



Abberior Instruments - STED microscopy from its inventors

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Work with the inventors of STED and RESOLFT microscopy



Dr. Gerald
Donnert
(# 10)



Dr.
Alexander
Egner
(# 27)



Dr. Benjamin
Harke
(# 18)



Prof. Dr.
Stefan W.
Hell
(# >300)



Nobel Prize in Chemistry
in 2014 for Stefan W. Hell



Dr. Lars
Kastrup
(# 16)



Dr. Matthias
Reuss
(# 9)



Dr. Andreas
Schönle
(# 37)



Dr. Christian
Wurm
(# 32)

**We have >100 years of experience
in developing super-resolution!**

What can you expect from us – nobel performance



KUNGL. VETENSKAPS AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2014 to Eric Betzig, Stefan W. Hell and William E. Moerner for the development of super-resolution microscopy.

The Nobel Prize 2014 in Chemistry



Their microscopes crossed the threshold

Optical microscopy had long been hindered by a presumed limitation: that it was impossible to achieve a resolution better than half the wavelength of light. Eric Betzig, Stefan W. Hell and William E. Moerner are awarded the 2014 Nobel Prize in Chemistry for ingeniously bypassing this limitation. Their revolutionary work has taken optical microscopy to nano dimensions.

Using what is now called nanoscopy, researchers can see the activity of individual molecules inside living cells. For example, they can see how proteins form synapses between the brain's nerve cells or how aggregating proteins in cases of Parkinson's, Alzheimer's or Huntington's disease.

Optical microscopy is one of the most important tools in the life sciences, allowing researchers to observe processes inside living cells. However, it had long been thought that it would be impossible to observe a cell in molecular detail. In 1928, microscopy's limit had been determined: a so-called Rayleigh microscope. Its resolution would never be better than half the wavelength of light, approximately 0.2 micrometres – but thanks to the 2014 Nobel Laureates in chemistry, optical microscopy can now be used to observe the nano world.

Key difficulties in principle are: a) a single molecule is too small to be seen by a microscope, and b) a microscope's light source is too broad, so that the light is scattered in all directions, blurring the image. To overcome these limitations, the Nobel Laureates have developed three different approaches to super-resolution microscopy. The first is the so-called STED microscope, which uses a second laser beam to focus the light on a single molecule. The second approach is the so-called PALM microscope, which uses a series of pulses of light to focus the light on a single molecule. The third approach is the so-called SIM microscope, which uses a series of pulses of light to focus the light on a single molecule.

The principle of STED microscopy

Regular optical microscope

STED microscope

1. A laser beam is focused on a sample. 2. A second laser beam is focused on the same spot, but with a different wavelength. 3. The two beams interact to create a super-resolution image.

Coherent microscopy

Super-resolution microscopy

2014 – Nobel Laureates in Chemistry

1. Eric Betzig, 1946, USA, is a chemist and physicist. He is currently a professor at the University of Michigan, Ann Arbor, Michigan, USA. He is also a member of the National Academy of Sciences, USA.

2. Stefan W. Hell, 1957, Germany, is a physicist and chemist. He is currently a professor at the University of Göttingen, Göttingen, Germany. He is also a member of the German Academy of Sciences, Berlin, Germany.

3. William E. Moerner, 1953, USA, is a physicist and chemist. He is currently a professor at the University of California, Berkeley, California, USA. He is also a member of the National Academy of Sciences, USA.

FURTHER READING:

• [The Nobel Prize in Chemistry 2014](#) (The Nobel Prize in Chemistry 2014)

• [The Nobel Prize in Chemistry 2014](#) (The Nobel Prize in Chemistry 2014)

• [The Nobel Prize in Chemistry 2014](#) (The Nobel Prize in Chemistry 2014)

Abberior Instruments
STED measurement
on the Nobel Poster

STED nanoscopy in a truck – is possible!



Abberior Roadshow



Abberior Demo-lab

– bringing the microscope to the users



Abberior DemoLab



Two nanoscope platforms existing...

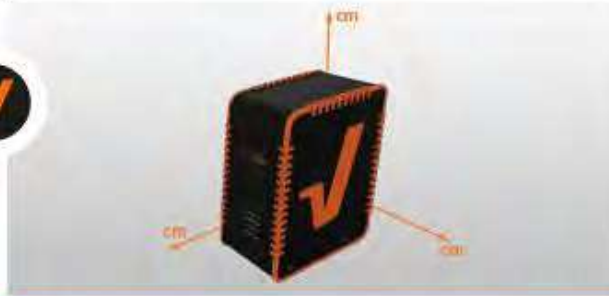
Expert line

STED / RESOLFT



Compact line

STEDYCON



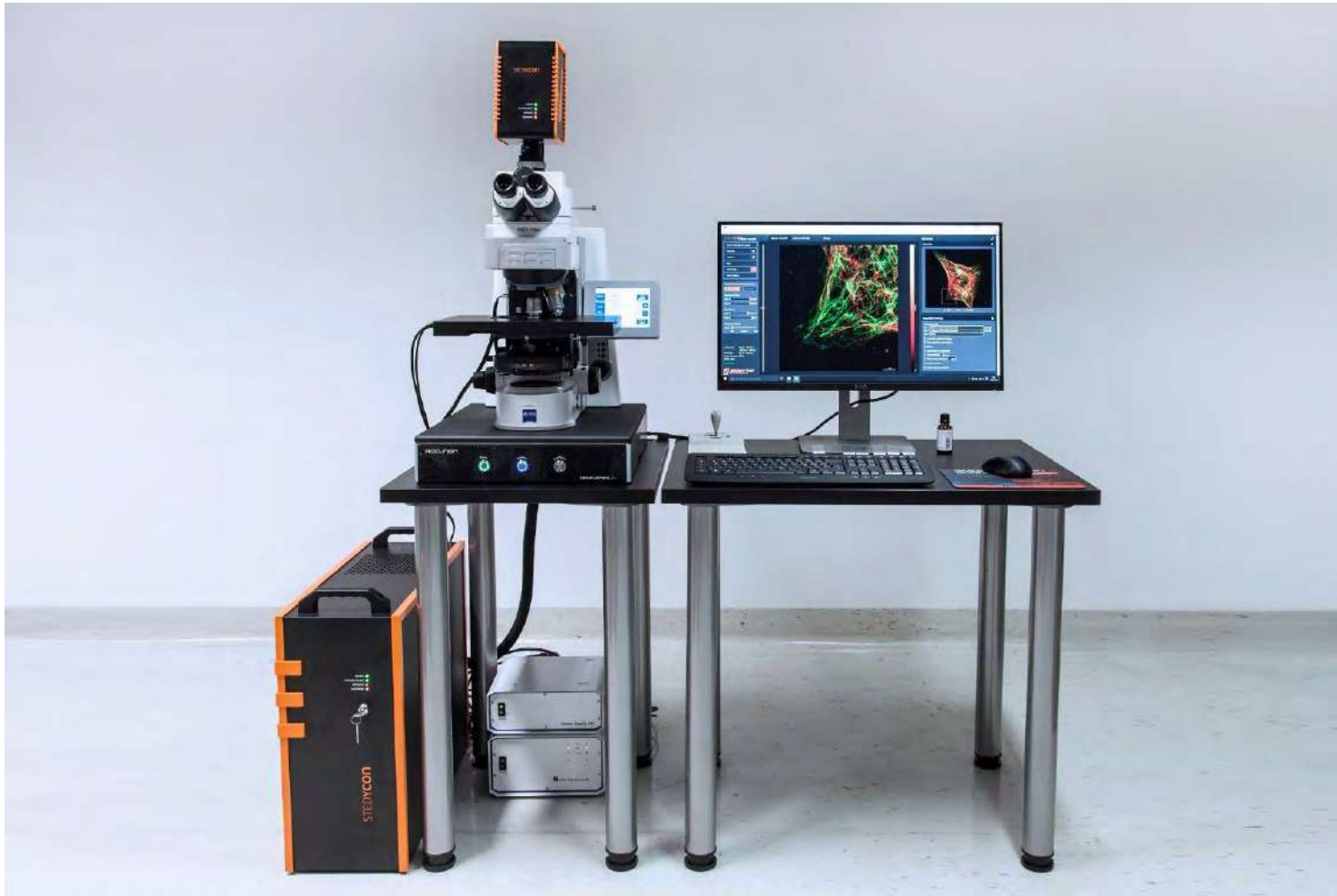
At the physical limits...

- ✓ High-end / customized
- ✓ Cutting-edge 2D & 3D STED
- ✓ Continuously upgradable
- ✓ Multiple STED options
- ✓ Powerful software

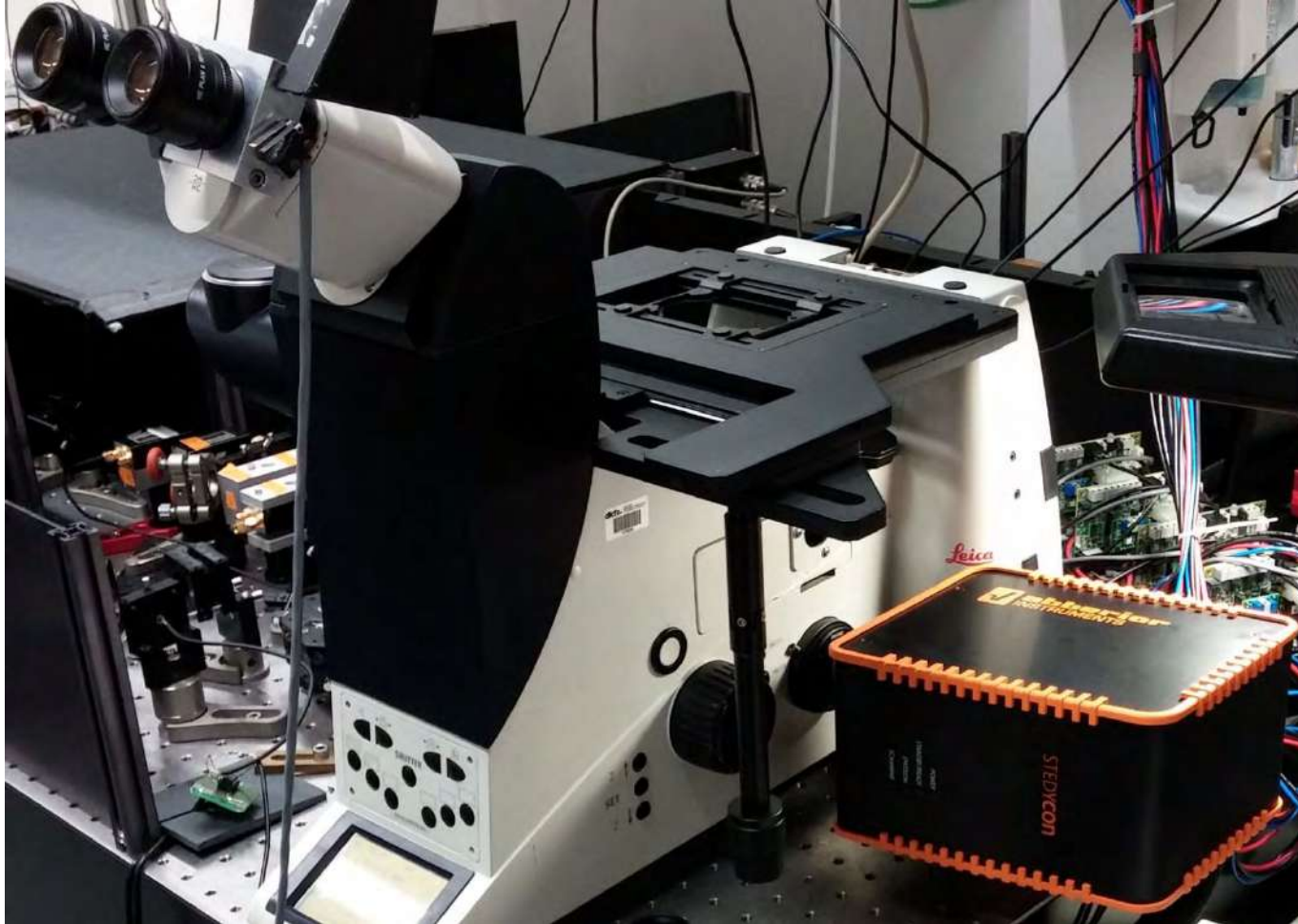
Expands any microscope stand to STED + confocal...

- ✓ Compact / rugged / economic
- ✓ Cutting-edge 2D STED
- ✓ Installation within minutes; plug & play STED + confocal imaging
- ✓ Fits any microscope body
- ✓ Intuitive software

STEDYCON – from unpacking to STED imaging in 3 minutes



STEDYCON - customer example



Leica DMI6000

STEDYCON - customer example



Nikon Ti-E

STEDYCON - customer example



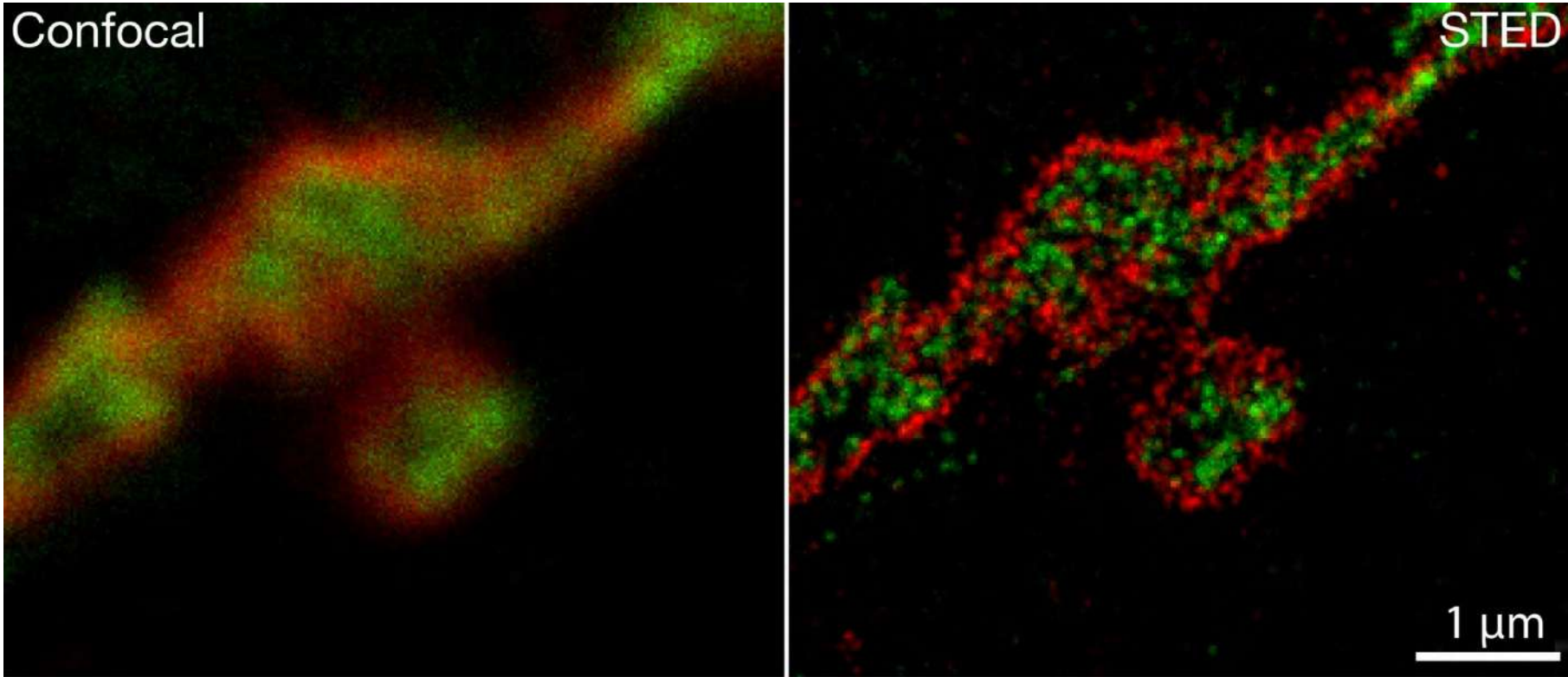
Zeiss Observer Z1

STEDYCON – Inverted example Olympus



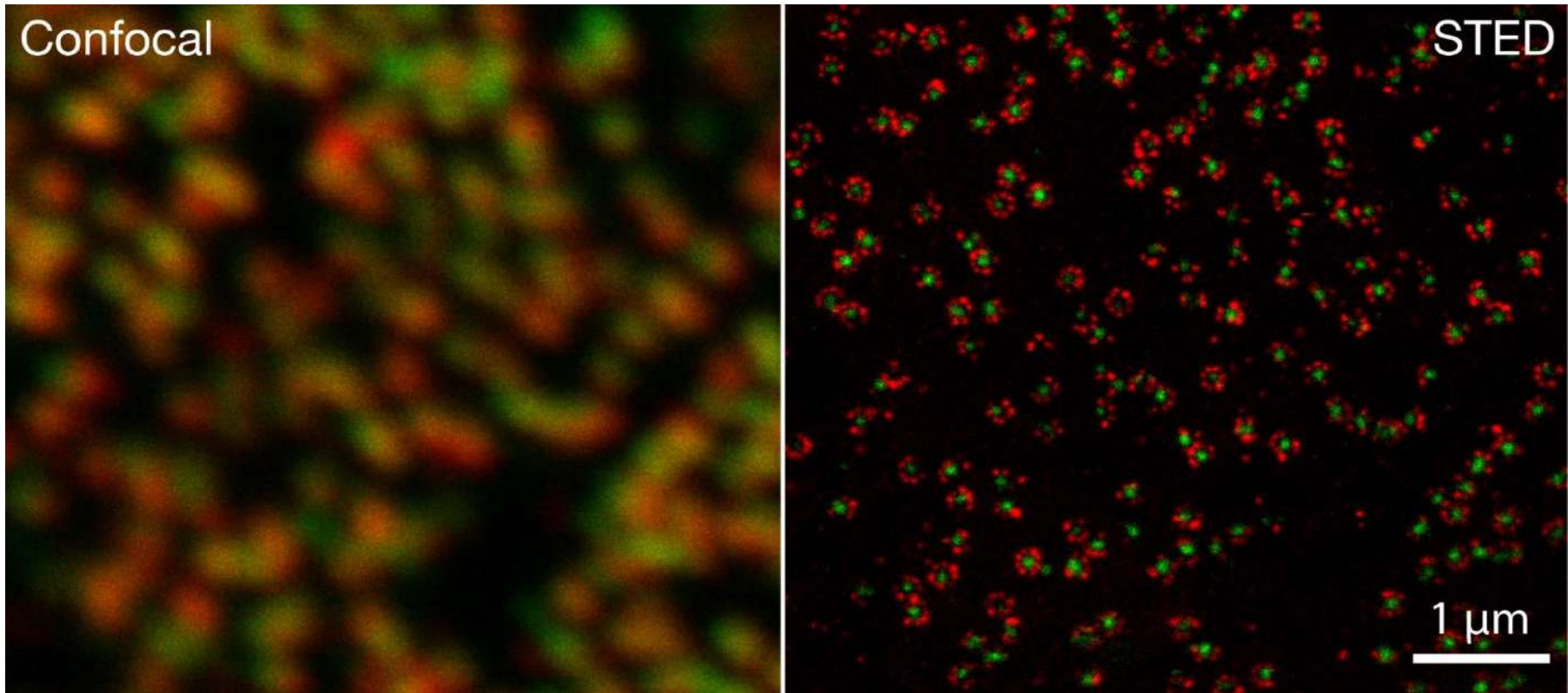
Olympus IX83

Microscopic Images



Two proteins in the Golgi apparatus were immunolabelled using primary antibodies specific for GM130 and Giantin and secondary antibodies coupled to Abberior STAR580 and Abberior STAR635P. Shown is a close-up of the image shown above.

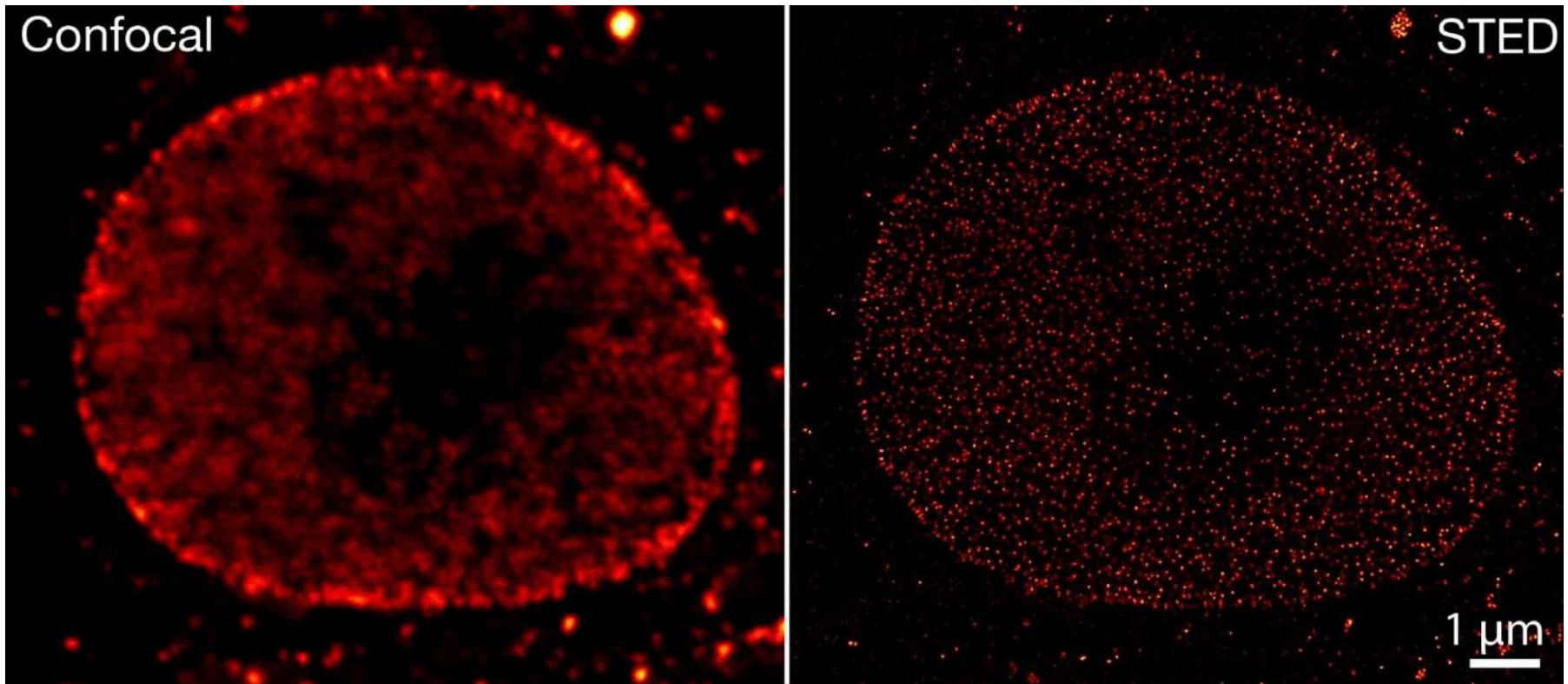
Microscopic Images



Two subunits of the nuclear pore complex were immunolabelled using antibodies against gp210 and antibodies with multiple specificities (PAN4/5) and secondary antibodies coupled to Abberior STAR580 and Abberior STAR635P. Note that gp210 is localized in an eightfold symmetric structure at the rim of the nuclear pore complex. Shown is RAW DATA.

Images were acquired using a STEDYCON attached to a Nikon Eclipse Ni.

Microscopic Images



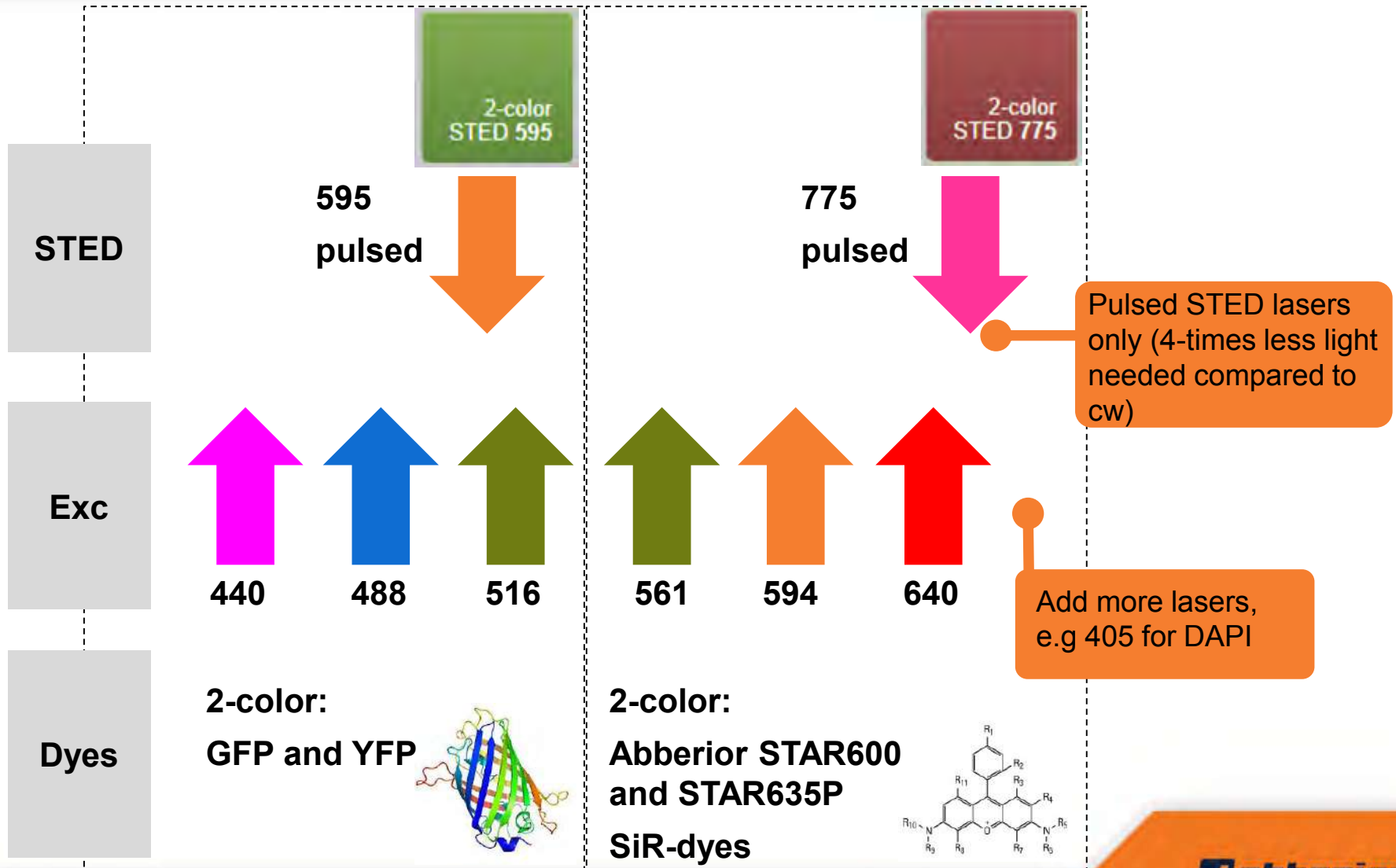
Nuclear pore complex subunits in the central channel (nup153) of mammalian cells immunolabelled using Abberior STAR635P. Shown is RAW DATA.

Images were acquired using a STEDYCON attached to a Zeiss Axio Imager Z2.

Abberior Instruments Expert Line

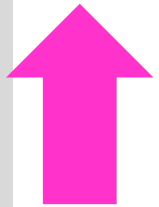


Schematic of the Abberior STED system with two pulsed STED laser @595 nm and @775 nm

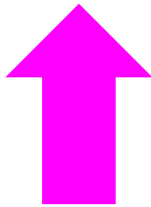


Confocal microscope system covering all relevant dyes and proteins

Conf.
lines



405



440



488



516



561



594



640

Selection
of
Markers

DAPI

STAR440Sx

STAR488

STAR580

STAR600

STAR635

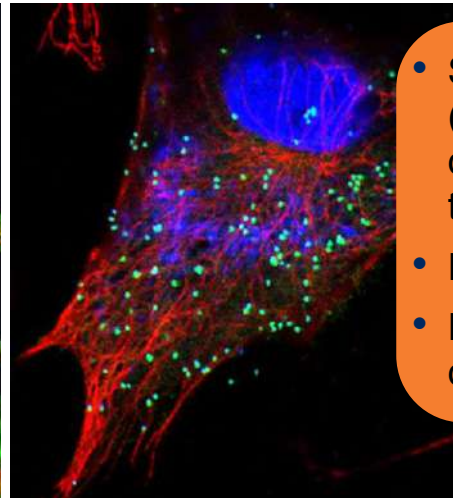
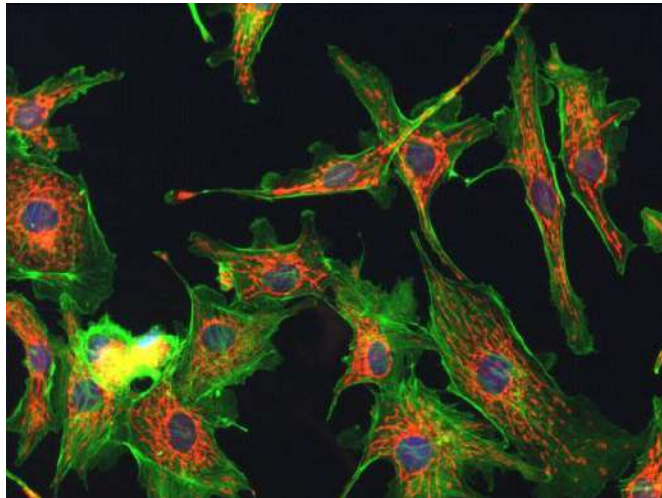
STAR635P

STARred

GFP

YFP

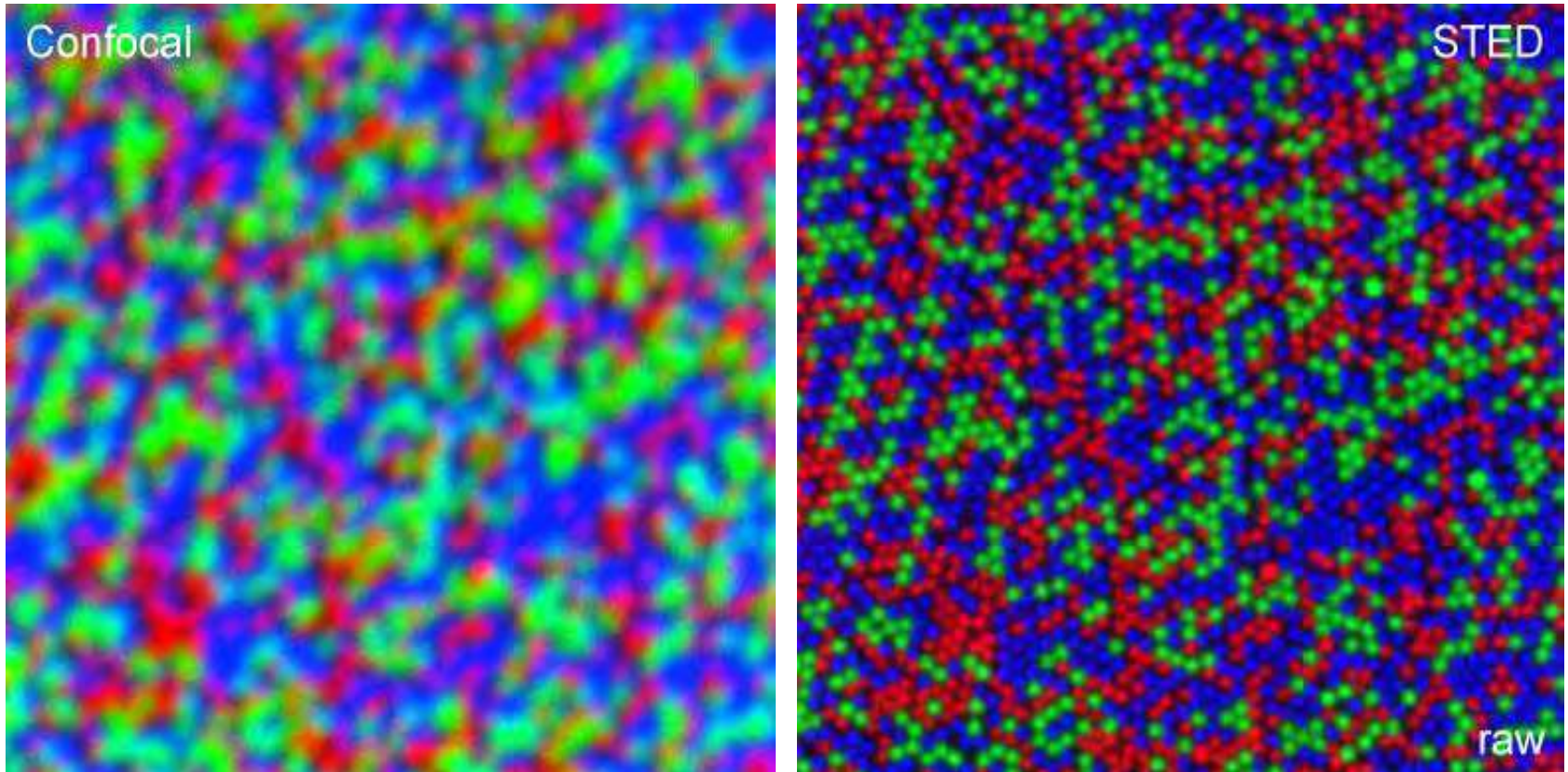
mCherry



- Single molecule detectors (APDs) with the highest detection efficiency (e.g. 62% in the red) among all detectors
- FLIM (also in STED mode)
- FRET, FRAP, transmitted light detection

Multicolor STED imaging using 2 pulsed STED lasers @595 nm and @775 nm

Resolution performance is equal to the current lab performance

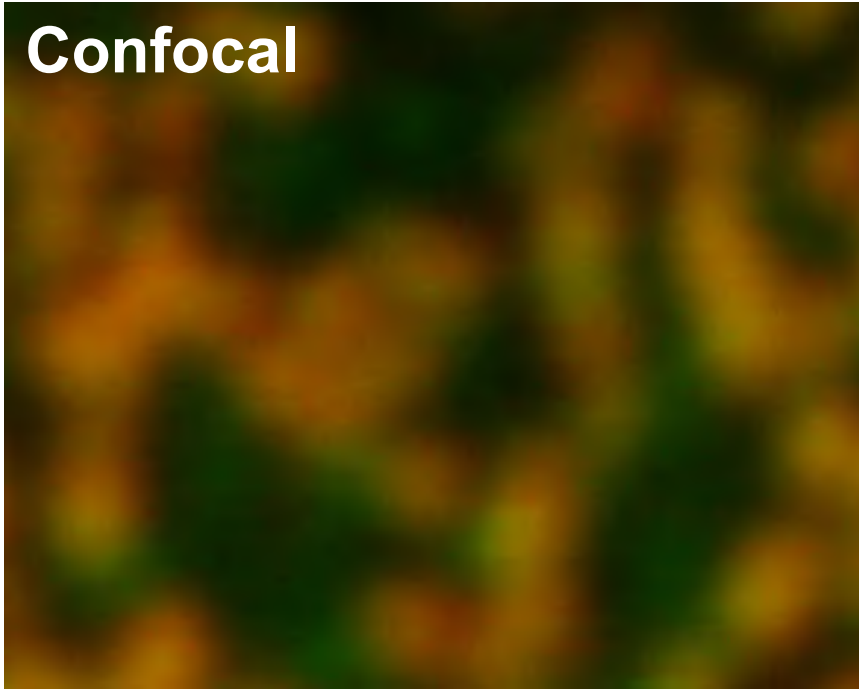


Monolayer of 3 species of fluorescent beads

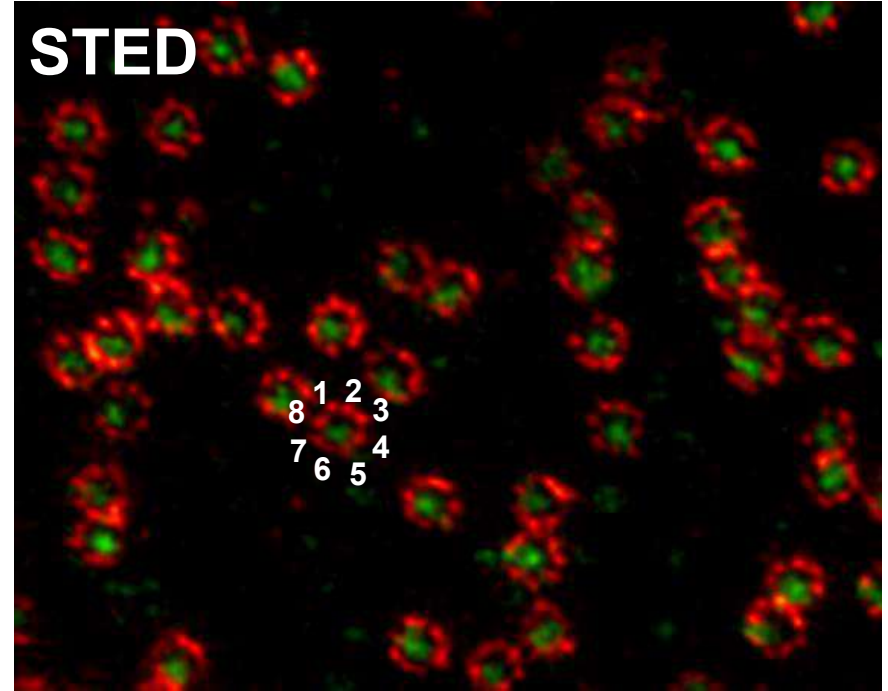
Microscopic images

Resolution performance is equal to the current lab performance

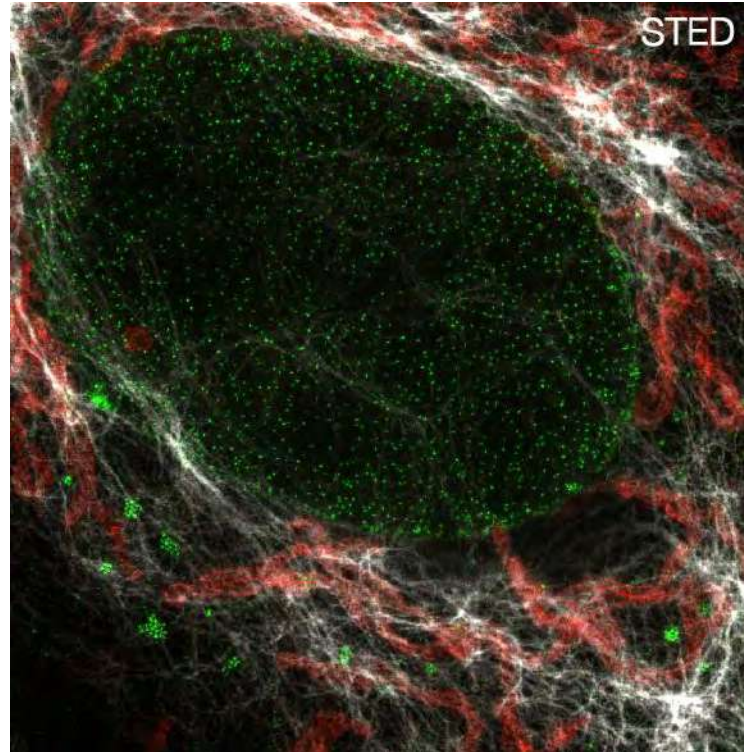
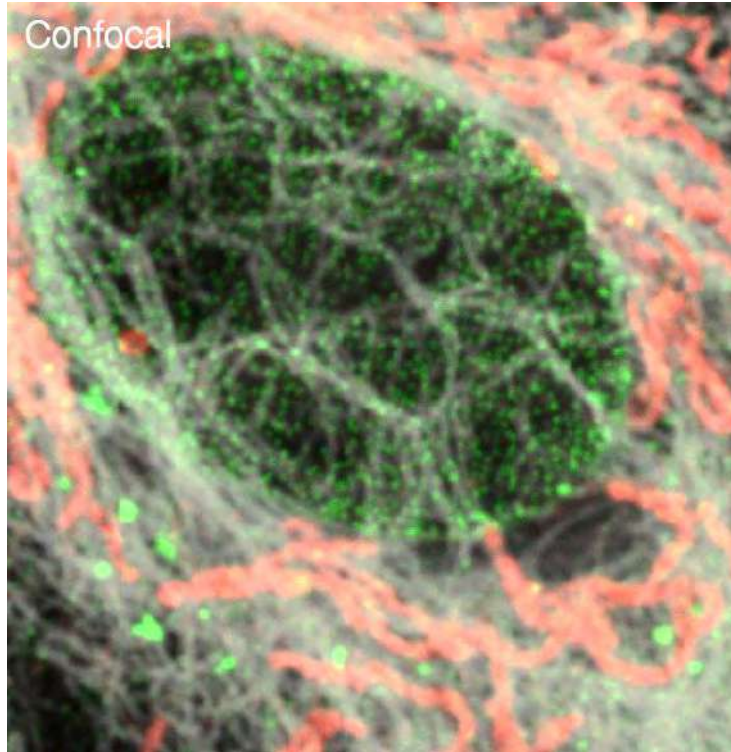
Confocal



STED

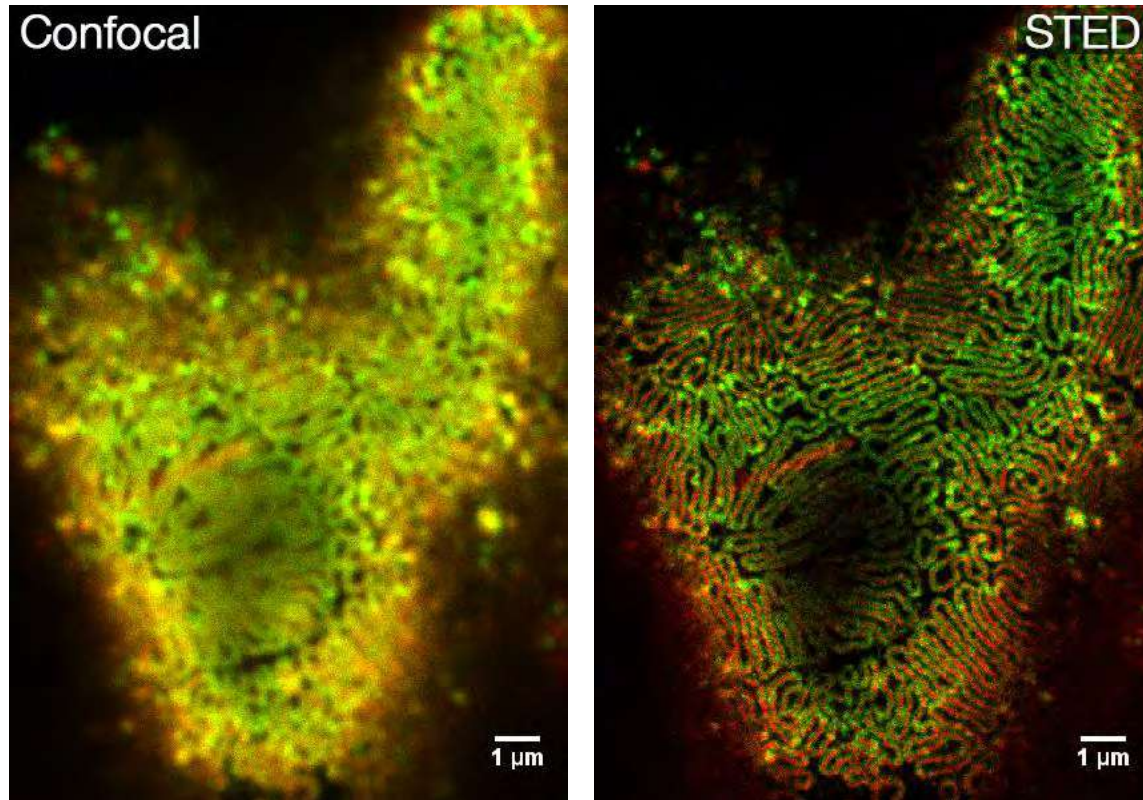


Microscopic images



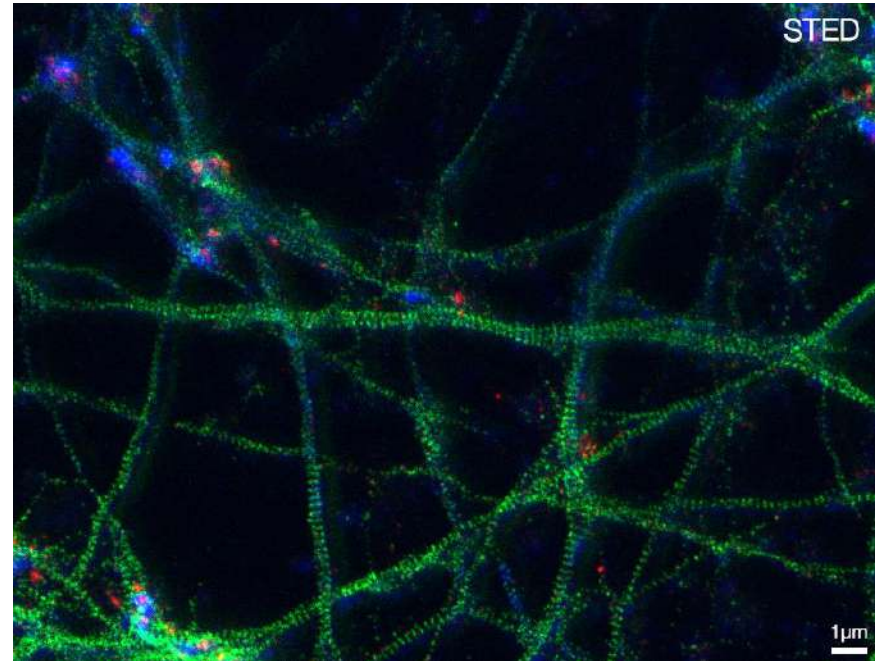
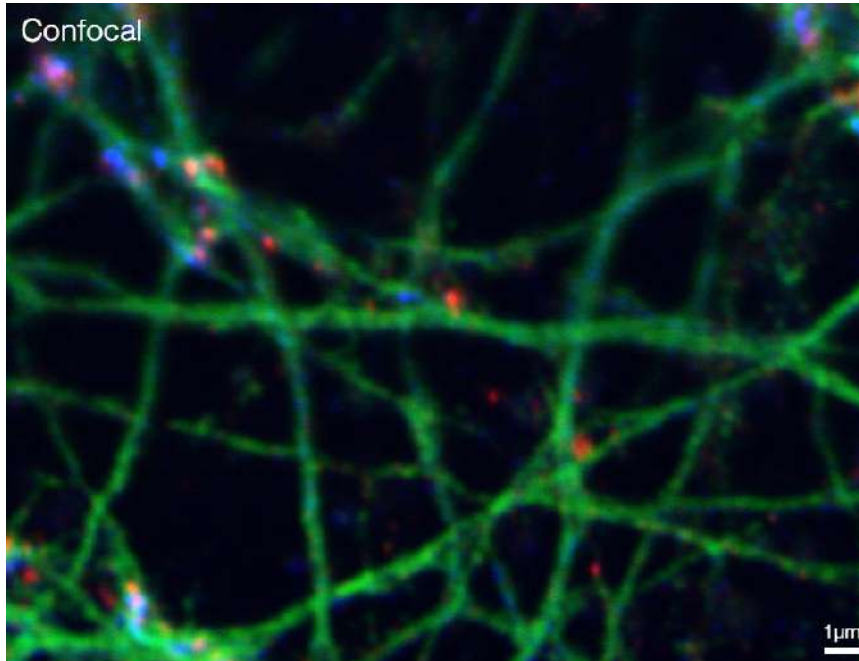
3-color RESCue STED images using both pulsed STED lasers @775nm and @595nm. Labelled structures: nuclear pore complex (green, nup153, Oregon green 488), vimentin (white, Abberior STAR635P) and mitochondria (red, Abberior STAR580).

Microscopic images



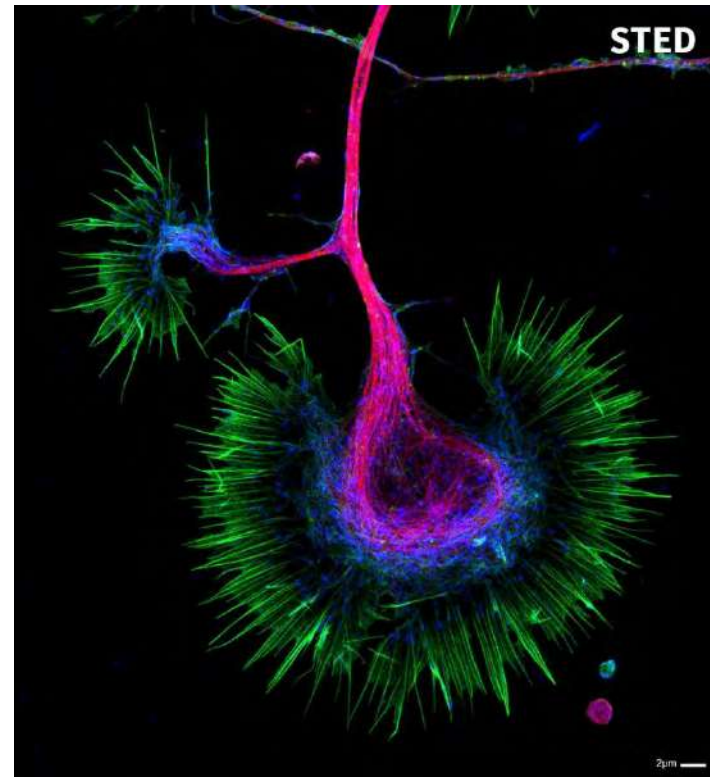
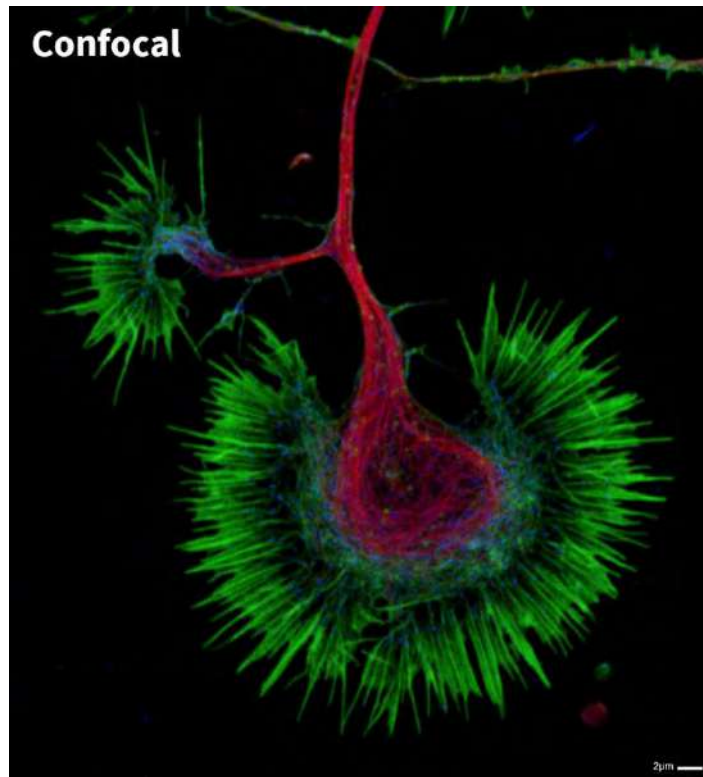
2-color 2D STED image of a cleared adult kidney sample of a rat. Shown is an image of a renal corpuscle showing Nephtrin (red, Abberior STAR635P) structures inbetween the Podocin slits (green, AlexaFluor594). Sample was prepared by D. Unnersjö Jess and H.G. Blom @ KTH Stockholm, Sweden.

Microscopic images



3 color STED image of primary hippocampal neurons. Please note the characteristic ~ 190 nm beta II spectrin periodicity along distal axons (green) which is only visible in the STED image. Labelled structures: beta II Spectrin (green, Abberior STAR635P), Bassoon (red, Abberior STAR580), Actin cytoskeleton (blue, Phalloidin, Oregon Green 488). Imaged with Abberior Expert Line with 595nm and 775nm STED laser. Sample was prepared by Elisa D'Este @ MPIBPC, Göttingen.

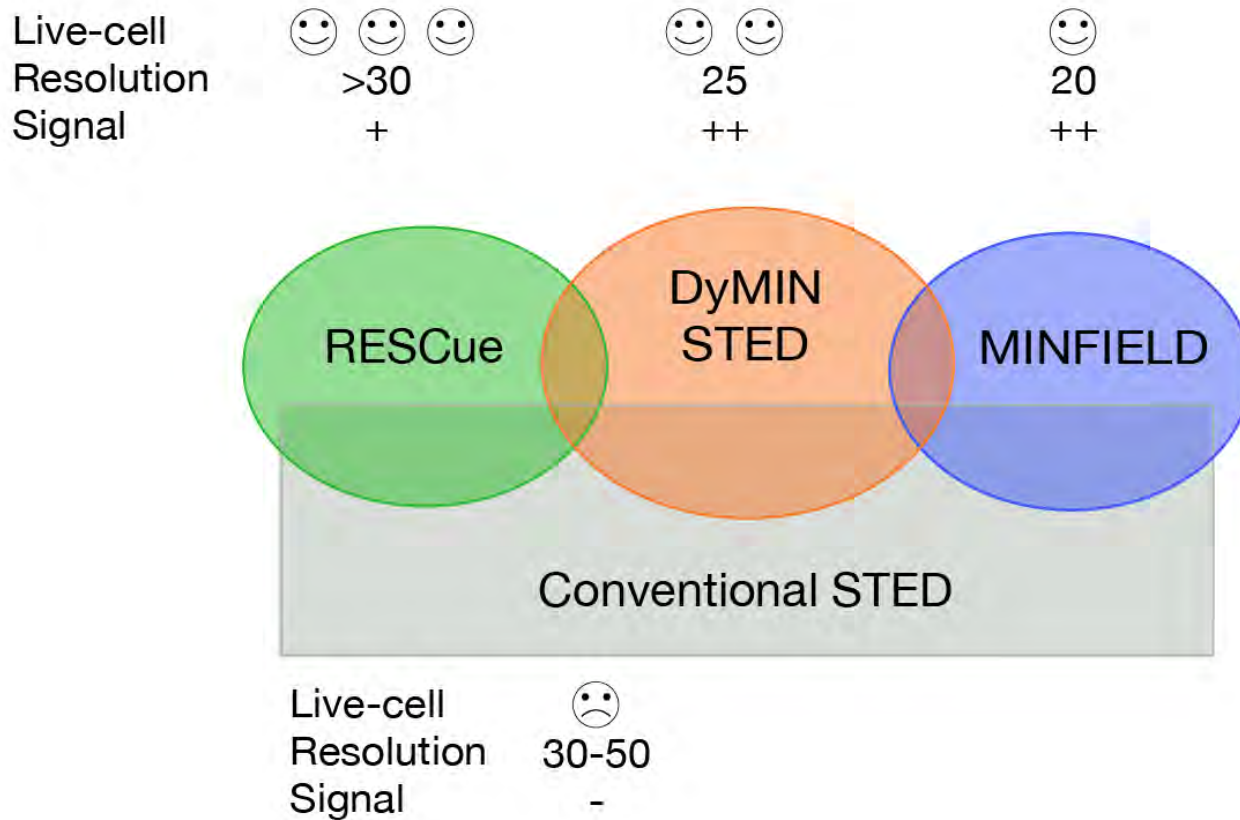
Microscopic images



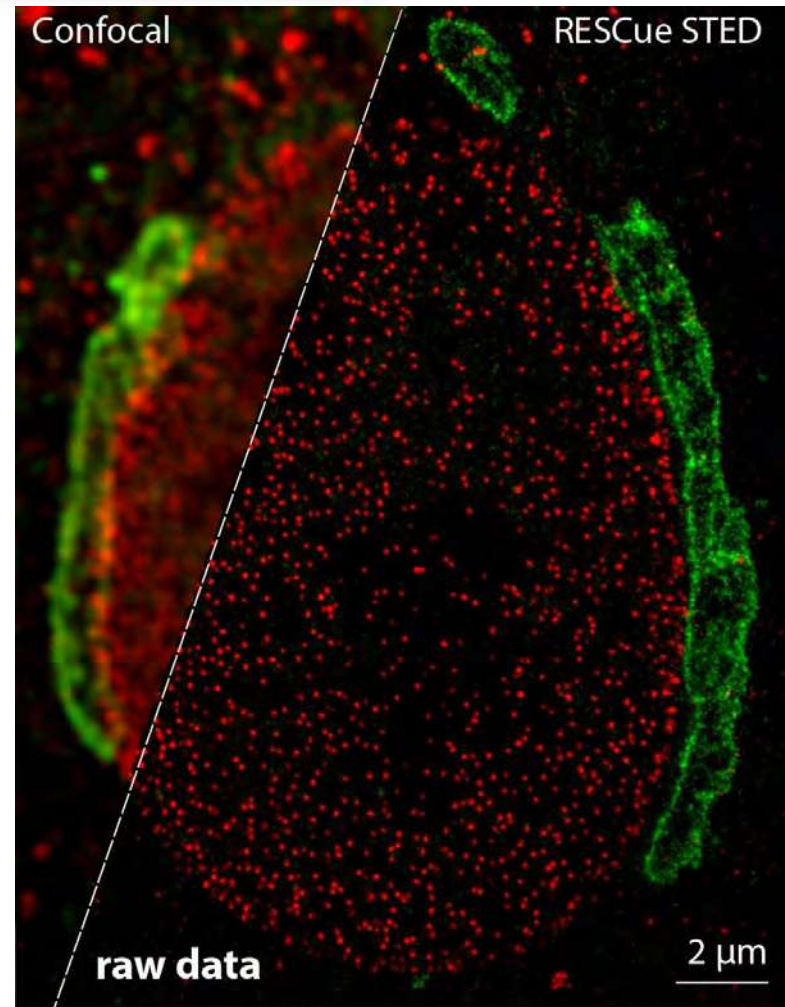
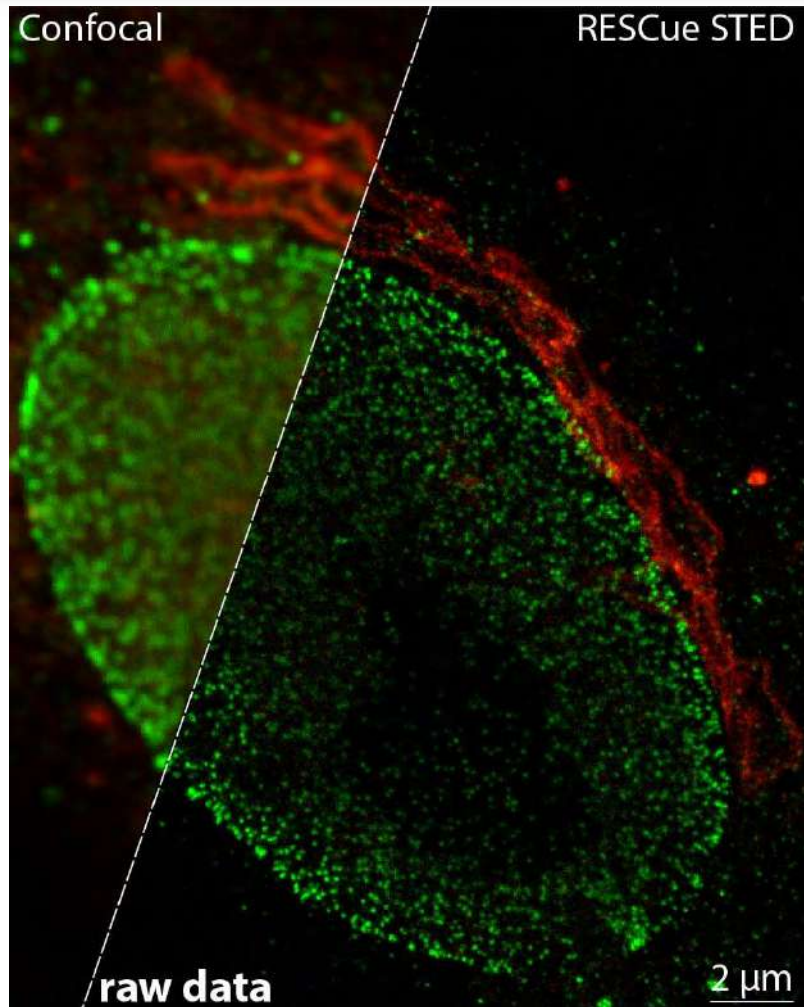
Growth cone at the tip of the axon of a primary hippocampal neuron at 1 day in vitro. Microtubules (Tuj1, Abberior STAR580, red) are bundled in the central-domain suggesting a pausing state. The molecular motor myosin IIB (confocal, Alexa488, blue) is enriched at the transition-zone, along the F-actin arcs. In the peripheral domain actin forms bundles in the filopodia (Phalloidin, Abberior STAR635, green). Imaged with Abberior Expert Line @ MPIbpc by Elisa D'Este.

Intelligent illumination schemes for STED

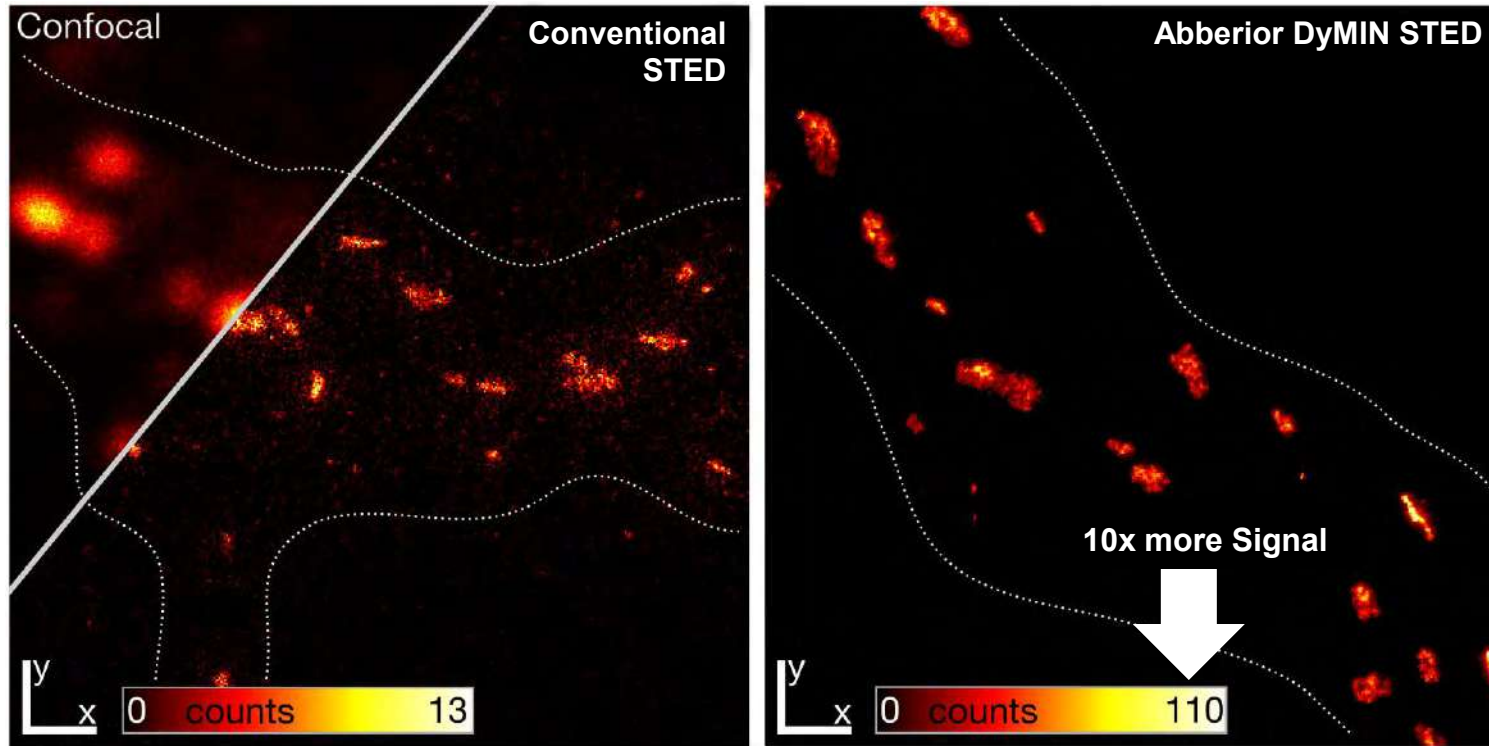
RESCue STED, DyMIN STED and MINFIELD STED greatly expand the performance of STED in terms of live-cell imaging, signal and resolution.



RESCue STED



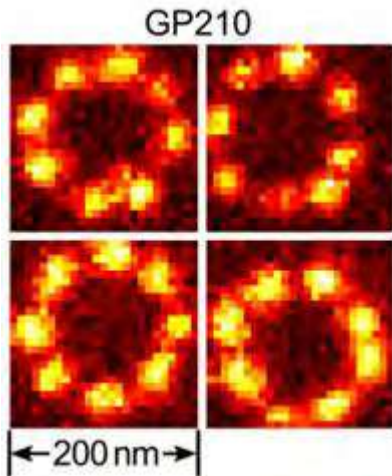
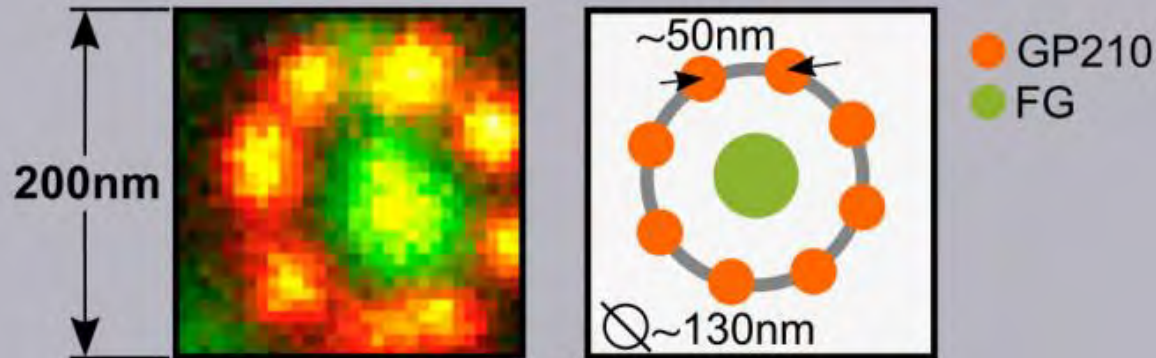
DyMIN STED – 10x more signal for the precise analysis of structures



J. Heine*, M. Reuss*, B. Harke*, E. D'Este, S. J. Sahl and S. W. Hell,
"[Adaptive-illumination STED nanoscopy](#)", PNAS (2017)

*Abberior Instruments GmbH

MINFIELD STED delivers STED resolutions < 20nm

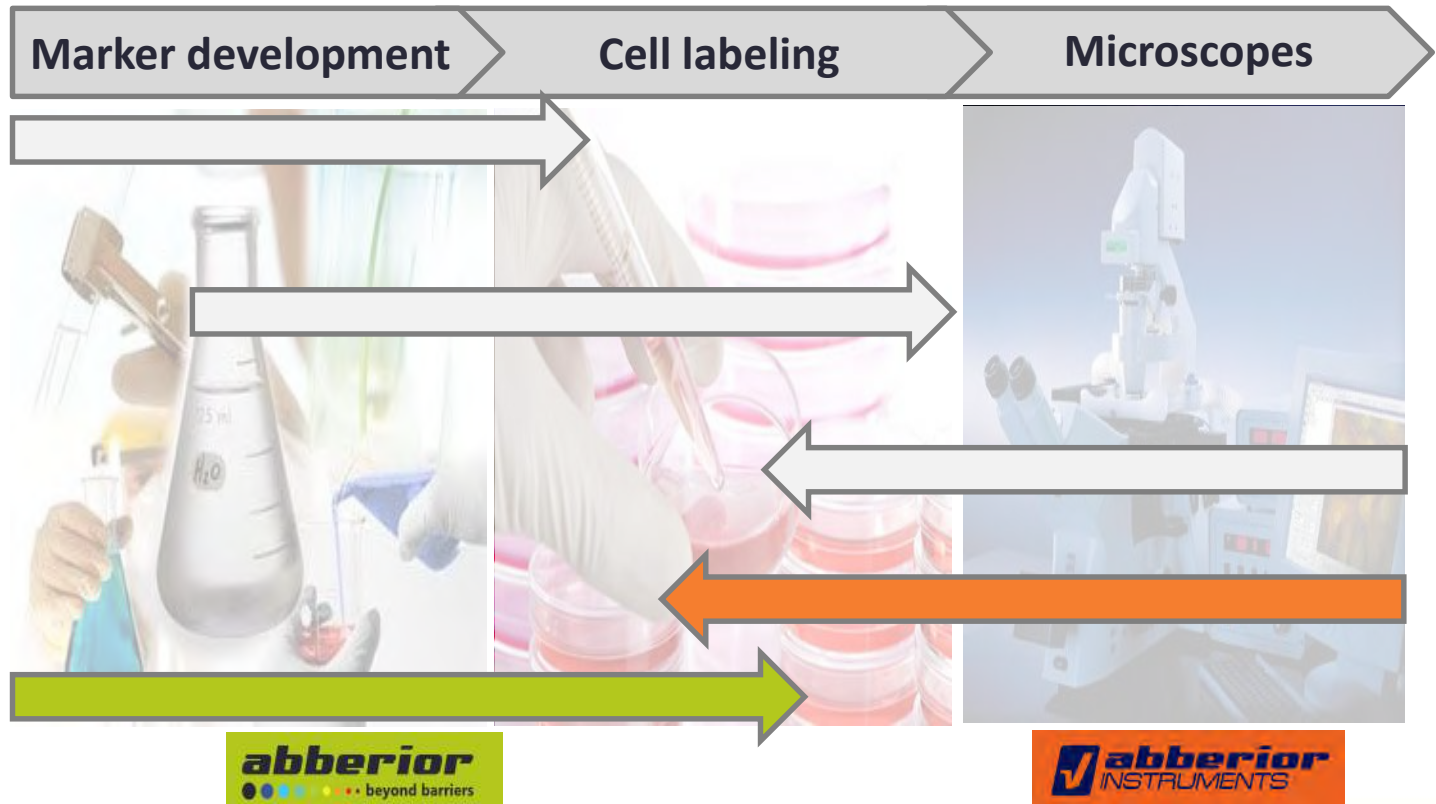


F. Göttfert, T. Pleiner, J. Heine*, V. Westphal, D. Görlich, S. J. Sahl, S. W. Hell, "[Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent](#)", PNAS 1621495114 (2017)

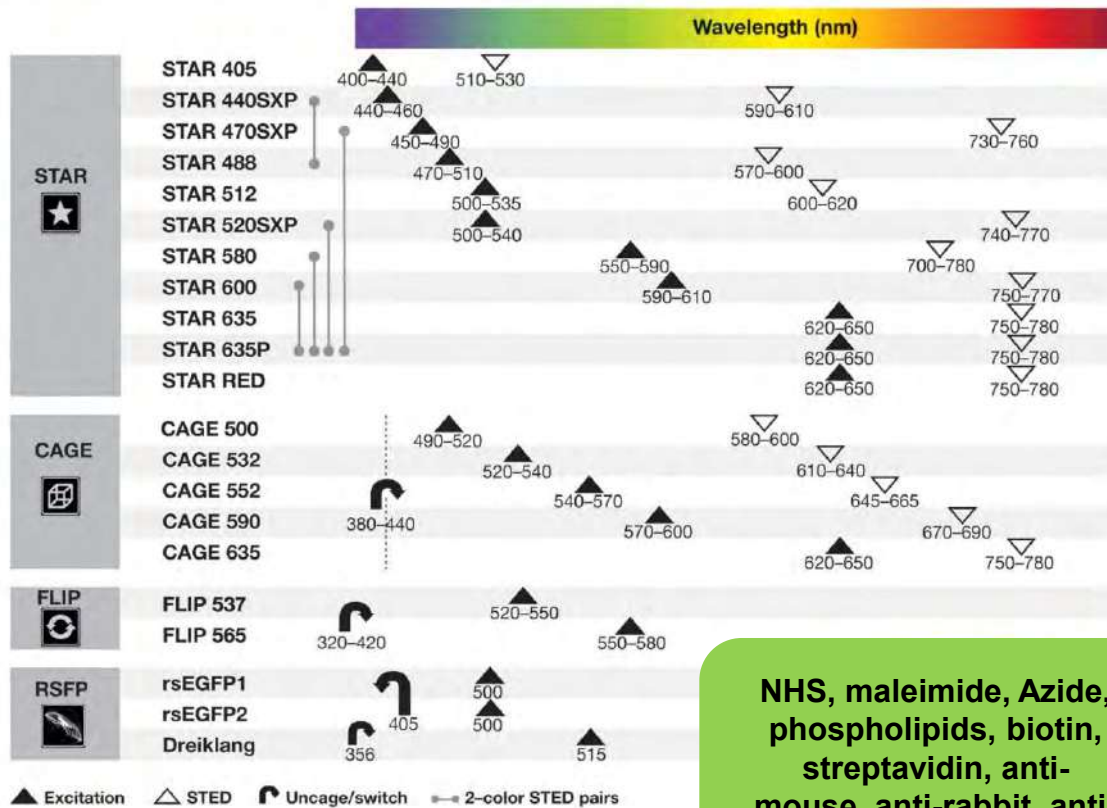
*Abberior Instruments GmbH

It is important to optimize both the superresolution dyes as well as the microscope hardware

⇒ Distinct expertise



Overview of Abberior markers



www.abberior.com

NHS, maleimide, Azide,
phospholipids, biotin,
streptavidin, anti-
mouse, anti-rabbit, anti-
rat, anti-chicken

Superresolution

Organic Dyes for STED microscopy

E: Expert Line
Y: STEDYCON

Recommended dyes to be used with the Abberior Instruments Expert Line & the STEDYCON

STED pulsed	775 nm		595 nm	
Exc.	640nm	594/561nm	485nm	450nm
Nanoscope	E/Y	E/Y	E	E/Y*
Always recommended	Abberior STAR RED Abberior STAR635P Abberior STAR635	Alexa 594 Down to 25nm** resolution	Oregon Green 488	Oregon Green 488
Sample dependent recommended	Atto 647N Atto 633 Alexa 637 Cy5	Abberior STAR580 Atto 594 Atto 530 Cy3	Abberior STAR488 Alexa 488 Atto 488 FITC	Abberior STAR488 Abberior STAR470SXP Alexa 488 Atto 488 FITC

abberior
beyond barriers

abberior
INSTRUMENTS

*STEDYCON with 450nm excitation for confocal imaging only

**only depending on the sample

Organic Dyes for live-cell STED microscopy

E: Expert line
Y: STEDYCON

Recommended dyes to be used with the Abberior Instruments Expert Line & the STEDYCON

STED pulsed	775 nm		595 nm	
Exc.	640nm	594/561nm	485nm	450nm
Nanoscope	E/Y	E/Y	E	E/Y*
Always recommended	SiR	Abberior LIVE580	Abberior LIVE510 Abberior LIVE515	Abberior LIVE510 Abberior LIVE515

abberior
beyond barriers



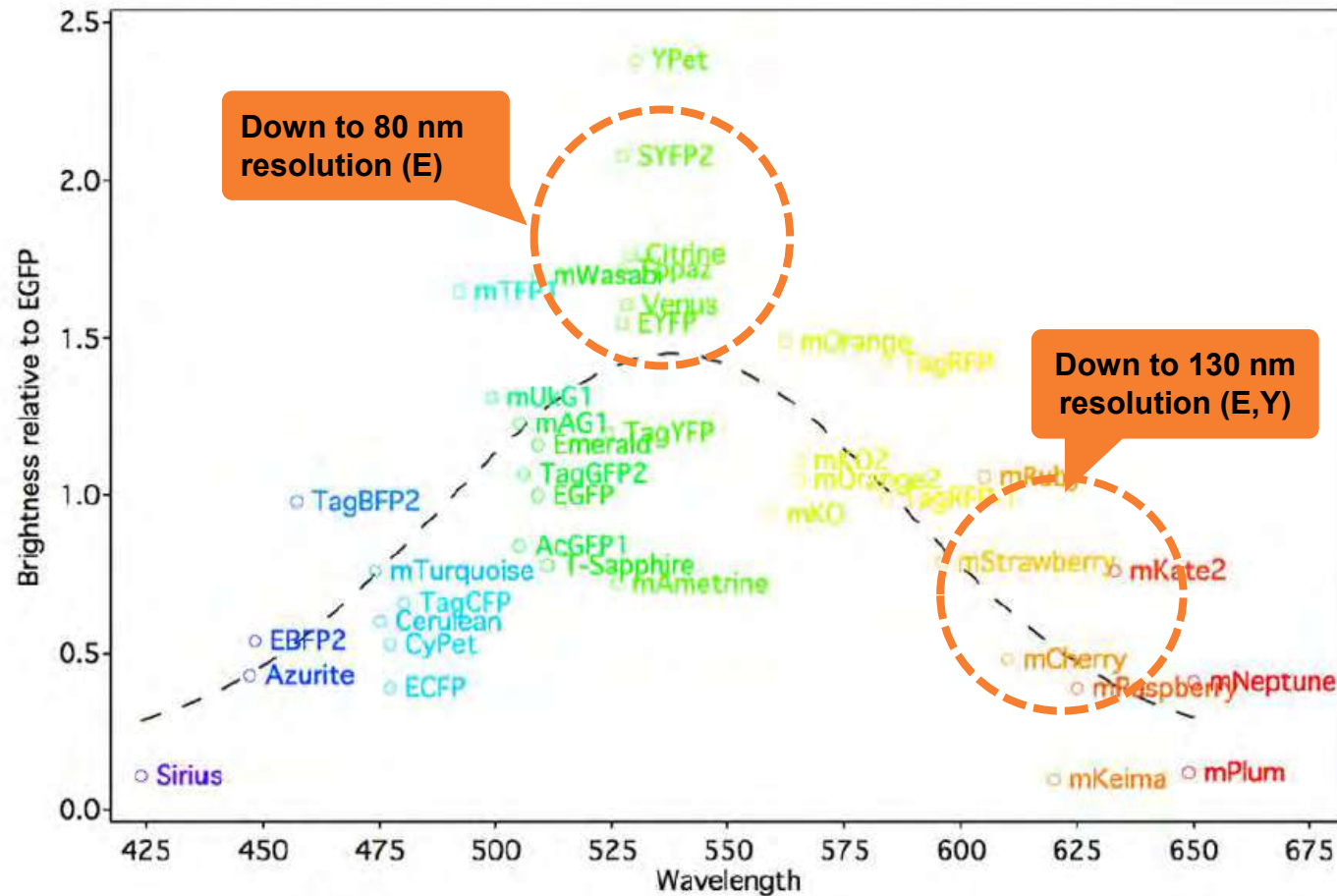
*STEDYCON with 450nm excitation for confocal imaging only
**only depending on the sample

Fluorescent Proteins for live-cell STED microscopy

E: Expert line
Y: STEDYCON

Recommended fluor. proteins to be used with the Abberior Instruments Expert Line & the STEDYCON

- Organic dyes throughout brighter & more photostable compared to fluorescent proteins
- Green fluorescent proteins superior to red fluorescent proteins
- Green proteins like EGFP, YFP & Citrine deliver ~ 80nm STED resolution (E)
- Red proteins like mCherry deliver ~ 130nm STED resolution (E,Y)



Meet part of our team...

