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.
   - Attach tissue sections to the coverslip, not the slide, for optimal imaging.

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.
   - Abberior recommends 1:100-1:200 dilution of their STAR dye secondary antibodies.
   - Confirm your labeling looks good with conventional confocal.

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

<table>
<thead>
<tr>
<th>Excitation/STED Depletion Lasers</th>
<th>Single Color</th>
<th>Dual Color</th>
<th>Triple Color</th>
<th>Four Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>640 / 775 nm</td>
<td>STAR RED</td>
<td>STAR RED</td>
<td>STAR RED</td>
<td>STAR RED</td>
</tr>
<tr>
<td>561 / 775 nm</td>
<td>STAR 580 OR Alexa594</td>
<td>STAR 580 OR Alexa594</td>
<td>STAR 580 OR Alexa594</td>
<td></td>
</tr>
<tr>
<td>485 / 595 nm</td>
<td>STAR 488 OR Alexa 488 OR Oregon Green</td>
<td>STAR 488 OR Alexa 488 OR Oregon Green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>440 / 595 nm</td>
<td></td>
<td></td>
<td></td>
<td>STAR 440 SXP</td>
</tr>
</tbody>
</table>

3. Mount sample in appropriate mounting medium
   - Mount fixed samples using a high refractive index mounting medium with antifade.
   - Position coverslip near the center of the slide so it lays flat on the stage.
   - Prolong is recommended.
   - Do not use Vectashield.

- We have aliquots of commonly used Abberior STAR secondary antibodies available with a KFS #.
- The facility also has coverslips, slides, and Prolong mounting medium available.
- A variety of Abberior STAR antibodies and reagents for STED can be purchased at Aberrior.com.
- Oregon Green and Alexa antibodies are available from ThermoFisher.