Computational methods for high resolution 3D fluorescence microscopy

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Epifluorescence microscopy results in blurry images with a very coarse optical sectioning; this limits its usefulness for cellular imaging, where resolving subcellular structures requires resolution close to or even beyond Abbe's diffraction limit. Techniques such as confocal microscopy (CLSM) and selective plane illumination microscopy (SPIM) have been proposed to reduce the out-of-focus light and to improve the resolution. Several other modalities, such as single-molecule localization microscopy (SMLM) and structured illumination microscopy (SIM) make the use of multiple acquisitions, trading time for resolution. These last techniques have been recently extended to 3D imaging.

These novel microscopy modalities rely highly on heavy computational post-processing to faithfully reconstruct the 3D image at high resolution. Although there exists commercial software for this task, the most innovative solutions are fostered by the academic community as open-source software. We shall review this essential computational step through three applications of 3D imaging systems: deconvolution, accurate localization in SMLM, and reconstruction in SIM.

Here, we present different algorithms to reconstruct an image by looking for the right trade-off between frame rate, photo-toxicity, resolution, contrast enhancement, and denoising. We formalize the numerical reconstruction task as an inverse problem based on knowledge of the image formation model, mainly summed up by the point-spread function (PSF). In practice, the choice of reconstruction software is driven by efficiency, ease of calibration, and overall usability. We show that it is important to preserve the quantitative signal while avoiding artificial artifacts in order to allow further image analysis.