DETERMINATION OF THE RANDOM ORIENTATION OF AN ELECTRIC DIPOLE FOR SINGLE MOLECULE DETECTION

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Single molecule detection has become an important technique to study dynamical processes such as bio-molecular motions and chemical reactions at a fundamental level. These techniques often employ fixed-site fluorescent probe molecules which, within the framework of classical electrodynamics, can be considered as ideal electric dipole emitters. The accuracy of this approach is however limited by the rapid orientational motion (or wobble) that the probes can typically exhibit [1]. Characterisation of the dipole orientation then becomes an important consideration for meaningful results.

Here we consider the determination of the orientation of an electric dipole using a high numerical aperture confocal microscope. We have further included random variations in the orientation of the dipole. Using exact vector wave optics we find an expression for the field on the image plane of the microscope [2] from which we calculate the Stokes vector. For an infocus transverse dipole with azimuthal “wobble” the Stokes parameters reduce to:

\[ S_0 = k^2 p^2 (|K_0|^2 + |K_2|^2 + (K_0 K_2^* + K_2 K_0^*) \cos 2\phi) \]

\[ S_1 = k^2 p^2 ((K_0 K_2^* + K_2 K_0^*) \cos 2(\phi - \chi - \psi) + |K_0|^2 \cos 2(\chi + \psi) + |K_2|^2 \cos 2(2\phi - \chi - \psi)) \]

\[ S_2 = k^2 p^2 \cos \left[ \arg \left( \frac{K_2 \sin(2\phi - \chi - \psi) - K_0 \sin(\chi + \psi)}{K_2 \cos(2\phi - \chi - \psi) + K_0 \cos(\chi + \psi)} \right) \right] \times |(-2K_0 K_2 \sin(2(\phi - \chi - \psi) + K_0^2 \sin(2(\chi - \psi) - K_0^2 \sin(2(2\phi - \chi - \psi)))| \]

\[ S_3 = k^2 p^2 \sin \left[ \arg \left( \frac{K_2 \sin(2\phi - \chi - \psi) - K_0 \sin(\chi + \psi)}{K_2 \cos(2\phi - \chi - \psi) + K_0 \cos(\chi + \psi)} \right) \right] \times |(-2K_0 K_2 \sin(2(\phi - \chi - \psi) + K_0^2 \sin(2(\chi - \psi) - K_0^2 \sin(2(2\phi - \chi - \psi)))| \]

where \( \phi, \chi \) and \( \psi \) are the azimuthal angle on the detector plane, mean dipole orientation and “wobble” angle respectively, \( k \) is the wavevector, \( p \) is the magnitude of the dipole moment and the \( K \) terms are as defined in [2]. We further find the probability density function for each Stokes parameter. Knowledge of this function allows us to then apply statistical signal processing techniques as a means to infer the mean dipole angle \( \chi \).