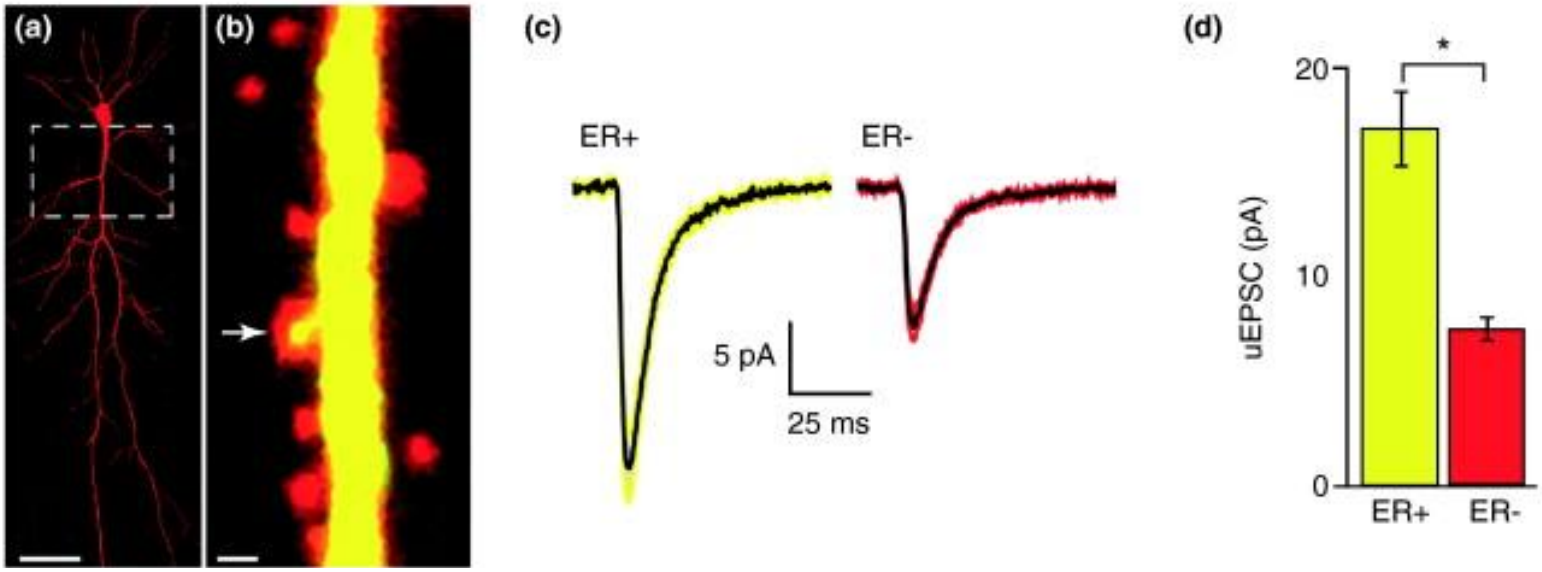
¿Cómo superar el límite de resolución en microscopia óptica?

Superresolution optical fluctuation imaging (SOFI)

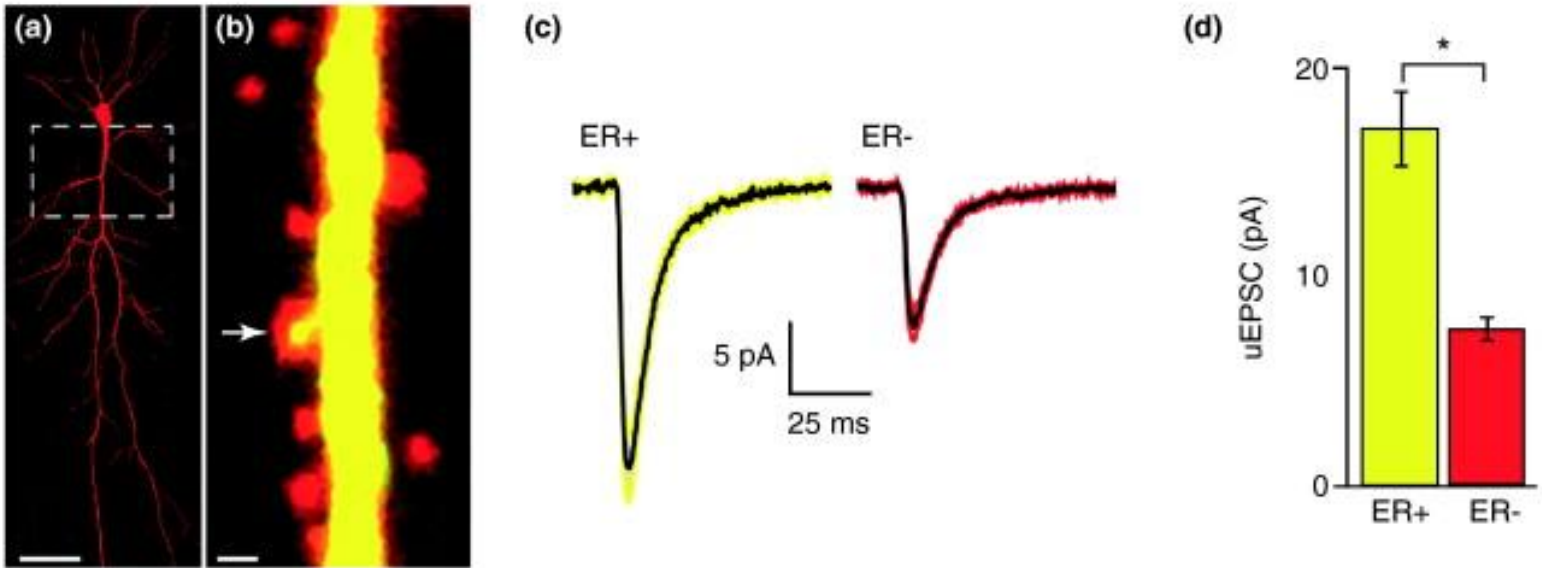
Omar Ramírez

The neuronal ER participates in synaptic potentiation



Holbro *et al.*, PNAS, 2009

The neuronal ER participates in synaptic potentiation

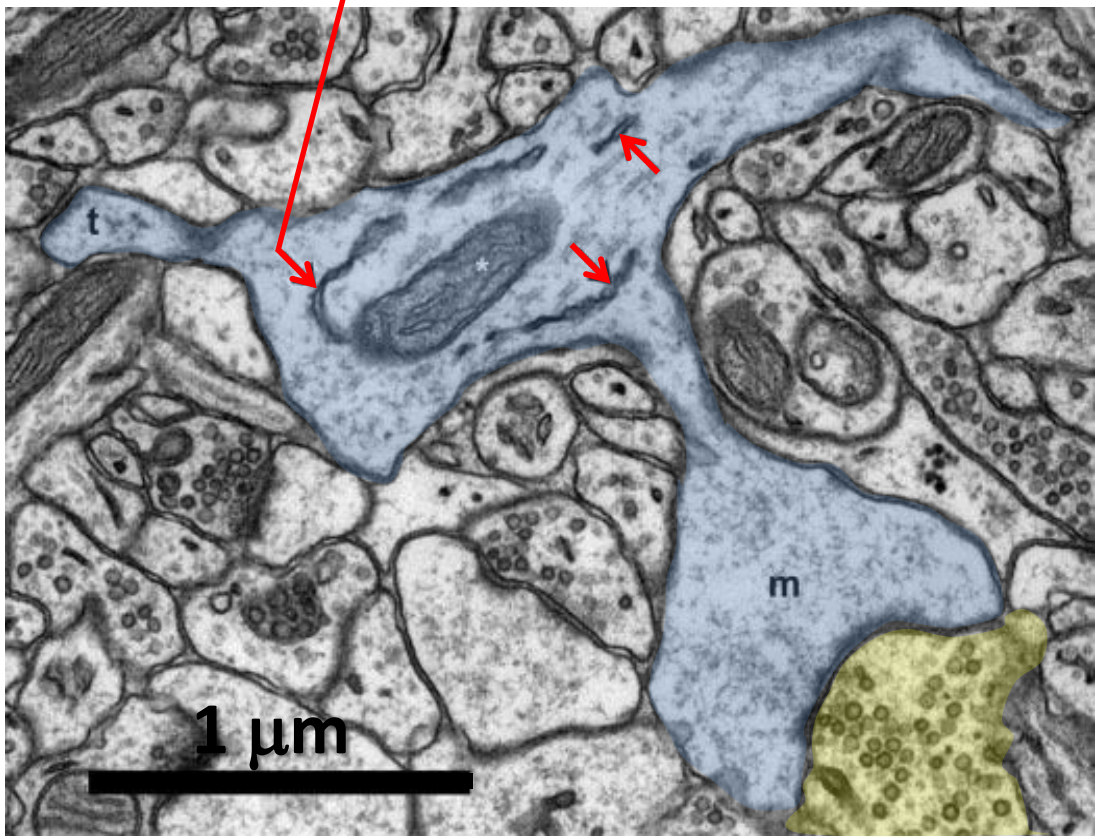


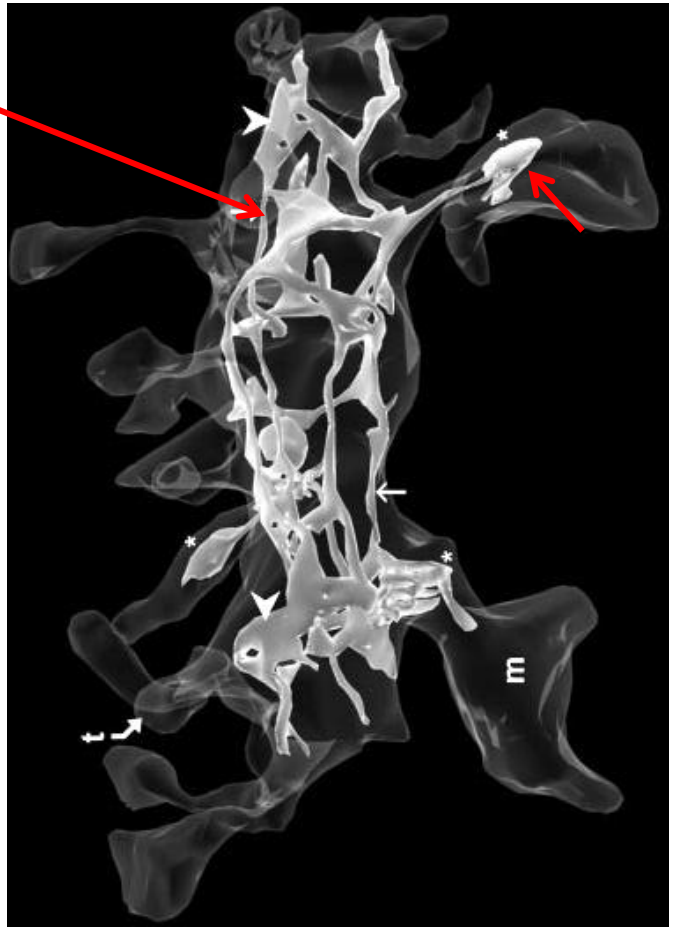
- Neurotransmitter receptor synthesis and transport
- Calcium storage/release

Holbro *et al.*, PNAS, 2009

The neuronal ER is a subdiffraction sized structure

The ER tubule diameter is < 40 nm, far below the ~ 250 nm resolution of conventional optical microscopes.

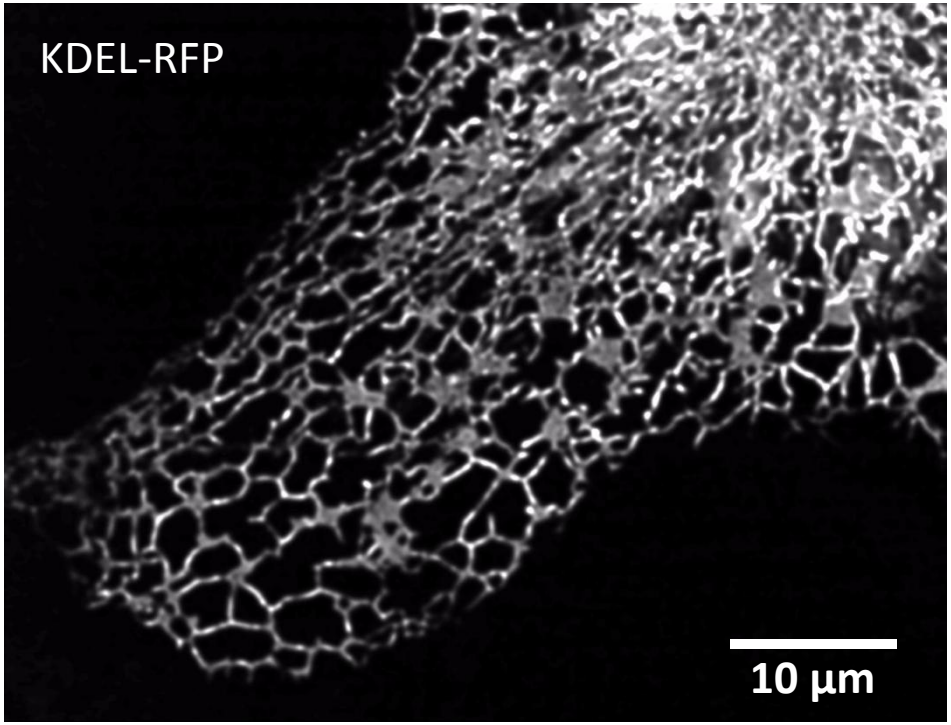




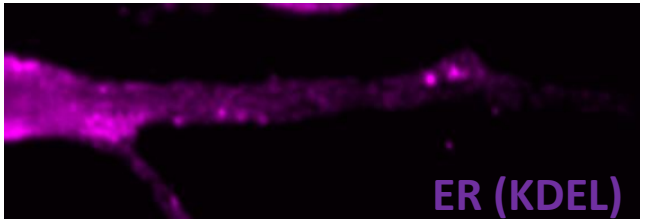
Cooney *et al.*, J Neuroscience, 2002

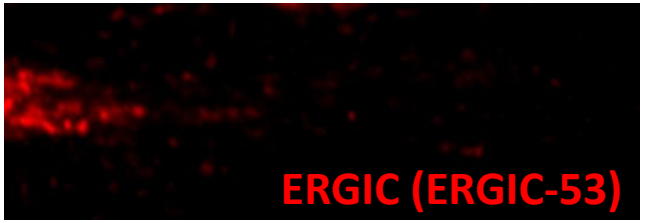
The neuronal ER is a subdiffraction sized structure

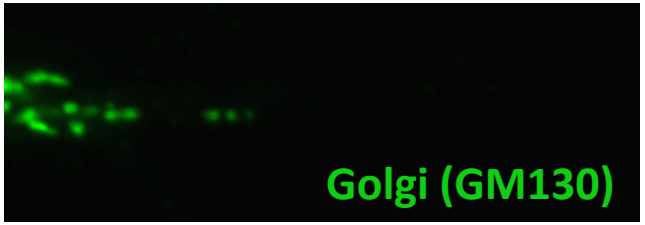
COS-7 cells



Primary hippocampal neurons

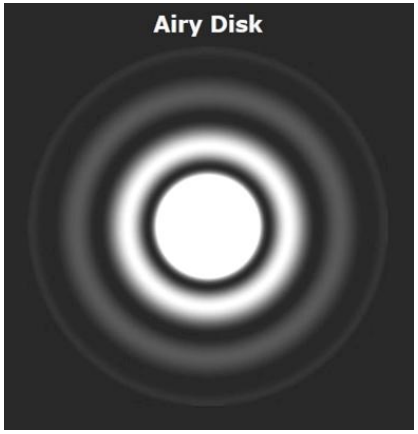


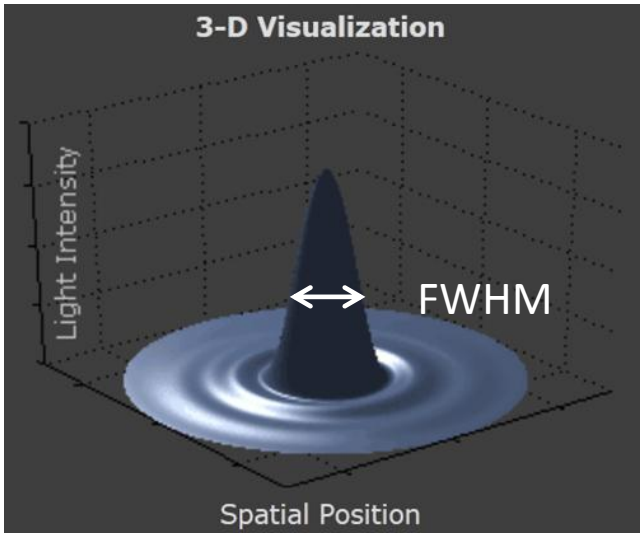


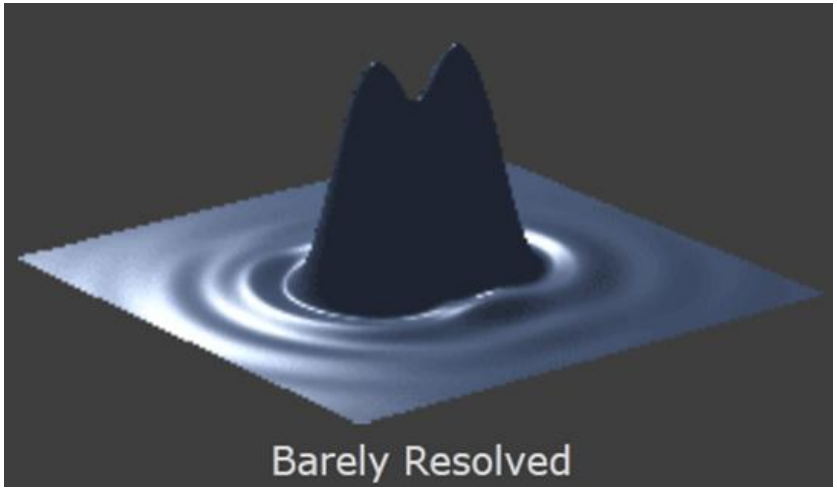


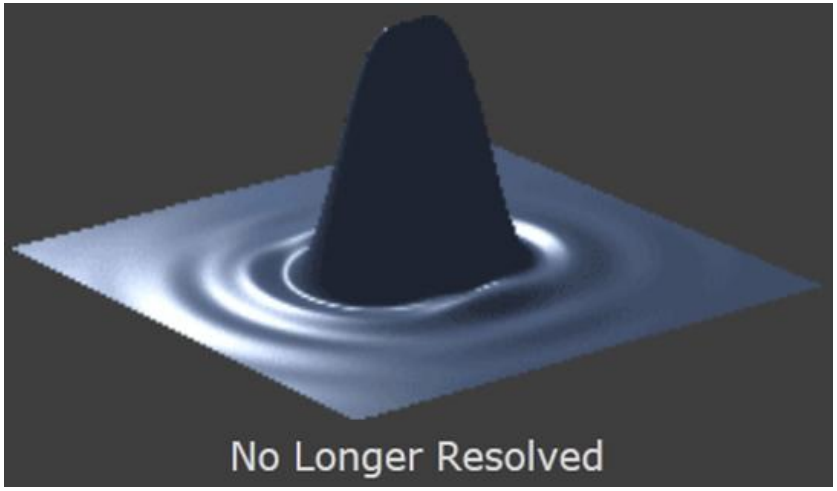
Light diffraction determines and limits resolution

Abbe's formula: $\Lambda_{\min} = \frac{\lambda}{2NA}$





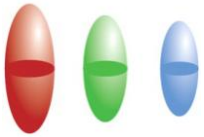




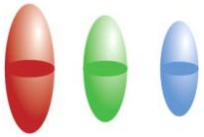
Current superresolution techniques

Technique	**Confocal**
Resolution limit	**~250 nm**
PSF	
Advantages	**Broadly available**
Drawbacks	**Diffraction limited (λ/2)**

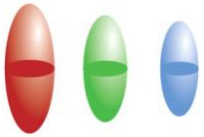
Current superresolution techniques

Technique	Confocal	SIM
Resolution limit	~250 nm	~120 nm
PSF		
Advantages	Broadly available	Multicolor Flexible labeling Live cell
Drawbacks	Diffraction limited (λ/2)	Resolution limited to (λ/4) Sensitive to alignment

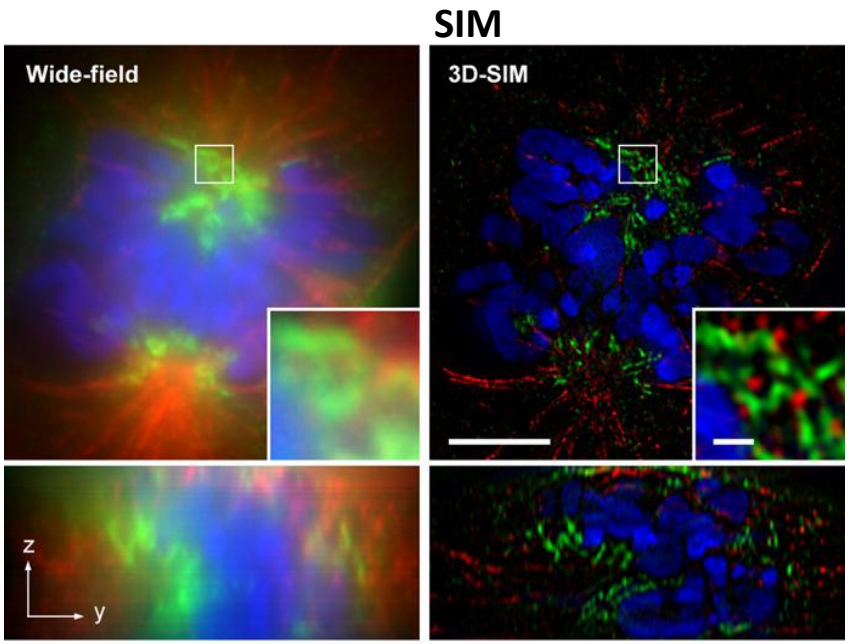
Current superresolution techniques

Technique	Confocal	SIM	STED
Resolution limit	~250 nm	~120 nm	~60 nm
PSF			
Advantages	Broadly available	Multicolor Flexible labeling Live cell	Two color Single scan imaging Live cell
Drawbacks	Diffraction limited (λ/2)	Resolution limited to (λ/4) Sensitive to alignment	Limited dye availability Sensitive to alignment Expensive

Current superresolution techniques

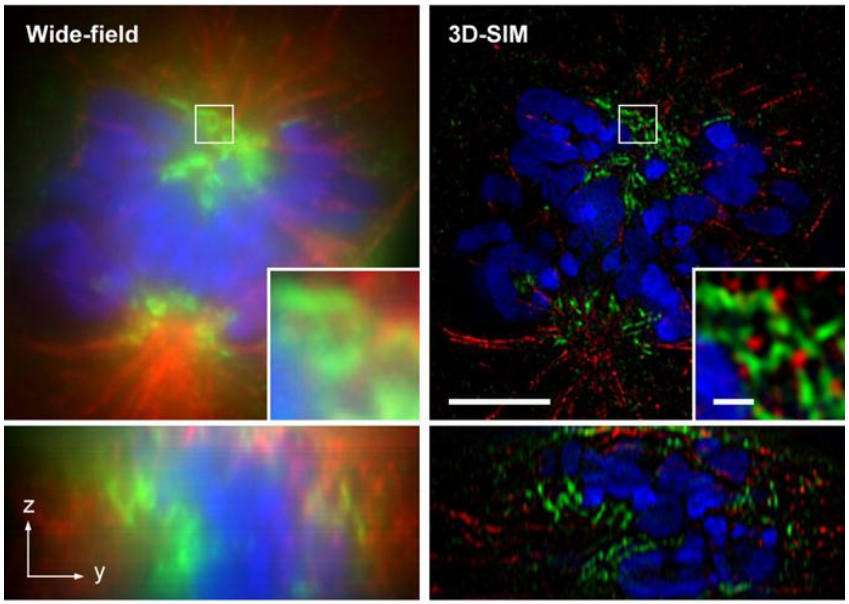
Technique	Confocal	SIM	STED	PALM
Resolution limit	~250 nm	~120 nm	~60 nm	~30 nm
PSF				
Advantages	Broadly available	Multicolor Flexible labeling Live cell	Two color Single scan imaging Live cell	Multicolor Single molecule tracking
Drawbacks	Diffraction limited (λ/2)	Resolution limited to (λ/4) Sensitive to alignment	Limited dye availability Sensitive to alignment Expensive	No endogenous labeling

Some examples

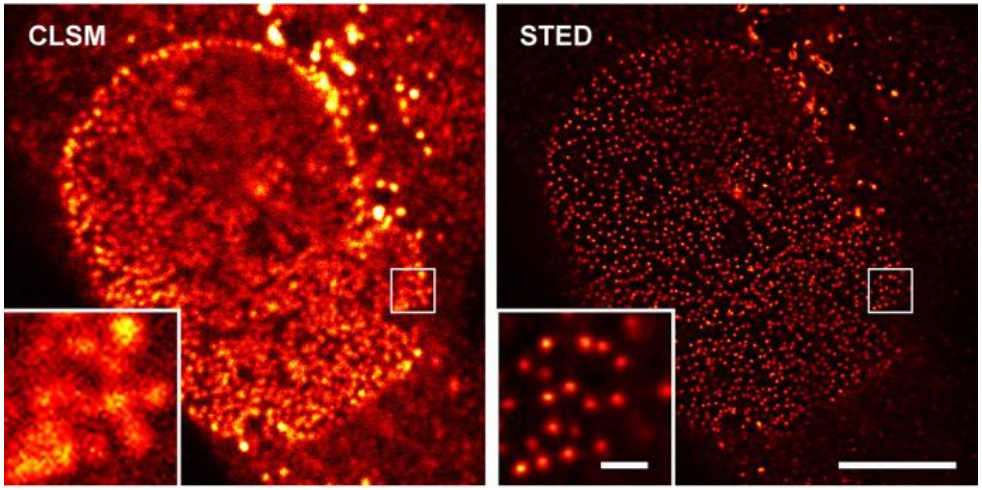


Some examples

SIM

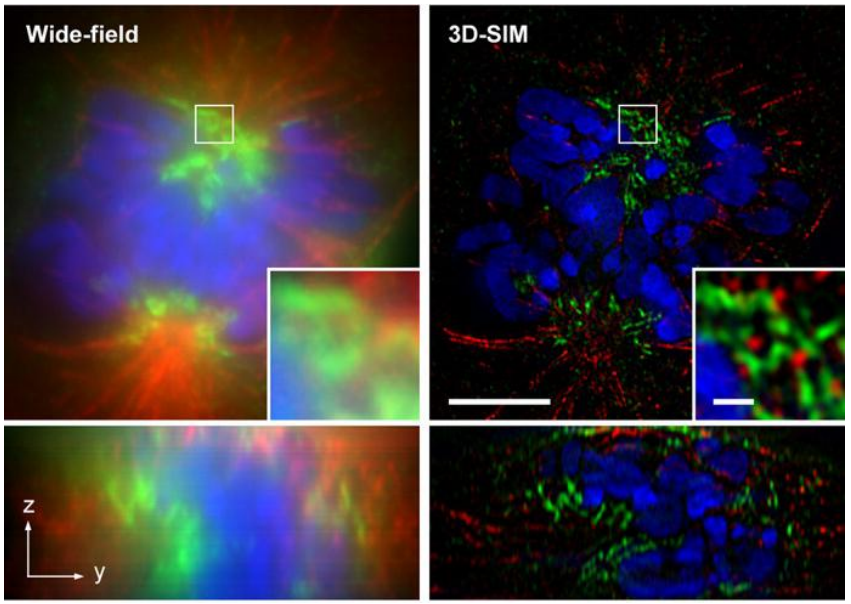


STED

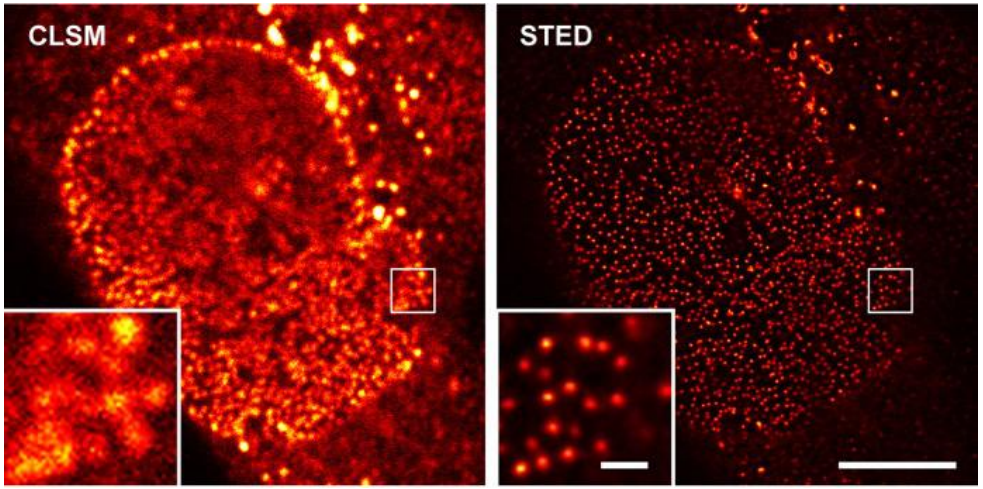


Some examples

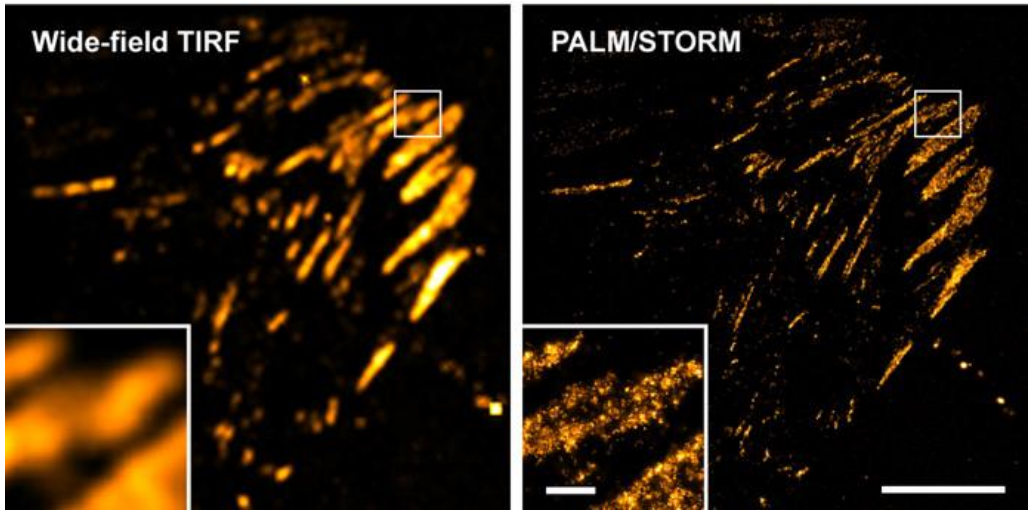
SIM



STED



PALM

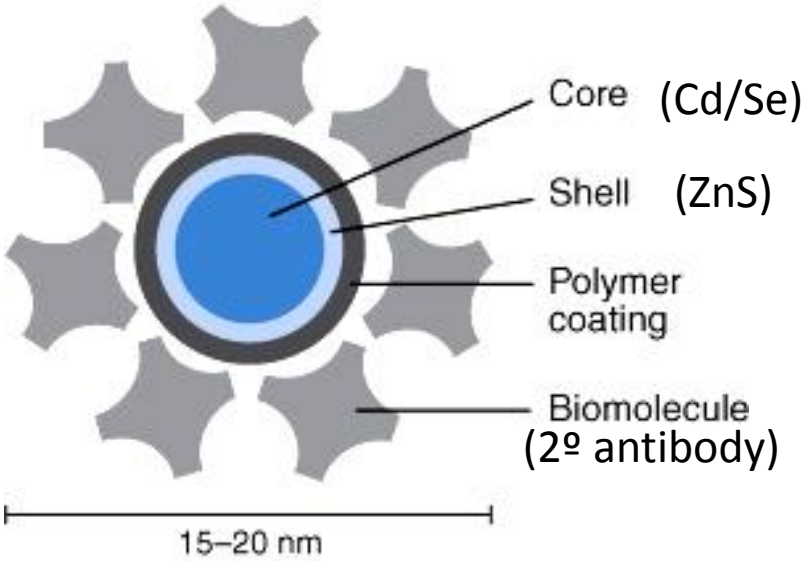


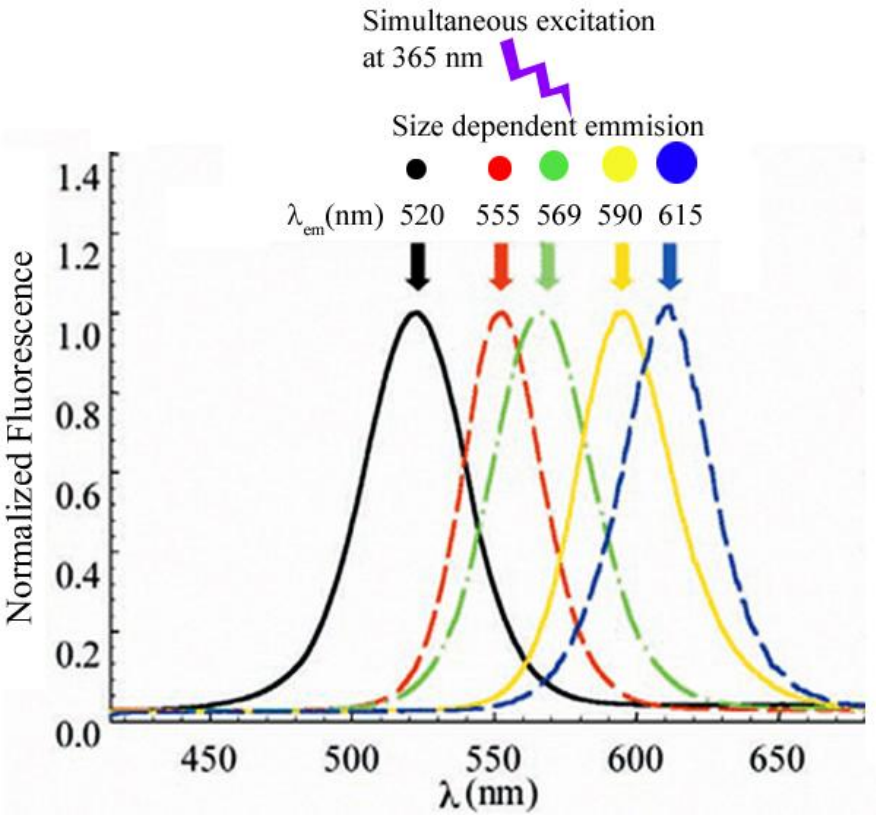
Bars:
5 µm (insets, 0.5 µm)

Superresolution optical fluctuation imaging (SOFI) method

We need a fluctuating light source:

Quantum dots





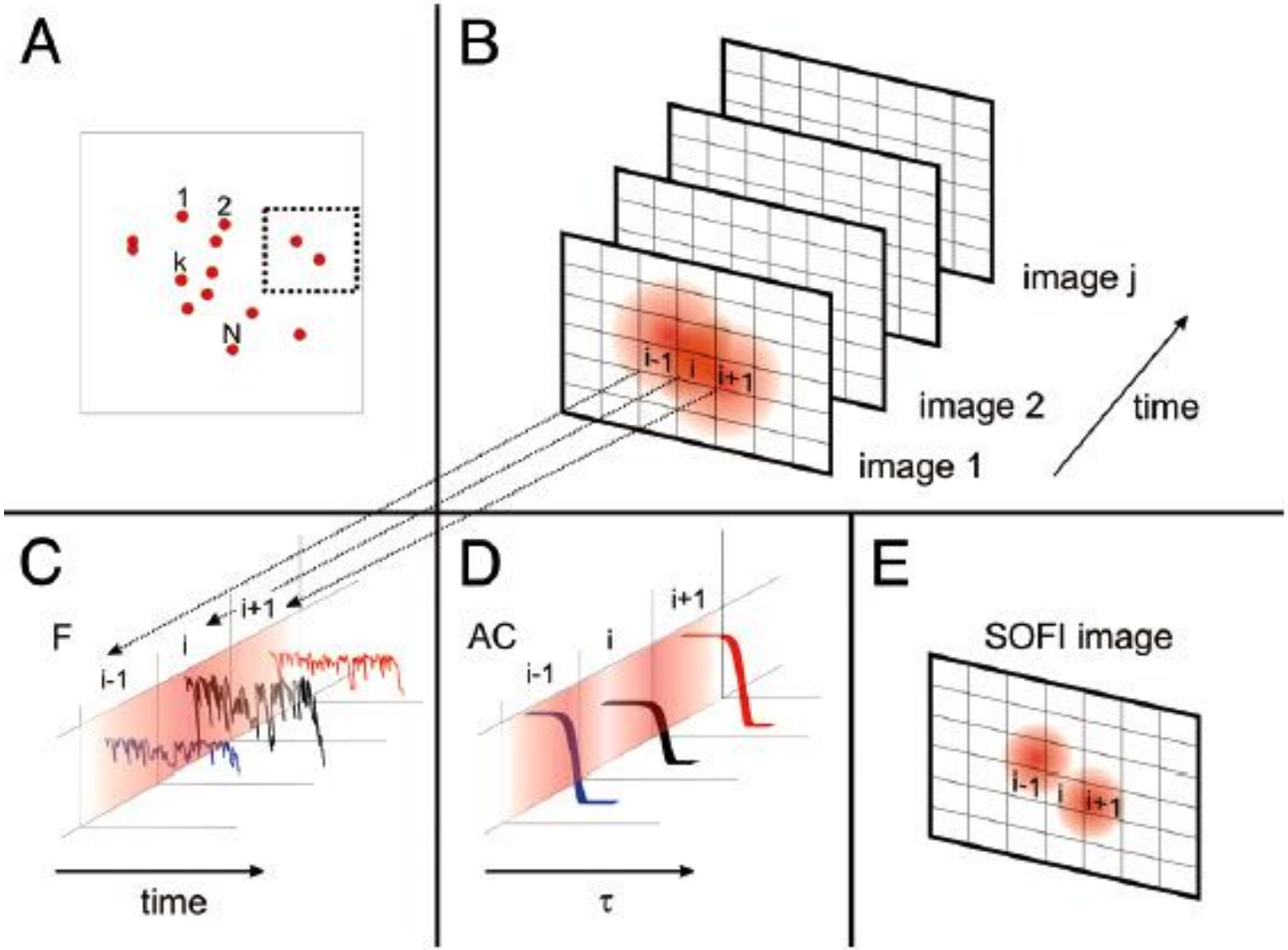
Joerg Enderlein

Superresolution optical fluctuation imaging (SOFI) method

ON/OFF fluctuation

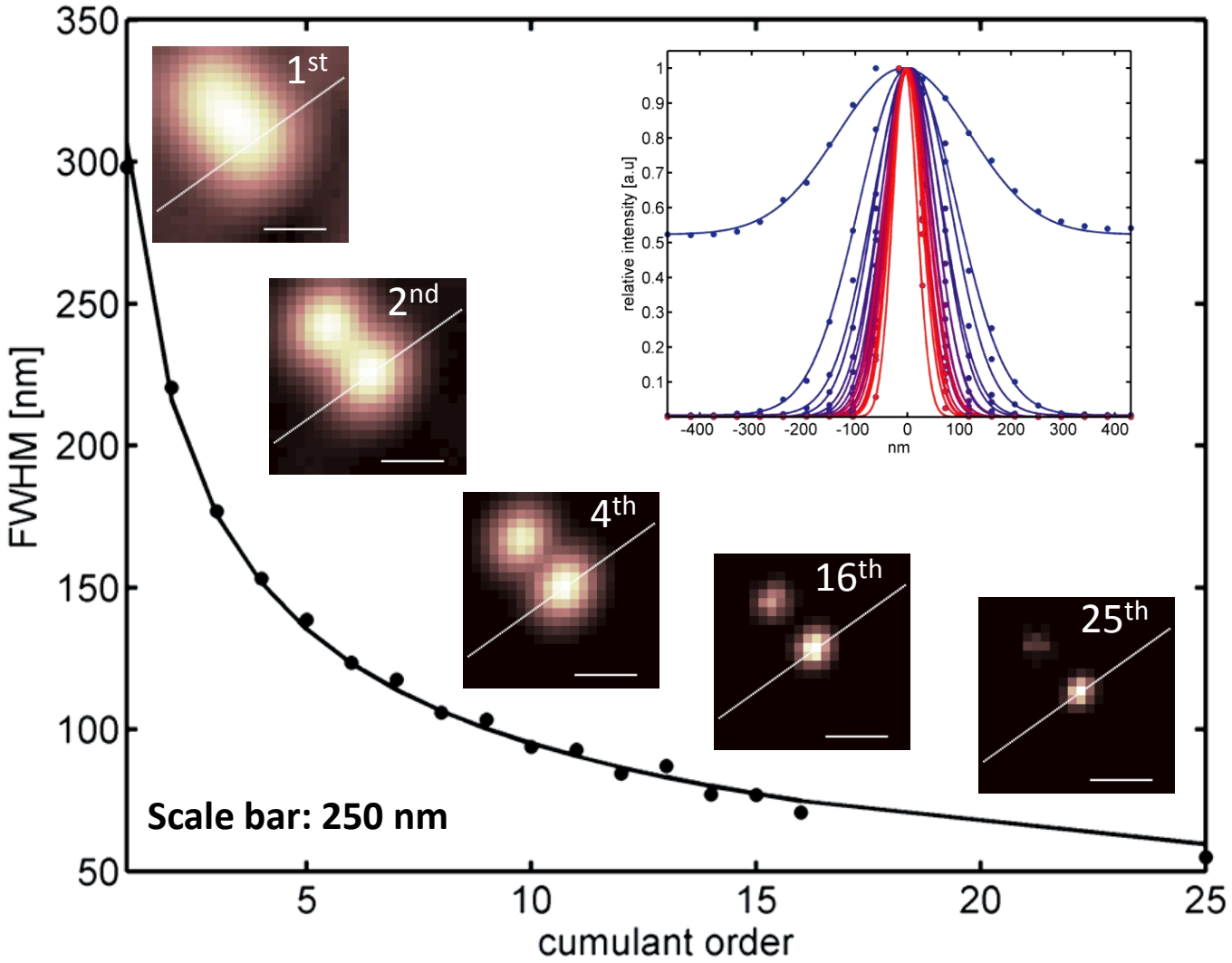
Qdot 525 nm emission wavelength,wide-field microscope, 10 Hz movie with a CCD camera

Image formation with SOFI



Dertinger T., Colyer R.; Iyer G.; Weiss. S.; **Enderlein J.**, PNAS, 2009

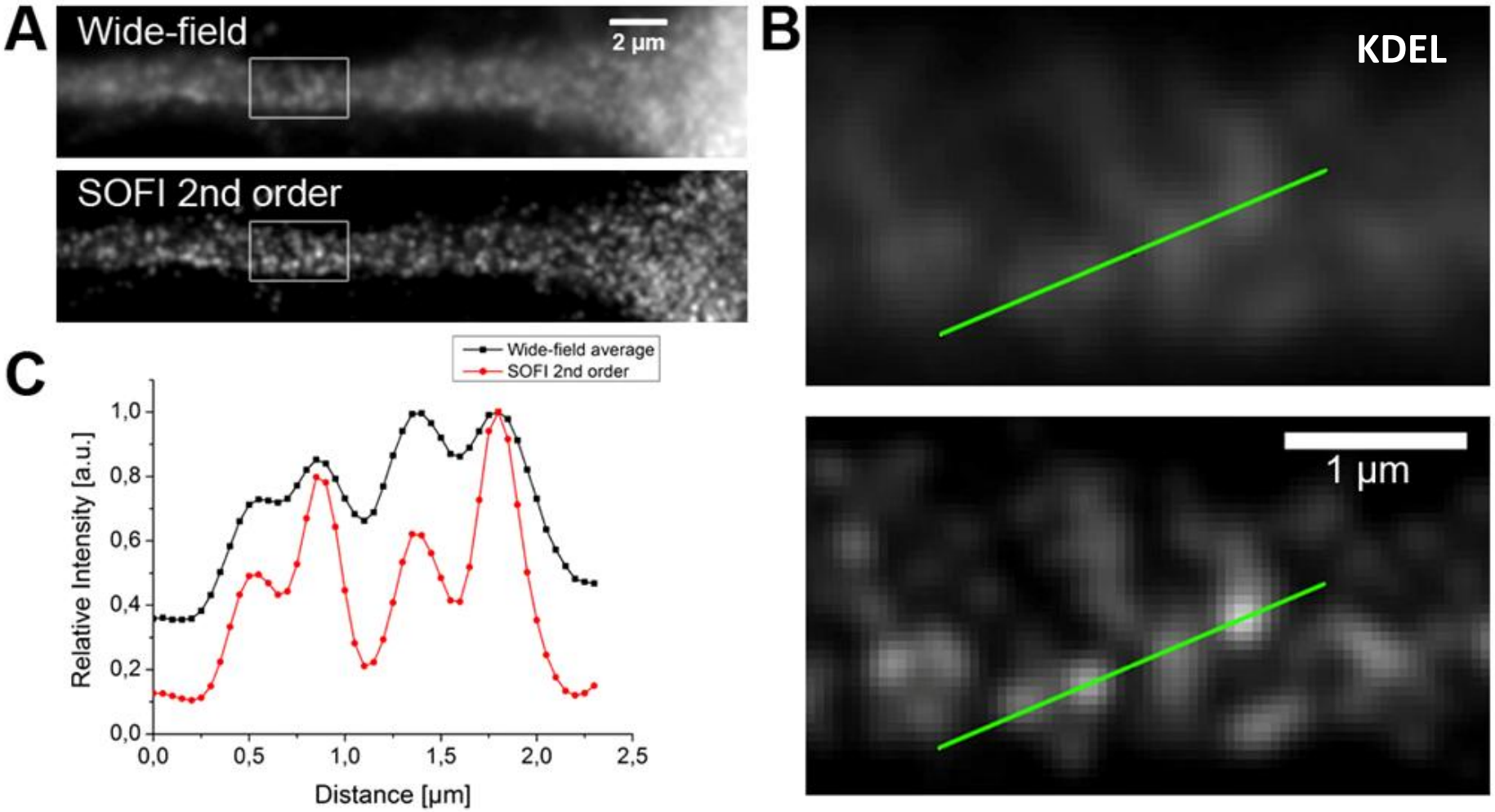
SOFI higher order cumulants



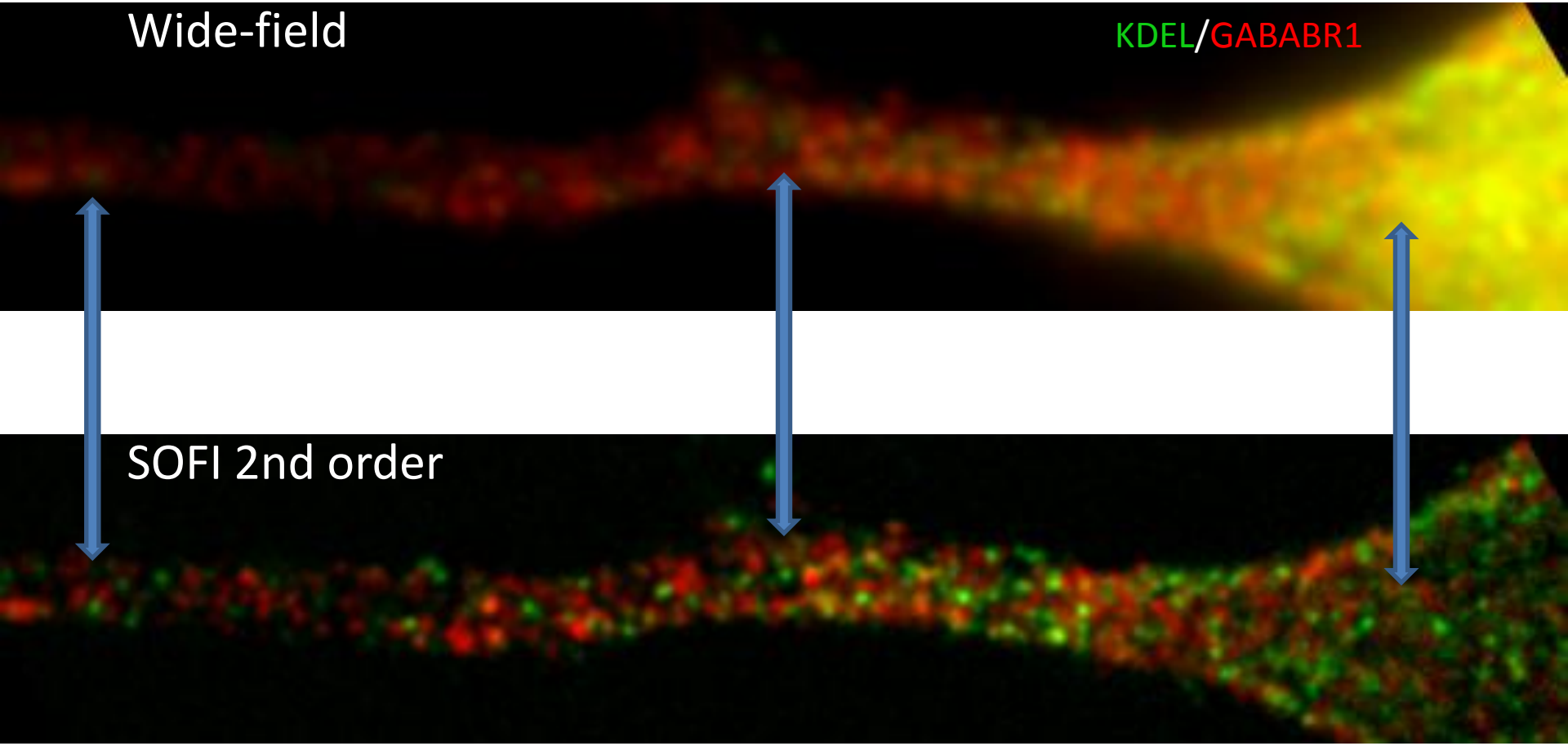
Resolution enhancement 5x = 60 nm

Dertinger T., Colyer R.; Iyer G.; Weiss. S.; **Enderlein J.**, PNAS, 2009

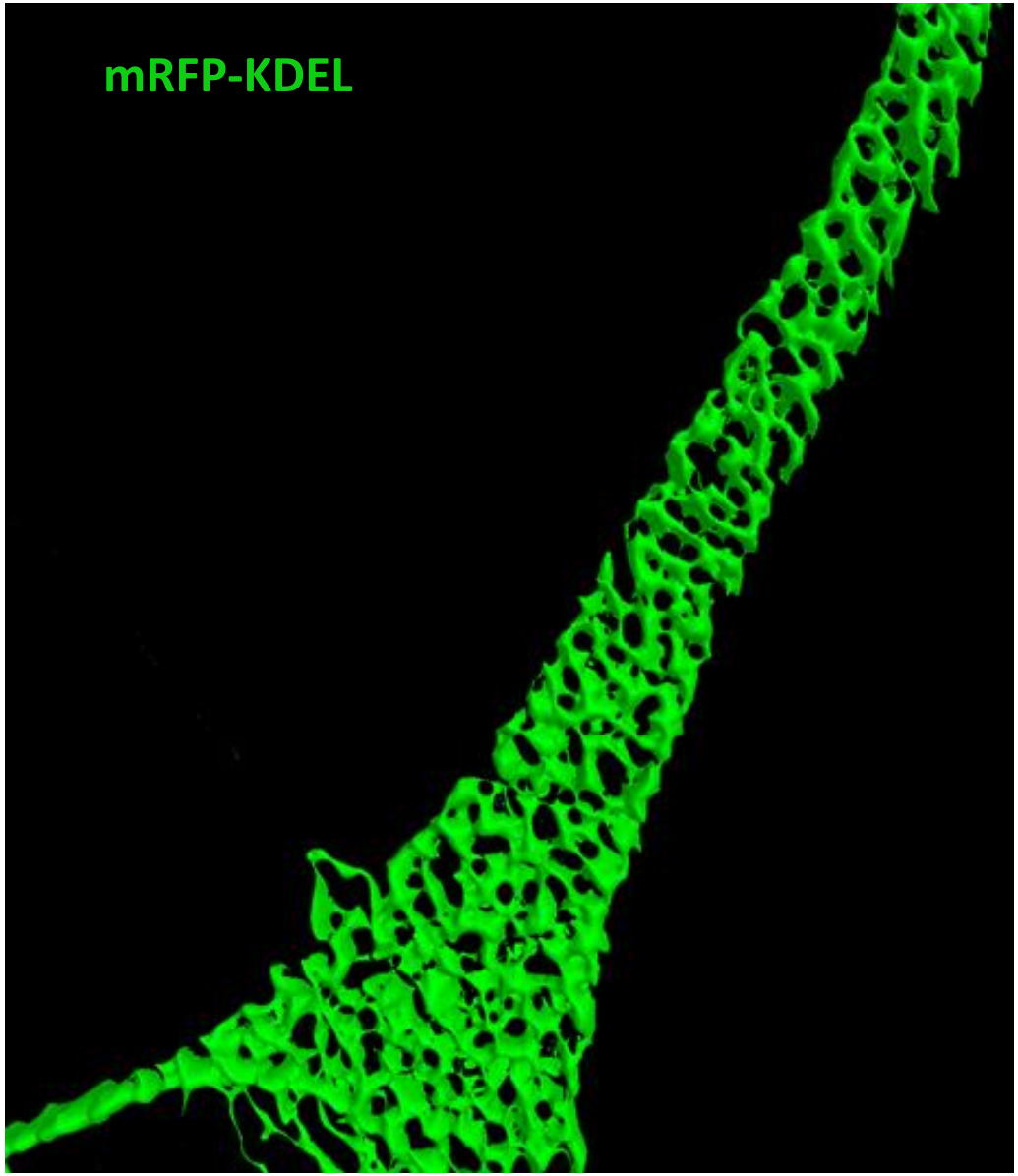
2nd order SOFI applied to a neuronal ER image

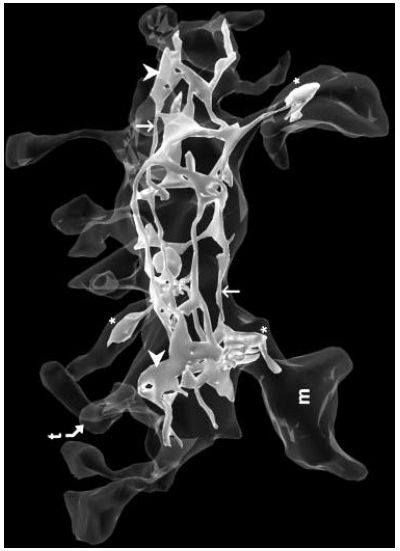


Two channel 2nd order SOFI



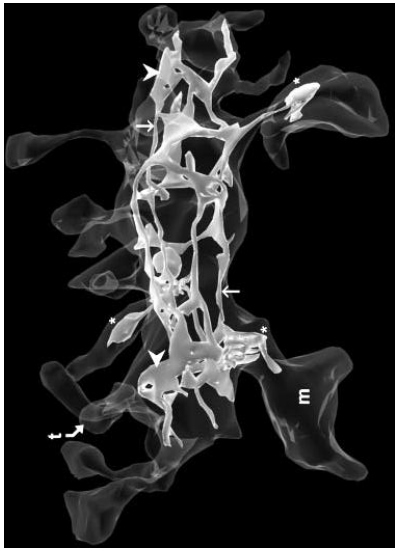
2nd order SOFI endoplasmic reticulum
3D reconstruction





2nd order SOFI endoplasmic reticulum 3D reconstruction

mRFP-KDEL

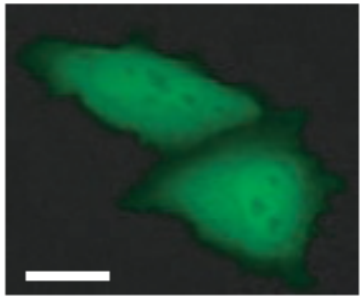


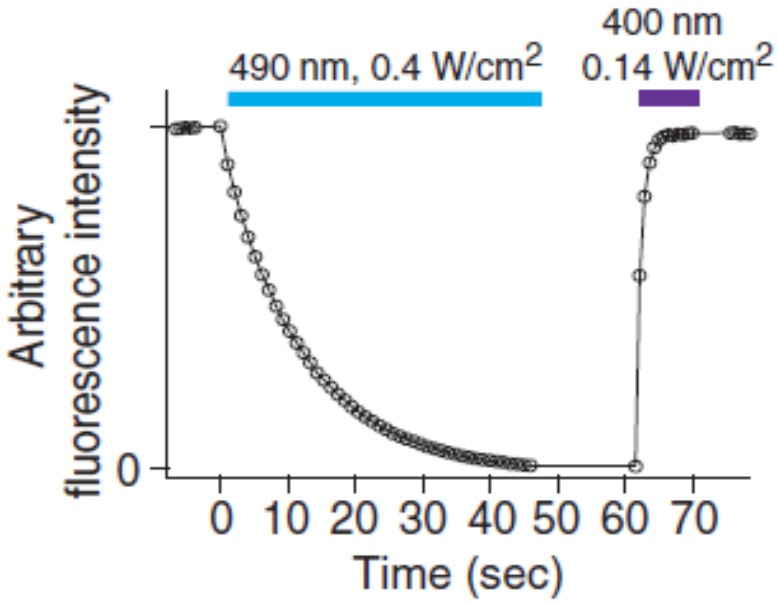
SOFI advantages:

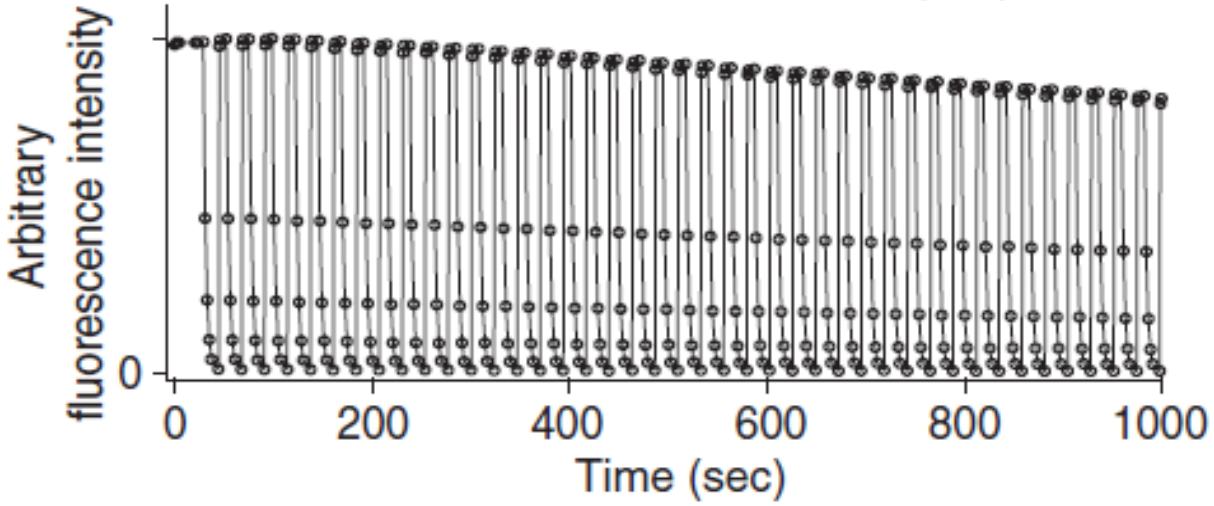
- Easy/cheap implementation
- Multiscale
- Multicolor

Preguntas

Photoswitchable fluorescent proteins for SOFI: Dronpa

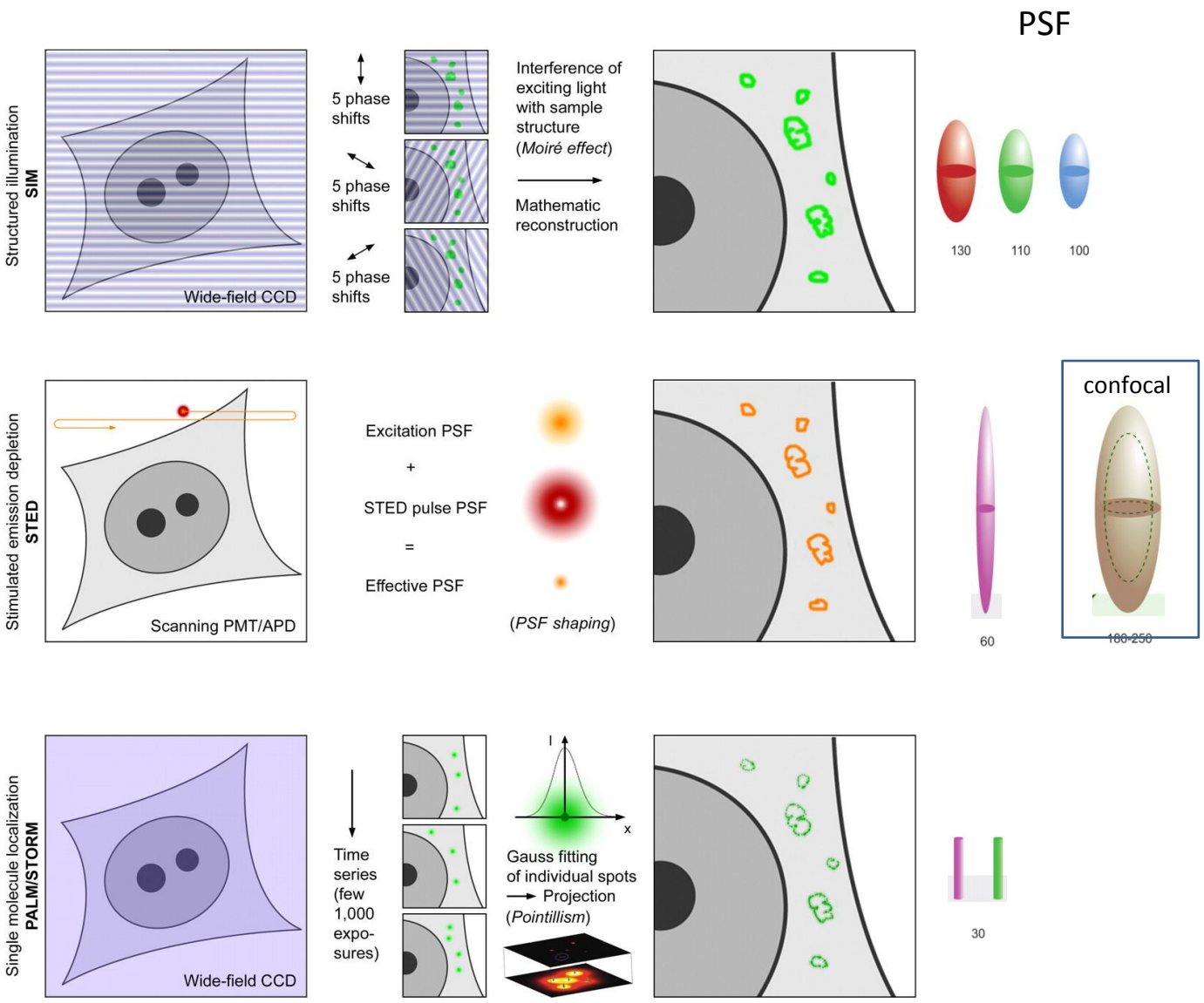






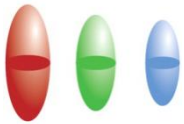
Ando, Science, 2004

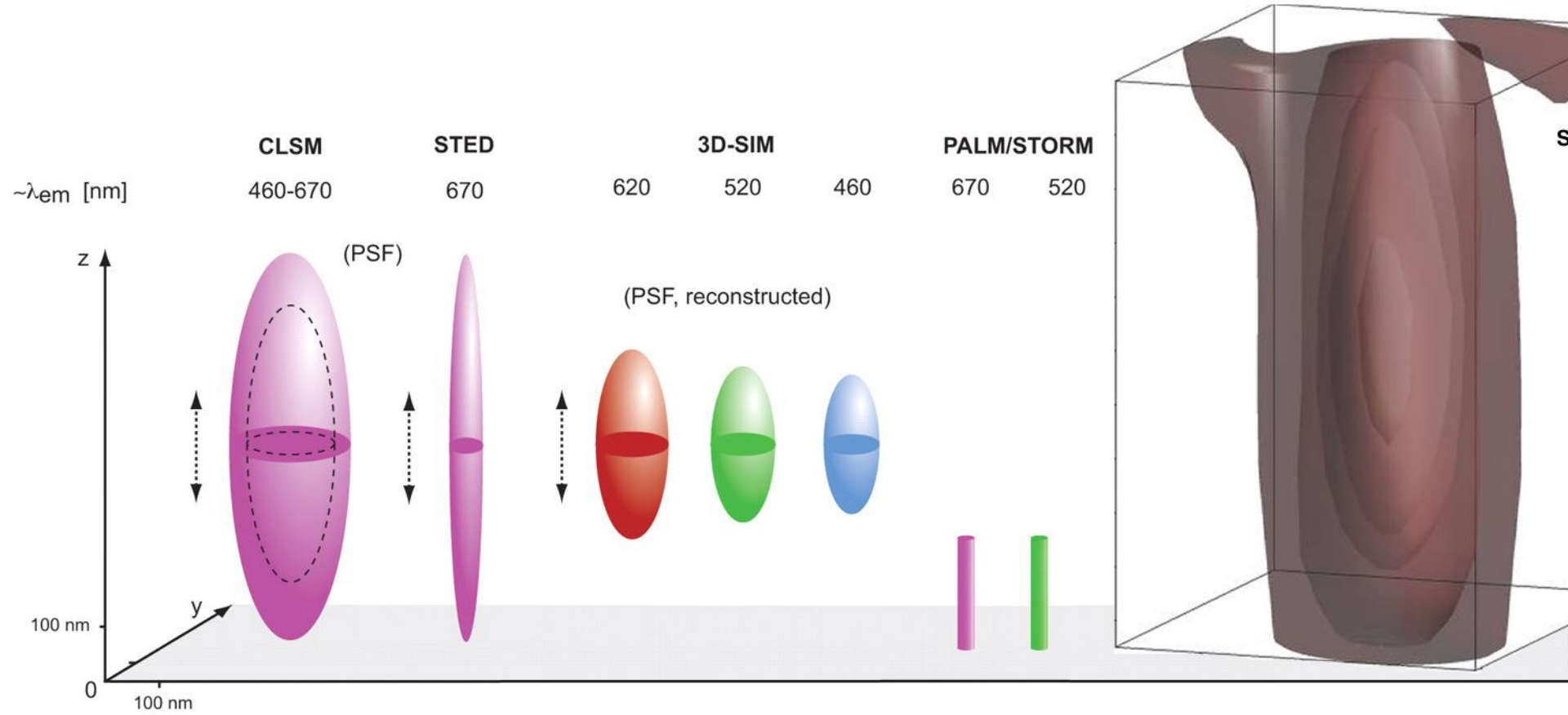
Current superresolution techniques



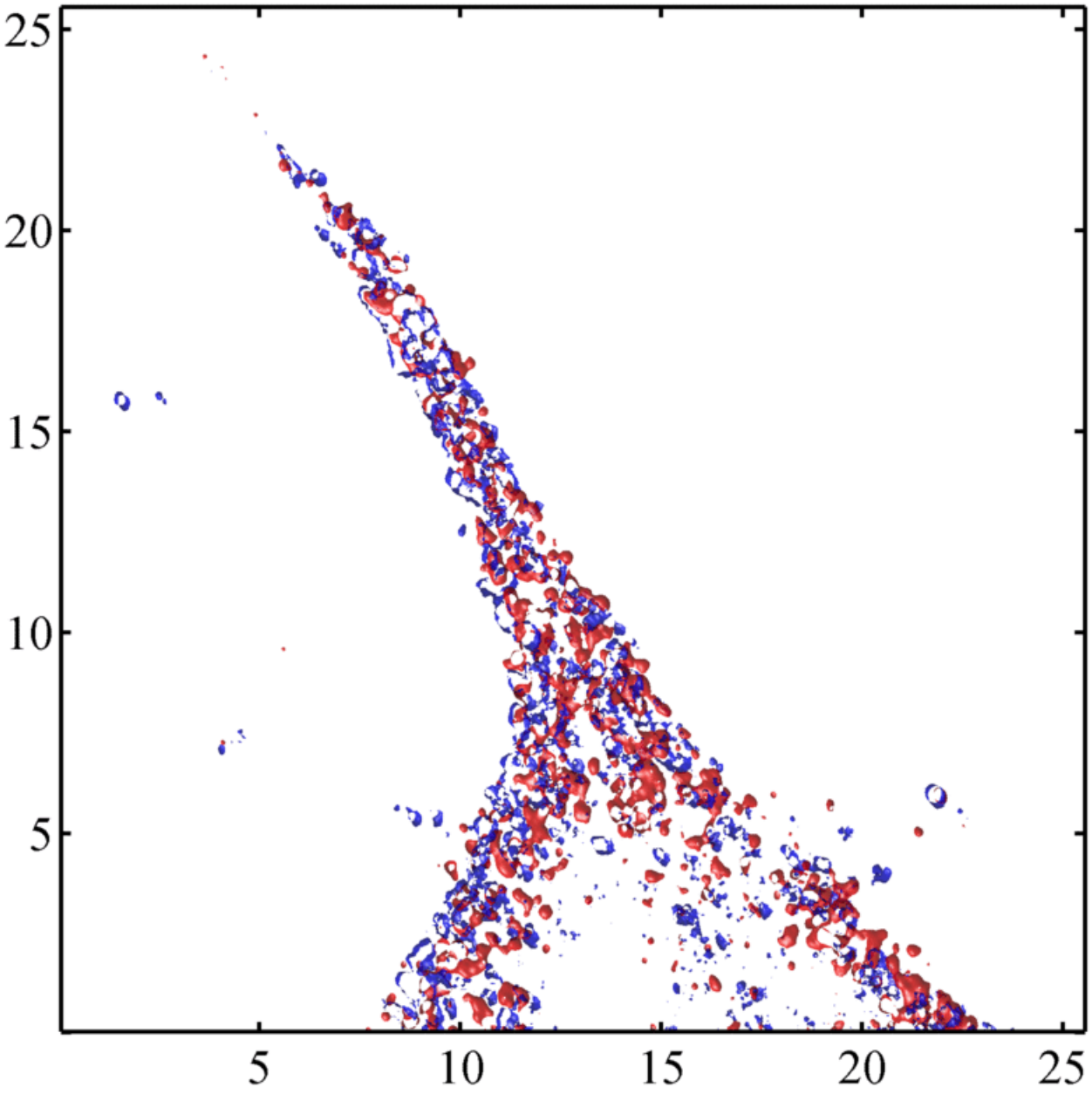
Schermelleh, JCB, 2010

Current superresolution techniques

Technique	Resolution Limit	PSF	Advantages	Drawbacks
Confocal	~250 nm		Broadly available	Diffraction limited (λ/2)
SIM	~120 nm		Multicolor Flexible labeling Live cell	Resolution limited to (λ/4) Sensitive to alignment
STED	~60 nm		Two color Single scan imaging Live cell	Limited dye availability Sensitive to alignment Expensive
PALM	~30 nm		High spatial resolution Single molecule tracking	No multicolor No endogenous labeling



~$D_{x,y}$ [nm]	180-250	60	130	110	100	30
~D_z [nm]	500-700	700	340	280	250	140
~$V_{x,y,z}$ [*10^{-3} µm^3]	10-23	1.3	3.0	1.8	1.3	0.1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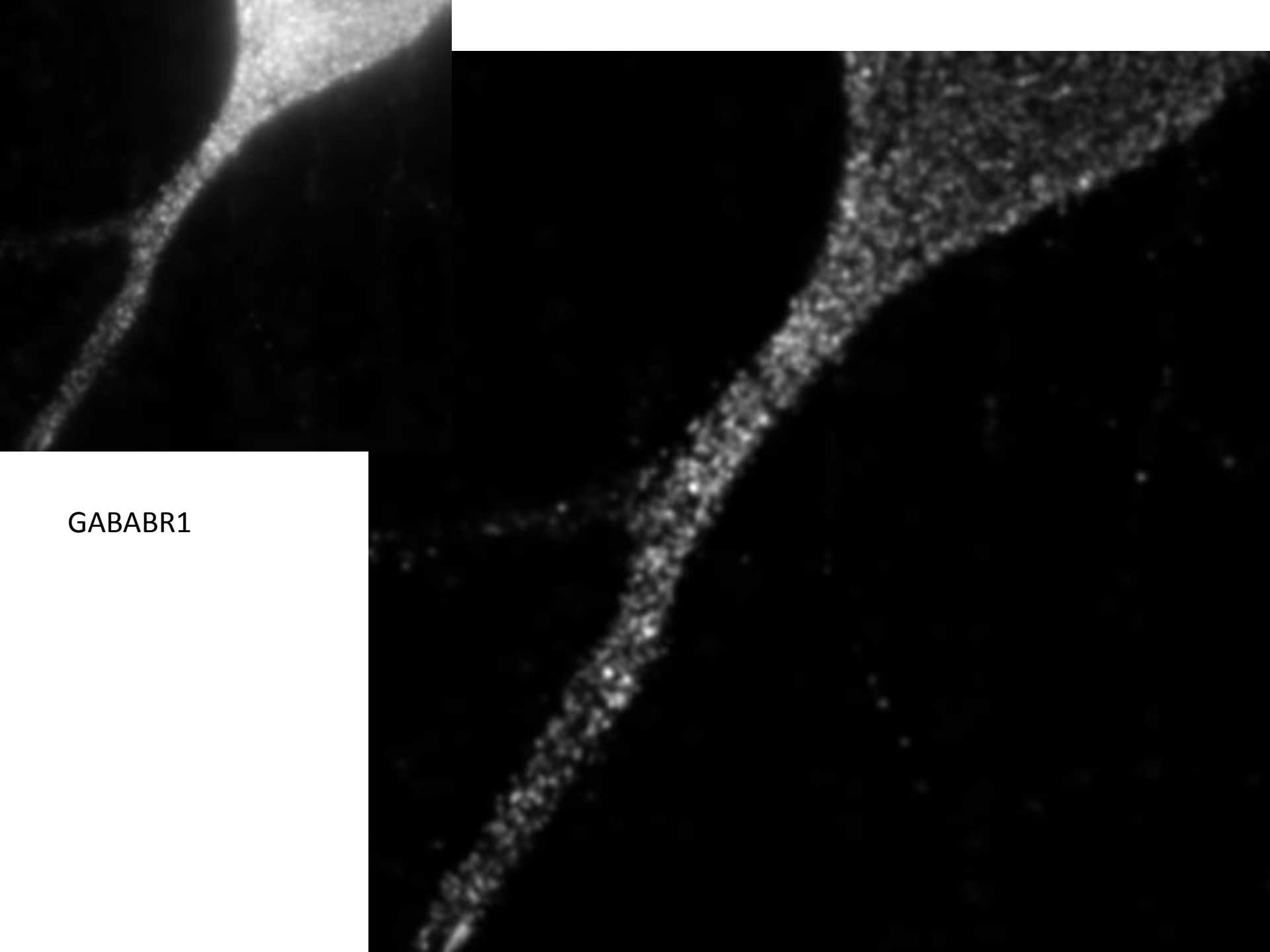
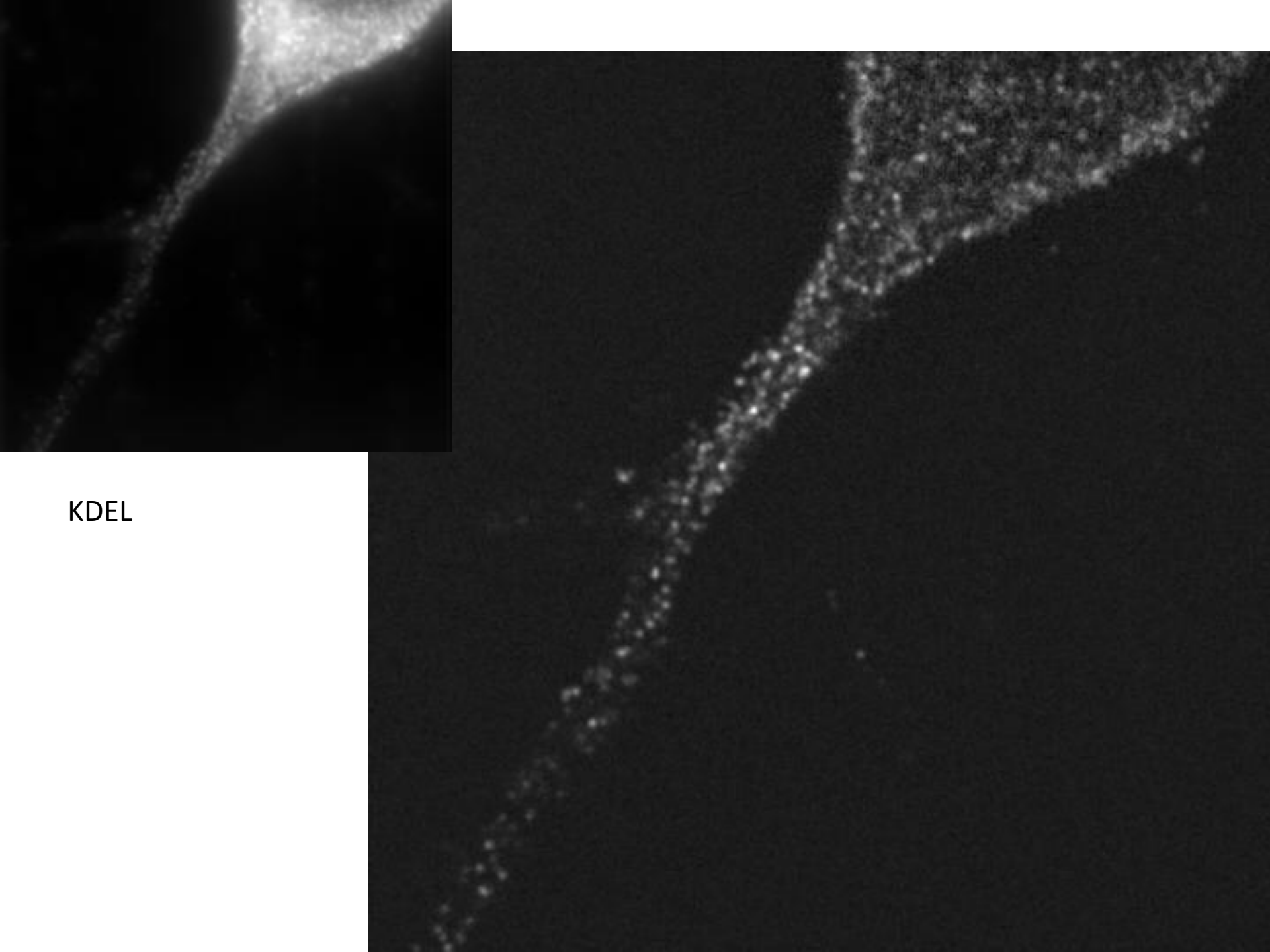
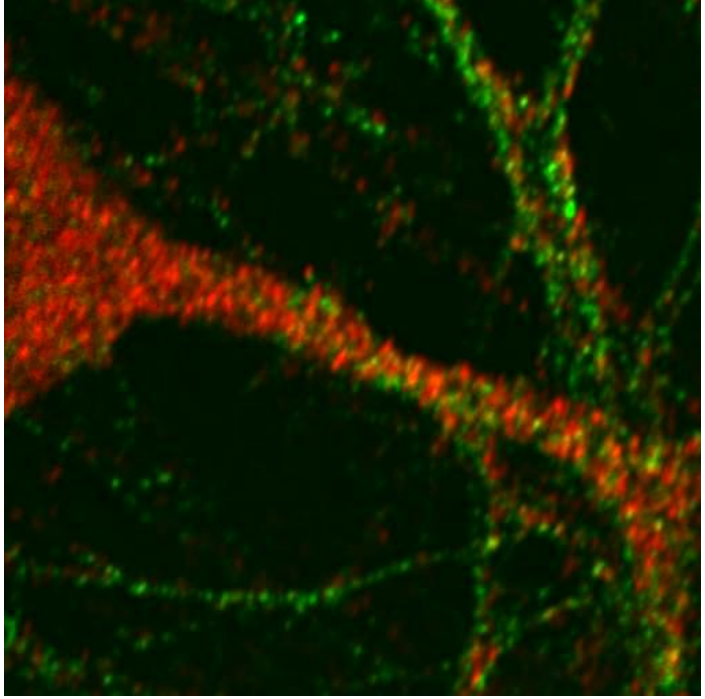


GABABR1

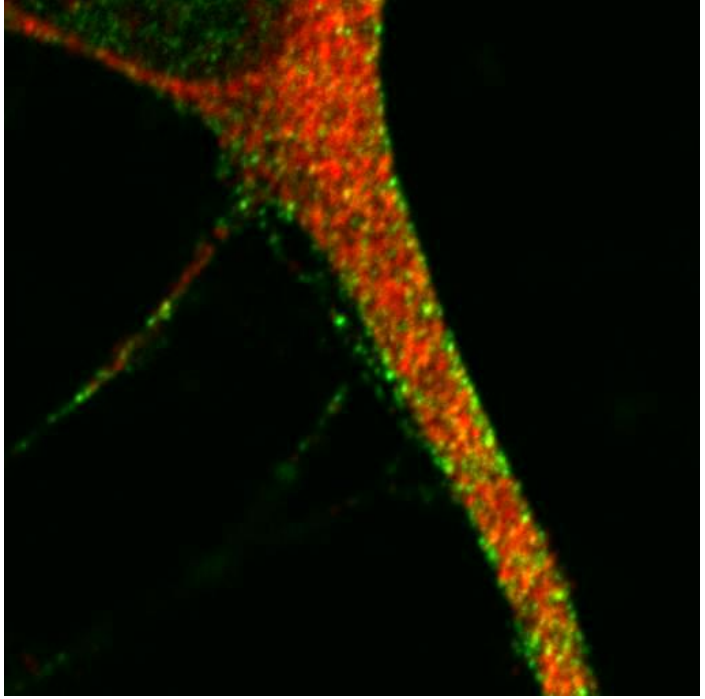


KDEL

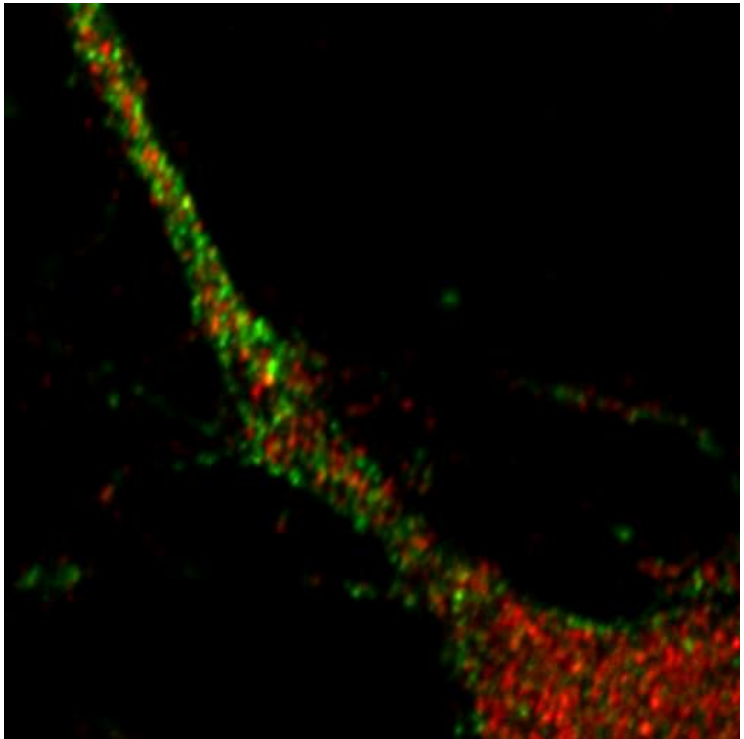


0’

30’

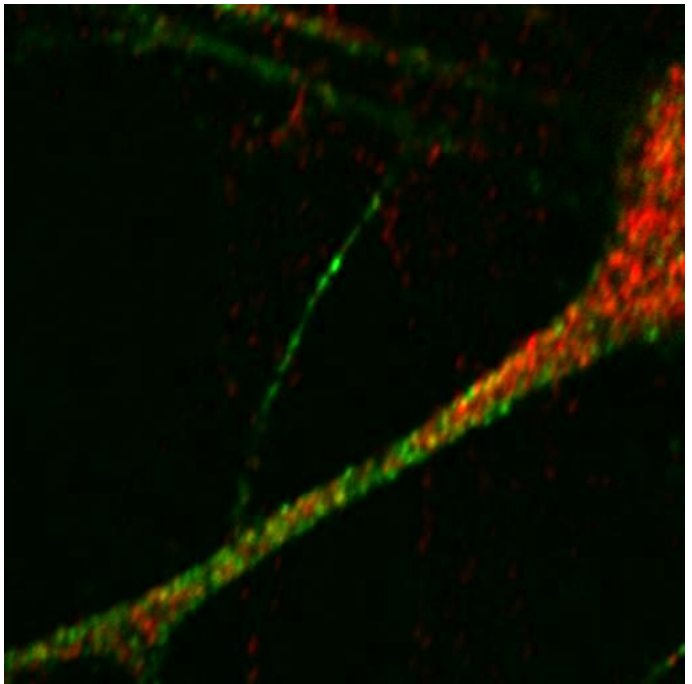


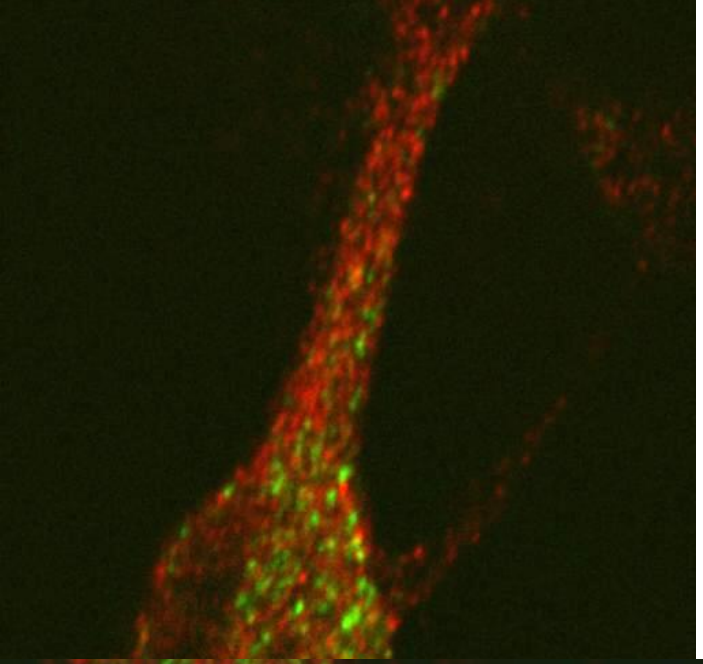
R1/R2



60’

90’



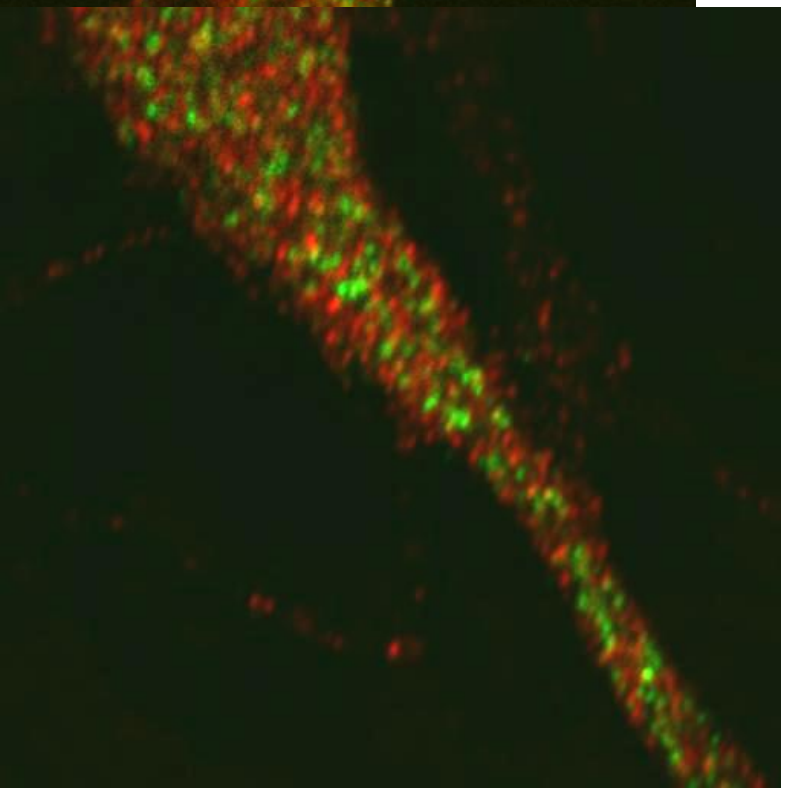


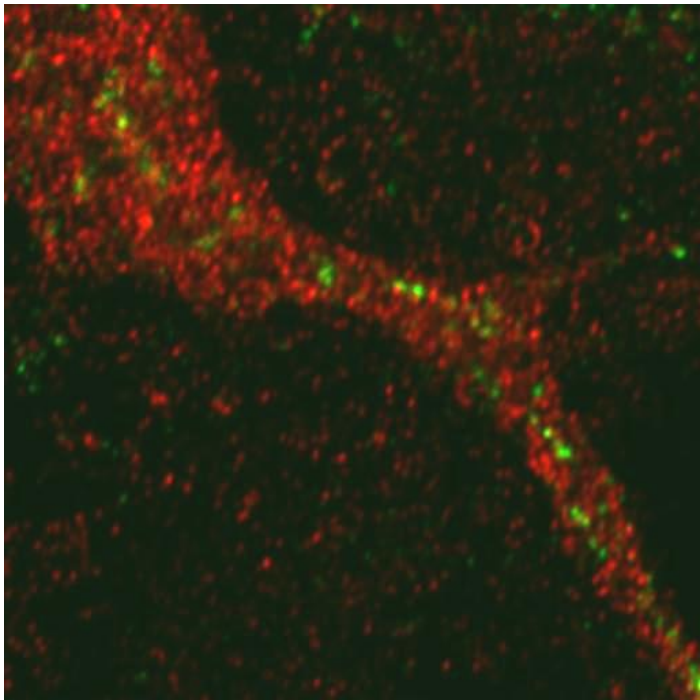
0’

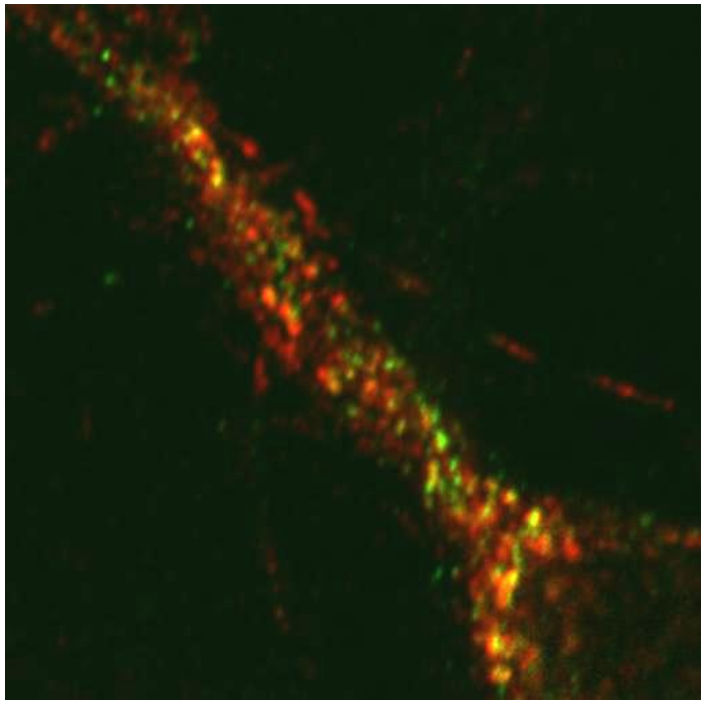
60’

R1/Golgi

120’

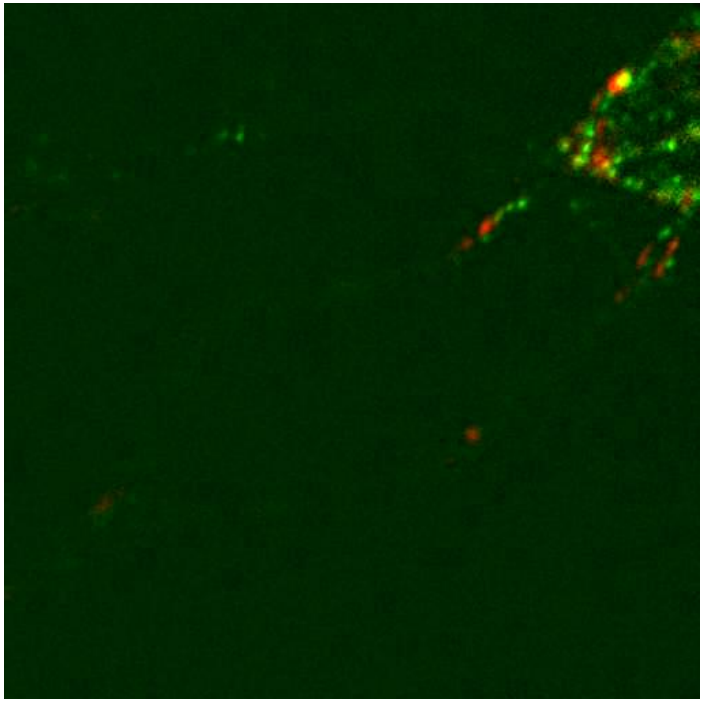


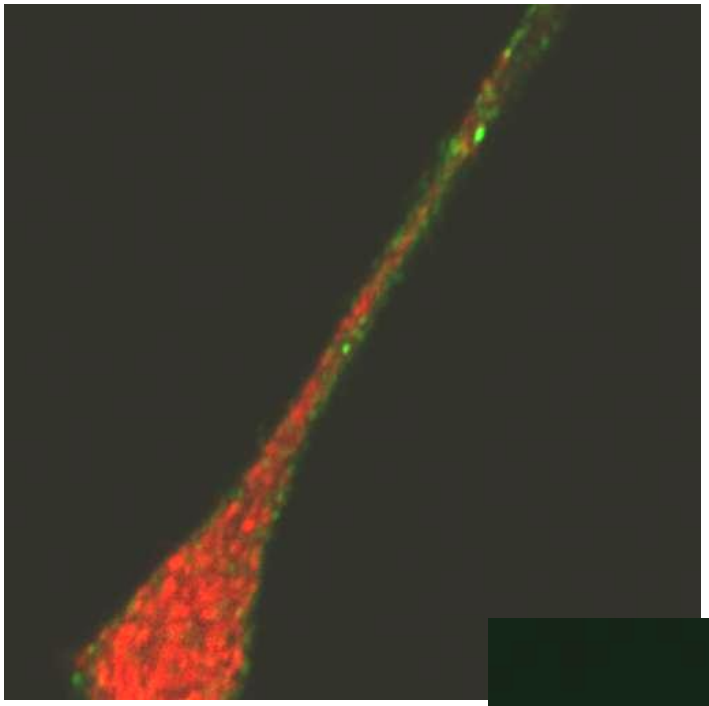




0’ 30’

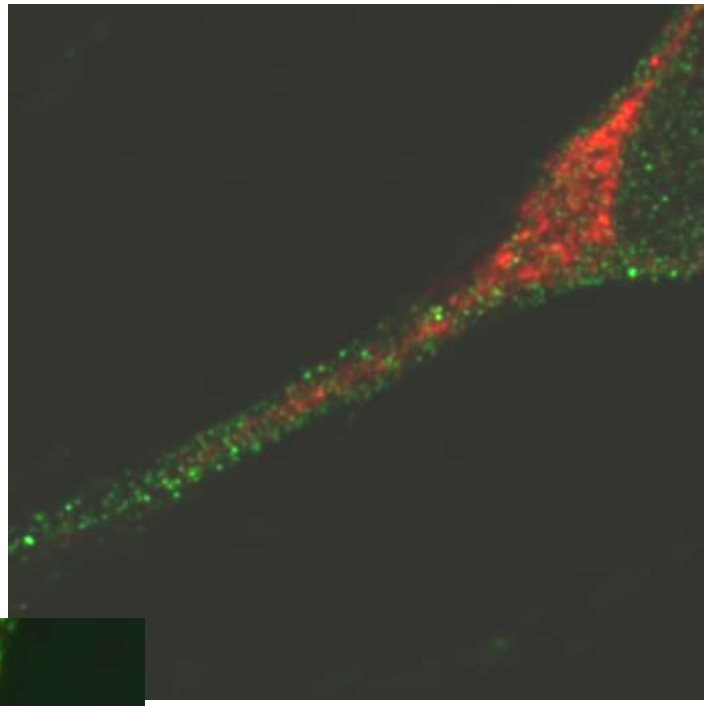
ERGIC/Golgi



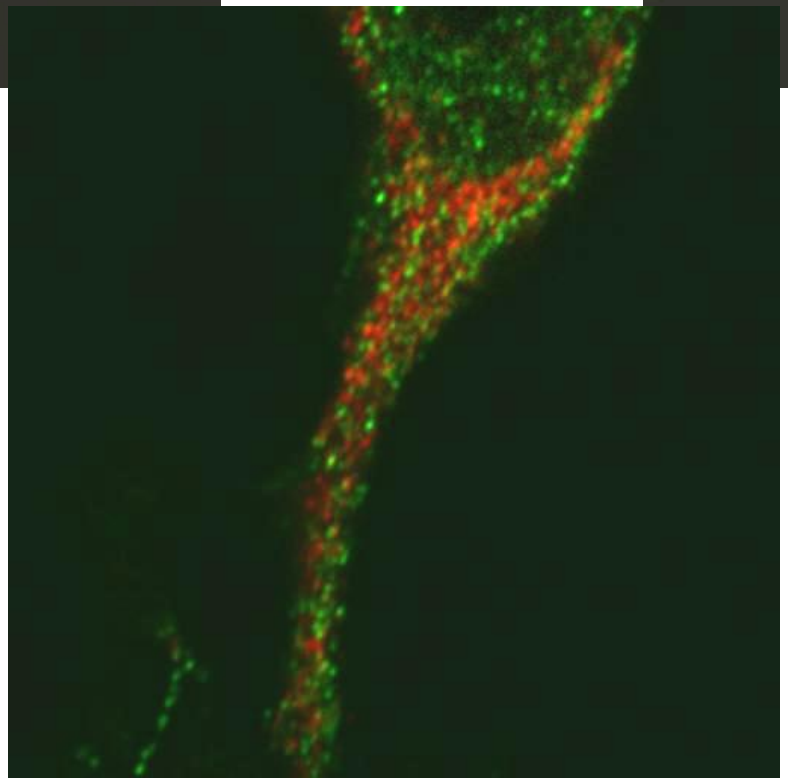


0’

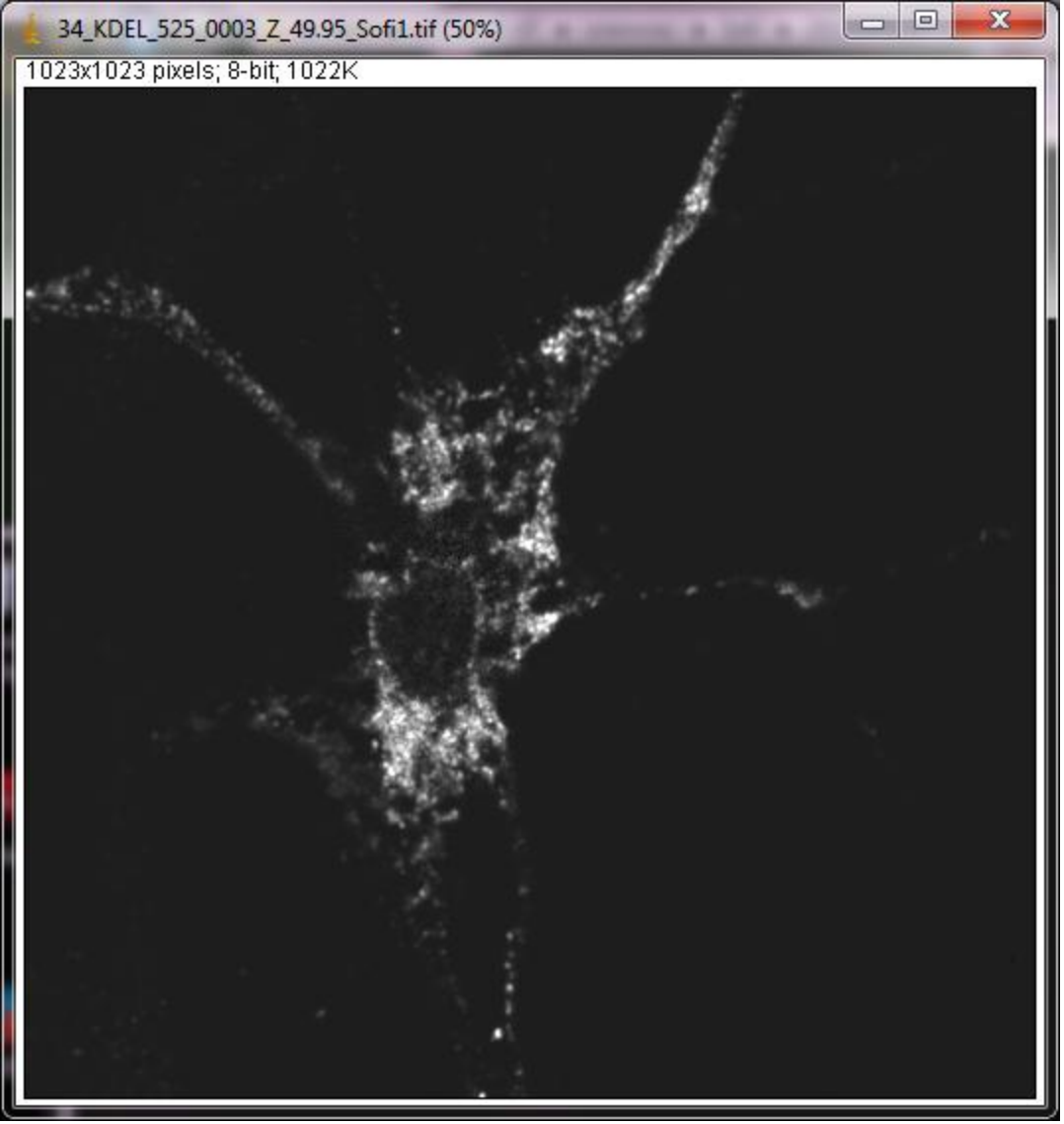
60’



ERGIC/R2

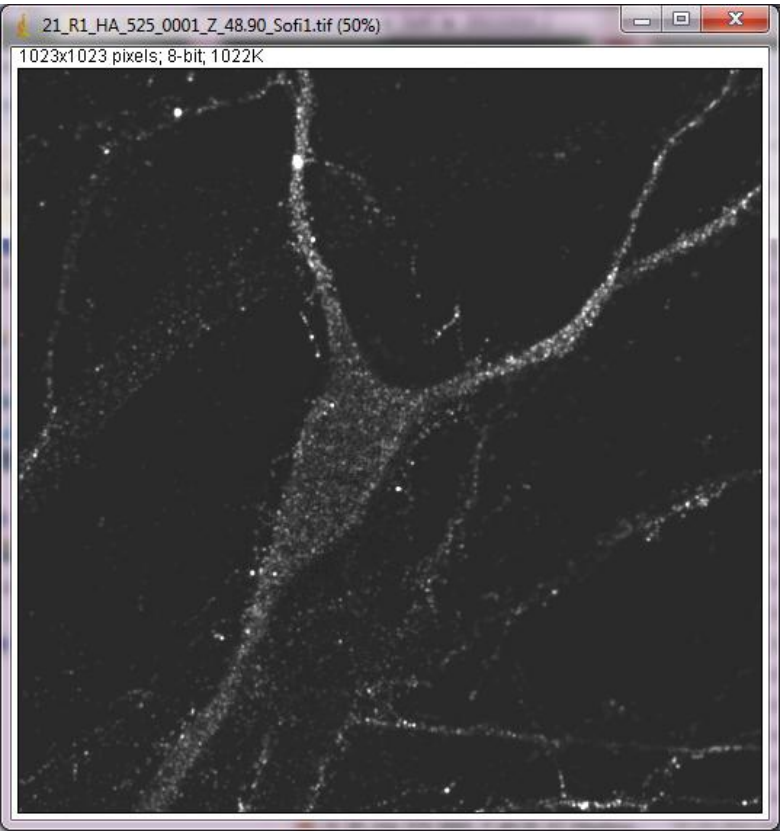


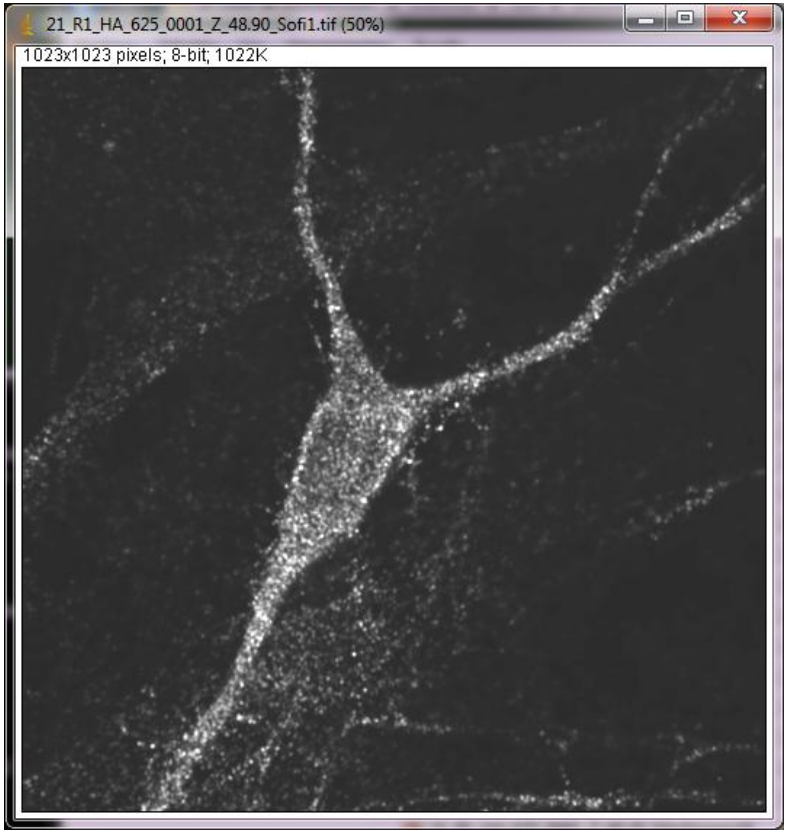
120’



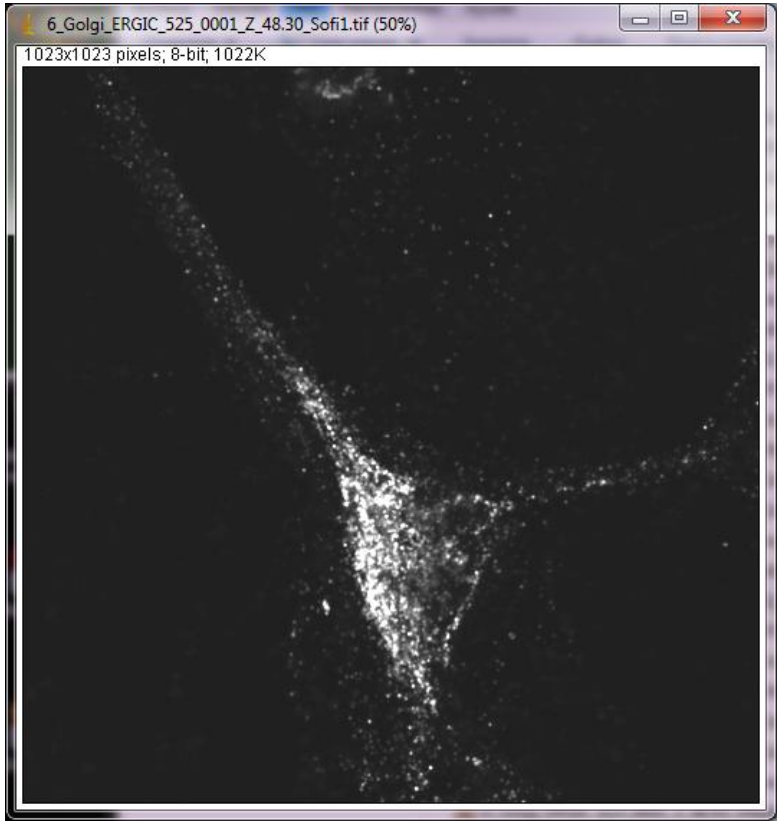
KDEL 30min glut
20111019_1

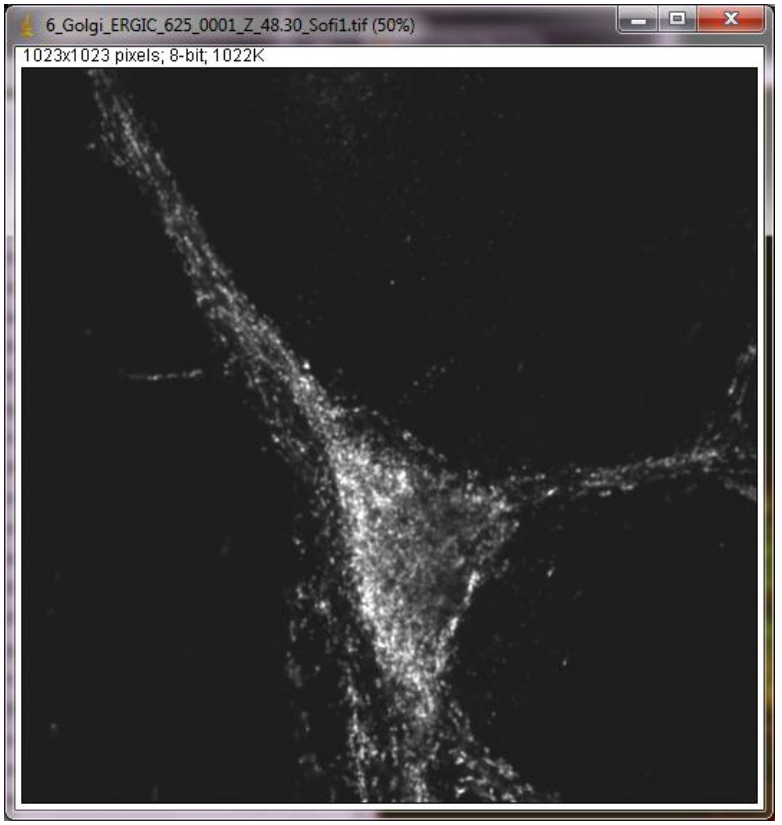
HAR2_R1
20111019_2

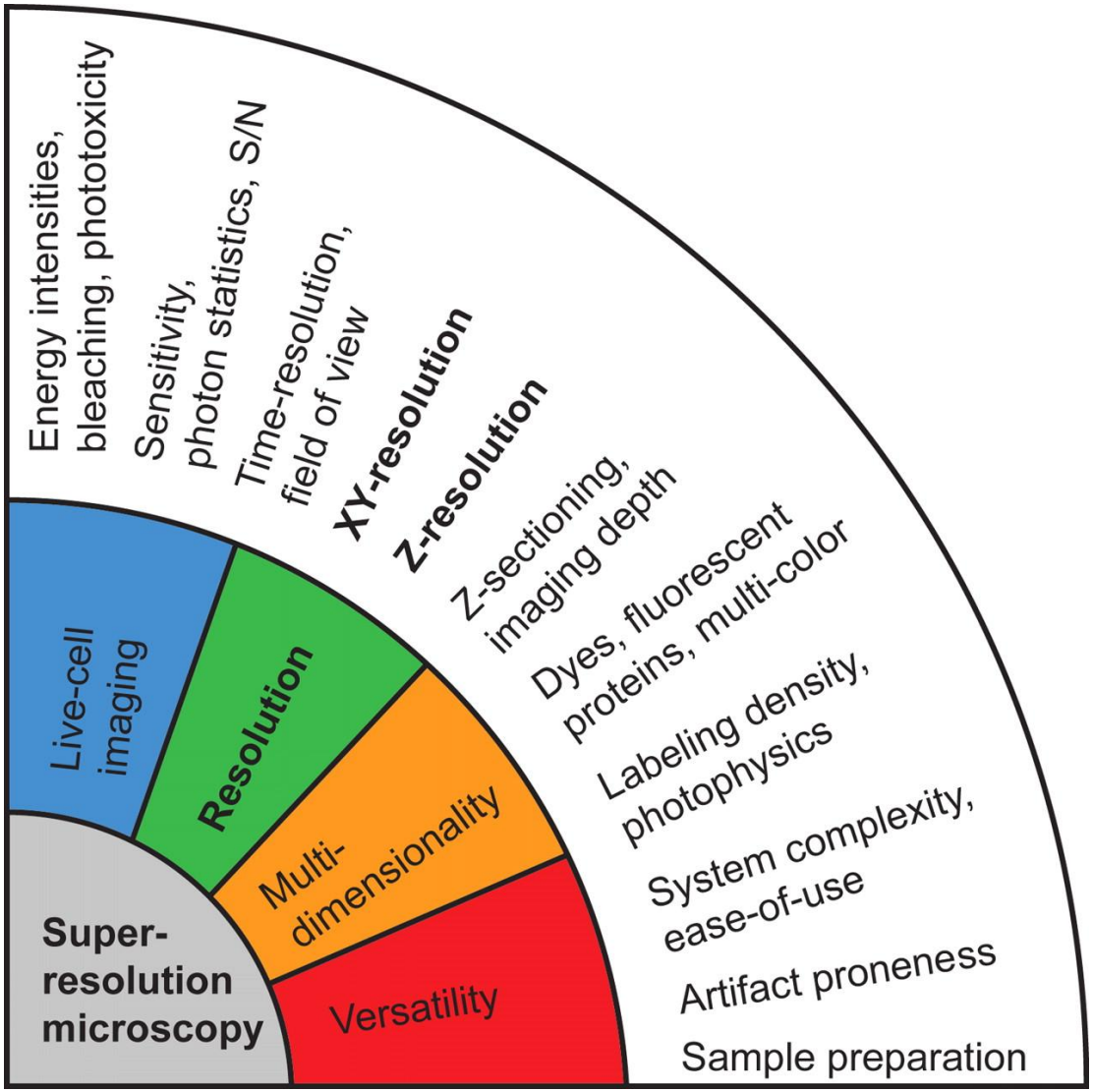


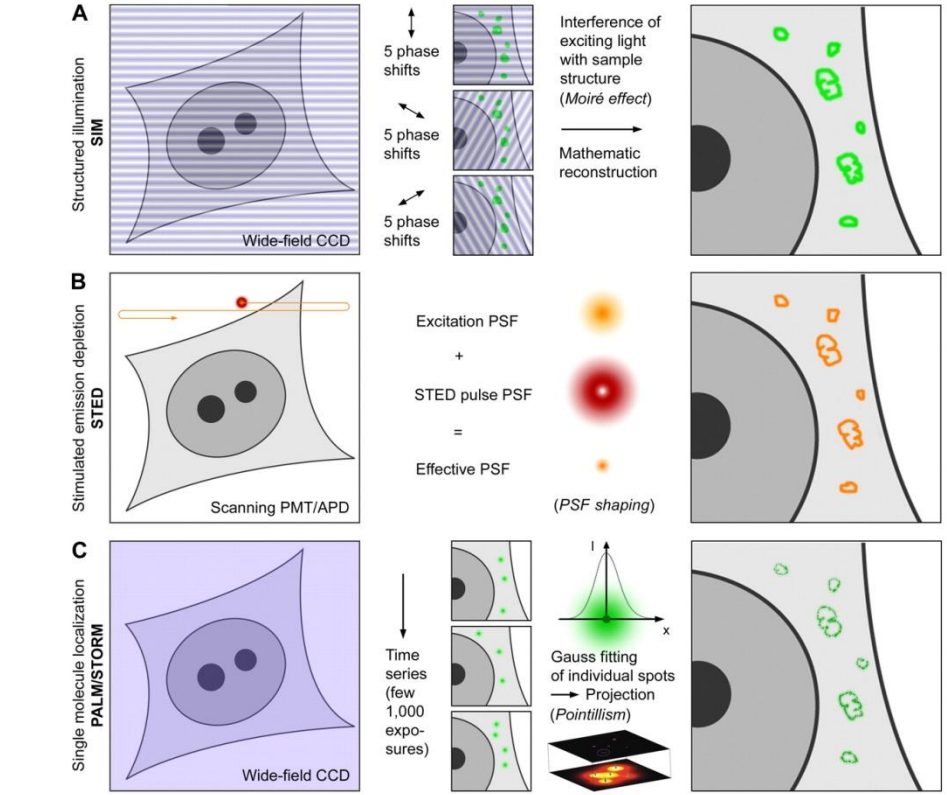


Golgi-ERGiC 30 min washout
20111019_2

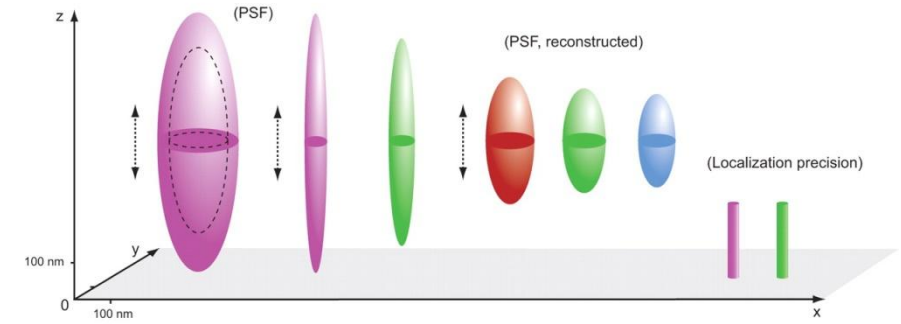




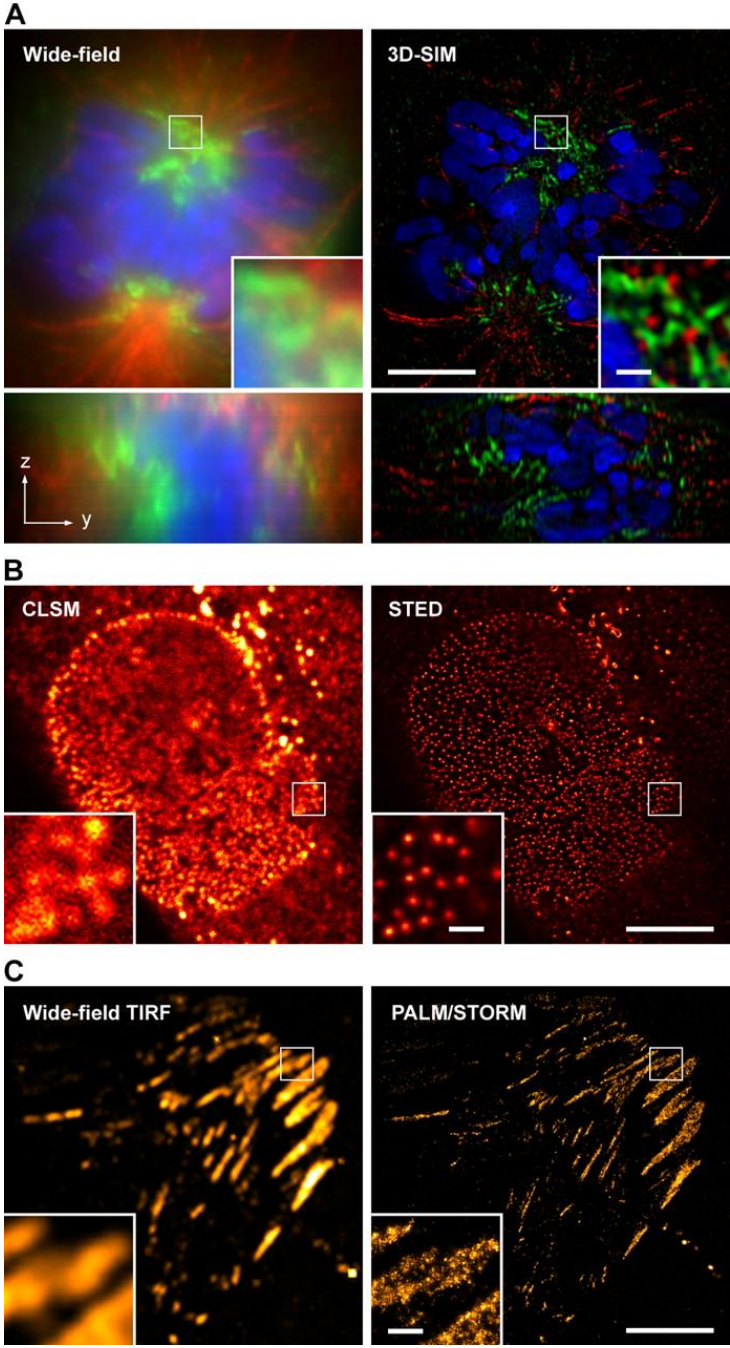

Super-resolution microscopy
Live-cell imaging
Resolution
Multi-dimensionality
Versatility
Energy intensities, bleaching, phototoxicity
Sensitivity, photon statistics, S/N
Time-resolution, field of view
XY-resolution
Z-resolution
Z-sectioning, imaging depth
Dyes, fluorescent proteins, multi-color
Labeling density, photophysics
System complexity, ease-of-use
Artifact proneness
Sample preparation



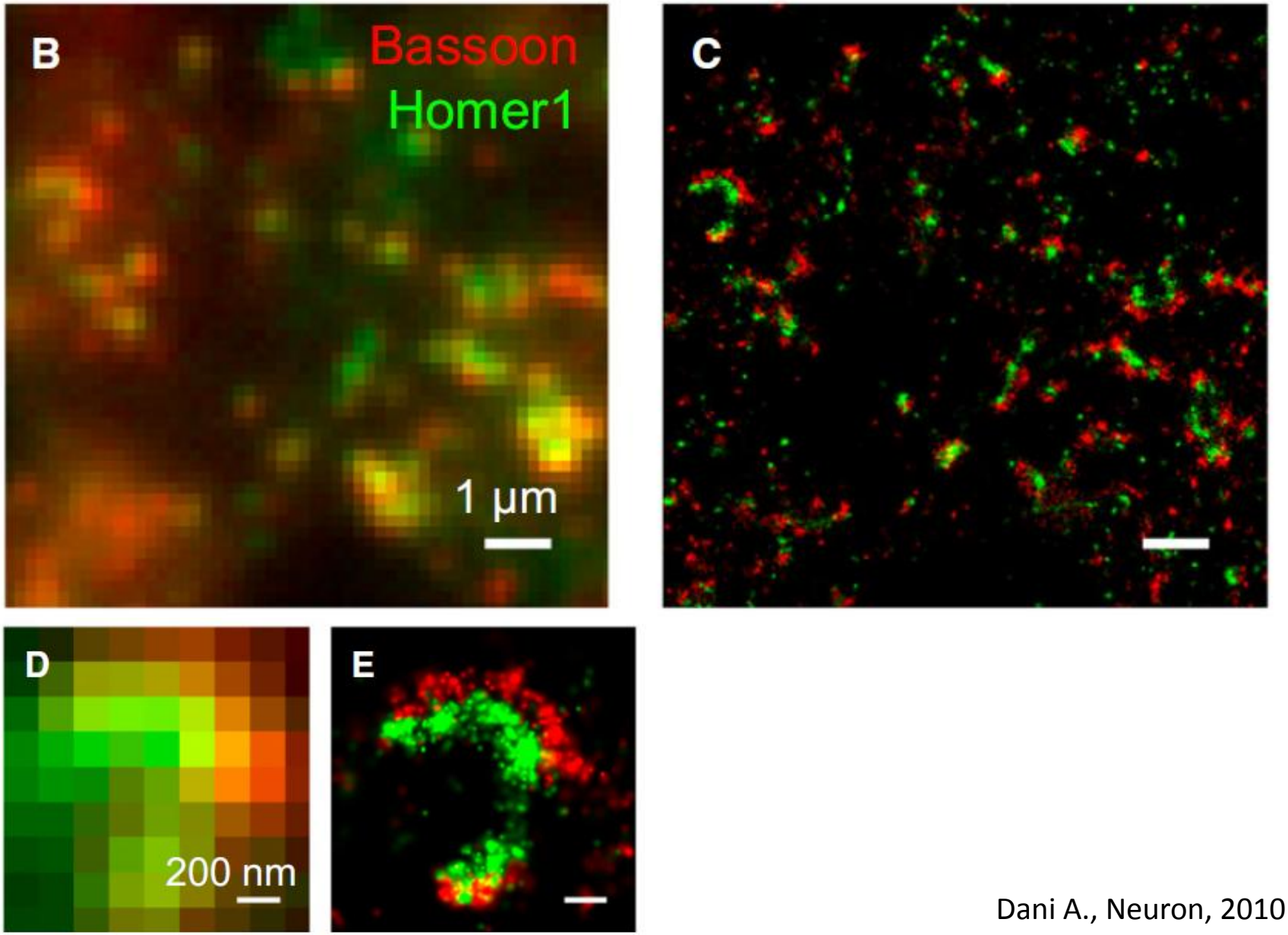
	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λem [nm]	460-670	670	520	620	520	460	670	520



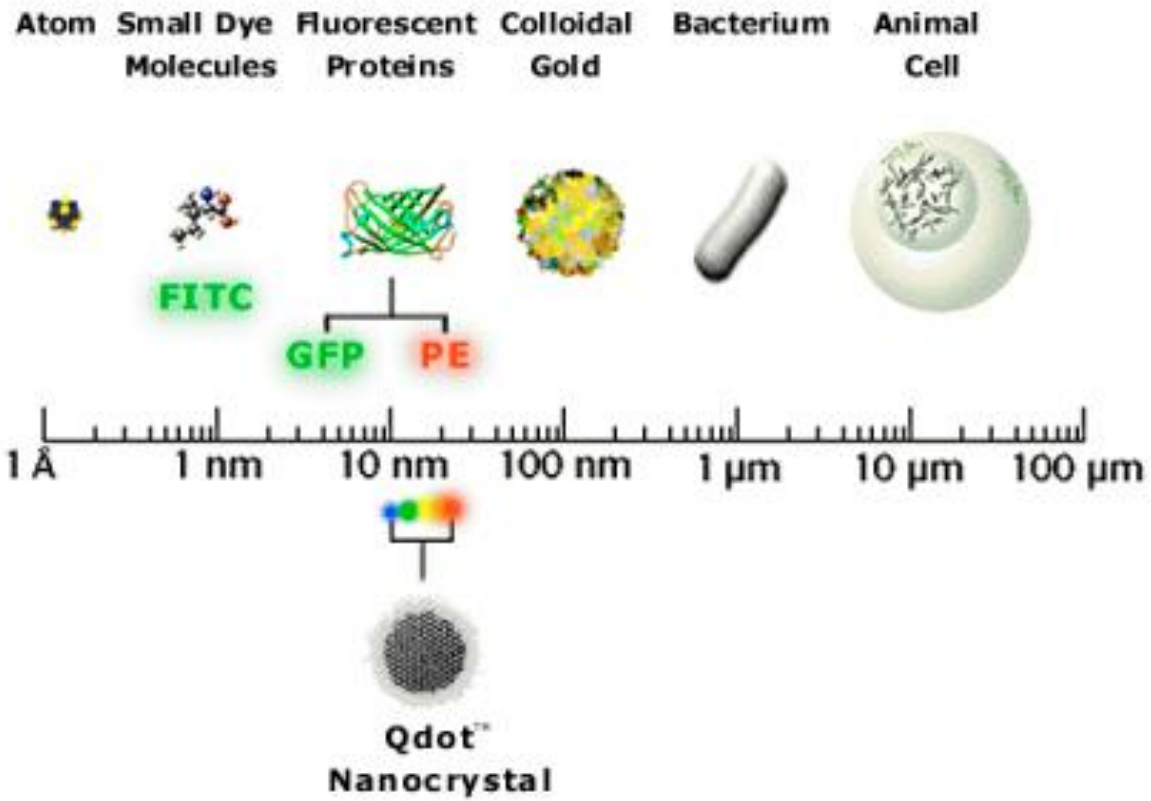
~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} µm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

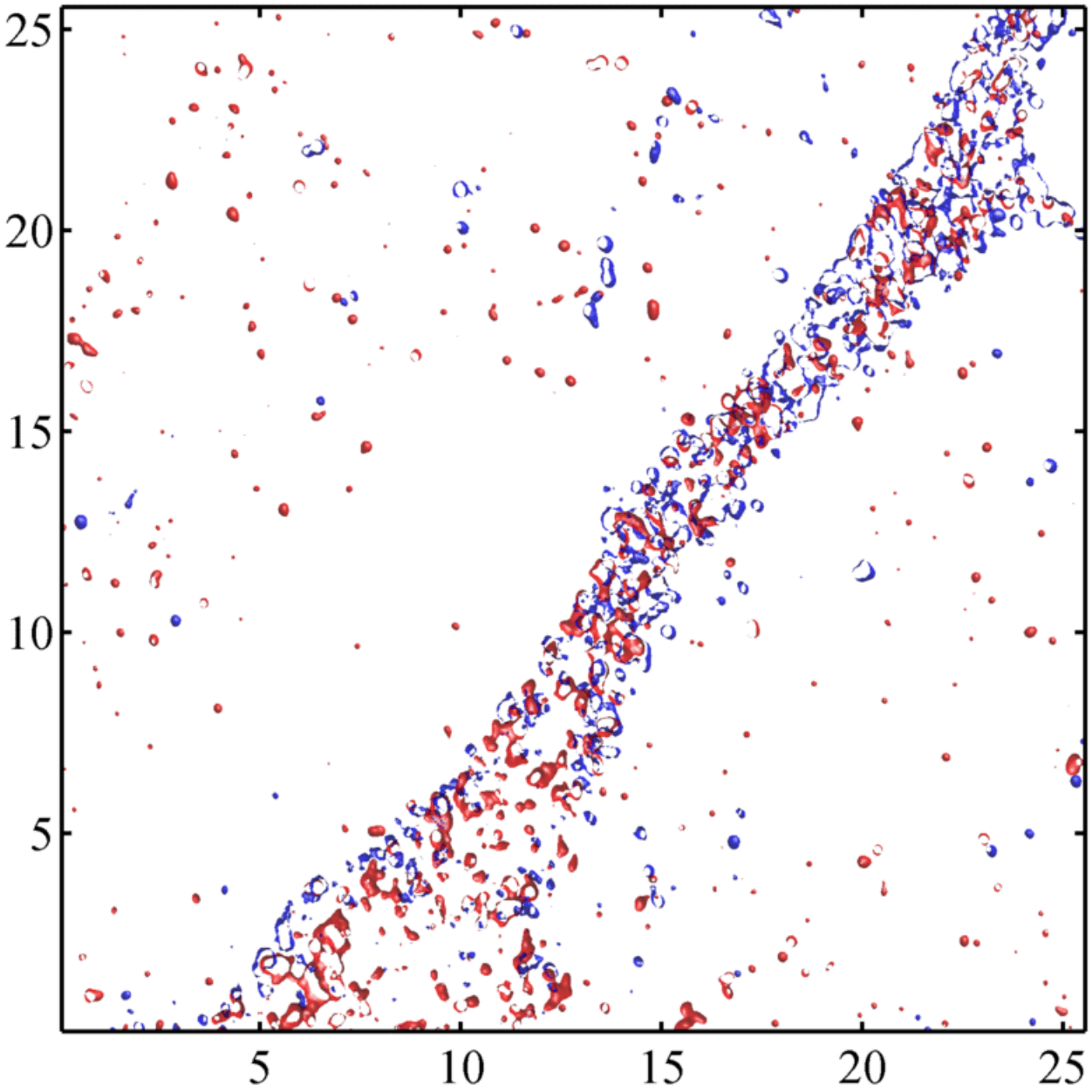


Synaptic organization and the need for superresolution imaging



Dani A., Neuron, 2010


Atom
Small Dye Molecules
Fluorescent Proteins
Colloidal Gold
Bacterium
Animal Cell
FITC
GFP
PE
1 Å
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
Qdot™ Nanocrystal


25
20
15
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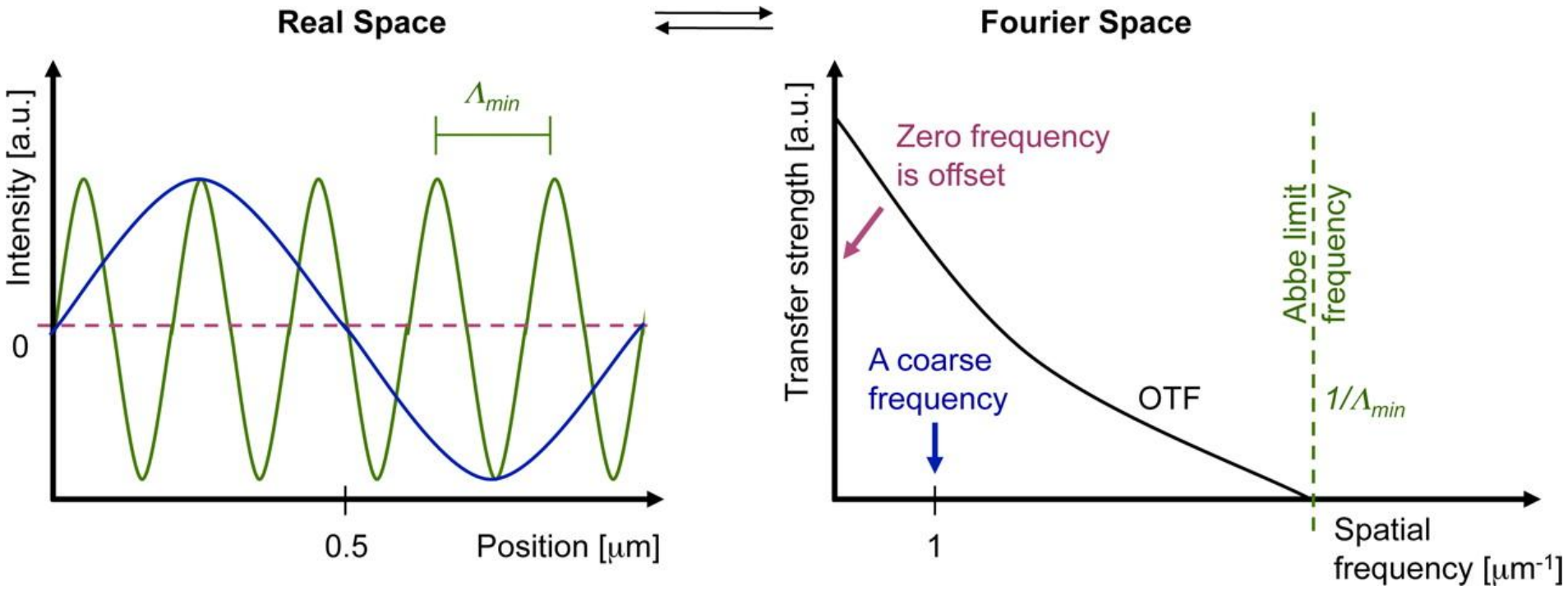
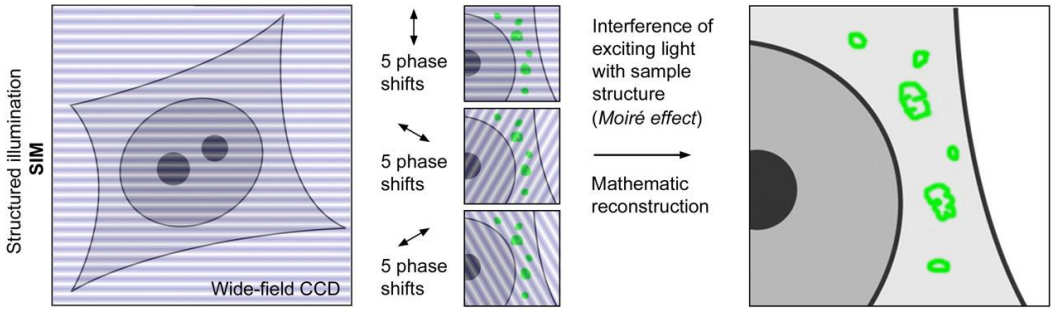
20
25

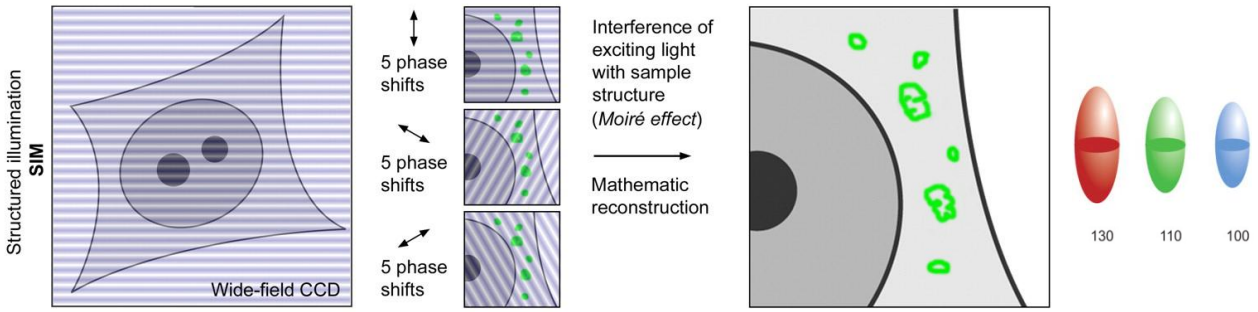
The diffraction limit of detection in the Fourier space



Current superresolution techniques

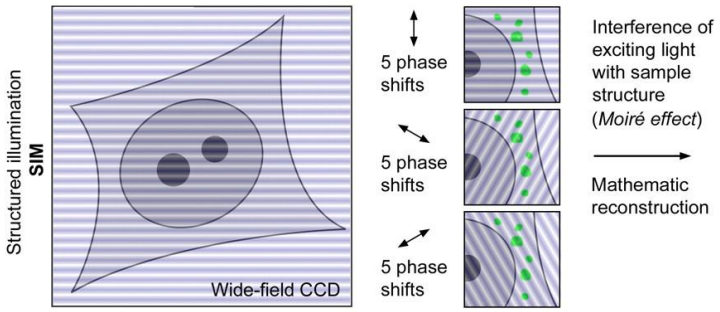


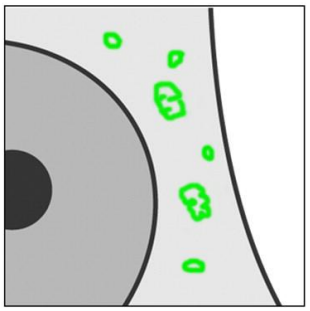
Current superresolution techniques



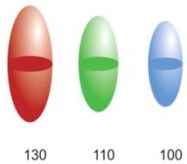
PSF

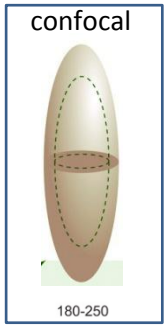
Current superresolution techniques





PSF





Features

Do not require special photophysics

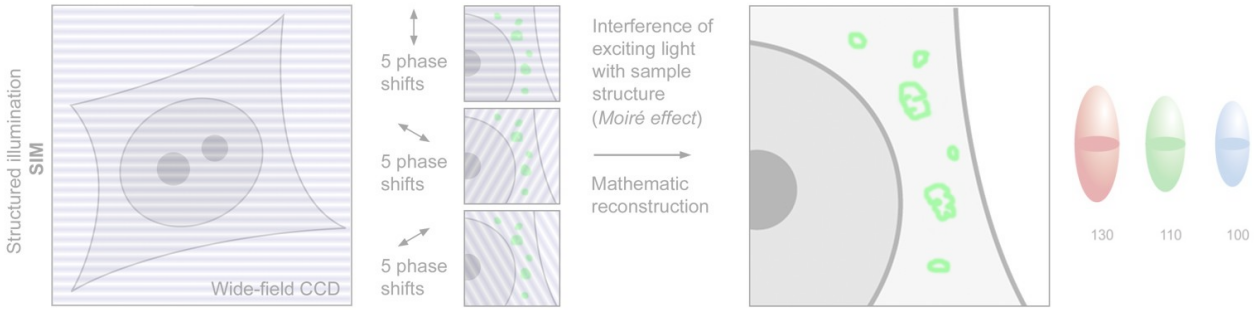
3D and multicolor capability

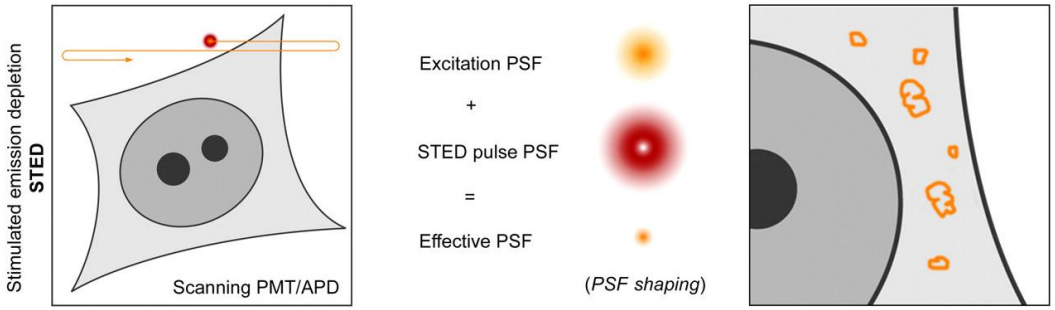
ms-s

Current superresolution techniques

PSF

Features



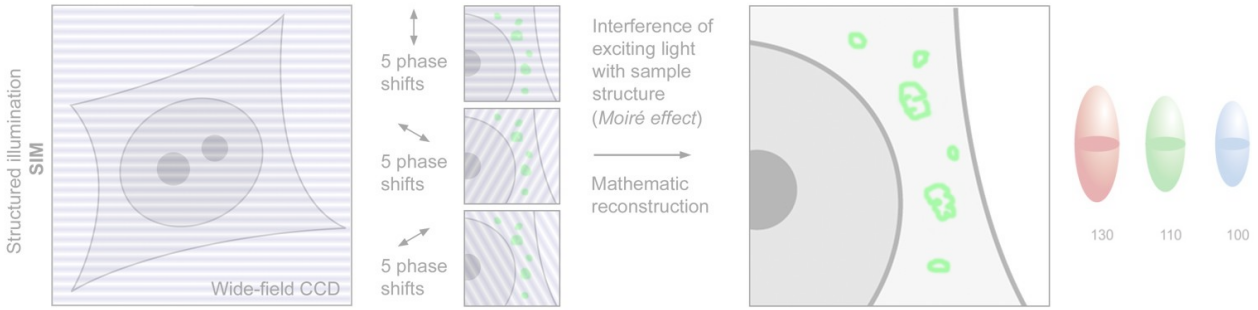


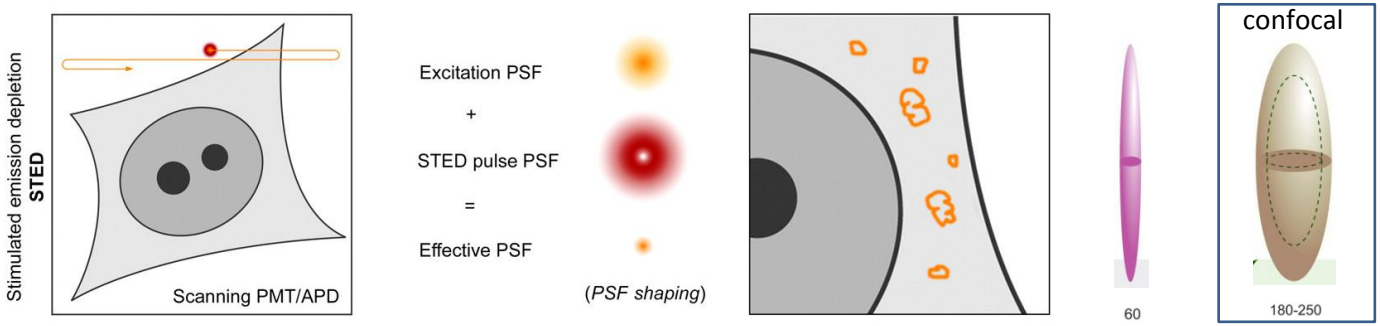
- Do not require special photophysics
- 3D and multicolor capability
- ms-s

Current superresolution techniques

PSF

Features



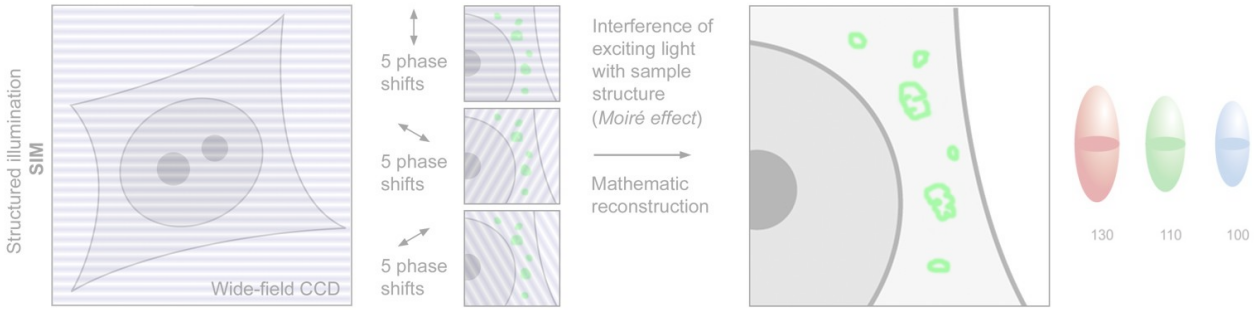


- Do not require special photophysics
- 3D and multicolor capability
- ms-s

Current superresolution techniques

PSF

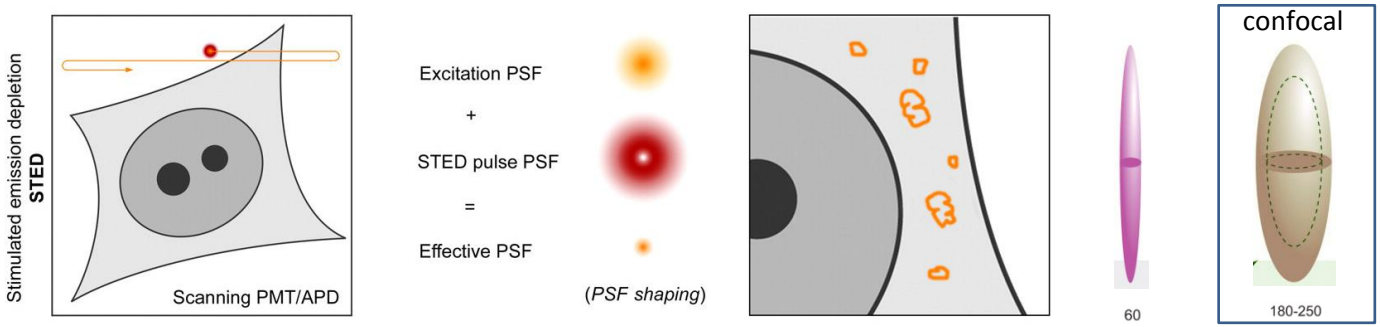
Features



Do not require special photophysics

3D and multicolor capability

ms-s



Expensive/complex equipment

Require special dyes

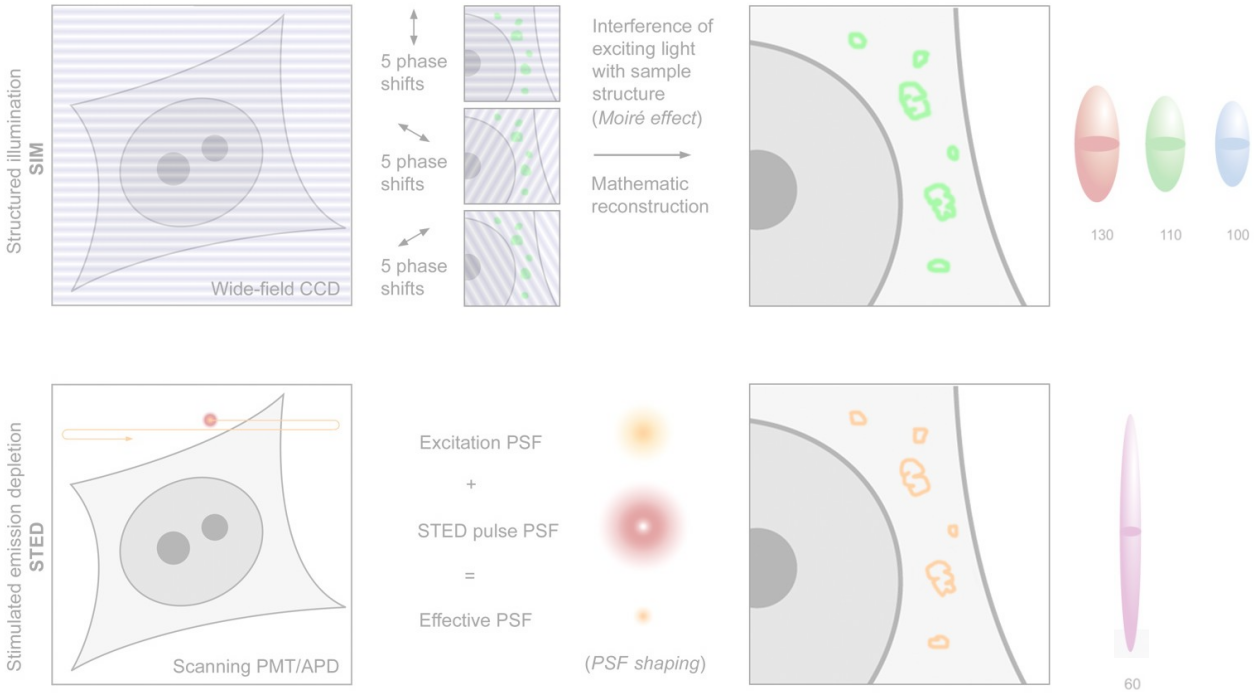
Limited flexibility for multilabaleing

ms-min

Current superresolution techniques

PSF

Features



Do not require special photophysics

3D and multicolor capability

ms-s

Expensive/complex equipment

Require special dyes

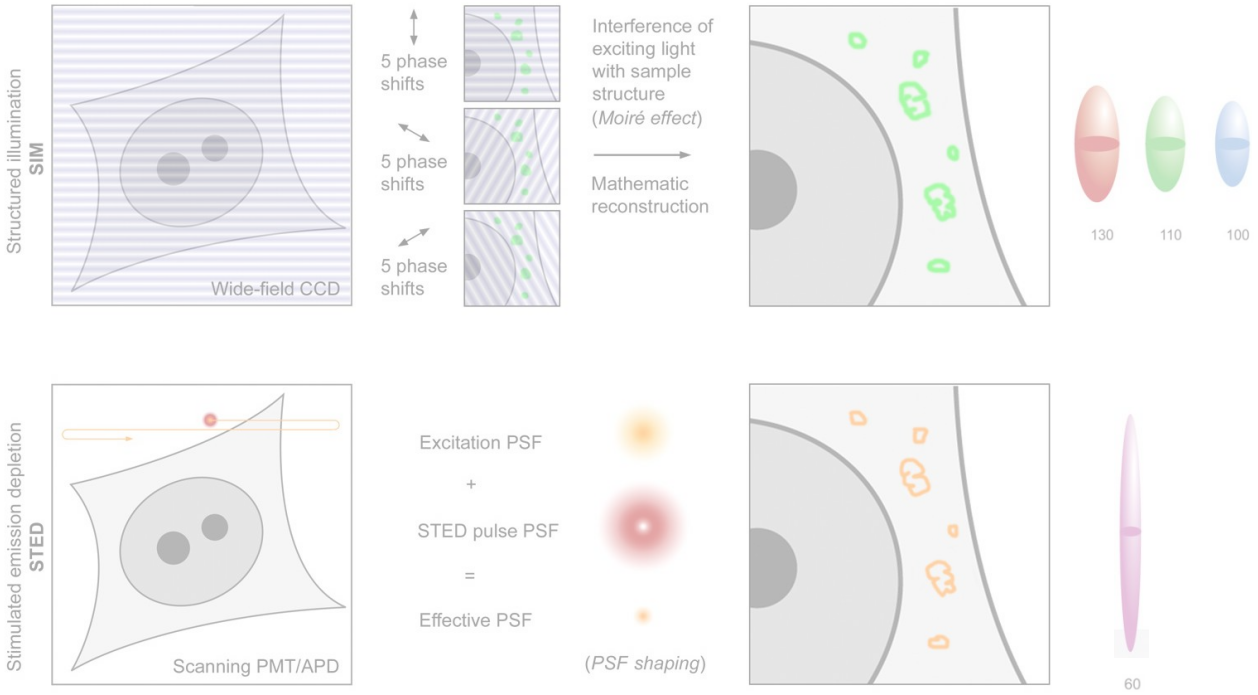
Limited flexibility for multilabaleing

ms-min

Current superresolution techniques

PSF

Features



Do not require special photophysics

3D and multicolor capability

ms-s

Expensive/complex equipment

Require special dyes

Limited flexibility for multilabaleing

ms-min

Require >1000 images per slice

Require special dyes

s-min