MULTIMODAL LOCALIZATION MICROSCOPY WITH EFFICIENT PHOTON COLLECTION

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A new imaging arrangement for localization based super-resolution microscopy was developed and tested. The proposed optical setup provides multimodalities (3D, polarization, spectral detection), and in the meantime overcomes the undesirable effect of lateral localization precision loss [1].

To achieve this, a secondary optical system was built atop an inverted microscope frame using an additional high NA oil immersion objective. The optical system was modelled and optimized using the OSLO optical design software. Image acquisition was done using a single Andor EMCCD camera. Our localization software, rainSTORM was used for data processing with additional custom tools for image registration, Z-calculation, polarization degree and 3D visualization. Measurements were taken using actin filaments in vitro, microtubules in Schneider 2 cells and sarcomer structures in Drosophila flight muscles (Figure 1) as samples.

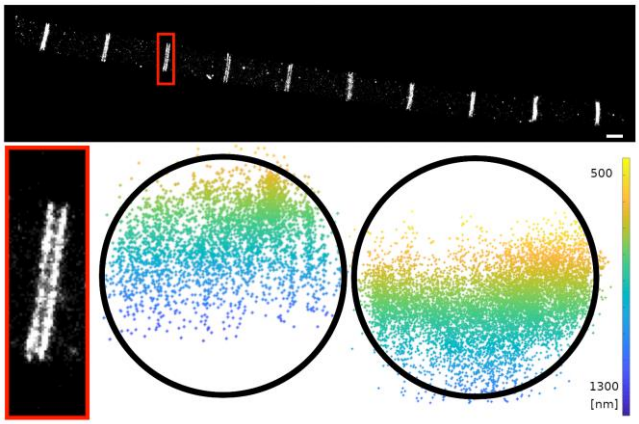

Figure 1: Tropomodulin labelled sarcomers were imaged using the asigmatic 3D modality implemented in our two objective setup.

We have found that using the proposed setup for volumetric imaging the lateral localization precision remains unchanged when using bi-plane or astigmatism modalities. The setup also works for fluorophore orientation measurements and for the simultaneous acquisition of two spectral channels. The optical setup does not require any modification of the detector path or the introduction of a second detector.

References:

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