Label Free FLIM-FRET Microscopy

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Imaging has become an indispensable tool in the study of cancer biology and in clinical prognosis and treatment. Microscopic and other intravital optical techniques have evolved over the last decade with the rapid advancement in technology and now allow experimental studies of genetic, molecular, and cellular events *in vivo*. Changes in energy metabolism, mitochondrial functions and of reactive oxygen species have been shown to induce alterations in cellular activities which are different in cancer vs. normal cells. Investigation of the metabolic activity at the molecular level would provide detection of cancer at the early stage. Multiphoton fluorescence microscopy has important advantages over conventional epi-fluorescence or confocal microscopy, especially for imaging thick biological specimens, accompanied by less photobleaching, less photodamage and deeper penetration. FLIM is sensitive to the local microenvironment of the molecule but insensitive to the change in its concentration or excitation intensity. Multiphoton microscopy in combination with FLIM allows us to investigate the molecular behaviour over time and space at different environmental conditions, e.g. metabolic changes in cancer cells. More importantly, we propose to implement FRET in combination of spectral FLIM to quantify the TRP-NADH interaction. FRET is an excited state process and it requires a significant overlap between the donor (TRP) emission and the acceptor (NADH) excitation spectra. Upon excitation of the donor, energy is non-radiatively transmitted to the acceptor via a long-range dipole-dipole coupling mechanism, and the efficiency of energy transfer depends on the donor-acceptor distance and the relative orientation of the donor emission and acceptor excitation dipoles. The binding of NADH and TRP results in changes in the TRP fluorescence lifetime. This relative quenching of TRP by NADH due to FRET measured by 2p FLIM correlates to the level of the cellular metabolic state. The results based on these investigations will be discussed.