

LIVE-CELL IMAGING AT 150 NM AND 100 HZ BY ROTATING COHERENT SCATTERING (ROCS) MICROSCOPY

Alexander Rohrbach
Lab for Bio- and Nano-Photonics, University of Freiburg,
Georges-Koehler-Allee 102, 79110 Freiburg, Germany
E-mail: rohrbach@imtek.de

KEY WORDS: Label-free, super-resolution imaging, 100Hz, dark-field, total internal reflection, actin cytoskeleton.

Biological structures are driven by thermal and active cellular forces. These movements become the faster the smaller the biological structures because of less friction and steric hindrance. This is an important aspect for super-resolution microscopy, since unfortunately the resolution of smaller structures requires more photons and time. Therefore, novel concepts enabling smart trade-offs between temporal and spatial resolution have to be developed.

Here, we present a variant of an oblique illumination super-resolution microscopy [1, 2] based on rotating coherent scattering (ROCS). The technique generates thousands of high contrast images without post-processing at frame rates of more than 100 Hertz and without labeling. The sample is scanned over all azimuthal illumination angles within a single camera exposure time and allows for variant illumination and detection modes such as bright-field, dark-field or total internal reflection (TIR). Thus, structures as small as 150 nm become separable through local destructive interferences. Within one rotation, bely suspects do not disappear but change to the object itself thereby improving contrast and resolution [3]. ROCS is applied to different cells revealing unexpected dynamic biological processes[4].

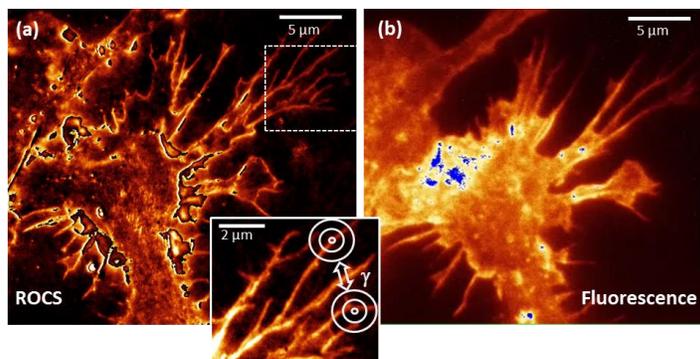


Figure 1: (a) ROCS image (DF mode) of a living mouse-macrophage recorded with a 405 nm laser within 9 ms. (b) The corresponding LifeAct GFP-actin labeled fluorescence image was recorded within 200 ms. Both images were recorded in TIR mode. Inset: The correlation between two spherical waves is quantified by the degree of coherence γ .

- [1] Jünger F, Olshausen P, Rohrbach A., Fast, label-free super-resolution live-cell imaging using rotating coherent scattering (ROCS) microscopy (2016) *Sci Rep*,6, 30393
- [2] Olshausen P, Rohrbach A., Coherent total internal reflection dark-field microscopy: label-free imaging beyond the diffraction limit (2013) *Opt Lett*, 38, 20, 4066 – 4069
- [3] D. Ruh, J. Mutschler, M. Michelbach, and A. Rohrbach, "Superior contrast and resolution by image formation in rotating coherent scattering (ROCS) microscopy," *Optica* 5, 1371-1381 (2018).
- [4] F. Jünger and A. Rohrbach, "Strong cytoskeleton activity on millisecond time scales during particle binding and uptake revealed by ROCS microscopy," *Cytoskeleton* 75, 410–424 (2018).