Quantifying the spatial resolution of an inverted tilted lightsheet fluorescence microscope

L. Dvinskikh[1,2], H. Sparks[1], N. Curry[1], C. Dunsby[1,3]
Department of Physics, Imperial College London, UK
[2]Department of Chemistry, Imperial College London, UK
[3]Centre for Pathology, Imperial College London, UK
liuba.dvinskikh13@imperial.ac.uk

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Abstract. The measurement of the spatial resolution of a fluorescence microscope is of key importance for characterizing its performance, identifying aberrations and interpreting the resulting images. It is typically determined by estimating the point spread function from the image of a point source. While software tools such as PSFj [1] successfully characterize ellipsoid shaped point spread functions by applying a 2D Gaussian fit laterally, and 1D Gaussian fit axially, they can't be directly implemented to quantify the tilted elongated point spread functions that are typical for light sheet fluorescence microscopy where the image stack is acquired by scanning the tilted light sheet illumination through the sample or where the stage translation direction is not perpendicular to the sheet [3].

We present a MATLAB-based tool for quantifying the spatial resolution of an optical system by characterizing the spatial extent of the images of sub-resolution fluorescent beads based on fitting a rotated 3D Gaussian distribution. First, binary image segmentation is applied using an intensity threshold above the estimated noise level. Second, connected components corresponding to images of spatially isolated beads are identified and selected. Third, the intensity distributions corresponding to each selected component are then individually fitted using a nonlinear least square fit of a 3D Gaussian distribution with a fixed angle of rotation and uniform weighting. Measured full-width half maxima in each of the three dimensions of each point spread image are then used to produce a 3D map of the spatial resolution as a function of position across the field of view of the microscope.

The method was tested by recovering the dimensions of simulated rotated ellipsoid PSF's and applied to characterize and compare the spatial resolution of two different light sheet fluorescence microscopes. Figure 1 shows exemplar images of sub resolution fluorescent beads and the corresponding 3D Gaussian distribution fits.
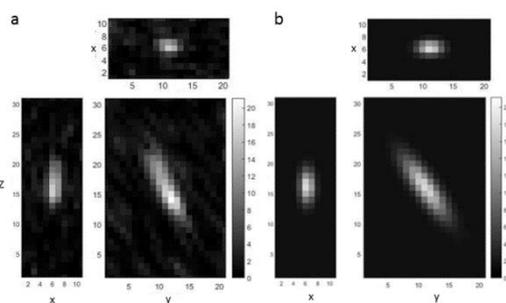
Figure 1: a) Three-way orthoslices through a subvolume of an image stack of 100 nm fluorescent beads acquired using an oblique plane light sheet fluorescence microscope. b) Three-dimensional Gaussian distribution fit to the bead image. Intensity bar units are photoelectrons, and Axes units are in pixels, each pixel sampling 0.24 μm in object space.

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