Fundamental limits in determining the orientation of single molecules

An information theoretic approach

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***Abstract*—Polarisation of light affords the means to perform robust orientational measurements of single molecules and sub-wavelength asymmetric scatterers. The precision with which such measurements can be made can be quantified using Fisher information and the Cramér-Rao lower bound. Specifically, a fundamental limit of 0.5/*N* radians (on average) is found where *N* is the number of detected photons. Measurement precision is shown to be lost in many realistic measurement scenarios, particularly for inference from null readings. The severity of these precision losses is however also shown to decrease as redundancy in the measurement is increased. Extraneous sources, such as a second fluorescing molecule, can contaminate measured data subsequently causing a further loss of precision. Results are hence also presented in this vein.**

Keywords-polarisation; single molecule; orientation; Cramér-Rao bound

I. INTRODUCTION

Single molecule localization techniques such as photoactivated localisation microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) have revolutionised microscopy in recent years. These methodologies have in turn lead to advances in, for example, study of cell dynamics and super-resolution microscopy. Measurement of the orientation of single molecules arguably promises equally useful gains, since this additional degree of freedom contains further information about the molecule, its micro-environment and history. Orientational measurements have for instance already allowed conformational changes and structure-function relationships of living cells and proteins to be examined by tracking fixed fluorescent tags [1-3]. Angular dependence of physical properties of single molecules, such as state transition probabilities can furthermore provide useful information about the photophysics of molecular sources [4].

To date optical measurements of molecule orientation have been realized using techniques based on structured illumination, defocused image fitting and total internal reflection microscopy [5-7]. These modalities can however exhibit low precision and restrictive experimental conditions. Directionality inherent in the polarisation state of light fortunately provides an alternative means by which robust orientation measurements can be made. Accordingly a number of super-resolution microscopy techniques using polarisation have been pursued and reported in the literature [8-10].

Benchmarking competing methodologies however plays an important role in experimental design and data analysis. Within the context of orientation studies however, conventional performance metrics such as signal to noise ratio and resolution are less appropriate, since they refer to intensity measurements. This work therefore aims to present a suitable formalism, derived from information theoretic principles, for describing precision limits in polarisation based measurements of single molecule orientation [11,12].

II. POLARISATION BASED ORIENTATIONAL MEASUREMENTS

Emission from a single molecule can be modeled as an electric dipole radiation pattern. In microscopy however the emitted field must practically be collected and imaged onto a detector, as can be achieved using a simple 4f lens arrangement. To determine the orientation of the source molecule the polarisation of the imaged field must then be measured. In this work restriction is made to division of amplitude polarimeters (DOAPs), in which light is split and each resulting beam simultaneously analysed, since they are suitable for dynamic studies and benefit from high light efficiencies. A basic DOAP considered in this work can be realized using a beam splitter, two polarisers (at arbitrary albeit distinct angles) and two detectors. Three additional DOAPs commonly found in the literature are also considered. A full description of each setup can be found in [13-15] (referred to as the Azzam, Compain and Lara DOAPs respectively), however schematics are shown in Figure 1.

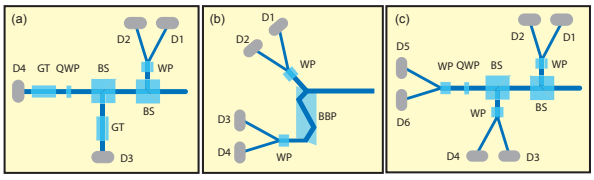


Figure 1. Schematics of three common polarimeter designs. Notation is as follows: BS - beam splitter, WP - Wollaston prism, QWP - quarter wave plate, GT - Glan Thompson polariser, BBP - broadband prism and D - detector

Analytical results for the input field can be found using vectorial ray tracing [12], and takes the form

$$\mathbf{E}_2(\boldsymbol{\rho}_2) = p_0 \begin{pmatrix} (K_0^A + K_2^A \cos 2\varphi_2)\cos\gamma + K_2^A \sin 2\varphi_2 \sin\gamma \\ K_2^A \sin 2\varphi_2 \cos\gamma + (K_0^A - K_2^A \cos 2\varphi_2)\sin\gamma \\ -2iK_1^B(\cos\varphi_2 \cos\gamma + \sin\varphi_2 \sin\gamma) \end{pmatrix}.$$

where φ_2 is the polar angle of the point in the image plane being analysed and the *K* factors are integrals dependent on the radial coordinate of the position being analysed and are given in [12]. Knowledge of this field allows an analysis of the measurement precision achievable as discussed below.

III. Limits on Orientational Measurements

Pivotal within the domain of statistical estimation was the formulation of a lower bound on the variance, K_γ, of *any* estimate that may be made of a parameter of interest (in this case molecule orientation γ) known as the Cramér-Rao lower bound (CRLB). Specifically this limit takes the form of the inequality $K_\gamma > J_\gamma$, where J_γ is the Fisher information and is derived from the noise statistics. Poisson noise is particularly prevalent in single molecule studies due to the low light intensities involved and is henceforth assumed. For a two polarizer DOAP in this noise regime it can be shown that when an on-axis in-focus dipole is considered the Fisher information for estimation of its orientation is given by,

$$J_\gamma = 2N \frac{\sin^2(\vartheta_1 - \vartheta_2)}{\cos^2(\gamma - \vartheta_1) + \cos^2(\gamma - \vartheta_2)}$$

where ϑ_1 and ϑ_2 are the orientations of the two analyzing polarisers and *N* is the total number of detected photons. Assuming the total intensity is known *a priori* gives

$$J_\gamma = 2N\left[\sin^2(\gamma - \vartheta_1) + \sin^2(\gamma - \vartheta_2)\right]$$

When the polarisers are crossed these expressions reduce to $0.5/N$. Figure 2(a) shows this result in conjunction with the equivalent results obtained when the DOAP architectures in Figure 1 are used.

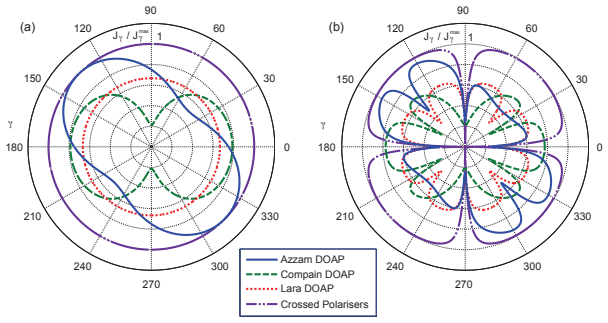


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Work funded by the European Union and the Engineering and Physical Sciences Research Council (UK).

The improved and orientation independent performance (for zero background) of the crossed polarizer arrangement is evident. This behaviour arises since the polarimeters of Figure 1 measure the ellipticity, in addition to the azimuthal angle of a general state of polarization. When a constant background count is present (see Figure 2(b)) significant losses in Fisher information are seen corresponding to a loss of measurement precision. It can be further shown that these dips arise at critical angles corresponding to null readings on one (or more) detector and originate from the Poisson nature of the noise. For a crossed polarizer arrangement the information loss is complete, however in more complicated DOAP architectures, in which a redundancy in polarisation space exists, non-zero Fisher information can be obtained, even at the critical dipole orientations. Further redundancy can be introduced by scanning the image field i.e. analyse the polarisation at multiple positions.

If multiple molecules are present in a detection volume localisation techniques are known to suffer large errors. Precision losses in orientational measurements can also be investigated by introducing a second dipole in the object plane of the imaging system. If incoherently radiating with respect to the first, this second molecule acts as a background source hence inducing information dips as found in the single molecule case. Although similar effects can be seen for a second coherently radiating dipole, the extent of precision losses becomes dependent on the relative configuration of the two dipoles producing a more complex behaviour. In essence however, information losses are again seen to occur when inference is based on null readings due to their susceptibility to perturbations. If estimation of the molecular orientation is based on an image (formed, for example, by use of a scanning microscope), a reduction in the complexity of the dipole crosstalk attributes is seen, partly due to the averaging introduced in such measurements, but also due to the larger number of measurements taken.

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