UConn STORM Sample Prep. Guide

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What you need for STORM imaging...

1. A fixed and immobile specimen
   • Imaging will take place over several minutes.
   • The sample cannot move during this time.

2. Samples prepared in a #1.5 glass bottom chamber or dish
   • Aqueous STORM buffer is added immediately before imaging.
   • Store labeled specimens in PBS at 4°.
   • Do not mount and/or seal coverslips on glass slides.
   • Use 35 mm glass bottom dishes, Lab-Tek chambered cover glass, or similar.

3. Labeling with the recommended dyes
   • STORM requires probes capable of photoswitching or “blinking” behavior.
   • For single channel experiments, use Alexa 647 or Cy5.
   • Add Alexa 568 as the second label for 2 channel experiments.
   • Do not label with DAPI or Hoechst.
   • Photoactivatable red proteins (PAmCherry, PAmKate) or green to red photoconvertible proteins (mEOS, Kaede) can be used for PALM

4. Optimized labeling
   • STORM requires the best immunofluorescence practices and usually involves fine tuning.
   • The goal is to get dense labeling of structures while minimizing background.
   • The tips that follow have been shown to improve results.
1. When possible, check performance of antibodies from different sources to find the cleanest labeling.

2. Optimize fixation (fixative concentration, permeabilization, etc.) to maximize structural preservation and antibody binding.

3. Minimize background signal levels by titrating primary antibody.

4. Block with heat-treated sterile filtered blocking serum.

5. Don’t skip washing steps and use 1% blocking serum to remove antibodies AT EVERY STEP.

6. Post-staining fixation is helpful if imaging will occur more than a couple days after labeling.

5. STORM Imaging Buffer
   - Volume depends on the size of the chamber. The imaging area must be fully immersed in buffer.
   - STORM buffer is available for purchase from the Facility using a KFS#.
   - Buffer is made fresh and added immediately before imaging.
   - When imaging fluorescent proteins (PALM) PBS is used instead.

<table>
<thead>
<tr>
<th>STORM Buffer # 1</th>
<th>STORM Buffer # 2</th>
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</thead>
<tbody>
<tr>
<td>10 mM Tris, pH 7.5</td>
<td>50 mM Tris, pH 8.0</td>
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<tr>
<td>10 mM NaCl</td>
<td>10 mM NaCl</td>
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<tr>
<td>10% D-glucose</td>
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<tr>
<td>10 mM Cysteamine (MEA)</td>
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<tr>
<td>50 mM Beta mercaptoethanol</td>
<td>40 ug/ml catalase</td>
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<tr>
<td>57 ug/ml catalase</td>
<td>0.5 mg/ml glucose oxidase</td>
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<tr>
<td>5 U/ml pyranose oxidase</td>
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<tr>
<td>2 mM Cyclooctatetraene (optional)</td>
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</tbody>
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6. Bead fiducials (optional)
   - Necessary for precise alignment of 2 channel acquisitions
   - Not needed for single channel acquisitions
   - Pre-coat coverslips using the following steps:
     1. Vortex a stock solution of 0.1 um Tetraspek beads (ThermoFisher)
     2. Dilute 1:2000 in distilled water and vigorously vortex
     3. Place 100 ul on the coverslip
     4. Air dry in the dark overnight