Which way is up? Nanophotonic calibration artefacts for accurate molecular orientation measurements

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Polarimetry – the analysis of the polarization components of an optical field – is a powerful tool capable of determining quantities ranging from the state of stress in a material to the relative orientations of molecules in a biomolecular complex. While the principles of optical design for polarimetry are well-understood, care and attention to detail are necessary to achieving high-quality measurements from which sound conclusions can be drawn.

Nowhere is this more true than in single-molecule fluorescence imaging, where we need to extract the maximum amount of information from a dim, sub-diffraction limit source. A super-resolution optical microscope comprises a complex train of optical components such as mirrors, beam splitters, dichroic filters, phase plates, and lenses. Each of these components will modify the state of polarization of light interacting with it, changing the relative amplitudes and/or phase relationships between the basis set of *s* and *p* polarizations that characterize the incident light. At the end of the optical train, the polarization state of the light reaching the detector may have been dramatically altered in a way that is difficult to detect, potentially leading to biased data and erroneous conclusions.

To address these challenges, we introduce a simple nanophotonic artefact that consists of a series of nanoscale slits, oriented at different angles to cover a 180° range. These slits are ion-beam milled into a thin gold film. When illuminated, each slit produces an emission pattern that closely matches the dipole emission of a single fluorophore, which is strongly polarized. Unlike single fluorophores, they are stable and of known orientation, enabling systematic analysis of the effect of each optical component in a microscope through both the illumination and imaging systems. With this information in hand, there are three options to mitigate polarization distortion effects component: 1] substituting a problematic component with a different one, 2] compensating for the effect of a component by adding another, and 3] calibration and correction when the first two options are not possible.

Here, we show how our artefact and methodology enable us to understand an eliminate bias in the measurement of single-molecule in-plane orientation measurement. We will discuss strategies to fabricate artefacts that will allow the measurement and correction of out-of-plane measurements.

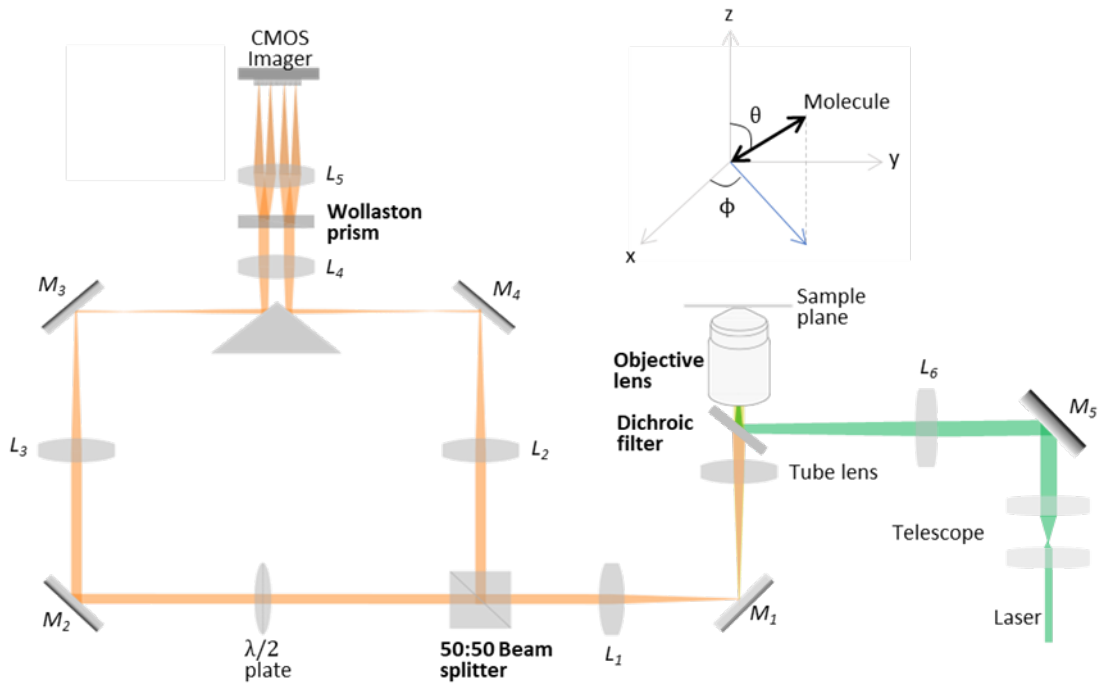

Fig. 1. A single-molecule polarimetry microscope system. The light emitted from a molecule is divided into two pairs of orthogonal polarizations. The relative intensities in the four channels can be used to determine the orientation of a molecule.

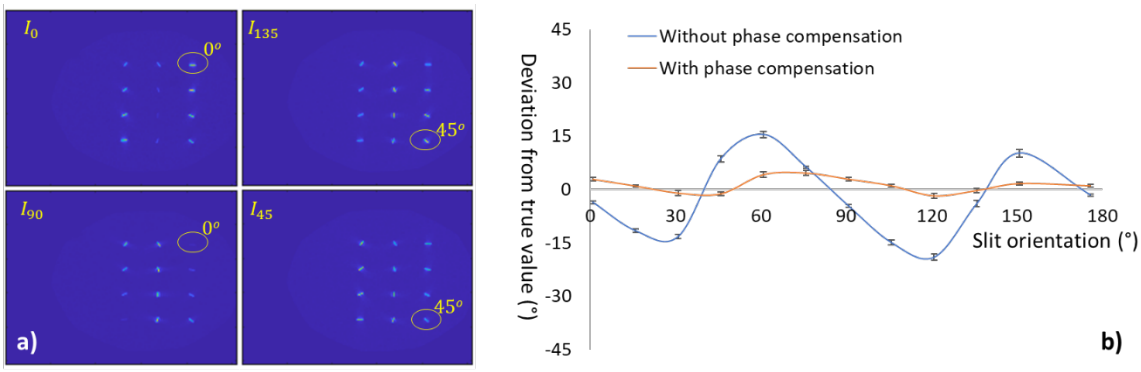

Fig. 2. a) Images of nanoslit arrays through each of the four polarization channels. As expected, the 0° slit shows bright and dark in the orthogonal 0° and 90° channels, respectively. However, the 45° slit shows up in *both* the 45° and 135° channels, indicating a polarization distortion. b) The bias in measured orientation is dramatically reduced following polarization compensation of a dichroic mirror using a matched dichroic mirror to correct for an induced phase error.

50-word summary: The polarization state of an optical signal can be altered by its passage through an imaging system, leading to biases in the detected signal. To measure these biases, identify their origin, and correct them, we introduce and demonstrate a nanophotonic calibration artefact and associated methodology.