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New techniques for fluorescence background rejection in microscopy and endoscopy

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Confocal microscopy is a popular technique in the bioimaging community, mainly because it provides optical sectioning. However, its standard implementation requires 3-dimensional scanning of focused illumination throughout the sample. Efficient non-scanning alternatives have been implemented, among which the simple and well-established incoherent structured illumination microscopy (SIM) [1]. We recently proposed a similar technique, called Dynamic Speckle Illumination (DSI) microscopy, wherein the incoherent grid illumination pattern is replaced with a coherent speckle illumination pattern from a laser, taking advantage of the fact that speckle contrast is highly maintained in a scattering media, making the technique well adapted to tissue imaging [2]. DSI microscopy relies on the illumination of a sample with a sequence of dynamic speckle patterns and an image processing algorithm based only on an a priori knowledge of speckle statistics. The choice of this post-processing algorithm is crucial to obtain a good sectioning strength: in particular, we developed a novel post-processing algorithm based one wavelet pre-filtering of the raw images and obtained near-confocal fluorescence sectioning in a mouse brain labeled with GFP, with a good image quality maintained throughout a depth of $\sim$100 μm [3]. In the purpose of imaging fluorescent tissue at higher depth, we recently applied structured illumination to endoscopy. We used a similar set-up wherein the illumination pattern (a one-dimensional grid) is transported to the sample with an imaging fiber bundle with miniaturized objective and the fluorescence image is collected through the same bundle. Using a post-processing algorithm similar to the one previously described [3], we obtained high-quality images of a fluorescein-labeled rat colonic mucosa [4], establishing the potential of our endomicroscope for bioimaging applications.

Ref:
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[2] C. Ventalon *et al*, Opt. Lett. **30**, 3350 (2005)
[3] C. Ventalon *et al*, Opt. Lett. **32,** 1417 (2007)
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