

DETERMINATION OF THE THREE DIMENSIONAL ORIENTATION OF SINGLE MOLECULES

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Single molecule detection and imaging techniques have become important in recent years for studying dynamic processes such as chemical reactions and molecular motions, since information is not lost by the ensemble averaging typical of more traditional methods. Of particular interest is the determination of the orientation of the emission dipole of single molecules since it can be used as a means to label biological structures and track their conformational changes and motions. Furthermore photophysical parameters of fluorophores, such as fluorescence lifetime, can depend on the molecule's orientation, a fact which can be used to study the molecule itself or its environment.

Many current techniques for determining the orientation of a dipole exist that are limited to finding the transverse angle, however uncertainty over the longitudinal dipole component can lead to large errors. Methods based on structured illumination, image fitting and total internal reflection e.g. [1, 2] can find the full three-dimensional orientation although they are often restricted to specific circumstances, subject to a poor signal-to-noise ratio (SNR) and are not suitable for real time measurements.

We present a novel technique [3] employing a π phase step in the back focal plane of the collector lens (see Figure 1), breaking the inherent symmetry (see Figure 2), and hence allowing the longitudinal dipole component to be measured. We then go on to consider the experimental tolerances associated with the setup including misalignments and the finite width of the molecule's emission spectrum.

- [1] B. Sick, B. Hecht and L. Novotny, Phys. Rev. Lett. **85**, 4482 (2000).
- [2] D. Patra, I. Gregor and J. Enderlein, J. Phys. Chem. A **108** 6836 (2004).
- [3] M. R. Foreman, C. Macías Romero, and P. Török, Opt. Lett. **33**, 1020 (2008).

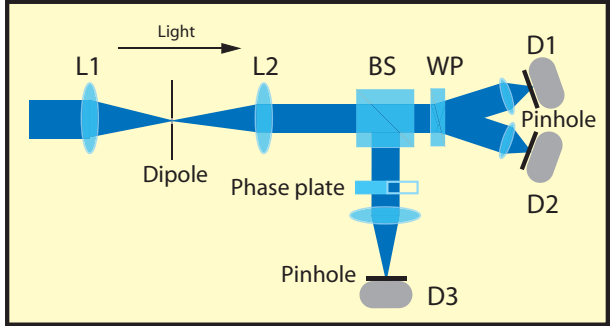


Figure 1: Proposed optical setup for determination of the full three-dimensional orientation of an electric dipole.

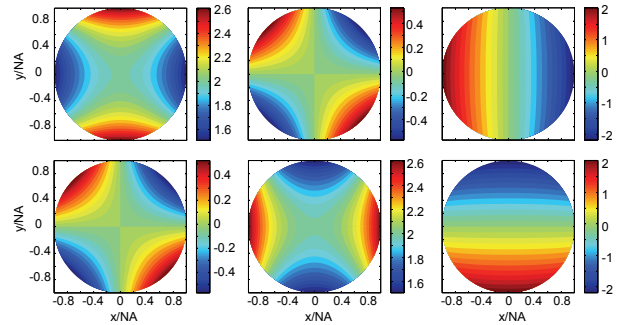


Figure 2: Field distributions for E_x (top) and E_y (bottom) in the back focal plane of a collecting lens ($NA = 0.966$) arising from orthogonal electric dipoles orientated along the x , y and z coordinate axes respectively (left to right).