Direct Supercritical Angle Localization Microscopy for Nanometer 3D Superresolution

Anindita Dasgupta[1,2,3], Joran Deschamps[1], Ulf Matti[1], Uwe Hübner[3], Jan Becker[3], Sebastian Strauss[4,5], Ralf Jungmann[4,5], Rainer Heintzmann[3,6,7], Jonas Ries[1*]

1. **Cell Biology and Biophysics, European Molecular Biology Laboratory, 69115 Heidelberg, Germany**
2. **Institute of Applied Optics and Biophysics, Friedrich-Schiller-University, 07743 Jena, Germany**
3. **Leibniz Institute of Photonic Technology, 07745 Jena, Germany**
4. **Faculty of Physics and Center for Nanoscience, Ludwig Maximilian University, 80799 Munich, Germany**
5. **Max Planck Institute of Biochemistry, 82152 Martinsried, Germany**
6. **Institute of Physical Chemistry, Friedrich-Schiller-University, 07743 Jena, Germany**
7. **Abbe Center of Photonics, Friedrich-Schiller-University, 07745 Jena, Germany**

email: anindita.dasgupta@uni-jena.de

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3D single molecule localization microscopy (SMLM) has emerged as a powerful method for structural cell biology, as it allows probing precise positions of proteins in cellular structures at a resolution of tens of nanometers in both 2D and 3D. Popular approaches to 3D imaging include PSF engineering to encode the position of single-emitters in its shape. However, such methods lead to worse localization precision axially than laterally. Complex localization microscopy techniques, such as iPALM, can provide isotropic resolution at the cost of a complex instrument. Supercritical angle fluorescence strongly depends on the z-position of the fluorophore and can be used for axial localization in a method called supercritical angle localization microscopy (SALM) [1-3]. Here, we realize the full potential of SALM by directly splitting supercritical and undercritical emission, using an ultra-high NA objective, and applying new fitting routines to extract precise intensities of single emitters, resulting in a several fold improved z-resolution compared to the state of the art [4]. We demonstrate nanometer isotropic localization precision on DNA origami structures, and on clathrin coated vesicles and microtubules in cells, illustrating the potential of SALM for cell biology.

References

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