FOURIER RING CORRELATION AS A TOOL TO ASSESS EFFECTIVE RESOLUTION IN POINT SCANNING MICROSCOPY

Giorgio Tortarolo[1,3], Marco Castello[1,3], Colin J. R. Sheppard[2], Sami Koho[1], Alberto Diaspro[2,4], Giuseppe Vicidomini[1]
[1]Molecular microscopy and spectroscopy, Istituto Italiano di Tecnologia, Genoa, Italy
[2]Optical nanoscopy, Istituto Italiano di Tecnologia, Via Morego 30, Genoa, Italy
[3]DIBRIS, Via Opera Pia 13, Genoa, Italy
[4]DIFI, Via Dodecaneso 33, Genoa, Italy
Email: giorgio.tortarolo@iit.it, giuseppe.vicidomini@iit.it

KEY WORDS: spatial resolution, Fourier Ring Correlation, STED microscopy, CLSM.

ABSTRACT:
Diffraction of light limits the spatial resolution of far-field conventional fluorescence to roughly 200 nm. Confocal laser scanning microscopy allows for a resolution enhancement of √2, while super resolution techniques such as stimulated emission depletion (STED) microscopy reach theoretically unlimited resolution.
However, the effective resolution of any microscopy experiment is affected by several factors, which influence the signal-to-noise ratio (SNR) of the image, such as photophysical properties of the fluorophore, acquisition time, intensity of excitation, collection efficiency of the system, nature and quality of the detector, and aberrations caused by the imperfection of the optical system. When considering STED microscopy, other critical parameters have to be taken into account: the choice of the fluorophore, STED laser intensity, temporal and spatial alignment between STED and excitation beams, and imperfections in the shape of the STED beam. Considering all these contributions in an *a-priori* model is prohibitive, thus effective resolution may differ from the predicted theoretical value. Conversely, in electron and single-molecule-localization microscopy, Fourier Ring Correlation (FRC) analysis is performed directly on measured data, in order to assess effective resolution in an *a-posteriori* fashion [1, 2]. For the above-mentioned reasons, we decided to extend the FRC analysis to point scanning microscopy techniques.
Fourier Ring Correlation evaluation requires two images of the same view of the sample, with statistically independent noise realizations, thus we performed FRC analysis alternatively on: a) two consecutively acquired images (which were drift-corrected) and b) two images obtained from a single scan, by splitting photons in two equal temporal windows for every pixel. We suppose that in the latter case eventual photo-bleaching or drifting effects between the two temporal windows are negligible.
We performed FRC analysis on confocal and STED measurements, showing a strong dependence between effective resolution and SNR. As expected, STED microscopy resolution is fundamentally limited by noise.
We demonstrated that FRC analysis is a valuable tool for assessing effective resolution also in point scanning microscopy.

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