UConn STORM Sample Prep. Guide

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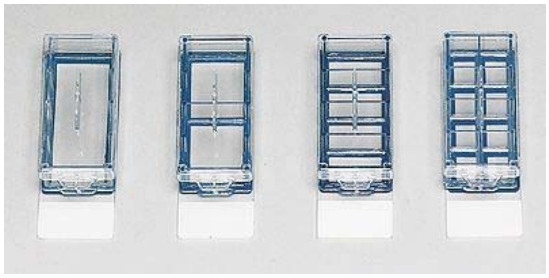
What you need for STORM imaging…

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.

STORM Buffer # 1	**STORM Buffer # 2**
10 mM Tris, pH 7.5	50 mM Tris, pH 8.0
10 mM NaCl	10 mM NaCl
10% D-glucose	10% D-glucose
10 mM Cysteamine (MEA)	100 mM Cysteamine (MEA)
50 mM Beta mercaptoethanol	40 ug/ml catalase
57 ug/ml catalase	0.5 mg/ml glucose oxidase
5 U/ml pyranose oxidase	
2 mM Cyclooctatetraene (optional)	

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