REAL TIME QUANTITATIVE ANALYSIS OF LIPID FLUX BY CONFOCAL SPECTRAL IMAGING OF INTRACELLULAR MICROPOLARITY

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In cells, fatty acids (FA) derived from the diet or through de-novo synthesis can be employed for energy production, energy storage and lipid biosynthesis. Their destination in cells is strictly regulated and depends on cellular energy status and/or biosynthetic requirements, to guarantee the full functionality of the cell. In the absence of demand for fatty acid β-oxidation, FA are stored in the form of triacylglycerols (TAG) in lipid droplets. When required, cells activate a lipolysis pathway that hydrolyses sequentially FA from TAG within LD for use in energy-yielding or biosynthetic reactions. These regulatory pathways are complex sets of sequential reactions that are finely regulated in different cell types. Here we present a real time imaging method for the quantification of the turnover of fatty acids into triglycerides in live cells. We performed confocal spectral imaging of intracellular micropolarity to detect micropolarity variations as they occur in time and at different pixels o microscope images. Acquired data are then analyzed in the framework of the spectral phasors technique. The method furnishes a metabolic parameter, which quantitatively assesses FA-TAG turnover and the activation of lipolysis and storage pathways. Moreover, it provides a polarity profile, which represents the contribution of hyperpolar, polar and non-polar classes of lipids. These three different classes can be visualized on the image at a submicrometer resolution, revealing the spatial localization of lipids in cells under physiological and pathological settings (Figure 1). This new method allows for a fine-tuned, real-time visualization of the turnover of fatty acids into triglycerides in live cells and tissues with submicrometric resolution. It also detects imbalances between lipid storage and usage, which may lead to metabolic disorders within living cells and organisms[1,2]

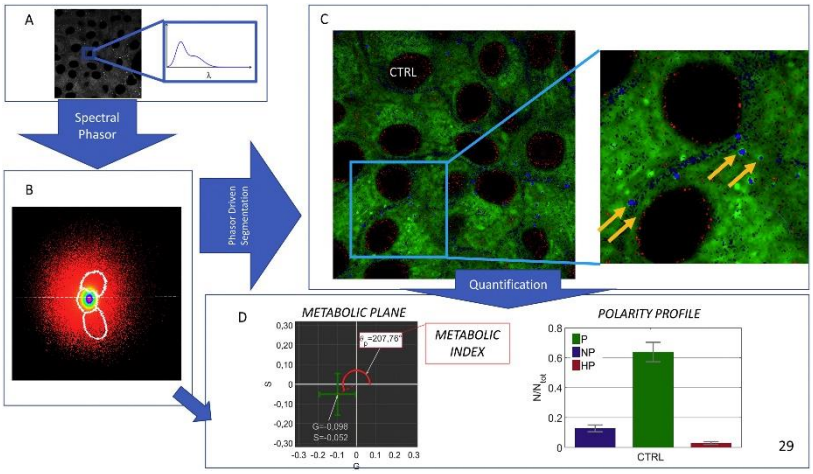

Figure 1. *Outline of the method for quantitative imaging of membrane micropolarity in living cells and tissues by spectral phasors analysis*

References

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