Comparison of spinning disc and structured confocal microscopy

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KEY WORDS: 3D-imaging, spinning disc confocal, structured illumination microscopy, confocality

1. Introduction

There are many different techniques for 3D fluorescence microscopy. Each having advantages and disadvantages for different samples and requirements [1][2]. The standard 3D tools are confocal and spinning disc confocal (SDC) microscopy [3].

We present a comparison of SDC and structured illumination microscopes (SIM). The SIM is improved with a hexagonal grating and line-confocal properties to suppress out of focus light in the phase images.

2. Results

Both instruments used a 40x0.95 objective and showed close to diffraction limited performance. Neither the SDC nor the SIM was optimized for higher resolution. The SDC can only achieve higher frame rates if the sample is strongly fluorescent and excitation power is not limited. An important parameter for 3D microscopy is the suppression of the out of focus background, which was measured with SIP-charts [4]. It shows that the width of the confocality is only slightly better in SIM with 1.6µm compared to 1.75µm for the SDC. But as shown in Fig. 1 the SDC has significant out of focus light that is not within the optical section but in its tails.

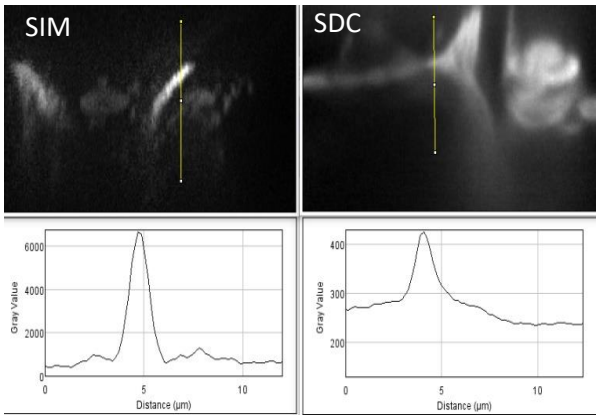

Fig. 1: xz-sections of Convallaria, λ_{ex}=488nm

3. Summary

Spinning disk images are smoother whereas structured confocal images show more contrast.

[1] Conchello, Lichtman, "Optical sectioning microscopy", *Nature Methods*, 2, 12, 920-931 (2005).
[2] Murry et al, "Evaluating performance in 3D fluorescence Microscopy" , *J. of Microscopy*, 228, 390-405 (2007).
[3] Wang et al, *J. of Microscopy*, 218, 148-159 (2005).
[4] Brackenhoff et al, *J. of Microscopy*, 219, 122-132 (2005).