Re-scan Confocal Microscopy: new applications and comparison with Confocal and Structured Illumination microscopy

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We present a new super-resolution technique, Re-scan Confocal Microscopy (RCM) [1], based on standard confocal microscopy extended with an optical (re-scanning) unit that projects the image directly on a CCD camera. This new microscope has improved lateral resolution and strongly improved sensitivity while maintaining the sectioning capability of a standard confocal microscope. This simple technology is typically useful for biological applications where the combination high-resolution and high sensitivity is required.

Confocal microscopy is potentially a super-resolution technique, giving improvement of lateral resolution by a factor of √2 compared to wide-field fluorescence microscopy. This resolution improvement can only be obtained if the dimension of the pinhole is ideally down to zero, reducing the signal-to-noise ratio in the acquired images dramatically. Re-scan Confocal Microscopy allows to obtain a √2 resolution improvement, but with an open pinhole (normally set at 2 AU) and a sensitive EMCCD camera for detection. These conditions are extremely convenient in applications where the photon economy is crucial. For example, measurements of 100 nm beads show that the FWHM for RCM with pinhole 2 AU is 170 nm compared to 245 nm in wide-field mode. A similar resolution improvement (measured 184 nm) can be obtained with confocal microscopy by squeezing the pinhole down to 0.2 AU which implies a reduction of photon collection by a factor of 100 compared to RCM.

In this talk we will focus on the comparison of RCM with some commonly used microscopy technique (Widefield, Confocal and Structured Illumination Microscopy). We will show a comparison in Point Spread Function shape, focusing on the lateral and axial resolution obtainable with the above listed techniques. In the same time, the signal-to-noise will be quantified for different imaging conditions. Imaging speed can be crucial for several biological applications: we will show that RCM and SIM allows similar resolution, but in RCM only a single image is needed. Moreover, RCM is a “optics only”-technology without image reconstruction procedures.

Keeping in mind the diverse biological applications, we will discuss a dual-color implementation of RCM and the combination of RCM with STED excitation.