IMAGING CELLULAR ORGANELLES USING PHOTOSWITCHING-BASED SUPERRESOLUTION MICROSCOPY

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Fluorescence microscopy has proven to be one of the most significant tools in the life sciences, owing to its high specificity and high modularity. However, the resolution of conventional fluorescence microscopy is limited by diffraction to about half the wavelength used. This is too large to resolve certain organelles, such as the centrosome, which is both the main organizing center of the microtubule network and a regulator of the cell cycle.

We use a combination of super-resolution techniques (PALM, STORM, dSTORM, GSDIM) that rely on photoswitching/photobleaching cycles of a subset of fluorophores on a continuously running camera, followed by the fitting of their positions with sub-diffraction resolution to investigate the structure of cellular organelles, with particular attention to the centrosome. Different modalities are used to take advantage of the properties (emission/absorption spectrum, number of photons...) of the existing dyes in the context of multidimensional imaging.

Figure1. Diffraction limited image (left, scale bar=2µm) and zoomed-in STORM image (right, scale bar=200nm) of a U2OS cell stained with a Cy3-Cy5 pair using a SNAP-tag