

## INTRAVITAL and *EX VIVO* MICROVASCULAR IMAGING USING DUAL FLUORESCENCE MULTIPHOTON MICROSCOPY

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Altered microvascular geometry is an important factor in abnormal oxygen transport in a variety of disease states including diabetes, cancer and sepsis (the latter being the leading cause of death in intensive care units in North America). With conventional intravital microscopy, analysis of the microcirculation is generally limited to surface (20-30 $\mu$ m) vessels and often fails to provide information on complete microvascular units – including feeding arteriole, capillary bed and collecting venule. Here we present preliminary data using a dual fluorescence laser scanning multiphoton microscopy (MPM) technique for the identification and quantification of microvascular geometry and perfusion at tissue depths of 150-200 $\mu$ m in skeletal muscle, heart and brain tissues. 3D angiograms are used to quantify functional microvascular geometry. Transgenic mice, constitutively expressing endothelial green fluorescent protein (GFP), were injected via the tail vein with Q-Tracker non-specific 5000 MW PEG coated fluorescent nanocrystals (655nm Quantum dots, QDs) and imaged using MPM. GFP endogenously labeled the microvascular networks while QDs acted as a microvascular perfusion marker.

900nm fs pulses (Ti-Sapphire laser) were focused through a 20x/0.7 NA objective (Leica AOBS SP2 microscope system) onto the exposed hindlimb skeletal muscle of anesthetized mice and later on harvested, but intact, clamped heart and brain tissue. Fluorescent images were acquired at 400Hz/3frame averages. Non descanned fluorescent signal was detected by two PMTs fitted with 525/50nm (GFP-signal) and 655/40nm (QD-signal) emission filters, respectively. Preliminary image analysis indicated that the correspondence between fluorescent GFP and QD signal was closest to unity in the heart. The difference in QD/GFP signal reflects differences in expression of endogenous GFP, especially in skeletal muscle, the extent of microvascular perfusion and the microvascular hematocrit. This dual fluorescence MPM imaging technique offers a promising approach to investigate abnormal microvascular perfusion in a number of disease states. Financial Support: Heart and Stroke Foundation Canada (HSFC), Michael Smith Foundation for Health Research, CIHR/HSFC IMPACT Fellowship.

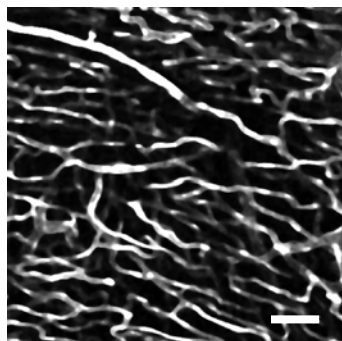


Figure 1. GFP image

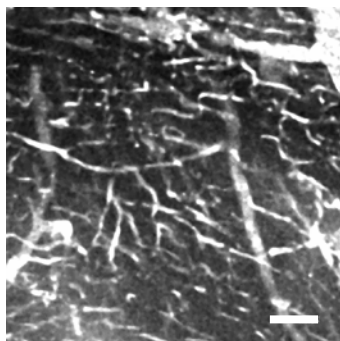


Figure 2. QD655 image

Fluorescent GFP and QD MPM images of microvascular networks in mouse heart. Figure 1: GFP image showing microvascular geometry. Figure 2: QD655 image showing perfused vessels. Images were acquired simultaneously at a depth of 70 microns. Bar = 45 microns.