SINGLE-PHOTON NIR IMAGING WITH RESCAN CONFOCAL MICROSCOPY

R.M.P. Breedijk[1], Venkataraman Krishnaswami[3], S.Hock[3], A.Kober[3], Carina Kommnick[3], E.M.M.Manders[2], F.Wouters[3] and G. Bunt[3]

[1] Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Science Park 904, 1098 XH, The Netherlands

[2] Confocal.nl BV, Amsterdam, The Netherlands

[3] Institute of Neuropathology, University Medical Center Göttingen, Göttingen, Robert-Koch Strasse 40, 37075, Germany.

E-mail: r.m.p.breedijk@uva.nl

KEY WORDS: rescan confocal microscopy, super resolution microscopy, near–infrared imaging

ABSTRACT

The use of longer wavelengths such as near-infrared (NIR) for imaging enables deeper investigation into tissue specimens. Multiphoton microscopy allows deep imaging by using non-linear excitation at longer wavelengths coupled with detection at shorter emission wavelengths. However, resolution drops at higher wavelengths resulting in loss of information. Additionally, such a system requires sophisticated instrumentation which also poses challenges for multicolor imaging.

Here, we use rescan confocal microscopy (RCM) [1] to improve the lateral resolution of images acquired with an excitation source in the near-infrared spectrum, by a factor of 1.4. We present a single-photon NIR-RCM specifically designed for deep-tissue imaging with low magnification and large working distance objectives. Improved lateral resolution was obtained for near-infrared excitation, at tissue penetration depths beyond those feasible for the visible spectrum. Furthermore, this technique scores with its ease of implementation, use and does not require any post-processing. We present the use of rescan confocal microscopy for performing high resolution, fluorescence imaging in the visible and near-infrared spectrum.

REFERENCES

[1] G. M. R. De Luca et all., Re-scan confocal microscopy: scanning twice for better resolution. Biomedical Optics Express. 4 (2013), p. 2644.

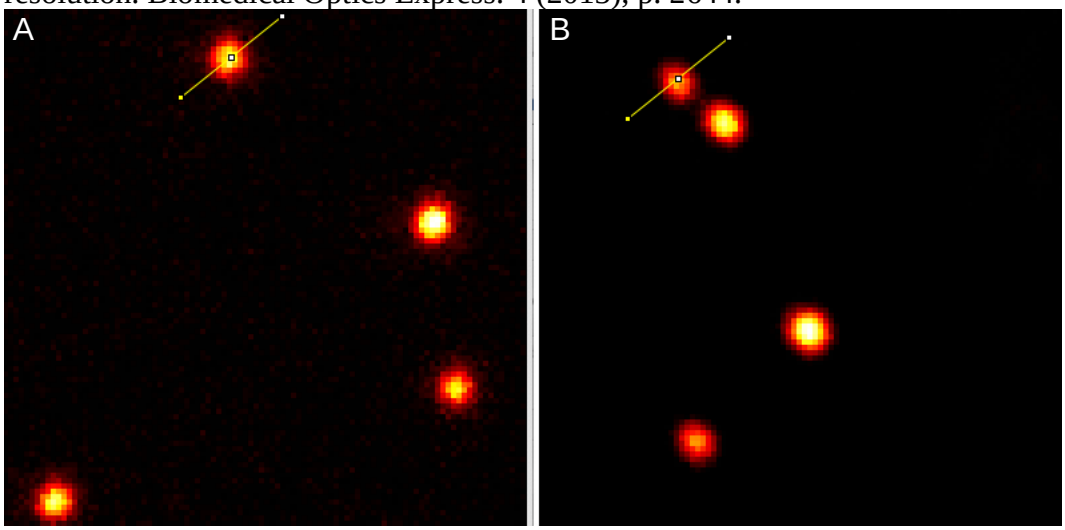

Figure 1. Images of 200 nm fluorescent beads recorded using (A)confocal mode excitation with 543nm laser and (B) RCM mode excitation with 785nm laser. Objective used is an 60x with an NA of 1.4. Measured FWHM for (A) is 274nm and for (B) is 270nm. Using RCM with NIR light compensates the resolution caused by the increased wavelength.