3D ANALYSIS OF LIPID DROPLETS IN DIC IMAGES AND FLUORESCENCE IMAGE STACKS

B. Heise¹, L. Muresan¹, H. Wolinski², S. Kohlwein²

¹ Department of Knowledge based Mathematical Systems, Johannes Kepler University, Linz, Austria
   Bettina.heise@jku.at, Leila.muresan@jku.at

² SFB Biomembrane Research Center, Institute of Molecular Biosciences
   University Graz, Austria, Heimo.wolinski@uni-graz.at

KEY WORDS: Lipid droplets, DIC, deconvolution, wavelets

The characterization of the spatial lipid droplet distribution and aggregation in yeast cells plays an important role for investigation of fat metabolism. To avoid multiple staining only lipid droplets are fluorescently marked, whereas the whole yeast cells are imaged by DIC microscopy simultaneously. By conventional DIC microscopy there is no linear relation between the measured intensity and the original phase gradient because of combined amplitude and phase response of this type of microscopy. Although several proposals for technical improvements of DIC microscopy based on phase shifting [1;2] or different shear directions [3] exist, commercial microscopes specialized for fluorescence imaging are often equipped only with a combined conventional DIC imaging modality. After linearization of the problem we can perform a deconvolution to get approximately quantitative values for the optical path length (OPL) map from the measured phase gradient. We use two different approaches for deconvolution: the first is based on a Maximum Likelihood deconvolution algorithm, the second approach is an iterative projection based method to reconstruct the phase values of the cells. Lipid droplets can be clearly recognized as peaks in OPL maps due to their slightly different refractive index.

3D OPL reconstruction of a yeast cell by ML deconvolution

3D OPL reconstruction of a yeast cell by iterative projection based deconvolution

Additionally to the DIC images, fluorescence image stacks of the stained lipid droplets are analyzed. The goal is to reconstruct the 3D configuration of the droplets inside the cell. À trous wavelets based techniques are successfully used for spot detection in 2D fluorescence microscopy images [4]. The technique is particularly well suited for the detection of isotropic features. Due to the spherical appearance of the lipid droplets, we apply the 3D version of the à trous wavelets to the image stack, combined with hard threshold shrinkage. A brief statistical analysis of the detected droplets features is performed. The potential for a more accurate analysis of the distribution of lipid droplets by combining the two techniques presented above is discussed in the conclusion of this work.


