The Power of SIM

Short intro to super-resolution microscopy

How structured illumination improves not only resolution ...

& how it is realised in OMX system

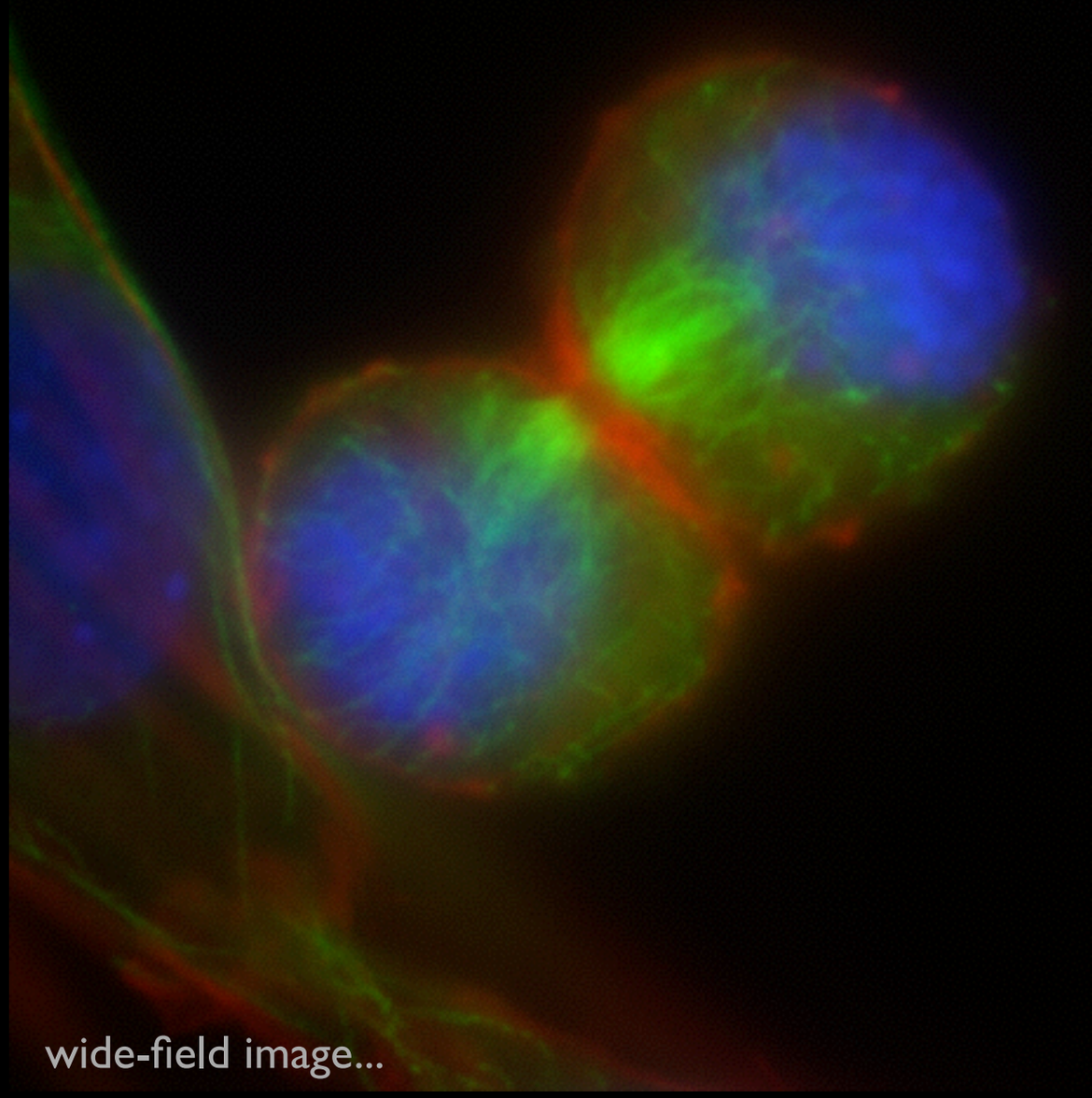
Comparison with other SR methods (Pros & Cons)

3D structured illumination microscopy of a mouse cell nucleus

Micron OXFORD

lothar.schermelleh@bioch.ox.ac.uk

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!

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lothar.schermelleh@bioch.ox.ac.uk

...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**	$d_{x,y} = 0.61\lambda / NA$	(~200-300 nm)
Axial Resolution **limit**	$d_z = 2\lambda / NA^2$	(~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)

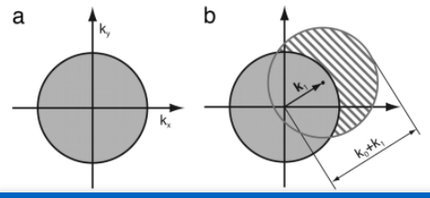
...improved to some extent by confocal imaging or deconvolution

Overcome the limit by super-resolution

3D structured illumination microscopy

Super-resolution microscopy - three major concepts

Structured illumination

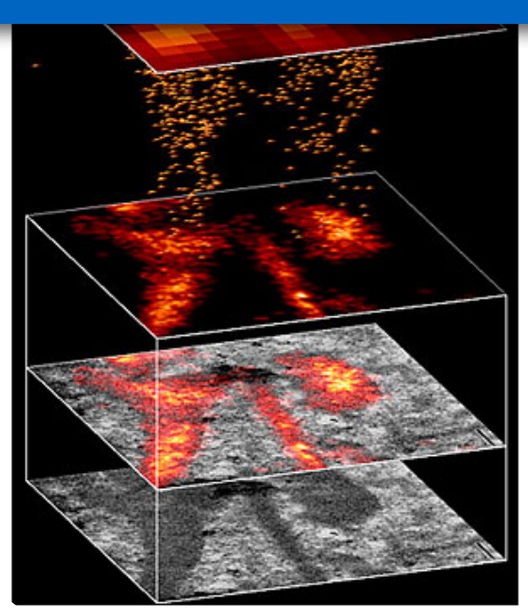


SIM-Methods:

Apotome (conventional SIM)

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

SSIM (non-linear SIM)
NL-SIM

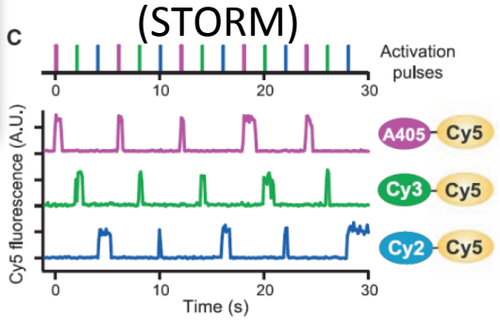


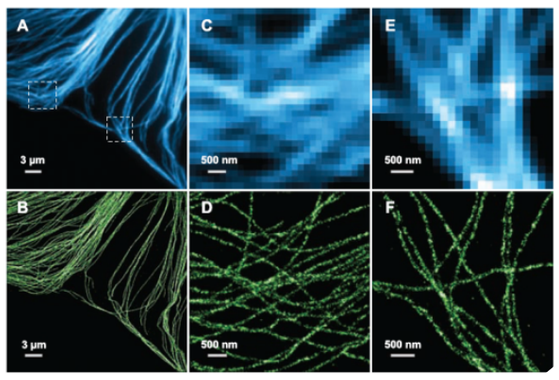
Abbe diffraction limit

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

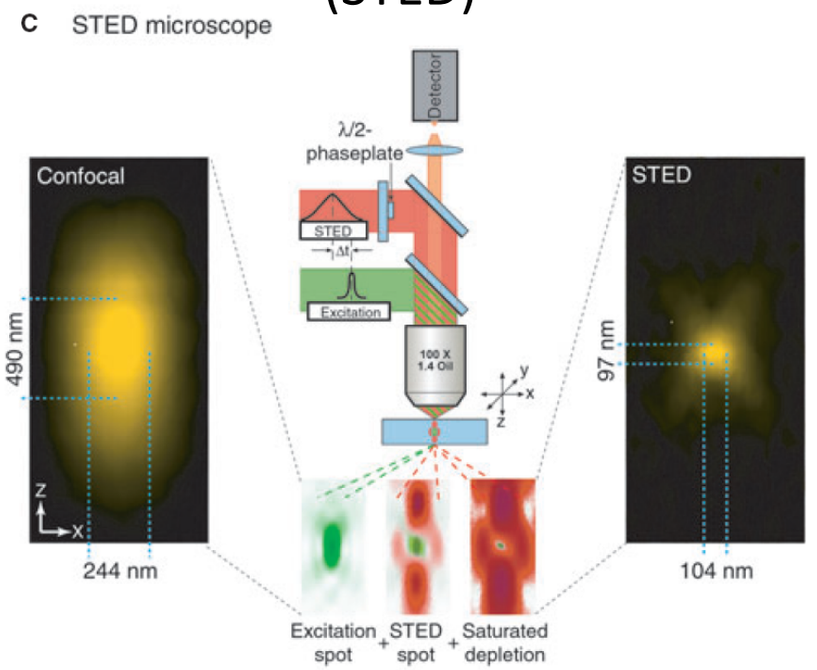
e localisation

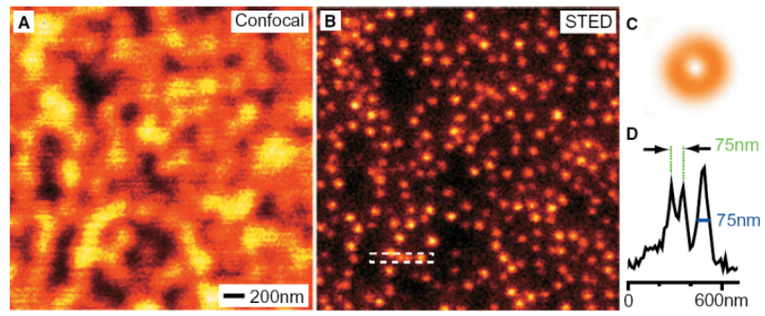
Stochastic optical
econstruction microscopy
(STORM)



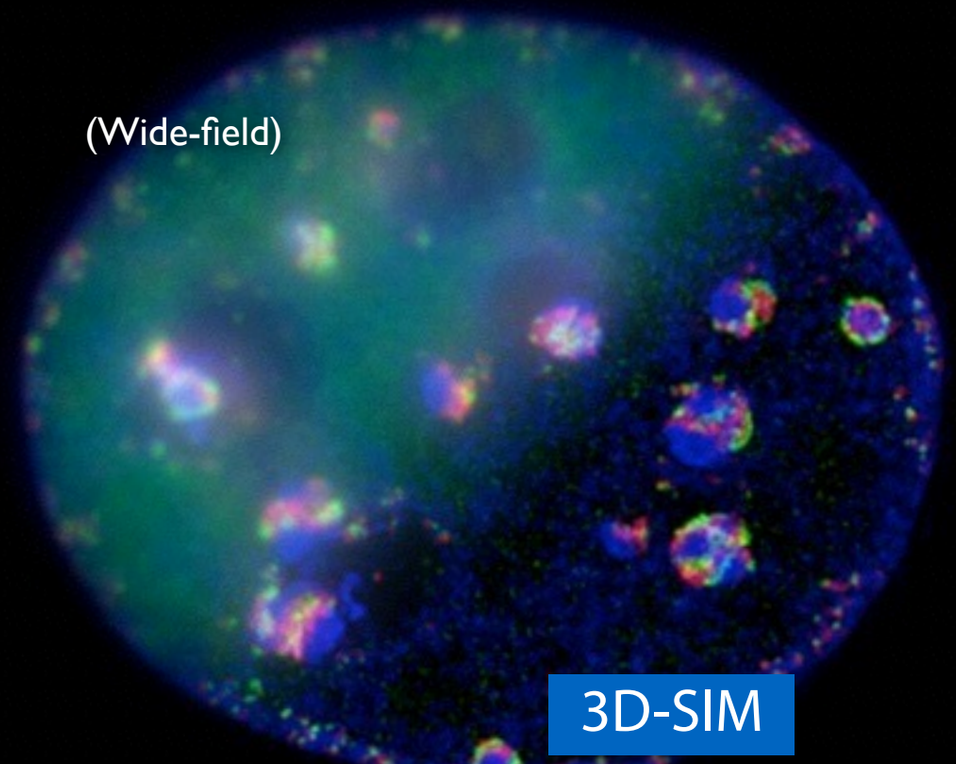


Stimulated emission depletion (STED)



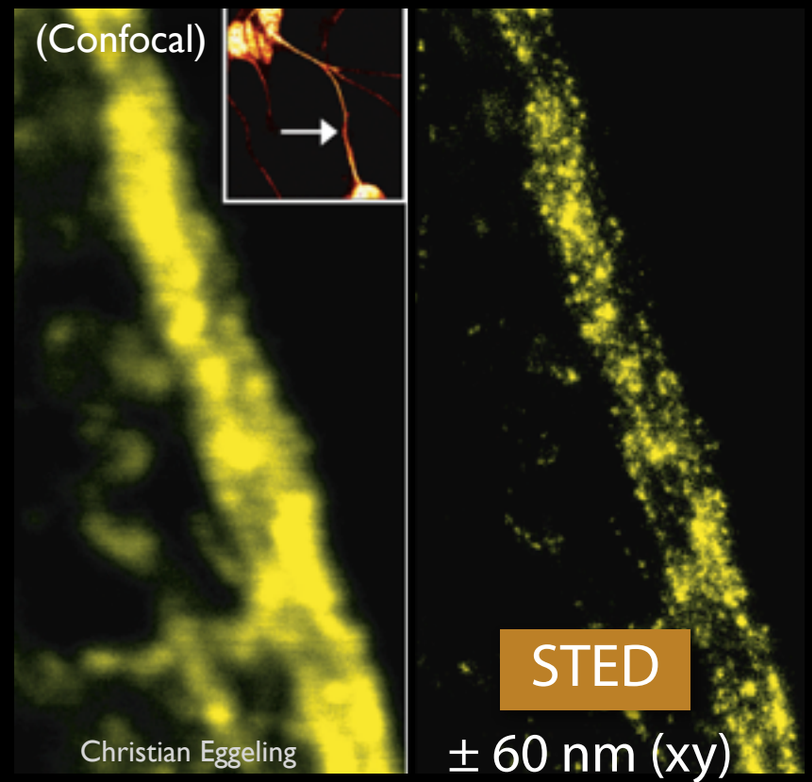


Super-resolution techniques to surpass the diffraction limit



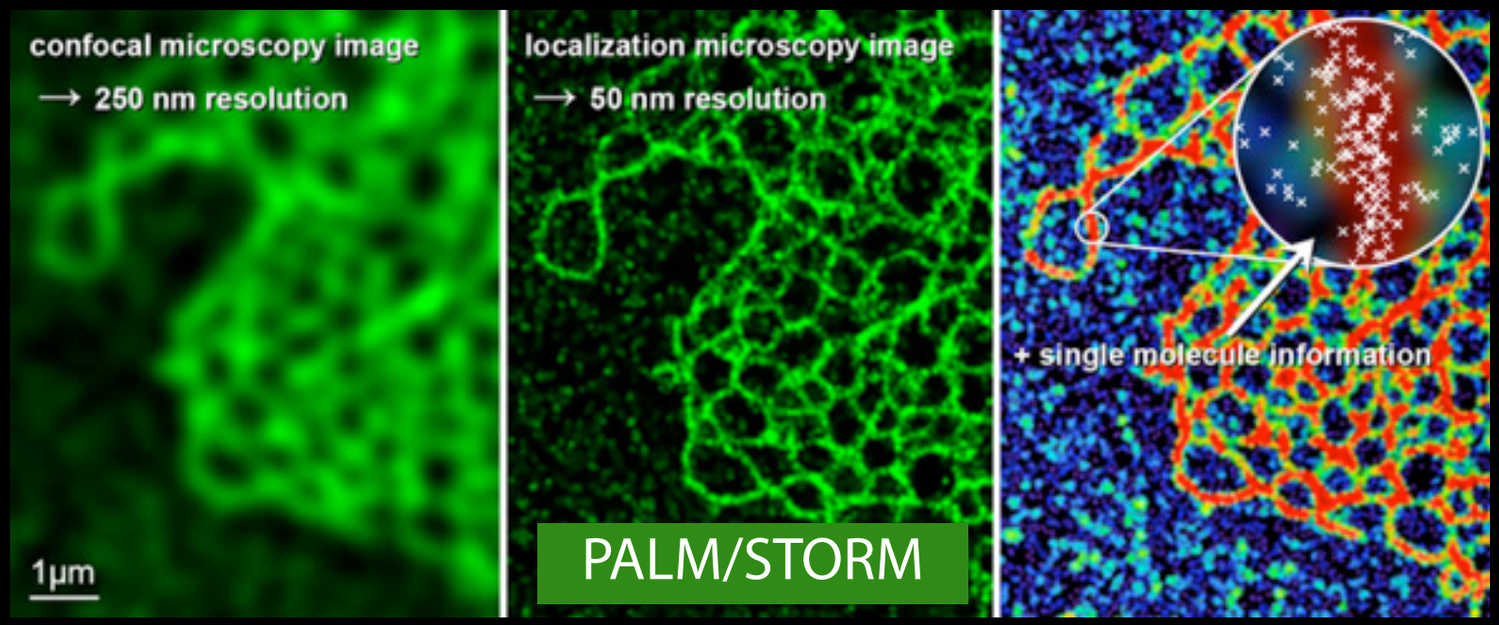
3D-SIM

100 nm (xy), 300 nm (z)



STED

± 60 nm (xy)



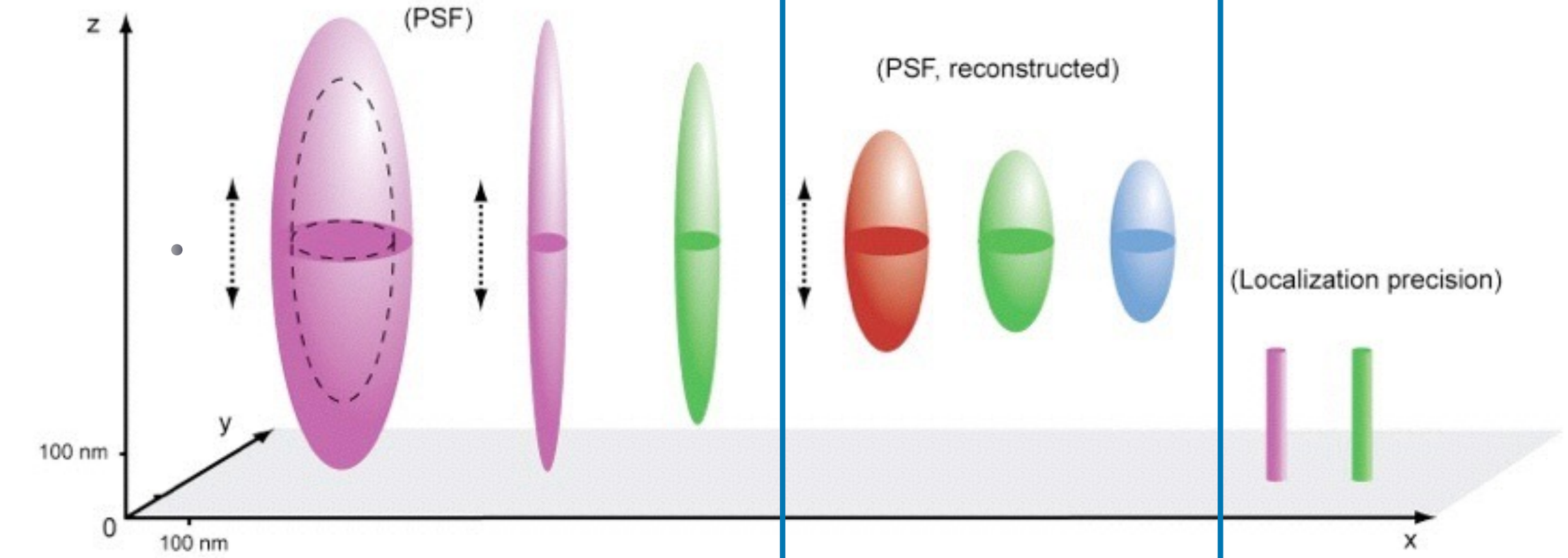
PALM/STORM

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	



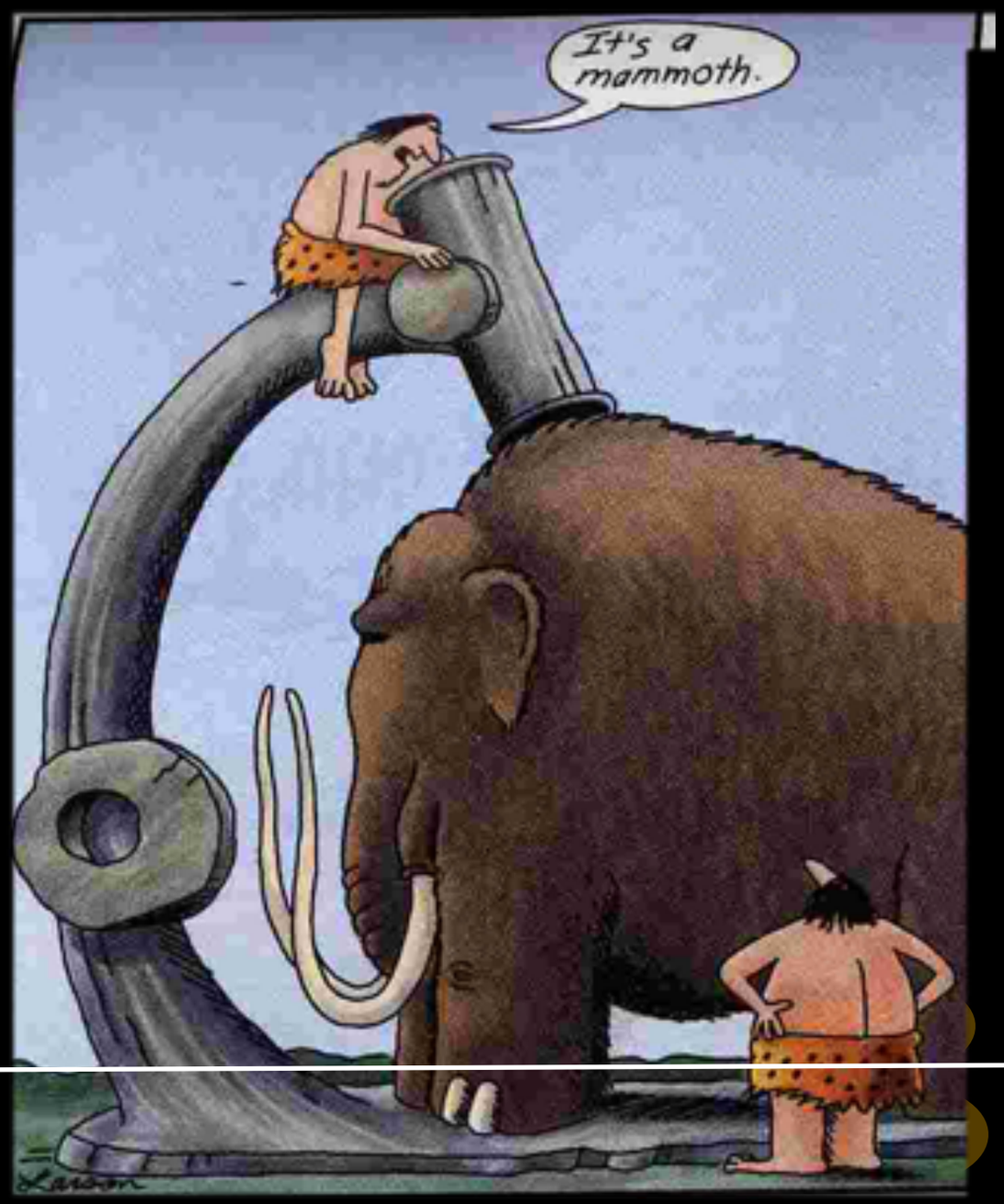
3D-SIM resolves ~8-fold smaller volumes than conventional microscopy

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)

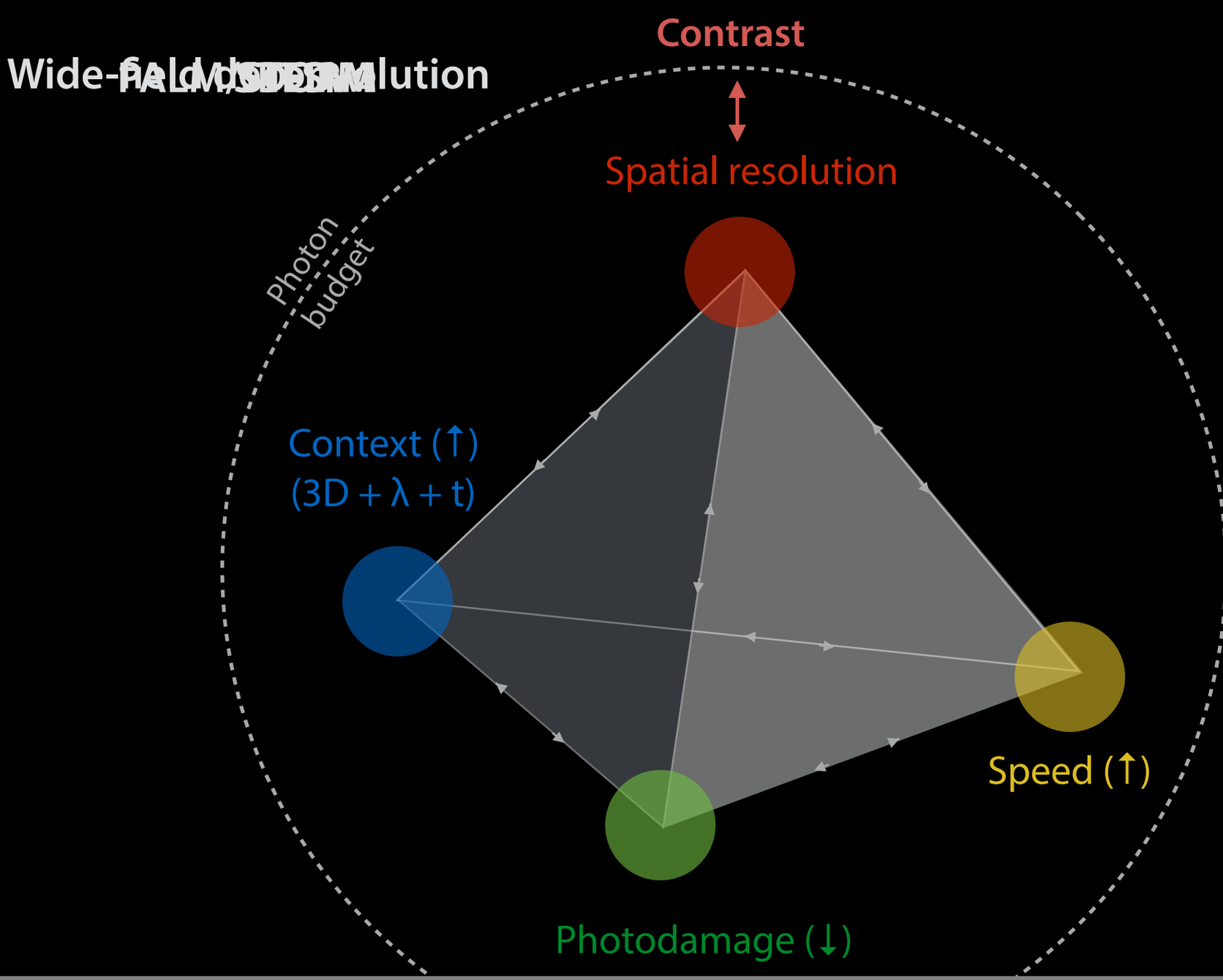
Not only resolution matters,

Multicolour & 3D information

Prague National Museum

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!

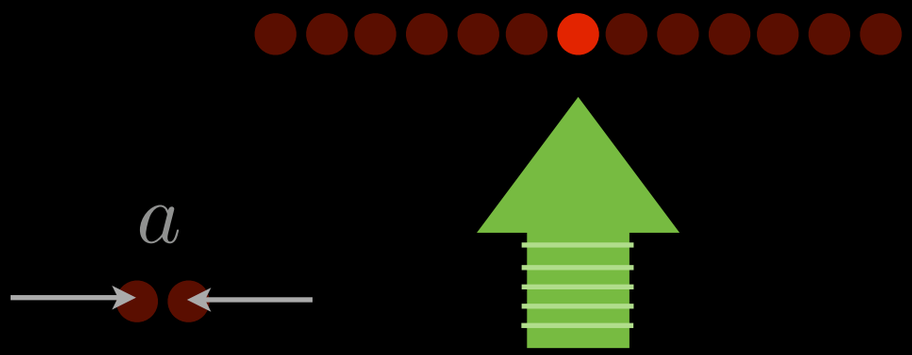
Contrast is the limit!!!

How to improve resolution with structured illumination?

The basic principle: Abbe‘s view

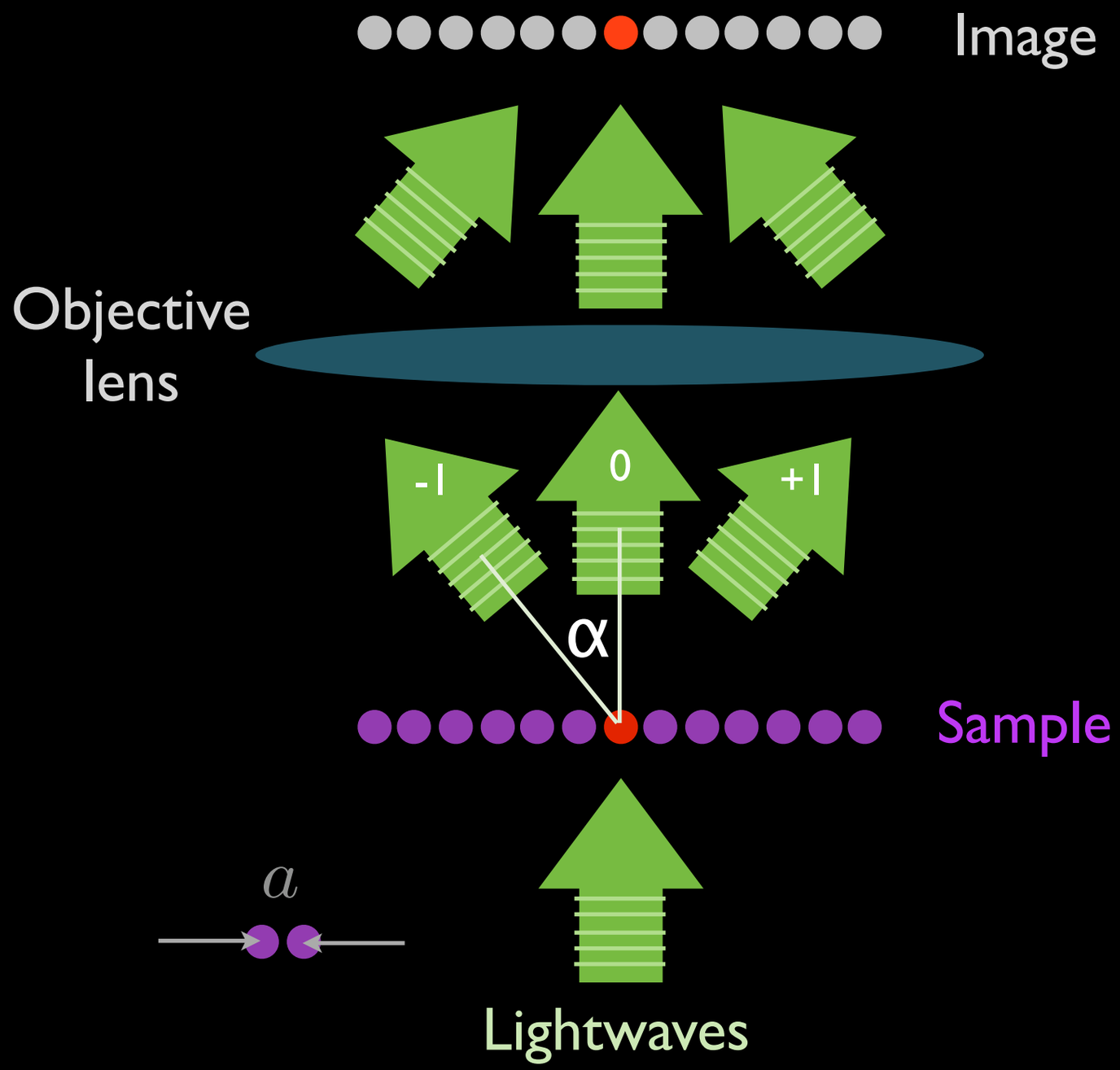
Sample = Structure

→ Periodicity



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

The basic principle: Abbe‘s view



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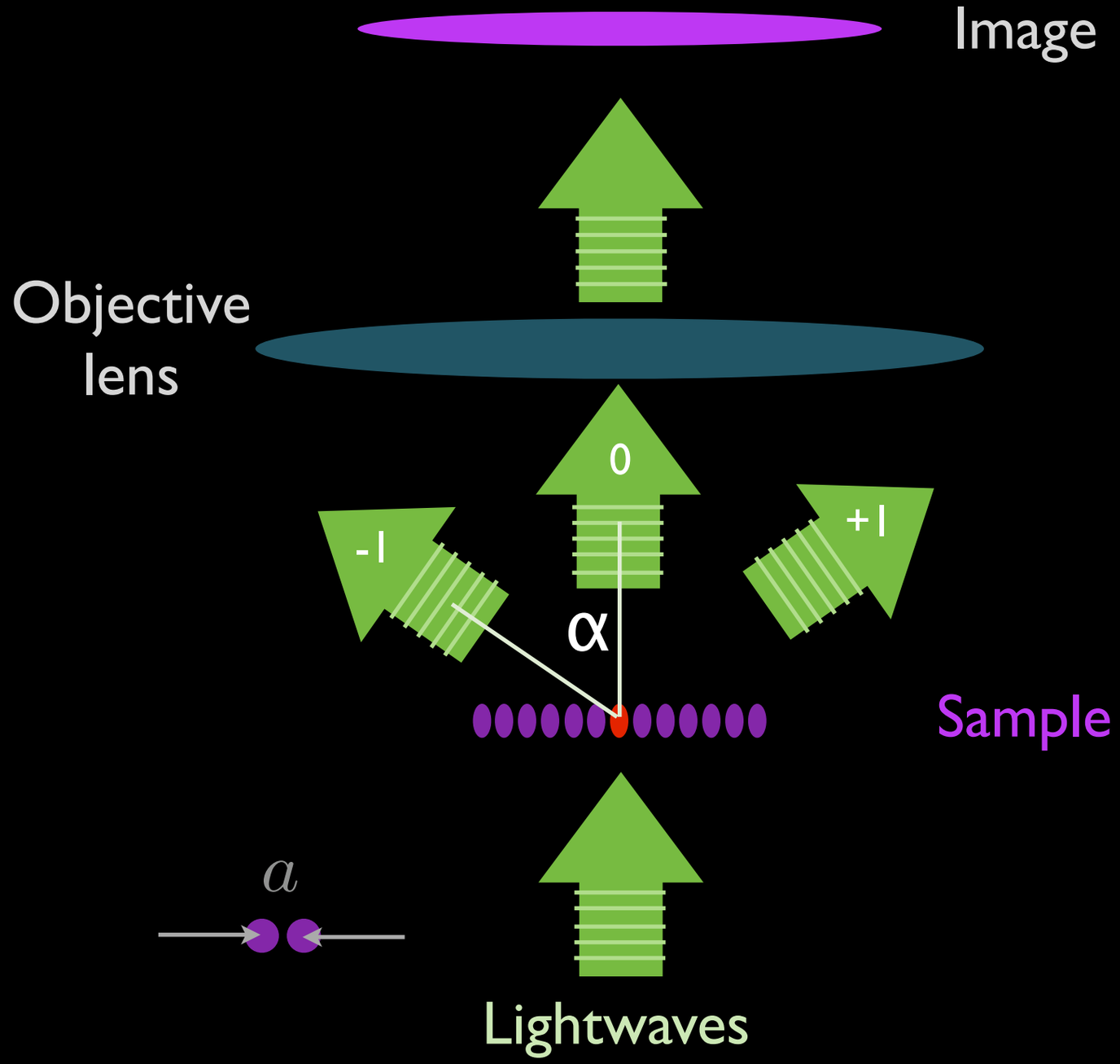
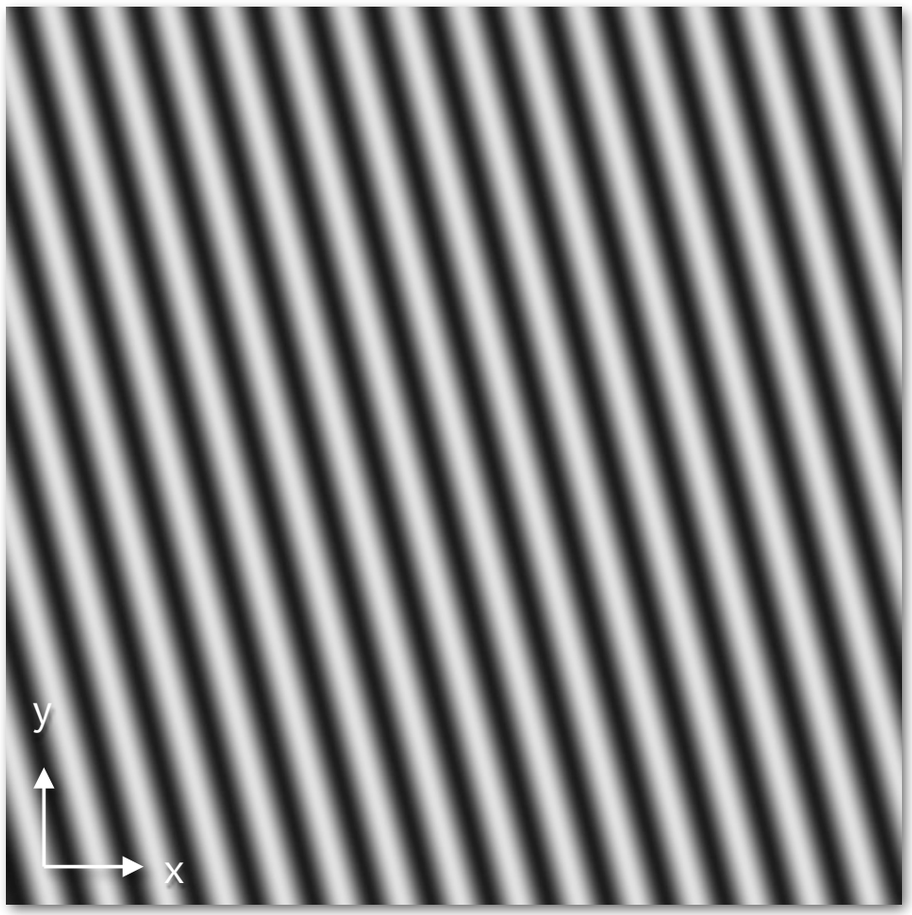


highest frequencies
(biggest α)
→
smallest structures

Fourier transformation in a nutshell

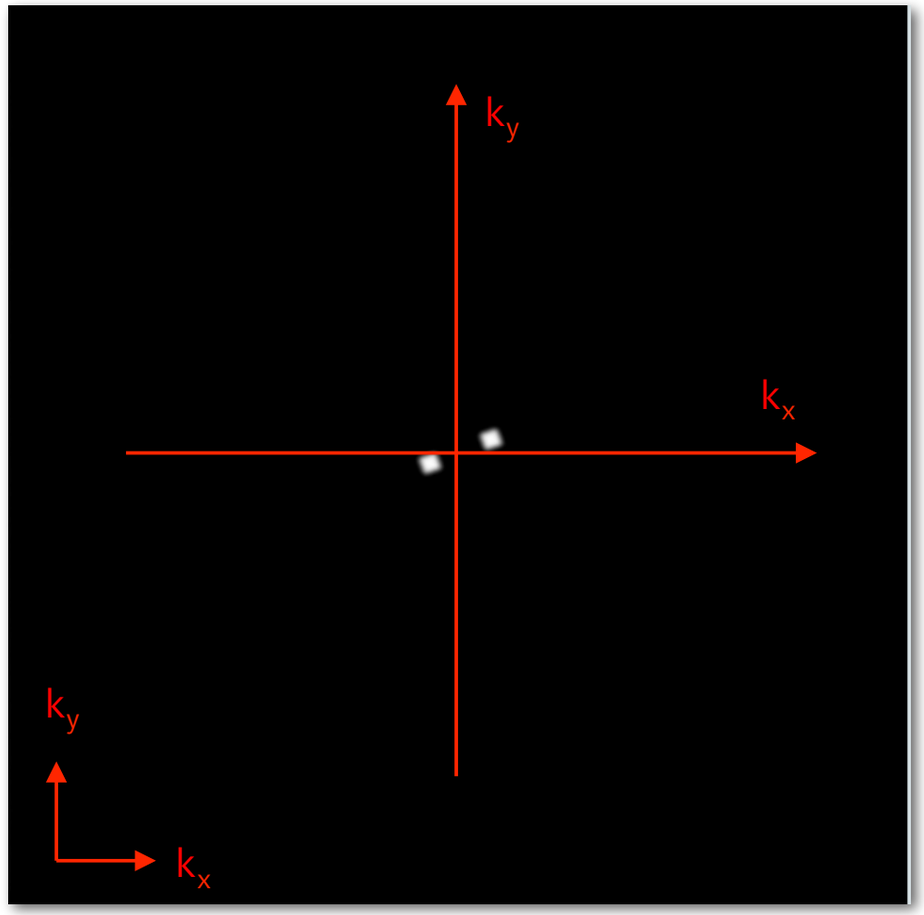
Real space (xy)



Fourier Transform

Inverse
Fourier Transform

Frequency space (k_x, k_y)
(a.k.a. Fourier space, reciprocal space)



Alternative representation of information
Low-resolution: near the origin
High-resolution: further out
kx, ky: Spatial frequencies, periods/μm

Image = superimposed periodicities

Real space (xy)

Fourier Transform

Inverse
Fourier Transform

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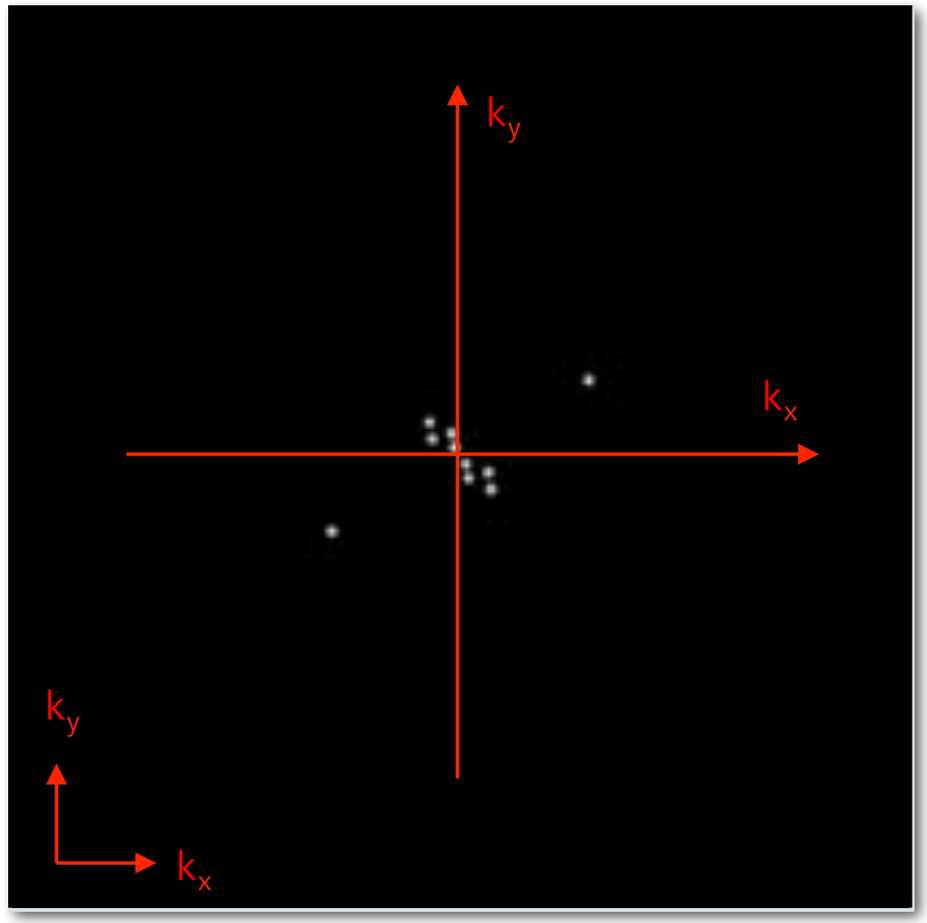

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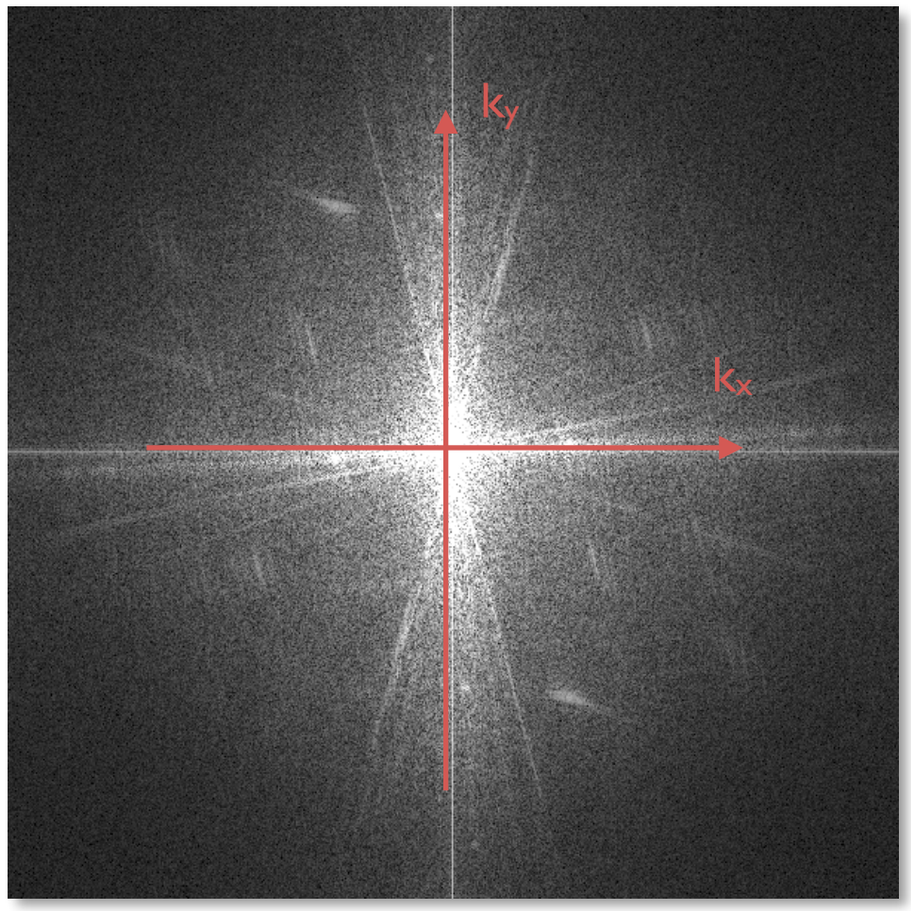
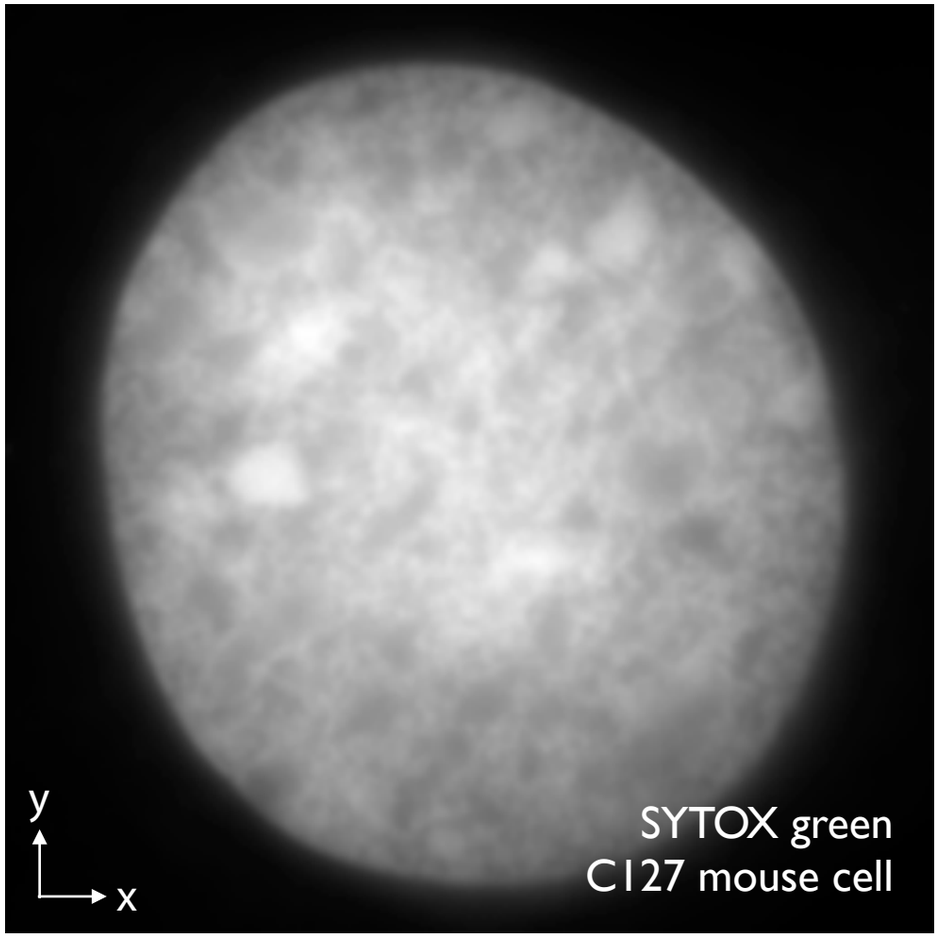


Alternative representation of information
Low-resolution: near the origin
High-resolution: farther out
kx, ky: Spatial frequencies, periods/μm

Frequency support in wide-field microscopy

Image = real space (xy)

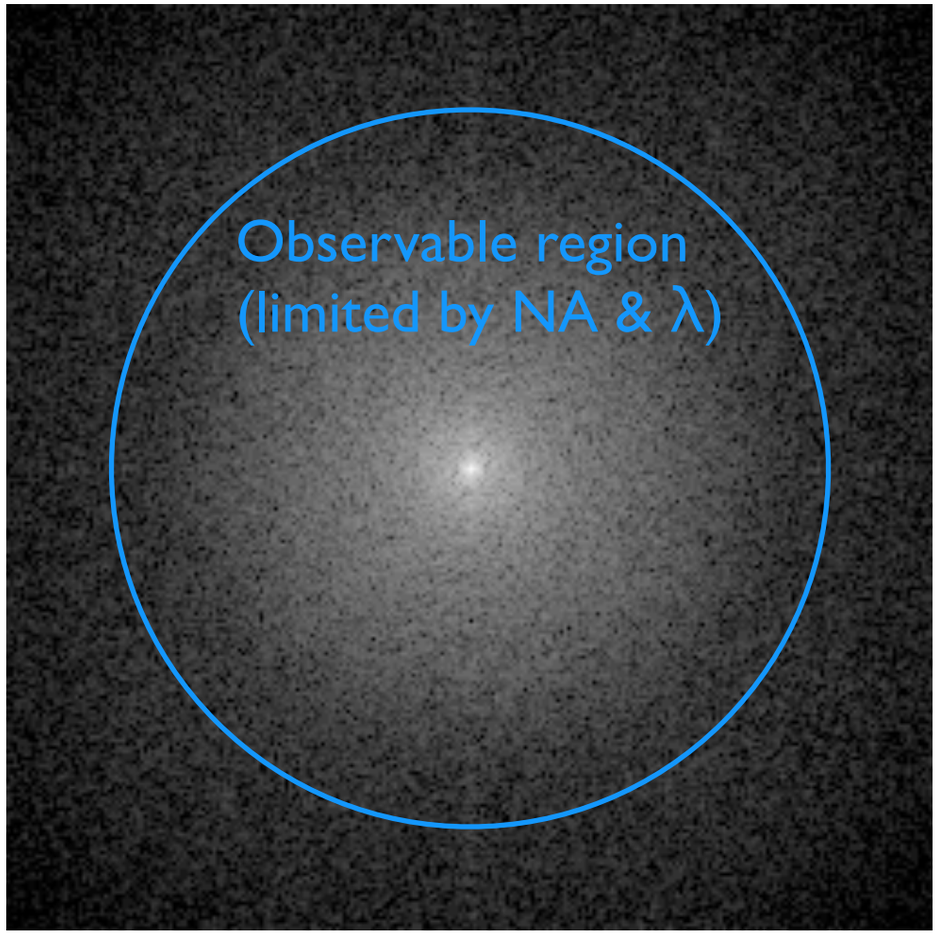


Fourier Transform

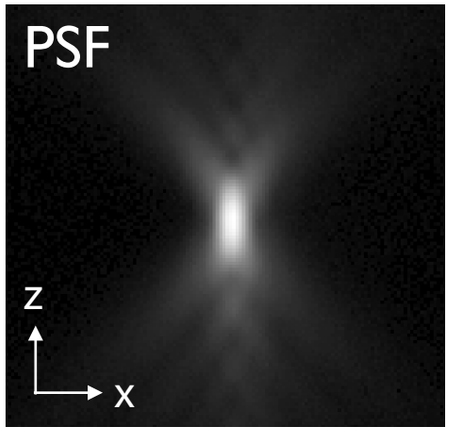
(inverse FT)

Frequency space (k_x, k_y)

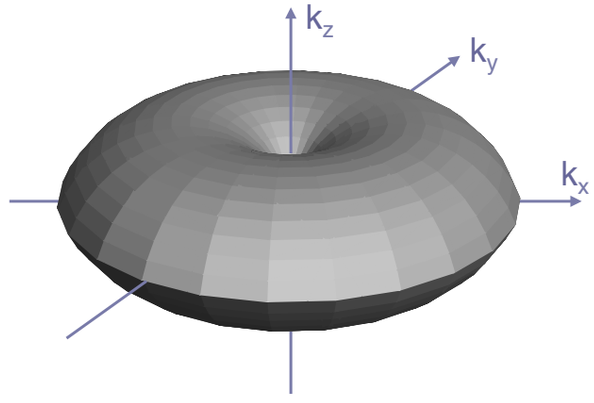
(a.k.a. Fourier space, reciprocal space)



"Real object" ⊗



Full frequency range ×



SIM principle: Moiré interference

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

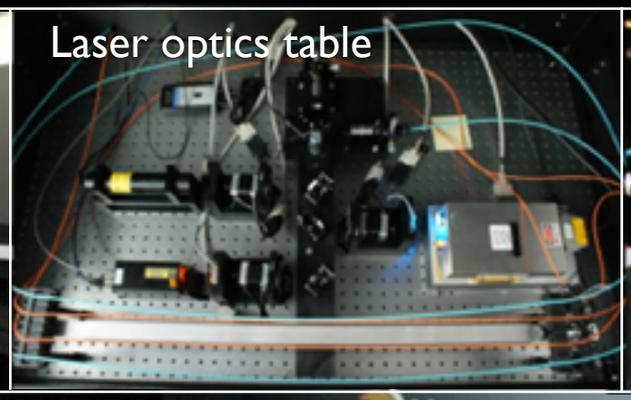
unknown structure

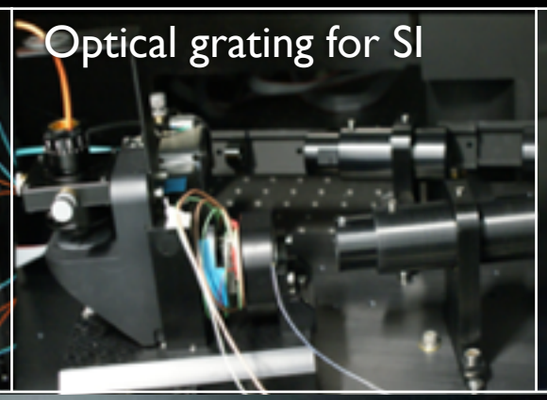
Fourier transform of the measured image

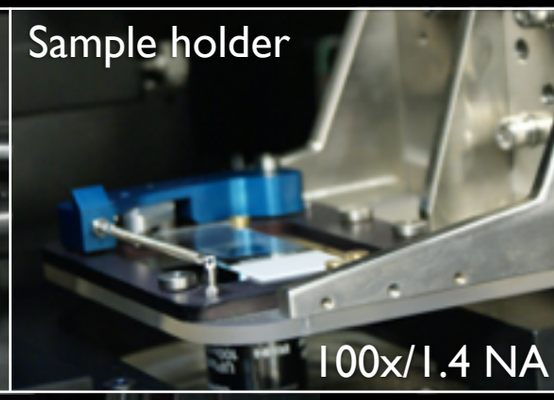
$$F\{f \times g\} = F\{f\} \otimes F\{g\} \longrightarrow F\{f\} = F\{f \times g\} \otimes^{-1} F\{g\}$$

known illumination function

OMX 3D-SIM microscope system

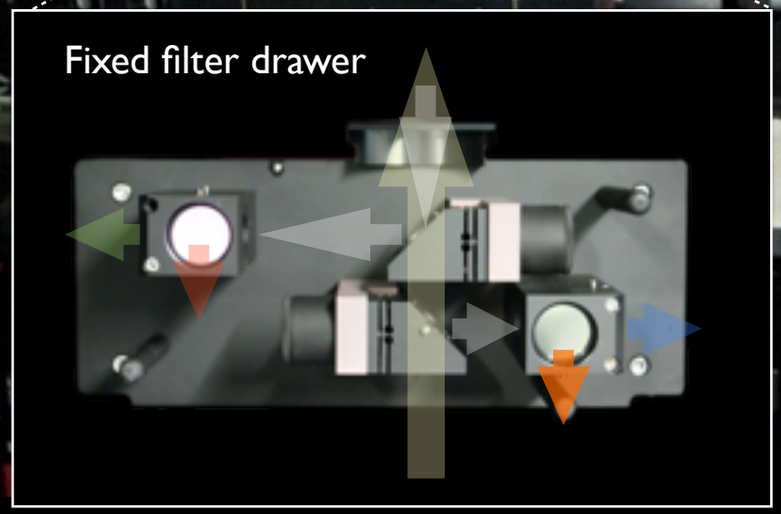






Max. mech. stability
Highest sensitivity

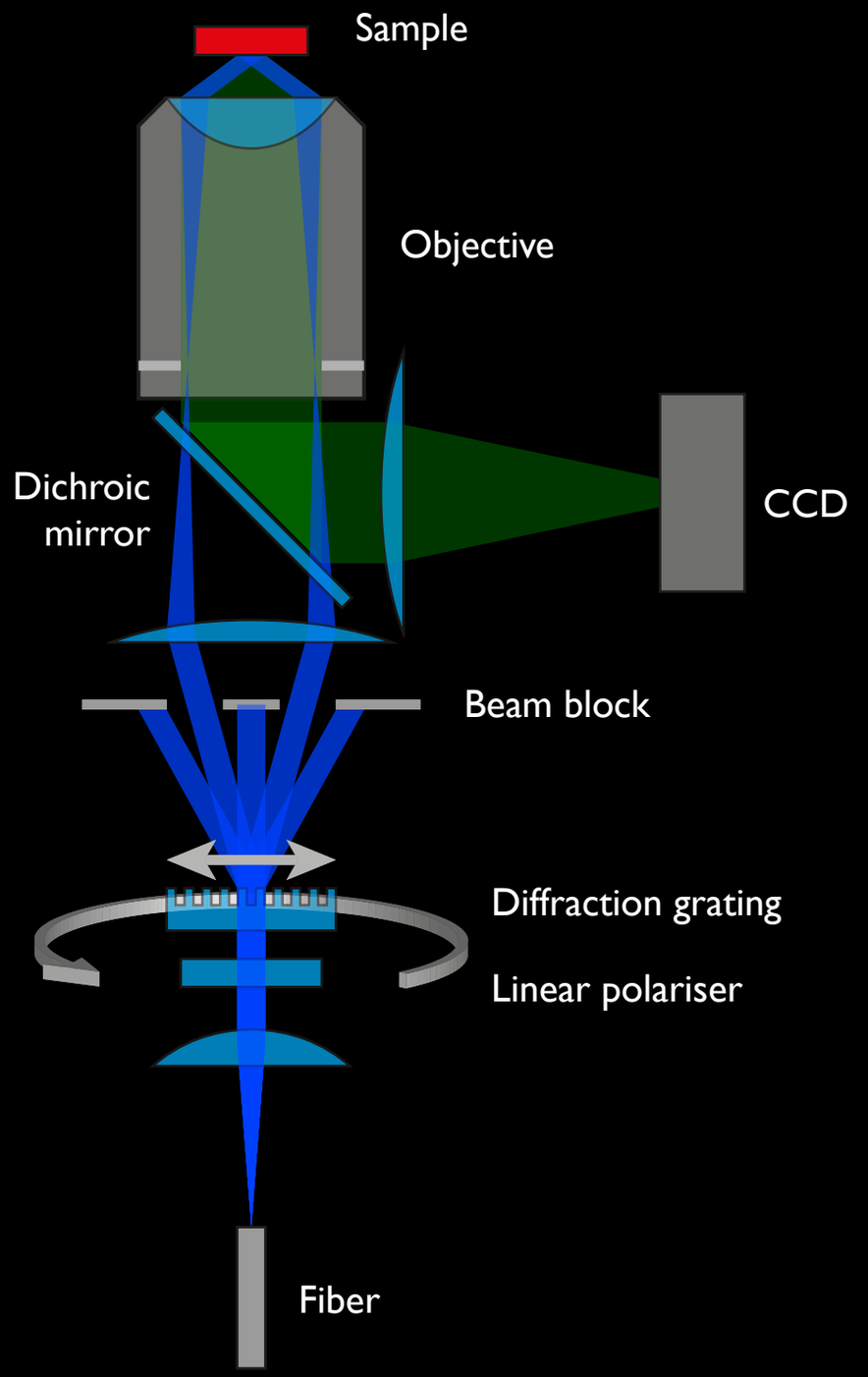
High-sensitive
EMCCD camera
(3x)



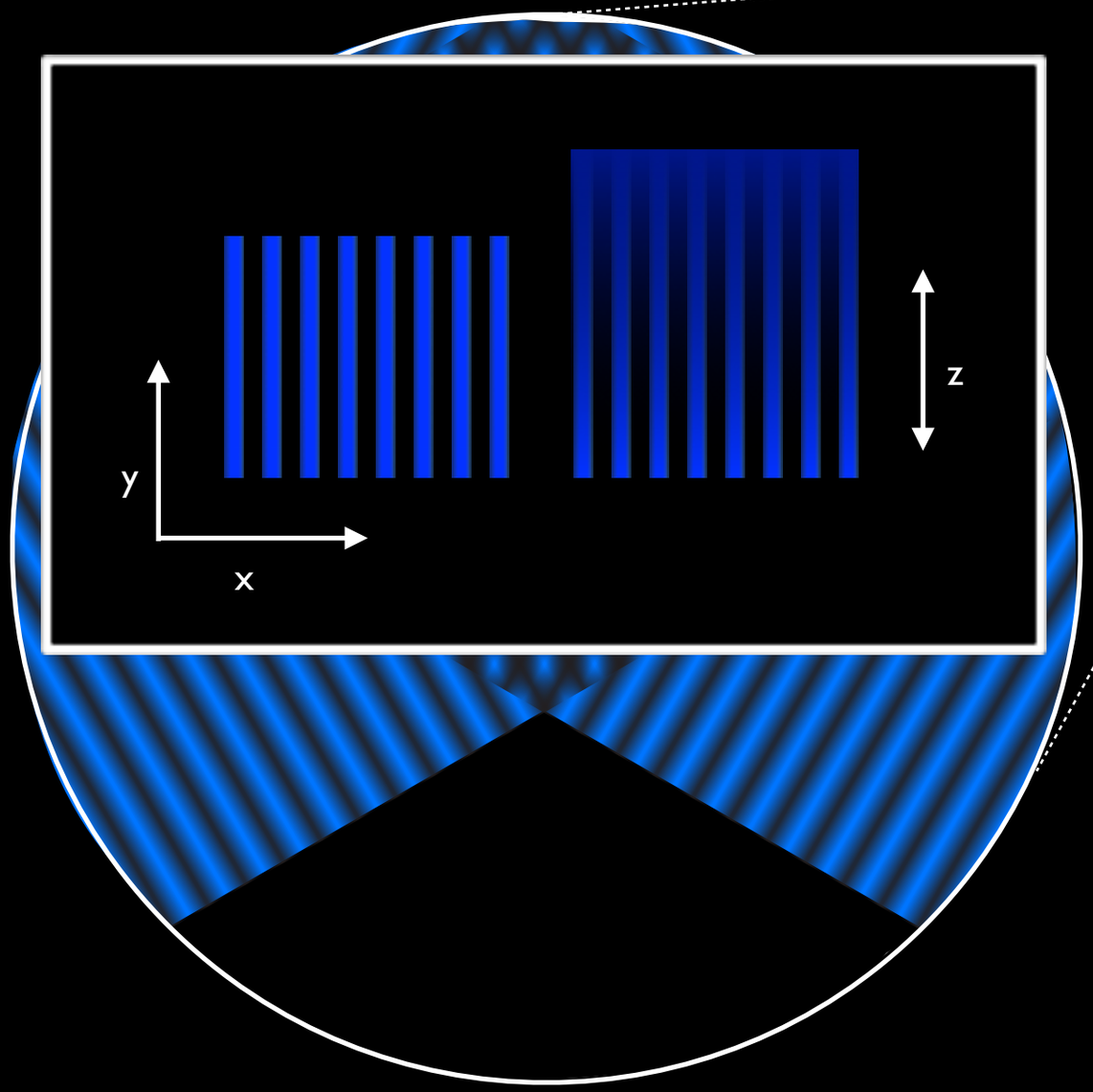
Mess

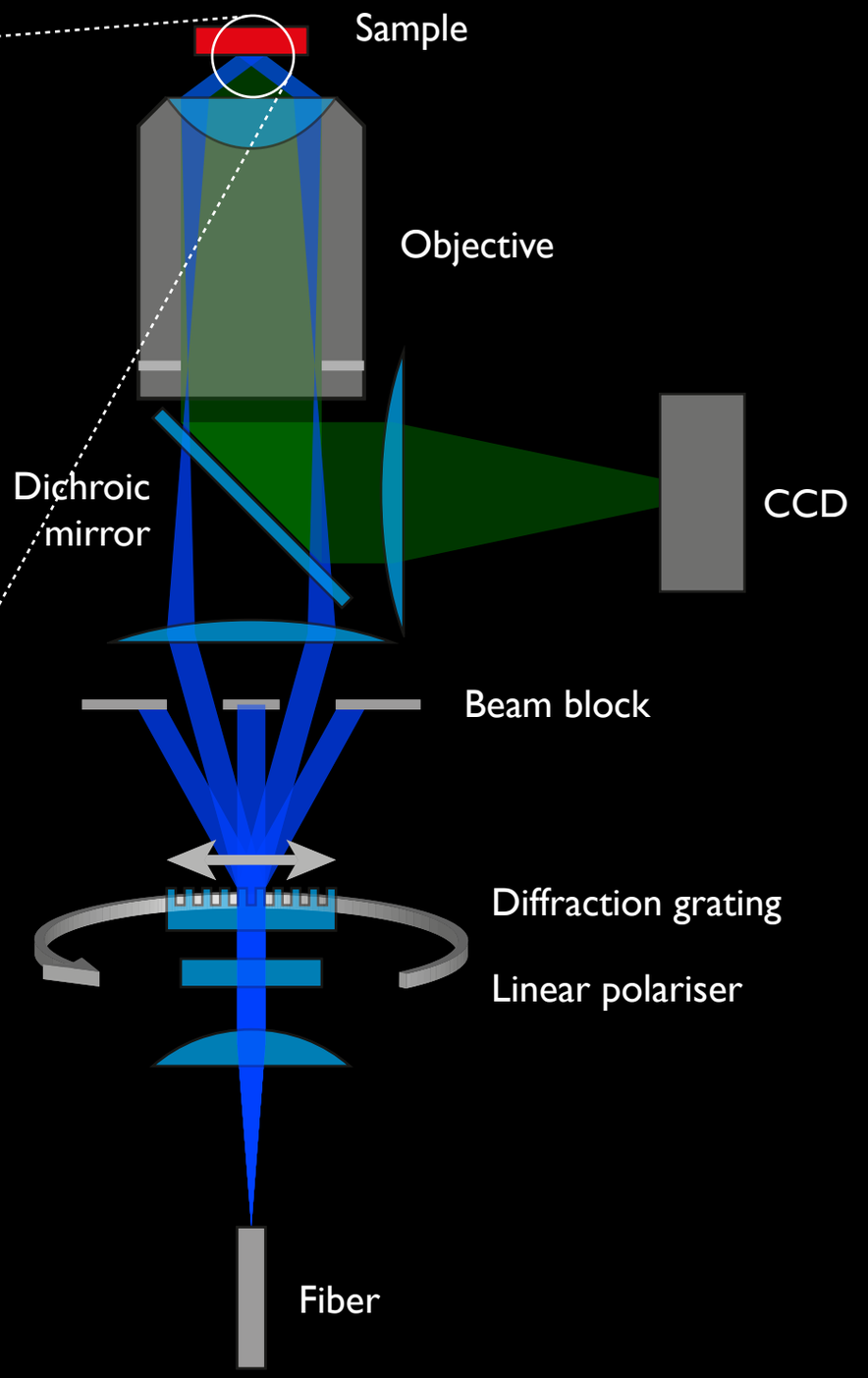
OMX V2

2D-SIM: microscope design



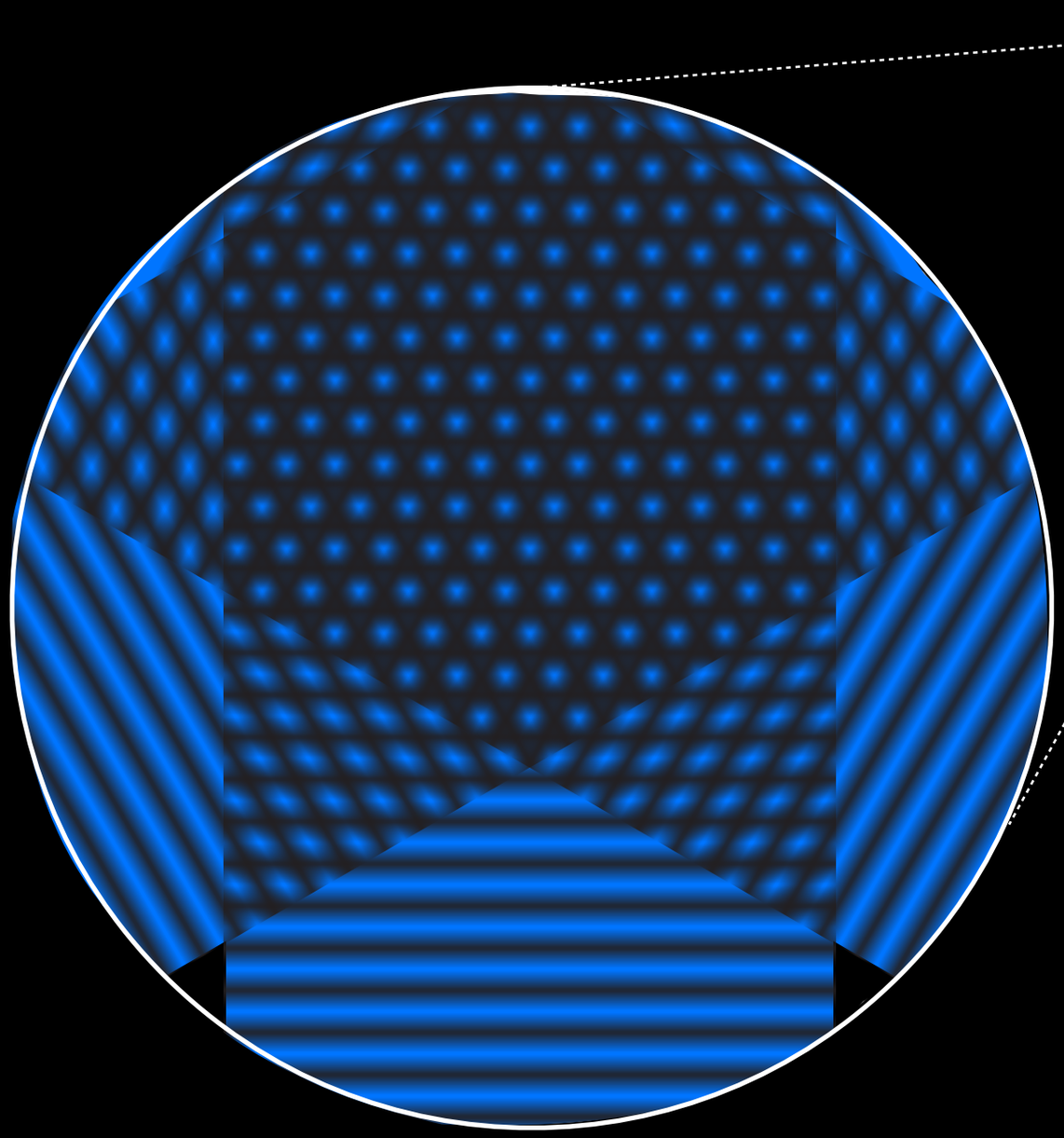
2D-SIM: microscope design

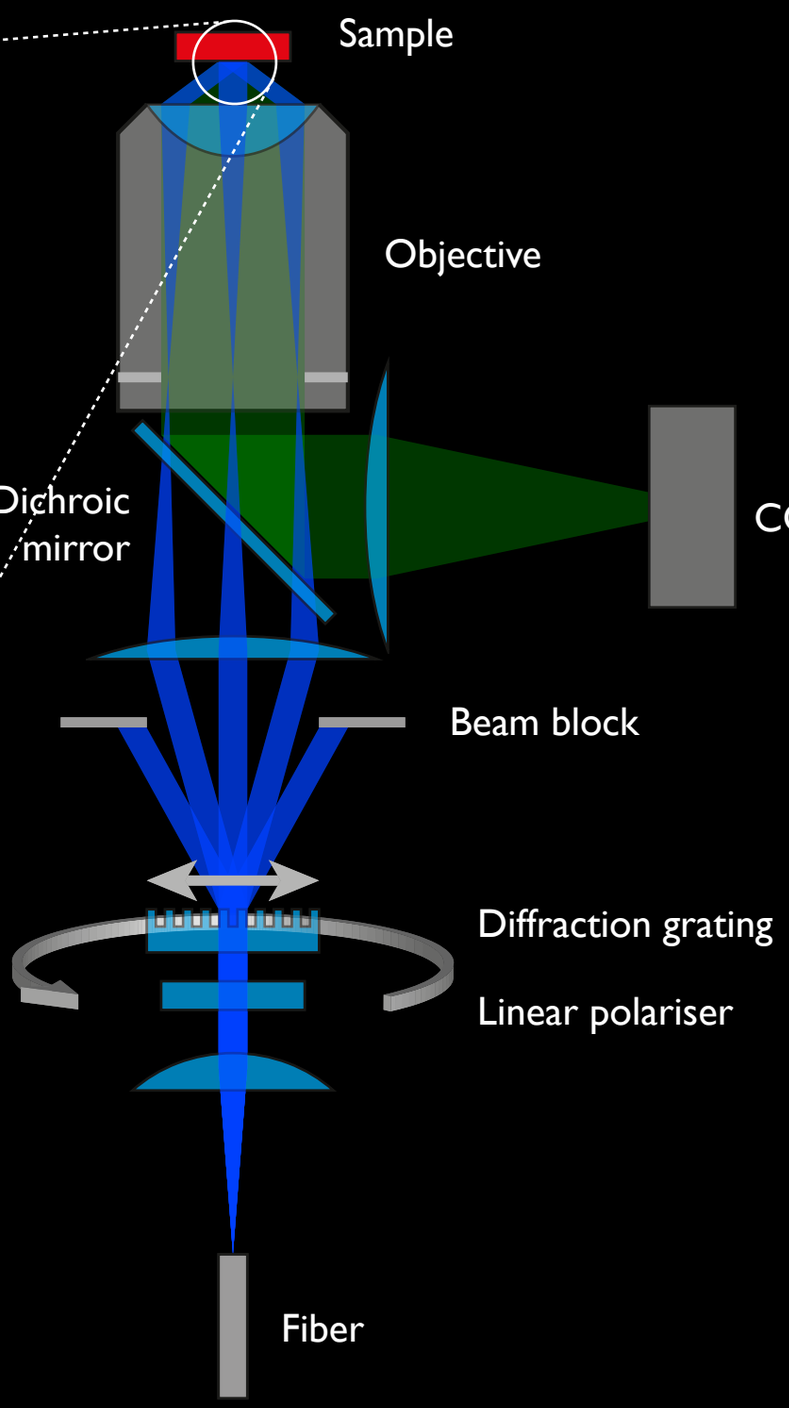




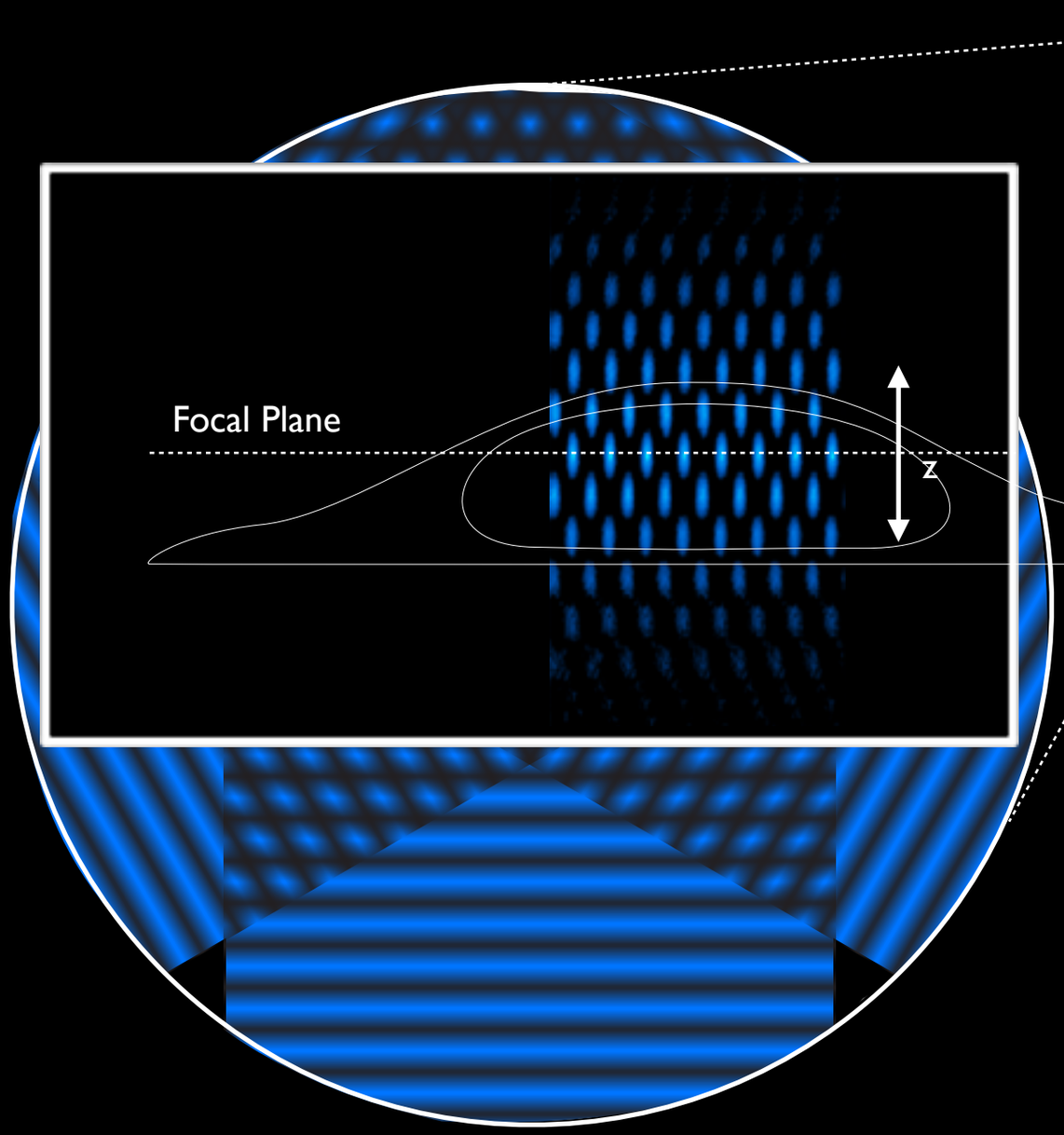
2-beam interference => modulation (stripes) in xy

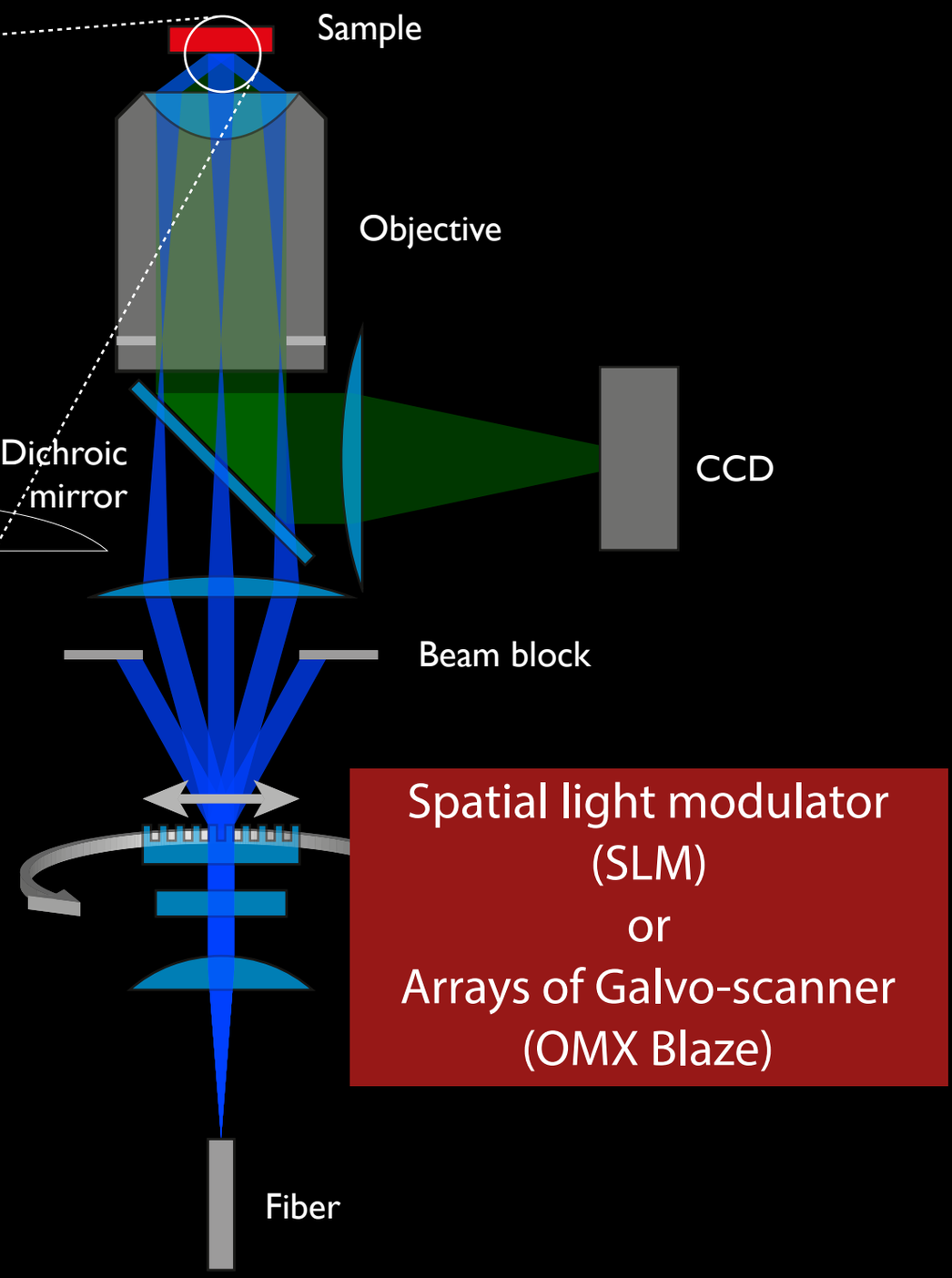
3D-SIM: microscope design





3D-SIM: microscope design

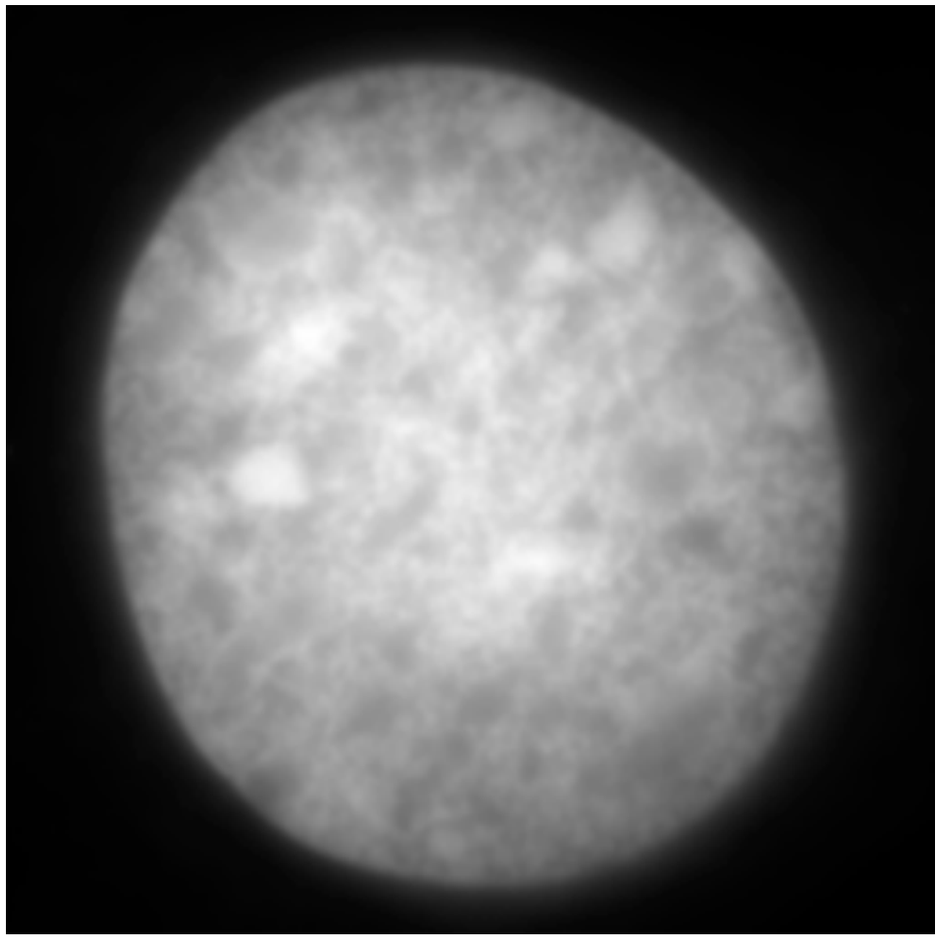




3-beam interference => modulation in xy and z

Frequency support in wide-field microscopy

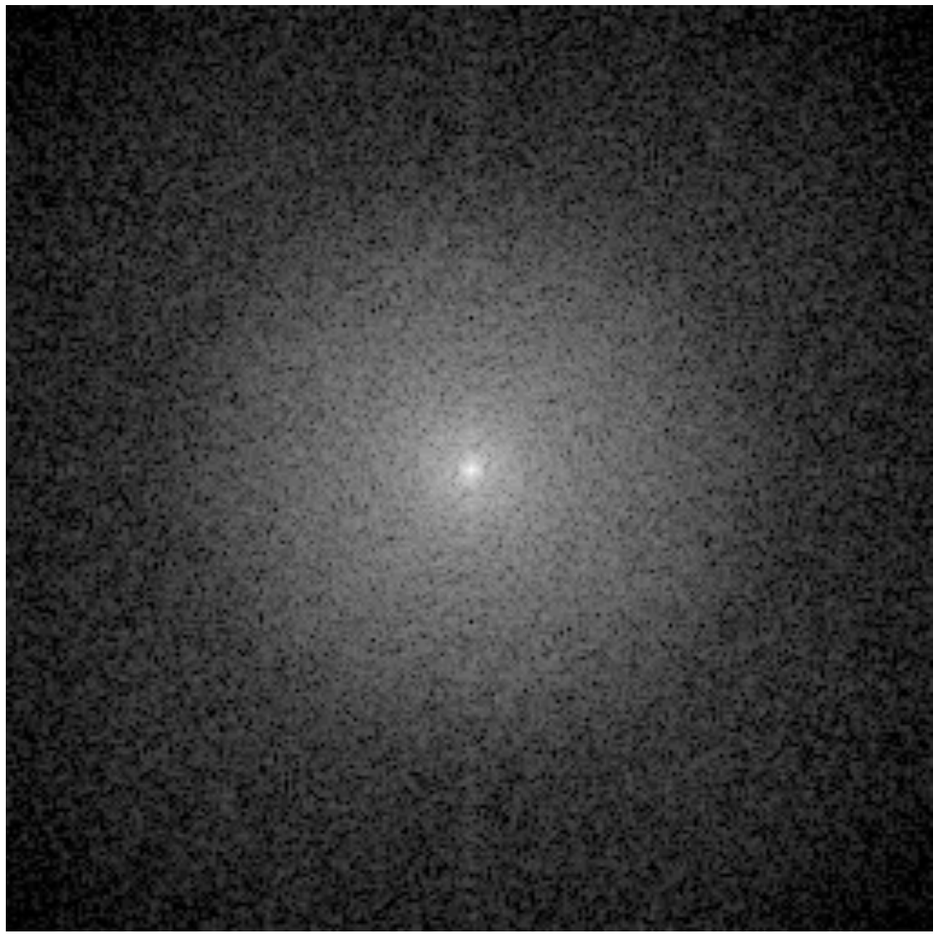
Real space



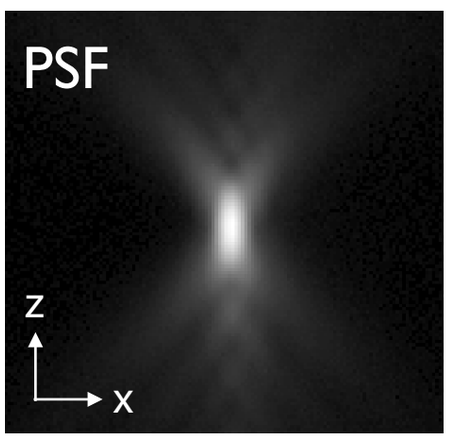
Fourier Transform

(inverse FT)

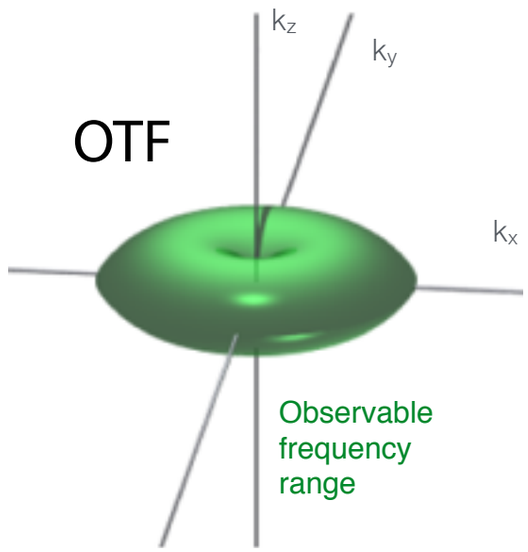
Reciprocal space



"Real object" ⊗ convolved

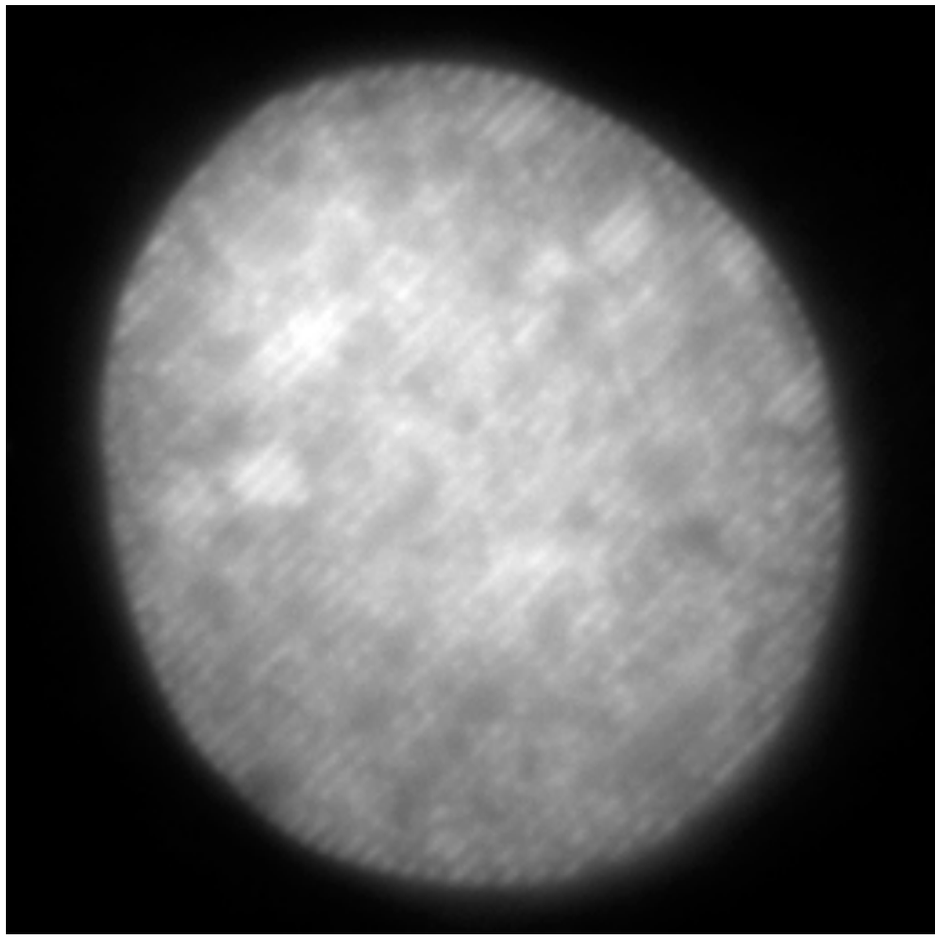


Full frequency range ×



Doubling frequency support in x-y and z

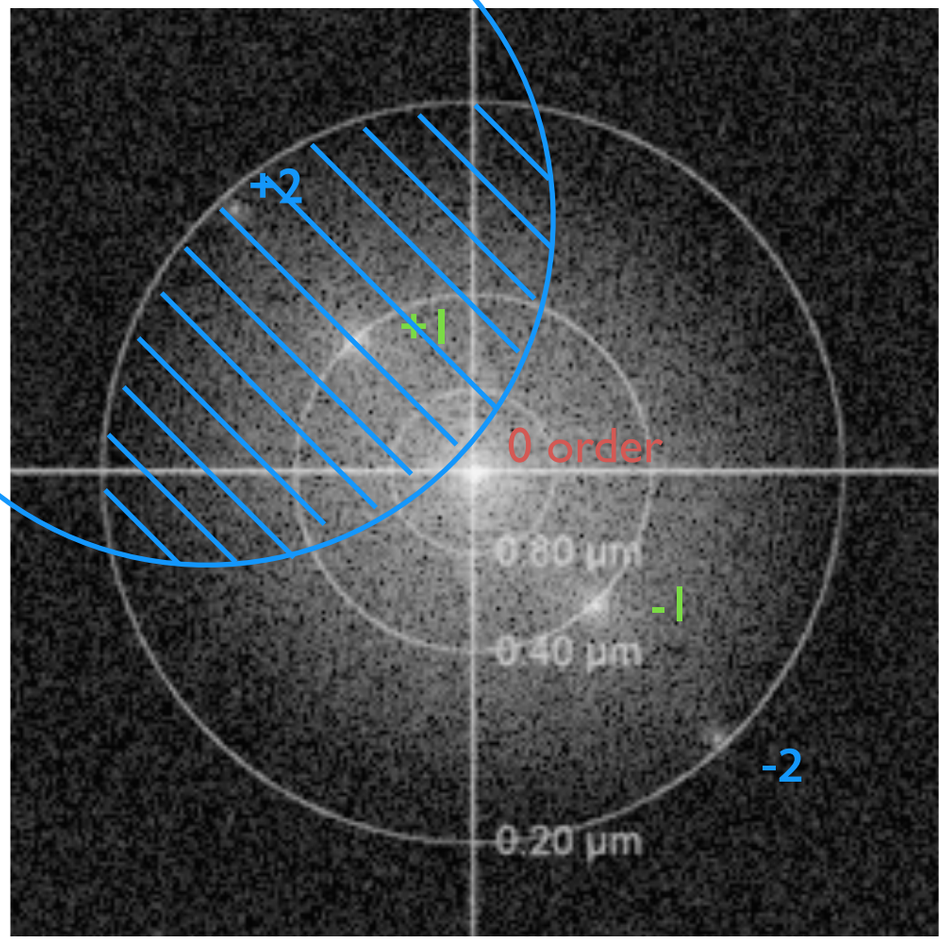
Real space

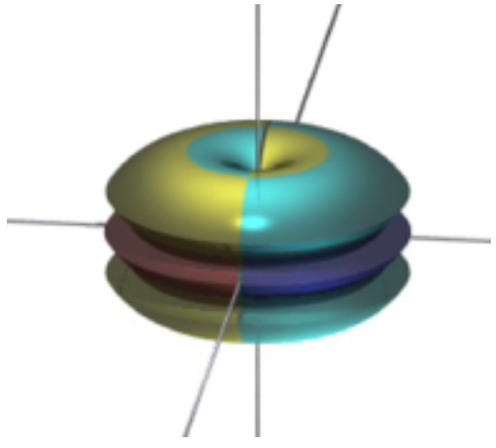


Fourier Transform

(inverse FT)

Reciprocal space

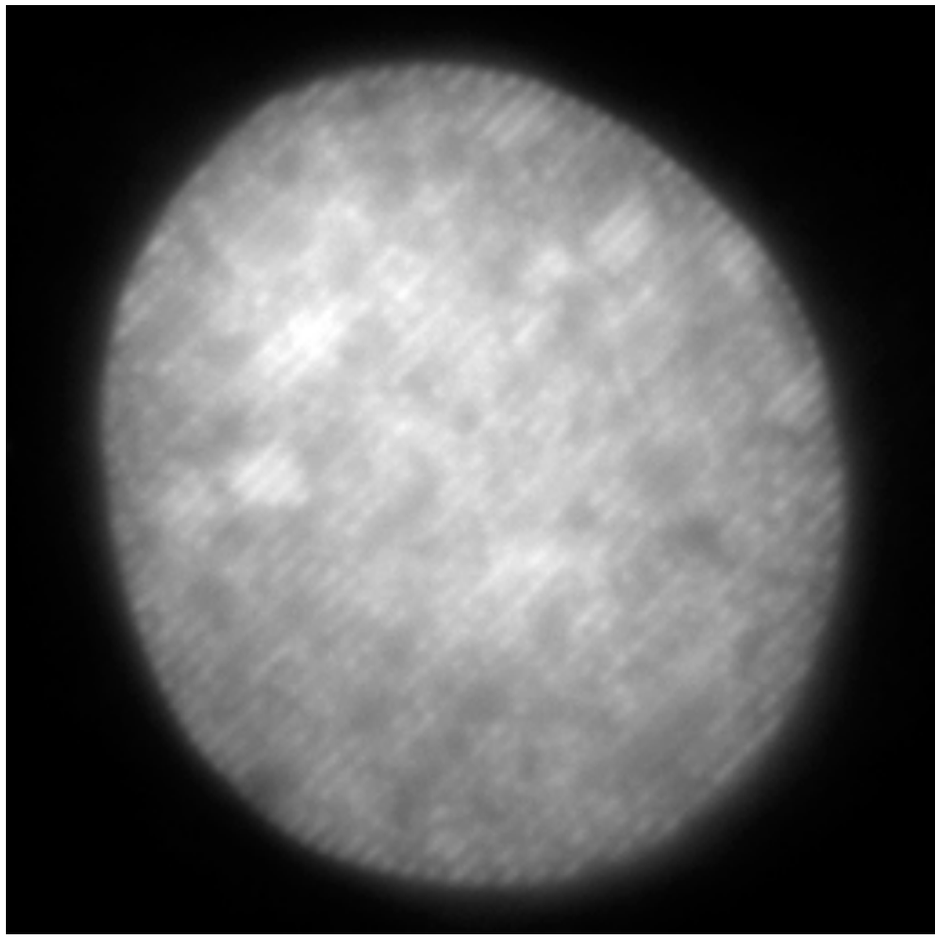




adapted from Gustafsson et al. (2008), *Biophys J* **94**

Doubling frequency support in x-y and z

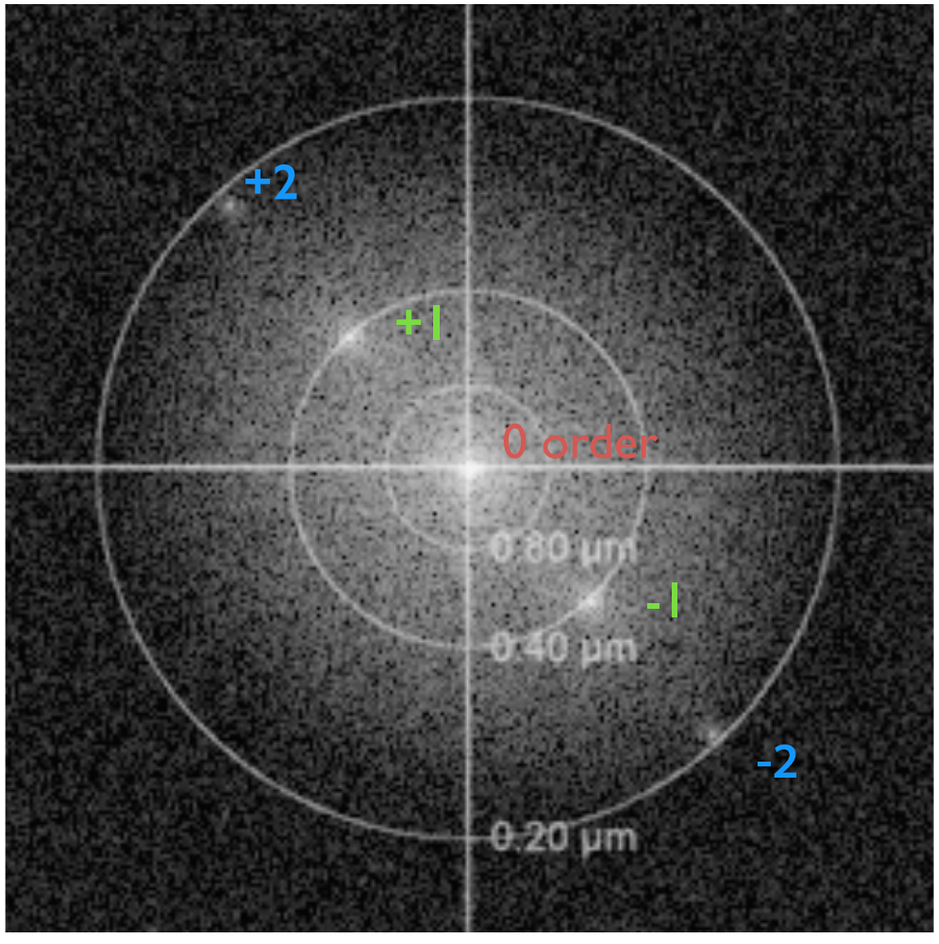
Real space



Fourier Transform

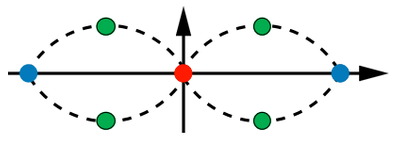
(inverse FT)

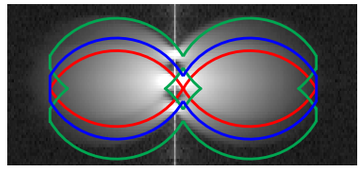
Reciprocal space

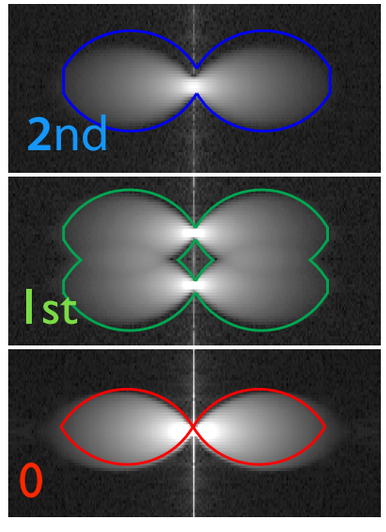


axial

(x-z)

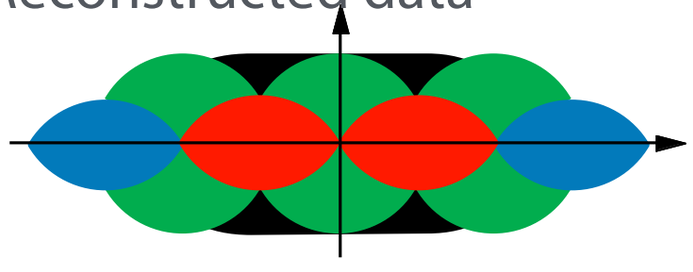






Band separation

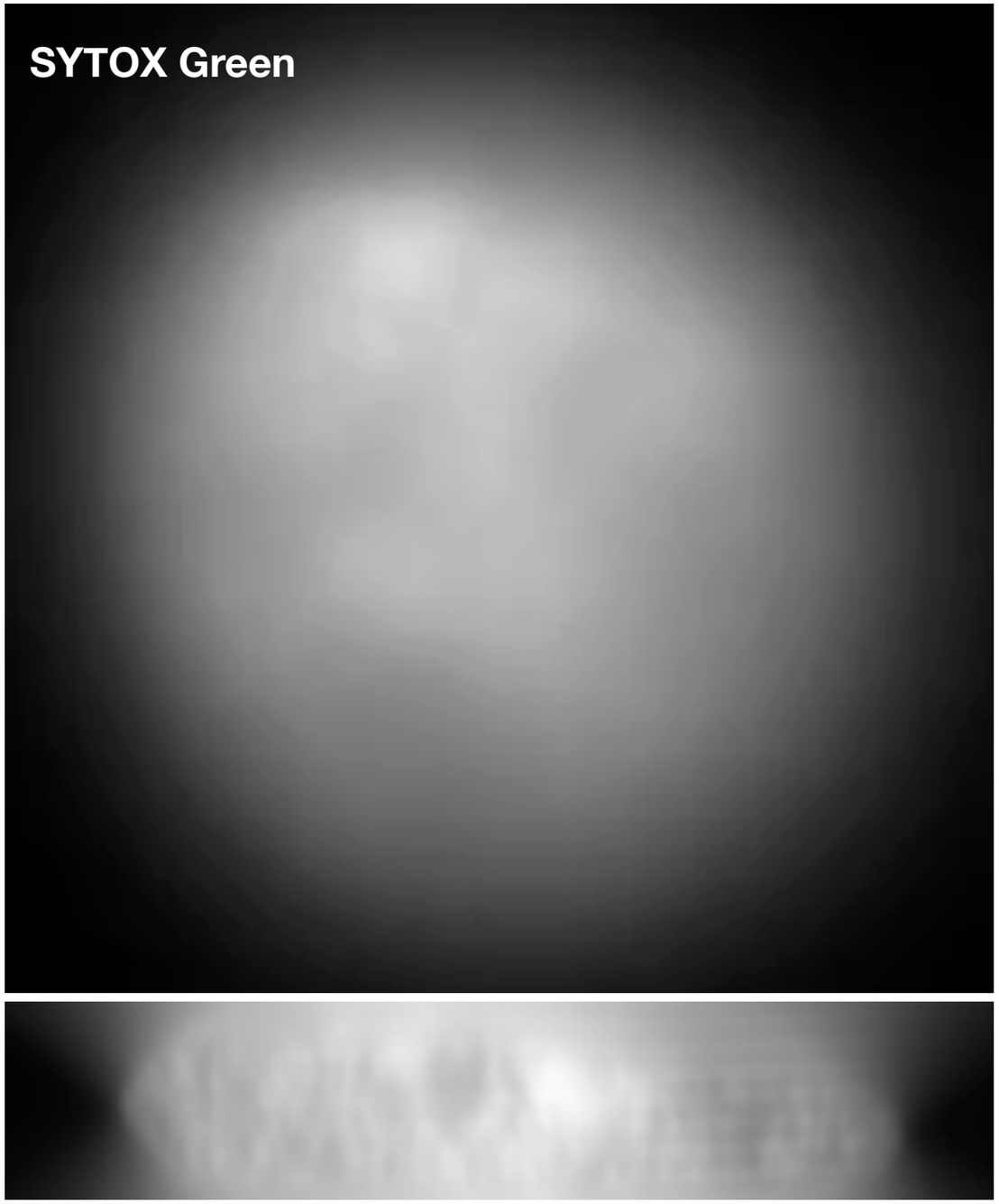
Reconstructed data

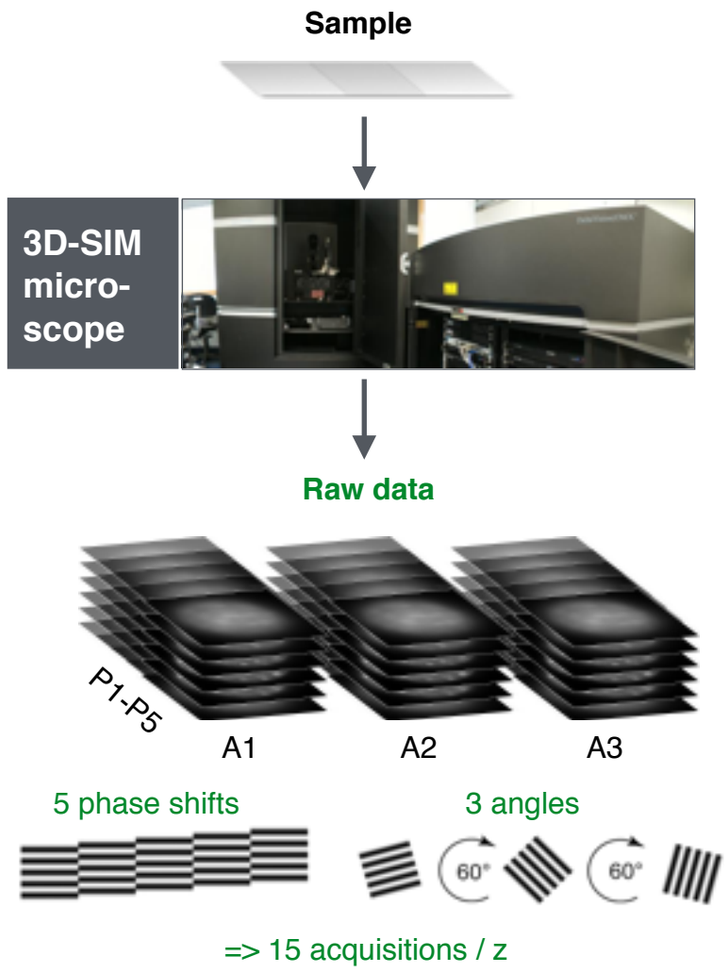


adapted from Gustafsson et al. (2008), *Biophys J* **94**

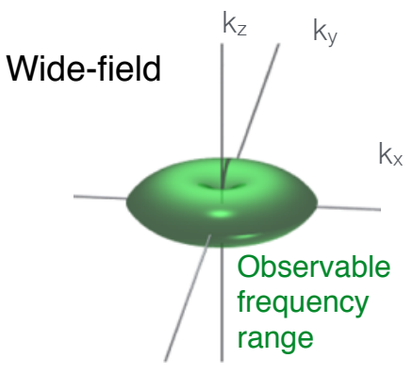
From wide-field to 3D-SIM

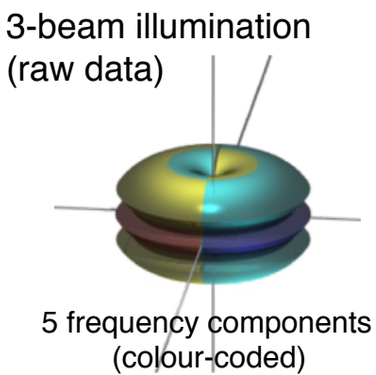
Mouse C127 cell



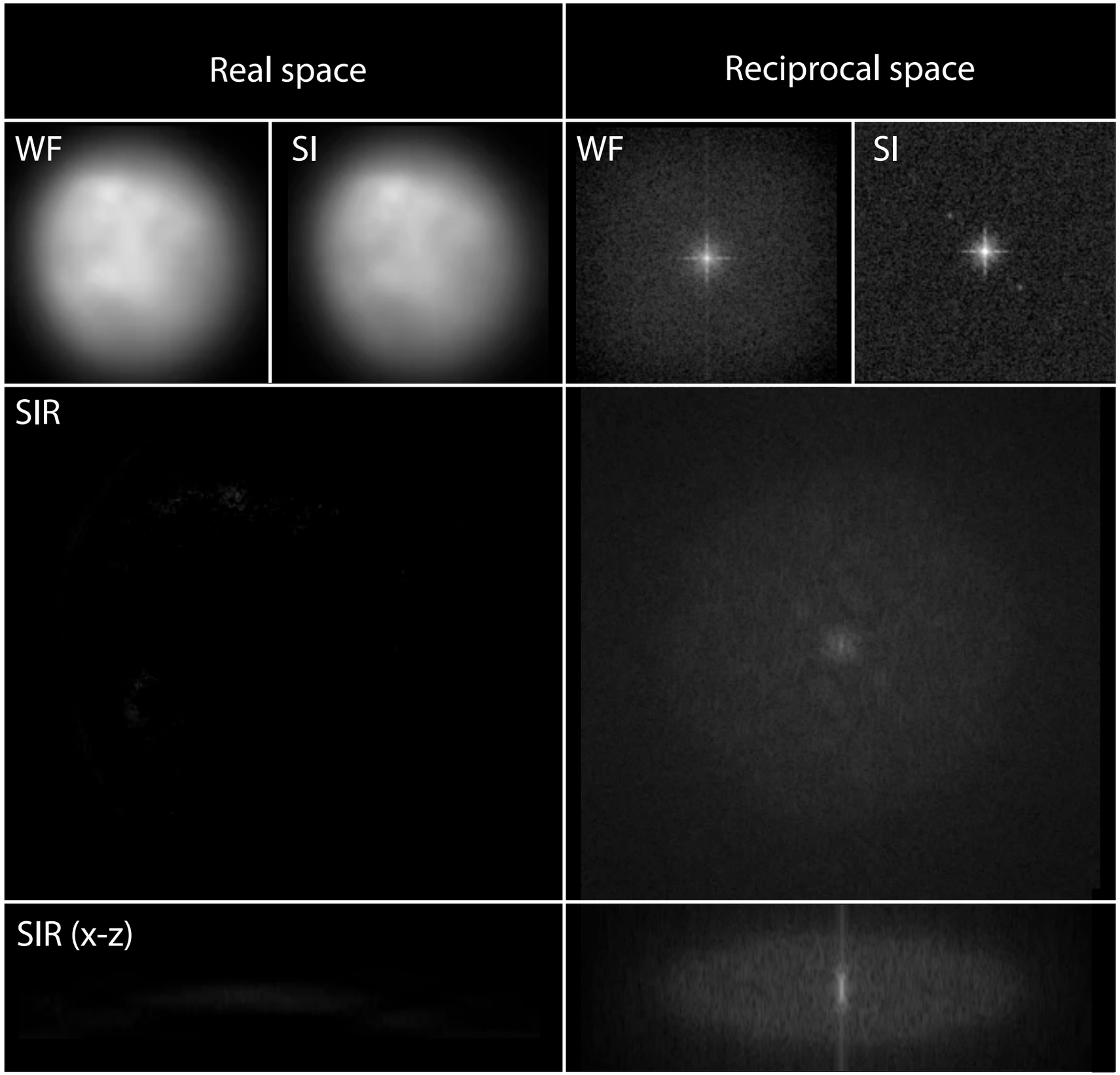


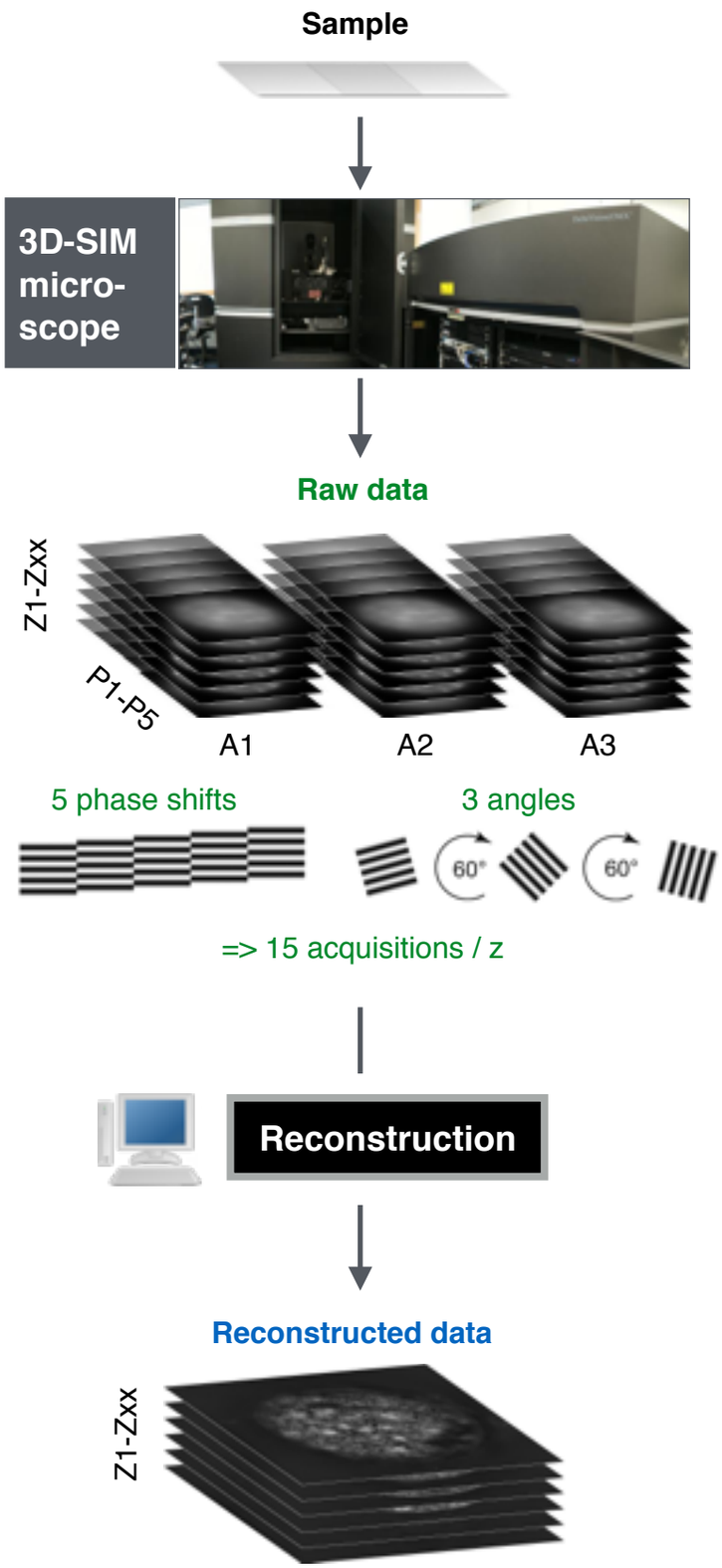
=> 15 acquisitions / z



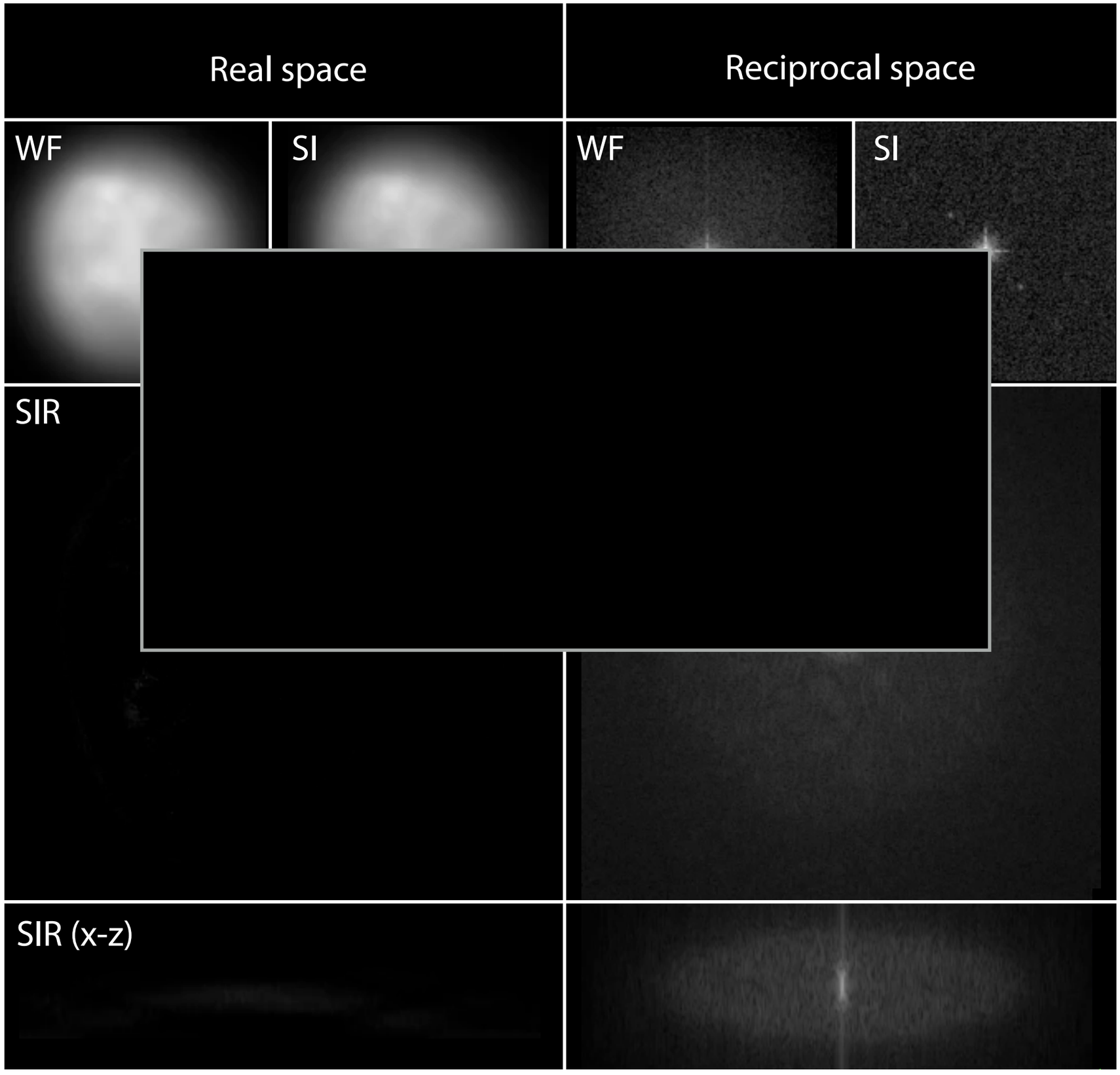


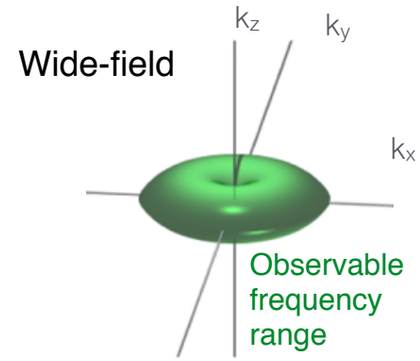
Overview of SIM processing

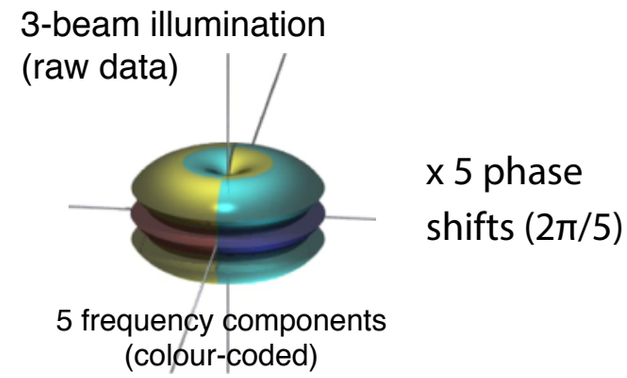


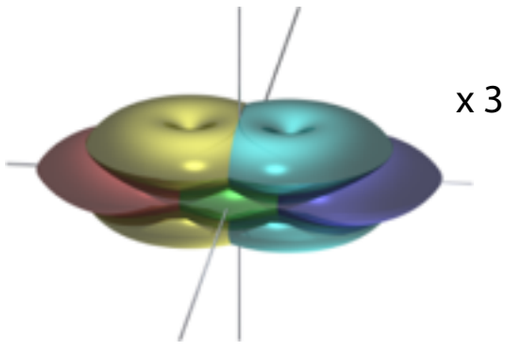


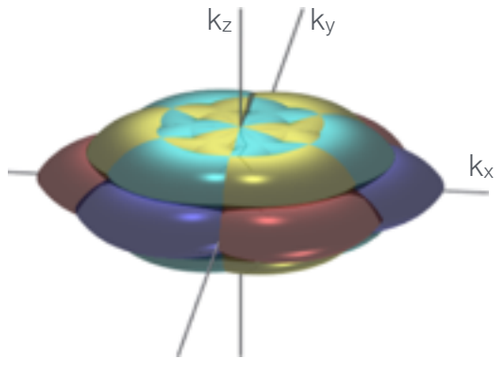
Overview of SIM processing











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

3D-SIM of a prophase nucleus

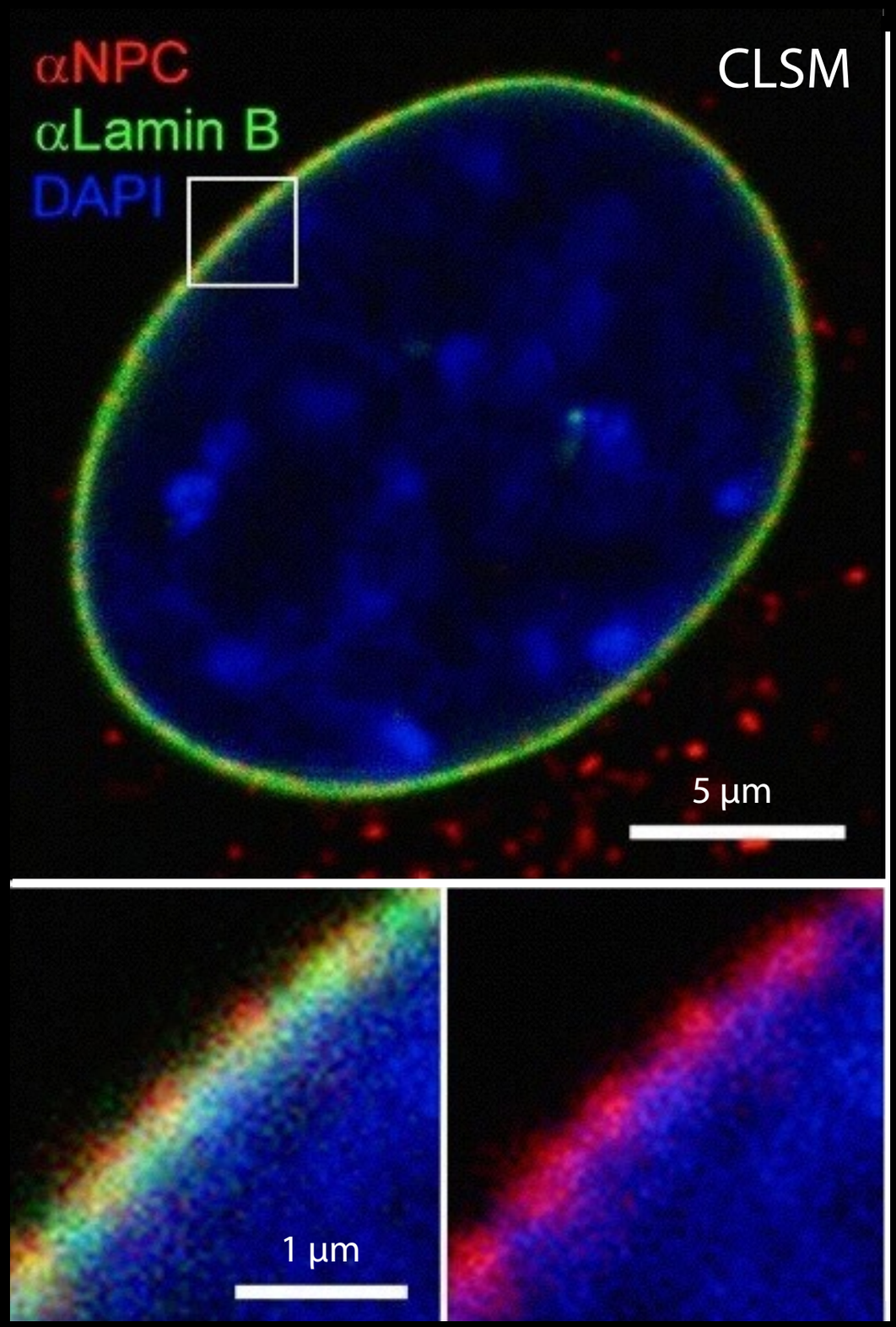
Lamin B
DAPI

3D volume
rendering

Mouse C2C12 cell

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

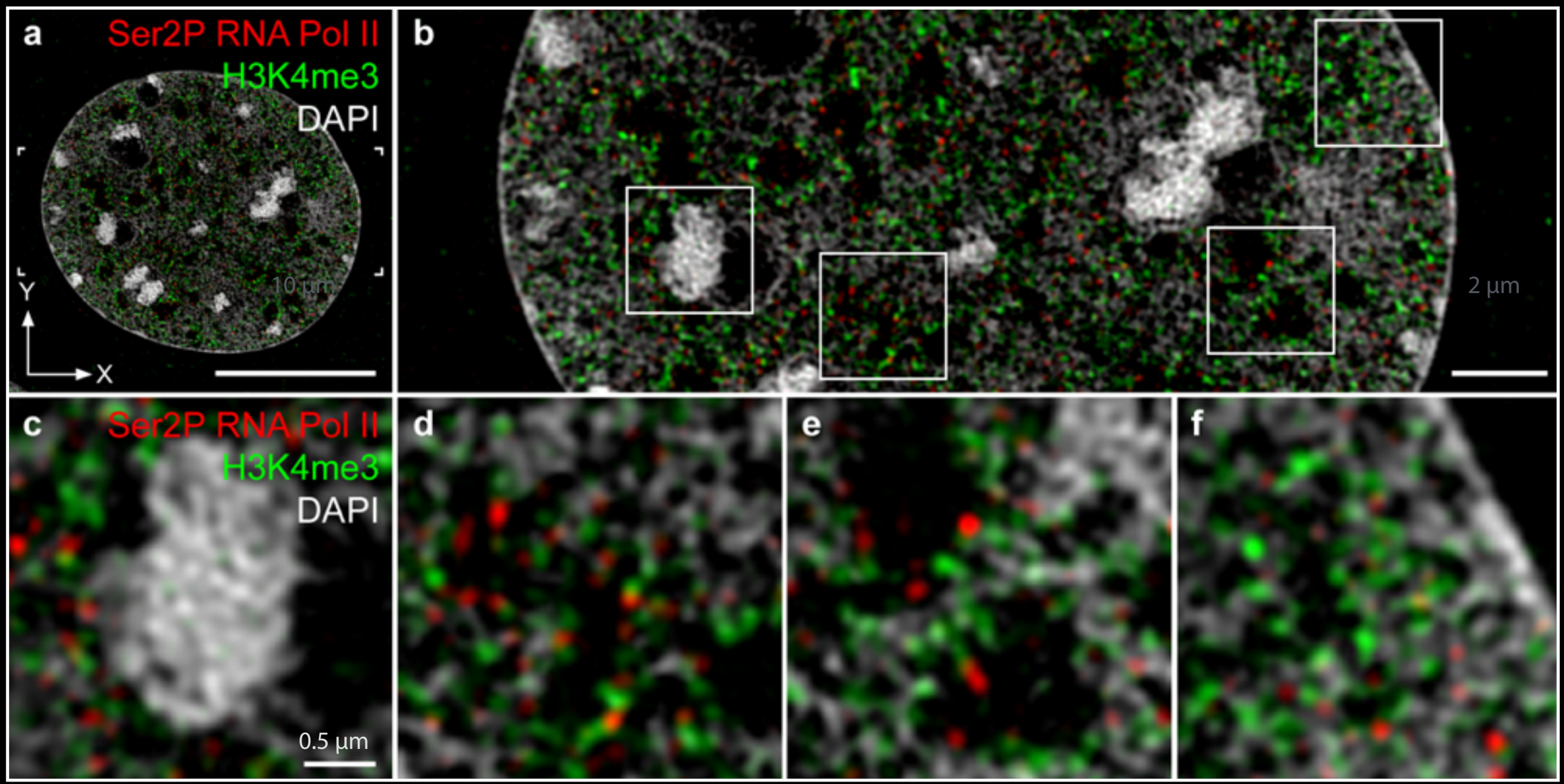
3D-SIM resolves chromatin domains and interchromatin channels



Mouse C2C12 cells

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

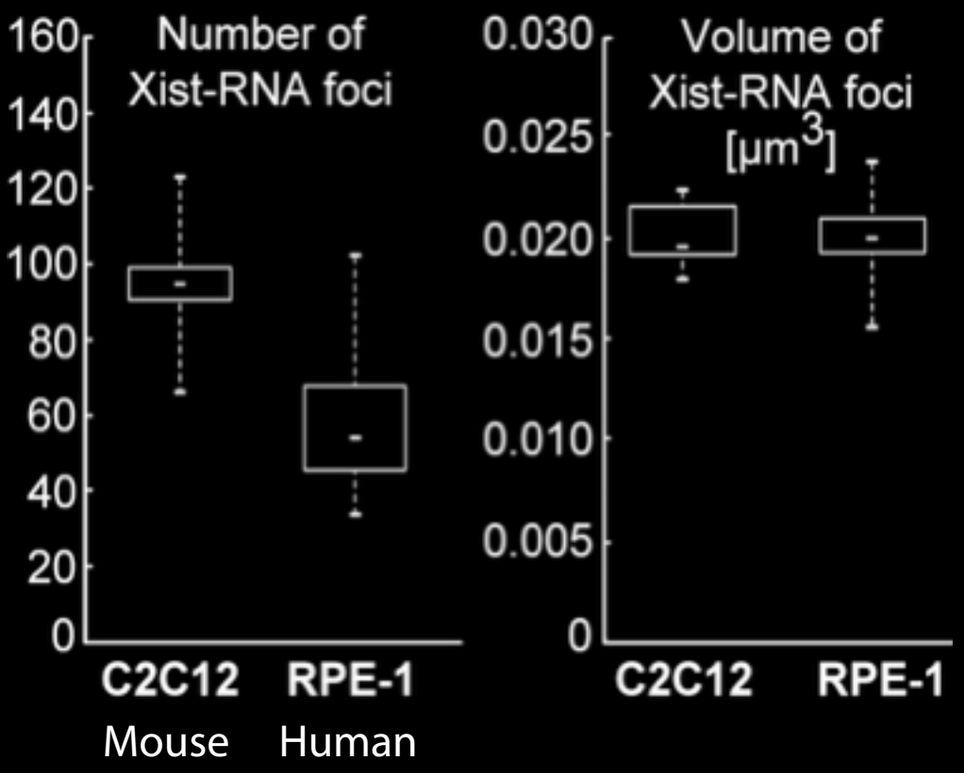
Active marker are constrained to chromatin domain boundaries



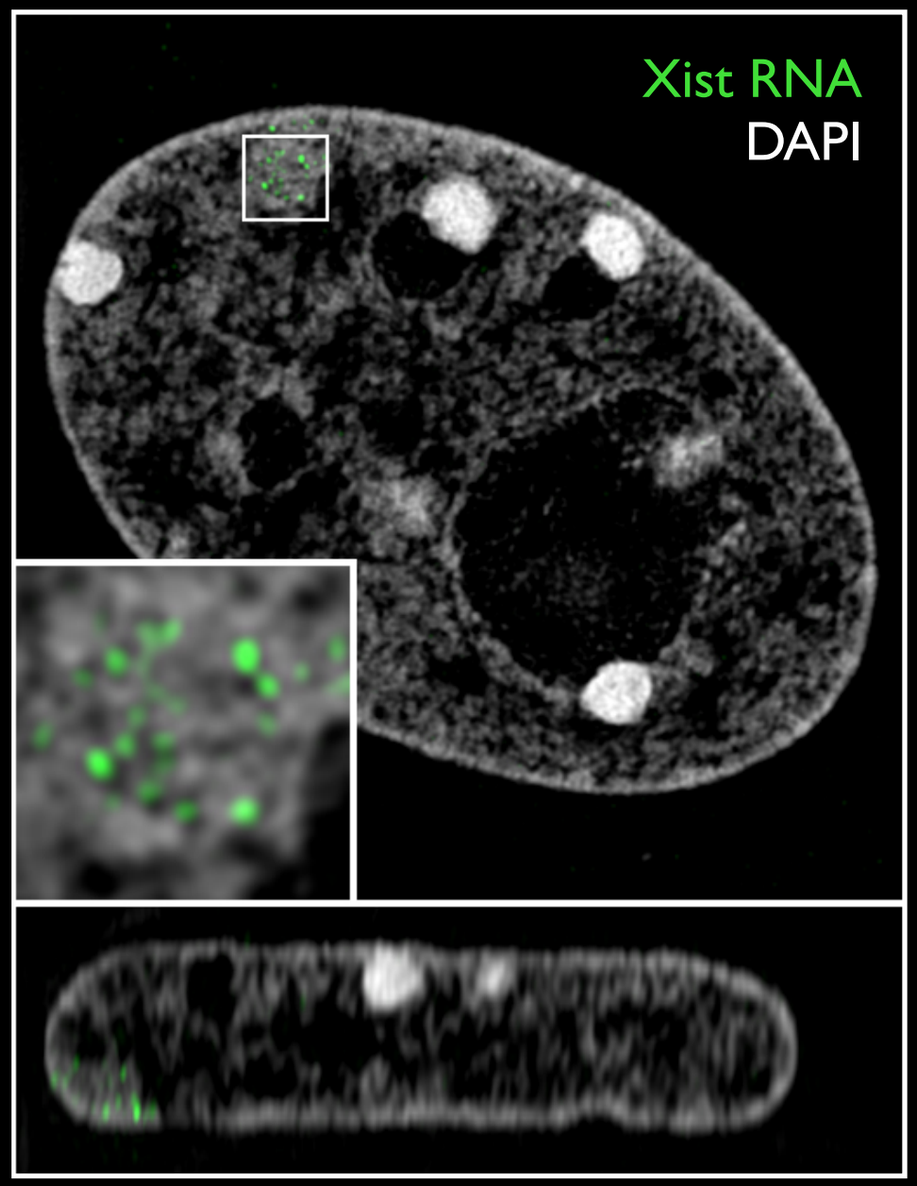
Mouse C127 cell

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

Super-resolution topology inactive X-chromosome



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



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

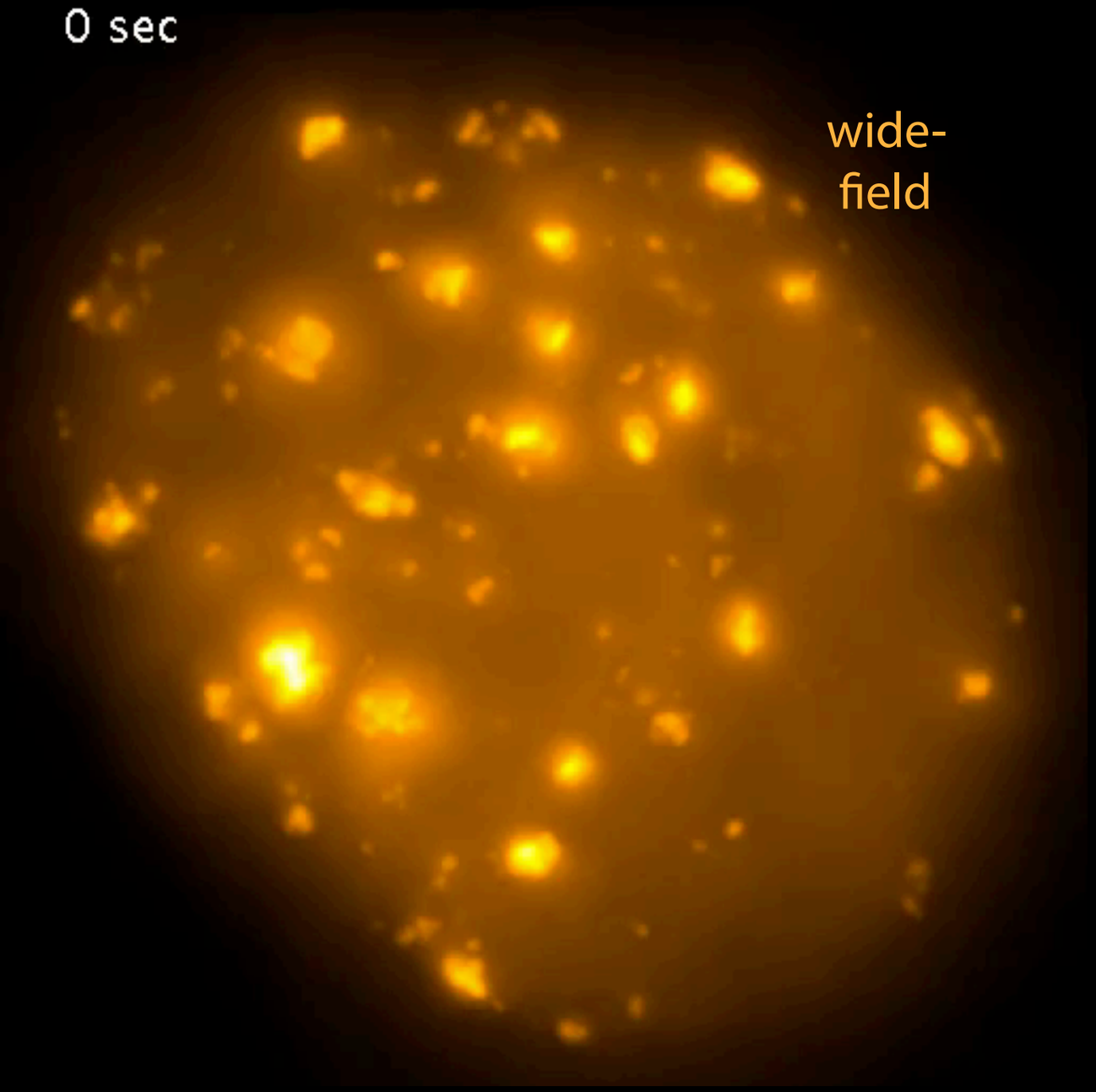
DNA replication foci
(GFP-PCNA in mouse C2C12 myoblast cell)

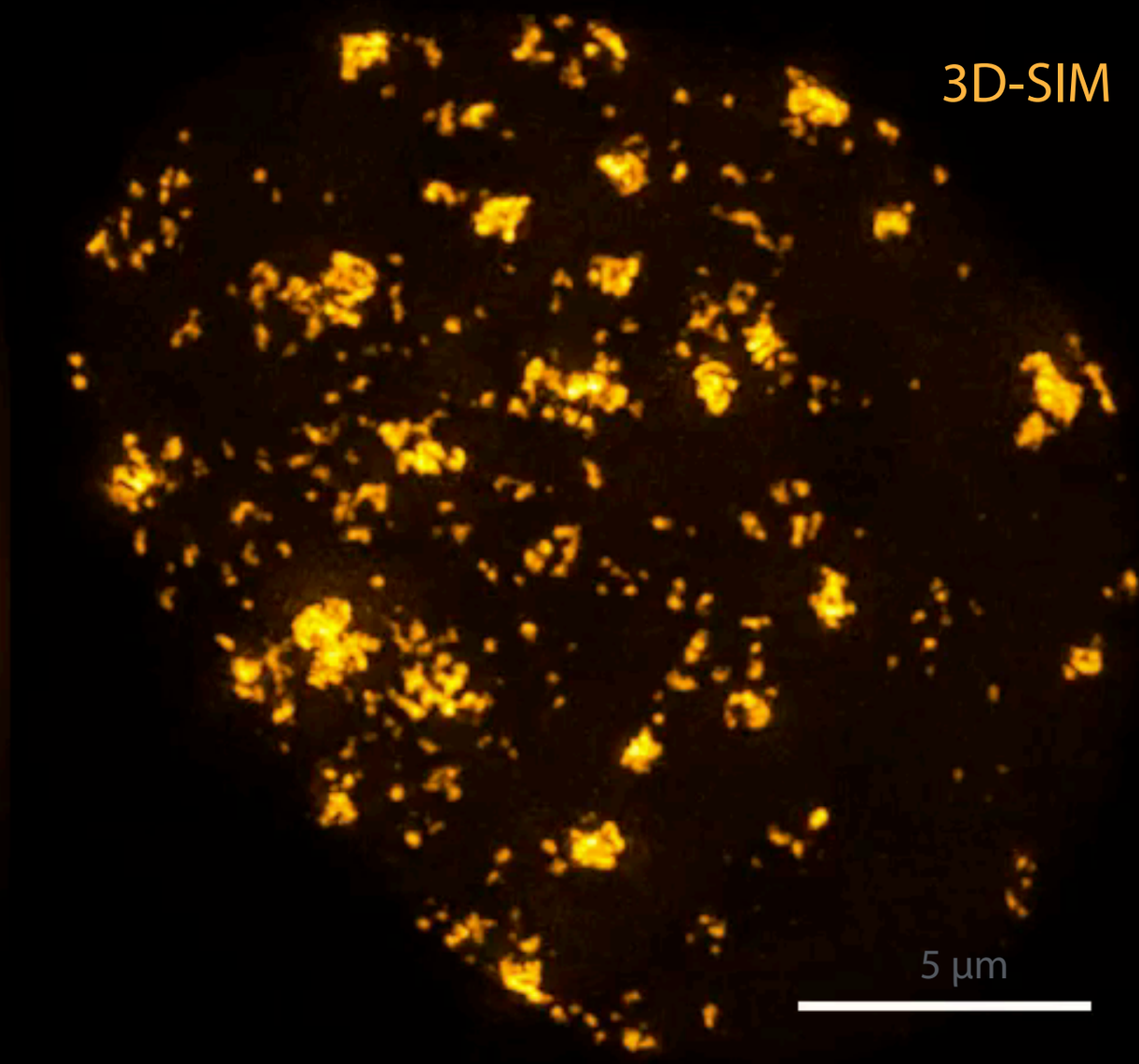
(OMX Blaze)

0 sec

wide-field

3D-SIM



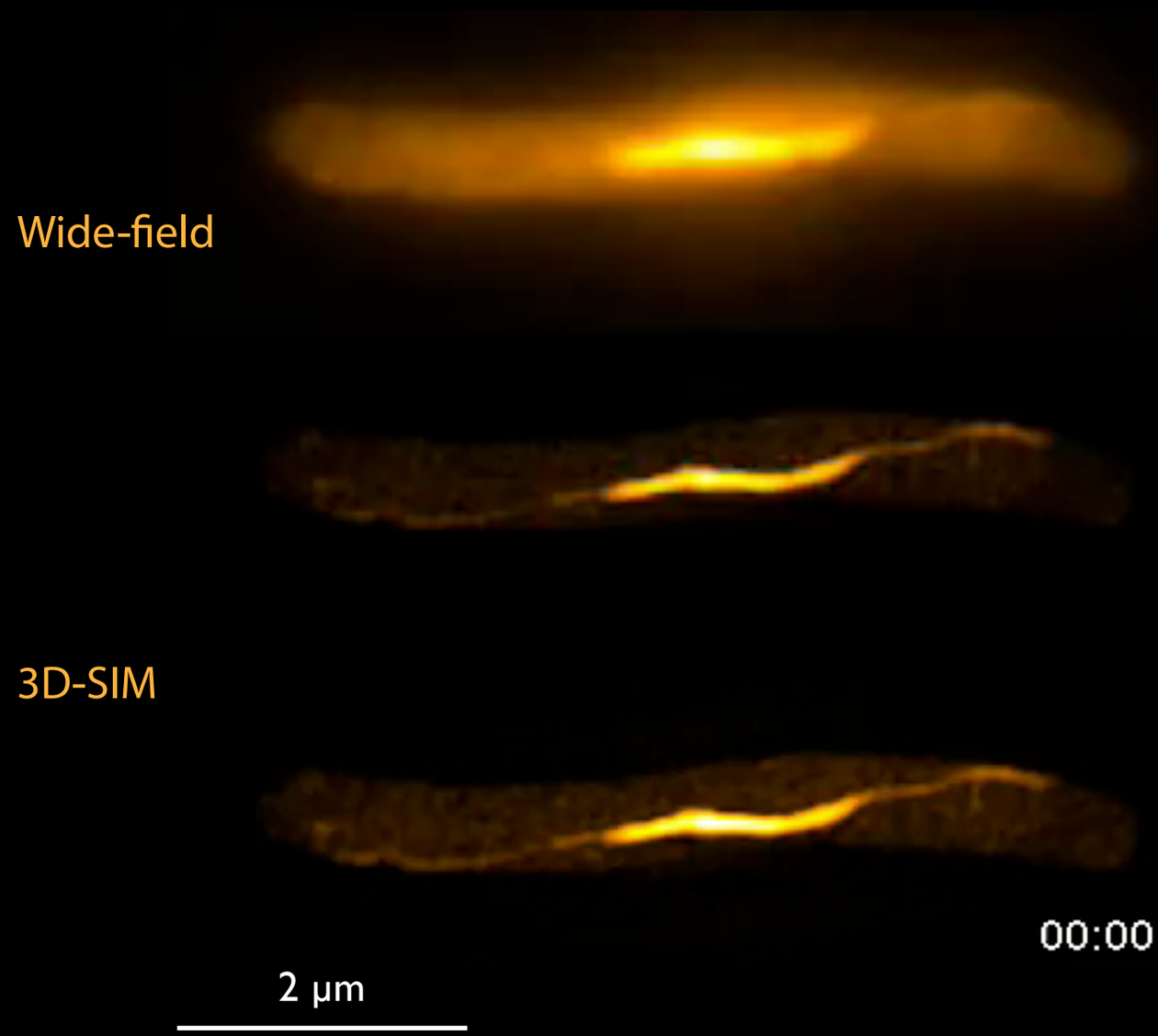


10 s / frame (5 µm z-stack = 600 images / frame)

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)

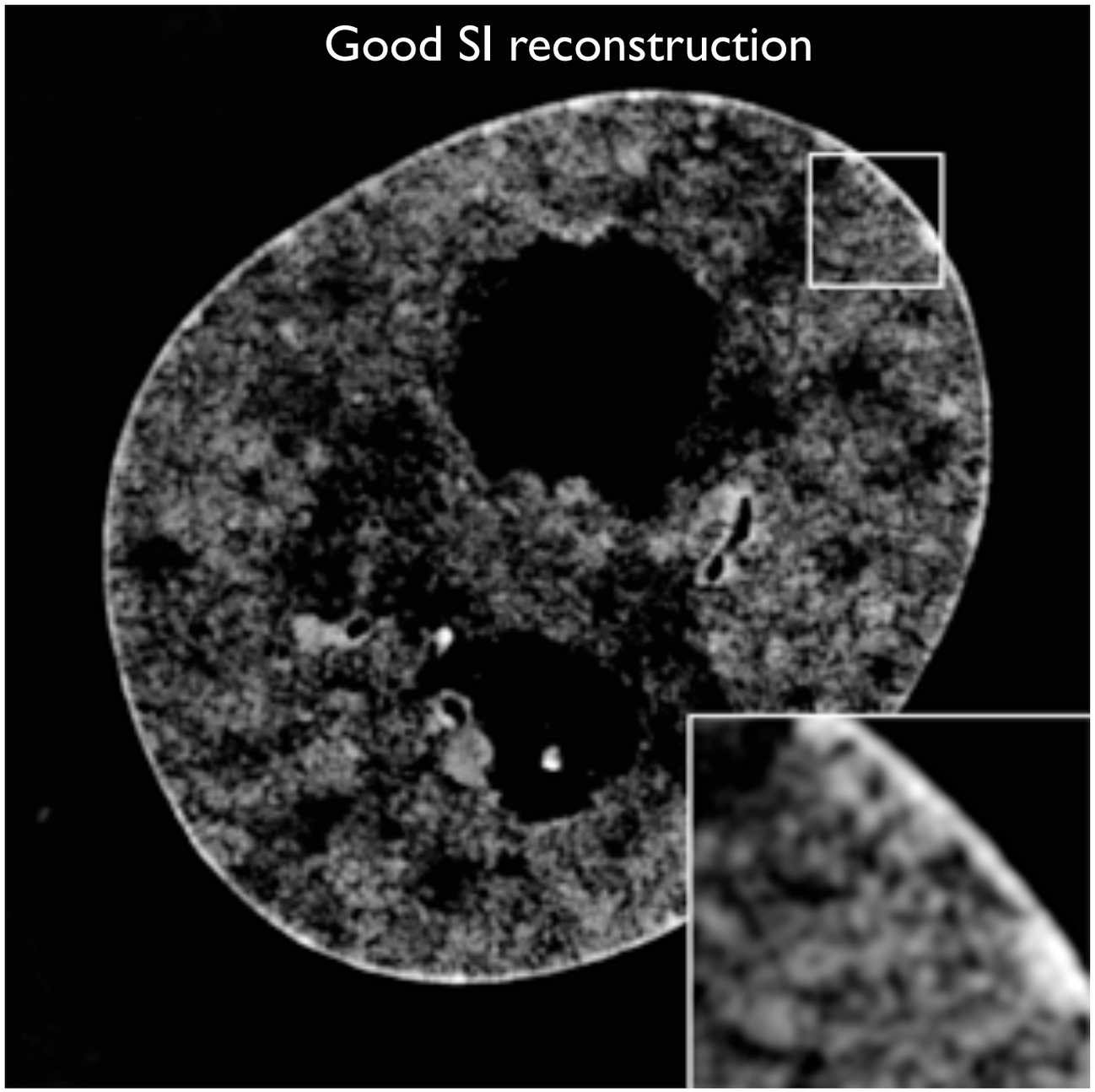
Lesterlin et al. , 2014, *Nature* (D. Sherratt Lab)

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

It‘s not that simple!

The untold story

SI reconstruction artifacts



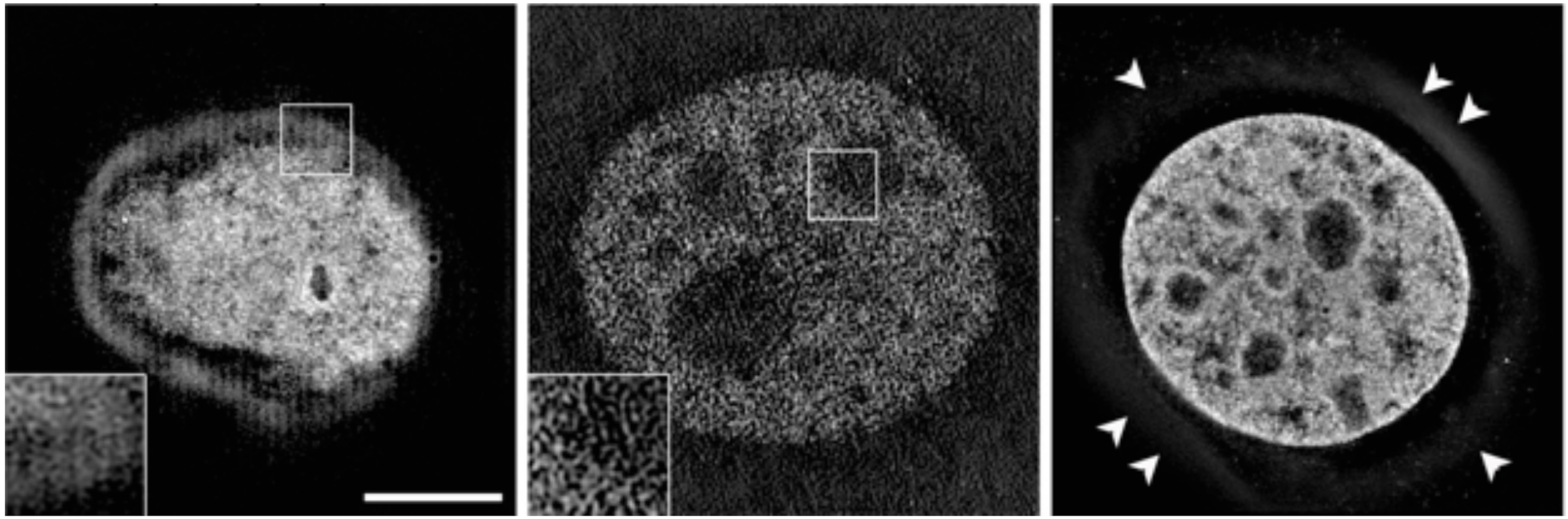
SI reconstruction artifacts

Stripes

High frequency noise

Halo / Doubling

HeLa clell nuclei, chromatin staining

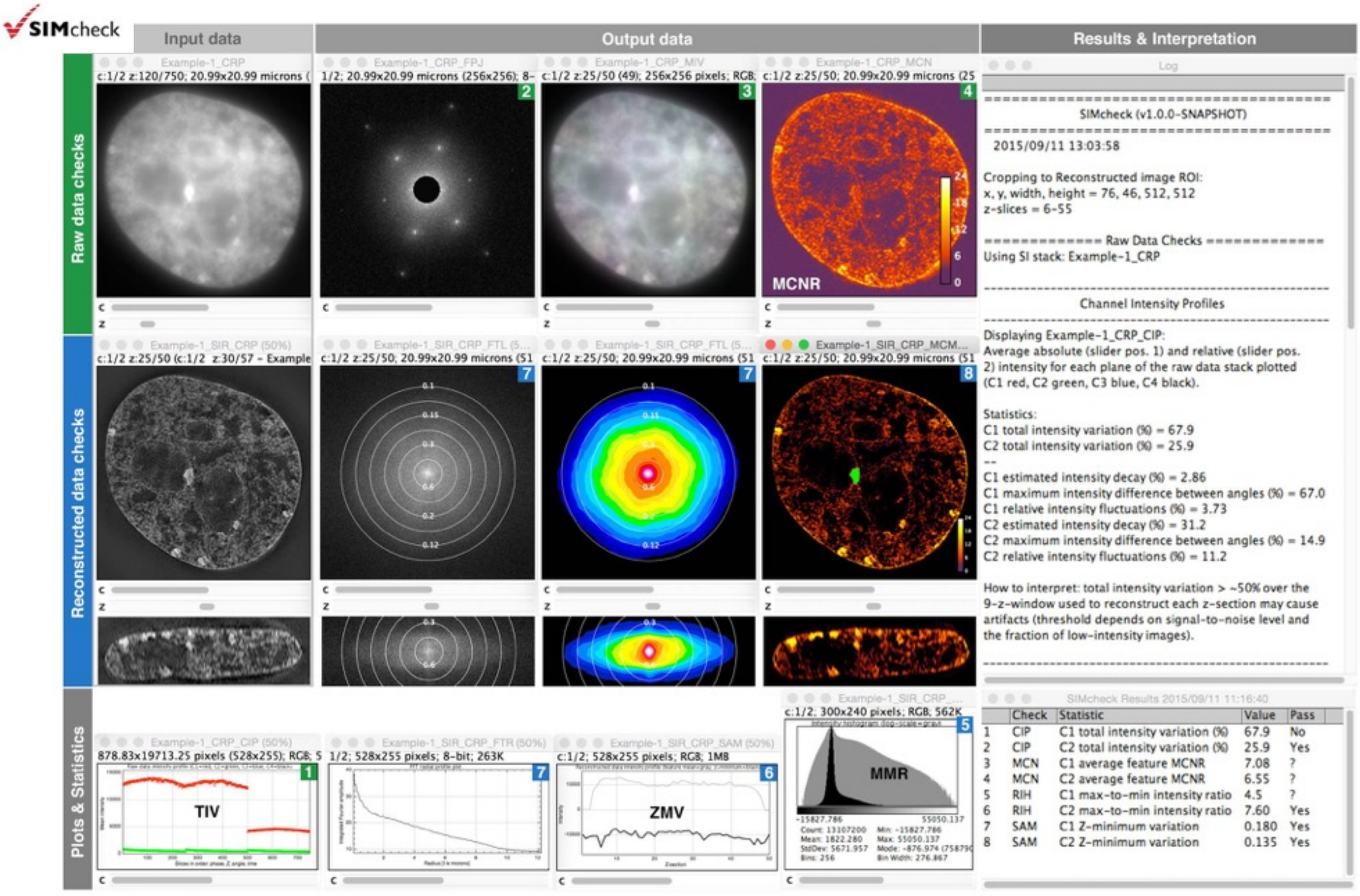


Bleaching,
Drift or vibrations
Moving particles
(locally constrained)

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

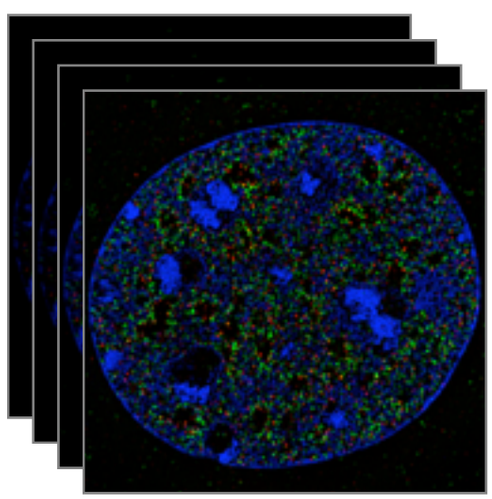
Microscope

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

Postprocessing

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

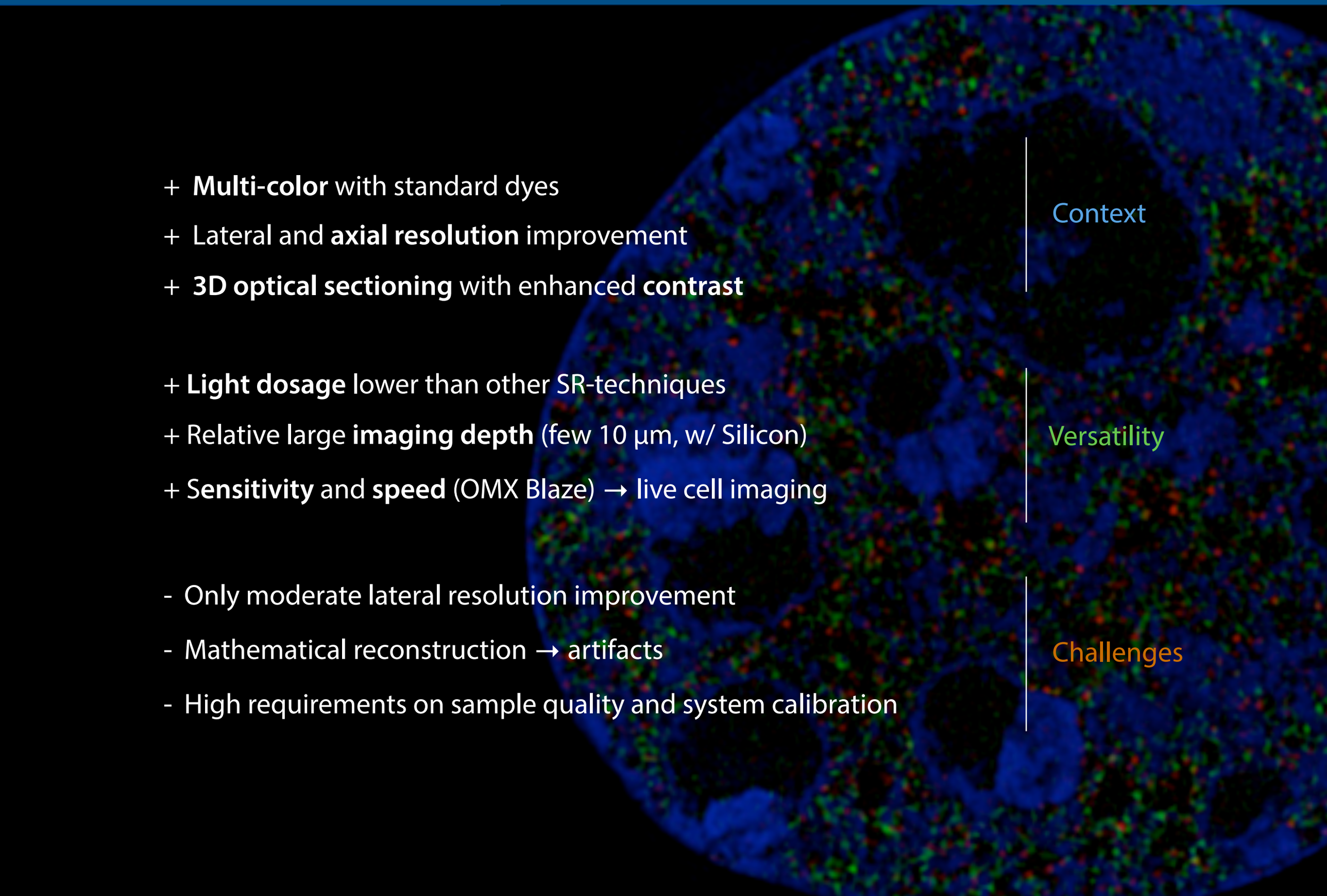
Dataset
x, y, z, λ,(t)



Quantitative Analysis

- Quality control
- Co-localisation
- Segmentation
- Distances

3D-SIM - pros & cons



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

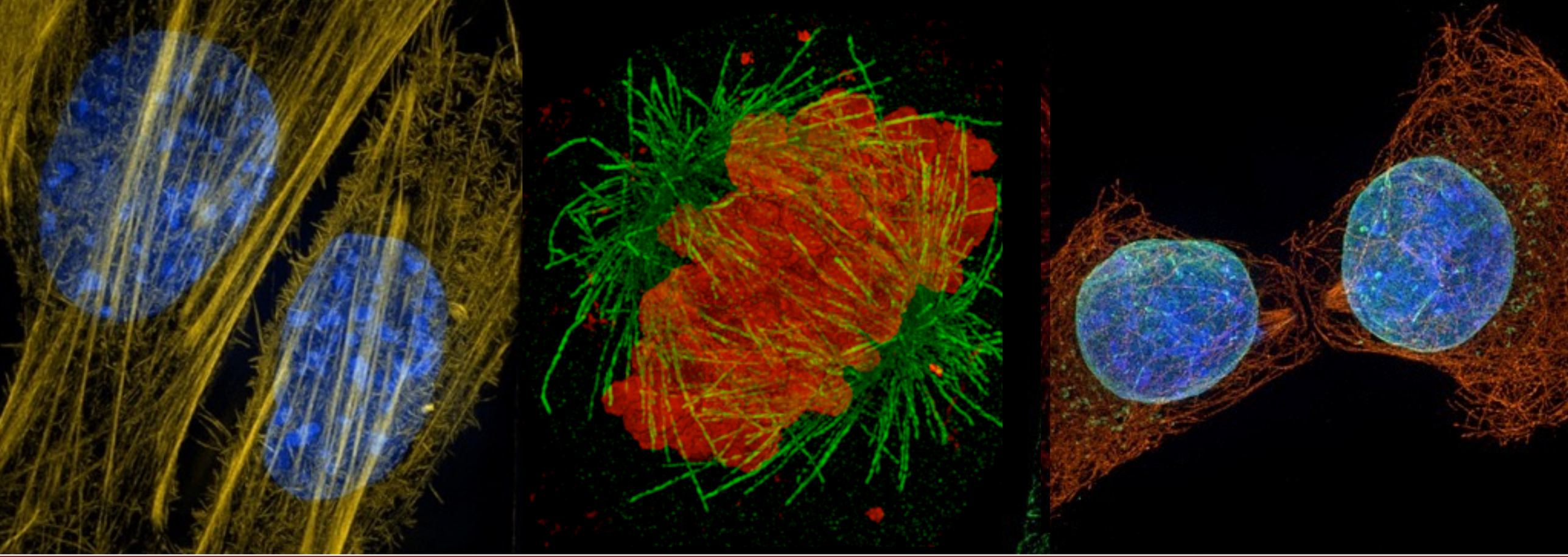
Context

+ **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