NUCLEAR PORES AS VERSATILE REFERENCE STANDARDS FOR QUANTITATIVE MICROSCOPY

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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.
Here we show that the use of reference standards can overcome these limitations and greatly improve quantitative microscopy. To this end we exploit the precise 3D arrangement and stoichiometry of proteins in the nuclear pore complex.
We present a set of genome edited cell lines in which we endogenously labeled the nucleoporin Nup96 with eGFP, SNAP- or HALO-Tag or the photoconvertible fluorescent protein mMaple. 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.
As a resource shared with the community, these cell lines will enable many groups to assess the quality of their microscopes and labels and to perform quantitative, absolute measurements.

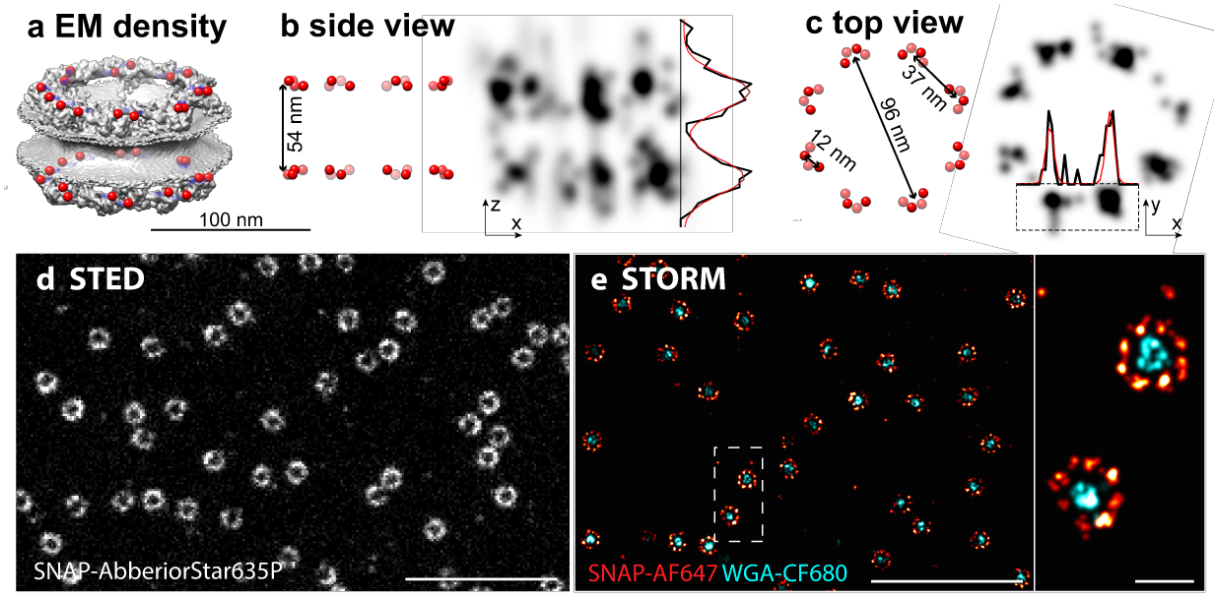

Figure: a: EM density and c-termini of Nup96. b: Side view schematic, STORM image of one nuclear pore and z-profile. c: Top view schematic and STORM image of one nuclear pore with x-profile. d: STED image. e: Dual-color STORM image.