 2 (lego units)



Resolution of 1 (lego units)

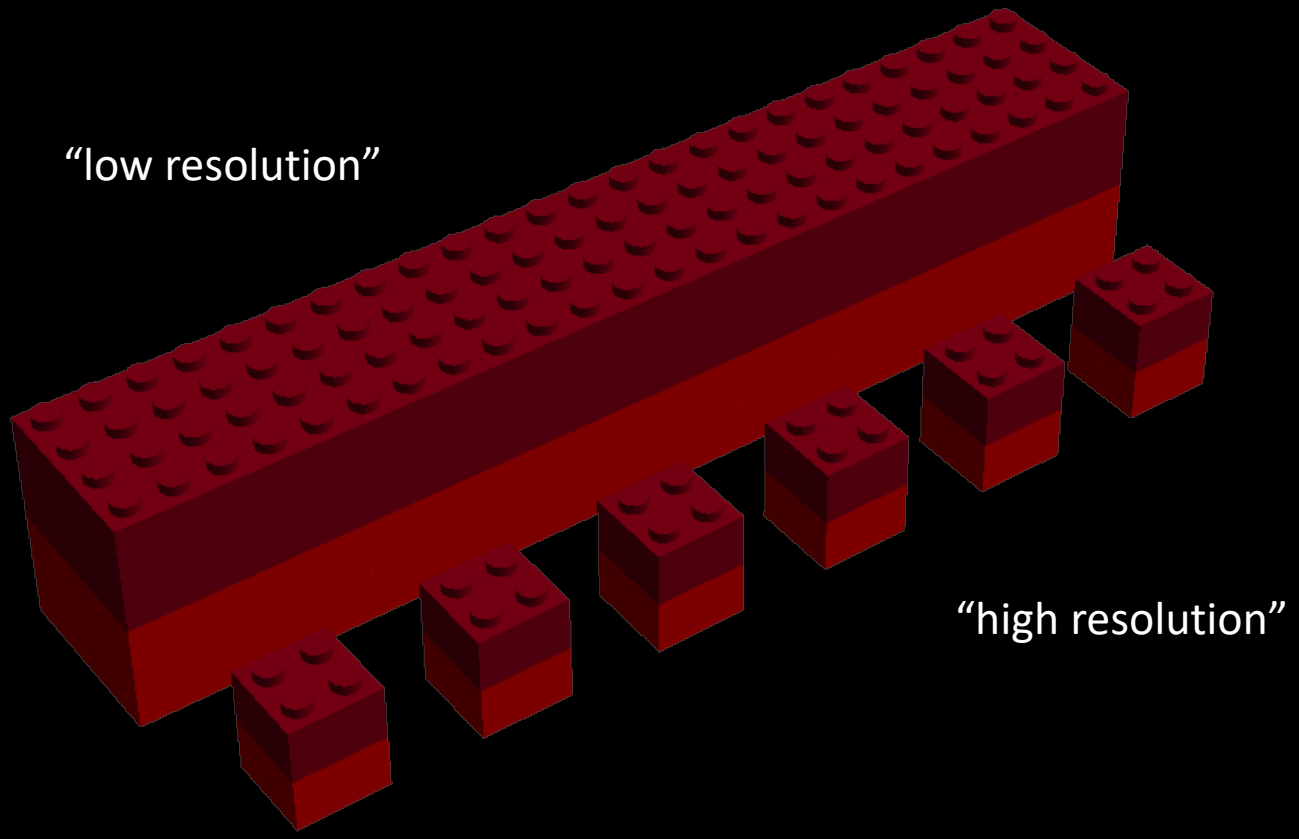
Resolution increase

- Objects appear 8 times smaller (2^3)
- Big implication for continuous structures



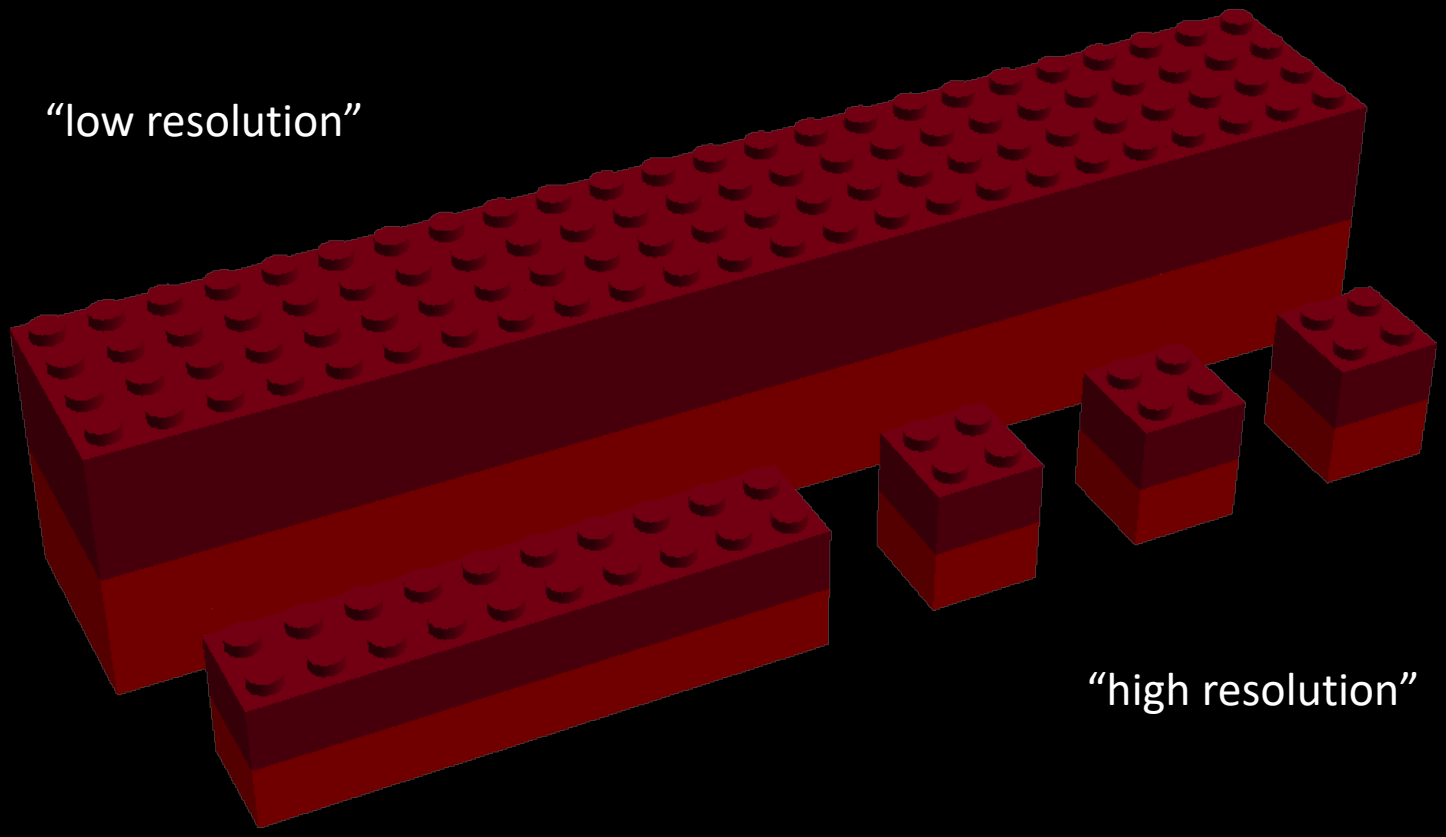
Resolution increase

- Objects appear 8 times smaller (2^3)
- Big implication for continuous structures



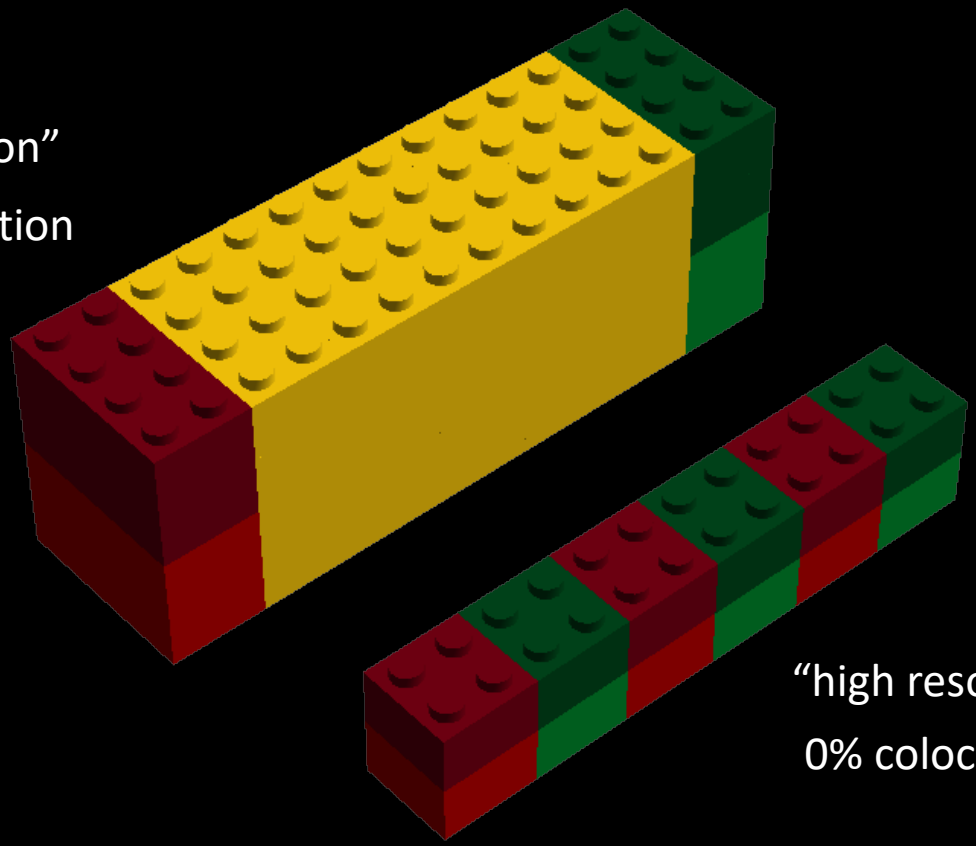
Resolution increase

- Objects appear 8 times smaller (2^3)
- Big implication for continuous structures



Resolution increase

- Big implication for continuous structures
- Big implication for colocalisation

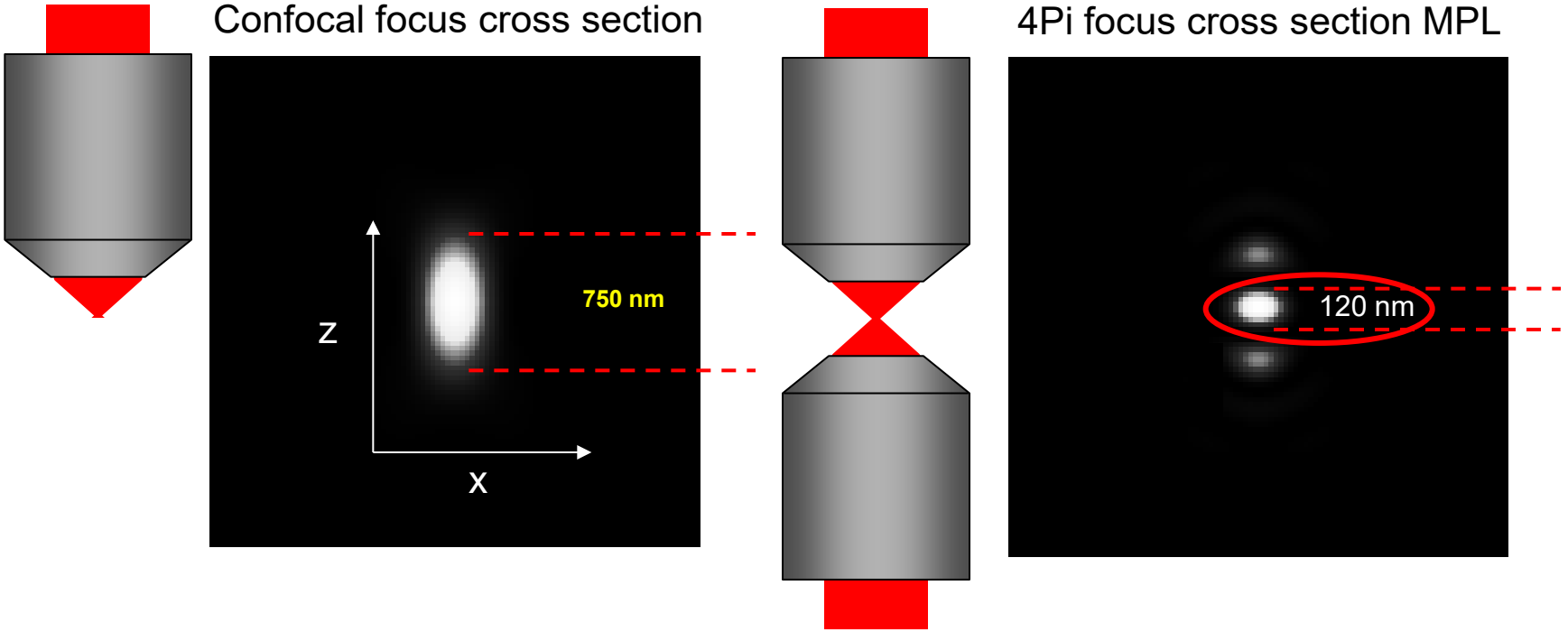


Adding one more lens

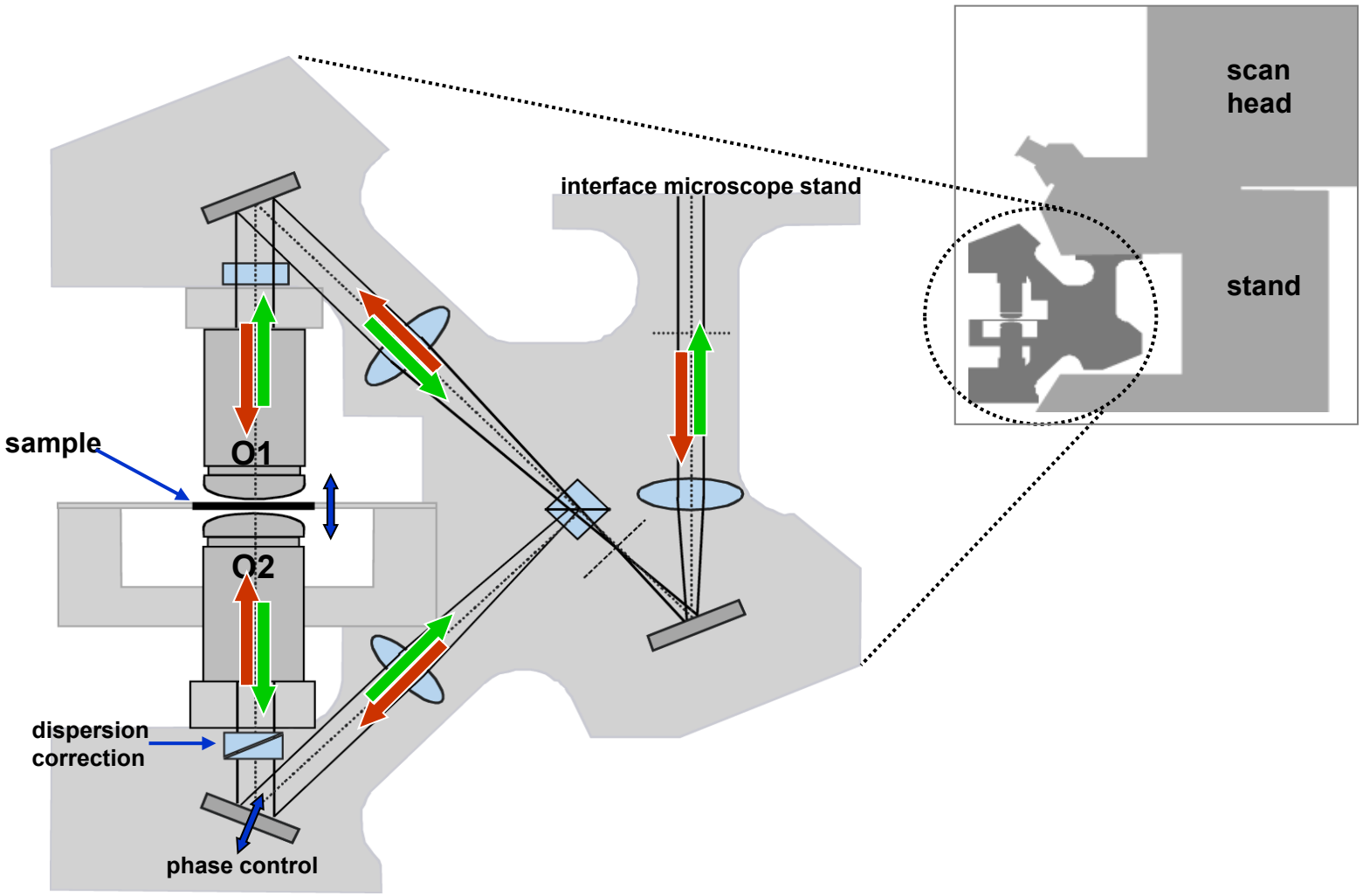
4PI MICROSCOPY

4Pi microscopy

- Confocal resolution XY ~ 200 nm, XZ ~ 600 nm
- 4Pi resolution XY ~ 200 nm, XZ ~ 120 nm
- 4Pi microscopy combines the Numerical Aperture of 2 opposing objectives through interference
- The axial resolution at the excitation wavelength of 780 nm is 120nm



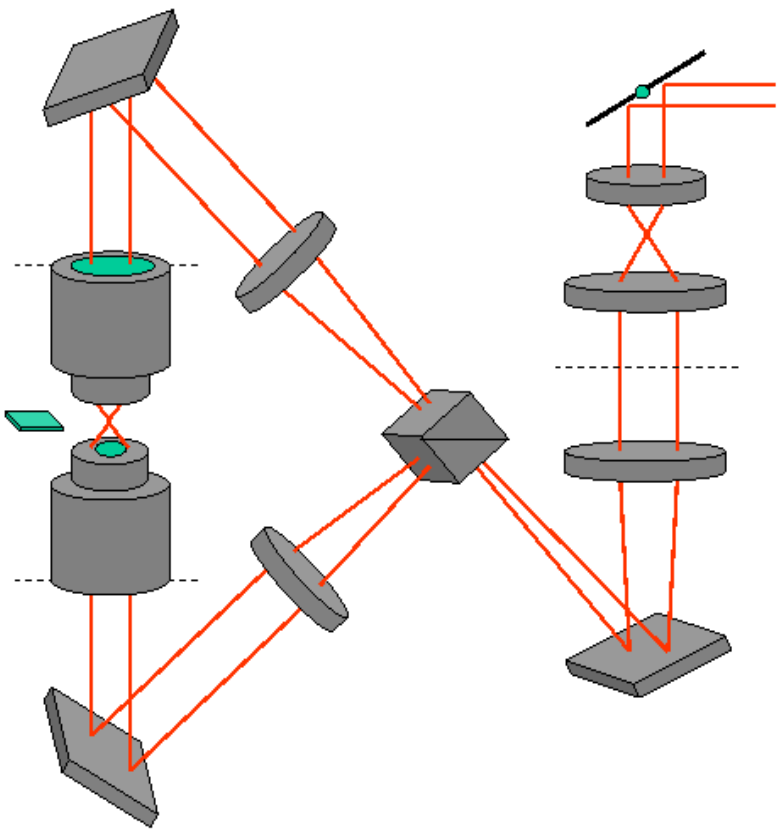
4Pi interferometer details



Compact 4Pi

Erasmus MC
Universitair Medisch Centrum Rotterdam

4Pi Microscopy Beam Scanning



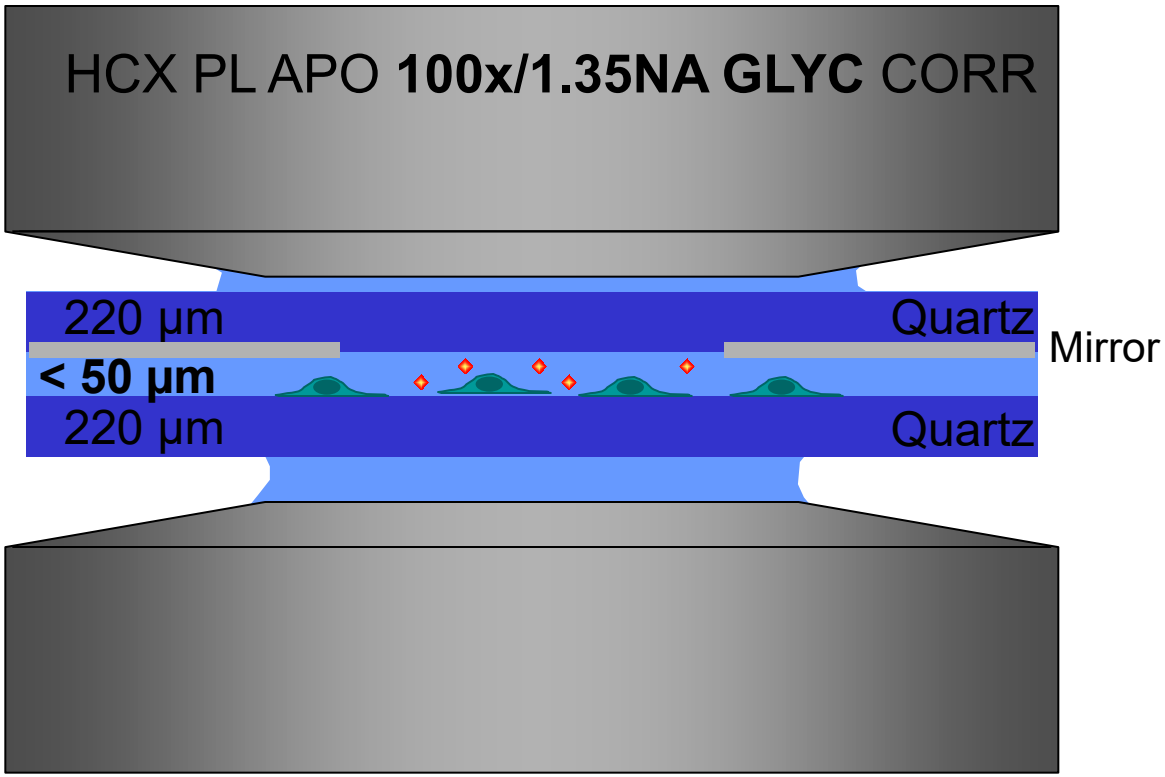
scanner

optics

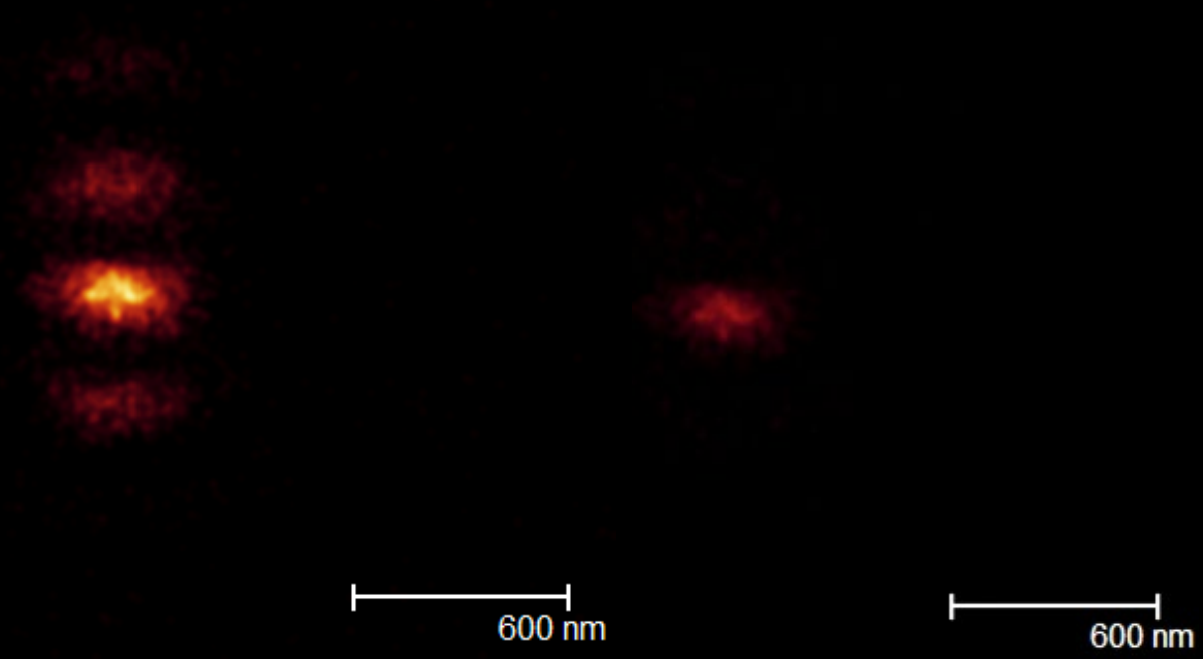
lens

sample

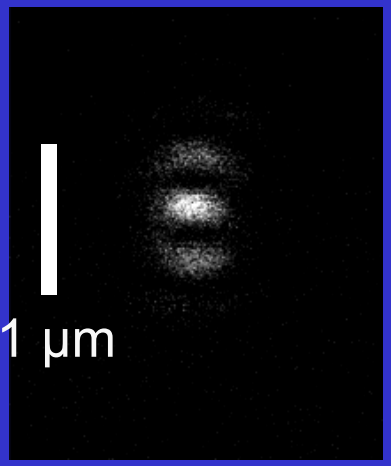
4Pi Microscopy: sample

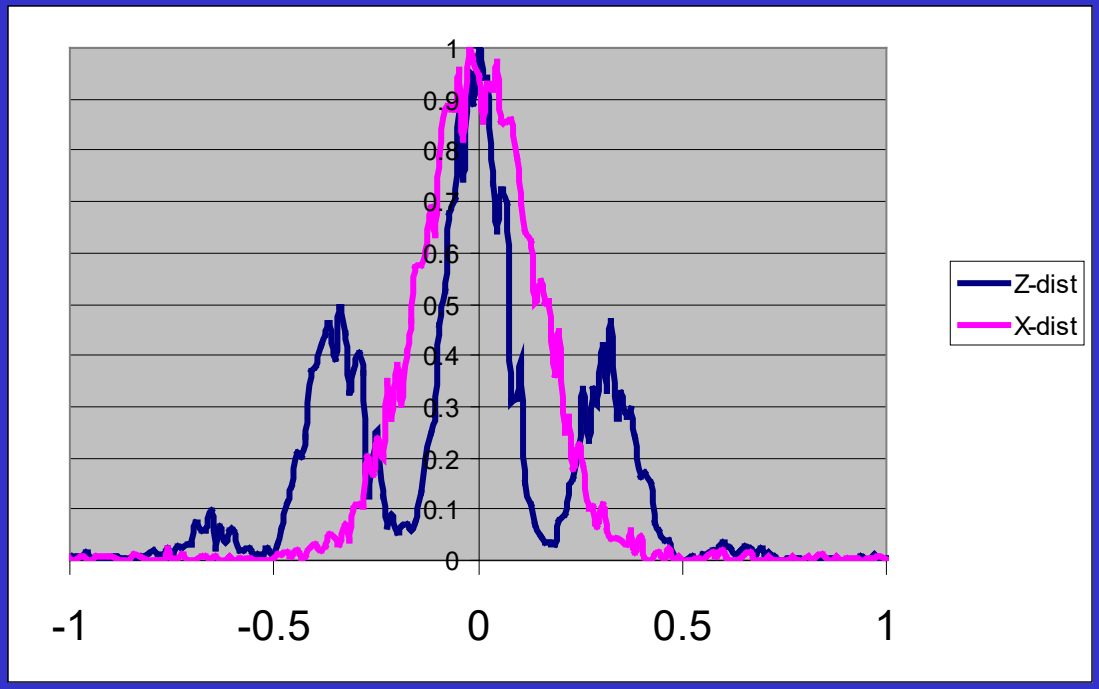


3-point-deconvolved image



100 nm bead



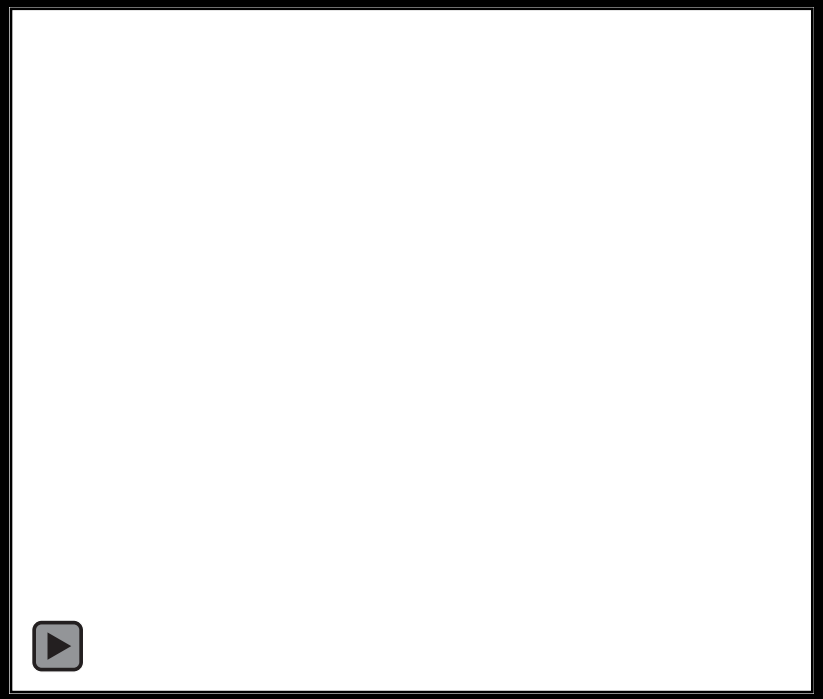


FWHM Z=130nm
FWHM X=250nm

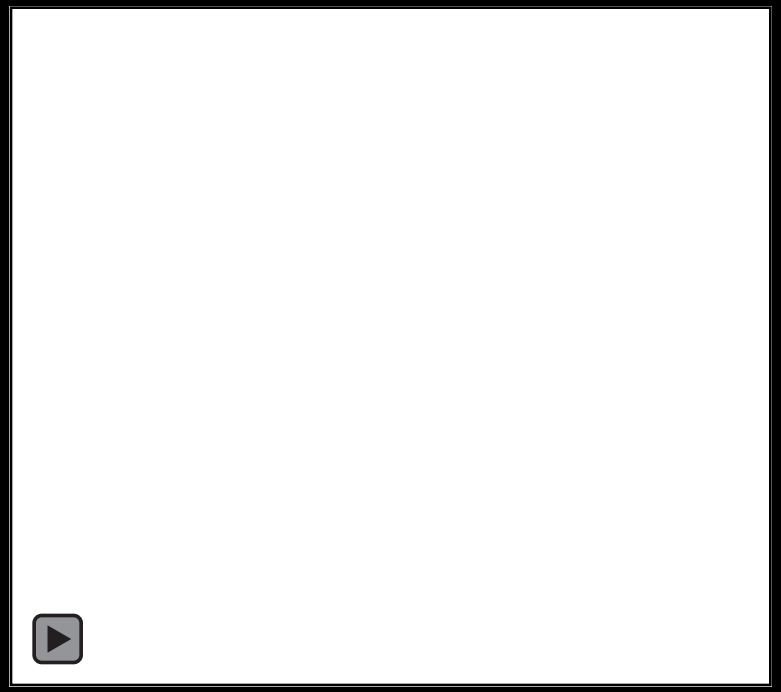
H2B-GFP in a prophase Hela cell

Rotation around x-ax

Confocal

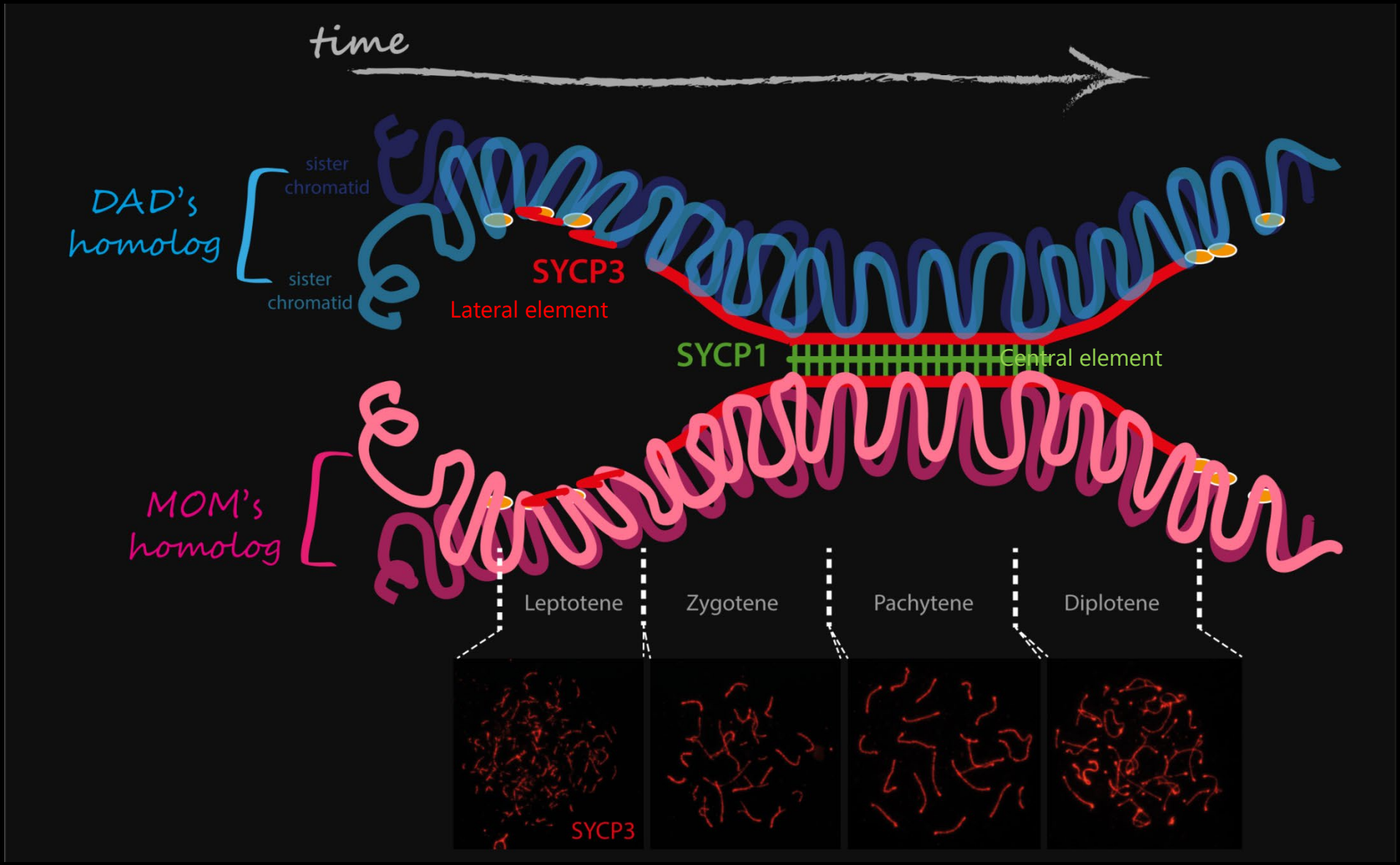


4Pi

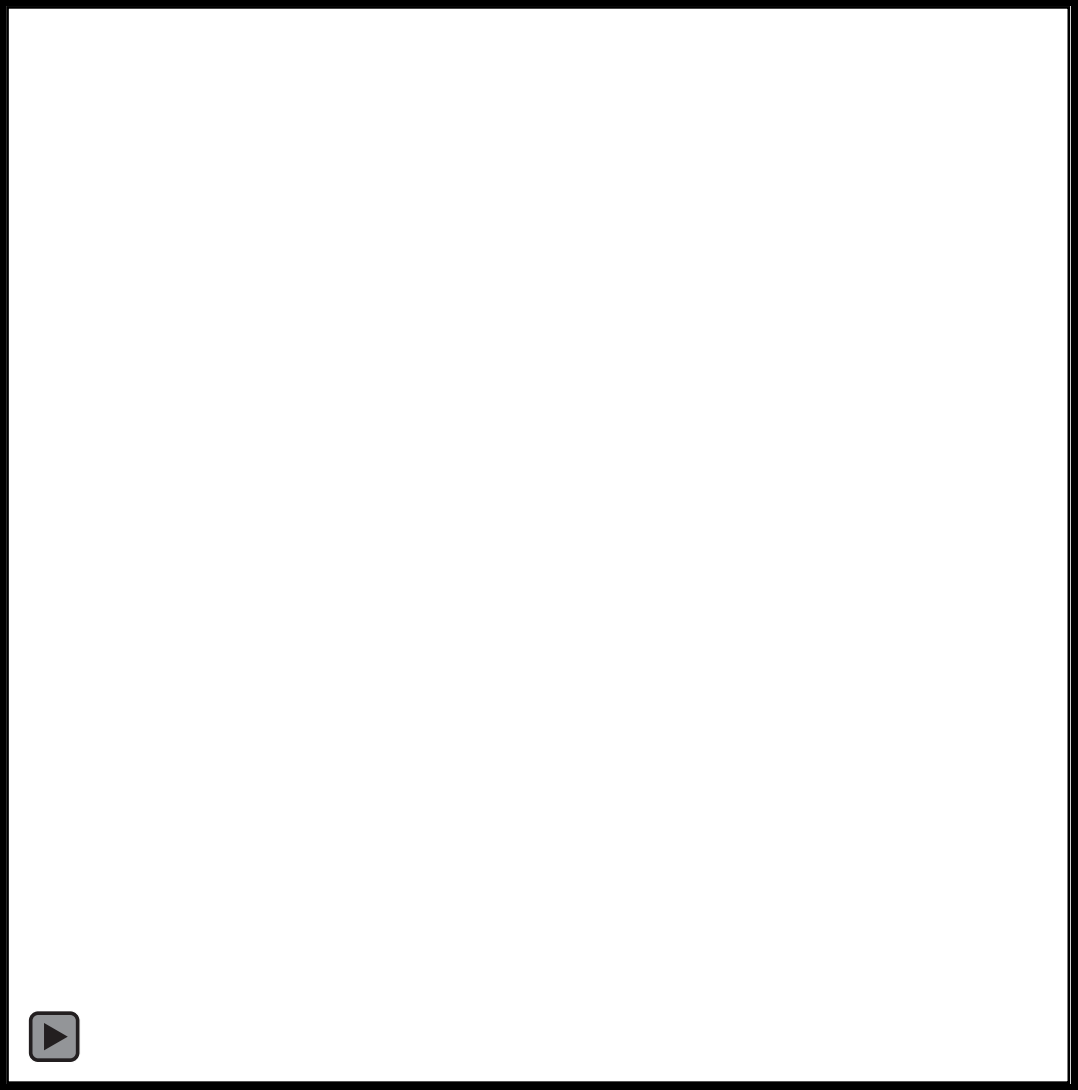


Synaptonemal complex

Meiotic prophase I

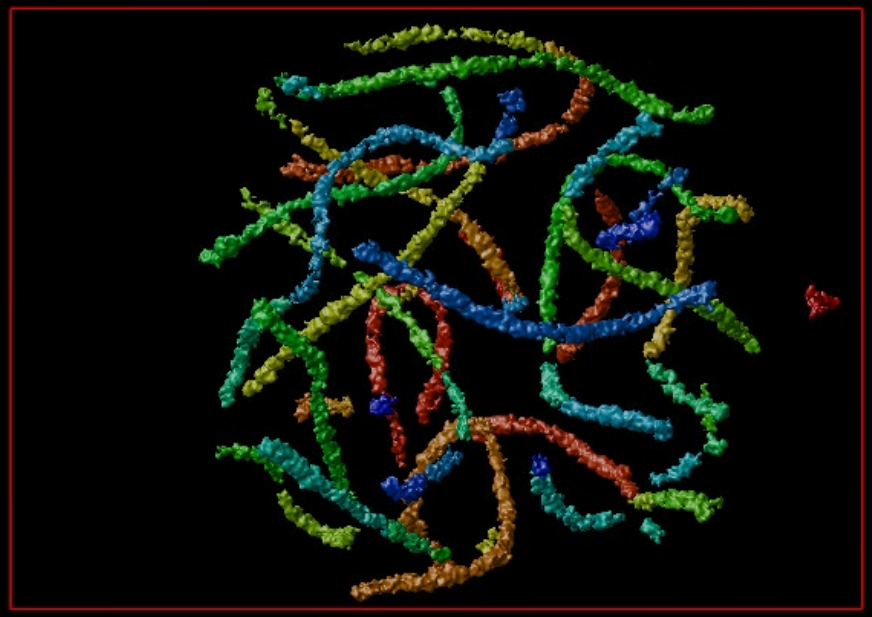


Spermatocytes SYCP3 4Pi deconvolved

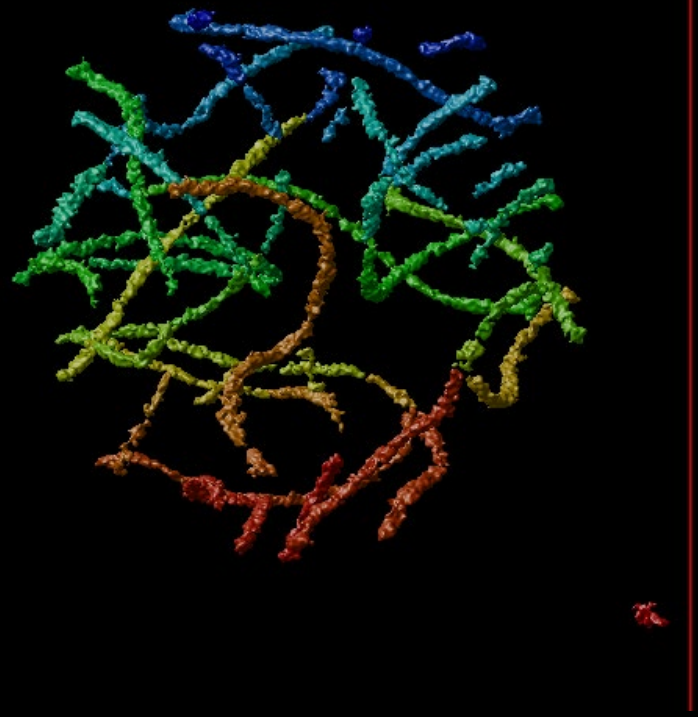


SYCP3-Alexa488 (ext MP 780) and PI (ext 561)

Spermatocytes SYCP3 4Pi



XY



XZ

Alexa488

Summary 4Pi

Increased Z resolution (<150nm)

multiple colors possible

Broad range of colors

Mainly in fixed samples

In our lab mainly used for imaging 3D nuclear structures