

Femtosecond High Resolution Near Infrared Non-linear Optical imaging (NIR-NLOI): Applications in Cell and Tissue Engineering

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INTRODUCTION: Current understanding and future advances in cell and tissue engineering require intricate knowledge of vital cellular, sub-cellular, extracellular (structural) and physiological (functional) changes in two-, three or even four-dimensions (in space and time). Precise high resolution monitoring of engineered cells and 3D tissue constructs can be accomplished by way of vital non-invasive near infrared (NIR) laser based optical techniques. Femtosecond NIR non-linear optical imaging (NIR-NLOI) is now emerging as a safe modality for imaging various living cells and tissue *in vivo* and *ex vivo* with minimal phototoxicity and photodamage. *In situ* NLOI enables simultaneous imaging of various endogenous and exogenous fluorophores by two- or multiphoton excitation¹ using a single NIR (700 – 1100 nm) wavelength. Importantly NLOI facilitates concomitant imaging of extracellular structural components such as collagen/elastin by utilizing their intrinsic optical signals such as second harmonic generation (SHG) as well².

METHODS: We have used the recently established (Mulholland, et al)³ state-of-art femtosecond multiphoton laser scanning optical system modified and configured for the non-invasive 3D imaging of thick biological specimens with microspectral imaging capability. Sub-femtolitre excitation was realised using a high-numerical-aperture (N. A. 1.3) 60x water or 100 x Oil immersion objectives, with a working distance of c.a 1 mm and 200 micrometers respectively. In the Bio-Rad multiphoton dedicated system, a single 670 nm ultraviolet optimised long-pass dichroic mirror, placed in the excitation path within the infinity focus of the microscope head directed fluorescence emission signal in the UV to visible wavelength range towards non-descanned bi-alkaline and multi-alkaline Photomultiplier Tubes (PMTs).

Images were loaded either into Imaris 4.2 or the LaserSharpe software for processing and analysis. The Imaris software suite offers a range of highly advanced 3D image processing capabilities including volume rendering, orthogonal plane

projections, and statistical analysis of three-dimensional surface-rendered objects. Each of these image analysis modalities was used to evaluate the *ex vivo* tissue samples and 3D cultures of stem cells and chondrocytes as well.

RESULTS:

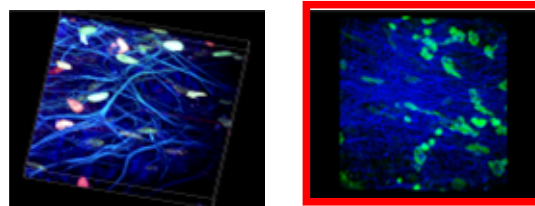


Fig. 1: Cell morphology and organisation of collagen in *ex-vivo* heart valve of pig (left) and mouse spleen (right).

DISCUSSION & CONCLUSIONS: During the meeting we will briefly describe the NIR-NLOI system-setup and present a few of the recent applications of this state-of-art imaging technology for non-invasive monitoring of (1) 3D orientation of cells and collagen in various living and *ex vivo* tissues by way of second harmonic generation imaging, (2) *in situ* changes in cell morphology and viability of chondrocytes cultured in 3D scaffolds; (3) 3D imaging of human bone marrow stem cells expressing eGFP, and (4) the unique possibility of optical gene transfer into target cells.

REFERENCES: ¹W. R. Zipfel., R. M. Williams, & W. W. Web (2003) *Nature Biotech.* **21**: 1369-1377. ² P. J. Campagnola & Loew, L. M, (2003) *Nature Biotech* **21**: 1356-1360. ³W. J. Mulholland, et al (2006 Apr 27). *J. Invest. Dermatol.* (Epub ahead of print)

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