HIGH-RESOLVED STRUCTURAL DYNAMICS IN LIVING BIOLOGICAL SAMPLES BY COMBINING ADVANCED LIGHT MICROSCOPY TECHNIQUES

Bernd Zobiak, Antonio Virgilio Failla
UKE Microscopy Imaging Facility (UMIF), University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
Email: a.failla@uke.de

KEY WORDS: Spectroscopic imaging, super-resolution, live imaging, biology, fluorescence microscopy, confocal microscopy

Our days, the further development of super resolution microscopy (SRM) enables researchers to investigate fixed and living biological samples, approaching the single molecule level. However, constraints on the image field of view and acquisition speed are still limiting the applicability of these techniques, especially regarding live imaging experiments. To circumvent these limitations, the combined utilization of high resolution live imaging techniques as Spinning Disk microscopy and TIRF microscopy, as well as high informative, multi-dimensional spectroscopic approaches like FLIM, FLIM-FRET, FCS and FCCS can provide valuable information on structural dynamics in cell biological experiments.

In this work, we summarize some relevant results achieved by advanced Spinning Disk-TIRF microscopy, FRET-FLIM and FCS/FCCS in fixed and living sample to demonstrate how these techniques can still present a valid alternative to SRM. Our aim is to combine these techniques with SRM methods to perform new and innovative imaging applications, for example STED-FLIM-FRET, or further implement powerful but not yet regularly used methods such as STED-FCS/FCCS [1]. We believe that the combination of all these techniques will generate cutting edge results in the frame of bio-medical research.

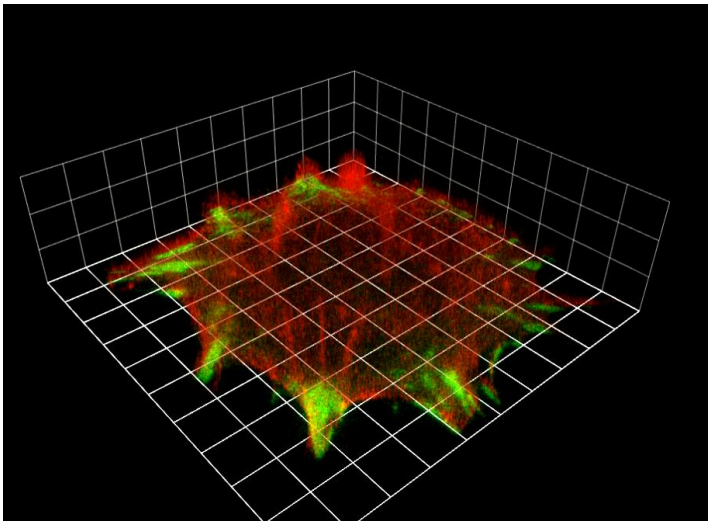

Figure 1: HeLa cell expressing RFP-Lifeact (red) and YFP-Vinculin (green) 40min after adhesion to fibronectin. Actin network and adhesion formation was visualized by SD-TIRF microscopy. 1 unit = 4.11µm.

[1] F. Schneider, D. Waithe, S. Galiani, J. Bernardino de la Serna, E. Sezgin, C. Eggeling, "Nanoscale Spatiotemporal Diffusion Modes Measured by Simultaneous Confocal and Stimulated Emission Depletion Nanoscopy Imaging." *Nano Lett.*, **18**, 4233-4240 (2018)