Quantitative Single Cell FLIM Image Analysis of Endogenous Molecules: FLIM-FRET Microscopy

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Fluorescence lifetime imaging microscopy (FLIM) is valuable tool and a sensitive techniques technique to quantitate the cellular response [1-2]. Few FLIM data analysis approaches are available in the literature but none of them is focused on quantitative data analysis of the autofluorescence signal of endogenous molecules to investigate the metabolism. Two- and three-photon excitation FLIM image requires complex data fitting and analysis [3-4]; we explored different ways to analyze the data to match diverse cellular morphologies. After non-linear least square fitting of the multi-photon TCSPC images by the SPCImage software (Becker & Hickl), all image data are exported and further processed in ImageJ. Photon images provide morphological, NAD(P)H signal based autofluorescent features, for which regions of interest (ROIs) are created. Applying these ROIs to all FLIM image data parameters with a custom ImageJ macro, generates a discrete, ROI specific database. Applying this highly automated assay we compared normal and cancer prostate cell lines with respect to their glycolytic activity by analyzing the NAD(P)H-bound fraction (a2%), NADPH/NADH ratio and efficiency of energy transfer (E%) for Tryptophan (Trp). Our results show that this data analysis assay is able to differentiate the effects of glucose stimulation and Doxorubicin in these prostate cell lines by tracking the changes in a2% of NAD(P)H, NADPH/NADH ratio and the changes in Trp E%. In this presentation we will discuss how this data analysis assay could isolate a large, ROI-based data set, reflecting the heterogeneous cellular environment and highlighting even subtle changes – rather than whole cell averages.

References:

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