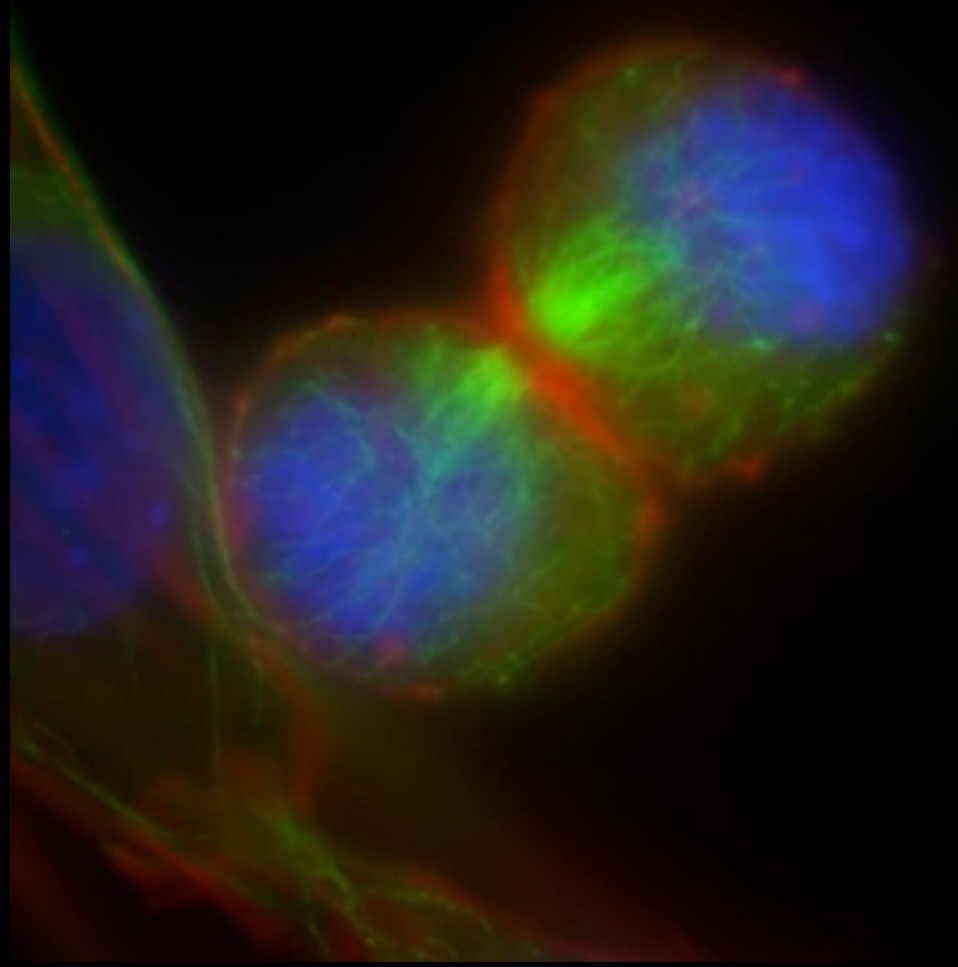
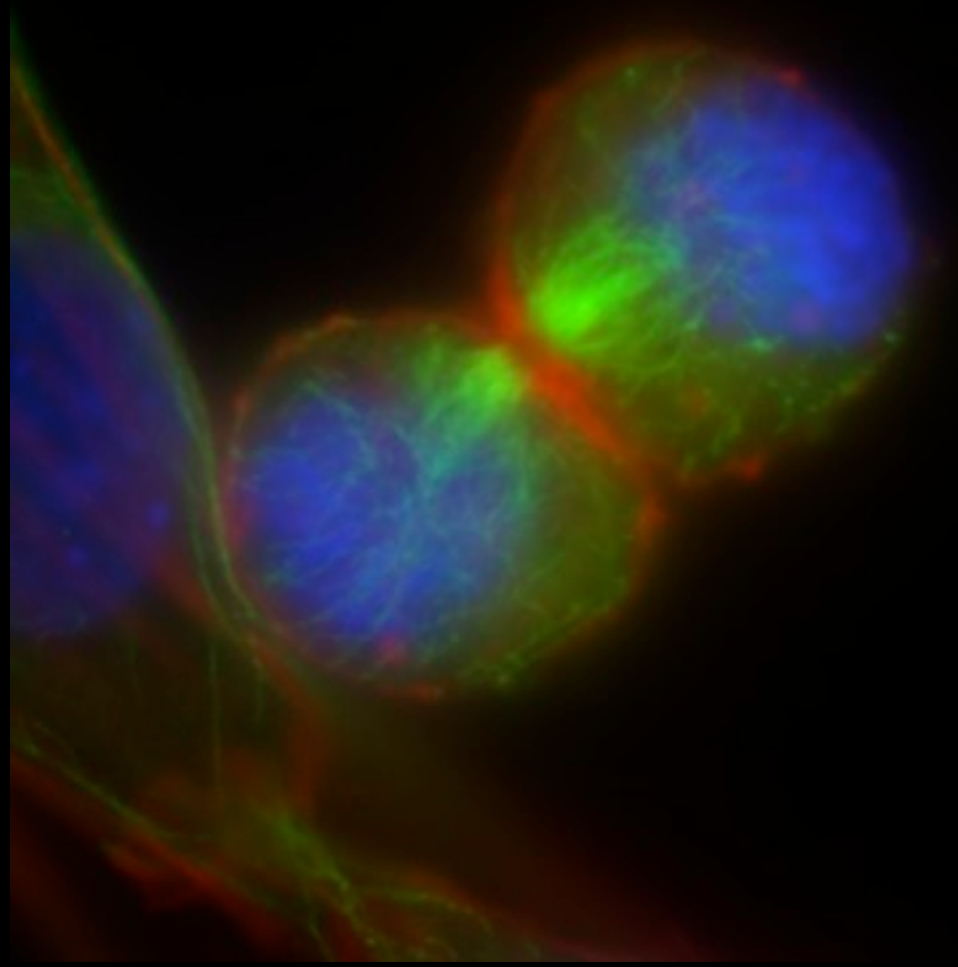
Lecture 13: Super-resolution microscopy



lothar.schermelleh@bioch.ox.ac.uk

Typical widefield image...

Lecture 13: Super-resolution microscopy



Optical resolution is diffraction limited!

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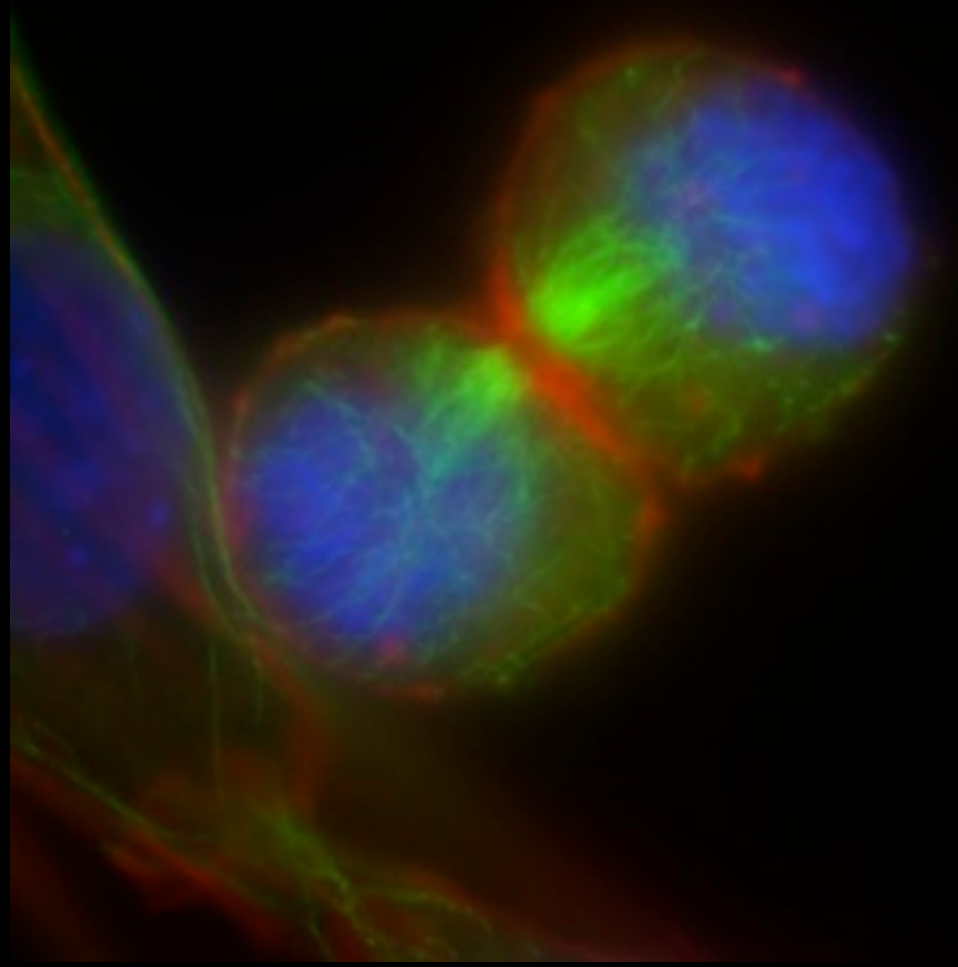
Typical widefield image...

Lecture 13: Super-resolution microscopy



Optical resolution is diffraction limited!

Magnification alone does not give more details!

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Typical widefield image...

Lecture 13: Super-resolution microscopy

Optical resolution is diffraction limited!

Magnification alone does not give more details!

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Typical widefield image...

... a little reminder:

"What determines the resolution of an optical microscope ?"

63x/1.25

100x/1.25

63x/1.4

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

... a little reminder:

"What determines the resolution of an optical microscope ?"

63x/1.25

€ 5.557,56

100x/1.25

€ 693,68

63x/1.4

€ 6.370,72

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

„... a bit more difficult...?“

25x/1.05

40x/1.0

40x/1.1

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

„... a bit more difficult...?“

25x/1.05

40x/1.0

40x/1.1

What's the difference in brightness ?

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

„... a bit more difficult...?“

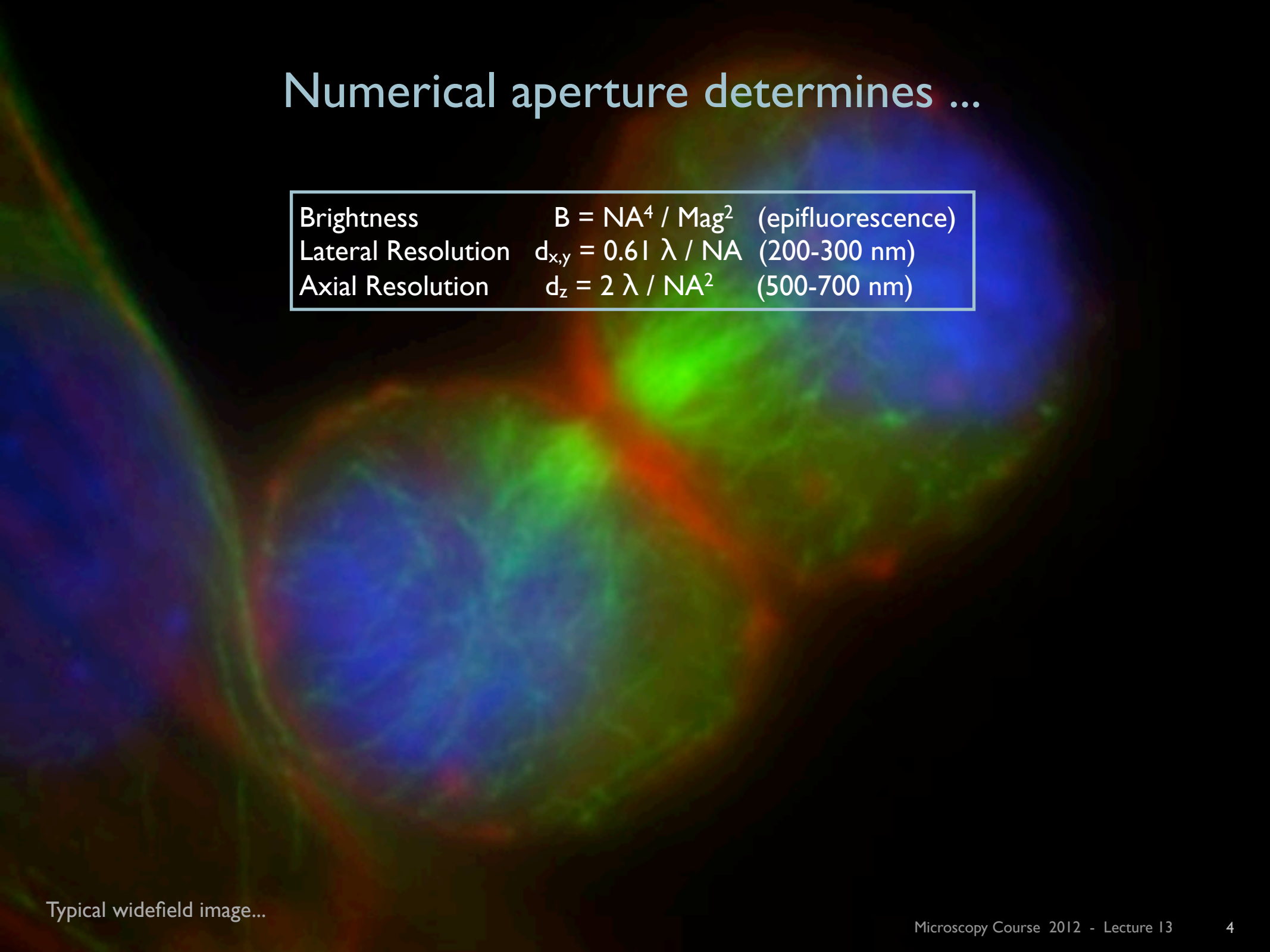
25x/1.05	40x/1.0	40x/1.1
€ 15.000,00	€ 3.786	€ 11.110,48

What's the difference in brightness ?

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

Numerical aperture determines ...

Brightness	$B = NA^4 / Mag^2$	(epifluorescence)
Lateral Resolution	$d_{x,y} = 0.61\ \lambda / NA$	(200-300 nm)
Axial Resolution	$d_z = 2\ \lambda / NA^2$	(500-700 nm)



Typical widefield image...

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Only applies under optimal conditions! BUT ...

spherical aberrations
chromatic aberrations
straylight
out-of-focus blur
noise
sample
...
Effective resolution is worse!
(max. 250 nm lateral and ≤ 1 µm axial)

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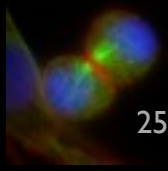
chromatic aberrations
straylight
out-of-focus blur
noise
sample
...
Effective resolution is worse!
(max. 250 nm lateral and ≤ 1 µm axial)

...improved to some extent by Confocal LSM or Deconvolution

Typical widefield image...

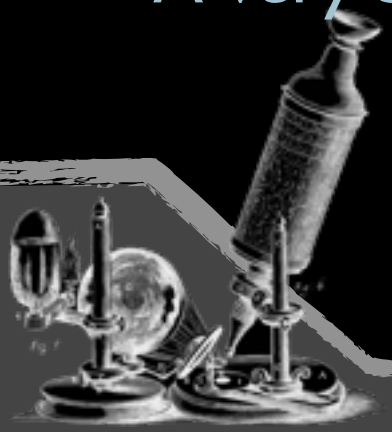
A very brief history of optical microscopy



1 µm

500 nm

250 nm



1600 1700 1800 1900 2000

R. Hooke

Z. Jansen

C. Huygens

Van Leeuwenhoek

Galileo Galilei

E. Abbe

A. Köhler

M. Minsky

F. Zernike

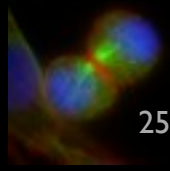
Abbe limit:

$$d_{xy} \approx \frac{\lambda_{em}}{2n \sin \alpha}$$

Super-resolution microscopy „breaks“ the limit

1 µm

500 nm



250 nm

65 nm

32 nm

16 nm

Abbe limit:

$$d_{xy} \approx \frac{\lambda_{em}}{2n \sin \alpha}$$

1600 1700 1800 1900 2000

R. Hooke

Z. Jansen

C. Huygens

C. M. Hall

Van Leeuwenhoek

Galileo Galilei

E. Abbe

A. Köhler

M. Minsky

F. Zernike

1991 / 1994 / 1999

1997

2006

SIM

J. Sedat
M. Gustafsson
D. Agard

STED

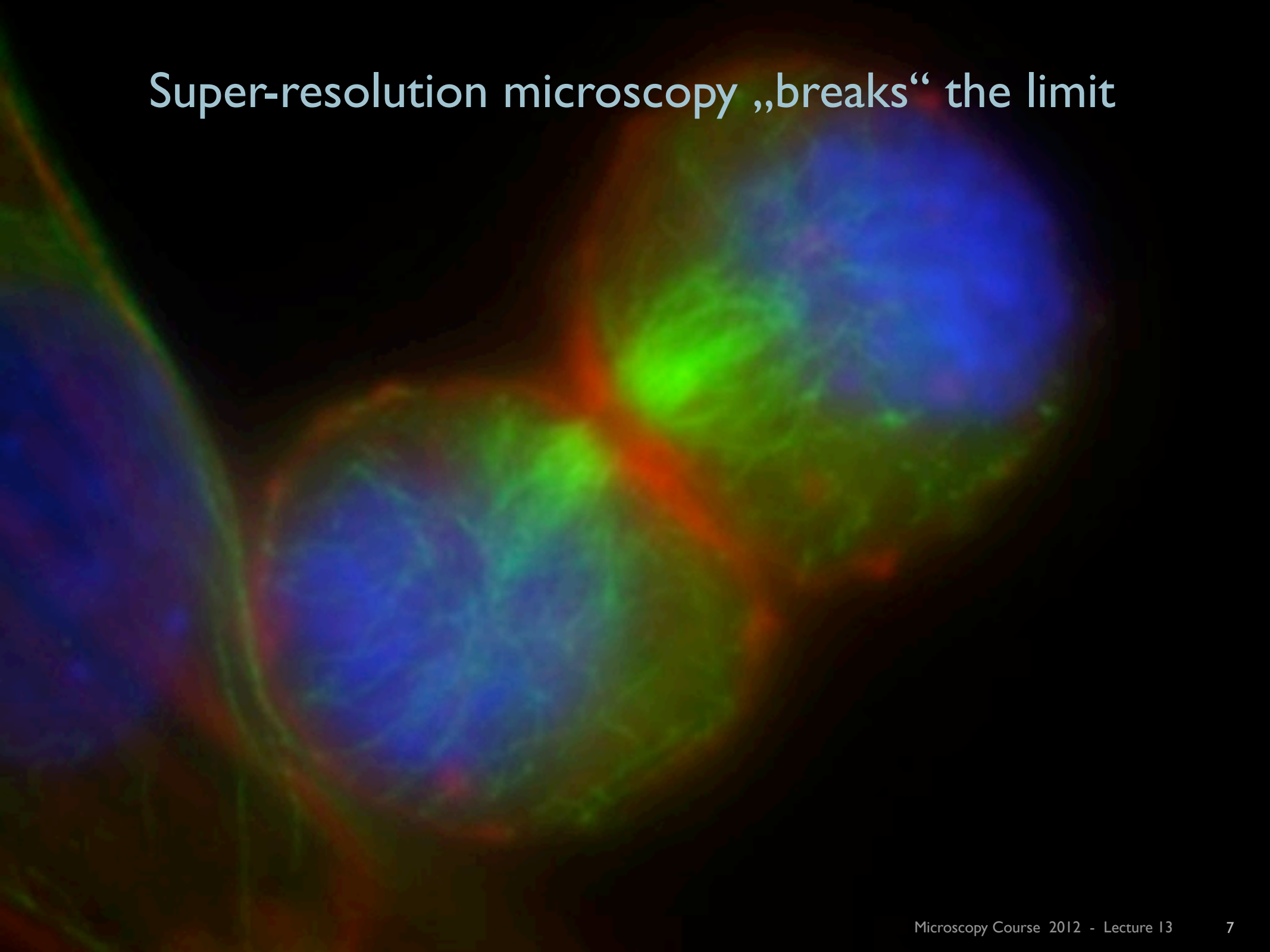
S. Hell

E. Betzig
S.T. Hess
X. Zhuang

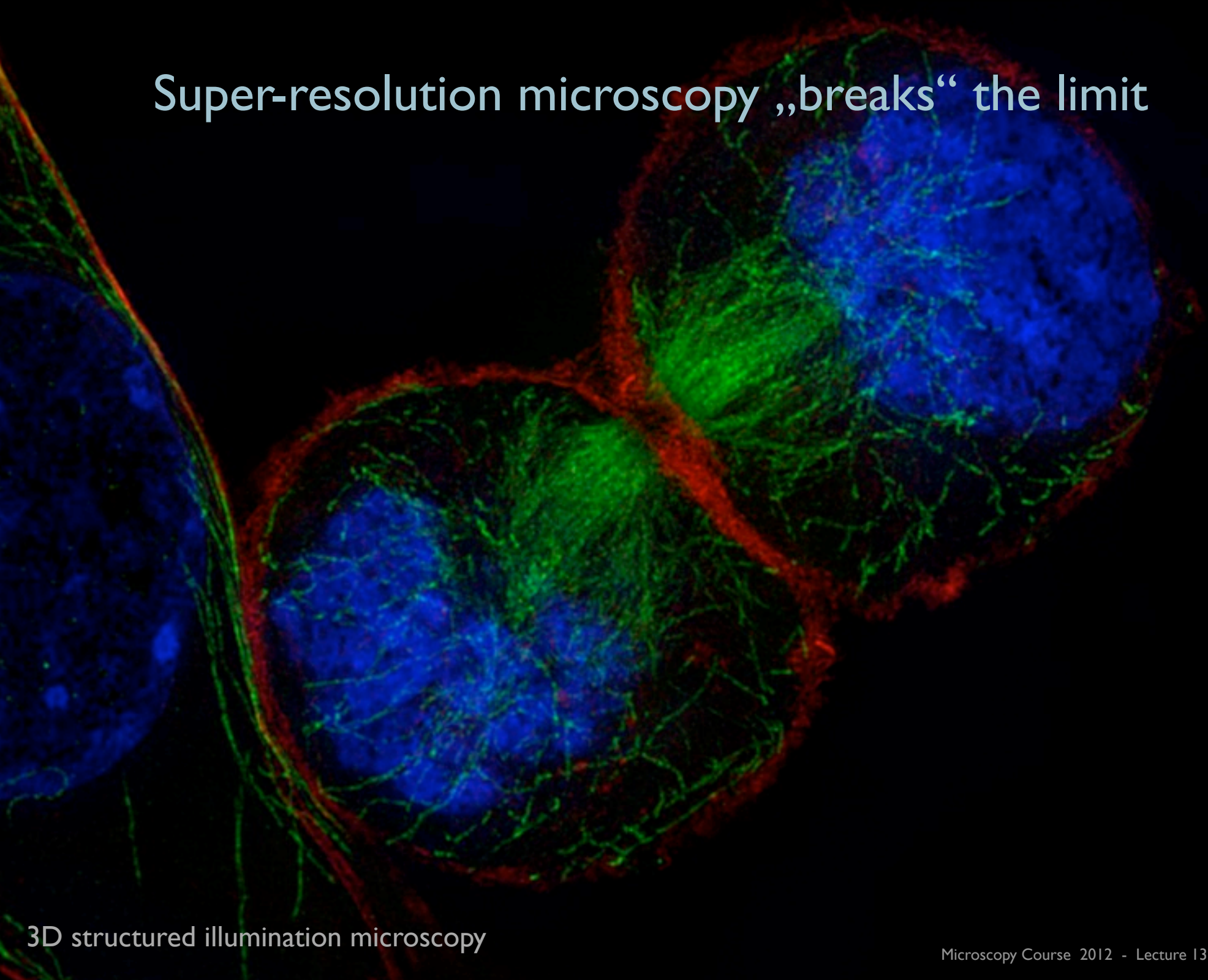
PALM
STORM

J. Neumann

Super-resolution microscopy „breaks“ the limit



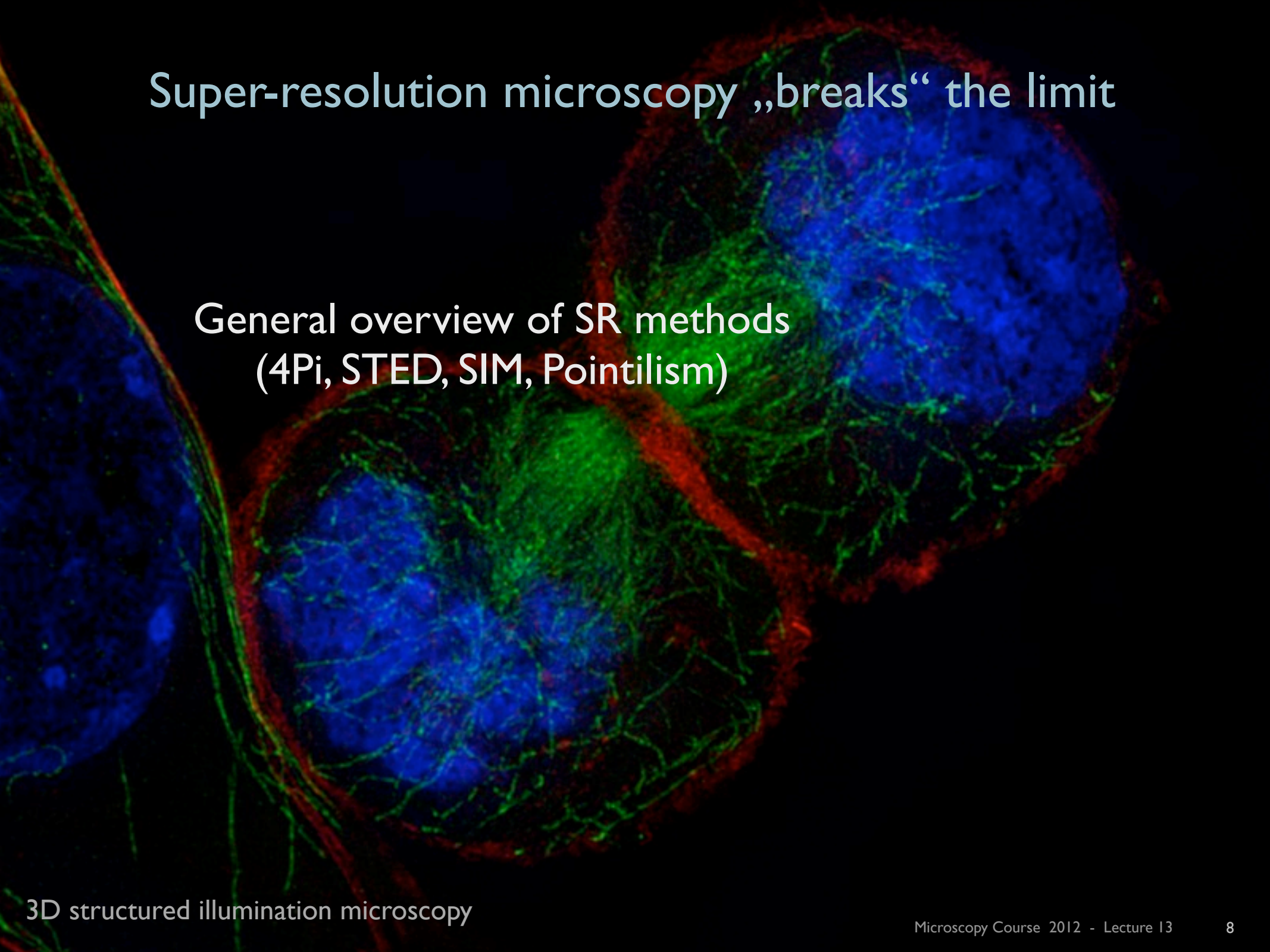
Super-resolution microscopy „breaks“ the limit



3D structured illumination microscopy

Super-resolution microscopy „breaks“ the limit

General overview of SR methods
(4Pi, STED, SIM, Pointilism)



3D structured illumination microscopy

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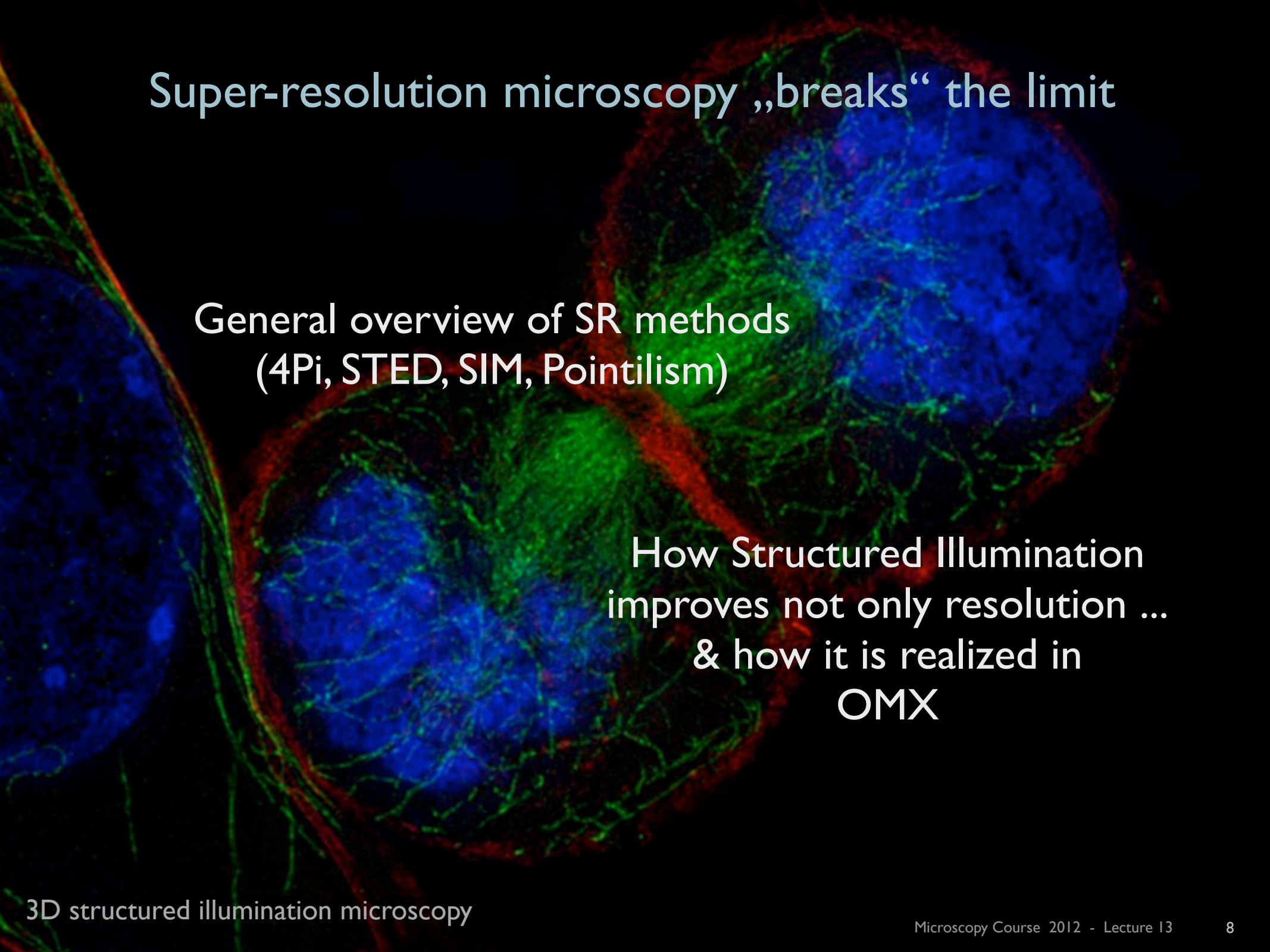

General overview of SR methods
(4Pi, STED, SIM, Pointilism)

How Structured Illumination
improves not only resolution ...
& how it is realized in
OMX

3D structured illumination microscopy

Optical super-resolution microscopy methods

	Near-field		Far-field					
Principle	Small aperture scanning (no lens)	Evanescent wave illumination	Wide-field + deconvolution	Confocal laser scanning	Moiré effect with structured illumination		PSF shaping with saturated emission depletion	Photoswitching and localization of single molecules (pointillism)
Acronym	**SNOM/NSOM**	**TIRFM**		**CLSM**	**SIM** (HELM, PEM) **3D-SIM**	**SSIM** (SPEM)	**STED/CW-STED**	**PALM/FPALM/STORM/ dSTORM/PALMIRA**
Illumination-emission dependence	Linear	Linear	Linear	Linear	Linear	Non-linear	Non-linear	Linear
Detector	Scanning PMT/APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/ APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/APD	Wide-field CCD/CMOS
XY-resolution	20–120 nm	200–300 nm	180–250 nm	180–250 nm	100–130 nm	50 nm	20–100 nm	20–50 nm
Z-resolution	10 nm (near-field range)	100 nm (near-field range)	500–700 nm	500–700 nm	250–350 nm	N.D.	560 nm (CW-STED) to 700 nm (100 nm with z-phase mask)	100 nm (TIRF) 20–30 nm (3D-STORM, TIRF) 75 nm (BP-FPALM, in plane)
Serial z-sectioning	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Z stack range	N.A.	N.A.	100 µm	100 µm	10–20 µm	N.A.	>20 µm	100 nm – few µm (BP-FPALM)
Dyes	Any	Any	Any	Any	Most conventional dyes (photostable)	Dyes require special characteristics	Dyes require special characteristics (CW-STED works with many conventional dyes)	Dyes require special characteristics
Simultaneous colors	2	3	>3	>3	3	1	2	2
Temporal resolution for 512 × 512 image	s-min	ms	ms	ms-s	ms-s	s-min	ms-min	s-min
Energy load/ intensity	Low	Low	Low	Medium	Medium	High	Medium-high	Medium-high
Live-cell imaging	Yes	Yes	Yes	Yes	Restricted (2D-TIRF)	No	Restricted	Restricted
Postprocessing required	No	No	Yes	No	Yes 9–25 raw images per slice	Yes ~100 raw images per slice	No	Yes >1,000 raw images per slice
Notes	No intracellular imaging	Restricted to region near the coverslip	Risk of artifacts; better for sparse samples		Reconstruction bears risk of artefacts	High excitation required; reconstruction bears risk of artefacts	Complex instrumentation; photobleaching	May require TIRF setup for best performance; labeling density is critical; performs better on particles and filaments as on volume stains
Dual lens implementation			**I^{5}M**	**4Pi**	**I^{5}S**		**4 Pi-STED/iso-STED**	**iPALM**
Z-resolution			70 nm	80 nm	100 nm		20–100 nm	10 nm (depth ~200 nm)

Schermelleh et al., JCB (2010)

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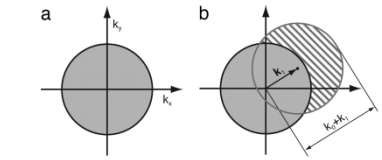
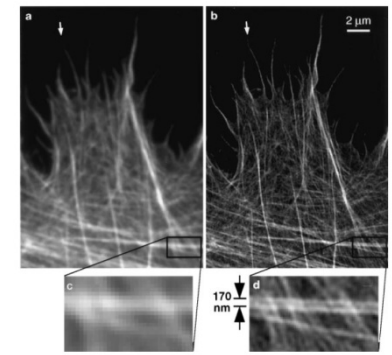
„Gold standard“

Super-resolution light microscopy: Imaging beyond Abbe's diffraction limit

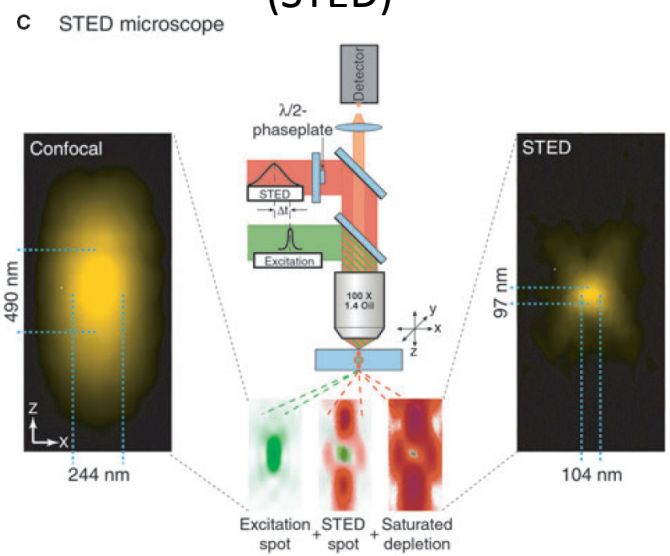
$$\Delta x, \Delta y = \frac{\lambda}{2n \sin \alpha}$$

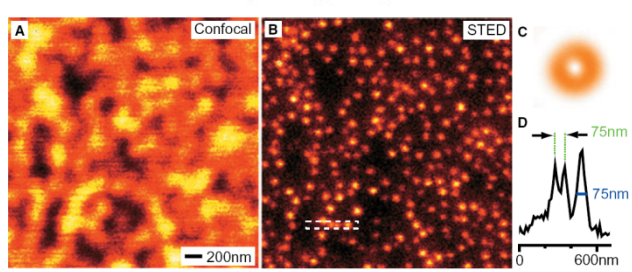
Structured illumination





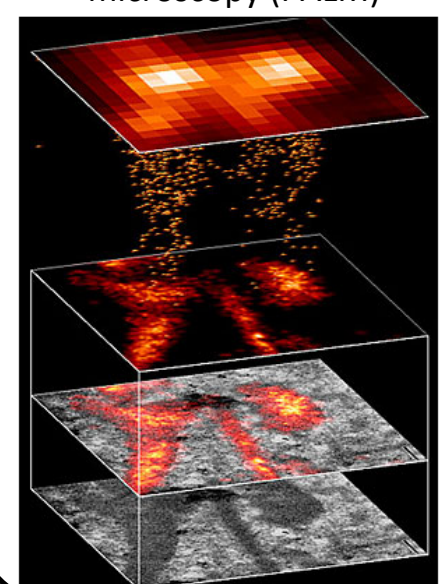
Stimulated emission depletion (STED)



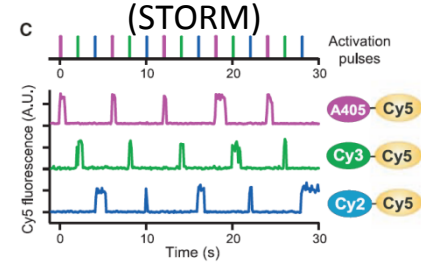


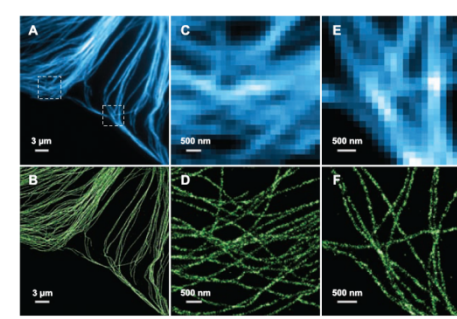
Localization microscopy (Pointilism)

Photoactivation localization microscopy (PALM)



Stochastic optical reconstruction microscopy (STORM)





Milestones in super-resolution microscopy

1873: Resolution limit, E Abbe

⋮

1992: 4Pi Microscopy, SW Hell, E Stelzer

(1994: STED Microscopy, SW Hell)

1995: I5M Microscopy, M Gustaffson, J Sedat

1998: Diffraction grating, R Heintzmann, C Cremer

2000: STED Microscopy, SW Hell

2000: Structured illumination, M Gustaffson

2006: PALM, E. Betzig, H. Hess

2006: FPALM, S. Hess

2006: STORM, X. Zhuang

2008: 3D SIM, M Gustaffson, J Sedat

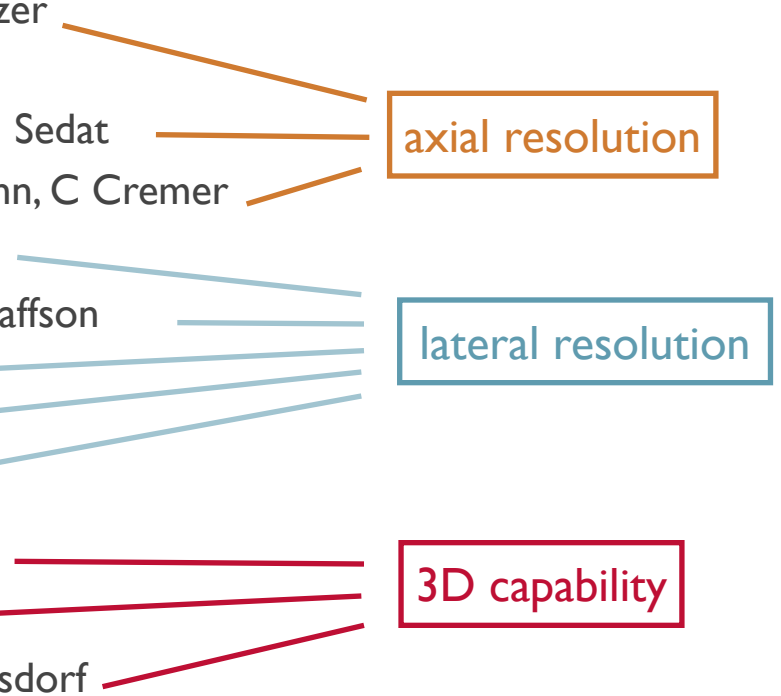
2008: 3D STORM, X. Zhuang

2008: Biplane FPALM, S Hess, J Bewersdorf

2008: Nature Methods: Method of the year

2009: 21st Nature Milestone for light microscopy

⋮



2003: Leica 4Pi (2P)

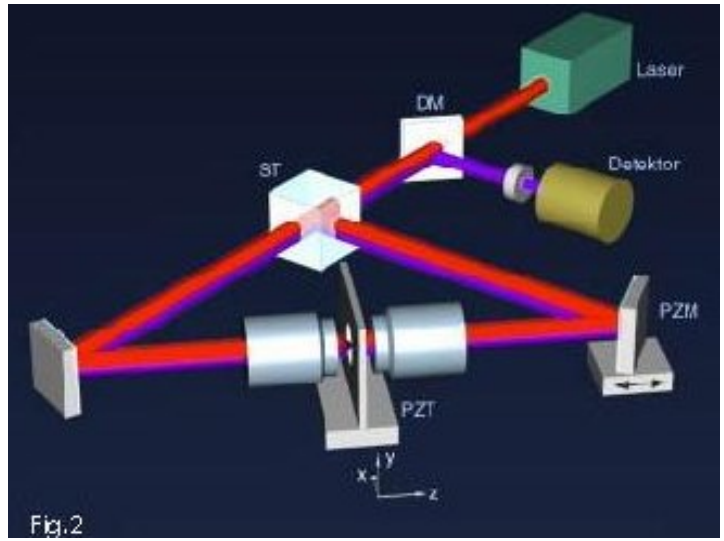
2006: Leica TCS STED

2009: DeltaVision OMX 3D-SIM
2009: Zeiss Elyra PALM/SIM
2009: Leica CW STED
2010: Nikon N-SIM/N-STORM
2012: OMX Blaze

4Pi Microscopy

(S. Hell)

- Phase and wave front corrected interferometer to generate counter propagating coherent wavefronts
- Illumination and imaging from both sides,
- Confocal/multiphoton system for image acquisition



4Pi Microscopy

(S. Hell)

- Phase and wave front corrected interferometer to generate counter propagating coherent wavefronts
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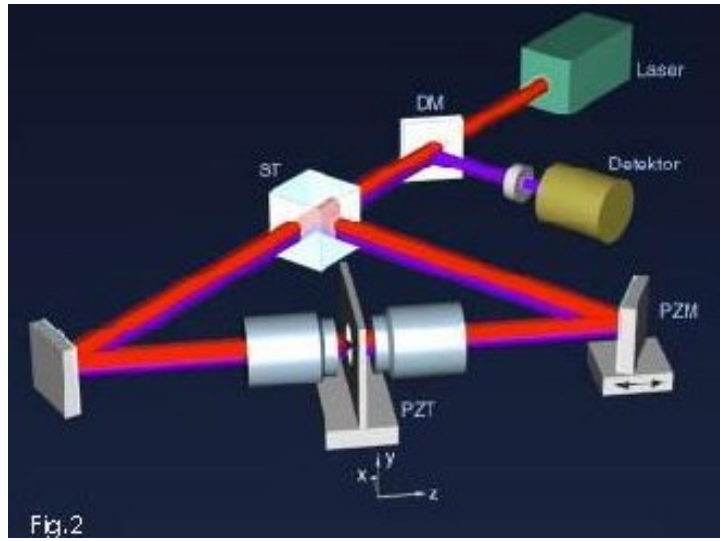
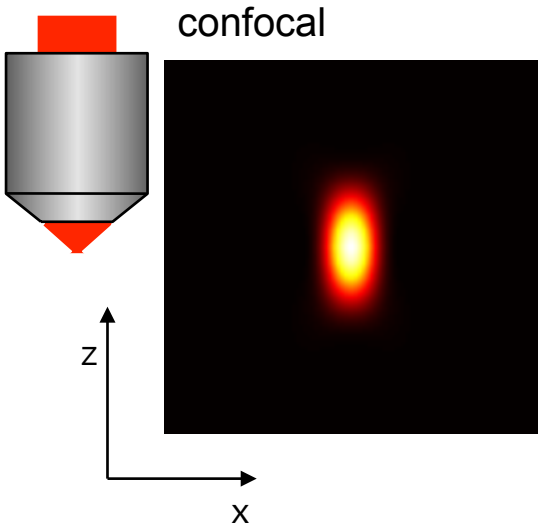
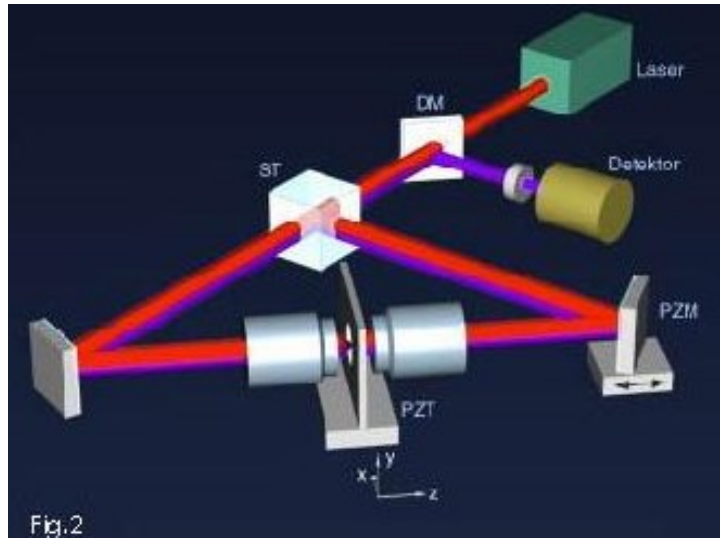




4Pi Microscopy

(S. Hell)



- Phase and wave front corrected interferometer to generate counter propagating coherent wavefronts
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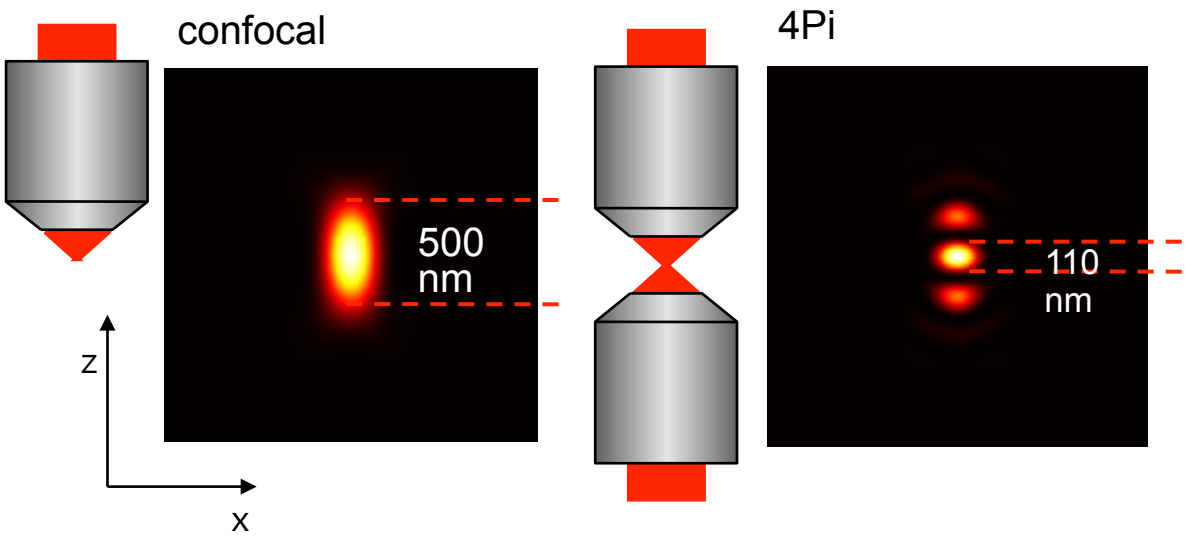

4Pi Microscopy

(S. Hell)

- Phase and wave front corrected interferometer to generate counter propagating coherent wavefronts
- Illumination and imaging from both sides,
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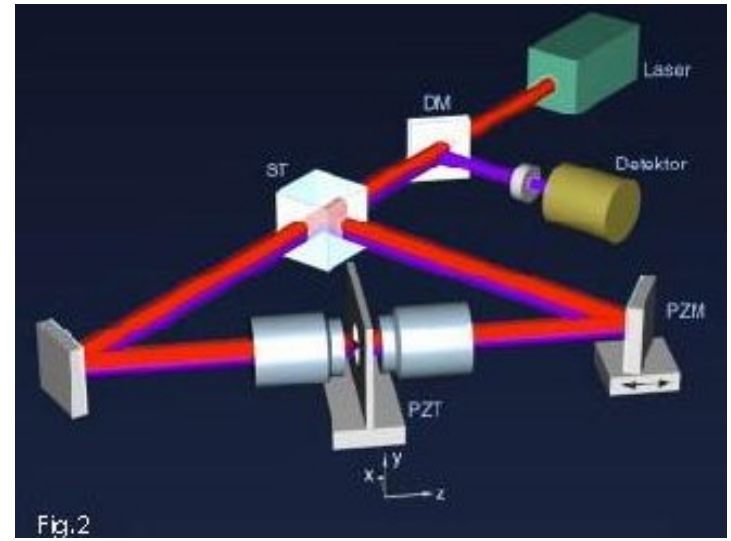
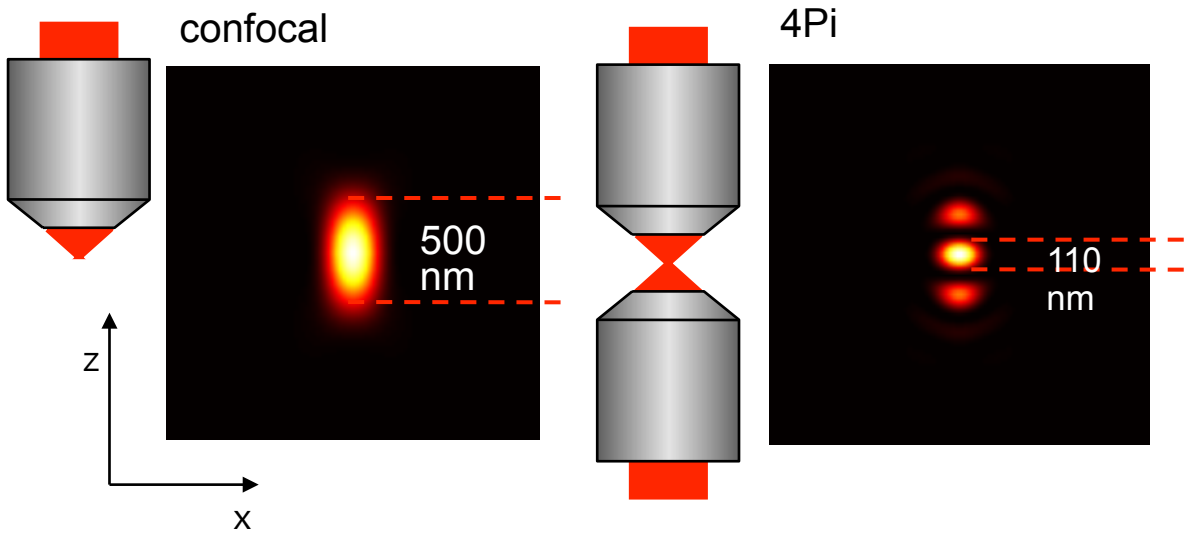


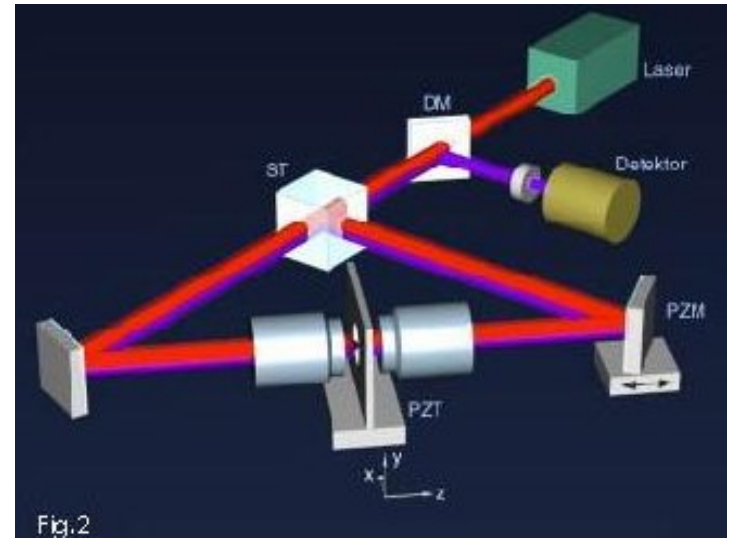


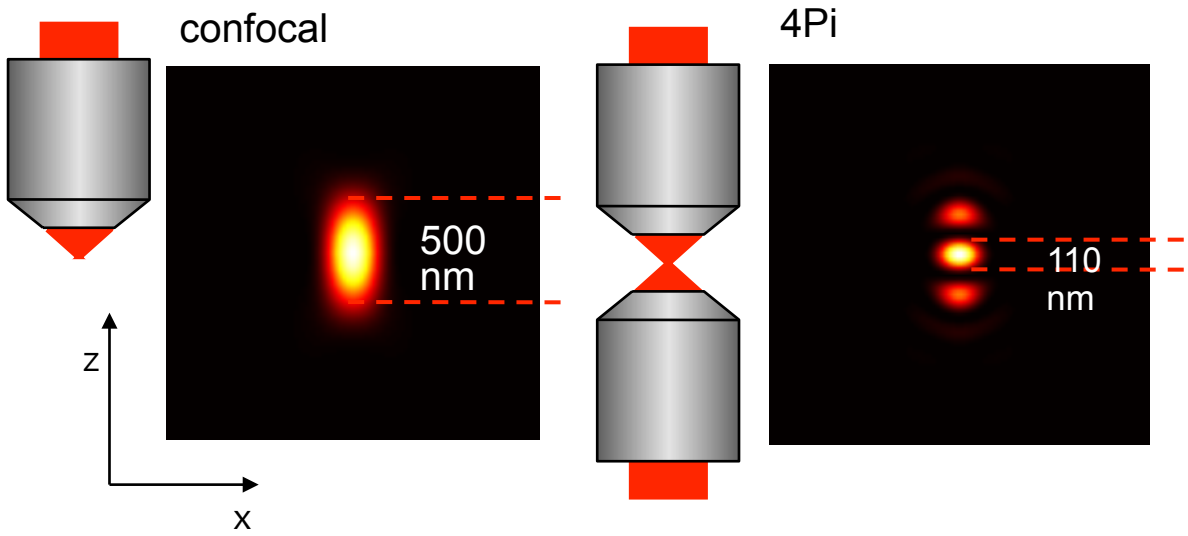
- constructive interference of excitation and emission light
- 5-8 times higher **axial resolution**: 110nm resolution @780 nm
- Side lobes can be eliminated by deconvolution

4Pi Microscopy

(S. Hell)

- Phase and wave front corrected interferometer to generate counter propagating coherent wavefronts
- Illumination and imaging from both sides,
- Confocal/multiphoton system for image acquisition





- constructive interference of excitation and emission light
- 5-8 times higher **axial resolution**: 110nm resolution @780 nm
- Side lobes can be eliminated by deconvolution

Problem: special sample requirements and need for physicist(s) to operate

Optical super-resolution microscopy methods

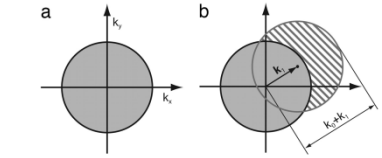
Principle	Near-field		Far-field					
	Small aperture scanning (no lens)	Evanescent wave illumination	Wide-field + deconvolution	Confocal laser scanning	Moiré effect with structured illumination		PSF shaping with saturated emission depletion	Photoswitching and localization of single molecules (pointillism)
Acronym	**SNOM/NSOM**	**TIRFM**		**CLSM**	**SIM** (HELM, PEM) **3D-SIM**	**SSIM** (SPEM)	**STED/CW-STED**	**PALM/FPALM/STORM/ dSTORM/PALMIRA**
Illumination-emission dependence	Linear	Linear	Linear	Linear	Linear	Non-linear	Non-linear	Linear
Detector	Scanning PMT/APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/ APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/APD	Wide-field CCD/CMOS
XY-resolution	20–120 nm	200–300 nm	180–250 nm	180–250 nm	100–130 nm	50 nm	20–100 nm	20–50 nm
Z-resolution	10 nm (near-field range)	100 nm (near-field range)	500–700 nm	500–700 nm	250–350 nm	N.D.	560 nm (CW-STED) to 700 nm (100 nm with z-phase mask)	100 nm (TIRF) 20–30 nm (3D-STORM, TIRF) 75 nm (BP-FPALM, in plane)
Serial z-sectioning	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Z stack range	N.A.	N.A.	100 µm	100 µm	10–20 µm	N.A.	>20 µm	100 nm – few µm (BP-FPALM)
Dyes	Any	Any	Any	Any	Most conventional dyes (photostable)	Dyes require special characteristics	Dyes require special characteristics (CW-STED works with many conventional dyes)	Dyes require special characteristics
Simultaneous colors	2	3	>3	>3	3	1	2	2
Temporal resolution for 512 × 512 image	s-min	ms	ms	ms-s	ms-s	s-min	ms-min	s-min
Energy load/ intensity	Low	Low	Low	Medium	Medium	High	Medium-high	Medium-high
Live-cell imaging	Yes	Yes	Yes	Yes	Restricted (2D-TIRF)	No	Restricted	Restricted
Postprocessing required	No	No	Yes	No	Yes 9–25 raw images per slice	Yes ~100 raw images per slice	No	Yes >1,000 raw images per slice
Notes	No intracellular imaging	Restricted to region near the coverslip	Risk of artifacts; better for sparse samples		Reconstruction bears risk of artefacts	High excitation required; reconstruction bears risk of artefacts	Complex instrumentation; photobleaching	May require TIRF setup for best performance; labeling density is critical; performs better on particles and filaments as on volume stains
Dual lens implementation			**I^{5}M**	**4Pi**	**I^{5}S**		**4 Pi-STED/iso-STED**	**iPALM**
Z-resolution			70 nm	80 nm	100 nm		20–100 nm	10 nm (depth ~200 nm)

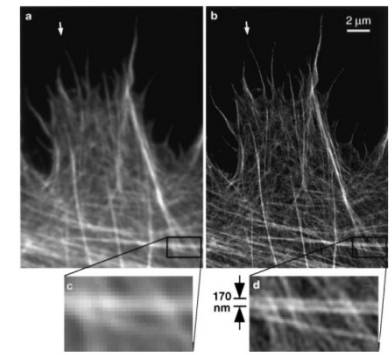
Schermelleh et al., JCB (2010)

Super-resolution light microscopy: Imaging beyond Abbe's diffraction limit

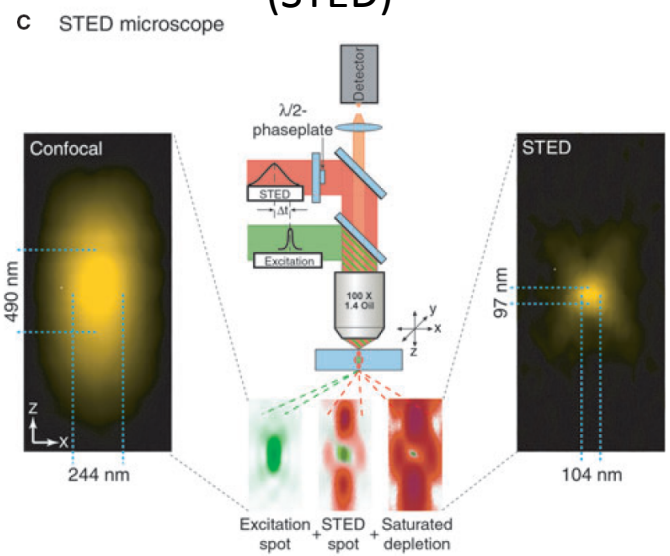
$$\Delta x, \Delta y = \frac{\lambda}{2n \sin \alpha}$$

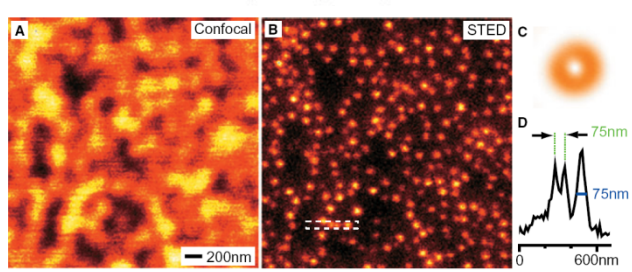
Structured illumination





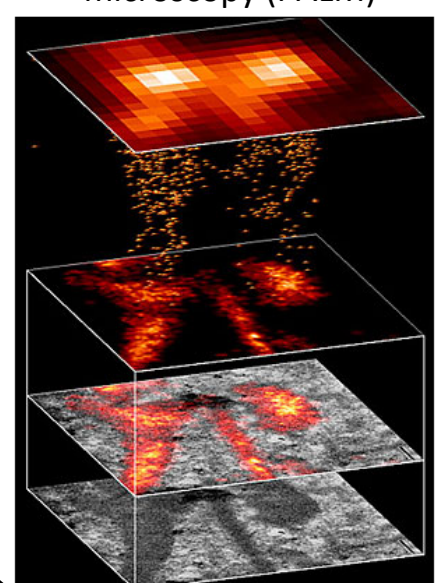
Stimulated emission depletion (STED)



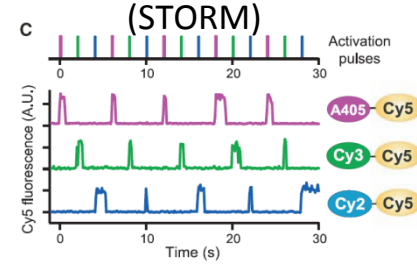


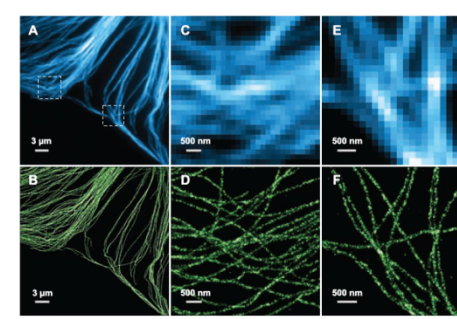
Localization microscopy (Pointilism)

Photoactivation localization microscopy (PALM)

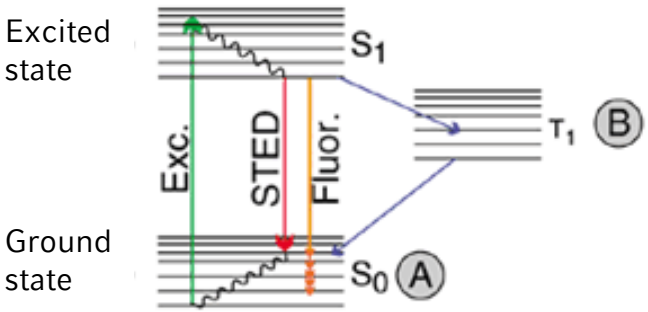


Stochastic optical reconstruction microscopy (STORM)

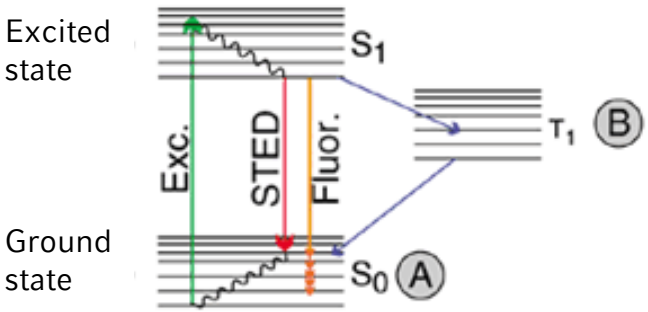




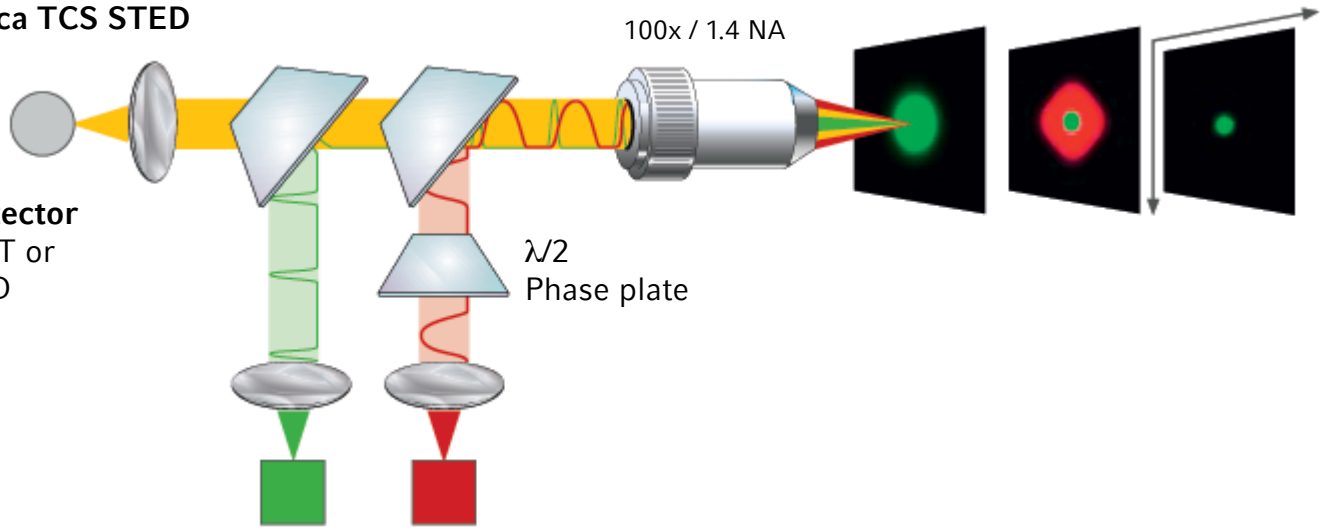
STED microscopy



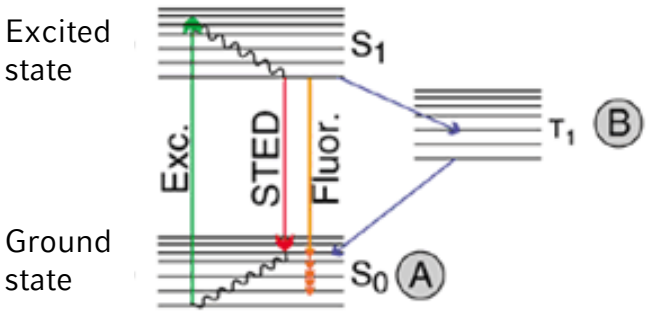
STED microscopy

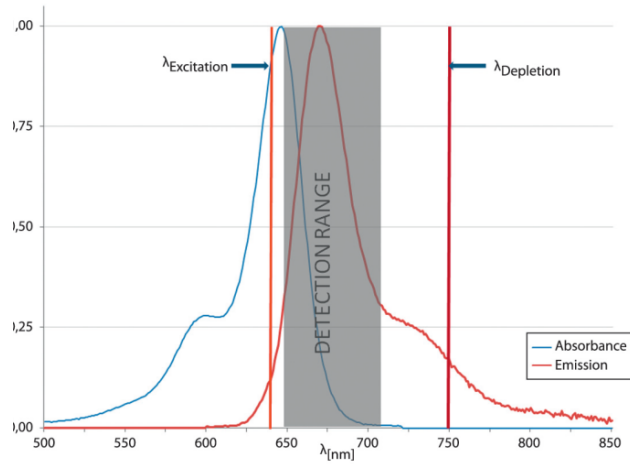


Leica TCS STED



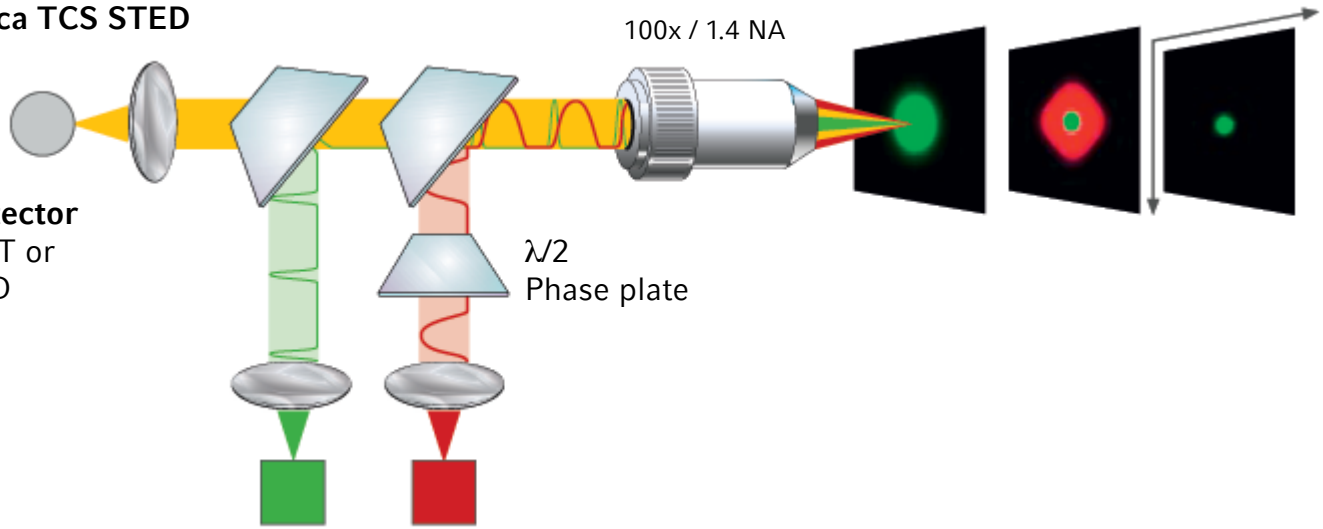
STED microscopy





Best dyes:
ATTO 647N
ATTO 655

Leica TCS STED



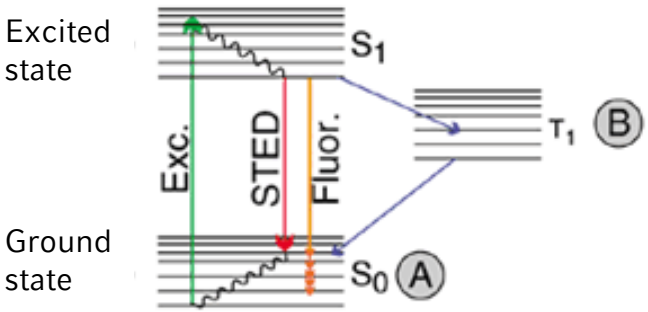
Detector
PMT or
APD

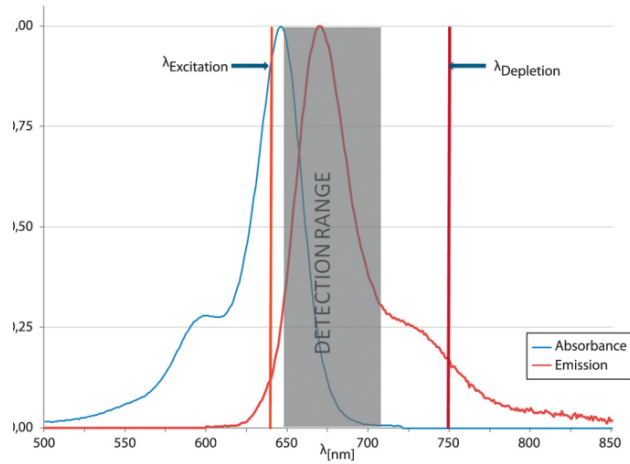
λ/2
Phase plate

Ex.
pulsed
640nm

STED
Ti:Sa-IR Laser
(~730-780 nm)

STED microscopy





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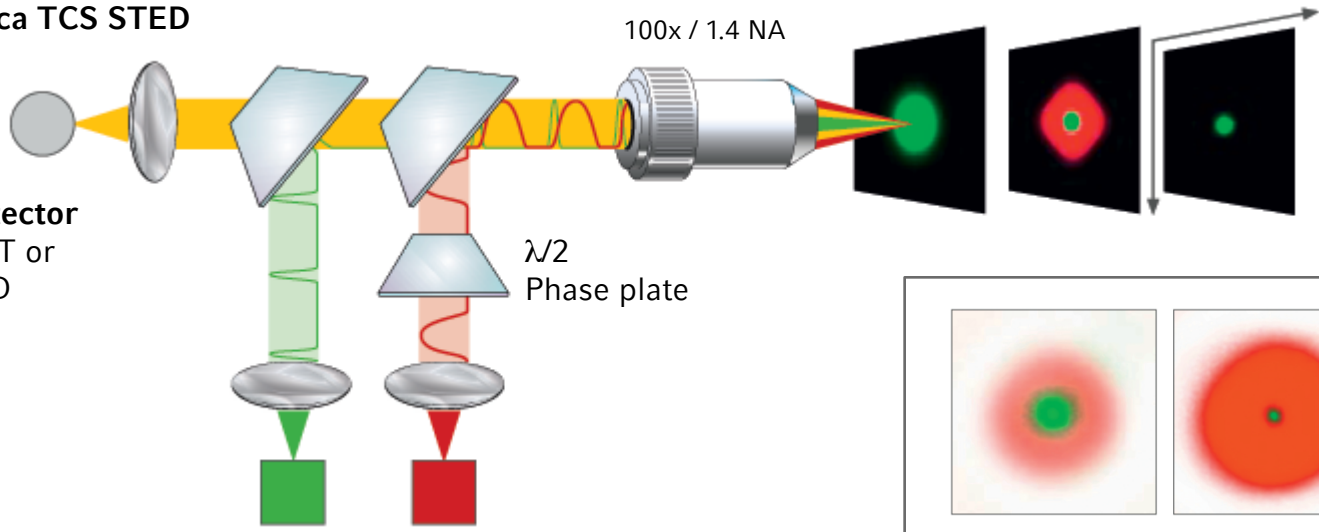


Detector
PMT or
APD

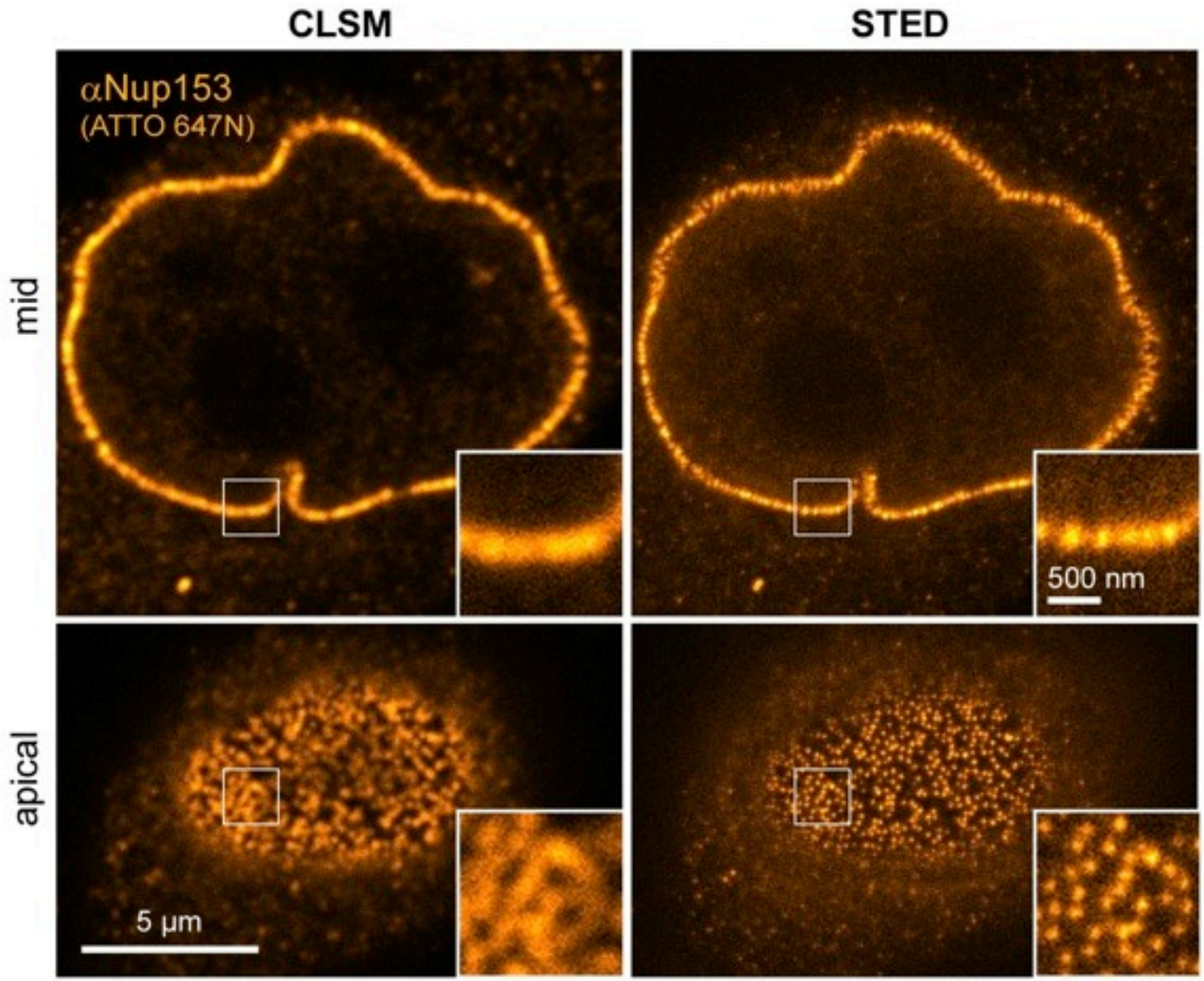
Ex.
pulsed
640nm

STED
Ti:Sa-IR Laser
(~730-780 nm)

$$\Delta x \approx \frac{\lambda}{2\,n \sin\alpha \sqrt{1 + I/I_s}}$$

Resolution depends on the ratio of the depletion intensity (I) and the saturation intensity (I_S)

STED microscopy (Leica TCS)



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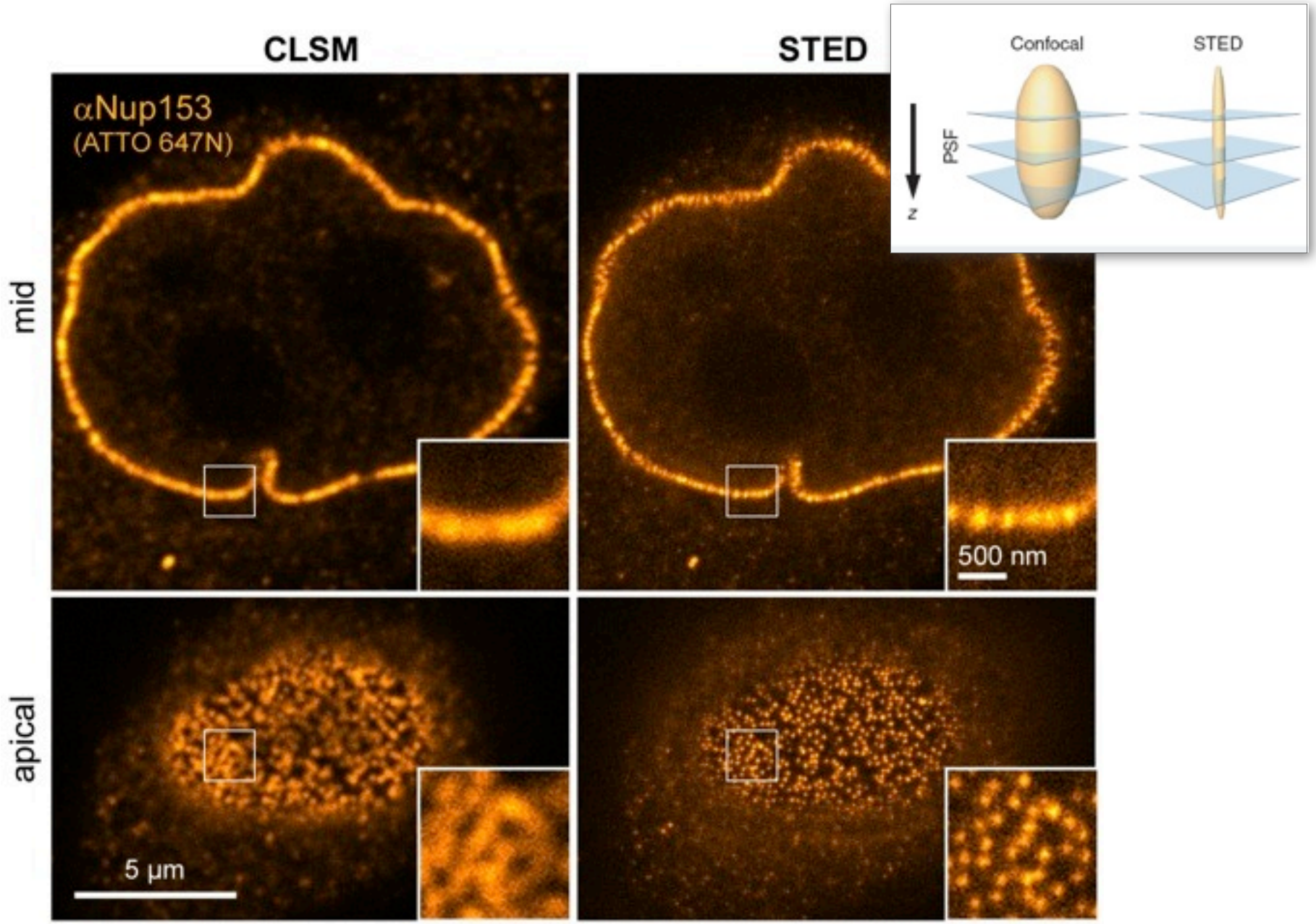
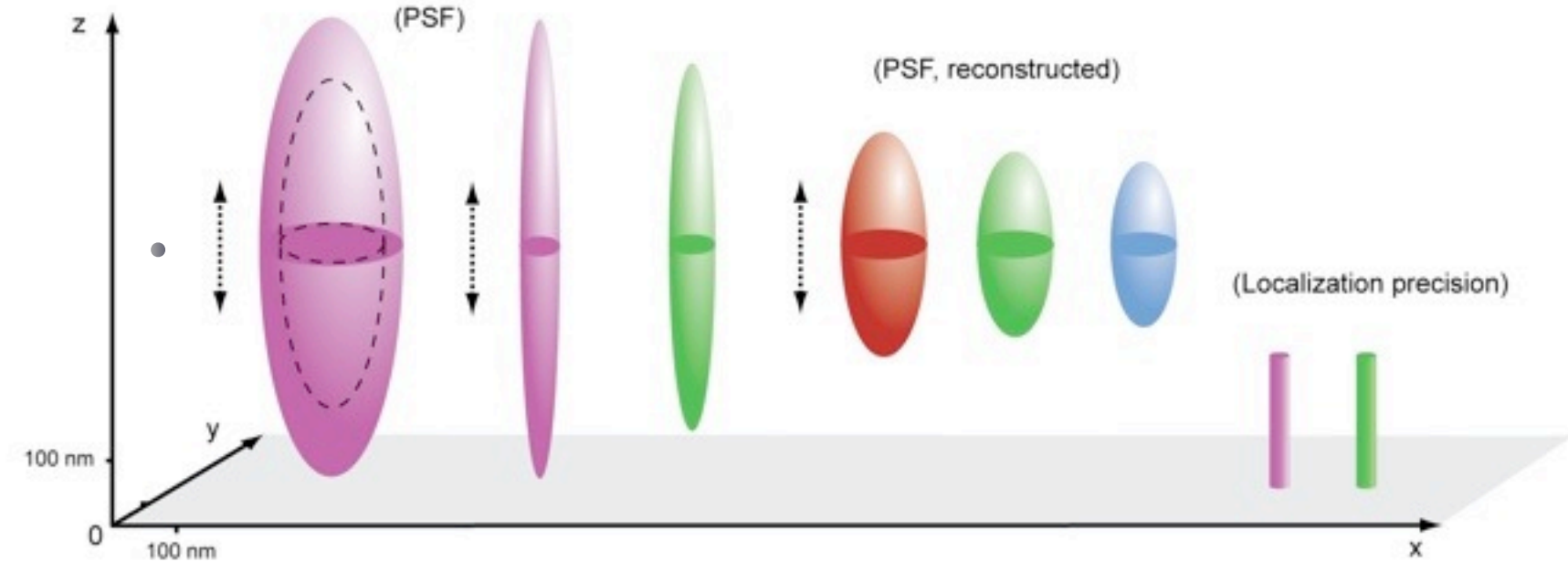


Resolving power of current commercial setups

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [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} µm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

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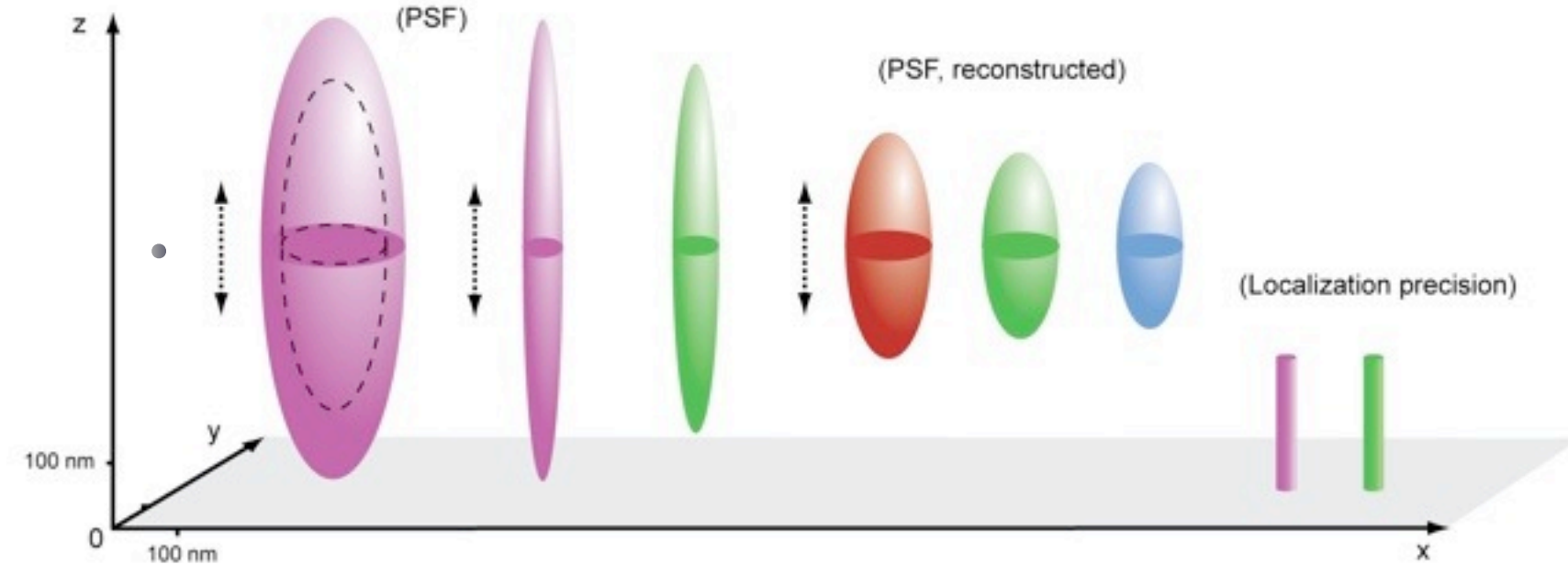

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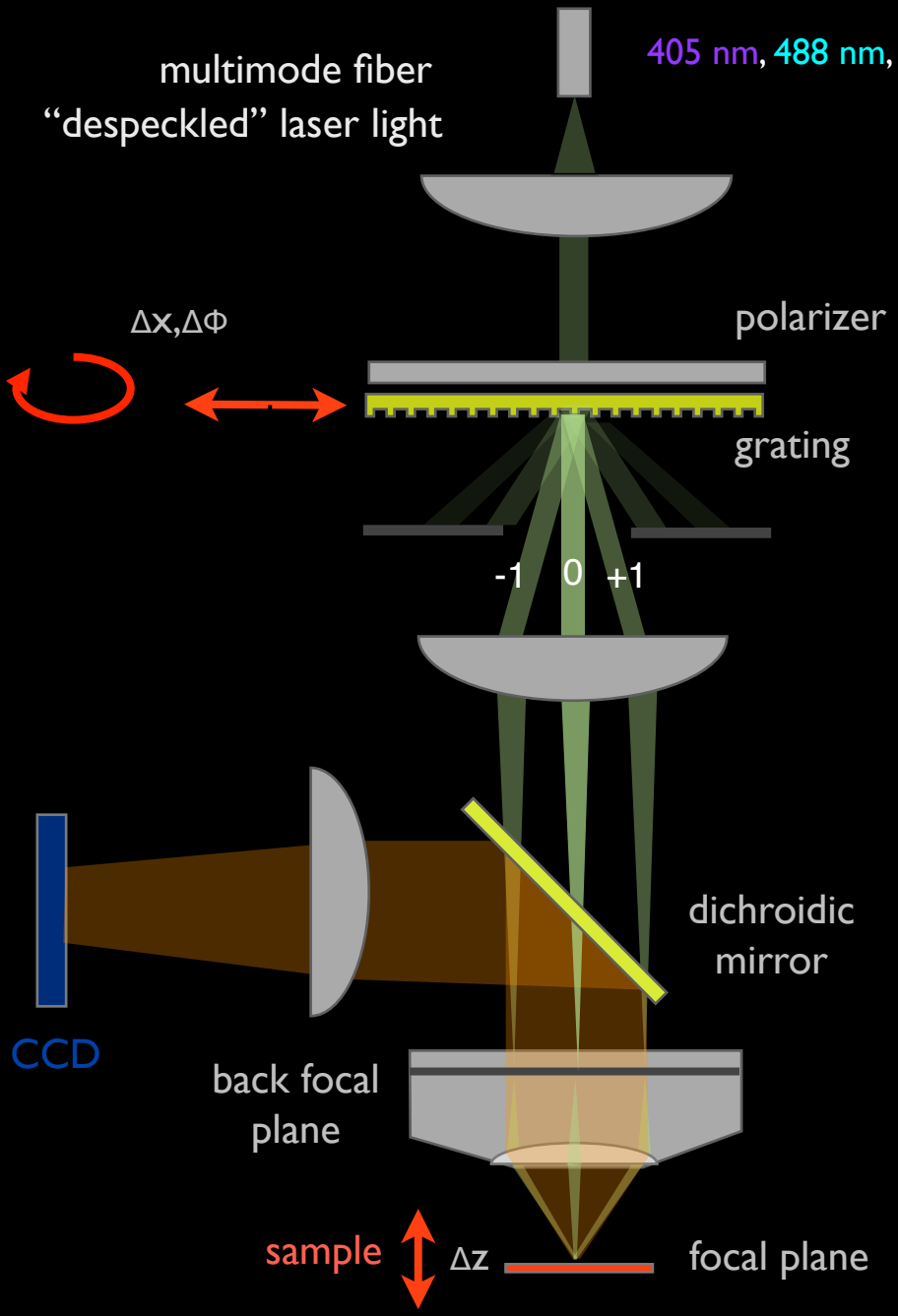
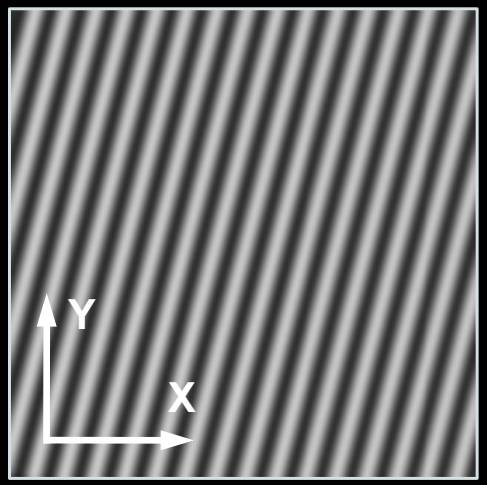
3D-SIM resolves ~8-fold smaller volumes than confocal laser scanning microscopy (CLSM)

Generating 3D-structured illumination

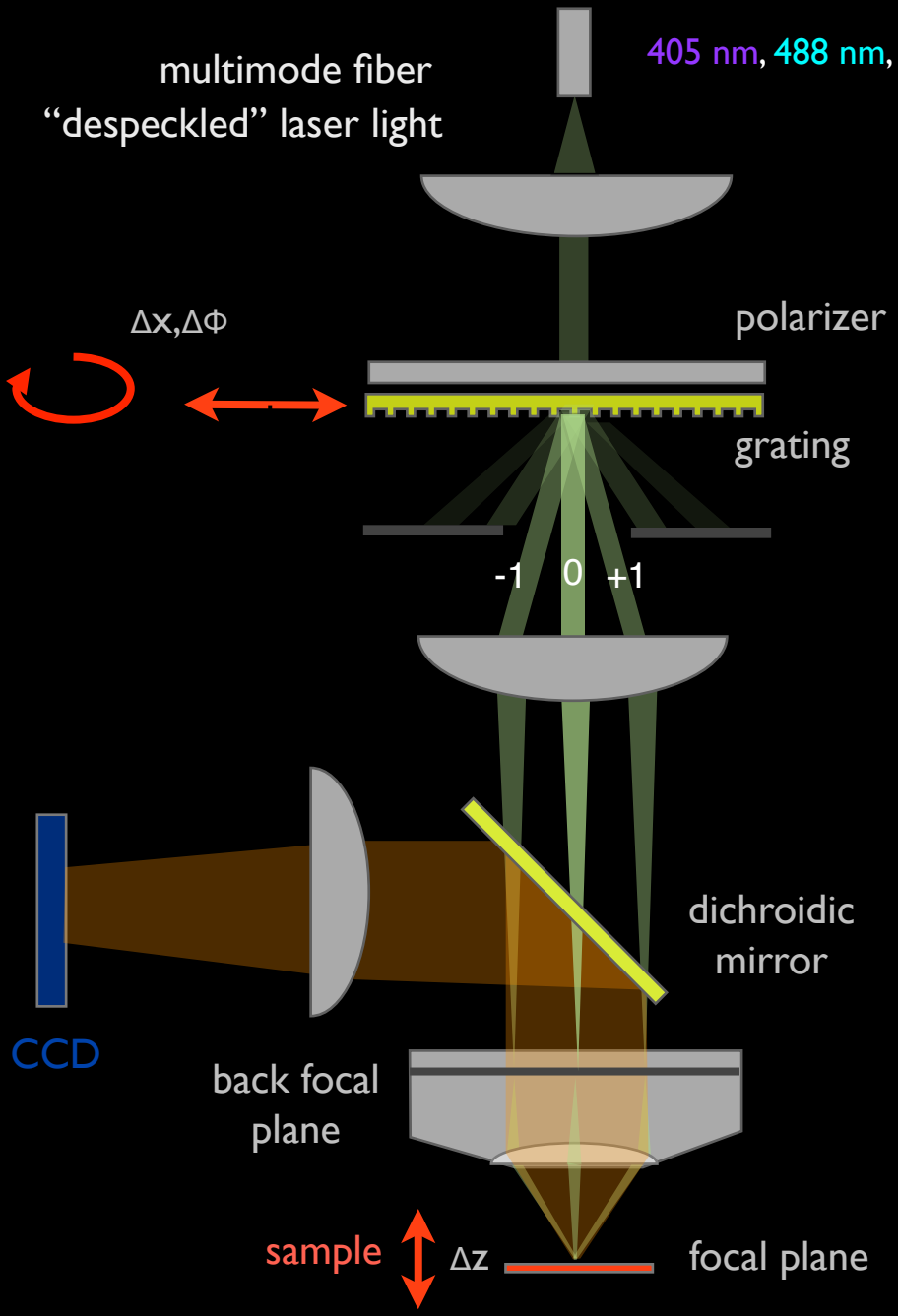


3 central diffracted beams interfere on the sample plane to generate sinusoidal stripes of ~220 nm width



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

Generating 3D-structured illumination



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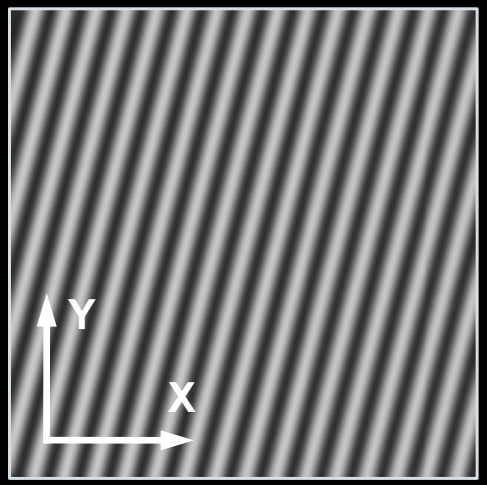
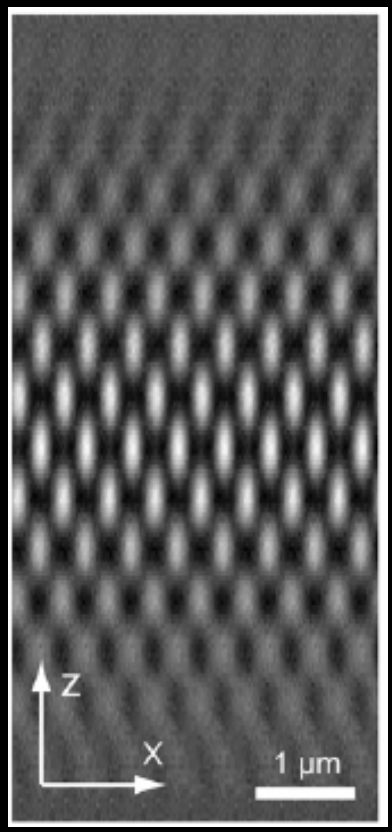


axial modulation



OMX: Housing

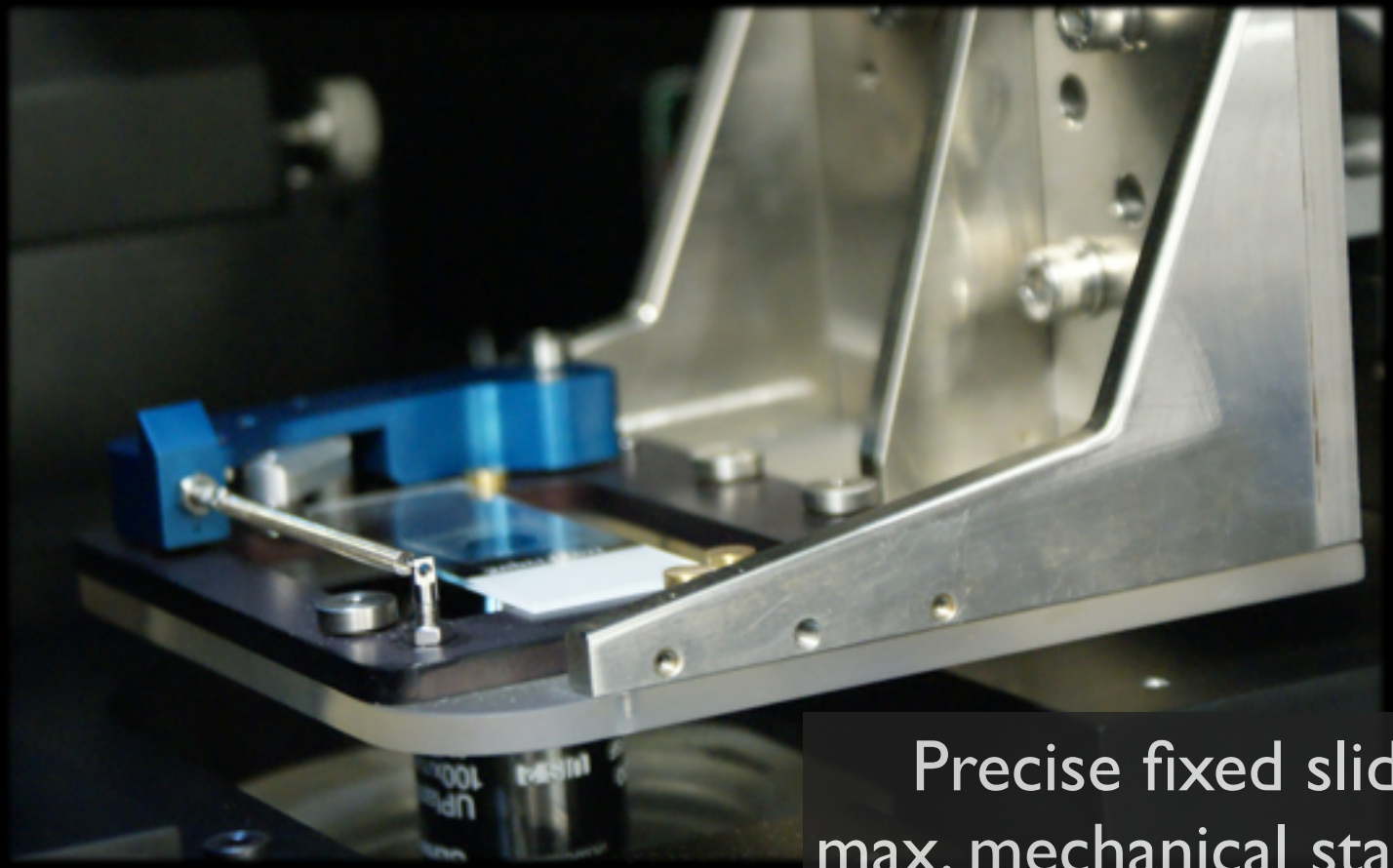
Dust filter

vibration-cushioned table & housing

Optical filters

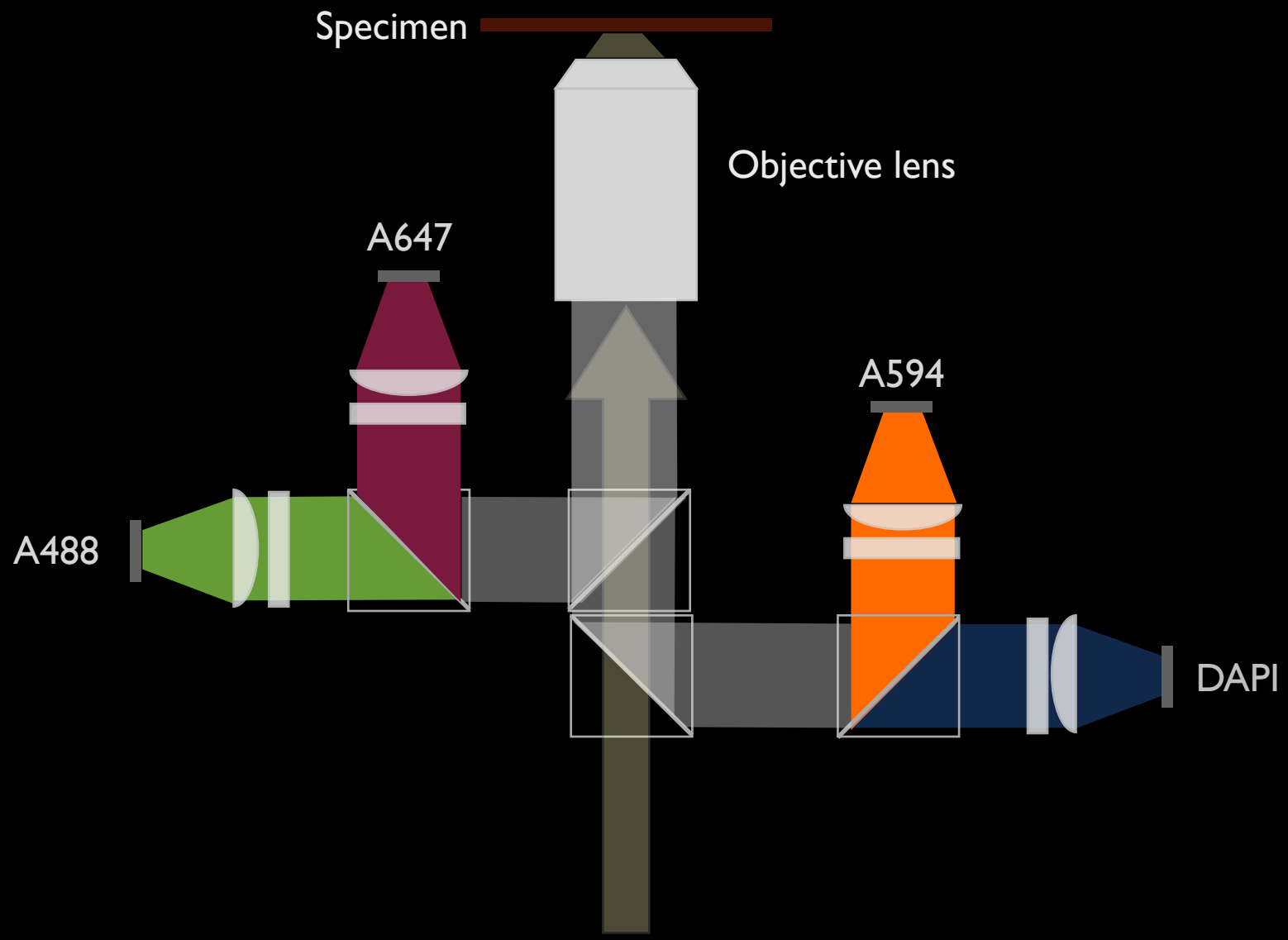
inside ?

OMX: Stage

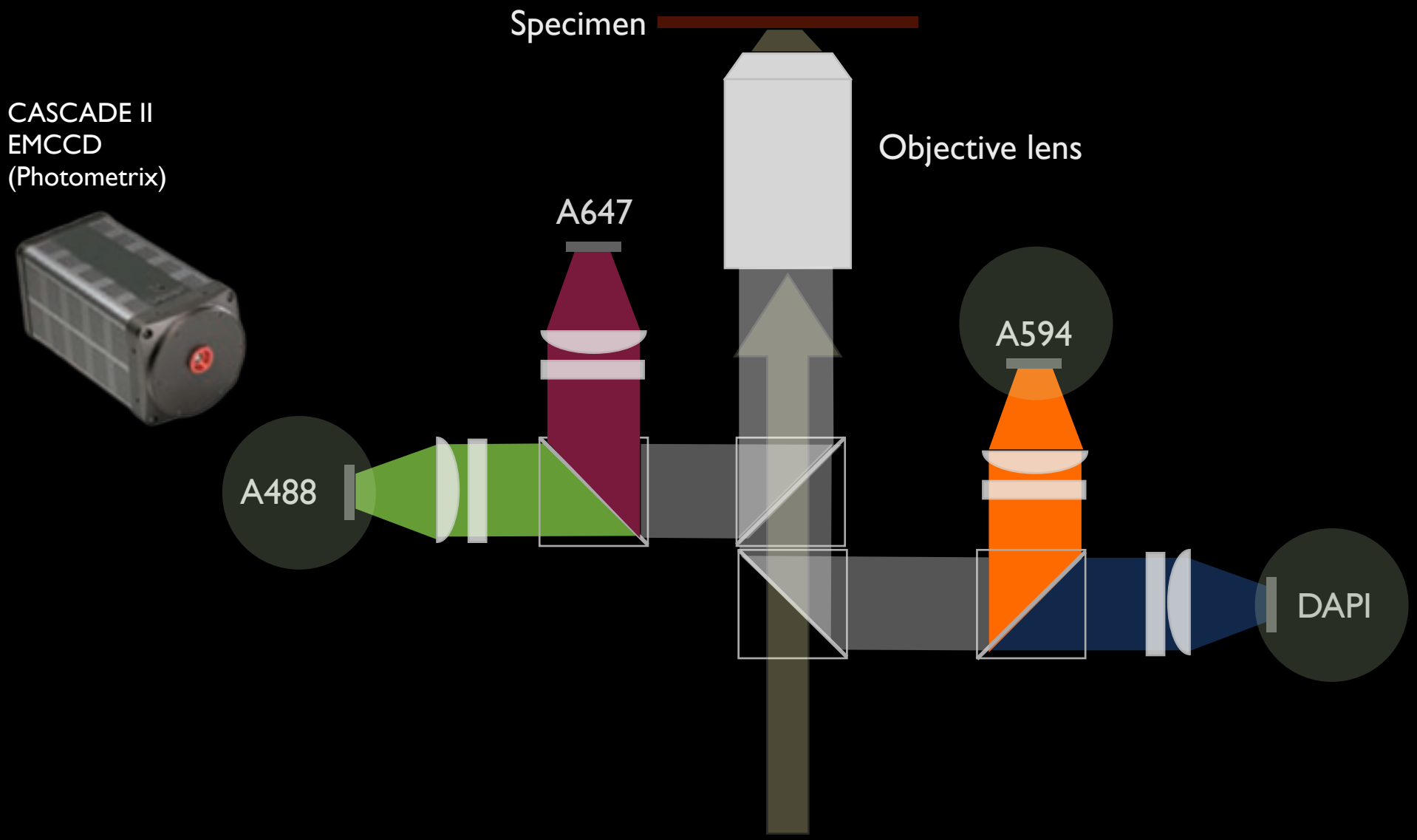


Precise fixed slide
max. mechanical stability
low thermal drift

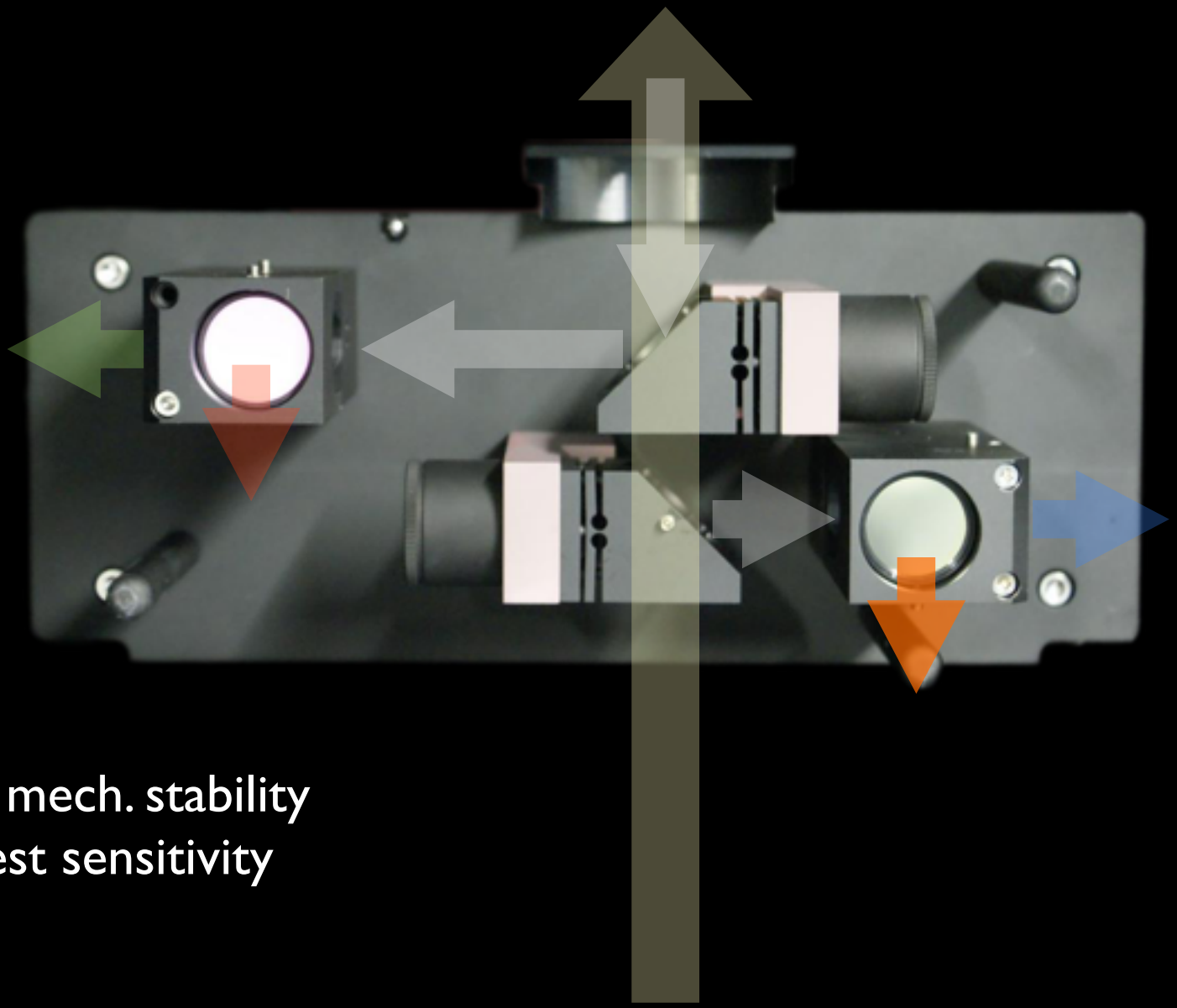
OMX: Filter Drawer Design



OMX: Filter Drawer Design

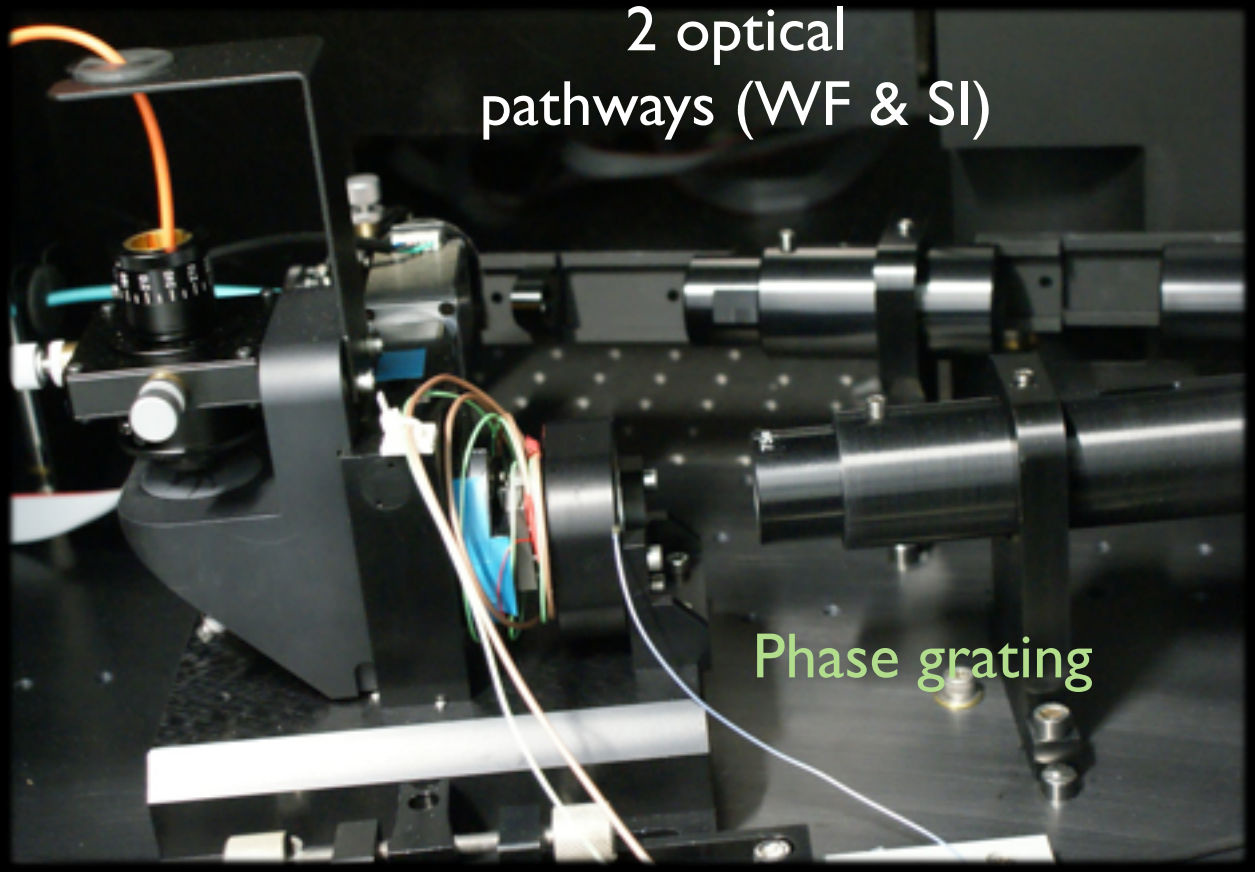


OMX: Filter Drawer Design

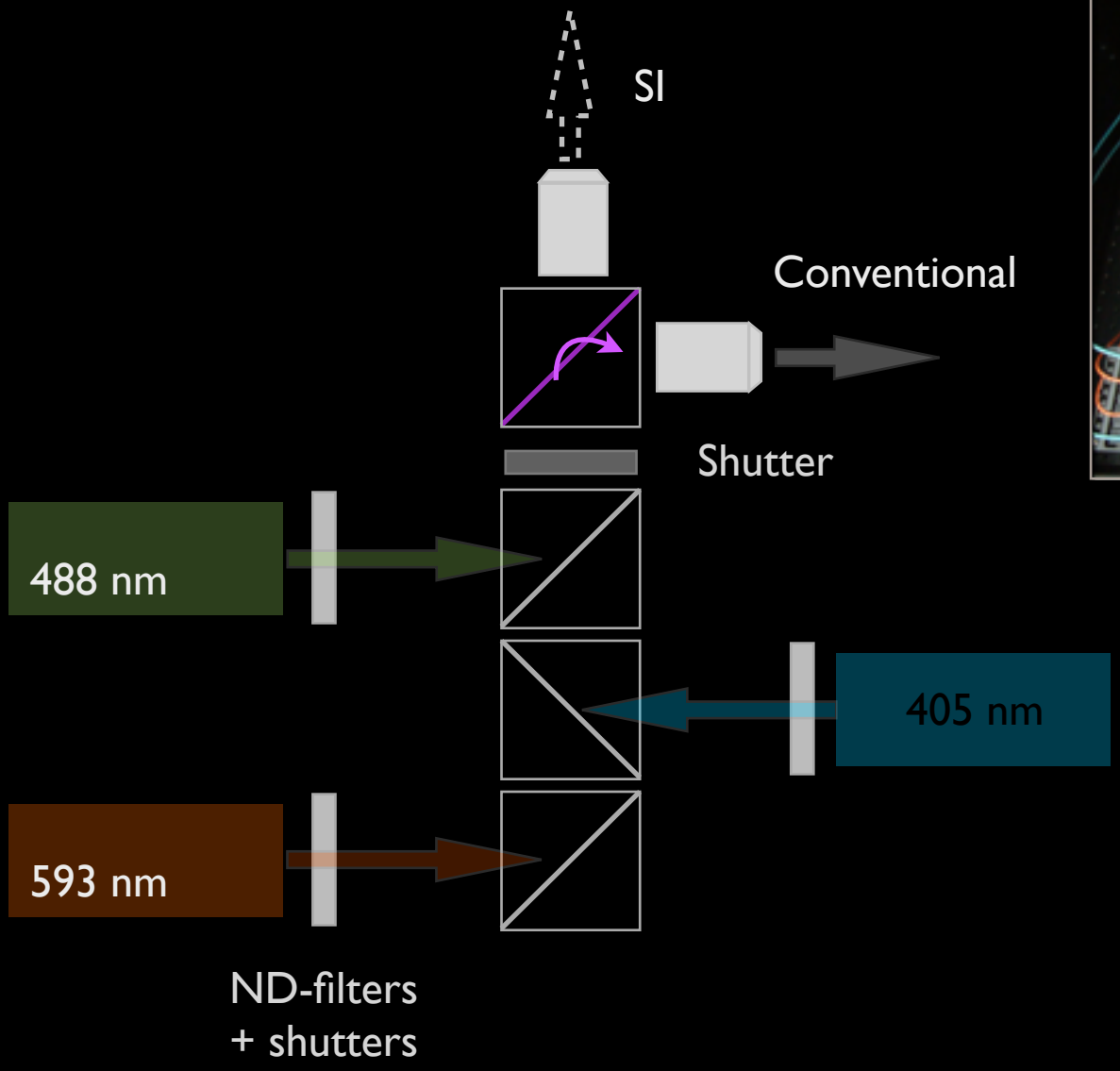


max. mech. stability
highest sensitivity

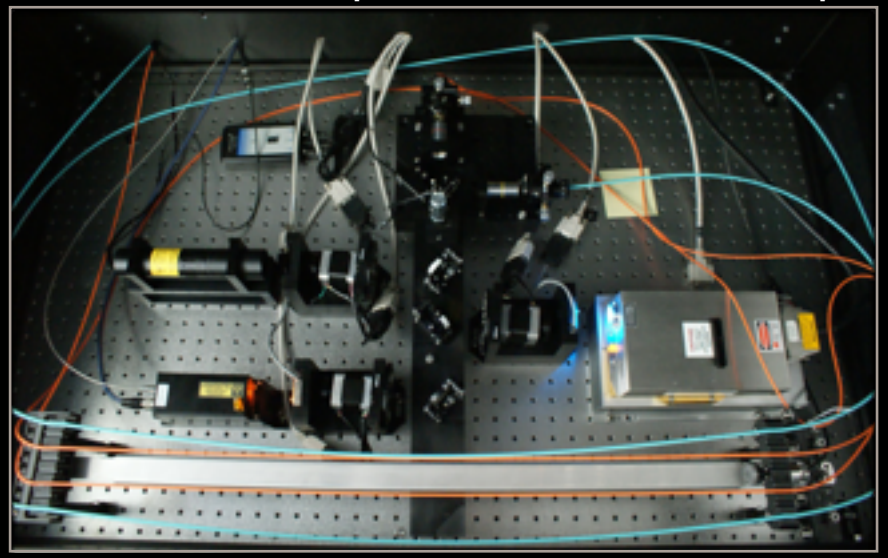
OMX: Dual illumination pathways



OMX: Laser unit



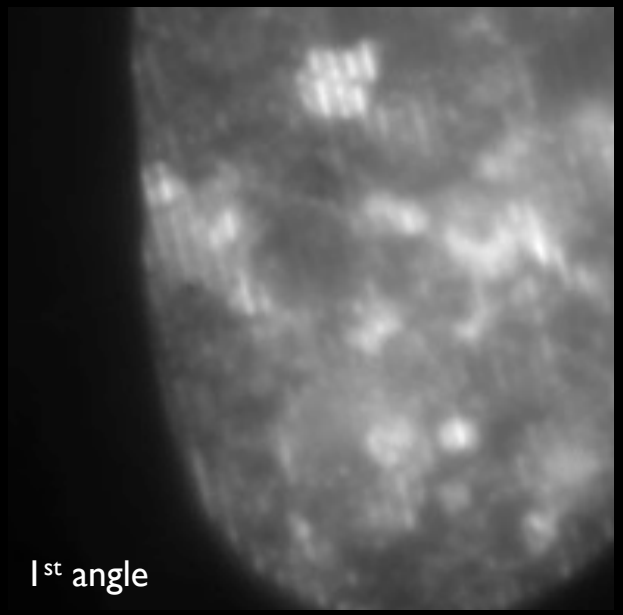
extendible / adaptable bread-board setup



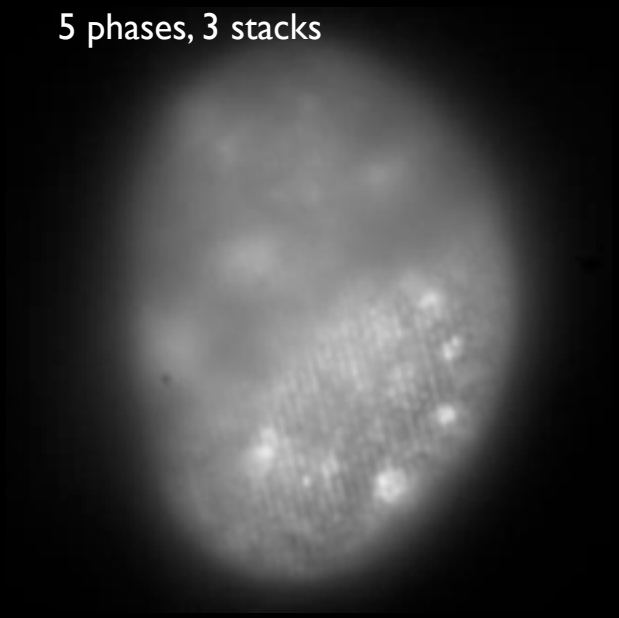
OMX: Electronics rack

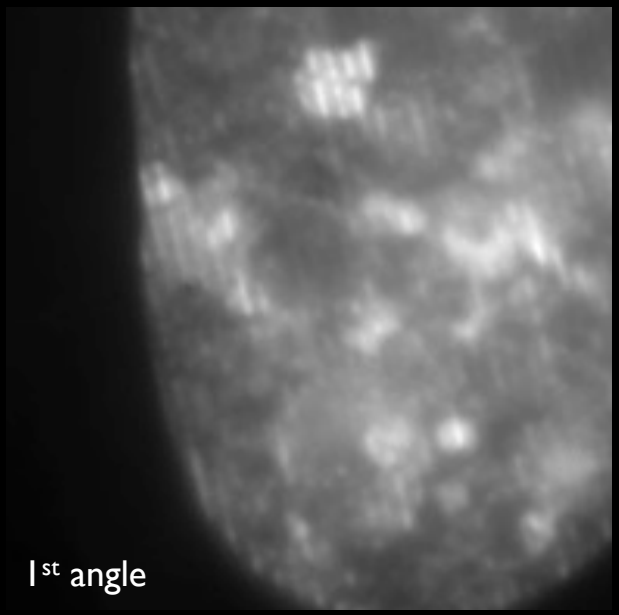
3D-SIM image acquisition and reconstruction

5 phases, 3 stacks

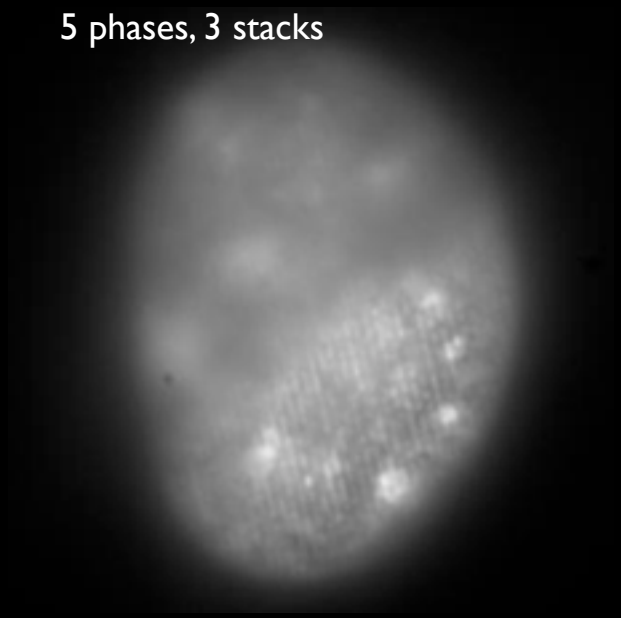


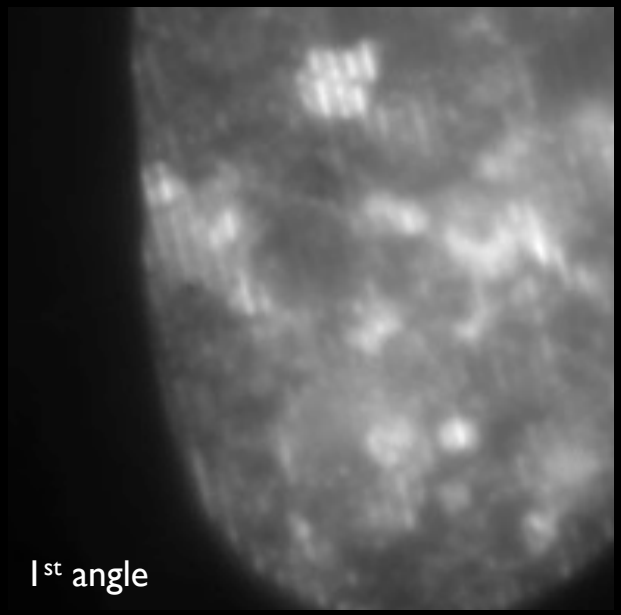
3D-SIM image acquisition and reconstruction

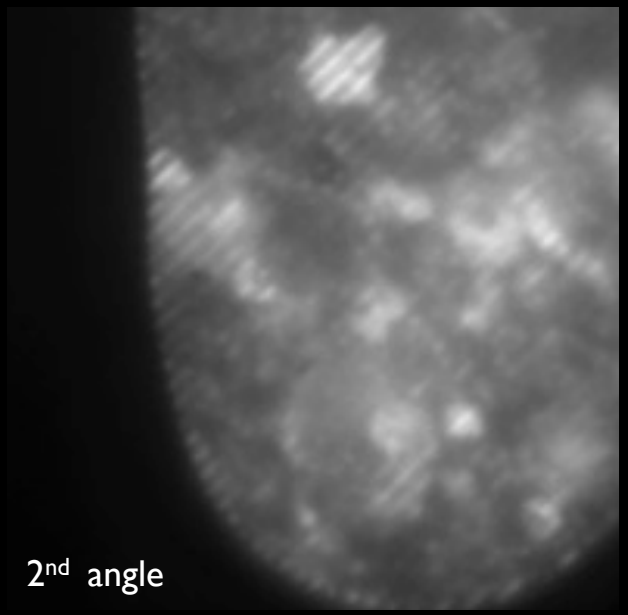




3D-SIM image acquisition and reconstruction







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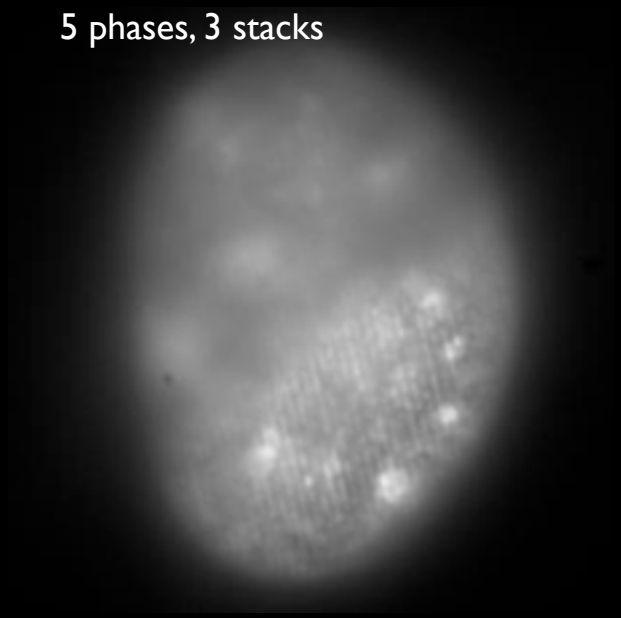

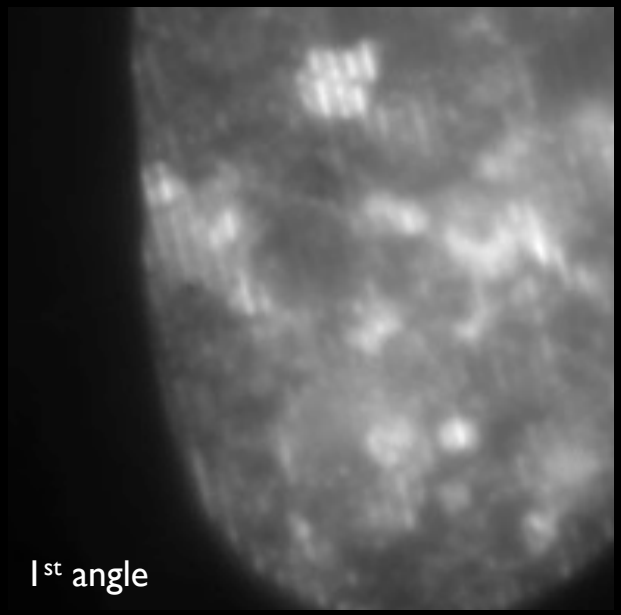

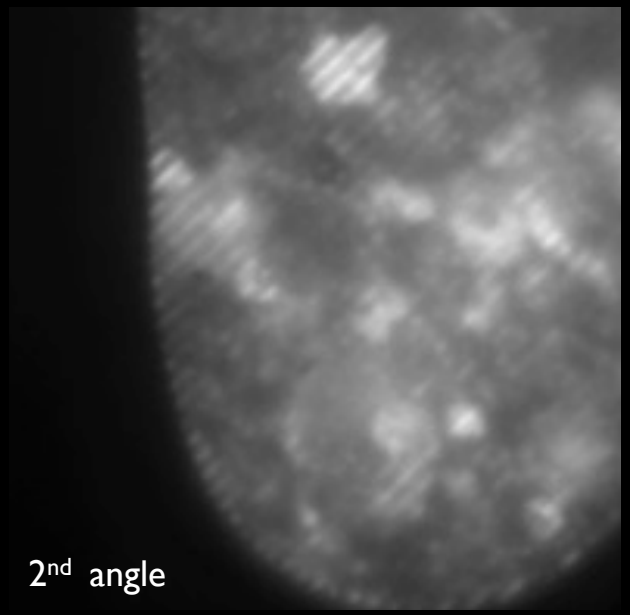

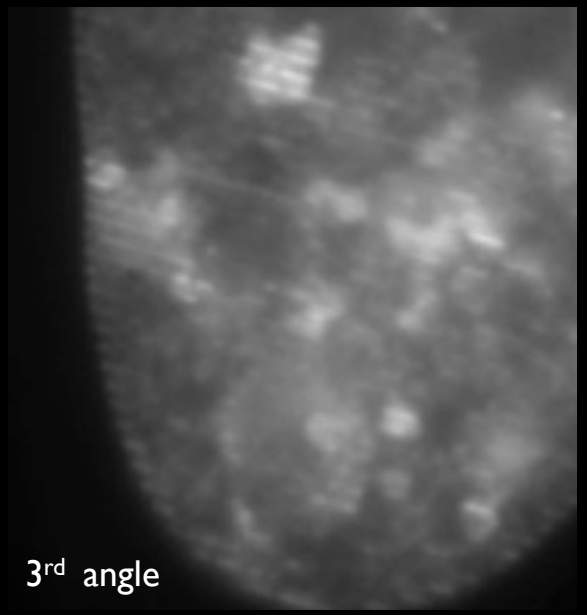

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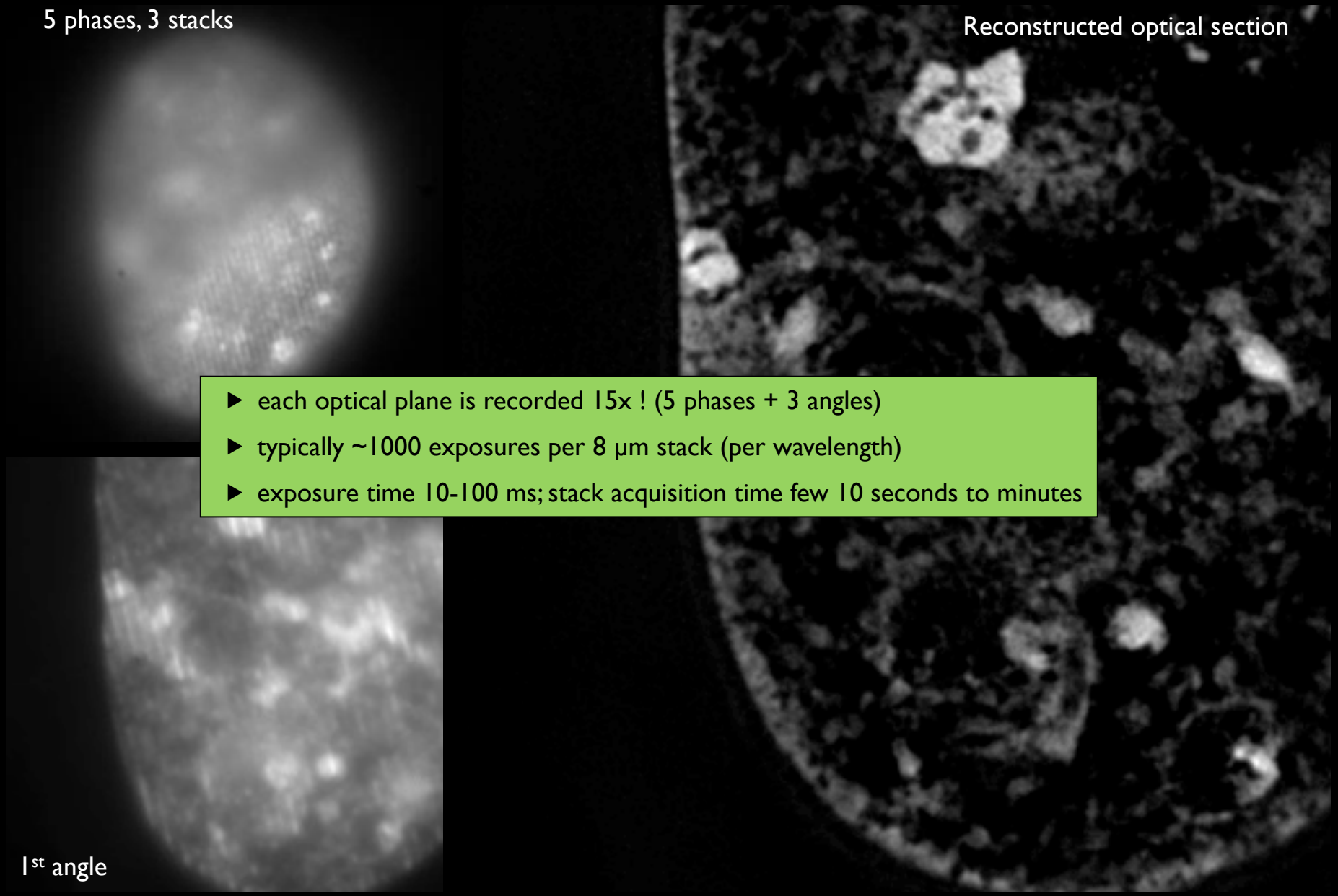
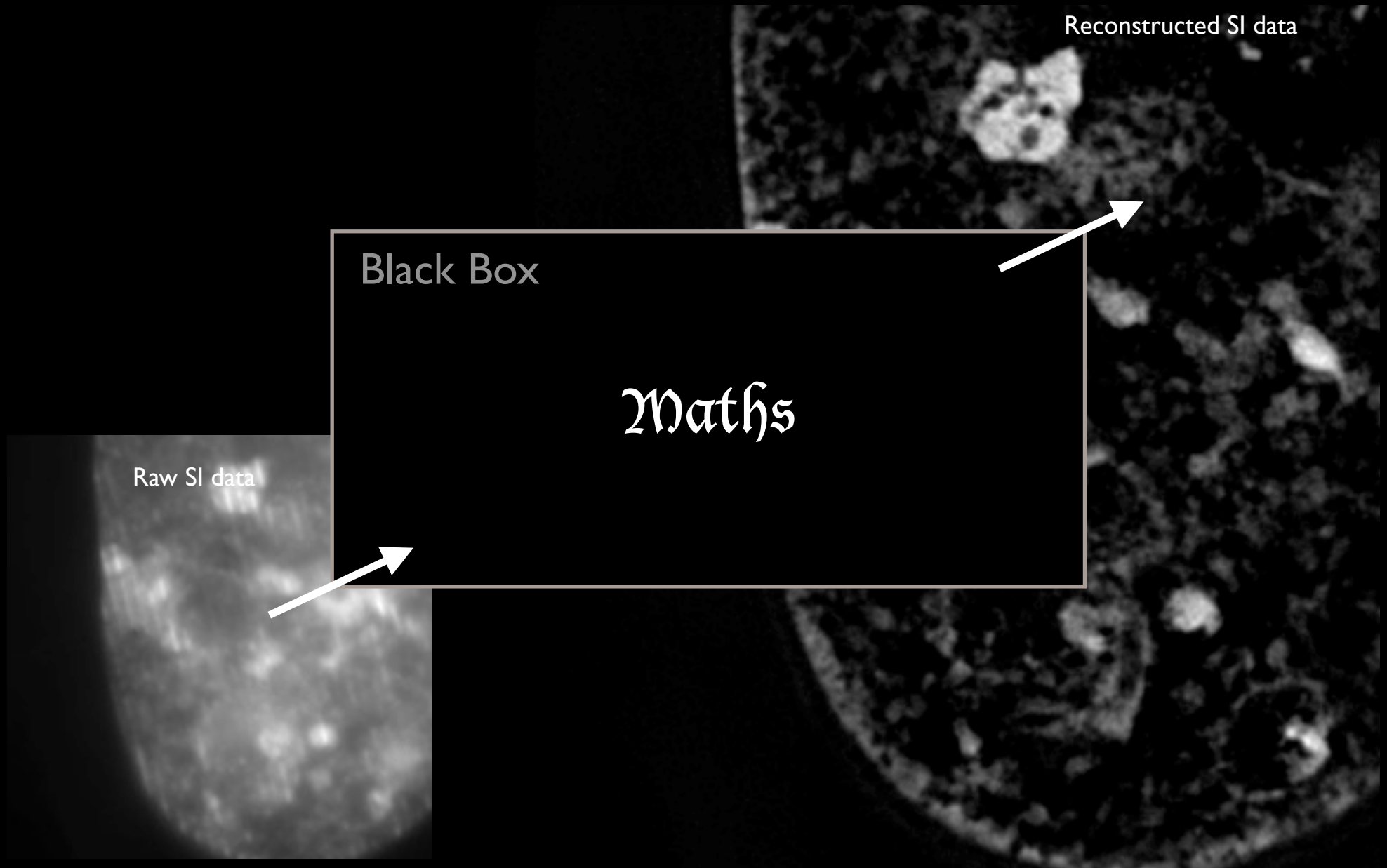
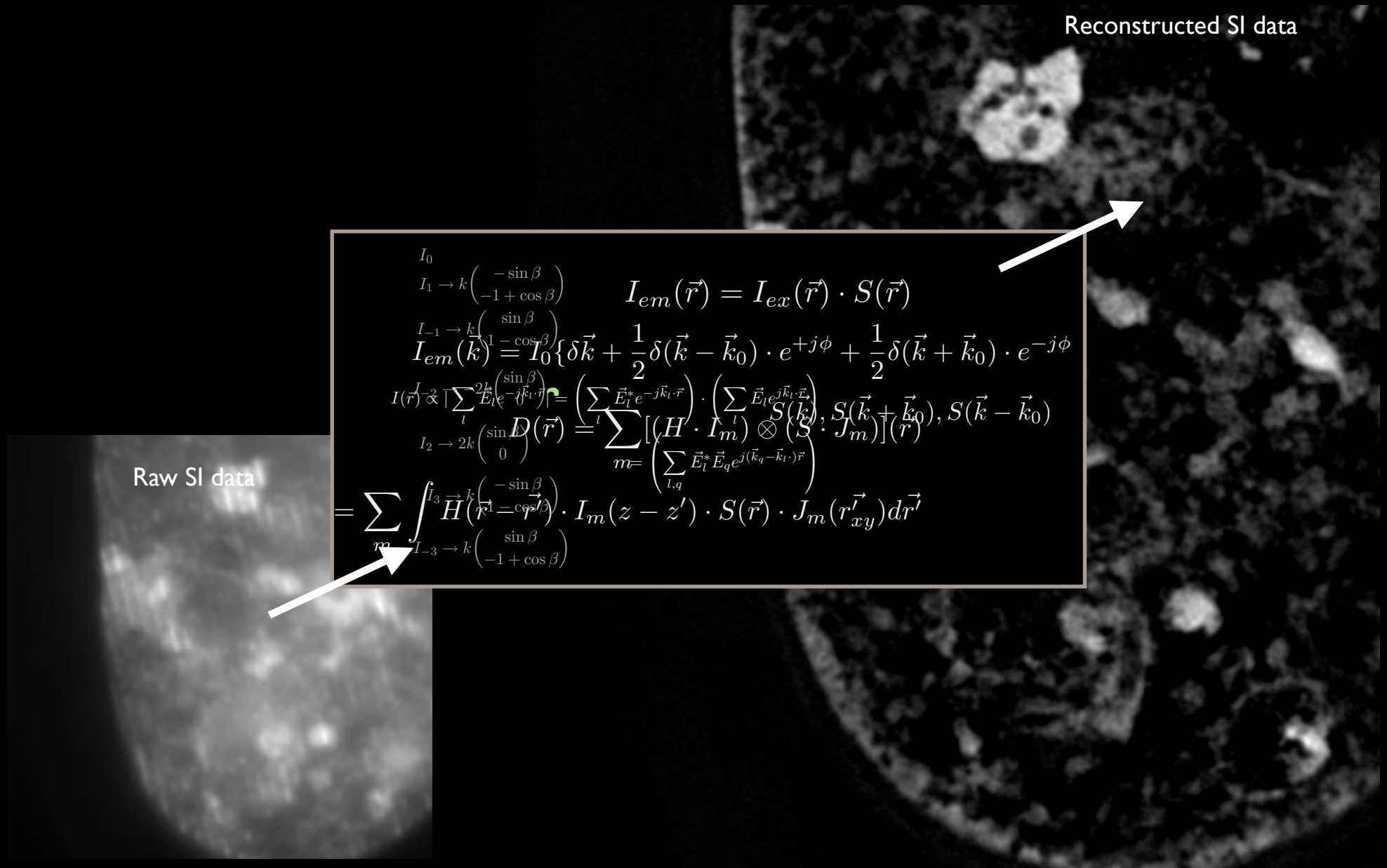


- each optical plane is recorded 15x ! (5 phases + 3 angles)
- typically ~1000 exposures per 8 µm stack (per wavelength)
- exposure time 10-100 ms; stack acquisition time few 10 seconds to minutes

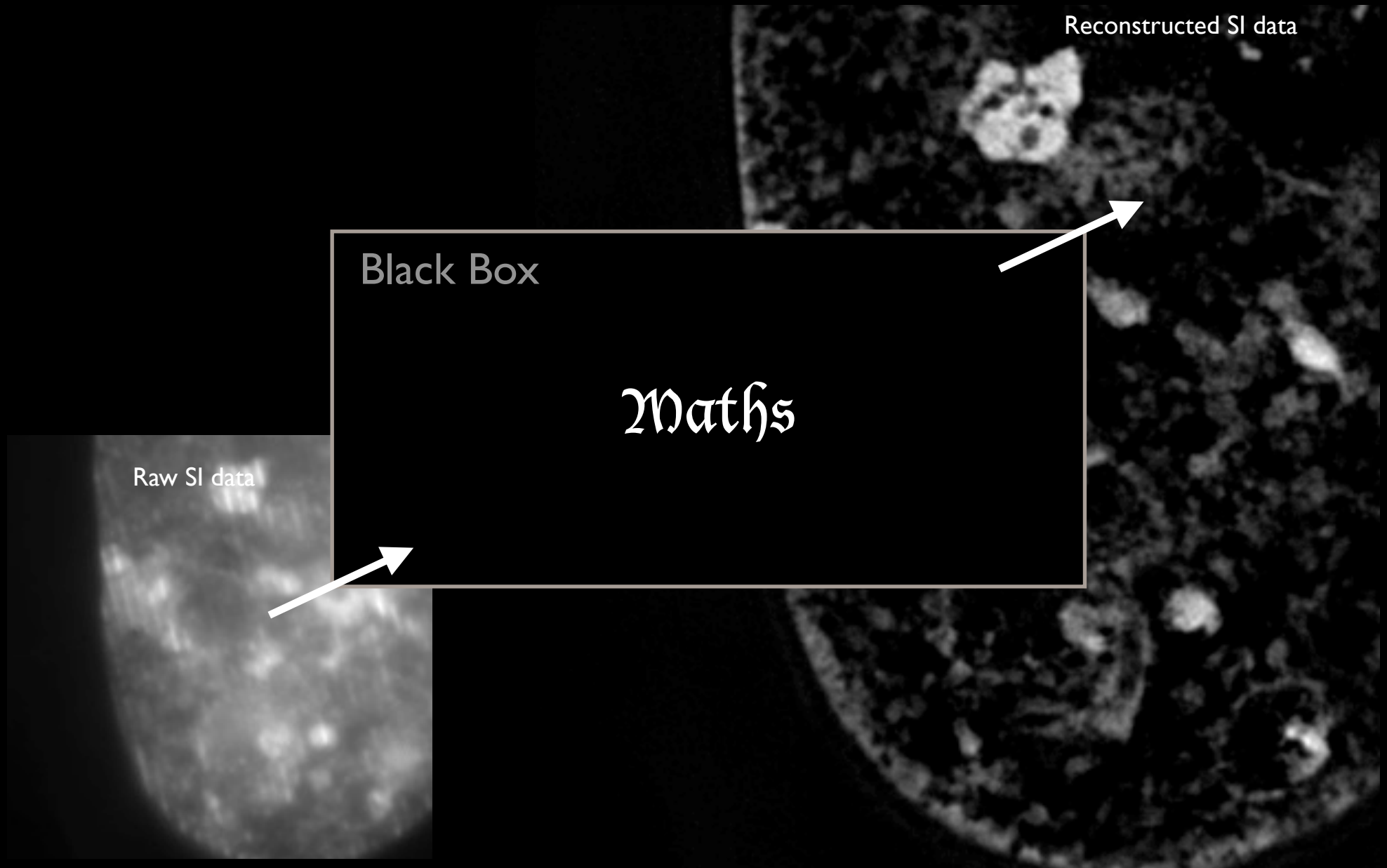
3D-SIM image acquisition and reconstruction



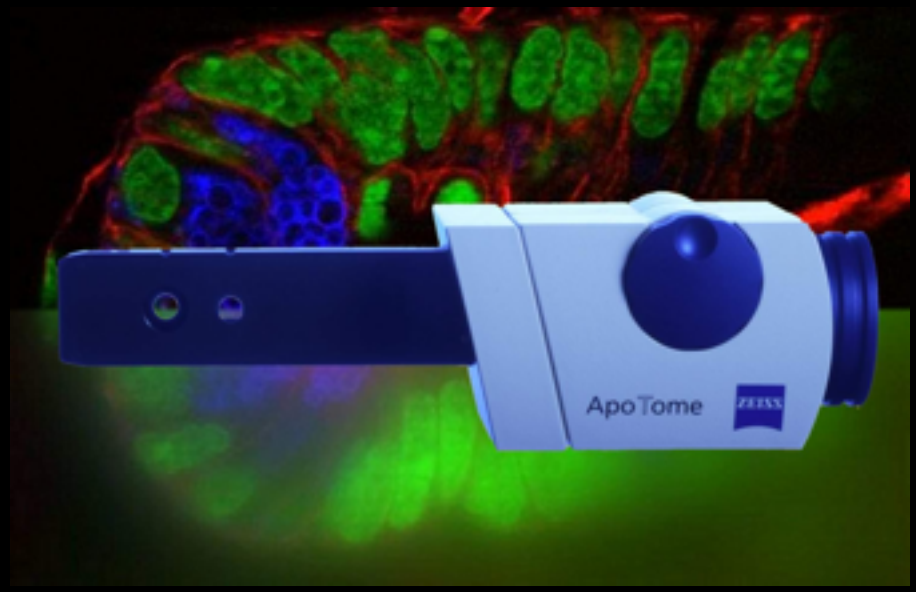
3D-SIM image acquisition and reconstruction



3D-SIM image acquisition and reconstruction



Apotome uses coarse SI to remove out-of-focus blur

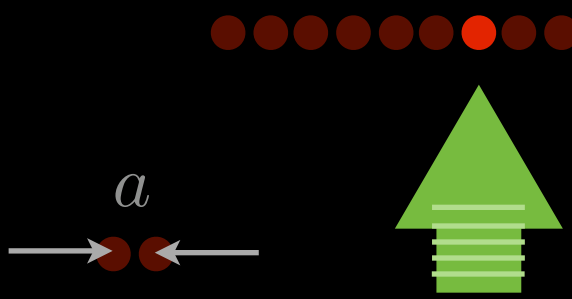


„Poor man‘s confocal“
No super-resolution!

The basic principle: Abbe's view

Sample = Structure

Periodicity

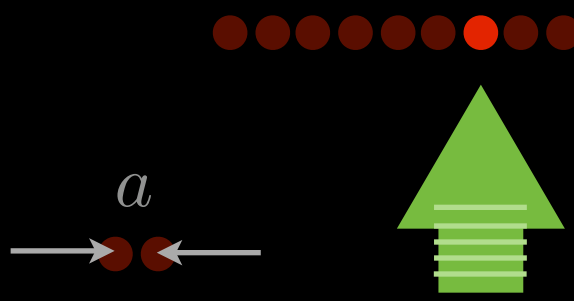


http://de.wikipedia.org/wiki/Ernst_Abbe

The basic principle: Abbe's view

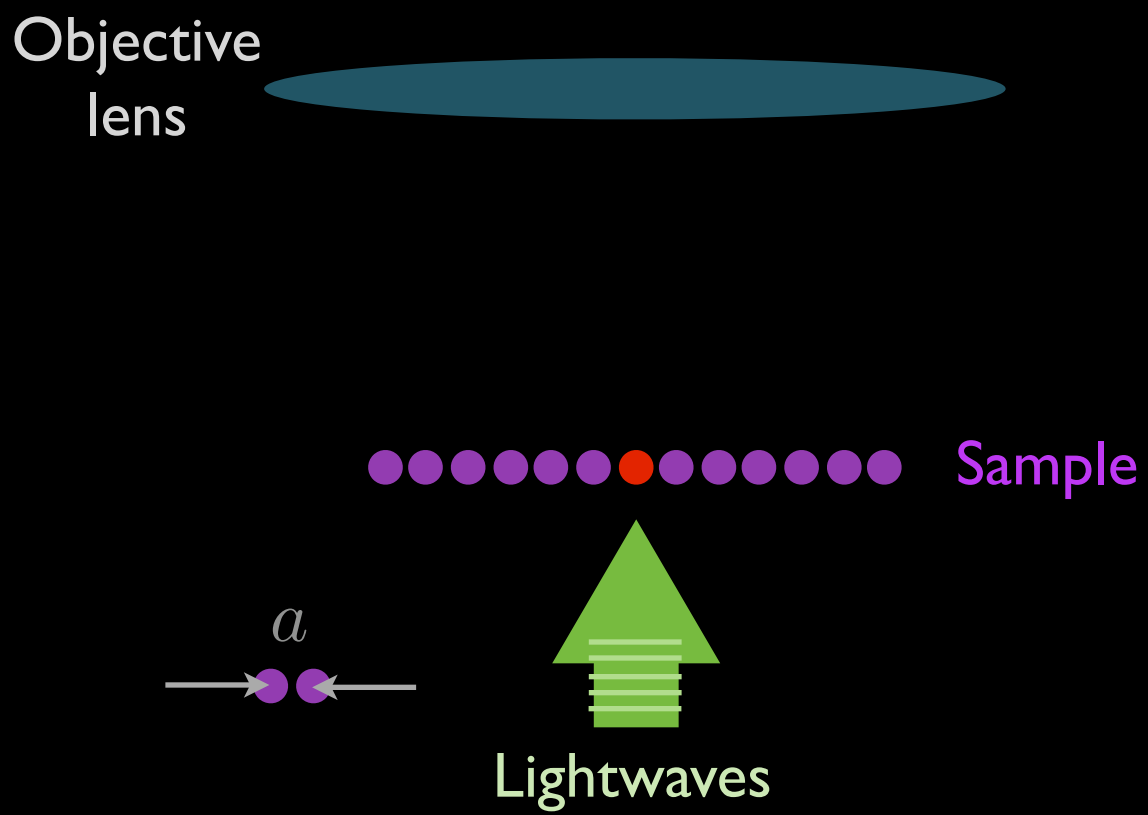
Sample = Structure

Periodicity

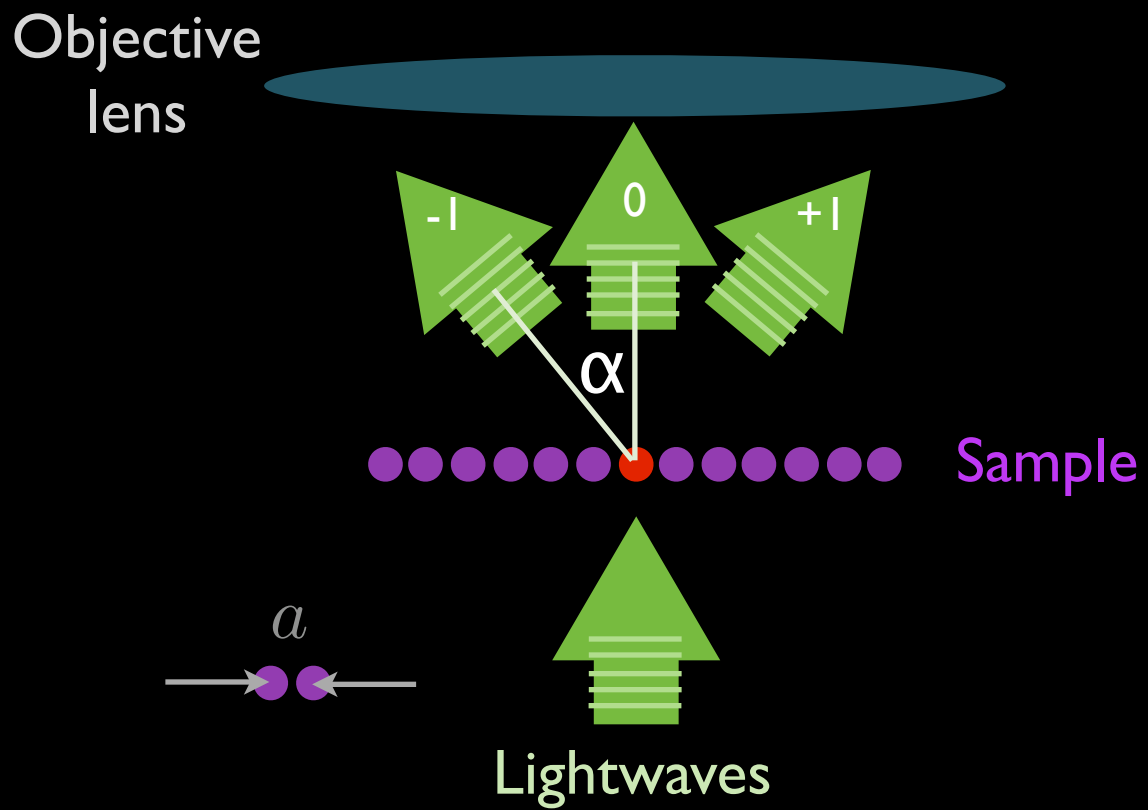


http://de.wikipedia.org/wiki/Ernst_Abbe

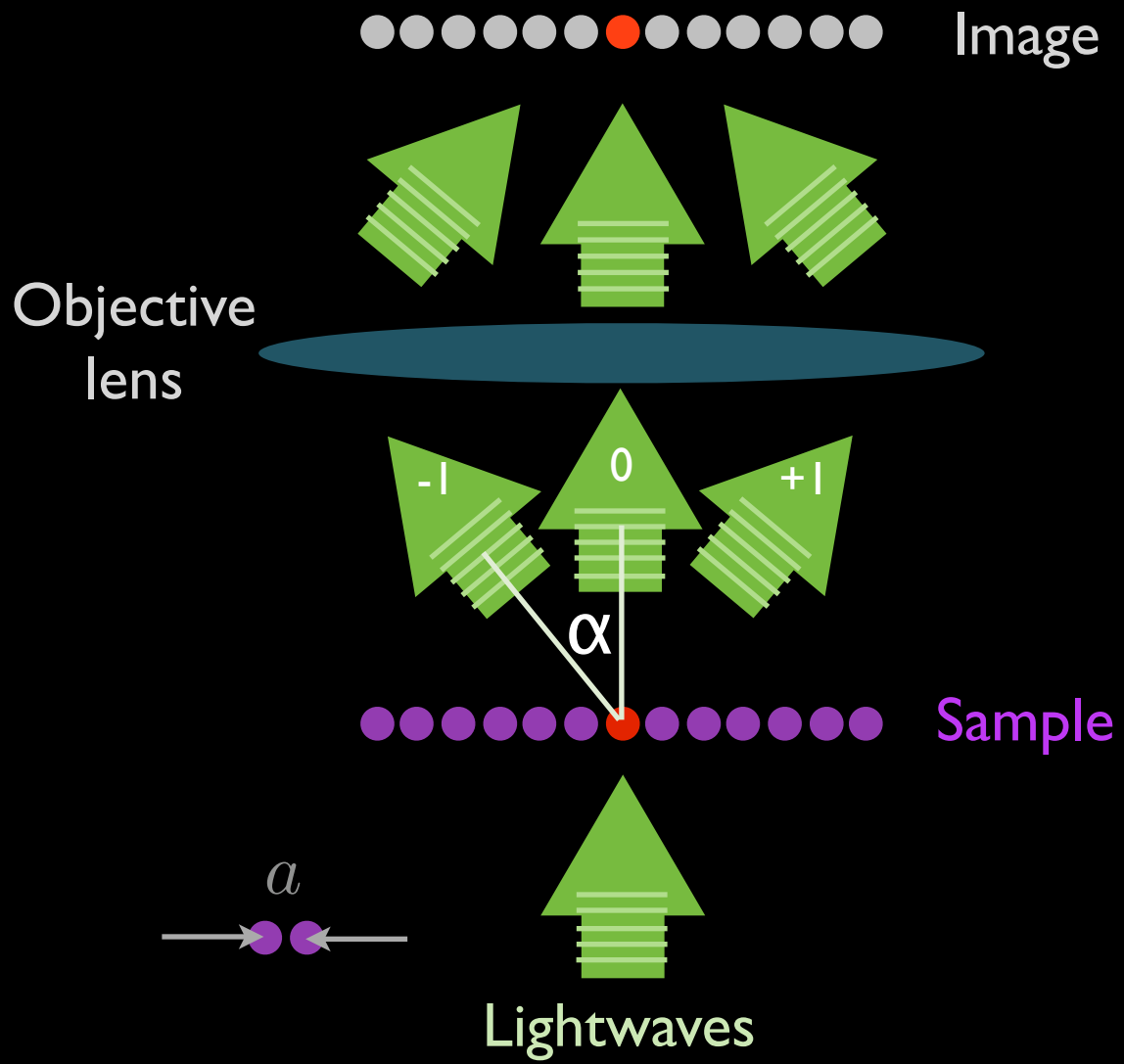
The basic principle: Abbe's view



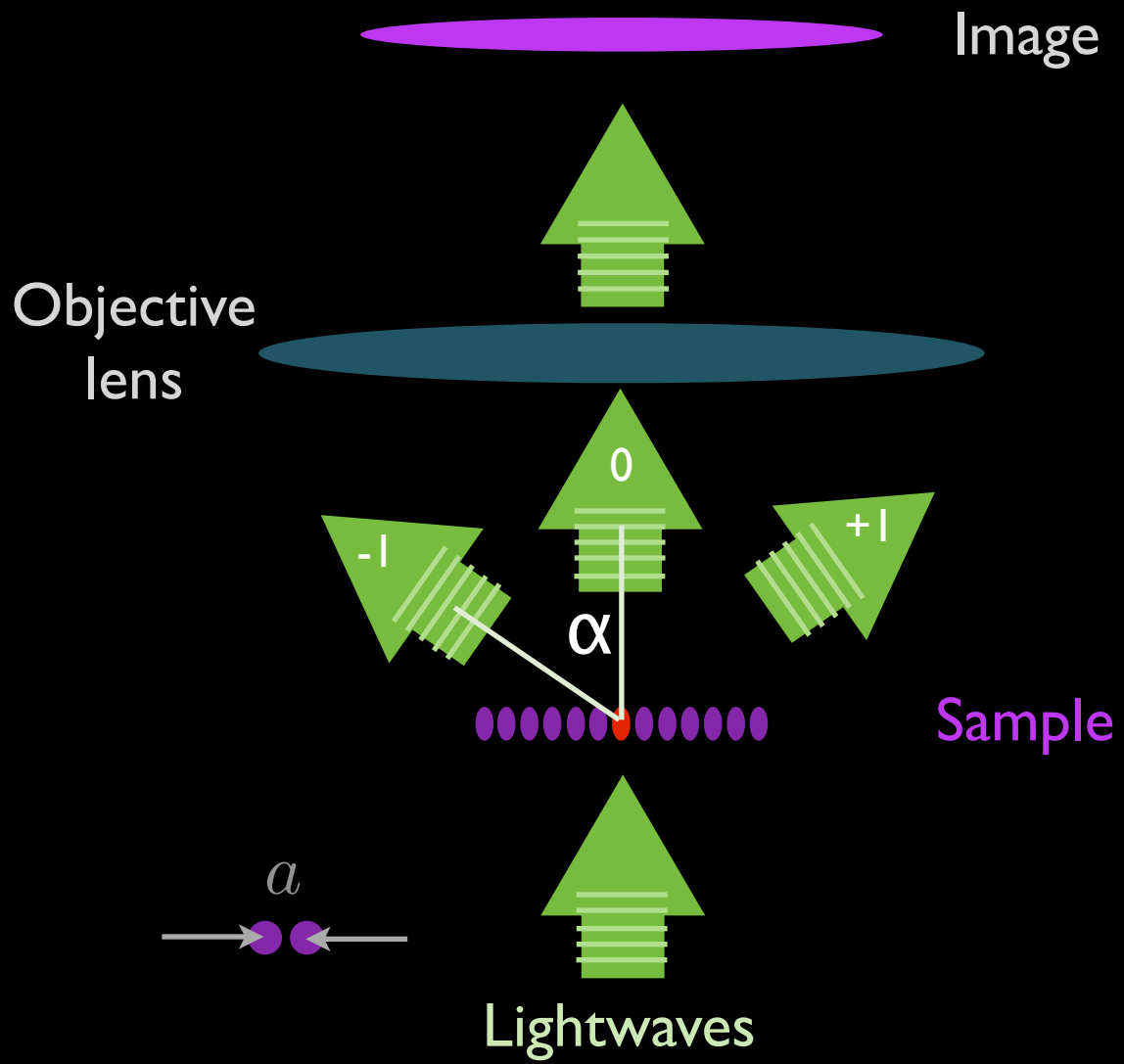
The basic principle: Abbe's view



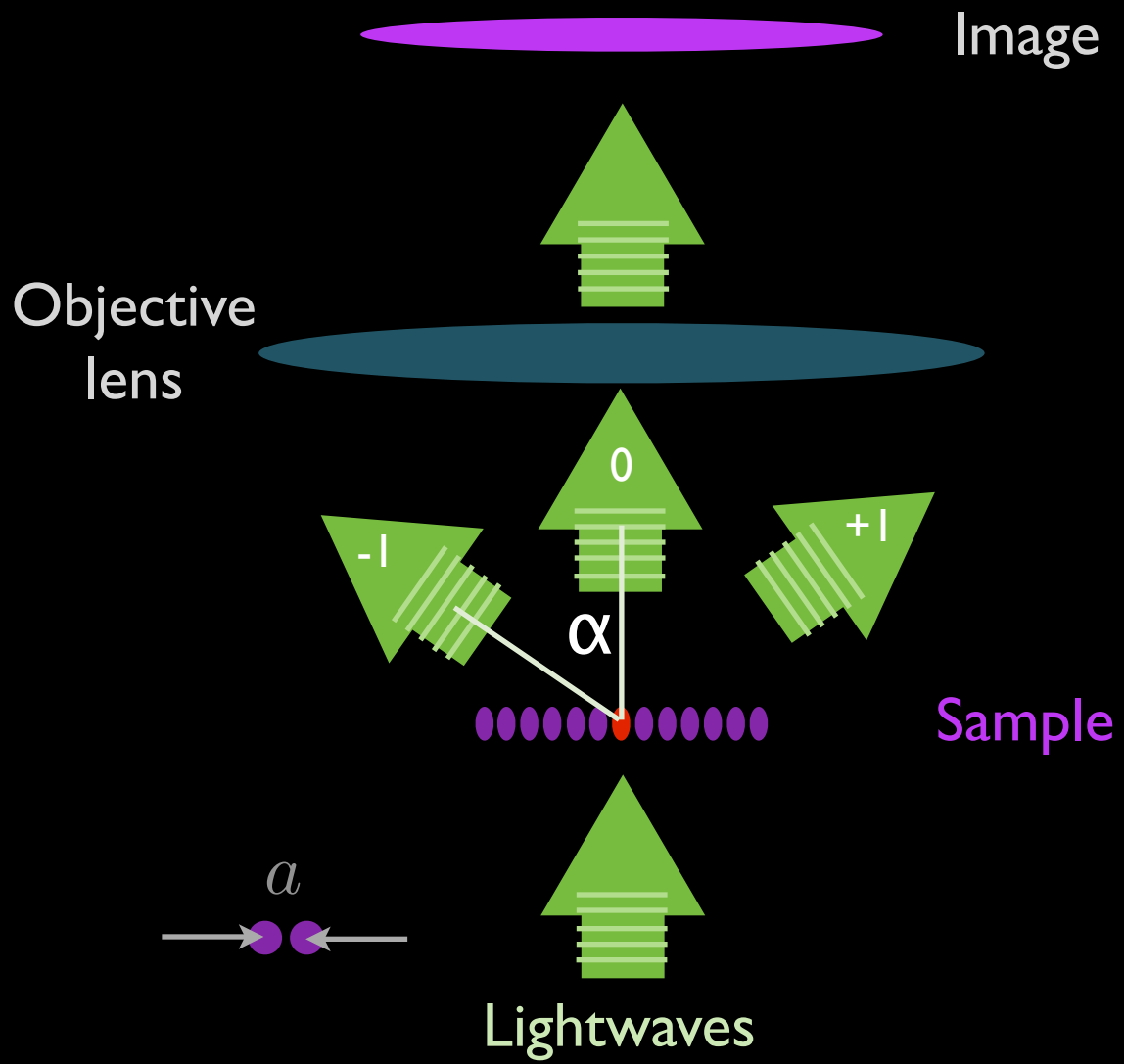
The basic principle: Abbe's view



The basic principle: Abbe's view



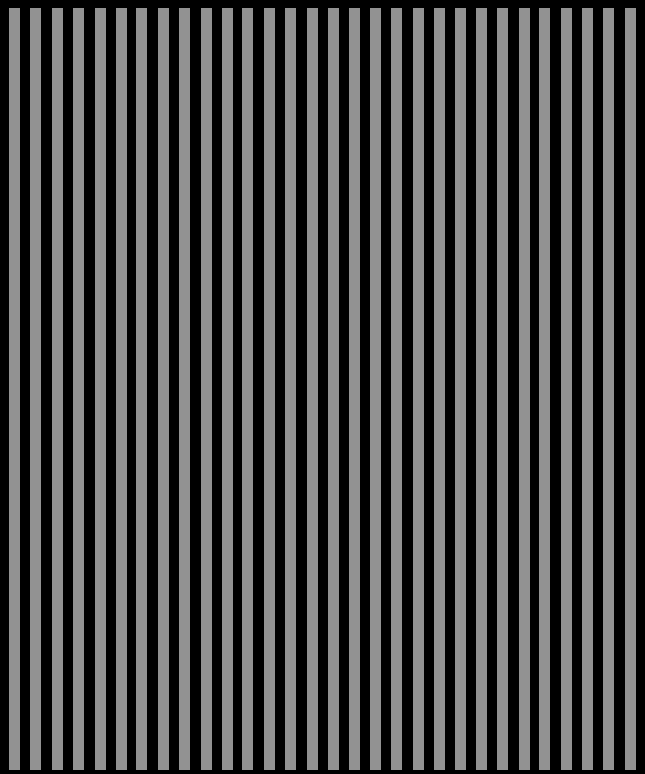
The basic principle: Abbe's view



highest frequencies
(biggest α)
→
smallest structures

The basic SI principle: Moiré fringes

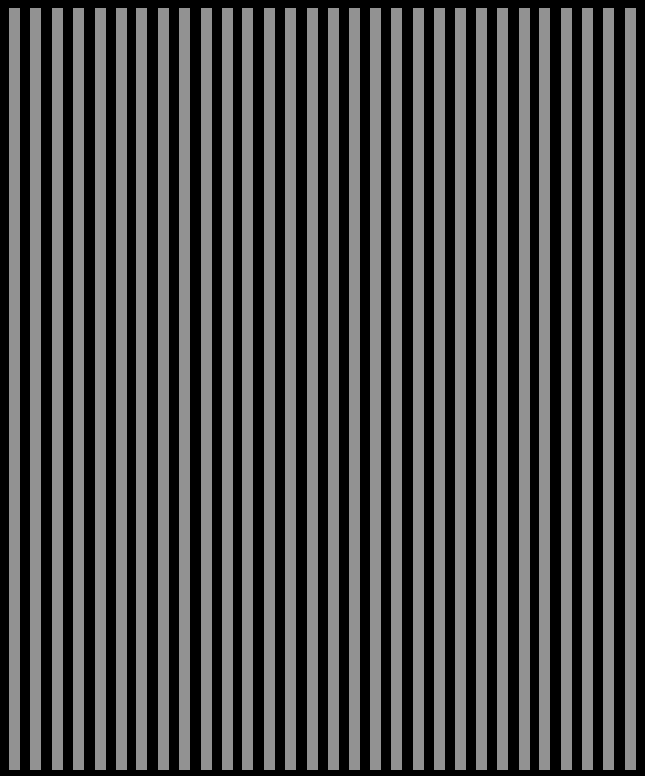
f_I = 30 Stripes / 300 Pixels = 0.1 S/P



Illumination with known periodicity

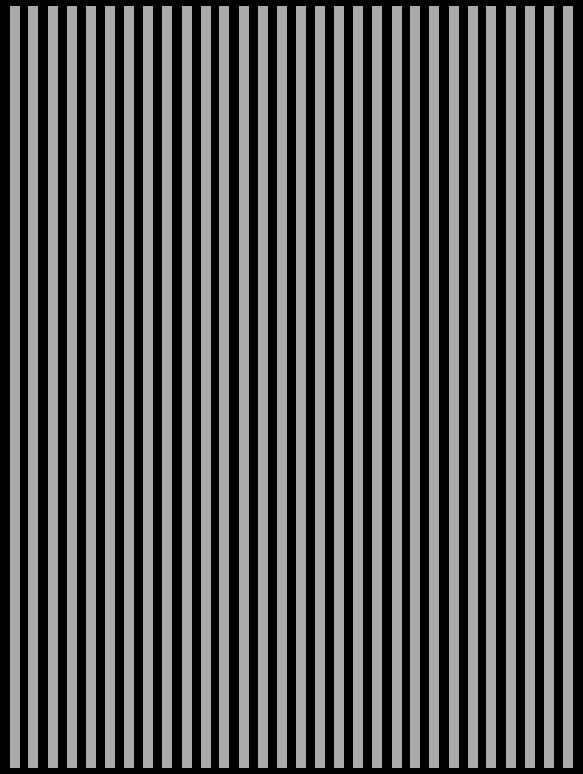
The basic SI principle: Moiré fringes

f_1 = 30 Stripes / 300 Pixels = 0.1 S/P



Illumination with known periodicity

f_2 = 30 Stripes / 270 Pixels = 0.11 S/P



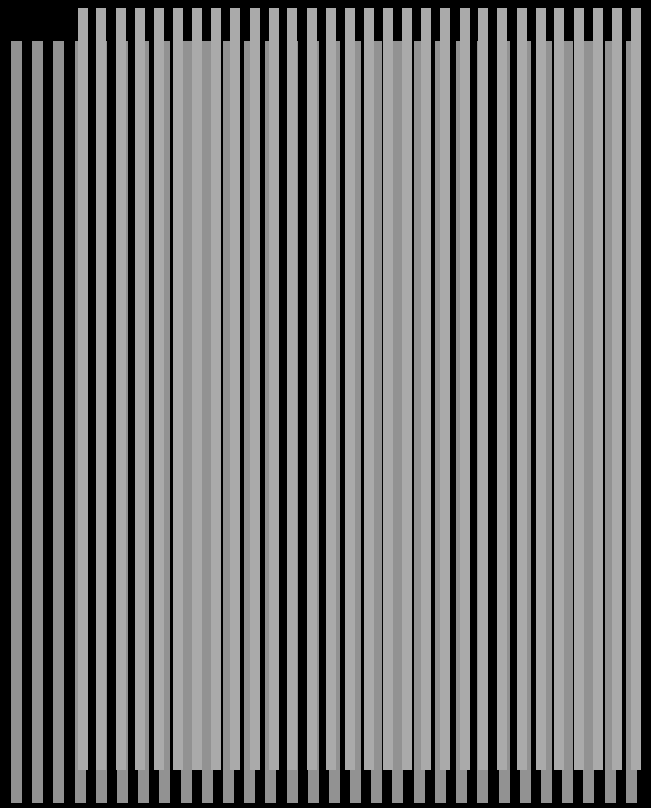
SAMPLE with "unknown" periodicity

The basic SI principle: Moiré fringes

f_1 = 30 Stripes / 300 Pixels = 0.1 S/P

f_2 = 30 Stripes / 270 Pixels = 0.11 S/P

Δf = 0.011 S/P



Illumination with known periodicity

SAMPLE with "unknown" periodicity

The basic SI principle: Moiré fringes

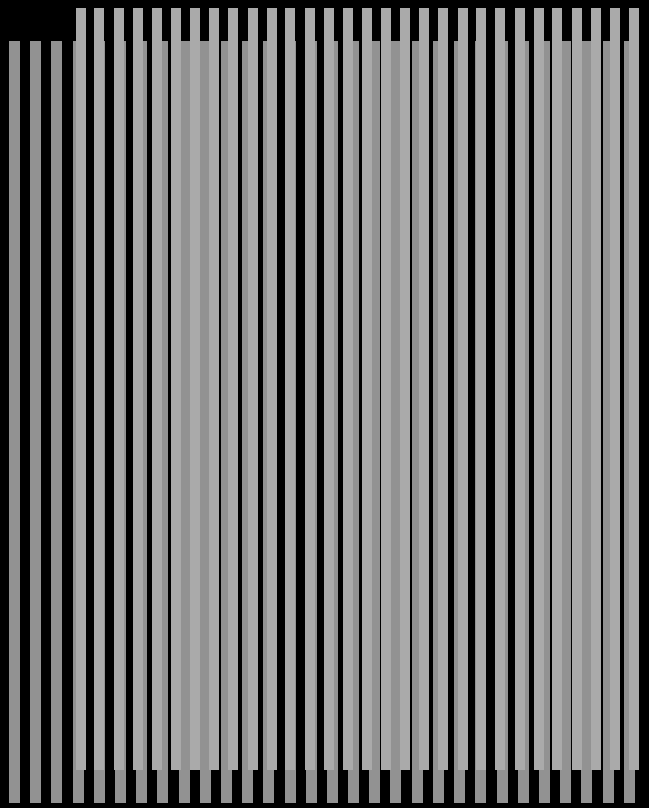
f_1 = 30 Stripes / 300 Pixels = 0.1 S/P

MOIRE-PATTERNS are generated by "multiplication" SAMPLE PERIODICITY can be calculated!

Illumination with known periodicity

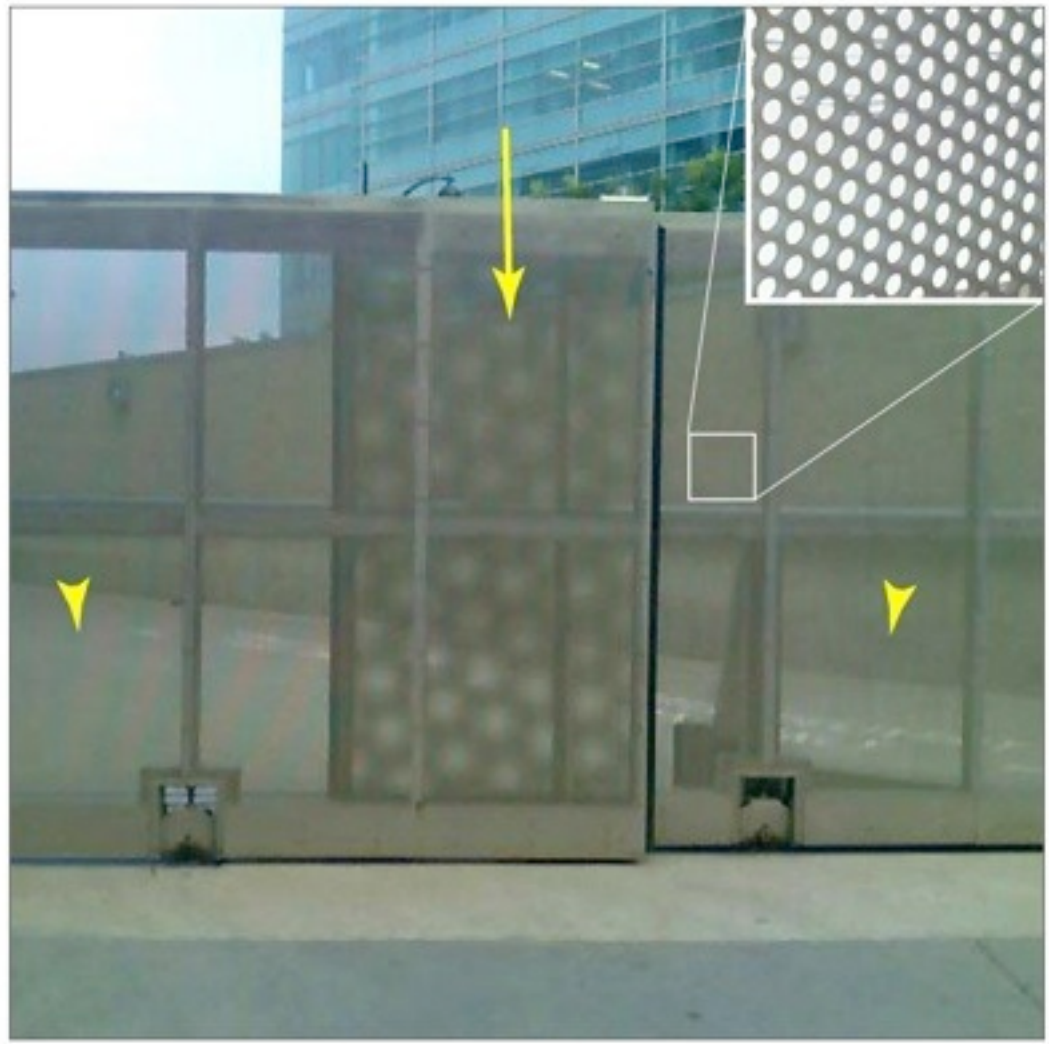
f_2 = 30 Stripes / 270 Pixels = 0.11 S/P

Δf = 0.011 S/P



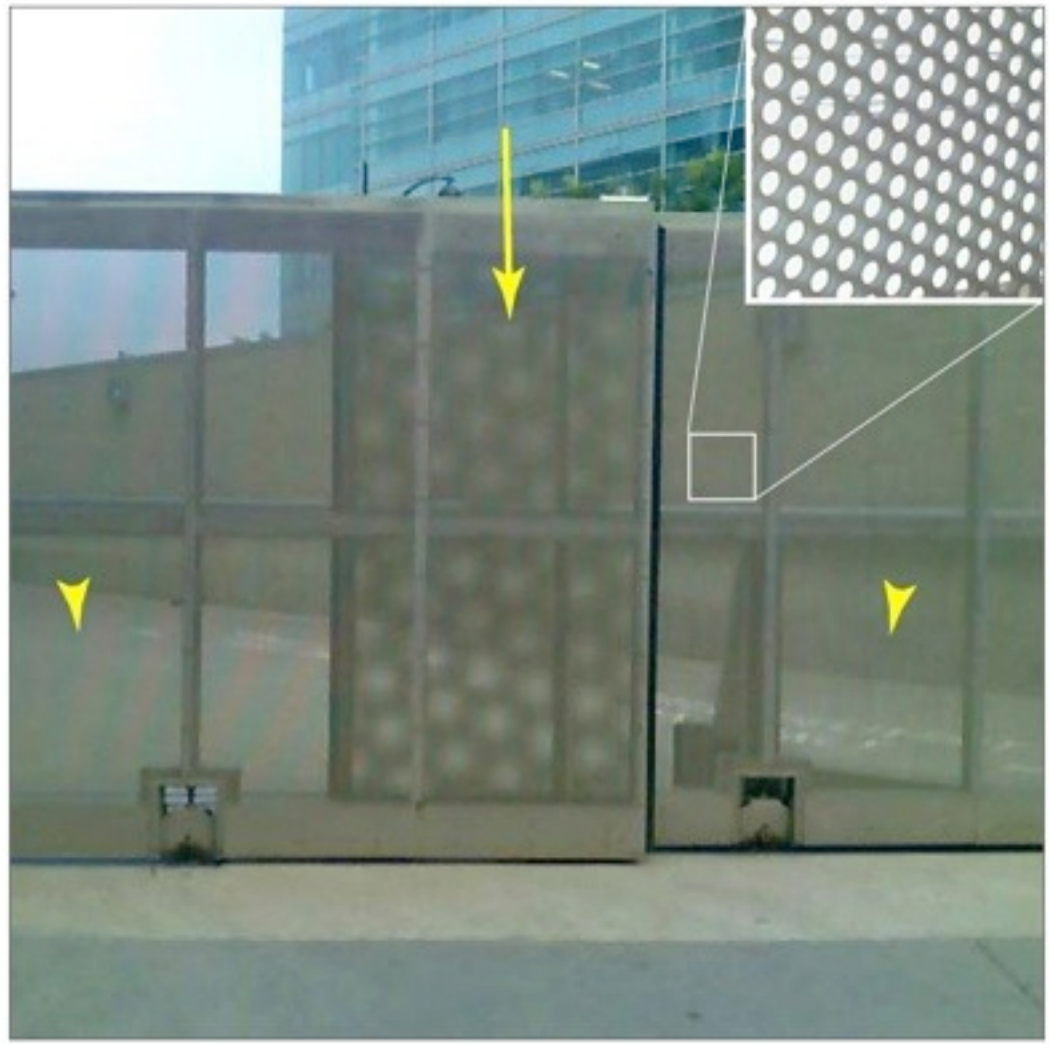
SAMPLE with "unknown" periodicity

The basic SI principle: Moiré fringes



high frequency information is shifted/encoded to a lower frequency, that can be resolved.

The basic SI principle: Moiré fringes



high frequency information is shifted/encoded to a lower frequency, that can be resolved.

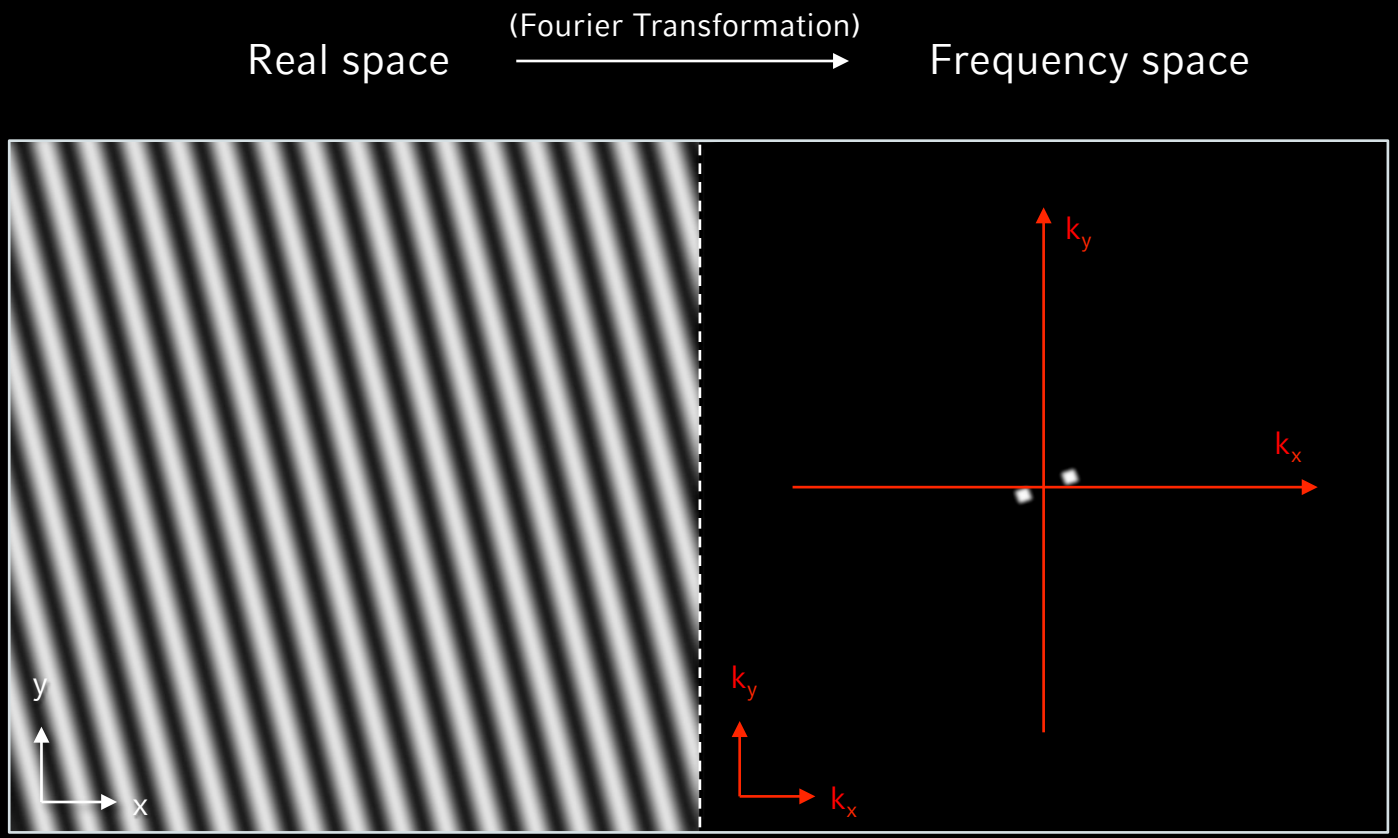
Fourier transform of the measured image

unknown structure

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

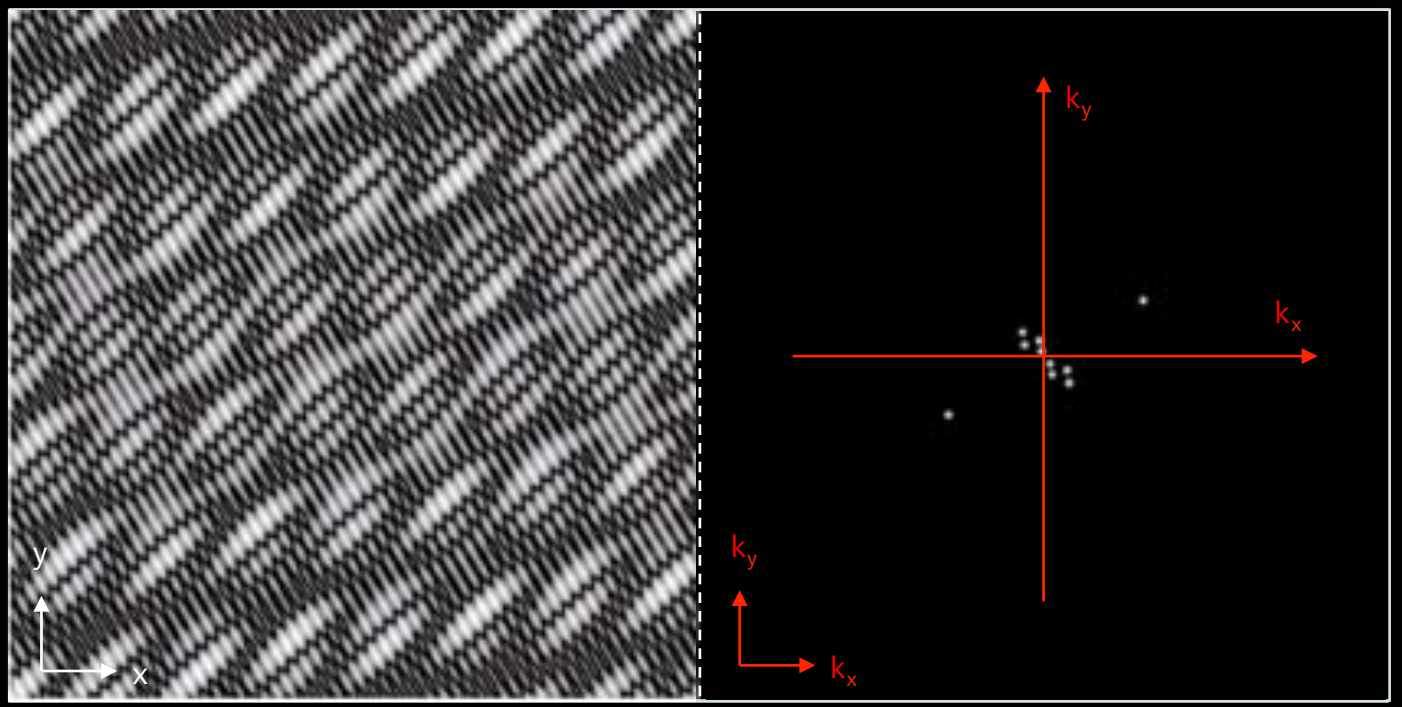
known illumination function

SI image reconstruction in Fourier space



SI image reconstruction in Fourier space

Real space (Fourier Transformation) → Frequency space



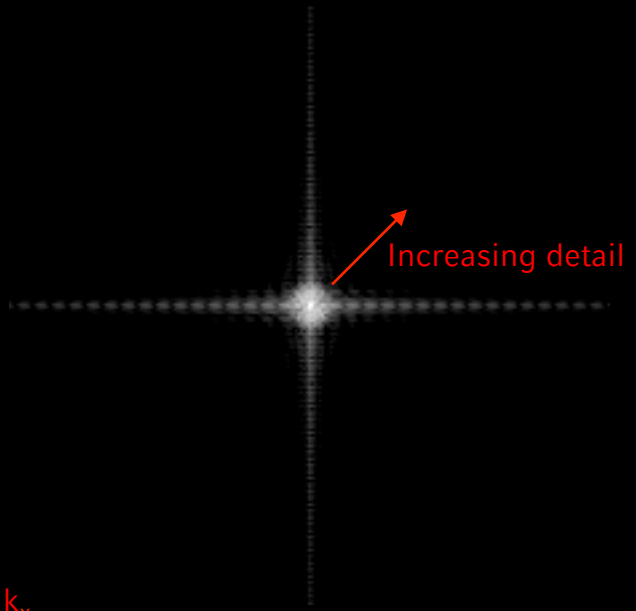
SI image reconstruction in Fourier space

Real space (Fourier Transformation) Frequency space

low NA objective

y

x



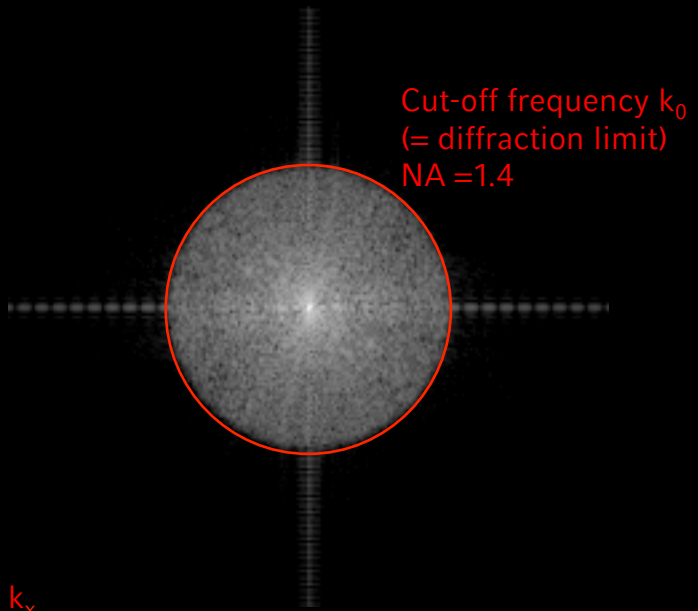
k_y

k_x

SI image reconstruction in Fourier space

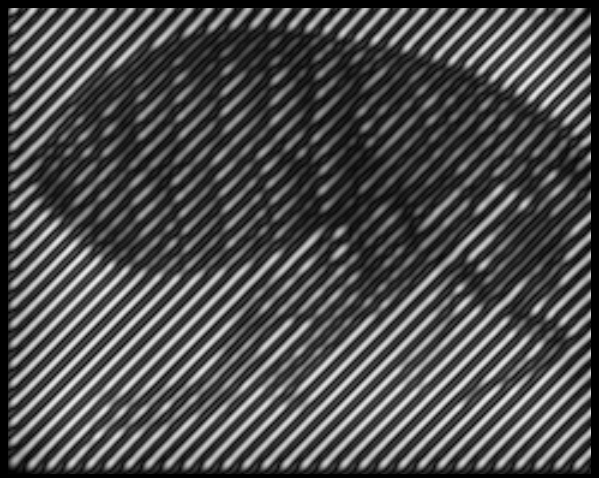
Real space (Fourier Transformation) → Frequency space

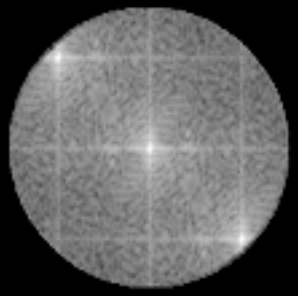
high NA objective



SI image reconstruction in Fourier space

Real space —(Fourier Transformation)→ Frequency space

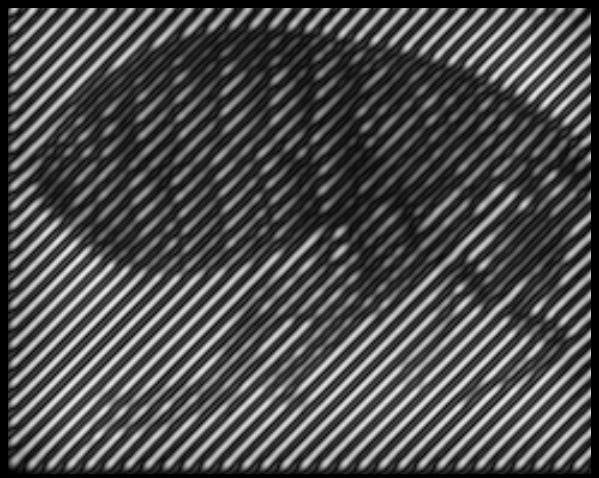


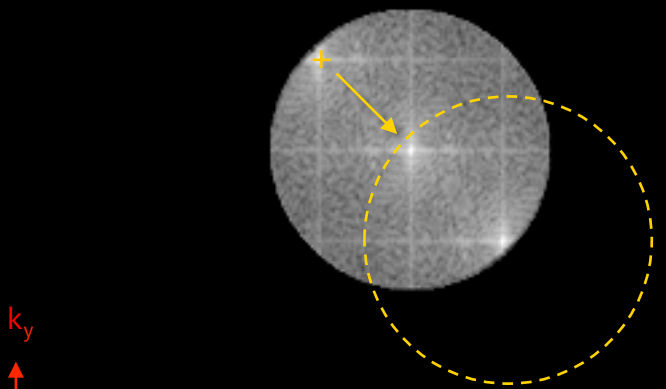


Illumination with known periodicity

SI image reconstruction in Fourier space

Real space (Fourier Transformation) Frequency space

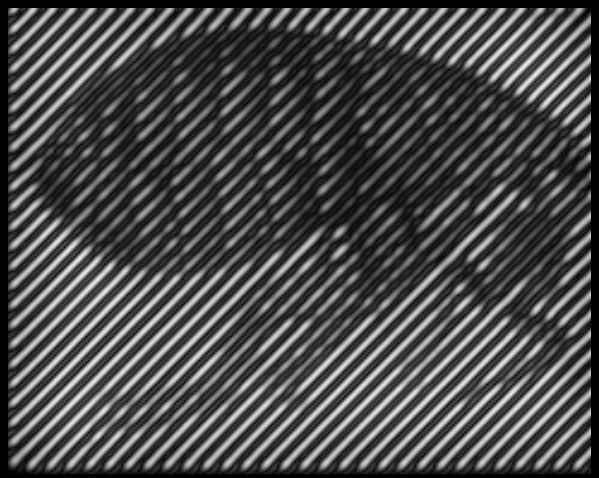


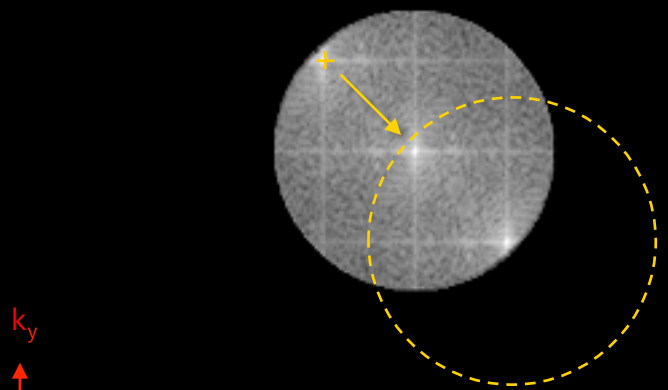


Illumination with known periodicity

SI image reconstruction in Fourier space

Real space —(Fourier Transformation)→ Frequency space



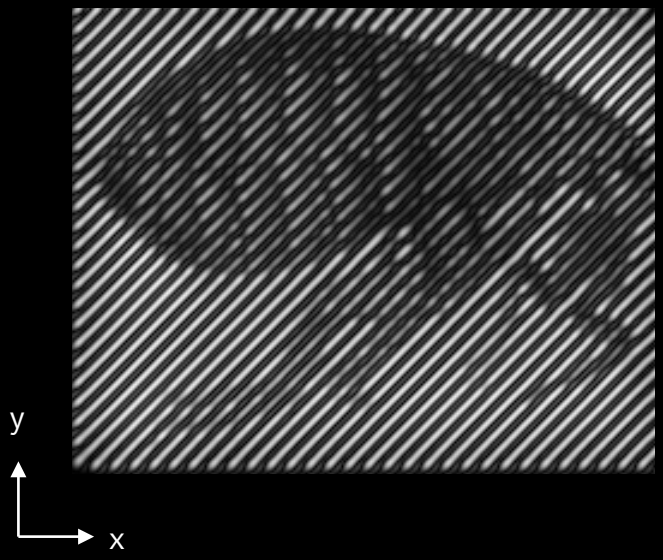


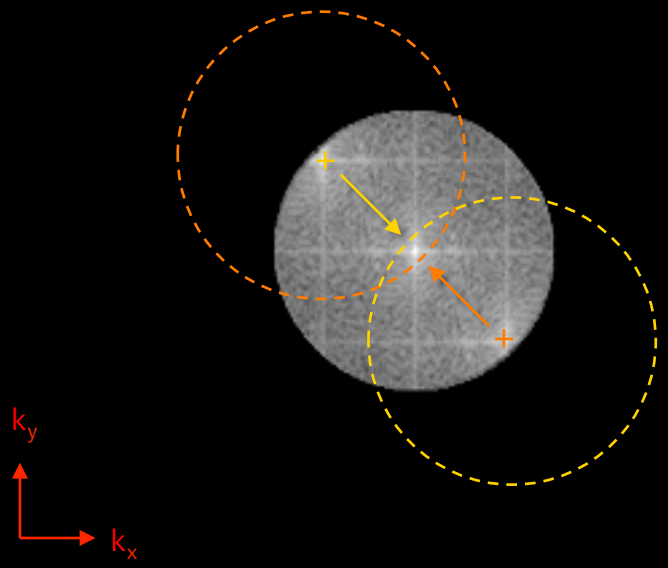
Illumination with known periodicity

"New" information is generated...

SI image reconstruction in Fourier space

Real space —(Fourier Transformation)→ Frequency space



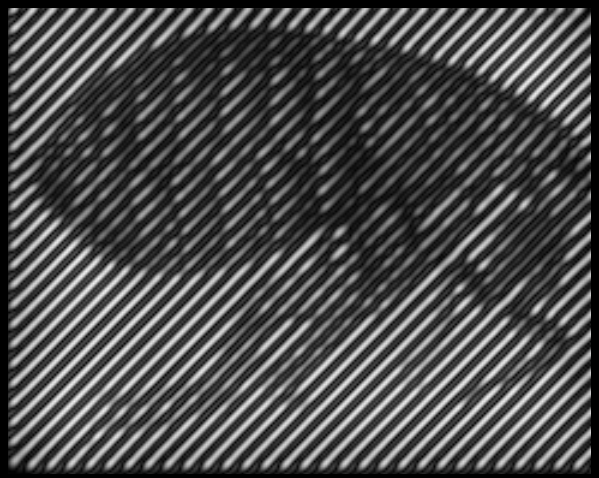


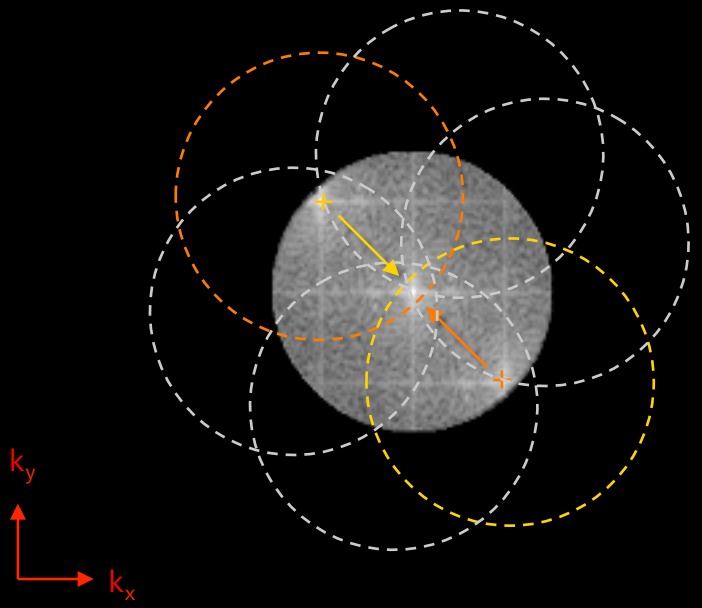
Illumination with known periodicity

"New" information is generated...

SI image reconstruction in Fourier space

Real space (Fourier Transformation) Frequency space





Illumination with known periodicity

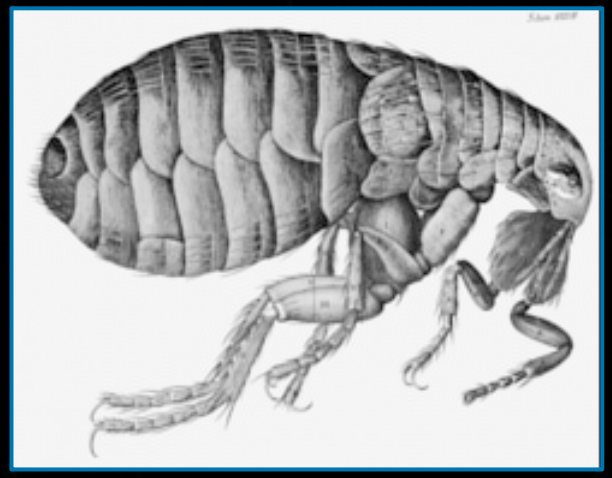
"New" information is generated...

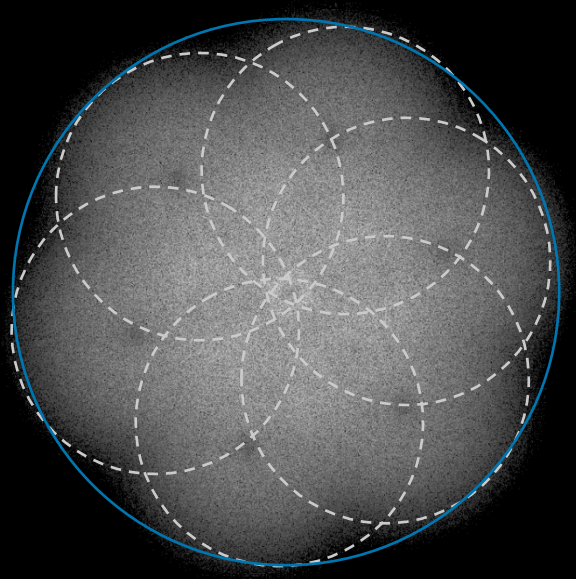
SI image reconstruction in Fourier space

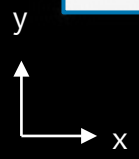
Real space ⟷ Frequency space

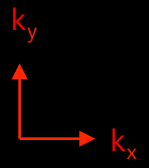
(Fourier Transformation) →

← (reverse FT)







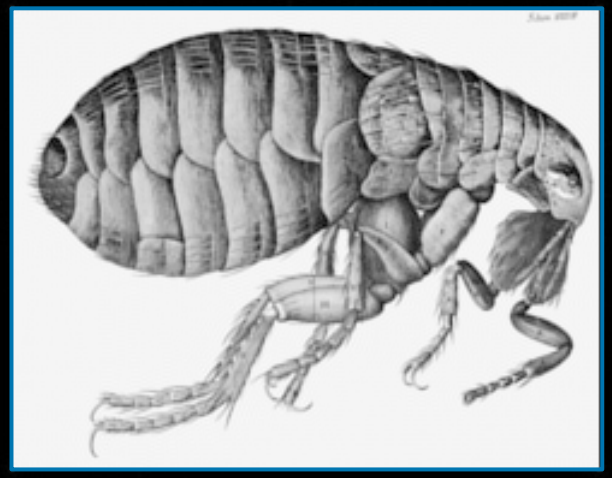


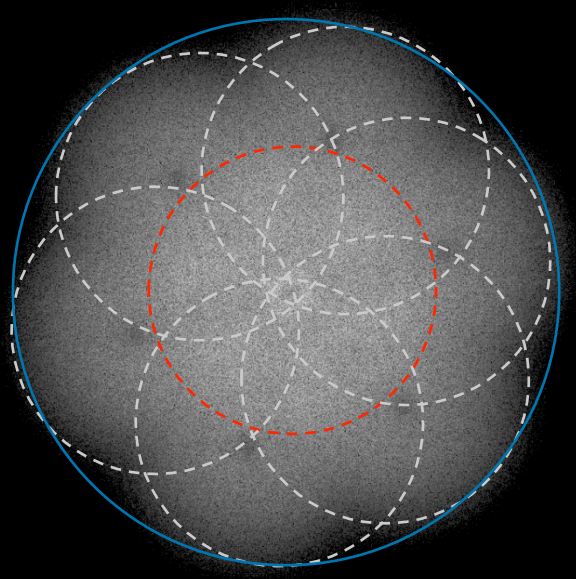
Equivalent to NA=2.35

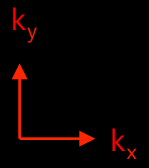
SI image reconstruction in Fourier space

Real space ⟶ (Fourier Transformation) ⟶ Frequency space

⟵ (reverse FT) ⟵





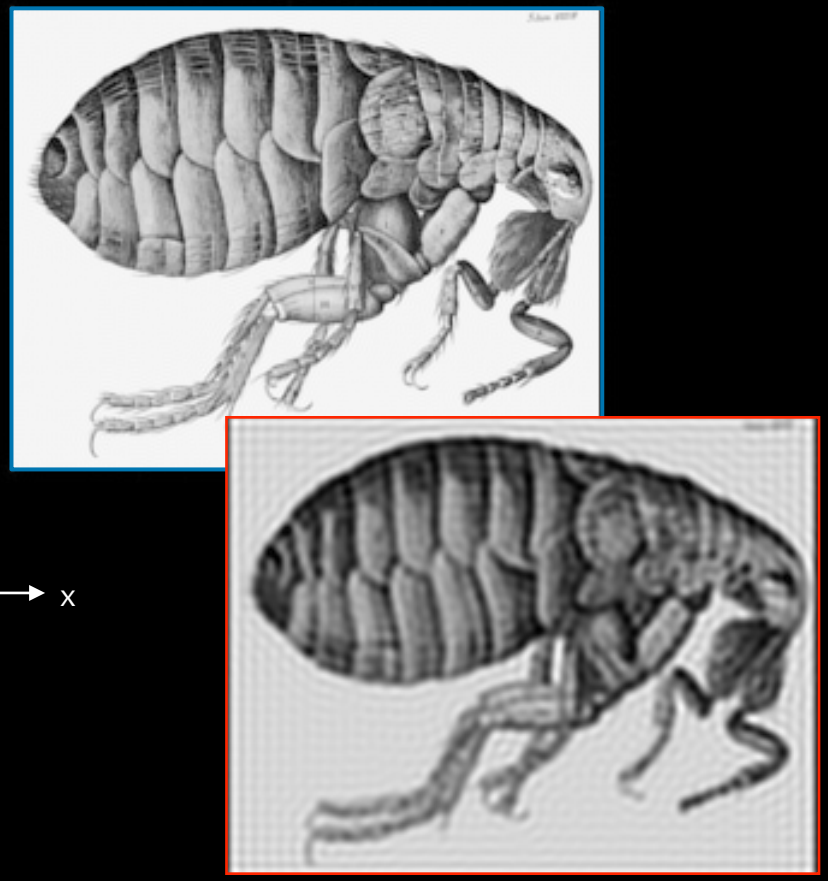


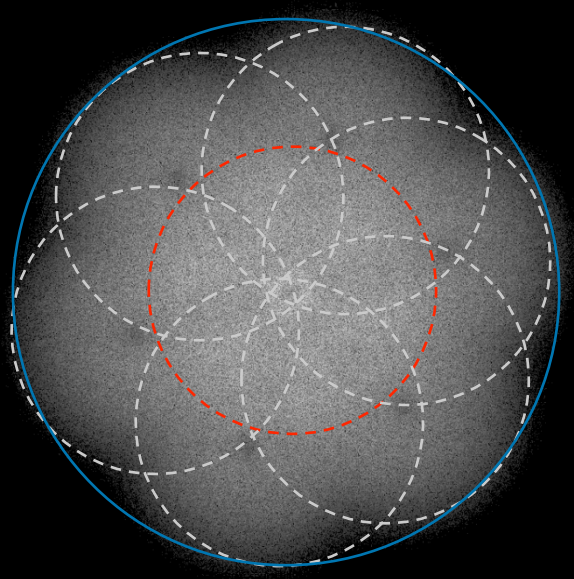
Equivalent to NA=2.35

SI image reconstruction in Fourier space

Real space ⟶ (Fourier Transformation) ⟶ Frequency space

⟵ (reverse FT) ⟵

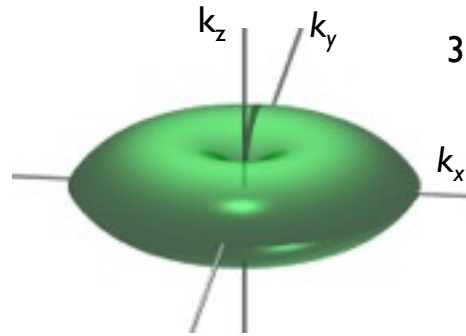




Equivalent to NA=2.35

Support in 3D-frequency space

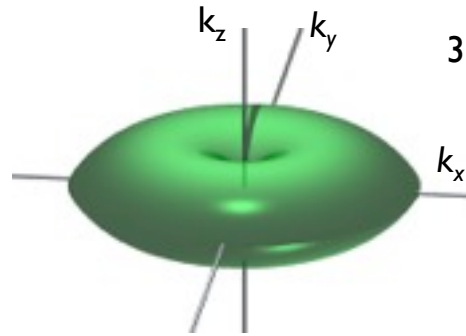
Widefield illumination



3D rendering illustrates cut-off frequency
(= Resolution limit)

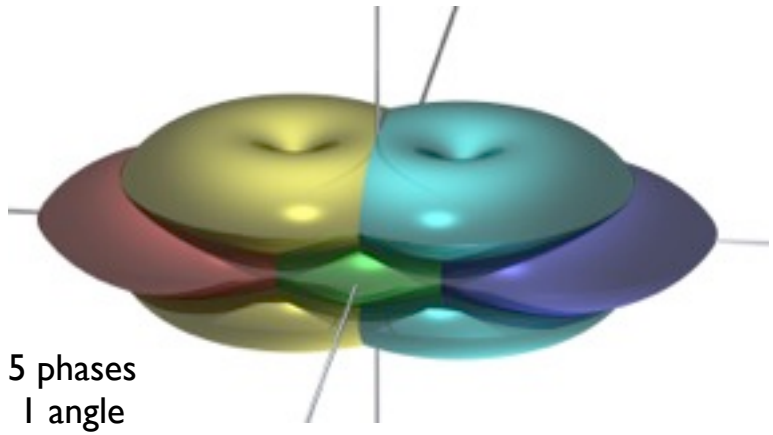
Support in 3D-frequency space

Widefield illumination

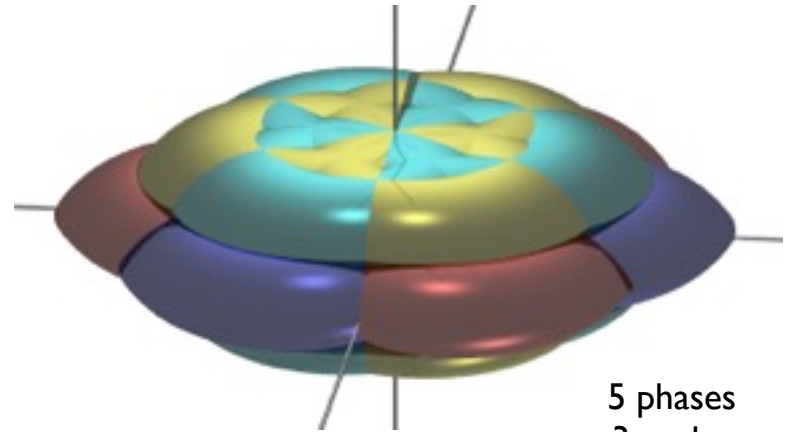


3D rendering illustrates cut-off frequency
(= Resolution limit)

3D Structured illumination



5 phases
1 angle

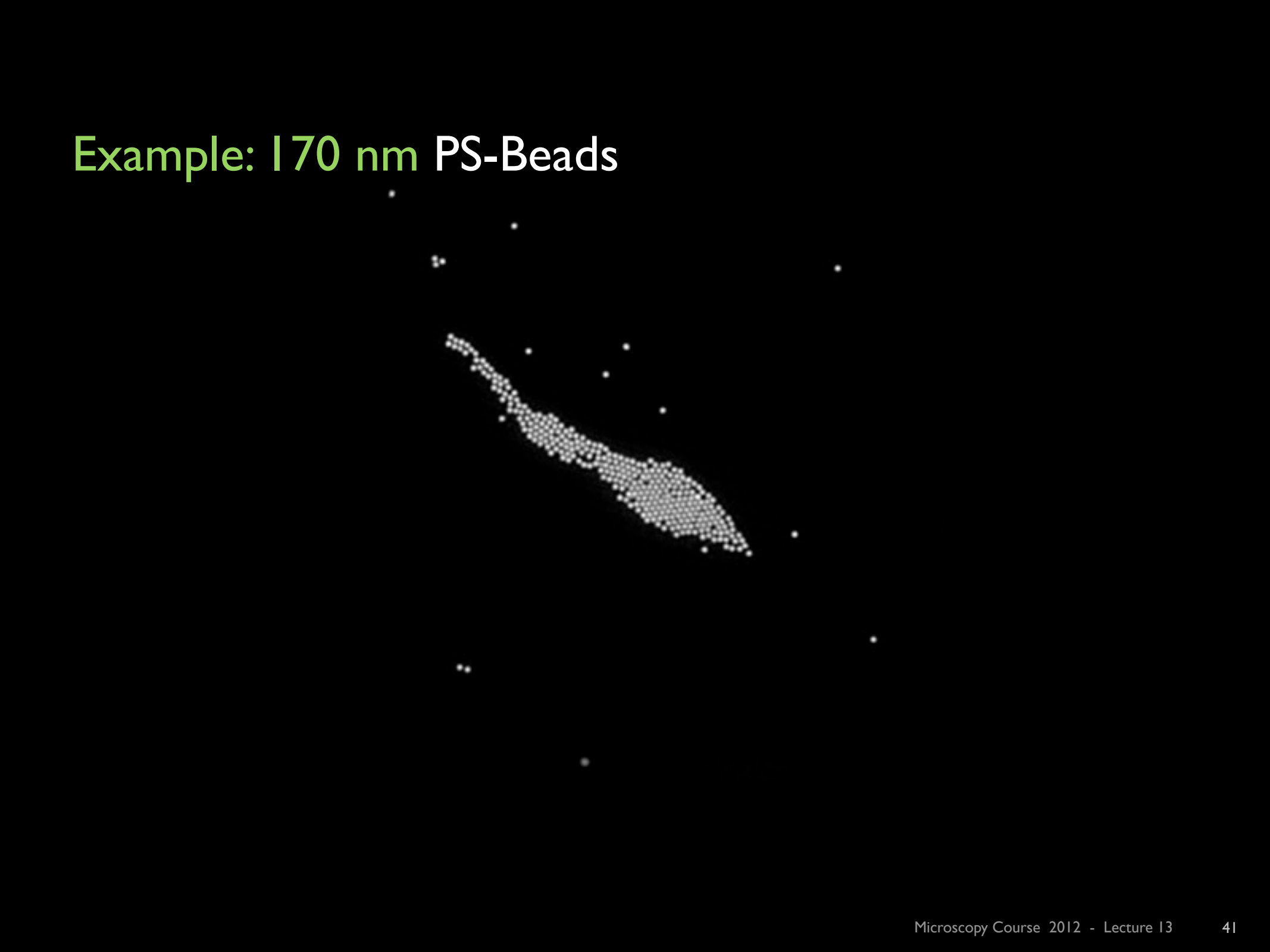


5 phases
3 angles

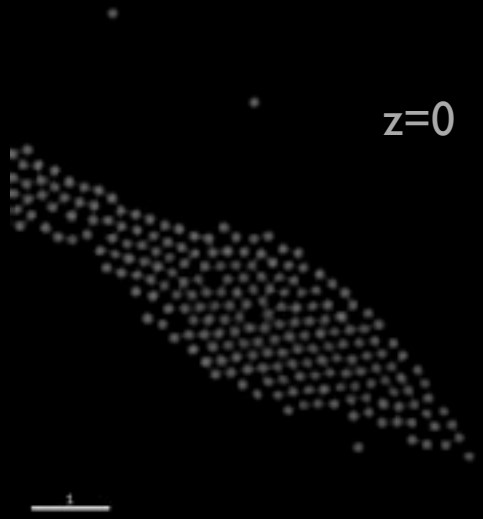
Filling of "missing cone" -> z-sectioning
2x higher lateral and axial resolution

Example: 170 nm PS-Beads

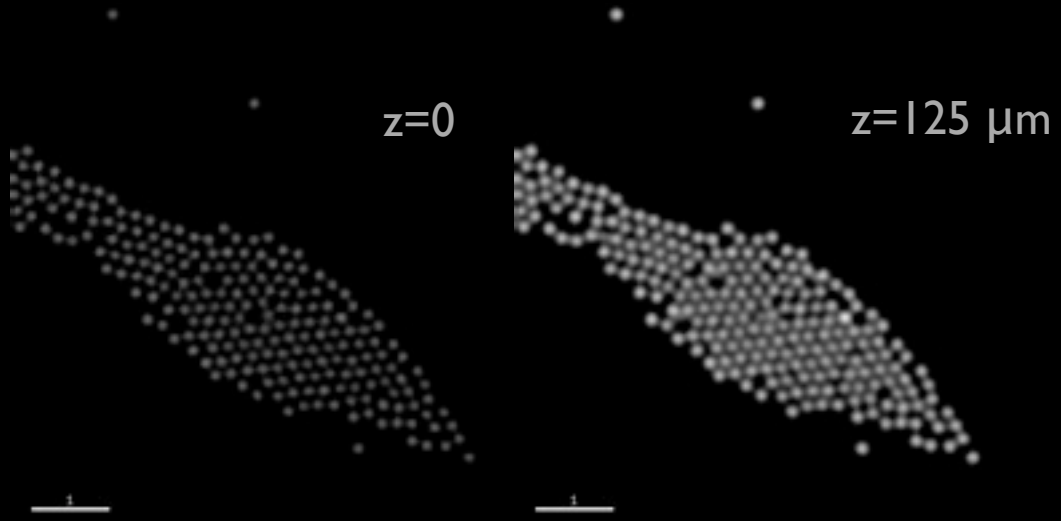
Example: 170 nm PS-Beads



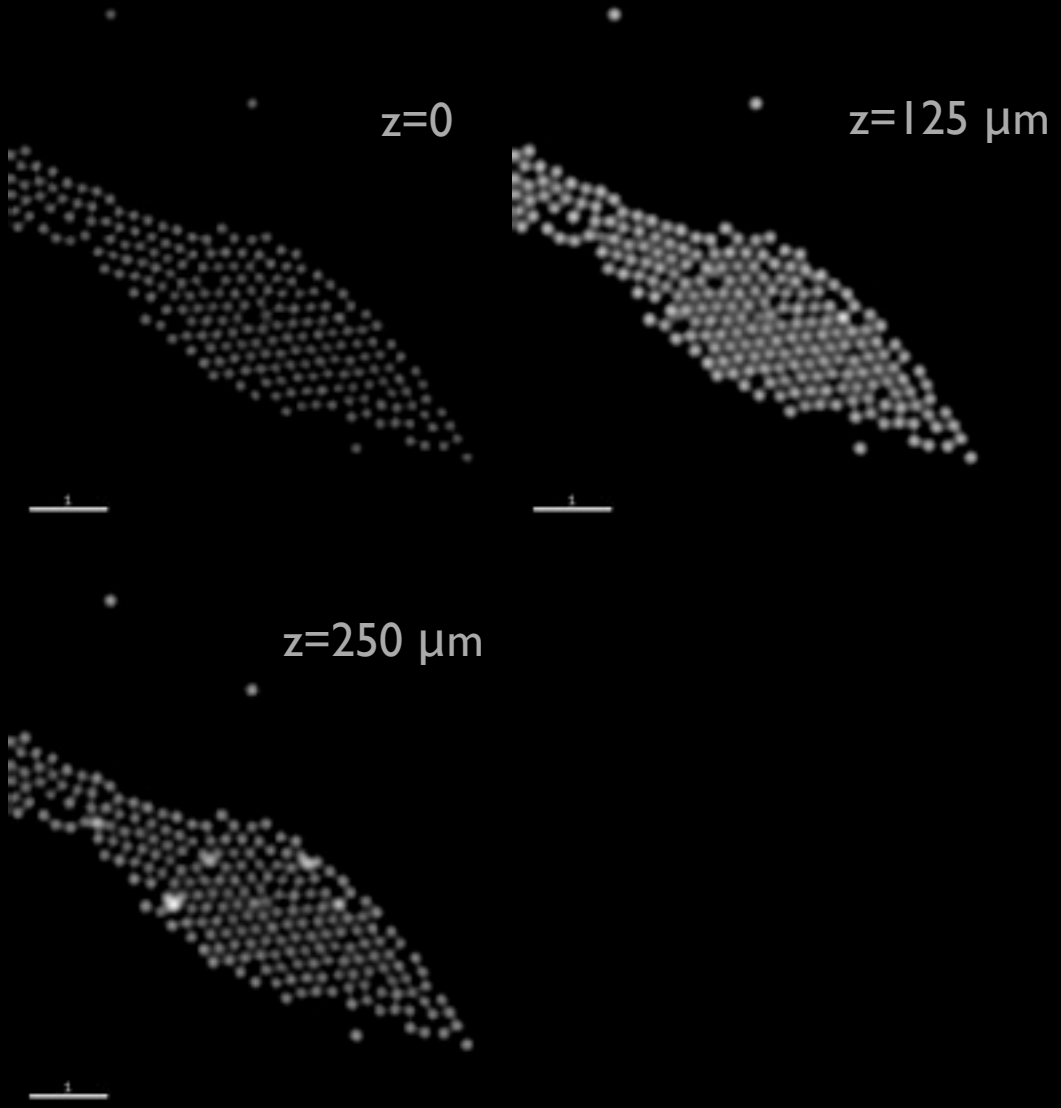
170 nm PS-Beads



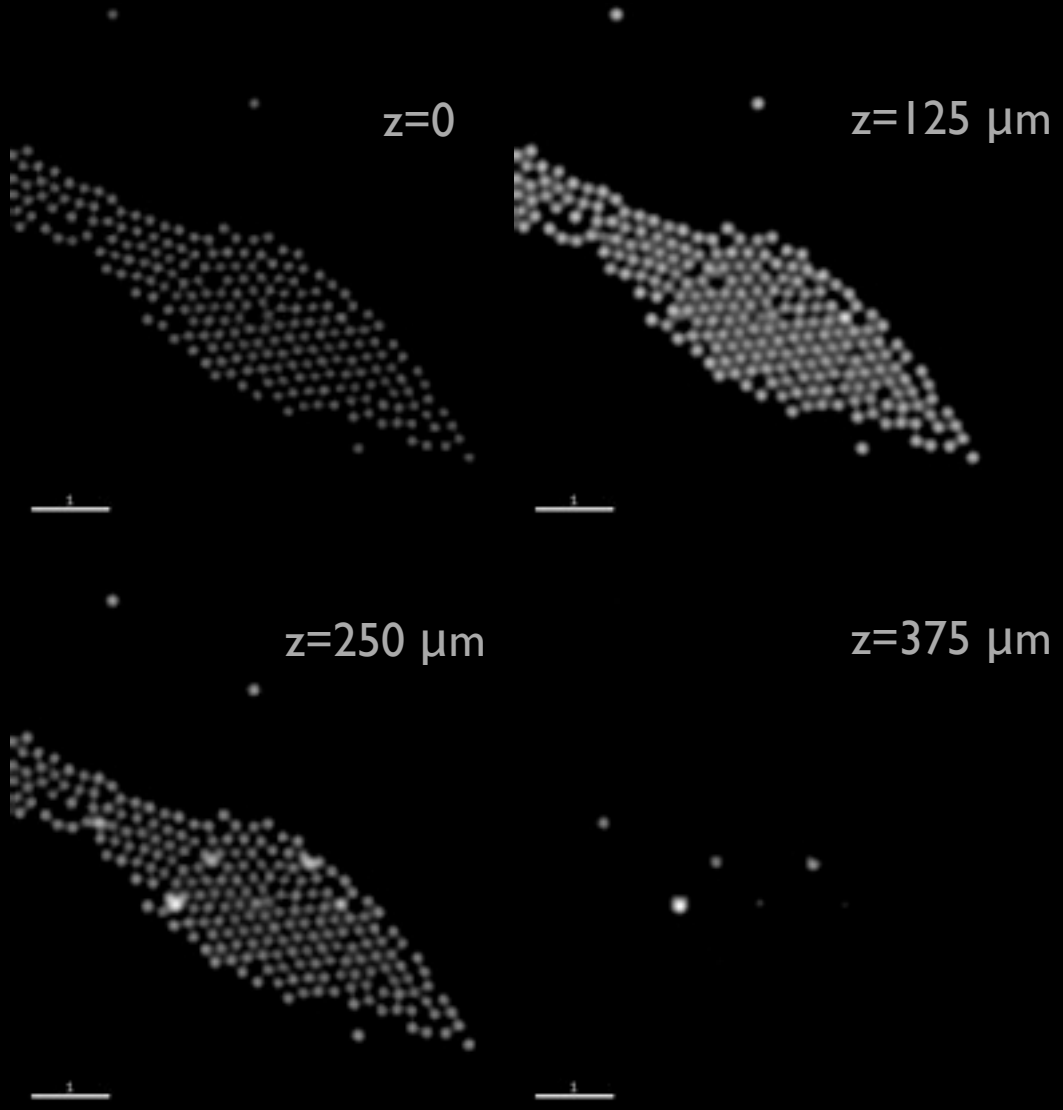
170 nm PS-Beads



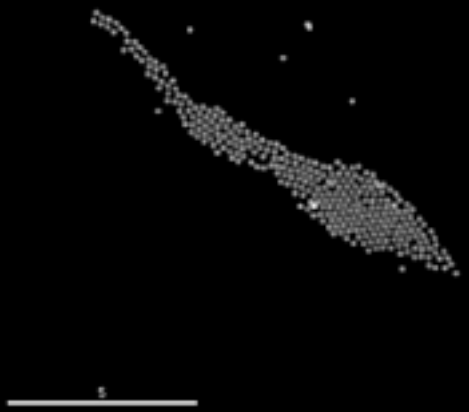
170 nm PS-Beads

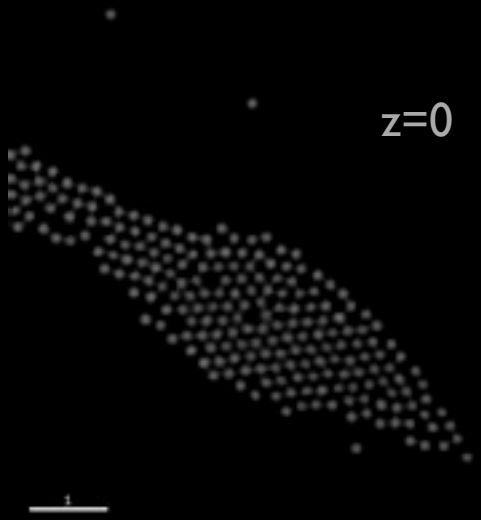


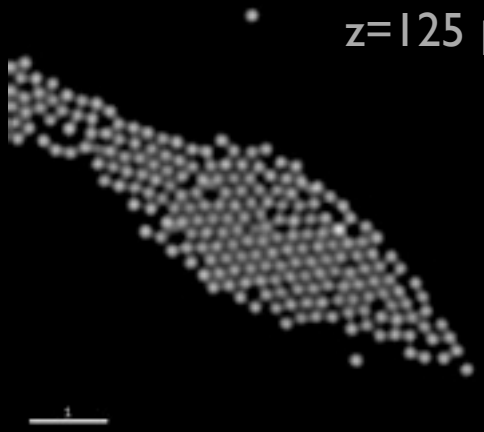
170 nm PS-Beads

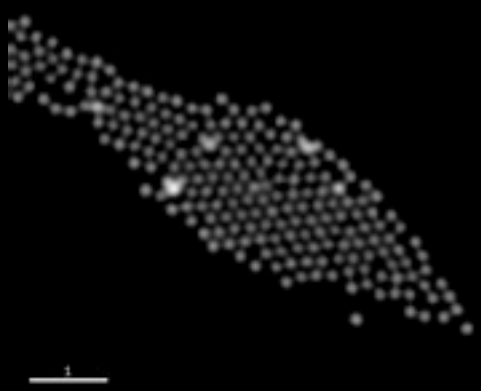


170 nm PS-Beads

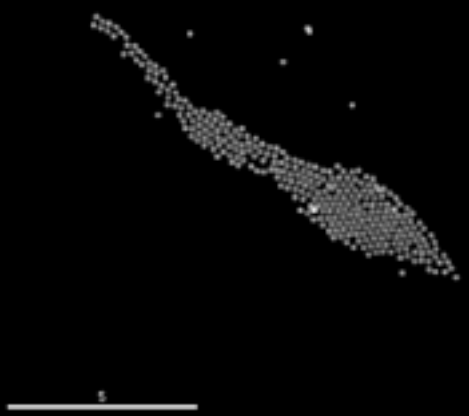


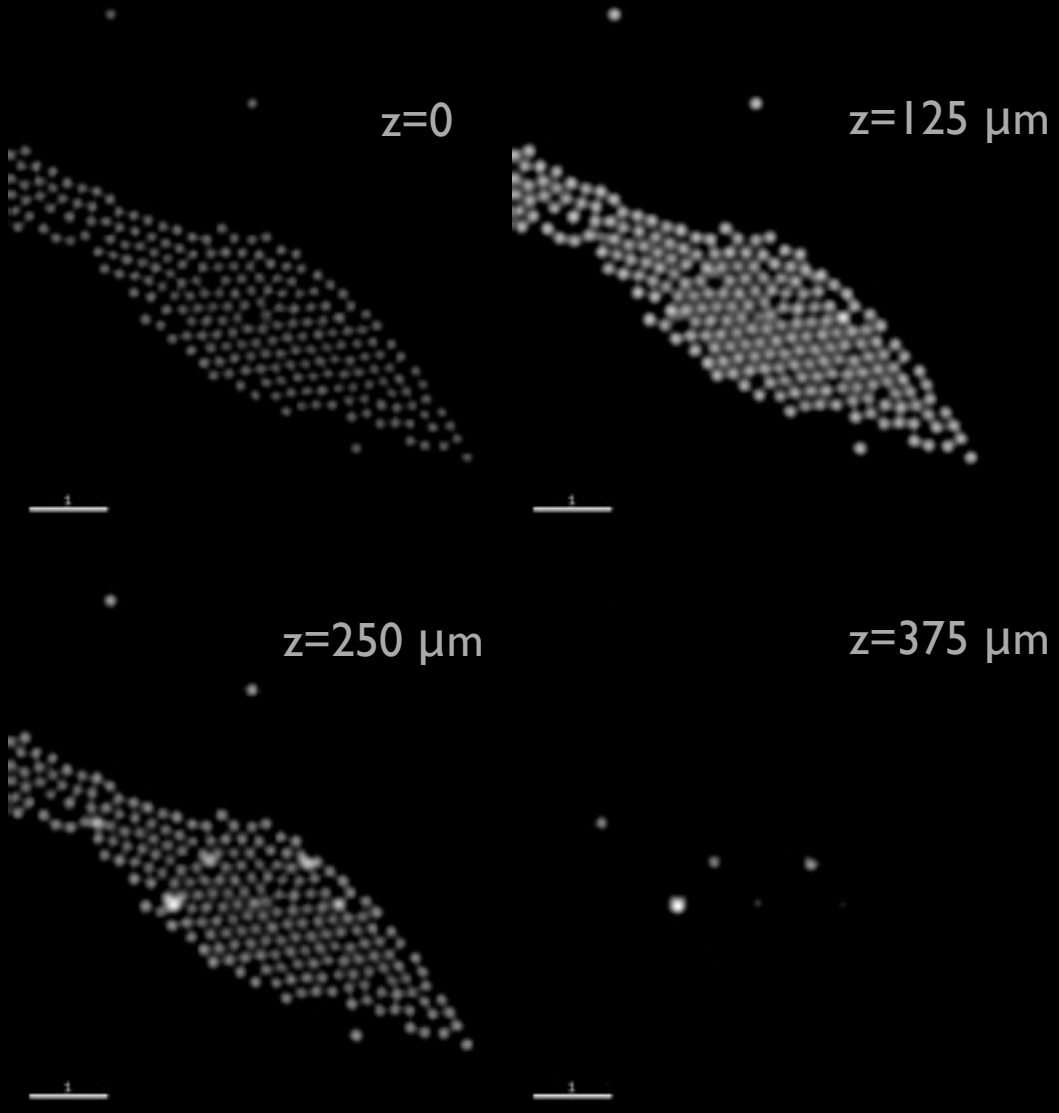






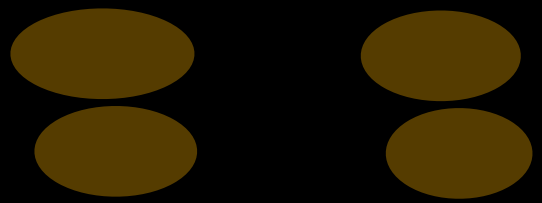
170 nm PS-Beads



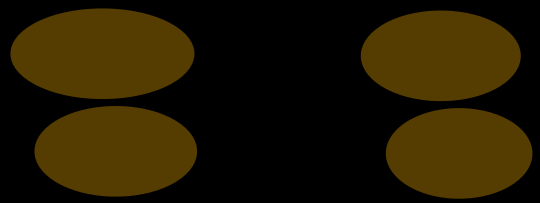


High sectioning quality!

What could this be?

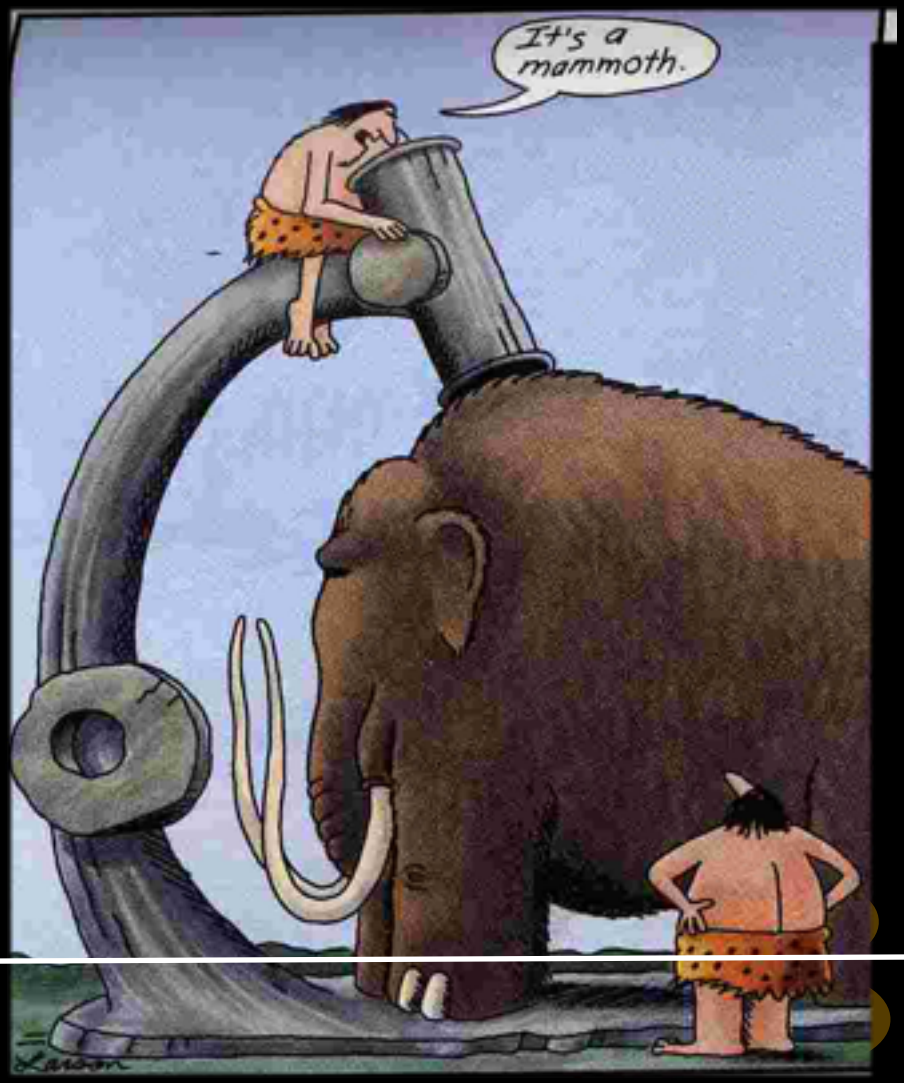


What could this be?



3D information (z-res., optical sectioning, z-depth) !

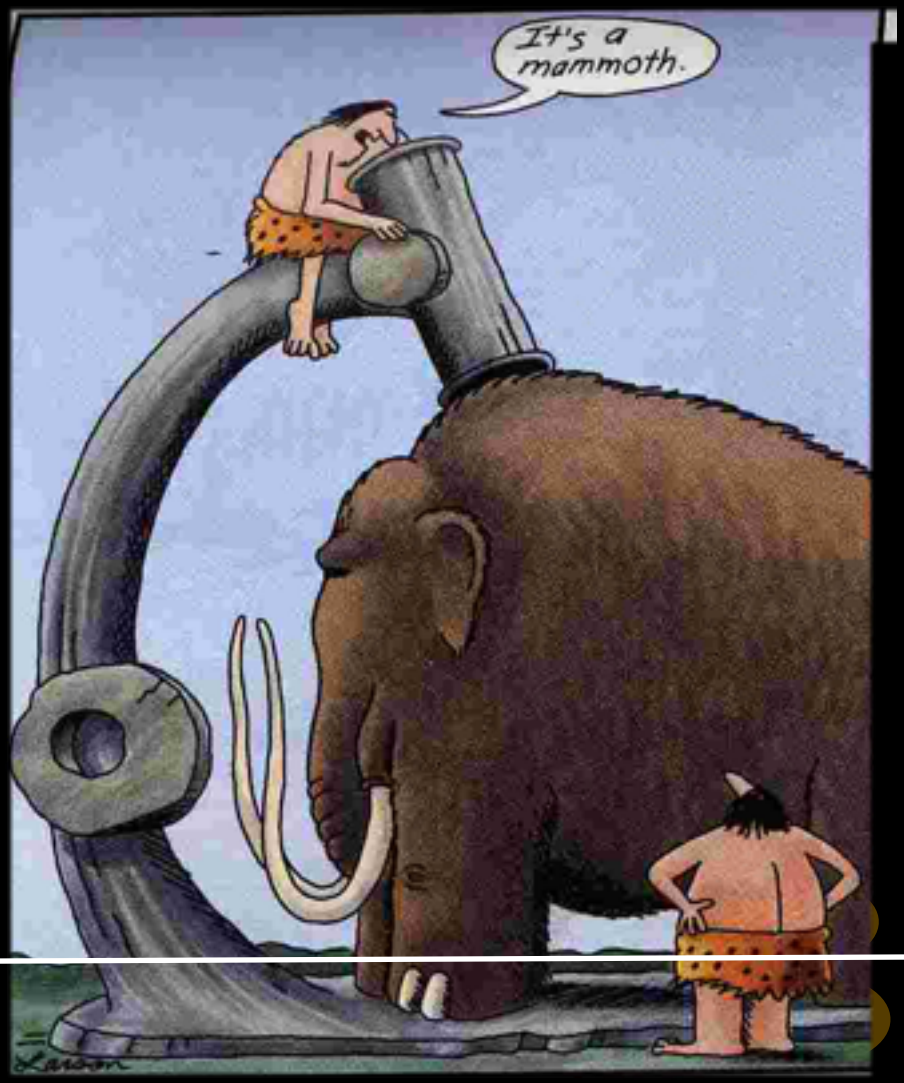
What could this be?



3D information (z-res., optical sectioning, z-depth) !

Not only resolution but also context matters! (I)

What could this be?

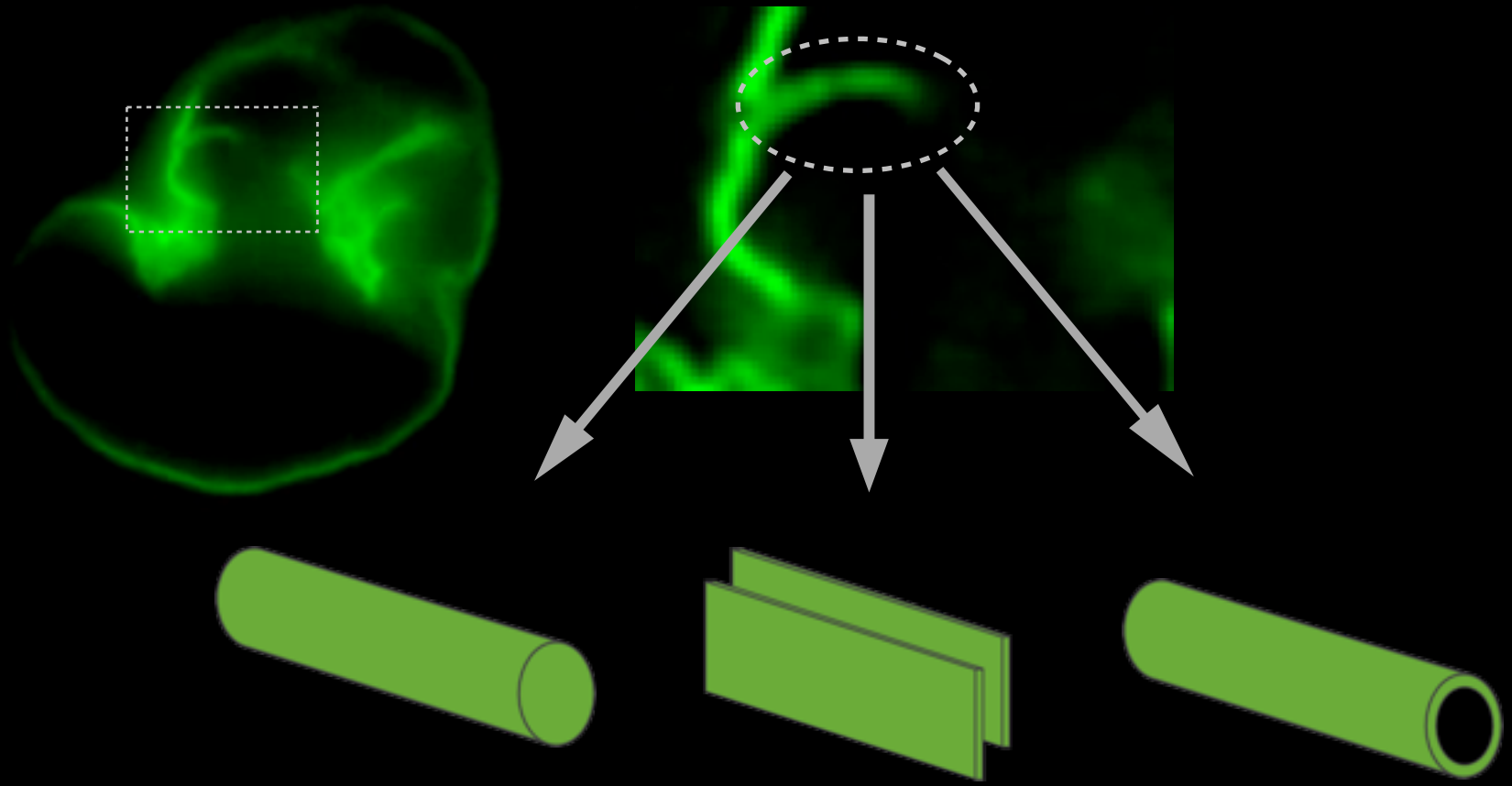


3D information (z-res., optical sectioning, z-depth) !

Not only resolution but also context matters! (III)

Wide-field

Lamin B



Mouse C2C12 cell

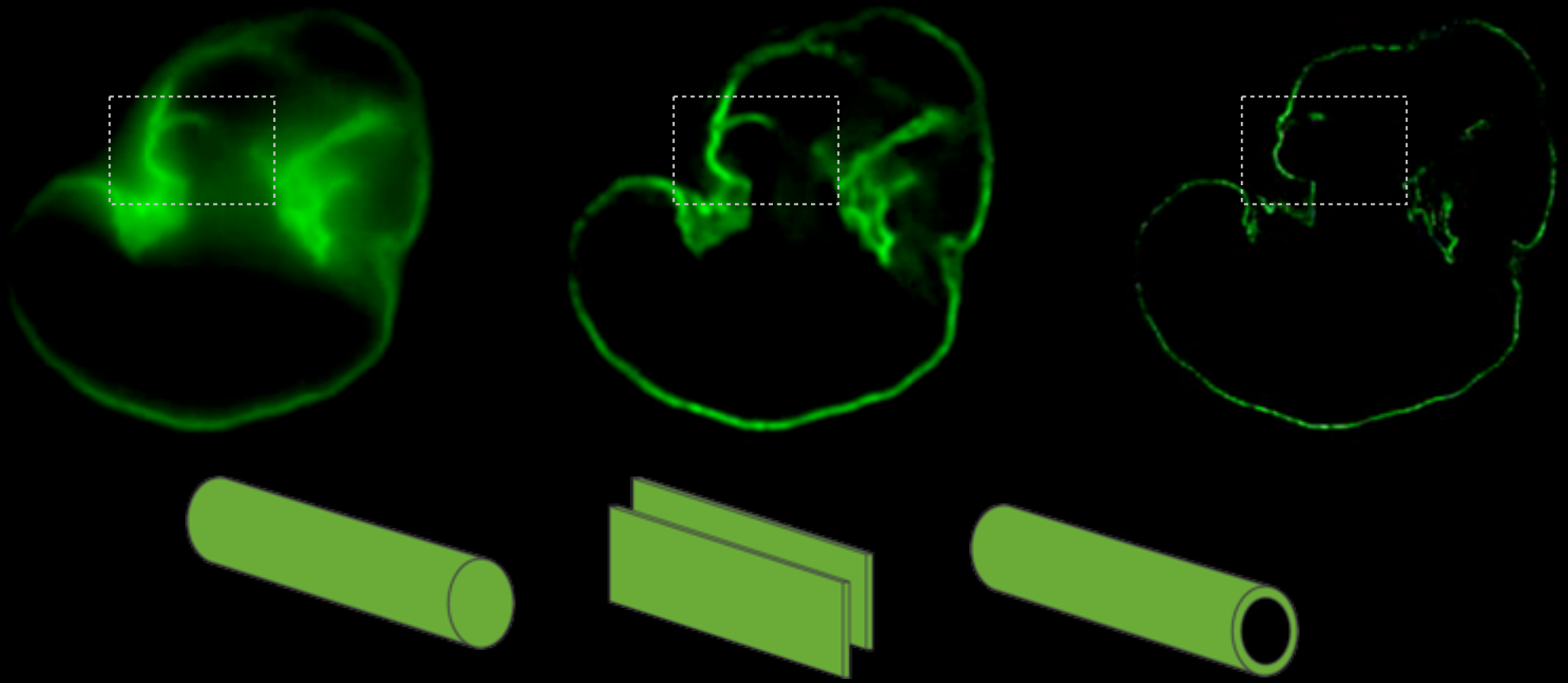
Not only resolution but also context matters! (III)

Wide-field

Wide-field deconvolution

3D-SIM

Lamin B



Mouse C2C12 cell

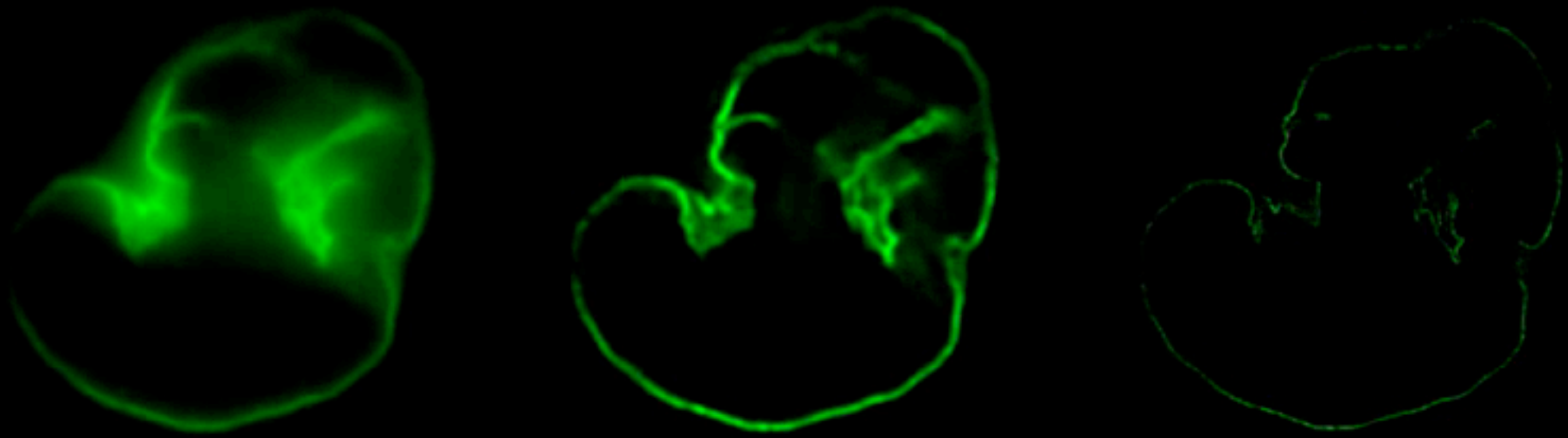
Not only resolution but also context matters! (III)

Wide-field

Wide-field deconvolution

3D-SIM

Lamin B



3D context by z-sectioning...

Not only resolution but also context matters! (III)

Wide-field

Wide-field deconvolution

3D-SIM

Lamin B
Tubulin
DAPI

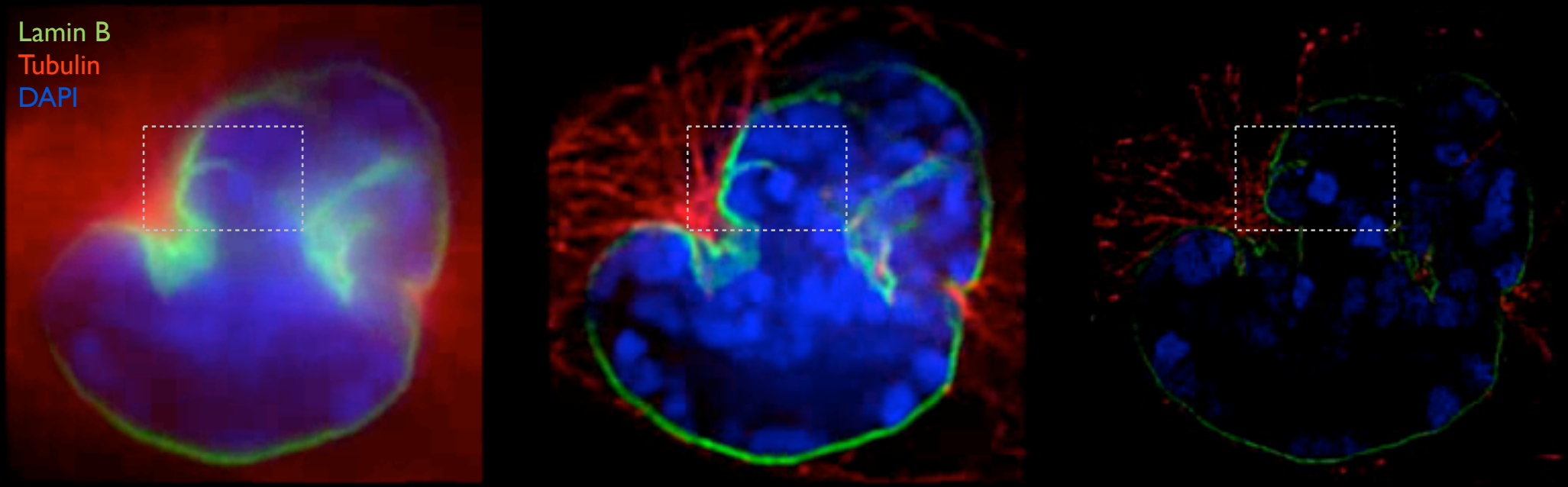
....more context by 3D multicolor sectioning

Not only resolution but also context matters! (III)

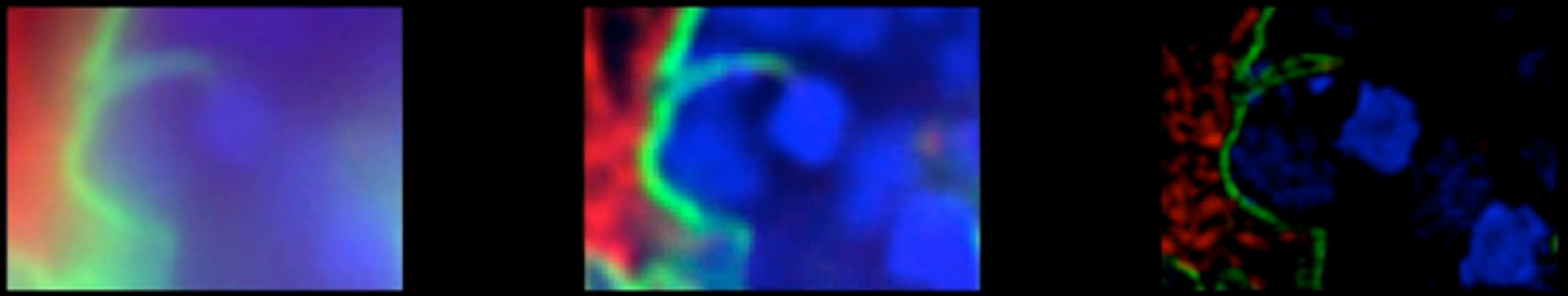
Wide-field

Wide-field deconvolution

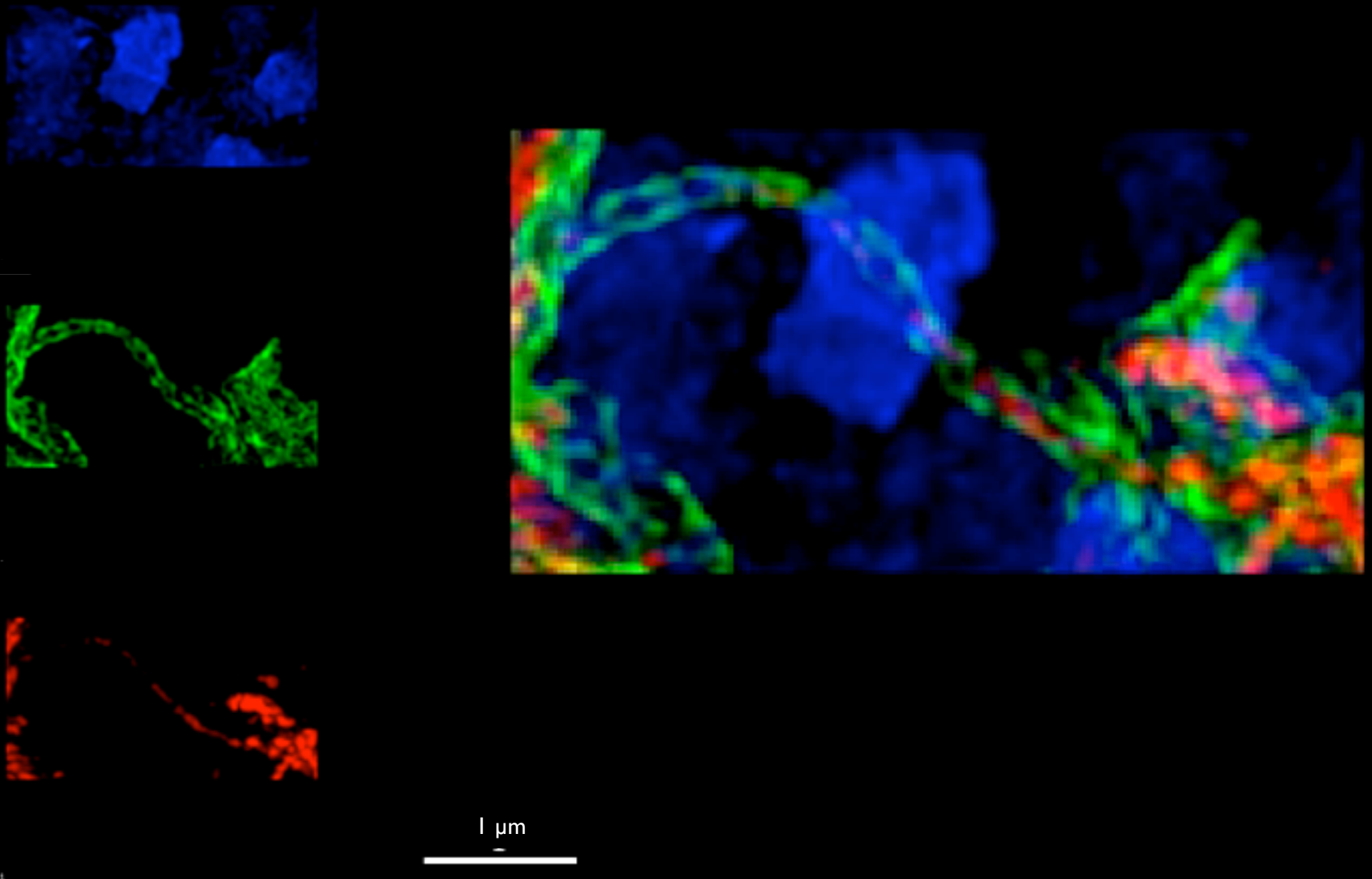
3D-SIM



....more context by 3D multicolor sectioning



Not only resolution but also context matters! (III)



3D SIM example: Prophase

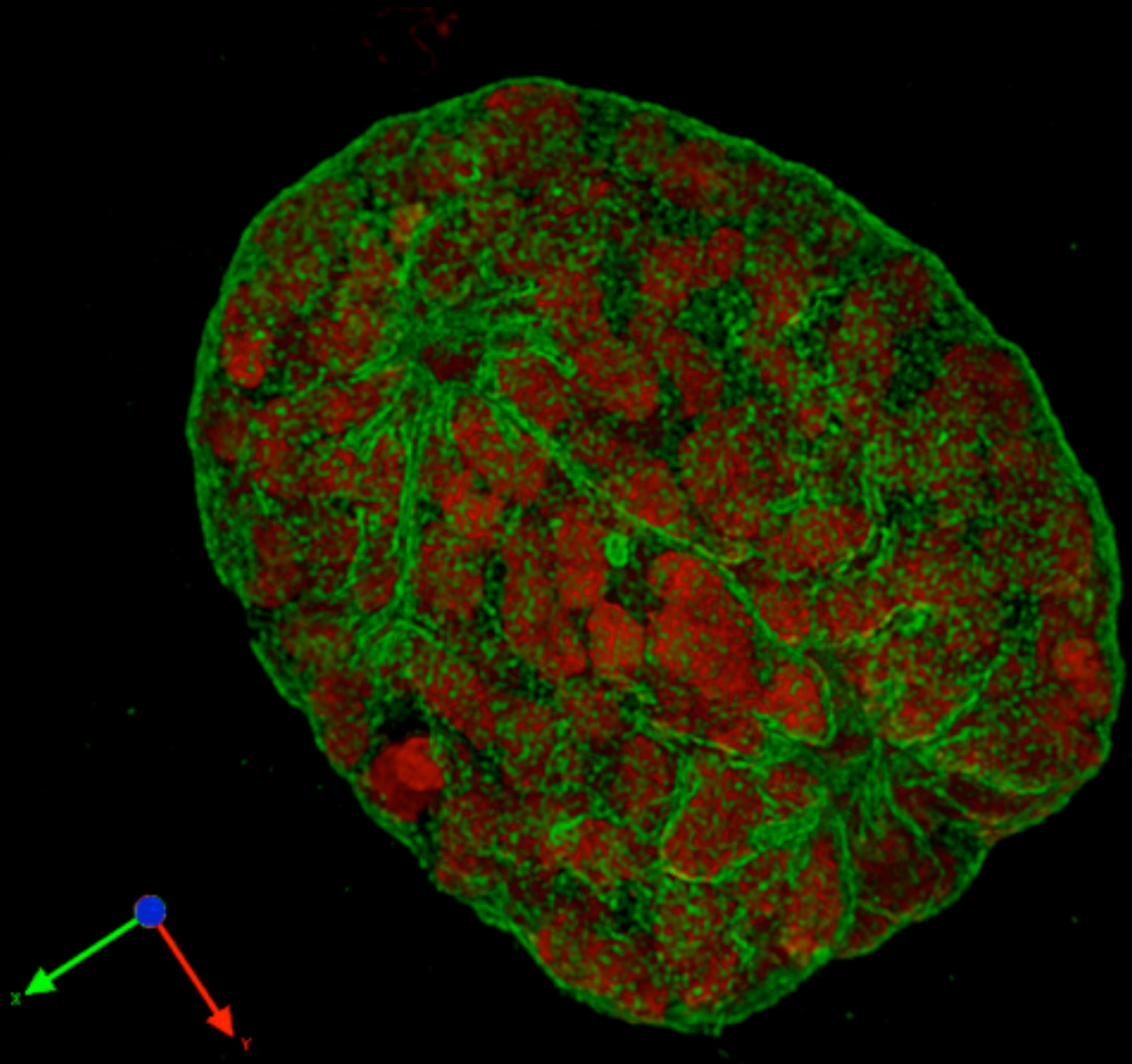
Lamin B
DAPI

3D volume
rendering

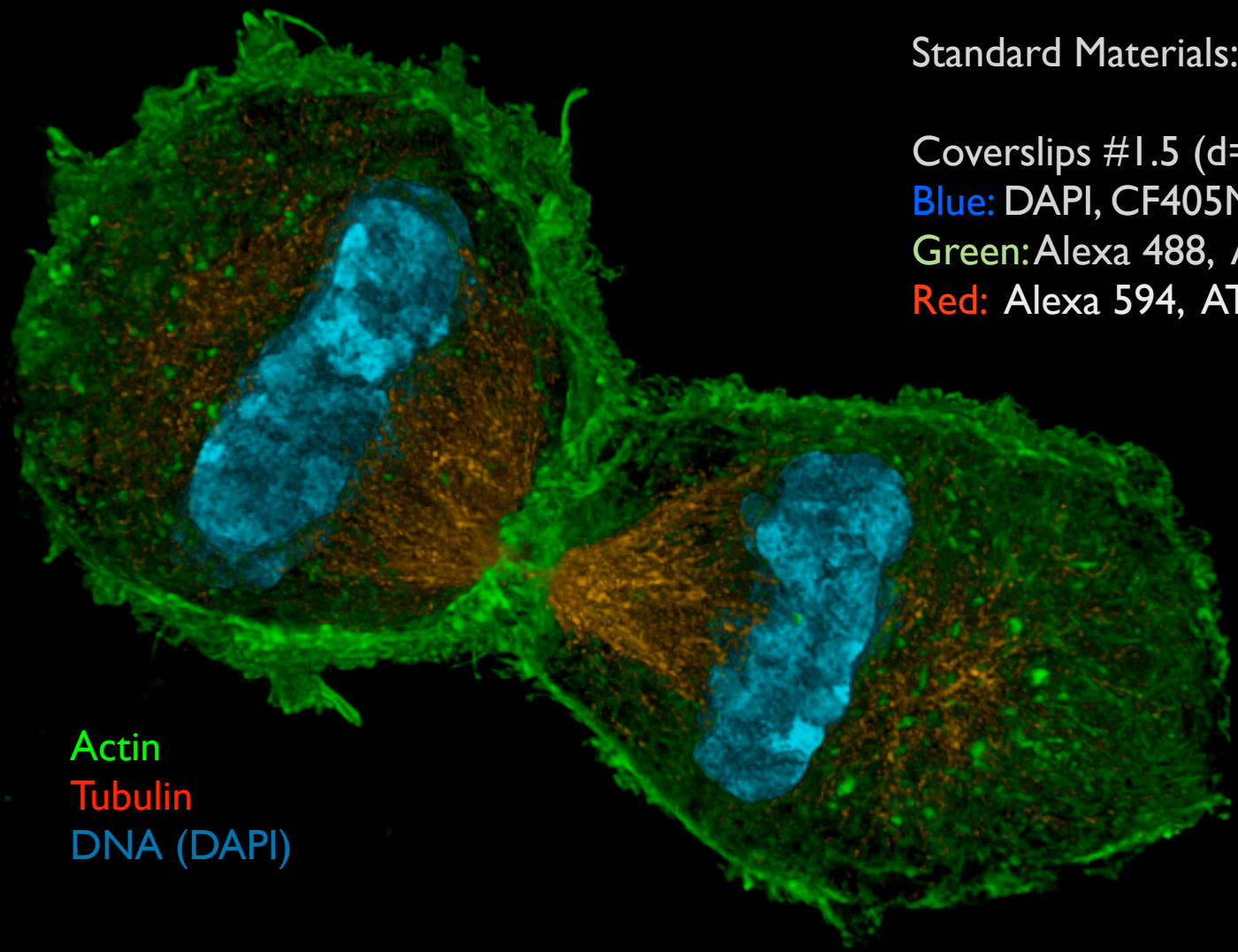
3D SIM example: Prophase

Lamin B
DAPI

3D volume rendering



3D SIM example: Telophase

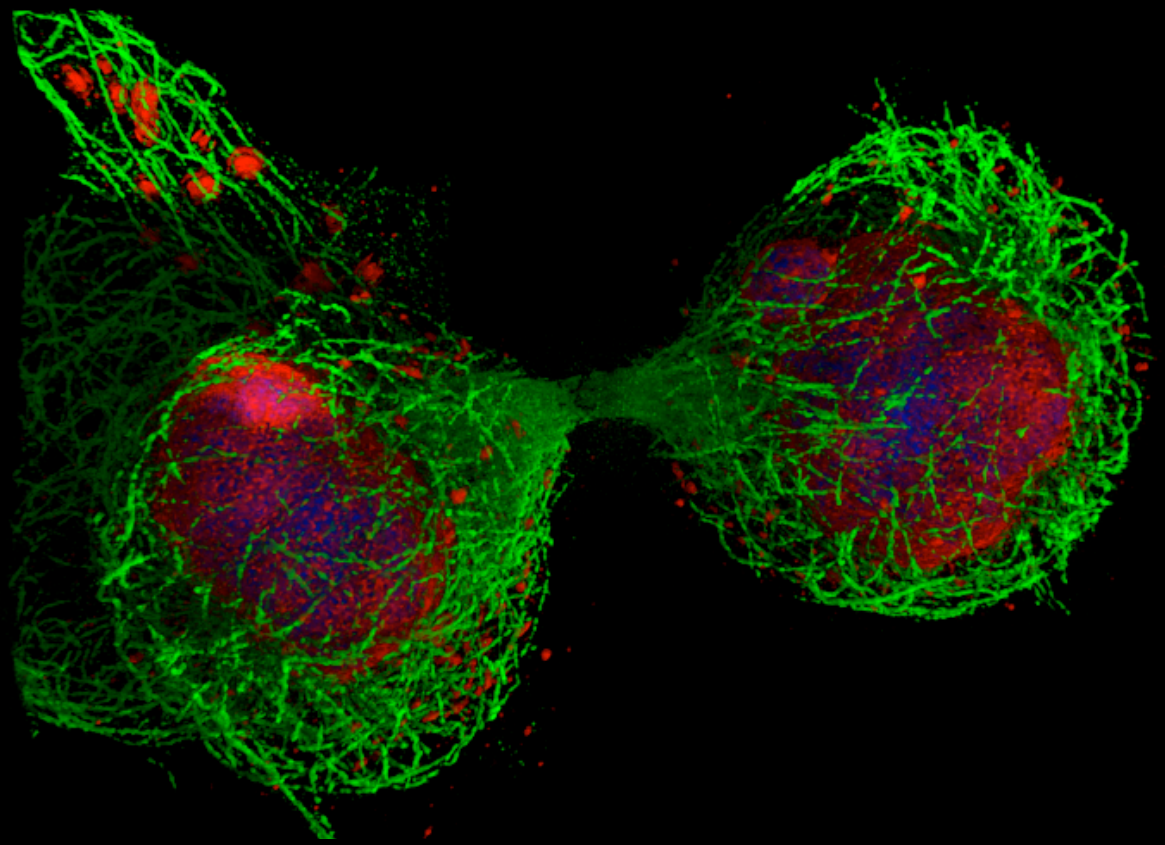


Standard Materials:

Coverslips #1.5 (d=0.170±0.005 mm)
Blue: DAPI, CF405M, ATTO425
Green: Alexa 488, ATTO 488, GFP (?)
Red: Alexa 594, ATTO 590, ATTO 594

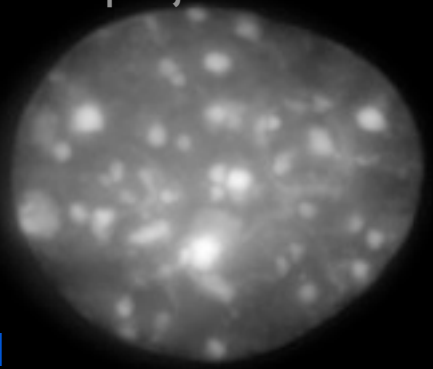
Tubulin
Lamin B
DAPI

Ray tracing rendering

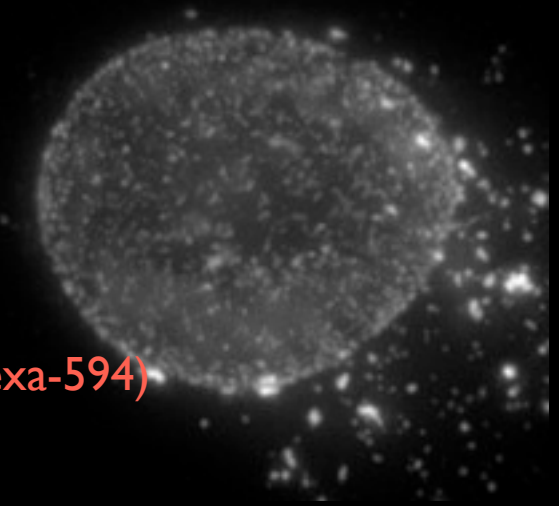


3D SIM example: Nuclear pores

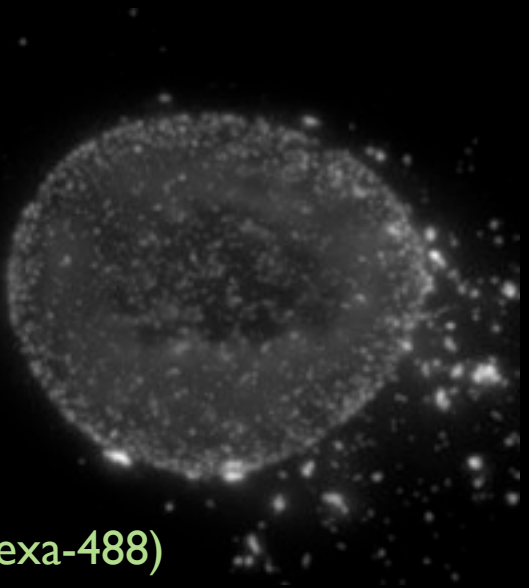
WF projections



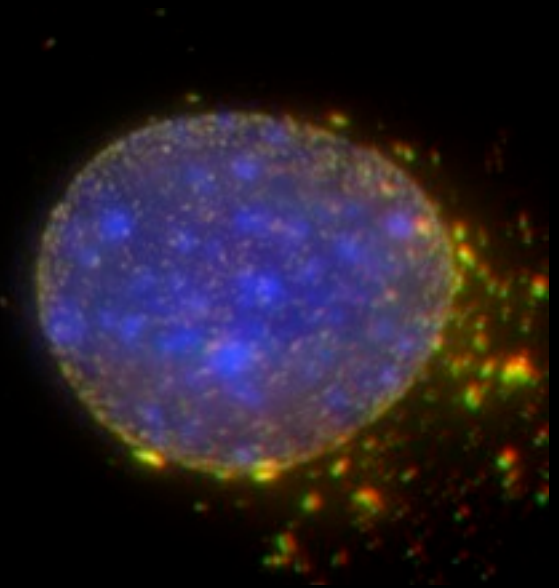
DAPI



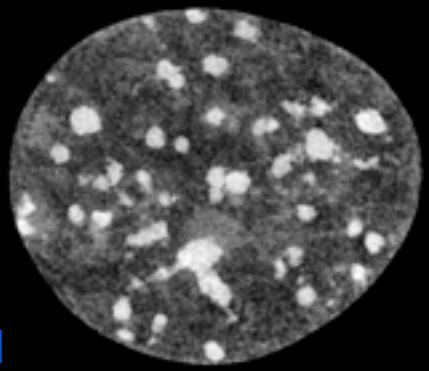
nup153 (Alexa-594)



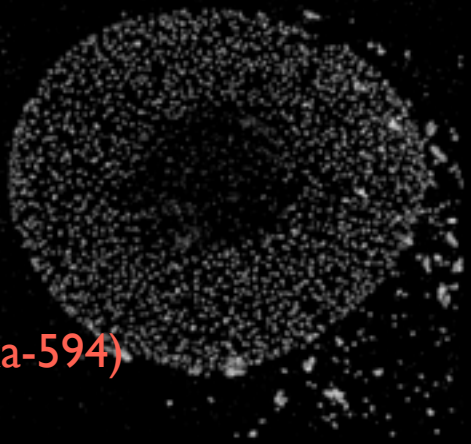
nup153 (Alexa-488)



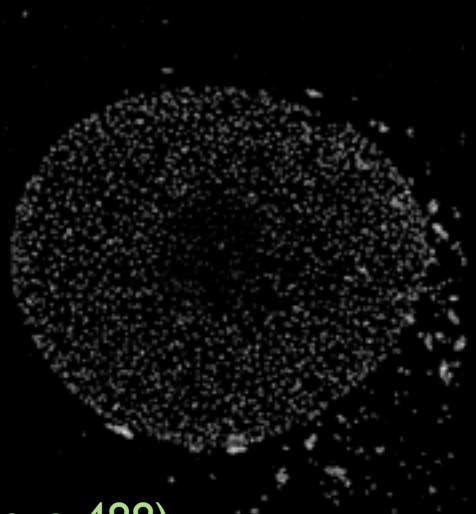
3D SIM example: Nuclear pores



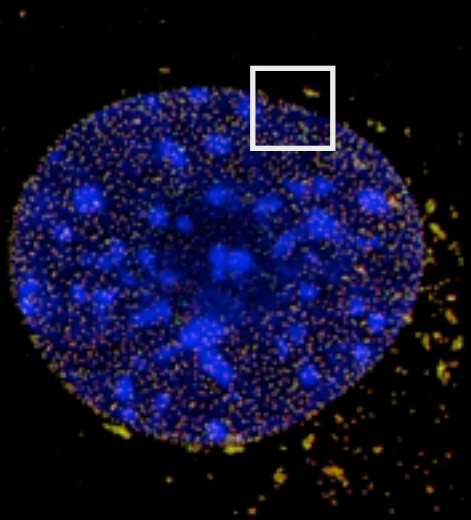
DAPI



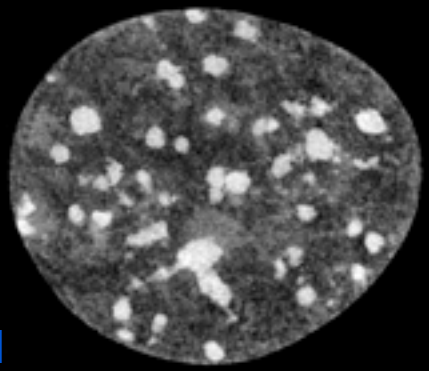
nup153 (Alexa-594)



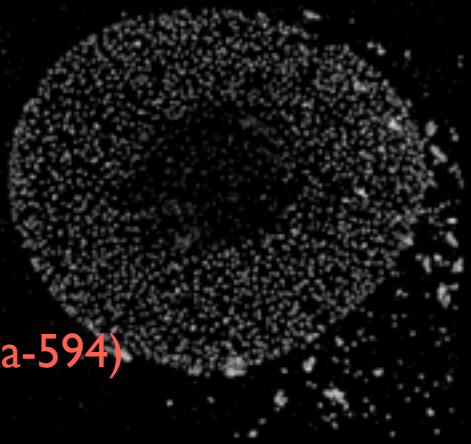
nup153 (Alexa-488)



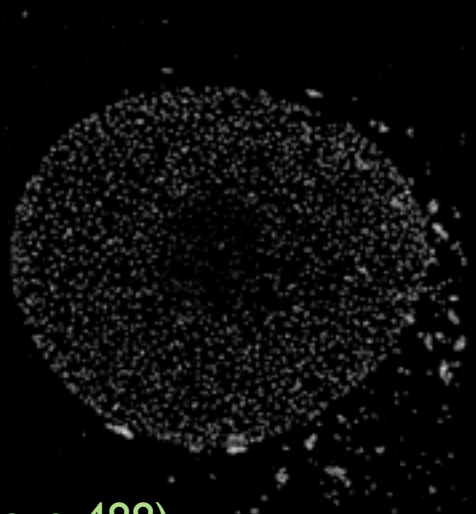
3D SIM example: Nuclear pores



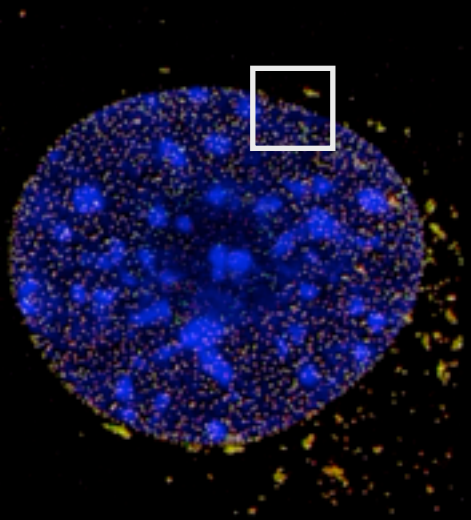
DAPI



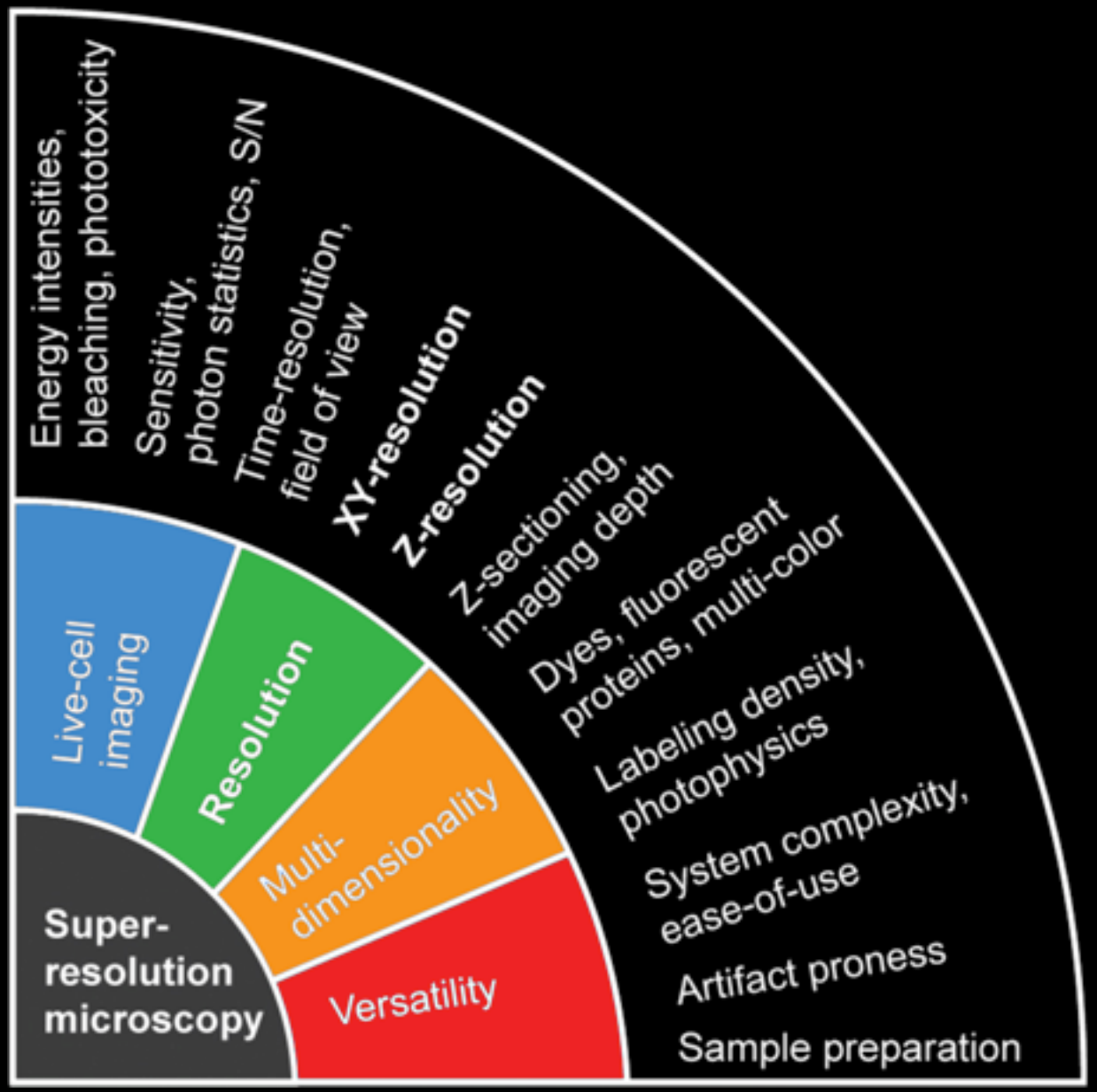
nup153 (Alexa-594)



nup153 (Alexa-488)



Super-resolution microscopy: a multi-dimensional challenge



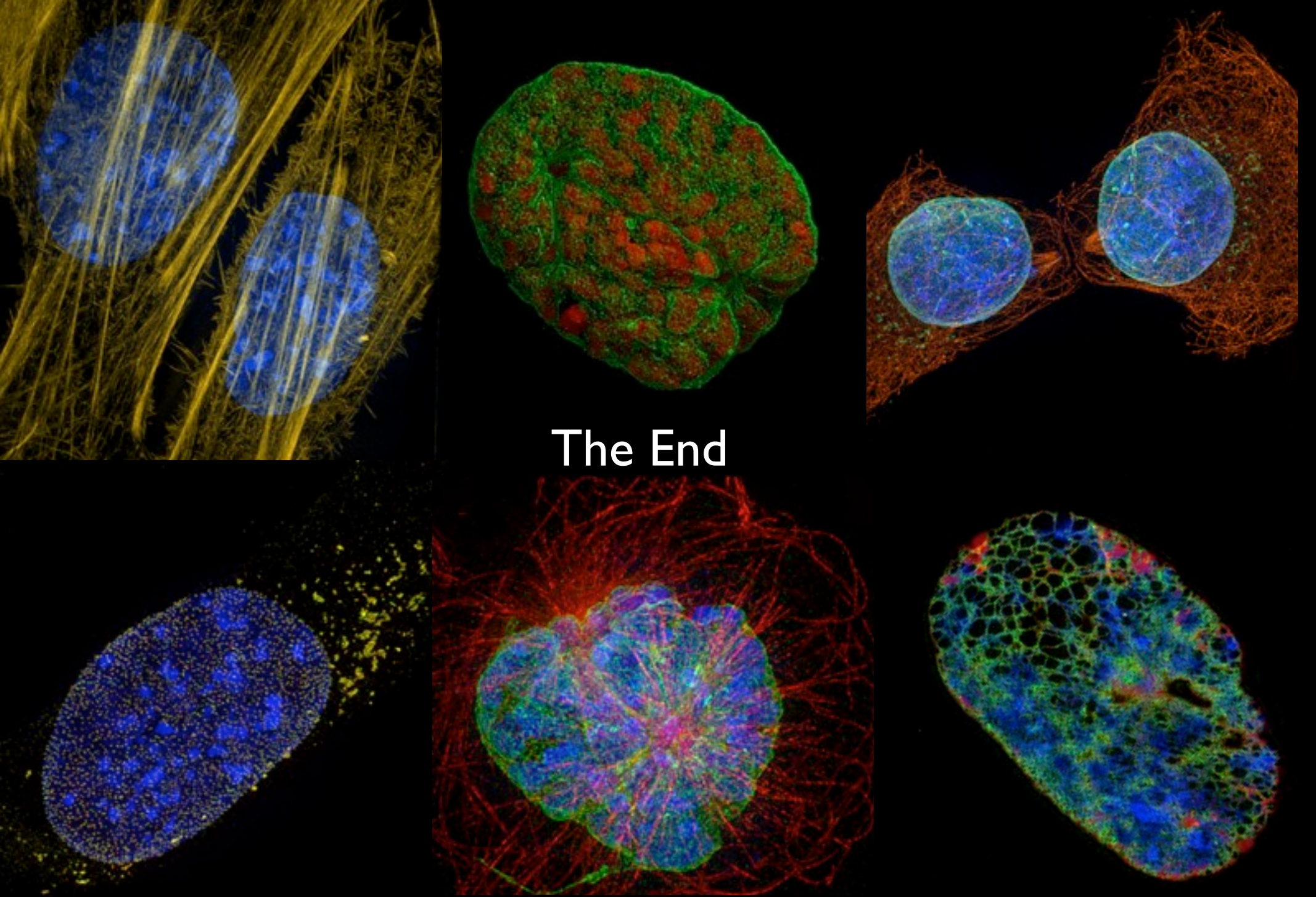
Schermelleh et al., JCB (2010)

Which super-resolution method to use ?

3D-SIM	general method - 3 colors, 3D sectioning, only modest (x2) resolution gain in xy and z (x8 volumetric) postprocessing, prone to artifacts
Live 3D-SIM	*OMX Blaze. Fast, large field-of-view (40 x40 µm), high sensitivity*
Non-linear SIM	*experimental setup, dx,y 40-50 nm, currently restricted to TIRF and one color only (Dronpa)*
TIRF	*only near-field, improved z-resolution only, high sensitivity and temporal resolution, „surface biology“*
SPIM	*isotropic resolution (~400 nm in xy, z) , deep imaging of large fields, large data, tissues and whole mounts*
STED	high resolution, theoretically unlimited, WYSWG, speed scales with field of view special dye requirements, bleaching issues, complex instrumentation, works best in Hell's lab.
4Pi	absolutely need a physicist. Little reported biology so far.
I5M	same specimen mounting difficulties as 4Pi, no commercial implementation.
PALM/STORM	simple hardware. software more complex, various flavours, high localization precision (~20 nm in x,y), structural resolution depends on labeling density, rather slow.

„All superresolution techniques excel in certain aspects and fail in others—the best technique will be determined by the demands of the application.“ Rego et al. 2012, PNAS


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



The End

Credits to Jürgen Neumann, Pete Carlton and Timo Zimmermann for sharing slides