IMAGING ORIENTATIONAL PROPERTIES OF ACTIN FILAMENTS BY SUPER-RESOLVED POLARIZED MICROSCOPY

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We present a polarized microscopy technique that allows the measurement of orientation properties of single fluorescent molecules, as well as their localization with tens of nm precision. Orientation properties encompass both mean orientation and orientational fluctuations extent (wobbling) averaged over the time scale of the image recording (30-100ms). These characteristics are not perceptible in single molecule super-resolution imaging techniques such as PALM or STORM, which rely on pure position localization. The measurement of orientational behaviour of single fluorescent probes in addition to their localization enriches these techniques, since providing that the fluorophores are rigidly linked to the proteins of interest, they can report protein organization at the nanoscale. In particular, the local conformational properties of proteins can be addressed with the orientation information, while wobbling can probe local constraints due to charges, viscosity and steric effects.

Our approach is based on polarization splitting of the imaging plane, which provides high precision determination of 2D in-plane orientation, wobbling, and 2D positions of single molecules. We have developed an inverse problem approach to retrieve the full orientation and wobbling information in a fast and robust way, from the intensity of the single molecules in four split polarized images.

The intrinsic off-plane orientation tilt of molecules can introduce a bias in the estimation of their wobbling. We have estimated this bias from simulations and propose a solution to reduce it, using a lower detection NA.

We have used this technique to report and classify the orientational behaviour of different fluorescent probes in *in vitro* reconstructed single F-actin filaments labelled with phalloidin conjugates. This study shows the importance of the probe structure in its orientational behaviour and permits now to evaluate the structural properties of F-actin in complex actin structures and networks in cells.

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