Non-invasive real-time visualization of multiple cerebral hemodynamic parameters in whole mouse brains using five-dimensional optoacoustic tomography

Sven Gottschalk, Ph.D.[1], Thomas Fehm, M.Sc., Dipl.-Phys. Univ.[1,2], Xosé Luís Deán-Ben, Ph.D.[1], and Daniel Razansky, Prof.[1,2,*]

[1]Institute for Biological and Medical Imaging (IBMI), Helmholtz Center Munich, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[2]Faculty of Medicine, Technical University of Munich, Ismaninger Straße 22, 81675 Munich, Germany

*Corresponding author:

Prof. Dr. Daniel Razansky

Helmholtz Center Munich, Institute for Biological and Medical Imaging (IBMI)

Ingolstädter Landstraße 1, Building 56

85764 Neuherberg, Germany

Phone: +49-(0)89-3187-1587

Fax: +49-(0)89-3187-3008

Email: dr@tum.de

Acknowledgements:

The research leading to these results has received funding from the European Research Council under grant agreement ERC-2010-StG-260991.

Running title: Non-invasive real-time 5D-optoacoustic neuroimaging

Disclosure: Nothing to disclose.

Abstract

Current functional neuroimaging methods are not adequate for high-resolution whole brain visualization of neural activity in real-time. Here, we demonstrate imaging of fast hemodynamic changes in deep mouse brain using fully noninvasive acquisition of five-dimensional optoacoustic data from animals subjected to oxygenation stress. Multispectral video-rate acquisition of three-dimensional tomographic data enables simultaneous label-free assessment of multiple brain hemodynamic parameters, including blood oxygenation, total hemoglobin, cerebral blood volume, oxygenized and deoxygenized hemoglobin, in real-time. The unprecedented results indicate that the proposed methodology may serve as a powerful complementary, and potentially superior, method for functional neuroimaging studies in rodents.

Keywords

Cerebral hemodynamics, functional neuroimaging, neuroimaing, optoacoustic tomography, real-time

Introduction

Cerebral hemodynamic changes are closely linked to neuronal activity by neurovascular coupling[1], and neuroimaging methods capable of assessing the concentrations of oxygenized (HbO) and deoxygenized (HbR) hemoglobin, cerebral blood volume (CBV) or cerebral blood flow (CBF) greatly contribute to a better understanding of the mechanisms underpinning this phenomenon.[2,3] Yet, the current imaging approaches are limited in their capacity of simultaneously monitoring all the aforementioned hemodynamic parameters with an adequate spatiotemporal resolution.[3,4] The mainstay of whole-brain functional neuroimaging, the blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI), offers noninvasive endogenous measurements of brain anatomy and function but struggles with resolving the cerebral microvasculature.[1] Furthermore, the temporal resolution of the method is considered insufficient to fully resolve neurovascular responses[5], while additional limitations stem from its basic contrast mechanism that purely relies on changes in HbR. Other imaging techniques, such as positron emission tomography (PET) and x-ray computed tomography (CT) are likewise not capable of real-time volumetric imaging and further require the application of exogenous contrast agents and exposure to ionizing radiation. Furthermore, the high costs associated with the whole-body clinical imaging methods limit their applicability in basic neuroscience research. Recently, functional neuroimaging of stimulus-evoked brain activation in the somatosensory cortex of the rat was showcased using pure ultrasound imaging.[6] While this method offered high spatiotemporal resolution, it was only sensitive to CBV and CBF and further required an invasive approach using cranial window.

Optical imaging methods are becoming increasingly attractive for in vivo brain imaging, mainly due to their unique capabilities to distinguish between HbO and HbR. Thereby, a large variety of biomedical optical techniques have been so far attempted for neuroimaging.[3] Here the main limitation is photon scattering that confines the effective imaging depth of even most advanced optical microscopy methods to superficial brain vasculature, also necessitating removal of the scalp and skull.[3] In turn, the intense light scattering in skin, skull and brain itself crumbles the spatial resolution of the non-invasive macroscopic whole-brain optical imaging approaches, like near-infrared spectroscopy (NIRS) and diffuse optical tomography (DOT).[3,7]

Amongst other available optical imaging techniques optoacoustics possibly combines the benefits of both optics and ultrasound as it provides the highly specific functional and molecular contrast of photons while not suffering resolution degradation because of photon scattering in deep tissues.[8] Although, optoacoustic imaging has recently demonstrated powerful performance in functional neuroimaging in rodents[5], significant limitations yet remain in terms of invasiveness,[5] inadequate penetration,[9] and lack of three-dimensional (3D) imaging capacity in real-time[10], hindering practical applications. More importantly, multispectral measurements have not yet been exploited to their full potential in simultaneous monitoring of multiple hemodynamic parameters. In this work, we demonstrate unprecedented performance in volumetric imaging of spectrally distinctive absorbers and fast hemodynamic changes in the brain using fully non-invasive acquisition of five-dimensional (5D) optoacoustic data (with 5D being three spatial dimensions, time and multispectral information). In particular, it is made possible, for the first time to our knowledge, to record and directly quantify blood oxygenation (SO_2), total hemoglobin (HbT) and CBV in deep brain structures and in real-time.

Materials & Methods

Animal preparation and breathing gas stimulation paradigm

Eight week old Female athymic nude-Foxn1^{nu} mice (Harlan Laboratories LTD, Itingen, Switzerland) were used for imaging, in full compliance with the institutional guidelines and with approval from the Government District of Upper Bavaria. Animals were anesthetized with isoflurane (1.5-2.5% v/v) in 100% O_2. Physiological parameters, including blood oxygenation, heart rate, and body temperature were continuously monitored throughout the experiments. The latter was kept constant using a rectal thermometer and a feedback-controlled heating pad (PhysioSuite^{TM}, Kent Scientific, Torrington, CT, USA). A custom-designed stereotactic mouse head holder (Narishige International Limited, London, UK) to avoid animal motion was positioned parallel to the surface of the imaging probe (Figure 1A). Optoacoustic recordings started under hyperoxic conditions (100% O_2) and the inhaled gas was changed between hyperoxia and normoxia (medical air, 20% O_2) every two minutes for a total duration of 10 minutes. The gas mixture was controlled manually using a multi-gas flowmeter (UNO, Zevenaar, Netherlands) keeping the isoflurane-level constant in the inhaled gas.

Optoacoustic tomography system and imaging paradigm

The spherical array probe used for real-time acquisition of volumetric optoacoustic image data has been previously described.[11] In short, a short-pulsed (~10ns) light beam from a custom-made optical parametric oscillator laser (Innolas Laser GmbH, Krailling, Germany), guided through a fiber-bundle (CeramOptec GmbH, Bonn, Germany) is used as excitation source. Pulse repetition frequency of the laser is 10Hz while it further possesses a unique fast tuning capability for precise per-pulse tuning of the excitation wavelengths within the entire near-infrared spectrum (700-900nm). The generated ultrasound waves are then picked up at different locations around the imaged volume by means of a 256-element detection array (4MHz central frequency, Imasonic SaS, Voray, France) and are simultaneously digitized with a parallel data acquisition system. In the current experiments, a total of eight wavelengths (Figure 1B) were used, thereby an entire multispectral dataset can be acquired within 0.8s.

Data Analysis

The acquired data were analyzed using Matlab (version 2013a, Mathworks Inc., Natick, MA, USA). In a first step, the signals were deconvolved with the impulse response of the transducer elements and then band-pass filtered between 80kHz and 8MHz. Volumetric data were reconstructed using a parallel GPU-based implementation of the 3D back-projection formula.[12] The reconstructed volume was corrected for light fluence attenuation using exponential decay function along the depth direction.[13] The distribution of HbO and HbR were retrieved by unmixing data at the eight measured wavelengths to a linear combination of these two components using their known extinction coefficient spectra (Figure 1B).[8] The blood oxygen saturation SO_2 was subsequently calculated voxel-vise via SO_2 = HbO / (HbO + HbR).

CBV was estimated as the number of voxels for which the total hemoglobin (HbT=HbO+HbR) signal is higher than a given threshold. HbT is taken from images reconstructed at 797 nm (isosbestic point of hemoglobin, Figure 1B) and the threshold is established as a heuristically-determined factor times the standard deviation of the background, i.e. the CBV was estimated as the number of voxels where the signal exceeds by a factor of at least 8.5 times the standard deviation of the background noise.

Results

A total of 750 unmixed frames were recorded during the experiments. The spatial resolution of the system of ~200µm enables visualizing 3D-distributions of major cerebral veins and their oxygenation status in real-time. Two different mouse head positions were used in two different mice to show visualizations of the cerebral vasculature (Figure 1C-D). The true benefits however of the 5D information (i.e. 3D multispectral data in real-time) can be best visualized in the Supplemental Video 1, in which quantitative changes of HbO and HbR in the whole brain are displayed over time.

Patterns of changes of HbO and HbR following the breathing gas stimulation paradigm can be readily analyzed in specific volumes of interest (VOI) located in the superior sagittal sinus and in deep cerebral veins, e.g. the longitudinal hippocampal vein (Figure 1D). The percentile changes in both mice are consistent with those observed in MRI for major cerebral veins under hyperoxic challenges.[14] Furthermore, VOIs placed outside the major vessels in the cortex allowed analyzing changes in HbO and HbR that followed the breathing gas stimulation paradigm (Figure 2A).

Vessel (hemoglobin) related voxels can be separated from background noise and sub-resolution voxels by applying the threshold criteria. A volumetric mask corresponding to the CBV can then be generated from positively identified voxels. This mask is further used to analyze changes in vessel-SO_2 (Figure 2B). This capacity is also dynamically visualized in Supplemental Video 2. Owing to the multispectral nature of our imaging paradigm, SO_2 can directly be quantified in any VOI from the spectrally-unmixed data. Figure 2B shows the analysis of SO_2-changes in the VOIs placed outside major vessels roughly inside the cortex.

Discussion

Optoacoustic imaging has recently demonstrated unique performance for measuring, visualizing and quantifying cerebral hemodynamic changes related to functional activity.[5] While state-of-the-art optoacoustic methods have prompted new prospects in neuroscience, the full capacity of the technology for evaluating brain activity has not yet been uncovered. Recent developments on transducer arrays, data acquisition systems and particularly fast wavelength-tunable lasers have enabled herein the acquisition of multispectral three-dimensional data from the entire mouse brain in real-time (5D), rendering simultaneous information on the underlying SO_2, HbT and CBV with high spatiotemporal resolution. Accuracy of the developed volumetric imaging approach has also facilitated quantification of fast dynamics in both HbO and HbR.

The measured changes of ~10% in venous SO_2 are in good accordance with reported values.[14] The newly introduced method thus offers benefits over non-optical neuroimaging techniques as SO_2 and other hemodynamic changes can be directly assessed in real-time without using any contrast agents. Most importantly for the application to functional neuroimaging, feasibility of analyzing hemodynamic changes outside major vessels has been demonstrated. Given the currently available spatial resolution of the system in the range of 200µm, one may consider two major cases: (1) The imaging voxel fully lies inside a blood vessel and thus its reconstructed value directly reflects hemodynamic changes in the vessel – this is the case for voxels located within large vessels, like the superior sagittal sinus; (2) The voxel only partially comprises vasculature, i.e. an unknown percentage of its volume contains blood vessels that are smaller than the resolution of the system – this would be the case for most of the recorded signals outside the major vessels. The two cases are common in all macroscopic imaging modalities, whose spatial resolution does not allow resolving microvasculature, and we were similarly able to follow hemodynamic changes under the above partial volume assumptions. Exemplarily, per imaged mouse blood oxygenation changes in two different cortex regions with low amounts of hemoglobin (outside major vessels) were successfully tracked. Moreover, the data-analysis procedures established in other imaging modalities can equally be adapted to process the 5D-optoacoustic data. This may further enhance performance of the technique to facilitate

functional neuroimaging studies (i.e. in presence of sensory stimuli), yet with temporal resolution superior to the other imaging techniques.

Several limitations remain to be addressed. First, significant light absorption in the brain imposes limitations on the achievable performance by having a profound influence on the local light fluence and hence on the amplitude of signals recorded from deep tissues. This effect was partially compensated here with the first order exponential correction factor but more accurate modeling of light distribution is expected to render further improvements in data quantification. The wavelength-dependent optical attenuation also contributes to spectral shifts in the generated signals that need to be considered for more accurate determination of oxygenation levels.[15] However, to achieve a higher accuracy in determining the distribution of the absorbers eight different wavelengths covering the near-infrared spectrum of HbO and HbR.

Finally, spatial resolution of the system prevents visualizing cerebral vasculature smaller than the major arteries and veins. It is however highly anticipated that the tremendous ongoing developments in the 3D detection array technology will soon make it possible to analyze the important functional parameters also in smaller deeply embedded singular vessels.

Supplementary Information

Supplementary information is available at the Journal of Cerebral Blood Flow & Metabolism website – www.nature.com/jcbfm.

Disclosure: Nothing to disclose.

References

1 Blockley, N. P., Griffeth, V. E., Simon, A. B. & Buxton, R. B. A review of calibrated blood oxygenation level-dependent (BOLD) methods for the measurement of task-induced changes in brain oxygen metabolism. *NMR Biomed* **26**, 987-1003, doi:10.1002/nbm.2847 (2013).

2 Buxton, R. B. Interpreting oxygenation-based neuroimaging signals: the importance and the challenge of understanding brain oxygen metabolism. *Front Neuroenergetics* **2**, 8, doi:10.3389/fnene.2010.00008 (2010).

3 Devor, A. *et al.* Frontiers in optical imaging of cerebral blood flow and metabolism. *J Cereb Blood Flow Metab* **32**, 1259-1276, doi:10.1038/jcbfm.2011.195 (2012).

4 Rodgers, Z. B., Jain, V., Englund, E. K., Langham, M. C. & Wehrli, F. W. High temporal resolution MRI quantification of global cerebral metabolic rate of oxygen consumption in response to apneic challenge. *J Cereb Blood Flow Metab* **33**, 1514-1522, doi:10.1038/jcbfm.2013.110 (2013).

5 Liao, L. D. *et al.* Transcranial imaging of functional cerebral hemodynamic changes in single blood vessels using in vivo photoacoustic microscopy. *J Cereb Blood Flow Metab* **32**, 938-951, doi:10.1038/jcbfm.2012.42 (2012).

6 Mace, E. *et al.* Functional ultrasound imaging of the brain. *Nature methods* **8**, 662-664, doi:10.1038/nmeth.1641 (2011).

7 Liao, L. D. *et al.* Neurovascular coupling: in vivo optical techniques for functional brain imaging. *Biomedical engineering online* **12**, 38, doi:10.1186/1475-925X-12-38 (2013).

8 Razansky, D. *et al.* Multispectral opto-acoustic tomography of deep-seated fluorescent proteins in vivo. *Nat Photonics* **3**, 412-417, doi:Doi 10.1038/Nphoton.2009.98 (2009).

9 Yao, J. *et al.* Noninvasive photoacoustic computed tomography of mouse brain metabolism in vivo. *Neuroimage* **64**, 257-266, doi:10.1016/j.neuroimage.2012.08.054 (2013).

10 Xiang, L., Wang, B., Ji, L. & Jiang, H. 4-D photoacoustic tomography. *Scientific reports* **3**, 1113, doi:10.1038/srep01113 (2013).

11 Deán-Ben, X. L. & Razansky, D. Adding fifth dimension to optoacoustic imaging: Volumetric time-resolved spectrally enriched tomography. *Light: Science and Applications* **3** (2014).

12 Dean-Ben, X. L., Ozbek, A. & Razansky, D. Volumetric real-time tracking of peripheral human vasculature with GPU-accelerated three-dimensional optoacoustic tomography. *IEEE transactions on medical imaging* **32**, 2050-2055, doi:10.1109/TMI.2013.2272079 (2013).

13 Laufer, J., Zhang, E., Raivich, G. & Beard, P. Three-dimensional noninvasive imaging of the vasculature in the mouse brain using a high resolution photoacoustic scanner. *Applied optics* **48**, D299-306 (2009).

14 Bulte, D., Chiarelli, P., Wise, R. & Jezzard, P. Measurement of cerebral blood volume in humans using hyperoxic MRI contrast. *Journal of magnetic resonance imaging : JMRI* **26**, 894-899, doi:10.1002/jmri.21096 (2007).

15 Tzoumas, S., Deliolanis, N., Morscher, S. & Ntziachristos, V. Un-mixing Molecular Agents from Absorbing Tissue in Multispectral Optoacoustic Tomography. *IEEE transactions on medical imaging*, doi:10.1109/TMI.2013.2279994 (2013).

Titles and legends to figures

Figure 1 - Five-dimensional (5D) imaging of mouse brain oxygenation under hyperoxia and normoxia

(A) Experimental setup. The mouse head is fixed inside a custom-made stereotactical frame to minimize motion artefacts and the mouse is placed in a supine position on top of the spherical array optoacoustic probe for volumetric imaging. DAQ: Data acquisition system.

(B) Extinction spectra of oxygenized (HbO) and deoxygenized (HbR) hemoglobin in the near-infrared range. Indicated are the eight wavelengths (dotted lines) that were used to acquire multispectral datasets.

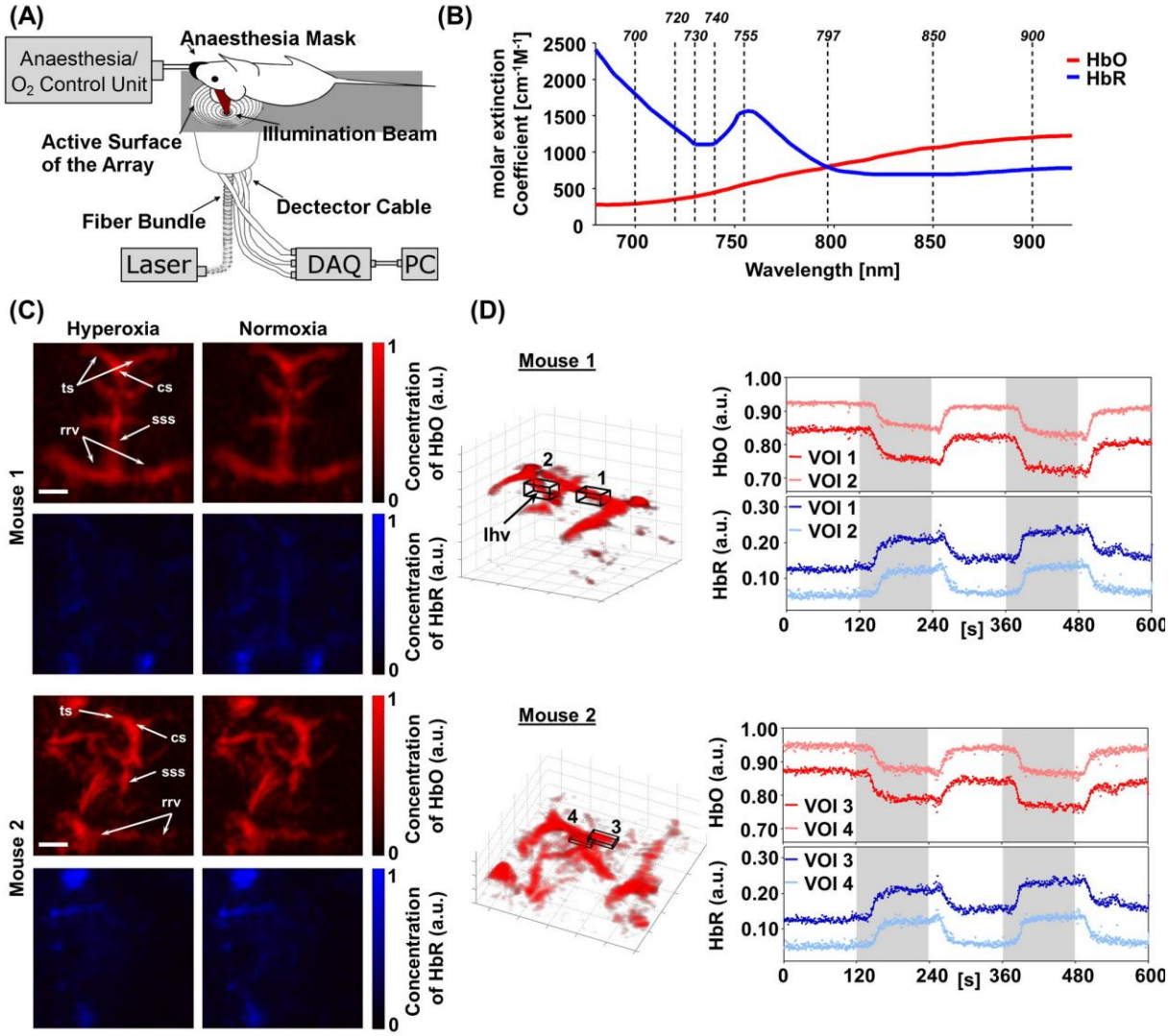
(C) Maximum intensity projection images (along the depth direction) of HbO and HbR distribution in the mouse brain under hyperoxia and normoxia. Data from the second mouse was recorded with the head angled in relation to the surface of the array probe, revealing more lateral vasculature of the brain. The main identifiable veins are indicated in the figure: Rostral rhinal vein (rrv), superior sagittal sinus (sss), confluence of sinuses (cs), transverse sinus (ts). Scale bars are 2mm.

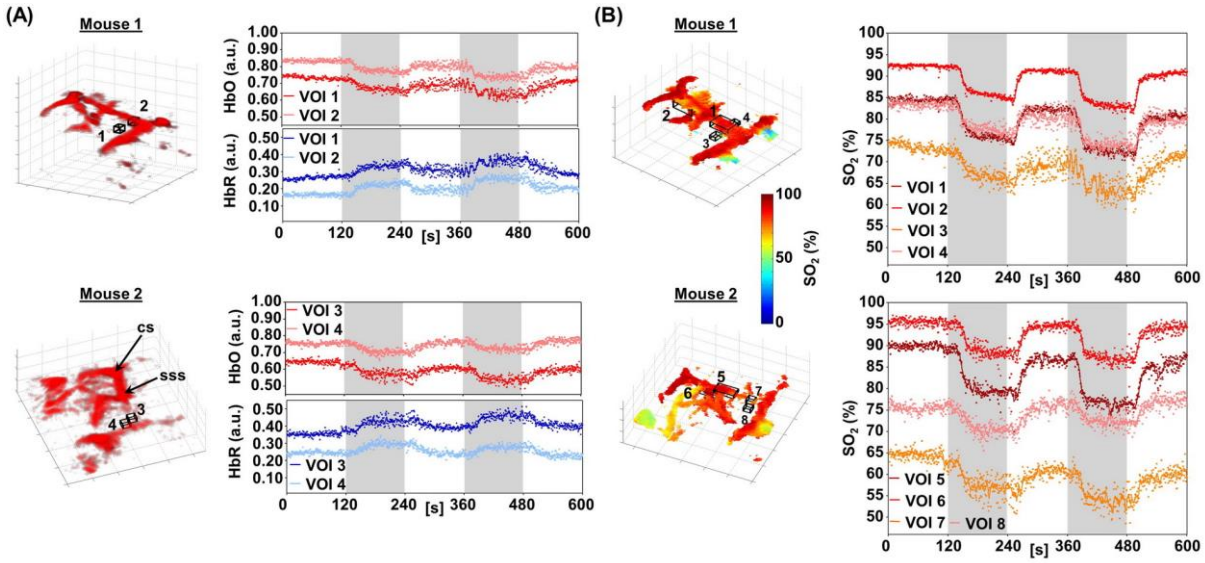
(D) 3D-visualization of HbO under hyperoxia. Volumes of interest (VOIs) were placed inside the anterior part of the sss and the longitudinal hippocampal vein (lhv). Time courses of HbO and HbR inside the VOIs are shown normalized to their respective total hemoglobin in all vessel voxels of a given frame (dots) along with the moving average over 5 frames (lines). The concentrations of HbO and HbR follow the changes from hyperoxia (white background) to normoxia (grey background) and vice versa. The benefits of the 5D information (multispectral three-dimensional data in real-time), provided by the system, can be best appreciated in the Supplemental Video 1, in which changes of HbO and HbR in the whole brain are displayed over time.

Figure 2 - Analysis of mouse brain hemodynamics

(A) VOI 1, 2 and VOI 3, 4 for mouse 1 and 2, respectively, were placed outside the major vessels inside the cortex. Variations of HbO and HbR inside these voxels are shown here as time-dependent curves (for details and abbreviations see Figure 1D).

(B) Blood oxygenation (SO_2) levels in the voxels were determined on a voxel by voxel basis from the spectrally unmixed data. Segmentation of major vessels was performed by thresholding the data acquired at 797nm. The generated volumetric vessel mask was applied to assess the SO_2-levels in the same VOIs as used in Figure 1D and SO_2 changes could be monitored in the sss (mouse 1: VOI 1; mouse 2: 5) and lhv (mouse 1: VOI 2; mouse 2: VOI 6). For the 3D-visualization only voxels exceeding the signal threshold were considered. See Supplemental Video 2 for a dynamic visualization.
In addition, SO_2 changes were analyzed in the same VOIs from Figure 2A, placed outside the major vessel inside the cortex (mouse 1: VOI 3, 4; mouse 2: VOI 7, 8).


(A)
Anaesthesia/
O_2 Control Unit
Anaesthesia Mask
Illumination Beam
Active Surface
of the Array
Fiber Bundle
Dectector Cable
Laser
DAQ
PC
(B)
molar extinction
Coefficient [cm^{-1}M^{-1}]
2500
2000
1500
1000
500
0
700
720
730
740
755
797
850
900
700
750
800
850
900
Wavelength [nm]
HbO
HbR
(C)
Hyperoxia
Normoxia
Mouse 1
Mouse 2
ts
cs
sss
rrv
Concentration
of HbO (a.u.)
Concentration
of HbR (a.u.)
1
0
(D)
Mouse 1
Mouse 2
2
1
lhv
4
3
HbO (a.u.)
HbR (a.u.)
1.00
0.90
0.80
0.70
0.30
0.20
0.10
VOI 1
VOI 2
VOI 3
VOI 4
0
120
240
[s]
360
480
600


(A)
Mouse 1
1
2
HbO (a.u.)
HbR (a.u.)
VOI 1
VOI 2
[s]
Mouse 2
cs
sss
3
4
VOI 3
VOI 4
(B)
Mouse 1
Mouse 2
100
50
0
SO2 (%)
VOI 5
VOI 6
VOI 7
VOI 8