Long-Term Monitoring of Capillary Flow to Measure Hypoxic Effects of Capillary Flow Disruptions

John Giblin[1,2], Kıvılcım Kiliç[1,2], John Jiang[1], Anderson Chen[1], Baoqiang Li[3], Sava Sakadžić[3], Anna Devor[1,2], David A. Boas[1,2]

[1]*Boston University Neurophotonics Center Boston, MA*
[2]*Boston University Department of Biomedical Engineering Boston, MA*
[3]*Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, United States*
Author e-mail address: jgiblin@bu.edu

Abstract: Cerebral blood flow is critical to supporting healthy brain metabolism. Here we demonstrate the ability to monitor capillary flux and oxygenation in up to 20 capillaries using Phosphorescence Lifetime Imaging (PLIM) for 10 minutes.

Introduction:
Cerebral blood flow is highly regulated in order to support proper brain function. Loss of flow for even brief periods can result in permanent damage. Recent work has shown that increases in transient flow disruptions at the capillary level can have deleterious effect on brain function [1,2]. While the reduction of these transient stalls in capillary flow was associated with improved cognitive performance [2], little is known about the impact of an individual "stall" on local oxygenation and microcirculatory flow. Phosphorescent Lifetime Imaging is well suited to address these questions [3,4]. A recently developed probe has been shown to be able to precisely measure the intravascular partial pressure of oxygen while also extracting capillary flux [5,6]. By monitoring sets of nearby capillaries for long periods of time, we can capture these stalling events and quantify their impact on microcirculation.

Methods:
Chronic cranial windows were surgically implanted in wild type mice as described previously (Erdener,2017) and allowed to recover for at least three weeks before acclimation to being head fixed. Baseline angiograms of ~800x800x400µm were acquired with two photon microscopy using 70kDa Alexa-680 Dextran. Mice were briefly anesthetized with 3% isoflurane and 0.1 mL of 34 µM Oxyphor-2P was injected retro-orbitally. For anesthetized experiments, 1.5% isoflurane mixed with air was maintained throughout the experiment. In awake experiments, animals were then briefly allowed to recover from anesthesia before being head fixed for imaging. 12-20 capillaries within the specified region of interest were selected and repeatedly and sequentially measured for 300 ms each for 10 minutes for each set of capillaries. Phosphorescence was excited by 10 µs of laser power at 950 nm followed by 290 µs of photon counting and binning based on arrival time. This was repeated 1000 times at each point and fit to a single exponential model for extraction of lifetime. Figure 1 shows the fitting of decays for extraction of pO2.

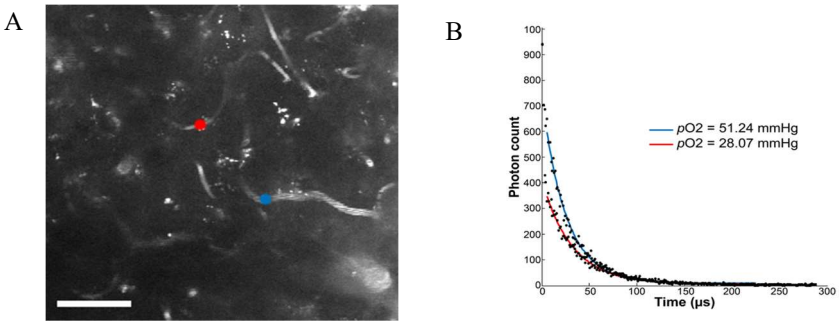

Figure 1 (A) Intensity image used to identify and select capillaries (B) Example of Phosphorescent decay fit to determine pO2

Results:

Figure 2 shows representative data capturing the stoppage of capillary flow. Points in a subregion are selected and monitored for 10 minutes. Since the Oxyphor-2P is contained only in the plasma, RBC flux is measured as dips in photon counts as RBCs pass through the point measurements. Large drops in measured pO2 are seen when RBC flux stops in a given capillary. Otherwise, intravascular pO2 appears to be very stable in capillaries with stable flux.

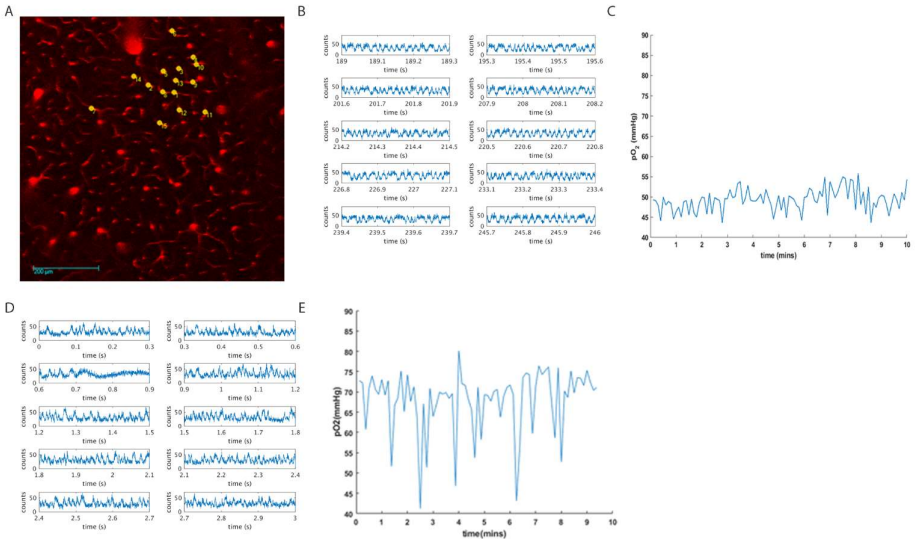

Figure 2 (B) Intensity scan used to identify and select capillaries for longitudinal imaging (B) Example intensity fluctuations as RBCs pass through the measurement pointing demonstrating stable flux (C) pO2 trace of the corresponding point in B show stable pO2 associated with stable uninterrupted flux (D) Intensity trace of a capillary who experiences an interruption in intensity dips due to a loss of flow (E) Corresponding pO2 trace of the capillary whose flow is regularly interrupted. The first large drop in pO2 was measured during the time in (D) where there is no RBC flux

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