

# Applying optical imaging to study neurovascular coupling in cerebral cortex: from populational scale to single-cell single-vessel measurements

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**Abstract:** We use a suite of optical imaging technologies in conjunction with electrophysiological recordings and fMRI to address the question of coupling between the hemodynamic response and the underlying brain neuronal activity.

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## 1. Introduction

Optical imaging methods allow full-field measurements of hemodynamic, metabolic and neuronal activity *in-vivo* with spatiotemporal resolution well beyond other neuroimaging modalities such as fMRI, PET or MEG. Optical methods can be easily combined with standard electrophysiological recordings or can be performed in MR environment without interference with either electrical or MR signals. In our group we use optical imaging in conjunction with other imaging/recording methods aiming to bridge the gap between human non-invasive fMRI studies measuring the hemodynamic response and the underlying electrical activity of neuronal circuits in the brain.

## 2. Methods

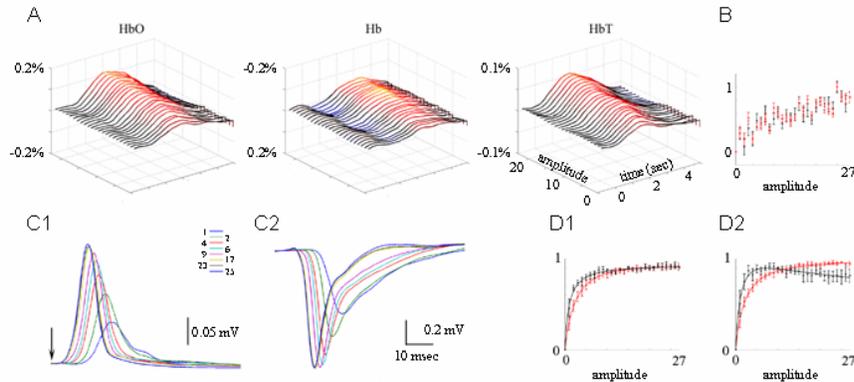
Our model system is rat cerebral cortex. We measure changes in neuronal and hemodynamic activity in response to a somatosensory stimulus. We use the following optical imaging technologies: (1) multi-wavelength (spectroscopic) imaging of deoxyhemoglobin (Hb), oxyhemoglobin (HbO) and total hemoglobin (HbT) [1-4], (2) laser speckle imaging of blood flow [3-5], (3) voltage-sensitive dyes imaging of neuronal activity, (4) Laminar Optical Tomography of Hb, HbO and HbT [6], (5) 2-photon imaging of single-vessel diameter and flow velocity [7].

These optical methods are combined with simultaneous electrophysiology using single electrodes and electrode arrays [1,2], or simultaneous fMRI.

## 3. Results and Discussion

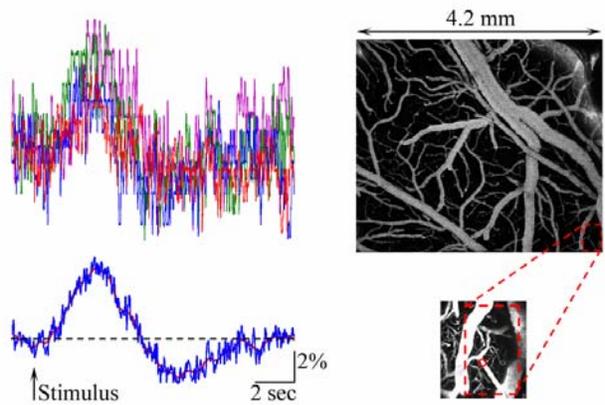
Simultaneous optical imaging and electrophysiology. Using simultaneous multi-wavelength optical imaging and electrophysiological recordings, we have demonstrated a strongly nonlinear relationship between electrophysiological measures of neuronal activity and the hemodynamic response (Fig. 1). Our data show that a point hemodynamic measure is influenced by neuronal activity across multiple cortical columns. As a result, positive hemodynamic response in one cortical column might be explained not only by neuronal activity in that column but also in the neighboring columns. This finding helps to explain the nonlinearity mentioned above. We also demonstrate that the hemodynamic response can be well approximated by space-time separable functions with an antagonistic center-surround spatial pattern. Using voltage-sensitive dyes we showed that the center-surround hemodynamic activity does not correspond to neuronal excitation surrounded by inhibition.

While multi-wavelength and laser speckle imaging methods do not possess depth resolution, a newly developed laminar optical coherence tomography (LOT) technology allows measuring of the hemodynamic response in 3D [6]. We used a combination of LOT, electrophysiological recordings and vascular corrosion casts to study the hemodynamic response in identified vascular compartments; arterial, venous and capillary. Our results show a smaller volume changes and significantly later onset times in veins, compared to arteries and capillaries. Arterial components show larger volume changes and earlier returns to baseline than capillary and venous compartments.



**Fig. 1. Nonlinear relationship between electrophysiological measures of neuronal activity and the hemodynamic response.** A. Timecourses of HbO, Hb and HbT in response to a deflection of a single whisker using different deflection amplitudes. B. Integral HbO (red) and HbT (black) responses as a function of stimulus intensity. The error bars reflect the inter-subject standard error. C. An example of MUA (C1) and LFP (C2) responses. Responses to different stimulus amplitudes (inset) are superimposed. The arrow denotes stimulus delivery. D. MUA (D1) and LFP (D2) peak (red) and integral (black) responses as a function of stimulus amplitude. The data were averaged across the same subjects as in A/B. The curves were fitted using the function  $ax/(1-bx)^c$ .

2-photon imaging of the hemodynamic response at the level of single vessels. We measured changes in vessel diameter and velocity of blood flow in single arterioles and venules in the rat cortex in a response to a somatosensory stimulus. Our first results are in agreement with LOT data and show profound dilation changes in the arterioles and velocity changes in the venules.



**Fig. 2. Single-vessel measurements of the hemodynamic response using two-photon microscopy.** Top left: a diameter change of a single arteriole in response to 2 sec electrical forepaw stimulation (3Hz, 1mA). Line scanning was performed at 1300 Hz across the vessel. Four individual stimulus presentations are superimposed. An average is shown below. Right: Maximal intensity projection of an image stack 0-300  $\mu$ m cortical depth. A zoomed-in image below features an arteriole corresponding to the measurements in A and B (red circle).

Optical imaging in MR environment. Simultaneous optical and MR measurement can serve as a link between electrophysiological recordings and fMRI measurements. Optical imaging will also allow direct 2D recording of neuronal activity performed simultaneously with fMRI. Our first results show a good spatial correspondence between the BOLD fMRI signal and optically recorded Hb. BOLD signal peaks earlier than Hb possibly reflecting a contribution from HbT.

#### 4. Conclusions

Optical technologies in conjunction with other imaging/recording modalities and fluorescent labeling are an important tool for studying *in-vivo* neuronal and vasular activity.

#### 5. References

1. Anna Devor, Andrew K. Dunn, Mark L. Andermann, Istvan Ulbert, David A. Boas, Anders M. Dale, "Coupling of total hemoglobin concentration, oxygenation, and neural activity in rat somatosensory cortex," *Neuron* **39**, 353-359 (2003).

2. Anna Devor, Istvan Ulbert, Andrew K. Dunn, Suresh N. Narayanan, Stephanie R. Jones, Mark L. Andermann, David A. Boas, Anders M. Dale, "Coupling of the cortical hemodynamic response to cortical and thalamic neuronal activity," *PNAS* **102**, 3822-3827 (2005).
3. Andrew K. Dunn, Anna Devor, Hyrunissa Bolay, Mark L. Andermann, Mike A. Moskowitz, Anders M. Dale, David A. Boas, "Dynamic imaging of cerebral blood flow using laser speckle," *Opt Lett* **28**, 28-30 (2003).
4. Andrew K. Dunn, Anna Devor, Anders M. Dale, David A. Boas, "Spatial extent of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex," *Neuroimage* **27**, 279-290 (2005).
5. Shuai Yuan, Anna Devor, David A. Boas, Andrew K. Dunn, "Determination of optimal exposure time for imaging of cerebral blood flow changes during functional activation using laser speckle contrast imaging," *Applied Optics* **44**, 1823-1830 (2005).
6. Elizabeth M. C. Hillman, David A. Boas, Anders M. Dale, Andrew K. Dunn, "Laminar optical tomography: demonstration of millimeter-scale depth-resolved imaging in turbid media", *Opt Lett* **29**, 1650-1652 (2003).
7. David Kleinfeld, Partha P. Mitra, Fritjof Helmchen, Winfred Denk, "Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layers 2 through 4 of rat neocortex," *PNAS* **95**, 15741-15746 (1998).