abbelight user guide

Protocol to image fixed cells with STORM nanoscopy

Material

- Coverslips n°1.5H (Marienfeld, precision cover glasses thickness No. 1.5H (tol. ± 5 µm), minimum size: 20*20 mm or 20 mm diameter, or equivalent)
- RBS 25 concentrate solution (Sigma, 83460).

Provided by abbelight during the demo:

- Cavity slides (BR475505 Sigma or equivalent)
- Sealing medium (Twinsil Rotec or equivalent)
- abbelight SMARTkit buffer

Sample preparation

- Wash coverslip (for example, RBS treatment: 2% RBS 25 in hot water (50-70°C) for 10 min with agitation - make sure coverslips do not overlap with each other -, followed by at least two washes with MilliQ water for 10 min with agitation).
- Seed cells on prewashed coverslips and perform biological experiment.
- Perform appropriate fixation. This step depends on the cell type, the structure of interest and the antibody, but the goal is to preserve the ultrastructure of the sample (for example, option 1: PFA 4% followed by neutralization step with glycine or ammonium chloride, option 2: PFA 4% with glutaraldehyde 0.2%, followed by neutralization step with $NaBH_4$).
- Perform staining following established protocol and using a fluorophore compatible with STORM nanoscopy (see abbelight leaflet for list of validated fluorophores. For 1 color, we recommend Alexa Fluor 647; for 2 colors: Alexa Fluor 647 and Alexa Fluor 555).
- Before moving on to STORM imaging protocol, check your samples with standard epifluorescence microscopy techniques (widefield, confocal…). If needed, optimize staining protocol to increase signal of interest and decrease background (for example: use specific antibodies, perform more stringent washes, add extraction step). Once the staining is optimized, we recommend to post-fix samples to immobilize antibodies (for example, PFA 4% followed by neutralization step with glycine or ammonium chloride).
- Keep your samples in PBS at 4°C in the dark.

On the day of the demo:

- Right before STORM imaging, prepare buffer using the abbelight SMARTkit:
  Step 1: add 10 µL of the green tube into the blue tube.
  Step 2: Add 6 µL of the red tube into the blue tube.
  Step 3: load 200 µL of this buffer on a cavity slide.
- Carefully mount your coverslip on the cavity slide avoiding bubbles in the buffer.

- Gently remove excess buffer with a kimtech wipe.
- Seal the coverslip with appropriate sealing medium. If using silicone glue (Rotec Twinsil or equivalent): mix blue and yellow medium 1:1 until it turns green and seal the coverslip. The sealing medium will solidify after a few minutes.
- The sample is ready for acquisition.