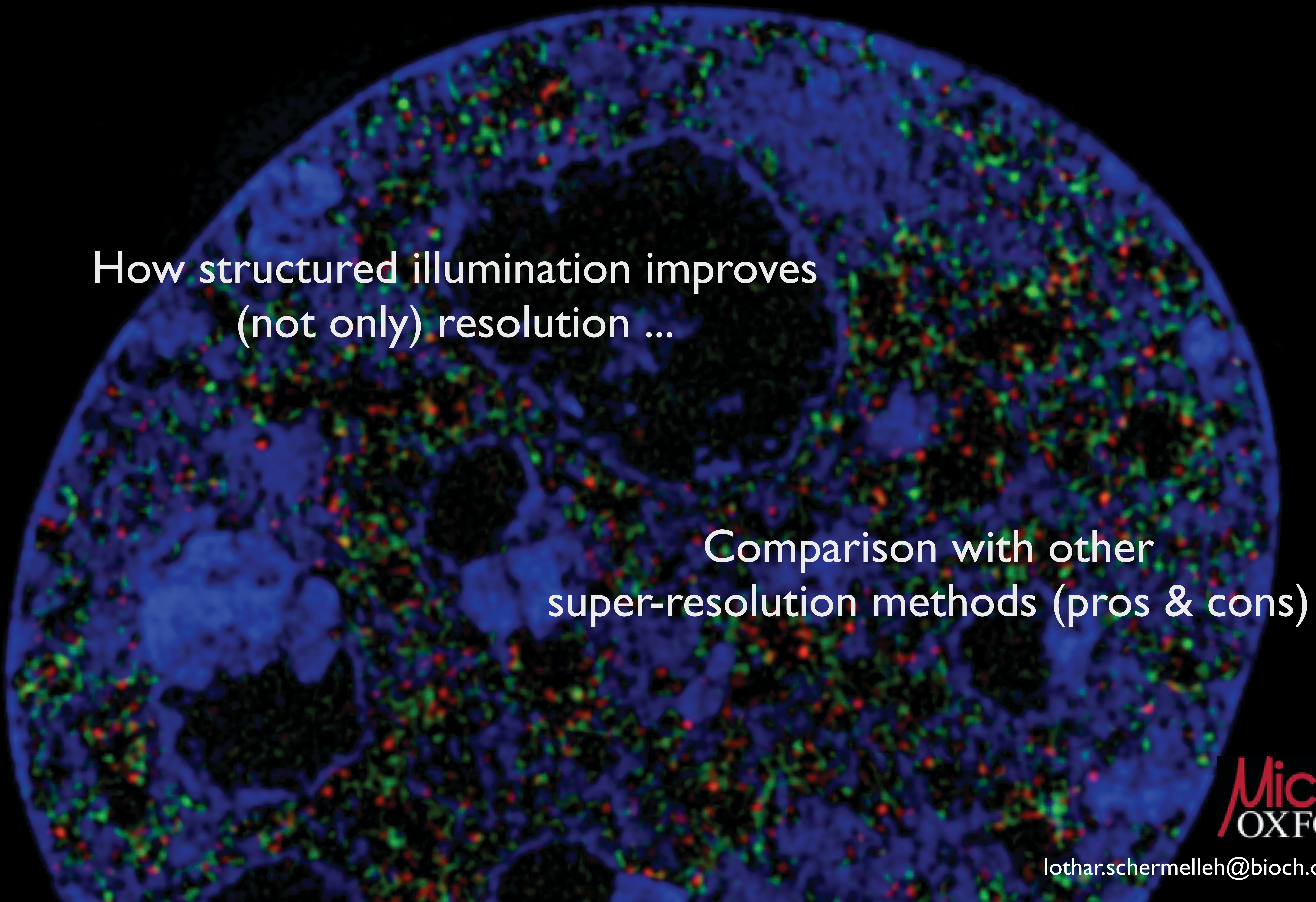
The Power of SIM



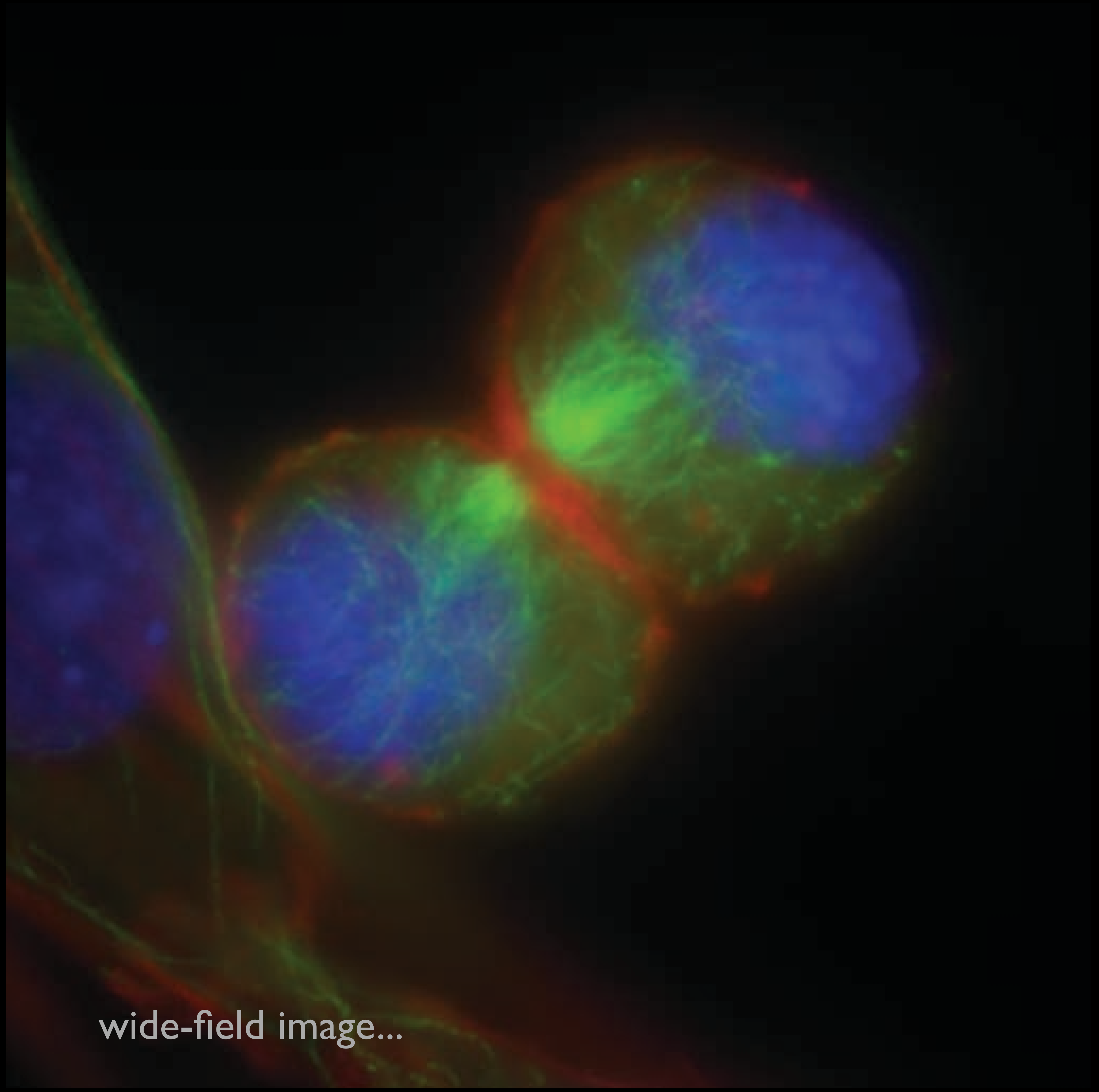
How structured illumination improves
(not only) resolution ...

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

Micron
OXFORD

lothar.schermelleh@bioch.ox.ac.uk

Super-resolution fluorescence microscopy



- 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

25x/1.05

£ 12,800

2

40x/1.0

£ 3,004

3

40x/1.1

£ 8,816

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

Numerical aperture determines ...

Lateral resolution limit:	$d_{x,y} = 0.61\ \lambda_{em}\ /\ NA$	(Rayleigh limit)
Axial resolution limit:	$d_z = 2\ \lambda_{em}\ /\ NA^2$	
Brightness	$F = (NA^4\ /\ Mag^2) \times 10^4$	

Only applies under ideal conditions! BUT ...

Spherical aberrations
Chromatic aberrations
Stray light
Out-of-focus blur
Detector noise
...

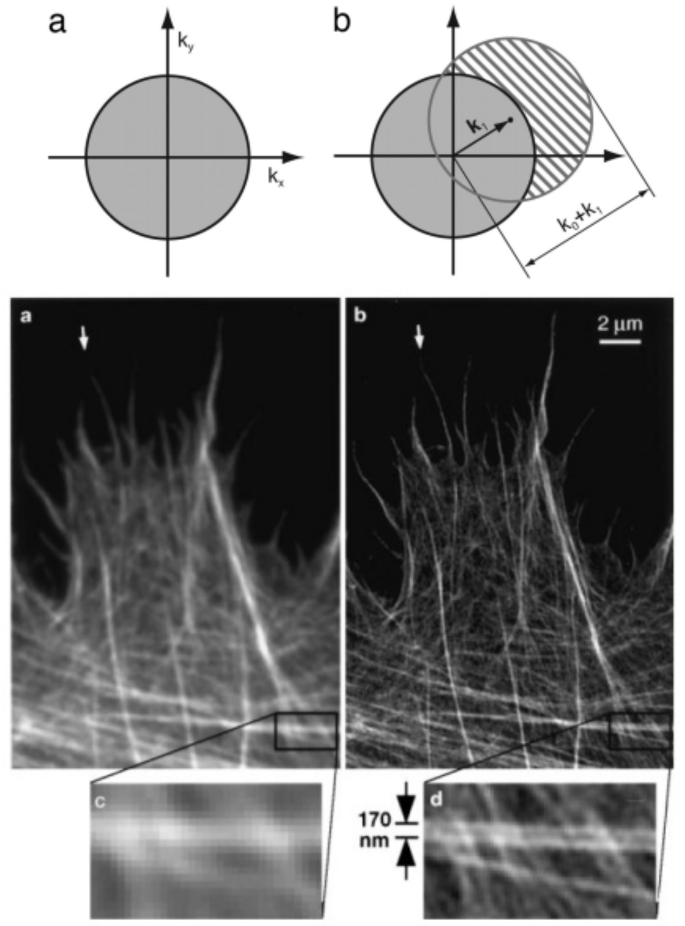
Real effective resolution is worse!
(rather >250 nm lateral and $\leq$ 1 µm axial)

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

“Breaking” the limit by super-resolution

Super-resolution microscopy - three major concepts

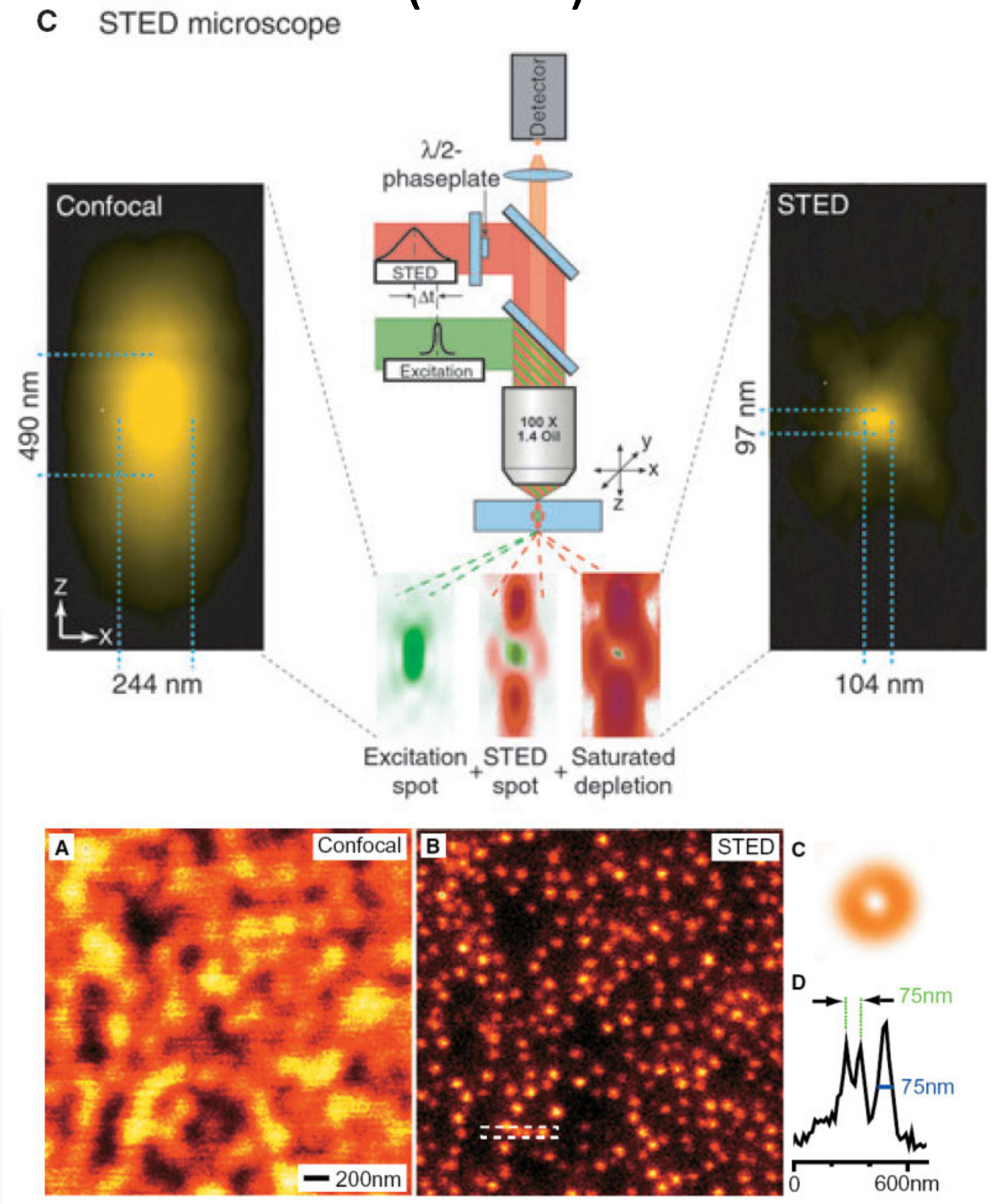
Structured illumination



Abbe diffraction limit

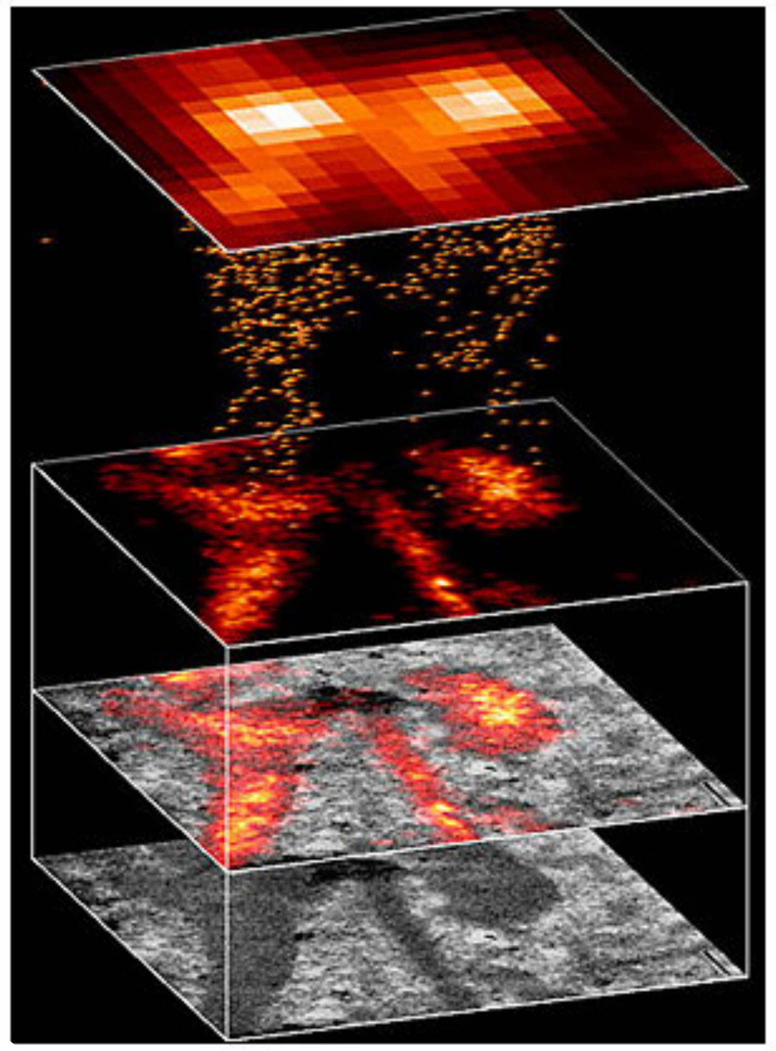
$$\Delta x, \Delta y = \frac{\lambda}{2n\sin\alpha}$$

Stimulated emission depletion (STED)

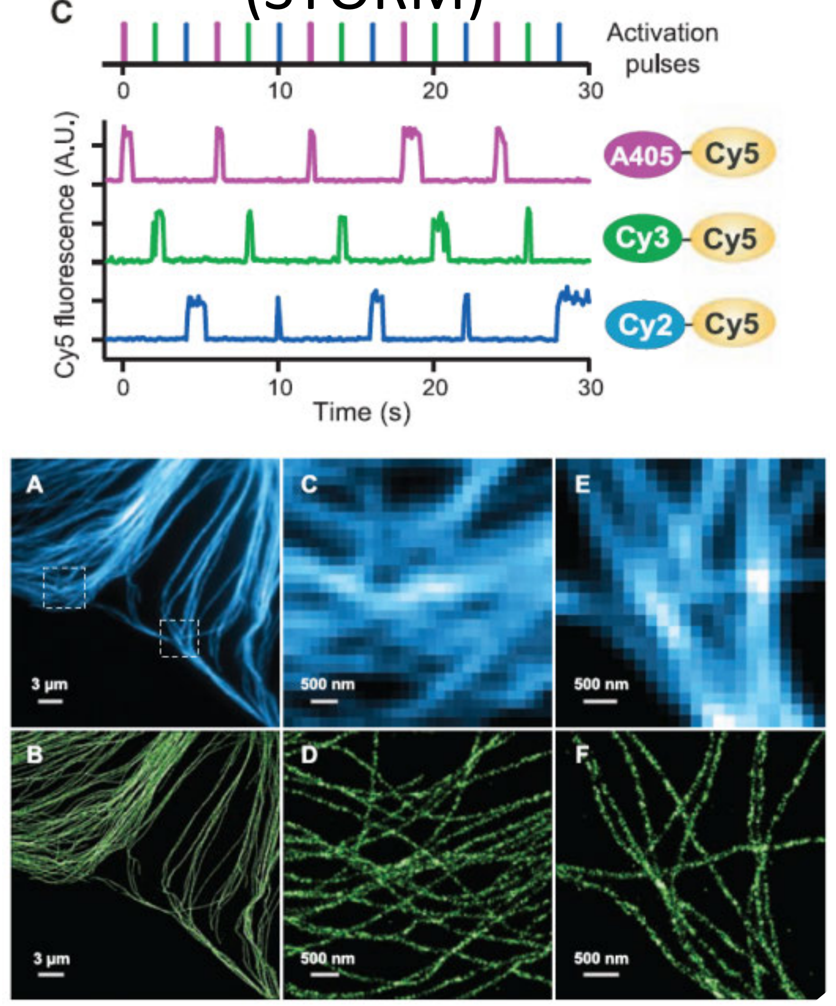


Single molecule localisation

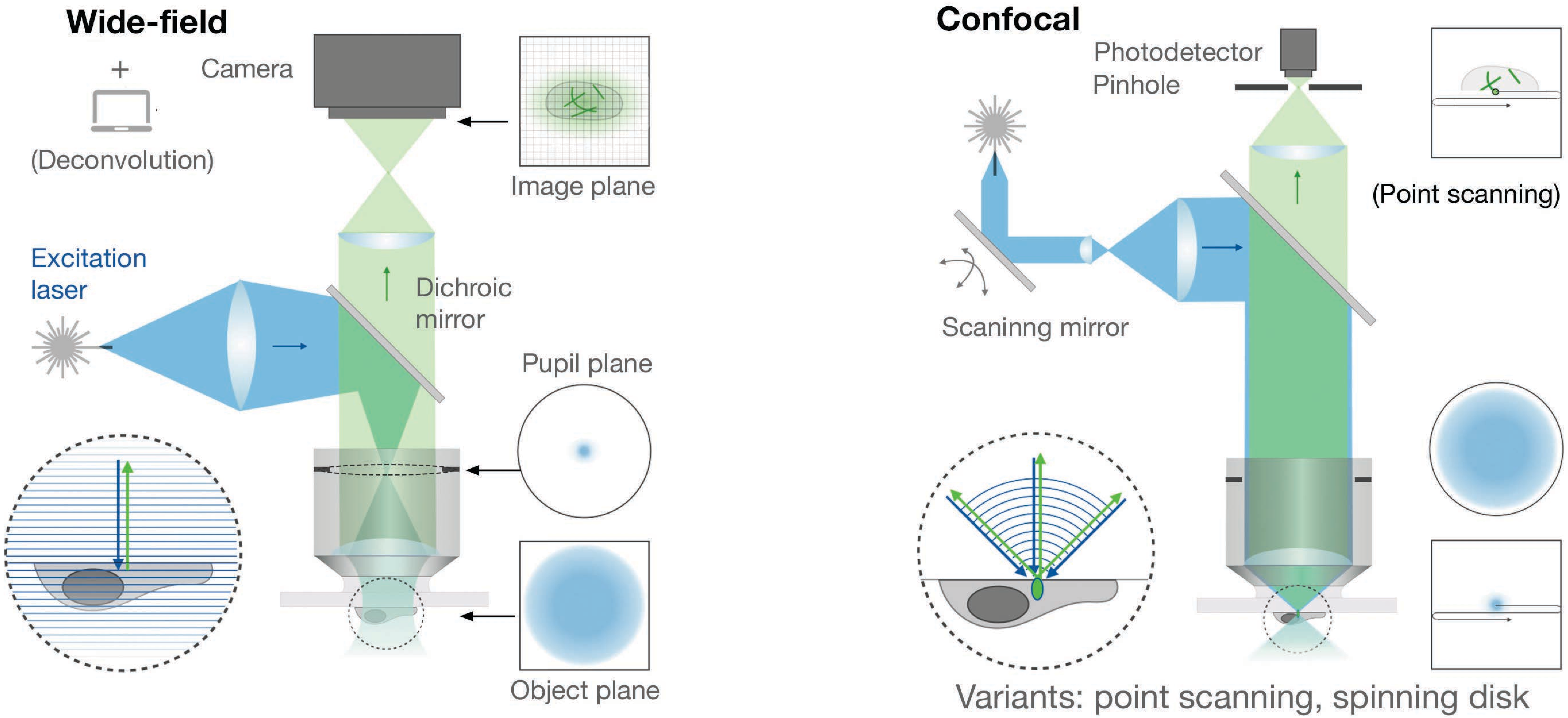
Photoactivation localization microscopy (PALM)



Stochastic optical reconstruction microscopy (STORM)



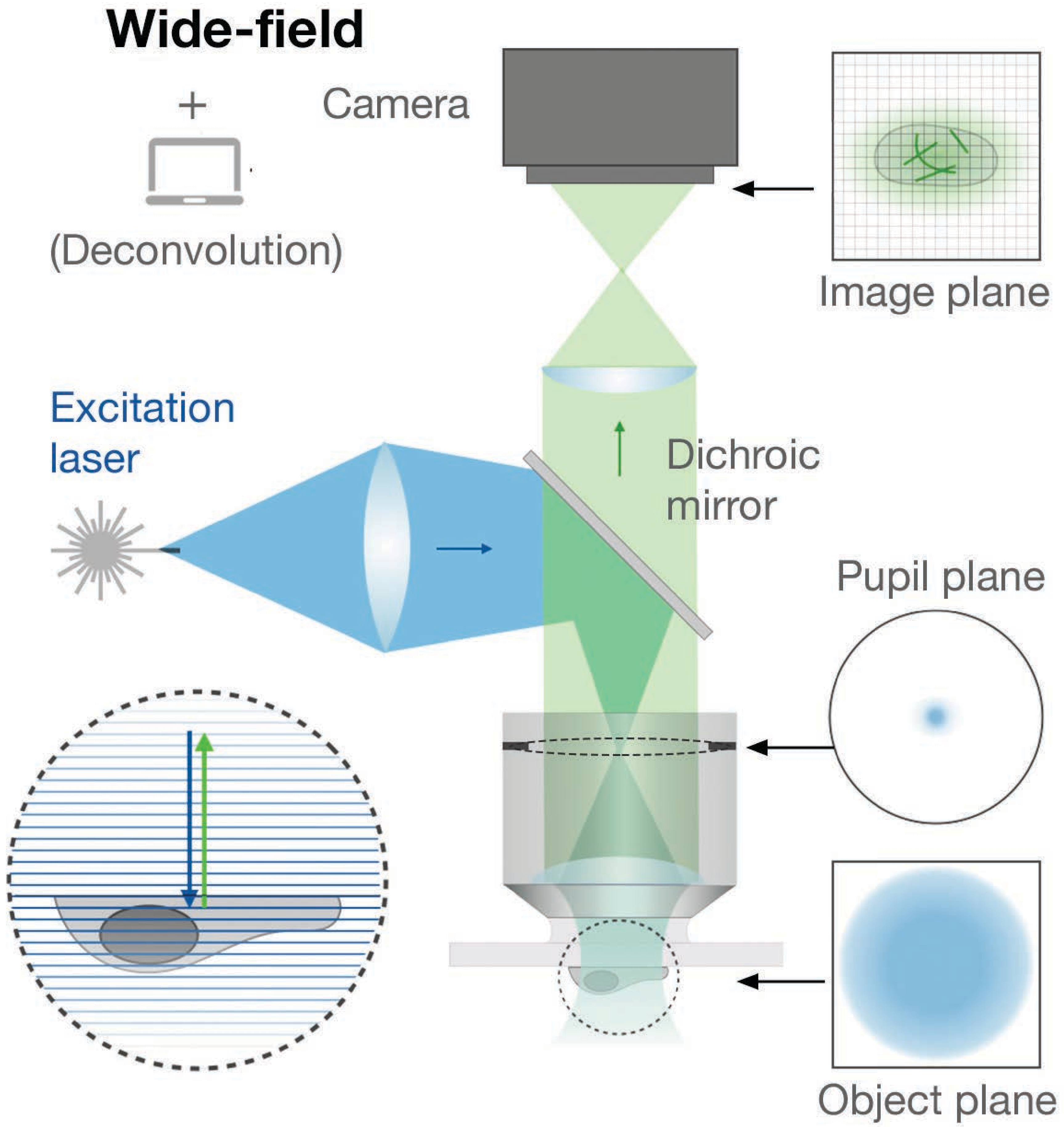
Comparison: Widefield vs Confocal

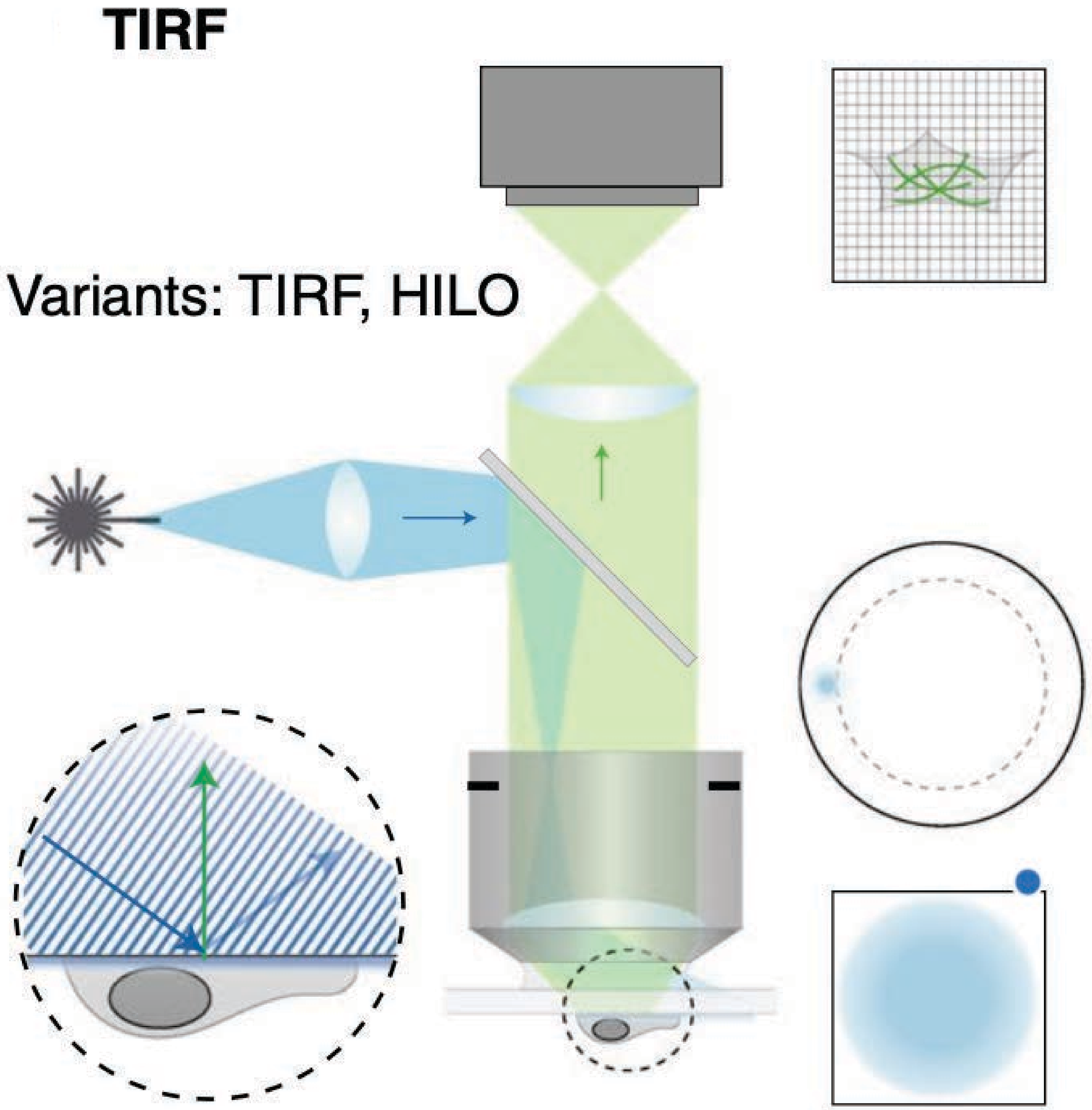


Variants: point scanning, spinning disk

Schermelleh et al. *Nat Cell Biol*, 2019

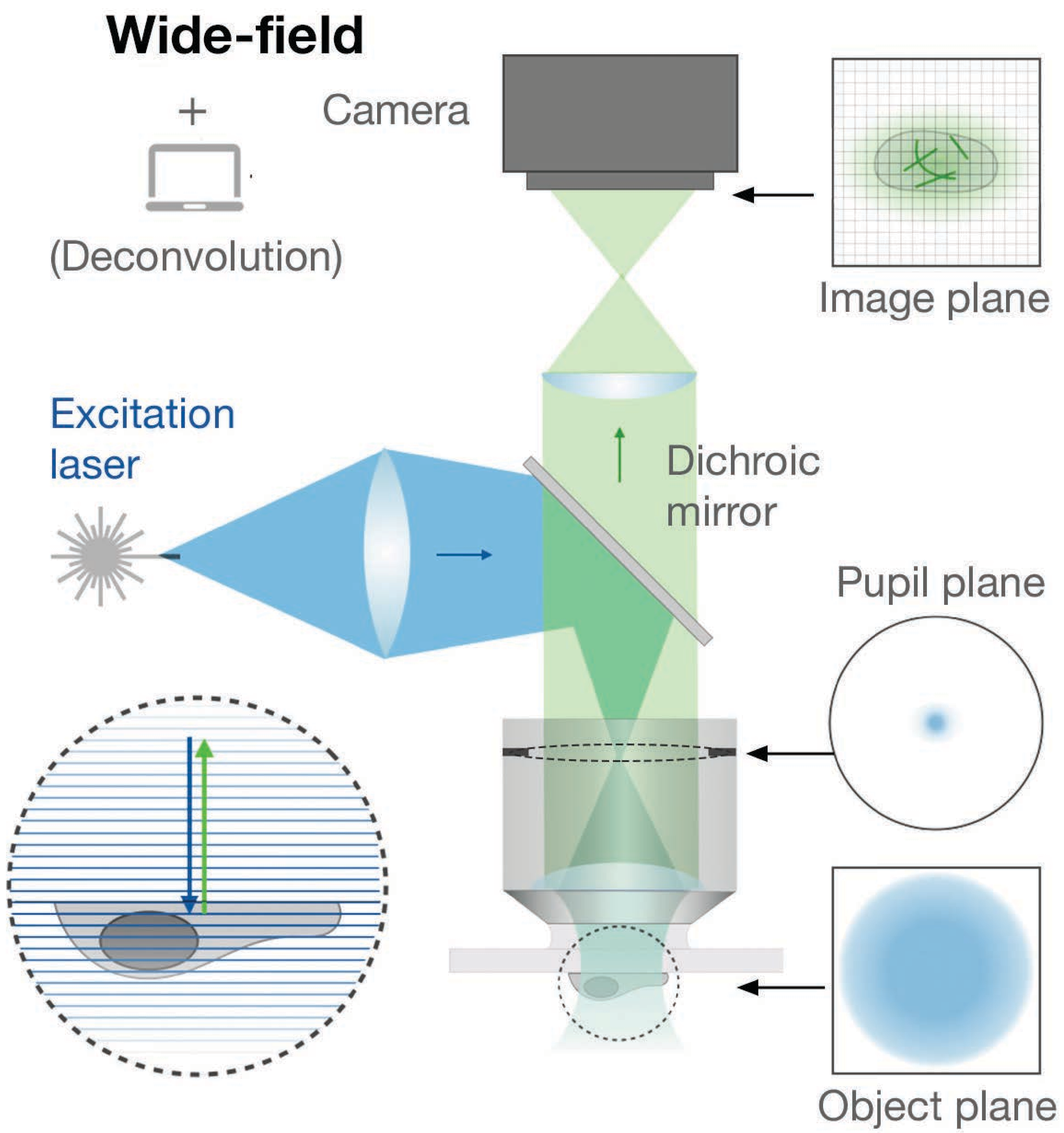
Comparison: Widefield vs TIRF

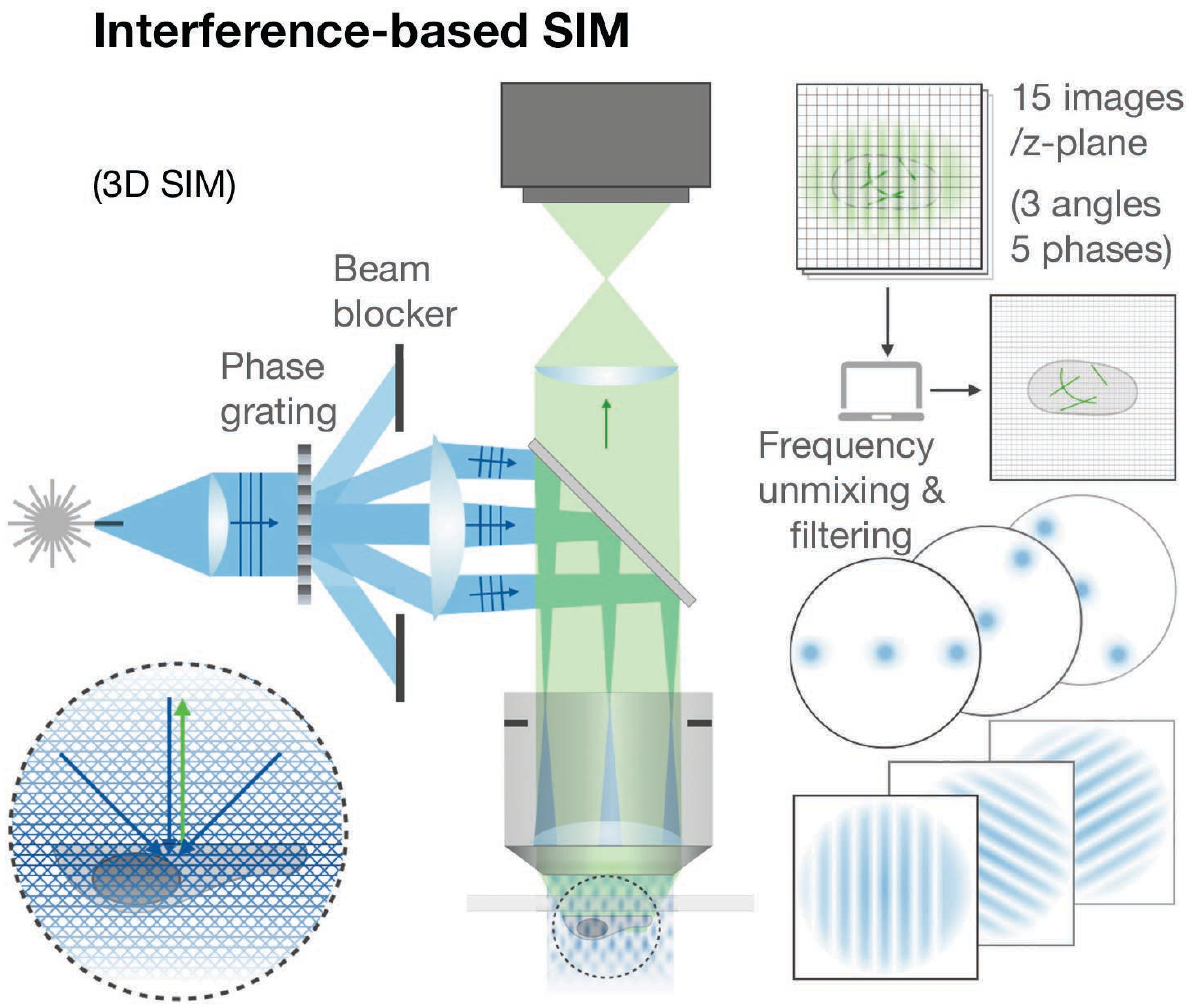




Schermelleh et al. *Nat Cell Biol*, 2019

Comparison: Widefield vs 3D-SIM

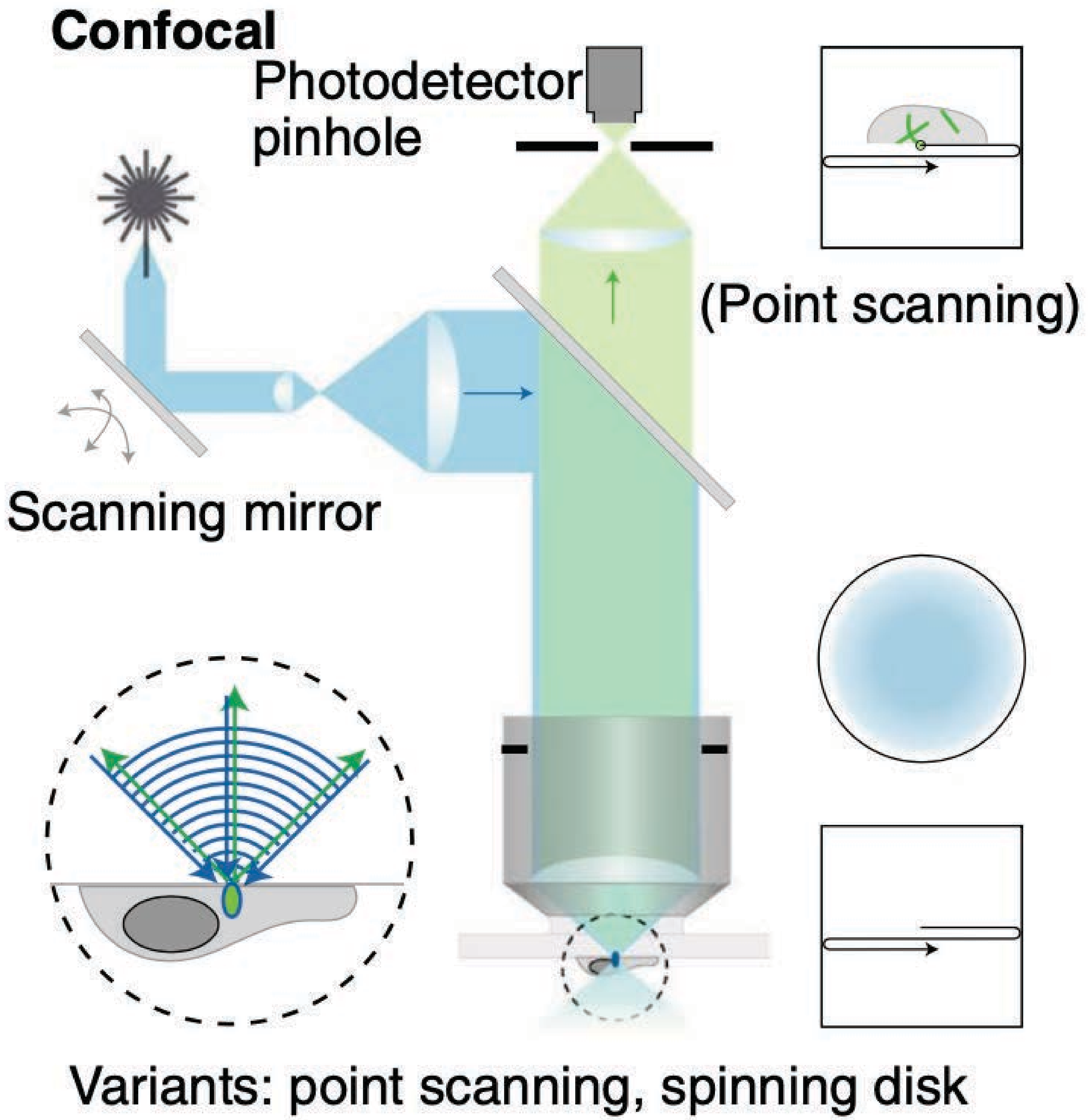


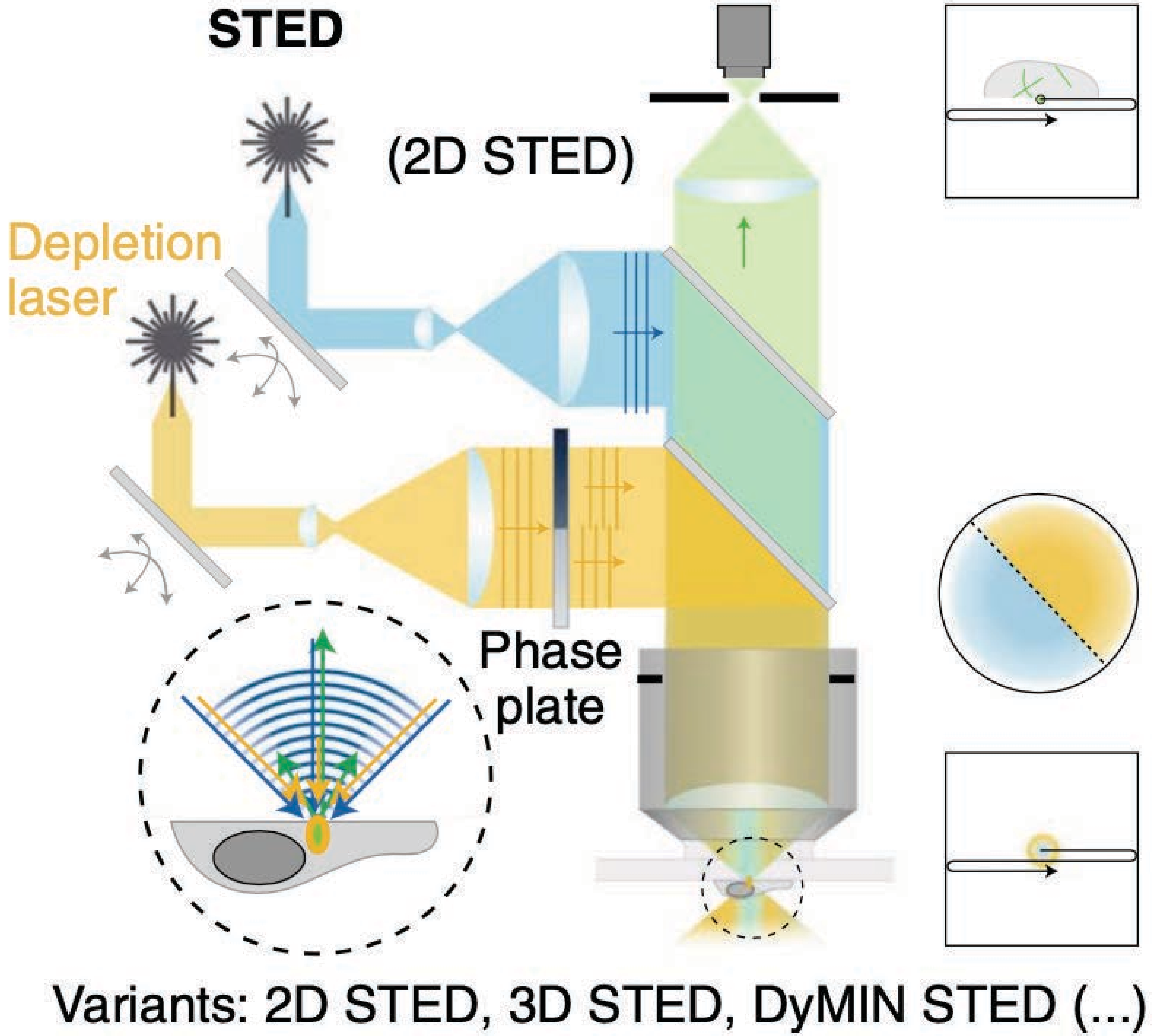


Variants: 3D SIM, 2D SIM, TIRF-SIM

Schermelleh et al. *Nat Cell Biol*, 2019

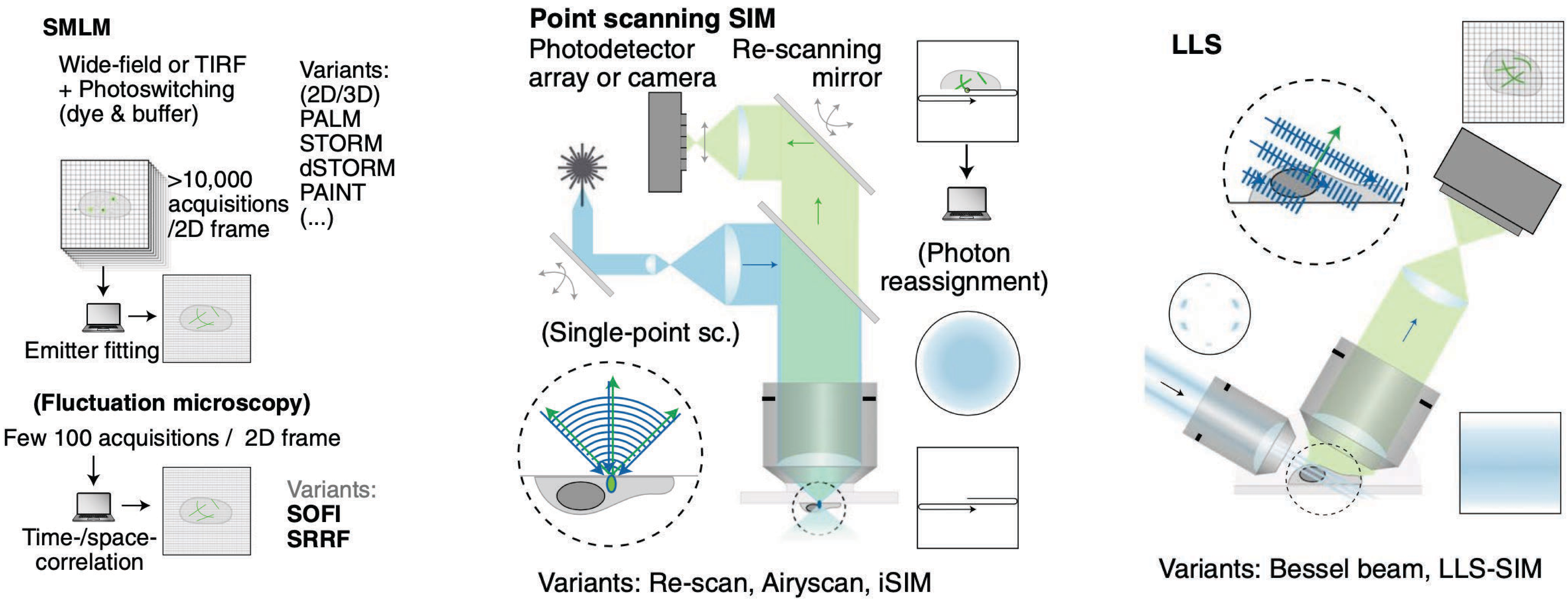
Comparison: Confocal vs STED





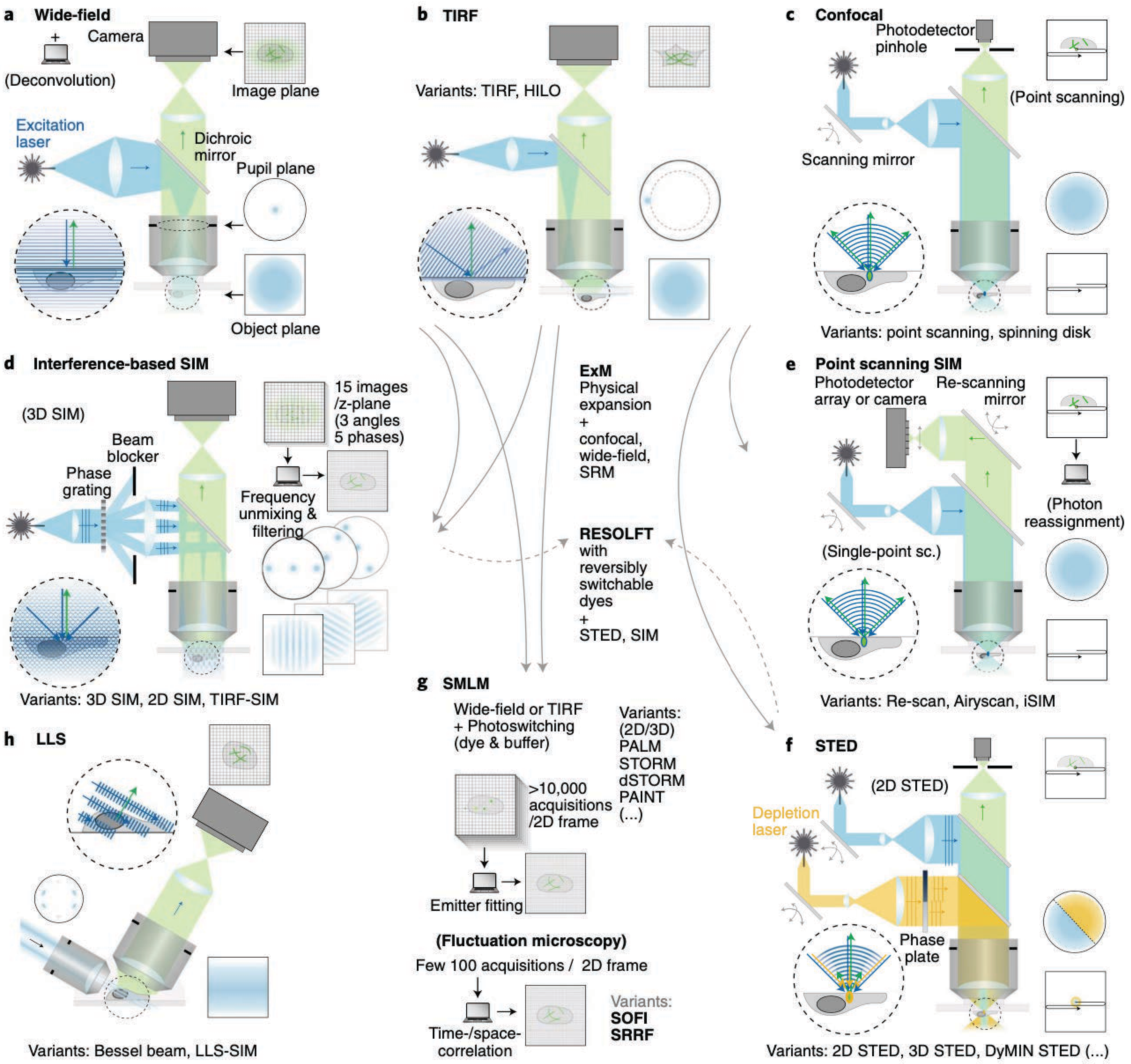
Schermelleh et al. *Nat Cell Biol*, 2019

Basic principles of SRM



Schermelleh et al. *Nat Cell Biol*, 2019

Relationship between SRM techniques



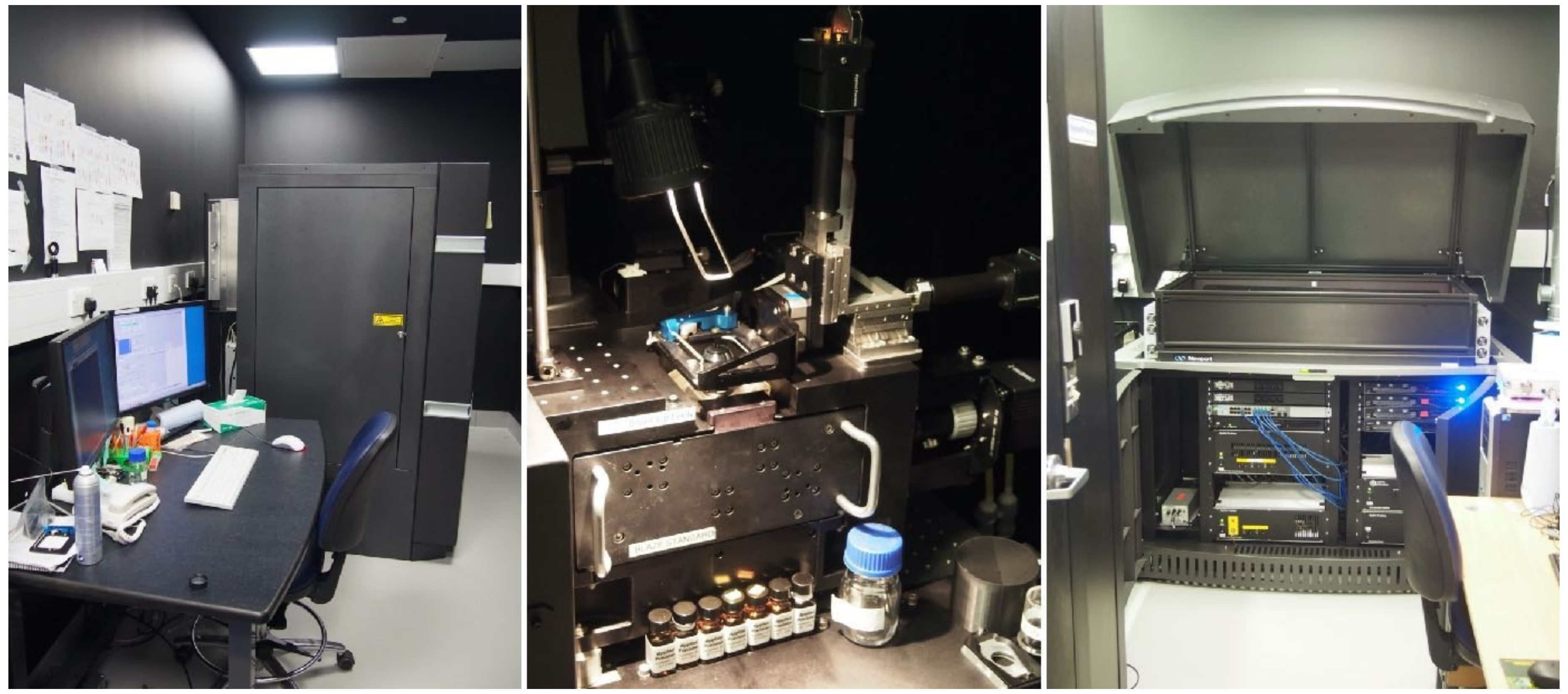
Schermelleh et al. *Nat Cell Biol*, 2019

Super-resolution microscopes

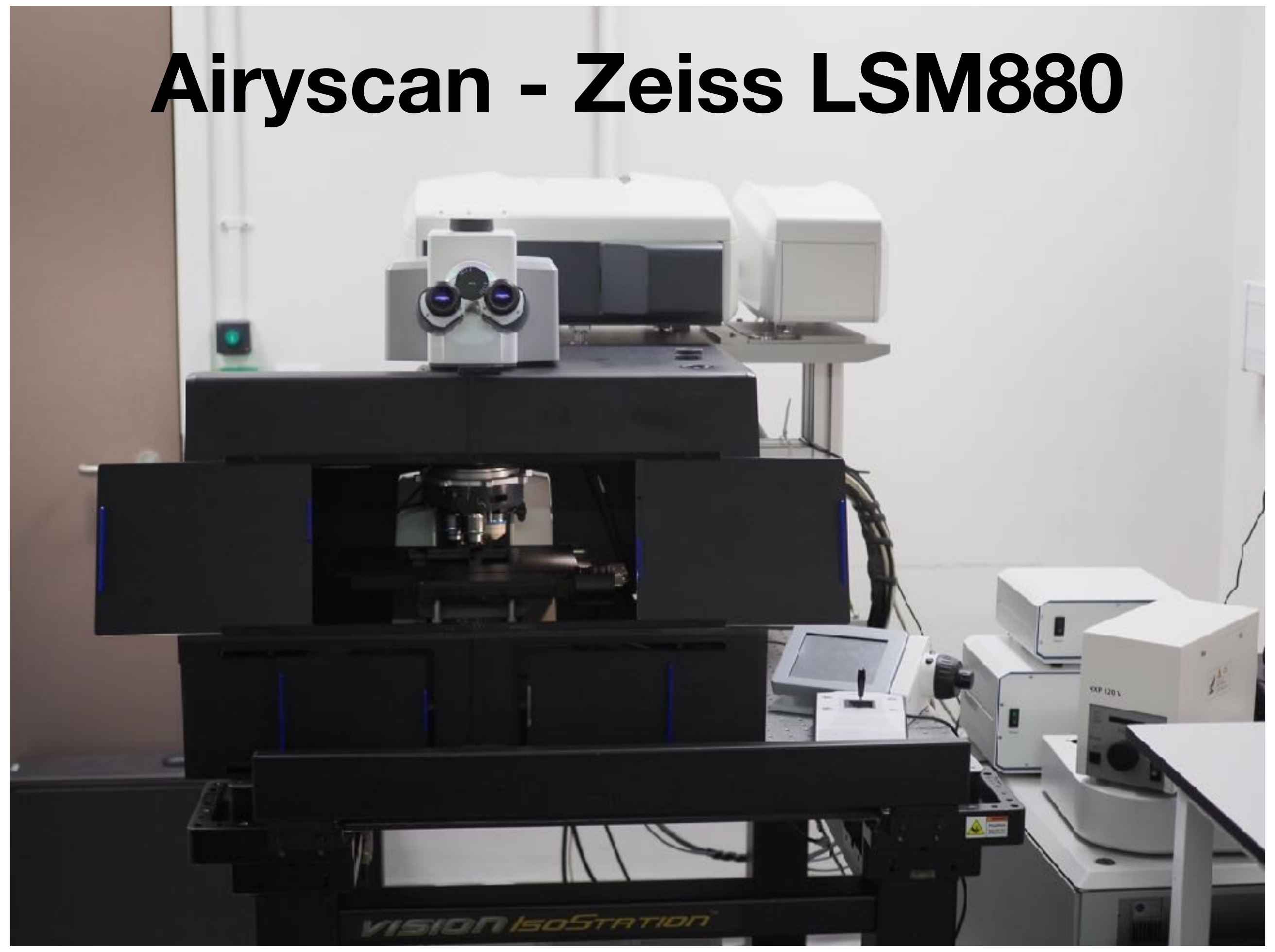
STORM - ONI Nanoimager

3D STED - Leica SP8X

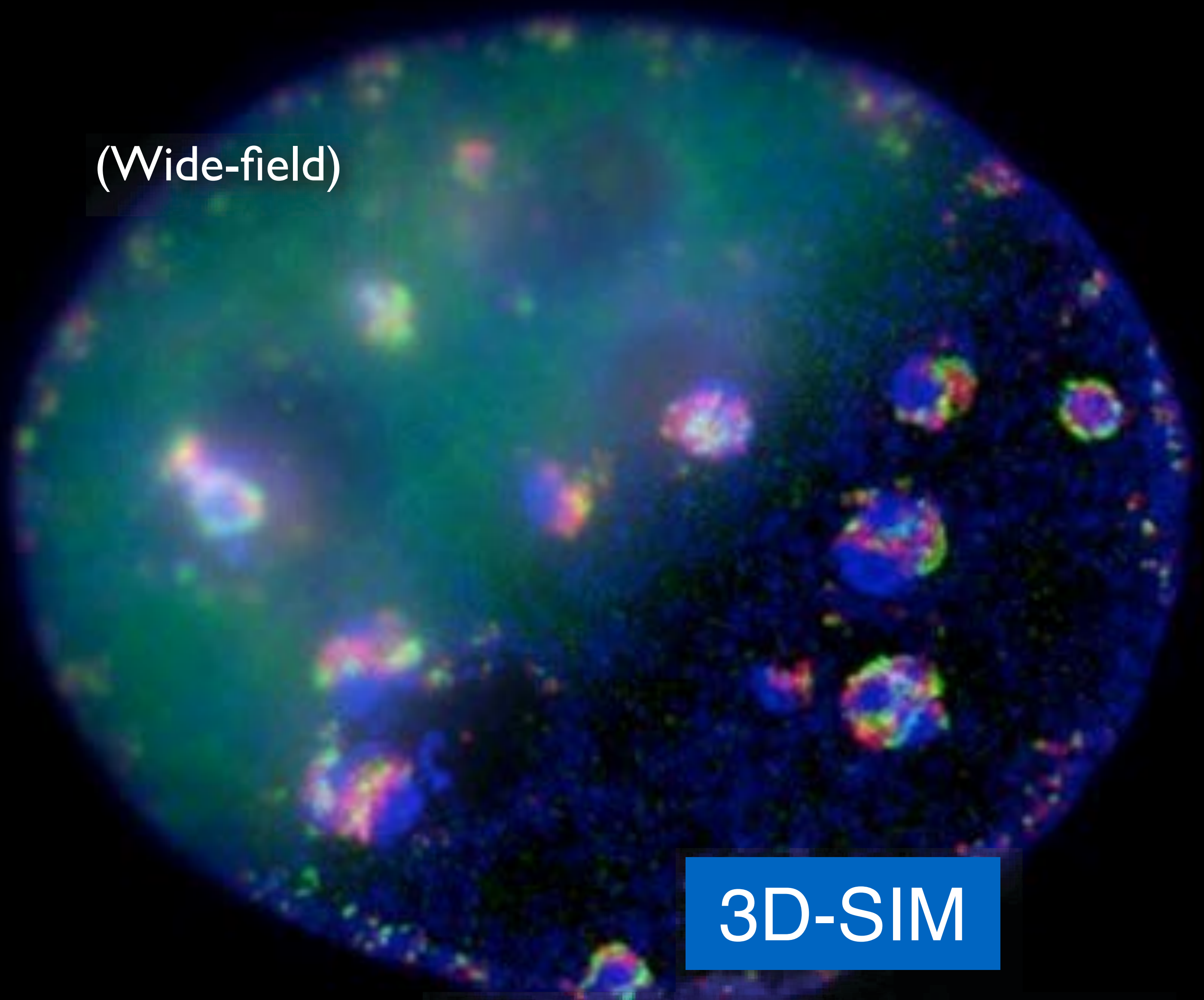
3D-SIM - OMX V3 Blaze



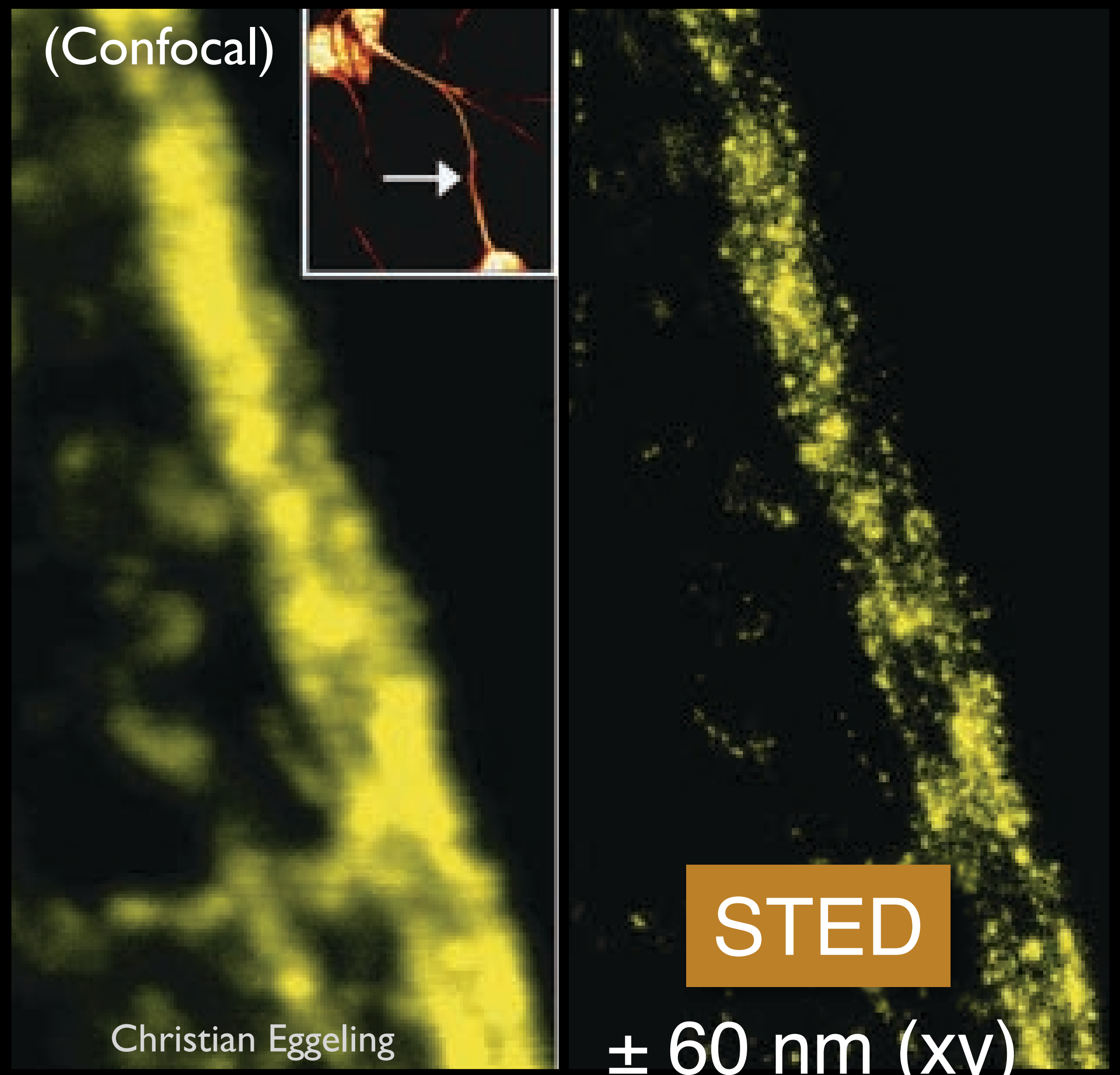
Airyscan - Zeiss LSM880



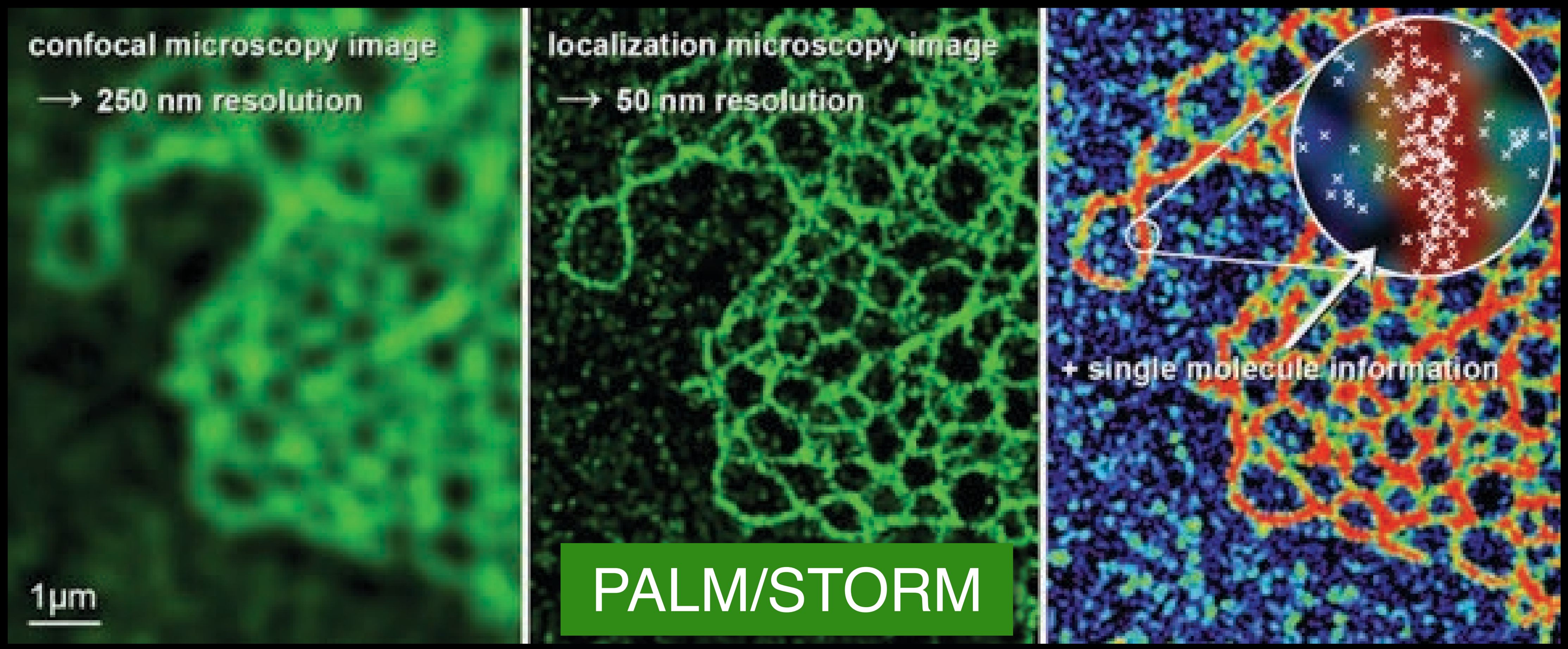
Super-resolution techniques to surpass the diffraction limit



100 nm (xy), 300 nm (z)



± 60 nm (xy)



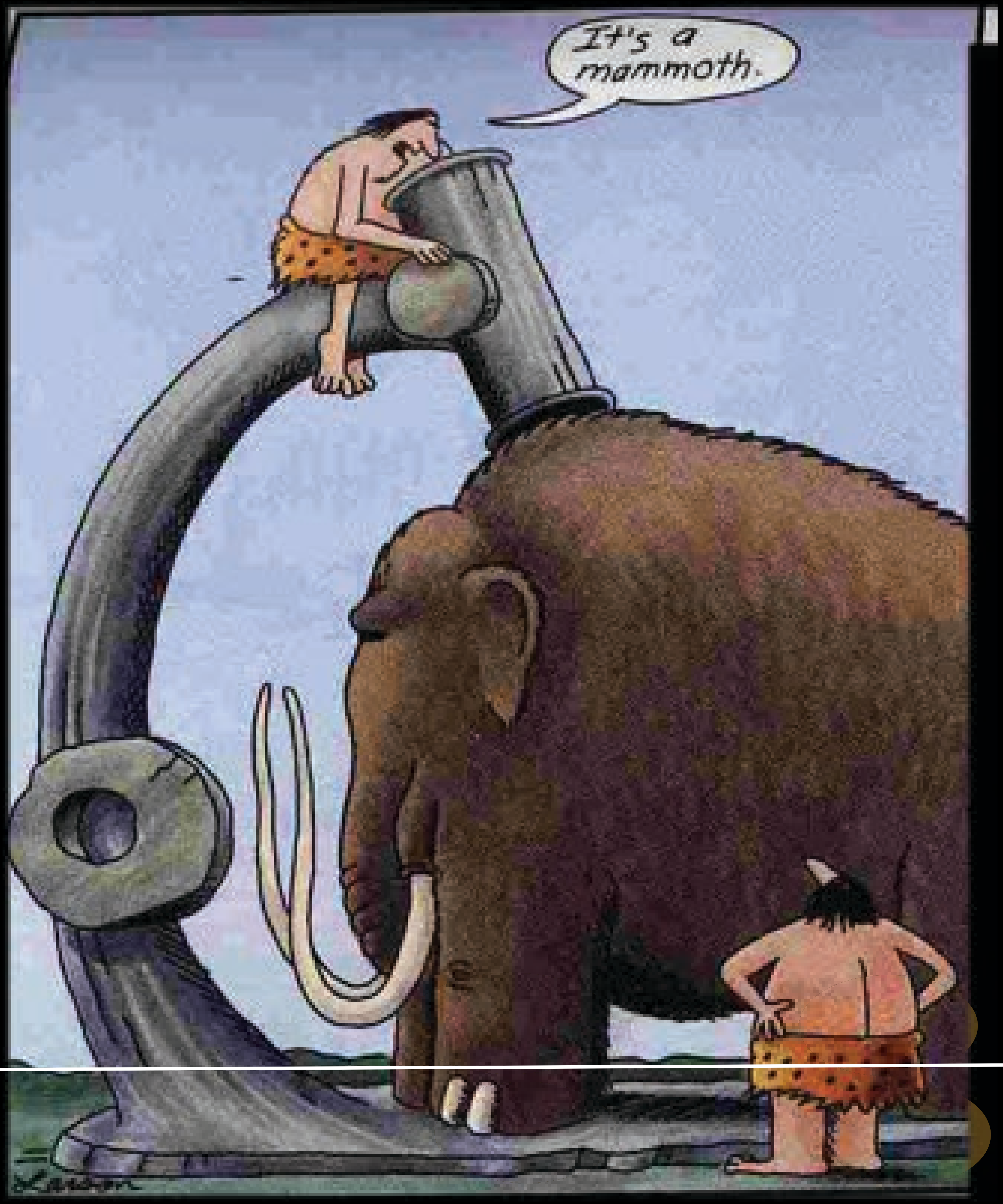
Rainer Kaufmann

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


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,

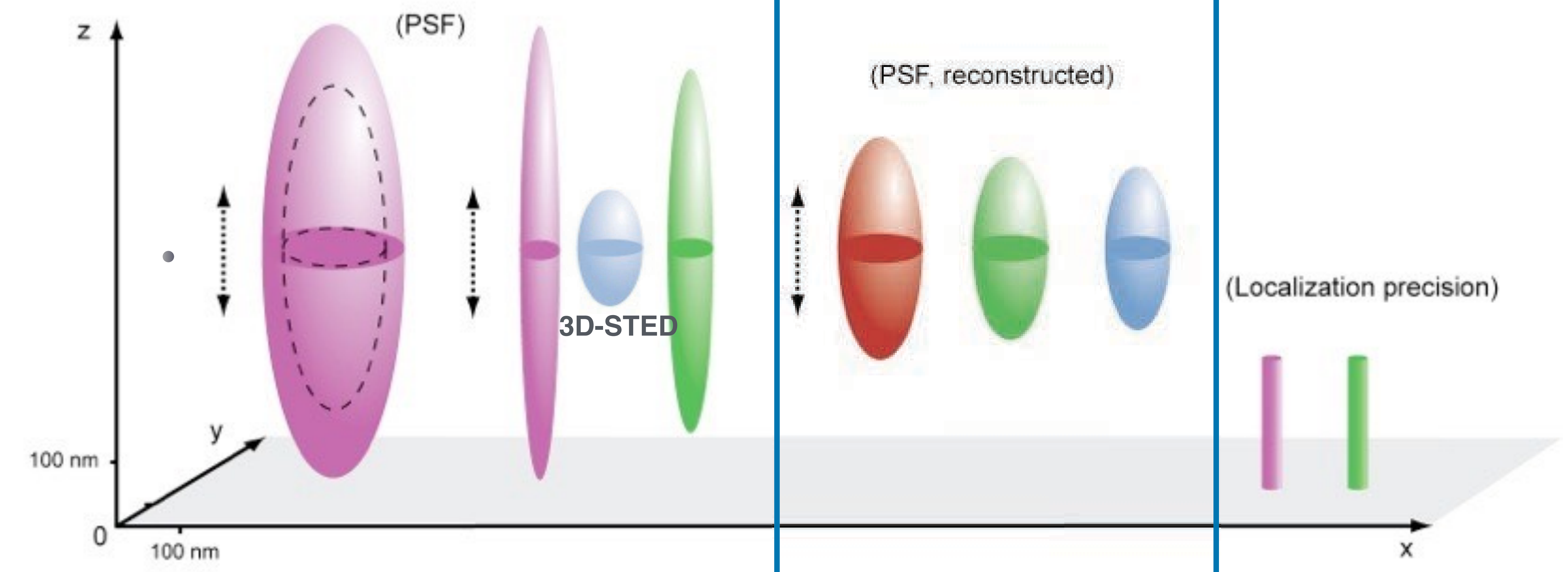
Prague National Museum

To understand the game you need to see the player move

Temporal information (live cell imaging)

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



	CLSM	STED	CW-STED	3D-SIM			PALM/STORM
$\sim D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
$\sim D_z$ [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
$\sim V_{x,y,z}$ [$*10^{-3}$ 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

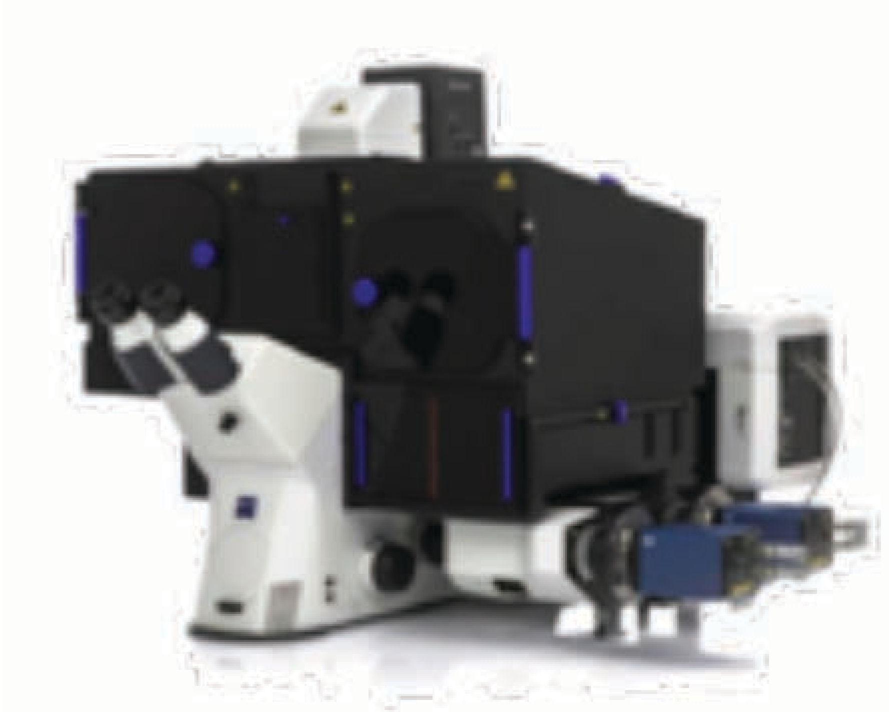
Schermelleh, Heintzmann, Leonhardt, *JCB*, 2010

Commercial SIM systems: Who does what?

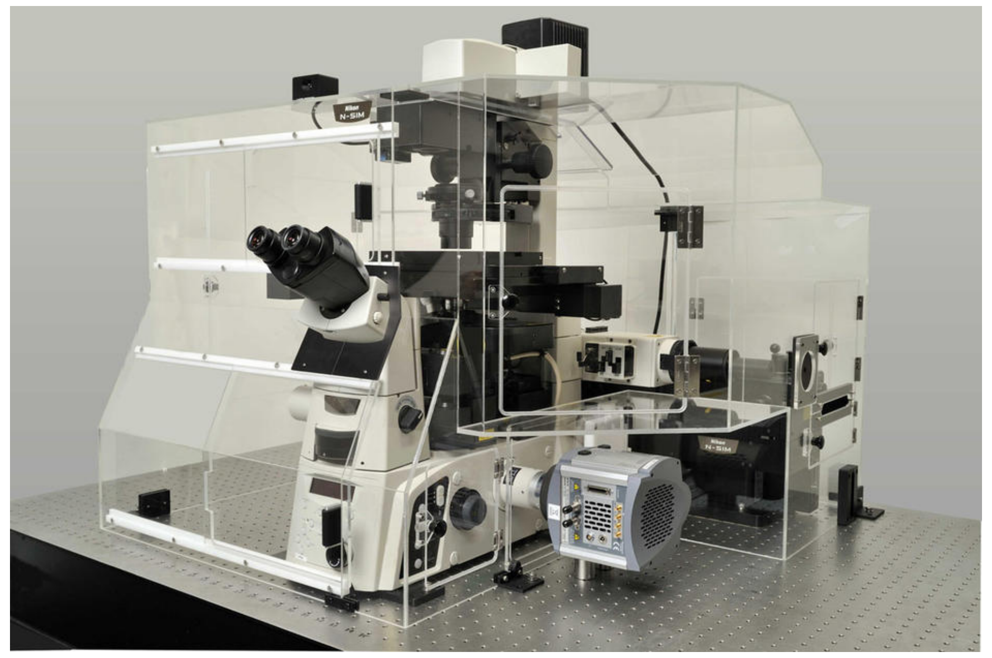
OMX V4
3D SIM (3-beam)
Blaze: fixed grating + galvos (fast)

OMX SR
3D SIM (3-beam)
2D SIM, TIRF-SIM (2-beam)
Blaze: fixed grating + galvos (fast)
Small foot print

Elyra S1
3D SIM (3-beam)
Rotating grating (slow)



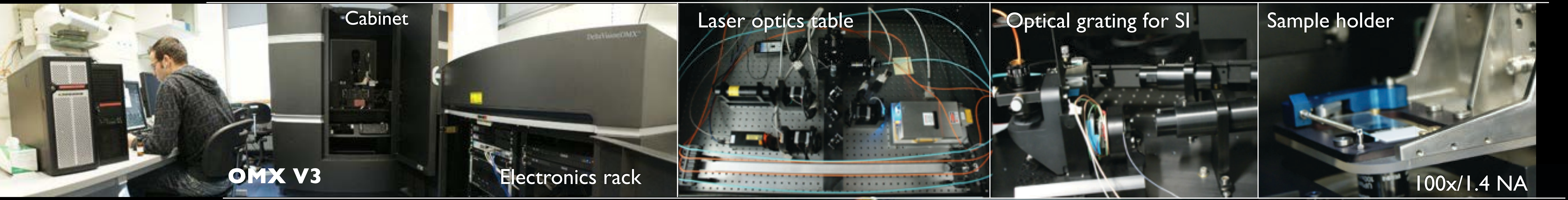
Elyra 7
3D SIM (5-beam)
No rotation (fast)

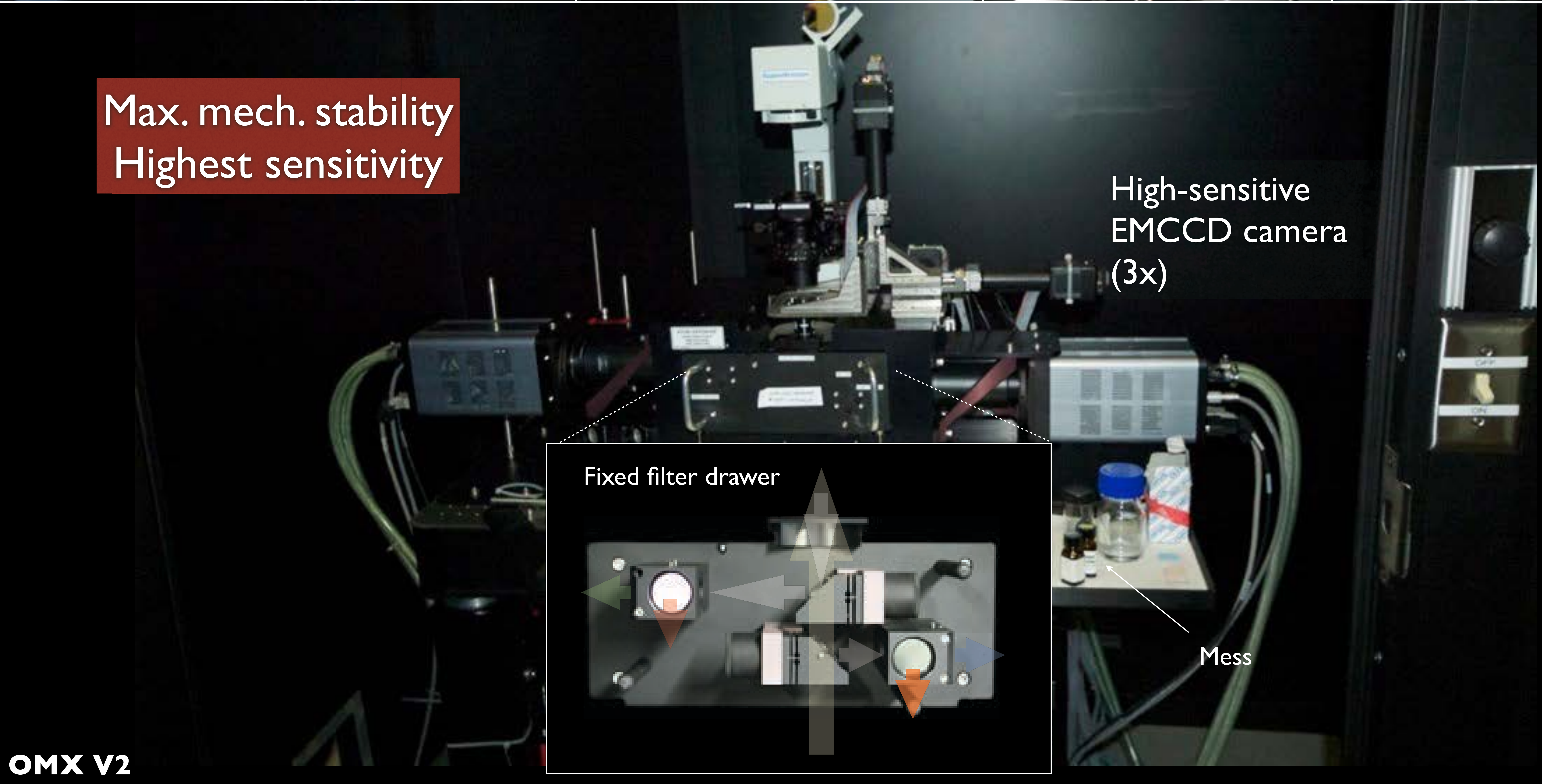


NSIM-E
3D SIM (3-beam)
Rotating grating (slow)

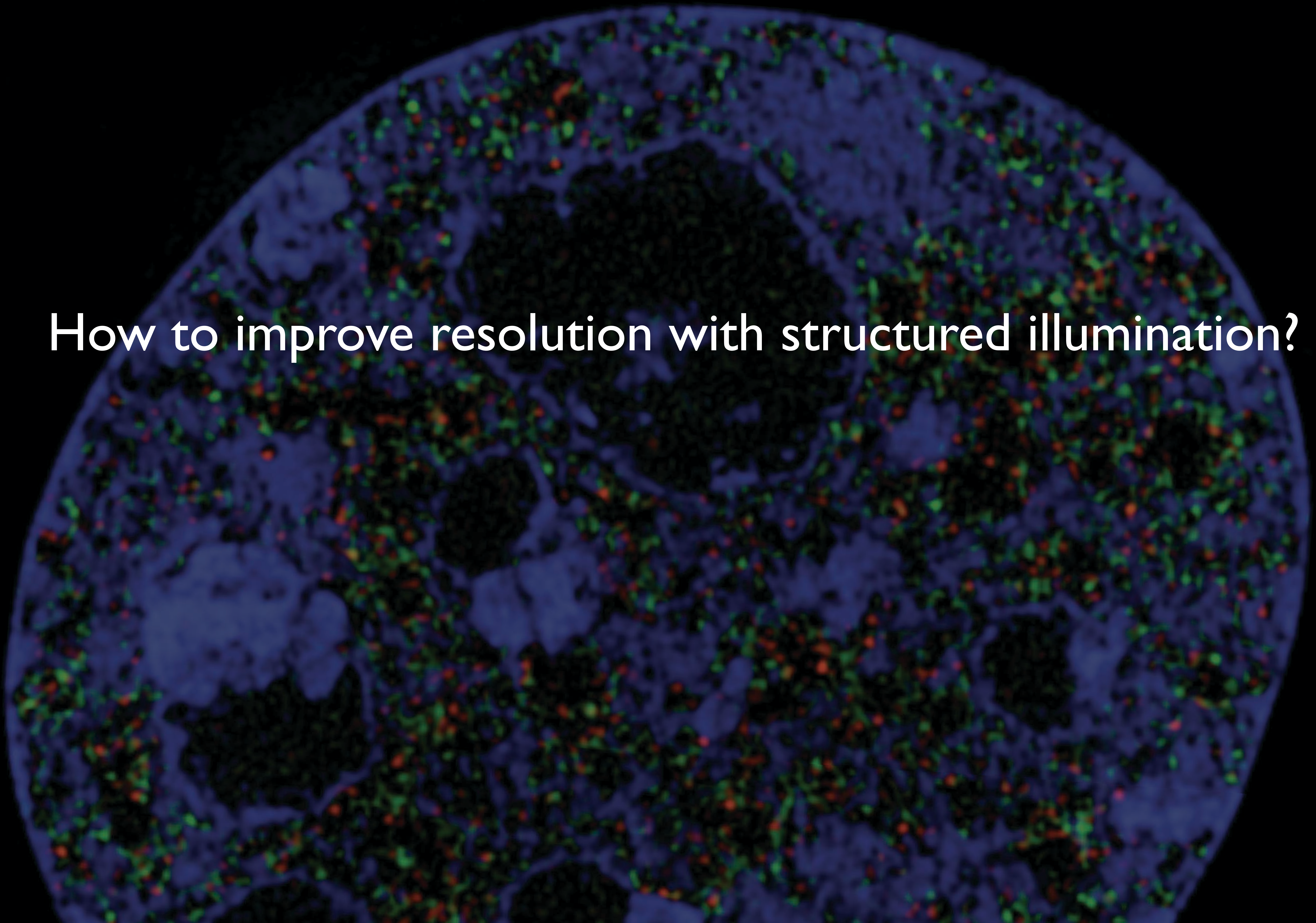
NSIM-S
3D SIM (3-beam)
2D SIM, TIRF-SIM (2-beam)
SLM (fast)

OMX 3D-SIM microscope system





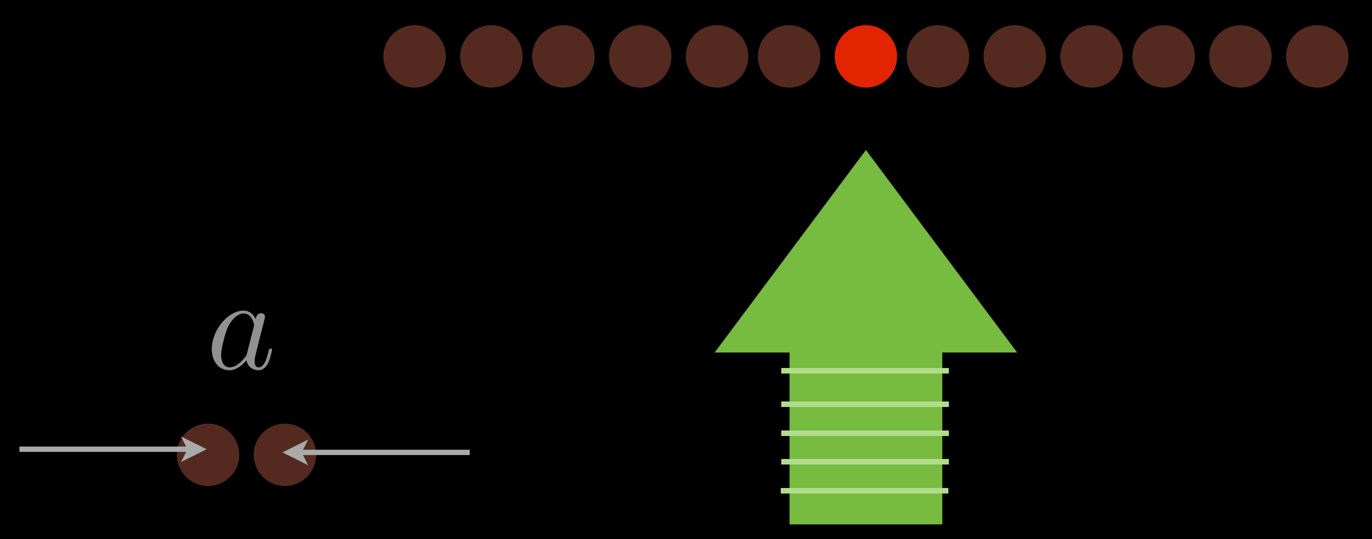
How to improve resolution with structured illumination?



The basic principle: Abbe's view

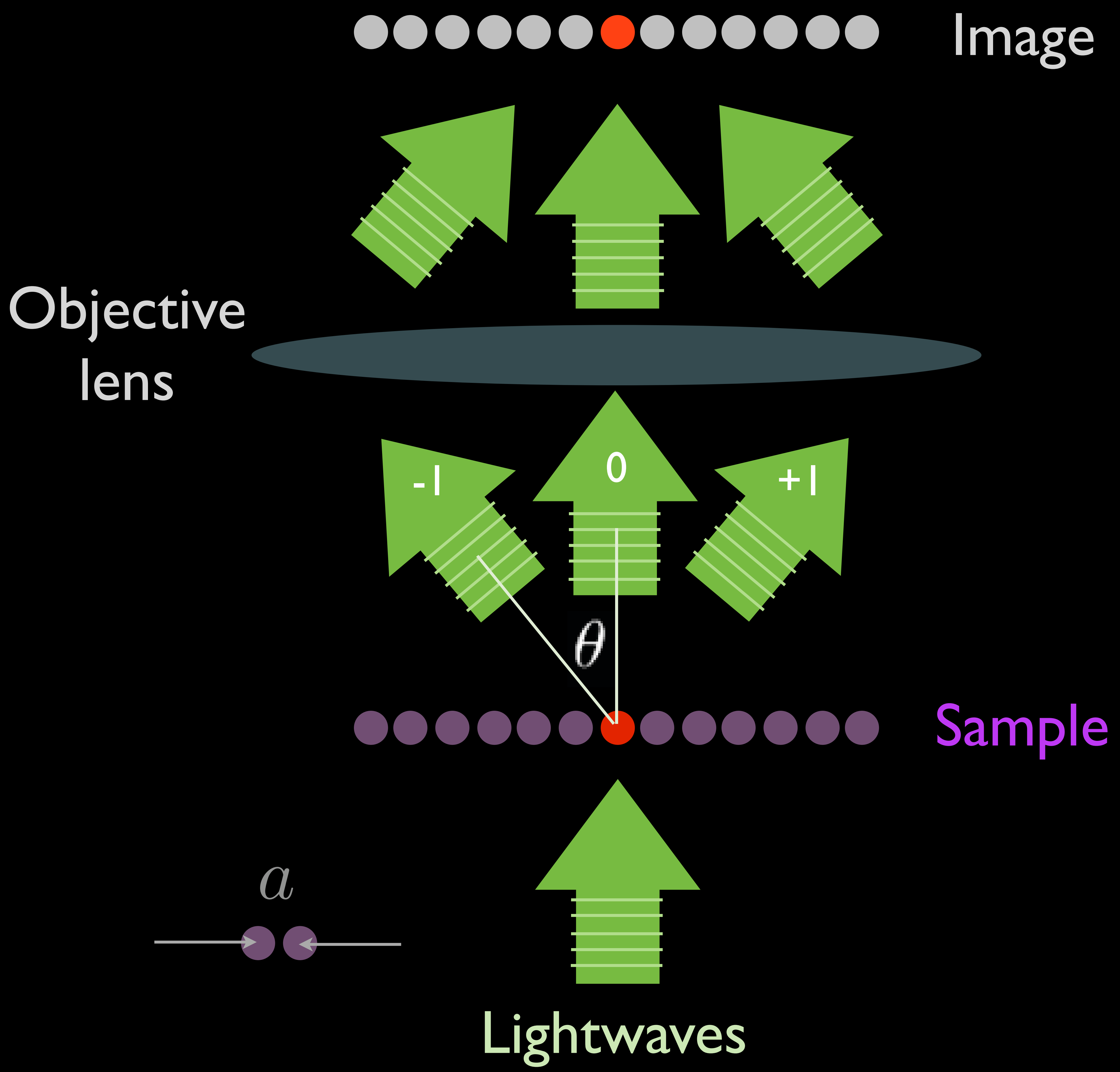
Sample = Structure

→ Periodicity

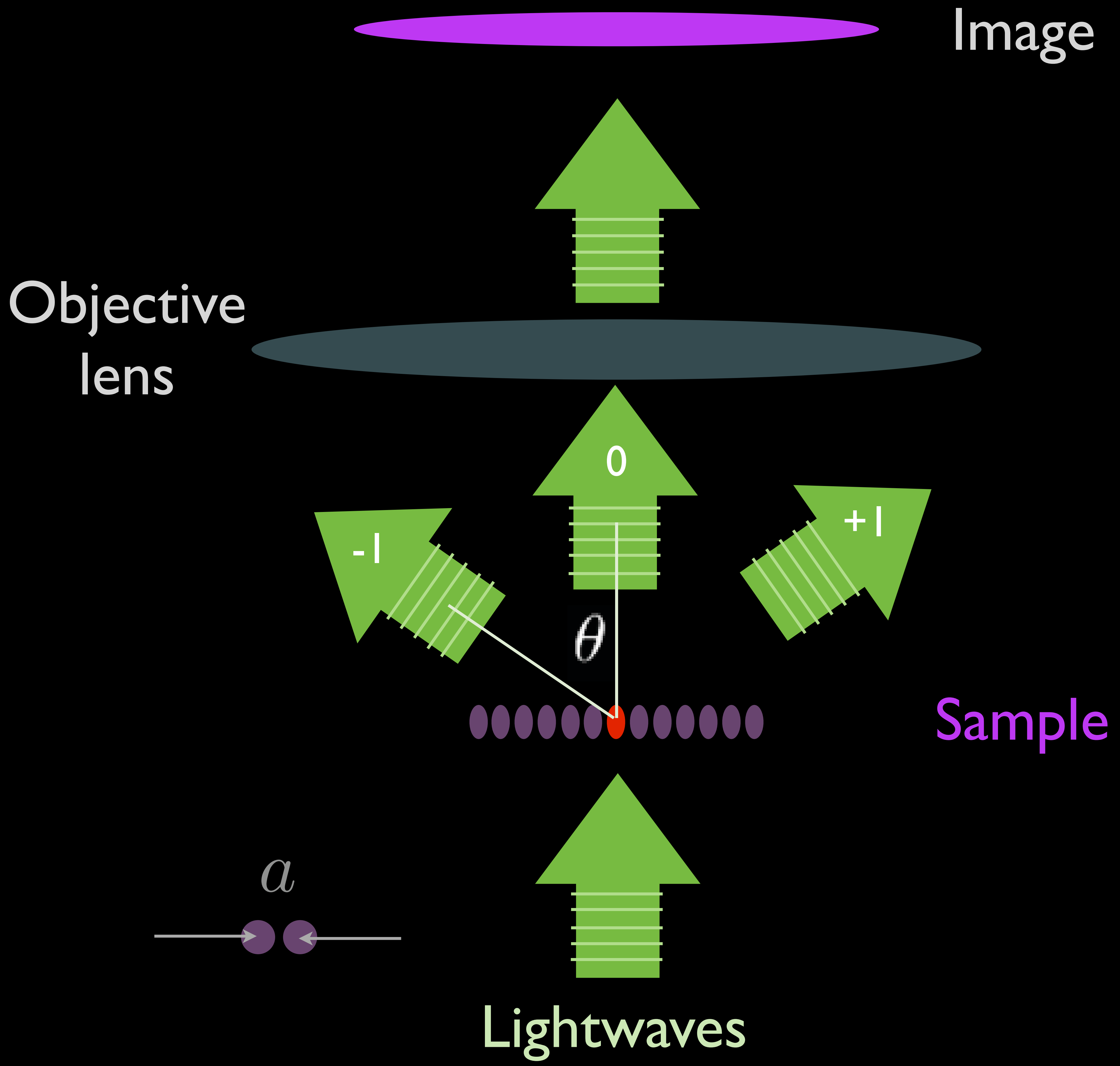


http://de.wikipedia.org/wiki/Ernst_Abbe

The basic principle: Abbe's view



The basic principle: Abbe's view

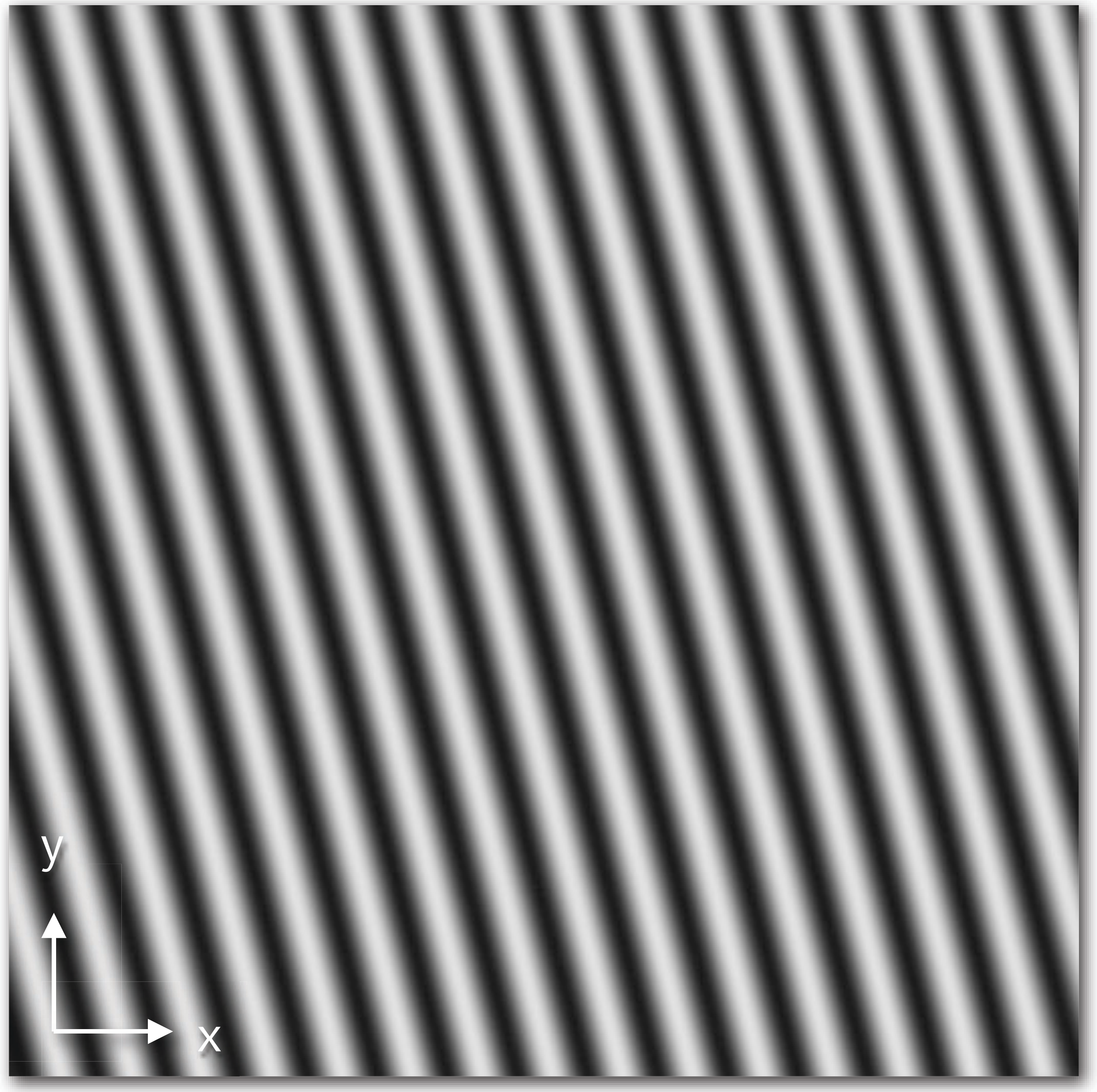


$$r = \frac{\lambda}{2n \sin \theta} = \frac{\lambda}{2\mathrm{NA}}$$

highest frequencies
(biggest α)
→
smallest structures

Fourier transformation in a nutshell

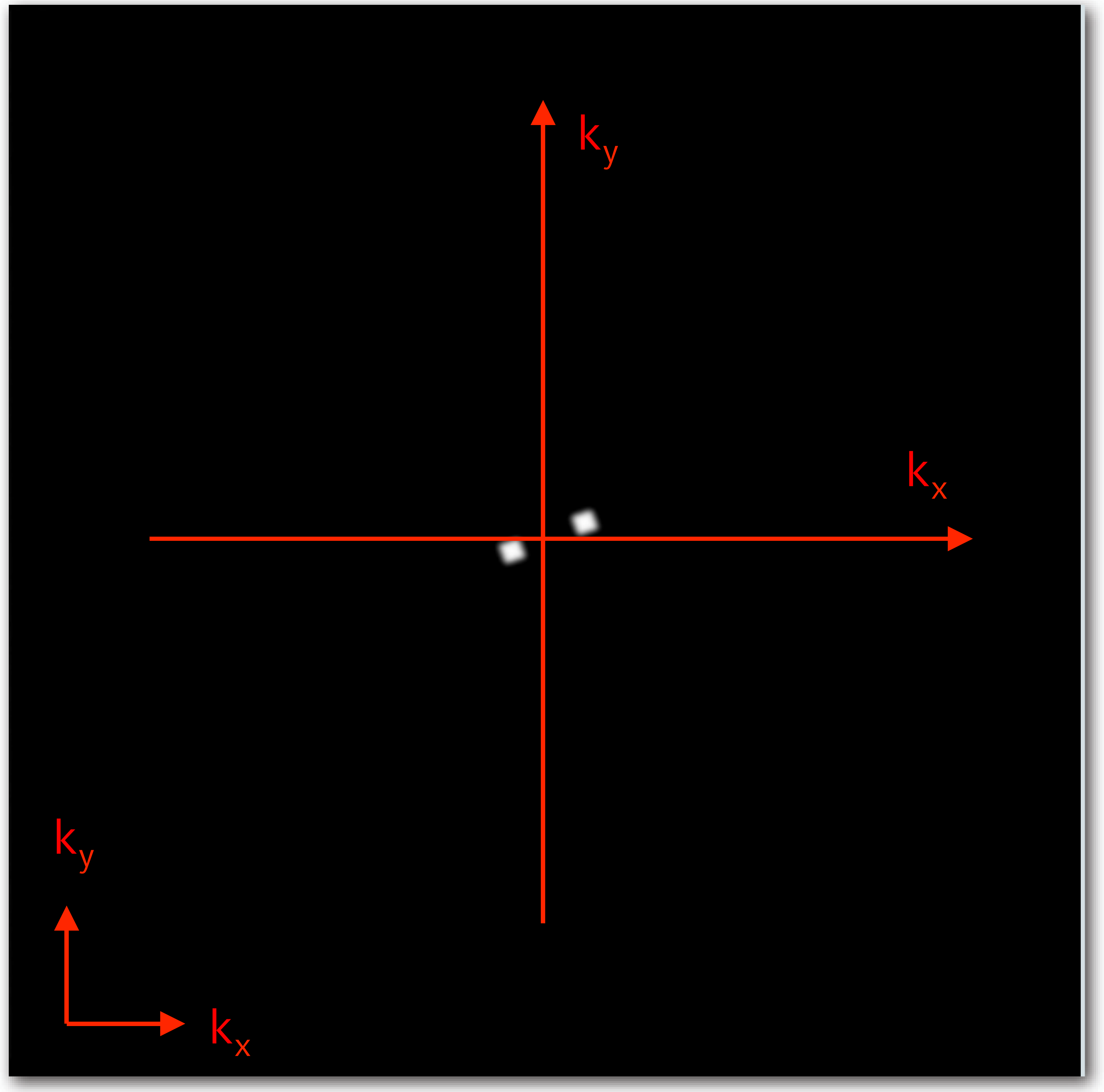
Real space (xy)



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

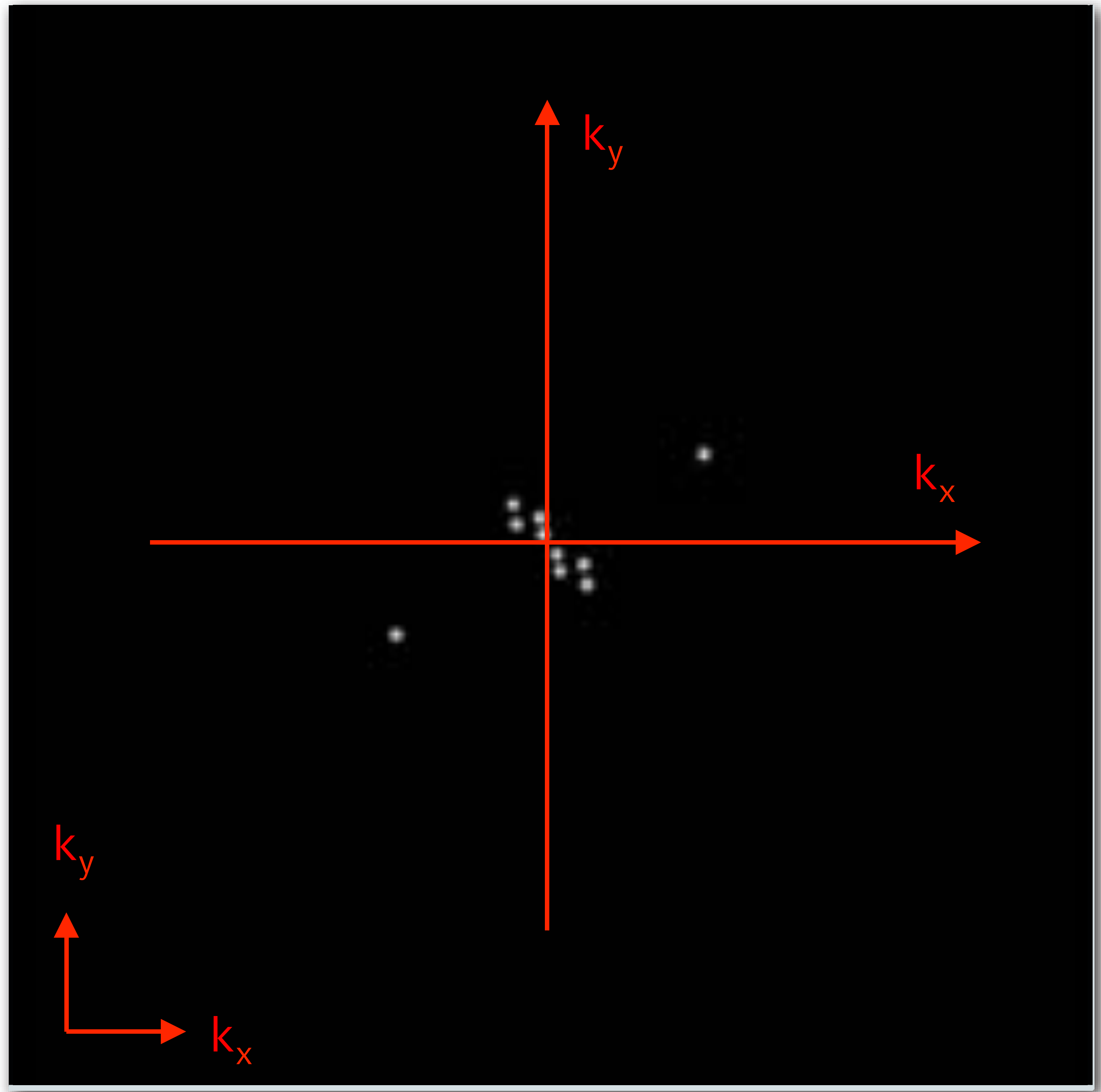
Image = superimposed periodicities

Real space (xy)

Fourier Transform

Inverse
Fourier Transform

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



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

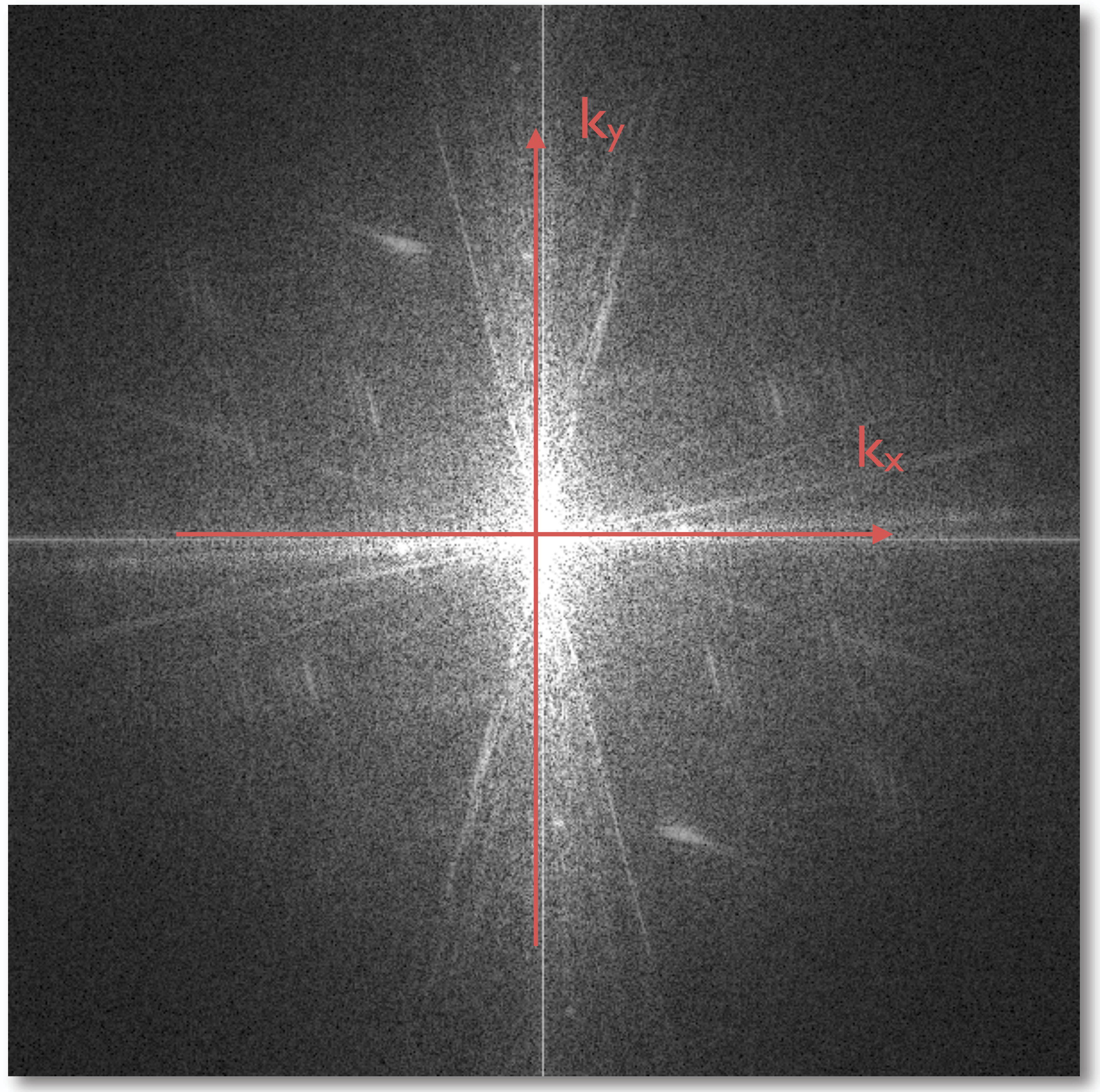
Image = superimposed periodicities

Real space (xy)

Fourier Transform

Inverse Fourier Transform

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



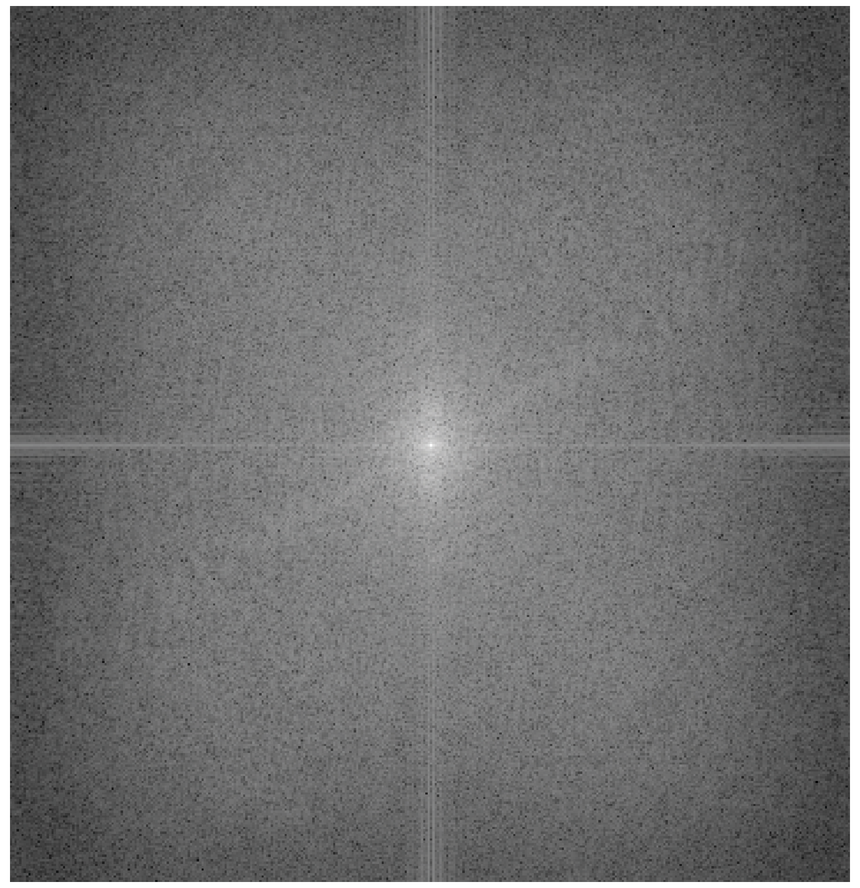
Alternative representation of information
Low-resolution: near the origin
High-resolution: farther out
kx, ky: Spatial frequencies, periods/μm

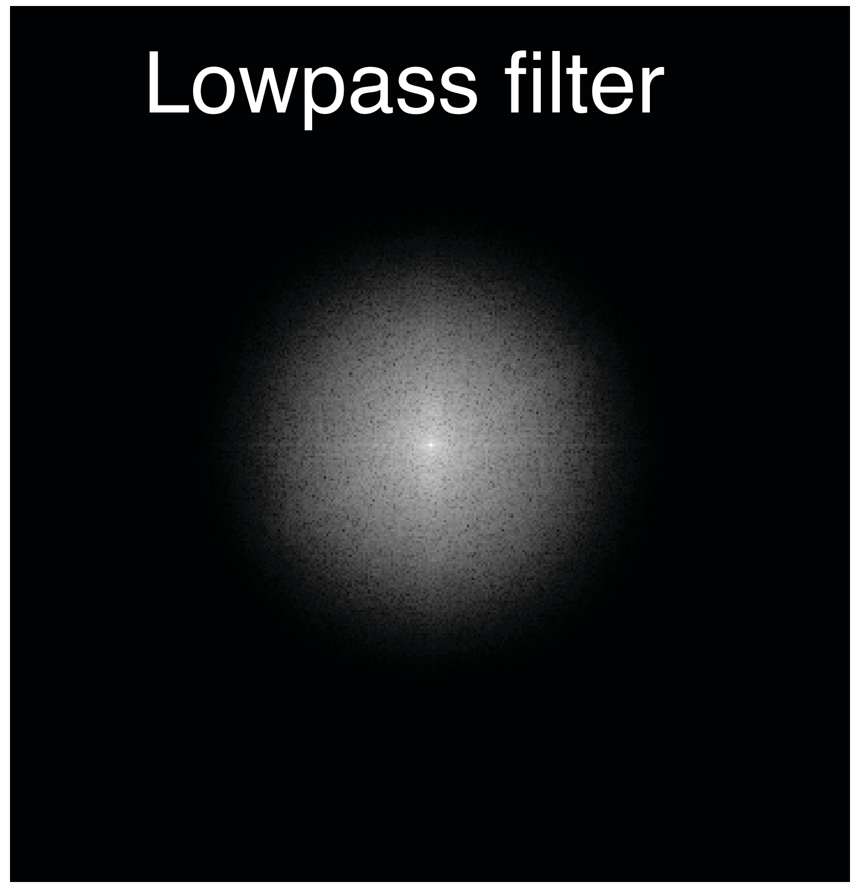
Image = superimposed periodicities

Real space (xy)

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

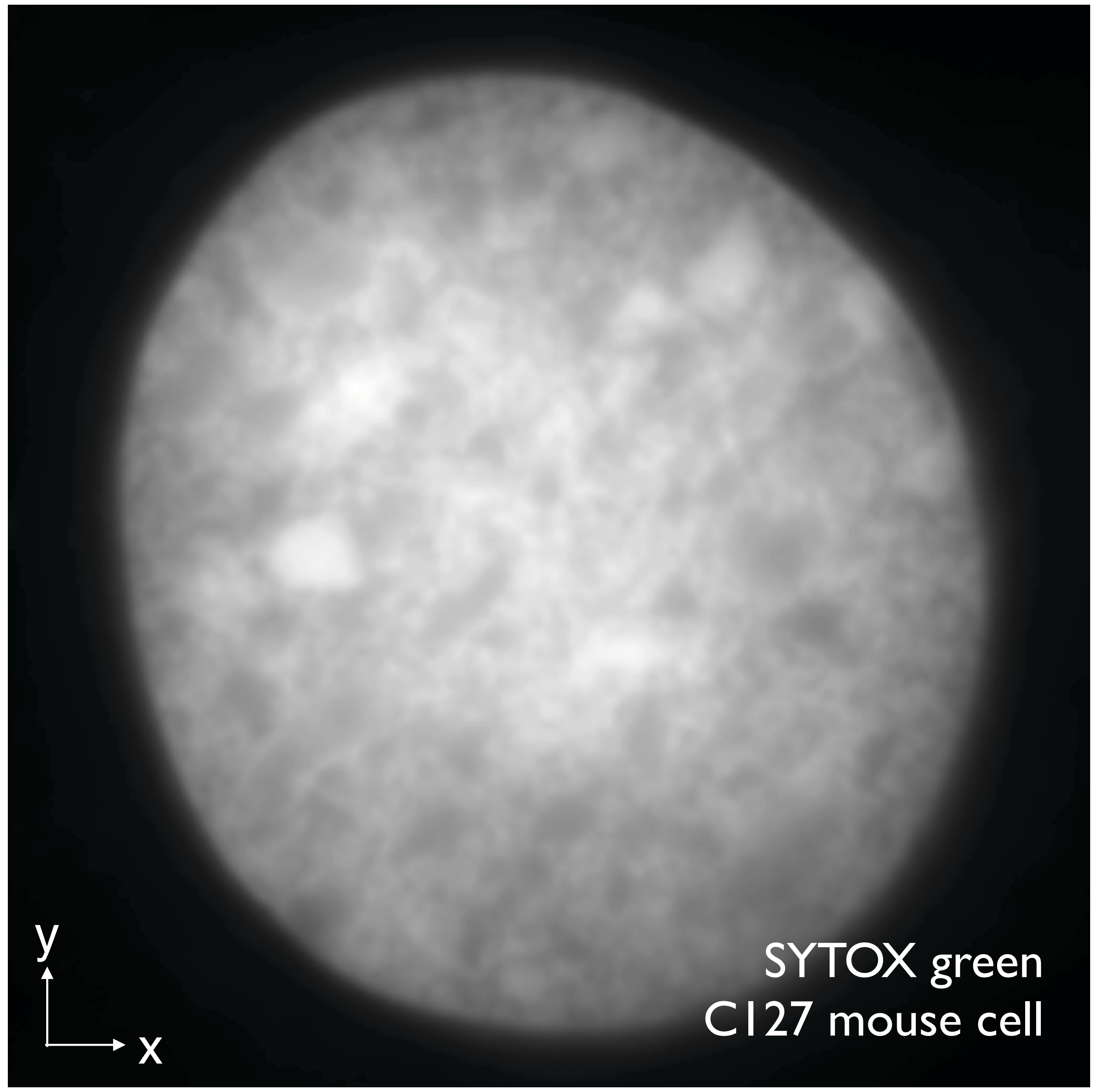
Fourier Transform





Frequency support in wide-field microscopy

Image = real space (xy)

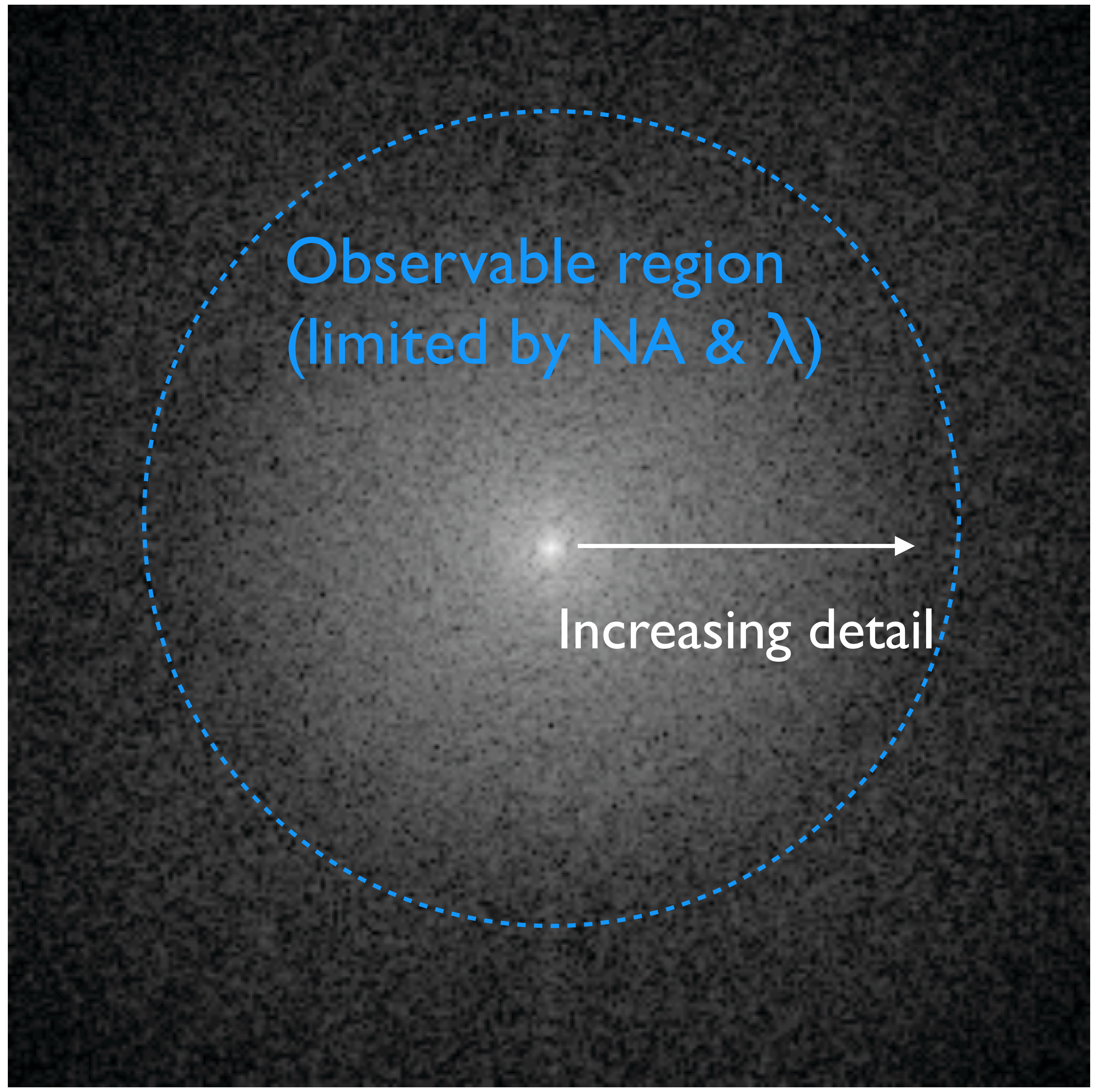


Fourier Transform

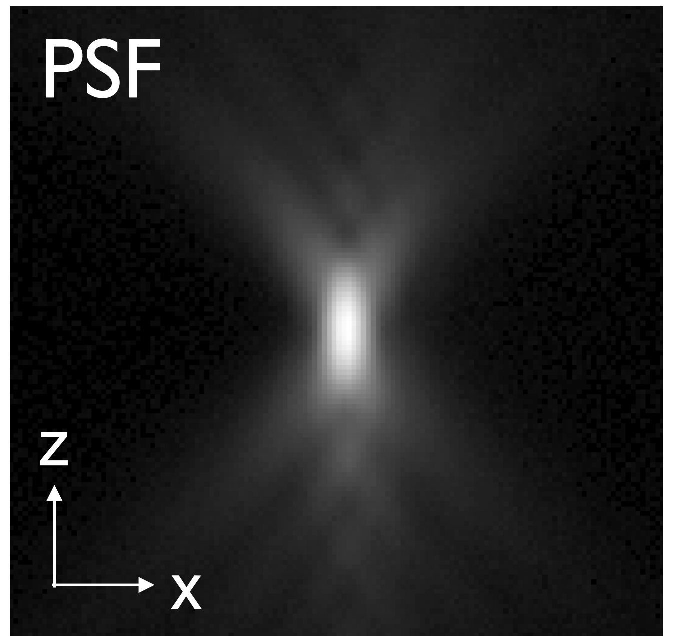
(inverse FT)

Frequency space (k_x, k_y)

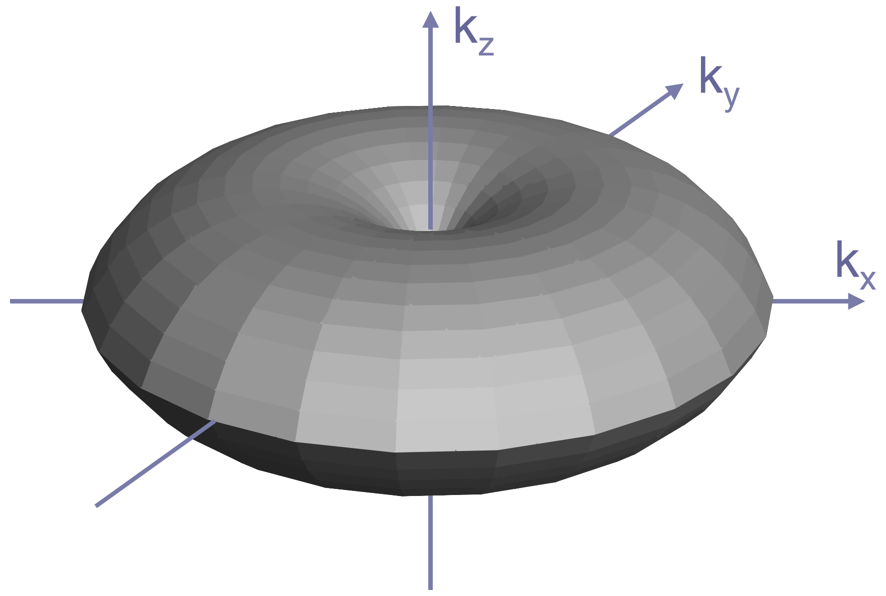
(a.k.a. Fourier space, reciprocal space)



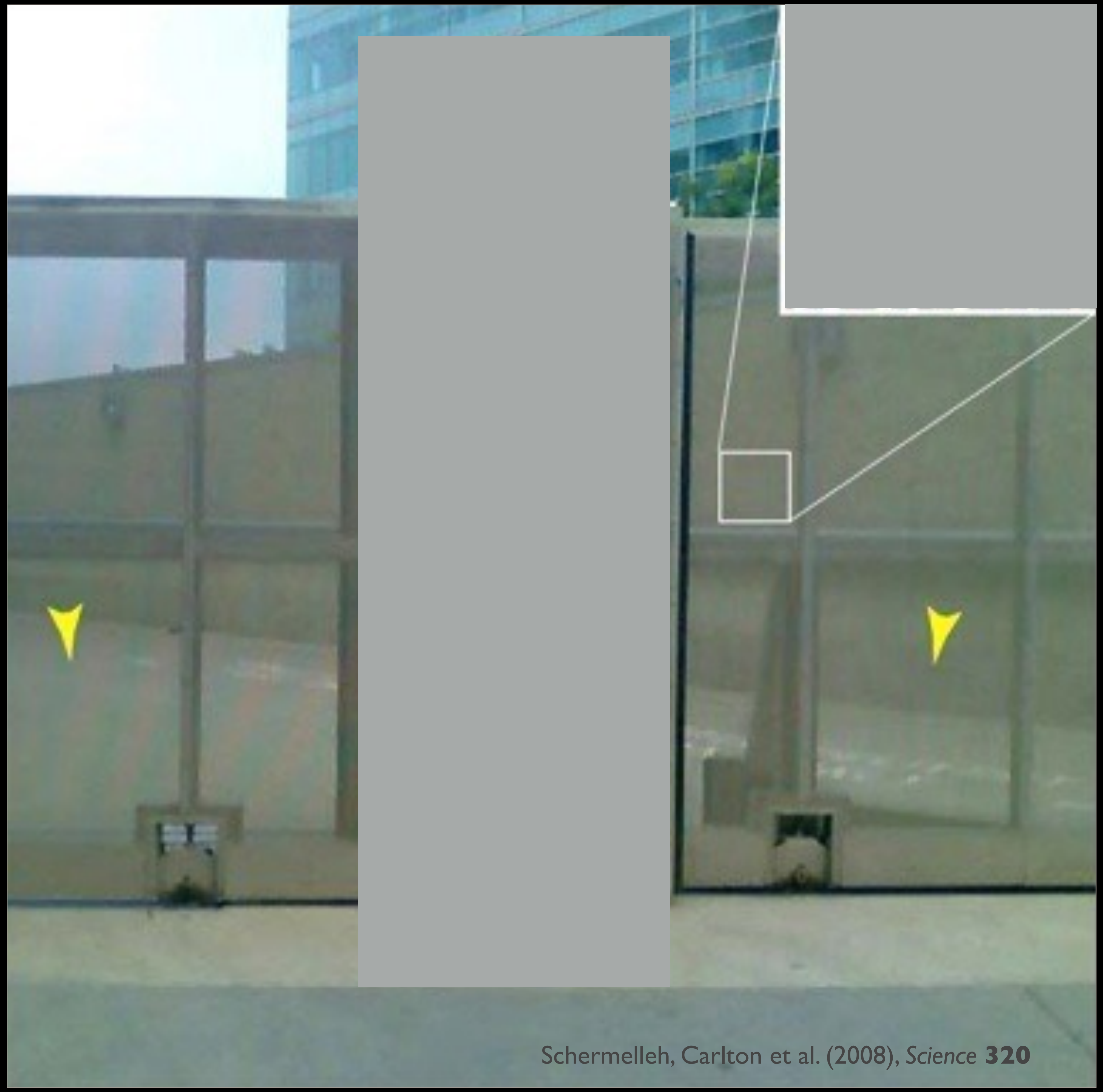
"Real object" ⊗



Full frequency range ×



SIM principle: Moiré interference



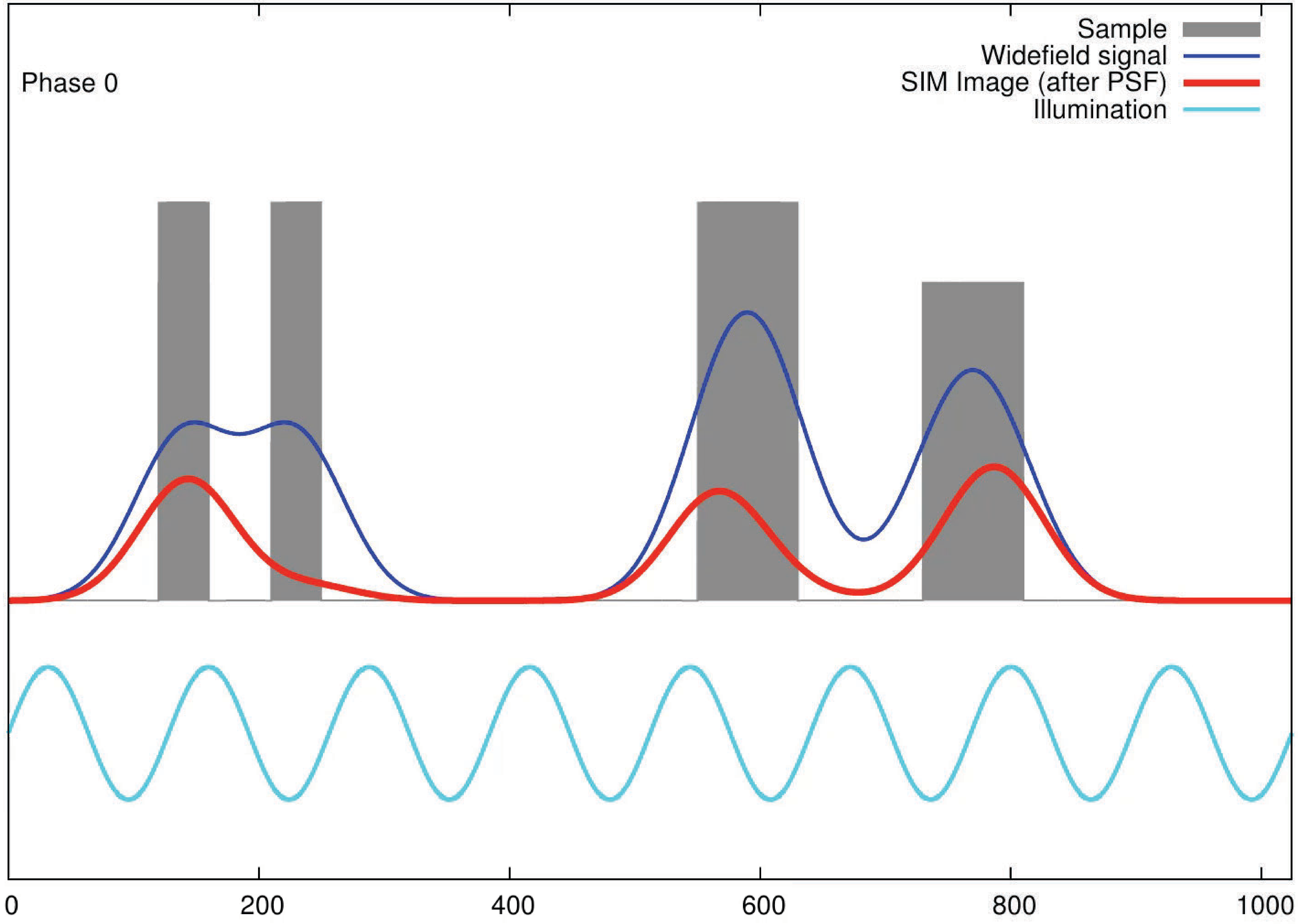
unknown structure

Fourier transform of the measured image

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

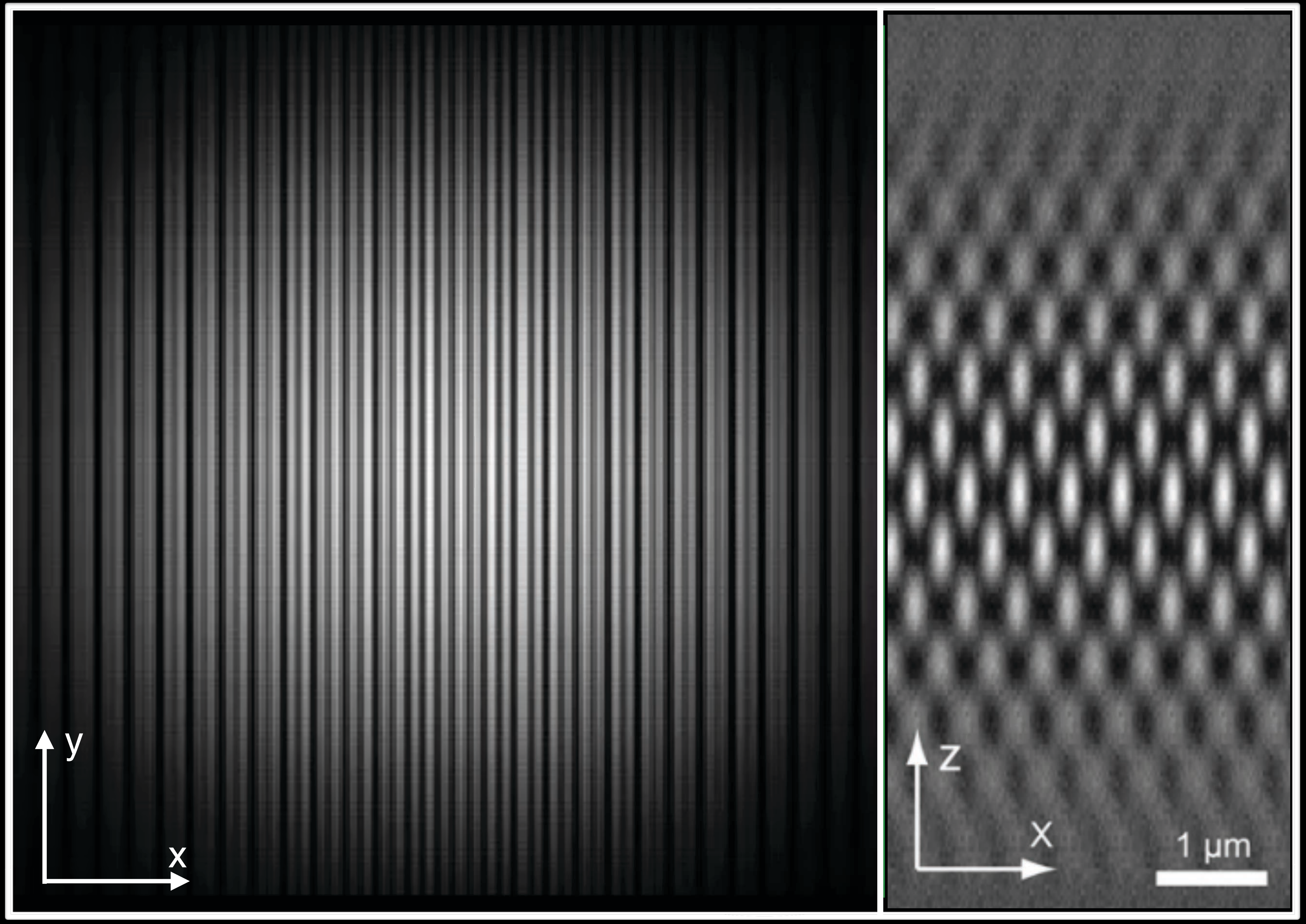
known illumination function

SIM extracts additional information

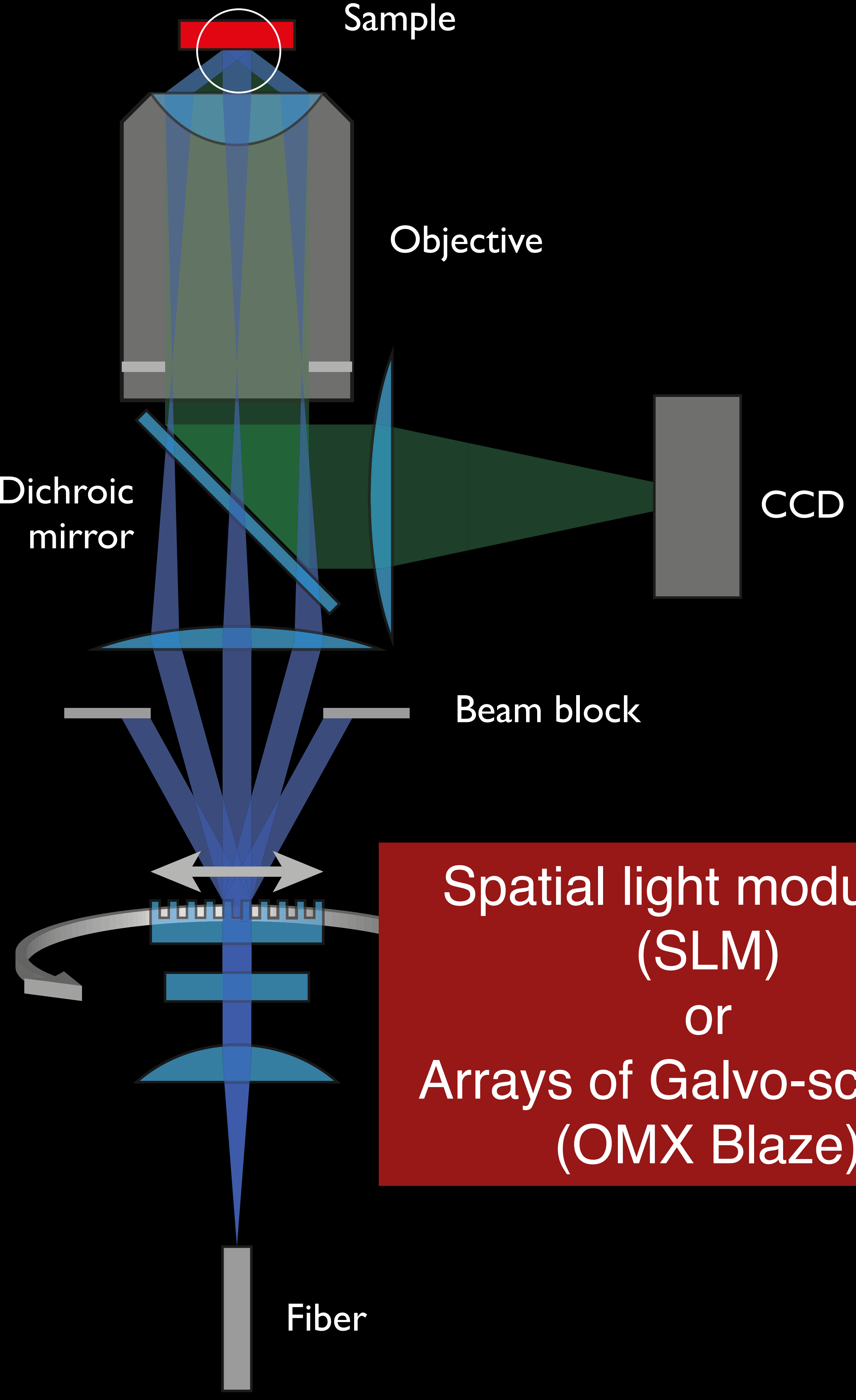


https://mueller-physics.github.io/SIM-Collection/

3D-SIM: microscope design



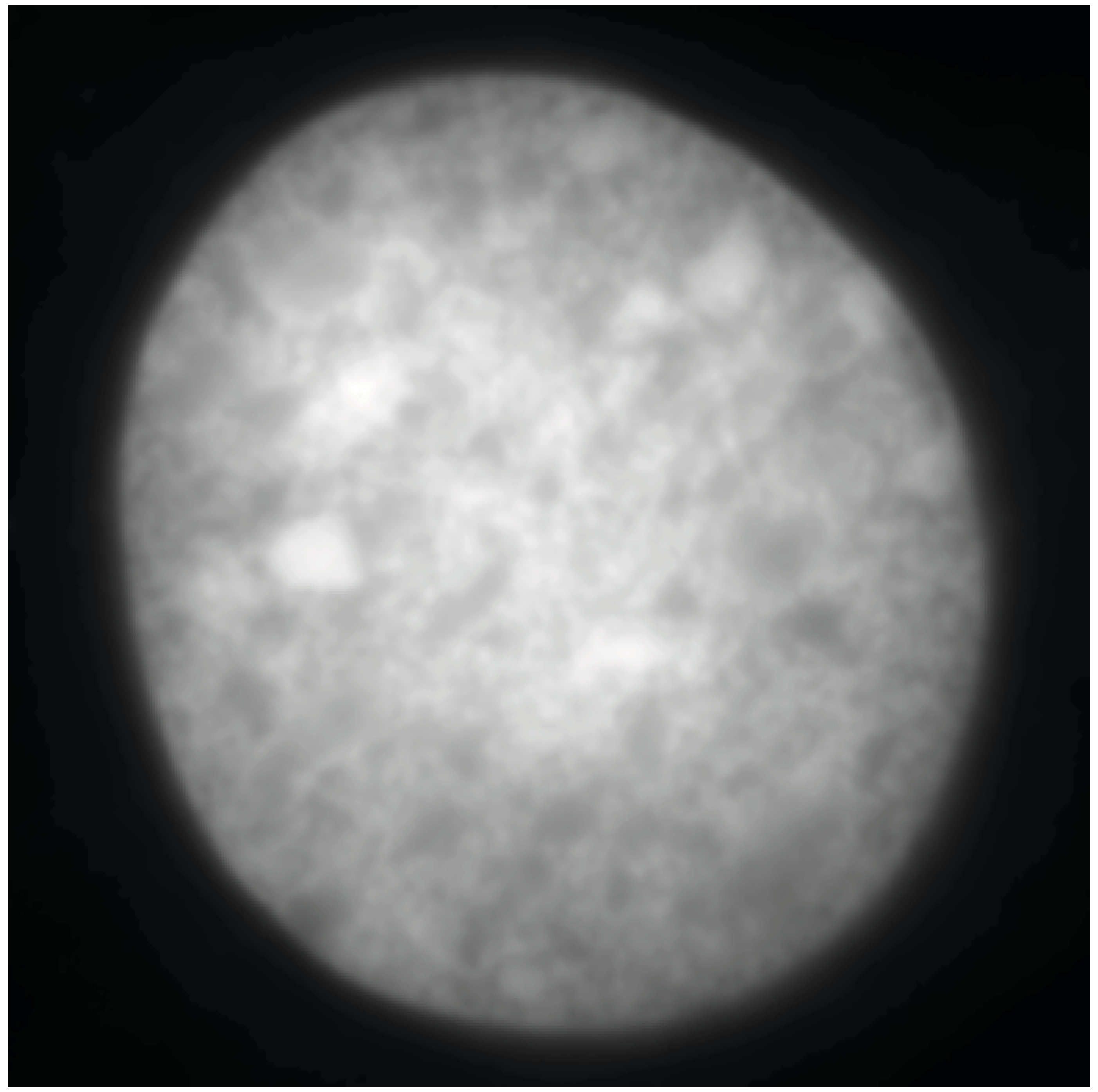
3-beam interference => modulation in xy and z



Spatial light modulator (SLM)
or
Arrays of Galvo-scanner (OMX Blaze)

Frequency support in wide-field microscopy

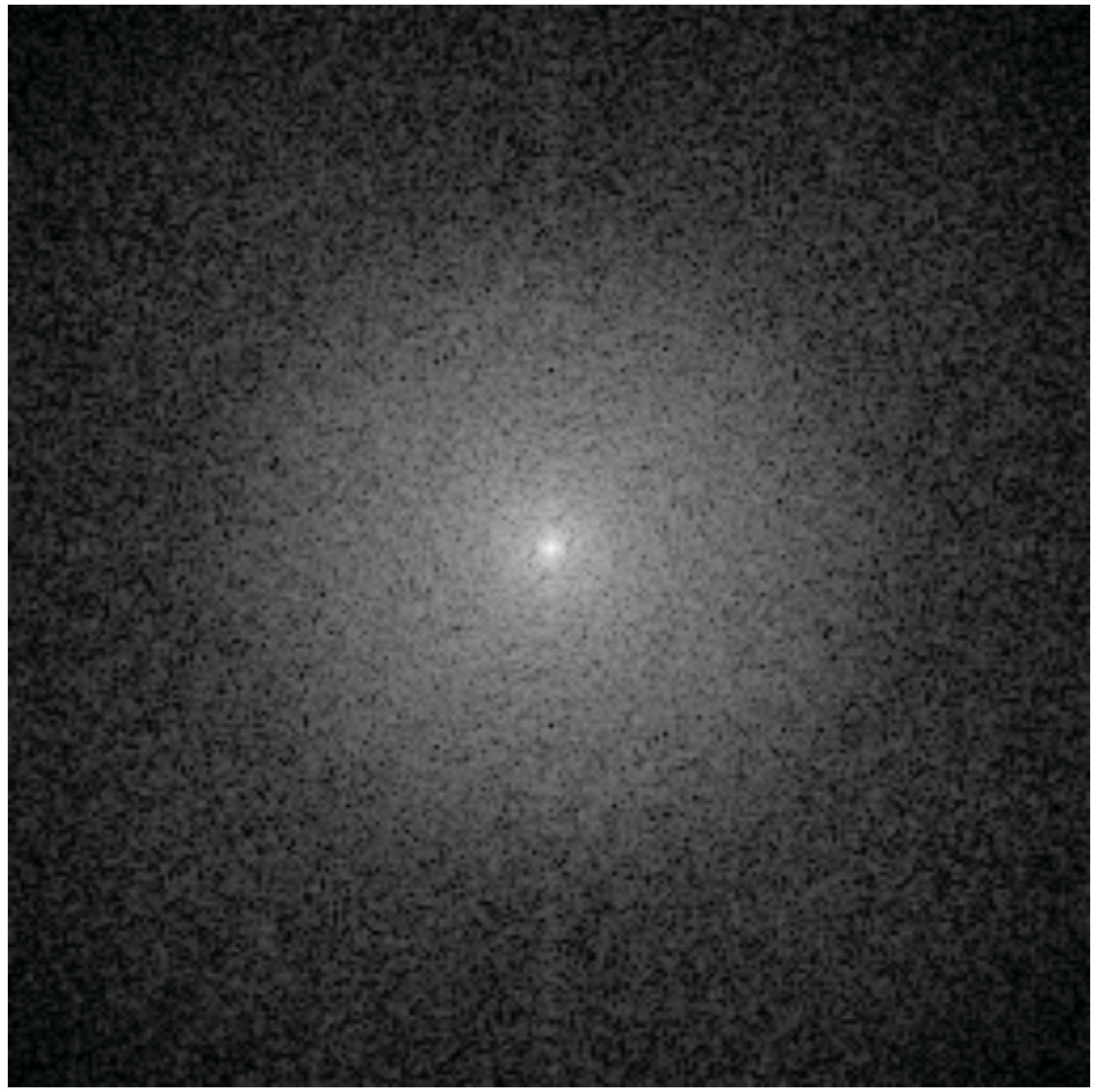
Real space



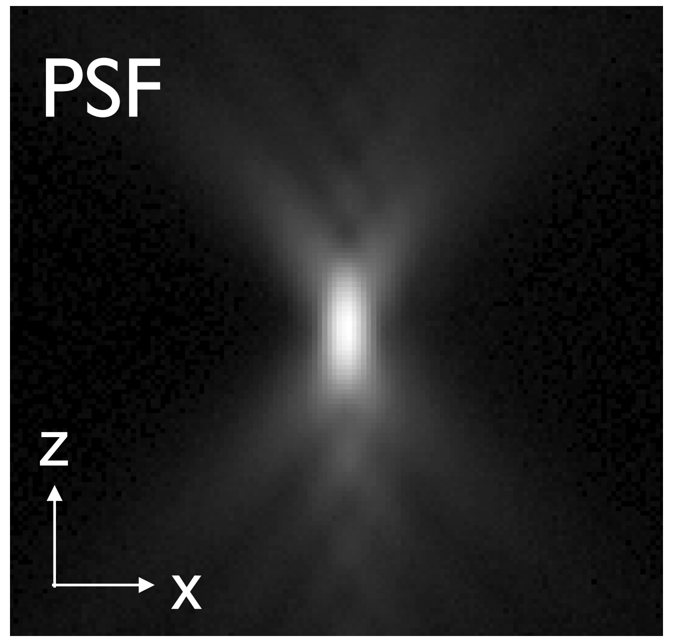
Fourier Transform

(inverse FT)

Reciprocal space

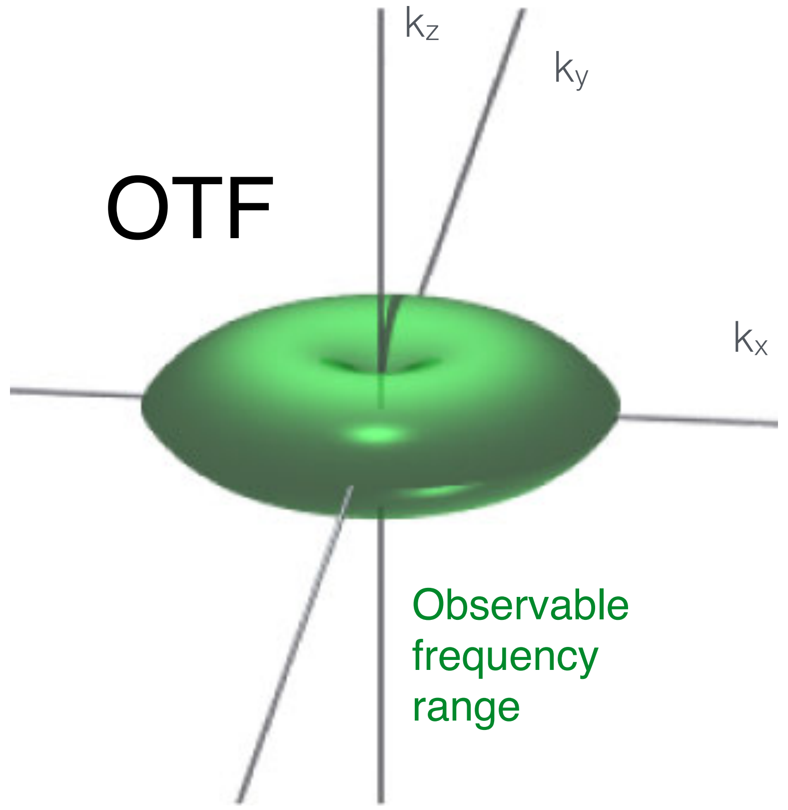


“Real object” ⊗ convolved



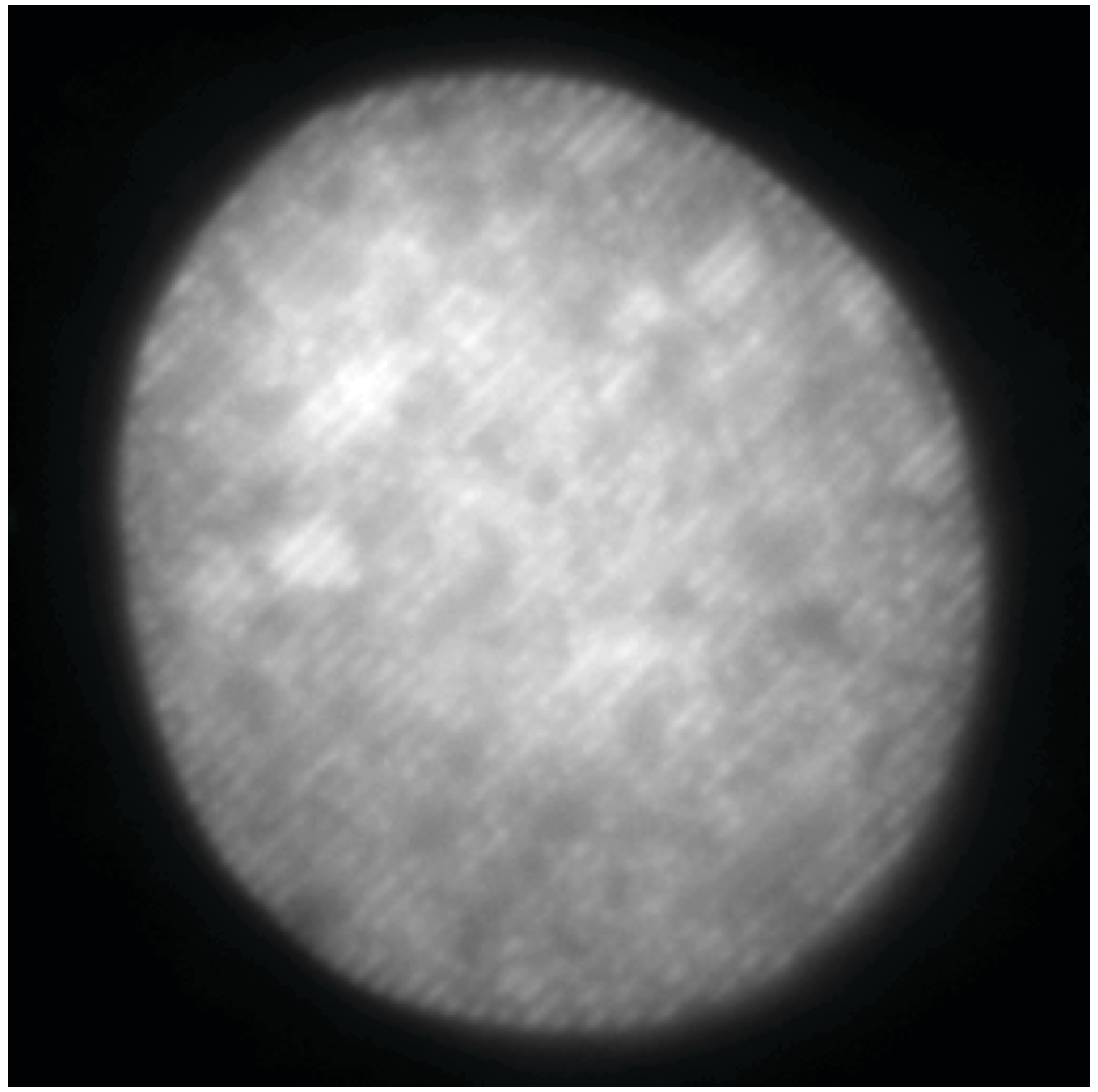
Full frequency range ×

OTF



Doubling frequency support in x-y and z

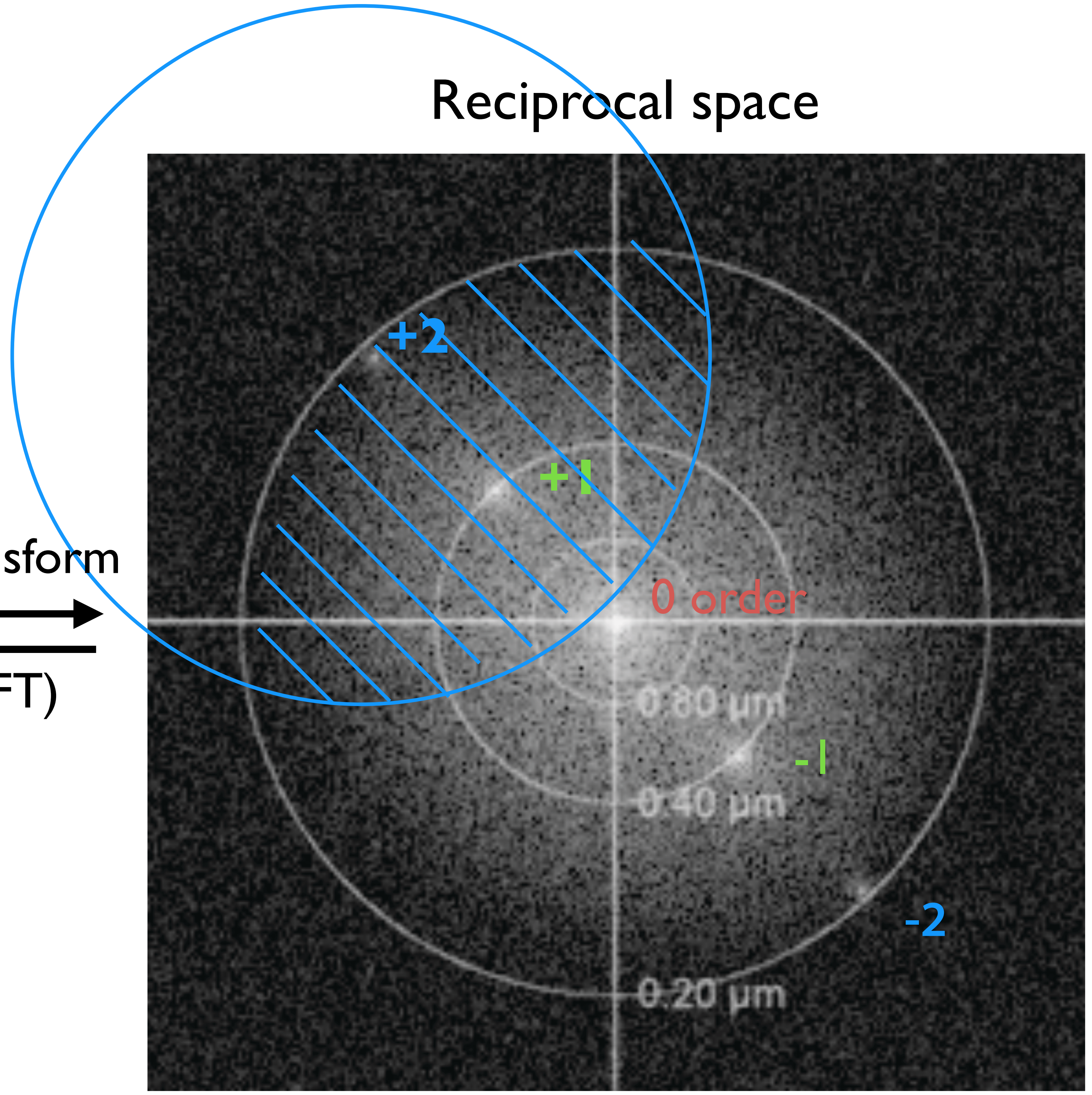
Real space

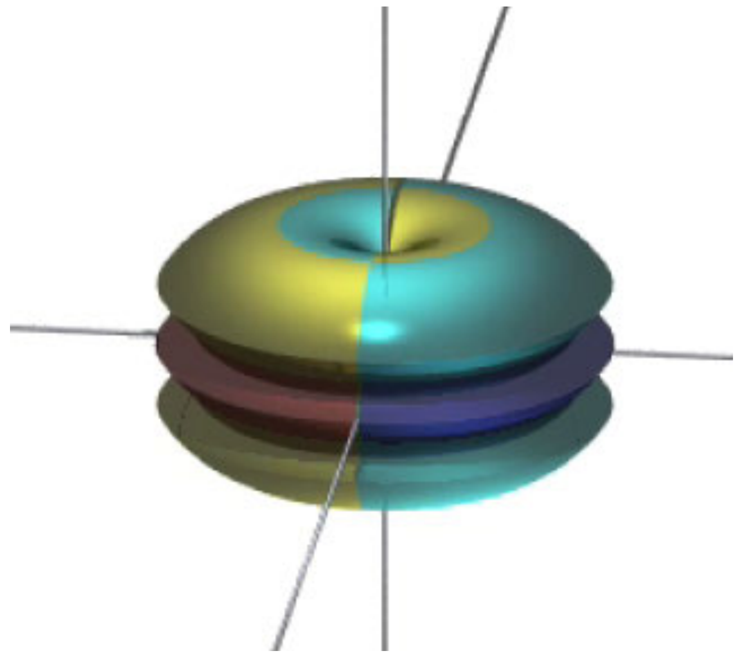


Fourier Transform

(inverse FT)

Reciprocal space

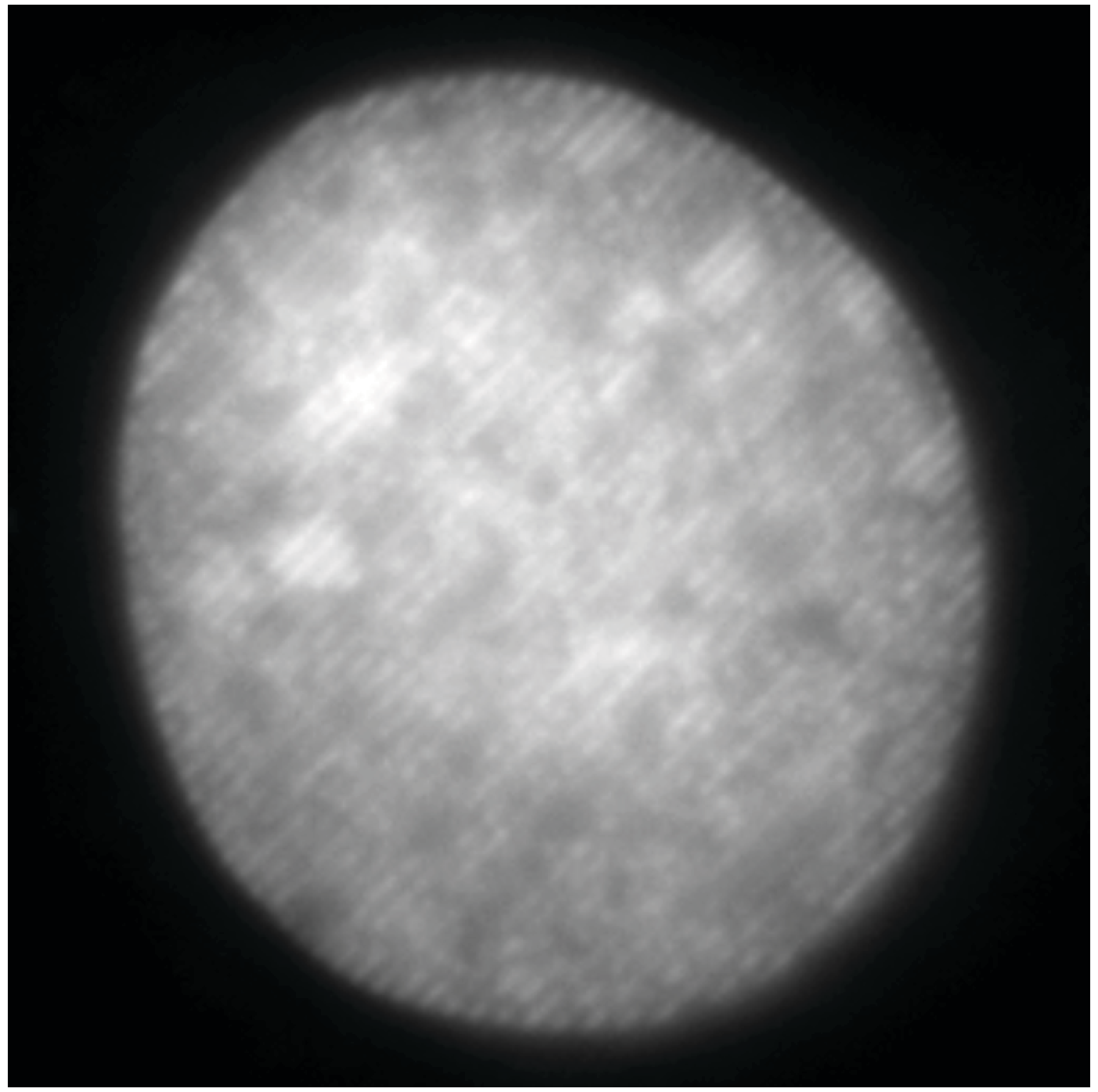




adapted from Gustafsson et al. (2008), *Biophys J* **94**

Doubling frequency support in x-y and z

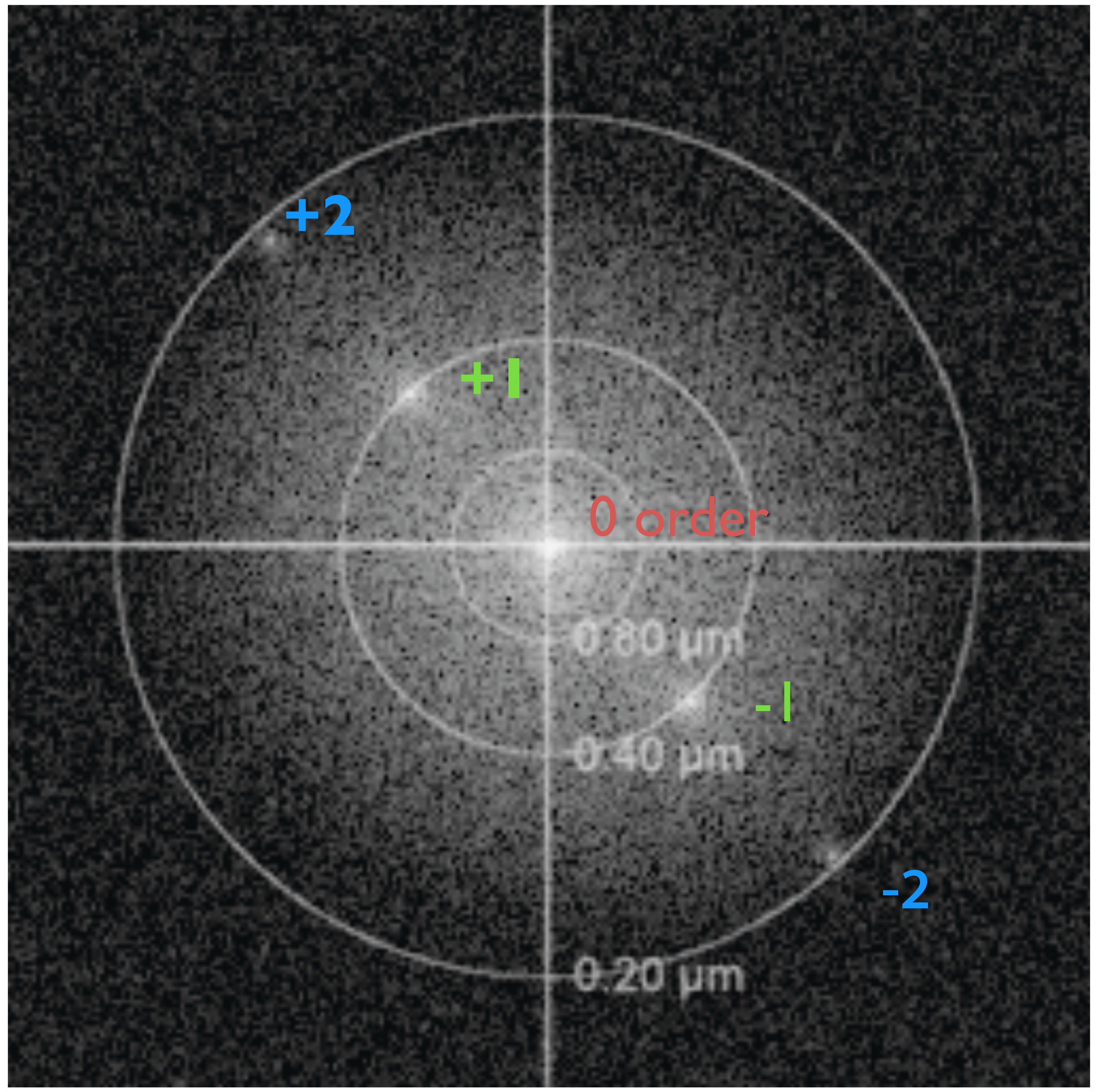
Real space



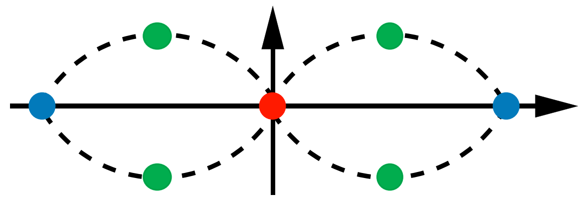
Fourier Transform

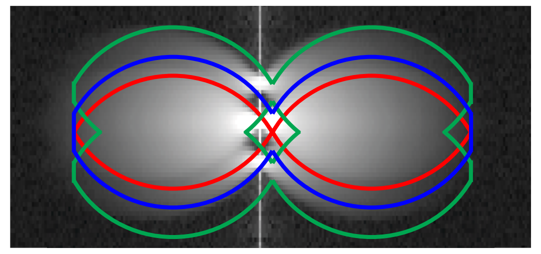
(inverse FT)

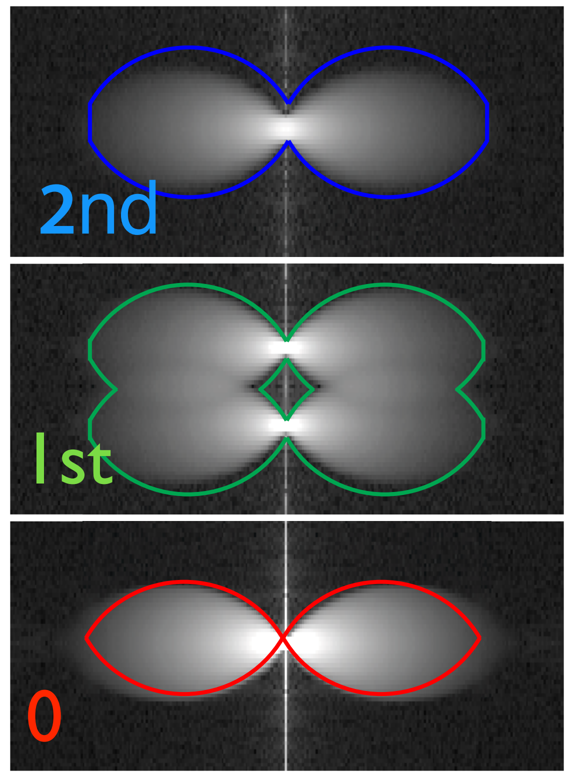
Reciprocal space



axial
(x-z)

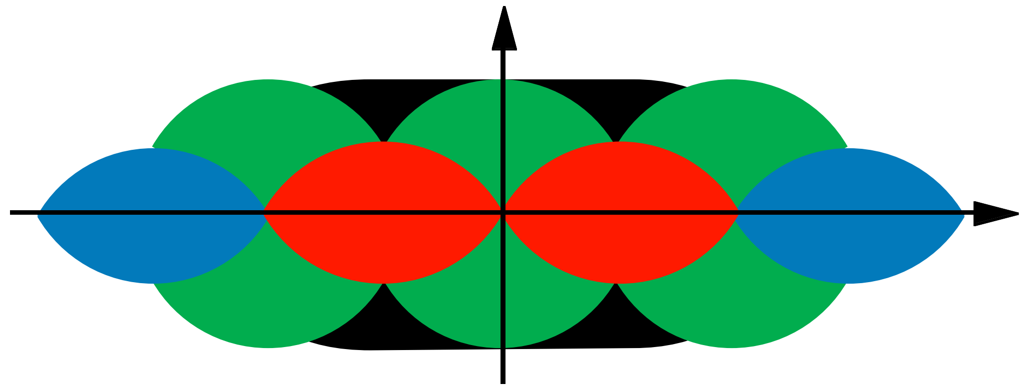






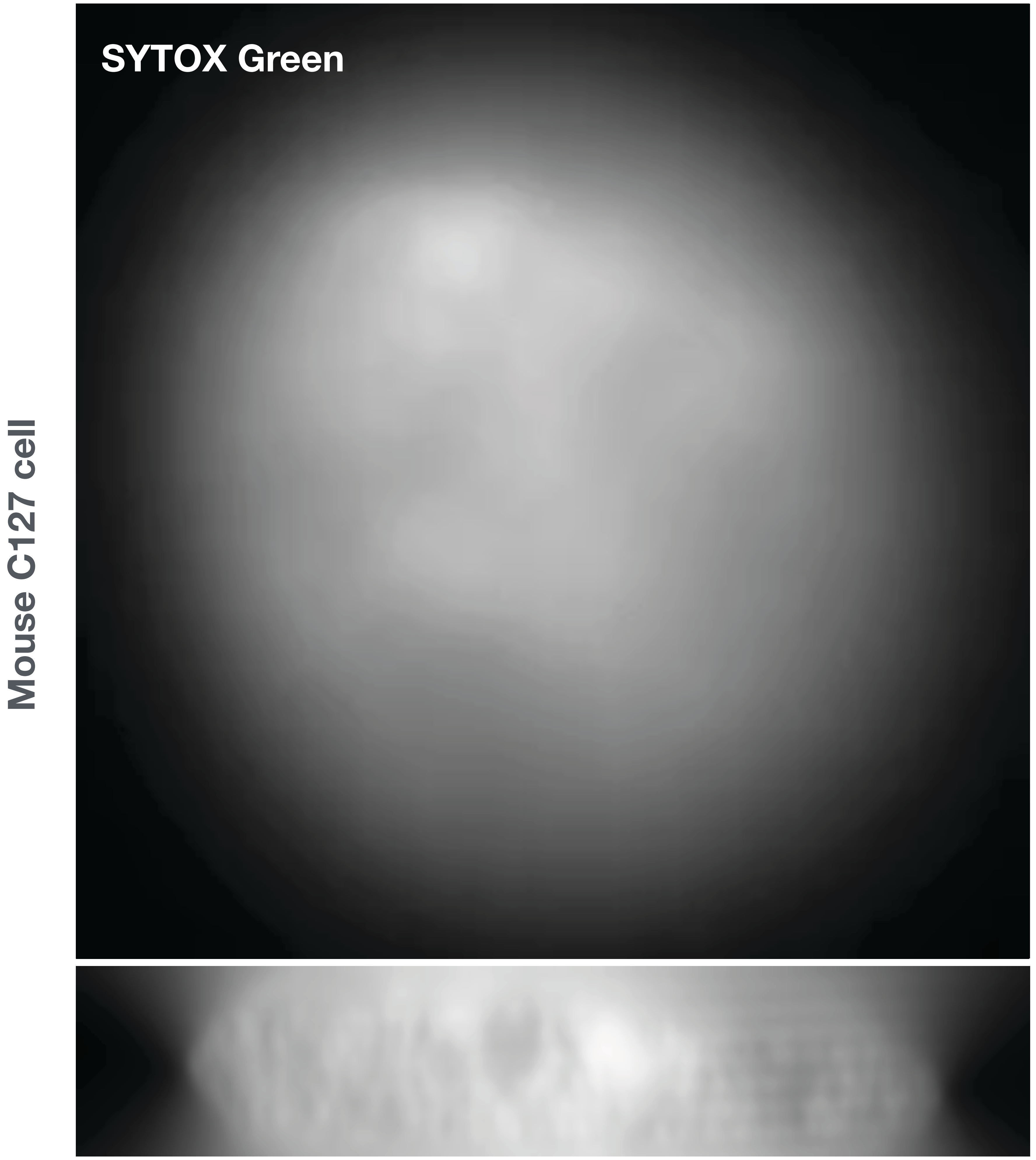
Band separation

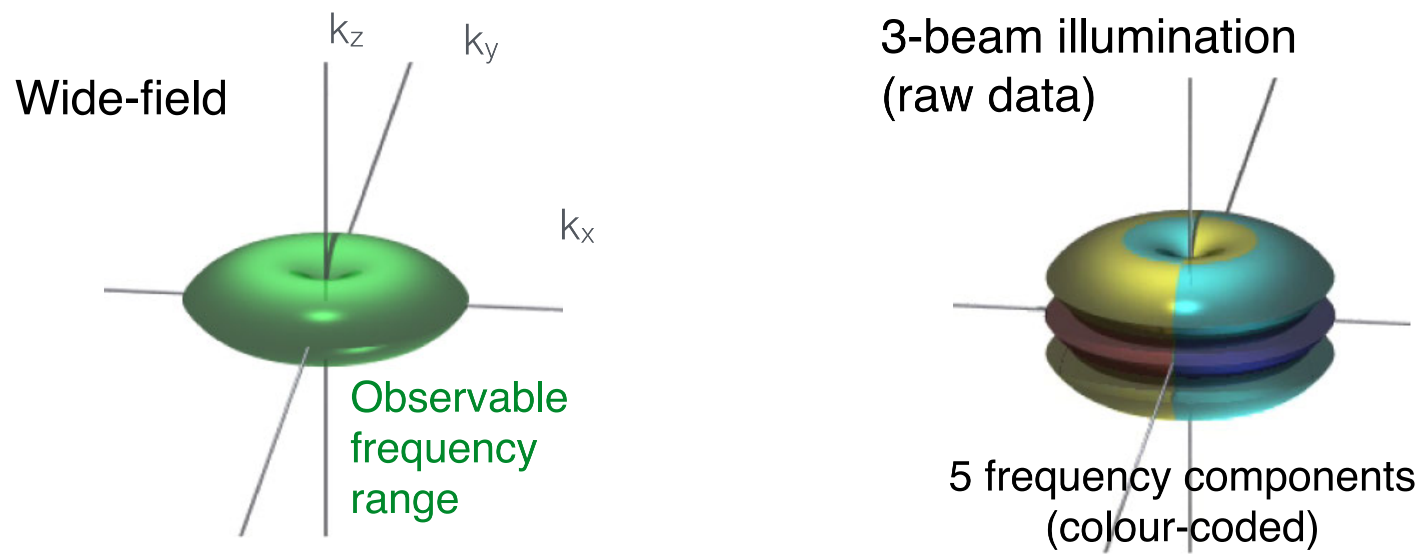
Reconstructed data

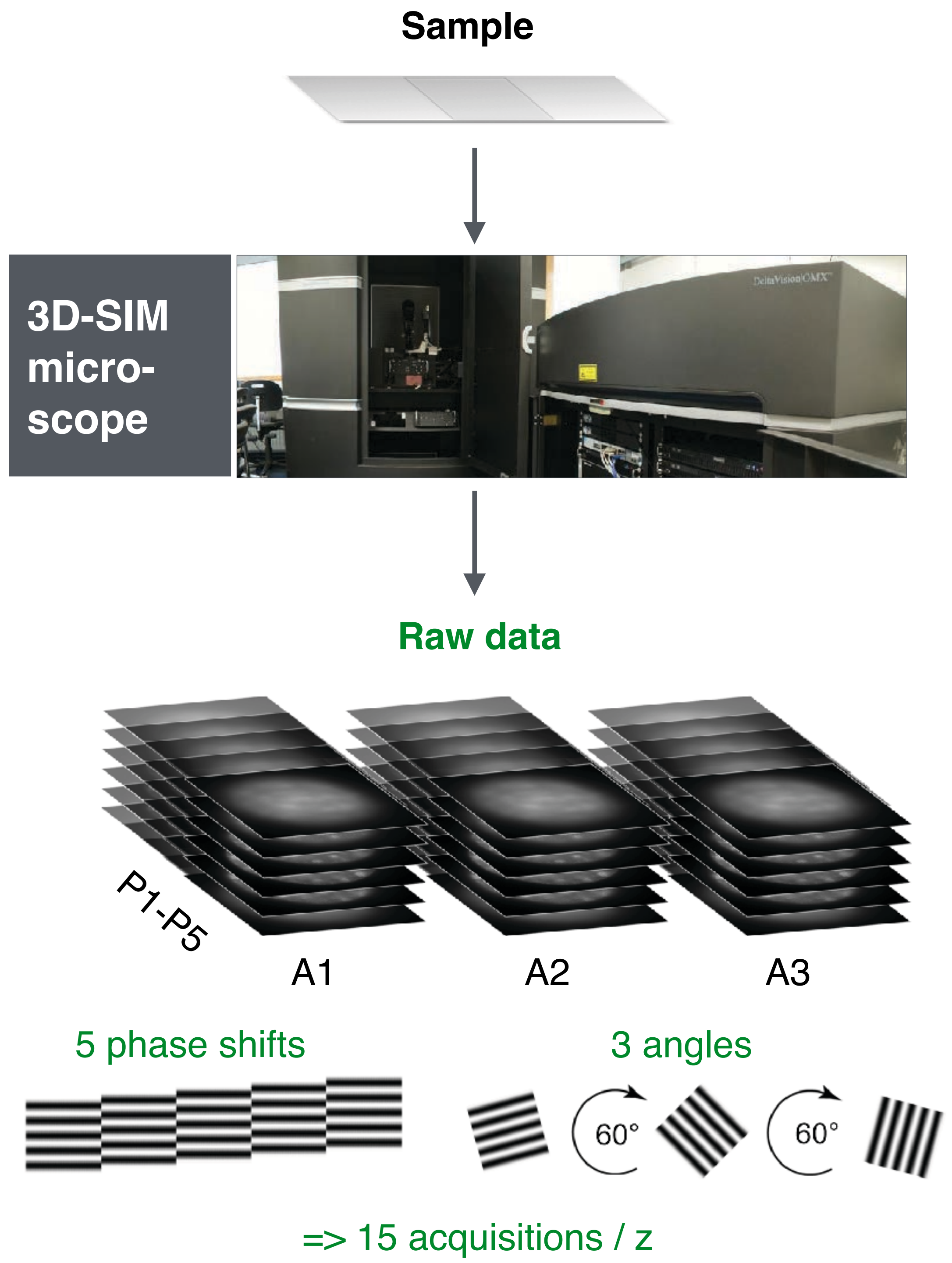


adapted from Gustafsson et al. (2008), *Biophys J* **94**

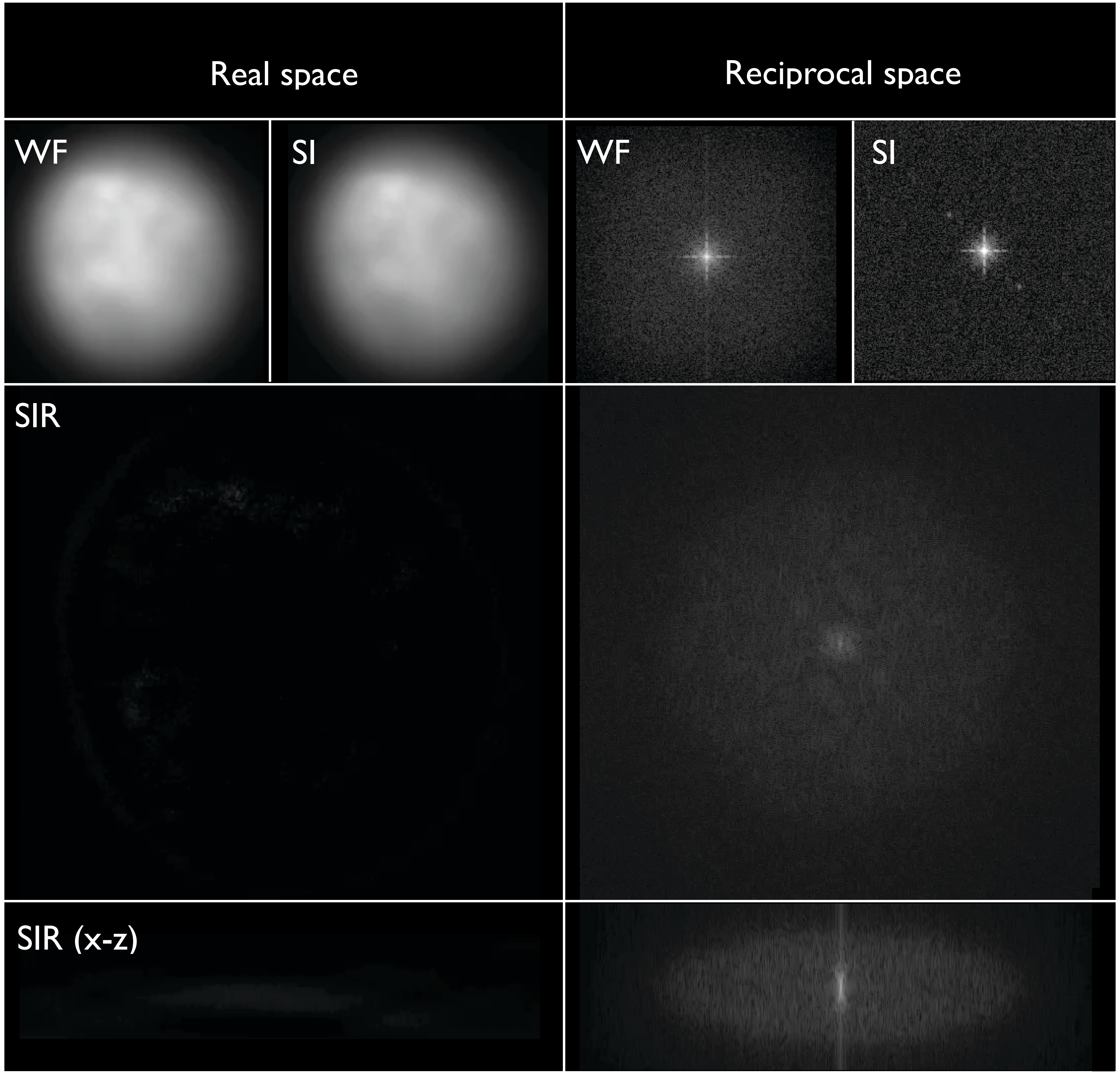
From wide-field to 3D-SIM

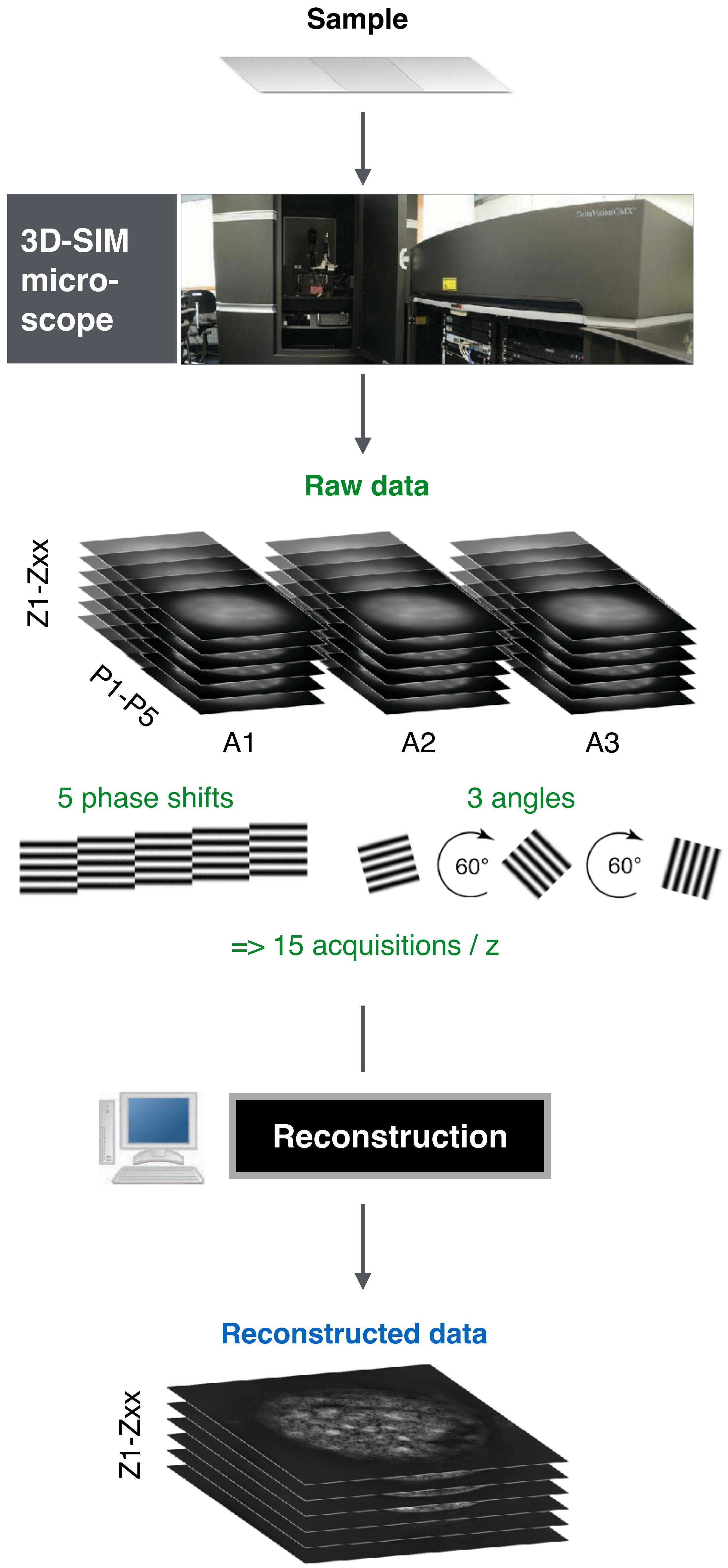




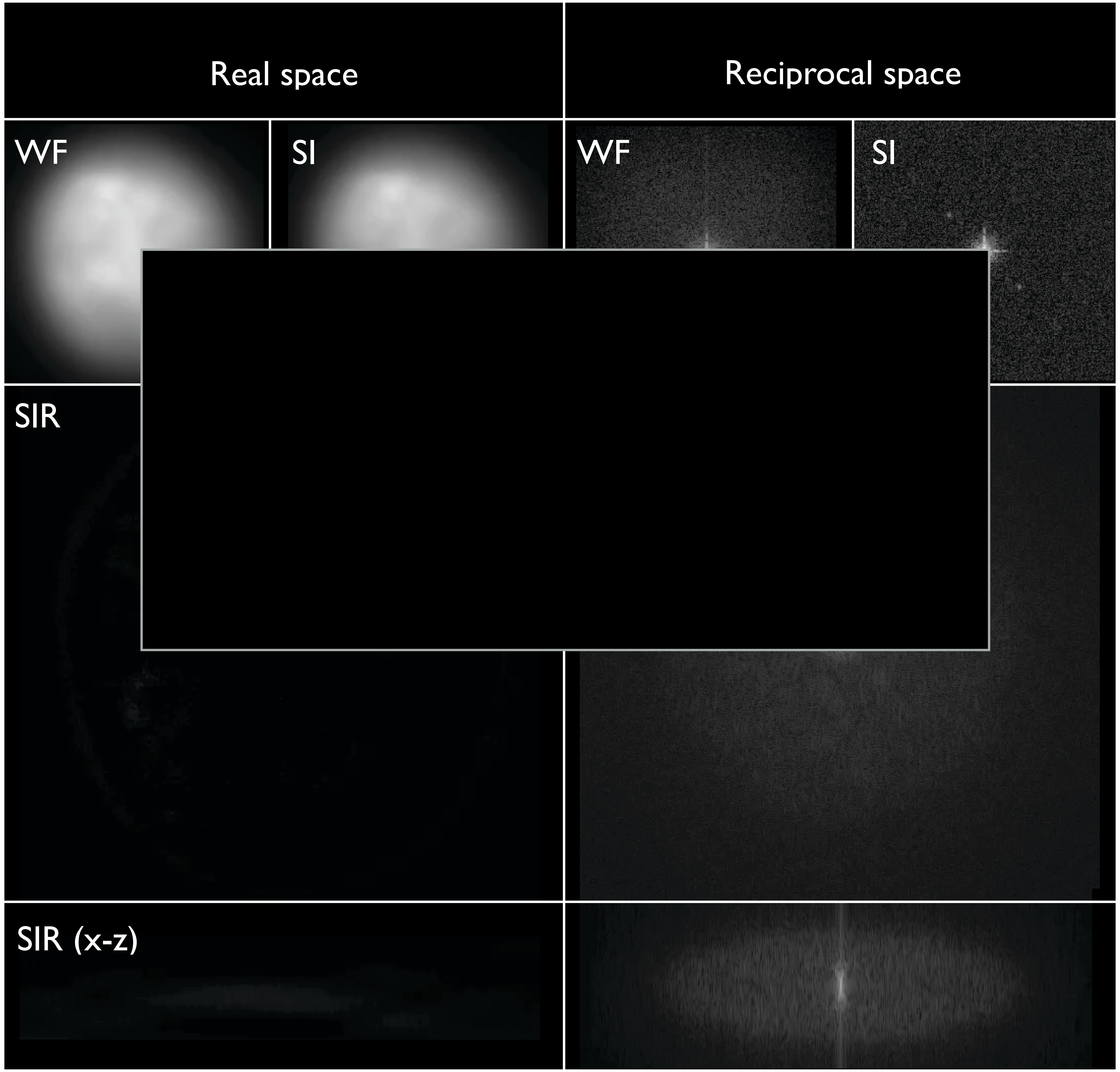


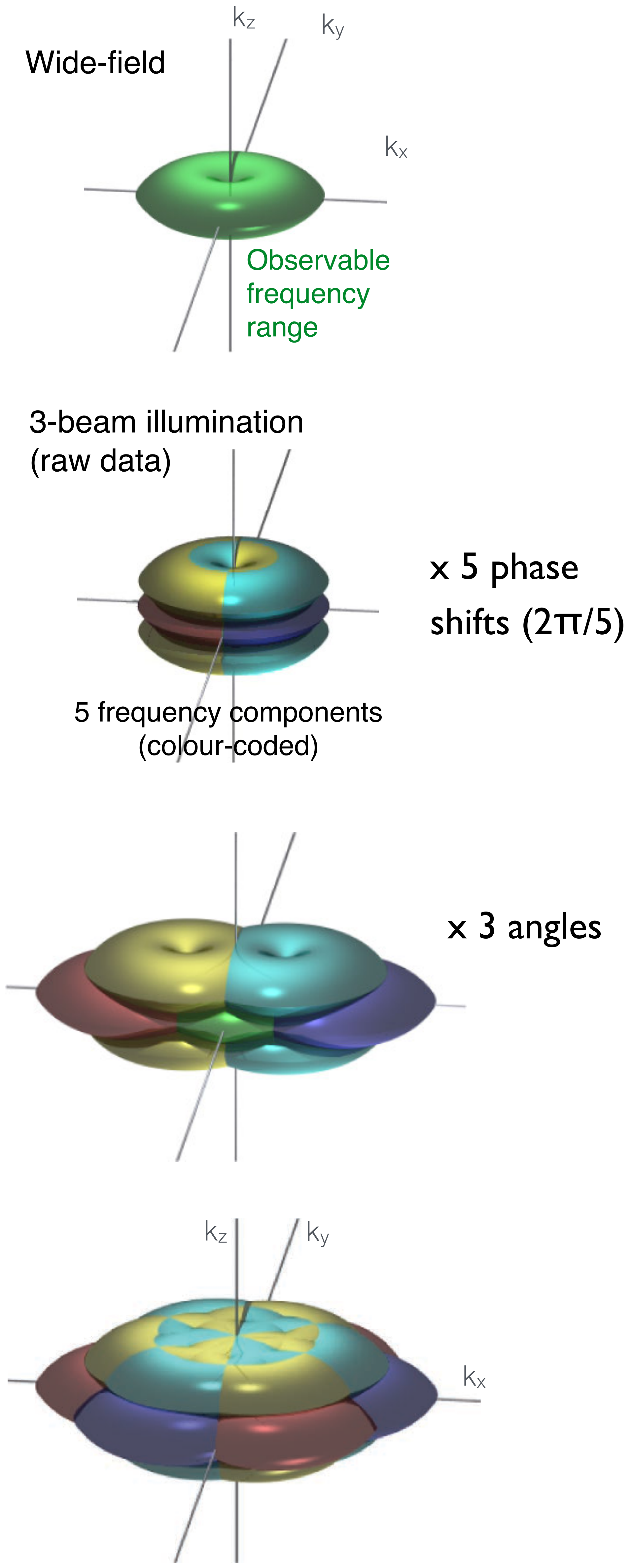
Overview of SIM processing





Overview of SIM processing

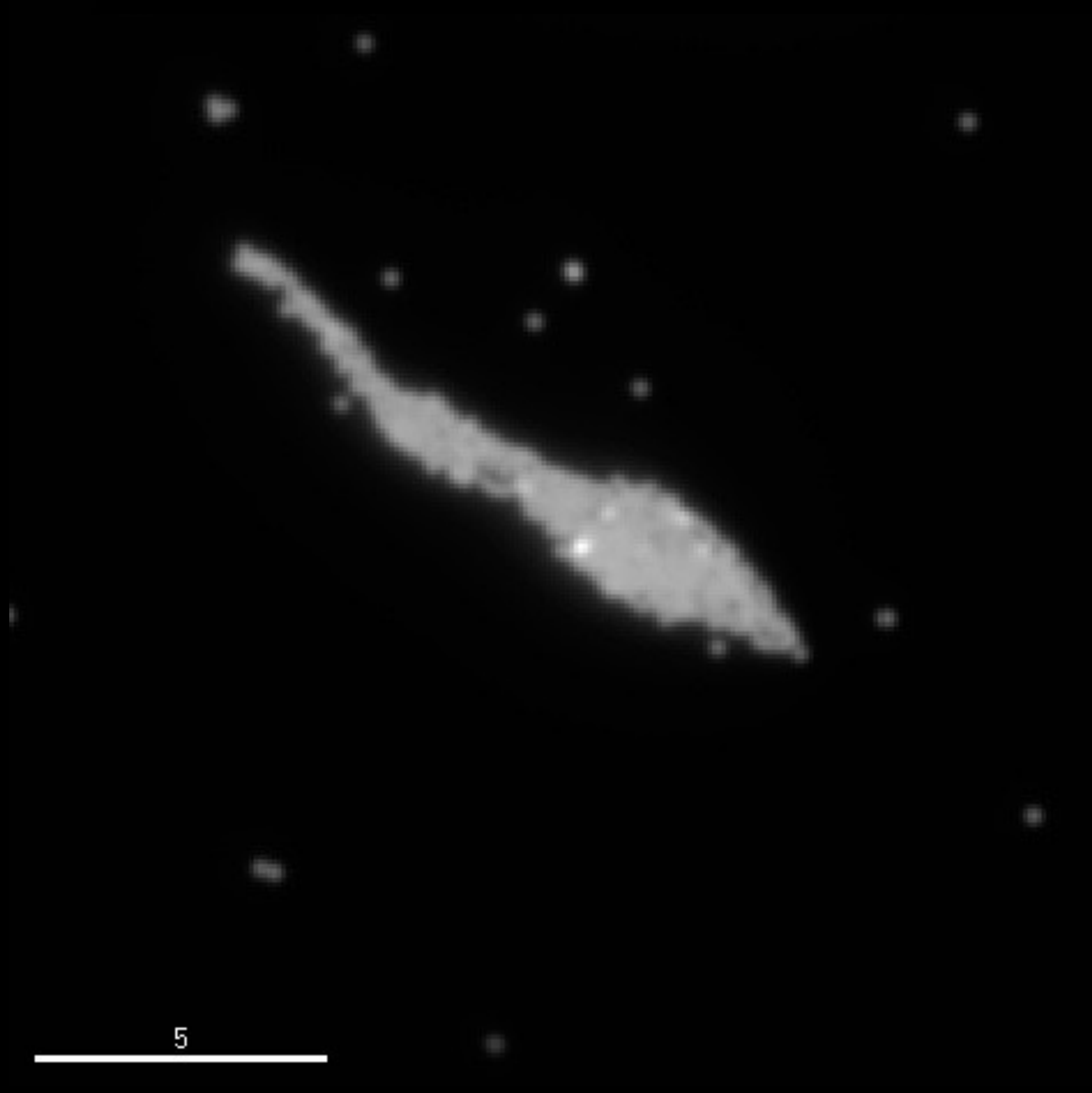


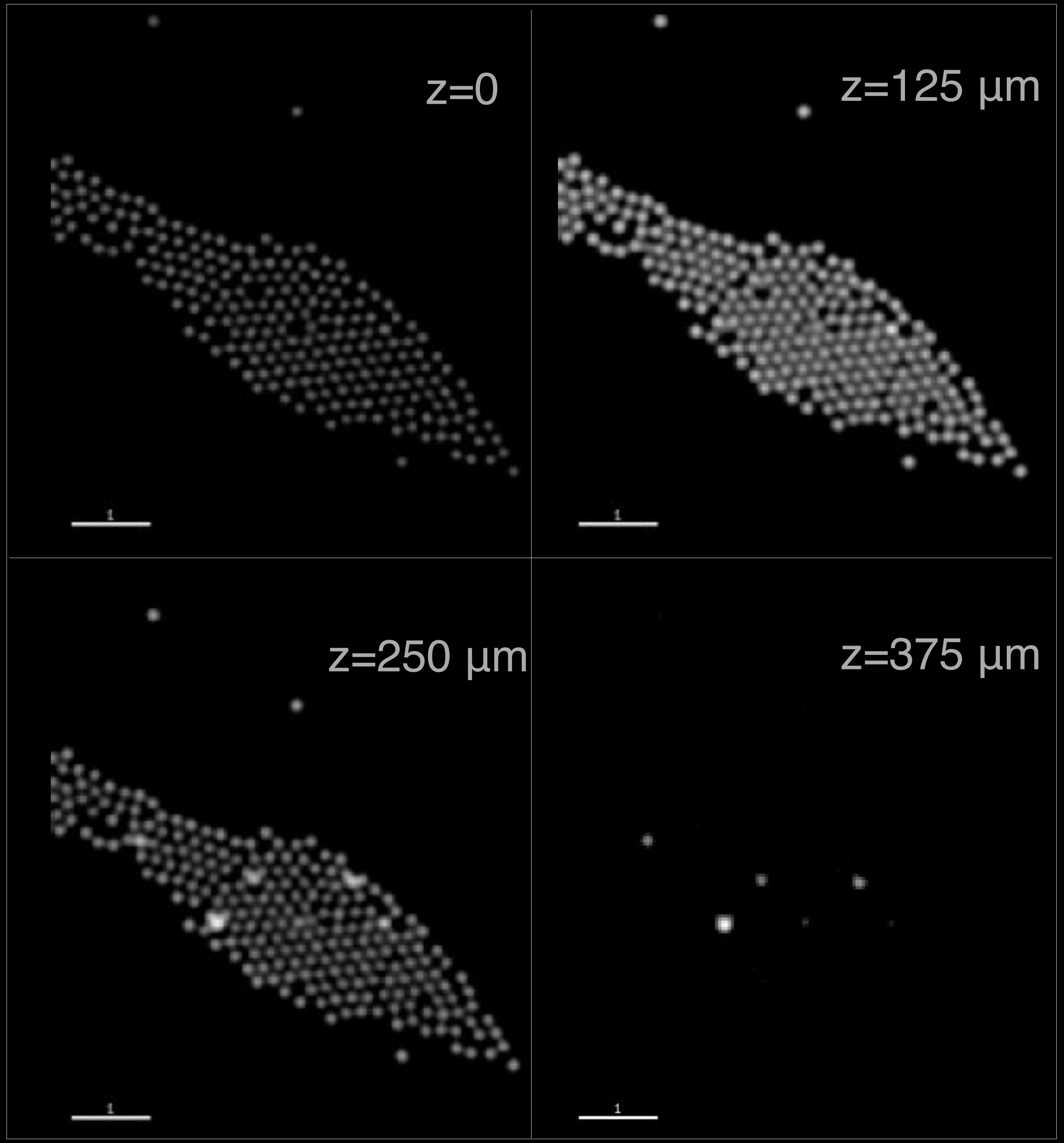


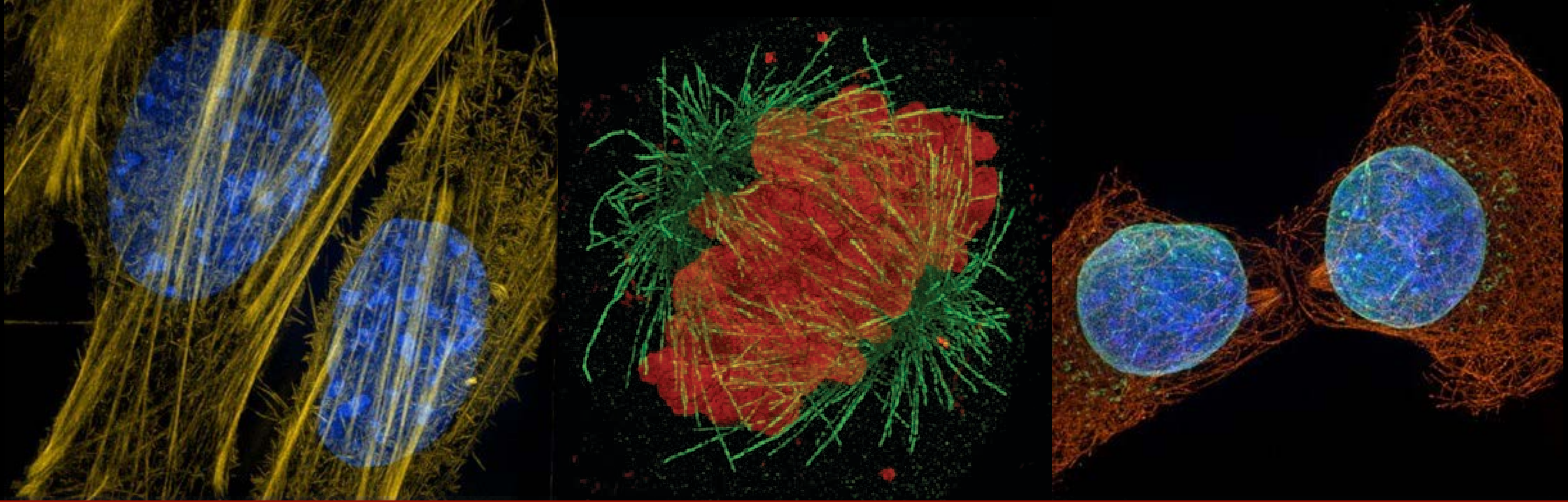
Schermelleh, Carlton et al. *Science* 2008; 320

3D optical sectioning capacity

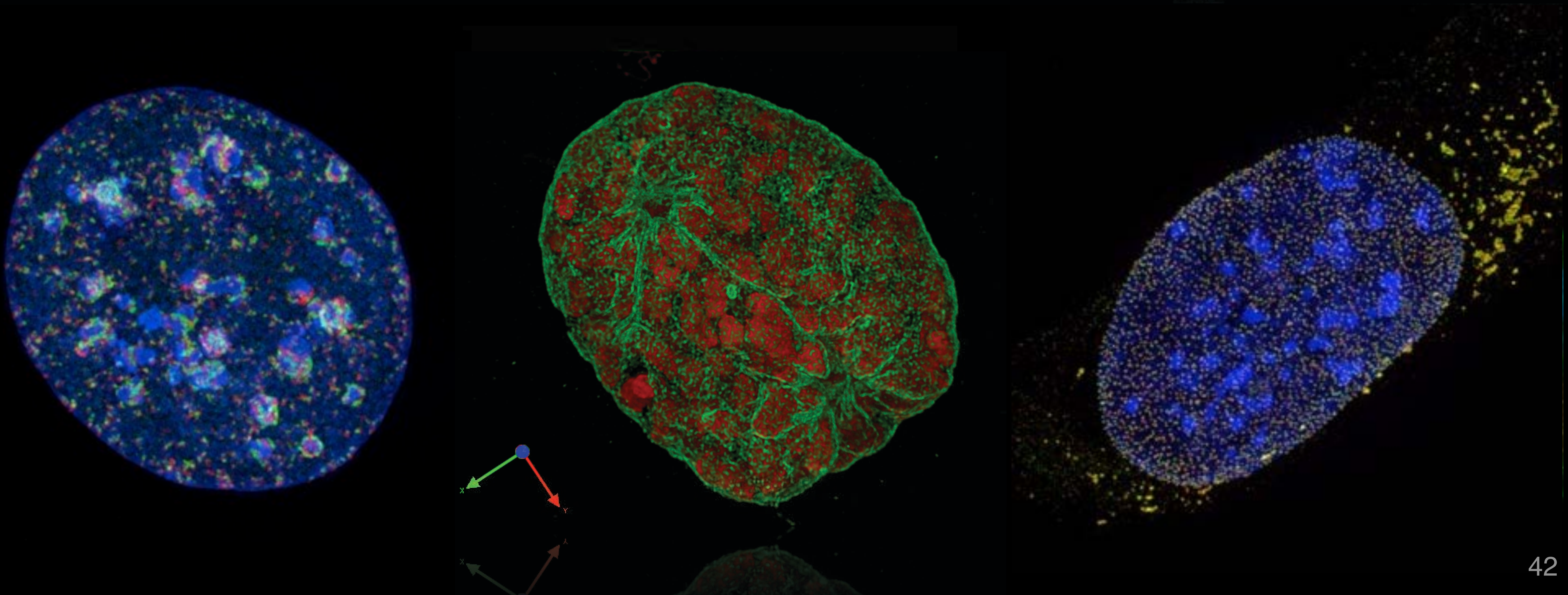
Example: 170 nm Fluospheres







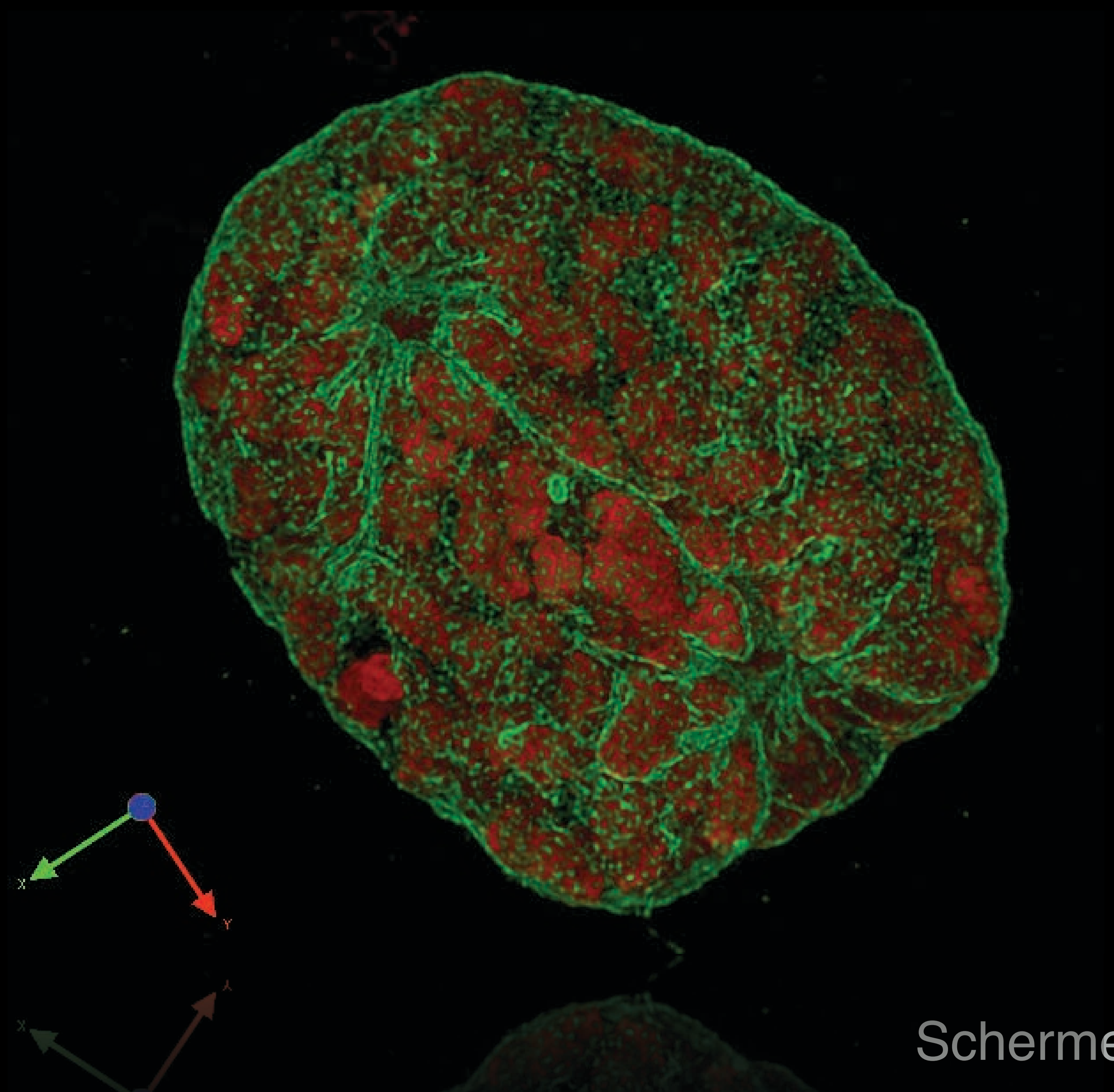
3D-SIM: Multicolour 3D optical sectioning • 8x enhanced volumetric resolution • 10-20 μm depth



3D-SIM of a prophase nucleus

Lamin B
DAPI

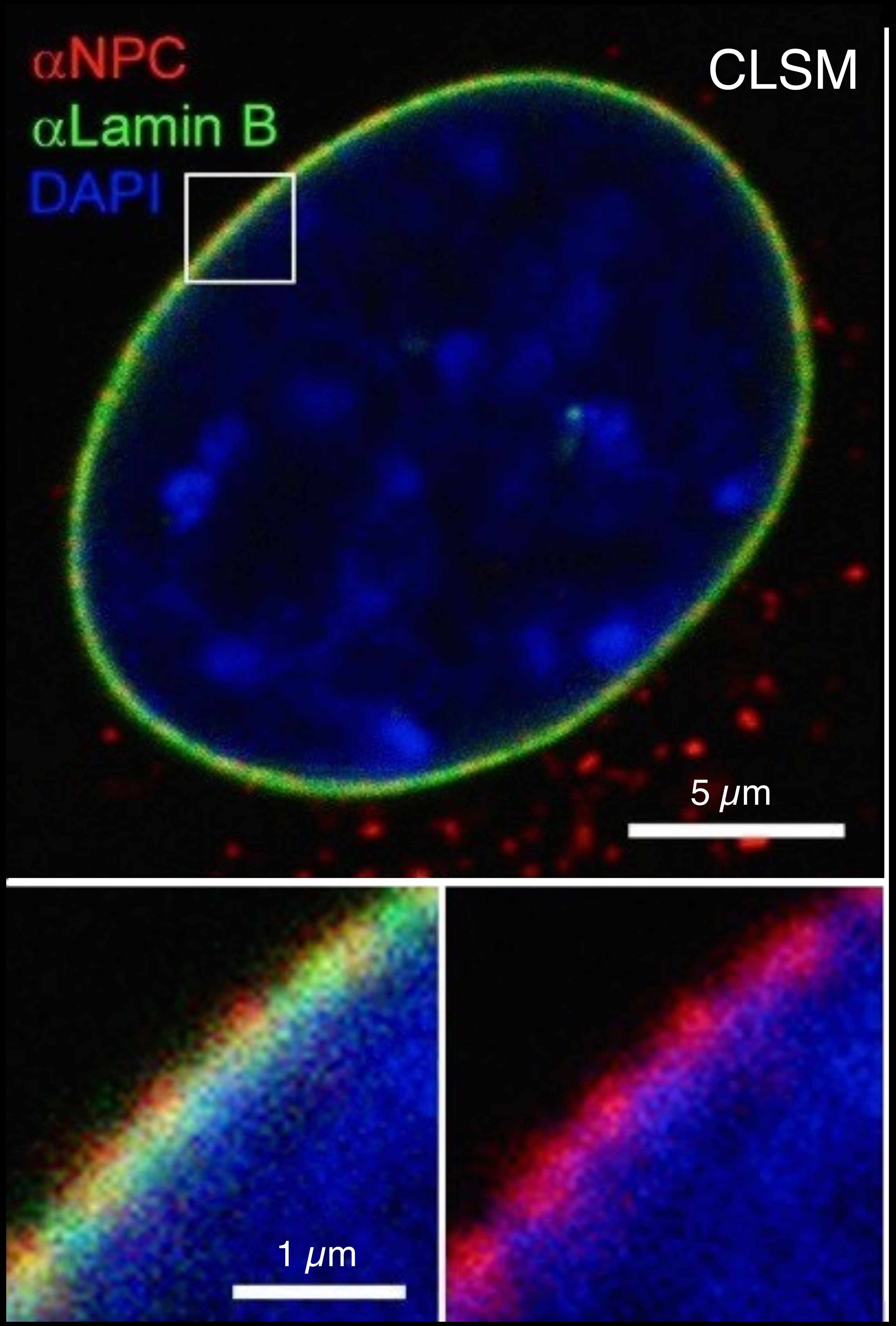
3D volume
rendering



Mouse C2C12 cell

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

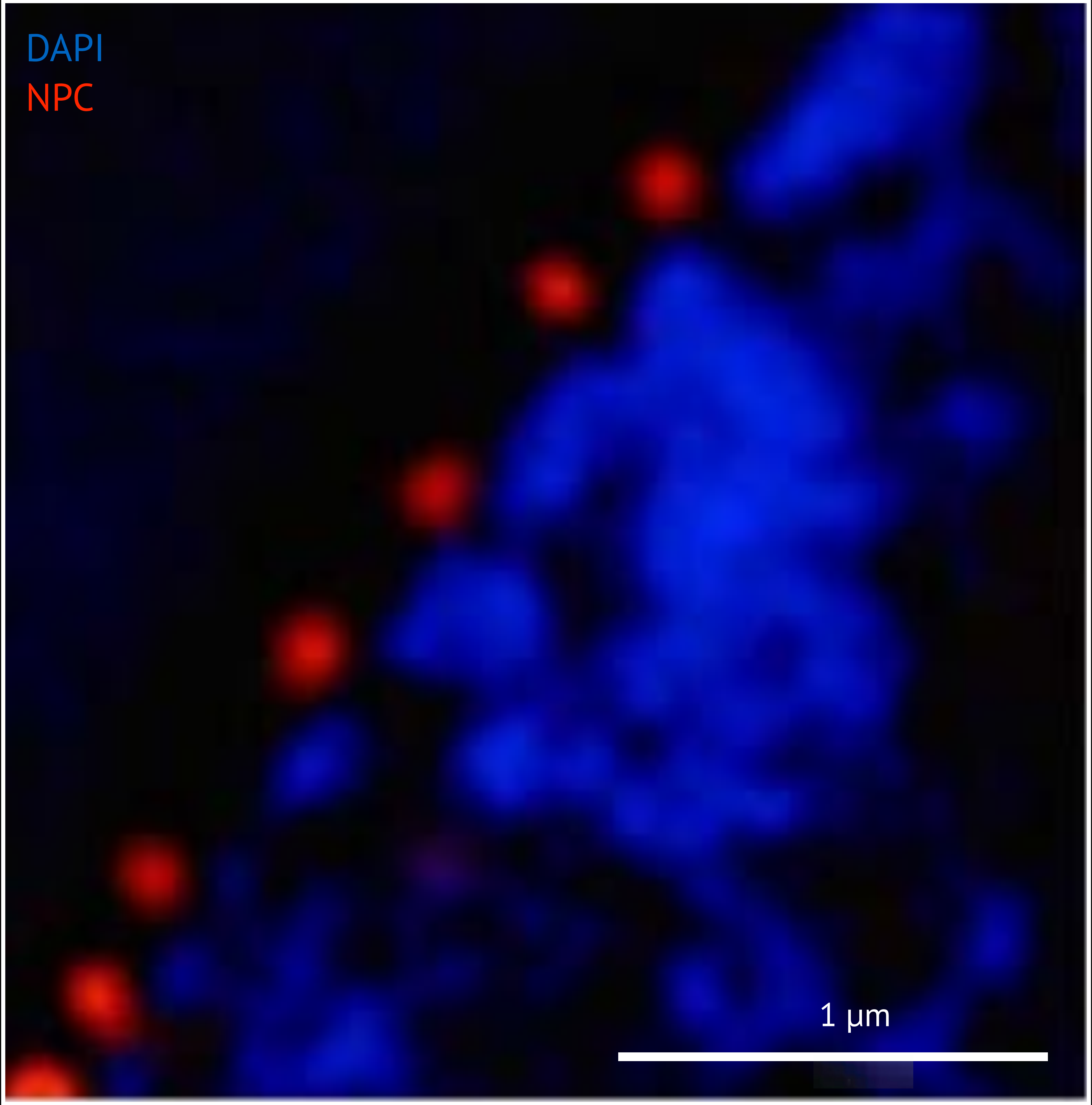
3D-SIM resolves chromatin domains and interchromatin channels



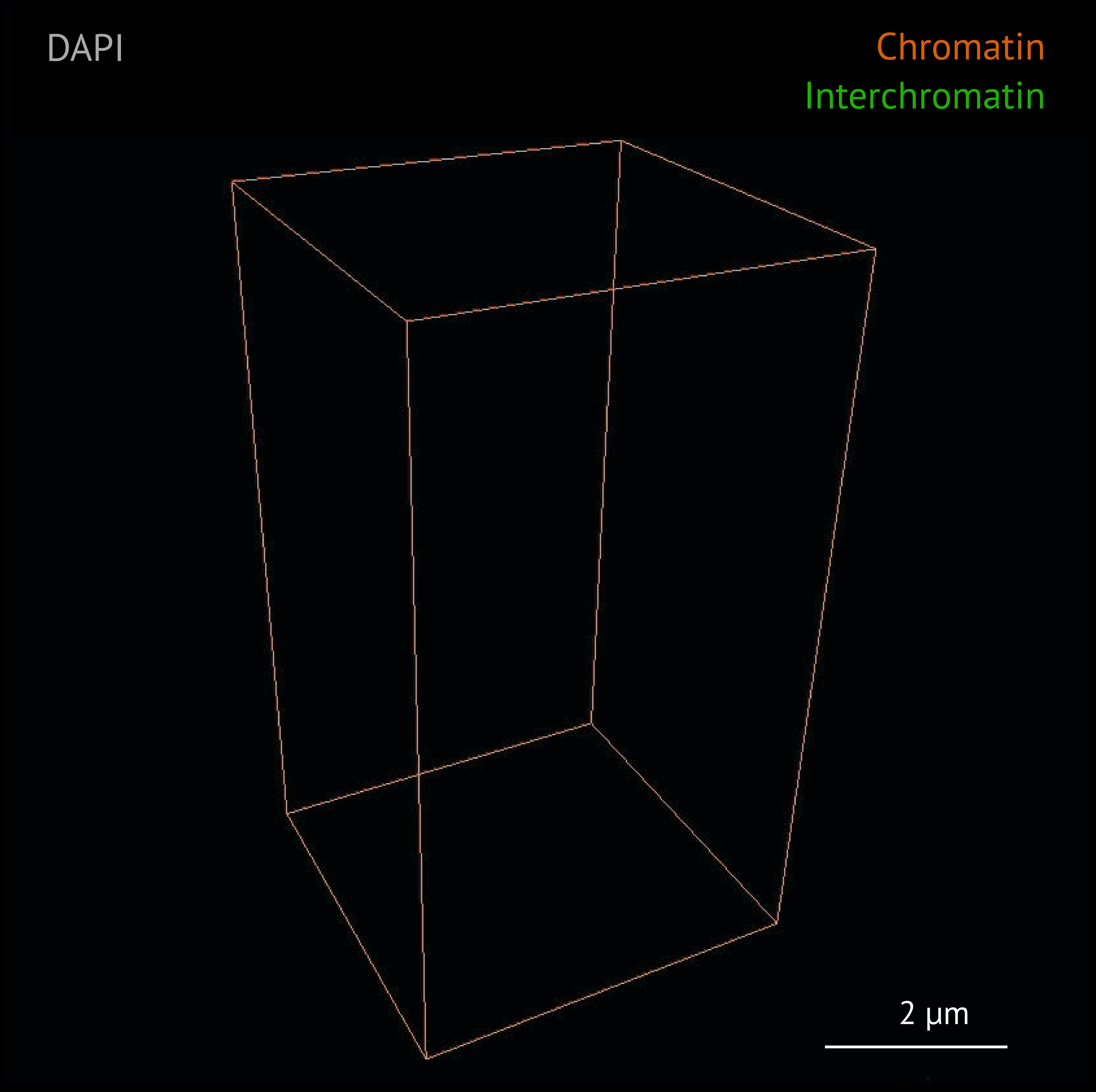
Mouse C2C12

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

3D-SIM resolves chromatin ‘domains’ & interchromatin compartment (IC)

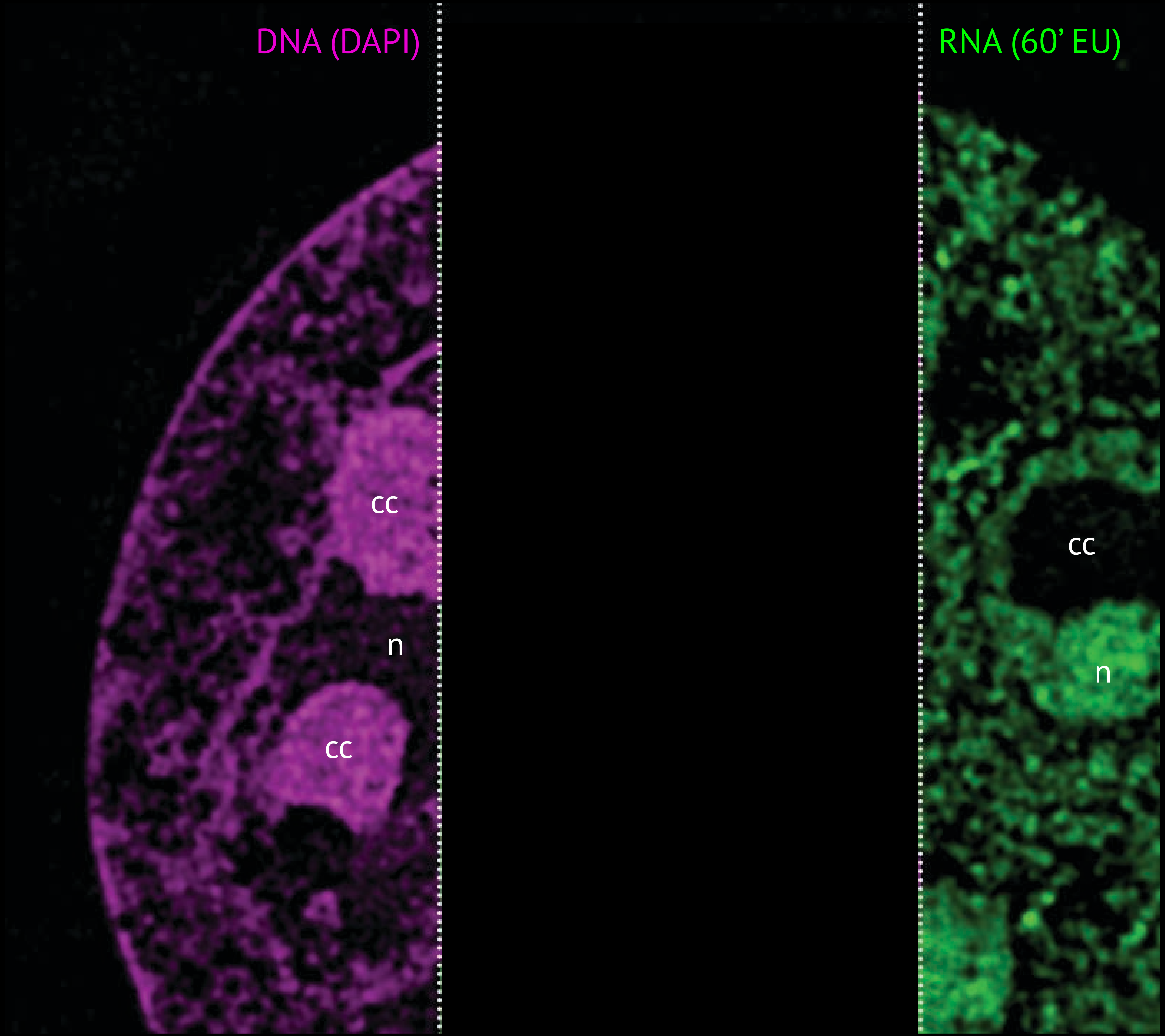


Schermelleh, Carlton et al. 2008, *Science*

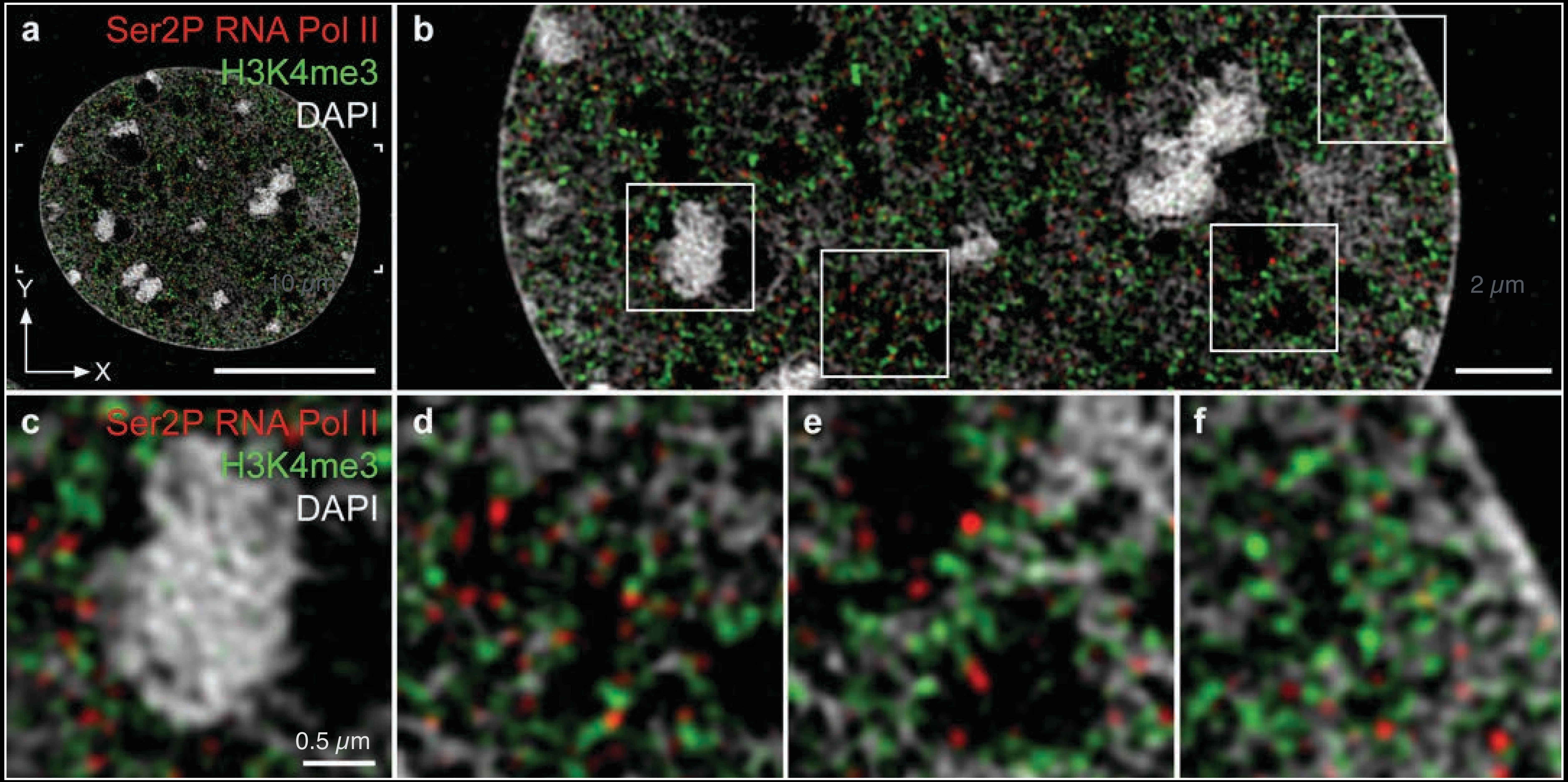


Smeets et al. 2014, *Epigenetics & Chromatin*

3D-SIM resolves chromatin ‘domains’ & interchromatin compartment (IC)



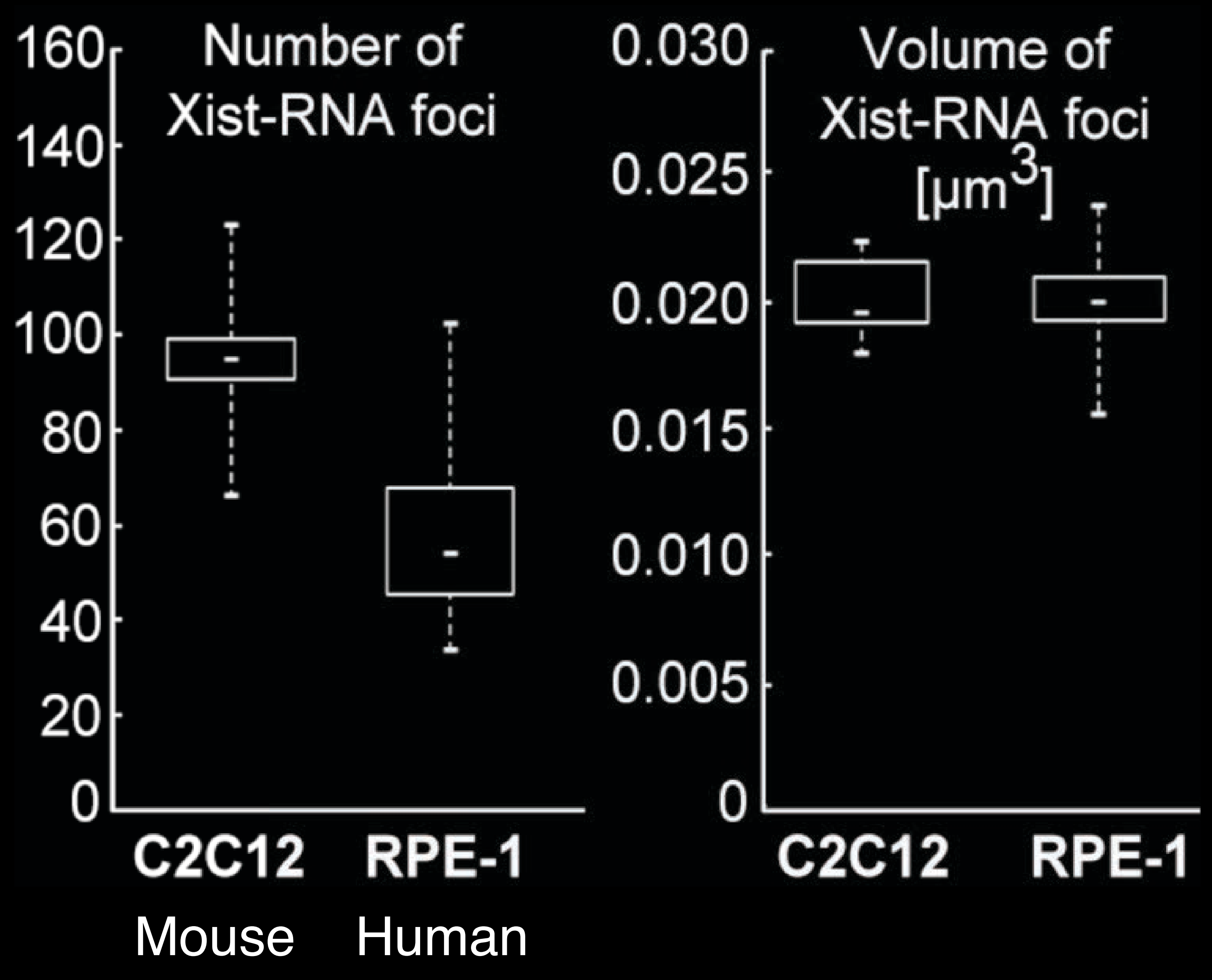
Active marker are constrained to chromatin domain boundaries



Mouse C127 cell

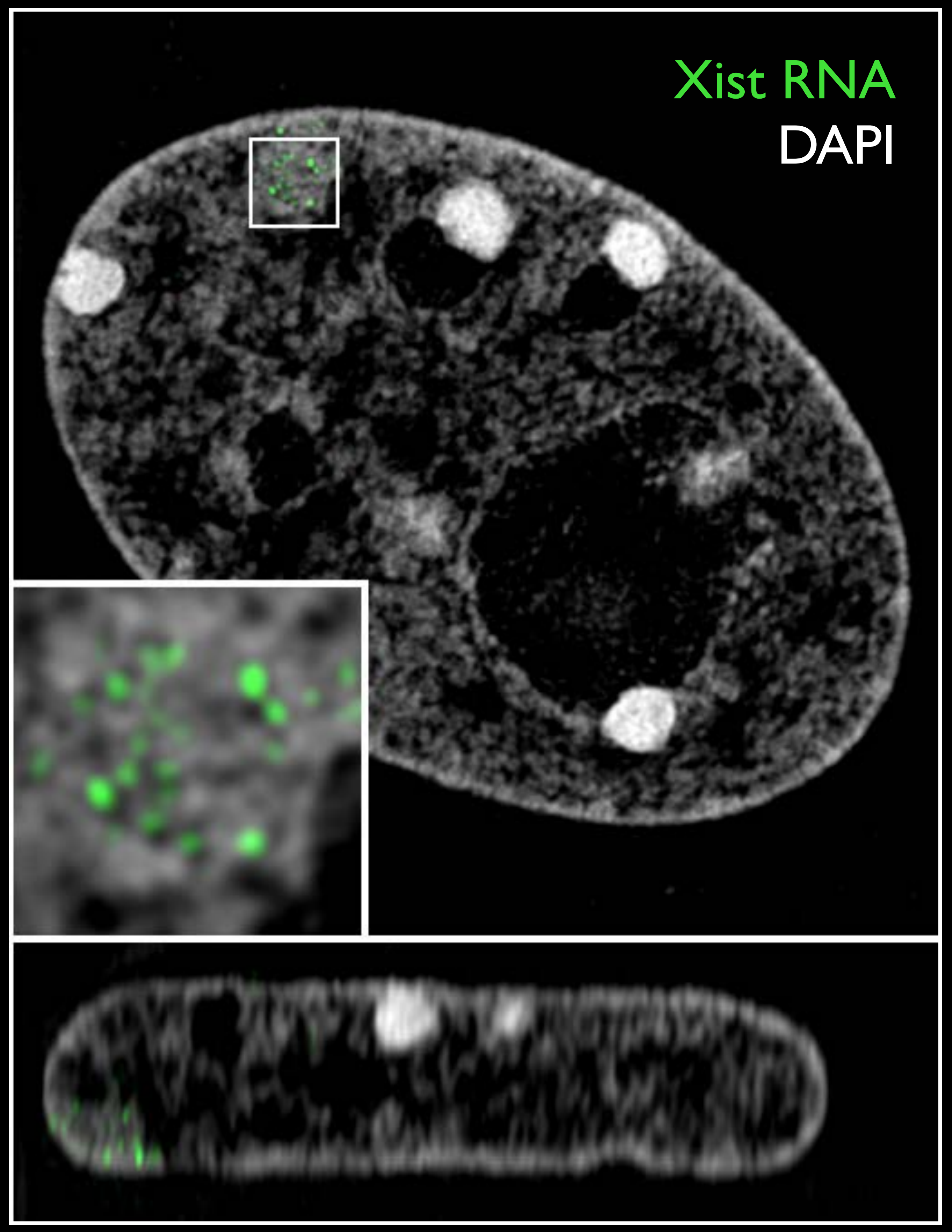
Markaki et al., 2011, *Cold Spring Harb Perspect Biol*, 75

Super-resolution topography of inactive X-chromosome



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

3D-SIM



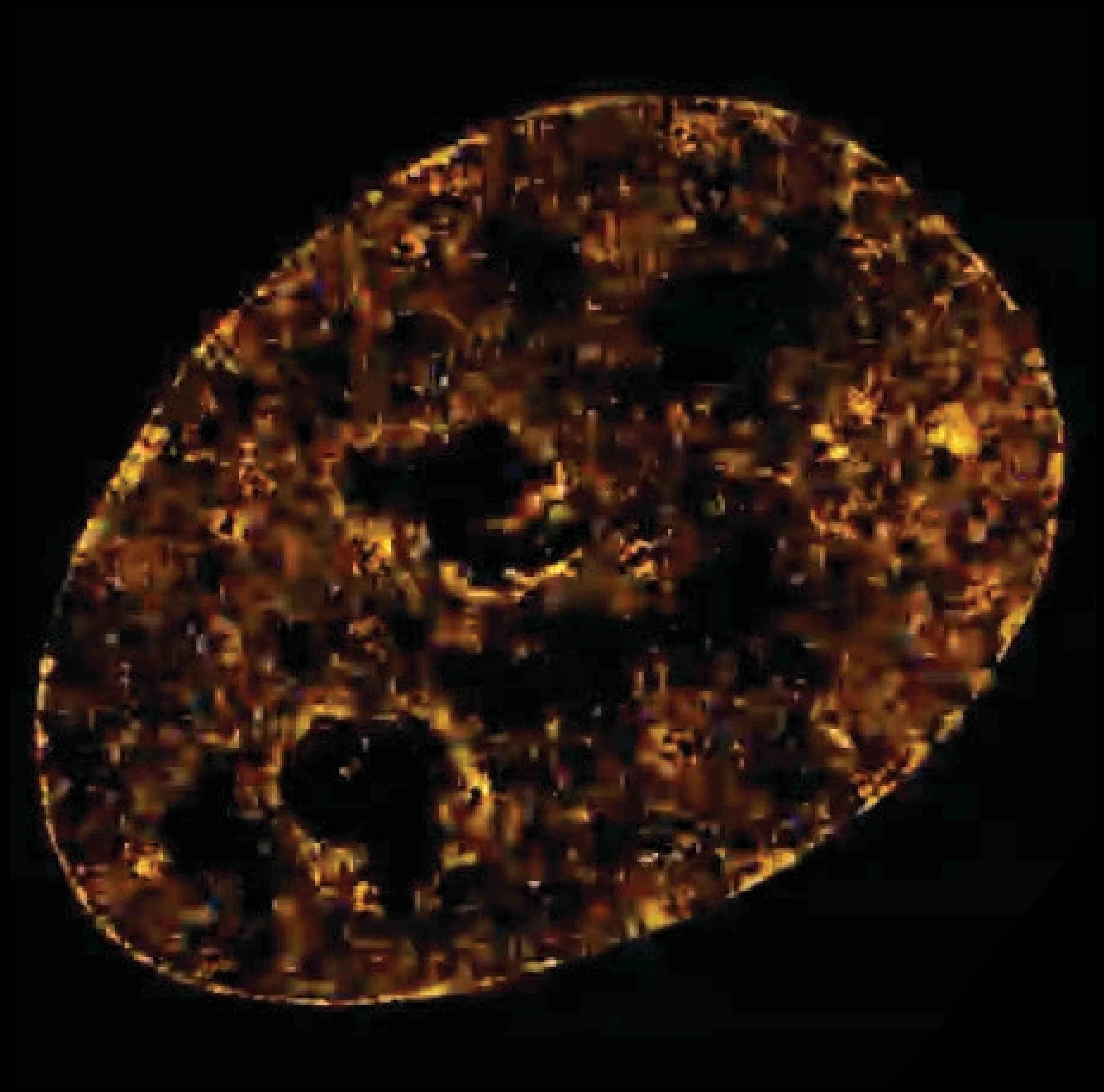
Markaki et al., (2013) *Methods Mol Biol*

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

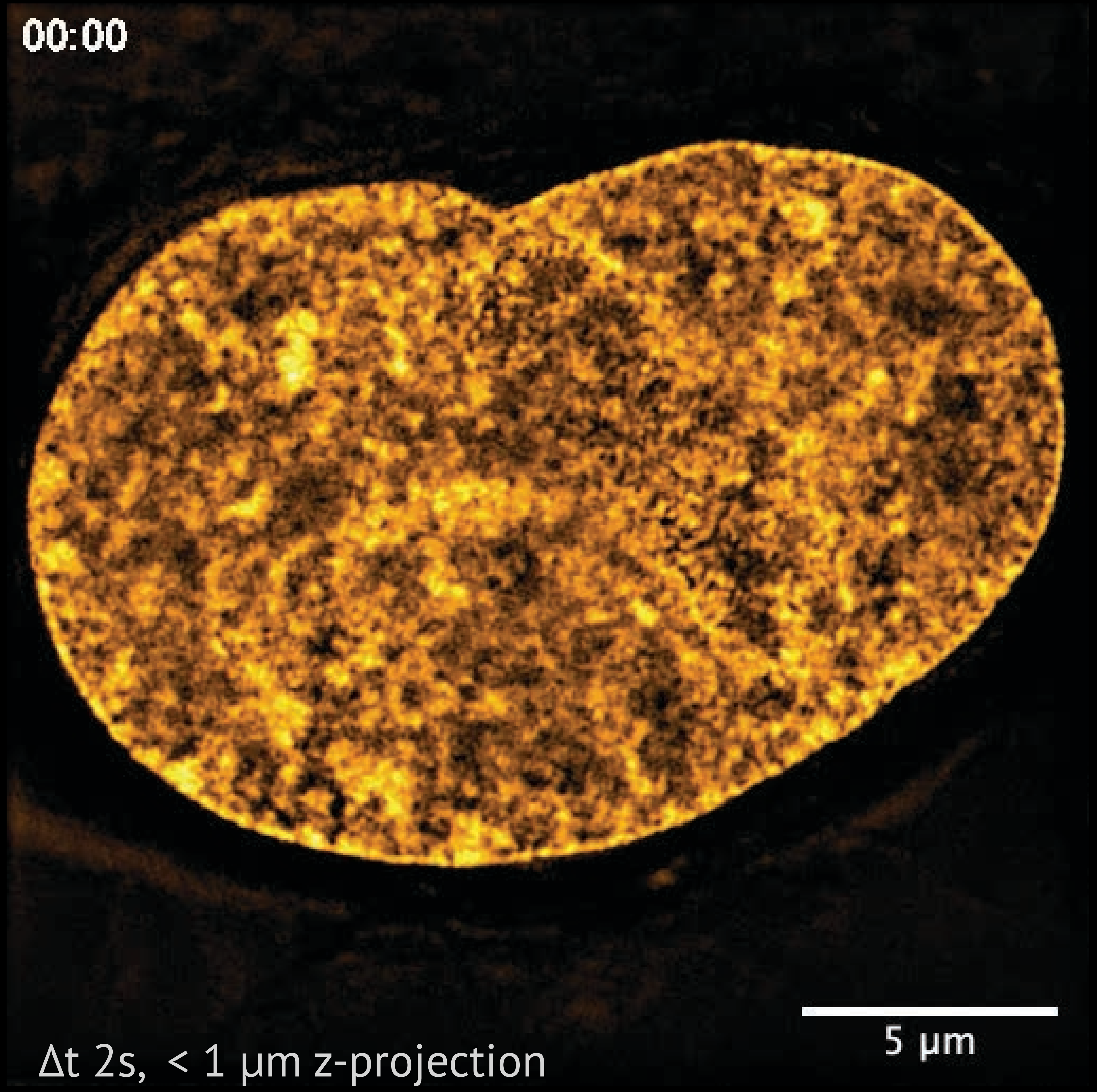
Can we go live?

Live cell 3D-SIM with OMX Blaze

H2B-GFP (unfixed)



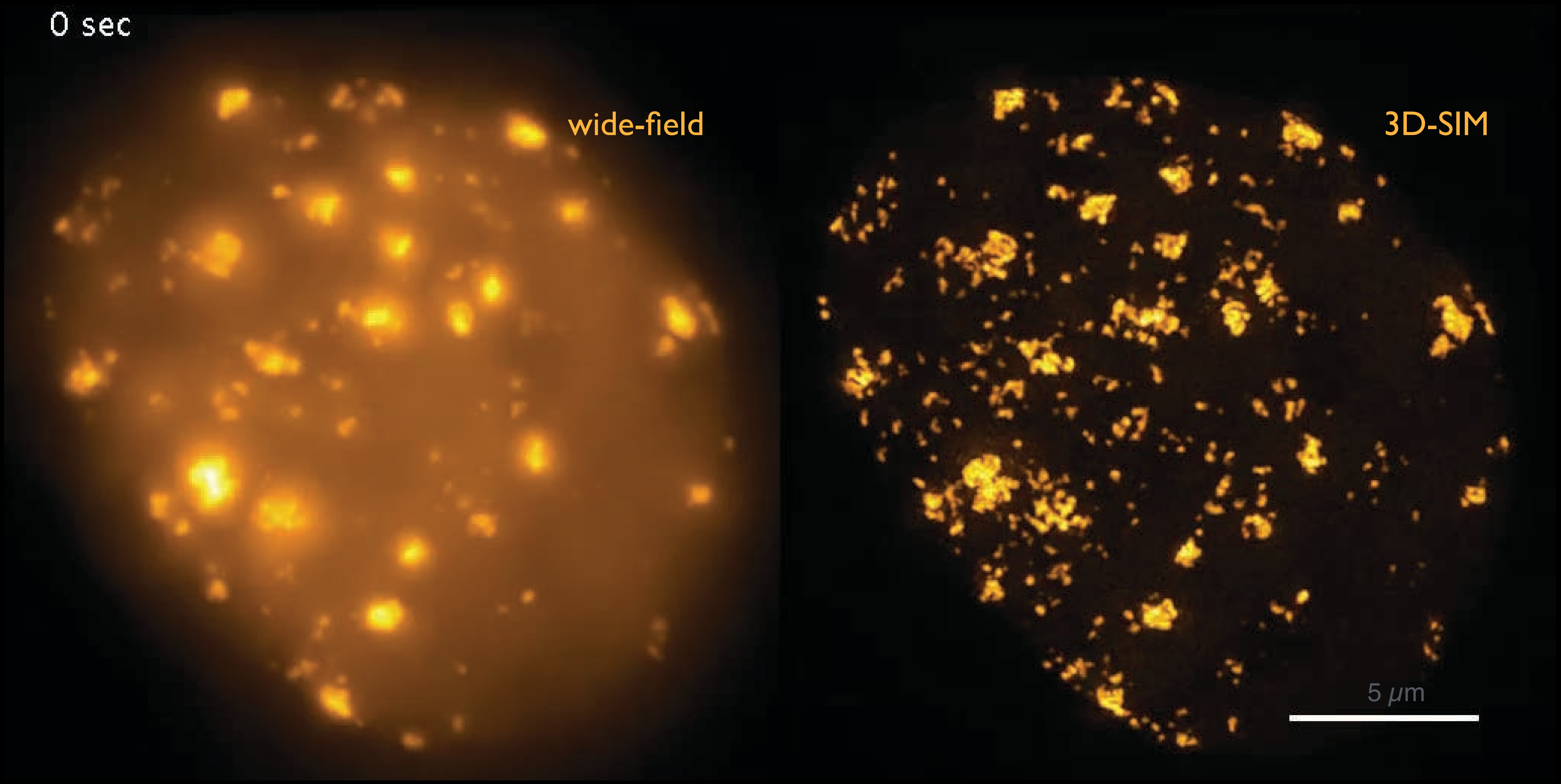
7 μm z-stack (56 sections, 5 ms exposure)



Live cell 3D super-resolution imaging of replication sites

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

(OMX Blaze)



(xz)

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

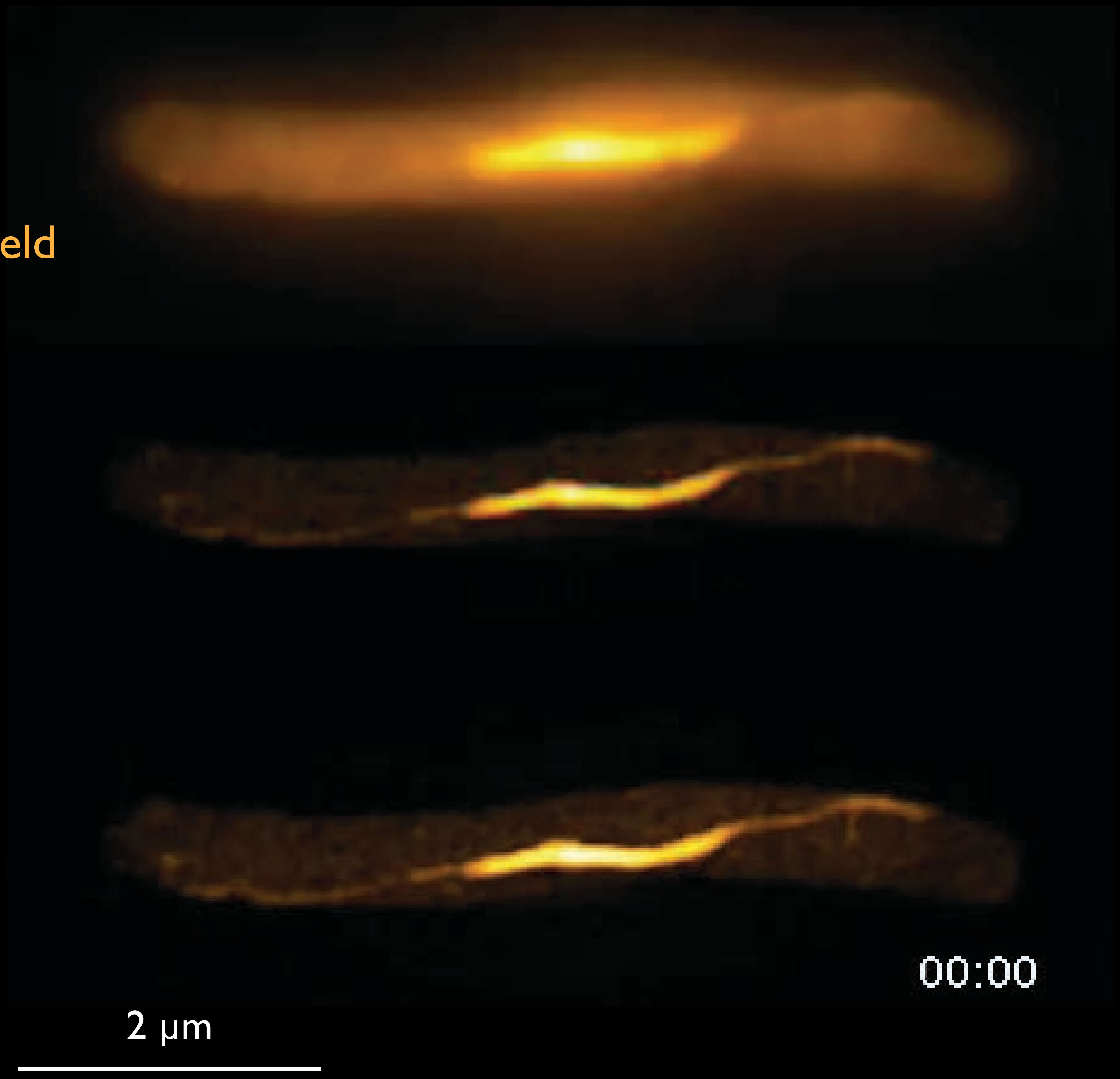
max. projection

Dynamics of RecA in DNA double strand break repair

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

Wide-field

3D-SIM

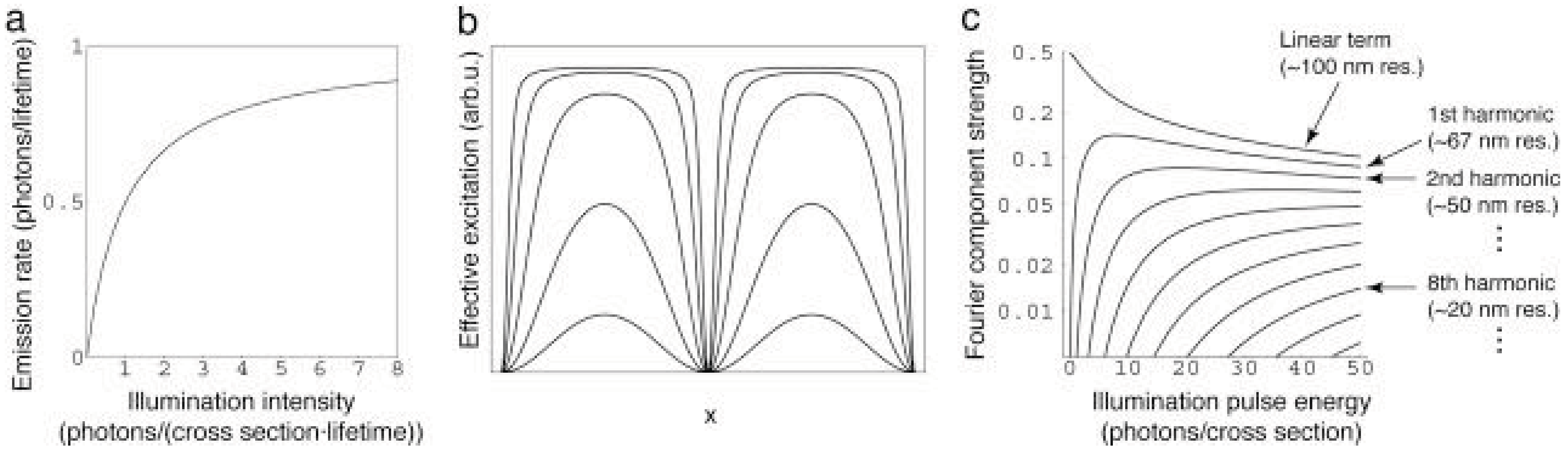


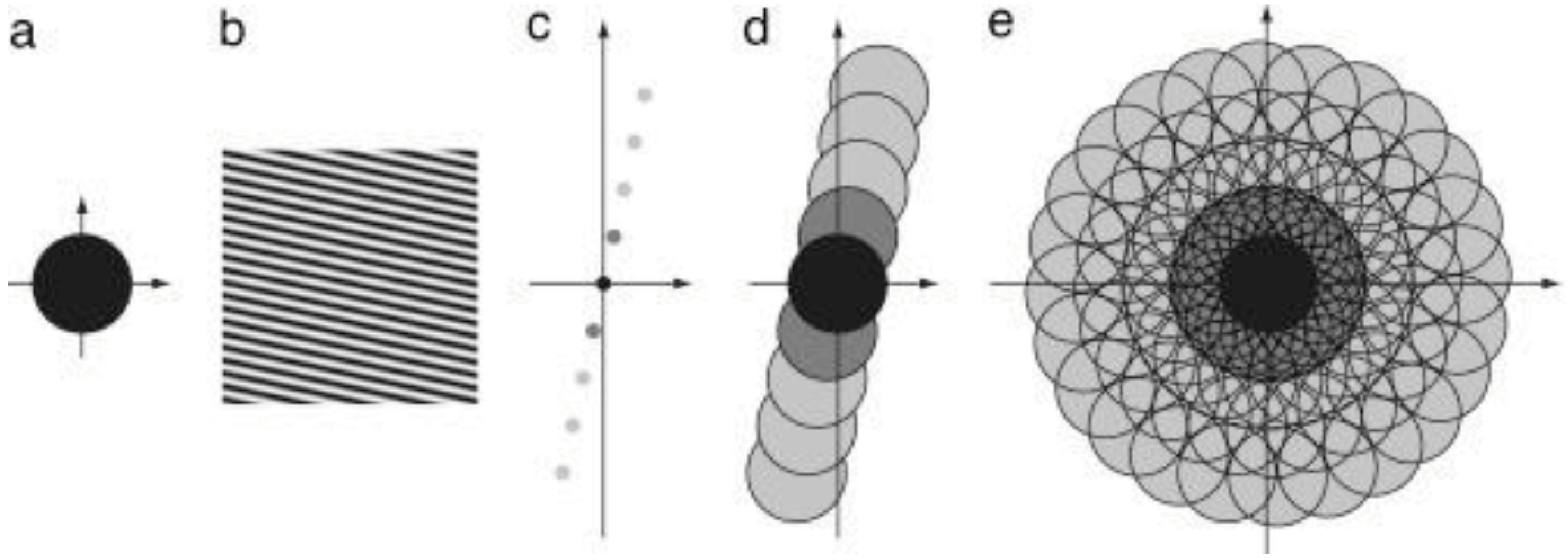
OMX Blaze: 2 s / frame (1.75 µm z-stack = 225 images, 100 time points)

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

2D/3D-SIM is still resolution limited!
Can we go beyond ?

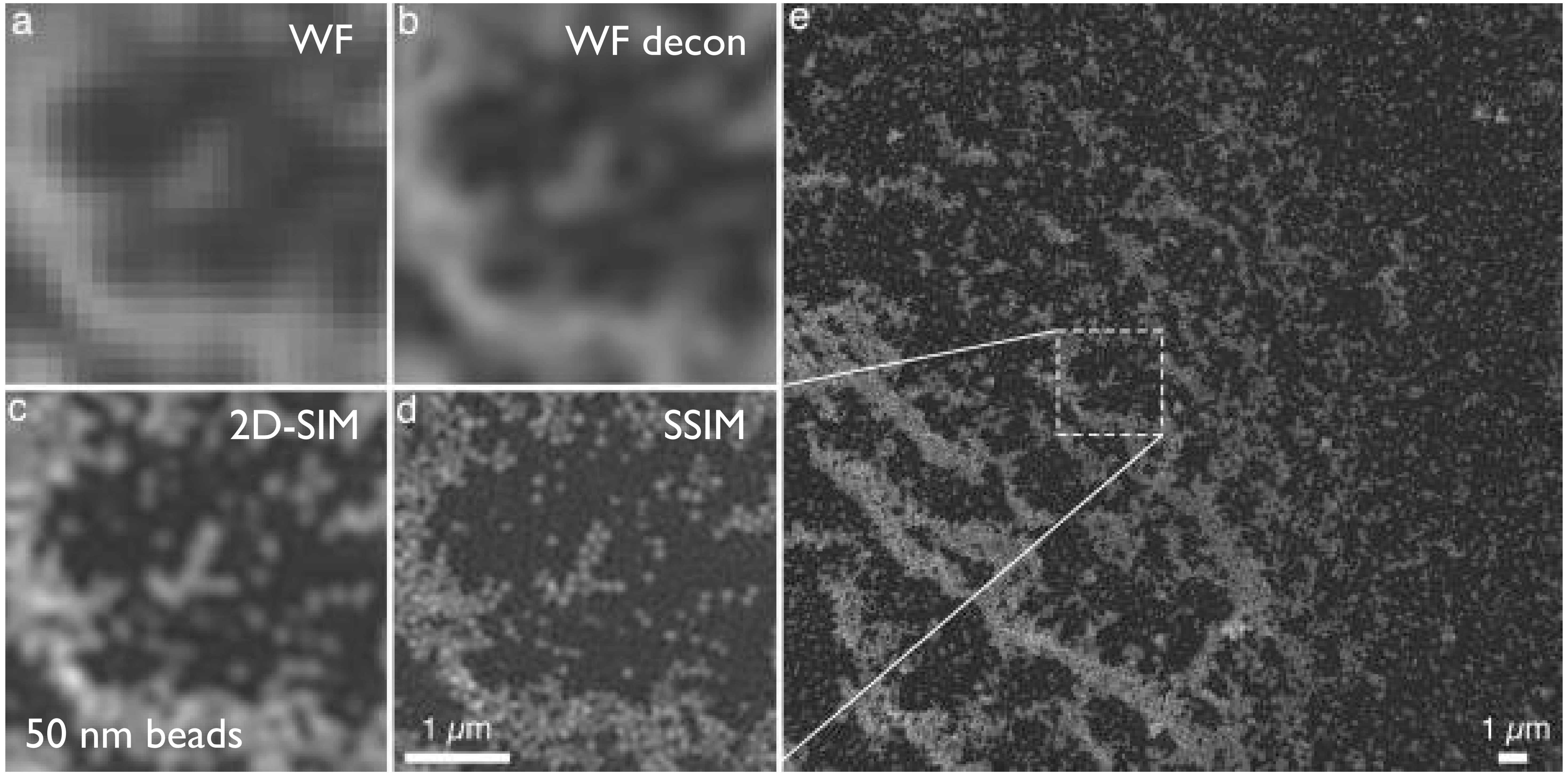
Non linear SIM - Saturated structured illumination microscopy (SSIM)





Gustafsson (2005), *PNAS* **102**

Non linear SIM - Saturated structured illumination microscopy (SSIM)

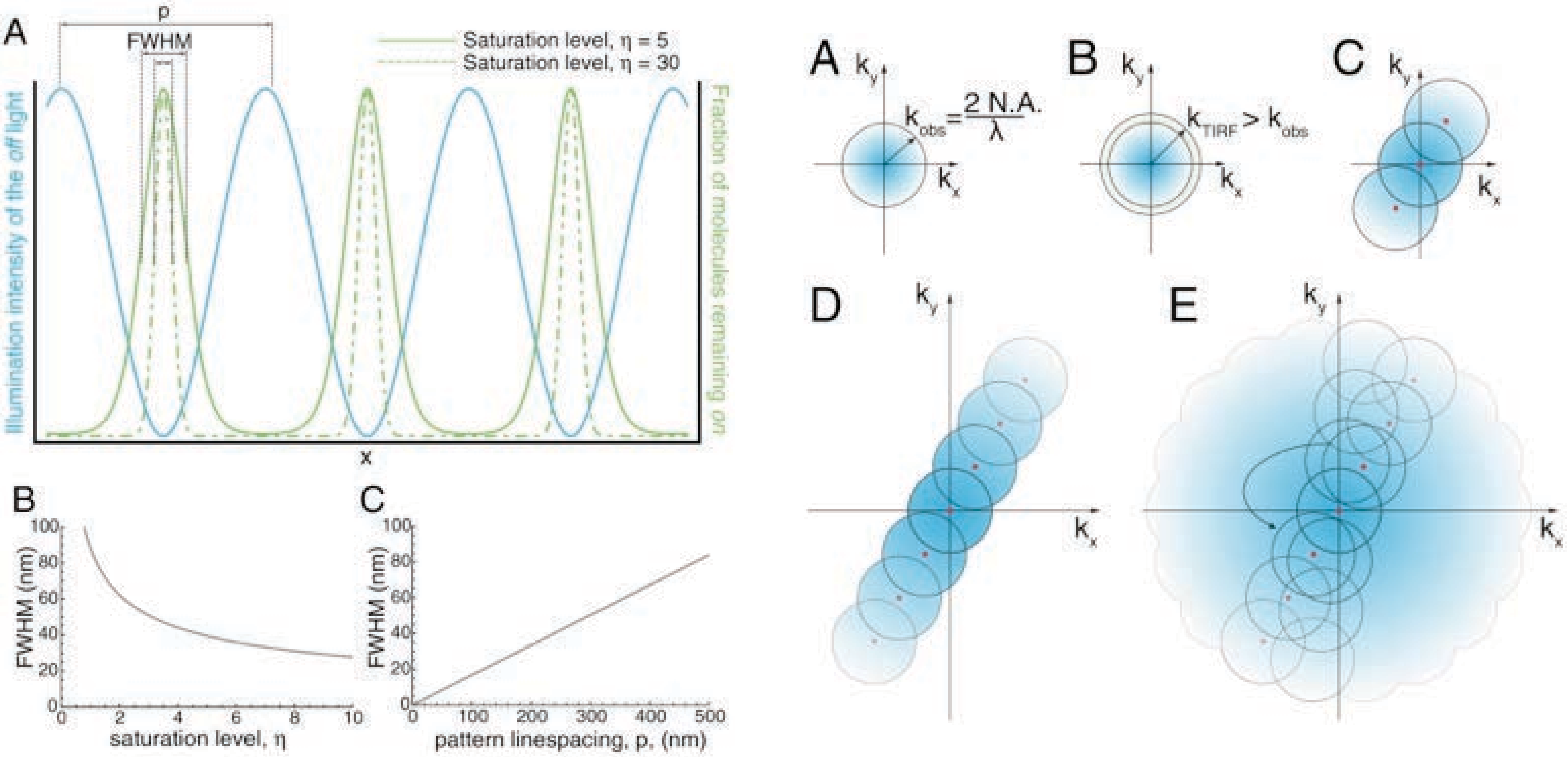


Resolution is theoretically unlimited!!!

Problem: photostability of the dye
=> works on beads but not on biological samples

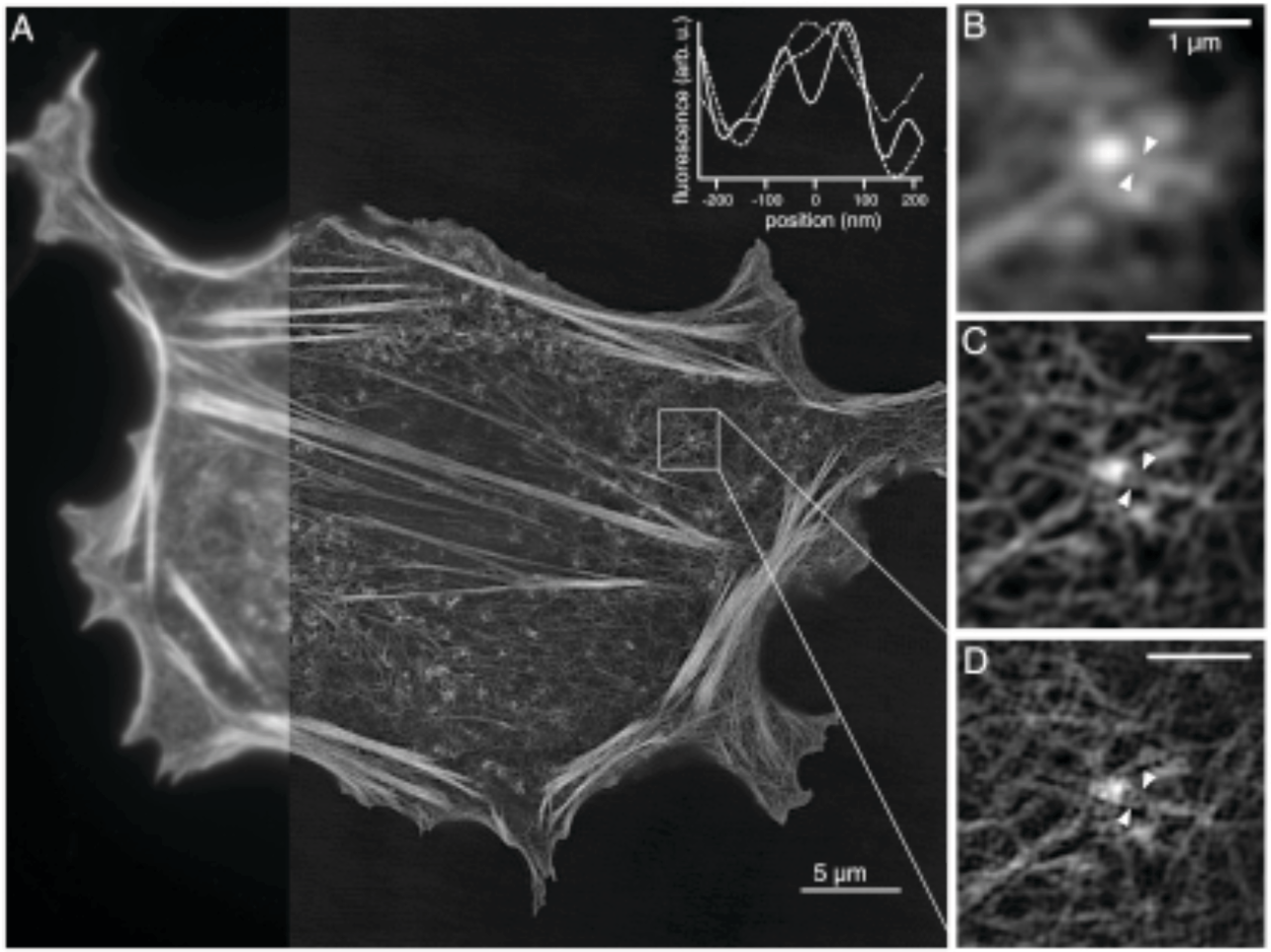
Gustafsson (2005), *PNAS* **102**

Non linear SIM with switchable fluorophores (Dronpa)



Rego et al. (2012), *PNAS* **109**

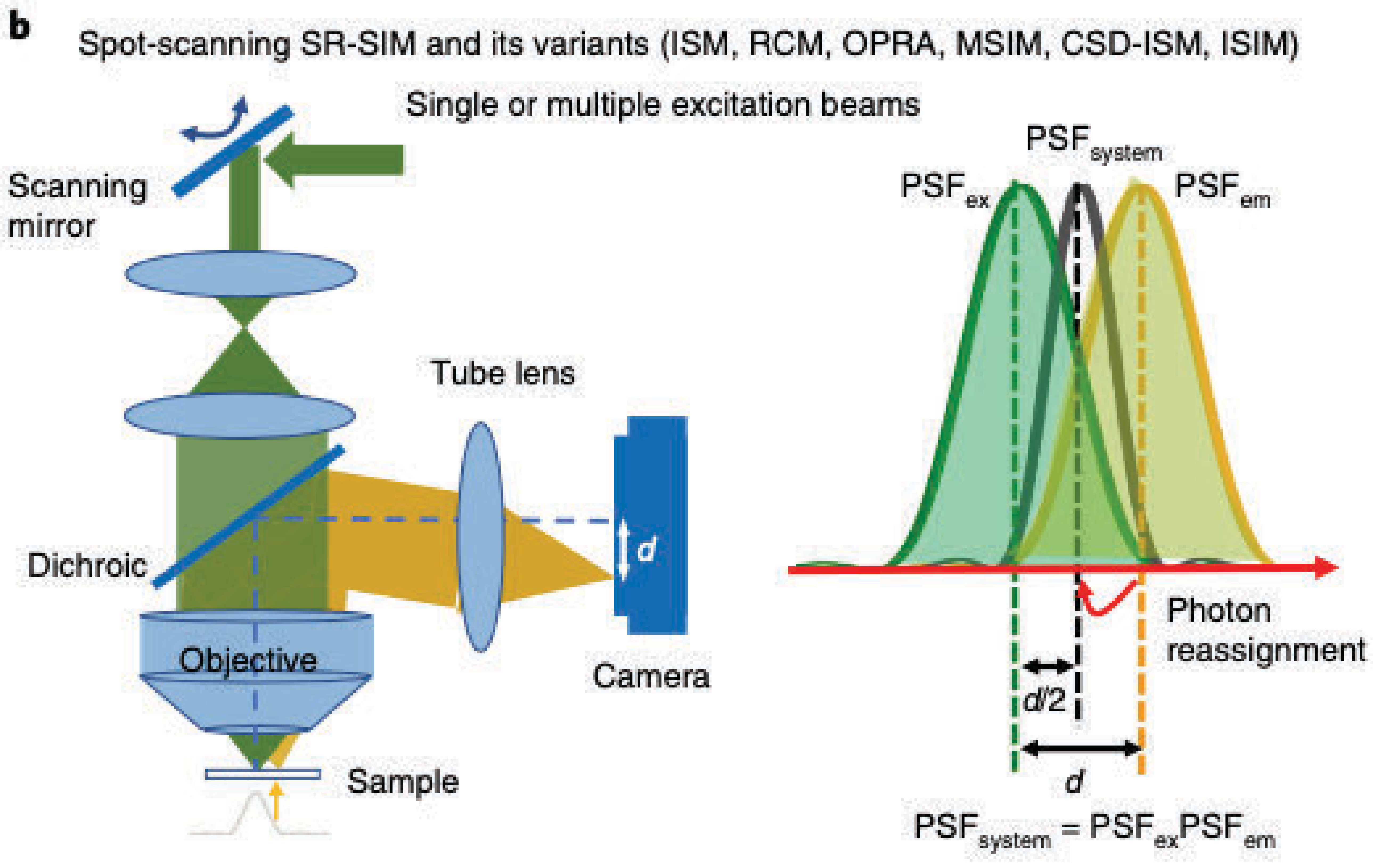
Non linear SIM with switchable fluorophores (Dronpa)

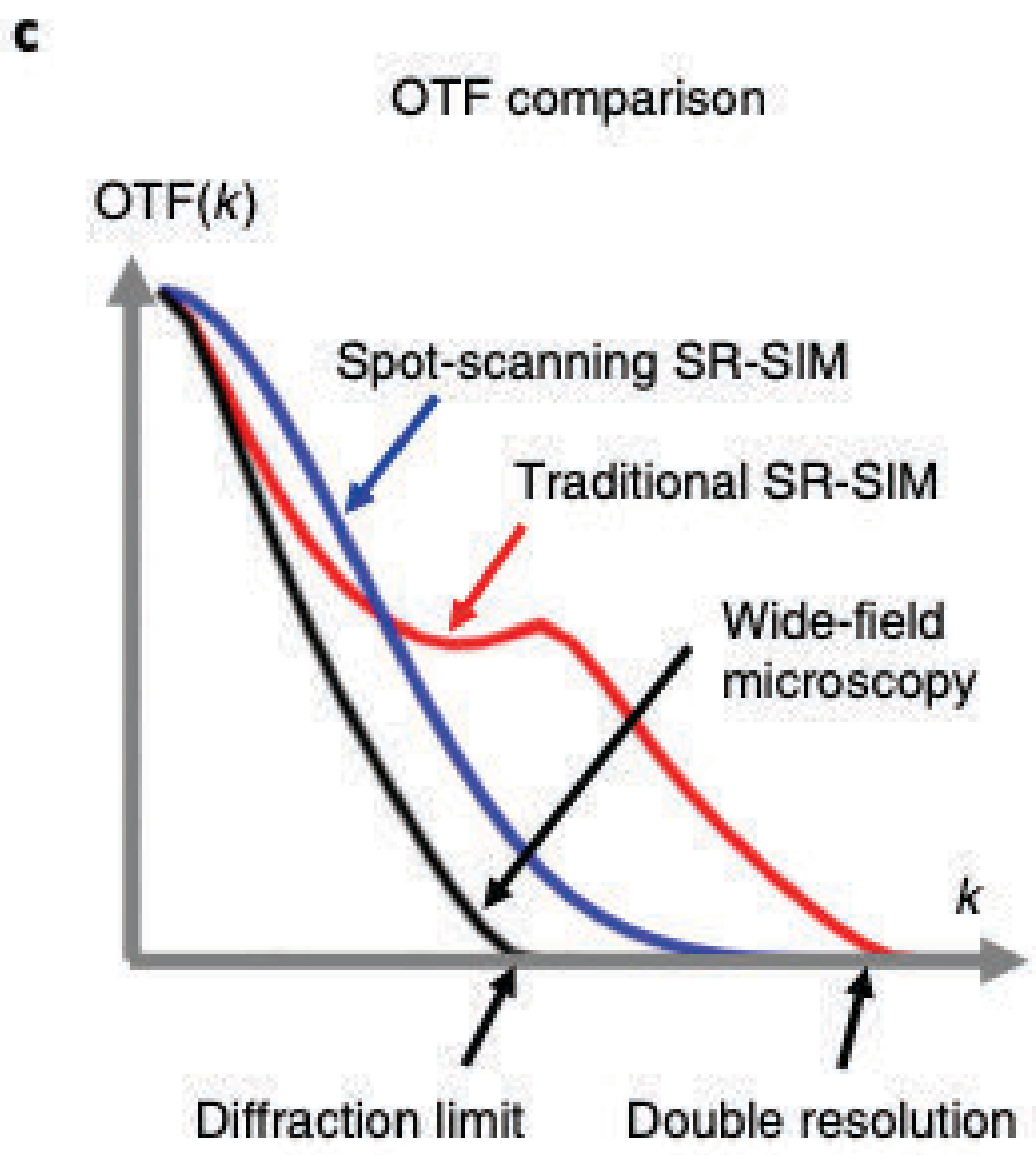


Biological imaging is possible!
but limited on the number of switching cycles
Only xy enhanced, requires TIRF

Rego et al. (2012), *PNAS* **109**

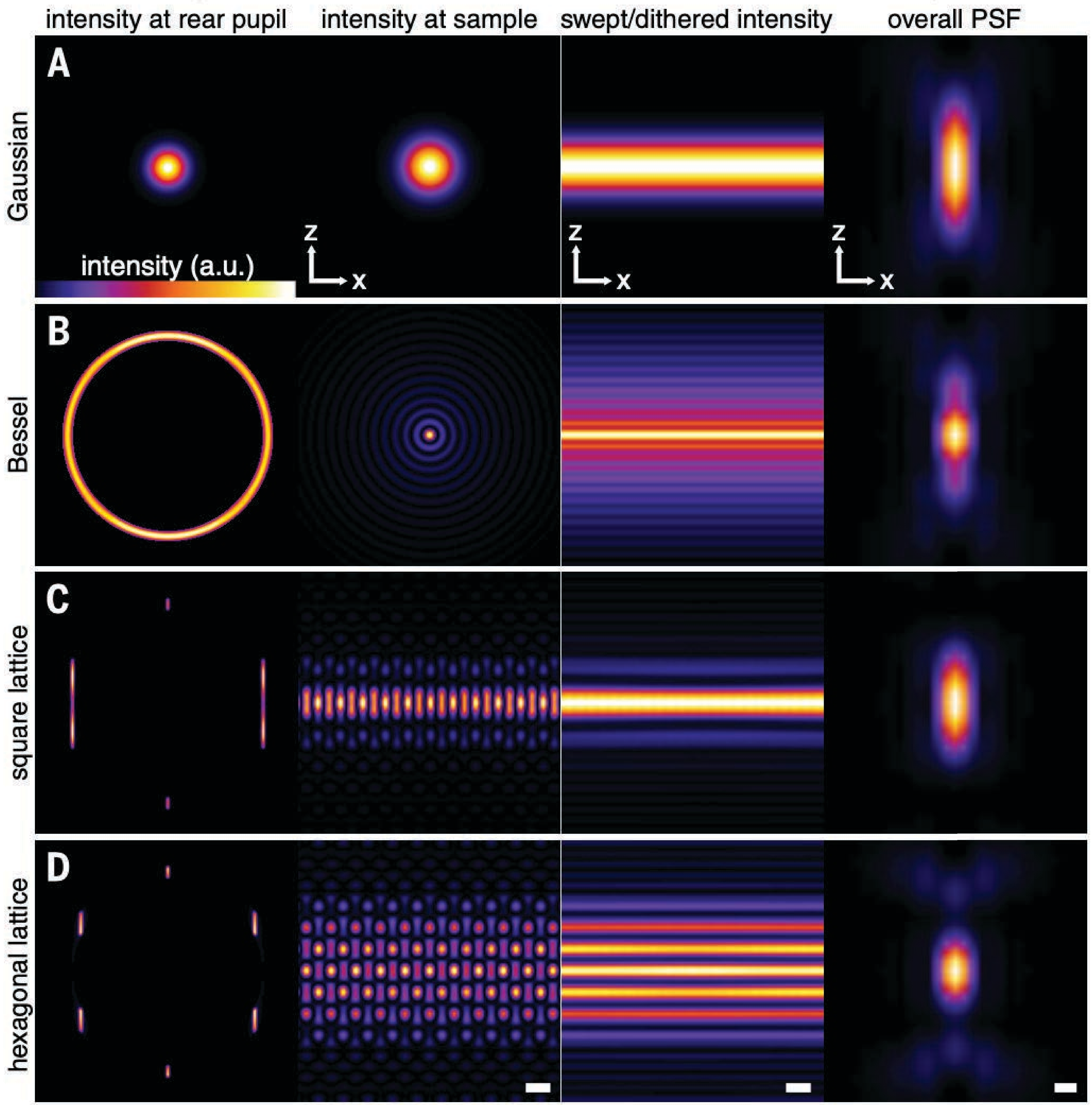
Point scanning SIM

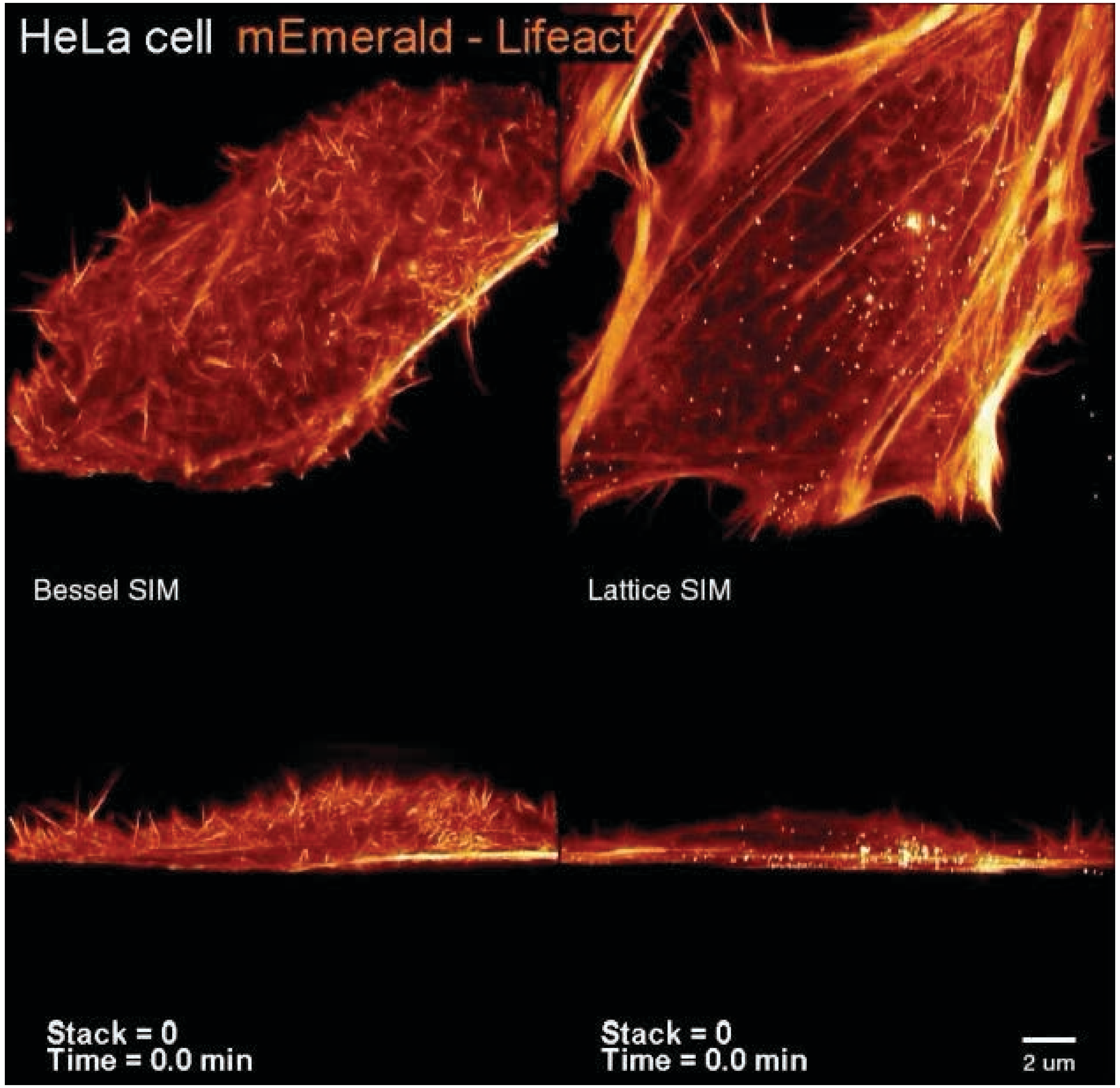




Wu & Schroff (2018), *Nat Methods*

Lattice light sheet microscopy - High resolution LSM





Chen et al. 2014, *Science*

3D-SIM - pros & cons

+ Multiple colours with standard dyes
+ Lateral and axial resolution improvement
+ 3D optical sectioning with enhanced contrast

Context

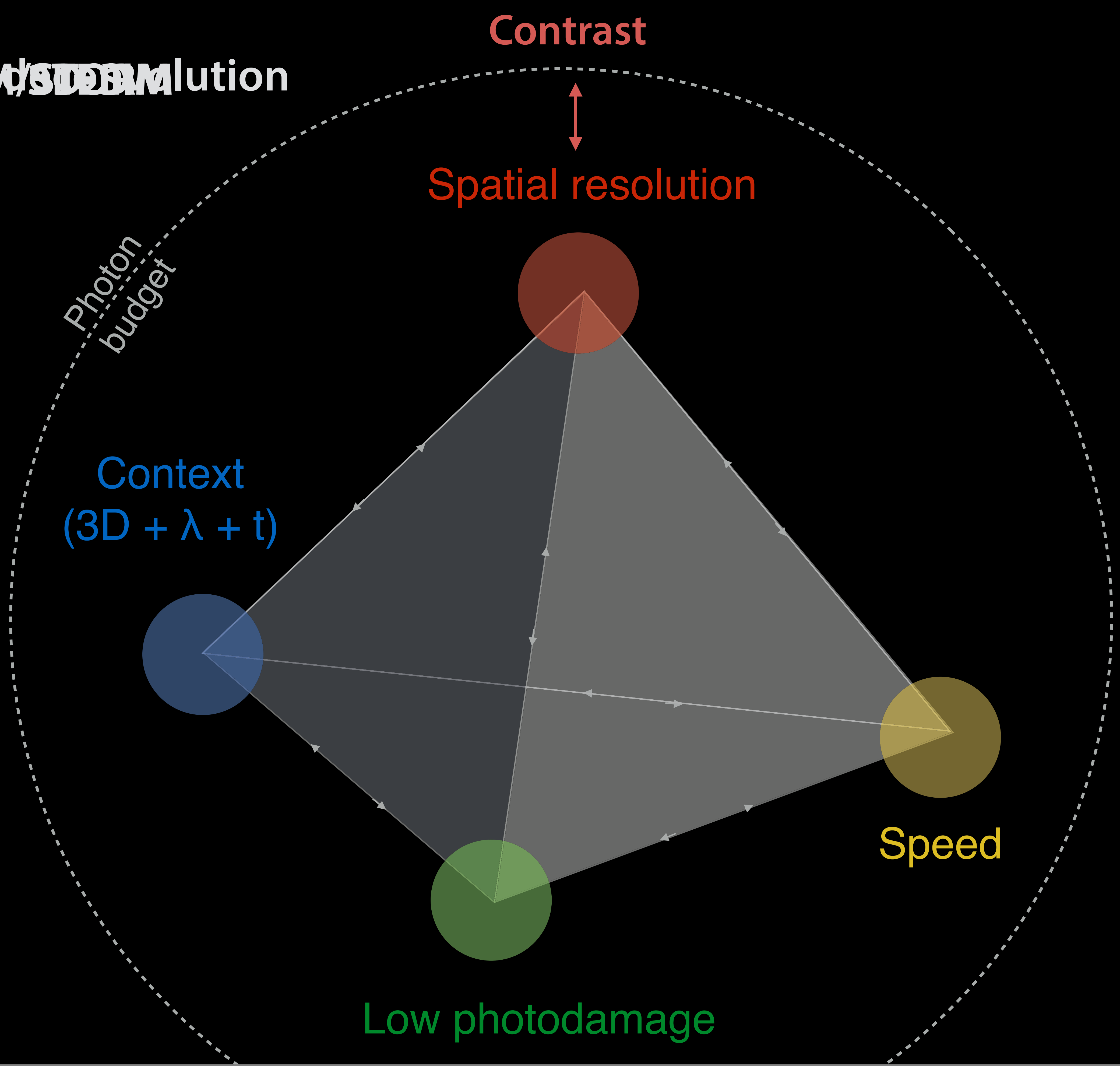
+ Light dosage lower than other SR-techniques
+ Linear response → quantitative
+ Relatively deep imaging (up to 10 µm, or 20 µm w/ Silicon)
+ Sensitivity and speed (SLM, Blaze) → live cell imaging

Versatility

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

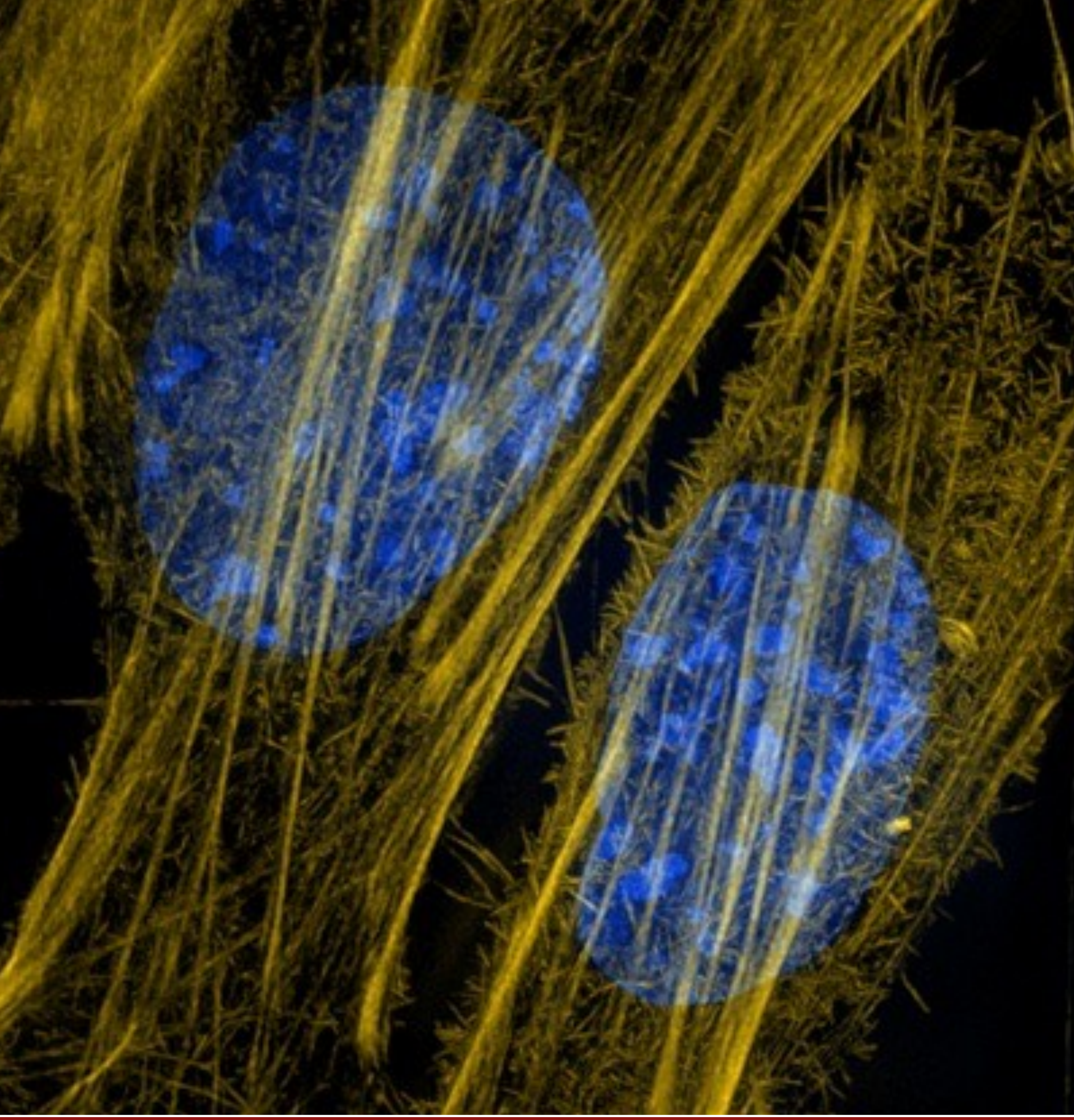
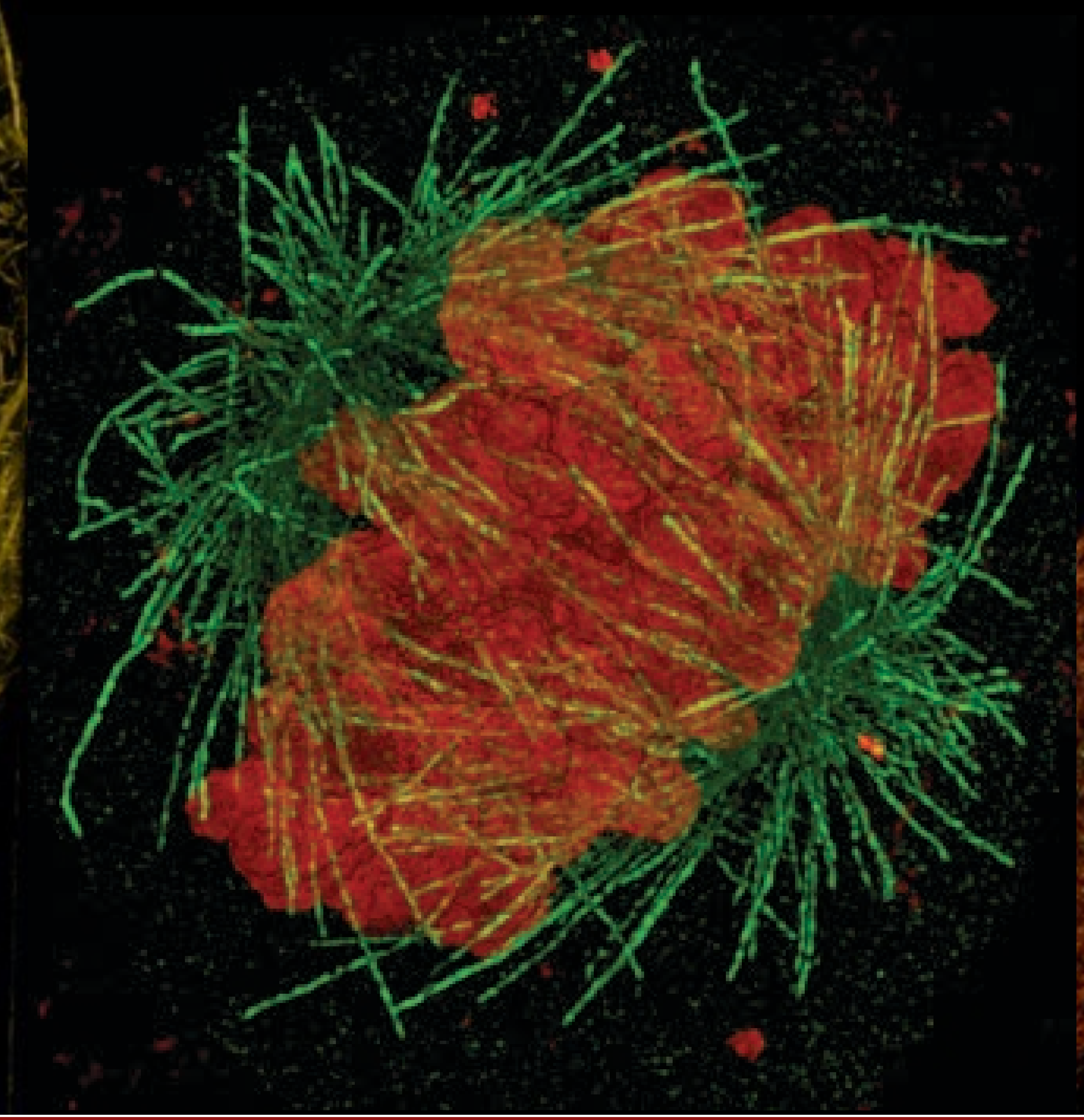
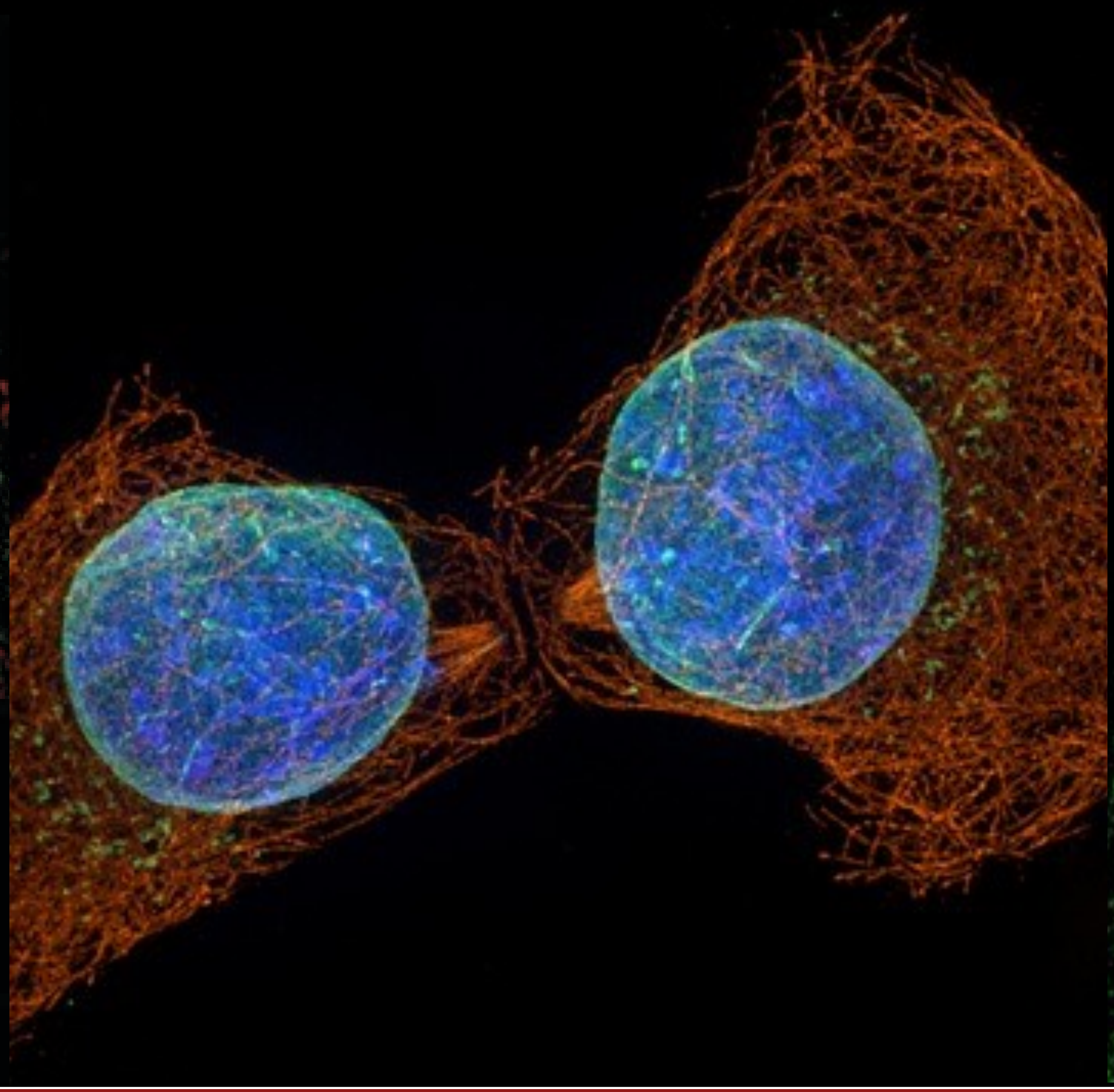
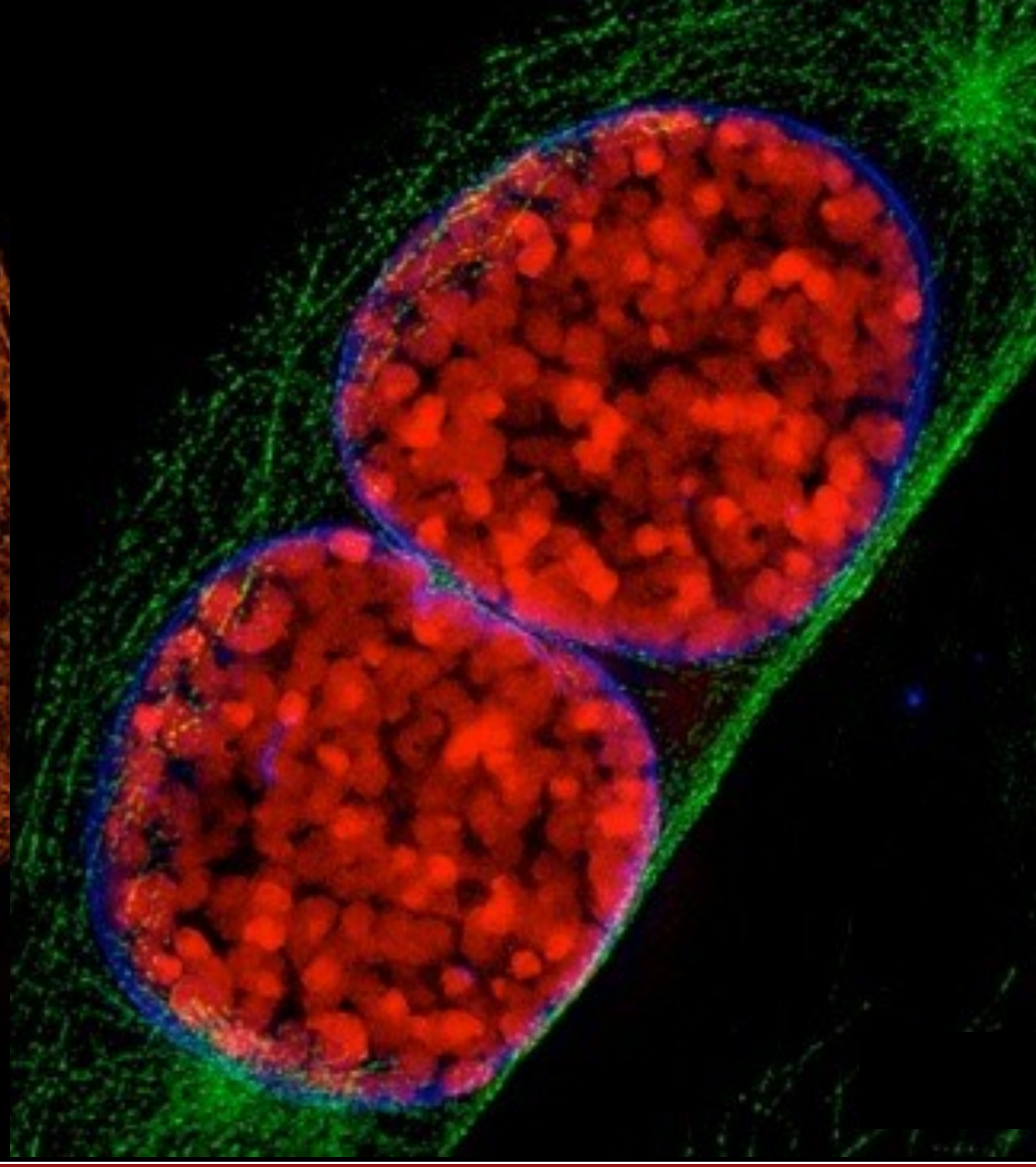
Challenges

Trade-offs in super-resolution microscopy

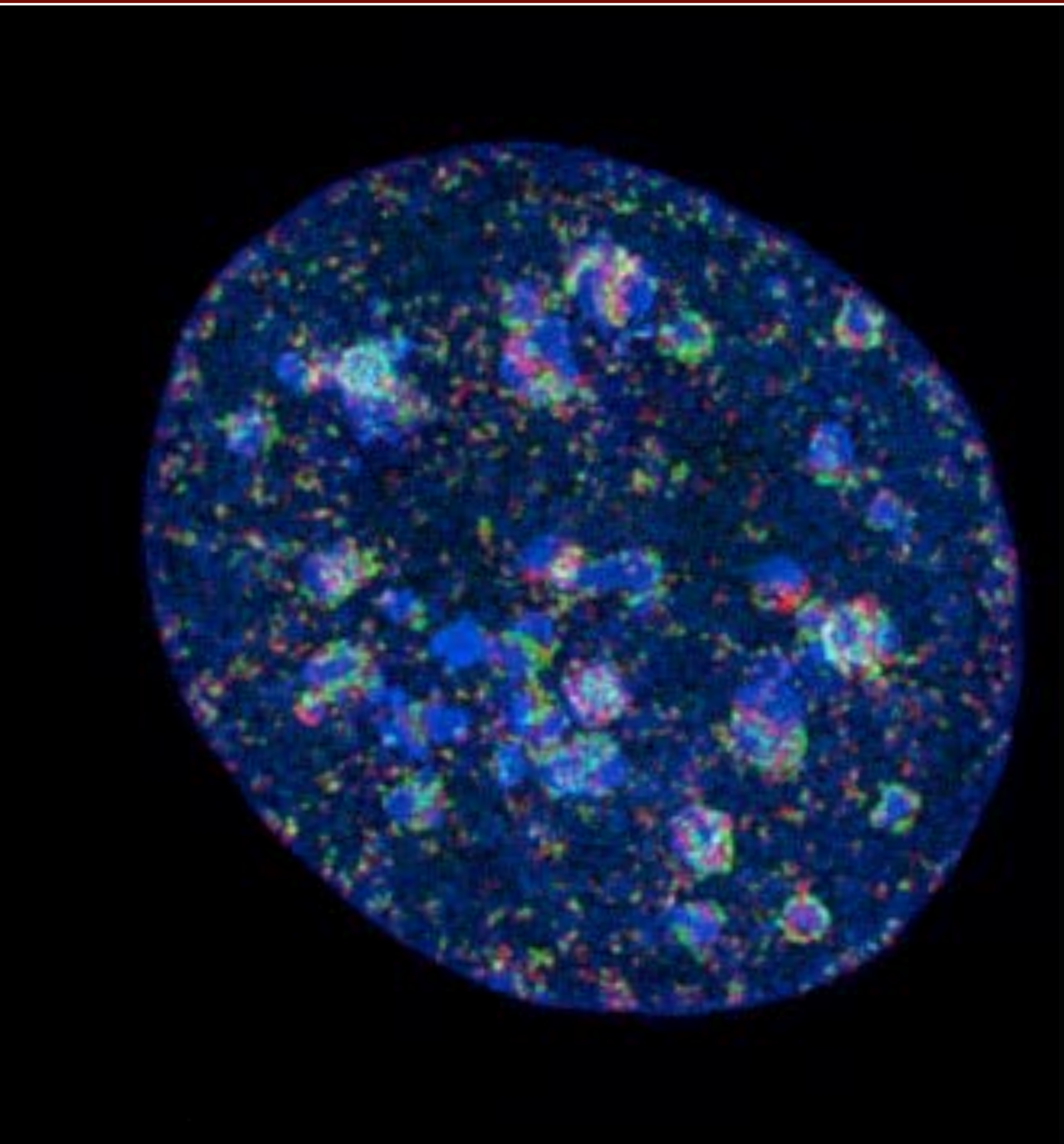
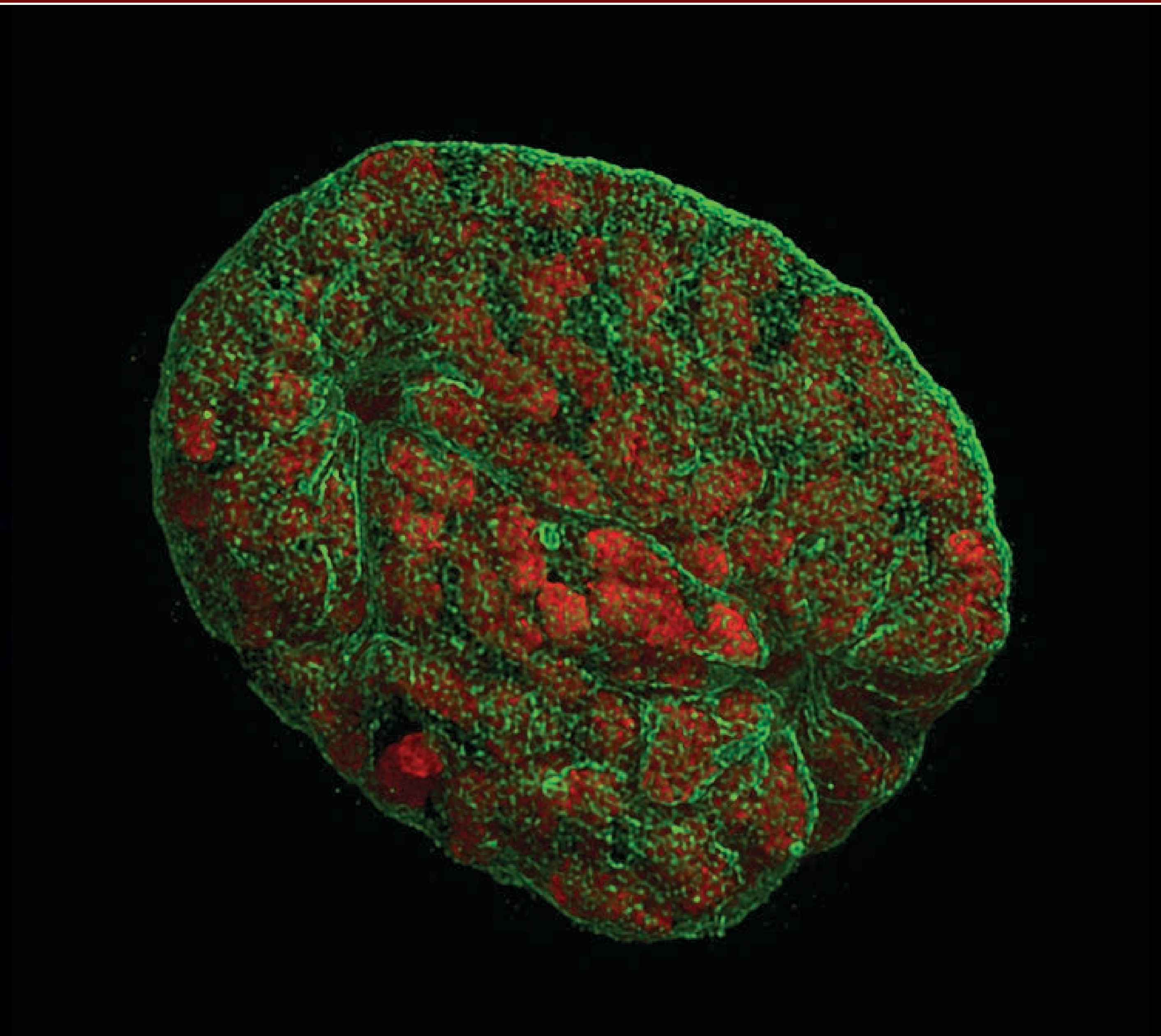
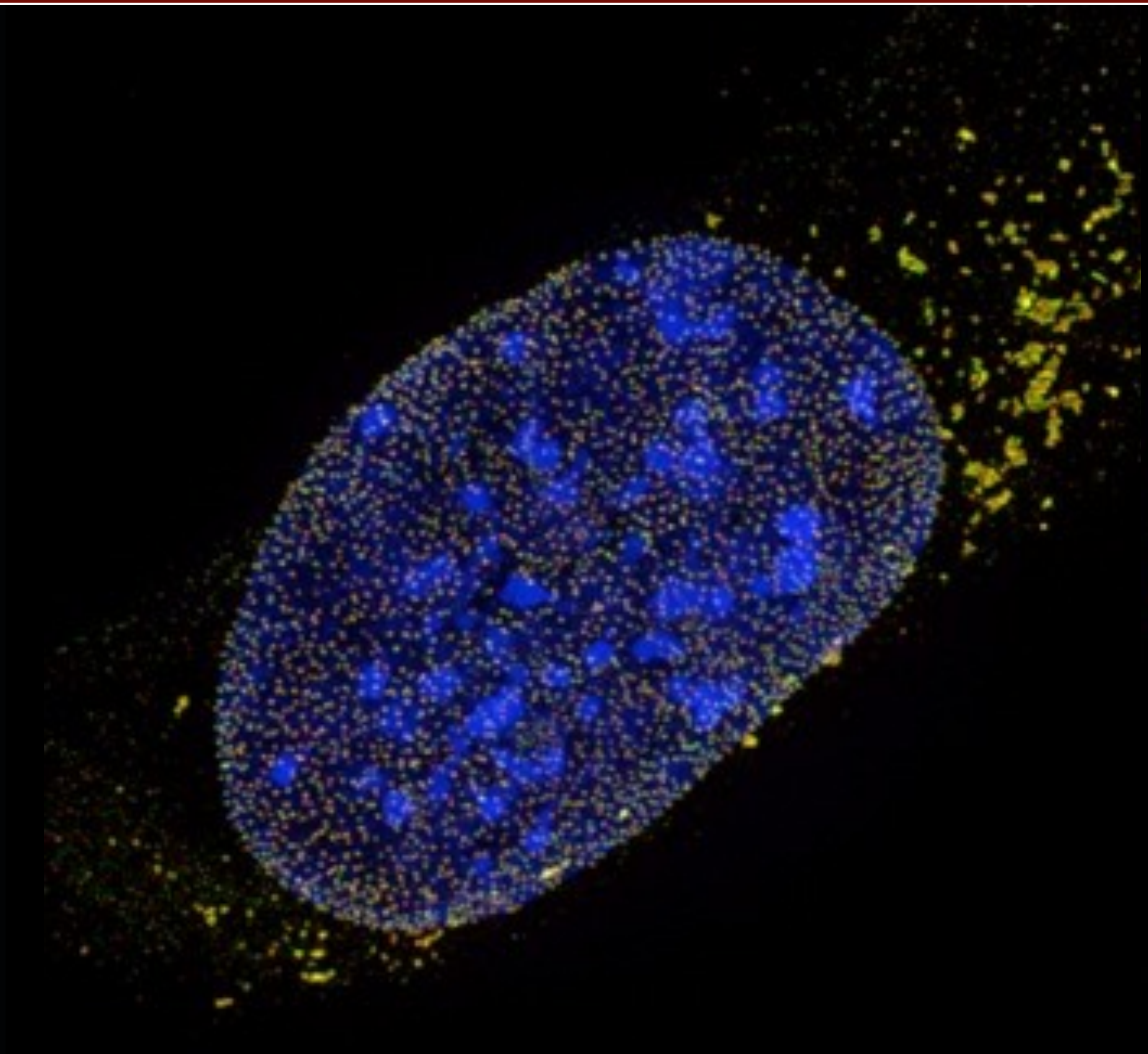
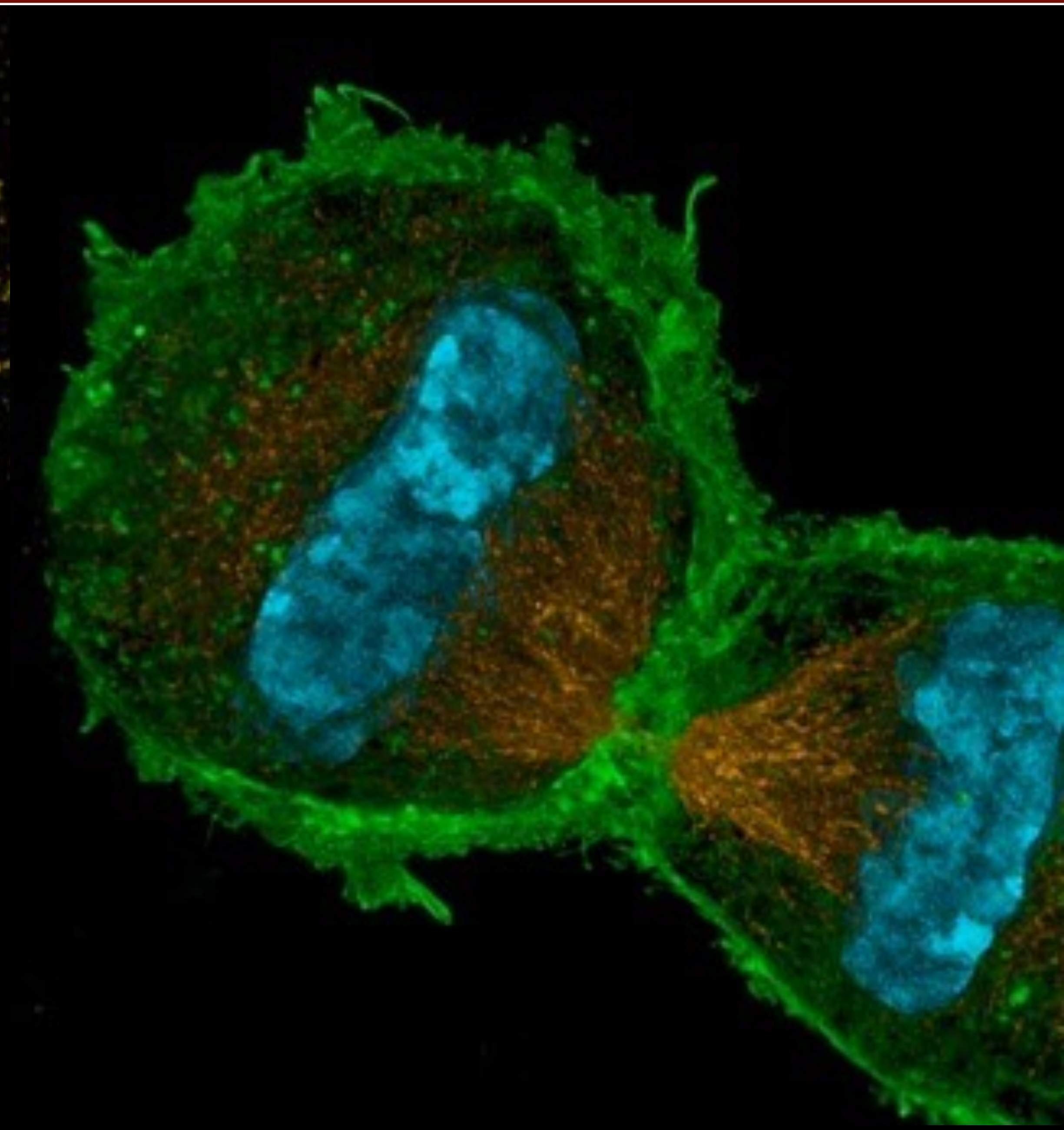


The optimal technique is determined by demands of the application!
Spatial resolution is only part of the equation!
Photon budget and **contrast** are the limiting factors in practice!

Contrast is the limit!!!

SIM rocks!

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

Thanks!

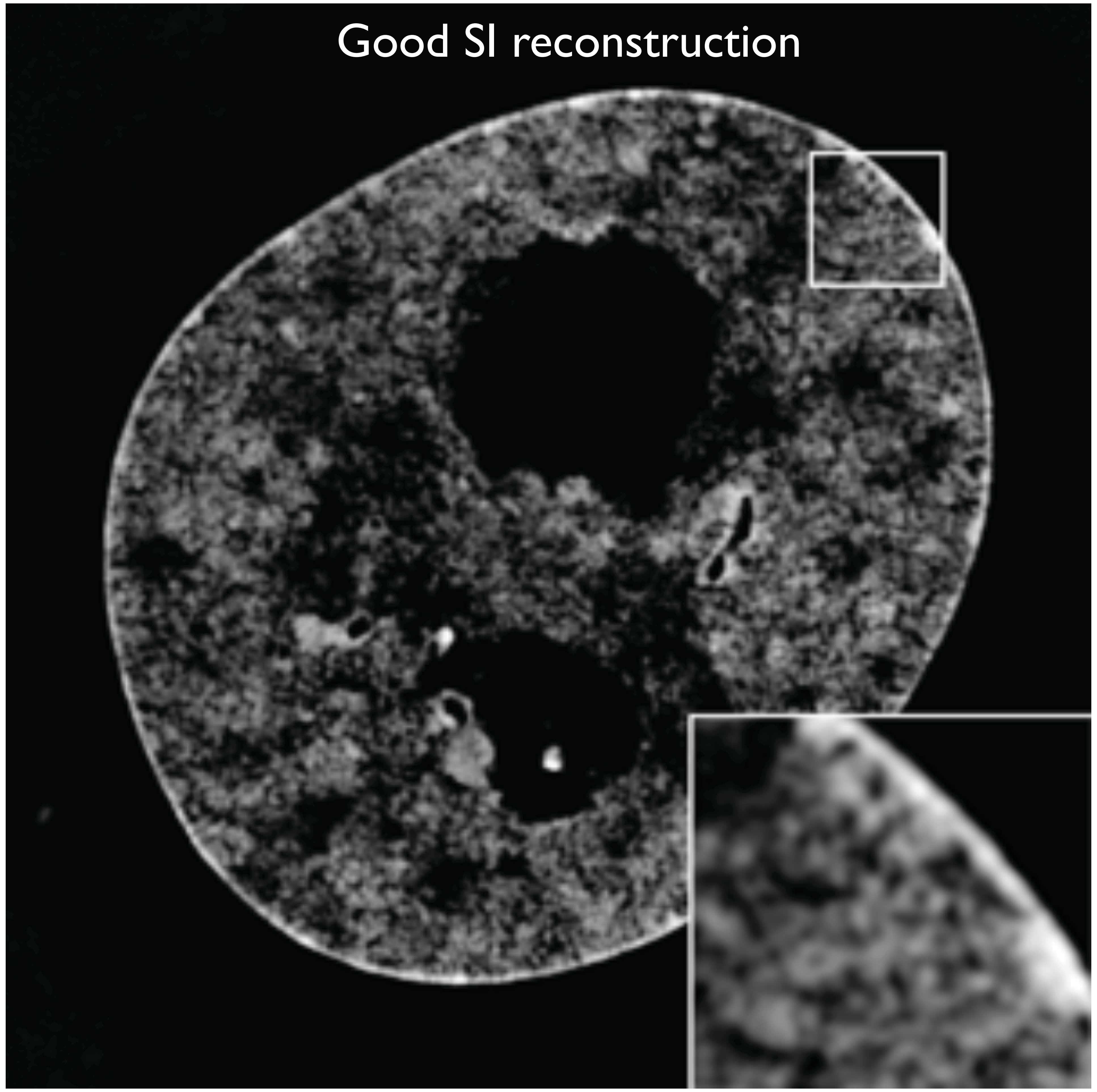
“DNA drop” Wellcome Trust Image Award 2017 Winning Entry (by Ezequiel Miron)

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

It‘s not that simple!

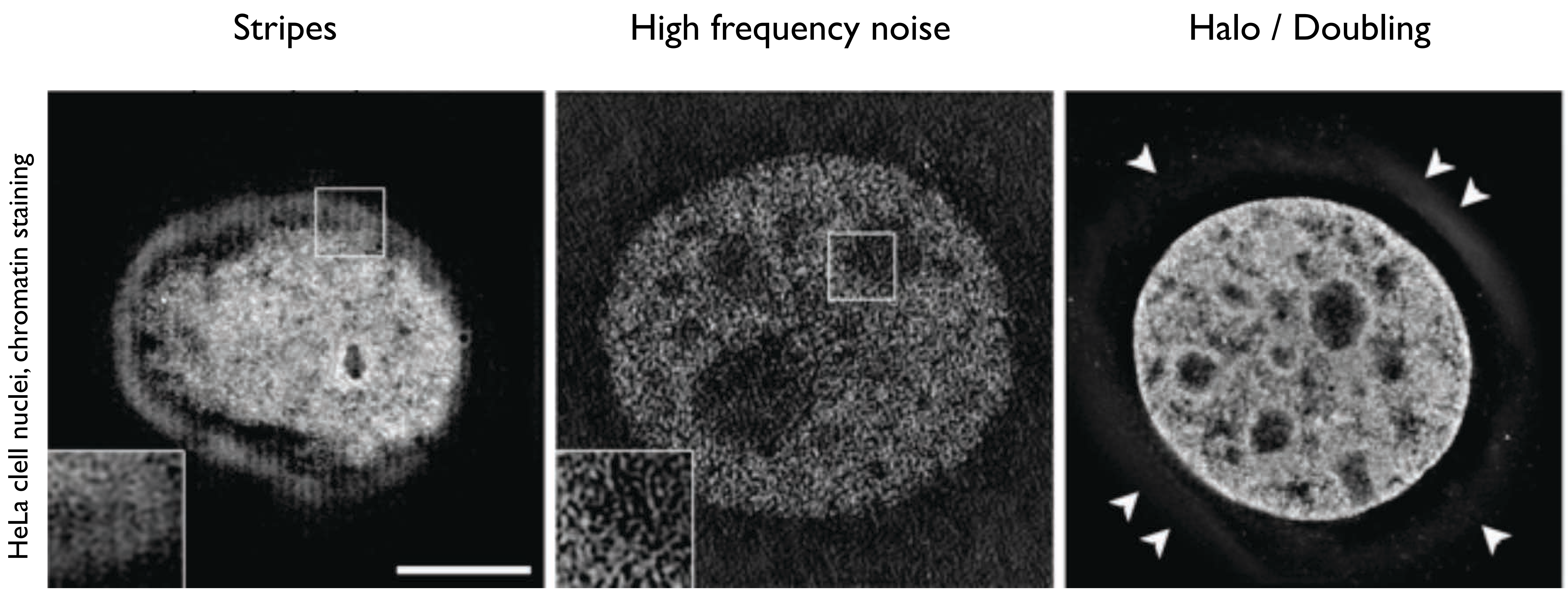
The untold story

SI reconstruction artifacts



RPE-1 cell, DAPI staining

SI reconstruction artifacts

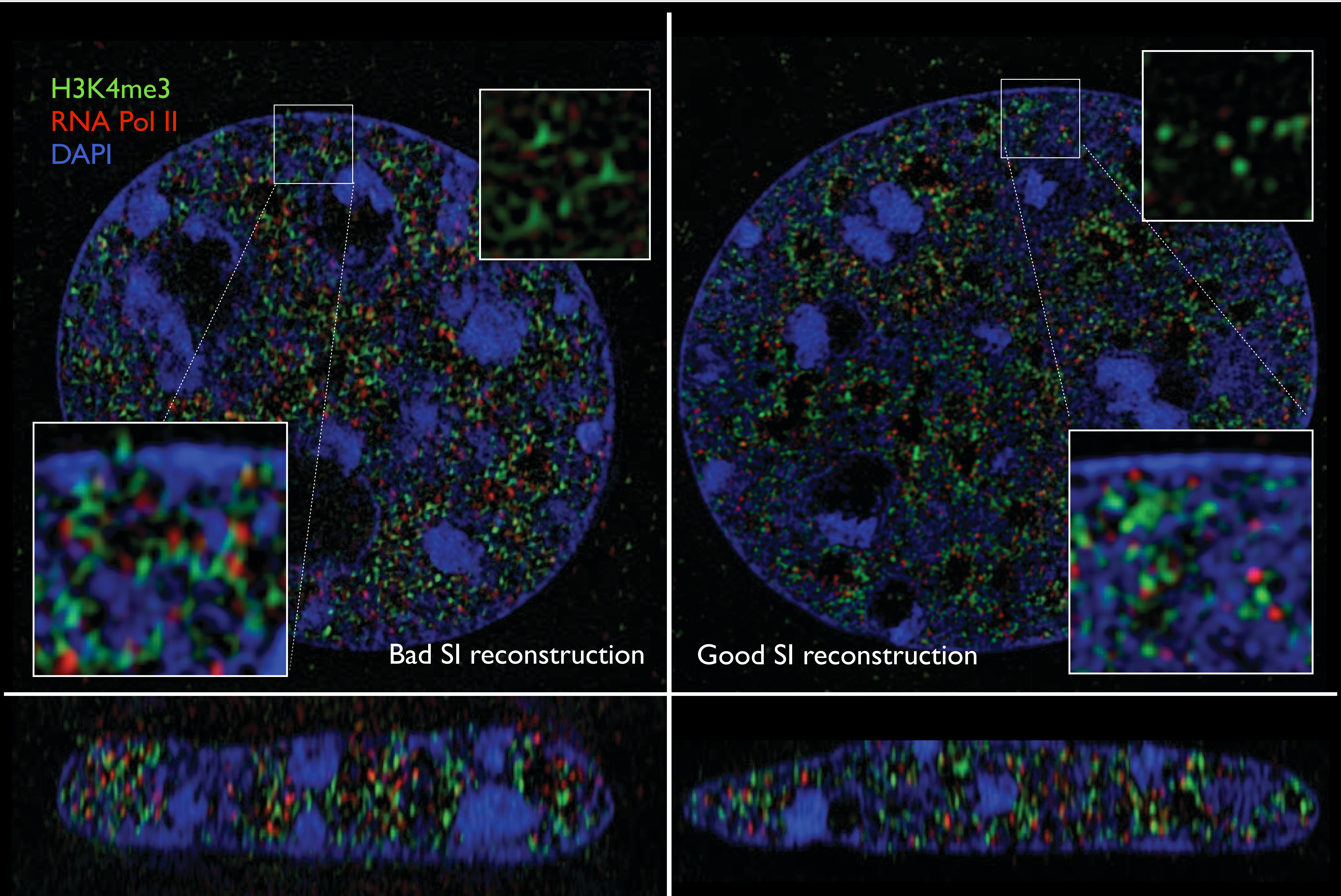


Bleaching,
Drift or vibrations
Moving particles
(locally constrained)

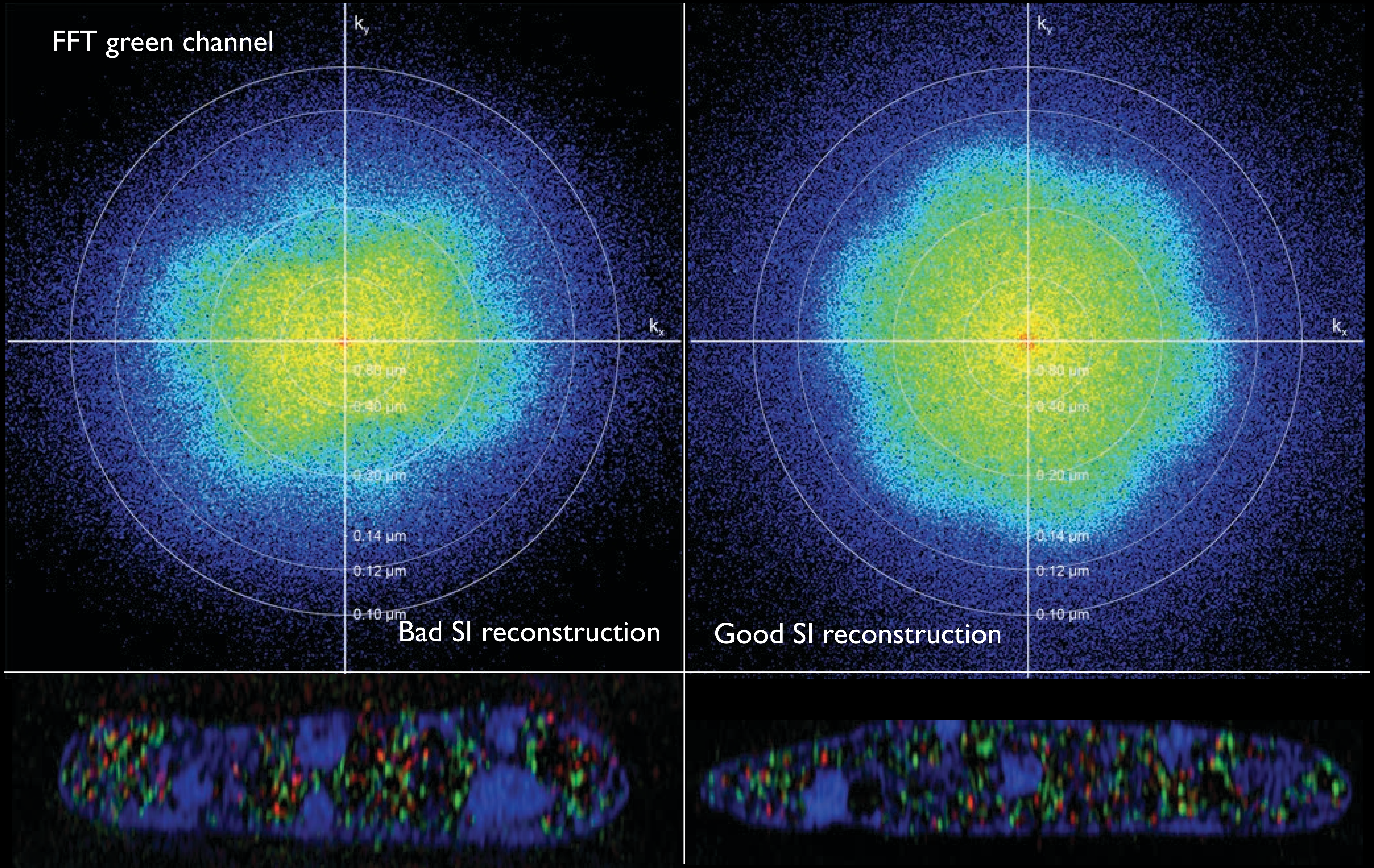
Low contrast-to-noise,
Low modulation contrast

Spherical aberration,
Refractive index mismatch

SI reconstruction artifacts

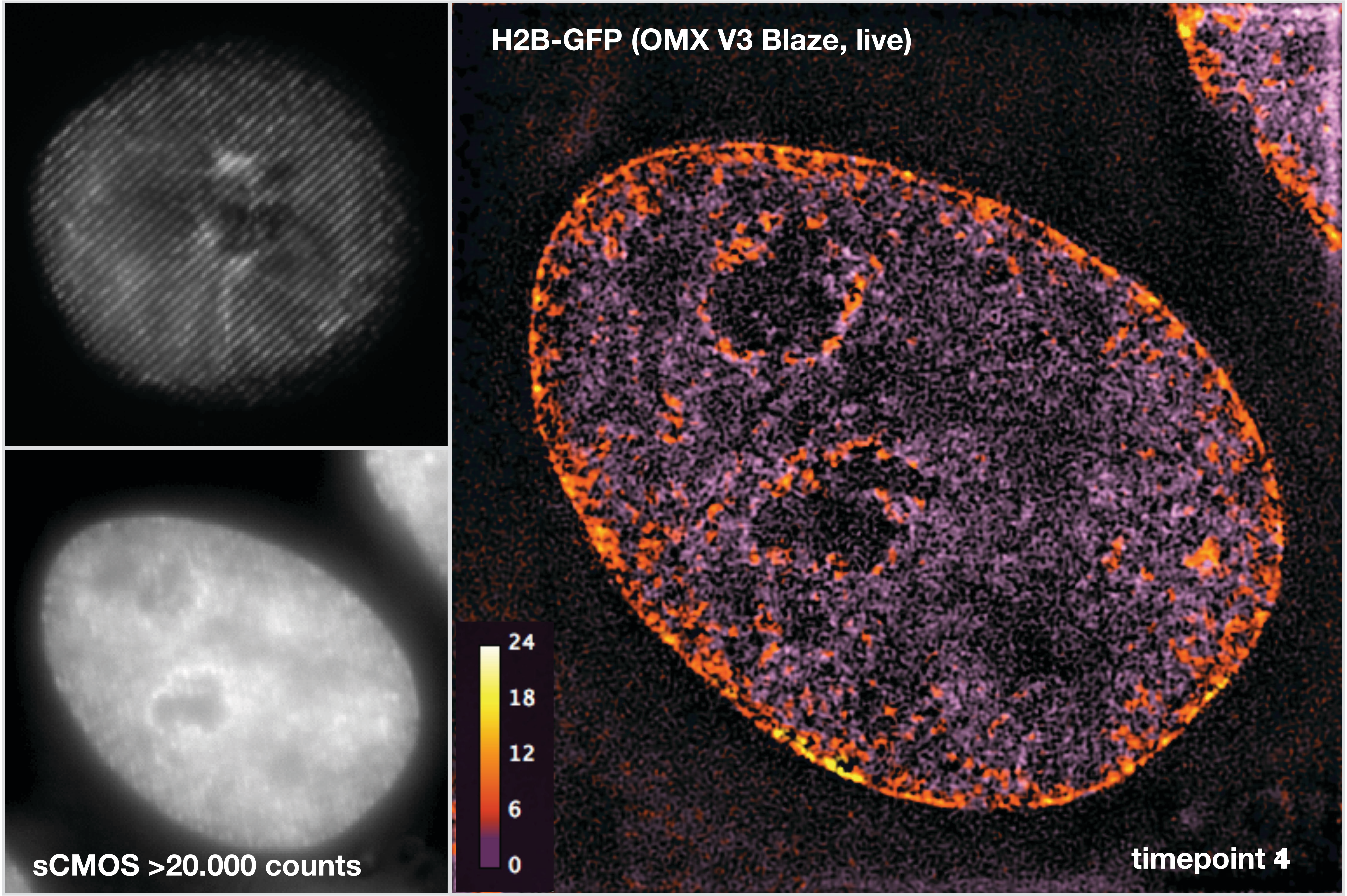


Quality control by Fourier analysis



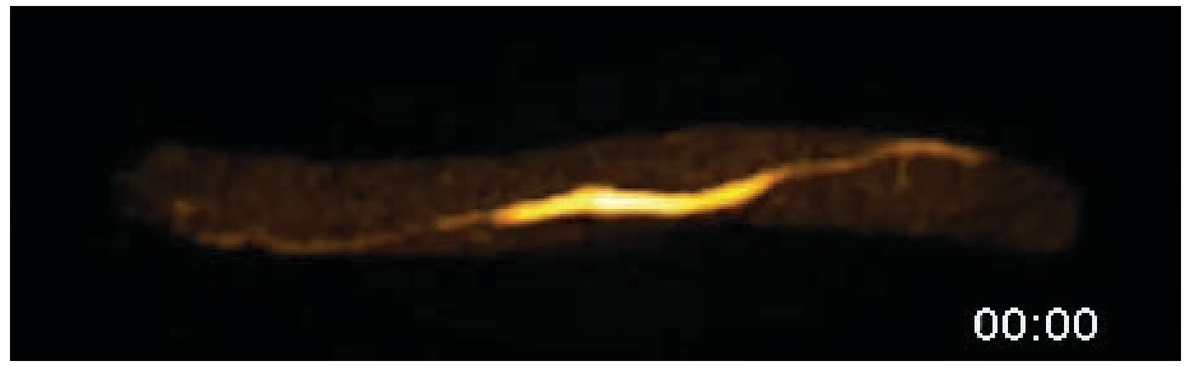
(Stripe) contrast is key!

Mapping of local modulation contrast variation (SIMcheck)

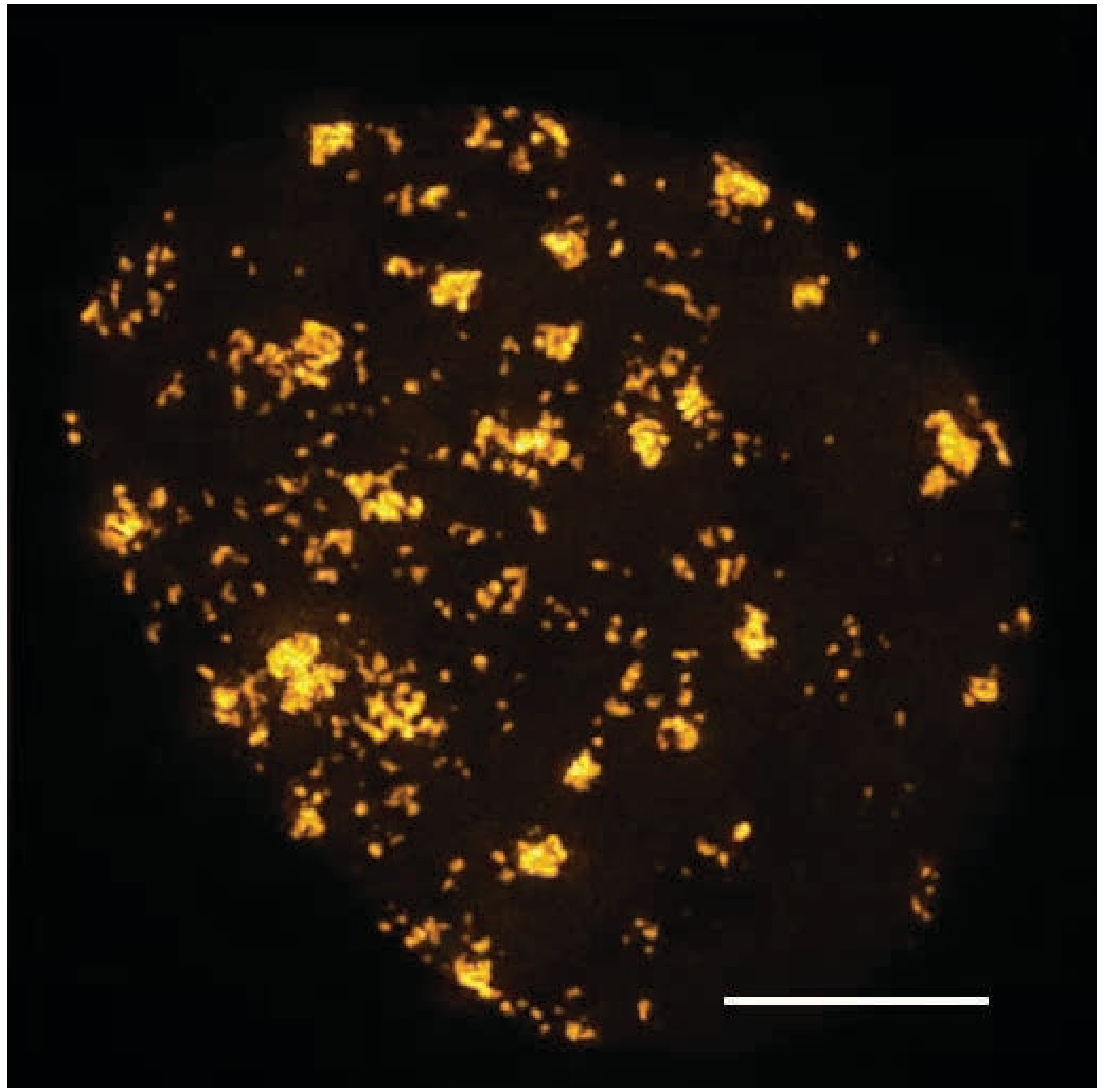


Photon budget is limited – spend it wisely!

Imaging multiple time points, requires trade off in other areas, e.g. z-height.

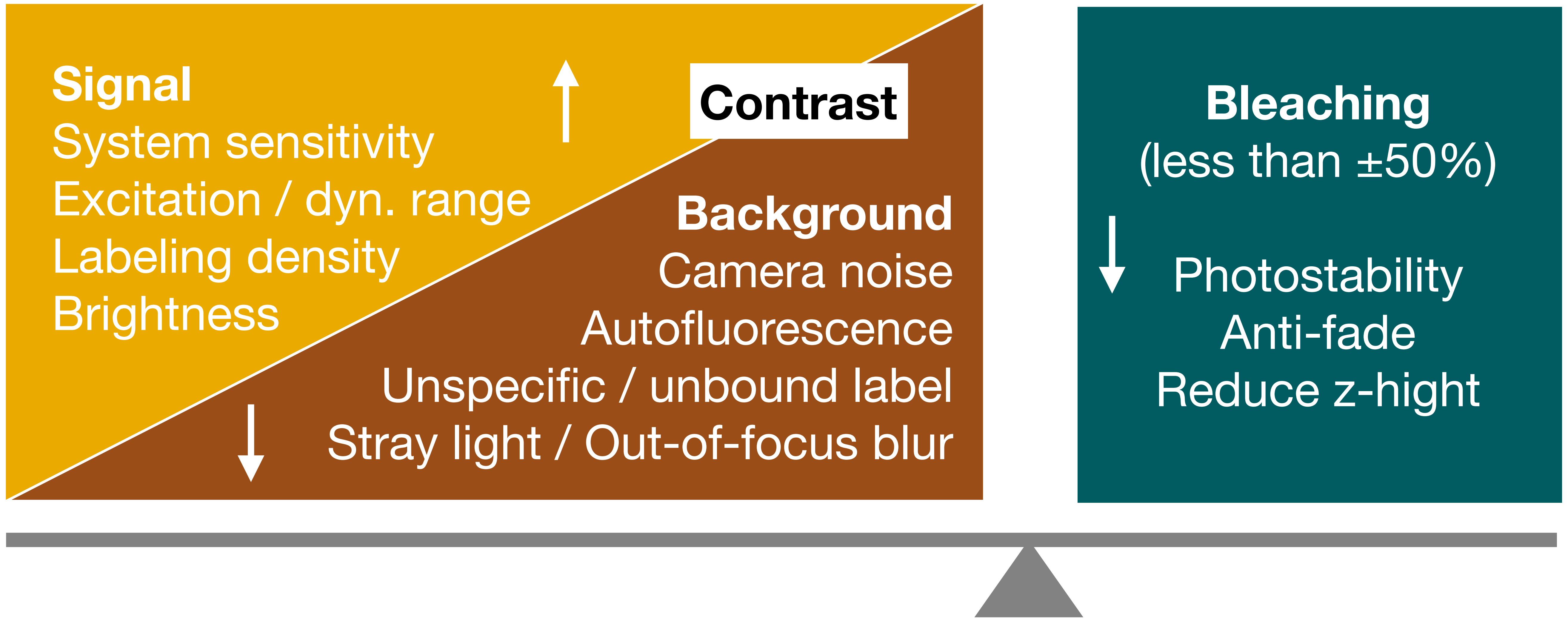


OMX Blaze: 2 s / frame
(1.75 *μ*m z-stack = 225 images /frame, 100 time points)



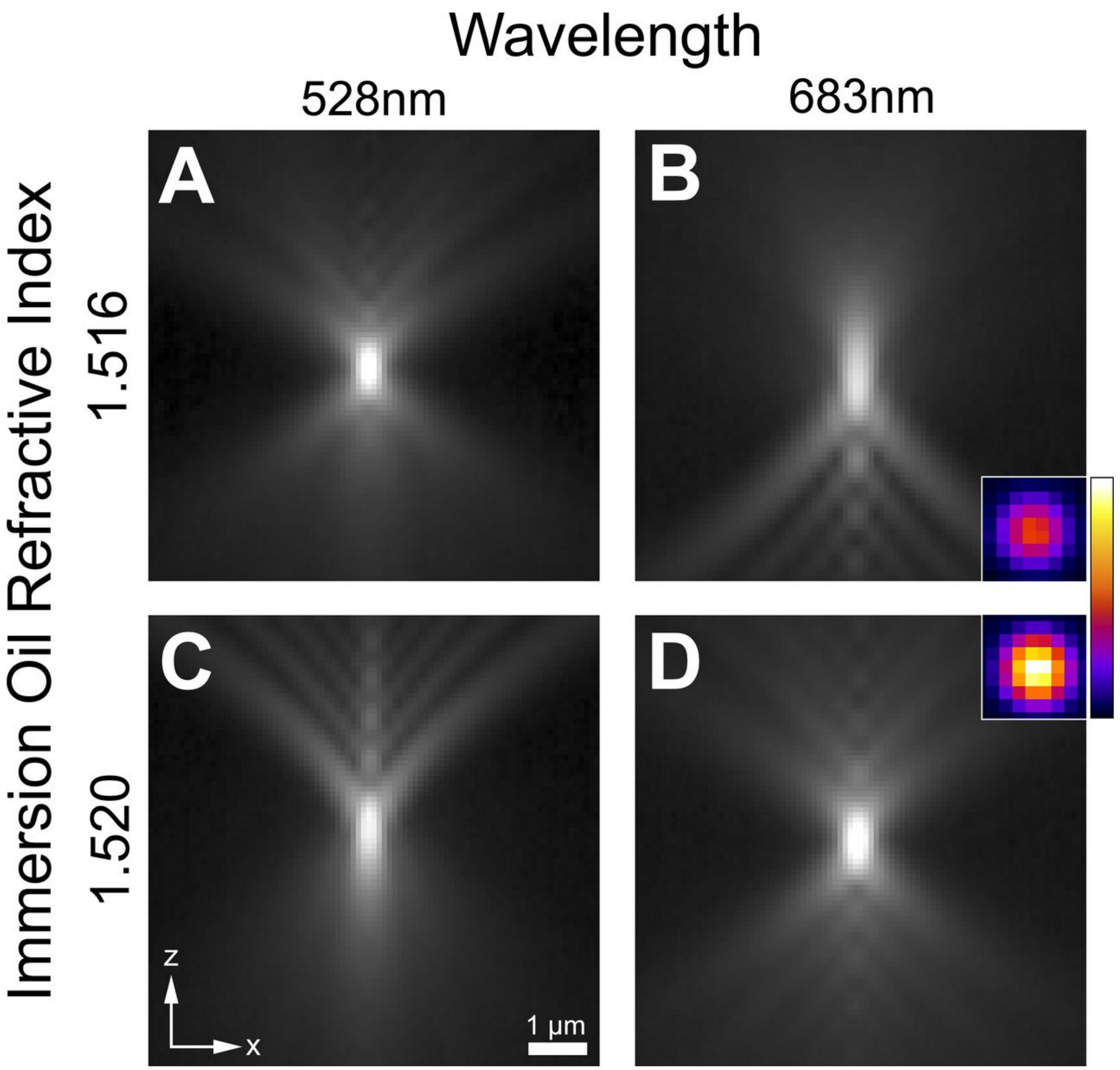
OMX Blaze 10 s / frame
(5 *μ*m z-stack = 600 images / frame, 10 time points

Optimal trade-off between signal-to-background and bleaching



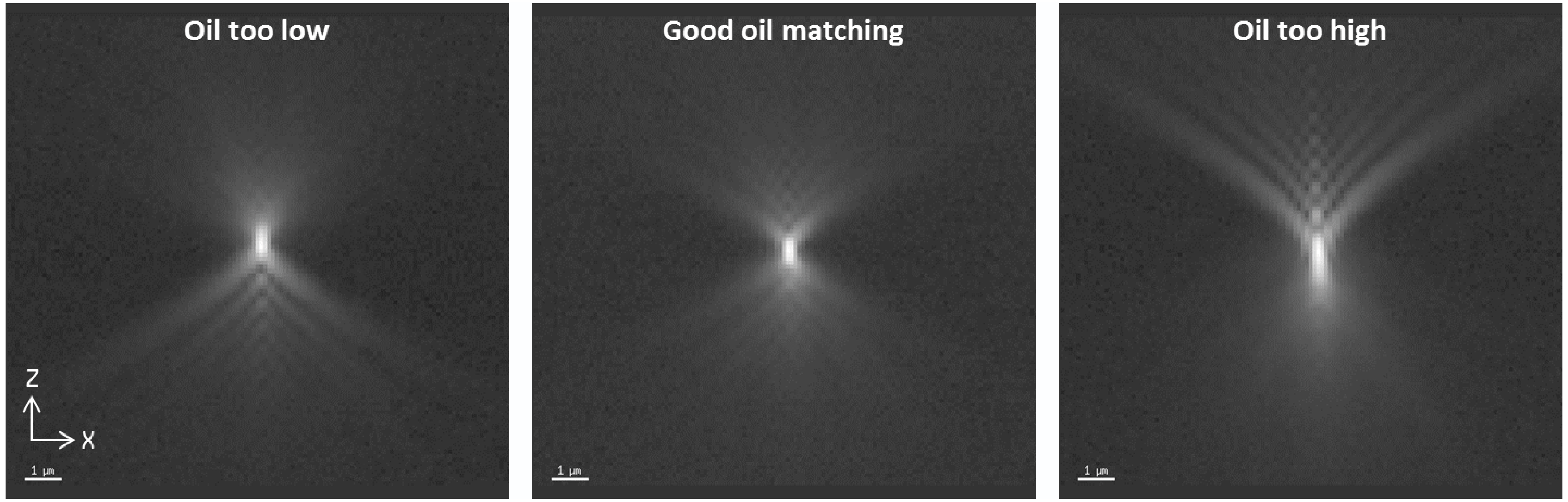
Strike an optimal balance of sufficiently high dynamic range and photobleaching,
No more than ~50% bleaching!

Spherical aberration - correction by oil refractive index



Talley et al. 2016, JCB

PSF

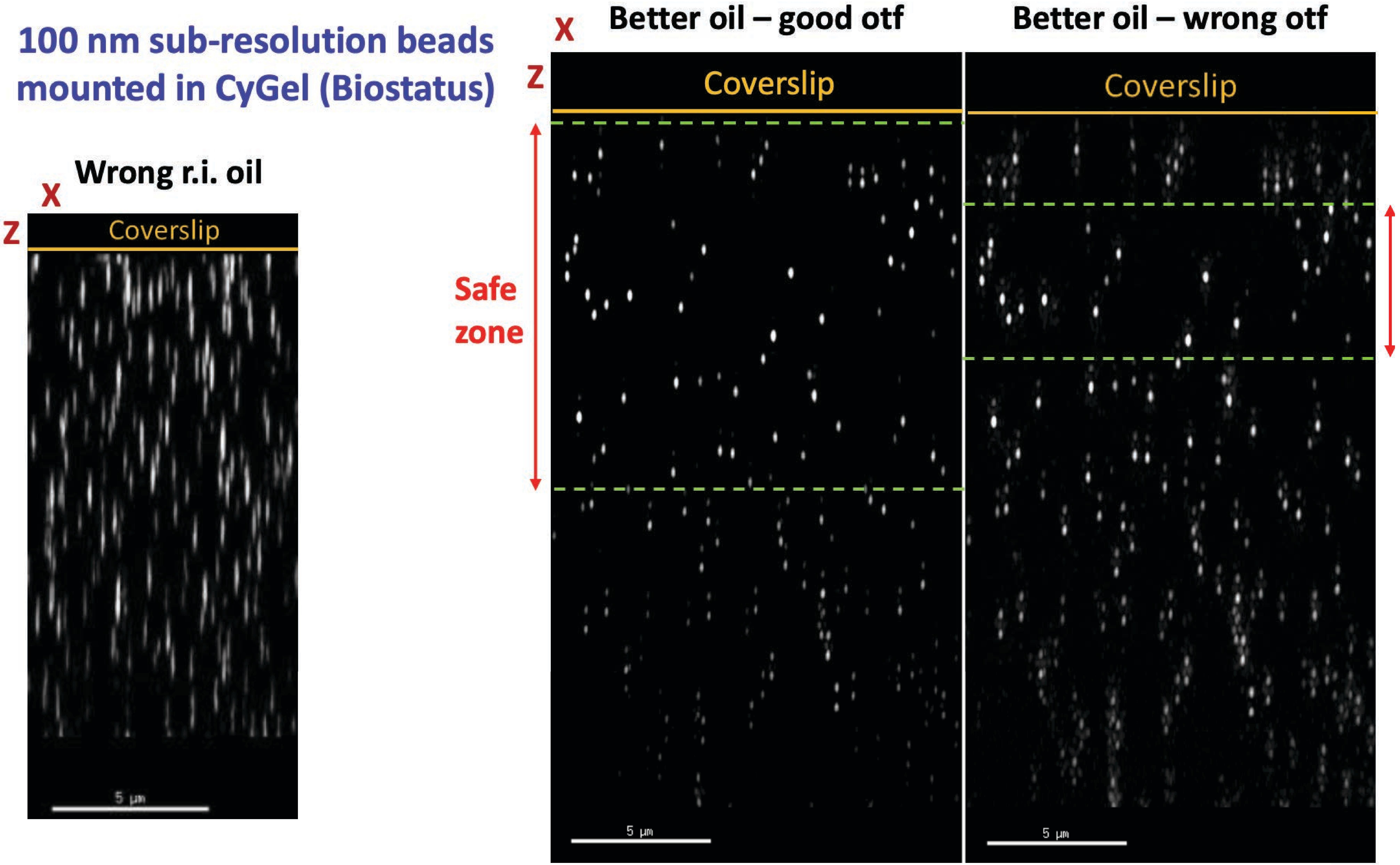


'bottom-heavy' symmetric 'top-heavy'

Courtesy of Dan White (GE)

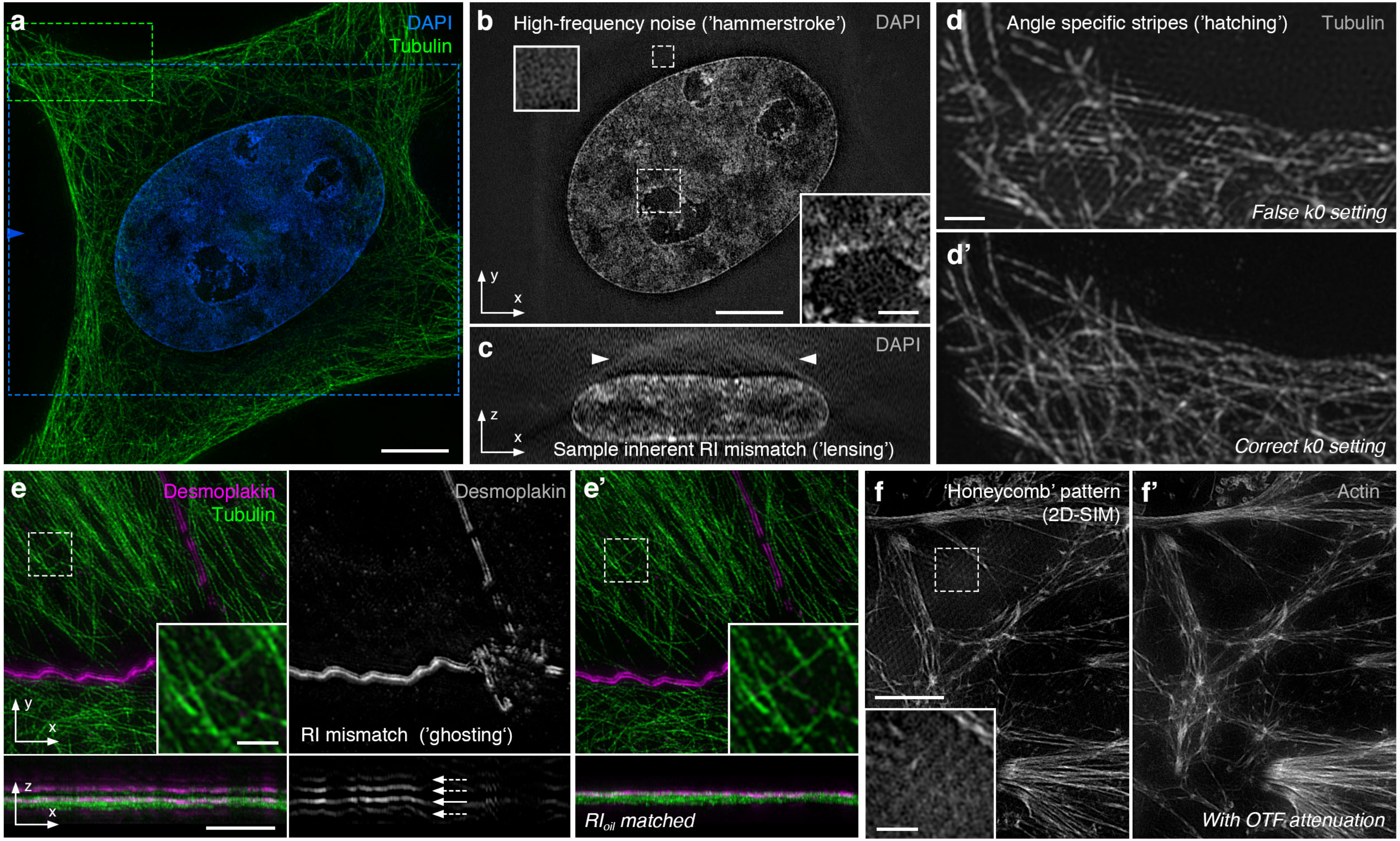
Spherical aberration - correction by oil refractive index

100 nm sub-resolution beads mounted in CyGel (Biostatus)



Alison North

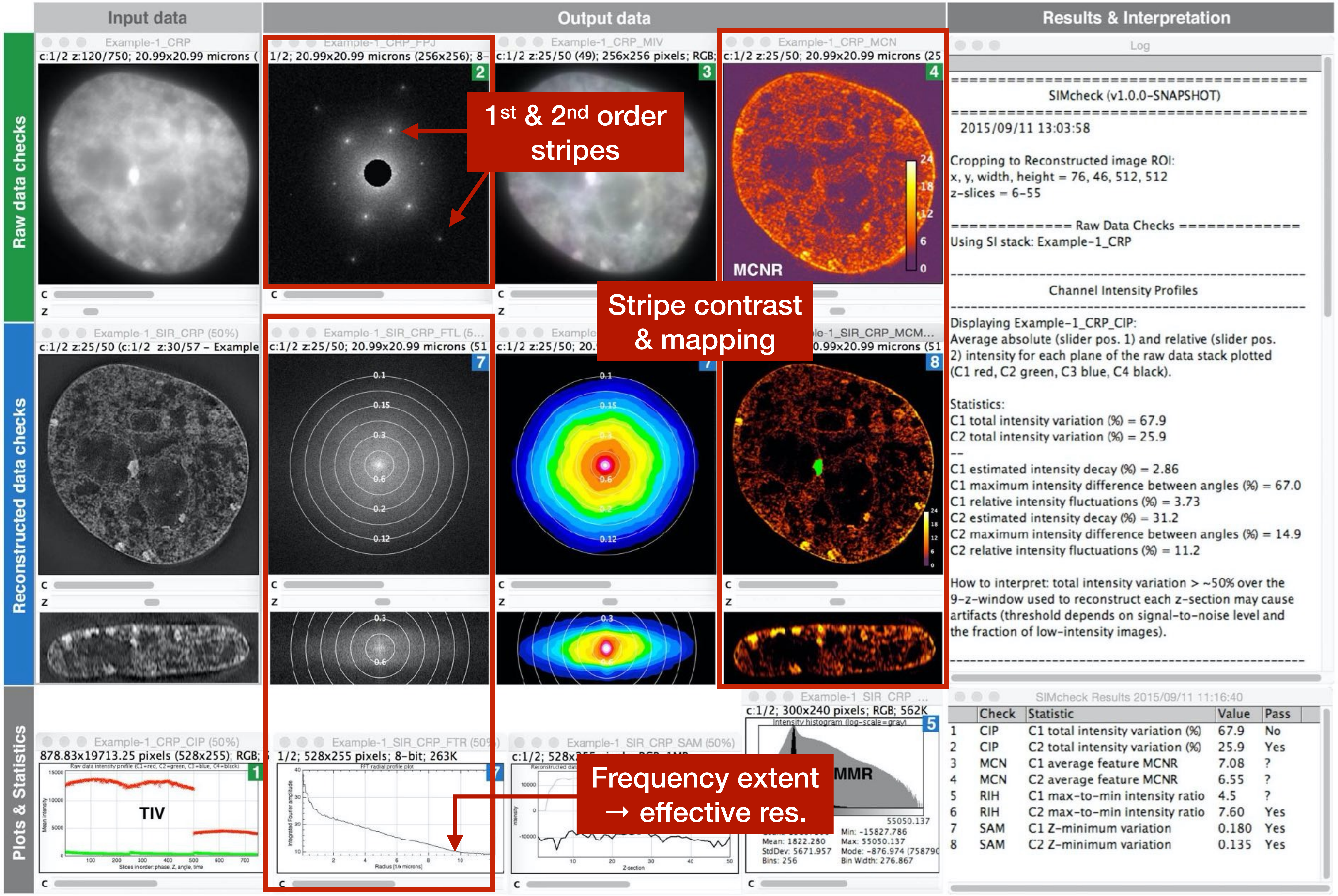
SI reconstruction artifacts



Demmerle et al. 2017, *Nat Protocols*

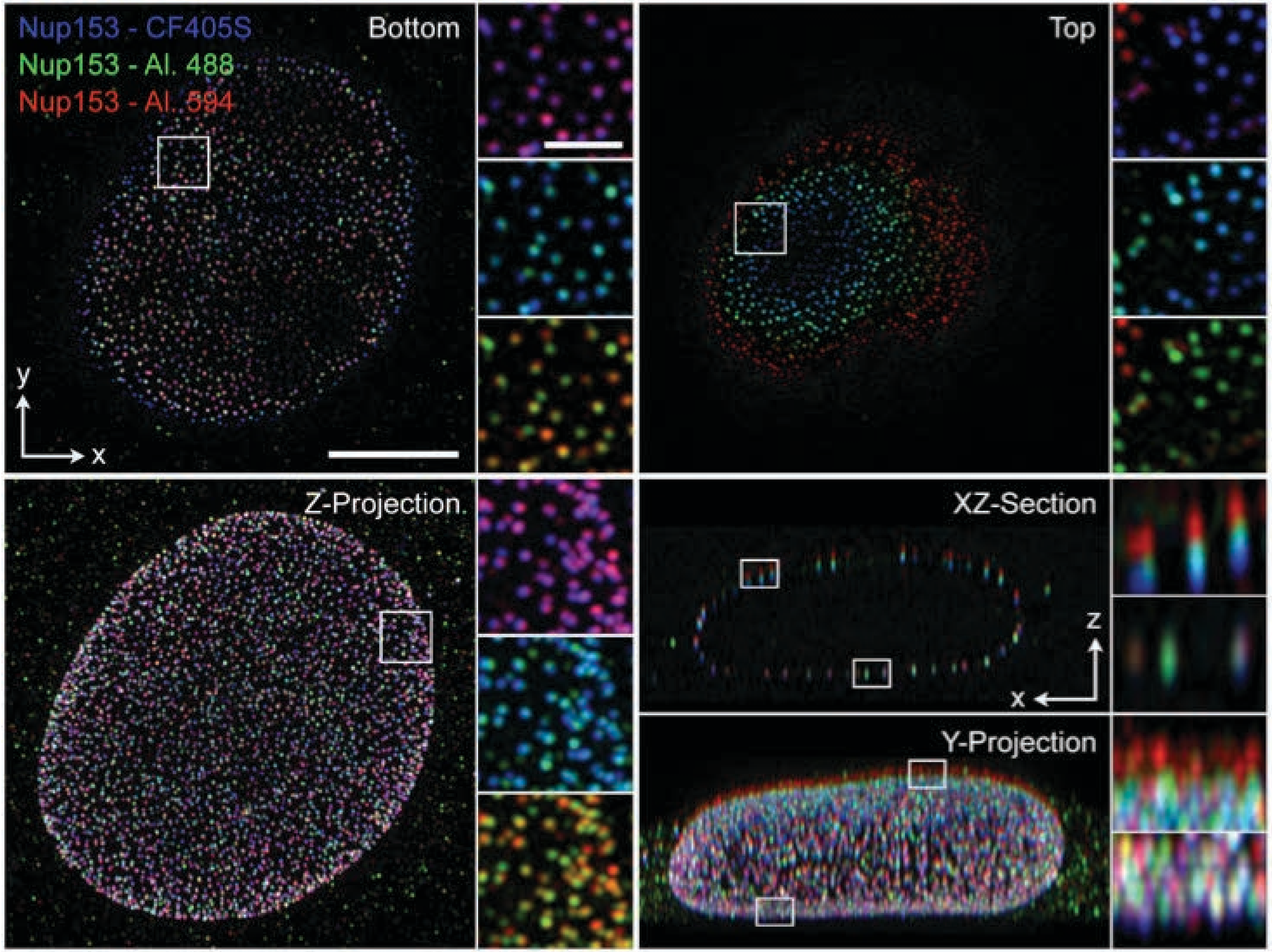
SIMcheck - Toolbox for Fiji/ImageJ

SIMcheck



Ball et al. 2015, *Sci Rep*

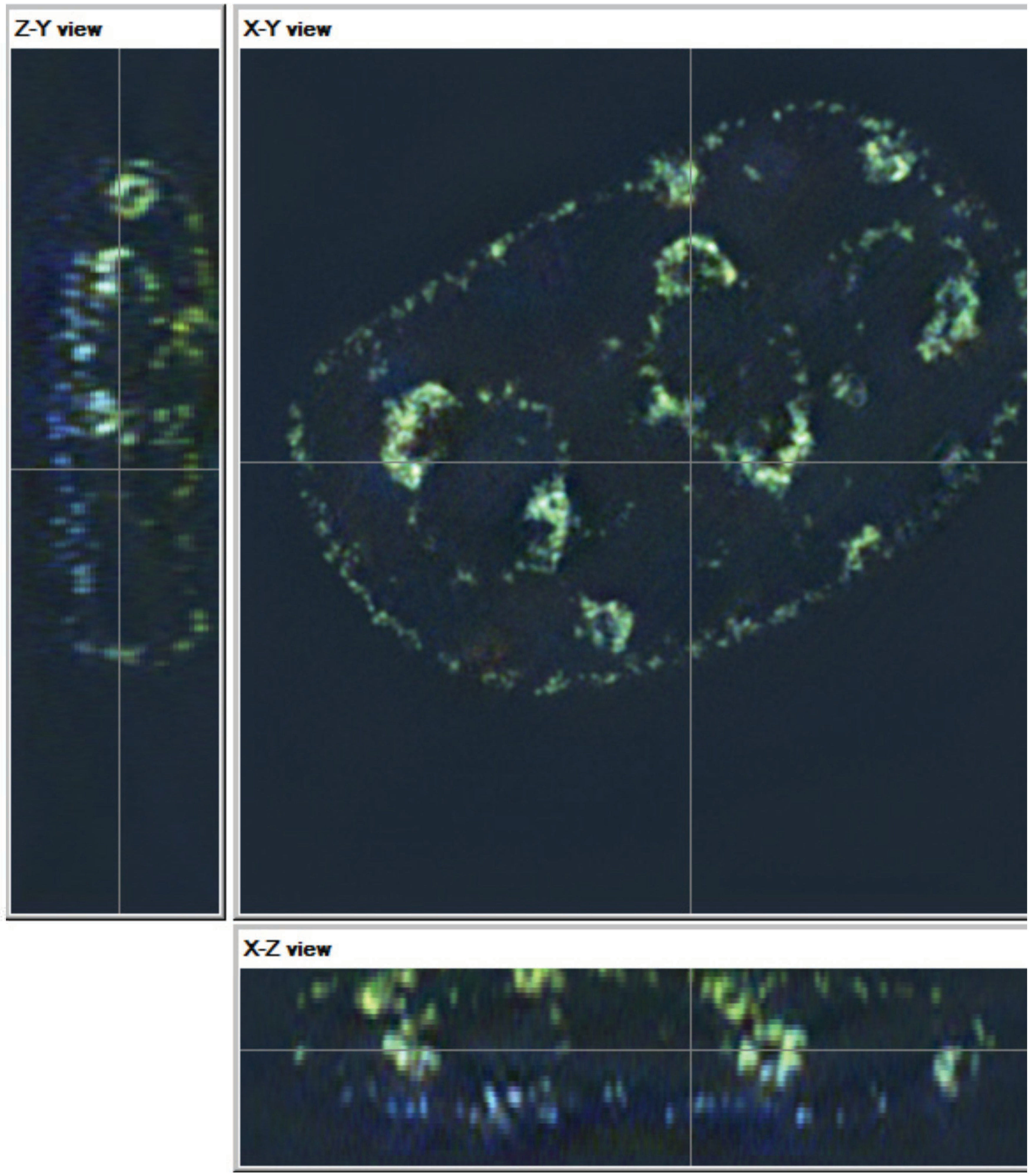
If you image 3D, register 3D!

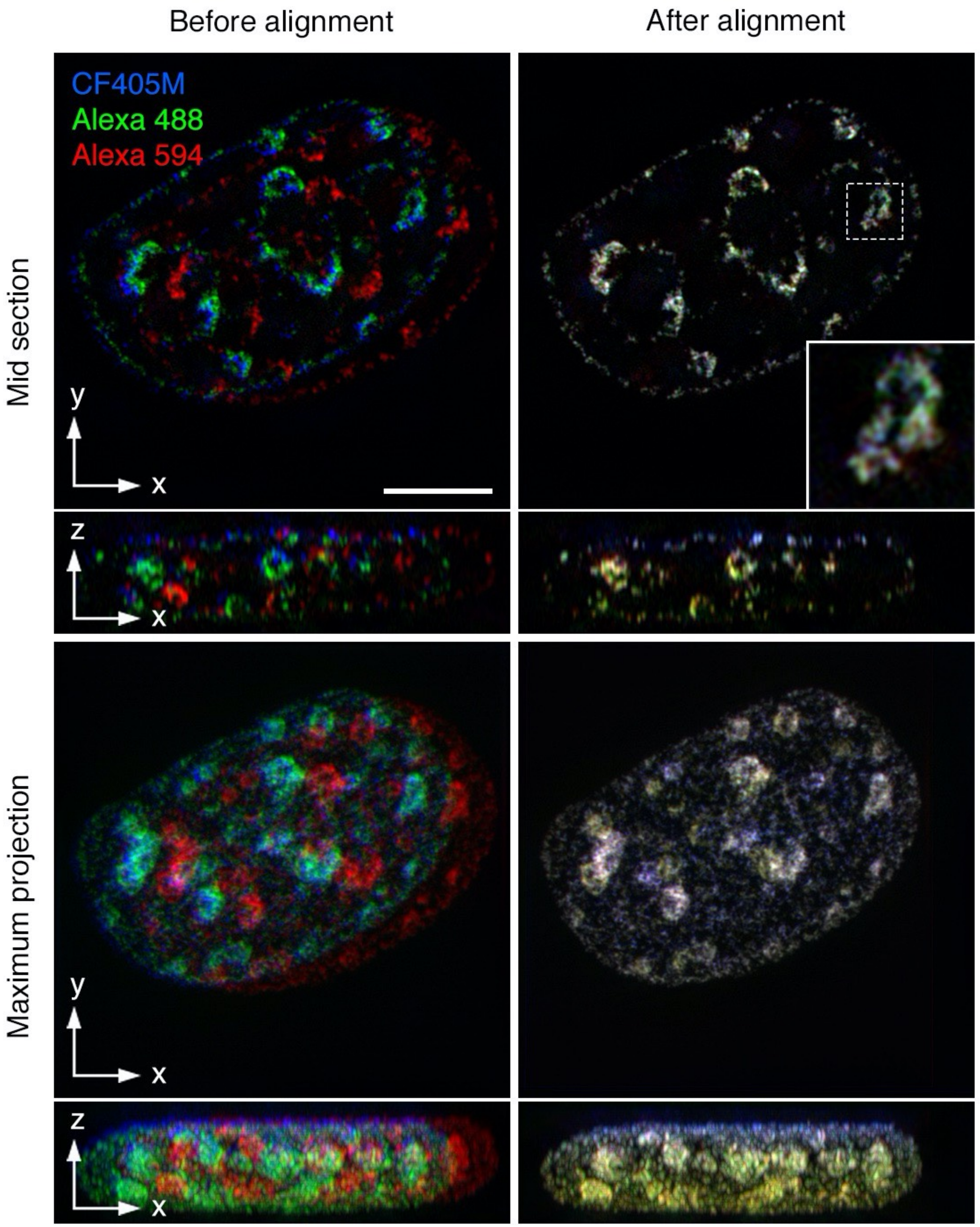


- Biological 3D calibration sample to determine alignment parameter
- Adjust z-shift to optimally match in the center of the sample

Smeets et al., 2014, *Neuromethods*

Channel registration in 3D using "biological" calibration slide





Kraus et al, 2017 NatProtocols

Golden rules (not only) for SIM

- Labeling **Specificity**
- Modulation **Contrast**
- Spherical **Aberration**
- Channel **Alignment**

Imaging workflow: quality is paramount!

Think 'holistically'!

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

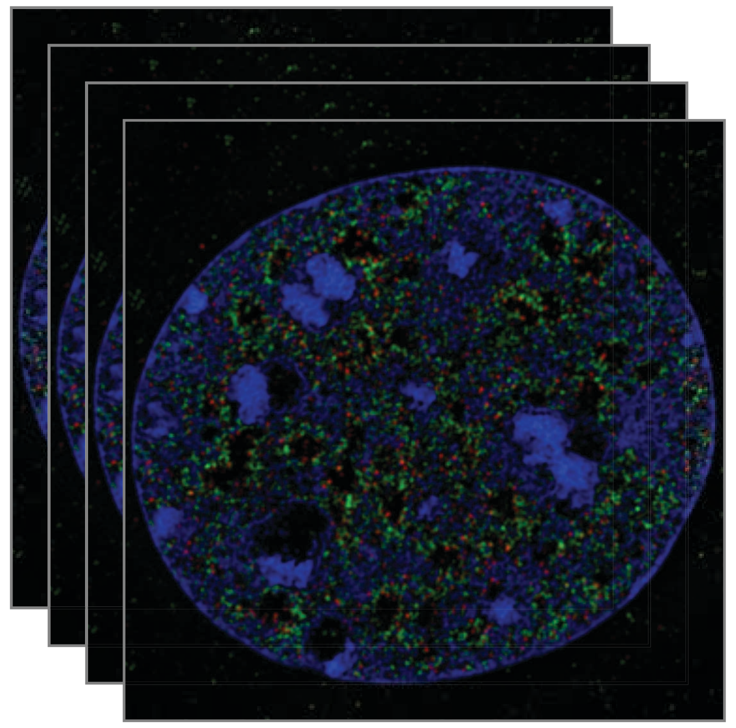
Microscope

- 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

Further reading: Tools and best practise protocols for SIM

PROTOCOL

Strategic and practical guidelines for successful structured illumination microscopy

Justin Demmerle[1,8], Cassandravictoria Innocent[1,8], Alison J North[2], Graeme Ball[1,7], Marcel Müller[3], Ezequiel Miron[1], Atsushi Matsuda[4,5], Ian M Dobbie[1], Yolanda Markaki[6] & Lothar Schermelleh[1]

NATURE PROTOCOLS | VOL.12 NO.5 | 2017

OPEN

SCIENTIFIC REPORTS | 5:15915 |

SIMcheck: a Toolbox for Successful Super-resolution Structured Illumination Microscopy

Received: 03 March 2015
Accepted: 01 October 2015
Published: 03 November 2015

Graeme Ball[1,†], Justin Demmerle[1], Rainer Kaufmann[1,2], Ilan Davis[1], Ian M. Dobbie[1] & Lothar Schermelleh[1]

PROTOCOL

Quantitative 3D structured illumination microscopy of nuclear structures

Felix Kraus[1,5], Ezequiel Miron[2], Justin Demmerle[2], Tsotne Chitiashvili[1,5], Alexei Budco[1], Quentin Alle[2], Atsushi Matsuda[3,4], Heinrich Leonhardt[1], Lothar Schermelleh[2] & Yolanda Markaki[1,5]

NATURE PROTOCOLS | VOL.12 NO.5 | 2017

OPEN

SCIENTIFIC REPORTS | (2018) 8:7583

Accurate and fiducial-marker-free correction for three-dimensional chromatic shift in biological fluorescence microscopy

Received: 11 May 2017
Accepted: 2 May 2018
Published online: 15 May 2018

Atsushi Matsuda[1,2], Lothar Schermelleh[3], Yasuhiro Hirano[2], Tokuko Haraguchi[1,2] & Yasushi Hiraoka[1,2]

REVIEW ARTICLE | FOCUS
https://doi.org/10.1038/s41556-018-0251-8
nature cell biology

Super-resolution microscopy demystified

Lothar Schermelleh[1*], Alexia Ferrand[2], Thomas Huser[3], Christian Eggeling[4,5], Markus Sauer[6], Oliver Biehlmaier[2] and Gregor P. C. Drummen[7,8*]

jove

J. Vis. Exp. (160), e60800, doi:10.3791/60800 (2020).

High-Accuracy Correction of 3D Chromatic Shifts in the Age of Super-Resolution Biological Imaging Using *Chromagnon*

Atsushi Matsuda[1,2], Takako Koujin[1], Lothar Schermelleh[3], Tokuko Haraguchi[1,2], Yasushi Hiraoka[1,2]

[1] Advanced ICT Research Institute Kobe, National Institute of Information and Communications Technology [2] Graduate School of Frontier Biosciences, Osaka University [3] Micron Advanced Bioimaging Unit, Department of Biochemistry, University of Oxford