ENTROPY-BASED SUPER-RESOLUTION IMAGING (ESI): USING DISORDER TO CREATE FINE DETAIL

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We introduce a novel iterative method for fast optical imaging with a spatial resolution below the diffraction limit, based on reconstructing super-resolved images from conventional image sequences containing signal fluctuations inspired by super-resolution optical fluctuation imaging (SOFI) [1]. Such sequences could be obtained from either single-molecule blinking experiments or rapid imaging sequences with fluorophores undergoing random signal fluctuations. By calculating entropy and cross-entropy values pixel-by-pixel, weighted with higher order-statistics, new pixel intensities representing the information content in a time series are obtained. We show that analyzing image sequences by this formalism enables the reconstruction of super-resolved images, e.g. the isolation of single particles and their subsequent localization with a precision of <20nm. We find that the acquisition of <100 frames per sequence is sufficient to reconstruct super-resolved images of entire cells. We demonstrate that not only on-off switching of the fluorescent dyes, but also other dynamic events, i.e. photobleaching, can be exploited for efficient and high-resolution reconstructions.

Figure 1: Super-resolution imaging by ESI reconstruction. (A) conventional image of an immunostained U2OS cell after acquisition of a 1000-frame image sequence at 18ms/frame. (B) 2nd-order ESI reconstruction at 10 original frames per ESI-frame resulting in a 100-frame ESI-stack. The white region of interest (ROI) shown in the upper left corner of (A) and (B) highlights an area which nicely demonstrates the improved spatial resolution. The cross-section along the dotted line is depicted in (i) and (ii) corresponding to the lines in (A) and (B). Within the first 1000 nm the original two peaks indicating tubulin filaments (A-i) break up into three peaks (B-ii). The distance of these two separated filaments is determined to be 260 nm, which is just below the theoretical diffraction limit of ~270 nm. The standard deviations of the peaks quantify the apparent resolution showing a tremendously increased degree of detail.