SpheroRuler

PROTOCOL

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1. Material you need

- The SpheroRuler suspension (7.10^8 particles/mL in PBS 1X, pH 7.4, 0.02% azide)
- A blinking buffer

2. Storage

- Keep the SpheroRuler suspension in the fridge (2-8°C) for 7 months.
- Keep it in the dark in its opaque bag.

Do not freeze.

3. SpheroRuler mounting

- Thaw the SpheroRuler suspension at room temperature.
- Sonicate for 1-2 minutes and vortex the SpheroRuler 5 minutes before using it – for homogeneous dispersion and to avoid aggregates.

Check that it is well resuspended: you should obtain an opalescent suspension of SpheroRuler after the sonication and vortex step.

- Add 5 µL of the resuspended SpheroRuler in a 22mm/500µL Willco dish.
- The SpheroRuler concentration can be adjusted according to your preferred bead density.
- Add the blinking buffer.
- Put the coverslip on the cavity filled with the blinking buffer without making any bubble.
- Apply the sealant.
- Wait for 10-15 minutes to let the SpheroRuler beads settle down.
- Proceed with image acquisition.

4. Image acquisition

- Find a field of view with isolated SpheroRuler beads.
- Do a gentle z-scan to focus on the larger diameter.
- Record reference images by standard epifluorescence and brightfield microscopy.
- Select a ROI and perform dSTORM acquisition.
- Apply dSTORM image processing to reconstruct the SpheroRuler beads images.

The SpheroRuler beads are coated with a high density of fluorophores. We recommend using a multi-emitter fitting approach, or any equivalent, to guarantee an efficient localization of individual blinking events.