ABERRATION-ACCOUNTING CALIBRATION FOR 3D SINGLE MOLECULE LOCALIZATION MICROSCOPY

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Single Molecule Localization Microscopy (SMLM) is now widely used for 2D imaging of biological samples, both for single particle tracking of particles in live samples, and for structural observation through blinking processes such as (d)STORM, (f)PALM or (DNA-)PAINT. While measuring the lateral positions of the fluorescent labels is quite straightforward, retrieving 3D information is more challenging, and thus requires combining 2D SMLM with a complementary axial detection method. Most of the time, this is done thanks to Point Spread Function (PSF) shaping methods. These techniques require a calibration to determine the correspondence between the PSF shape and the axial position. Such a calibration is often performed by using fluorescent beads deposited on a coverslip and scanning the objective position thanks to a piezoelectric stage. However, this method does not take into account the effect of the spherical aberration induced by the index mismatch between the sample and the glass coverslip, which affects the shape of the PSFs.
We propose a straightforward, fully experimental method that accounts for the effect of the detection system by imaging a sample of known geometry in the nominal imaging conditions [1], i.e. for a given focus plane position and a given fluorescence wavelength. More specifically, we use 15 µm diameter latex microspheres coated with fluorescent dyes: thus, simply by measuring the lateral positions of the molecules, their depths can be known. This provides the calibration curve of the axial detection, which we compare to that obtained using the previously mentioned technique using an axial scan of the object plane. The discrepancy is found to be as large as 250 nm in a 1.2 µm imaging depth range. We will highlight the precision and the versatility of our calibration, and we will illustrate its interest on biological samples.

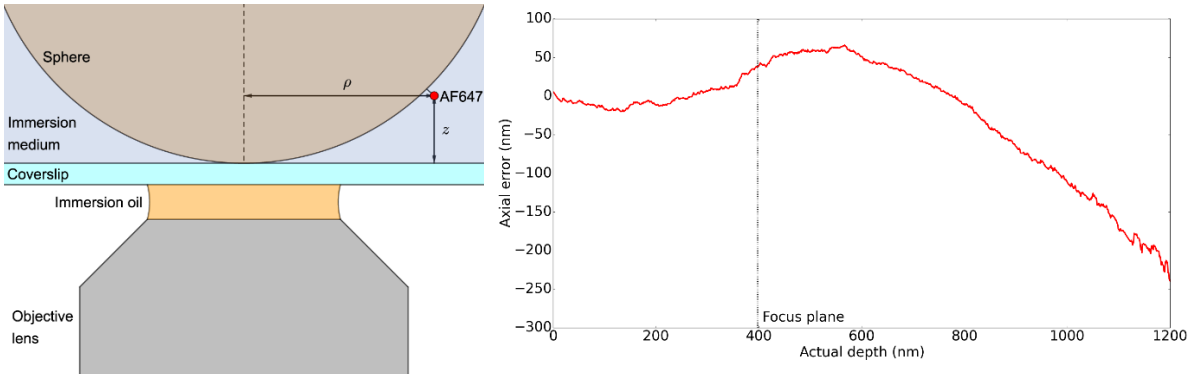

Figure 1: (left) Schematic of the sample used for the calibration, (right) Axial bias induced by the aberrations when using beads deposited on a coverslip to perform the calibration.

[1] Cabriel, C., Bourg, N., Dupuis, G. & Lévêque-Fort, S. Aberration-accounting calibration for 3D single-molecule localization microscopy, *Optics Letters* **43**, 174-177 (2018).