Mapping tissue chromophore changes in cerebral ischemia: A Pilot Study

David Abookasis,[a] Marlon S. Mathews,[a,b] Christopher Lay,[c] David J. Cuccia,[a] Ron D. Frostig,[c] Mark E. Linskey,[b] and Bruce J. Tromberg [a,d]

[a] Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA 92612.
[b] Department of Neurological Surgery, University of California, Irvine, Medical Center, Orange, CA 92868.
[c] Department of Neurobiology and Behavior, University of California, Irvine, CA 92697.
[d] Department of Biomedical Engineering, the Henry Samueli School of Engineering, University of California, Irvine, CA 92697.

ABSTRACT

We describe the projection of spatially modulated light for quantitatively mapping changes in oxyhemoglobin, deoxyhemoglobin, and oxygen saturation in two pilot studies in the rat barrel cortex during both permanent and temporary cerebral ischemia. The approach is based on the projection of spatial modulation of white light onto the brain. The reflected light is captured on a CCD camera, which is then processed to obtain the concentration and distribution of chromophores over a wide field. Preliminary results confirm a measurable and quantifiable increase in tissue molecular concentration of deoxy-hemoglobin and decrease in hemoglobin oxygen concentration in both experimental settings. Our preliminary data from our pilot studies demonstrate that spatial modulation of light can provide quantitative chromophore mapping of the brain and has a potential role in monitoring the course and severity of cerebral ischemia in cerebrovascular disease patients.

Keywords: Brain Injury, Stroke, Spatially Modulate Light, Optical Properties, Cerebral Hemodynamics.

1. INTRODUCTION

Brain ischemia is the damage to brain tissue that occurs when blood flow to a region of the brain suddenly stops or decreases below tissue viability thresholds resulting in tissue dysfunction or death with subsequent temporary or permanent loss of brain function.[1] During ischemia there are changes in the concentrations of oxyhemoglobin, deoxyhemoglobin, and water in brain tissue.[2-4] In this study we propose a new technique to detect and quantitatively map changes in oxyhemoglobin, deoxyhemoglobin and oxygen saturation, during acute ischemic injury of the brain using spatial modulation of white light.[5] In order to demonstrate the ability of the technique to quantify hemodynamic activity in the brain during ischemia, we focused our experiments on two different types of acute injury in the rat brain: permanent stroke and transient ischemic attack (TIA). Strokes (cerebrovascular accident or CVA), result from vascular disease affecting the arteries supplying blood to the brain and occurs when one of these vessels bursts or is clogged.[6] The most commonly involved artery is the Middle Cerebral Artery (MCA) which when occluded causes a large hemispheric stroke.[7] TIA is reversible ischemic injury to the brain that occurs when a blood clot temporarily clogs an artery, and blood supply to part of the brain is temporarily compromised.[8] The symptoms, which are the same as stroke symptoms, occur rapidly and last for a few minutes to a few hours, but reolve within 24 hours. Clinically, TIA's are major risk factors for subsequent strokes, and are associated with eventual cognitive deterioration and vascular dementia.

Photonic Therapeutics and Diagnostics III, edited by Nikiforos Kollias, Bernard Choi, Haishan Zeng, Reza S. Malek, Brian J. Wong, Justus F. R. Ilgner, Kenton W. Gregory, Guillermo J. Tearney, Henry Hirschberg, Steen J. Madsen, Proc. of SPIE Vol. 6424, 64242J, (2007) · 1605-7422/07/$18 · doi: 10.1117/12.718430

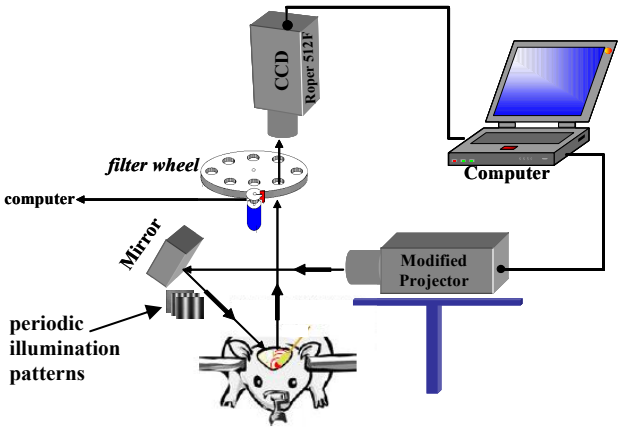


Figure. 1: Schematic of the optical configuration.

2. MATERIALS AND METHODS

2.1 Imaging Instrument

A schematic diagram of our experimental setup is shown in Fig. 1. Periodic illumination patterns of two spatial frequencies with a 120º phase shifting between three adjacent patterns are projected onto the brain from a conventional projector based digital micromirror device (DMD). However in order to fit the projector to our demands such as illumination stability, high intensity, and small region of interest several modification steps were required. Further technical details about these changes are beyond the scope of this paper and will be published separately. The diffusely reflected light (the deformed fringe pattern) passes through 10nm-wide bandpass filters (Andover Corp.) and is then recorded by a CCD camera (Roper Scientific, 512F), which features an 512×512 imaging array covering the spectral range between 400nm and 1000nm. Five bandpass filters (680, 720, 760, 800 and 980nm, at times we used 880nm instead of 980nm) are placed on a five position automatic filter wheel (Spectral Product, AB302). The demodulation of the reflectance spatially-modulated light characterizes the modulation transfer function of the diffuse reflectance of the brain and contains structural and functional information of the brain. To determine quantitatively the absorption (μ_a) and reduced scattering (μ_s') coefficients, and hence also the chromophores parameters, we used the diffusion approximation to the radiative transport theory with Monte Carlo simulation[9] to relate the measured reflected light intensity to the optical coefficients through MATLAB technology (Ver 7.04). The entire system is controlled by a personal computer through the LabView platform (Ver 7).

2.2 Surgical Procedure

Adult male Sprague-Dawley rats were anesthetized and their heads were fixed on a stereotactic apparatus in order to create an imaging window. A midline skin incision was made and 5x5mm square area overlying the left somatosensory cortex was outlined. The skull over this area was thinned with a dental drill (to about 100μm) until the middle cerebral artery and superior cerebral veins were visible. A thin film of agarose was made over the thinned area of skull. The agarose film was covered with a coverglass and the entire set up served as an imaging window. The agarose is filled in the cranial window to prevent the skull from drying and to increase its transparency to the light. Specific procedures in each experimental group are discussed under the following section.

2.2.1 Permanent Middle Cerebral Artery Occlusion (MCAo)

In addition to the following surgical procedures outlined above, a craniotomy was performed on the left side of the brain as the imaging window to expose the MCA. The MCA was surgically coagulated using monopolar electric cautery to produce MCA occlusion.

2.2.2 Temporary Middle Cerebral Artery branch occlusion - TIA

Adult male Sprague-Dawley rats underwent the same surgical procedure to create an optical imaging window as outlined in the Section 2.2.1. 3% (wt/vol) Rose Bengal dye (Sigma, St. Louis, MO) in isotonic saline was injected through the tail vein, following which, four branches of the MCA supplying the barrel cortex were identified, using a CCD camera and a green illumination. To optically excite Rose Bengal and produce temporary thrombosis[10] a continuous wave 532nm laser beam (EO, NT53-768) with 1mw average power (after attenuation) was directed into the aforementioned surface branches of the MCA with illumination of 10 minutes at each location. Using this technique, re-canalization of the artery can be expected in approximately 2 hours.[10]

2.3 Imaging Procedure

Imaging was performed through the surgically created imaging window on the anesthetized rats held in a stereotactic frame. The reflected image of the ROI of the cortex was acquired on a CCD camera while projecting spatially modulated light onto the ROI. The modulated images were obtained from ROI selected by the investigators, and processed to obtain quantitative oxy and deoxy hemoglobin maps. These maps were generated every minute to study the changes in cortical perfusion with time following experimental manipulation. Imaging was started before induction of experimental ischemia to establish baseline concentrations of oxyhemoglobin, deoxyhemoglobin, and hemoglobin oxygen saturation and repeated during and after experimental intervention. The animal remained anesthetized during the entire imaging procedure.

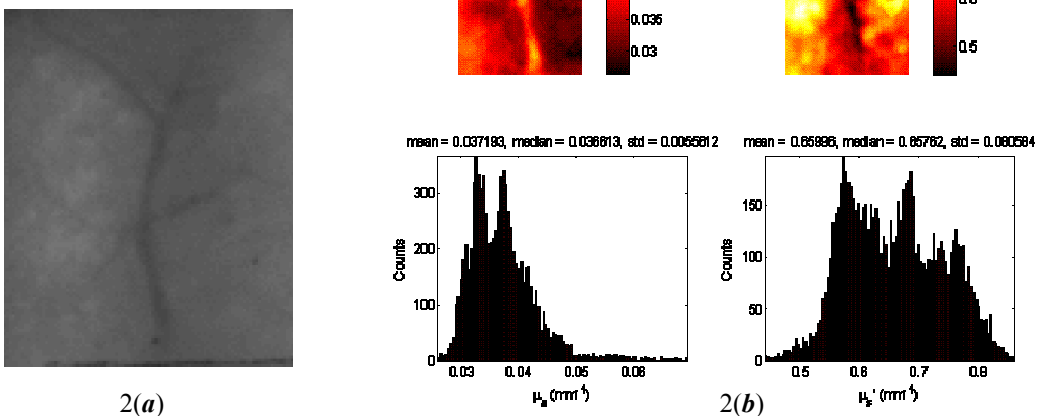
Animal housing, care, and experimental protocols were carried out in conformity with the guidelines of the United States National Institutes of Health. The laboratory animal protocol for this work was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine and by the U. S. Department of Defense (DOD).

3. RESULTS AND DISCUSSION

3.1 MCAo Results

The demonstration of the absolute optical properties (μ_a and μ_s' in units of mm^{-1}) maps recovered at 680nm after the occlusion at the end of the experimental session obtained from the ROI is shown in Fig. 2. The ROI photograph of the rat cortex is shown in Fig. 2(*a*). The size of this ROI is about 5×5 mm. Optical coefficients were retrieved from measurements using the solution of the steady state diffusion equation for a semi-infinite medium for spatial illumination source. Seen in the upper left of Fig. 2(*b*) is strong absorption at this wavelength in a vein from deoxy-hemoglobin in venous blood. Below each map is histogram distribution of the corresponding quantitative maps, highlighting the spatial variation in recovered optical properties. To validate the accuracy of these results we used the Monte Carlo computation of the inverse problem. The resulting optical properties were found to agree well between the two methods. By mapping the absorption coefficient at each wavelength, we can perform quantitative spectroscopy of tissue. Knowledge of the extinction coefficients of the tissue chromophores allows us to fit these spectra to a linear Beer-Lambert absorption model.[11] Consequently, we arrive at the quantitative concentrations of each chromophore after the occlusion, shown in Fig. 3. Notice the low and high concentration of oxy and deoxy-hemoglobin, respectively, over the vein regions. This effect can be emphasized by calculating the tissue-level oxygen saturation (S_tO_2 =

100*(HbO_2/[HbO_2+Hbr])), highlighting the effect of tissue oxygen extraction. The summation of HbO_2 and Hbr yields the total hemoglobin concentration (THC). Note that this quantitative, micromolar concentration is a direct, absolute measure of blood volume. The average results obtained from these maps yield concentrations for HbO_2, Hbr, THC and StO_2 of 35μM, 54μM, 89μM and 40%, respectively.



2(***a***) 2(***b***)

Figure. 2: (***a***) ROI photograph of the rat cortex selected by the investigator for data processing. (***b***) Top: Quantitative Absorption (left) and scattering (right) maps at 680nm over the ROI. Bottom: histograms corresponding to above maps showing statistical distribution of recovered map values.

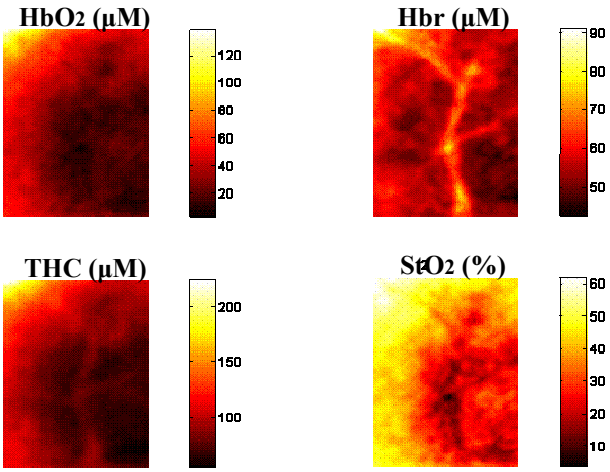


Figure. 3: Quantitative hemodynamic mapping of the cortex after the occlusion at the end of the experimental session. Higher concentration values correspond to brighter pixel scale values represents by the scale bar in the right of each panel.

A comparison of the changes in the Hbr and S_tO_2 maps pre and post the occlusion are shown in Figure 4. Averaging over each of these maps we found a 15% increase in absolute tissue molecular concentrations of deoxy-hemoglobin concentration and a 20% decrease in hemoglobin oxygen saturation from a baseline measurement respectively following MCA occlusion. These changes reflect the pathophysiologic state of the brain and the ability of spatially modulated light to quantify changes in chromophore concentration with time.

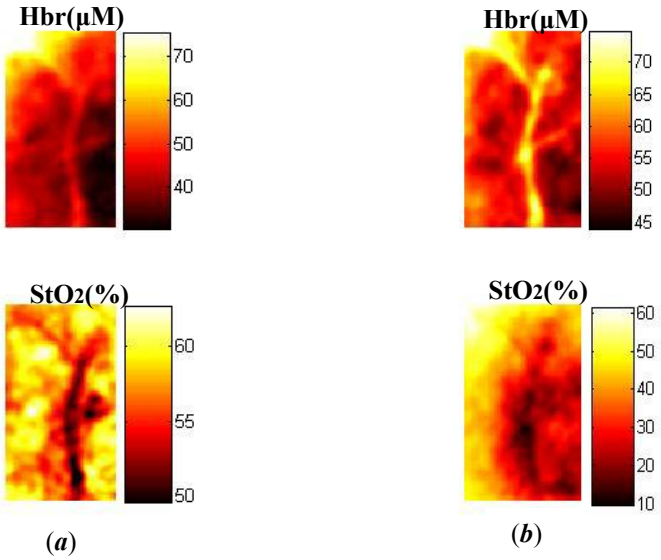


Figure. 4: Deoxyhemoglobin and oxygen saturation maps pre(***a***) (baseline) and post (***b***) MCAo.

3.2 TIA Results

Thrombosis was introduced by optical excitation of Rose Bengal using green laser in the TIA experiment. This is an established model for inducing reversible ischemia already demonstrated by Schaffer *et. al*.[10] To assist with visualizing the blood flow while inducing the TIA, we inject through the tail-vein a fluorescent contrast agent Fluorescein-conjugated Dextran (FITC) prior to the Rose Bengal injection. Since excitation of FITC by blue light causes a fluorescent emission in the green region, we used a blue LED source and green filter in the front of the camera to visualize and to confirm the production of thrombosis. Clots are characterized by non-fluorescent region of densely packed cells. Under the CCD camera with green LED illumination, candidate vessels for photochemical blockage were identified and their position noted. Image of the cortex sixty-three minutes after 10 min of 532nm laser illumination for phototrombosis were acquired and analyzed. An example of the changes in the average deoxyhemoglobin concentration over time after the veins occlusion are depicted in Fig. 5. Photothrombosis was followed by an increase (68%) in tissue deoxy-Hb concentration from the baseline and a decrease in tissue oxygen saturation by 35 %. Between 84-86min after photothrombosis we observed an 11% decrease in tissue Hbr concentration which plateaued at 56μM for the remainder of the imaging session. This reversal was probably from spontaneous thrombolysis occurring in one or more of the four illuminated vessels. Further study is on the way to validate this observation. An interesting finding in the relative changes of tissue Hbr concentrations is that following MCA occlusion tissue Hbr increased by 15%, however in the TIA a 68% increase was seen. At this point the reason for differences in magnitude of changes in chromophore concentrations between the two models is not clear. Possible mechanisms include variations in tissue response to temporary or permanent ischemia as well as technical variability from device calibration. Further study is necessary to determine if this observation is due to physiologic, technical or calibration issues.

4. CONCLUSION

In this paper, we have demonstrated a powerful technique that provides quantitative information on the spatiotemporal distribution of physiologic parameters during brain ischemia in rodents using structured light. Two different pilot studies to map both permanent and temporary ischemic injury in the rat barrel cortex were explored. Although, a number of studies relating to the application of optical modalities in the brain have been presented during the last thirty years including NIR spectroscopy (NIRS),[12] optical intrinsic signal imaging (OISI),[13] and laser speckle contrast imaging(LSCI),[14] to the best of our knowledge, this is the first work describing the use of spatial modulation of light for mapping acute changes in tissue concentrations of physiologic chromophores over time in response to ischemia and separates absorption from scattering. The results in this study are consistent with known directions of change in oxy-,deoxy- hemoglobin and oxygen saturation mentioned in the literature.[15-17] We found that the values of the chromophore concentrations determined here agree with published data.[18,19] Thus we believe that spatial modulation of light can be useful for quantitative chromophore mapping of the brain and has great potential for assessing and monitoring cerebral ischemia. The results presented in this work are preliminary, and more work is currently in progress.

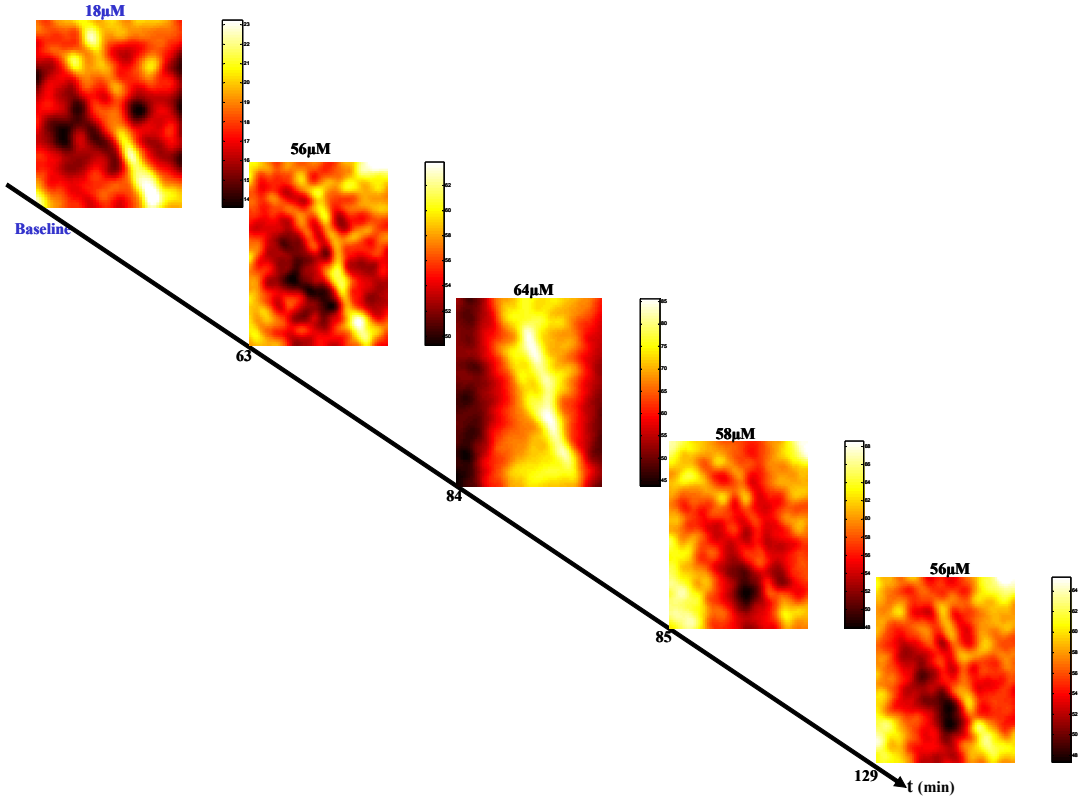


Figure. 5: Quantitative chromophore distribution maps changes in space and time for deoxyhemoglobin. In each panel the average Hbr (top) and the time after the occlusion (bottom) are shown.

ACKNOWLEDGMENTS

This study has been funded by Laser Microbeam and Medical Program (LAMMP), a NIH Biomedical Technology Resource, grant #P41-RR01192, the U.S. Air Force Office of Scientific Research (AFOSR), Medical Free-Electron

Laser (MFEL) Program (F49620-00-2-0371 and FA9550-04-1-0101) and the Beckman Foundation. Mr. Abookasis gratefully acknowledges the support of the Rothschild Foundation (Yad Hanadiv) Postdoctoral Research Fellowship Program, Israel. The authors would also like to thank Zhou *et.al.*[20] for permission to use the image of the rat shown in Fig. 1. E-mail may be addressed to dabookas@uci.edu

REFERENCES

1. U. Dirnagl, C. Iadecola, and M.A. Moskowitz, "Pathobiology of ischaemic stroke : an integrated view," Trends Neurosci. **22**, pp. 391-397 (1999).
2. J. S. Wyatt, M. Cope, D. T. Delpy, S. Wray, and E. O.R. Reynolds, "Quantification of cerebral oxygenation and homodynamic in sick newborn infants by near infrared spectrophotometry," Lancet. **2**, pp. 1063-1066 (1986).
3. J. P. Culver, T. Durduran, T. Furuya, C. Cheung, J. H. Greenberg, and A. G. Yodh, "Diffuse optical tomography of cerebral blood flow, oxygenation, and metabolism in rat during focal ischemia," J. Cereb. Blood Flow Metab. **23**, pp. 911-924 (2003).
4. C. S. Robertson, S. P. Gopinath, and B. Chance, "Use of near-infrared spectroscopy to identify traumatic intracranial hemotomas," J. Biomed. Optics. **2**, pp. 31-41 (1997).
5. D. J. Cuccia, F. Bevilacqua, A. J. Durkin, and B. J. Tromberg, "Modulated imaging: quantitative analysis and tomography of turbid media in the spatial-frequency domain," Opt. Lett. **30**, pp. 1354-1356 (2005).
6. L. R. Caplan, *Stroke*, (AAN Press, 2006).
7. S. T. Chen, C. Y. Hsu, E. L. Hogan, H. Maricq, and J. D. Balentine," A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction," Stroke. **17**, pp. 738-743 (1986).
8. S. Chaturvedi, and S. R. Levine, *Transient Ischemic Attack*, (Blackwell Publishing, 2004).
9. F. Bevilacqua and C. Depeursinge, "Monte Carlo study of diffuse reflectance at source-detector separations close to one transport mean free path," J. Opt. Soc. Am. A. **16**, pp. 2935-2945 (1999).
10. C. B. Schaffer, B. Friedman, N. Nishimura, L. F. Schroeder, P. S. Tsai, F. F. Ebner, P. D. Lyden, and D. Kleinfeld, "Two-Photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion," PLoS Biol. **4**, pp. 258-270 (2006).
11. D. T. Delpy, M. Cope, P. van der Zee, S. Arridge, S. Wray, and J. Wyatt, "Estimation of optical pathlength through tissue from direct time of flight measurement," Phys. Med. Biol. **33**, pp. 1433-1442 (1988).
12. A. Villringer, J. Plank, C. Hock, L. Schleikofer, and U. Dirnagl, "Near-infrared spectroscopy (NIRS): a new tool to study hemodynamic changes during activation of brain function in human adults," Neurosci. Lett. **154**, pp. 101-104 (1993).
13. A. Grinvald, E. Lieke, R. D. Frostig, C. D. Gilbert, and T. N. Wiesel, "Functional architecture of cortex revealed by optical imaging of intrinsic signals," Nature. **324**, pp. 361-364 (1986).
14. A. K. Dunn, H. Bolay, M .A. Moskowitz, and D. A. Boas, "Dynamic imaging of cerebral blood flow using laser speckle," J. Cereb. Blood Flow Metab. **21**, pp. 195-201 (2001).
15. D. K. Hill, and R. D. Keynes, "Optical changes in stimulated nerve," J. Physiol. **108**, pp. 278-281 (1949).
16. F. F. Jöbsis, "Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters," Science. **198**, pp. 1264-1267 (1977).
17. J. E. Brazy, D. V. Lewis, M. H. Mitnick, and F. F. Jöbsis, "Noninvasive monitoring of cerebral oxygenation in preterm infants: preliminary observations," Pediatrics. **75**, pp. 217-225 (1985).
18. J. Choi, M. Wolf ,V. Toronov, U. Wolf, C. Polzonetti, L. P. Safonova, R. Gupta, A. Michalos, W. Mantulin, and E. Gratton, "Noninvasive determination of the optical properties of adult brain: near-infrared spectroscopy approach," J. Biomed. Opt. **9**, pp. 221-229 (2004).
19. J. Zhao, H. S. Ding, X. L. Hou, C. L. Zhou, and B. Chance, "*In vivo* determination of the optical properties of infant brain using frequency-domain near-infrared spectroscopy," J. Biomed. Opt. **10**, pp. 0240281-0240287 (2005).
20. C. Zhou, G. Yu, D. Furuya, J. Greenberg, A. Yodh, and T. Durduran, "Diffuse optical correlation tomography of cerebral blood flow during cortical spreading depression in rat brain," Opt. Exp. **14**, pp. 1125-1144 (2006).