The Power of SIM

How structured illumination improves
(not only) resolution ...

Comparison with other
super-resolution methods (pros & cons)

3D structured illumination microscopy of a mouse cell nucleus

Micron
OXFORD
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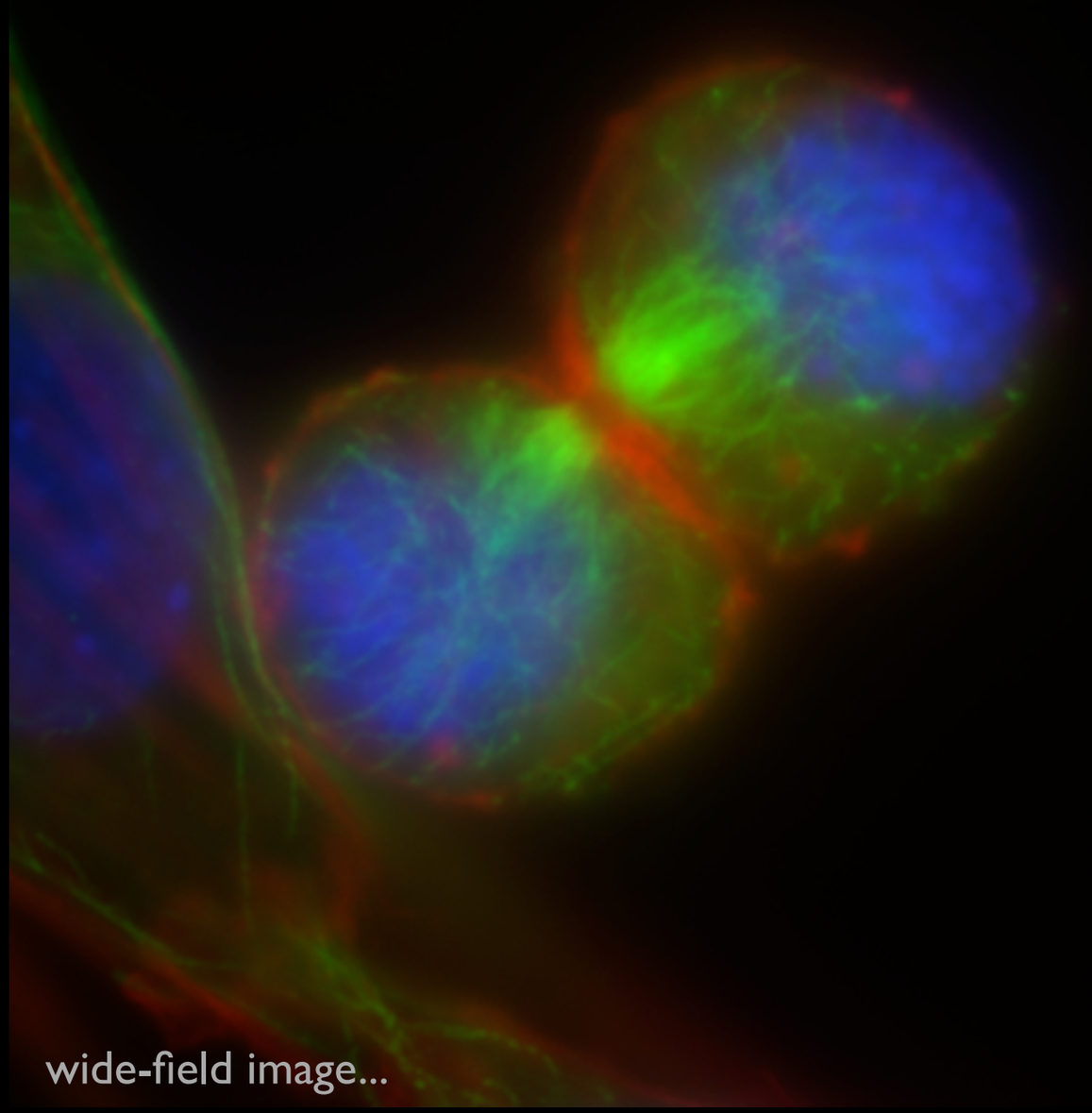
Super-resolution fluorescence microscopy



wide-field image...

- Specificity
- Sensitivity
- Non-invasive (*in situ* & *in vivo*)
- Multi-dimension (x, y, z, λ, t,...)
- Relative localisation & dynamics
- "Single cell" to "high throughput"

Spatial resolution is
diffraction limited!

Magnification alone does not give
more details!

...warmup:

"What determines the resolution of an optical microscope ?"

1	2	3
		
63x/1.25	100x/1.25	63x/1.4
£ 3 618.00	£ 550.00	£ 5 055.00

„... what objective would you take..."

„... a bit more difficult...?“

1	2	3
		
25x/1.05	40x/1.0	40x/1.1
£ 12,800	£ 3,004	£ 8,816

What's the difference in image brightness = light gathering power ?

„... what objective would you take...“

Numerical aperture determines ...

Brightness index	$F = (NA^4 / Mag^2) \times 10^4$	
Lateral resolution limit	$d_{x,y} = 0.61\ \lambda_{em} / NA$	(~200-300 nm)
Axial Resolution limit	$d_z = 2\ \lambda_{em} / NA^2$	(~500-700 nm)

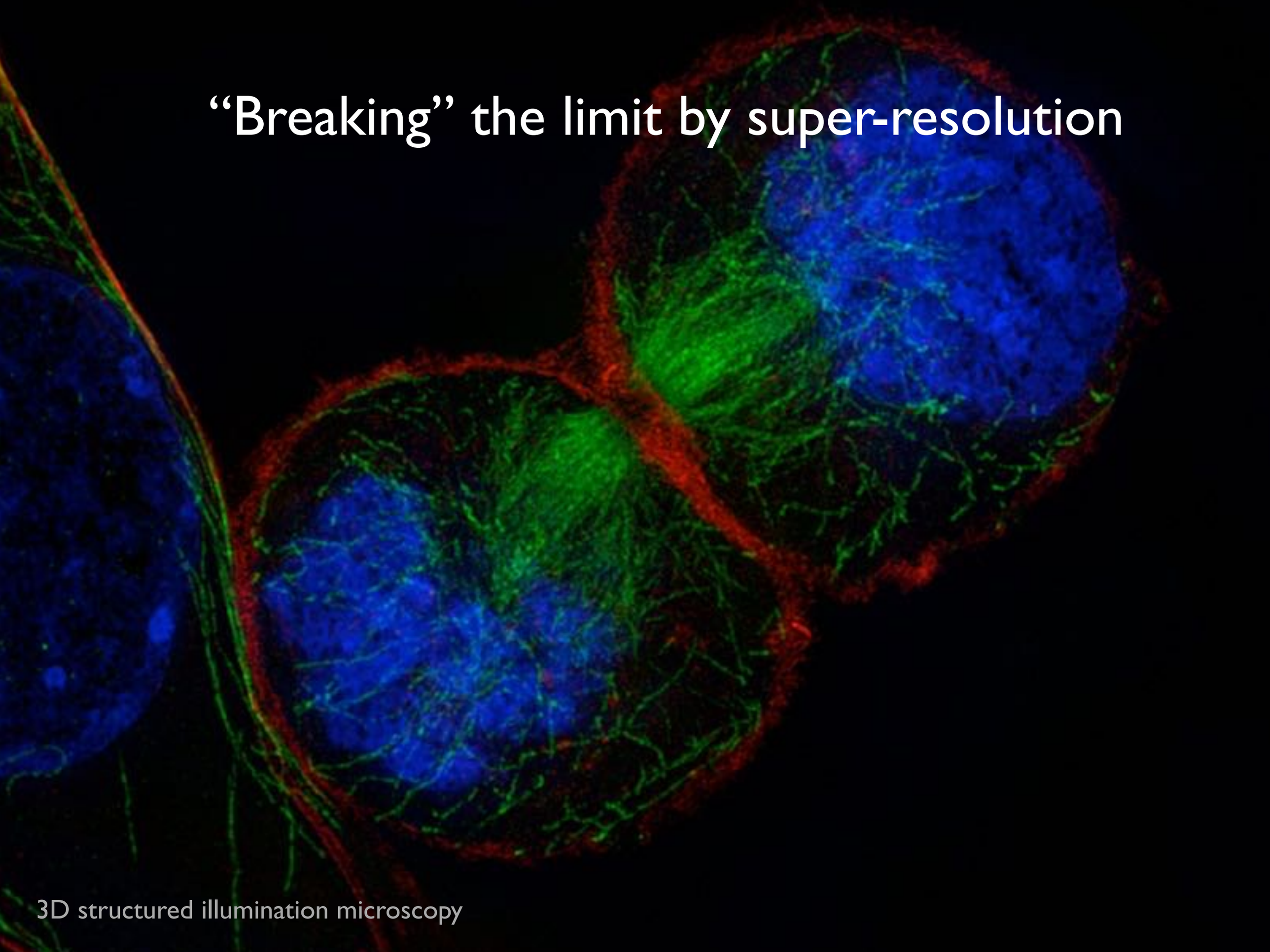
Only applies under ideal conditions! BUT ...

Spherical aberrations
Chromatic aberrations
Straylight
Out-of-focus blur
Detector noise
...

Real effective resolution is worse!
(rather >250 nm lateral and ≤ 1 µm axial)

...improved to some extent by confocal imaging or deconvolution

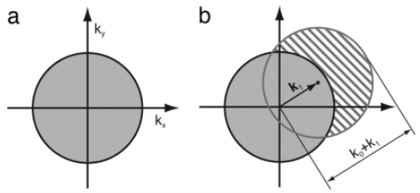
“Breaking” the limit by super-resolution



3D structured illumination microscopy

Super-resolution microscopy - three major concepts

Structured illumination

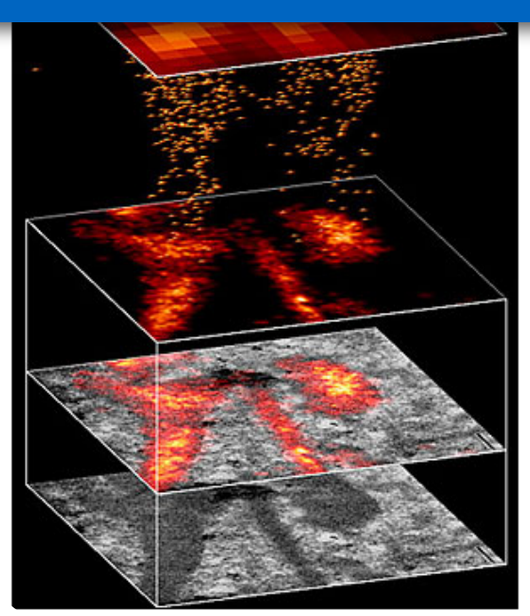


SIM-Methods:

Apotome (conventional SIM)

2D-SIM
3D-SIM (linear SIM)
TIRF-SIM

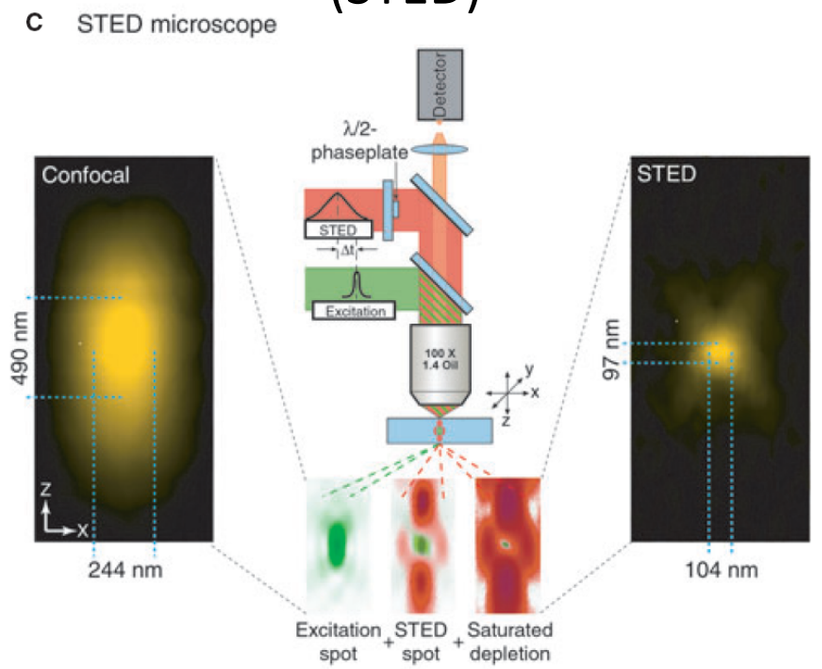
SSIM (non-linear SIM)
NL-SIM

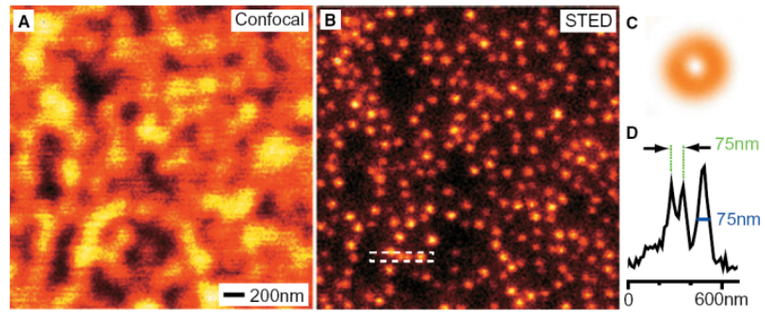


Abbe diffraction limit

$$\Delta y = \frac{\lambda}{2n\sin\alpha}$$

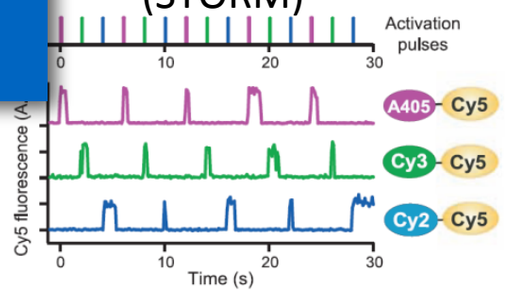
Stimulated emission depletion (STED)

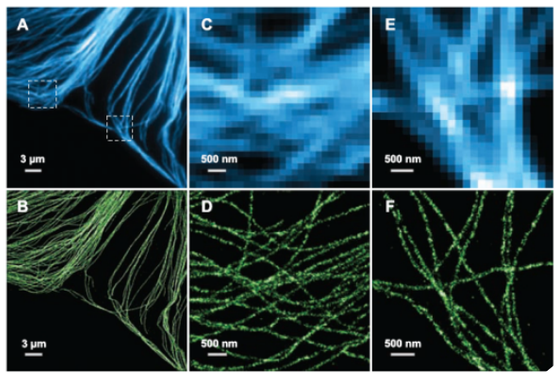




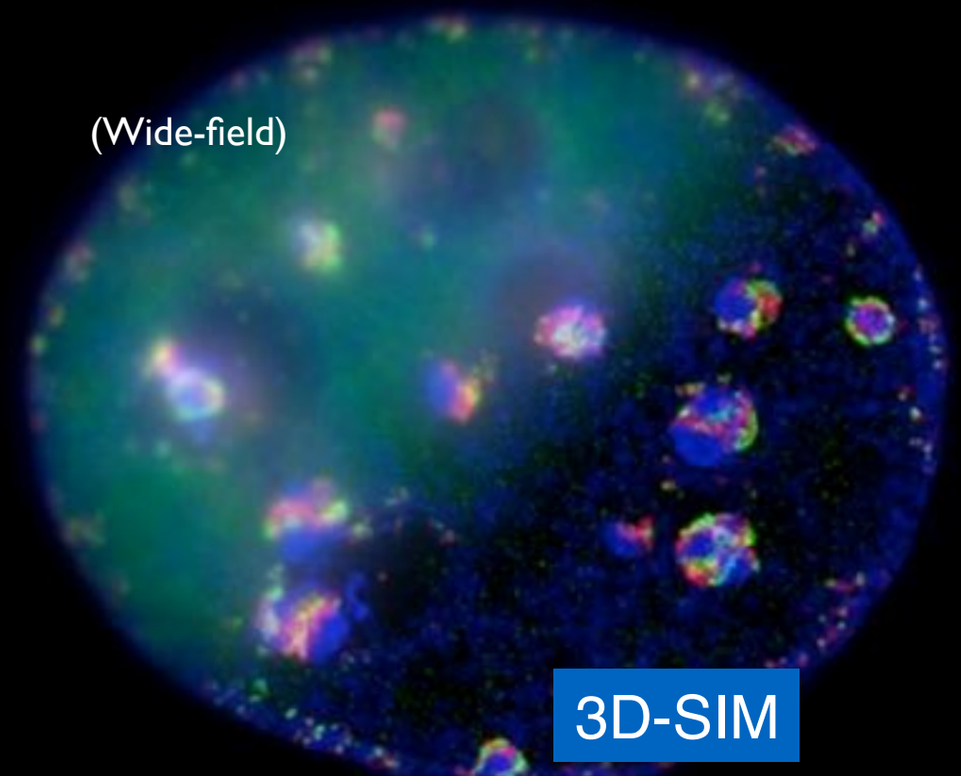
ocalisation

Stochastic optical
onstruction microscopy
(STORM)

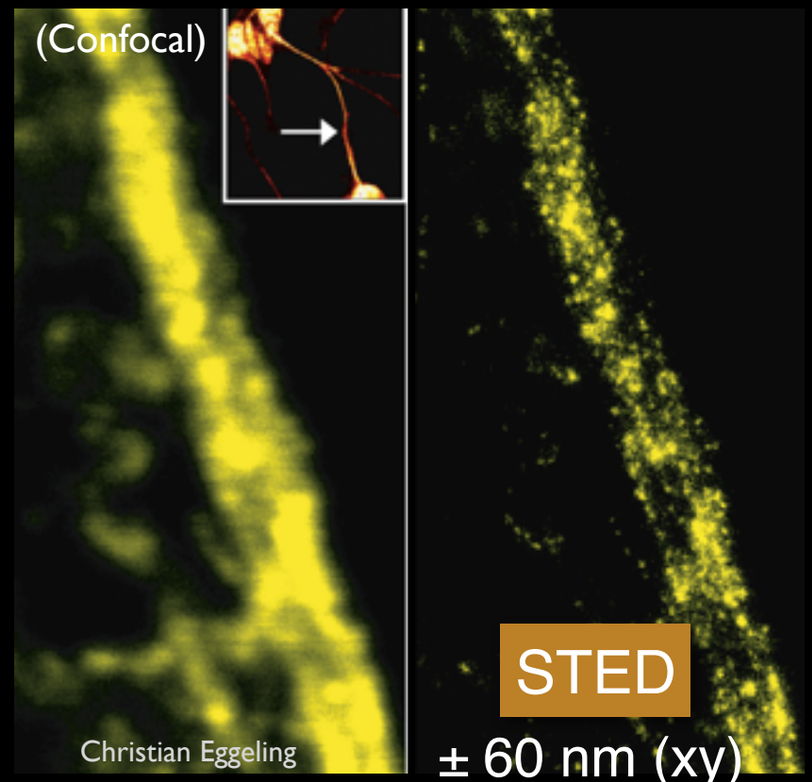




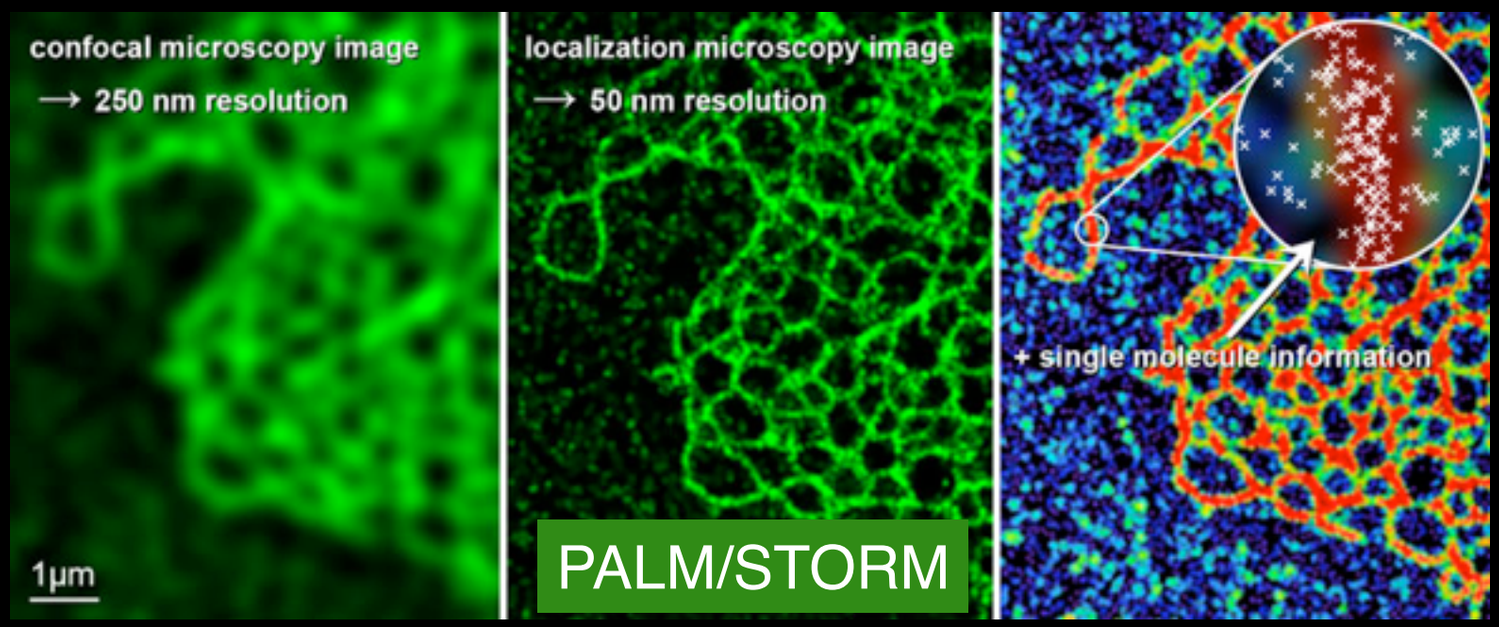
Super-resolution techniques to surpass the diffraction limit



100 nm (xy), 300 nm (z)



± 60 nm (xy)

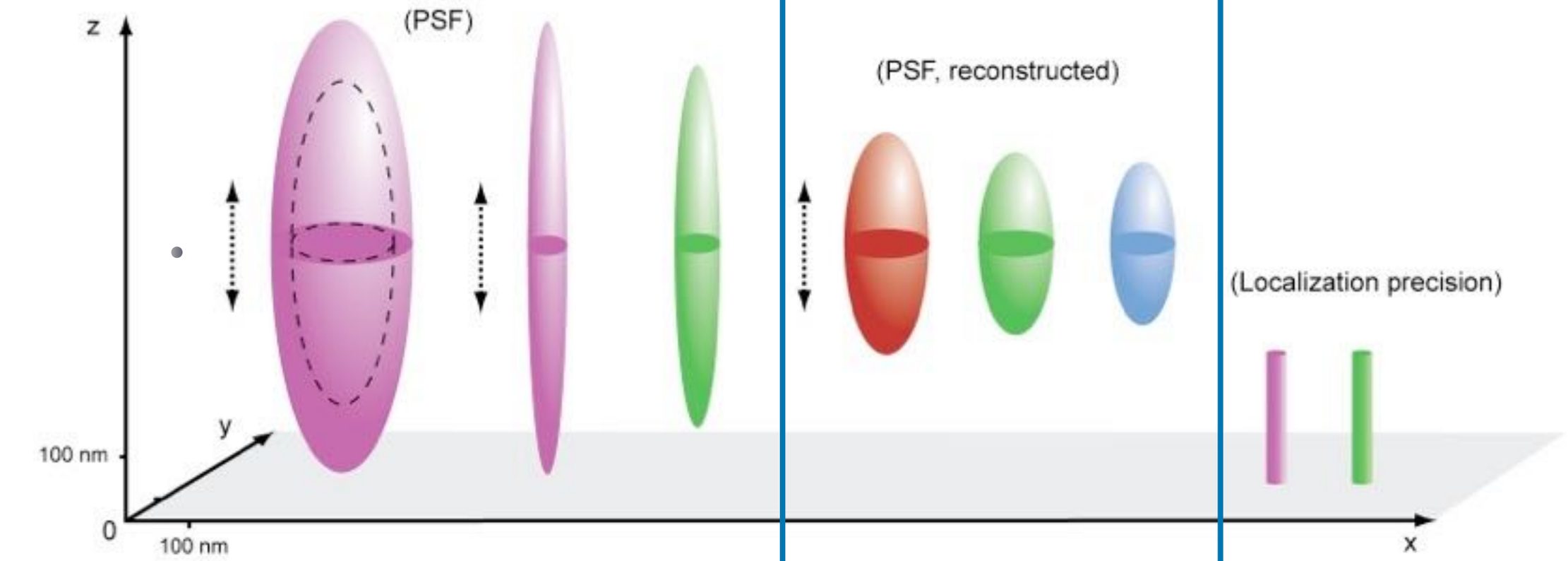


Rainer Kaufmann

± 20 nm (xy localisation precision); ± 50 nm (structural resolution)

Resolving power of commercial super-resolution systems

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λem [nm]	460-670	670	520	620	520	460	670	520
~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30	
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)	
~$V_{x,y,z}$ [*10^{-3} µm³]	10-23	1.3	1.5	3.0	1.8	1.3	0.1	



3D-SIM resolves ~8-fold smaller volumes than conventional microscopy

Schermelleh, Heintzmann, Leonhardt, *JCB*, 2010

8D

EDO COMPETITION / BENTLEY

Edo Speed GT

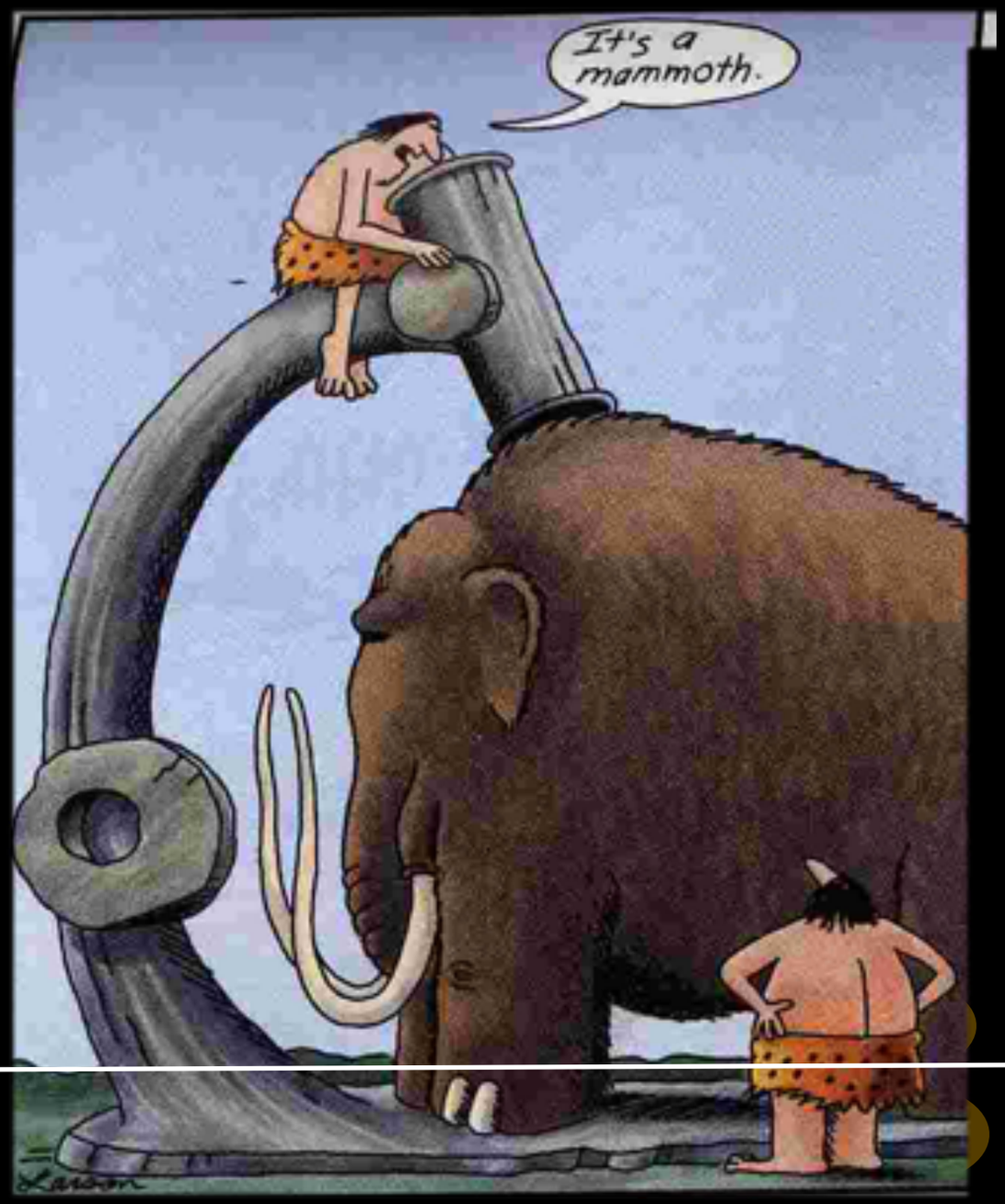
Unverb. Preisempfehlung: Auf Anfrage

Hubraum:	5 998 ccm
Leistung:	500 kW / 680 PS
Geschwindigkeit:	342 km/h
0-100 km/h:	4,2 sec
Gewicht:	2 350 kg

a) MC12 XX
b) GT2 RS
c) GT

Not only resolution matters, ...

What could this be?



3D information (z-resolution, optical sectioning, imaging depth)

Not only resolution matters,

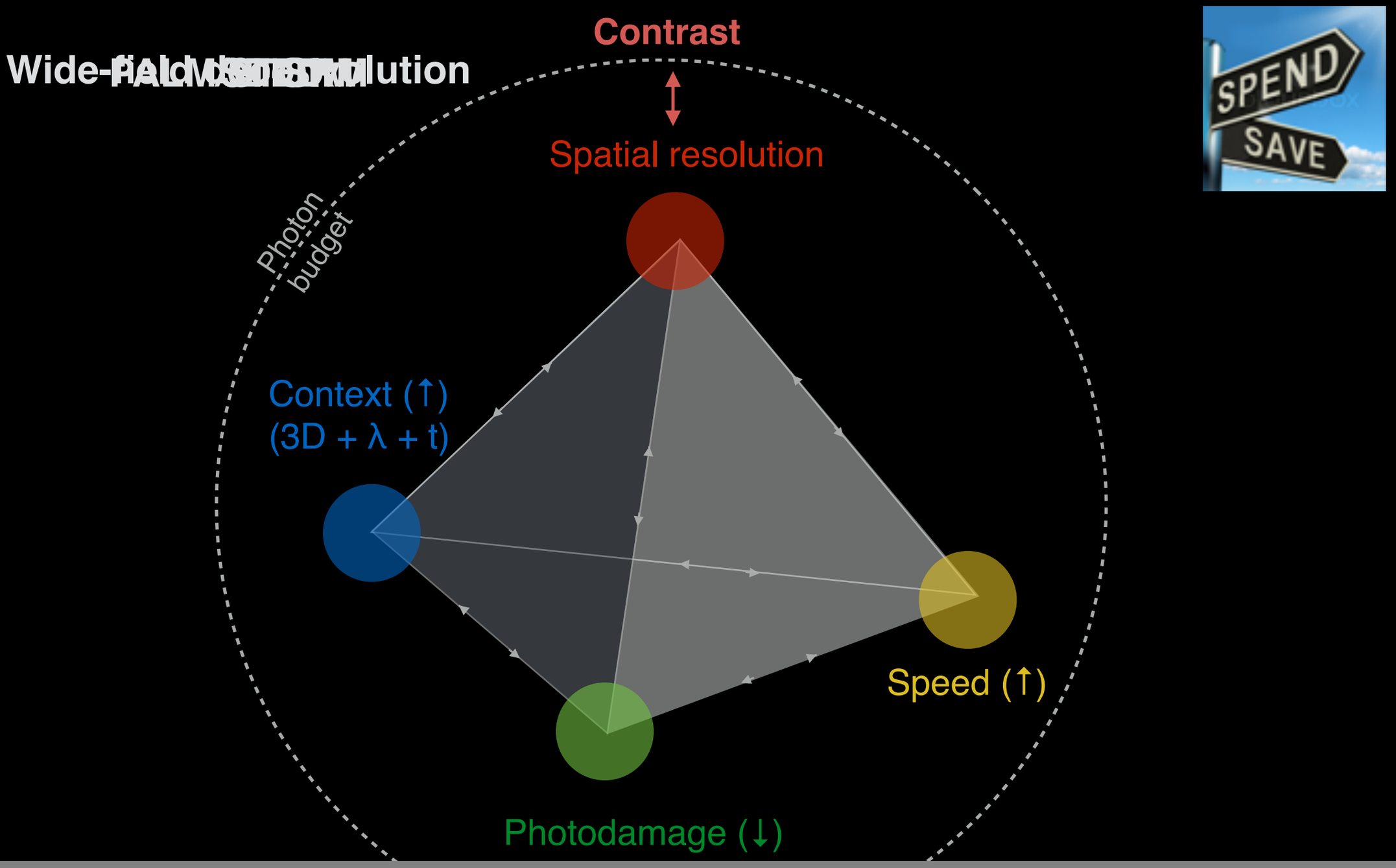
Multicolour & 3D information

Prague National Museum

To understand the game you need to see the player move

Temporal information (live cell imaging)

Trade-offs in super-resolution microscopy



The optimal technique is determined by demands of the application!
Spatial resolution is only part of the equation!
Photon budget and **contrast** are the limiting factors in practice!

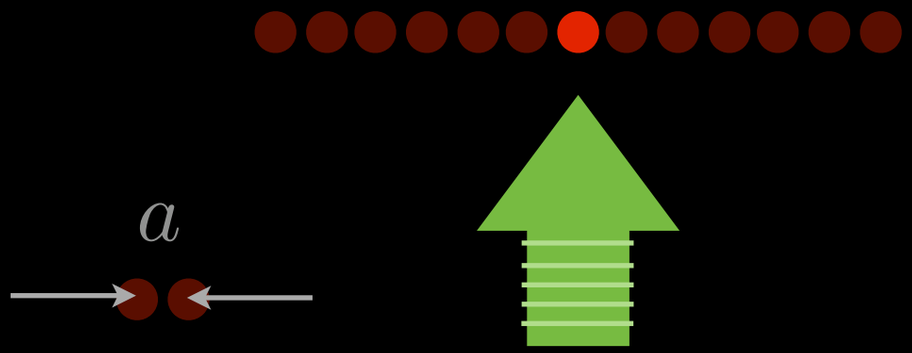
Contrast is the limit!!!

How to improve resolution with structured illumination?

The basic principle: Abbe‘s view

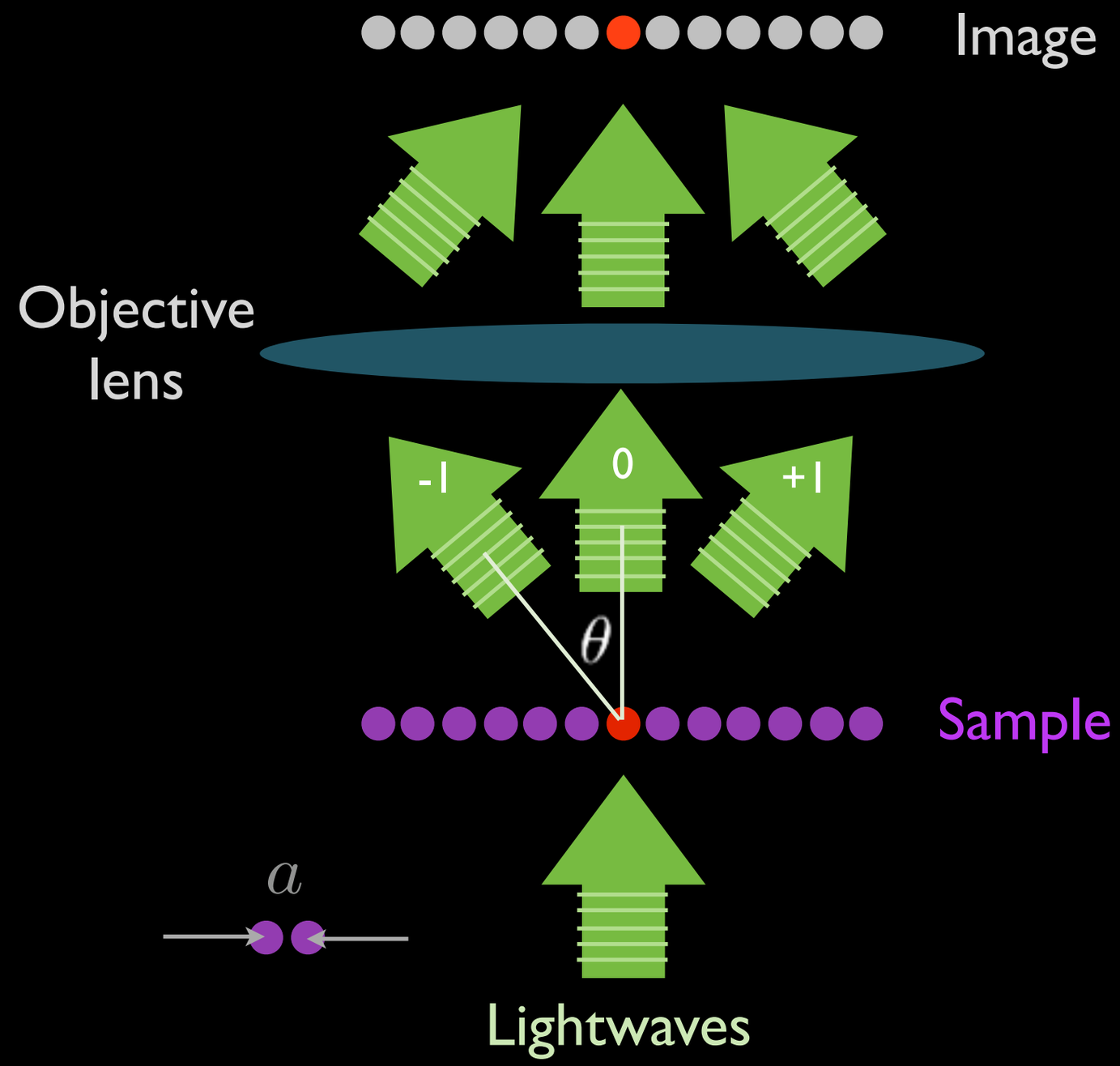
Sample = Structure

→ Periodicity

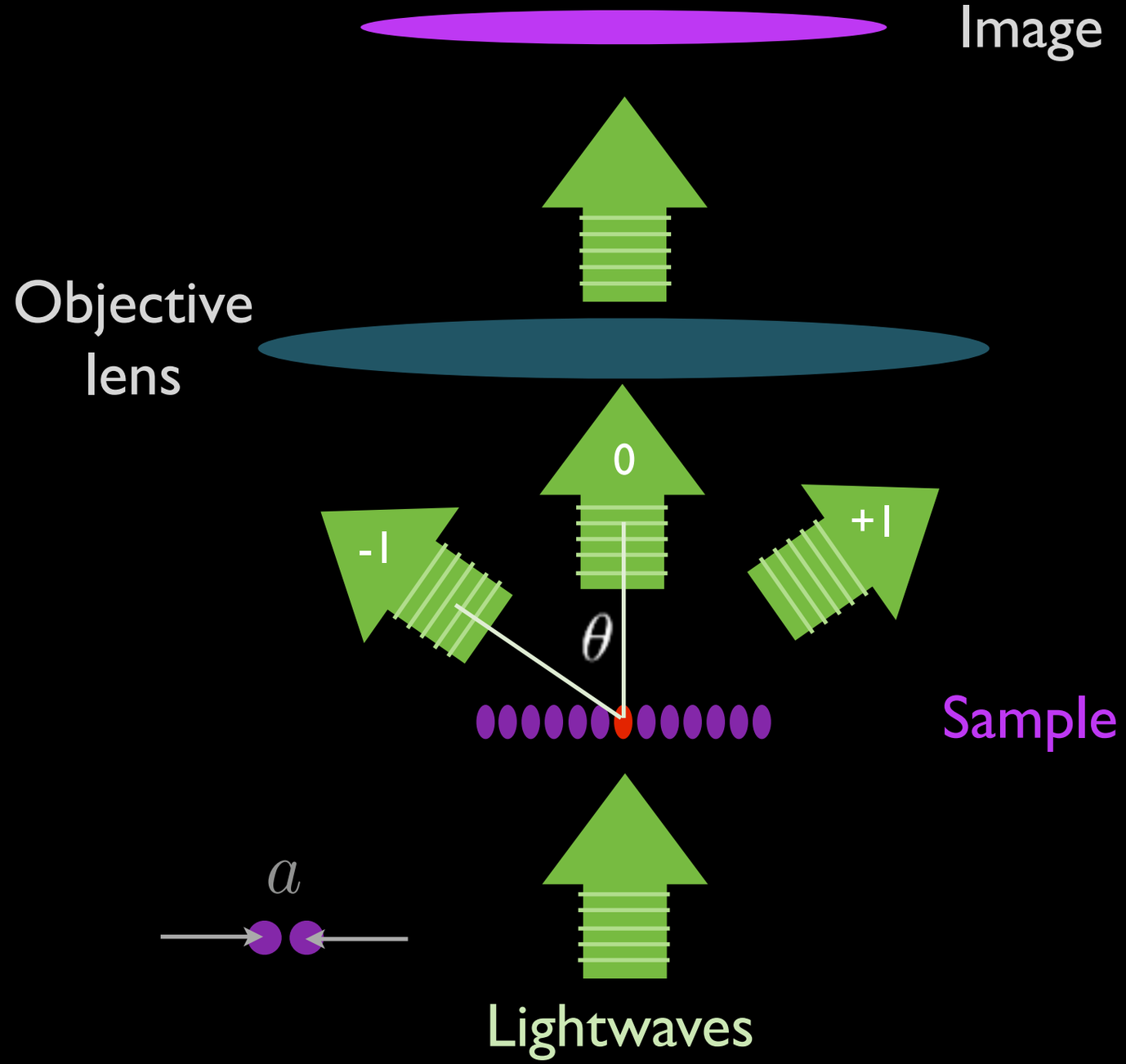


http://de.wikipedia.org/wiki/Ernst_Abbe

The basic principle: Abbe‘s view



The basic principle: Abbe's view

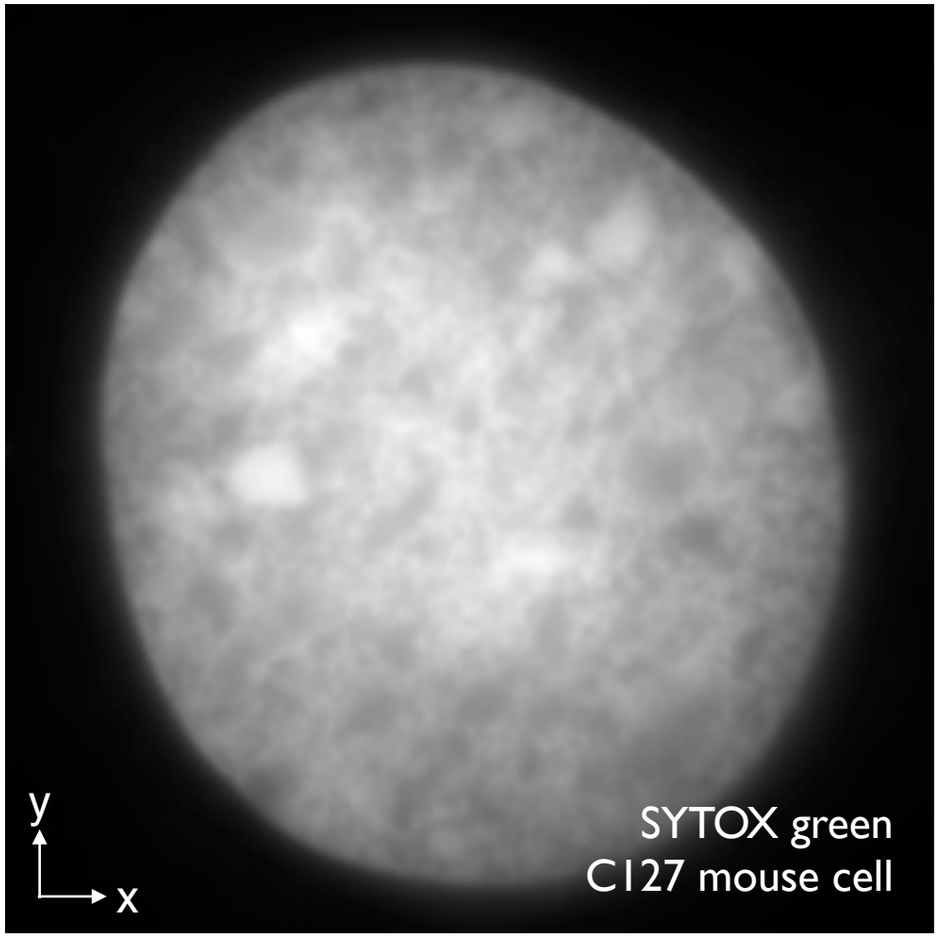


$$r = \frac{\lambda}{2n \sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$

highest frequencies
(biggest α)
→
smallest structures

Frequency support in wide-field microscopy

Image = real space (xy)

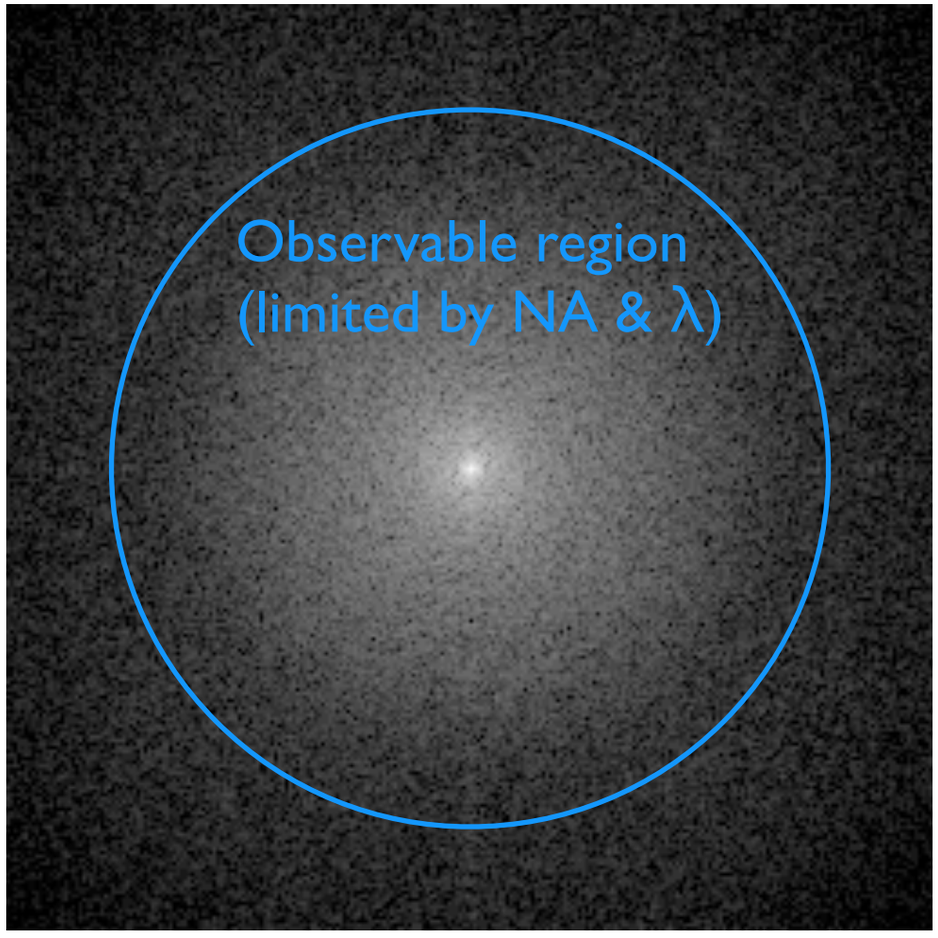


Fourier Transform

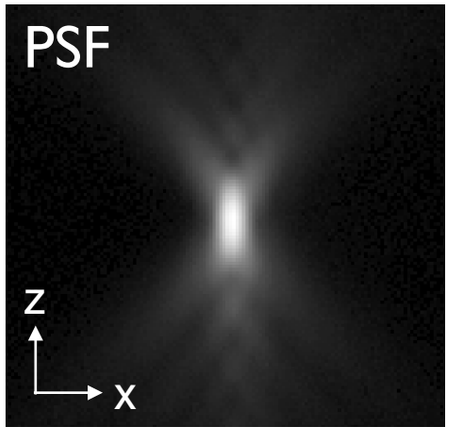
(inverse FT)

Frequency space (k_x, k_y)

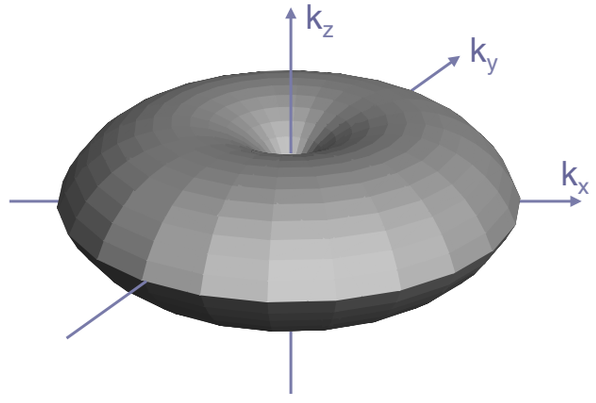
(a.k.a. Fourier space, reciprocal space)



"Real object" ⊗



Full frequency range ×



SIM principle: Moiré interference

Schermelleh, Carlton et al. (2008), *Science* **320**

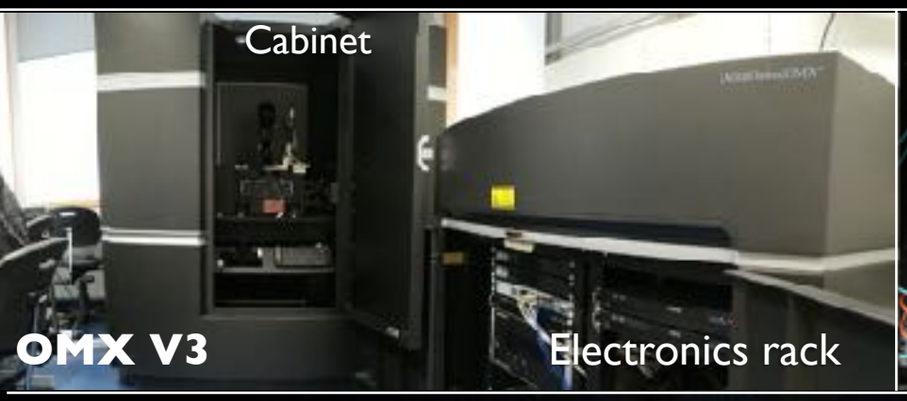
Fourier transform of the measured image

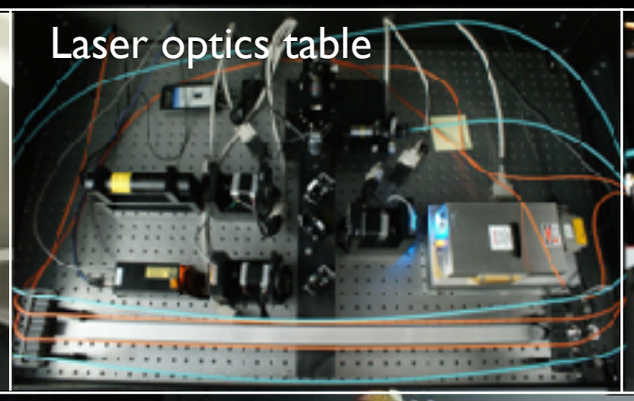
unknown structure

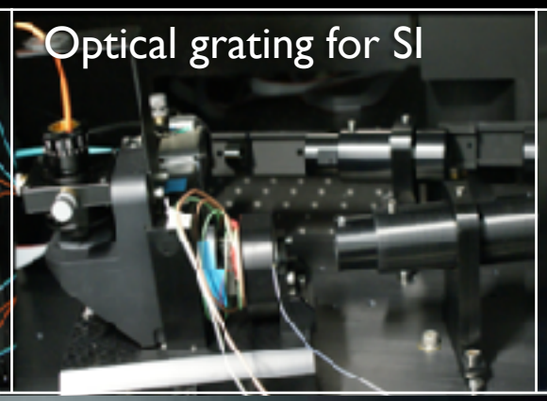
$$F\{f \times g\} = F\{f\} \otimes F\{g\} \longrightarrow F\{f\} = F\{f \times g\} \otimes^{-1} F\{g\}$$

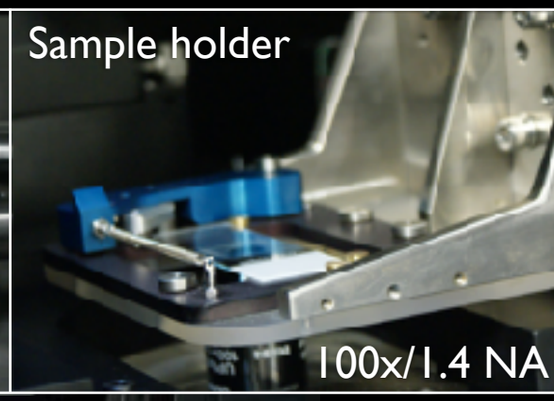
known illumination function

OMX 3D-SIM microscope system





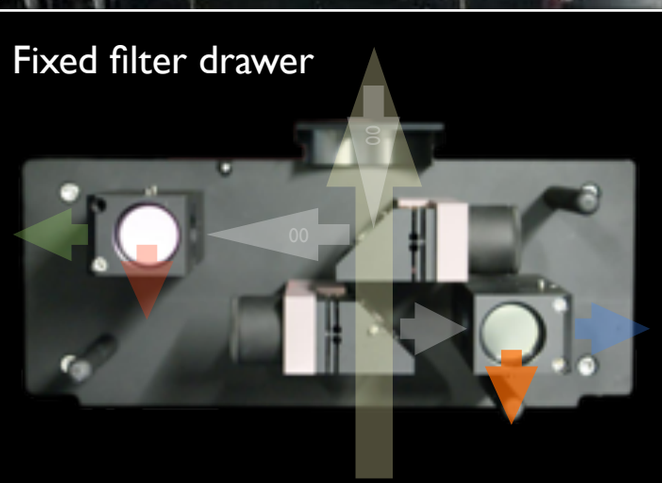




Max. mech. stability
Highest sensitivity

High-sensitive
EMCCD camera
(3x)

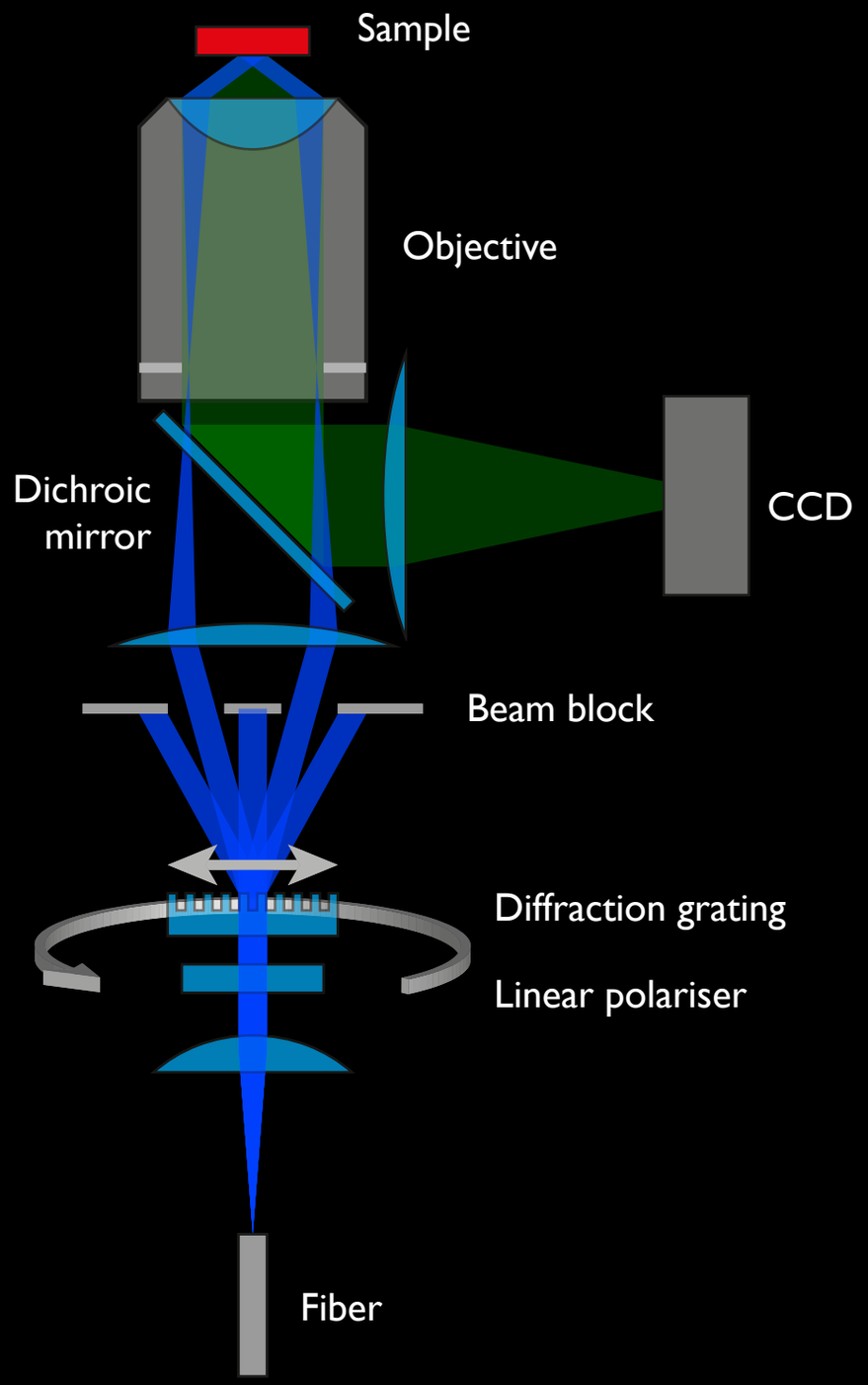
Fixed filter drawer



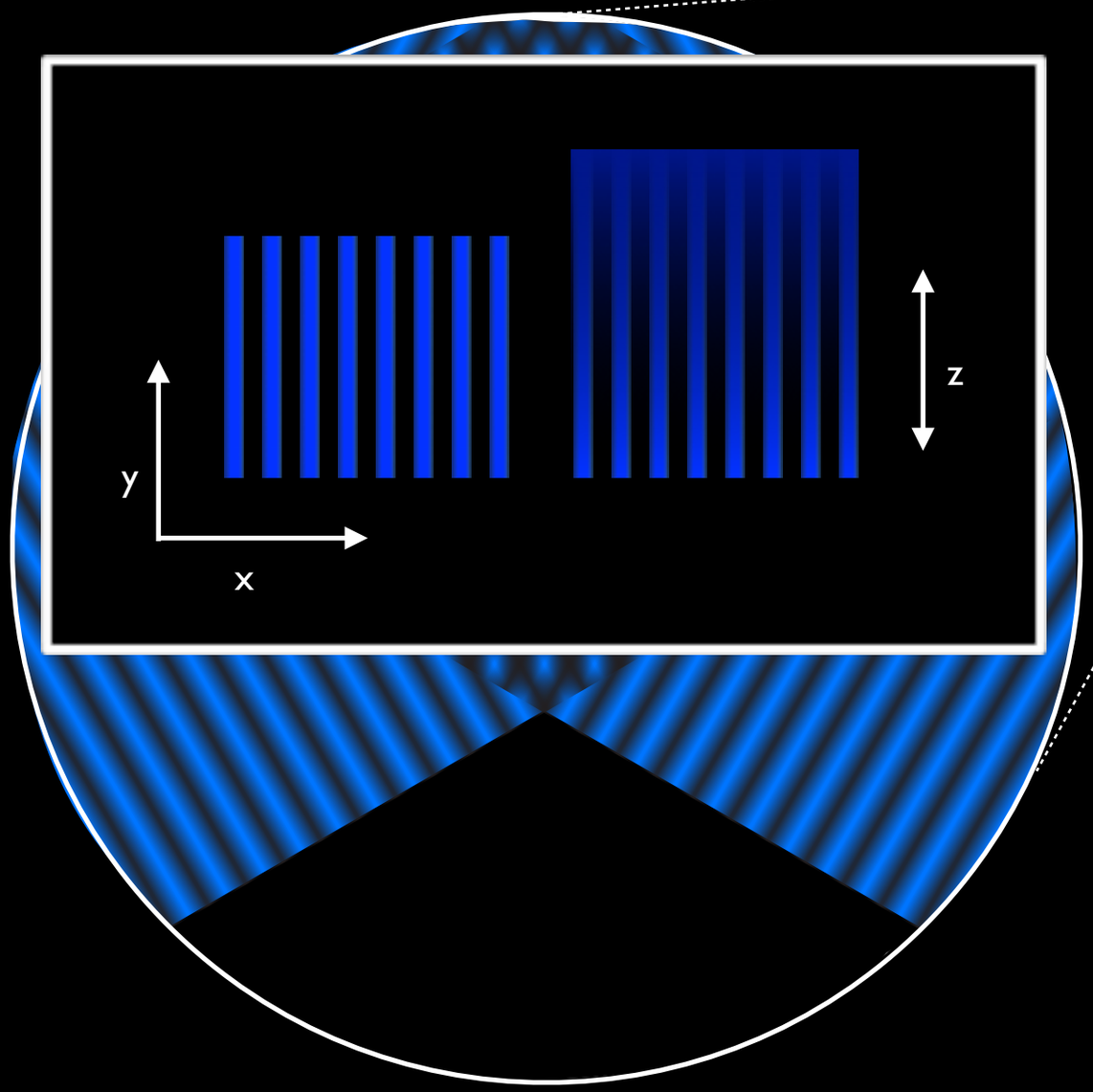
Mess

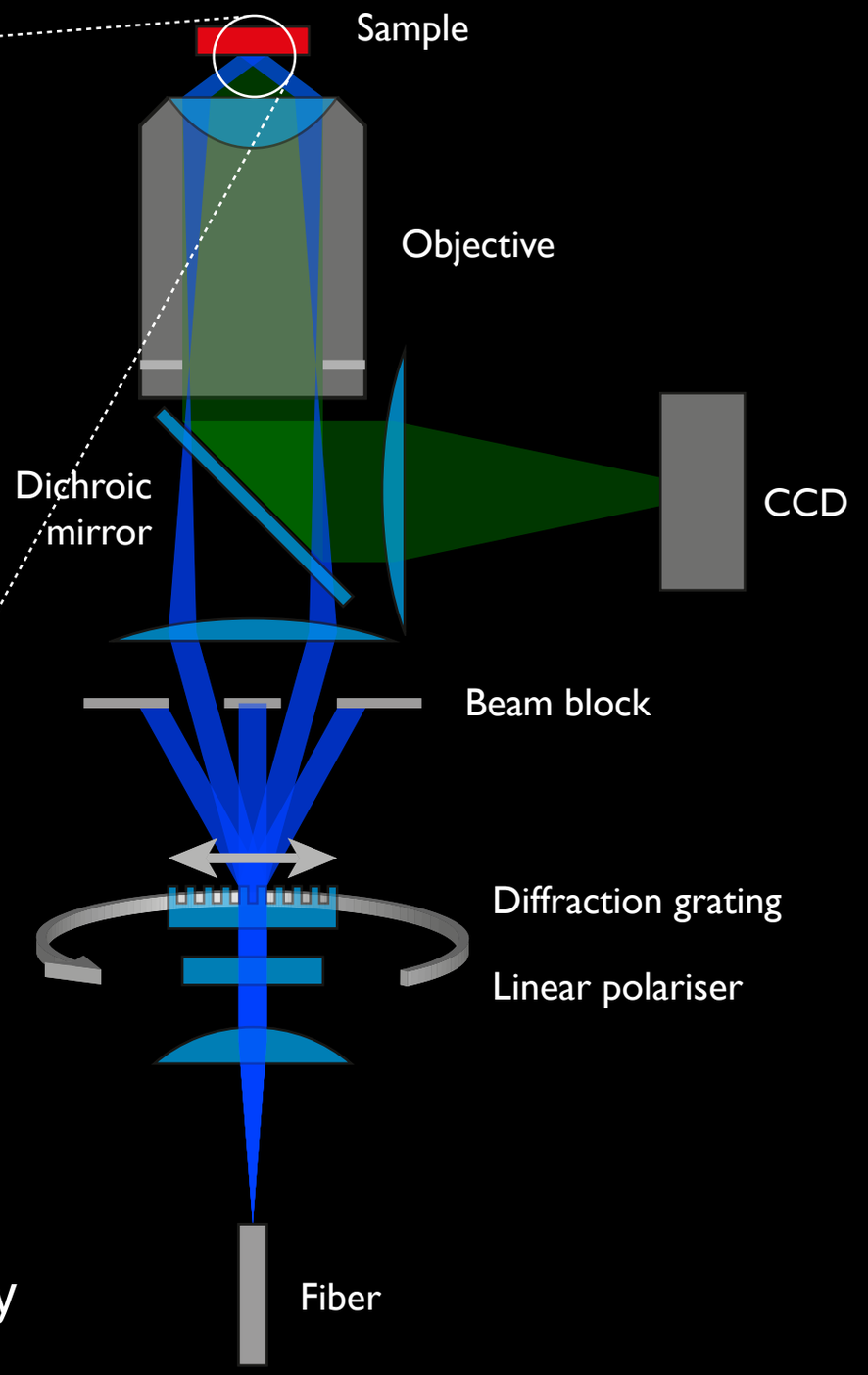
OMX V2

2D-SIM: microscope design



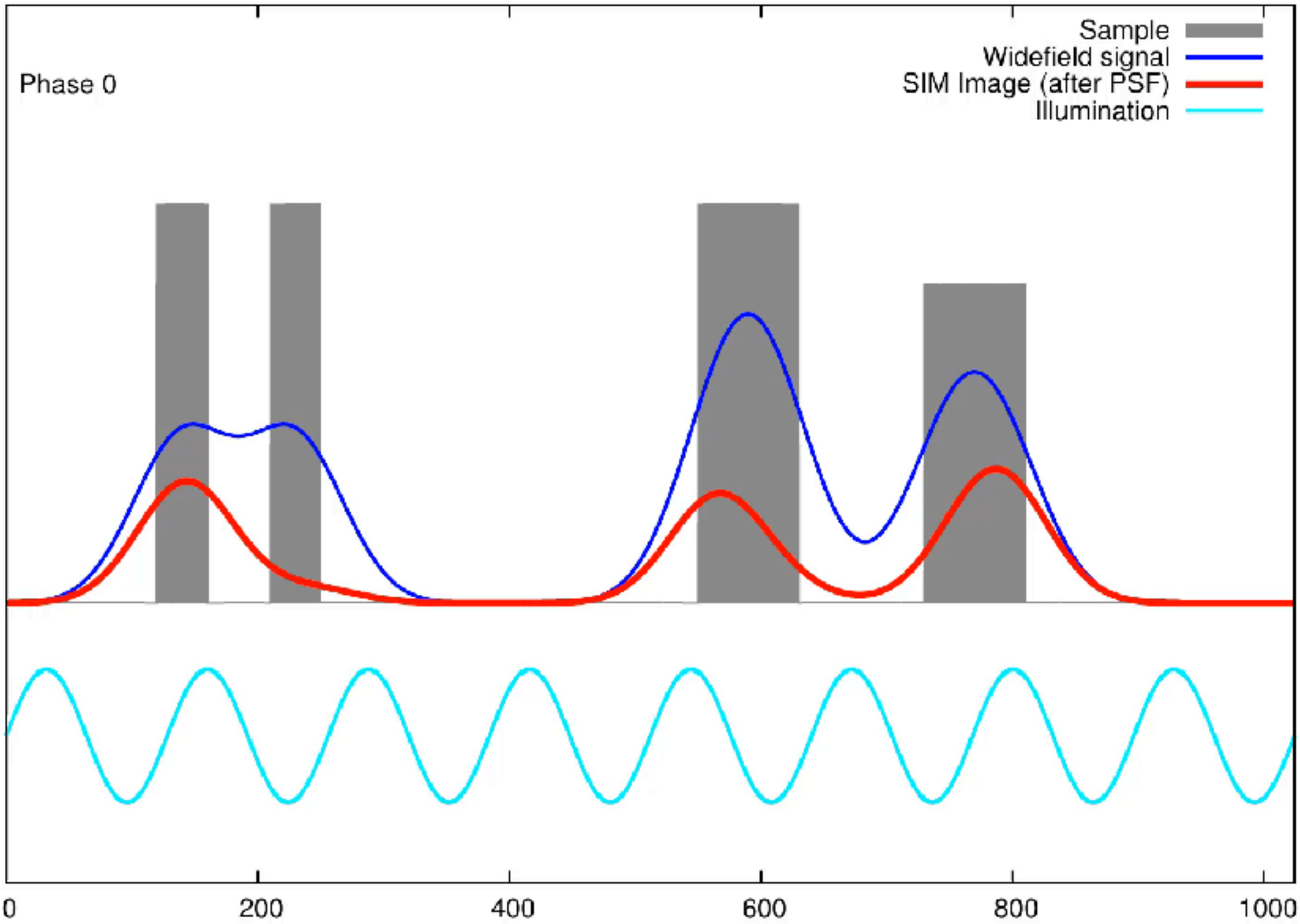
2D-SIM: microscope design





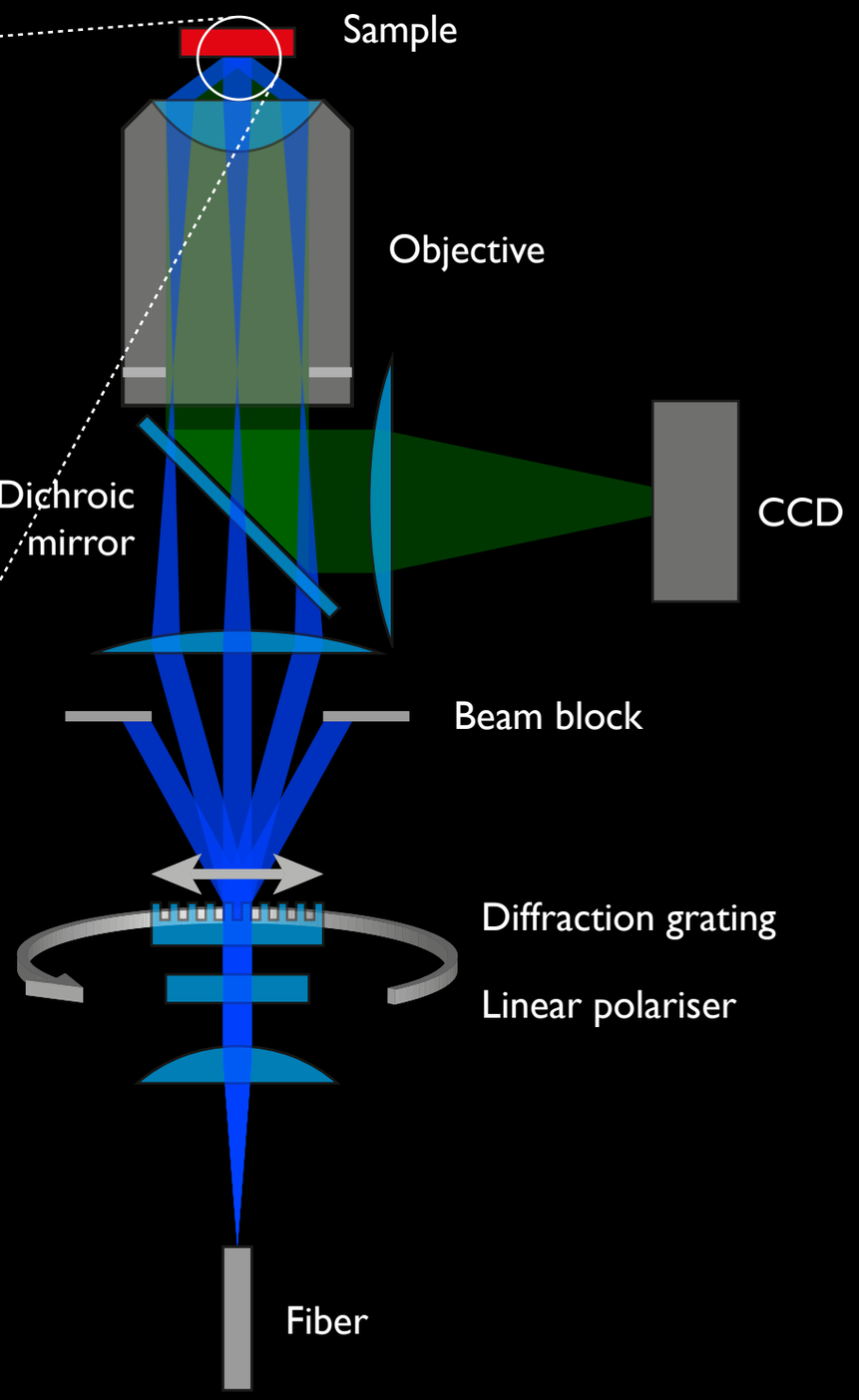
2-beam interference => modulation (stripes) in xy

SIM principle: Moiré interference

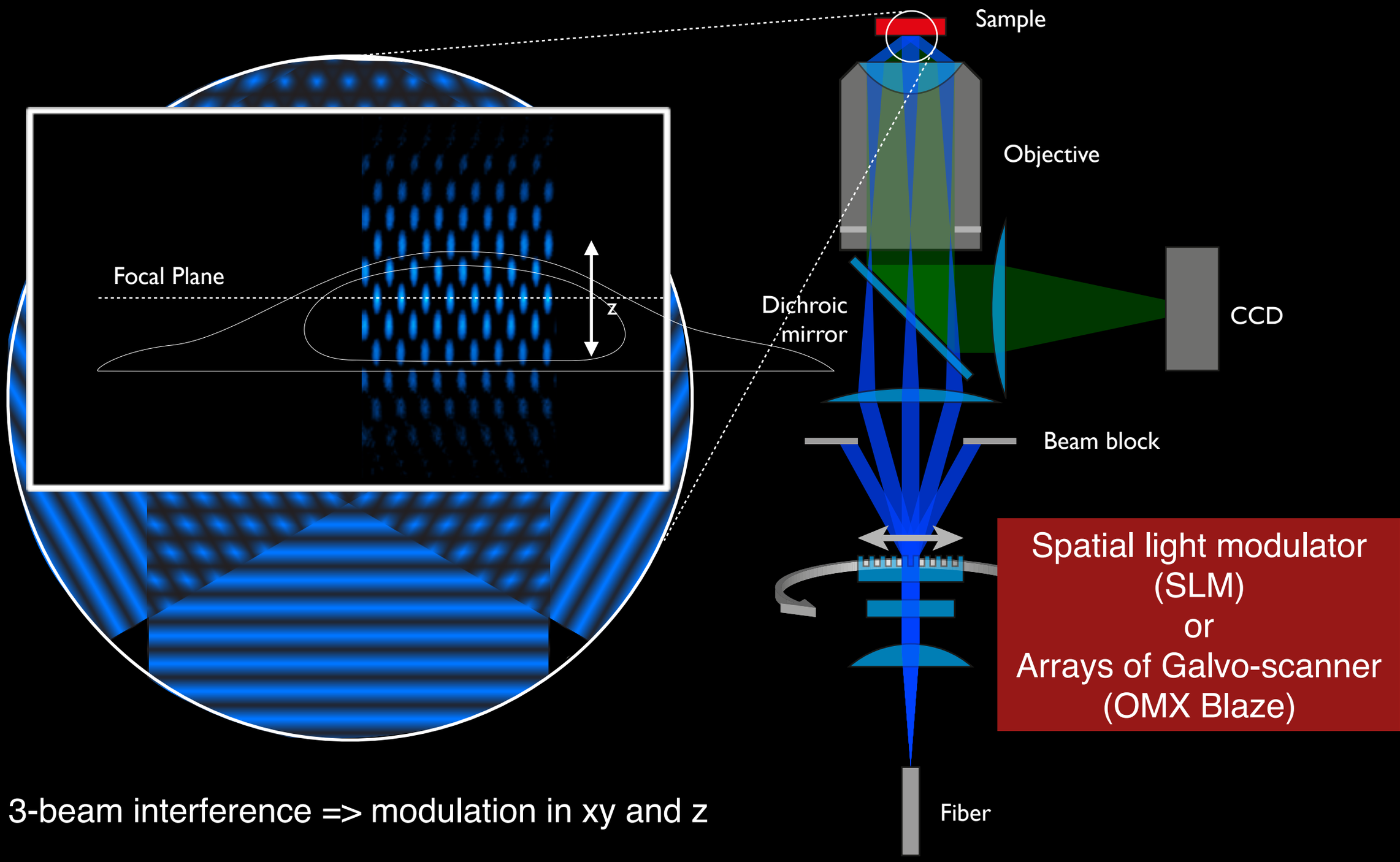


https://mueller-physics.github.io/SIM-Collection/

3D-SIM: microscope design



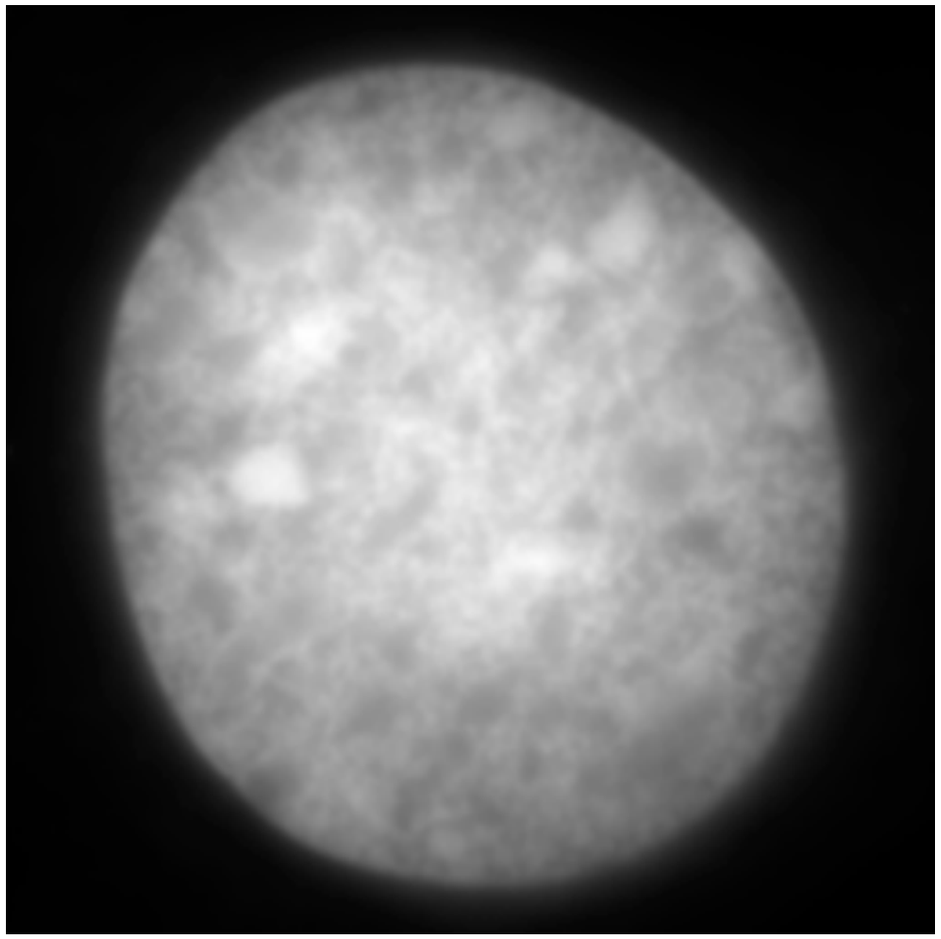
3D-SIM: microscope design



3-beam interference => modulation in xy and z

Frequency support in wide-field microscopy

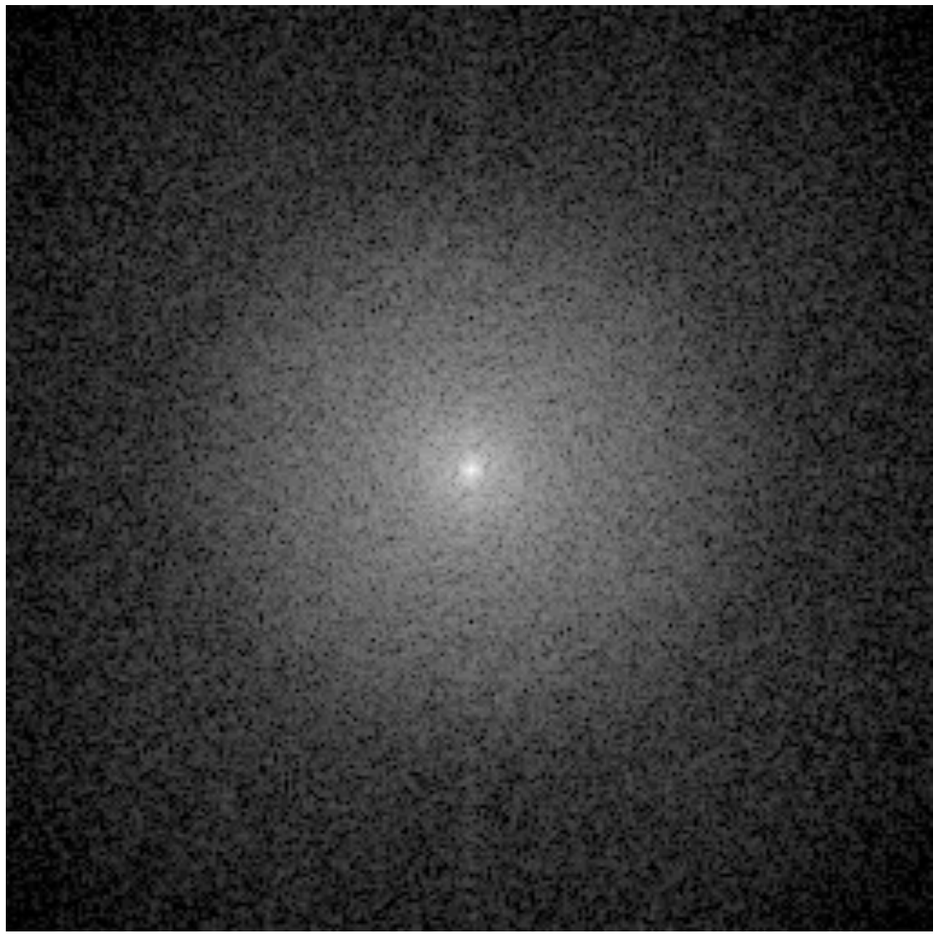
Real space



Fourier Transform

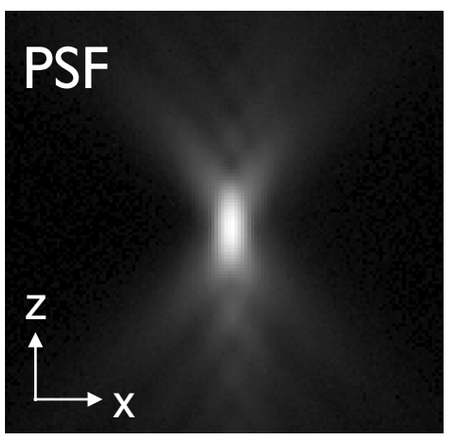
(inverse FT)

Reciprocal space

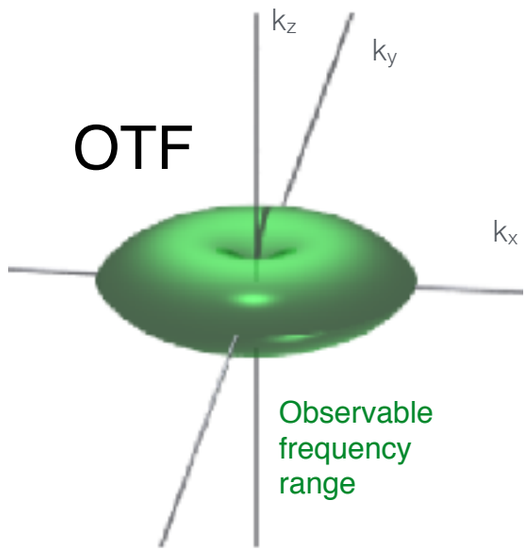


“Real object” ⊗

convolved

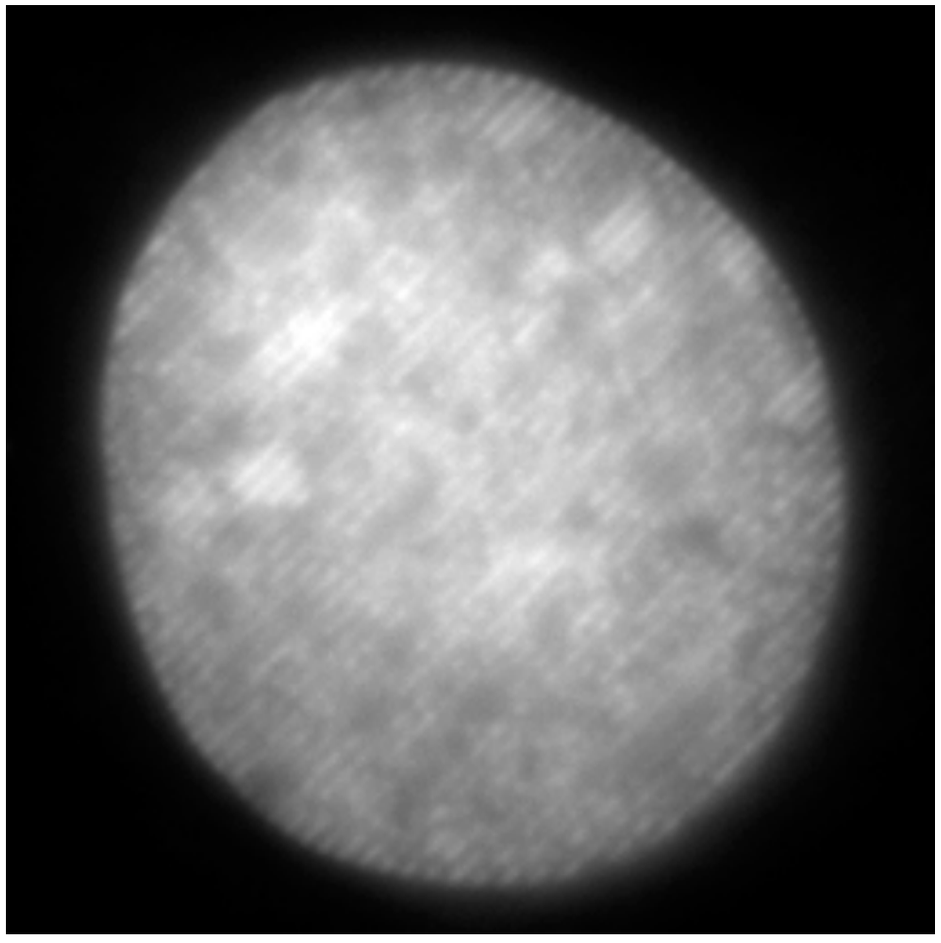


Full frequency range ×



Doubling frequency support in x-y and z

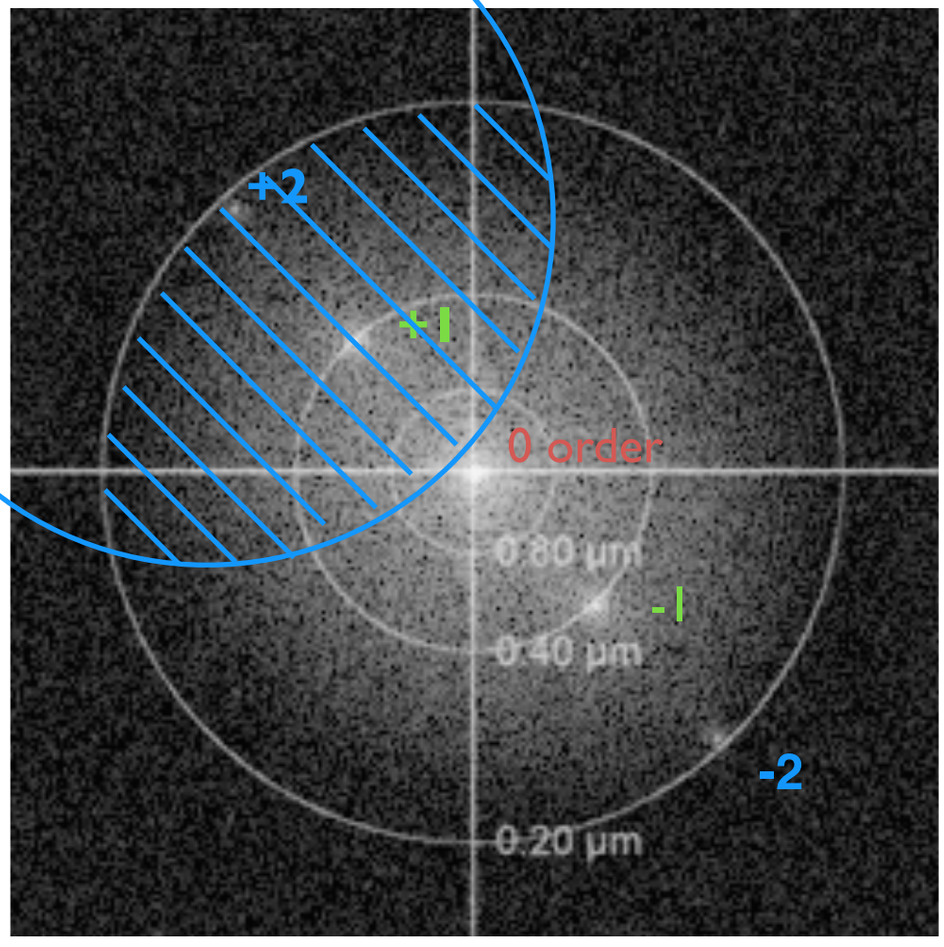
Real space

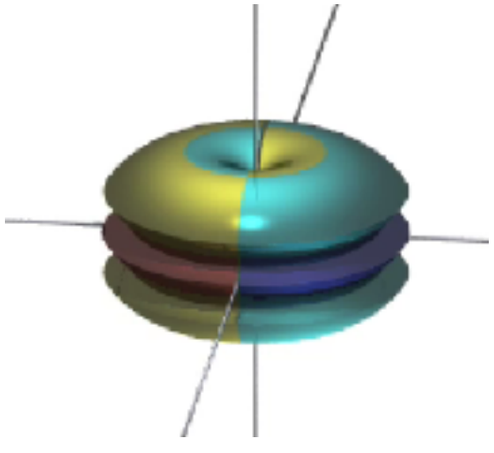


Fourier Transform

(inverse FT)

Reciprocal space

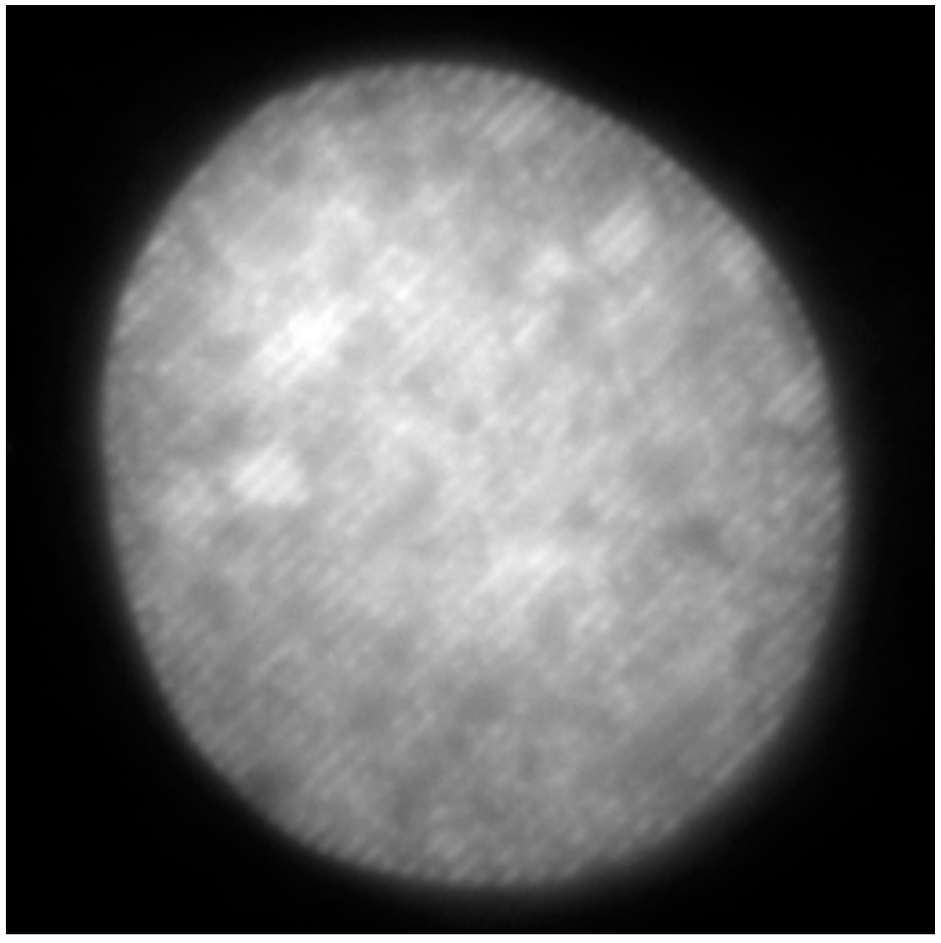




adapted from Gustafsson et al. (2008), *Biophys J* **94**

Doubling frequency support in x-y and z

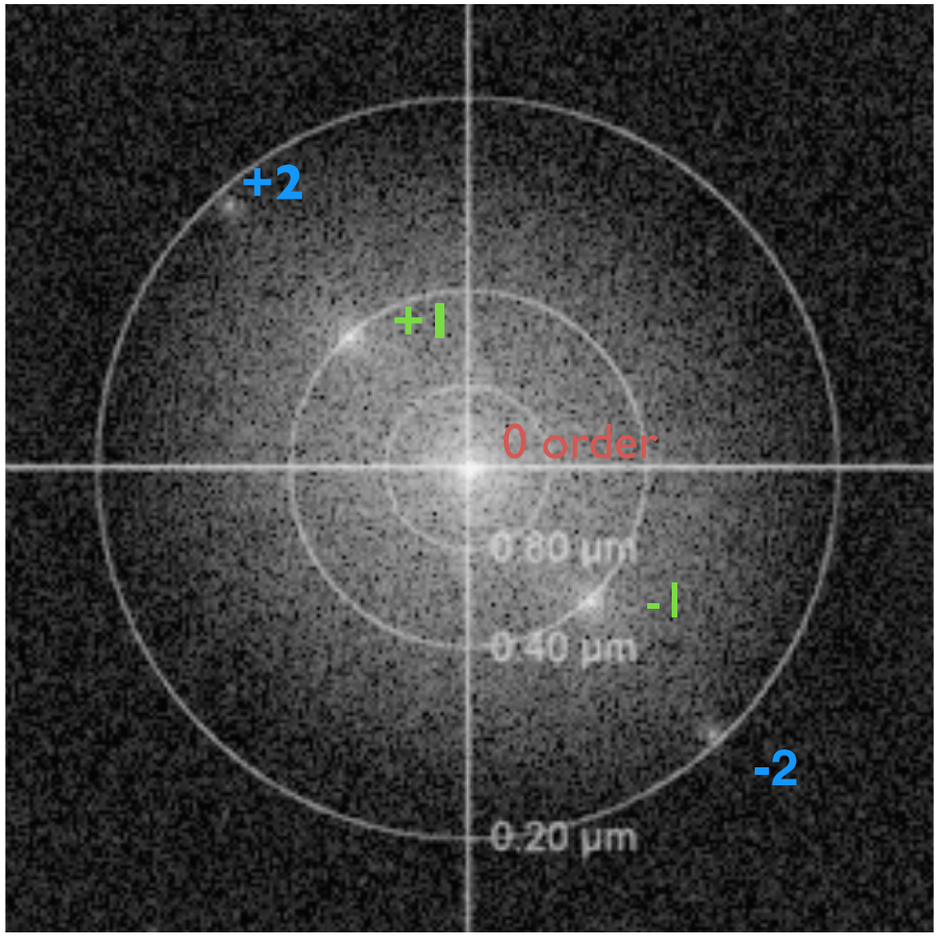
Real space



Fourier Transform

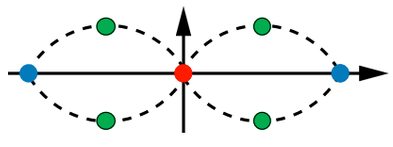
(inverse FT)

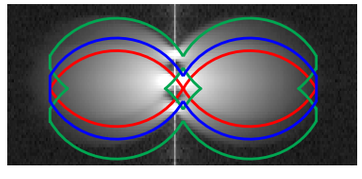
Reciprocal space

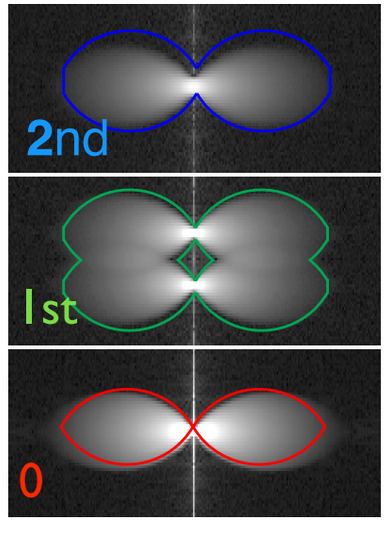


axial

(x-z)

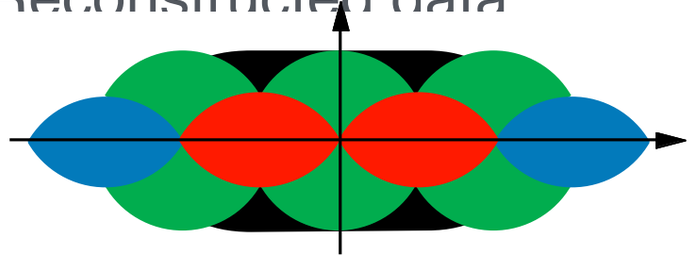






Band separation

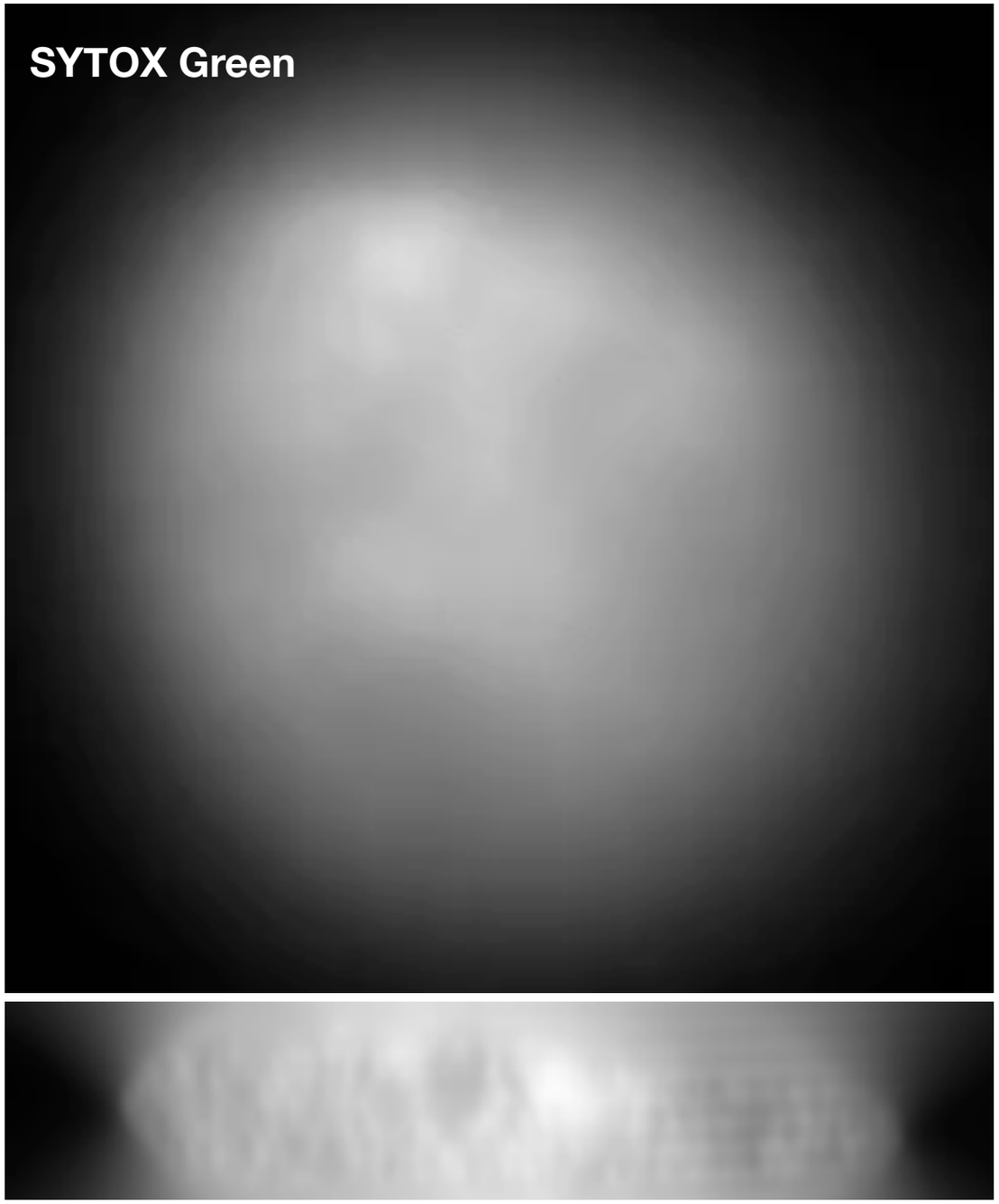
Reconstructed data

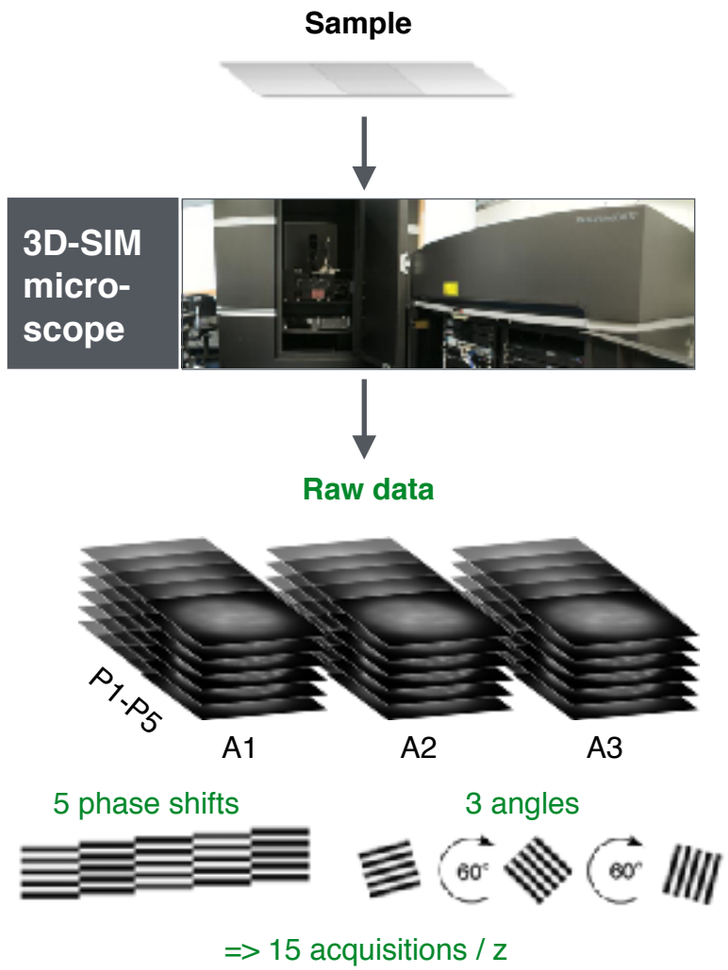


adapted from Gustafsson et al. (2008), *Biophys J* 9

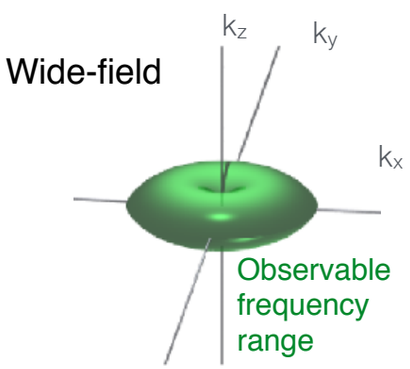
From wide-field to 3D-SIM

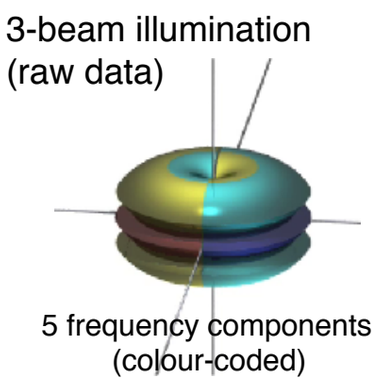
Mouse C127 cell



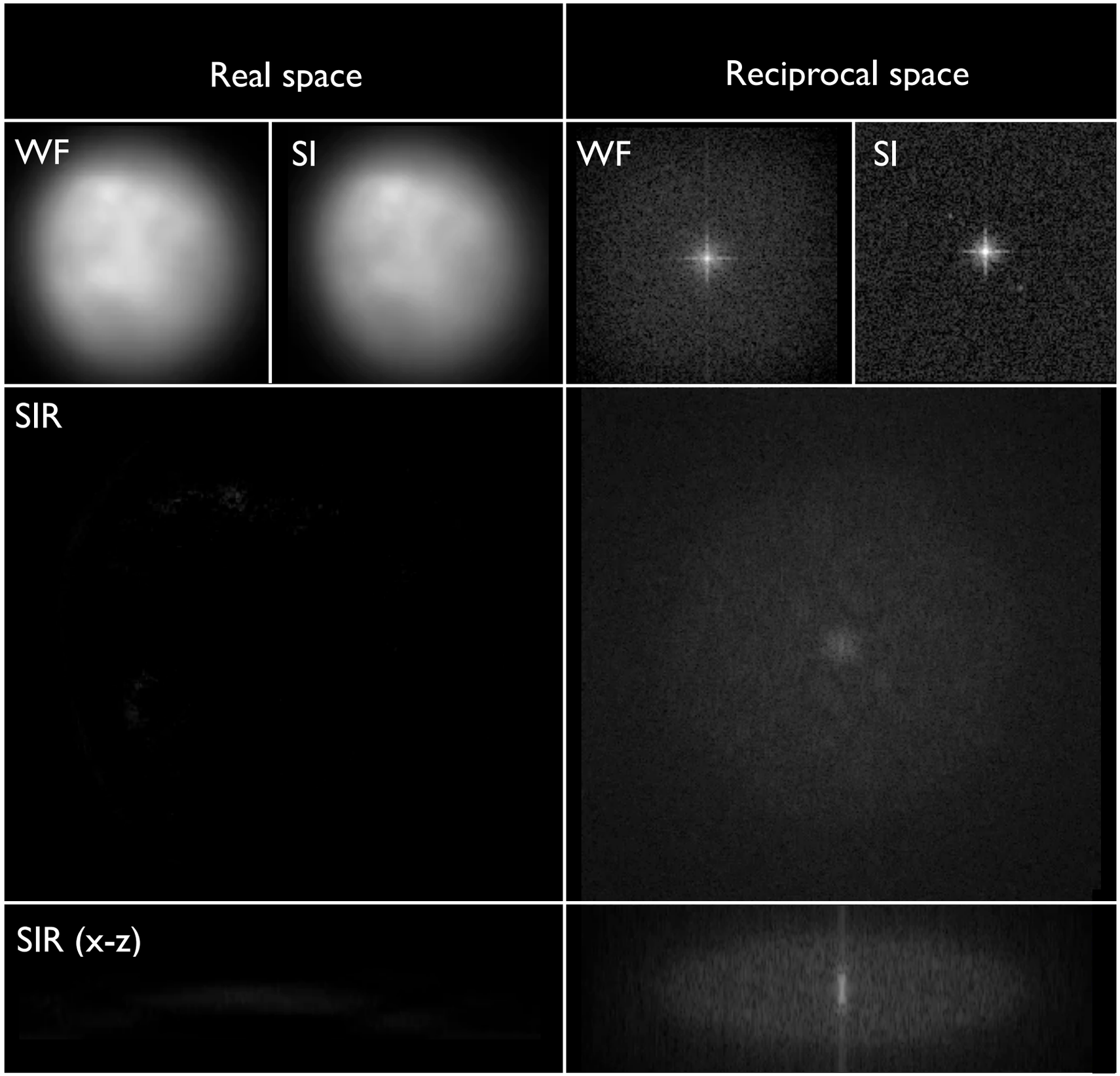


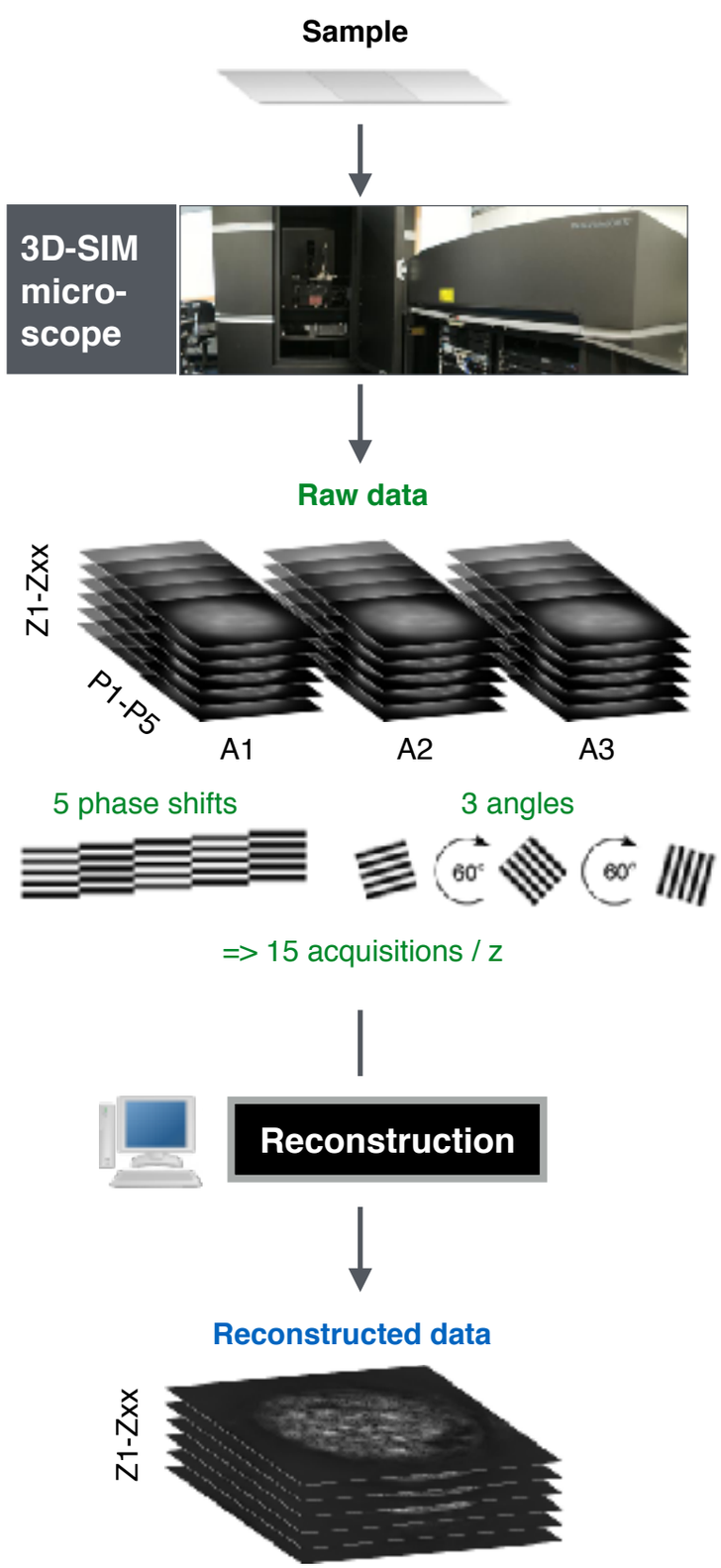
=> 15 acquisitions / z



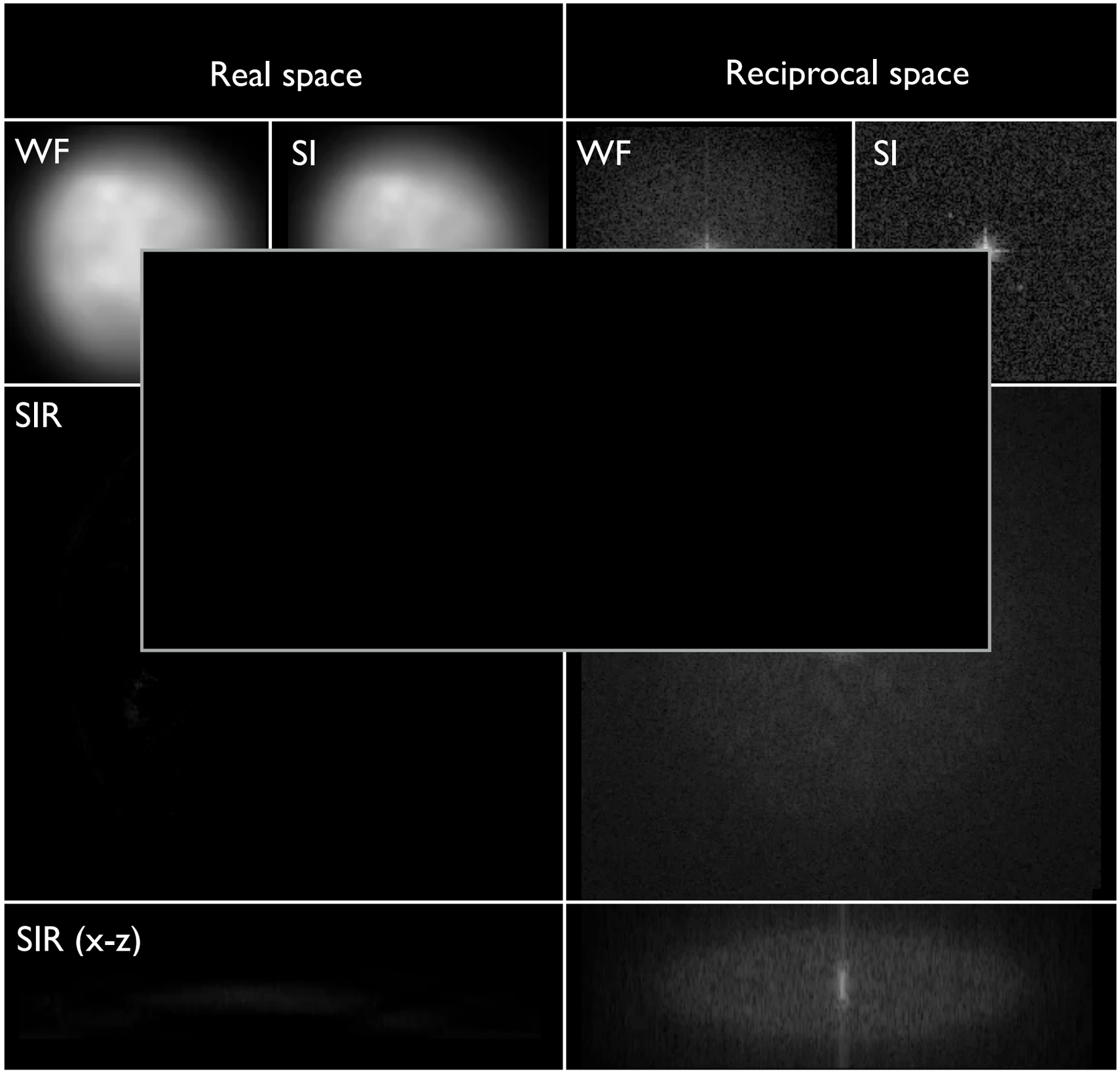


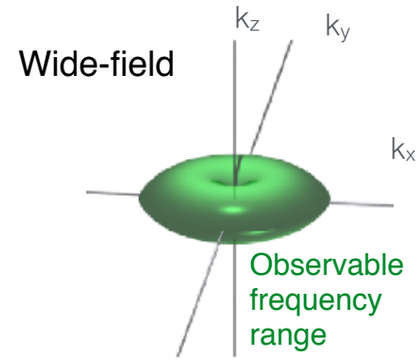
Overview of SIM processing



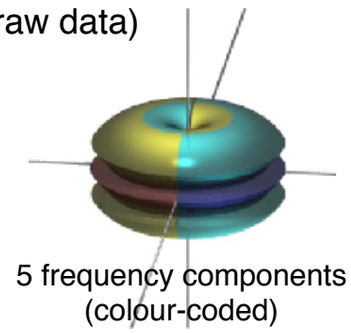


Overview of SIM processing

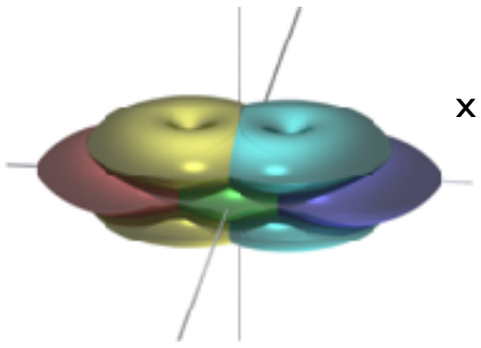




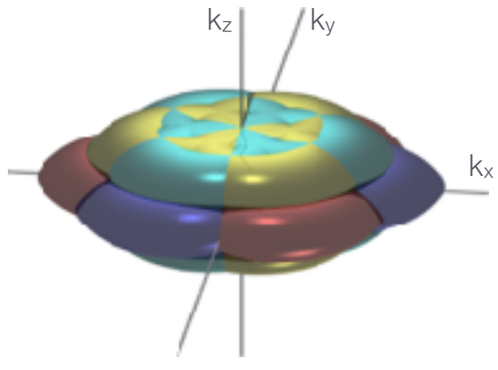
3-beam illumination (raw data)



x 5 phase shifts (2π/5)



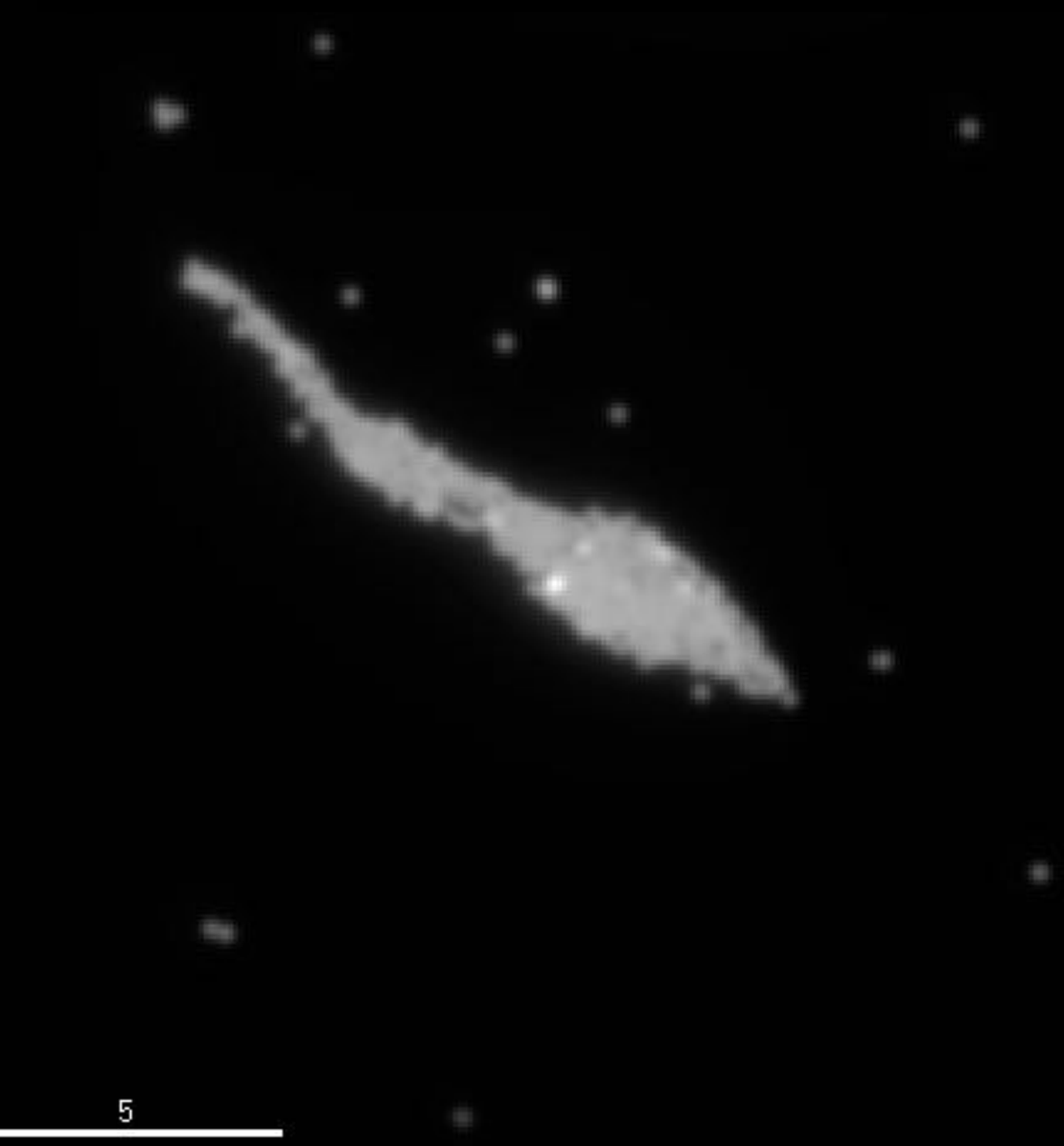
x 3 angles

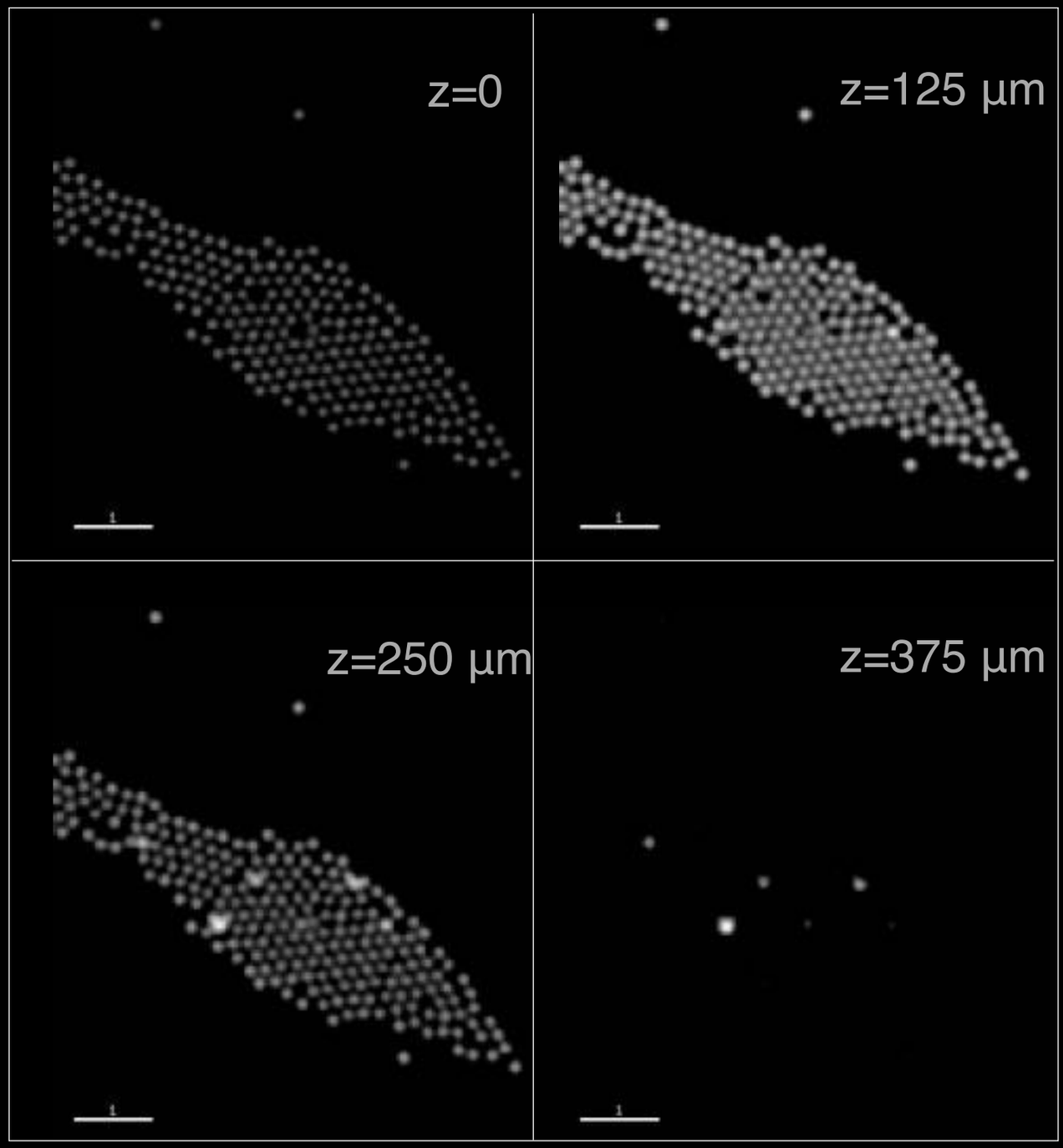


Schermelleh, Carlton et al. *Science* 2008; 320

3D optical sectioning capacity

Example: 170 nm Fluospheres

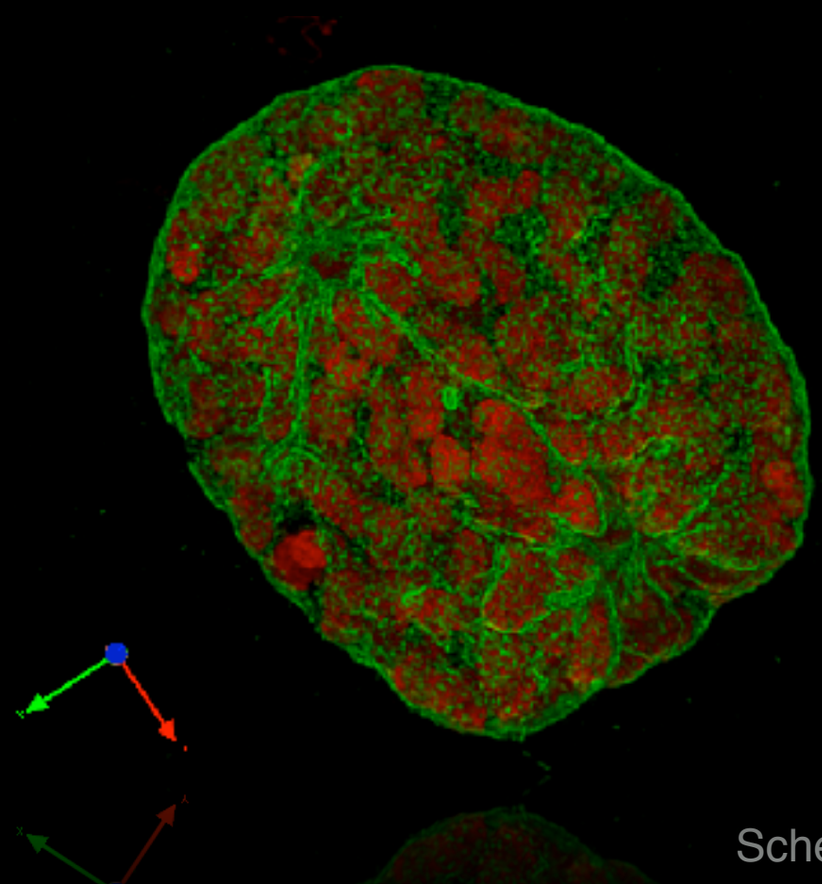




3D-SIM of a prophase nucleus

Lamin B
DAPI

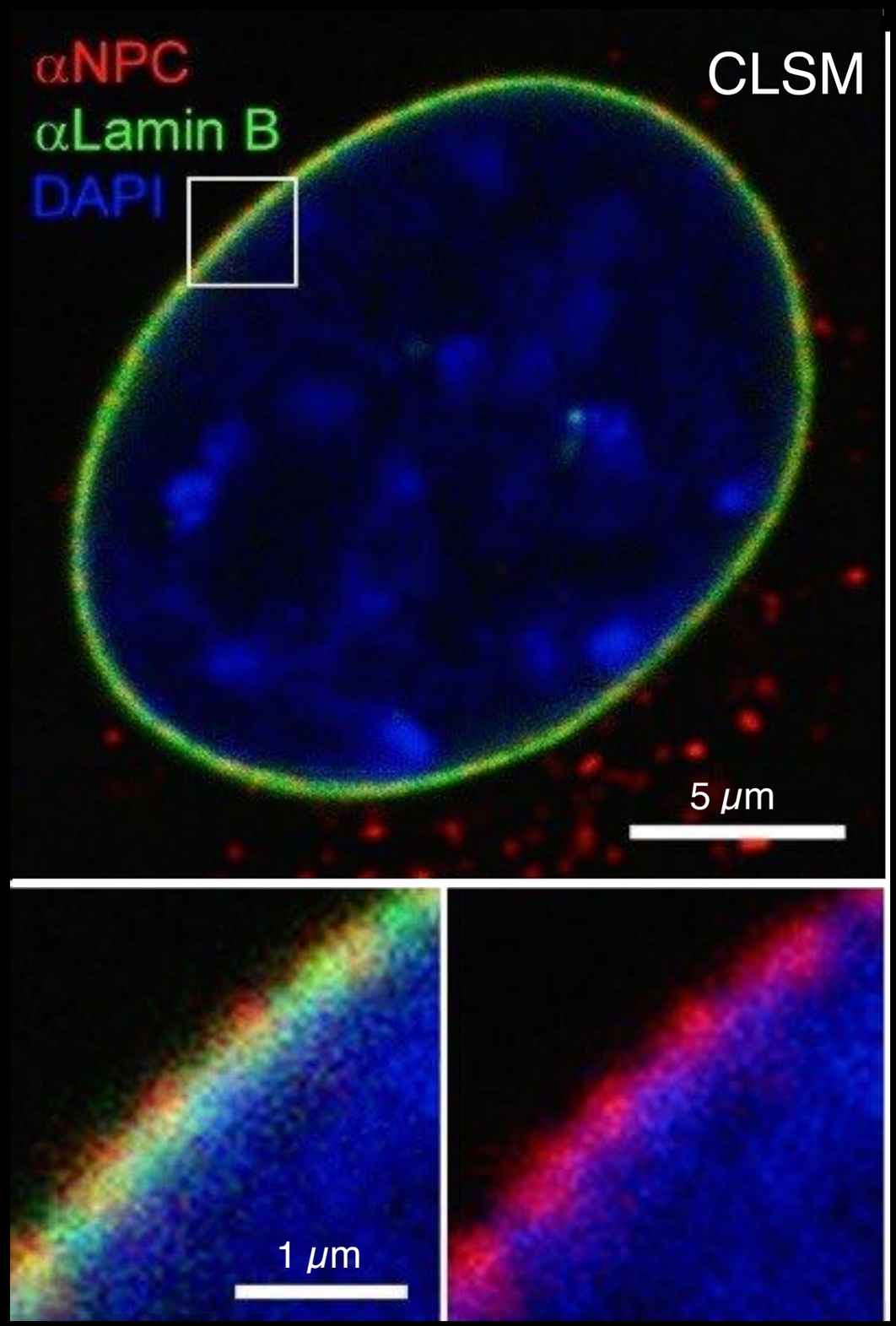
3D volume rendering



Mouse C2C12 cell

Schermelleh, Carlton et al. (2008), *Science* **320**

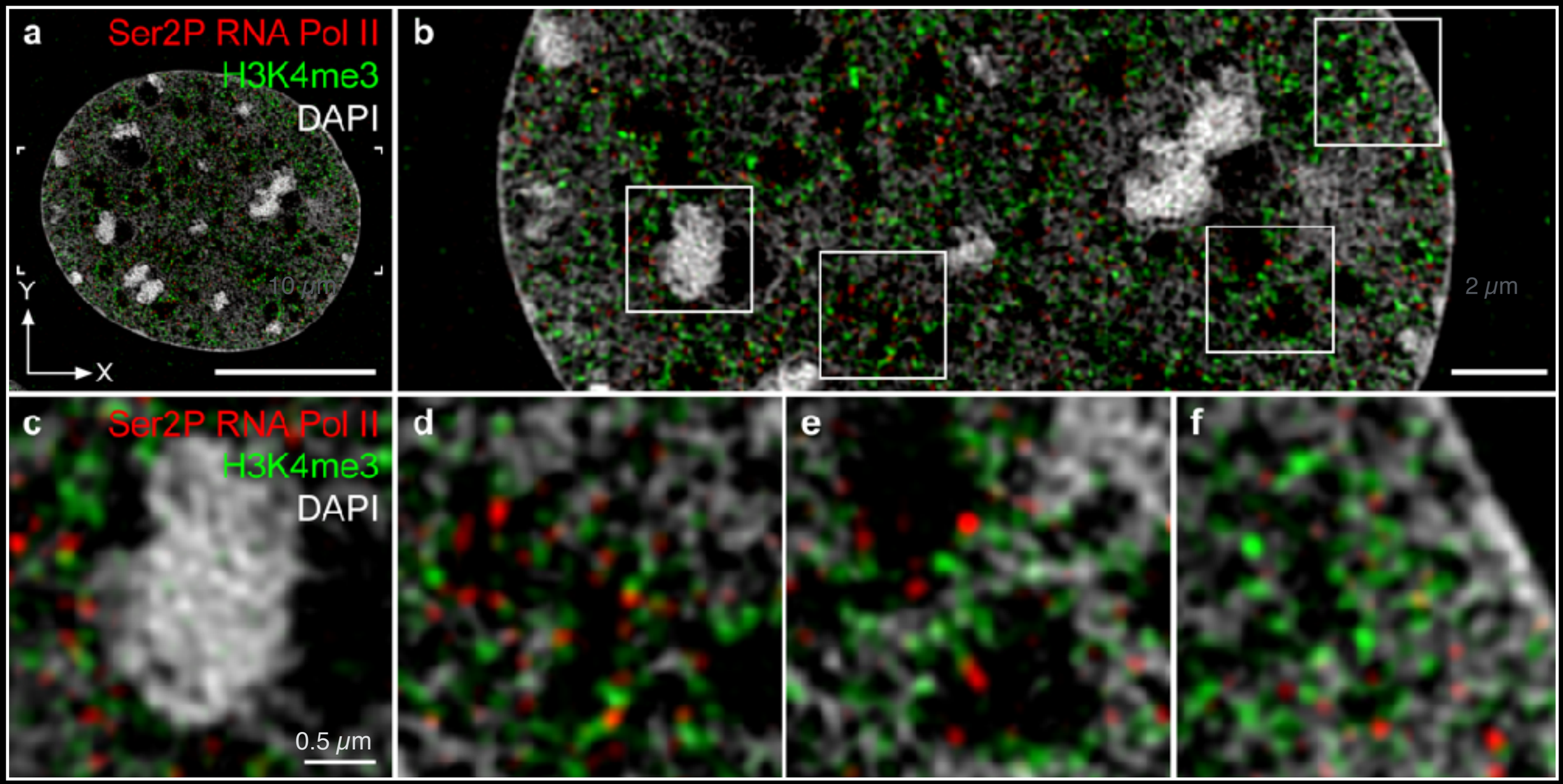
3D-SIM resolves chromatin domains and interchromatin channels



Mouse C2C12

Schermelleh, Carlton et al. (2008), *Science* **320**

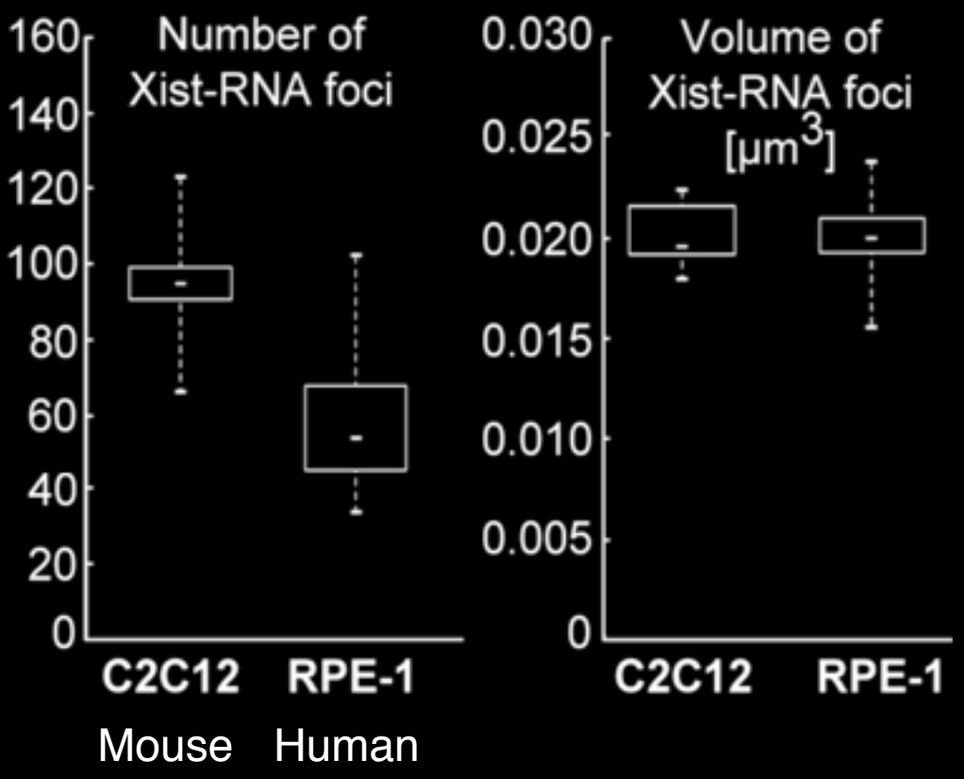
Active marker are constrained to chromatin domain boundaries



Mouse C127 cell

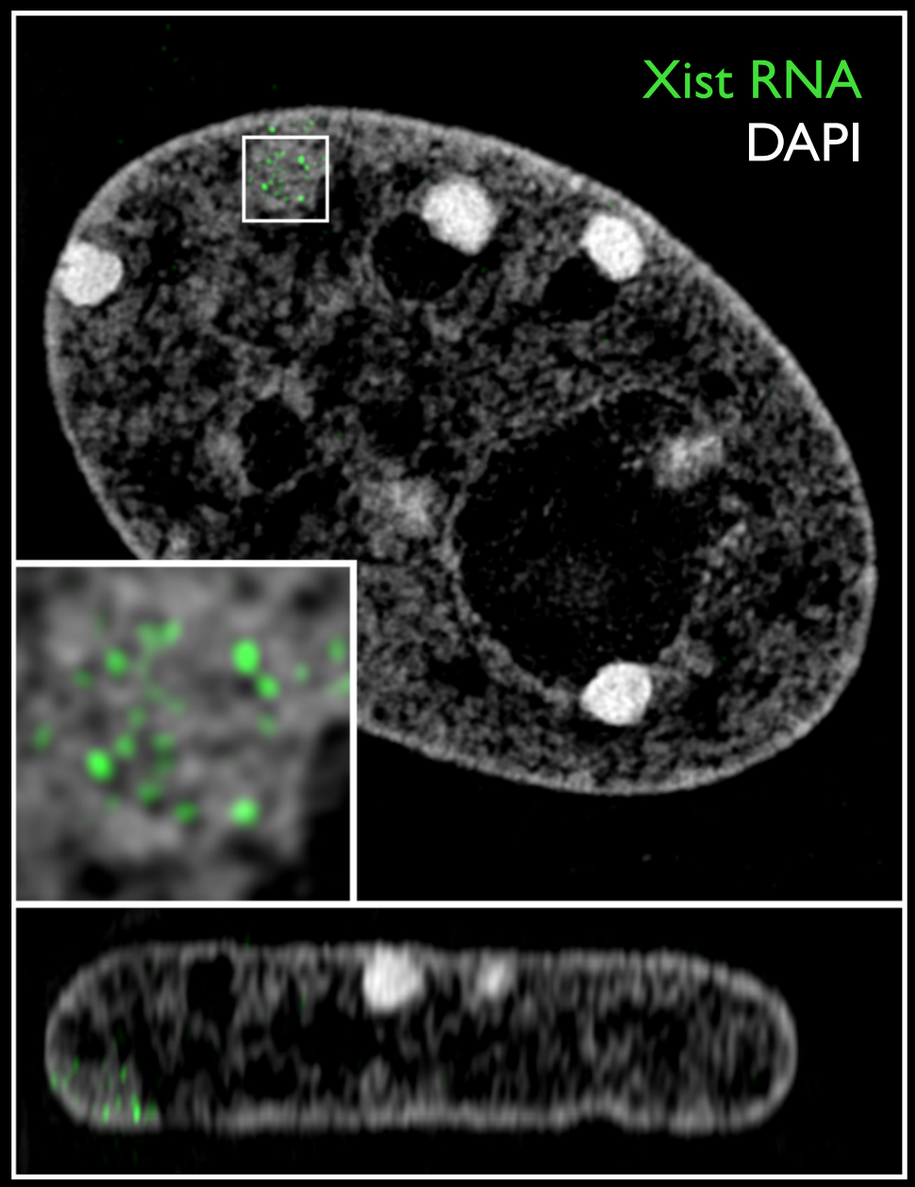
Markaki et al., 2011, *Cold Spring Harb Perspect Biol*, 75

Super-resolution topology inactive X-chromosome



Smeets et al. (2014), *Epigenetics & Chromatin*

3D-SIM



Markaki et al., (2013) *Methods Mol Biol*

Xist RNA forms distinct domains within the Barr Body
Evidence for multimerisation (3-10 Xist RNAs/focus)

Can we go live?

Live cell 3D super-resolution imaging of replication sites

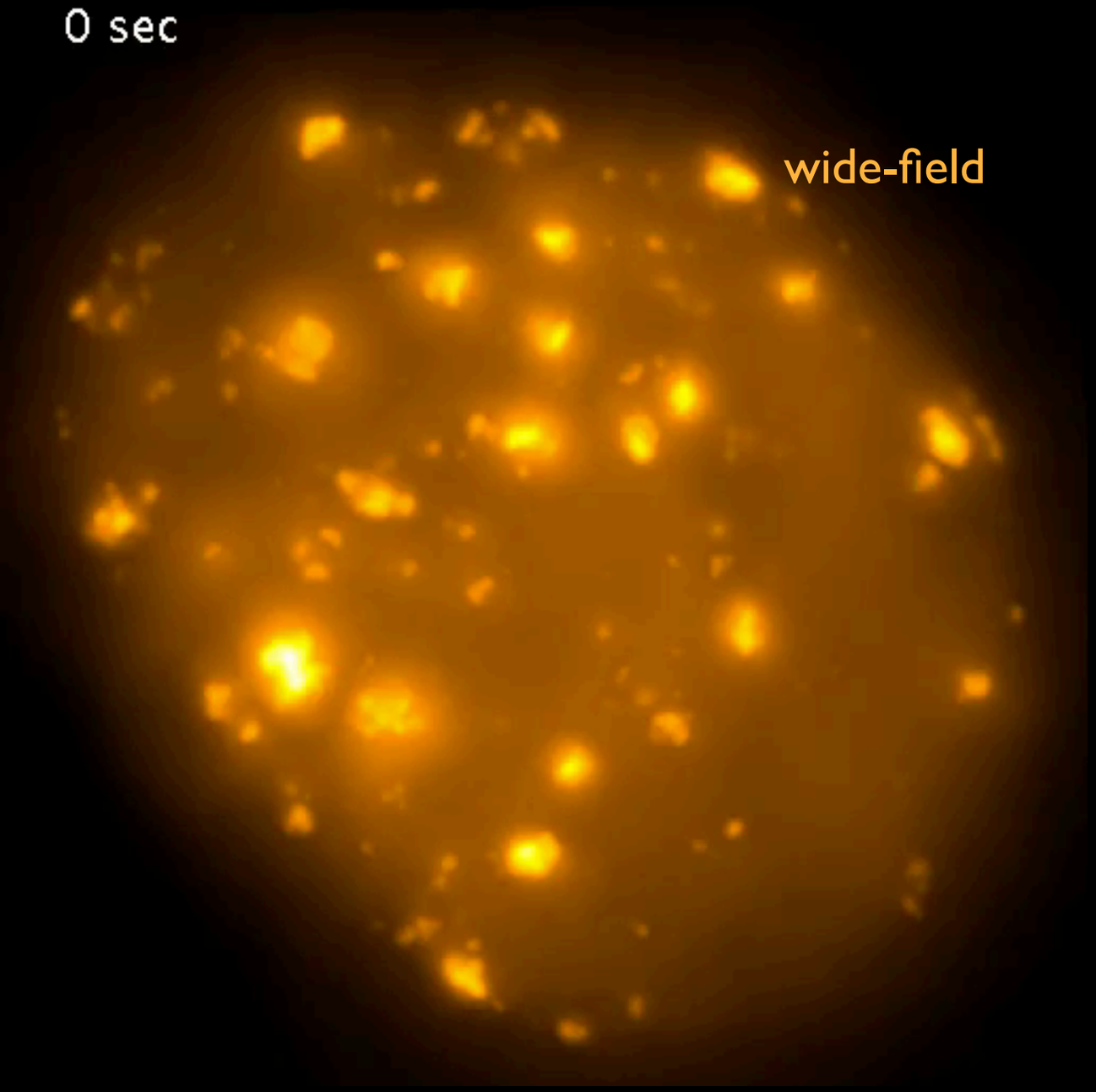
DNA replication foci
(GFP-PCNA in mouse C2C12 myoblast cell)

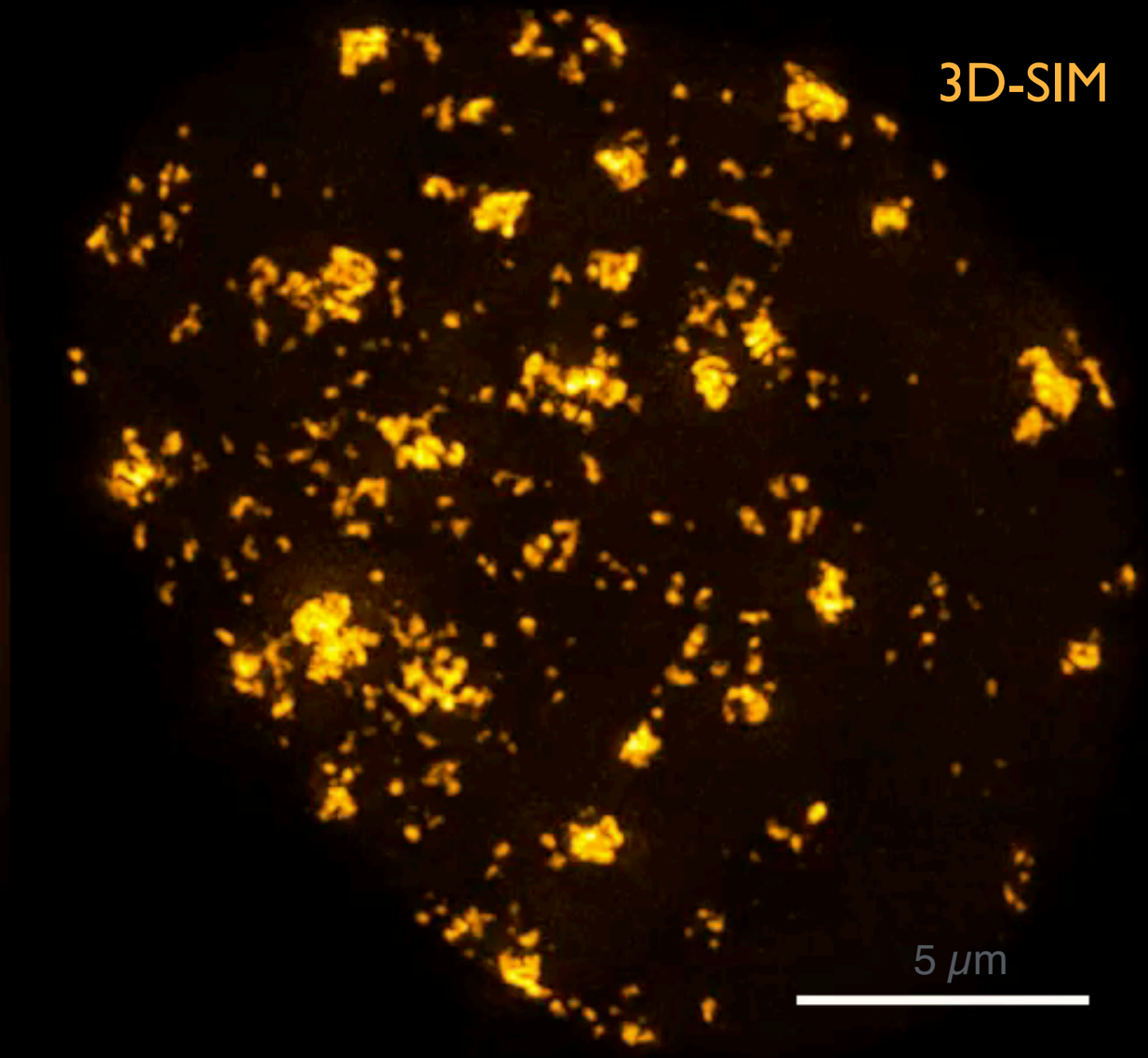
(OMX Blaze)

0 sec

wide-field

3D-SIM



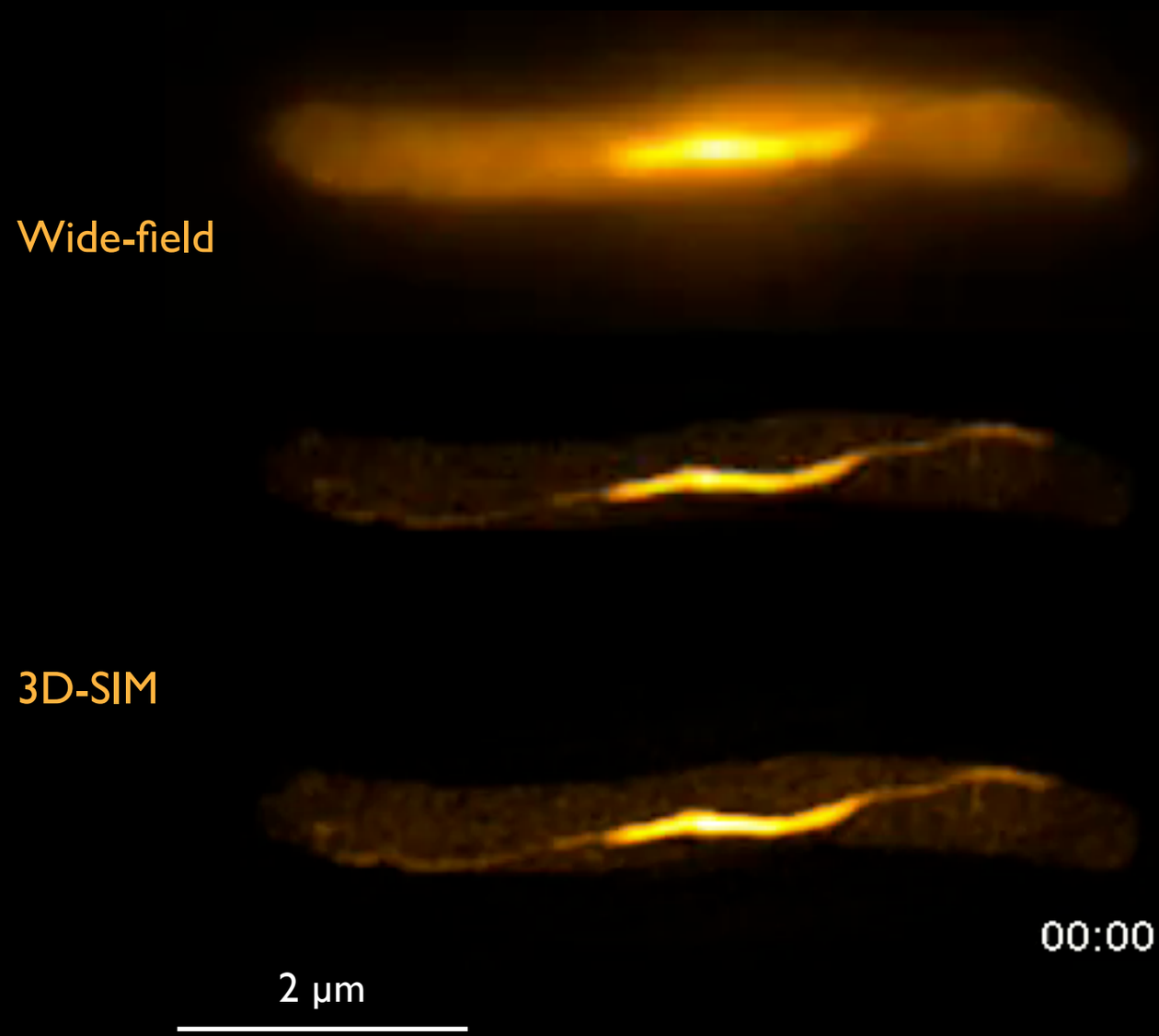


10 s / frame (5 μm z-stack = 600 images / frame)

max. projection

Dynamics of RecA in DNA double strand break repair

RecA-GFP in *E.coli* after DSB induction



OMX Blaze: 2 s / frame (1.75 µm z-stack = 225 images, 100 time points)

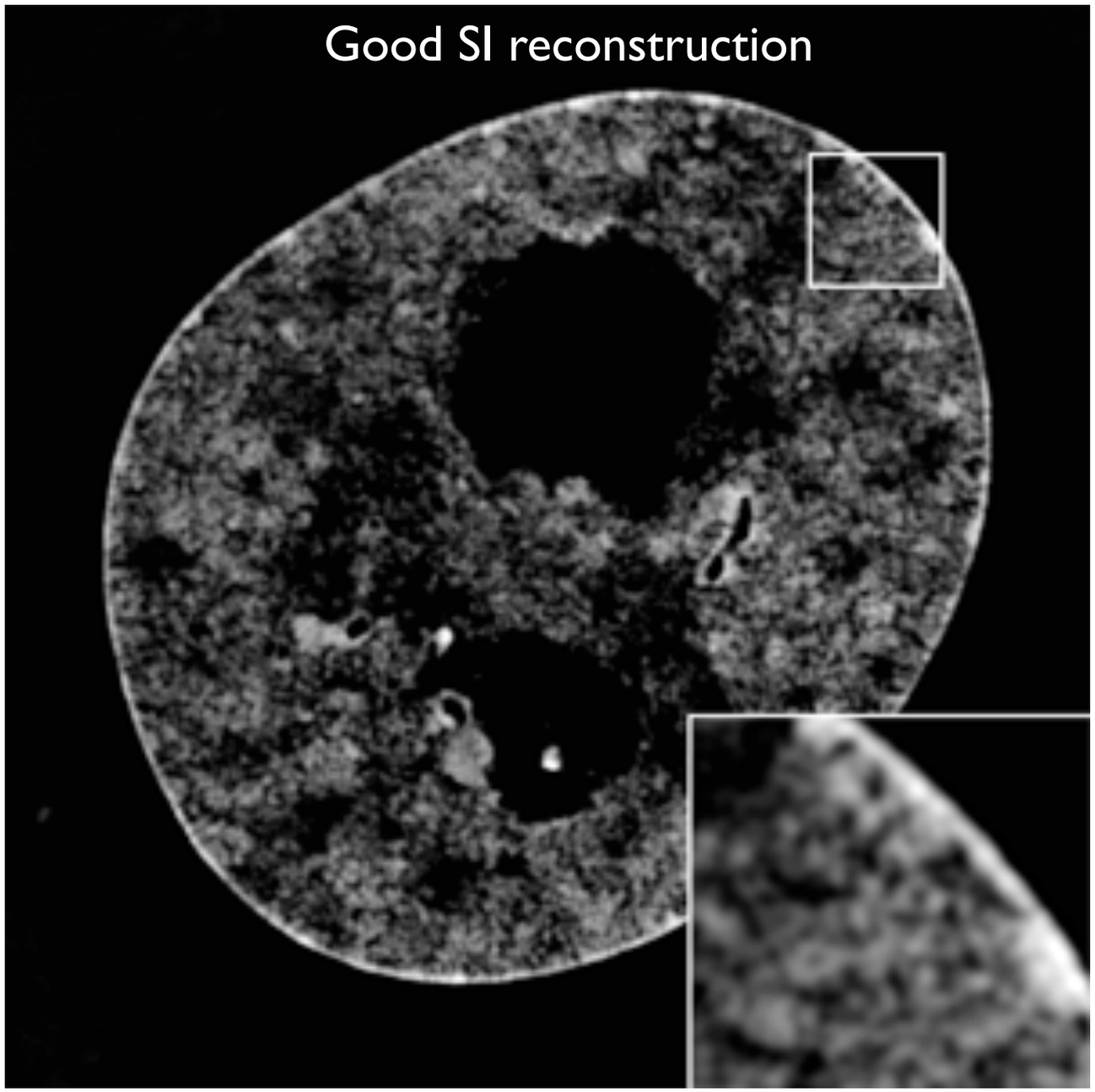
Lesterlin et al. , 2014, *Nature* (D. Sherratt Lab)

3D-SIM,
just another tool in the repertoire ?

It‘s not that simple!

The untold story

SI reconstruction artifacts



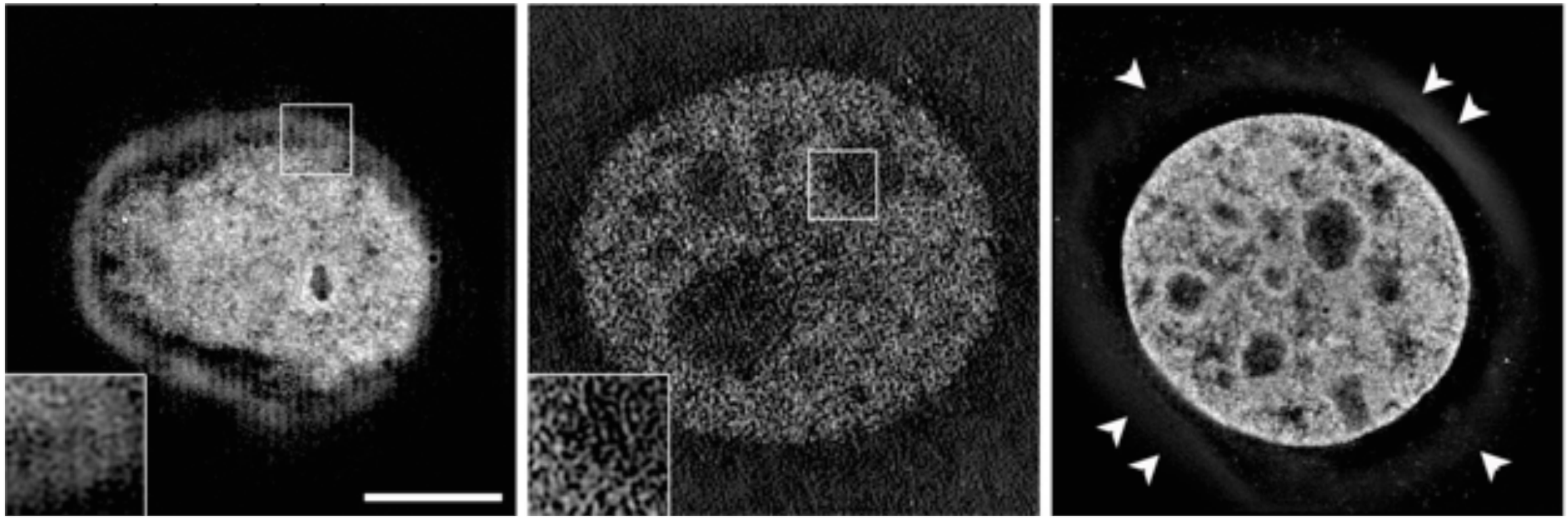
SI reconstruction artifacts

Stripes

High frequency noise

Halo / Doubling

HeLa clell nuclei, chromatin staining

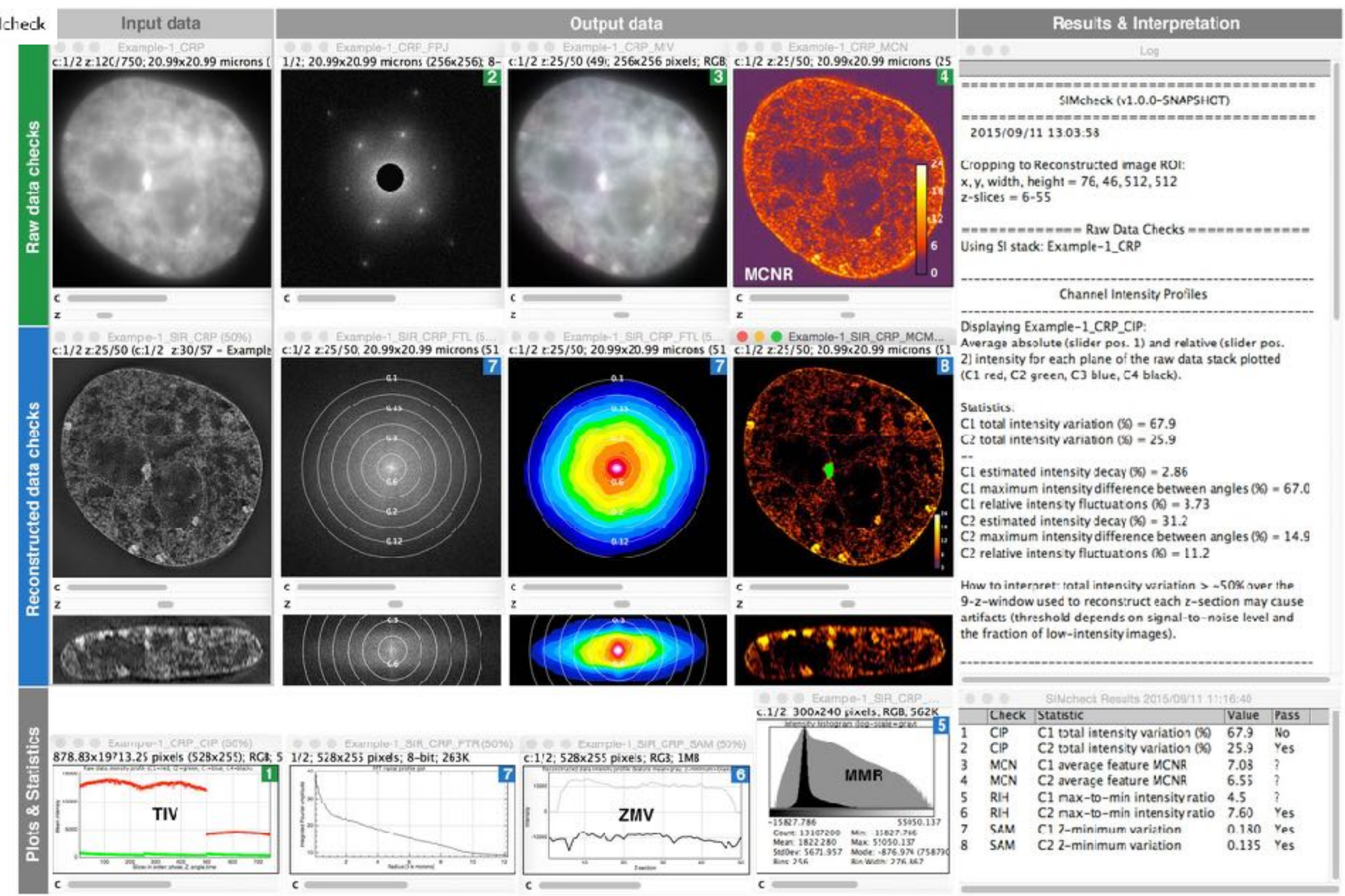


Bleaching,
Drift or vibrations
Moving particles
(locally constrained)

Low contrast-to-noise,
Low modulation contrast

Spherical aberration,
Refractive index mismatch

SIMcheck - Toolbox for Fiji/ImageJ



Ball et al. 2015, *Scientific Rep* 5

3D-SIM workflow: quality is paramount !!!

Labelling

- Dyes (spectra, photo-stability)
- Labelling method (FPs, IF, FISH,....)
- Labelling specificity (antibodies)
- Signal-to-noise / background

Sample

- Optical quality (coverslip, cleanness)
- Refractive index mismatch
- Embedding medium, RI immersion
- Imaging depth

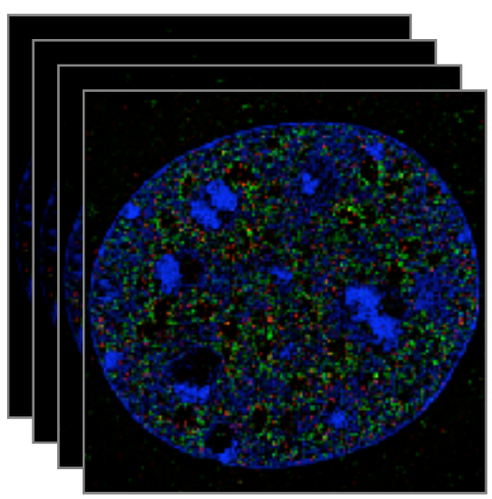
Microscope

- Mechanical stability
- Photon efficiency
- Modulation contrast / calibration
- Camera: (EM)CCD / sCMOS

Postprocessing

- PSF/OTF (λ-, depth-, RI-dependent)
- Channel alignment
- Quality control

Dataset
x, y, z, λ,(t)

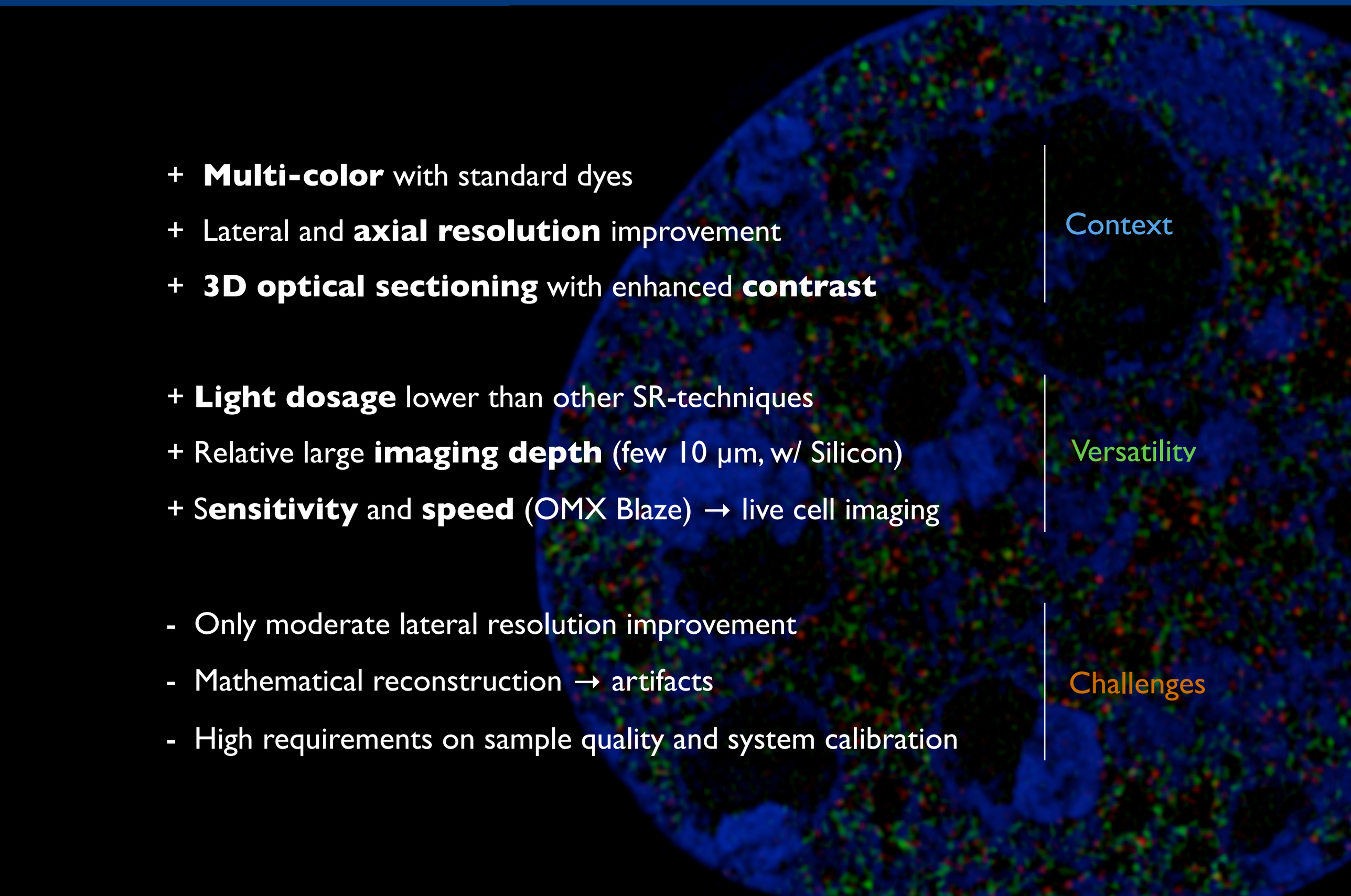


Quantitative Analysis

- Quality control
- Co-localisation
- Segmentation
- Distances

Demmerle et al. 2017, *Nature Protocols,* in press & Kraus et al. 2017, *Nature Protocols,* in press

3D-SIM - pros & cons



+ **Multi-color** with standard dyes
+ Lateral and **axial resolution** improvement
+ **3D optical sectioning** with enhanced **contrast**

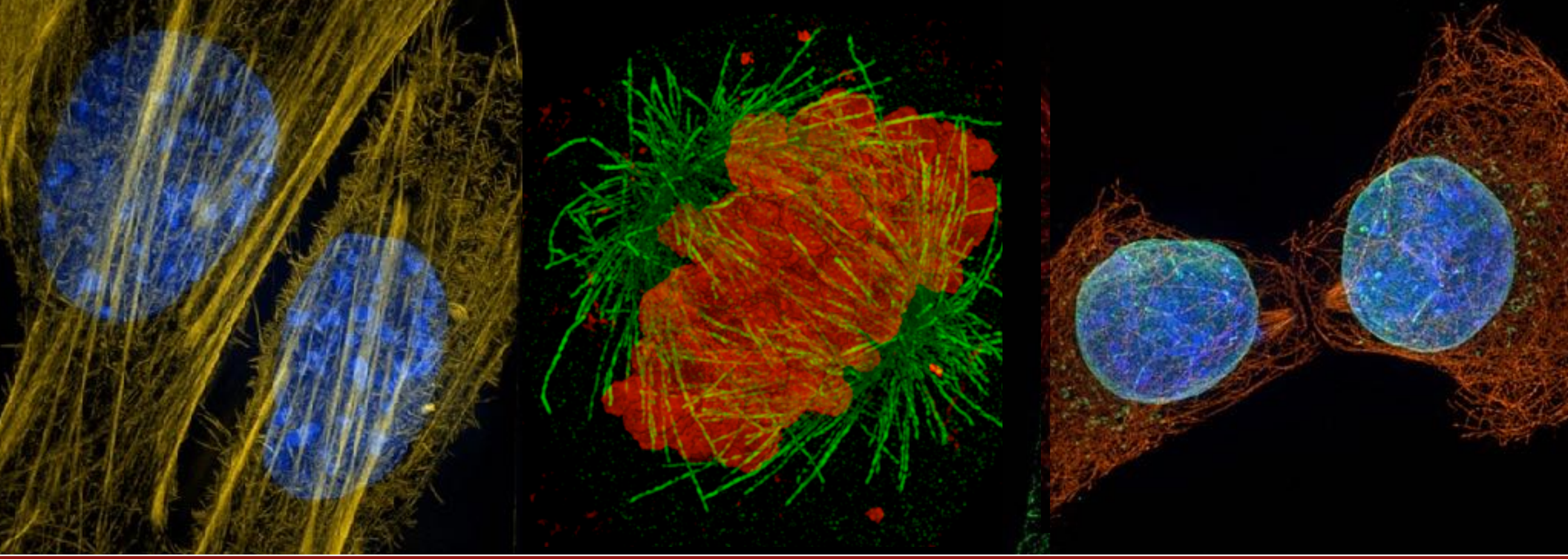
Context

+ **Light dosage** lower than other SR-techniques
+ Relative large **imaging depth** (few 10 µm, w/ Silicon)
+ S**ensitivity** and **speed** (OMX Blaze) → live cell imaging

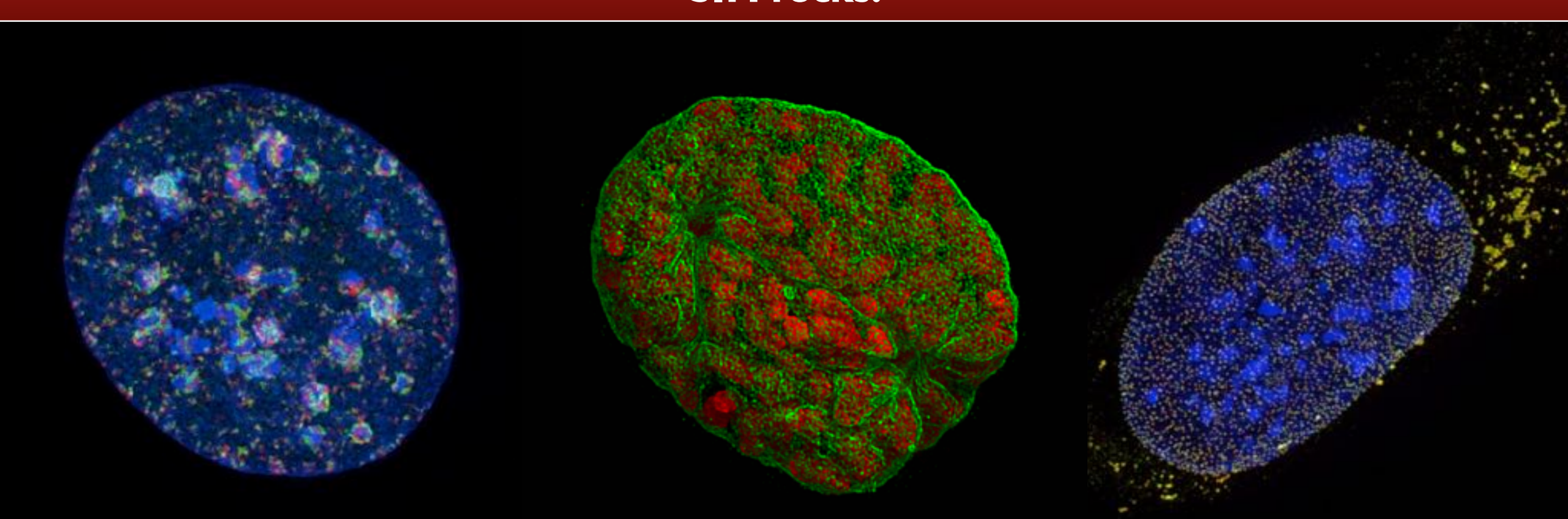
Versatility

- Only moderate lateral resolution improvement
- Mathematical reconstruction → artifacts
- High requirements on sample quality and system calibration

Challenges



SIM rocks!



Thanks to Jürgen Neumann, Lin Shao, Julio Mateo Langerak for sharing slides


What, all?!
How many are there?
...let's go through the image, point-by-point...
79.345!
Pontillism sucks!