SIMPLE DETERMINATION OF MICROSCOPE OBJECTIVE FEATURES IN FLUORESCENCE MICROSCOPY

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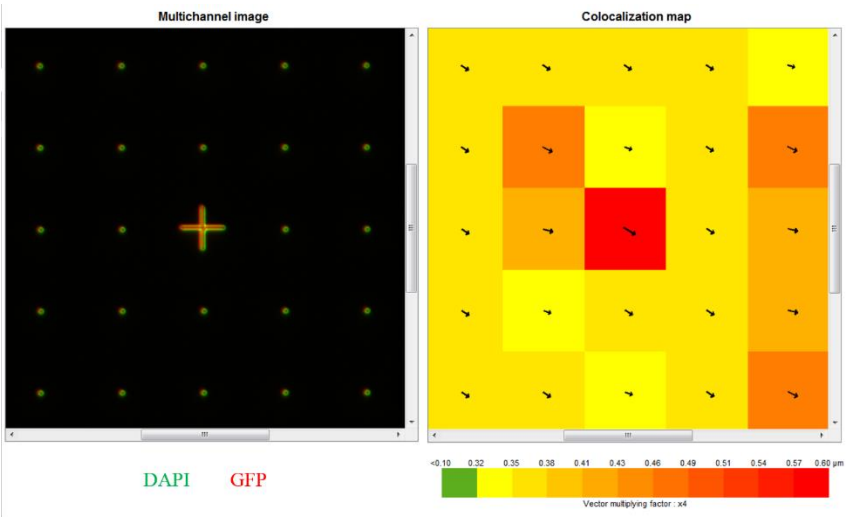
Optical features of modern fluorescence microscopes are usually unknown in a quantitative way. Although the main optical elements in a microscope, the objectives, are qualified by their correction for optical aberrations, their actual performances are not provided. For example, a Plan Apochromat objective is supposed to be corrected for field curvature, spherical aberration and chromatic aberration. But who can tell how flat the field is, how much spherical aberrations are left, and what the chromatic shits are for different wavelengths, and this in the entire field of view (FOV)?

Sub-resolution beads, tagged or not with different dyes, can be used to answer these questions. But the result is partial, as the point spread function and the chromatic aberration determinations are usually performed only in the center of the FOV. The ideal tool would be a sample containing a 2D matrix of sub-resolution beads, tagged with different dyes, which position is perfectly known in the FOV. Unfortunately, this tool does not exist, to our knowledge.



Based on a process that enables the etching of long-term stable multicolor fluorescent patterns with sub-micrometer sizes in 2D and 3D inside glass, we have fabricated a sample that contains a 2D matrix of fluorescent emitters, continuously excitable from 325 to 650 nm, spatially distributed in a ring shape, and which position is known. With such a sample, optical features of microscope objectives can be easily determined in the entire FOV. As an example, the present figure shows the chromatic shifts obtained from two images of a matrix of fluorescent rings acquired with a wide-field microscope, a Plan Apochromat 63×/1.4 oil objective, on the GFP and DAPI channels. Other information can be extracted from this matrix of fluorescent rings, such as shading, distortion, contrast, etc.