Recommendations for STED nanoscopy: 5 key points for sample preparation

1. As secondary antibodies (optimized for STED imaging), please use one or several shown in the list below:
[Antibodies surrounded in red are available at the Bioimaging, contact us for more information]

Secondary Antibodies	Alternative to	Excitation	Depletion
Alexa Fluor 488	ATTO488 or Dylight 488	@488 nm	@ 592 nm
Abberior STAR 488	ATTO488, AF488, Dylight 488	@488 nm	@ 592 nm
AlexaFluor 568, 555, 532		from 532 to 568 nm	@ 592 nm
Abberior STAR 580	ATTO590/594, AF 584/594	from 550 to 590 nm	@ 775 nm
Alexa Fluor 594	ATTO590/594, STAR 580	from 550 to 590 nm	@ 775 nm
Alexa Fluor 647	STAR RED, ATTO647N, STAR 635P, AF647, Cy5	diode lasers @635 or 650 or @647 with a Krypton laser	@ 775 nm
Abberior STAR 635P	STAR RED, ATTO647N, AF647, Cy5	diode lasers @635 or 650 or @647 with a Krypton laser	@ 775 nm
Abberior STAR RED	ATTO647N, AF647 or Cy5	diode lasers @635 or 650 or @647 with a Krypton laser	@ 775 nm

*** Examples of Dyes combinations: STAR580 +STAR635P (2 colors), AF488 + AF594 (2 colors), STAR580 + STAR RED + AF488 (3 colors), AF488 + AF594 + STAR RED (3 colors), etc....***

*** Good results have been reported with the following fluorescent proteins: **mTurquoise2, mTFP1, eGFP, EmGFP, eYFP, Venus, mCitrine, DsRed/ mRFP, mStrawberry, mCherry...*****

2. **Do not use "Blue dyes" such as DAPI, Hoechst or even Propidium ionide or ethidium Bromide for labeling or in embedding media.** These dyes may be excited by the STED laser and thereby might lead to high background and blurred images. As a good alternative, please use **PICOGREEN** *(available at the Bioimaging)*, **YOYO-3** or **TO-PRO-3** (*InVitrogen*) to label the nucleus.

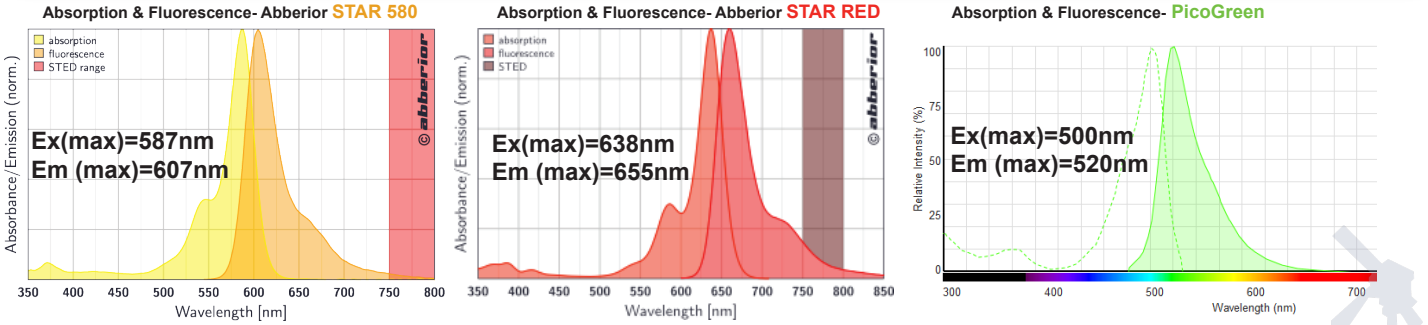
3. Use one of the following mounting medium: **Glycerol/ PBS with a fresh antifading (NPG or Dabco), Prolong Anfifade Gold** *(available at the Bioimaging)* **or Diamond, Fluoromount G, Mowiol, Abberior Liquid Antifade and Solid Antifade, Roti©-Mount FluorCare.**

*** Please do not use Vectashield, Vectashield Hard set or other embedding media containing p-phenylenediamine as antifading reagent.***

4. Choose the correct coverglass: use the **1.5 coverslips (0.170 +/- 0.01 mm thick, Hecht-Assistent, cat. Number: 1014/ 2424)** *(available at the Bioimaging)*.

5. **Seal the coverslips with nail polish**.

The Bioimaging core facility can provide you a kit - if necessary- which contains the following components:
- secondary Ab (anti-rat, anti-mouse or anti-rabbit): Abberior **STAR RED** and Abberior **STAR 580**
- **PicoGreen** stock solution
- Coverslips
- Aliquots of mounting medium (Prolong Antifade Gold)



More information about STED principle at :

* https://www.leica-microsystems.com/science-lab/sted-super-resolution-microscopy-nanoscopy-principles-and-photophysics/

* http://www.abberior.com/knowledge/microscopy-tutorials/sted/

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