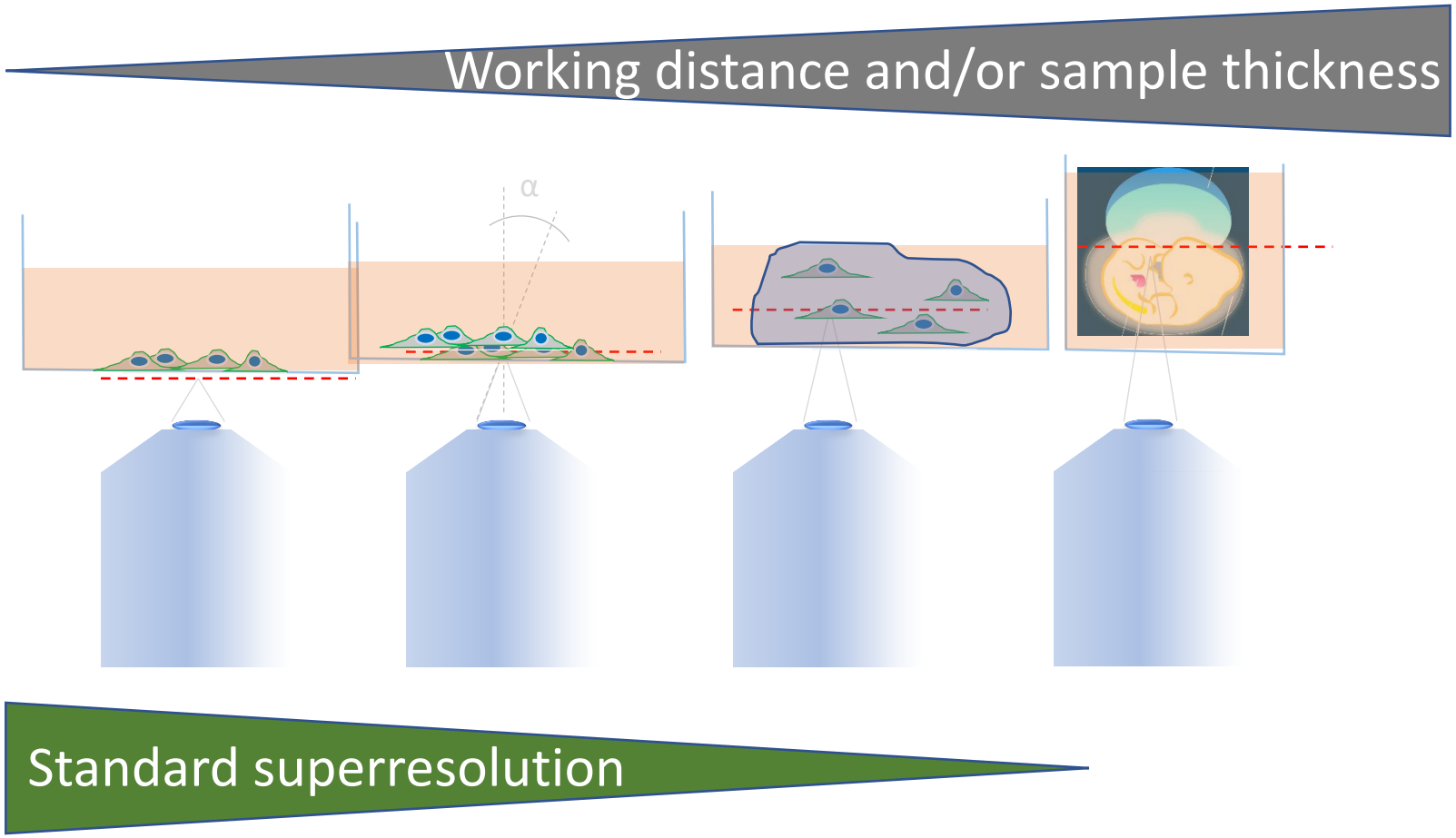
Live cell superresolution imaging – important aspects

Critical checkpoint to choose the suitable SR method:

- The type of the sample
- Fluorophore selection = visualization
- Photo-toxicity
- Live structure motility

The type of the sample - which superresolution method is good for me?



STED	**STED**	**STED**
SIM	**SIM**	SIM
localization	localization	localization

Live cell superresolution imaging – important aspects

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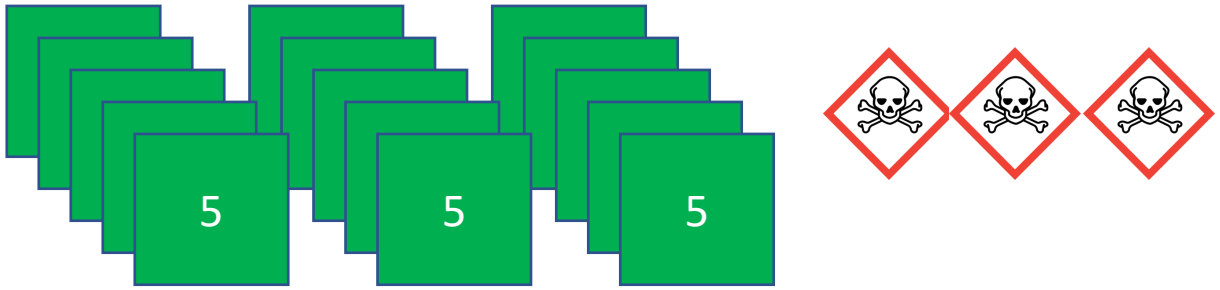
- Photo-toxicity
- Live structure motility

Photo-toxicity: how to effectively kill your live sample

Wide-field image creation -

3D-SIM image creation -



localization image creation -

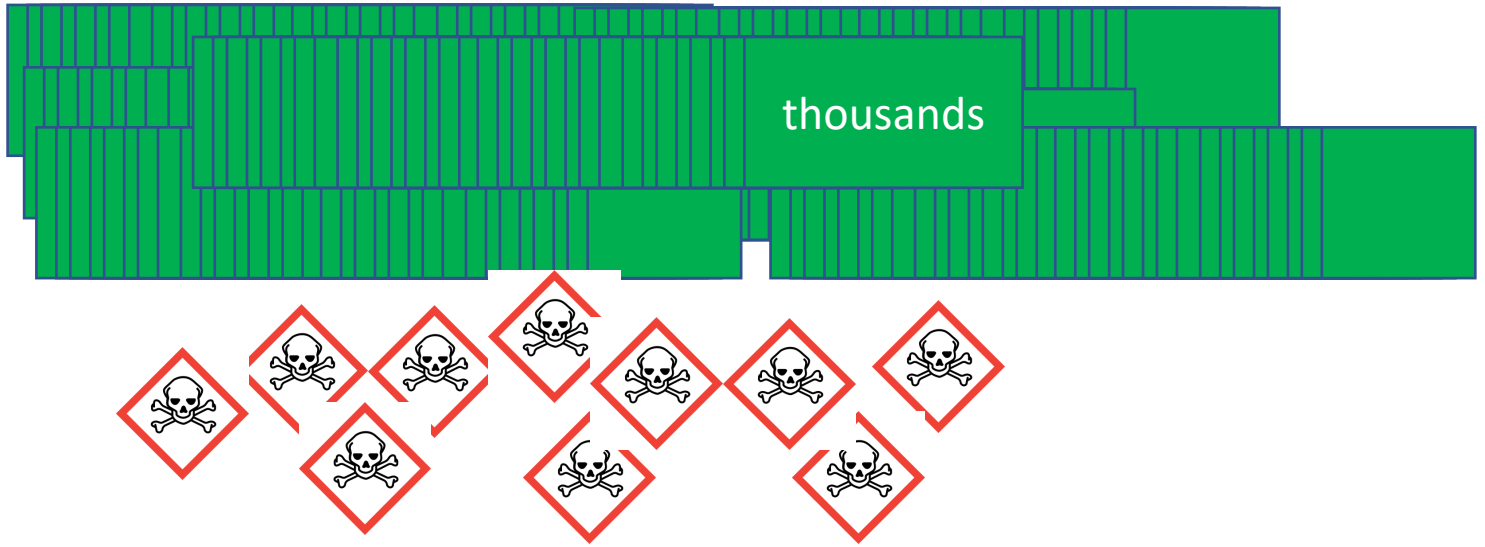


Photo-toxicity: how to effectively kill your live sample

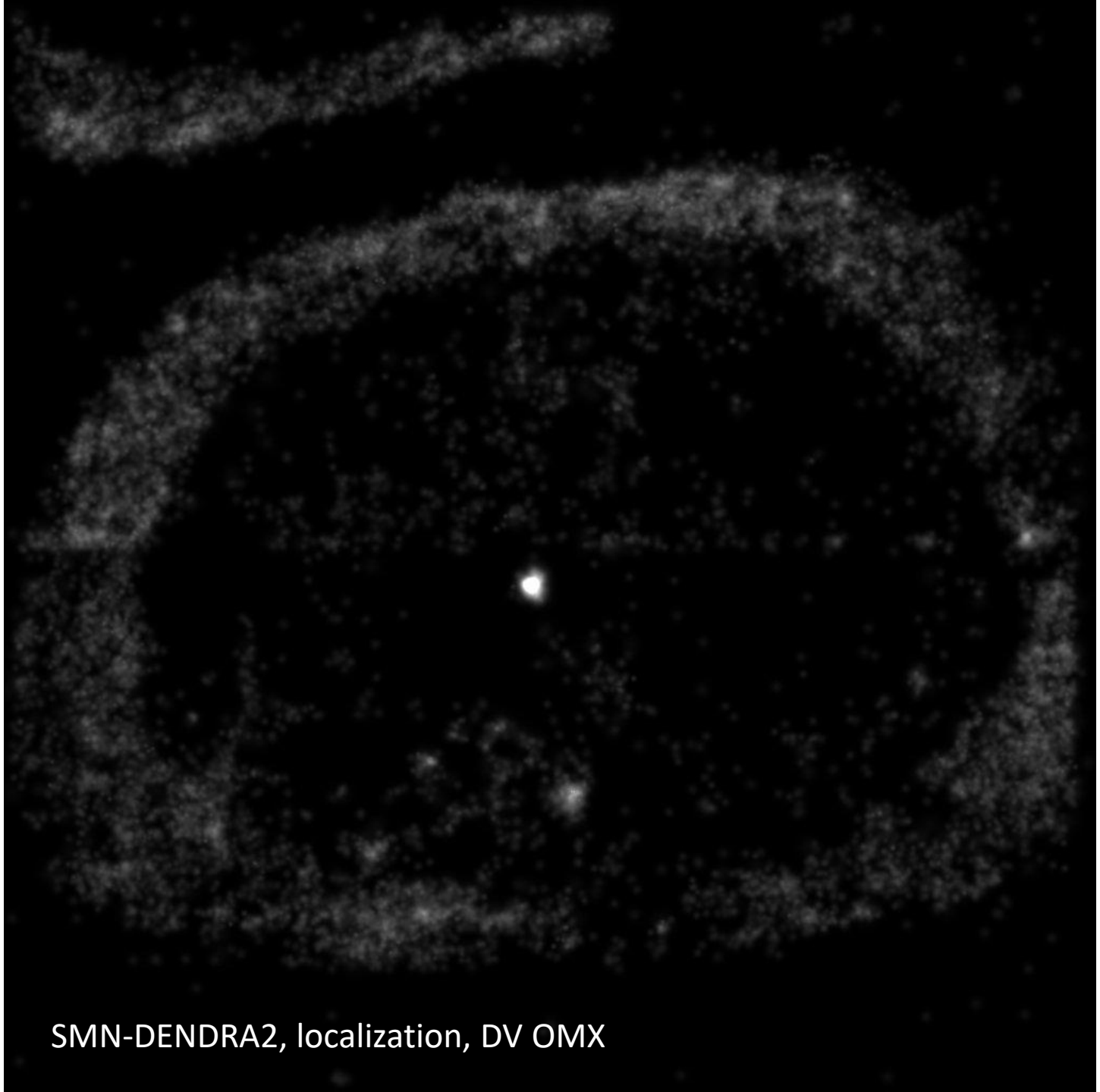
Up to 1 000-10 000x higher intensity of depletion laser

STED image creation - 1

Live cell imaging – Localization..

Very limited, high photo-toxicity and
Long time for one single time frame .. it could be advantage!

Blinking dyes for live cells:
Dendra2
Oregon green
TMR
SiR/HMSiR
Janelia dyes



SMN-DENDRA2, localization, DV OMX

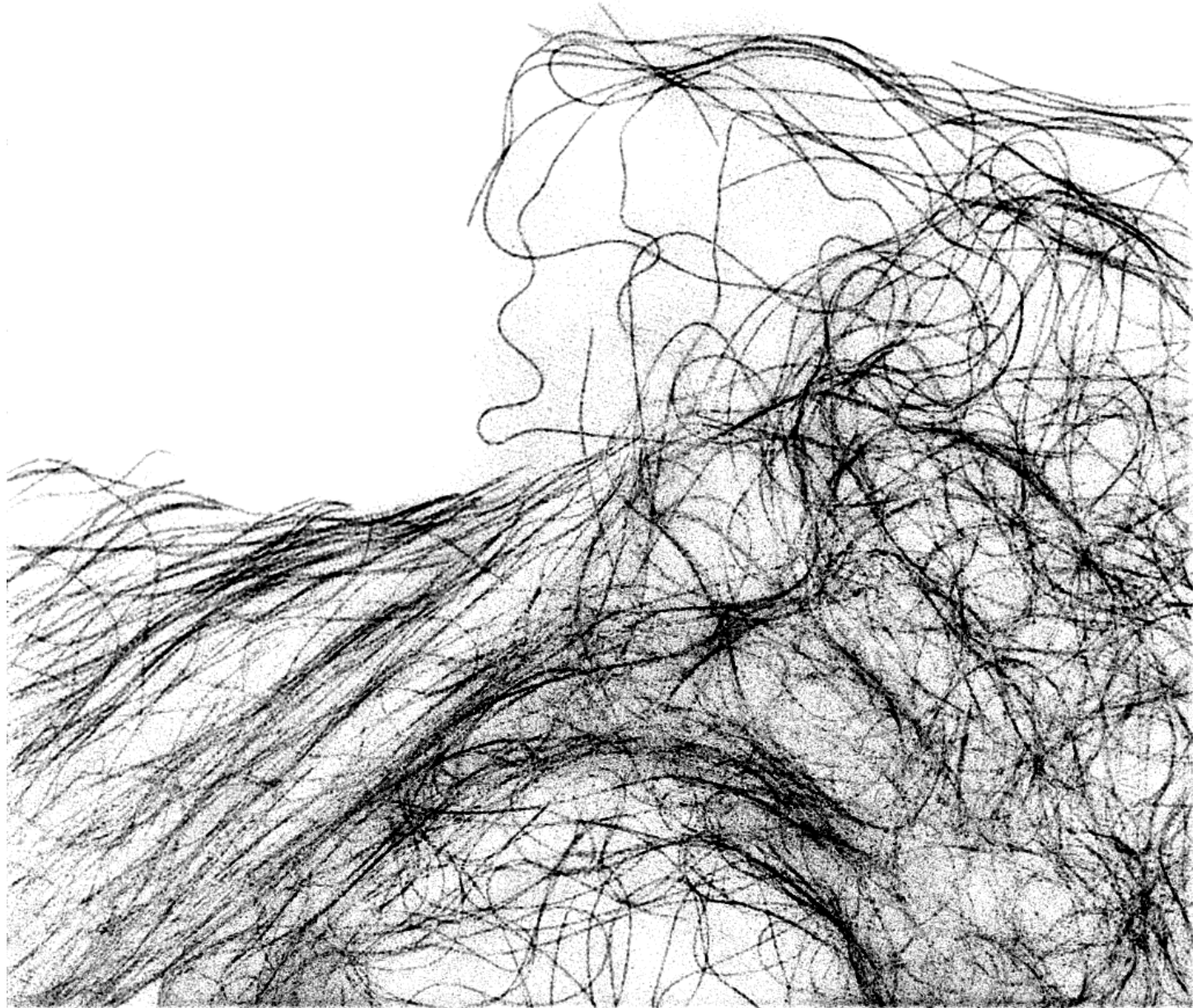
Live cell imaging - SIM

SIM Strengths

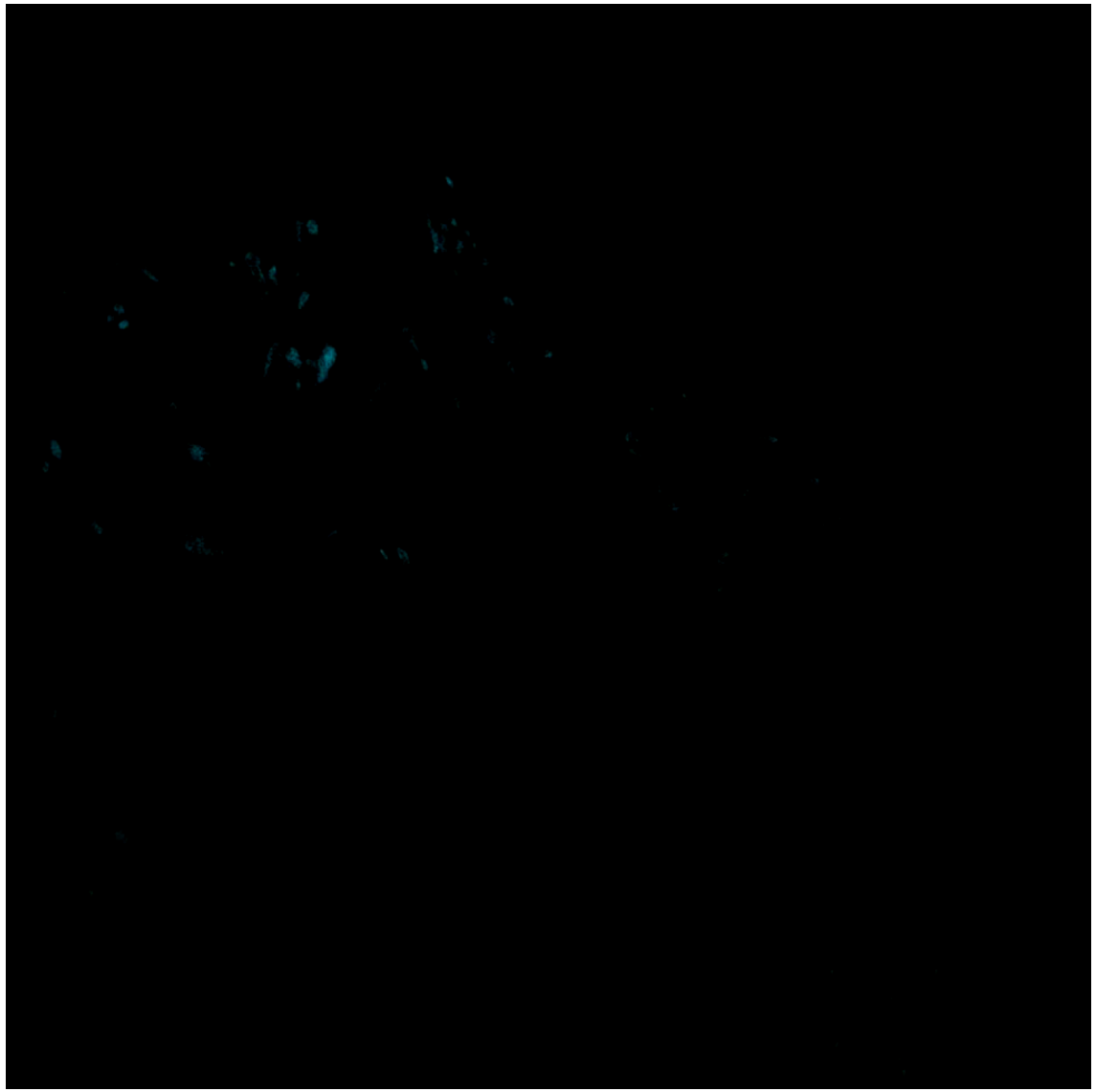
X-Y-Z resolution of 110 × 110 × 360 nm for 488nm excitation in the 3D mode; lateral resolution of 100 nm in the TIRF mode
No special sample preparation or fluorophore requirements
2-color 3D live cell imaging capability

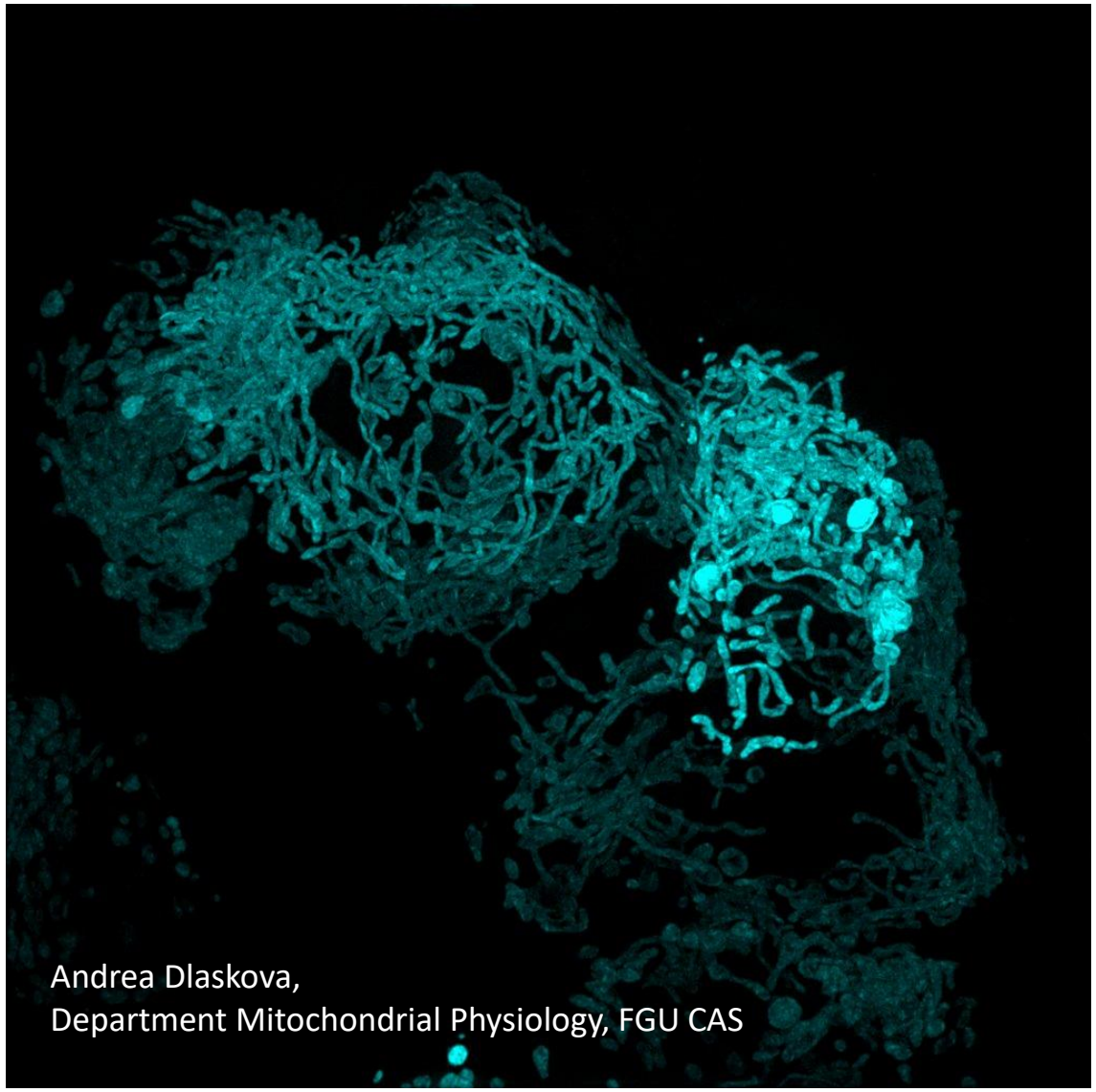
SIM Limitations

Samples for 3D SIM should be thinner than 12 µm.
The SIM reconstruction - motion artifacts when **sample moves while the illumination pattern changes** more than 100nm per image.
Photobleaching and photo-toxicity can really restrict imaging time.

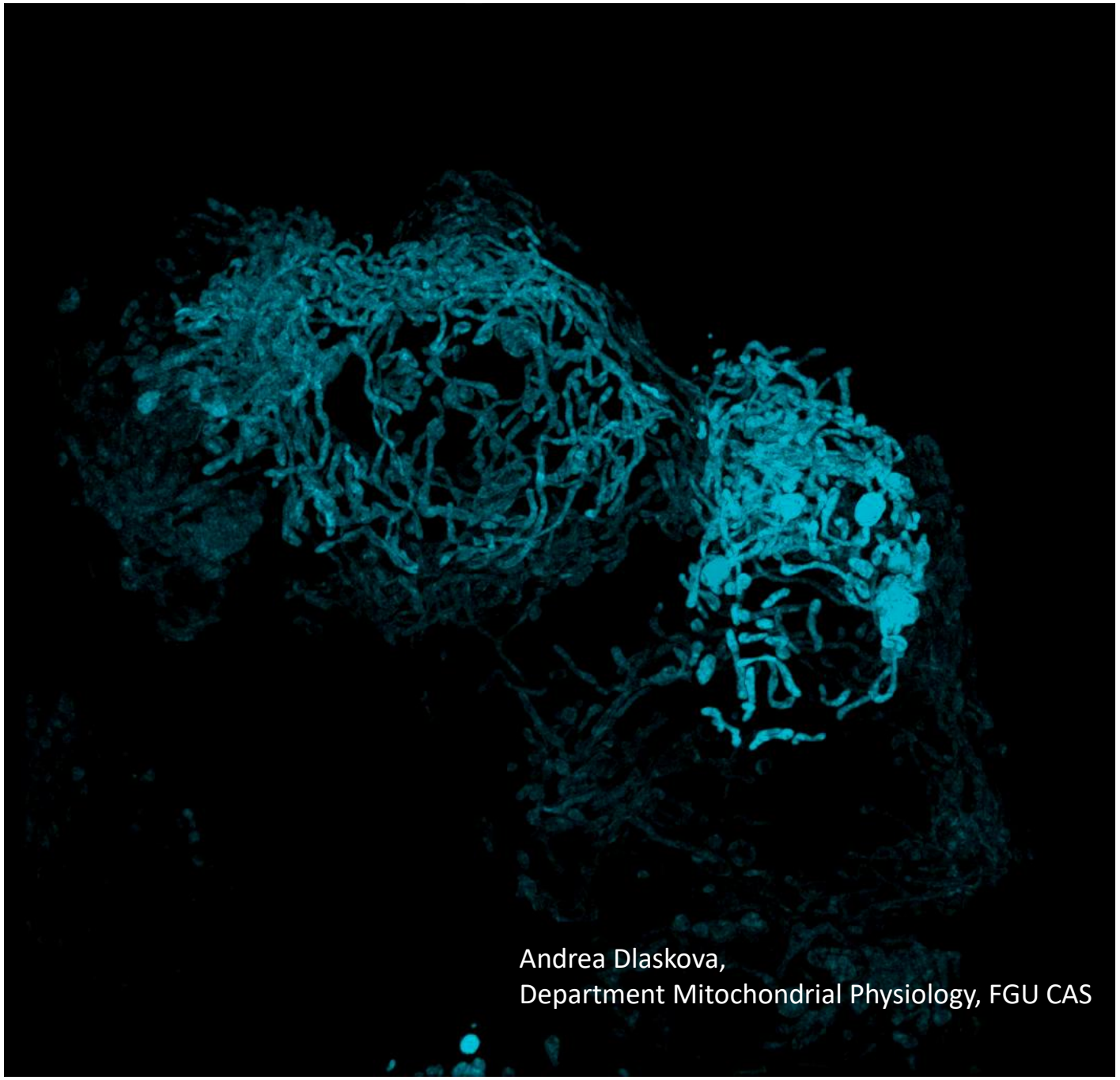


SIM image, live cells, full z-stack and projection





SIM image, live cells, 3D projection



Live cell imaging - STED

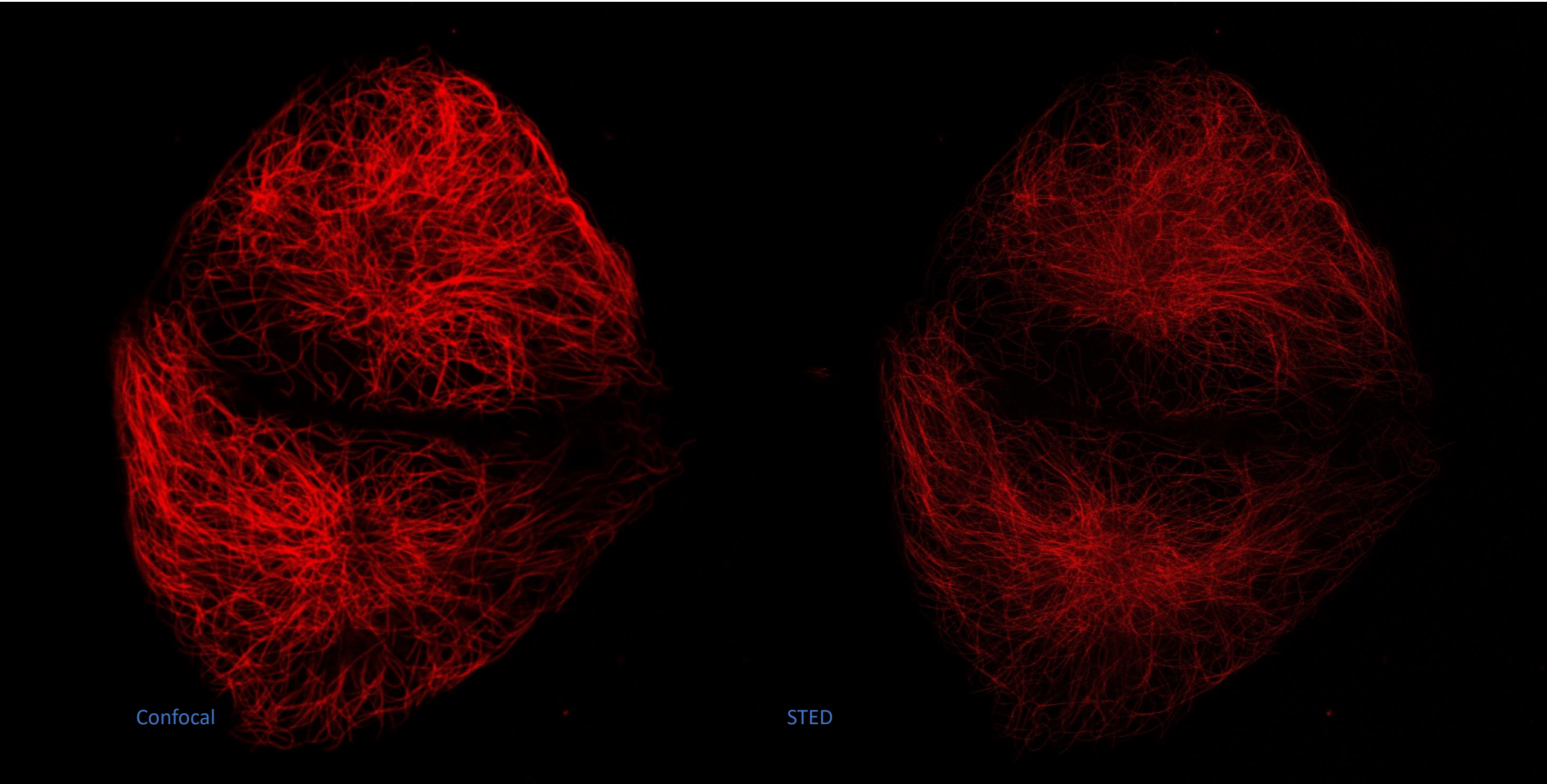
STED Strengths

X-Y-Z resolution of 40 × 40× 300 nm with optimal settings
No mathematical reconstruction of the final image
Can go deeper to the specimen and acquire thicker z-stack (theoretically)

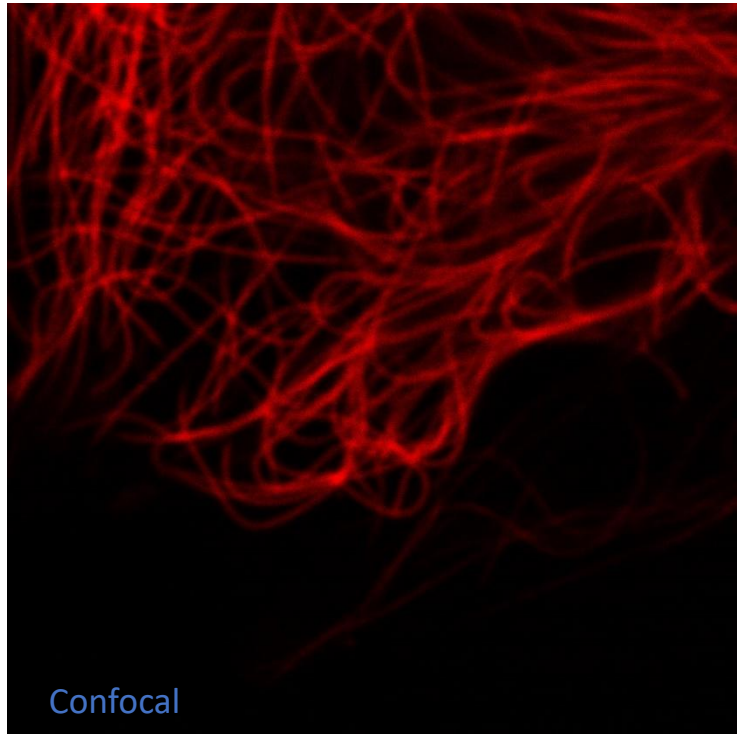
STED Limitations

Very high energy – influence the cell viability
Relatively slow acquisition
Limitation in fluorophores/depletion laser

SiR anti-Tubulin, pulse 775



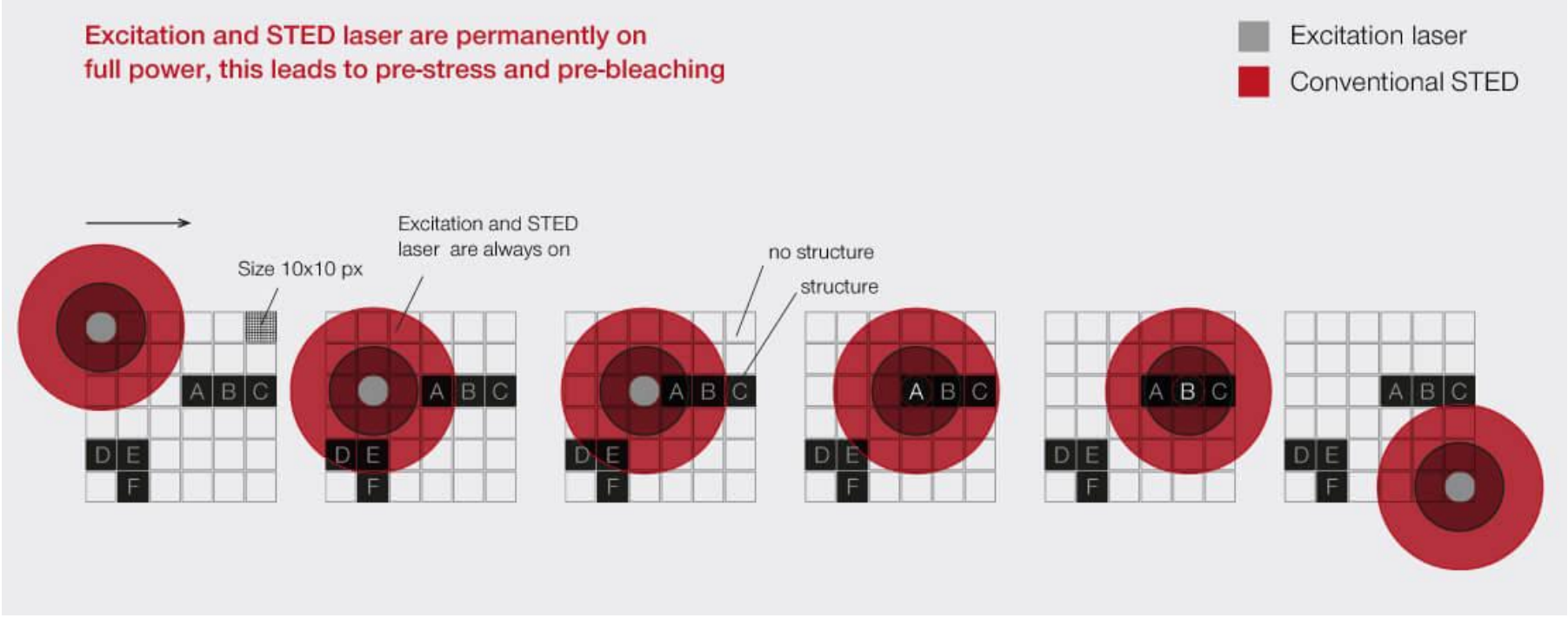
SiR anti-Tubulin, pulse 775

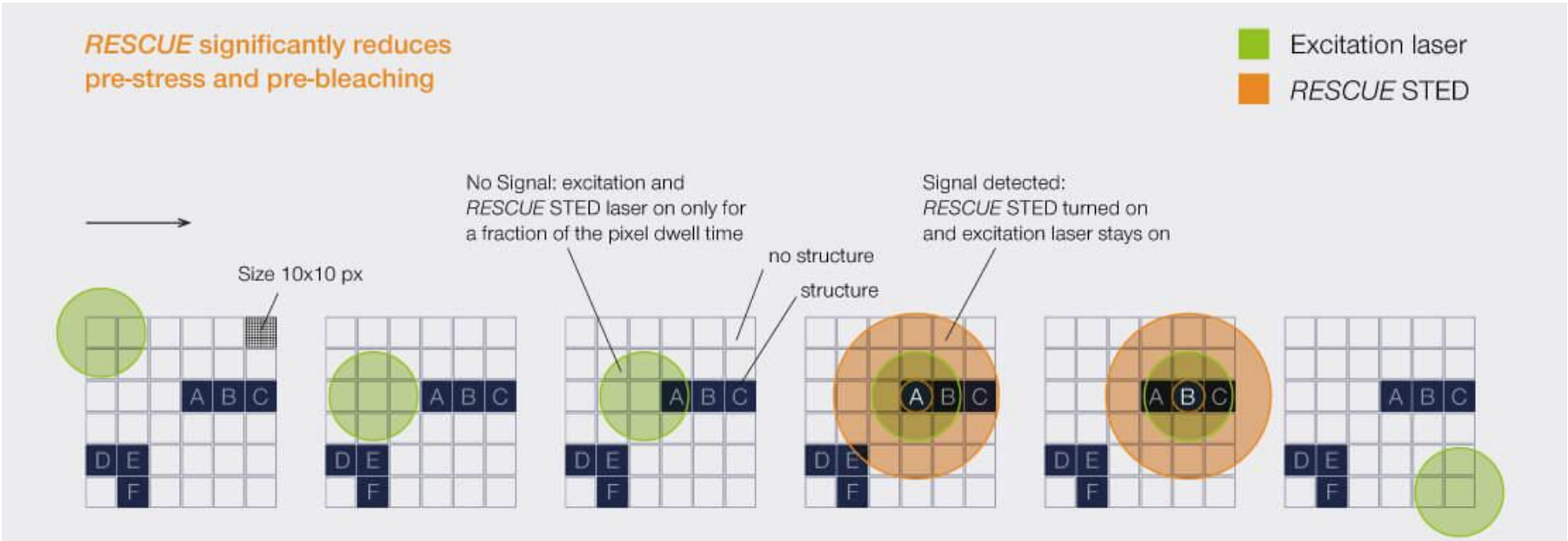


Confocal

STED, 20 time frames

Confocal probing to the RESCUE STED

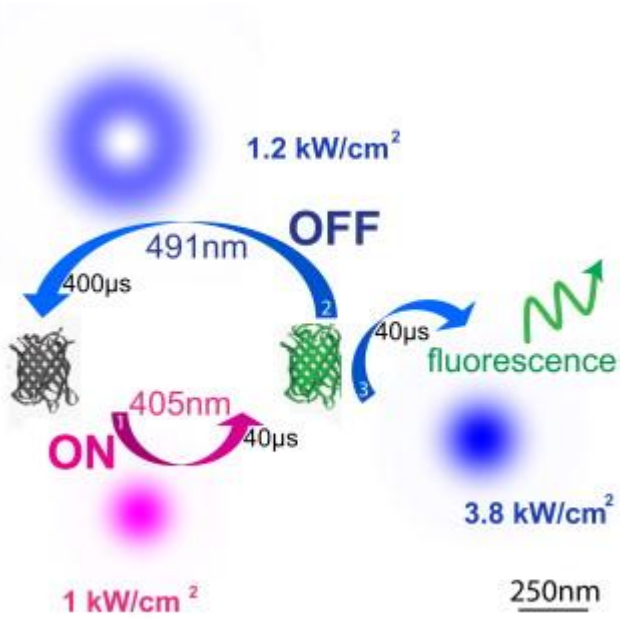




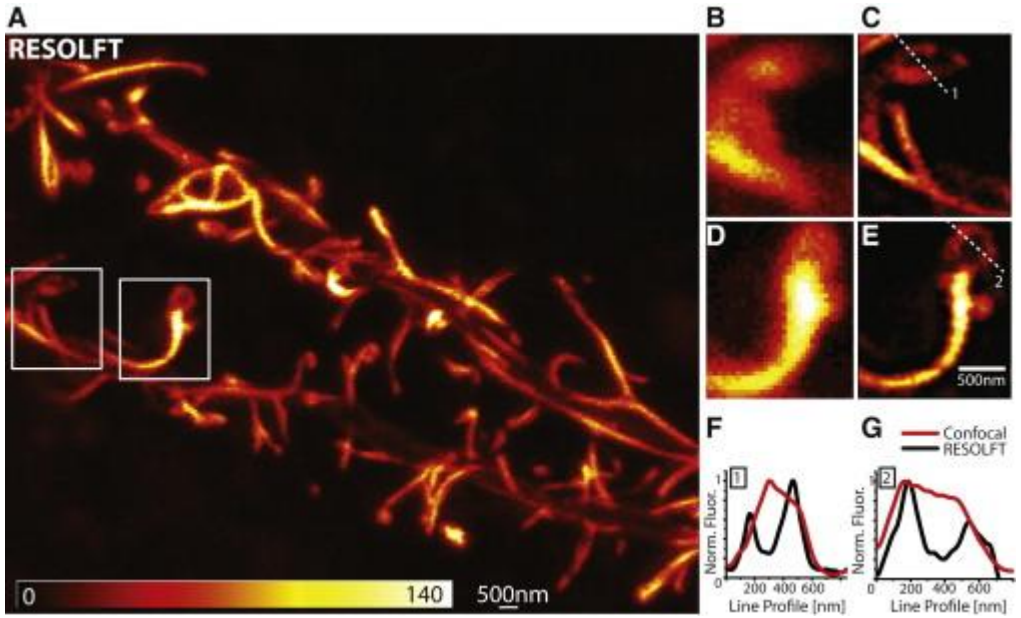
https://abberior.rocks/superresolution-confocal-systems/modules/adaptive-illumination/

RESOLFT

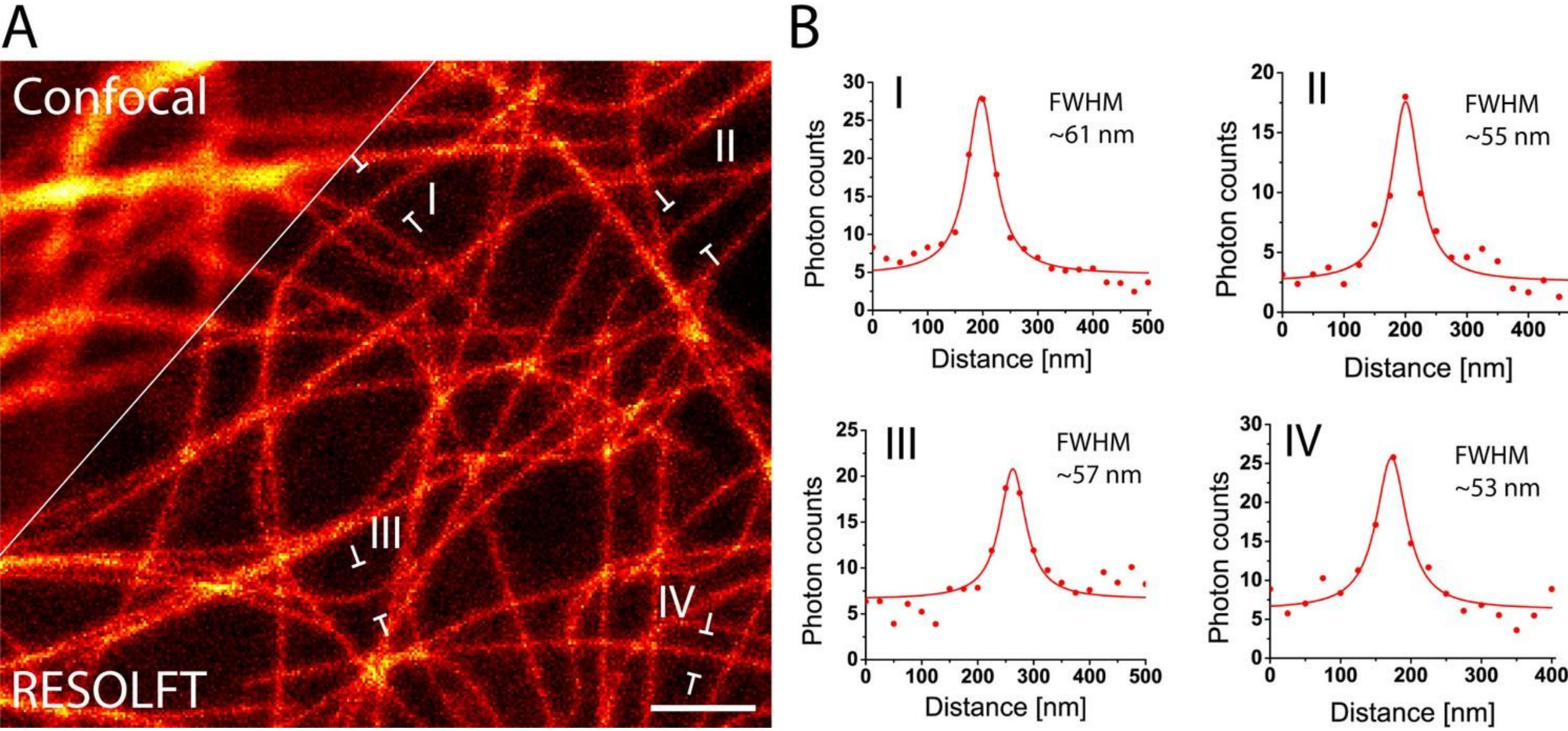
REversible **S**aturable **O**ptica**L** **F**luorescence **T**ransitio



Reversibly switchable fluorescent proteins (RSFPs)



laria Testa, Nicolai T. Urban, Stefan Jakobs, Christian Eggeling, Katrin I. Willig, Stefan W. Hell,
Nanoscopy of Living Brain Slices with Low Light Levels,
Neuron,
Volume 75, Issue 6,
2012



Sebastian Schnorrenberg, Tim Grotjohann, Gerd Vorbrüggen, Alf Herzig, Stefan W Hell, Stefan Jakobs (2016) In vivo super-resolution RESOLFT microscopy of Drosophila melanogaster eLife 5:e15567https://doi.org/10.7554/eLife.15567

Live cell imaging - STED

SiR F-Actin labelling

Abs/Em: 652/674 nm

50 nmol SiR-actin Kit

SiR-DNA labelling

Abs/Em: 652/674 nm

50 nmol SiR-DNA Kit

SiR-Lysosome labelling

Abs/Em: 652/674 nm

50 nmol SiR-lysosome Kit

SiR-Microtubule labelling

Abs/Em: 652/674 nm

50 nmol SiR-tubulin Kit

SiR-Cytoskeleton Kit

Abs/Em: 652/674 nm

50 nmol SiR-actin +
50 nmol SiR-tubulin

https://spirochrome.com/products/

Live cell imaging – general rule:

The sample MUST NOT move within the single time-frame acquisition!