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High-Throughput Super-Resolution Microscopy for Reconstructing Molecular Architecture

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Super-resolution microscopy is an important tool because it enables imaging beyond the diffraction limit, while maintaining the specificity and live-cell possibilities of fluorescence microscopy. Yet, many important biological questions remain out of reach due to challenges in acquiring and analyzing statistically significant datasets. Previously, we created high-throughput photoactivated localization microscopy (PALM) by building an automated microscope to image hundreds of bacteria cells, live, 3D, and across cell cycle. To complement this, we created a uniform illumination scheme to enable large field of view PALM/STORM, and now PAINT and iSIM images. We achieved this by engineering a light shaping module inspired by the Koehler Integrator. This required different engineering principles to be applied to wide-field microscopy (as for PALM or STORM) compared with multi-point scanning confocal microscopy (iSIM). For PAINT, we needed to introduce an evanescent field to illuminate only molecules near the coverslip. We achieved this by developing waveguides with robust coupling and even propagation, resulting in millimeter-scale, highly uniform total internal reflection fluorescence.

We demonstrate the power of this approach for studying large, multi-protein complexes within cells, using the centriole as a case study. To study the organization of such complexes, particle-based analysis has proven to be powerful, but has been limited so far by difficulties in generating large multi-color particle libraries, as well as the complexity of orientational alignment. We have addressed both challenges and, as a result, present a novel framework for deciphering the 3D organization of protein complexes composed of multiple components. This allows us to assemble multi-color, 3D volumetric reconstructions of centriolar proteins as well as post-translational modifications to the tubulin scaffold around which proteins assemble. Our future outlook includes going beyond automation and high-throughput, to develop microscopes that incorporate computer vision to adapt acquisitions to sample dynamics.

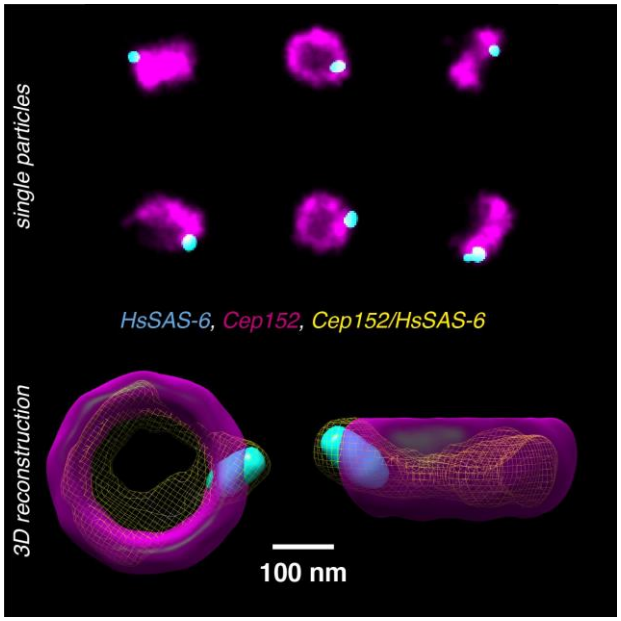

Figure 1. Individual two-dimensional STORM images of centrioles (top) and volumetric reconstructions based on computational inference from many projections (bottom) of centriolar proteins, showing the early procentriole marker HsSAS-6 (cyan) and the proximal toroidal protein Cep152 (magenta).

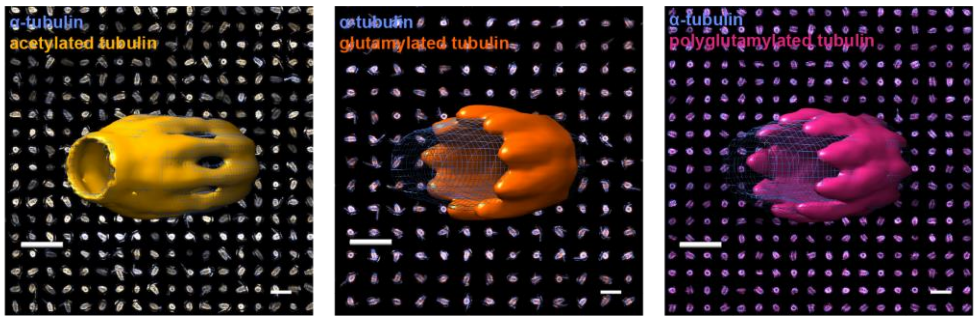

Figure 2. Individual three-dimensional iSIM images (background montage) are aligned to create volumetric reconstructions of tubulin post-translational modifications in the centriole. All scale bars are 100 nm.

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