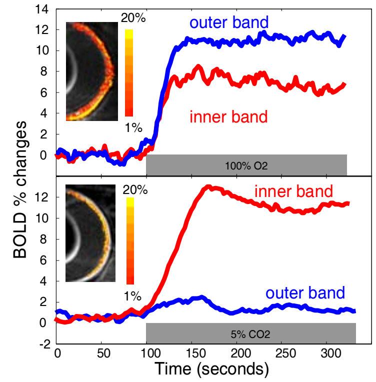
Imaging oxygenation, blood volume, and blood flow changes in the retina

H. Cheng[1], M. Kohl-Bareis[2], L. Wang[1], G. Nair[1], T. Nagaoka[1], and T. Q. Duong[1]

[1]Yerkes Imaging Center, Neurology, Emory Univesity, Atlanta, GA, United States, [2]University of Applied Sciences Koblenz, Remagen, Germany

Introduction We recently reported a surprising observation that the two vascular layers bounding the retina responded differently to 100% O2 and 5% CO2 stimuli using layer-specific BOLD fMRI [1] (see figure on the right). The BOLD response to 100% O2 was larger in the outer layer than the inner band, whereas the BOLD responses to 5% CO2 was larger in the inner band (retinal vascular layer) than the outer band (choroidal vascular layer), suggesting differential blood flow and oxygenation regulation in the two vascular supplies in the retina. The likely explanation is that oxygen strongly vasoconstricts retinal vessels (60% compared to 10% in the brain) but it has little or no effect on the choroidal vessels [2]. Similarly, CO2 strongly vasodilates retinal vessels but has little effect on the choroidal vessels [2].

In this study, we developed and applied *combined* laser speckle imaging (LSI) and intrinsic optical imaging (IOI) to measure blood flow and oxygenation in the rat retina in order to corroborate our BOLD fMRI findings.



Methods Six male rats (300-400g) were imaged under ~1% isoflurane, paralyzed and mechanically ventilated. End-tidal CO_2, heart rate and O_2 saturation and rectal temperature were maintained within normal physiological ranges unless purposefully altered. 100% O_2 and 5% CO_2 challenges were used with air as baseline.

A combined LSI and IOI modality was developed by modifying a commercial video imaging system (Imager 3001). Images of LSI were obtained using a diode laser beam (780nm) and IOI was acquired utilizing four different wavelengths (546, 605, 630 and 700nm) in an interleaving fashion. Images were acquired at 25Hz at 7 μm resolution with an image area of 3-4 mm. Blood flow (BF) changes detected by the LSI was computed as described in [3]. A modified Lambert-Beer law with differential path-length factors for different wavelengths, which was proposed by Kohl et al [4], was used to calculate changes in deoxy-Hemoglobin (Hb), oxyhemoglobin (HbO2) and total hemoglobin (HbT, blood volume) for IOI

Results LSI revealed that BF decreased under 100% O2 and BF increased under 5% CO2 relative to baseline (air) over the entire retina [Fig.1]. Vessel diameters were also observed to increase under CO2 and decrease under 100% O2. Under 100% O2, IOI showed a concentration decrease in Hb, increase in HbO2 and decrease in HbT. Under 5% CO2, IOI showed a decrease in Hb, increase in HbO2 and HbT [Fig.2].

Discussion We developed a combined LSI and IOI approach to cross validate our MRI findings. The implementation of these optical techniques on rat retinas was challenging because of the poor optical property of the rat's lens and small pupils. LSI provides two-dimensional BF images without the need for line scanning and it has high spatio-temporal resolution comparing with scanning laser Doppler technique. A modified Lambert-Beer law with a wavelength dependent differential path-length factors was used in the calculation of Hb and HbO2 because it corrects for the absorption and scattering properties and its effect on the optical path length [4]. While we can clearly identify visible retinal vessels which showed a response to O2 and CO2 challenges as expected, a major limitation is that LSI and IOI cannot unambiguously resolve the choroidal vessels. The observed LSI and IOI signal changes in general arise from both the retinal and choroidal vasculatures.

Conclusion LSI and IOI confirmed part of our layer-specific fMRI findings. While coupling of blood flow, oxygenation and metabolism in the brain has been well described, experimental evidence of such coupling in the retina was reported only recently and blood flow regulations of the retinal and choroidal vasculatures appear unique. Combined LSI and IOI along with fMRI study could help better understand the neural-vascular coupling in the retina under various perturbed conditions. These techniques can also apply to study retinal diseases. Further improvement in image quality and sensitivity are expected. Future studies will involve adaptive optics, con-focal techniques to resolve different retinal layers, better understanding of the optical/fMRI signal sources, visual stimulation and retinal disease models.

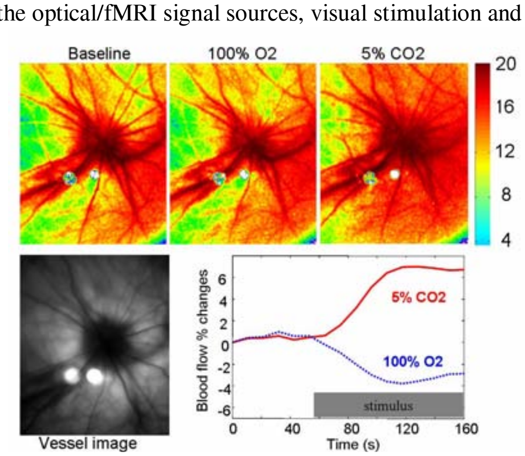


Fig 1. Retinal laser speckle imaging at 7 μm. Blood flow decreased under 100% O2 but increased under 5% CO2 relative to baseline.

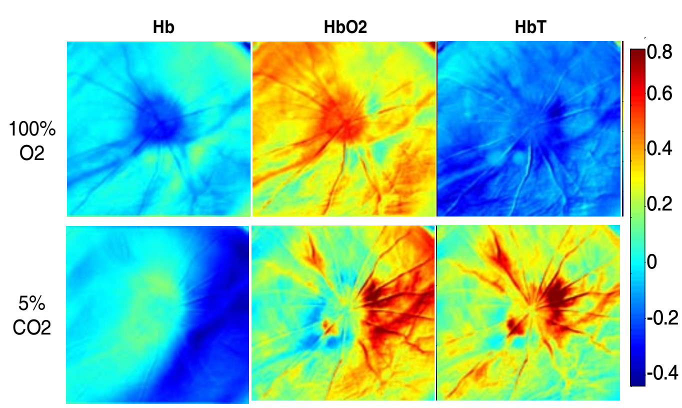


Fig 2. Retinal intrinsic optical imaging at 7 μm. Hb, HbO2 and HbT responses to 100% O2 and 5% CO2.

References.

[1] Cheng et al., PNAS 130:17525 (2006).

[2] Trokel S, *Arch Ophtham* 38: 838 (1965).

[3] Cheng et al., Phys Med Biol 49:1347 (2004).

[4] Kohl et al. Phys Med Biol 45:3749 (2000).