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Assessment of inflow and washout of indocyanine green in the adult human brain by monitoring of diffuse reflectance at large source-detector separation

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Abstract. Recently, it was shown in measurements carried out on humans that time-resolved near-infrared reflectometry and fluorescence spectroscopy may allow for discrimination of information originating directly from the brain avoiding influence of contaminating signals related to the perfusion of extracerebral tissues. We report on continuation of these studies, showing that the near-infrared light can be detected noninvasively on the surface of the tissue at large interoptode distance. A multichannel time-resolved optical monitoring system was constructed for measurements of diffuse reflectance in optically turbid medium at very large source-detector separation up to 9 cm. The instrument was applied during intravenous injection of indocyanine green and the distributions of times of flight of photons were successfully acquired showing inflow and washout of the dye in the tissue. Time courses of the statistical moments of distributions of times of flight of photons are presented and compared to the results obtained simultaneously at shorter source-detector separations (3, 4, and 5 cm). We show in a series of experiments carried out on physical phantom and healthy volunteers that the time-resolved data acquisition in combination with very large source-detector separation may allow one to improve depth selectivity of perfusion assessment in the brain. [DOI: 10.1117/1.3574018]

Keywords: spectroscopy; near-infrared imaging; medical imaging; light diffusion; brain perfusion; indocyanine green.

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1 Introduction

Brain perfusion is a measure of brain vitality which is of special importance for treatment of patients with insufficiencies of cerebral circulation. Several methods allowing for 3-D imaging of brain perfusion like single photon emission computed tomography (SPECT)[1] or contrast agent–mediated computed tomography (CT)[2] and magnetic resonance imaging (MRI)[3] improved treatment of critically ill patients suffering from brain perfusion deficits.[4–6] However, these methods are difficult to apply in patients treated in the intensive care unit, whereas transporting patients in critical condition to the imaging facility is particularly dangerous. Additionally, these imaging methods are expensive, which makes frequent repetition of the measurement economically unjustified or even impossible. The more easy-to-apply and cheaper method based on intracranial ultrasound imaging is limited to evaluation of blood flow in the large brain arteries. It can be applied only through a small bone window and cannot give information about perfusion of the brain cortex.[7,8] As an alternative to the above-mentioned methods, near-infrared spectroscopy (NIRS) was proposed in middle 1980s for assessment of brain oxygenation at the bedside.[9,10] NIRS is an optical modality based on emission of light into the tissue and analysis of light remitted at the distance of several centimeters. Because of the low absorption and high scattering coefficient of the tissue in the near-infrared wavelength region, the probability of reemission of the photons is rather high. Differences in spectral properties of the oxygenated and deoxygenated hemoglobin can be utilized in order to estimate changes in concentrations of these chromophores.[11] The method was validated in many physiological and clinical studies carried out in adults and neonates.[12] A serious problem connected with application of the NIRS technique is contamination of the measured signals with the components originating from the extracerebral tissue layers (skin, skull).[13] It was shown that an increase of interoptode distance leads to better determination of intracerebral changes in tissue absorption.[14] It was also reported that utilization of time-resolved measurement, which allows one to differentiate early (superficially traveling) and late photons (traveling with higher probability to the brain), may allow one to study brain oxygenation with depth discrimination.[15]

A method for assessment of brain tissue vitality with the use of an optical contrast agent bolus tracking technique based on NIRS was proposed and extensively tested in the last decade.[16–23] It was shown that monitoring of inflow of indocyanine green (ICG) allows for evaluation of cerebral blood flow (CBF)[18,22,24–27] and cerebral blood volume.[16,17] These reports were based mostly on continuous-wave near-infrared spectroscopy in which the signal from the brain was contaminated

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by the extracerebral tissue perfusion. Hongo et al. showed with a multidistance continuous-wave system that the amplitude of the change of optical signal caused by inflow of the dye increased with source-detector separation when ICG was injected into the internal carotid artery.[28] Recently, it was also shown that frequency-domain and time-resolved near-infrared reflectometry may reduce influence of contaminating signals related to the perfusion of extracerebral tissues and allow for better discrimination of information originating from the brain.[18,19,22,24,29,30] A methodology for time-resolved detection of fluorescence, which has good potential of improvement of depth discrimination, was also proposed.[31–34]

In the present paper, we continue the studies related to the monitoring of inflow and washout of ICG in adult humans. We show that the near-infrared light can be detected noninvasively on the surface of the tissue at very large interoptode distance. In most of the NIRS instruments, the distance between source and detector on the surface of the tissue varies between 3 and 5 cm. Measurements at larger distances are difficult because of the fast decay of the signals with the interoptode distance. In theoretical studies, it was shown that the probability of the reemitted photon penetration in the brain tissue increases at large source-detector separations. Thus, such measurement may lead to better selectiveness of the signals originating from the brain.

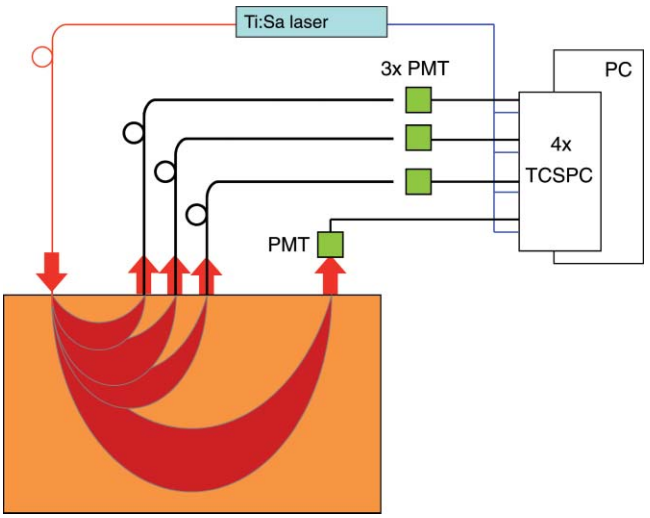

Fig. 1 Construction of a combined laboratory setup for measurement of diffuse reflectance at source-detector separations of 3, 4. and 5 cm (using fiber bundles marked by thick black lines) and at 9 cm using the PMT located directly on the surface of the tissue. The laser light is generated by a Ti:Sa laser and transmitted to the tissue with the use of optical fiber (red line). Single-photon pulses from the PMTs are transferred to four TCSPC cards mounted in an industrial PC. Synchronization pulses are provided from the laser source (blue lines).

2 Methods

2.1 *Instrumentation*

A time-resolved laboratory setup based on a femtosecond MaiTai laser (Spectra Physics, Irvine, California) was constructed. Laser pulses were generated at a wavelength of 760 nm with frequency of 80 MHz. The light was delivered to the subject's head with the use of an optical fiber (400 μm diam). On the surface of the tissue, a beam expander was applied in order to distribute the laser light on the area of the tissue that was 15 mm diam. The laser light power measured on the tip of the fiber was 300 mW.

A small-size photomultiplier tube (PMT) detector (R7400U-02, Hamamatsu Photonics, Hamamatsu City, Japan) was positioned directly on the surface of the head. The detector photocathode was ~8 mm diam. The center of the detector was located at a distance of 9 cm from the center of source position. The PMT was equipped with a high-voltage power supplier (C4900–01, Hamamatsu Photonics, Hamamatsu City, Japan) and a preamplifier (HFA-D, Becker & Hickl, Berlin, Germany). All these elements were mounted in a metallic box, which prevented influence of external high-frequency noise sources. In order to avoid any contact between the subject's head and the surface of the PMT photocathode, a glass-shielding plate (5 mm thick) was mounted in front of the PMT.

In order to compare signals obtained at large source-detector separation with data collected at shorter interoptode distances, the setup was combined with the time-resolved brain imager, for which construction details can be found elsewhere.[35] The combined laboratory setup is presented in Fig. 1. This monitoring system allowed one to perform measurements at distances of 3, 4, and 5 cm with the use of detecting bundles (4 mm diam) and at a 9-cm distance with the use of the PMT located directly onto the surface of the tissue. The single-photon pulses were processed by a set of four time-correlated single-photon counting cards (SPC-134, Becker & Hickl, Germany). The cards were mounted in an industrial PC. The distributions of times of flight of photons (DTOFs) were acquired using a LabView (National Instruments, Austin, Texas) software with repetition of 10 Hz.

2.2 *Signal Processing*

The DTOFs were acquired synchronously in four measurement channels with a sampling frequency of 10 Hz. The DTOFs were analyzed by calculation of their statistical moments:[36] total number of photons N_{tot}, mean time of flight $\langle t \rangle$ and variance V. A Matlab 13 (Mathworks Inc., Natick, Massachusetts) environment was applied for signal processing. The signals representing the changes in moments of the DTOFs were smoothed with a 3-s moving average. It should be noted that all the signals of moments acquired at the different source-detector separations were preprocessed in the same way. Thus, qualitative analysis of noise level difference in the measured signals is possible.

It was reported that higher moments of the DTOF (specifically variance) are increasingly sensitive to absorption changes appearing in the deeper tissue compartments and have lower sensitivity to the changes located superficially.[37] A combination of measurements at large source-detector separation, which will allow the measurement of the signals from the deep tissue compartment, with the utilization of variance of the DTOF will assure proper condition for monitoring of the inflow of ICG to the brain.

2.3 *Phantom Experiments*

A fish tank (size 41×26×30 cm) was filled with a solution of milk (3.2% fat content) and water (1:3) with a small amount of black ink (ca. 0.125‰), which led to optical properties of the mixture close to these observed in living tissues

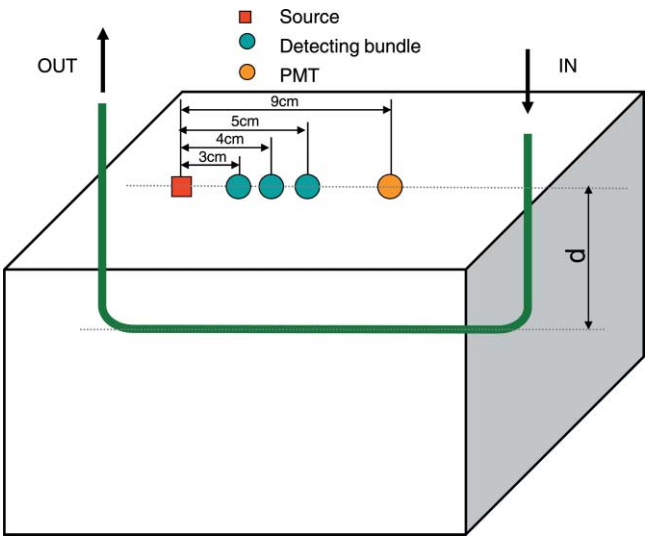

Fig. 2 Geometry of the laboratory setup for measurement of diffuse reflectance at various source-detector separations. The tube was located at various depths d in the optically turbid medium mimicking human tissue (mixture of milk and ink with optical properties of $\mu_s' \approx 12.5\ \mathrm{cm}^{-1}$ and $\mu_a \approx 0.035\mathrm{cm}^{-1}$.

($\mu_s' \approx 12.5\ \mathrm{cm}^{-1}$ and $\mu_a \approx 0.035\ \mathrm{cm}^{-1}$). The same mixture was pumped from the container through a transparent plastic tube (3 mm i.d., 4 mm o.d.) using a peristaltic pump with speed of ~0.9 ml/s. The configuration of the optodes and geometry of the phantom are shown in Fig. 2. Measurements were carried out for a single-source position and four detection positions, which formed four source-detector pairs (with source-detector separation of 3, 4, 5, and 9 cm) in analogy to the optode setup used in the *in vivo* studies. The tube was located parallel to the surface of the liquid and to the line formed by the optodes (as shown in Fig. 2). There were four different depths at which the tube was fixed in the phantom: 1, 3, 5, and 7 cm below the surface of the liquid. The beam expander, tips of fiber bundles, and the PMT were fixed within black rubber, which covered the surface of the liquid in order to prevent any reflections of light. Additionally, the optode setup was positioned on the surface of the fish tank in such a way that the distance between any of the detecting bundles and source optics and the wall of the fish tank was not shorter than 10 cm, which prevented the influence of the reflections of light on the surface of the walls on the signals measured.

In order to mimic injection of the bolus, a mixture of ICG with the above-mentioned water-milk-ink solution was rapidly injected into the tube and the time-resolved signals revealing inflow of the dye were monitored. Each bolus contained 2 mg of ICG dissolved in 2 ml of the milk-water-ink mixture.

2.4 *In Vivo Measurements*

The *in vivo* measurements were carried out on three healthy subjects (the coauthors of the paper). The subjects were examined in supine position. The optodes were fixed on the surface of the tissue with the use of rubber foam and Velcro stripes. The optodes were positioned on the forehead close to the line of hairs. A dose of 10 mg of ICG (Pulsion, Germany) dissolved in 5 ml of aqua pro injectione was administrated rapidly (injection time of ~1 s) into the forearm vein and flushed by a consecutive quick injection of 10 ml of normal saline.

3 Results

3.1 *Phantom Measurements*

Signals of moments of the DTOFs obtained for different depths of the tube locations in the phantom are shown in Fig. 3. It can be noted that when the tube is located superficially ($d = 1$ cm), all the considered moments reveal sensitivity to the inflow of the dye at all used source-detector separations. The inflow of the dye leads to a sudden drop of all considered signals. For N_{tot} and $\langle t \rangle$, the amplitude of the drop increases gradually with increase of the interoptode distance. This effect for N_{tot} was already reported by other authors.[28] When the tube was located at the depth of 3 cm, detection of the bolus in N_{tot} signals was possible only with the source-detector separation of 9 cm. For this depth of the tube fixation, only signals of variance V allow one to detect the bolus for the interoptode separations shorter than 9 cm. However, for the source-detector of 3 cm (typically used in *in vivo* studies), detection of the bolus is impossible. In analysis of mean time-of-flight changes, it can be observed that even larger source-detector separations reveal only a small amplitude of the signal drop caused by inflow of the dye. For the interoptode distance of 9 cm, the amplitude of the signal drop was ~50 ps, which is far above the noise level typically observed in such measurements. For the tube located at depth of 5 cm, the bolus inflow can be detected only for source-detector separation of 9 cm. Moreover, this detection was possible only with the use of variance of the measured distribution of times of flight of photons. However, it should be noted that the amplitude of signal decrease was much smaller in comparison to the cases when the tube was located superficially (note the difference in scaling of the amplitude for lower two rows in Fig. 3). For the other two moments (the number of photons and mean time of flight), changes in the signal connected with inflow of the dye were not observed, even at large source detector separation. Finally, analysis of the signals obtained for the tube fixed at the depth of 7 cm shows that for all source-detector separations and all considered moments of the DTOFs, the inflow of the dye was nondetectable.

3.2 *In Vivo Measurements*

Time courses of change in statistical moments of the DTOFs acquired during the *in vivo* tests are presented in Fig. 4. It can be noted that the amplitude of drop of the moments of the DTOF increases with the interoptode distance. This effect was previously reported in a multidistance continuous-wave study.[28] It can be also observed in the tail of the signal that variance of the DTOF tends to return faster to the initial level at larger interoptode distances than at shorter ones. At a large interoptode distance, the noise level is relatively high for variance V in third subject, in which the count rate of photons was much lower than in both other measurements. Nevertheless, in all statistical moments obtained by using the proposed setup, the drop of the signal caused by inflow of ICG is clearly visible also for large source-detector separation.

It can be also observed that the minima reached in the signals of moments appear at different times. We studied these

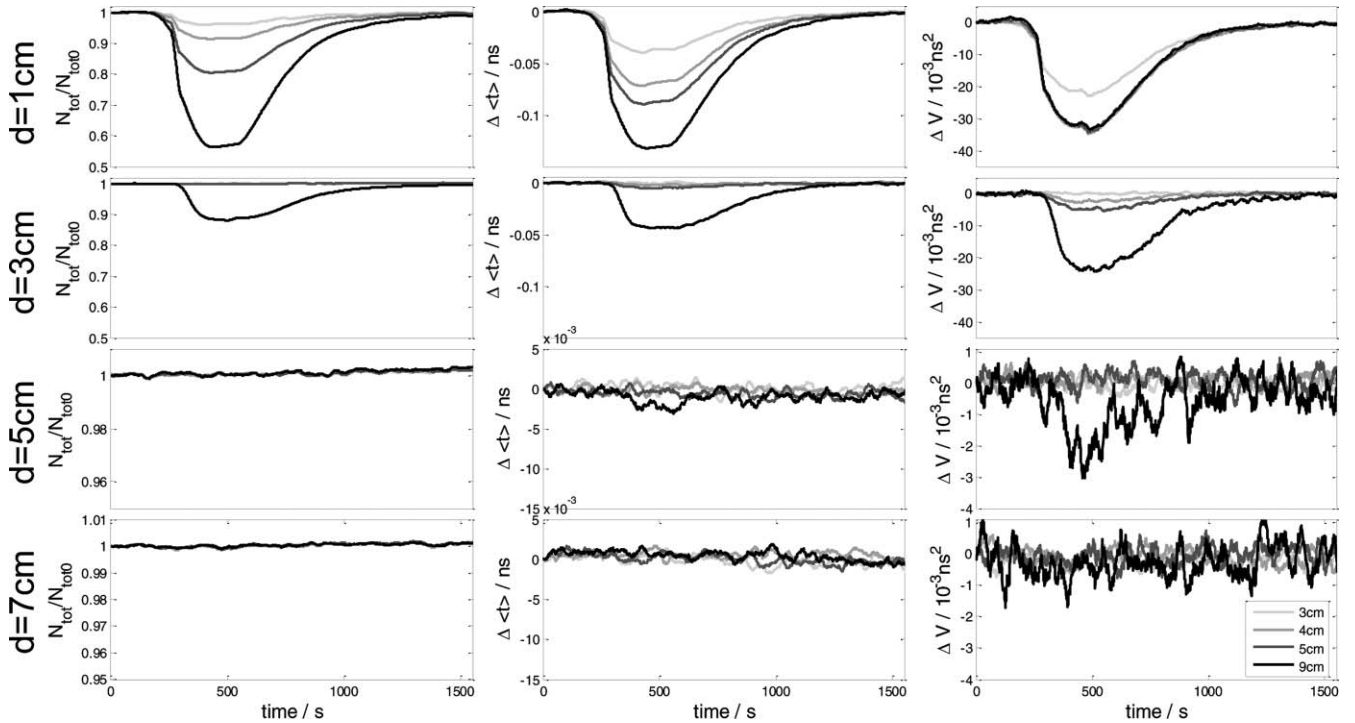

Fig. 3 Changes in the moments of DTOFs (N_{tot}, $\langle t \rangle$, and V) (shown in the columns of panels) measured during injection of the ICG in a physical phantom mimicking human tissue at four different source-detector separations (3, 4, 5, and 9 cm). The bolus was injected into a tube located at various depths d (1, 3, 5, and 7 cm), and results were shown in the rows of panels. The signals of N_{tot} were normalized by the average of the first 10 s of the measurement (N_{tot0}). For the signals of $\langle t \rangle$ and V the values of average of the first 10 s of the measurement were subtracted. Note difference in scaling of the amplitudes in lower two rows.

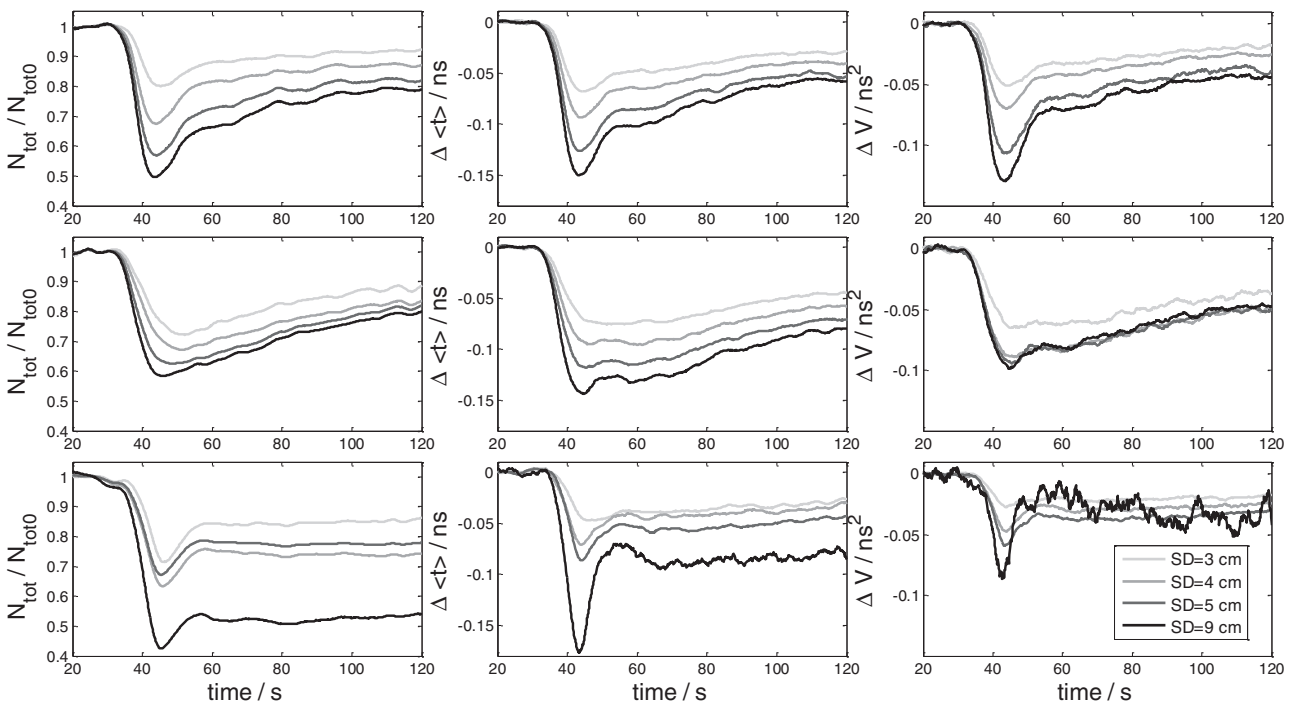

Fig. 4 Changes in the moments of DTOFs (N_{tot}, $\langle t \rangle$, and V) (shown in columns of panels) measured during injection of ICG in three healthy volunteers (rows of panels) at four different source-detector separations. The bolus injection at $t = 20$ s.

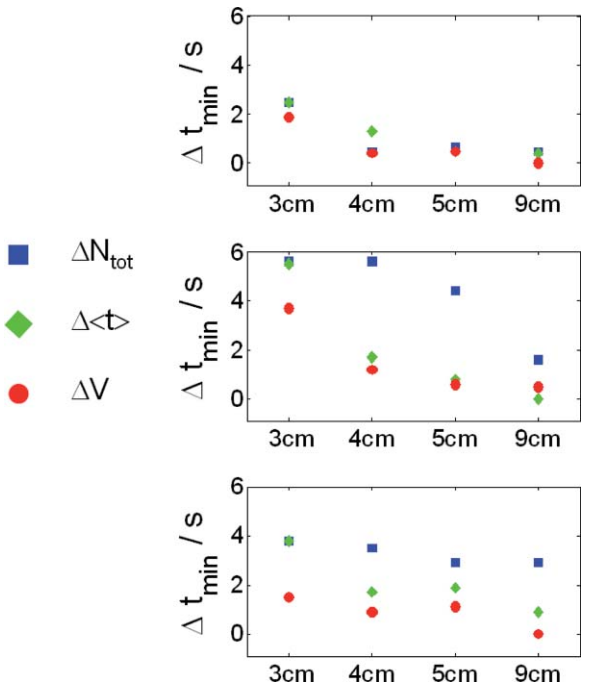

Fig. 5 Normalized delays of temporal positions of the minimum t_{min} presented for different moments and source-detector separations for three healthy volunteers.

phenomena of change in the timing of the minima of the signals in more detail. Some authors suggest using this timing for evaluation of CBF or cerebral perfusion index.[17,22,23,38] Thus, the temporal position of the minimum in the signal measured may have influence on robustness of the technique based on ICG bolus tracking. In Fig. 5, results of analysis of the temporal position of the minimum t_{min} were presented. Because of lack of arterial input function, we normalized all the delays to the shortest one observed in all signals measured in a certain subject.

It can be observed that the temporal position of the minimum depends strongly on the source-detector separation for two first subjects. For the last one the differences are smaller. However, it should be noted that even small (<1 s) deviations of t_{min} (or a related quantity, such as time to peak) may be used in assessment of brain perfusion.[39] In all subjects, at larger source-detector separations t_{min} is reduced. Besides the first subject, it can also be observed that the t_{min} is shorter for the higher moments ($\langle t \rangle$ and V).

4 Discussion and Conclusions

We presented construction of the laboratory setup allowing for acquisition of distributions of times of flight of diffusely reflected photons at large source-detector separation. This configuration of optodes can be successfully used in *in vivo* measurements. We showed that measurement of diffuse reflectance signals at a very large source-detector separation of 9 cm is feasible. The method was validated in measurements on the physical phantom and during the *in vivo* test carried out on healthy volunteers during injection of ICG.

Detection of diffuse reflectance at such large source-detector separation with a high signal-to-noise ratio was possible because of the use of powerful laser source in combination with the sensitive photodetector, which was positioned directly on the surface of the head. Application of a beam expander and distribution of the light on a large tissue area assures that the measurement is safe and does not lead to energetic stimulation of the skin tissue. The power of light used is about two orders of magnitude higher than in the time-resolved devices proposed for brain studies.[35,40–42] On the other hand, application of the sensitive photodetector (PMT) positioned directly on the surface of the head allowed one to reduce light losses. These losses are related to the application of fibers or fiber bundles in which the light is attenuated. Additionally, they limit numerical aperture of the detection system.[43] The filling factor of the fiber bundles lead to another limitation of the number of photons transmitted from the tissue to the detector. Light-coupling optics between fiber bundle and detector is used in most cases, which introduces light losses caused by reflections on optical elements. A simple comparison of light-detection efficiency with the use of the directly applied PMT and the fiber-bundle–based solution was carried out. The measurements showed that the number of photons diffusely transmitted through an optically turbid phantom collected by the PMT fixed on the surface of the phantom was 10 times higher than the number of photons collected from the same phantom with the use of fiber-bundle setup.

The optical signals measured at large source-detector separations may potentially suffer from increased noise caused by a limited number of photons that can be detected on the surface of the tissue. However, in our experiments, the number of photons detected at the source-detector separation of 9 cm was similar to that typically detected in *in vivo* studies at interoptode distance of 3 cm (count rate of 10×10^6 photons/second). This fact leads to the conclusion that the photon noise in large distance measurements is not affected by the number of photons detected. However, the width of the DTOF (which is much larger at large source-detector separation) may lead to an increase of the photon noise.[36] Additionally, it should be noted that the increased depth of light penetration and volume of the tissue interrogated by the photons at large source-detector separation may lead to lower sensitivity of the method to the small nonhomogeneities of the vascular net and perfusion in the tissue under investigation. Detailed analysis of the signal-to-noise ratio in the signals of moments of DTOFs measured at different source-detector separation needs further study.

The proposed emission-detection setup based on high-power light source and sensitive detector positioned directly on the head of the subject introduces two sources of potential risk for the patient. The first is connected with light power, and the second with influence of the high-voltage electric field. However, we ensured that the light power density on the surface of the tissue is lower than allowed by the safety regulations. On the other hand, the danger connected with presence of a high-voltage source located close to the surface of the tissue can be limited by the proper shielding of the device. It should be noted that the power delivered to the detector is very limited, which causes that the potential electrical shock would not be very dangerous for the human subject. To avoid this risk, we provided the additional glass-plate shielding the photocathode, which limits probability of the contact of the photodetector with the tissue under investigation.

The measurements were carried on a physical phantom mimicking inflow of the dye at different depths in the tissue. These studies show that the bolus of ICG can be detected even when the dye inflows at a depth of 5 cm in the model. However, this detection was possible only for source-detector separation of 9 cm and with utilization of variance of the measured DTOFs. This observation matches well with the results of *in vivo* studies. The measurements carried out on the healthy volunteers suggest that the signals measured at source-detector separation of 9 cm contain a larger contribution from the brain than the signals measured simultaneously at the shorter interoptode distances (3–5 cm). It can be observed that, at a larger source-detector distance, return of the signals to the initial level is faster than that noted at shorter distances. This effect is in line with the fact that, in healthy subjects, the dye in the brain does not undergo extravasation because of the proper brain-blood barrier function. The ICG, which is a blood pool agent, is washed out from the brain very quickly. The signals measured represent superposition of changes in absorption originating from the inflow of dye to the brain and to the overlying extracerebral tissues in which the dye may undergo extravasation. Thus, the signals measured contain a fast inflow-washout pattern typical for the brain and delayed, broader pattern characteristic for extracerebral tissues.[18,37] The results of analysis of t_{min} show that the temporal position of the minimum depends strongly on source-detector separation. It was noted that the signals monitored at a very large emitter-detector distance reached their minima in the shortest times. The delay between intra- and extracerebral contributions was estimated to be a few seconds in healthy subjects by MRI experiments.[18] In this respect, the differences in t_{min} observed in the *in vivo* experiments suggest that the content of the extracerebral contamination in the signals measured is smaller for large source-detector separations. The results obtained suggest also that, at large source-detector separation, the fast inflow-washout pattern is visible with higher probability when the higher order moments (mean time of flight and variance) of the DTOF are analyzed in comparison to the analysis of number of photons typically measured in continuous-wave NIRS devices. This observation is in line with our previous reports on advantages of monitoring of higher order moments during injection of the ICG.[35,37,38]

It should be noted that the NIRS measurements carried out at very large source-detector separation will suffer from limited spatial resolution. Nevertheless, the effect of suppression of contamination of the measured signals by the influence of extracerebral tissues at large source-detector separation may be very important for clinical usefulness of the NIRS instrumentation. It should be also considered that at extremely large source-detector separations, the depth of light penetration is much larger than for a typically used separation of 3–5 cm. In consequence, the measurement carried out at vary large emitter-detector distance may allow one to probe deeper layers of the brain tissue. Thus, one of the most critical limitations of the NIRS technique may be eliminated with the use of the technique proposed in this paper.

Acknowledgments

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