

Cell biophysics of fluorescent probes for super-resolution optical microscopy

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Superresolution microscopy

- **PALM** - photoactivation localization microscopy, ~20 nm lateral resolution, fixated samples only, low intensity staining sufficient
- **STORM** - stochastic optical reconstruction microscopy
- **STED** - stimulated emission depletion microscopy, ~60 nm lateral resolution, living cells, high intensity needed (expression high enough)
- **SIM** - structured illumination microscopy, ~ 100 nm lateral resolution, fixated samples only



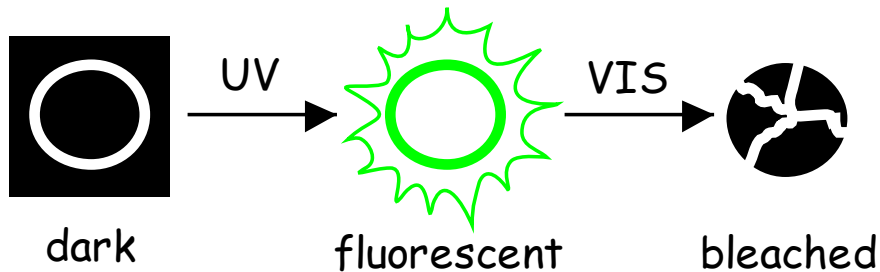
PALM/STORM - Principle

- Only small fraction of fluorescence molecules population is activated
- The localization is calculated by mathematical algorithms
- Result: table of numbers with precise location of the fluorophore molecule

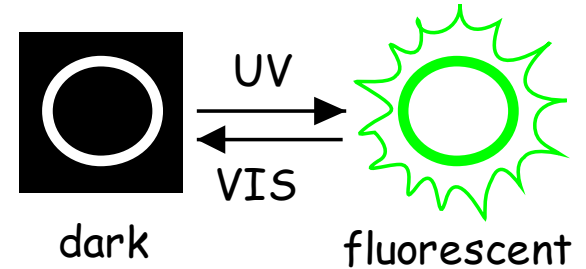


Photoactivation, photoconversion and photoswitching

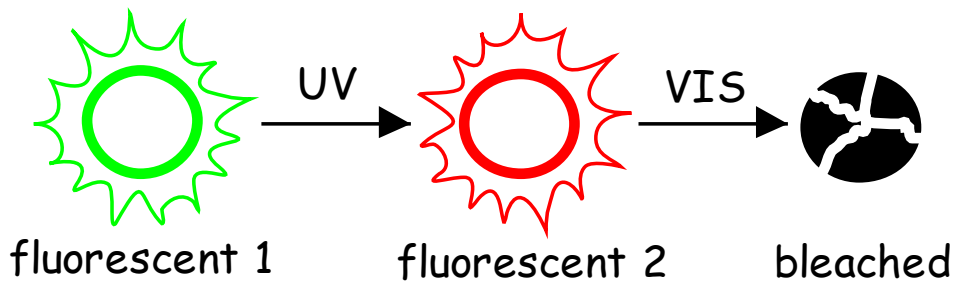
photoactivation irreversible



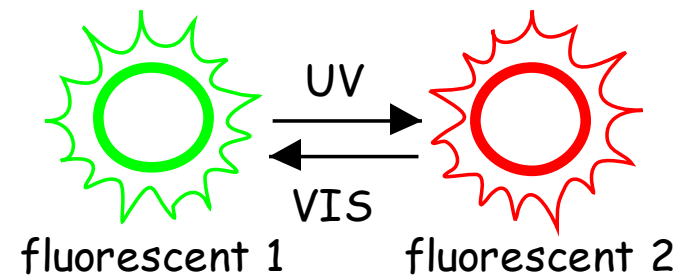
photoactivation reversible



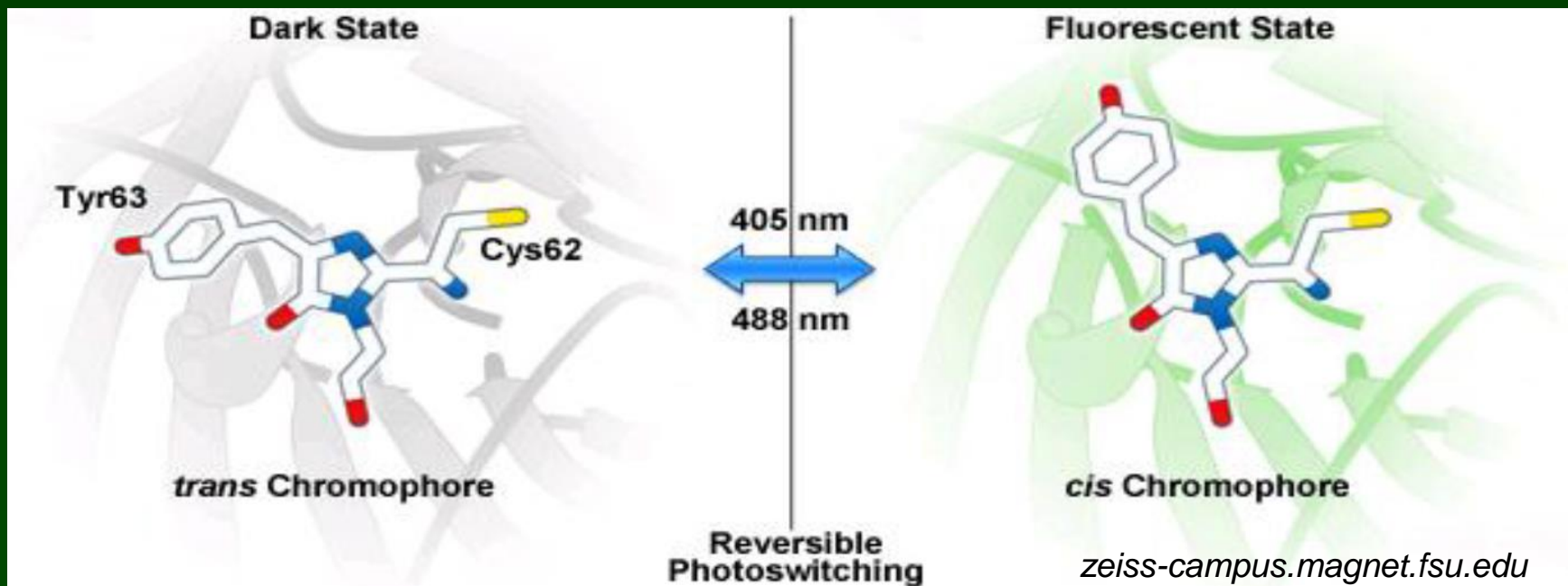
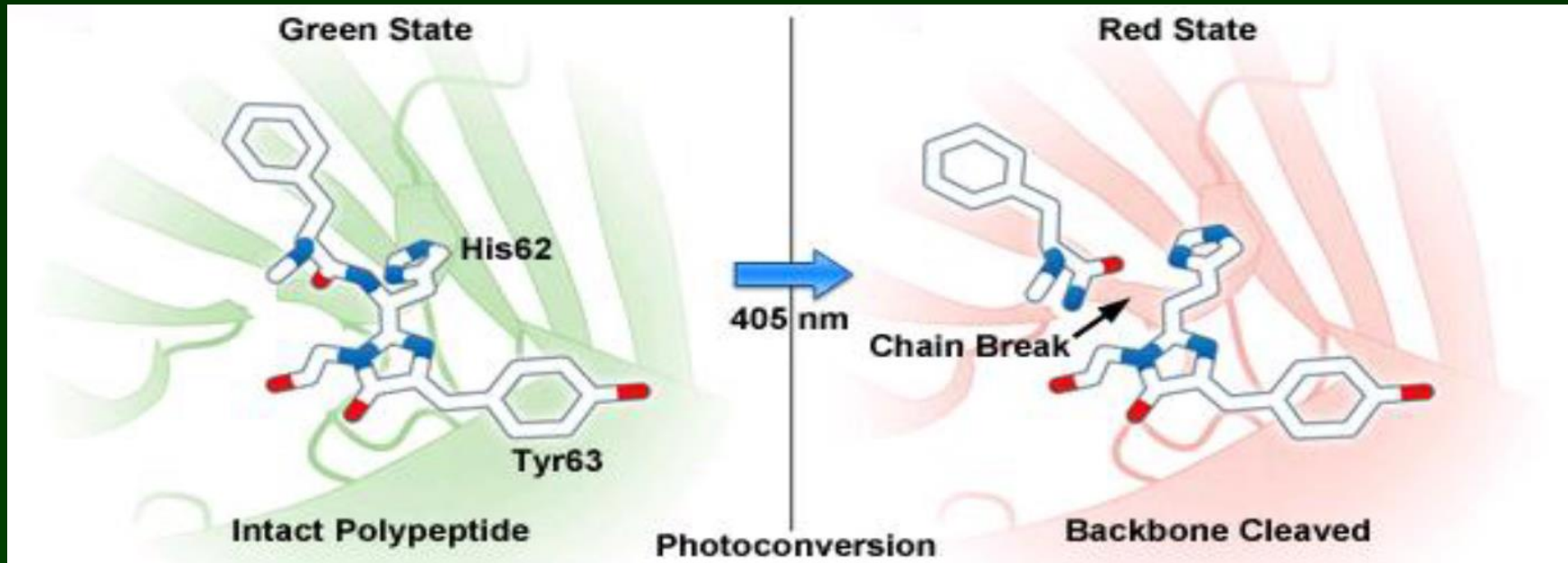
photoconversion irreversible



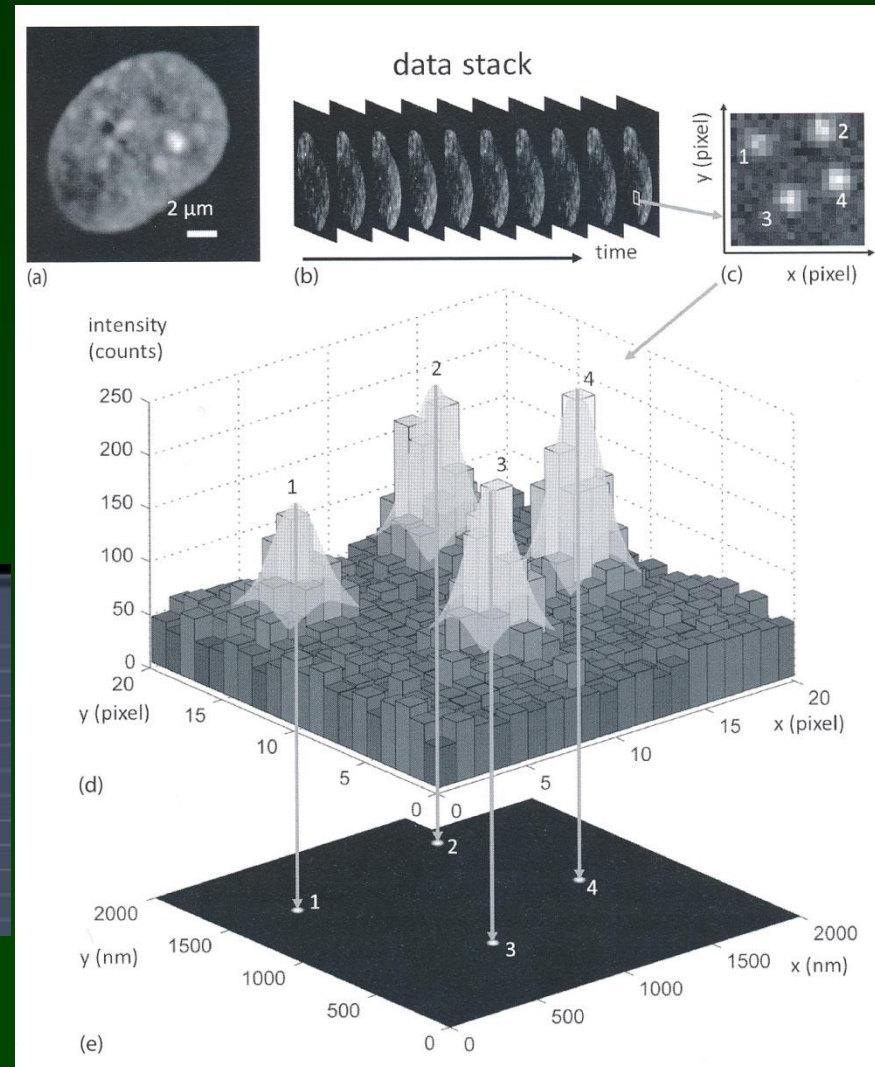
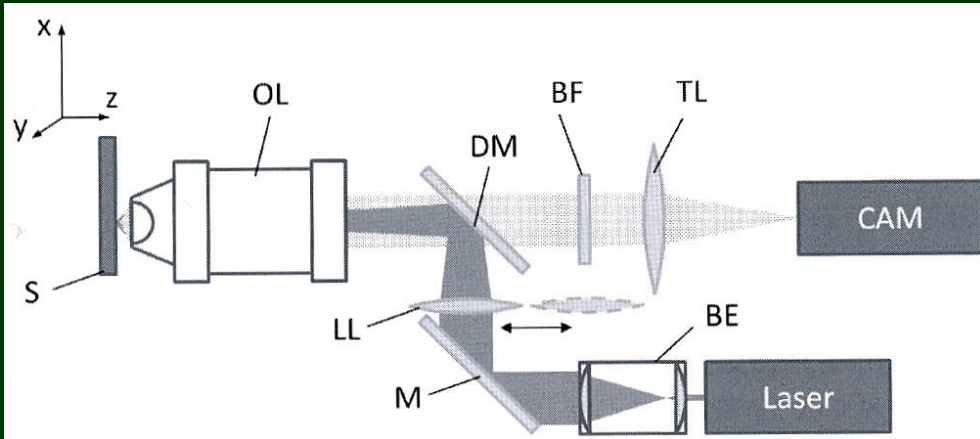
photoconversion reversible



Photoconversion and photoswitching



PALM/STORM



Position X [nm]	Position Y [nm]	Precision [nm]	Number Photons	Background variat	Chi square	PSF width [nm]
12295.3	20075.8	18.7	840	37.94	0.61	177.9
19228.8	24263.9	18.4	1292	94.72	0.97	177.9
18971.3	23727.5	14.7	1645	94.72	0.97	177.9
19487.6	23735.2	11.3	2207	94.72	0.97	177.9
10060.3	18738.4	36.3	428	42.30	0.50	177.9
12471.8	24750.5	31.7	641	73.86	0.54	177.9
19893.1	24530.7	15.0	1605	94.72	0.97	177.9
11072.9	19337.3	13.0	1335	42.30	0.50	177.9
3605.5	14952.1	35.1	475	49.19	0.47	177.9
17266.6	18409.1	27.7	711	67.88	0.63	177.9
10029.7	18751.6	20.6	799	43.41	0.42	177.9
18967.0	23680.8	15.4	1353	66.87	0.97	177.9



PALM/STORM

ZEN 2012

File View Acquisition Maintain Macro Window Help

Workspace Zoom [Reset]

Workspace Configuration [Meta]

Processing Maintain


Method

- Maximum intensity projection
- Color-coded projection
- Image calculator
- Average
- Filter
- Linear unsmearing
- Ion Concentration
- Correlation
- Modify Series
- HDR- Imaging
- Stack
- PALM**

Method Parameters

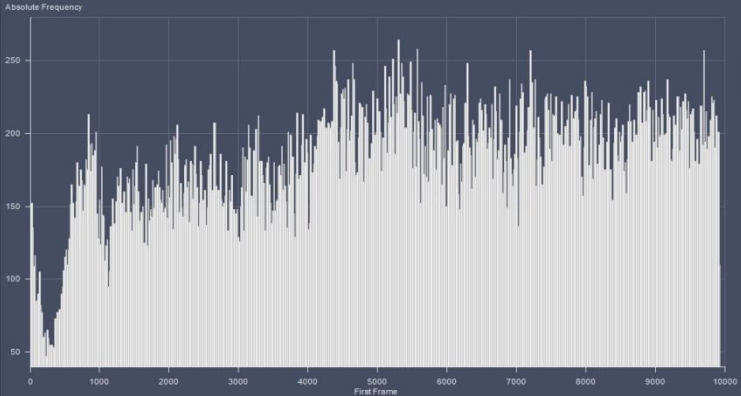
Input Image [Select]

2D
3D
Split
3.5D
Info
Cubic
Profile
PALM
Info



Index	First Frame	Number Frames	Frames Missing	Position X [nm]	Position Y [nm]	Precision [nm]	Number Photons	Background vari	Chi square	PSF width [nm]	Channel
1	1	1	0	12295.3	20075.8	18.7	840	37.94	0.61	177.9	
2	1	1	0	19228.8	24283.9	18.4	1292	94.72	0.97	177.9	
3	1	1	0	18971.3	23727.5	14.7	1645	94.72	0.97	177.9	
4	1	1	0	18487.6	23735.2	11.3	2207	94.72	0.97	177.9	
5	1	1	0	10060.2	18788.4	26.3	428	42.30	0.50	177.9	
6	1	1	0	12471.8	24760.5	31.7	641	73.86	0.54	177.9	
7	1	1	0	18893.1	24530.7	15.0	1605	94.72	0.97	177.9	
8	1	1	0	11072.9	19337.2	13.0	1335	42.30	0.50	177.9	
9	1	1	0	3605.5	14952.1	35.1	475	49.19	0.47	177.9	
10	1	1	0	17266.6	18409.1	27.7	711	67.88	0.63	177.9	
11	2	1	0	19029.7	18751.6	20.8	799	43.41	0.42	177.9	
12	2	1	0	18967.0	23680.8	16.4	1263	66.87	0.92	177.9	
13	2	1	0	18356.5	24275.9	15.2	1356	66.87	0.92	177.9	
14	2	1	0	18428.6	23697.8	9.2	2400	66.87	0.92	177.9	
15	2	1	0	12499.8	24755.4	34.6	438	39.84	0.57	177.9	
16	2	1	0	16814.5	18548.8	33.4	424	34.30	0.44	177.9	
17	2	1	0	17318.9	18497.2	18.3	825	34.30	0.44	177.9	
18	2	1	0	11155.9	19449.8	23.6	631	35.95	0.45	177.9	
19	3	1	0	13775.4	23357.6	19.9	1048	72.43	1.01	177.9	
20	3	1	0	17203.5	22490.8	26.7	466	50.07	0.43	177.9	
21	3	1	0	17433.4	22967.4	18.9	965	52.07	0.43	177.9	
22	3	1	0	13765.7	20681.1	14.7	1004	30.45	0.41	177.9	
23	3	1	0	18824.5	23650.7	18.3	1385	123.48	0.95	177.9	
24	3	1	0	18294.9	23527.9	22.5	1178	123.48	0.95	177.9	
25	3	1	0	18206.2	24128.6	30.1	866	123.48	0.95	177.9	
26	3	1	0	19209.6	22034.6	10.3	2736	123.48	0.95	177.9	
27	4	1	0	10468.8	19152.2	12.5	971	16.40	0.47	177.9	
28	4	1	0	15172.1	21756.7	30.9	477	36.93	0.46	177.9	
29	4	1	0	20002.7	24389.2	37.6	456	52.28	0.76	177.9	
30	4	1	0	19028.7	24086.4	17.4	1049	52.28	0.76	177.9	
31	4	1	0	18921.2	24454.9	17.1	1086	52.28	0.76	177.9	
32	4	1	0	19491.6	23018.5	11.0	1707	52.28	0.76	177.9	
33	4	1	0	17173.2	18514.8	36.8	581	83.19	0.68	177.9	
34	4	1	0	13629.5	23323.2	26.1	831	82.89	0.68	177.9	
35	4	1	0	5559.7	15840.5	14.0	1088	32.04	0.54	177.9	

Absolute Frequency



Dimensions Display Graphics

PALM-DR PALM-Grouping PALM-Statistics PALM-Filter PAL-Rendering

Pixel Resolution XY 38 nm/pixel

Display Mode Basic

Expansion factor 3.00 x PSF

Render auto dynamic range HR Scale 99.00 %

Render auto dynamic range SWP Scale 88.00 %

Render Localized Precision x/y: 40.0 nm z: 100.0 nm

Render Best Quality

CPU 0% Free HD 3.2 TB
Free Ram 54 GB

EN 912
6.09.2016

PALM/STORM

ZEN 2012

File View Acquisition Maintain Macro Window Help

Workspace Zoom | Reset

Workspace Configuration | Merge

Processing | Maintain


Method

- Maximum intensity projection
- Color-coded projection
- Image calculator
- Average
- Fiber
- Linear unmixing
- Ion Concentration
- Correlation
- Modify Series
- HDE- Imaging
- Stack
- PALM**

Method Parameters

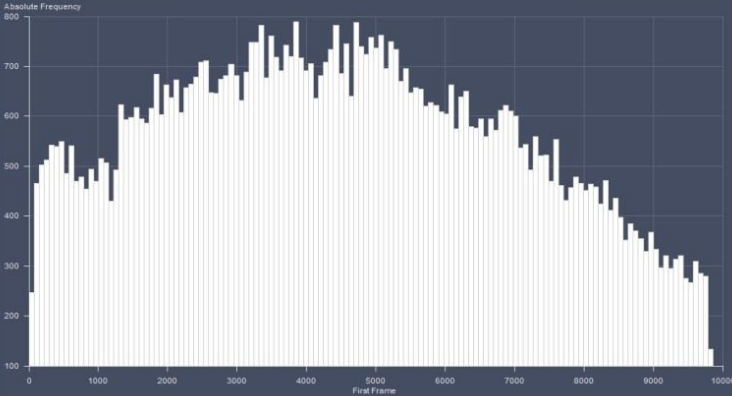
Input Image: [Select]

2D
3D
3.5D
Histo
Profile
Mean ICR
FMAP
PALM
Info



Index	First Frame	Number Frames	Frames Missing	Position X [nm]	Position Y [nm]	Precision	Number Photons	Background var	Chi square	PSF width [nm]	Channel
1	1	1	0	19048.1	4055.3	23.2	2083	134.05	0.57	235.8	-
2	1	1	0	11136.6	10781.6	24.3	3354	237.42	0.60	255.4	-
3	1	1	0	10277.3	17346.5	36.7	4614	303.57	0.95	365.1	-
4	1	1	0	9453.8	17358.5	26.2	2870	277.87	0.99	247.6	-
5	1	1	0	10720.3	1524.2	35.5	1008	250.12	1.10	175.3	-
6	1	1	0	7507.6	12507.1	28.5	6615	290.00	0.91	387.9	-
7	1	1	0	18599.8	8850.1	36.0	3070	225.18	0.74	317.1	-
8	1	1	0	18401.8	10743.3	32.6	8904	289.03	0.87	485.6	-
9	1	1	0	18277.4	11826.2	35.3	6729	244.60	0.65	454.6	-
10	2	1	0	5977.6	13524.0	16.6	1585	82.95	0.63	191.9	-
11	2	1	0	16742.4	1981.7	22.5	2004	220.45	0.85	239.6	-
12	2	1	0	6420.7	23541.2	29.6	3432	218.48	0.81	305.6	-
13	2	1	0	8287.5	10523.2	38.5	2089	269.29	0.78	259.5	-
14	2	1	0	9331.2	17533.4	34.9	1221	263.94	0.87	189.4	-
15	3	1	0	19842.8	9720.9	19.5	4507	288.91	1.14	266.8	-
16	3	1	0	16727.2	1956.6	26.2	1307	257.87	1.12	164.0	-
17	3	1	0	7530.3	17232.2	23.9	5575	264.75	0.84	327.0	-
18	3	1	0	19079.2	4166.0	21.4	1562	99.51	0.53	209.6	-
19	3	1	0	18579.2	10066.1	37.7	6330	254.68	0.86	451.0	-
20	4	1	0	7992.8	15188.1	27.9	731	198.94	0.99	142.6	-
21	4	1	0	19102.4	4076.6	15.7	2209	101.88	0.51	209.5	-
22	4	1	0	12011.5	16743.4	27.6	4308	199.58	0.69	339.2	-
23	5	1	0	7437.2	17187.9	16.8	6305	268.93	0.93	285.1	-
24	5	1	0	7981.4	15174.3	18.8	707	96.21	0.91	131.3	-
25	5	1	0	19080.8	4117.4	15.9	2019	84.97	0.46	210.5	-
26	5	1	0	7591.5	13002.2	36.1	6452	292.05	1.03	430.3	-
27	5	1	0	8140.7	23548.3	37.0	407	212.49	0.89	118.8	-
28	5	1	0	13131.6	16588.6	38.8	876	257.67	1.04	170.3	-
29	5	1	0	13474.3	13992.2	37.3	490	223.12	0.85	129.3	-
30	6	1	0	15826.5	13988.8	19.4	1598	177.28	1.00	175.6	-
31	6	1	0	15387.2	15525.9	32.3	3395	269.29	0.98	302.0	-
32	6	1	0	9707.3	15221.4	22.3	4941	273.28	0.78	298.6	-
33	6	1	0	12540.0	1487.2	25.3	976	189.28	0.90	155.4	-
34	6	1	0	10320.7	16223.9	36.0	2776	167.08	0.78	324.3	-
35	6	1	0	19087.7	4055.4	18.5	1580	89.84	0.46	189.5	-

Absolute Frequency



Dimensions | Display | Graphics

Time: 10000 | 5001 | 222 | Interpolation

Zoom: [Buttons]

Tools: [Buttons]

Modify: Source Image | PALM Image

Channels: [Single Channel] | Range Indicator | Quick Color Setup

PALM-ORF | PALM-Grouping | PALM-Statistics | PALM-Fiber | PALM-Rendering

Pixel Resolution XY: 35 | nm/pixel

Display Mode: Gauss

Expansion factor: 1.00 | A, PSF

Render auto dynamic range HR | Scale: 99.00 | %

Render auto dynamic range SWF | Scale: 99.00 | %

Render Localized Precision x/y: 40.0 nm | 100.0 nm

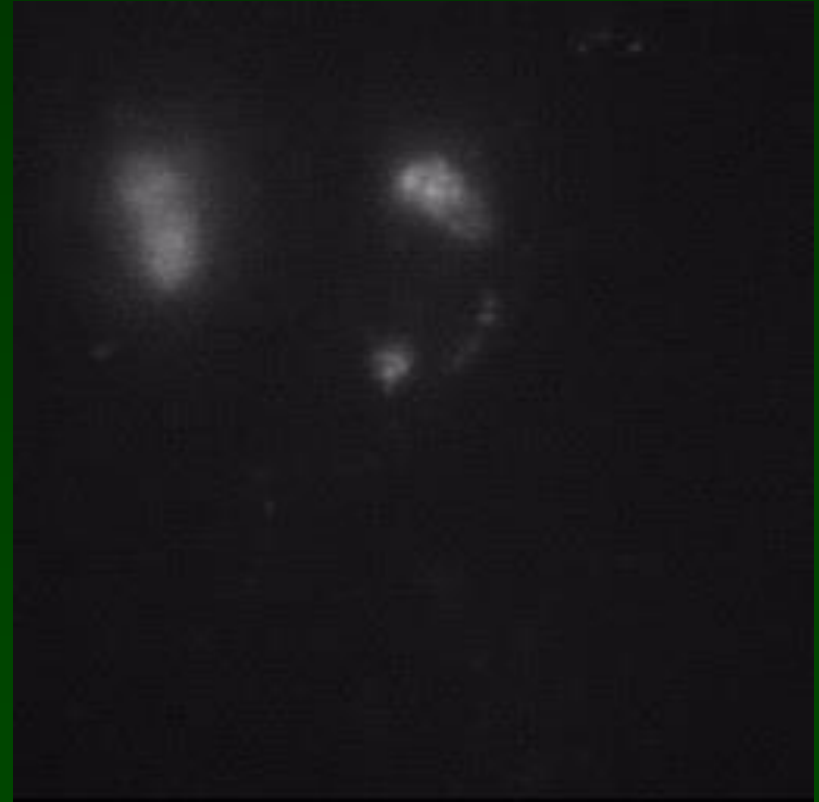
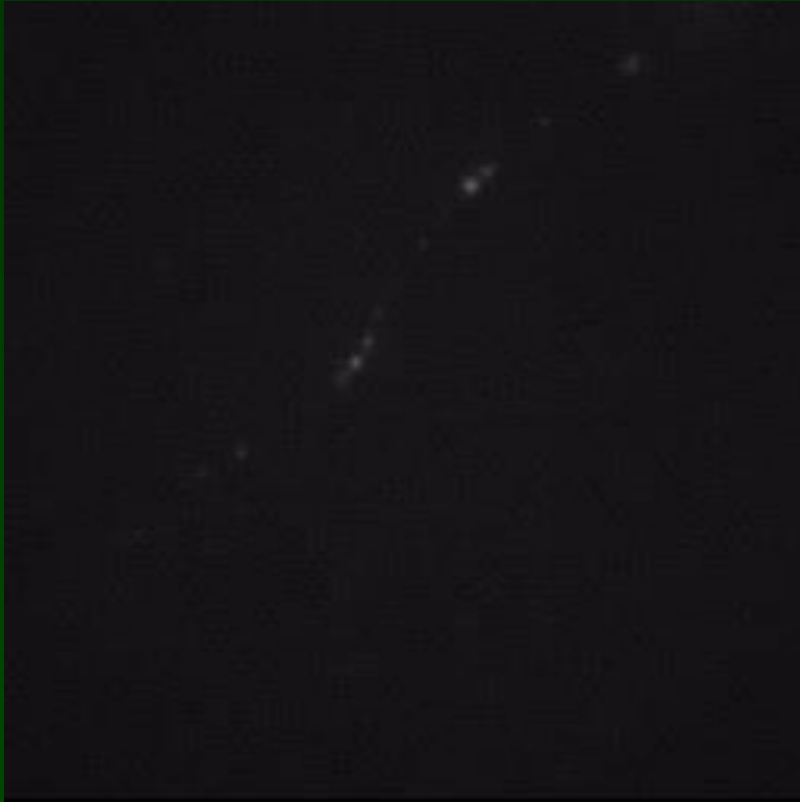
Display Raw Image

Render Best Quality

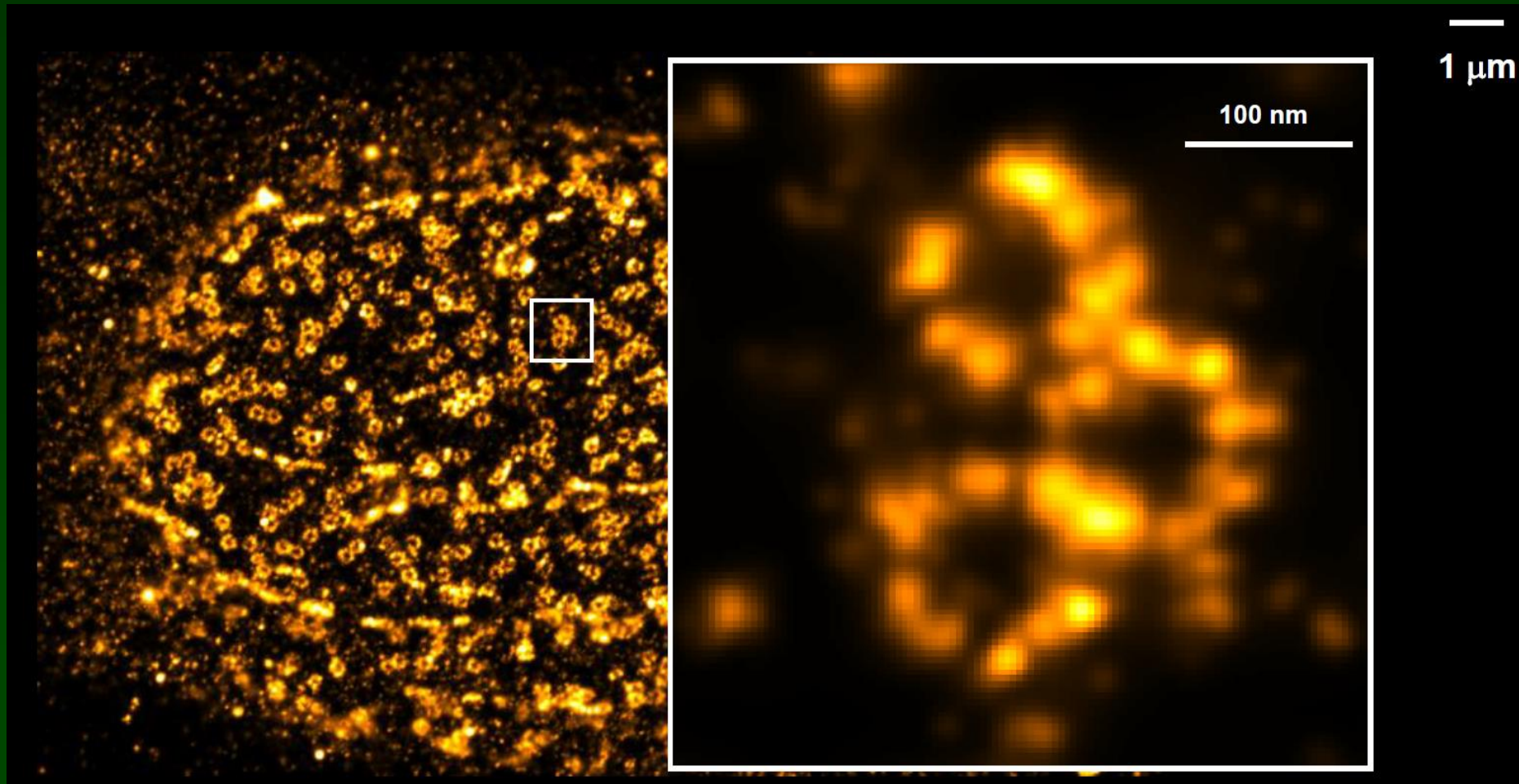
Cancel

EN | 811 | 6.09.2015

PALM/STORM - Recording



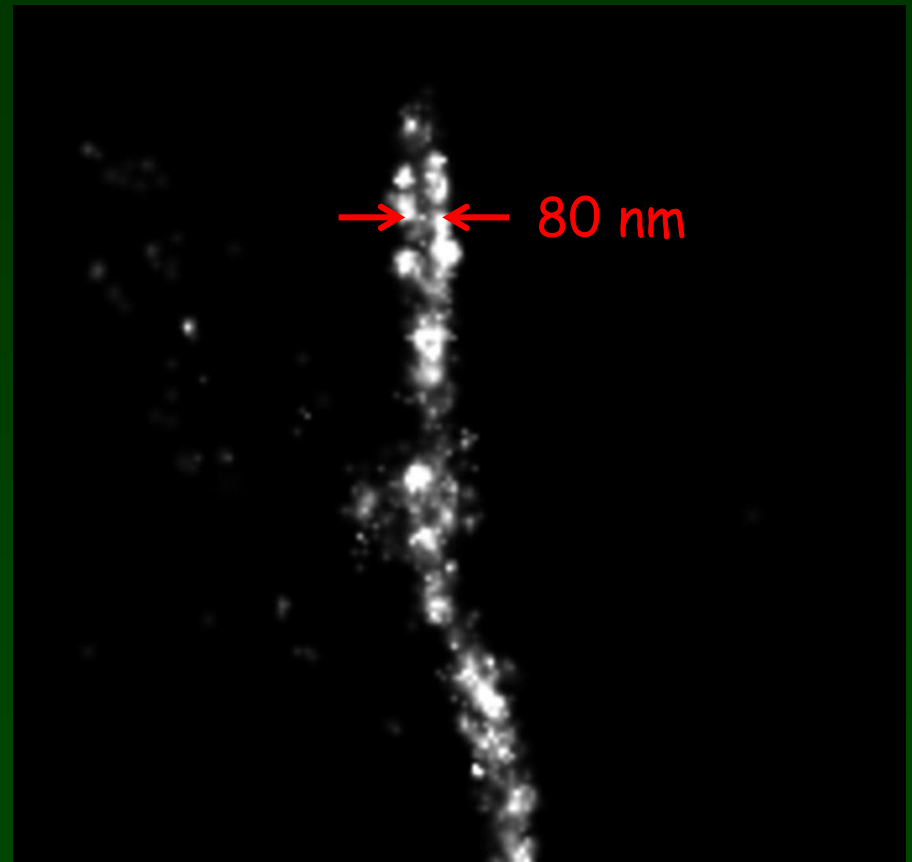
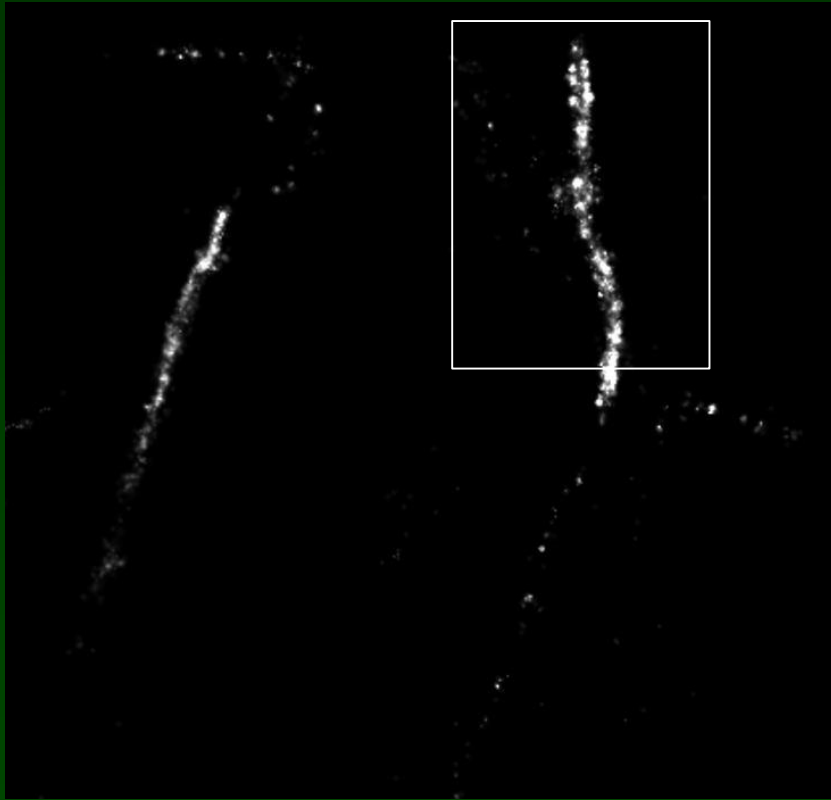
PALM



A. Löschberger, M. Sauer, University of Würzburg, Germany



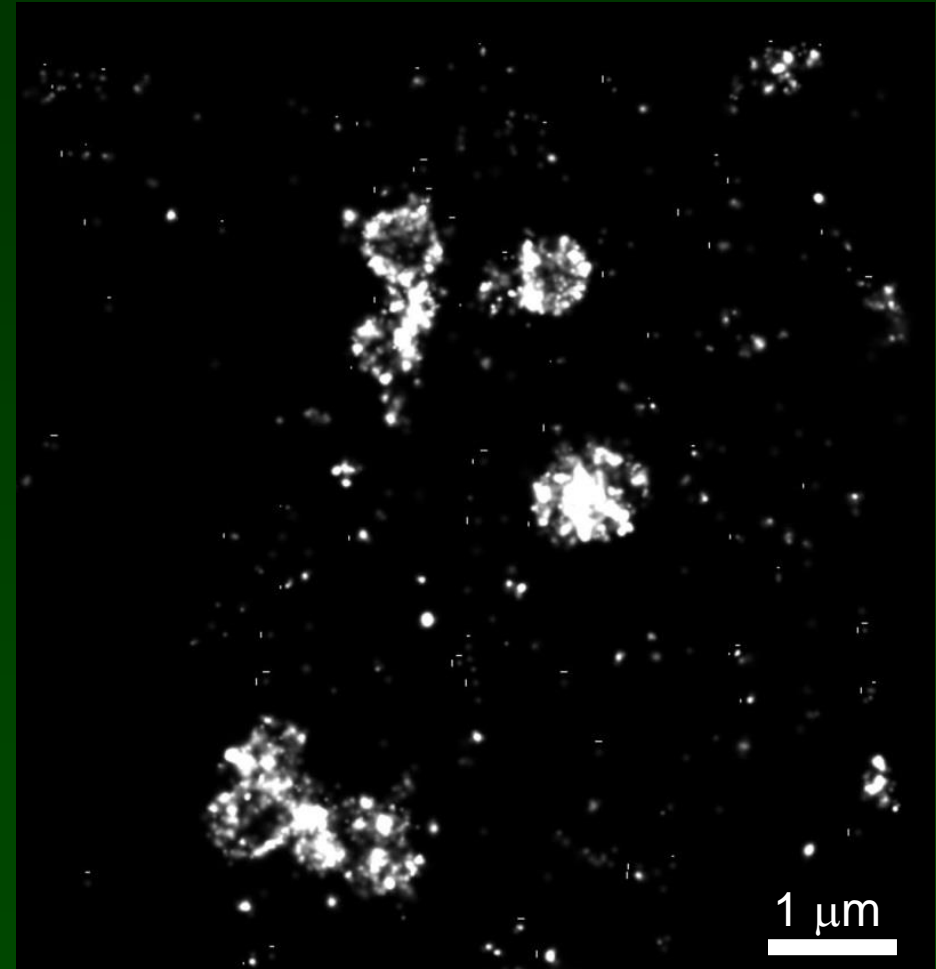
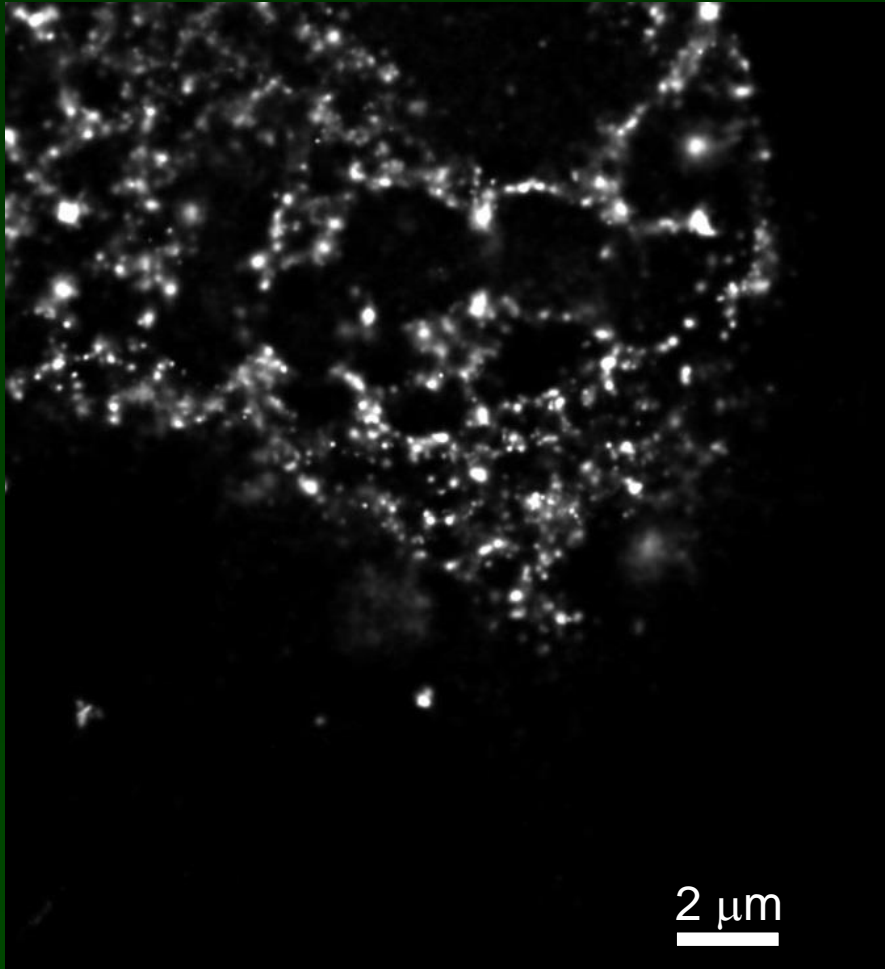
PALM - Zeiss ELYRA P.1



td-EOS-TOMM20



PALM - Zeiss ELYRA P.1

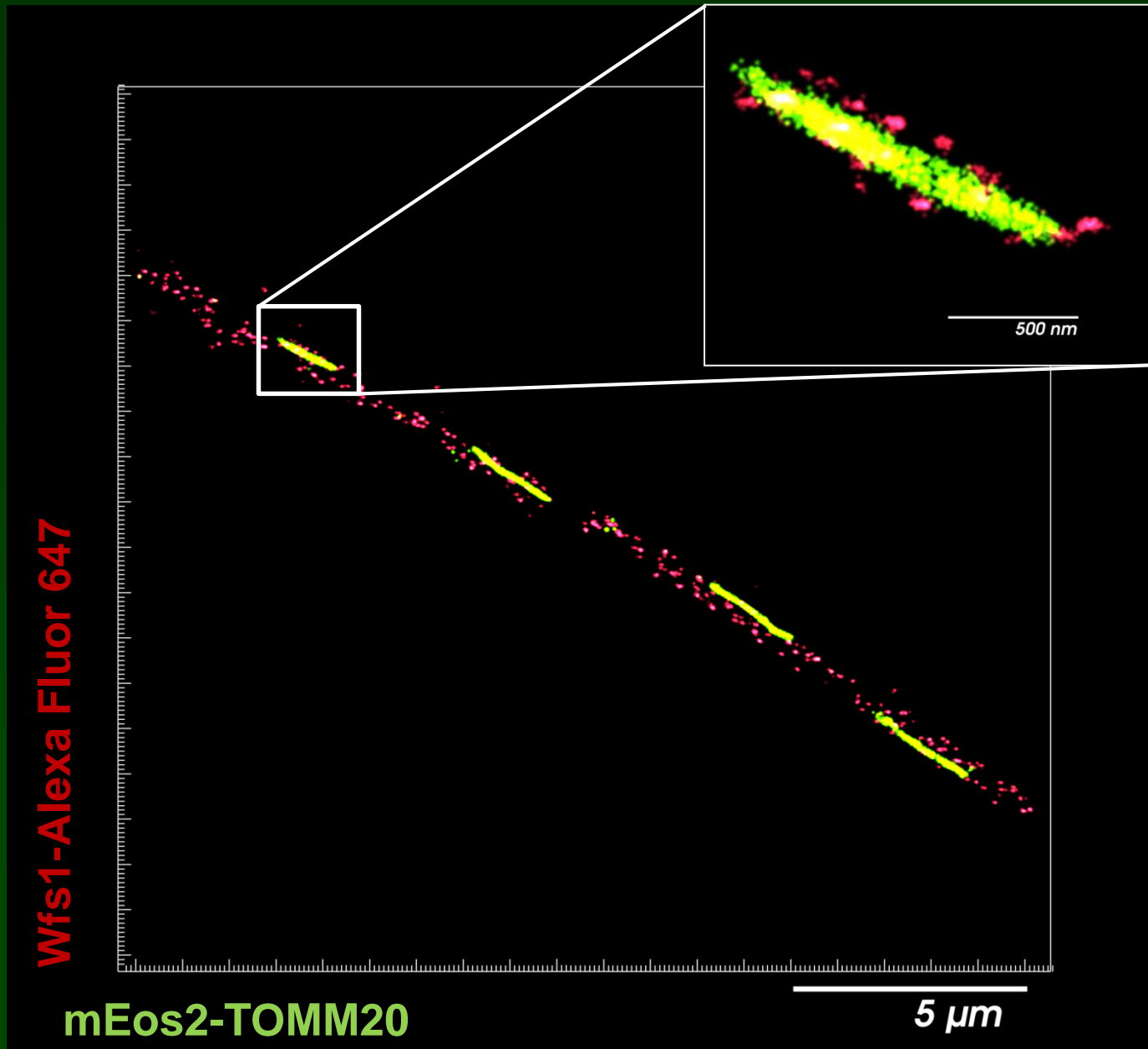


ER-EOS, TIRF

tdEos-Lysosomes-20



PALM - Double staining



By: T. Spacek



PALM/STORM

- Practical tips&tricks:
- Optimal for membrane bound or filamentar structures
- Acquisition of one image takes ~ 20 min
- Acquisition in TIRF mode
- No crop or rotation of the image
- One image ~ 1.5 GB
- Temperature stability is crucial
- Atto antibodies

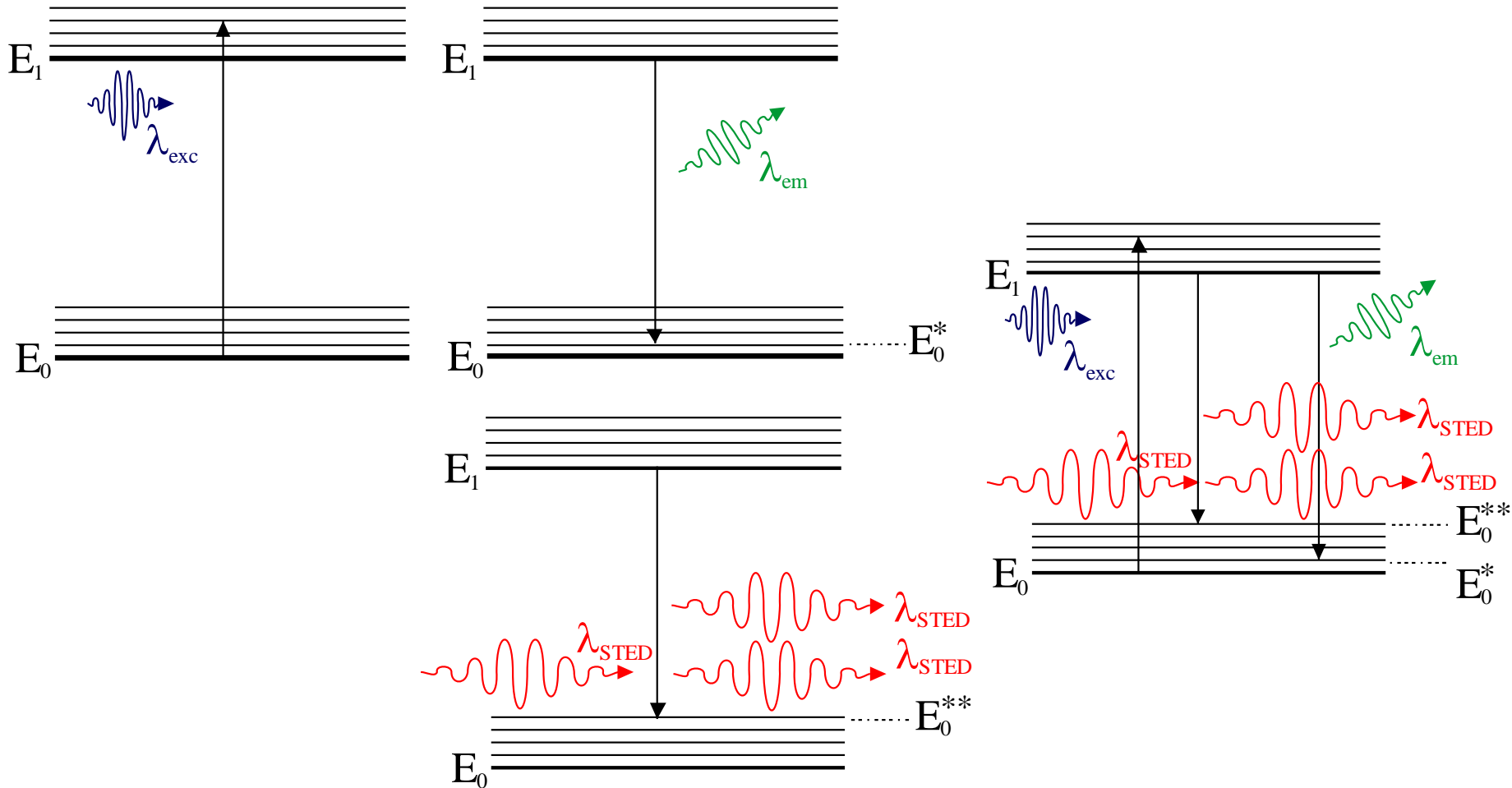


PALM/STORM

- PROs
- Highest lateral and axial resolution
- No need for high expression of proteins
- CONS
- Fixated samples only
- Longer acquisition time
- to find proper image for acquisition - tricky



STED - Principle

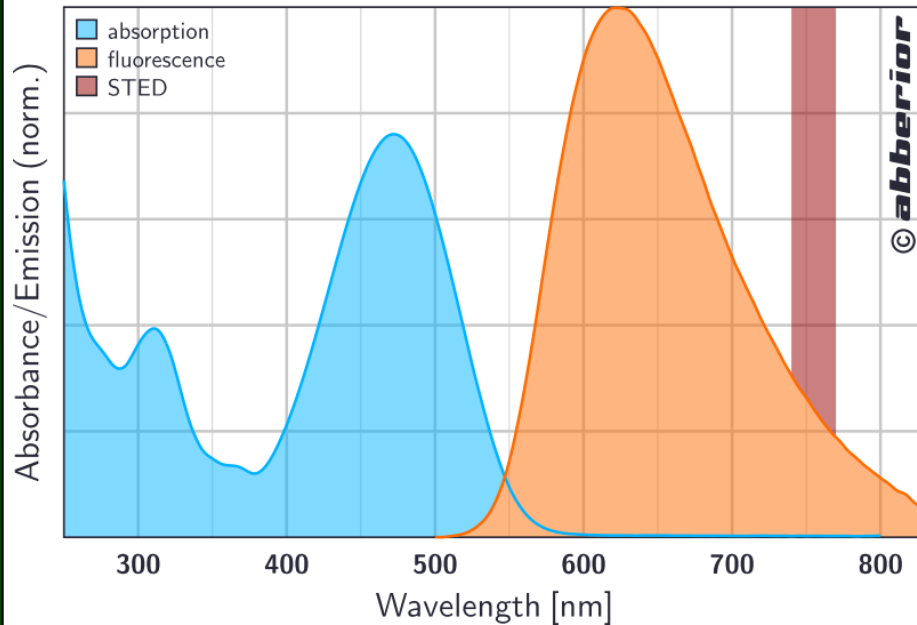


Modified from Udo J. Birk: Super-Resolution Microscopy



STED - Principle

Abberior STAR 470SXP



$$p_{STED} = \exp\left(-\frac{I_{STED}}{I_{sat}}\right)$$

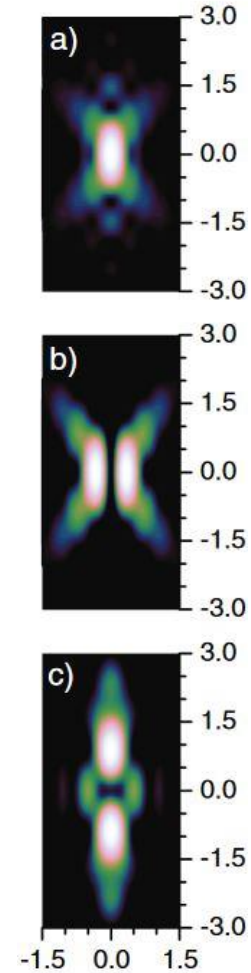
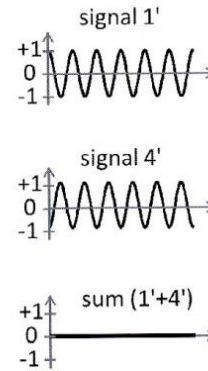
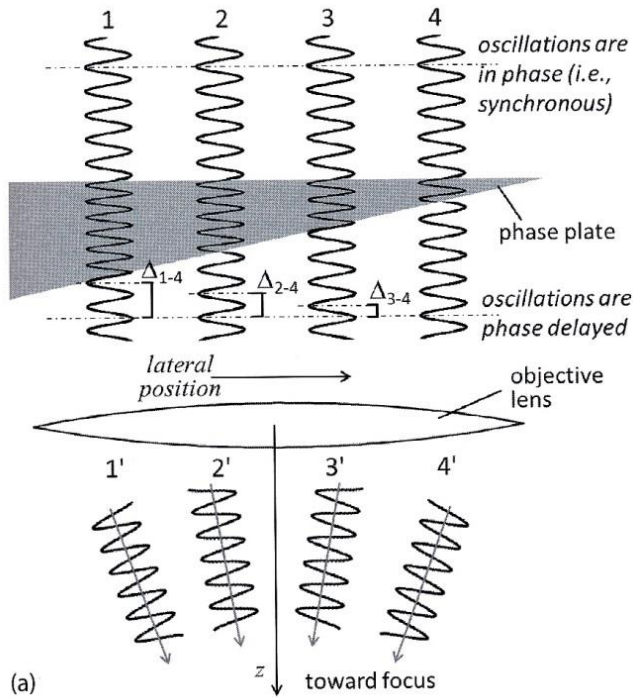
$$d_{STED} = \frac{\lambda}{2NA} \frac{1}{\sqrt{1 + I_{STED}/I_{sat}}}$$

<http://www.abberior.com/shop/Labels-by-Application/Confocal-Epi-SIM/Abberior-STAR-470SXP::2.html>

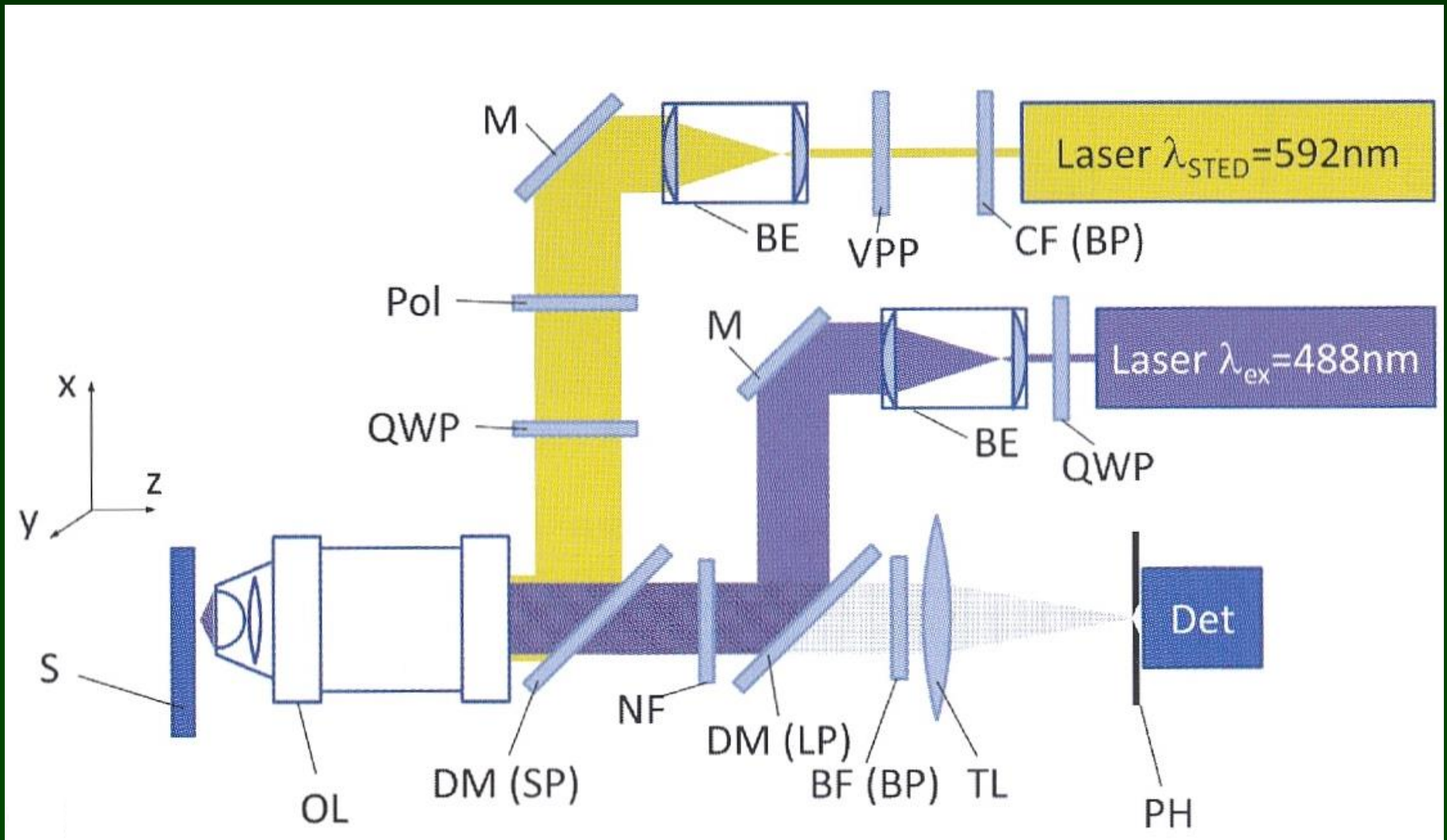
Udo J. Birk: Super-Resolution Microscopy



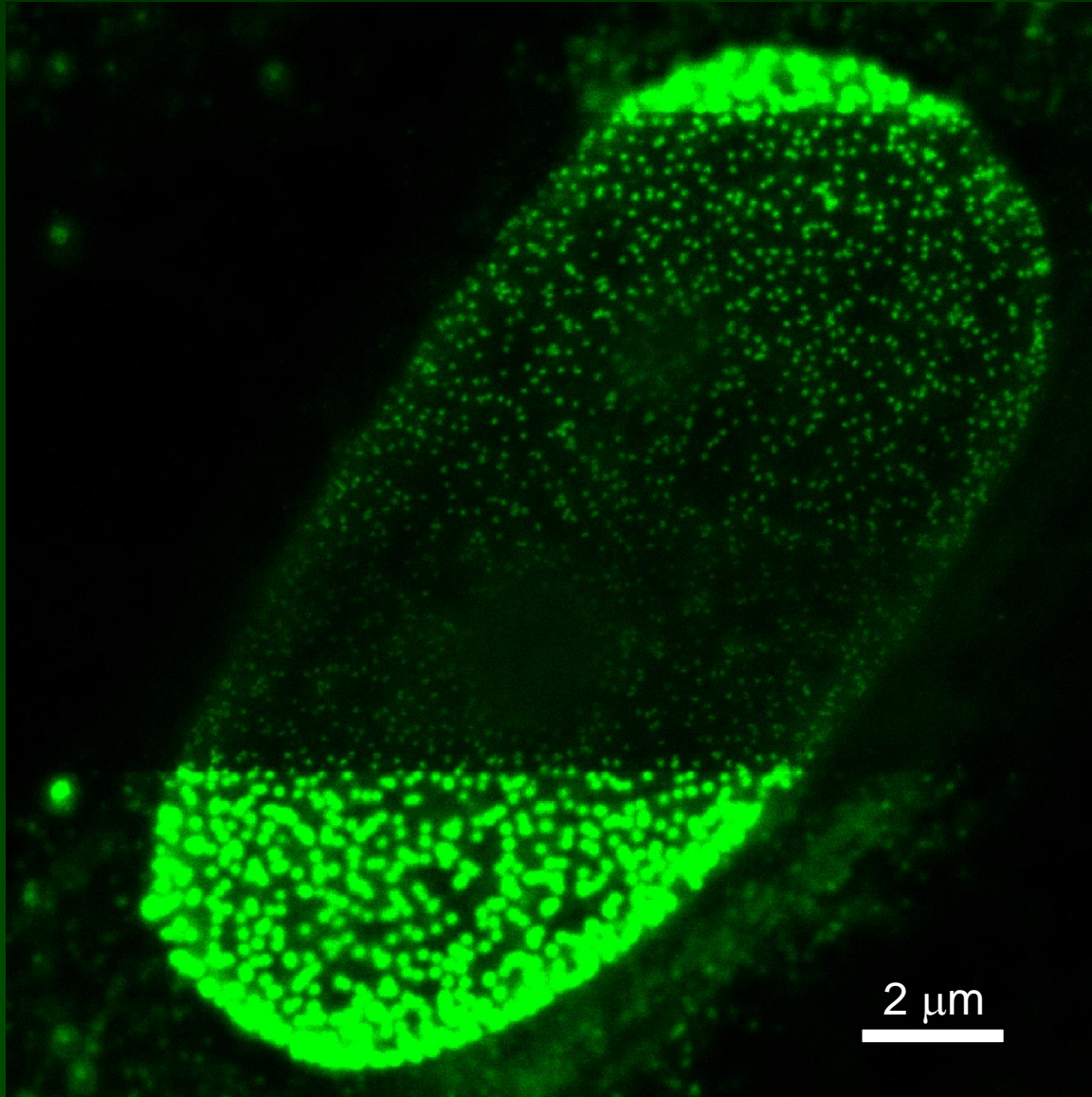
STED - Principle



STED - Principle



STED - Leica SP8

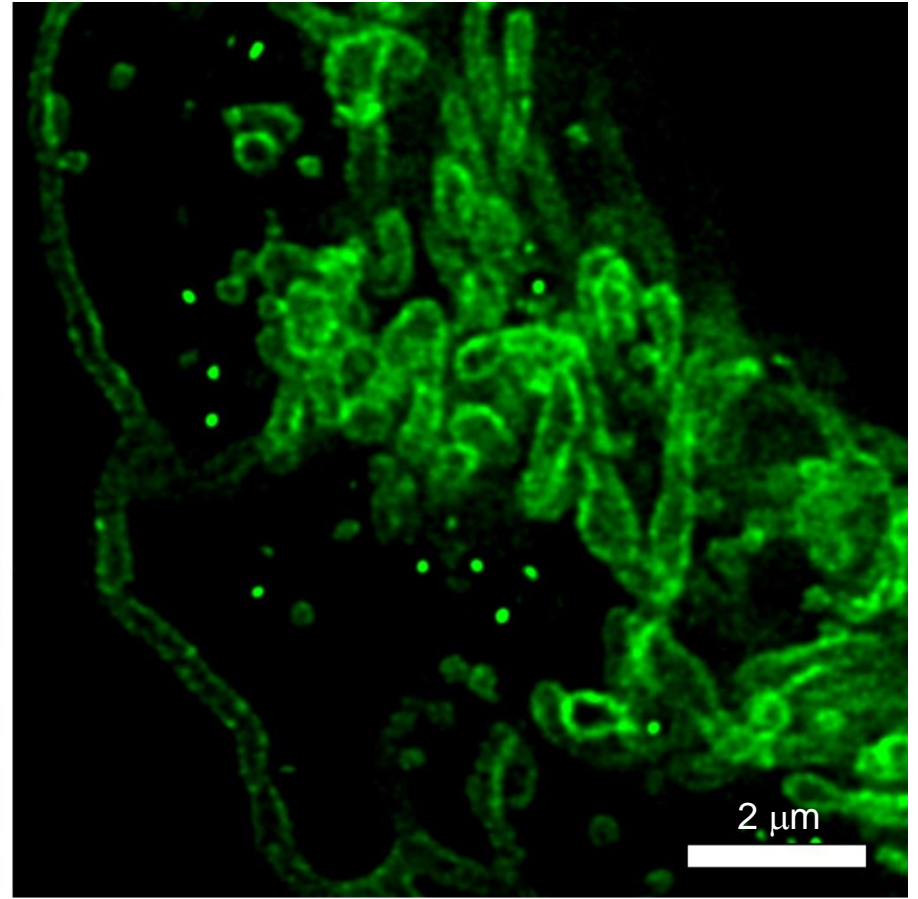
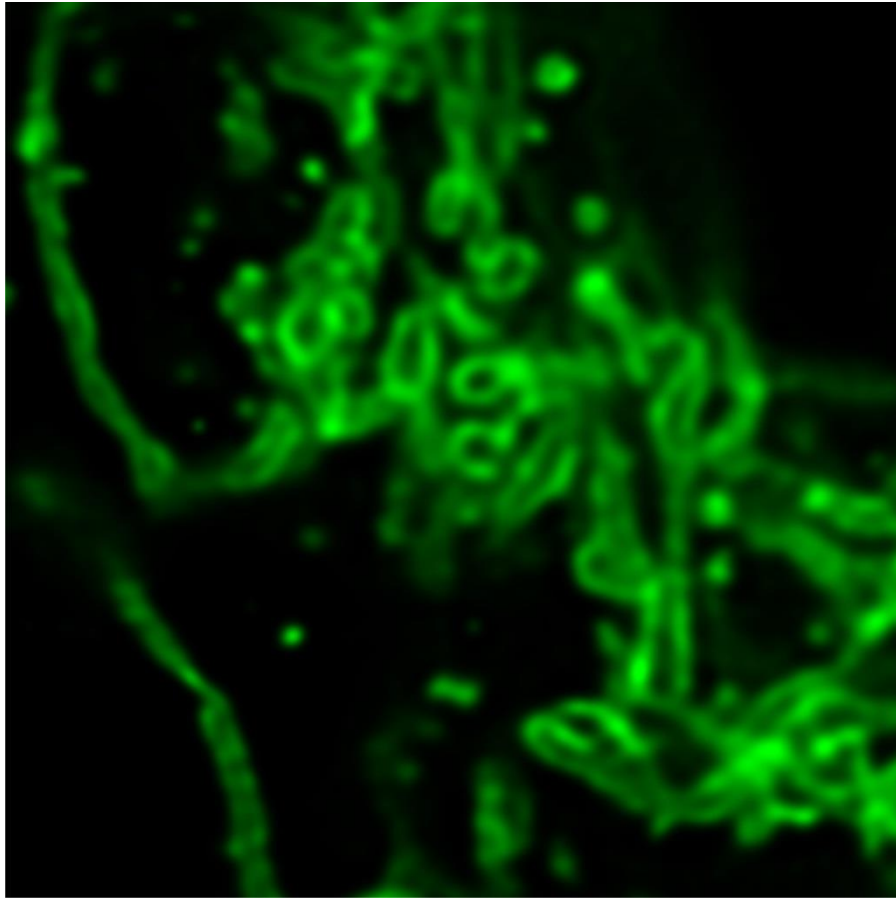


nuclear pore
complex
protein
(nup153)

By: D. Smeets



Visualization of outer mitochondrial membrane

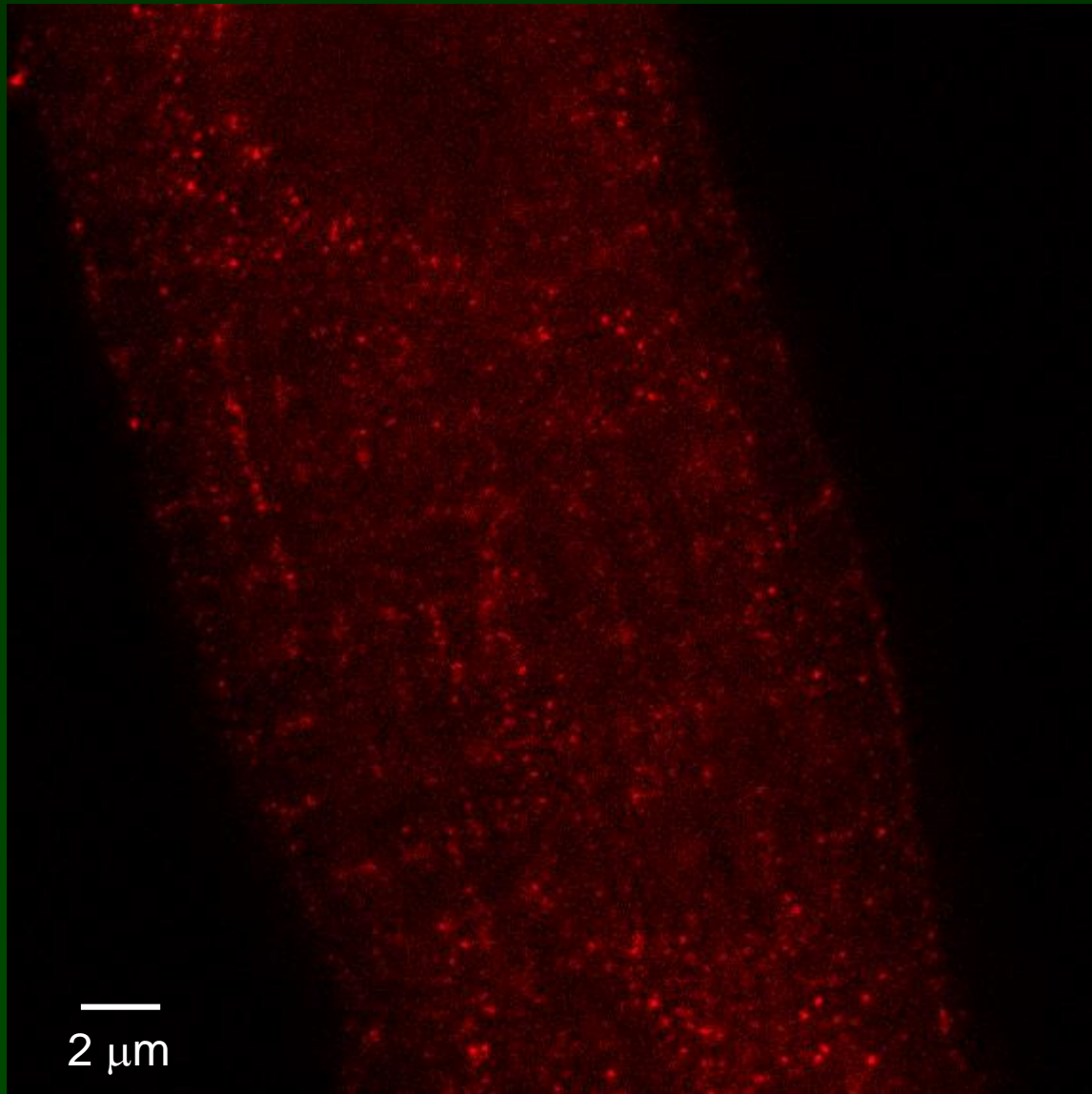


AKAP-eYFP



Calsequestrin

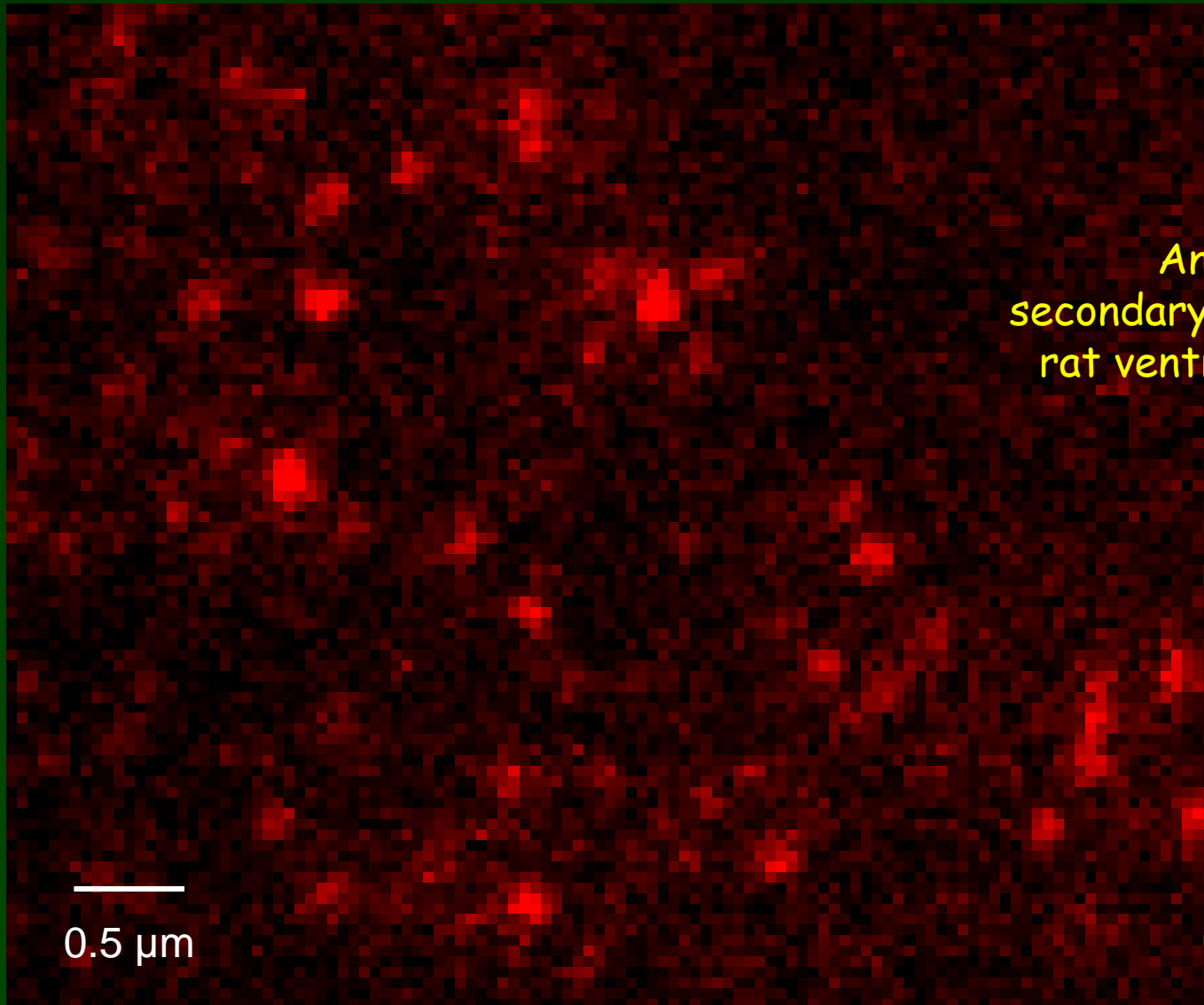
Anti-CSQ2,
secondary Ab: DyLight550
rat ventricular myocyte



By: A. Zahradnikova



Calsequestrin



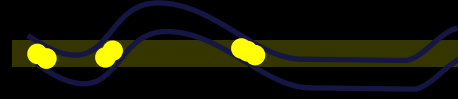
Anti-CSQ2,
secondary Ab: DyLight550
rat ventricular myocyte

0.5 μm

By: A. Zahradnikova



3D-STED

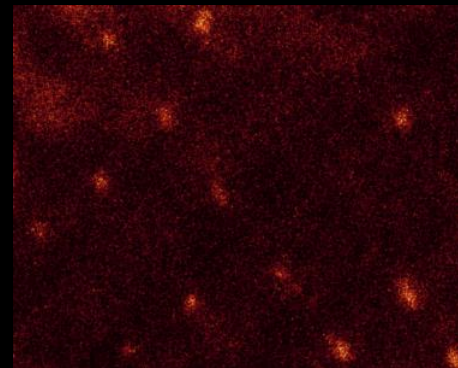
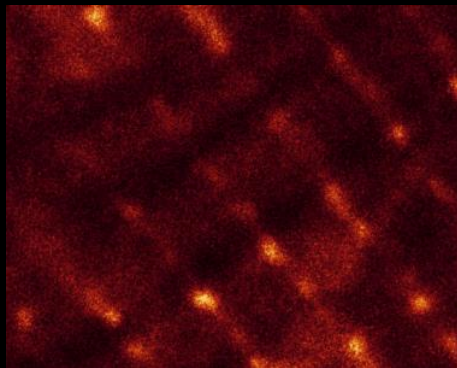


Z-resolution

confocal

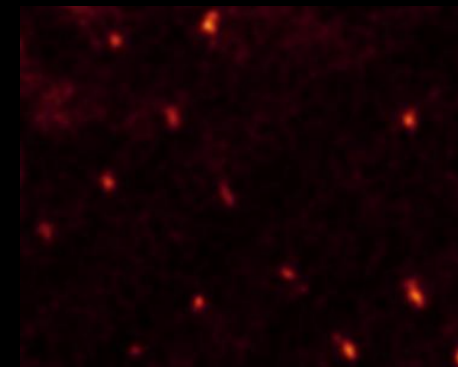
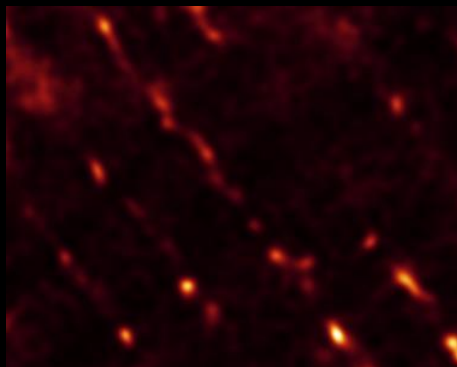
3D-STED

raw



T-tubules,
mCling-ATTO635
Rat ventricular myocyte

deconvolved



2 μ m

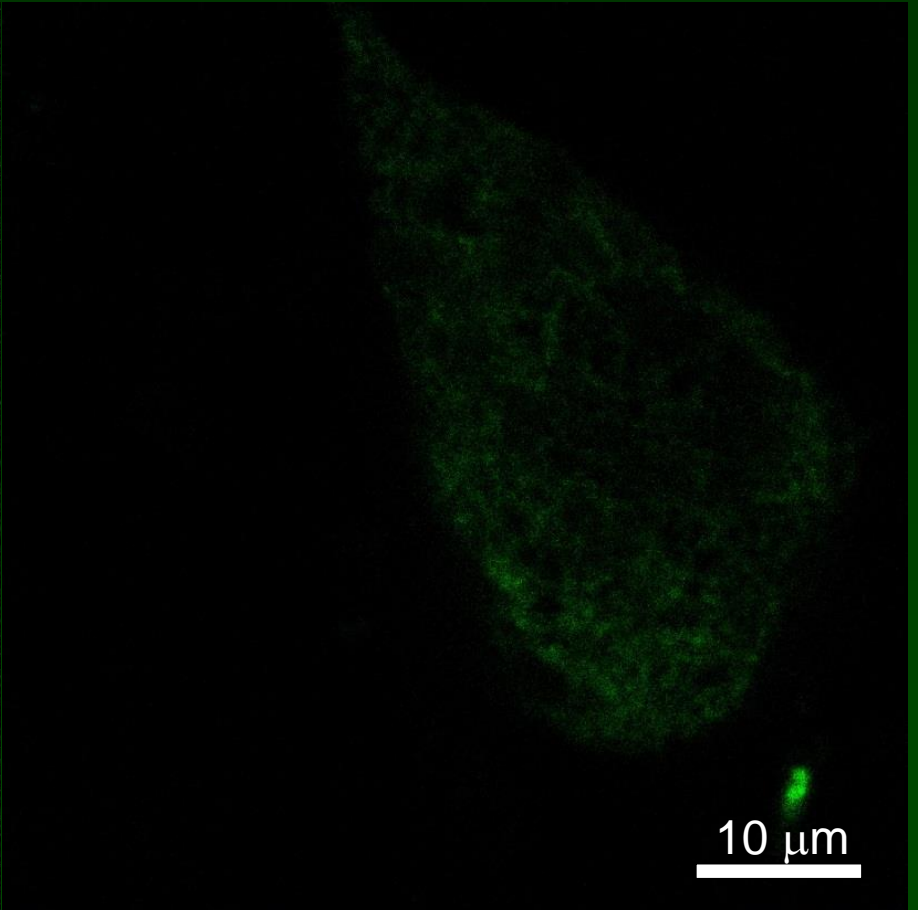
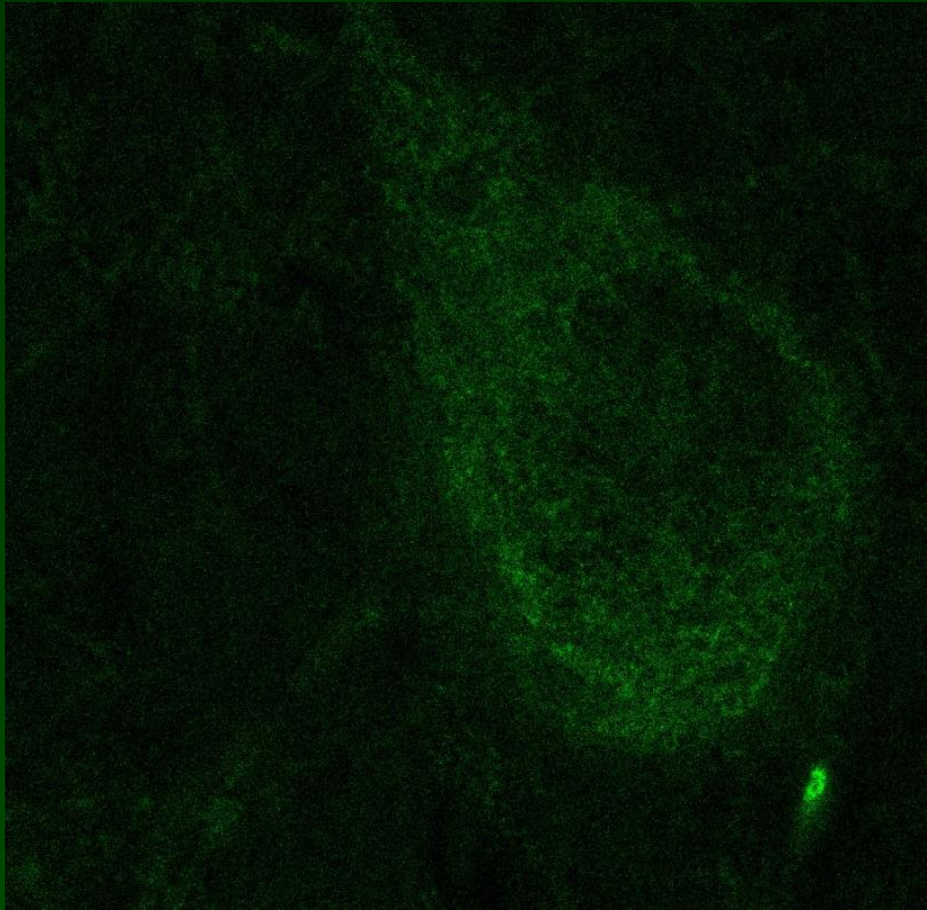


By: A. Zahradnikova

STED - Leica SP8

confocal

STED

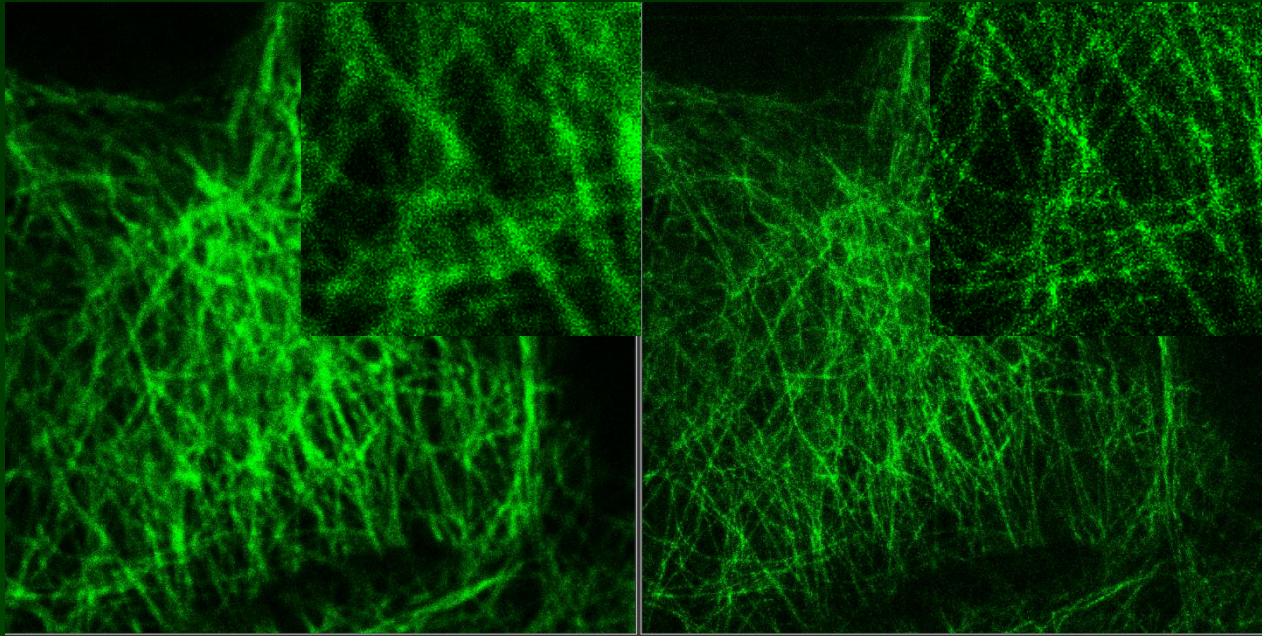


10 μm

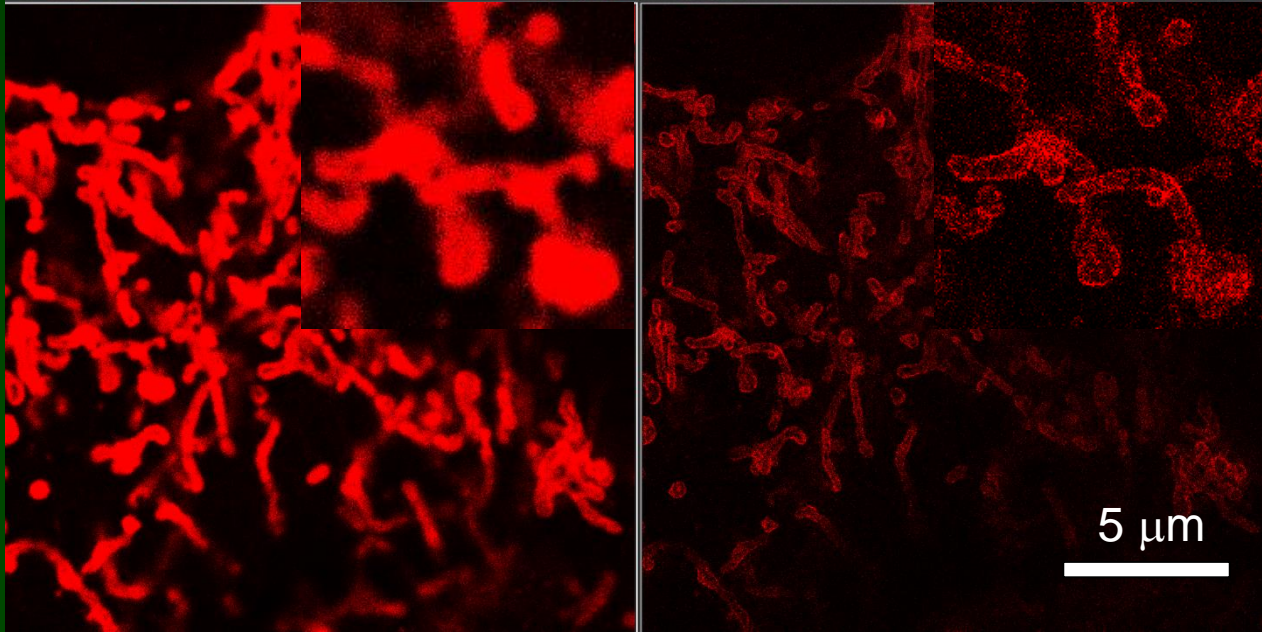
WFS1-GFP



STED - Double staining



Tubulin



TOMM20

5 μm

By: D. Smeets



STED

PROs

- Acquisition of living cells possible
- In one image STED/non STED
- Shorter acquisition time when compared to PALM/STORM

CONs

- Lower lateral and axial resolution when compared to PALM/STORM
- High expression of proteins necessary
- Autofluorescence



People, acknowledgements

- **SAS, Slovakia:** Ivan Zahradnik, Alexandra Zahradnikova, Alexandra Zahradnikova Jr
- **ASCR, Czech Republic:** Tomas Smolek, Katarina Smolkova, Petr Jezek
- **UT, Estonia:** Allen Kaasik



Eesti Teadusagentuur
Estonian Research Council



Slovak Academy of Sciences Programme

S A S P R O

 **VEGA**
Vedecká grantová agentúra
Ministerstva školstva SR a SAV



Superresolution needs
superoptimization!

