Title : Characterisation and calibration of a 3D super-resolution microscopy system

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Abstract

Nanometre-scale molecules such as proteins or DNA assemble in biological structures responsible for every function of life. Conventional light microscopy is limited by the diffraction barrier around 250 nm as described by Ernst Abbe in 1873. Abbe's limit remained unbroken until the end of the twentieth century. E. Betzig, W. E. Moerner and S. Hell were awarded the Nobel Prize in chemistry in 2014 for the development of the techniques that overcome Abbe's limit and for developing this new field now known as super-resolution microscopy or nanoscopy.

Our work concentrates on dSTORM (direct STochastic Optical Reconstruction Microscopy), a single molecule localisation microscopy (SMLM) technique, that consists of the photoswitching of fluorescent molecules and their localisation with nanometre precision. dSTORM also relies on labelling techniques, chemical buffer conditions and data analysis.

A reliable and well-understood microscope system is paramount to achieve the best increase of resolution permitted by dSTORM, down to a few nanometres. The characterisation of our system comprises several steps that will be developed in this work. This characterisation shows the imperfections found in any optical system and enables us to develop correction measures to reduce their impact on the quality of future experiments.

The third spatial dimension can be explored on a dSTORM system by the insertion of an additional optical system in the emission path. Biplane three-dimensional microscopy uses a beam splitter to divide the image of the sample into two focally shifted parts. Alternatively, an additional cylindrical lens in the emission path introduces an optical aberration (astigmatism) dependent on the axial position of each fluorescent molecule. Both these three-dimensional techniques require the recording of a z-dependent function that precisely reflects how the setup produces an image of a given sample along the optical axis.

In this work, we present the characterisation of our dSTORM system on known structures, i.e. fluorescent beads. This deep assessment of our system will allow us to be more confident in the data we will obtain in the future system and the three-dimensional calibration will allow us to easily extract more information by the addition of a lens in the optical setup and a supplementary data analysis step.