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SpheroRuler

Questions & Answers

Q/ What are SpheroRuler beads made of?

The SpheroRuler beads are 1µm-diameter polymer particles surrounded by 647-fluorophores covalently anchored to their surface. Beads will be visible as hollow rings or spheres when reconstructed in 2D or 3D SMLM experiments respectively.

Q/ Which types of imaging is SpheroRuler compatible with?

SpheroRuler beads are coated with 647-fluorophores giving a stable blinking in SMLM micrsocopy, and have been initially developed for dSTORM imaging. Since then, they have also been successfully used in SFFR, SEM, confocal, Airyscan confocal and SEM microscopy.

Q/ How accurate is the SpheroRuler bead size?

The spherical particles making up Spheroruler beads have been selected based on very good monodispersity properties. The accuracy and reproducibility of the bead diameter have been characterized by SEM on 25 independent microspheres and showed a standard deviation of 1 +/- 0.05µm.

Q/ What is included in the SpheroRuler kit? Is there anything I need that is not provided?

The SpheroRuler kit contains a 50µL suspension of SpheroRuler beads. All you need to have on your side is some blinking buffer, coverslips and imaging vessels of your choice.

Q/ How many experiments can I carry out with one SpheroRuler kit?

One kit contains a 50µL suspension of SpheroRuler beads, allowing for 10 experiments when using the recommended 5µL volume in 35mm glass-bottom dishes.

Q/ Can I use it alongside my biological samples?

Yes, SpheroRuler beads are resuspended in PBS and can be loaded together with biological specimens (cells, tissue sections, etc).

Q/ For how long can I keep my SpheroRuler solution?

The SpheroRuler suspension is stable for at least 7 months when stored at 4°C.

Q/ Is there any specific reconstruction algorithm I should use?

SpheroRuler beads are highly fluorescent beads 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. Although the available options will vary on each imaging system used, examples of algorithms that were successfully used include the "account for overlap" function on the ZEN software, or the “high-density” mode on the Zeiss software and the freely available UNLOC software.

Q/ How should fluorophore thickness be taken into account in the diameter measurement?

Fluorophores are directly coated on the bead surface without linkers or antibodies, and their thickness is therefore negligible compared to the measured diameter. The apparent thickness of the fluorophore ring will depend on the resolution of your imaging system (i.e. around 160-200nm when measured in dSTORM). The external periphery of the beads should be taken into account when measuring diameter.

Q/ I am not retrieving a perfectly spherical shape when reconstructing the beads in 3D. Is it normal?

Although 2D reconstructions of SpheroRuler beads should be accurately circular, obtaining an elongated shape in z is a common artefact that will depend on the system used for imaging and for 3D reconstruction (biplane reconstruction, astigmatism, etc). The measured diameter in z and its distance from the actual 1µm can therefore be used as a robust indicator to evaluate the fidelity of the 3D reconstruction for a given system. As an example, measured z diameter was 1.3 µm when tested on a Vutara VXL system using biplane imaging.

A Q&A written in April 2023.