Bi-plane Calibration in Super-resolution Microscopy

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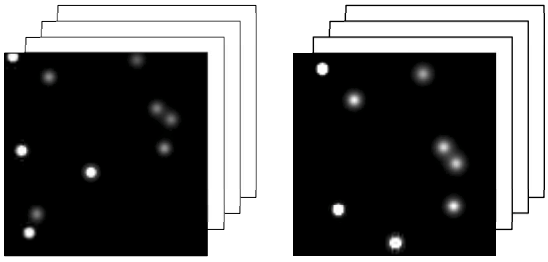
We consider the task of aligning the imaging detectors of a bi-plane microscope for super-resolution applications [1]. Such a microscope consists of two separate focal planes and the optical misalignment is modelled as an affine transform. In particular, $\vec{u}_1 = \mathbf{A}\vec{u}_2 + \vec{\mathbf{b}}$ accounts for translation, rotation and scaling operations (see illustration for an example of misaligned data). Here, $\vec{u}_1$, $\vec{u}_2$ describe a single point in space in terms of the two coordinate systems, **A** is a 3x3 matrix, and $\vec{\mathbf{b}}$ is a translation vector. There are 12 misalignment parameters that need to be found, i.e. the matrix **A** and the vector $\vec{\mathbf{b}}$.



The calibration data consists of two z-stacks, one for each plane. The microscopic sample is composed of fixed fluorophores beads that are located at unknown lateral and axial positions. The algorithm has two main stages: particle localisation and affine transform estimation.

In the localisation stage, the two stacks are processed separately. Local maxima are assumed to originate from fluorophore beads, and a threshold value is then used to keep the prominent ones only [2]. Each one of these local maxima is then fitted with a PSF model, yielding the lateral and the axial coordinates of each fluorophore bead. The fitting error is further compared with a threshold value for obtaining reliable localisation results. The algorithm fits the local maxima with the Gibson and Lanni PSF [3]. This model considers aberrations that are due to refractive indices mismatches at the sample - cover slip - immersion interfaces, as well as for the thickness of these layers. In particular, axial stage displacements correspond to different values of the immersion layer thickness. The output of the localisation stage is two lists of 3D beads locations.

The next stage of the algorithm consists of a least square estimation of the matrix **A** and the vector $\vec{\mathbf{b}}$

$$\min_{\mathbf{A},\vec{\mathbf{b}}} \sum_n \left\| \vec{u}_1^n - \mathbf{A}\vec{u}_2^n - \vec{\mathbf{b}} \right\|_{l_2}^2 ,$$

where the *n*-th subscript is a running index for the two localisation lists. The minimum least square solution is analytically described by a set of linear equations. It assumes, however, that the two lists are properly ordered in the sense that $\vec{u}_1^n$ and $\vec{u}_2^n$ correspond to the same fluorophore bead. This does not hold true in practice and the algorithm uses the nearest neighbour criterion for ordering the two lists as a preliminary operation. The nearest neighbour criterion works well in this case as the affine transform introduces small discrepancies between the two coordinate systems. Once the 12 parameters of the affine transform are estimated, the algorithm interprets them in terms of translation, scaling and rotation operations that need to be applied to one of the imaging planes. Alternatively, the calibration parameters can be used in localization algorithms that mathematically overcome misalignment.

We developed an ImageJ plugin and validated it for both synthetic and real data. It consists of fast multi-threaded calculations of the PSF and it possesses a simple user interface.

References: [1] M. Juette et al., "Three-dimensional sub–100 nm resolution fluorescence microscopy of thick samples", *Nature Methods* - **5**, 527 – 529, 2008 [2] Eric Betzig et al., "Imaging Intracellular Fluorescent Proteins at Nanometer Resolution", Science, Vol. 313, no. 5793 pp. 1642-1645, 2006. [3] S. F. Gibson and F. Lanni, "Experimental test of an analytical model of aberration in an oil-immersion objective lens used in three-dimensional light microscopy," J. Opt. Soc. Am. A, vol. 8, no. 10, pp. 1601–1613, 1991.

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