Novel imaging-based tools to investigate neutral lipid metabolism in budding yeast

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The synthesis, storage and turnover of neutral lipids, i.e. triacylglycerols (TG) and steryl esters (SE), are critical cellular processes to maintain vital functions of eukaryotic organisms. All eukaryotic species accumulate TG and SE within specialized subcellular structures, which are termed “lipid droplets” (LD). Serving to store excess fatty acids and sterols, LD are essential for a balanced cellular lipid metabolism and energy homeostasis. In mammals, dysregulation or dysfunction of these processes cause highly prevalent metabolic diseases such as obesity, type-2 diabetes or atherosclerosis. Due to the importance of neutral lipid metabolism for vital functions, LD and proteins associated with their biogenesis, metabolism and inheritance are subject of extensive investigations in different model systems including the yeast, *Saccharomyces cerevisiae*.

Yeast is a widely used model organism to study lipid metabolism at the cellular and molecular levels because of the significant functional and structural conservation of proteins involved in related molecular processes. In addition to “traditional” experimental techniques such as chromatographic methods, mass spectrometry, molecular biology techniques or biochemical assays to address lipid related biological processes of yeast, high resolution quantitative fluorescence microscopy has become an important tool for analysis of yeast lipid metabolism [1-3]. A challenge for quantitative analysis of high-resolution yeast image data, however, is the precise extraction of acquired image objects for quantitative assessment and particularly the reliable detection of yeast cell boundaries in dense populations of this unicellular organism. Recent efforts focus on the development of efficient mathematical tools for the underlying image-processing steps such as reconstruction, enhancement and extraction of features [4-6].

Here we present novel software tools and an experimental setup for statistically relevant quantitative analysis of fluorescently labeled yeast LD. The approach includes optimized labeling of LD in heterogeneous cell populations, high-resolution multi-dimensional confocal imaging, a novel software tool for automated segmentation and quantification of imaged LD in batch mode, and non-invasive automated registration of yeast cell boundaries in simultaneously acquired conventional transmission images (fig. 1). We applied the procedure for detailed statistical analysis of the time-depended degradation of yeast neutral lipids and lipid droplets, respectively. The obtained quantitative data provides evidence that lipolysis in yeast is a highly coordinated process consisting of the controlled interplay of specific subcellular positioning of LD and their degradation in distinct stages of the cell cycle.

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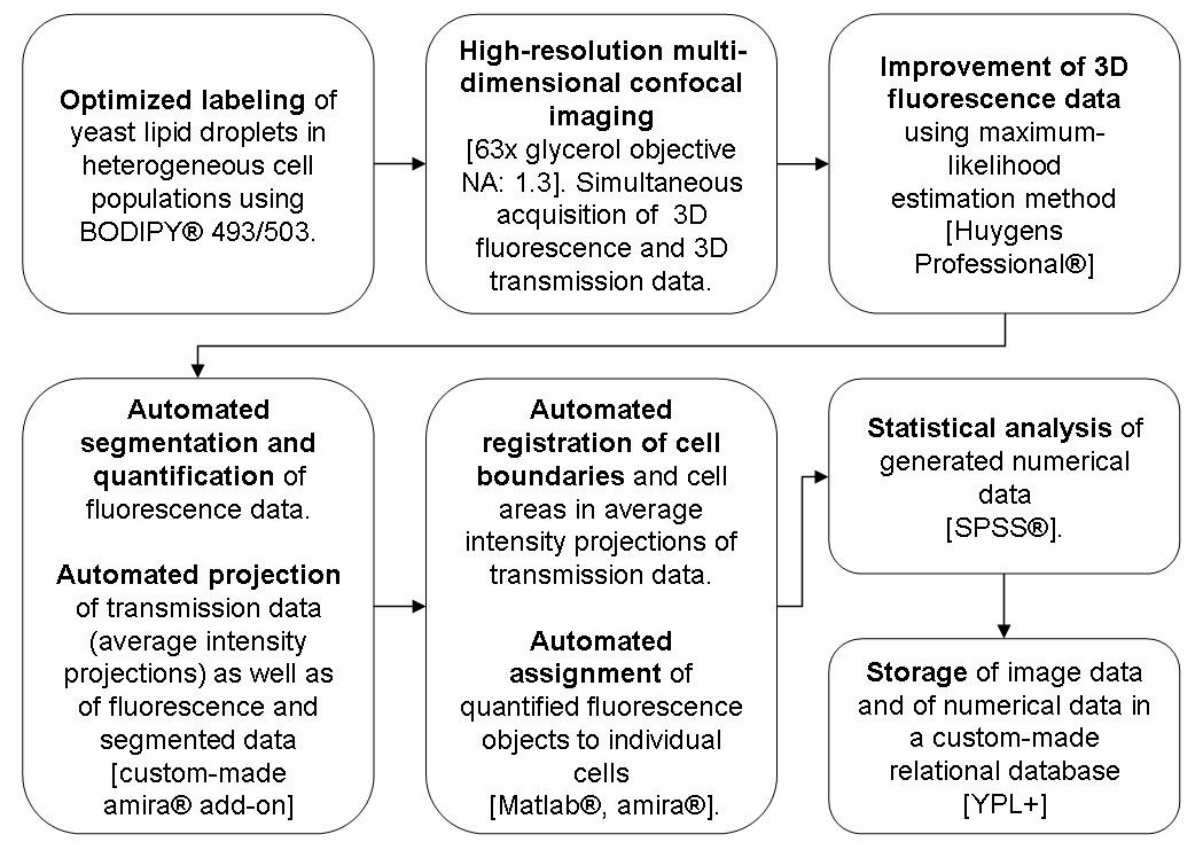

Figure 1. Workflow of the designed procedure for quantitative analysis of yeast lipid droplets.

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