QUANTITATIVE ANALYSIS OF YEAST SUBCELLULAR STRUCTURES

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In the post-genomic era, large-scale microscopic analyses of protein localization patterns or morphological traits provide powerful tools towards functional characterization of proteins. Live cell imaging using fluorescent protein fusions has become a standard tool for studies on protein localization and the organization of subcellular structures in the three-dimensional context of the living cell, but also to investigate the impact of mutations on organelle morphology and inheritance. We employ GFP-tagging and vital dyes to investigate lipid droplet formation and inheritance in the yeast Saccharomyces cerevisiae. Lipid droplets (LD) are the storage compartment for fat in yeast, as in plants and mammalian cells (e.g. adipocytes). However, despite their important physiological function in lipid and energy homeostasis, very little is known about the mechanisms of lipid droplet formation and inheritance. Due to the ease of genetic manipulation and the availability of knock-out mutant collections of all non-essential genes, and the similarities in many central metabolic pathways, yeast is a highly attractive model system to study lipid droplets biogenesis with relevance also to mammalian biology and lipid storage disease. Although yeast cells are rather small (5-10 µm) resolution by confocal laser scanning microscopy in 3d is sufficiently high for identifying morphological alterations as a consequence of mutations or drug treatment; large cell populations that are imaged at once and short generation times (90 min) provide statistically relevant information of morphological alterations and unprecedented options for studies on organelle dynamics, respectively.

Here we present a novel quantification procedure to acquire statistically relevant quantitative information of green-fluorescent-protein (GFP) labelled structures in large and heterogeneous populations of asynchronously growing yeast cells. We present a scripting-customized software tool for processing of microscopic image data and for extraction of spatial objects from volumetric fluorescence data sets. In addition, we introduce an efficient image reconstruction algorithm for automated registration of yeast cells in differential interference contrast (DIC) images and for assignment of isolated image objects to individual cells. We exploit the capabilities of confocal imaging to acquire reliable 3D image data for subsequent quantitative analysis of yeast populations. The application of this imaging based analysis to the nutrient dependent dynamic behaviour of lipid storage compartments (lipid droplets) of yeast will be presented.