Photon efficient orientation estimation using polarization modulation in single-molecule localization microscopy

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Single-molecule localization microscopy (SMLM) is an established super-resolution microscopy technique for providing high spatial resolution (20-40 nm). A next step is to extract more information from each molecule than just its position. The orientation of label molecules in cases of restricted or absent rotational diffusion provides unique insights into a diverse set of biological structures [1]. Here, we report on a new technique for measuring the orientation of single-molecules that extends conventional SMLM. Our method combines the standard spot centroid estimation with polarization modulation induced photon count variation to extract orientation information. Previous work was restricted to measuring the azimuth angle in the focal plane with an empirically found precision of about 5.8° [2]. We extend this work to molecular orientations over the full 4π solid-angle and analyze its precision limit by means of a Cramér-Rao lower bound (CRLB) analysis.

Our method is based on modulating the polarization of the illumination over a sequence of recorded camera frames. This approach is inspired by our recent work on modulating illumination patterns to improve the localization precision with a factor of two [3]. We show that typically 5 different illumination polarization states, using a series of obliquely incident plane waves near the critical angle, lead to good results. The method reaches the CRLB in both azimuth and polar angles for a wide range of photon counts, indicating azimuthal and polar precisions of 2.5° and 1.5° at 1000 total signal photons and 10 background photons/pixel.

In the presentation, we will explain the method in detail, characterize its capabilities through simulations, and describe its potential in probing the conformation of individual DNA strands with high precision, even in low light conditions.

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