3D-SRRF: REAL-TIME LIVE-CELL-COMPATIBLE VOLUMETRIC SUPER-RESOLUTION MICROSCOPY

Romain F. Laine1,2, Tommaso Galgani3, Bassam Hajj3,*, Ricardo Henriques1,2,*

1 MRC-Laboratory for Molecular Cell Biology, University College London, London, UK
2 The Francis Crick Institute, London, UK
3 Laboratoire Physico-Chimie, Institut Curie, CNRS UMR168, Paris, France

Email: bassam.hajj@curie.fr, r.henriques@ucl.ac.uk

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The direct and real-time observation of cellular mechanisms in live biological systems at high resolution in 3D remains a challenge for the microscopy community. This is mainly because conventional super-resolution microscopy (SRM) techniques require intense illumination or long acquisition times, limiting their compatibility with live-cell imaging. Recently, some of us pioneered the SRRF approach [1], which only requires low illumination and few frames to enable SRM.

Figure 1. 3D-SRRF. Left: Principles of 3D-SRRF. MFM data can be analysed using the SRRF approach. Right: U2OS cells expressing TOM20-Halo, loaded with JF549.

SRRF exploits the spatial (radial pattern) and temporal (intensity fluctuations) features of fluorescent emitters in the sample to extract spatial resolution beyond the diffraction-limit. The original method, however, can only perform 2D (lateral) resolution improvement.

To be able to extend this approach in the axial dimension, it is necessary to acquire multiple axial planes simultaneously. Multifocus microscopy (MFM), developed by some of us, uniquely offers true simultaneous volumetric imaging [2,3], where different focal planes are acquired in parallel on the same camera. Hence, the emitters’ 3D spatial patterns can be acquired in a single camera frame, allowing for the capture of its volumetric fluctuations.

Here, we present an approach to extend SRRF to true 3D-SRM where the resolution is improved throughout the volume, including in the axial direction, thanks to MFM.

We demonstrate the approach on simulated data, ArgoLight slide as well as on mitochondrial dynamics in living cells. This method is currently able to provide true 3D SRM in living cells every ~2s, within an imaging volume of 20 x 20 x 3.6 µm³. This constitutes an invaluable tool for the dynamic study of numerous biological mechanisms.