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Cellular metabolism reveal through NADH autofluorescence lifetime in response to laser engraved geometrical constrains

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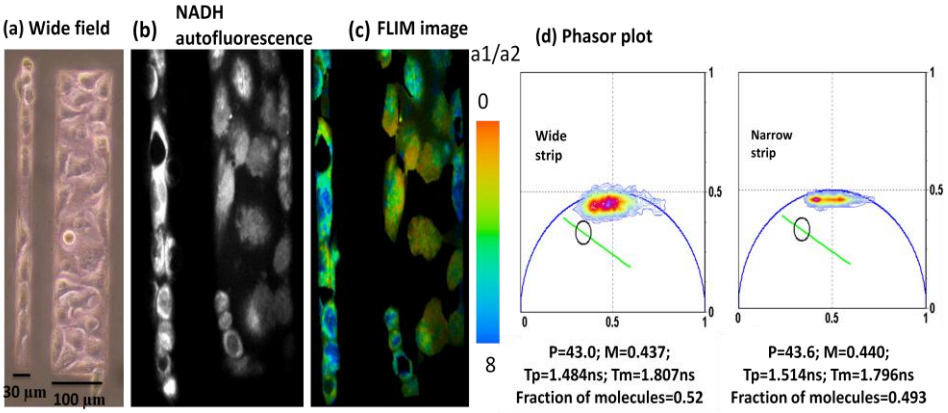
We reports a fluorescence lifetime imaging (FLIM) based mapping of NADH cellular metabolic activity in response to geometrical constrains. A novel Q-switched pulsed neodymium-doped yttrium ortho-vanadate (Nd:YVO_4) laser directed *in situ* micro-fabrication technique was implemented to make the different geometrical micro-domains. The uniquely designed platform was comprised of thin gold coating over a glass surface that functions as a thermal transducer and was overlayered by a cell repellant polymer layer. Micro-patterns were engraved on the platform, subsequently exposing specific cell adhesive micro-domains by ablating the gold-polymer coating photo-thermally [1]. Rectangular micro-patterns of three different sizes of widths 200 µm, 100 µm, 30 µm were made. The morphology of those cells in response to different geometry was observed. Experimental results indicated that cells were aligned along the length of the geometry when the width was comparable to the size of a cell around 30 µm for HeLa cells. In contrast, for micro domain with higher width length, cells were in normal morphology. FLIM technique has been used to study the NADH metabolic activity of the cells in response to these three geometries. Ti:sapphire laser was used to achieve two photon autofluorescence excitation of NADH from an intracellular region. Upon binding to mitochondrial membrane proteins, NADH molecules get associated with the energy generation pathway [2]. Therefore, evaluating the ratio between free and enzyme-bound forms of the fluorophore (NADH) by FLIM can provide further insight into the relative metabolic state of cells [3]. We have used the phasor approach to study the FLIM data and compare the average lifetime (τ_m) and free and enzyme bound ratio of NADH for the cells in different domains. The results indicated that cells those are in narrower domain (30 µm) have lower metabolic activity than those are in wider geometry (100 µm, 200 µm).



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[1] J. Nakanishi, T. Takarada, K. Yamaguvhi, and M. Maeda, "Recent advances in cell micropatterning techniques for bioanalytical and biomedical sciences," Anal. Sci. 67-72 (2008).
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[3] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Plenum Press, New York (1999).