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Planar Implantable Sensor for In Vivo Measurement of Cellular Oxygen Metabolism in Brain Tissue

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Abstract

Background—Brain imaging methods are continually improving. Imaging of the cerebral cortex is widely used in both animal experiments and charting human brain function in health and disease. Among the animal models, the rodent cerebral cortex has been widely used because of patterned neural representation of the whiskers on the snout and relative ease of activating cortical tissue with whisker stimulation.

New Method—We tested a new planar solid-state oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating on the working side, to monitor dynamics of oxygen metabolism in the cerebral cortex following sensory stimulation.

Results—Sensory stimulation led to changes in oxygenation and deoxygenation processes of activated areas in the barrel cortex. We demonstrate the possibility of dynamic mapping of relative changes in oxygenation in live mouse brain tissue with such a sensor.

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Comparison with Existing Method—Oxygenation-based functional magnetic resonance imaging (fMRI) is very effective method for functional brain mapping but have high costs and limited spatial resolution. Optical imaging of intrinsic signal (IOS) does not provide the required sensitivity, and voltage-sensitive dye optical imaging (VSDi) has limited applicability due to significant toxicity of the voltage-sensitive dye. Our planar solid-state oxygen sensor imaging approach circumvents these limitations, providing a simple optical contrast agent with low toxicity and rapid application.

Conclusions—The planar solid-state oxygen sensor described here can be used as a tool in visualization and real-time analysis of sensory-evoked neural activity *in vivo*. Further, this approach allows visualization of local neural activity with high temporal and spatial resolution.

Keywords

Barrel field; In vivo optical imaging; Functional brain mapping; Quenched-phosphorescence oxygen sensing; Planar oxygen sensor; Somatosensory cortex; Whisker stimulation

Introduction

Localization and real-time monitoring of spontaneous and evoked neuronal activity in the brain are important in understanding the functional characteristics of neural networks and brain function (Bouchard et al, 2009; Inyushin et al, 2013; Lenkov et al, 2012; Silasi et al, 2016; Zhang et al, 2014).

Over the last two decades a number of optical methods have been developed for functional brain imaging. Most of them are based on the detection of changes in blood flow, tissue oxygenation and energy usage (Lenkov et al, 2012; Liao et al, 2013). VSDi method was used in several studies, including novel 3D visualization of the neural activity, however due to cytotoxicity of the indicator dye it cannot be used in clinical practice (Tang et al, 2016). Changes in local oxygenation and glucose consumption induced by changes in neuronal activity (Tsytarev et al, 2011 and 2013) allow indirect monitoring of neural activity with these metabolic markers. Two-photon enhanced phosphorescent nanoparticles in combination with two-photon in vivo imaging have been successfully used for monitoring of the oxygen partial pressure in the cortical tissue *in vivo* (Sakadzic et al, 2010, 2011). It is also possible to use single photon excitation using phosphorescence oxygen sensitive dye, for example Oxyphor R3 (Sakadzic et al, 2011). Each method has its advantages and limitations, but only a few can be used in long-term experiments and in clinical settings. This motivated us to develop a new approach, which overcomes toxicity side effects of sensor dyes and which is better suited for functional brain mapping applications.

The rodent whisker neural system (which consists of a patterned array of whisker follicles on the snout, the trigeminal ganglion neurons that innervate them, whisker-specific neural modules, the barrelettes in the brain stem, the barreloids in the thalamus, and the barrels in the primary somatosensory cortex) is a useful model for studying peripherally evoked neural activity in the central nervous system (Erzurumlu and Gaspar, 2012). Mechanical stimulation of a single whisker evokes neural activity all along the system and ultimately in the corresponding barrel of the contralateral hemisphere. Barrels are located in cortical layer

IV, so they are accessible for optical monitoring of whisker-evoked neural activity *in vivo*, through a cranial window (Tsytarev et al, 2013).

Optical imaging methods based on the monitoring of local brain oxygenation are useful tools for studying different cortical regions and physiological processes in these regions (Chen et al, 2003). Activity patterns obtained in our experiments are similar to the blood oxygenation level dependent signals (BOLD) produced in fMRI or activity patterns obtained by near-infrared spectroscopy (NIRS) (Fukuda et al, 2016; Lenkov et al, 2012). Both BOLD fMRI and NIRS methods employ hemoglobin as an endogenous contrast agent and visualize neuronal activity indirectly via correlations with local metabolism (Arthurs and Boniface, 2002). In contrast, the use of a planar solid-state oxygen sensor together with phosphorescence imaging described here is direct and measures the concentration of free oxygen dissolved in the tissue.

Fluorescence based imaging, using different probes and contrast agents, has grown considerably in recent years (Baker et al, 2015; Jaafari et al, 2015; Mutoh et al, 2015; Tsytarev et al, 2012; Liao et al, 2013). VSDi allows direct monitoring of excitatory neural activity (Tsytarev et al, 2012), while IOS (Inyushin et al, 2001) reflects optical changes in cortical tissue associated with differential absorption of light by oxy- and deoxyhemoglobin (Hu et al, 2009; Kalchenko et al, 2014, 2015).

In this study, we describe the use of a planar solid-state oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating on the working side. This sensor, when applied topically on mouse dura mater surface, allows dynamic real-time *in vivo* imaging on a commercial fluorescent imager of deoxygenation and reoxygenation in specific areas of cortical tissue, with high spatial and temporal resolution. Obtained data demonstrate that the planar sensor provides simple and efficient means for the dynamic 2D imaging of the oxygen levels in cortical tissue *in vivo*.

Materials and Methods

The planar oxygen sensor was produced by applying a polymeric cocktail of PtBP dye (Luxcel Biosciences) on the surface of polyester film, Mylar® (Du Pont, 22×22 mm pieces) using a 6800 Spin Coater machine (SCS) at 3000 rpm (0.2 ml per sample) (Figure 1, B). The coated films were then cut into 5×5 mm pieces of planar oxygen sensors, which were used in the animal experiments. Planar sensor was also tested in Ringer solution alone, which is similar in composition to the vertebrate body fluids. The fluctuation of fluorescence signal did not exceed 0.05 F/F(%) (Figure 1, C)

The *in vivo* imaging experiments were performed in six male and female mice (B6, weight 20–35 g, aged 3–6 months) using a MiCAM-02 optical imaging system (SciMedia, Ltd., Figure 1, A). The subjects used here were C57BL/6N (B6) line, which was originally obtained from Charles River Laboratory (Frederick, MD) and maintained in the University of Maryland School of Medicine animal facility. All animal handling was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH

Publications No. 80-23) revised in 1996 and a protocol approved by the University of Maryland School of Medicine Institutional Animal Use and Care Committee.

Animals were anesthetized with an intraperitoneal injection of urethane (1.15 g/kg). The anesthetized mouse was fixed on a stereotaxic frame, the skin and muscles of the dorsal part of the head were removed surgically, and an approximately 10 mm² part of the skull above the left parietal cortex was removed using a dental drill. The exposed area was washed with a hemostatic sponge with the artificial cerebrospinal fluid (ACSF).

A planar sensor was applied to the exposed cortex, with the oxygen-sensitive surface facing down to dura mater surface in immersion contact with the tissue. In the imaging session the CCD camera was positioned above the recording area and directed such that its optical axis was perpendicular to the area of recording. The sensor was excited with 630±10 nm light and emission (maximum at 760 nm) was recorded with a 650 nm long-pass filter. At the start of each imaging session a grayscale image of the monitored area was captured and saved as a graphic file. The imaged session consisted of 100 trials with 200 frames per trial measured at 50 ms per frame and inter-trial intervals of 30s.

Mechanical stimulation of the E2 whisker was delivered by specially designed electromechanical stimulator at the 50th frame. Each stimulation consisted of ten 25-ms deflections with a 25-ms inter-pulse interval.

Phosphorescent signals in the recording area were analyzed using the Brain Vision Analyzer (Brain Vision Inc., Tokyo, Japan) and presented as $\Delta F/F$ (%). One of the limiting factors in functional brain mapping is low-frequency biological noise (<0.3 Hz (Grinvald et al, 1999)). When signal measurement time is significantly longer (10 s in our case), biological noise is present in all of the imaging frames acquired during that trial. However, the first frame taken prior to the whisker stimulation, contains only this biological noise and no other components that relate to stimulus elicited activity. Therefore, subtraction of the first frame from all subsequent frames can significantly reduce the overall noise and improve data analysis (Grinvald et al, 1999). As a result of this procedure, phosphorescence response at each time point is presented as $\Delta F/F$ (%) for the whole area of imaging, where F is mean pixel intensity in this area. This is a common way to analyze quite noisy intrinsic (Grinvald et al, 1999) and extrinsic (Grinvald and Hildesheim, 2004) brain optical imaging data.

After the measurement session animal was euthanized by overdose of urethane. Immediately after this sensor phosphorescence was monitored for ~1 minute to assess the optical signal in anoxic tissue.

Results

Functional pseudocolor images of recorded phosphorescence signal were constructed using the first frame analysis (Tsytarev et al, 2008). As a result of this analysis regions of activated areas of the cortex were identified in each frame. These activated areas of the brain surface corresponded to pixels exhibiting a change in fluorescence ($\Delta F/F$) greater than (for the deoxygenation) or lower than (for the oxygenation) the half-maximal or half-minimal,

respectively. This operation, called thresholding, is commonly used to detect borders of activity patterns when analyzing optical imaging data (Tsytarev et al, 2013).

We found that brain surface areas activated by the whisker stimulation were clearly identifiable. The kinetics of tissue re-oxygenation and deoxygenation in these areas is shown in Figure 2. One can see that deoxygenation area appears first, and then it is followed by re-oxygenation (marked in blue and red colors, respectively). The re-oxygenation and deoxygenation patterns have the same location; however re-oxygenation spans over larger areas than deoxygenation.

Time course of the observed signal in responding area shows two peaks (Figure 3, A), negative and positive. After the second peak the optical signal returns to a new baseline, which corresponds to an O_2 level slightly higher than before the stimulation. The integrated change in phosphorescence in the center of activity pattern ($\Delta F/F(\%)$, 7×7 pixels area) was calculated for each frame and experimental session. We used these values to plot time courses of the fluorescence signals, which are shown in Figure 3. Error bars show standard deviation for all the experiments.

At the end of experiment the animal was euthanized by an overdose injection of urethane, and the optical signal in the area which responded to whisker stimulation was recorded for ~ 1 min after the injection (Figure 3, B). During this measurement a large increase in sensor phosphorescent signal was observed, reflecting the transition of brain tissue to a state of anoxia. The change of phosphorescence during mouse euthanasia was global and homogeneous in the recorded area (Figure 4).

Discussion

In all experimental animals changes in phosphorescence signals evoked by whisker stimulation were recorded. These changes were in the areas that correspond to the barrel field of primary somatosensory cortex, and they reflected the processes of initial deoxygenation and subsequent reoxygenation of the corresponding regions of brain tissue located beneath the sensor. The increases in phosphorescence signal correspond to local deoxygenation, while decreases – to reoxygenation of brain tissue adjacent to the sensor. When deoxygenation and reoxygenation patterns were analyzed, we found that corresponding activity spots had the same location, but the oxygenation area was considerably larger.

We expected the deoxygenation signals to reflect neural activity, while reoxygenation signals reflect the delayed changes in tissue oxygenation due to increased cerebral blood flow which occurs more slowly. Therefore, activated areas in oxygenation imaging had a larger size.

In all the experiments, the activated areas initially produced a deoxygenation peak with a maximum 0.6 ± 0.1 s after stimulus onset, then a reoxygenation peak with a minimum at 1.1 ± 0.1 s, and subsequent gradual return to the baseline.

Thus, activation of the barrel field initially causes rapid local deoxygenation, and then delayed reoxygenation of broader area.

The large increase of the phosphorescence immediately after euthanasia (induced by injection of overdose of urethane), demonstrated the large drop in oxygen concentration in the dying brain tissue. For technical reasons we could not record optical signals longer than 1 min. However, it is well known that brain death is accompanied by rapid depletion of oxygen and establishment of the state of tissue anoxia (Arthurs, 2002). This experiment also indicates that live cortical tissue analyzed by us had basal oxygenation close to physiological, due to efficient supply of oxygen via cerebellar blood flow.

In the indirect oxygenation imaging techniques and in our direct method, measured relative changes in the optical signals inform on the changes in local oxygenation. This is a relatively slow process, which proceeds on the scale of hundreds of milliseconds and seconds, which is related to the evoked neural activity that occurs within a few tenths of milliseconds. The biphasic character of the signal observed by us reflects the processes of tissue deoxygenation and re-oxygenation that have also been demonstrated by NIRS - Transcranial magnetic stimulation (Akiyama et al. 2006). These authors attributed the first component of the response to an increase in deoxygenated hemoglobin concentration, while the second component - to an increase in oxyhemoglobin (Akiyama et al, 2006).

NIRS and fMRI are established techniques for functional brain imaging, but have specific disadvantages: low spatial resolution for NIRS and high costs for fMRI. The IOS imaging is less expensive, has good spatial resolution but does not provide the required sensitivity and direct quantitative readout of free oxygen available in the tissue. Imaging using voltage-sensitive dyes also has limited applicability due to their significant toxicity and poor suitability for long-term use (Kim and Jun, 2013; Tsytarev et al, 2012).

In case of VSDi, signal responses to neuronal activity usually range between 0.1%–1%, so to get an appropriate signal to noise ratio the noise level must be ten times lower (Lippert et al, 2007; Liang et al, 2015). In the case of IOS noise reduction it is even more complicated: since IOS is low in signal to noise ratio, more sensitive systems or larger number of averaging data is required for adequate mapping of the neural activity (Kim and Jun, 2013).

Our method overcomes many of these limitations, providing good spatial resolution, low toxicity and direct sensing of free O₂ concentration in surface areas of cortical tissue. As a functional analog of the IOS, this method provides higher signal to noise ratio and better sensitivity to relative changes in oxygen concentration. It allows monitoring of the local oxygenation with high temporal and spatial resolution, so it can be used in different studies of neurovascular coupling. The IOS signals recorded from the cortical surface are rather weak: changes in reflected light intensity due to neuronal activity never exceed 0.1–6.0% of the total intensity of the reflected light. Strong signal is usually more difficult to use for the functional brain mapping (Grinvald et al, 2001). IOS signal can be higher for green light, but it is less useful for functional brain mapping. In our experiments signal changes usually exceed 0.2% in either negative or positive components, so the total change is >0.4%. The sensor is thin, transparent, and it allows high sensitivity without losing spatial resolution, which is mainly determined by the type of CCD-camera.

The dura mater in mammals is permeable to water, oxygen and carbon dioxide (Bernards, 1994; Maikos et al, 2008; Reina et al, 1994), and its thickness varies from tens of microns in mice to hundreds of microns in primates (Galashan et al, 2011). The relatively permeability of the dura mater allow the use of oxygen-sensitive materials on the outer surface of the dura mater, to monitor O₂ dynamics in the brain tissue adjacent to it.

Further development of the planar imaging sensor approach and measurement methodology described in this study can lead to other applications, including potential clinical use. In the future we will aim to produce planar sensors on more flexible film support, so that plastic foil oxygen sensor can better adhere to the dural surface or durotomized cortical surface.

Planar O₂ sensors have been known for many years, but used mostly for point measurements as spot sensors and as pressure sensitive paints (Wang and Wolfbeis, 2014; Anyoji et al, 2015). More recently they have found use in biomedical imaging (Babilas et al, 2005; Schreml et al, 2011). So far, they have not been used in high spatial and temporal resolution imaging and for monitoring of functional activity of live animal brain.

Advancements of functional brain mapping over the last years have opened new opportunities for fundamental and clinical investigators. IOS allows imaging of hemodynamics and metabolism in clinical settings: it offers high spatial resolution, does not make contact with the cortical surface, and has no potentially harmful compounds (Cannestra et al, 2001; Pouratian et al, 2002; Suh et al, 2006). IOS is relatively inexpensive, compared to other brain imaging modalities, but has disadvantages. One of them is long duration of the imaging session - tens of minutes to get a functional map of the cortical area. This time should be reduced significantly to make IOS compatible with brain opening during neurosurgical operations. Our planar O₂ sensor is much more sensitive than the IOS, therefore imaging session and brain opening can be kept short.

We believe our planar sensor can be used for chronic experiments that do not require micron scale resolution and where cortical neural or vascular signals are recorded from the few square millimeters cortical surface. Planar sensor, described here, does not have intrinsic toxicity, and therefore can be used in long-term experiments. It seems easy to install the sensor into the skull and keep for many weeks, like cranial window in case of chronic IOS (Ji et al, 2016). Being non-toxic, in contrast with VSDi, and much more sensitive, unlike IOS, our technology combine advantages of these methods and can be used in many experimental neuroscientific goals.

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References

- Anyoji M, Numata D, Nagai H, Asai K. Pressure-sensitive paint technique for surface pressure measurements in a low-density wind tunnel. *J Vis (Tokyo)*. 2015; 18(2):297–309. [PubMed: 25937803]

- Arakawa H, Erzurumlu RS. Role of whiskers in sensorimotor development of C57BL/6 mice. *Behav Brain Res*. 2015; 287:146–55. [PubMed: 25823761]
- Arthurs OJ, Boniface S. How well do we understand the neural origins of the fMRI BOLD signal? *Trends Neurosci*. 2002; 25(1):27–31. [PubMed: 11801335]
- Akiyama T, Ohira T, Kawase T, Kato T. TMS orientation for NIRS-functional motor mapping. *Brain Topogr*. 2006 Winter;19(1–2):1–9. [PubMed: 17136594]
- Babilas P, Liebsch G, Schacht V, Klimant I, Wolfbeis OS, Szeimies RM, Abels C. In vivo phosphorescence imaging of pO₂ using planar oxygen sensors. *Microcirculation*. 2005 Sep; 12(6): 477–87. [PubMed: 16147465]
- Baker B, Gao X, Wolff BS, Jin L, Cohen LB, Bleau CX, Wu JY. In Vivo Voltage-Sensitive Dye Imaging of Mammalian Cortex Using “Blue” Dyes. *Cold Spring Harb Protoc*. 2015; 2015(11): 1000–2. [PubMed: 26527769]
- Bernards CM. Human dura mater permeability. *Can J Anaesth*. 1994; 41(11):1125.
- Bouchard MB, Chen BR, Burgess SA, Hillman EM. Ultra-fast multispectral optical imaging of cortical oxygenation, blood flow, and intracellular calcium dynamics. *Opt Express*. 2009; 17(18):15670–8. [PubMed: 19724566]
- Cannestra AF, Pouratian N, Bookheimer SY, Martin NA, Beckerand DP, Toga AW. Temporal spatial differences observed by functional MRI and human intraoperative optical imaging. *Cereb Cortex*. 2001; 11(8):773–82. [PubMed: 11459767]
- Chen Y, Tailor DR, Intes X, Chance B. Correlation between near-infrared spectroscopy and magnetic resonance imaging of rat brain oxygenation modulation. *Phys Med Biol*. 2003; 48(4):417–27. [PubMed: 12630739]
- Erzurumlu RS, Gaspar P. Development and critical period plasticity of the barrel cortex. *Eur J Neurosci*. 2012; 35(10):1540–53. [PubMed: 22607000]
- Fukuda M, Poplawsky AJ, Kim SG. Submillimeter-resolution fMRI: Toward understanding local neural processing. *Prog Brain Res*. 2016; 225:123–52. [PubMed: 27130414]
- Galashan OF, Rempel HC, Meyer A, Gruber-Dujardin E, Kreiter AK, Wegener D. A new type of recording chamber with an easy-to-exchange microdrive array for chronic recordings in macaque monkeys. *Journal of Neurophysiology*. 2011; 105(6):3092–3105. [PubMed: 21451061]
- Grinvald, A., Shoham, D., Shmuel, D., Glaser, D., Vanzetta, I., Shtoyerman, E., Slovlin, H., Sterkin, A., Wijnbergen, C., Hildesheim, R., Arieli, A. *Modern Techniques in Neuroscience Research*. Springer; Berlin Heidelberg: 1999. In-vivo Optical imaging of cortical architecture and dynamics; p. 893-969.
- Hu S, Maslov K, Tsytarev V, Wang LV. Functional transcranial brain imaging by optical-resolution photoacoustic microscopy. *J Biomed Opt*. 2009; 14(4):040503. [PubMed: 19725708]
- Inyushin MY, Volnova AB, Lenkov DN. Use of a simplified method of optical recording to identify foci of maximal neuron activity in the somatosensory cortex of white rats. *Neurosci Behav Physiol*. 2001; 31(2):201–5. [PubMed: 11388374]
- Jaafari N, Vogt KE, Saggau P, Leslie LM, Zecevic D, Canepari M. Combining Membrane Potential Imaging with Other Optical Techniques. *Adv Exp Med Biol*. 2015; 859:103–25. [PubMed: 26238050]
- Ji N, Freeman J, Smith SL. Technologies for imaging neural activity in large volumes. *Nat Neurosci*. 2016; 19(9):1154–64. [PubMed: 27571194]
- Kim SA, Jun SB. In-vivo Optical Measurement of Neural Activity in the Brain. *Exp Neurobiol*. 2013; 22(3):158–66. [PubMed: 24167411]
- Kalchenko V, Israeli D, Kuznetsov Y, Harmelin A. Transcranial optical vascular imaging (TOVI) of cortical hemodynamics in mouse brain. *Sci Rep*. 2014; 4:5839. [PubMed: 25059112]
- Kalchenko V, Israeli D, Kuznetsov Y, Meglinski I, Harmelin A. A simple approach for non-invasive transcranial optical vascular imaging (nTOVI). *J Biophotonics*. 2015; 8(11–12):897–901. [PubMed: 25924020]
- Lenkov DN, Volnova AB, Pope AR, Tsytarev V. Advantages and limitations of brain imaging methods in the research of absence epilepsy in humans and animal models. *J Neurosci Methods*. 2013; 212(2):195–202. [PubMed: 23137652]

- Li Z, Roussakis E, Koolen PG, Ibrahim AM, Kim K, Rose LF, Wu J, Nichols AJ, Baek Y, Birngruber R, Apiou-Sbirlea G, Matyal R, Huang T, Chan R, Lin SJ, Evans CL. Non-invasive transdermal two-dimensional mapping of cutaneous oxygenation with a rapid-drying liquid bandage. *Biomedical Optics Express*. 2014; 5(11):3748–3764. [PubMed: 25426308]
- Liang J, Xu W, Geng X, Wu JY. Monitoring Population Membrane Potential Signals from Neocortex. *Adv Exp Med Biol*. 2015; 859:171–96. [PubMed: 26238053]
- Lippert MT, Takagaki K, Xu W, Huang X, Wu JY. Methods for voltage-sensitive dye imaging of rat cortical activity with high signal-to-noise ratio. *J Neurophysiol*. 2007; 98(1):502–12. [PubMed: 17493915]
- Liao LD, Tsytarev V, Delgado-Martinez I, Li ML, Erzurumlu R, Vipin A, Orellana J, Lin YR, Lai HY, Chen YY, Thakor NV. Neurovascular coupling: in vivo optical techniques for functional brain imaging. *Biomed Eng Online*. 2013; 12:38. [PubMed: 23631798]
- Maikos JT, Elias RA, Shreiber DI. Mechanical properties of dura mater from the rat brain and spinal cord. *J Neurotrauma*. 2008; 25(1):38–51. [PubMed: 18355157]
- Mutoh H, Mishina Y, Gallero-Salas Y, Knopfel T. Comparative performance of a genetically-encoded voltage indicator and a blue voltage sensitive dye for large scale cortical voltage imaging. *Front Cell Neurosci*. 2015; 9:147. [PubMed: 25964738]
- Pouratian N, Cannestra AF, Martin NA, Toga AW. Intraoperative optical intrinsic signal imaging: a clinical tool for functional brain mapping. *Neurosurg Focus*. 2002; 13(4):e1.
- Ricard C, Stanchi F, Rougon G, Debarbieux F. An orthotopic glioblastoma mouse model maintaining brain parenchymal physical constraints and suitable for intravital two-photon microscopy. *J Vis Exp*. 2014; 21(86)
- Reina MA, Lopez Garcia A, de Andres JA, Sellers F, Arrizabalaga M, Mora MJ. *Rev Esp Anestesiología y Reanimación*. 1999; 46(8):344–9. [PubMed: 10563140]
- Sakadzic S, Roussakis E, Yaseen MA, Mandeville ET, Srinivasan VJ, Arai K, Ruvinskaya S, Devor A, Lo EH, Vinogradov SA, Boas DA. Two-photon high-resolution measurement of partial pressure of oxygen in cerebral vasculature and tissue. *Nat Methods*. 2010; 7(9):755–9. [PubMed: 20693997]
- Sakadzic S, Roussakis E, Yaseen MA, Mandeville ET, Srinivasan VJ, Arai K, Ruvinskaya S, Wu W, Devor A, Lo EH, Vinogradov SA, Boas DA. Cerebral blood oxygenation measurement based on oxygen-dependent quenching of phosphorescence. *J Vis Exp*. 2011; 4(51)
- Silasi G, Xiao D, Vanni MP, Chen AC, Murphy TH. Intact skull chronic windows for mesoscopic wide-field imaging in awake mice. *J Neurosci Methods*. 2016; 267:141–9. [PubMed: 27102043]
- Schreml S, Meier RJ, Wolfbeis OS, Maisch T, Szeimies RM, Landthaler M, Regensburger J, Santarelli F, Klimant I, Babilas P. 2D luminescence imaging of physiological wound oxygenation. *Exp Dermatol*. 2011 Jul; 20(7):550–4. [PubMed: 21443617]
- Suh M, Bahar S, Mehta AD, Schwartz TH. Blood volume and hemoglobin oxygenation response following electrical stimulation of human cortex. *Neuroimage*. 2006; 31(1):66–75. [PubMed: 16480899]
- Tang Q, Tsytarev V, Frank A, Wu Y, Chen CW, Erzurumlu RS, Chen Y. In Vivo Mesoscopic Voltage-Sensitive Dye Imaging of Brain Activation. *Sci Rep*. 2016; 6:25269. [PubMed: 27125318]
- Tsytarev V, Arakawa H, Borisov S, Pumbo E, Erzurumlu RS, Papkovsky DB. In vivo imaging of brain metabolism activity using a phosphorescent oxygen-sensitive probe. *J Neurosci Methods*. 2013; 216(2):146–51. [PubMed: 23624034]
- Tsytarev V, Bernardelli C, Maslov KI. Living Brain Optical Imaging: Technology, Methods and Applications. *J Neurosci Neuroengineering*. 2012; 1:180–192.
- Wang XD, Wolfbeis OS. Optical methods for sensing and imaging oxygen: materials, spectroscopies and applications. *Chem Soc Rev*. 2014 May 21; 43(10):3666–761. [PubMed: 24638858]
- Zhang T, Zhou J, Carney PR, Jiang H. Towards real-time detection of seizures in awake rats with GPU-accelerated diffuse optical tomography. *J Neurosci Methods*. 2014; 30(240):28–36.

Highlights

- We visualized local oxygenation in the mice barrel field in response to the whisker stimulation using a new planar solid-state oxygen sensor comprising a polymeric film with a phosphorescent oxygen-sensitive coating on the working side.
- We demonstrate the possibility of dynamic mapping of relative changes in oxygen concentration and consumption in live mouse brain tissue with such a sensor
- Our results provide evidence that our planar solid-state oxygen sensor can be used as a tool in visualization and analysis of sensory-evoked neural activity in vivo

Figure 1.

A. Experimental setup for *in vivo* optical imaging using planar phosphorescence based oxygen sensor. The cortex is illuminated with light 630 nm wavelengths, and the emitted photons with wavelength above 690 nm are acquired with CCD camera. B. The photograph of the sensor. C. Left: sensor located on the drop of the Ringer solution, above the millimetric scale. Right – fluctuation of the fluorescence signal, recorded from the sensor at the left figure.

Figure 2.

Continuously recorded cortical images (left hemisphere) showing changes in oxygenation in response to single whisker stimulation. Time after stimulus onset is indicated in top left corner. Fluorescence changes ($\Delta F/F(\%)$) are shown in pseudocolors. Activity spot at the medial part of the imaging area, which appears 1500 ms after the stimulation, is probably caused by blood vessel activity which is usually delayed by >1 s after sensory stimulation.

Figure 3.

A. Time course of the oxygen-sensitive signals, $m=6$. Stimulation indicated by arrow. B. Change of fluorescence signal after euthanasia $n=6$, the moment of overdose injection of the urethane indicated by arrow. Error bars show standard deviation for all the experiments.

Figure 4.

Continuously recorded cortical images (left hemisphere) showing changes in sensor phosphorescence after injection of urethane overdose. The time after injection in seconds is indicated in the top left corner. Phosphorescence changes ($\Delta F/F(\%)$) are shown in pseudocolors.