Abberior Instruments -
STED microscopy from its inventors

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

Nobel Prize in Chemistry in 2014 for Stefan W. Hell

Work with the inventors of STED and RESOLFT microscopy
What can you expect from us – nobel performance

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 new dimensions.

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

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**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

Nikon Ti-E
STEDYCON - customer example

Zeiss Observer Z1
STEDYCON – Inverted example Olympus

Olympus IX83
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.
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.
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.
Schematic of the Abberior STED system with two pulsed STED laser @595 nm and @775 nm

STED

Exc

Dyes

595 pulsed

440

488

516

561

594

640

2-color:
GFP and YFP

2-color:
Abberior STAR600 and STAR635P
SiR-dyes

Pulsed STED lasers only (4-times less light needed compared to cw)

Add more lasers, e.g. 405 for DAPI
Confocal microscope system covering all relevant dyes and proteins

<table>
<thead>
<tr>
<th>Conf. lines</th>
<th>405</th>
<th>440</th>
<th>488</th>
<th>516</th>
<th>561</th>
<th>594</th>
<th>640</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection of Markers</td>
<td>DAPI</td>
<td>STAR440Sx</td>
<td>STAR488</td>
<td>STAR580</td>
<td>STAR600</td>
<td>STAR635</td>
<td>STAR635P</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Live-cell</th>
<th>Resolution</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESCue</td>
<td>&gt;30</td>
<td>+</td>
</tr>
<tr>
<td>DyMIN STED</td>
<td>25</td>
<td>++</td>
</tr>
<tr>
<td>MINFIELD</td>
<td>20</td>
<td>++</td>
</tr>
</tbody>
</table>

Conventional STED

Live-cell: ☹️
Resolution: 30-50
Signal: -
RESCue STED
DyMIN STED – 10x more signal for the precise analysis of structures


*Abberior Instruments GmbH
MINFIELD STED delivers STED resolutions < 20nm


*Abberior Instruments GmbH
It is important to optimize both the superresolution dyes as well as the microscope hardware.
Overview of Abberior markers

| STAR 405 | 400-440 | 510-530 | 590-610 | 730-760 |
| STAR 440SXP | 440-460 | 510-530 | 590-610 | 730-760 |
| STAR 470SXP | 450-490 | 510-530 | 590-610 | 730-760 |
| STAR 488 | 470-510 | 500-535 | 570-600 | 600-620 |
| STAR 512 | 500-540 | 550-590 | 600-620 | 740-770 |
| STAR 520SXP | 500-540 | 550-590 | 600-620 | 740-770 |
| STAR 580 | 550-590 | 600-620 | 700-780 | 770-780 |
| STAR 600 | 590-610 | 620-660 | 700-780 | 750-780 |
| STAR 635 | 620-650 | 700-780 | 750-780 | 750-780 |
| STAR 635P | 620-650 | 700-780 | 750-780 | 750-780 |
| STAR RED | 620-650 | 700-780 | 750-780 | 750-780 |


| FLIP 537 | 320-420 | 520-650 | 550-580 |
| FLIP 565 | 320-420 | 520-650 | 550-580 |

| RSFP | 356 | 500 | 515 |
| rsEGFP1 | 356 | 405 | 500 |
| rsEGFP2 | 356 | 405 | 500 |
| Dreiklang | 356 | 405 | 500 |

NHS, maleimide, Azide, phospholipids, biotin, streptavidin, anti-mouse, anti-rabbit, anti-rat, anti-chicken
Organic Dyes for STED microscopy

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

<table>
<thead>
<tr>
<th>STED pulsed</th>
<th>775 nm</th>
<th>595 nm</th>
</tr>
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<tbody>
<tr>
<td>Exc.</td>
<td>640nm</td>
<td>594/561nm</td>
</tr>
<tr>
<td></td>
<td>485nm</td>
<td>450nm</td>
</tr>
<tr>
<td>Nanoscope</td>
<td>E/Y</td>
<td>E/Y</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>E/Y*</td>
</tr>
</tbody>
</table>

Always recommended

- Abberior STAR RED
- Abberior STAR 635P
- Abberior STAR 635

Alexa 594
Oregon Green 488
Oregon Green 488

Sample dependent recommended

- Atto 647N
- Atto 633
- Alexa 637
- Cy5
- Abberior STAR 580
- Abberior STAR 488
- Abberior STAR 488
- Abberior STAR 470 SXP

- Atto 594
- Atto 530
- Cy3
- Alexa 488
- Abberior STAR 488
- Abberior STAR 488

- Atto 488
- FITC
- Atto 488
- FITC

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

Down to 25nm** resolution
## Recommended dyes to be used with the Abberior Instruments Expert Line & the STEDYCON

<table>
<thead>
<tr>
<th>STED pulsed</th>
<th>Exc.</th>
<th>Nanoscope</th>
<th>SiR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>640nm</td>
<td>E/Y</td>
<td>Abberior LIVE580</td>
</tr>
<tr>
<td></td>
<td>594/561nm</td>
<td>E/Y</td>
<td>Abberior LIVE510</td>
</tr>
<tr>
<td></td>
<td>485nm</td>
<td>E</td>
<td>Abberior LIVE515</td>
</tr>
<tr>
<td>775 nm</td>
<td>450nm</td>
<td>E/Y*</td>
<td>Abberior LIVE510</td>
</tr>
<tr>
<td>595 nm</td>
<td></td>
<td></td>
<td>Abberior LIVE515</td>
</tr>
</tbody>
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*STEDYCON with 450nm excitation for confocal imaging only
**only depending on the sample
Fluorescent Proteins for live-cell STED microscopy

- 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)

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

E: Expert line
Y: STEDYCON
Meet part of our team...