3D tracking of single molecules using spatial light modulation

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Application Note

Introduction

Single-molecule fluorescence spectroscopy and microscopy (SMFSM) is a powerful method which can detect dynamics with local resolution close to the molecular scale without averaging or the need for models to extract data. Thus, SMFSM yields valuable information on heterogeneities which together with other techniques and simulations will contribute to an increased understanding of dynamics in polymers. Several optical SMFSM investigations have already been performed to study rotational and translational motion of dye molecules in polymers and glassy liquids. The use of highly fluorescent and photo-stable dyes at low concentrations allowed us to position single molecules with lateral accuracy in the 10 nm range [1], far below the diffraction limit of optical microscopy.

Experiment

After having established optimized 2D positioning and tracking methods, our goal was to achieve good localization in all three dimensions. Since the point spread function (PSF) is symmetric relative to the focus, introducing axial asymmetry is a suitable way to gain information about the z-position. We reached this goal by optical engineering of the PSF using a spatial light modulator (SLM). We implemented different phase masks to introduce an astig-matism [2] and a double-helix PSF [3, 4]. To calibrate our setup concerning the axial position, we investigated fluorescent nanoparticles of different size (20 nm, 100 nm, 200 nm). These nanoparticles have the advantage of being bright and photostable, thus allowing the collection of a lot of photons to yield low noise calibration patterns which are presented in Fig. 1a. It demonstrates the rotation of the double helix point spread function and the change of signal lengths in the two lateral directions, respectively, for different axial positions which were set with a piezotable.

We used the an EMCCD camera (iXon Ultra DU897 UCS-BV from Andor Technology) as a detector. The excellent sensitivity of the iXon Ultra not only allows for the detection of immobilized single emitters, but also for the tracking of single emitters in viscous media (see Fig. 1b).

References

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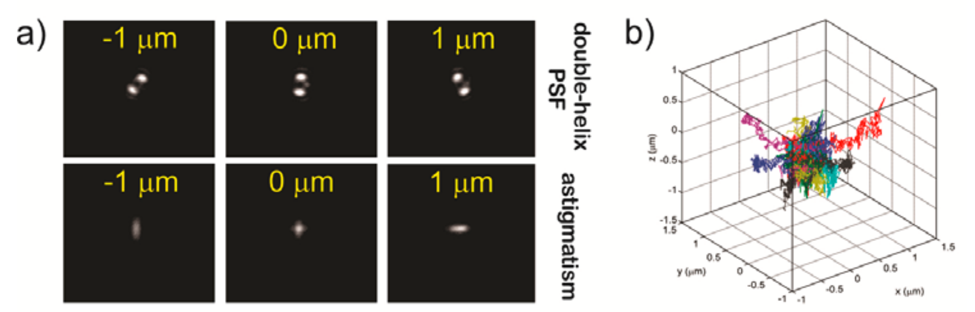

Figure 1. (a) Different types of PSFs. (b) 3D-tracking of perylenediimide molecules in a polyethylacrylate film.