Laboratory for optics and biosciences
Ecole Polytechnique – CNRS – INSERM – IP Paris

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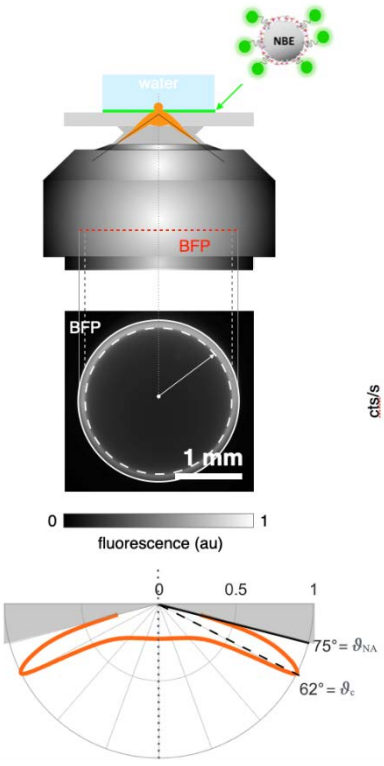
https://www.sppin.fr/the-teams/team-1-biophysics-of-the-brain/

From Emile Bertrand to smart coverslips: Why combinatorial front- and back-focal plane imaging is a more than a gadget



In a microscope, the back-focal plane (BFP) of the objective lens is where light rays are sorted by angle rather than position. The BFP contains the average fluorophore radiation pattern of all excited fluorophores within the field-of-view (FOV). This radiation pattern encodes information about the fluorophore orientation, nanometric axial distance from the surface and the local refractive index (RI) of the the fluorophore-embedding medium, information that is complementary to what can be retrieved from a sample plane (SP) image. However, when imaging the BFP spatial (x,y) information is generally lost, or it can only be obtained indirectly - either by reducing the FOV to a tiny excitation spot and implementing stage scanning, or — in biological experiments — by genetically addressing the fluorophore to defined sub-cellular targets.

Applied to wide-field fluorescence microscopy, SAF is the optical reciprocal and experimental twin of objective-type TIRF. With their large collection numerical aperture (NA > refractive index, RI), highly corrected oil-immersion objectives capture both under- and supercritical fluorescence components. Supercritical fluorescence (SAF), which is forbidden by Snell's law for most dipoles, occurs when fluorophores are very close to the dielectric interface, producing high-angle emission. The SAF/UAF ratio can provide a nanometric height map of fluorophores over the entire FOV, allowing axial fluorophore localisation with unprecedented precision on a standard inverted microscope. BFP images also aid in optical metrology, helping with aberration correction and optical misalignment detection. They provide a simple way to measure the effective numerical aperture (NAeff) of high-NA objectives and it can improve TIRF penetration-depth calibration and quantify optical sectioning in super-resolution microscopies.

In my talk, I will present recent and ongoing work using combinatorial front- and BFP imaging for biological fluorescence. I will argue that this approach not only offers advantages compared to TIRF microscopy, but offers completely new experimental paradigms that result from the unique physical properties of near-interface fluorophores.