

New modalities in Re-scan Confocal Microscopy (RCM)

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In the last few years the Re-scan Confocal Microscope [1] has become a well-known and simple technology to obtain images with an improved resolution (170 nm) as compared to standard confocal microscopy (240 nm). The technology is based on a standard confocal microscope with an additional scanner (the re-scanner) that directs the emitted light to a sensitive (CMOS or CCD) camera. Precise control of the scanner (that “reads” the sample) and re-scanner (that “writes” the image on the camera) allows super-resolution imaging without closing the pinhole to a minimum. This highly photon-economical way of detection (no losses at the pinhole) and the use of a highly sensitive camera reduce the noise level in RCM images. Apart from imaging with better resolution and less noise the RCM microscope has a lot more potential.

In the last few years we have exploited the possibilities of the RCM technology. We have adjusted the microscope for sequential and simultaneous multi-color imaging, FRET imaging, pH-imaging, ratio-imaging and FRAP measurements [2]. Recently, we added to this list deep-tissue imaging, SCIM-RCM (low-phototoxicity high-resolution imaging) [3,4], life-time imaging and other techniques to further improve the resolution. In this presentation we present our latest results.

[1] De Luca, Giulia MR, et al. "Re-scan confocal microscopy: scanning twice for better resolution." *Biomedical optics express* 4.11 (2013): 2644-2656.

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[4] Krishnaswami, V., et al., Spatially-controlled illumination microscopy: For prolonged live-cell and live-tissue imaging with extended dynamic range. *Quarterly Reviews of Biophysics*, 49 (2016).