Introduction: The cerebral metabolic rate of oxygen consumption (CMR\textsubscript{O2}) is a key factor in brain function. Oxygen is consumed in the mitochondria of cells and is delivered to tissue by blood, mostly at the capillary level. Hemoglobin-sensitive imaging methods, such as BOLD fMRI, partly rely on this sensitivity to map brain function. The dynamic relationship between brain function and oxidative metabolism remains largely unknown because direct measurements of tissue CMR\textsubscript{O2} are very difficult. Changes in the intrinsic auto-fluorescence of tissue have been shown to stem from changes in metabolism since much of it stems from proteins that participate in the metabolic cascade (1,2,3). In this work, the temporal evolution of the changes in cellular oxidative metabolism, tissue oxygen tension and blood oxygenation were investigated using flavoprotein auto-fluorescence imaging (FAI), oxygen microelectrodes and deoxyhemoglobin-sensitive optical imaging of intrinsic signal (OIS), respectively. The impact of these findings on MRI-based CMR\textsubscript{O2} measurements is discussed.

Methods: Nine Sprague-Dawley rats (200-500g) were used in this study with experimental protocols approved by the University of Pittsburgh’s IACUC. The animals were anesthetized with isoflurane (5% for induction, 2% for surgery) via vaporizer. Catheters were inserted in the femoral artery and vein. An area 5x7mm on the skull centered over the somato-sensory cortex was thinned and removed. The dura was resected and the exposed area was resealed with 1% agarose gel. Upon completion of the surgeries, the isoflurane level was decreased to 1.1-1.4% for the remainder of the experiment. The default condition established after the surgery was considered to be the control condition in which stimulation evoked both vascular (CBF) and metabolic responses (CMR\textsubscript{O2}). In order to evoke only metabolic responses, a vasodilator (sodium nitroprusside) was used to dilate the blood vessels and suppress the CBF and CBV responses (4,5). This condition has been demonstrated to not alter brain electrical activity (4,5) and the experimental data were recorded under this condition. To evoke a sensory response, two needles were inserted between digits 2 and 4 of the left forepaw. Forelimb stimulation consisted of 1ms, 1.5mA pulses delivered at a frequency of 3 Hz for 15 s every 45 s or at 12 Hz for 4 s.

Results and Discussion: A fast and robust increase in FAI signal was observed with stimulation onset due to increases in CMR\textsubscript{O2} on average reaching 90% of its peak in 1.5s (Fig. 1A, 2). The increases were temporally sustained and spatially consistent during stimulation. A significant decrease was also observed following stimulation. Significant but slower decreases in tissue P\textsubscript{O2} were observed during stimulation due to increases in CMR\textsubscript{O2}, on average reaching 90% of its minimum in 4.1s (Fig. 1B). Significant decreases in blood oxygenation were observed using OIS during stimulation, similar to the decreases in tissue P\textsubscript{O2}, and on average reaching 90% of its minimum in 4.4s (Fig. 1C). The FAI and OIS maps were found to be highly coincident although the FAI maps spanned a slightly larger area.

Fast increases in cellular oxidative metabolism were observed with increases in neural activity using FAI. The results showed that the increase in CMR\textsubscript{O2} prompted the need for oxygen from the surrounding tissue and blood shortly after, indicating that, temporally, the dynamic changes in blood oxygenation (e.g. BOLD fMRI) reflect the average changes in tissue oxygen but less so the changes in cellular CMR\textsubscript{O2}.