QUANTITATIVE ANALYSIS OF LIPID METABOLISM BY CONFOCAL SPECTRAL IMAGING OF INTRACELLULAR HYDROPHOBICITY.

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ABSTRACT:

Nile Red (9-diethylamino-5H-benzo [α] phenoxazine-5-one) is a fluorescent lipophilic dye characterized by a shift of emission from red to yellow according to the degree of hydrophobicity of lipids. Polar lipids (phospholipids) emit mainly in the red part of the emission spectrum, whereas neutral lipids (esterified cholesterol and triglycerides), which are present in lipid droplets, emit mainly in the yellow part [1].

In this work, in order to improve this analysis based on a qualitative contrast between polar and neutral lipids, we assessed small differences of the hydrophobic strength by a confocal spectral imaging approach. Throughout a global spectral phasor analysis algorithm, the fluorescence spectrum of each pixel in the image is Fourier transformed, and the real and imaginary components of the first and second harmonic of the transform are employed as X and Y coordinates in a scatter plot. The spectral phasor representation allows for real time semi-blind spectral unmixing of the contribution of several lipids and classes of lipids in the image. The Nile Red spectral phasor approach enables discrimination of different lipids in live cells, allowing a fine-tuned real time monitoring of processes as lipid droplets formation and lipid remodeling [2].

REFERENCES:

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