

Re-scan Confocal Microscopy (RCM): toward SIM-resolution

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In the last few years the Re-scan Confocal Microscope (RCM) [1-3] has become a well-known and simple technology to obtain images with improved resolution (170 nm; FWHM) as compared to standard fluorescence microscopy (240 nm). The RCM 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 (resolution beyond the Abbe limit) 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 explored different imaging modes and how the resolution of the RCM microscope can further be improved. As expected, dedicated deconvolution techniques (Huygens, SVI) improves the sharpness of the image (FWHM of sub-resolution objects) and strongly reduces the noise. Another way to improve the resolution of RCM is by combining RCM with SIM. In this presentation we will explore how RCM-SIM and other new techniques can help to further improve RCM resolution.

[1] De Luca, Giulia MR, et al. "Re-scan confocal microscopy: scanning twice for better resolution." *Biomedical optics express* 4(11), 2644-2656 (2013)

[2] De Luca, Giulia MR, et al. Configurations of the Re-scan Confocal Microscope (RCM) for biomedical applications. 2016, *Journal of Microscopy*, 266(2), 166-177 (2017)

[3] De Luca, Giulia MR, Ronald Breedijk, Ron Hoebe, Sjoerd Stallinga and Erik Manders. “Re-scan Confocal Microscopy (RCM) improves the resolution of confocal microscopy and increases sensitivity. *Methods and Applications in Fluorescence*, 5, 15002, (2017)