**3D multiphoton autofluorescence imaging of fixed tissues: feasibility and potential values for biomedical applications**

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**Abstract:** The value of optical redox imaging (ORI) of cells/tissues based on the intrinsic fluorescences of NADH and oxidized flavoproteins (containing flavin adenine dinucleotide, i.e., FAD) has been demonstrated for potential biomedical applications including diagnosis, prognosis, and determining treatment response. However, the Chance redox scanner (a 3D cryogenic tissue imager) is limited by speed and spatial resolution (~50µm), and tissue ORI using fluorescence microscopy (single or multiphoton) is limited by the light penetration depth. Furthermore, viable or snap-frozen tissues are usually required. In this project we aim to study whether ORI may be achieved for fixed tissue using a state-of-the-art modern two-photon (2P) whole tissue optical scanner (WTOS) that can rapidly acquire 3D images at micron resolution. Tissue specimens of mouse muscle, liver, brain and xenografts of human tumors were harvested and fixed (4% paraformaldehyde) for 24 hours. Scanning by the TissueCyte® 1000 WTOS was conducted under room temperature (NADH channel: 2P excitation 750nm, blue emission filter; FAD channel: 2P excitation 850 or 860nm, green emission filter). Tissue structures (e.g. muscle fibers) are clearly visible and single subcellular structures such as nuclei are readily discernible. Some spatial heterogeneities may indicate new biological information. We will present 2D/3D WTOS images of fixed tissues and discuss the feasibility and potential value of fixed tissue ORI for biomedical applications.

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