- **Title:**
  Simultaneous two-photon imaging of cerebral oxygenation and capillary blood flow in atherosclerotic (ATX) mice module
- **Trigger:**
  The link between major vascular events, such as acute stroke, and their subsequent impact on brain function has long been established. However the impact of gradual changes in vascular function, occurring over a lifetime with age or due to long-term habits, is more difficult to characterize. There is mounting evidence that modifications in vascular function operating on longer time scales and with increasing age have subtle sub-clinical effects and contribute to significant health burden. In epidemiologic studies of vasculopathies, aco-occurrence of neurological manifestations is observed (mood changes, dementia and migraine). Cognitive impairment is also often prodromal to dementia in patients with risk factors for cardiovascular diseases. In neurological diseases and degenerative dementias, such as Alzheimer disease (AD), there is an active debate on the origin of the pathology (neural or vascular) and vascular changes remain a staple of these pathologies.
  Brain tissue is strongly dependent on a continuous delivery of nutrients and oxygen to maintain its activity. With aging, brain hypo-perfusion, vessel rarefaction and tissue volume reductions suggest that oxygen delivery may be compromised. A reduction in blood flow and ensuing tissue hypoxia may then lead to tissue adaptation through the hypoxia-inducible factor-1 (HIF-1). Associated increase in inflammation, Blood Brain Barrier (BBB) permeability, ion redistribution and oxidative stress are then hypothesized to contribute to secondary injury potentially leading to neuronal death. Brain tissue oxygenation and oxygen delivery may thus yield potent biomarkers of neurological diseases, either to capture deviations from normal physiology or to monitor treatment efficacy. Indirect indicators of resting oxygen delivery obtained with positron emission tomography (PET) display increased oxygen extraction fractions (OEFs) with age in humans [1]. Non-invasive MRI techniques to quantify CMRO2 are also emerging [2], [3] and support the potential of oxygen consumption as a biomarker for neurological diseases [4]. In a recent study using multispectral optical imaging in otherwise healthy aging animals, we showed reduced flow-volume and flow-metabolism ratios during stimulation suggesting altered oxygen delivery with age [5]. With the long-term aim of finding a substrate for the hypoxia-driven tissue damage hypothesis, a relation between these macroscopic imaging observations and explicit microscopic oxygen delivery measures is required. However, such information on oxygen delivery at the level of the neurovascular unit is lacking.
  Until recently, our ability to probe tissue oxygenation at the microscopic level was limited to punctual measures from oxygen electrodes [6]. As such, very limited data exist on the impact of vascular and neurological diseases on brain tissue oxygenation. The recent emergence of oxygen-quenching phosphorescent dyes with high two-photon absorption cross-sections has given new opportunities to investigate oxygen delivery in these pathologies in much greater details [7], [8]. In turn, linking measured microscopic changes to macroscopic phenomenon also has the potential to link clinical imaging biomarkers with specific micro-vascular remodeling processes.
- **Frontier of knowledge**
  Oxygen and glucose supply is essential for normal functioning of the brain and needs to be finely regulated, both spatially and temporally, to meet metabolic demand. Neurovascular coupling describes how local neuronal activity through the coordinated

action of smooth muscle cells, endothelial cells, and pericytes, adjusts local cerebral blood flow (CBF) to meet these changing needs. In hypertension, Alzheimer's disease, age and stroke, documented disruptions in neurovascular coupling suggest that CBF may become uncoupled with the underlying tissue metabolic needs, with potential deleterious consequences on cognition. Aging and vascular diseases are associated with changes in brain vascular characteristics including thickening of endothelial basement membranes, variable capillary diameters, reduced capillary density, and pericyte loss. These changes are exacerbated in hypertension and Alzheimer's disease with the addition of pericyte degeneration and swelling of the endothelium surrounding astrocytic end-feet. Persistent pericyte constriction has been implicated in impairing capillary reflow during arterial reperfusion following ischemic stroke. All of these results suggest a close association between cerebral vascular dysfunction and the onset and progression of different neurodegenerative diseases. Oxygen delivery and consumption, quantified by CBF, OEF and the cerebral metabolic rate of oxygen (CMRO2), are central to this association and potentially represent important markers of healthy versus unhealthy aging. To establish these markers in the clinical setting, investigating and confirming expected age-related changes in oxygen delivery at the micro-vascular level is essential.

- **Objectives**

  AIM1: Quantify in control (WT) and atherosclerotic mice (ATX), the changes in the spatial distribution of resting tissue oxygenation during aging

  AIM2: Assess and investigate changes in microscopic tissue oxygenation during brain activation

  AIM3: Establish a link between macroscopic clinical imaging using fMRI-BOLD and direct microscopic measures of flow and oxygen distribution in micro-vascular networks

- **Expected results**

  Hypothesis **1.1**: Older WT and ATX mice will show lower total tissue PO2 at rest when compared to young controls and increased microscopic tissue PO2 spatial heterogeneity.

  Hypothesis **1.2**: In these older mice, the tissue PO2 radial profile from pre-capillary arterioles will display a lower ordinate value at the vessel wall and slower decay with radial distance.

  Hypothesis **2.1**: Older WT and ATX mice will display a lower flow/tissue-PO2 ratio change during stimulation signaling decreased vascular O2 reserve when compared to young WT controls.

  Hypothesis **2.2**: In these older mice, investigation of the spatial distribution of PO2 in activated tissue will be associated with micro-pockets of hypoxia at high stimulation intensities.

  Hypothesis **3.1**: Macroscopic imaging observations of increased OEF, and decreased flow-metabolism ratio changes during aging will be predicted with biophysical modeling of microscopic measures.

- **Originality**

  Very little is known about the link between vascular dysfunction, brain tissue oxygenation and pathogenesis. This knowledge void has thus far been mostly due to a lack of techniques to measure brain tissue oxygenation in relation to blood flow in micro-vascular environments. Our study will be the first to provide such information. Furthermore, validated biophysical model will root observations of macroscopic signal changes into known physiological modifications. Linking both spatial scales, we will be

able to predict the impact of specific micro-vascular modifications on imaging data and validate these changes through hypothesis-driven research. While validated on two animal models, technological and modeling advances will offer new opportunities to investigate similar biomarkers in other models through collaborations.

- **Anticipated impact**

  In 2010, the total number of people with dementia was estimated at 35.6 million worldwide with a projection to double every 20 years. According to current demographic projections, one Canadian in 4 will be 65 years or older in 2031 (Statistics Canada, 2005). While new therapies are being investigated and are in various stages of clinical trials, no treatment is currently available to cure or even alter the progressive course of age-related dementia. Early detection and the identification of sub-clinical biomarkers form the cornerstone of a successful intervention. A case for focusing on vascular changes and oxygenation is supported by studies displaying a high rate of incidental vascular findings which in turn correlate to cognitive dysfunction. The use of oxygen delivery as a biomarker is supported technically by the emergence of fMRI and fNIRS techniques quantifying OEF and CMRO2 reliably.

- **Anticipated risks and approach to manage/mitigate them**

  1-While the somatosensory cortex is not the most affected brain region during aging when compared to others e.g. hippocampus, the technical challenges associated with imaging deeper regions and the potential for tissue damage guided us to investigate the somatosensory cortex. That being said, this region has been shown to be very responsive when investigated by our team in ATX mice and during the aging process. We are hence comfortable with pursuing our studies using this anatomic region.

  2-The level of anesthesia affects tissue PO2 at baseline and needs to be controlled. Monitoring anesthesia with brain electrodes and a power spectrum analysis in specific bands will be investigated.

  3-Our focus in these studies is the vascular component of the neurovascular unit; we are aware that other manifestations of brain dysfunction may originate in astrocyte or neuronal signaling but these are outside the scope of this proposal, which aims to focus on oxygenation. However, we expect that through collaborations, some of these will be addressed.

- **Required resources (the main ones)**

  Two-photon absorption cross-sections microscope; macroscopic clinical imaging using fMRI-BOLD; control (WT) and atherosclerotic mice (ATX) mice; positron emission tomography (PET) display; oxygen delivery probes.

- **Proposed timeline(Months)**

Combine 2 Photon microscope and OCT, validate on phantoms and control animals.	3M
Simulations and modeling on control scans	3M
Start 50% WT/ATX animals: G1 (mid-year), continuous monitoring	6M
Start 50% WT/ATX animals: G2, continuous monitoring	6M
Validate forepaw on control animals in MRI, sequence optimization	3M
Full cohort data analysis (PD1 for microscopy, PD2 for MRI	3M
Validation of BOLD modeling with MRI data	6M
Data analysis and consolidation, papers	6M

- **References**

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