

UConn STED: Fixed Sample Prep. Guide

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1. Prepare samples on #1.5 glass coverslips (0.17 mm thickness)

- Coverslips come in various thicknesses. For STED, it must be #1.5.
- The specimen holder fits standard glass slides, 35 mm glass bottom dishes, or chambered coverglass like LabTek II.

2. Label with recommended dyes

- STED requires bright, stable dyes with optimized photophysical properties.
- STAR RED is a far red probe and is the best performing dye.
- Preferred dye combinations for multi-channel imaging are in the table below.
- Do not label with DAPI or Hoechst. If you need DNA labeling talk to Chris.
- Confirm your labeling looks good with conventional confocal.

Preferred dye combinations for Abberior STED (see notes at the bottom)

Excitation/STED Depletion Lasers	Single Color	Dual Color	Triple Color
640 / 775 nm	STAR RED	STAR RED	STAR RED
561 / 775 nm		STAR ORANGE <u>OR</u> STAR 580 <u>OR</u> Alexa594	STAR ORANGE <u>OR</u> STAR 580 <u>OR</u> Alexa594
488 / 775 nm			Atto490LS <u>OR</u>
488 / 595 nm			STAR GREEN <u>OR</u> STAR 488 <u>OR</u> Alexa 488 <u>OR</u> Oregon Green
440 / 775 nm			STAR 460L

3. Mount sample in appropriate mounting medium

- Mount fixed samples using a high refractive index mounting medium with antifade.
- Position coverslip only near the center of the slide.
- Prolong Gold or Diamond are recommended.
- Do not use Vectashield or Prolong Glass.

- We have aliquots of commonly used Abberior STAR secondary antibodies available with a KFS #.
- Abberior recommends 1:100-1:200 dilutions of their antibodies
- A variety of Abberior STAR antibodies and reagents for STED can be purchased at Abberior.com.
- The facility also has coverslips, slides, and Prolong mounting medium available.