

STRUCTURED ILLUMINATION MICROSCOPY IN 2D AND 3D CELL ASSEMBLIES

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An experimental setup for Structured Illumination Microscopy (SIM) has been established using the first diffraction orders of a spectral light modulator (SLM) to create an interference pattern in two dimensions [1, 2]. This pattern is recorded for three phases (0 , $2\pi/3$, $4\pi/3$) at rotation angles of 0° , 60° and 120° in order to obtain an optical transfer function (OTF) of rotational symmetry, which results in a lateral resolution slightly above 100 nm. The setup has now been validated with fluorescent nano-beads and further applied to living cells for imaging the cytoskeleton (Figure 1), mitochondria or the distribution of a cytotoxic drug (doxorubicin) in cell nuclei. Light exposure in SIM exceeds that of conventional wide-field microscopy only slightly, and is considerably lower than exposures needed for other super-resolution techniques. This favors SIM for live cell microscopy, in particular, if longer exposure times or repeated measurements are required.

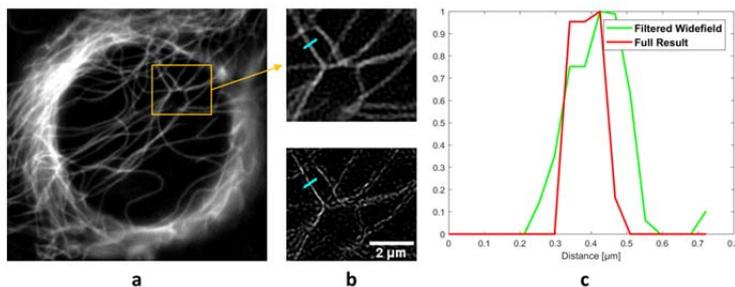


Fig. 1. 3T3 fibroblast incubated with Tubulin Tracker™ Green (250 nM, 20min.): (a) wide-field microscopy with Wiener filter, (b) comparison of wide-field image and SIM, (c) line scan over 0.8 μm for wide-field and SIM (“full result”); $\lambda_0 = 488 \text{ nm}$; $\lambda_D \geq 515 \text{ nm}$;

While SIM works reliably for 2-dimensional samples (e.g. cell monolayers), the application of this method remains a challenge for 3-dimensional specimens, e.g. cell multilayers, spheroids or tissue biopsies. In this case working distances are larger, and long distance objective lenses of lower aperture have to be used. The increase of SIM resolution compared to conventional wide-field microscopy, however, is maintained. Furthermore, precise positioning of the specimen in axial direction (not available in many customary microscopes) is cogent for imaging 3D samples. Finally, light scattering in the depth of larger specimens may destroy the interference pattern. We suggest, therefore that the interference pattern may be created within a light sheet, so that scattering out of this light sheet can be suppressed more easily.

References

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