Karlsruhe Institute of Technology

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# Speaker Abstracts Karlsruhe Days of Optics & Photonics


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### Towards structural cell biology using superresolution microscopy

Recent advances in superresolution microscopy now allow us to address structural questions in cell biology with optical methods. A quantitative interpretation however is often limited by sub-optimal performance and calibration of the microscope, undetermined performance of the fluorescence label and imaging conditions, unknown labeling efficiencies and systematic errors in counting protein numbers. To overcome these limitations, we developed reference standards based on the precise 3D arrangement and stoichiometry of proteins in the nuclear pore complex. We demonstrate their use as a) simple and robust resolution standards for calibration and quality control, b) accurate assays to quantify absolute labeling efficiencies in superresolution microscopy and c) precise counting reference standards for absolute stoichiometry measurements.

In the second part of this talk I will show how superresolution microscopy can be used to gain mechanistic insights into the structural organization of a complex protein machine, namely the machinery involved in clathrin-mediated endocytosis. We developed a high-throughput superresolution microscope to reconstruct the nanoscale structural organization of 23 endocytic proteins from over 100,000 endocytic sites in yeast. This allowed us to visualize where individual proteins are localized within the machinery throughout the endocytic process and resulted in a model of how the force is produced to pull in the membrane and form a vesicle.