3D ”COLOR” SIM FOR OPHTHALMOLOGIC RESEARCH

Florian Schock [1,2,3], Gerrit Best [1,3], Nil Celik [4], Yangyi Wang [2,3], Alena Bakulina [5,9], Saadettin Sel [4], Udo Birk [1,3], Rainer Heintzmann [6,7], Jürgen Hesser [5,9], Stefan Dithmar [4,8], Christoph Cremer [1,2,3,9]

[1] Institute of Molecular Biology, University of Mainz
[2] Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg
[3] Kirchhoff Institute for Physics, University of Heidelberg
[4] Department of Ophthalmology, University-Hospital Heidelberg
[5] Experimenatal Radiation Oncology, University Medical Center Mannheim, University of Heidelberg
[6] Institute for Physical Chemistry and Abbe Center of Photonics, University of Jena
[7] Leibniz Institute of Photonic Technology
[8] Department of Ophthalmology, Hospital Wiesbaden
[9] Interdisciplinary Center for Scientific Computing (IWR), University of Heidelberg
Email:c.cremer@imb-mainz.de

Key Words: SIM, autofluorescence, ophthalmology, ex-vivo, human tissue, medical research

While super-resolution-microscopy (SRM) techniques have become widely available, application in the clinical context is still mostly restricted to cultivated cells. Here, we present the application of Structured Illumination Microscopy (SIM) for clinical research on extracted tissues as well as quantitative analysis of over 300 human RPE cells and their granules (intracellular particles) regarding a correlation to age related macular degeneration. So far we have shown that in human retinal tissue, SIM improves the resolution and the contrast. [1-3]
In contrast to other SRM approaches, SIM can be used without modification of the specimen in all cases where conventional wide field fluorescence microscopy approaches can be applied. Since autofluorescence is a characteristic of several chorioretinal conditions, SIM offers the potential for further clinical imaging at illumination intensities that allow application to living patients.
We present quantitative data, resulting from over 300 human RPE cells and over 25.000 single intracellular particles. Further we will show, that by using 3D SIM, not only automated segmentation of these granules will become statistically reliable, but also the quantitative analysis on granule density per cell for different donor ages and regions.
In contrast to other SRM methods, SIM not only improves resolution, but by using autofluorescence, it is able to use excitation/emission spectra (in our case up to 3 spectral channels) to obtain information on the composition of the particles.
All investigations were in accordance to the declaration of Helsinki on good clinical practice.

[1] G. Best, R. Amberger, D. Baddeley, T. Ach, S. Dithmar, R. Heintzmann, C. Cremer; “Structured illumination microscopy of autofluorescent aggregations in human tissue”, *Micron* 42 (2011);
[2] T. Ach, G. Best, S. Rossberger, R. Heintzmann, C. Cremer, S. Dithmar ;”Autofluorescence imaging of human RPE cell granules using structured illumination microscopy”; *British Journal of Opthalmology* (2013)
[3] S. Rossberger, G. Best, D. Baddeley, R. Heintzmann, U. Birk, S. Dithmar, C. Cremer; “Combination of structured illumination and single molecule localization microscopy in one setup”, *JoO* (9/2013)