Sample Preparation & Labelling for STED Microscopy

Labelling Protocols

A good starting point is a protocol that you have confirmed works well for confocal microscopy, as it is likely to work well for STED microscopy too, as long as the dyes used are (replaced with) STED compatible dyes (see Table 1.)

When staining tissue, increase the incubation times typically used when staining cells (though actual times will vary depending on tissue type, thickness etc.).

For detailed protocols, please see https://abberior-instruments.com/knowledge/protocols/.

We also recommend the following methods article:
C. A. Wurm, D. Neumann, R. Schmidt, A. Egner, S. Jakobs (2009) 'Sample Preparation for STED Microscopy' Methods Mol. Biol. 591, 185–199.

Coverslips

We recommend using glass coverslips with a specified thickness of 170 um (#1.5 or #1.5H). Most objective lenses on Abberior systems are corrected for this thickness of glass.

Please do NOT use plastic coverslips or chambers with a plastic coverslip base. Plastic can cause aberrations and polarization changes in the STED beams which will compromise imaging quality.

We do NOT recommend using coverslips with grids, gratings or similar structures on the surface as these will interfere with the shapes of the excitation and depletion foci, which in turn will compromise the imaging resolution.

When growing cells, please do so on the coverslip surface (not on the slide surface) to minimize the distance between the cells and the objective lens.

Embedding Media

The following embedding media are recommended for 2D STED microscopy:

- ✓ Abberior Mount Solid (Abberior GmbH, Göttingen, Germany)
- ✓ Abberior Mount Solid Antifade (Abberior GmbH, Göttingen, Germany)
- ✓ Mowiol/DABCO
- ✓ Prolong Antifade Gold (Life Technologies Inc., Carlsbad, CA, USA)
- ✓ Prolong Antifade Diamond (Life Technologies Inc., Carlsbad, CA, USA)

The following embedding media are recommended for 2D and 3D STED microscopy:

- ✓ Abberior Mount Liquid (Abberior GmbH, Göttingen, Germany)
- ✓ Abberior Mount Liquid Antifade (Abberior GmbH, Göttingen, Germany)
- ✓ TDE (Abberior GmbH, Göttingen, Germany)

Please do NOT use Vectashield, Vectashield HardSET or any embedding medium that contains p-phenylenediamine as an antifade reagent.

When using ProLong Glass, please note that some orange dyes may become red-shifted, causing cross-talk with the red channel.

Please do NOT include DAPI, Hoechst, Propidium Iodide or Ethidium Bromide in your embedding medium, as these dyes may be excited by the STED laser, leading to high background and blurred images. If you need a nuclear counter stain, you may use **DAPI or HOESCHT in VERY LOW amounts**!

Dyes for STED Microscopy

See Table 1 below for some of our recommended dyes for STED Microscopy.

Please note that the 595 nm depletion line is not available on the STEDYCON and is a feature of the FACILITY and INFINITY STED systems only. However, all 3 systems offer confocal imaging in up to 4 channels.

Table 1.

775 Depletion	**Red dyes (640 nm excitation line)**	
	Abberior Dyes: **STAR RED**, **STAR 635P**, STAR 635 Alternatives: **ATTO647N**, ATTO633, *Alexa 647*, *Cy5*	Live-cell Organic Dyes: **Silicon Rhodamine (SiR)**, JF646, LIVE 610 Fluorescent Proteins: *mGarnet*
	Orange dyes (561 nm excitation line)	
	Abberior Dyes: **STAR ORANGE**, STAR 580 Alternatives: **AF594**, **ATTO594**, AF568, *Cy3*	Live-cell Organic Dyes: **ATTO590**, JF549, LIVE 560, LIVE 580, LIVE 590 Fluorescent Proteins: *mNeptune2*
595 Depletion	**Green dyes (485 nm excitation line)**	
	Abberior Dyes: **STAR GREEN**, STAR 488 Alternatives: Oregon Green, AF488, ATTO488	Live-cell Organic Dyes: LIVE 510 Fluorescent Proteins: mNeon Green, GFP, YFP etc.
Confocal	**Blue Dyes (405 nm excitation line)** DAPI, Hoescht (please use very low amounts!), AF405	

Legend

Highly recommended for STED

Increased photobleaching with STED

For STED imaging, please use organic dyes whenever possible. Organic dyes are superior to fluorescent proteins (FPs), especially with respect to brightness and photostability.

As an alternative to FPs in live-cell experiments, cell-permeable organic dyes (e.g. SiR or ATTO590) may be used in conjunction with self-labelling protein tags e.g. SNAP- and/or Halo-tag.

Choosing Colors

Single-color STED Samples

- ✓ For best results (especially with respect to resolution), choose a red organic dye e.g. Abberior STAR RED, Abberior STAR635P or ATTO647N
- ✓ Second best option is an orange dye e.g. Abberior STAR ORANGE or AF594

Two-color STED Samples

- ✓ Choose a red + orange combination as this is ideal for colocalization experiments and images can be acquired in line sequential mode
- ✓ Use the red dye to label the structure for which you require the highest resolution

Three-color STED Samples

- ✓ Red + orange + green is recommended
- ✓ Red and orange will give the best resolution, so use these for the structures you care most about resolving
- ✓ You may acquire the red and orange images in a single acquisition using line sequential mode
- ✓ Green must be acquired in a separate frame acquisition only AFTER you are done acquiring red and orange – the 595 depletion laser will bleach the red and orange dyes

Recommended Live-Cell Imaging Media

In general, imaging media for STED microscopy must be non-absorbing in the excitation channels and non-fluorescent in the detection channels.

For mammalian cells, HDMEM (Dulbecco's Modified Eagle's Medium) buffered with HEPES (Invitrogen, USA) or DMEMgfp-2 (Evrogen, Moscow, Russia) may be used.

For yeast cells, many Synthetic Complete media (without yeast extract and peptone) may be used.

Contact

If you have any further questions, please do not hesitate to contact us at info@abberior-instruments-america.com.