Anatomical and Functional Images of *in vitro* and *in vivo* Tissues by NIR Time-domain Diffuse Optical Tomography*

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Near infra-red (NIR) diffuse optical tomography (DOT) has gained much attention and it will be clinically applied to imaging breast, neonatal head, and the hemodynamics of the brain because of its noninvasiveness and deep penetration in biological tissue. Prior to achieving the imaging of infant brain using DOT, the developed methodologies need to be experimentally justified by imaging some real organs with simpler structures. Here we report our results of an *in vitro* chicken leg and an *in vivo* exercising human forearm from the data measured by a multi-channel time-resolved NIR system. Tomographic images were reconstructed by a two-dimensional image reconstruction algorithm based on a modified generalized pulse spectrum technique for simultaneous reconstruction of the μ_a and μ'_s . The absolute μ_a - and μ'_s -images revealed the inner structures of the chicken leg and the forearm, where the bones were clearly distinguished from the muscle. The $\Delta\mu_a$ -images showed the blood volume changes during the forearm exercise, proving that the system and the image reconstruction algorithm could potentially be used for imaging not only the anatomic structure but also the hemodynamics in neonatal heads.

Key Words: Diffusion, Biomechanics, Inverse Problem, Finite Element Method, Image Processing, Muscle and Skeleton, Optical Measurement

1. Introduction

Near infra-red (NIR) diffuse optical tomography (DOT), which aims at providing both anatomical images or functional information of organs, has been increasingly receiving interests in the past decade because it can be used safely and continuously for bedside intensive care⁽¹⁾⁻⁽³⁾. To date, the applications of NIR DOT are mostly focused on the potential of

imaging woman breast, human head hemodynamics and neonatal head⁽⁴⁾⁻⁽⁶⁾. For the neonates, who suffers from ischaemia or hemorrhages in brain, bedside monitoring of the cerebral perfusion situation, e.g., the blood oxygen saturation and blood volume, is inevitable for avoiding permanent injure. Due to the absorption and scattering nature of the tissue to NIR light, light emitted from head is both intensity attenuated and temporally and spatially expanded, and consequently NIR DOT systems are complicated in both image reconstruction and measurement. Now, studies for the neonate head imaging with NIR light are still concentrated on developing imaging reconstruction algorithm and measurement systems suitable for continuous bedside monitoring.

For the measurement system, the equipment should have the capability of detecting very weak light diffused by the tissue. One of the promising systems is so-called a time-resolved (TR) system using ultra-short laser and very fast light detectors

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probing the transmitted transient light that contains the information of the absorption and scattering of light in the tissue⁽⁷⁾⁻⁽⁹⁾.

The ultimate goal of NIR imaging system is to utilize the experimental data and some kind of image reconstruction algorithm for revealing the optical property distributions inside the tissue, which is so called the inverse problem. In TR system, researchers have developed nonlinear image reconstruction algorithms that use the featured-data of the temporal profiles, such as intensity and mean time, as data types to effectively reduce the computation time⁽¹⁰⁾. A generalized pulse spectrum technique (GPST), which is based on converting the time-dependent signals by Laplace transform to complex frequency domain and then inverting the complex domain components, is also attractive in solving the inverse problem. To avoid the absolute intensity scaling, one can use the ratio R of Laplace-transformed time dependent photon flux at two distinct frequencies as the data type. In the GPST, the nonlinear inverse problem posed by TR DOT can be linearized by a Newton-Raphson scheme, forming the outer-loop of the iterative procedure,

$$[\mathbf{R} - \mathbf{F}(\mathbf{x}_k)] = \mathbf{J}(\mathbf{x}_k) \delta \mathbf{x}_k, \quad \mathbf{x}_{k+1} = \mathbf{x}_k + \delta \mathbf{x}_k \quad (1)$$

where $\mathbf{R}$ is a column vector of measurement regarding R with length of $S \times D$ (S and D are the number of source and detection fibers, respectively); $\mathbf{F}(\mathbf{x}_k)$ is the solution to the diffusion equation (DE) by finite-element-method (FEM) at k th iteration to predict R ; $\mathbf{x}_k$ is the vector denoting the optical properties (both the absorption (μ_a) and scattering (μ_s) coefficient) at the nodes of the FEM mesh and $\delta \mathbf{x}_k$ their perturbations at the k th iteration; $\mathbf{J}(\mathbf{x}_k) = [\mathbf{J}_{\mu_a}(\mathbf{x}_k), \mathbf{J}_{\mu_s}(\mathbf{x}_k)]$ is the Jacobian matrix of $\mathbf{F}(\mathbf{x}_k)$ constructed using a perturbation technique to DE⁽¹⁰⁾. Due to the requirement for calculating the gradients of Green's function and flux density in $\mathbf{J}_{\mu_s}(\mathbf{x}_k)$, the standard GPST algorithm suffers from heavy computation load, numerical instability, μ_s -reconstruction insensitivity, and evident cross-talks from μ_s in μ_a -image. To further cut off the computation load and improve the image quality, we have developed a modified GPST algorithm for TR DOT that combines the standard GPST with an approximate calculation of the $\mathbf{J}_{\mu_s}(\mathbf{x}_k)$ that is directly related to $\mathbf{J}_{\mu_a}(\mathbf{x}_k)$ ⁽¹¹⁾⁻⁽¹³⁾. In the inner loop of the algorithm, $\delta \mathbf{x}_k$ in Eq. (1) is solved by a weight-scaled conjugate gradient method. The outer-loop of the iteration is truncated after which no evident improvement in the image quality is observed and the optical property values at that stage $\mathbf{x}_{k+1}$ are considered as those of the target. In order to avoid the serious influence of the systematic and statistic noise on the image reconstruction, the differential image recon-

struction method is usually adopted, in which the ratio of R between the two states is used as the datatype.

In this paper, we report our image reconstruction results for some realistic models as the step toward the anatomical and functional imaging of neonate head by using modified GPST algorithm and the multi-channel time-resolved NIR measurement system. Experiments using an *in vitro* chicken leg immersed in the Intralipid solution and using an *in vivo* human forearm, which have the similar size and heterogeneity as the neonate head, demonstrate the potentials of the NIR TR DOT system and the corresponding image reconstruction algorithm for showing the anatomic structure and hemodynamic changes.

2. Experimental Setup

The diagrammatic sketch of the imaging system is shown in Fig. 1. The system contains three pulsed laser diodes (High power picosecond light pulsers, Hamamatsu Photonics, Japan) emitting ultra-short light pulses to the target with a pulse duration of about 100 ps at 759 nm, 799 nm and 834 nm, respectively. The pulses have a repetition rate of 5 MHz and a peak power of about 300 - 400 mW resulting in an average power of about 0.2 mW. 16 fibers are attached with equal spacing along the circumference of the target through a metal fiber holder. A coaxial structure is adopted for the source and detection fibers, i.e. a single fiber of 400 μm in diameter at the inner core for irradiation and a bundle of small fibers with a diameter of 30 μm at the annulus for detection⁽⁷⁾. When one of the 16 fiber bundles is irradiated as the source, the re-emissions at the boundary were time-resolved recorded simultaneously by 9 fibers located at the opposite side to the source. Then, a set of temporal profiles is recorded by the measurement system. With these temporal profiles, the images of both μ_a and μ_s are simultaneously reconstructed using the nonlinear reconstruction algorithm as described

Fig. 1 Time resolved NIR tomography system for imaging the inner structure of targets. $\leftrightarrow$ is the symbol of the concentric fiber with the inner core for the source and annular part for the detector.

Fig. 2 Experimental setup for chicken leg measurement. 40 ml Intralipid-10% was diluted by 550 ml distilled water to make 7% Intralipid-10% solution. The imaging plane is in the half height of the solution and the chicken leg area on the imaging plane is about 30 mm $\times$ 40 mm.

above. During the experiment, the room was kept quiet, constant temperature and constant humidity.

The experimental setup for imaging the chicken leg is as shown in Fig. 2. In order to acquire data for the irregularly shaped chicken leg, a white plastic bottle with a diameter of 79 mm was used as the container, which was filled with 7% Intralipid-10% solution with a height of 120 mm as background. The imaging plane located at half the height of the solution and so that the calculation can be performed under a two dimensional model. A measurement was first taken for the homogenous solution to obtain the optical properties of the background (reference measurement) and, with all the experimental conditions fixed, another measurement (target measurement) was performed after the chicken leg was immersed in the solution. At the image plane, the distance between the longitudinal center of the chicken leg and the axis of the cylindrical container was about 15 mm; the size of the chicken leg was about 30 mm in the X direction and 40 mm in the direction perpendicular to X , as indicated in Fig. 2. The solution was mildly stirred by a magnetic stirrer during the measurements.

Human forearm experiment was carried out on a healthy male subject aged 37, as shown in Fig. 3. Informed consent was obtained from the subject prior to the experiment. During the experiment, the subject was at a sitting position with the forearm being supported at the height of his heart. The fibers were fixed in the fiber holder and kept in good contact with the forearm. Black rubber was inserted between the fiber holder and the forearm both for forming a regular circular boundary and for blocking the light leakage from the source fiber directly to the detection fibers. The diameter of the forearm confined by the rubber at the imaging plane was about 65 mm. After 10 min rest, the reference data were firstly collected

Fig. 3 Experimental setup for imaging human forearm. Optical fibers were fixed in the fiber holder and in good contact with the forearm. During exercise task measurement, the subject executed a stable 50% MVC handgrip.

while the forearm was at rest and the subject's blood pressure was normal. Then the subject started a consecutive 50% maximum voluntary contraction (MVC) handgrip exercise that caused the oxygen saturation and blood volume changes in the tissue. 1 min after, the task measurement was performed with all the experimental conditions fixed.

Time for obtaining the reference data was about 10 min when integration time was 10 s. The system adjusted the attenuation degree of the attenuators for every source-detector pair to satisfy the requirement of single photon counting. Time for collecting task data was less than 3 min at the same integration time once the attenuators were fixed.

In order to obtain the anatomic structure of the forearm for validation of our results, co-registered MRI images were taken on the subject's forearm by using a MRI imaging system (BRUKER, Germany). The image slice thickness was selected to be 5 mm for enhancing the spatial resolution.

3. Results and Discussions

All the following reconstructions were truncated at 15 iterations after which no evident improvement would be observed according to the errors between the estimated and measured data. The execution time of the reconstruction at one wavelength was less than 50 seconds for 15 iterations when performed on a 1 GHz Pentium III personal computer with memory of 1 GB. For evaluating the reconstructed images, we use the full width at half maximum (FWHM) change in gray scale of a boundary for measuring the target size.

3.1 Reconstructed images of the *in vivo* chicken leg

The optical properties of Intralipid solution were obtained by curve fitting the temporal profiles of the

background measurement, which were 0.0030 mm^{-1} and 0.62 mm^{-1} for μ_a and μ'_s at the three wavelengths and used as the optical properties of the background in the target measurement.

Figure 4 illustrate the μ_a - and μ'_s -images of the *in vitro* chicken leg reconstructed by using the temporal profiles measured at the three wavelengths. In the images, the chicken leg is clearly distinguished from the Intralipid solution background and the muscle and bone of the chicken leg that have very close μ_a can be distinguished by the aid of μ'_s -image (the extremes or the black spots appearing in the μ'_s -image). From the reconstructed μ_a -image at 759 nm (Fig. 4(a)), the size of the chicken leg is estimated about 26 mm in X -axis (the same as the X -direction in Fig. 2) and 35 mm in Y -axis; the separation between the chicken leg centroid and the axis of the container is estimated about 17 mm. All these values conform to the experimental conditions as illustrated in Fig. 2.

μ_a and μ'_s of the chicken leg muscle at 759 nm estimated from the image are about 0.022 mm^{-1} and 0.62 mm^{-1} , respectively, while those reviewed by Cheong et al.⁽¹⁴⁾ are $0.012 - 0.017 \text{ mm}^{-1}$ and $0.33 - 0.80 \text{ mm}^{-1}$ (at 633 nm), respectively. The discrepancy existing between the literature and this paper is believed mainly because of the different wavelengths and the sample preparation. μ_a and μ'_s estimated from the image for the chicken leg bone are about 0.026 mm^{-1} and 0.59 mm^{-1} , respectively. The higher μ_a and lower μ'_s of the chicken leg bone than the muscle maybe caused by the anatomical structure of chicken leg. The intact chicken bone has only a thin wall of compact bone and is filled with marrow, which can be considered to have larger μ_a and smaller μ'_s because of high concentration of blood. An artifact existing in the μ'_s -image might be caused by either the model mismatch between the two dimensional (2-D) algorithm and the three dimensional (3-D) photon migration in the real target or some measurement noise.

Though it was reported that μ'_s of Intralipid solution decreases 14% with the increase in wavelength from 759 nm to 834 nm⁽¹⁵⁾, we observed almost constant μ'_s in this wavelength range. The possible reasons of the discrepancy are the different Intralipid types as mentioned in Ref.(15) and the inaccuracy of the NIR optical image in quantification, which is caused by both the noise of measurement system and the model mismatch between the image reconstruction algorithm and the real target. Considering the μ'_s of human muscle decreases less than 3% in the wavelength range of 759 nm - 834 nm⁽¹⁶⁾ and the influence of the sample preparation, the almost constant μ'_s of the chicken muscle and bone in Fig. 4 is reasonable.

Fig. 4 Reconstructed μ_a - and μ'_s -images of an *in vitro* chicken leg at (a) 759 nm (b) 799 nm (c) 834 nm

3.2 Results of the human forearm

The co-registered transverse MRI image of the forearm is shown in Fig. 5 with the protrusion out of the arm boundary marking the position of fiber holder and the orientation of the forearm in NIR measurement. The two predominant parts in the MRI image are Ulna and Radius while the black spots distributing inside the muscle are believed to be the blood vessels. The sizes of Radius and Ulna are $13.9 \text{ mm} \times 11.5 \text{ mm}$ and $11.8 \text{ mm} \times 10.6 \text{ mm}$, which were measured in the direction connecting the maximum diameter of the two bones (direction 1) and the direction perpendicular to direction 1 (direction 2), respectively.

Figure 6 are the reconstructed absolute μ_a - and

Fig. 5 Transverse MIR image of human forearm for showing the inner structure. The protrusion out of the arm boundary is the marker of fiber holder position in NIR measurement. The two predominant parts in the image are Ulna and Radius while the black spots distributing inside the muscle are believed to be the blood vessels. Line 1 and 2 indicate the two directions for measuring the size of the bones.

μ_s -images of the forearm in the rest and exercise (hand gripping) state. Considering that one usually only adopt two wavelengths for calculating the changes in hemoglobin concentration, only the images at 759 nm and 834 nm are presented here. In the images, the higher μ_a regions probably correspond to some of the arteries and veins due to the higher absorption coefficient of blood. During NIR measurement, the arm was squeezed to some extent in the fiber holder to form a round boundary. So, the region with the maximum absorption in the μ_a -image may be not only the arteries and veins near but also those a little bit far from the skin. For NIR DOT having a lower spatial resolution, these arteries and veins may be blurred or connected in the image as shown in Fig. 6. μ_a of this maximum absorption region and other part are estimated about 0.1 mm^{-1} and $0.04 - 0.06 \text{ mm}^{-1}$, which correspond to the μ_a of blood and bone/muscle⁽¹⁴⁾, respectively. In the μ_s -image, the bones are clearly differentiated from the muscle as the region of increased scattering, which is almost exactly the same position as revealed by MRI. The size of Radius and Ulna corresponding to the direction 1 and 2 of Fig. 5 are $11.0 \text{ mm} \times 13.5 \text{ mm}$ and $11.2 \text{ mm} \times 11.2 \text{ mm}$ respectively, which are consistent with those measured from the MRI image but showing a slight shape distortion in Radius. The estimated μ_s are $1.4 - 1.8 \text{ mm}^{-1}$ for the bone and $0.6 - 1.4 \text{ mm}^{-1}$ for other tissue respectively, which are the same ranges of the μ_s of bone and muscle/blood in the literatures^{(14),(17)}.

For observing the changes in absorption in response to the oxygen concentration changes resulted by exercise, we ignore the scatter change of tissue during the activation and adopt the differential image reconstruction method as described in the introduc-

Fig. 6 Absolute μ_a - and μ_s' -images of a human forearm in the cases of rest and exercise (hand grip) at 759 nm and 834 nm, respectively

Fig. 7 Changes in absorption of the forearm between rest and excise at 759 nm and 834 nm, which are directly calculated from the differential imaging scheme.

tion. For utilizing the algorithm for absolute image reconstruction, a set of optical properties is needed as the initial values in the calculation. In this paper, the initial values used are $\mu_a = 0.04 \text{ mm}^{-1}$ and $\mu'_s = 0.8 \text{ mm}^{-1}$, which are about the optical properties of muscle. Figure 7 shows the reconstructed $\Delta\mu_a$ images between rest and excise at 759 nm and 834 nm, which are from -0.005 mm^{-1} to 0.02 mm^{-1} and from -0.005 mm^{-1} to 0.03 mm^{-1} , respectively.

Considering that, in the 700–900 nm range, the strongest absorber is hemoglobin and the blood volume is proportional to the hemoglobin concentrations^{(18),(19)}, the change in blood volume, oxy- and deoxy-hemoglobin concentration can be calculated with the extinction coefficients and the changes in absorption coefficients at the two wavelengths. Because the wavelengths of 759 nm and 834 nm are at the either side of the hemoglobin absorption isobestic point, the positive $\Delta\mu_a$ regions at both 759 nm and 834 nm in Fig. 7 represent the increment of blood volume happened around the arteries when the forearm was excising. The regions with positive $\Delta\mu_a$ at 759 nm but negative $\Delta\mu_a$ at 834 nm represent the decrease of oxygenation happened in the muscle.

4. Conclusions

This study demonstrates that the combination of a NIR TR DOT measurement system with a simultaneous μ_a and μ'_s reconstruction algorithm based on the modified GPST can not only qualitatively reveal the inner structure of tissue but also reasonably quantify the optical properties; the simultaneously reconstructed μ'_s -images is necessary to the differentiation between the organ constituents, especially for distinguishing bones, in some clinical situations when TR DOT is adopted. The differential images ($\Delta\mu_a$ -image) showed the potential of NIR DOT in detecting the oxygenation changes and perfusion situations. By further cutting off the measurement time, improving

the reconstruction algorithm for adapting arbitrary boundary, and enhancing the spatial resolution, it is hopefully for NIR DOT technology to be applied in bedside anatomic and functional imaging of the neonatal head.

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References

- (1) Reich, D., Emerging Technologies, J. of Cardiothoracic and Vascular Anesthesia, Vol. 10, No. 3 (1996), pp. 406–418.
- (2) Villringer, A. and Chance, B., No-invasive Optical Spectroscopy and Imaging of Human Brain Function, Trends Neurosci., Vol. 20, No. 10 (1997), pp. 435–442.
- (3) Boas, D.A., Brooks, D.H., DiMarzio, C.A., Kilmer, M., Gaudette, R.J. and Zhang, Q., Imaging the Body with Diffuse Optical Tomography, IEEE Signal Processing Magazine, November (2001), pp. 57–75.
- (4) Hillman, E.M.C., Hebden, J.C., Schweiger, M., Dehghani, H., Schmidt, F.E.W., Delpy, D.T. and Arridge, S.R., Time Resolved Optical Tomography of the Human Forearm, Phys. Med. Biol., Vol. 46, No. 7 (2001), pp. 1117–1130.
- (5) Boas, D.A., Gaudette, T., Strangman, G., Cheng, X., Marota, J.A. and Mandeville, J.B., The Accuracy of Near Infrared Spectroscopy and Imaging during Focal Changes in Cerebral Hemodynamics, Neuroimage, Vol. 13, No. 1 (2001), pp. 76–90.
- (6) McBride, T.O., Pogue, B.W., Gerety, E.D., Poplack, S.B., Osterberg, U.L. and Paulsen, K.D., Spectroscopic Diffuse Optical Tomography for the Quantitative Assessment of Hemoglobin Concentration and Oxygen Saturation in Breast Tissue, Appl. Opt., Vol. 38, No. 25 (1999), pp. 5480–5490.
- (7) Eda, H., Oda, I., Ito, Y., Wada, Y., Oikawa, Y., Tsunazawa, Y., Takada, M., Tsuchiya, Y., Yamashita, Y., Oda, M., Sassaroli, A., Yamada, Y. and Tamura, M., Multichannel Time-resolved Optical Tomographic Imaging System, Rev. Sci. Instr., Vol. 70, No. 9 (1999), pp. 3595–3601.
- (8) Hebden, J.C., Venstra, H., Dehghani, H., Hillman, M.C., Schweiger, M., Arridge, S.R. and Delpy, D.T., Three-dimensional Time-resolved Optical Tomography of a Conical Breast Phantom, Appl. Opt., Vol. 40, No. 19 (2001), pp. 3278–3287.
- (9) Zint, C.V., Gao, F., Torregrossa, M. and Pullet, P., Near Infrared Optical Tomography of Scattering Cylindrical Phantoms Using Time-resolved Detection, Proc. SPIE, Vol. 2, No. 7 (2001), pp. 109–120.
- (10) Arridge, S.R., Optical Tomography in Medical Imaging, Inverse Problems, Vol. 15, No. 2 (1999),

- pp. R41-R93.
- (11) Gao, F., Poulet, P. and Yamada, Y., Simultaneous Mapping of Absorption and Scattering Coefficients from a Three-dimensional Model of Time-resolved Optical Tomography, *App. Opt.*, Vol. 39, No. 31 (2000), pp. 5898-5910
 - (12) Gao, F., Zhao, H., Tanikawa, Y. and Yamada, Y., Time-resolved Diffuse Optical Tomography Using a Modified Generalized Pulse Spectrum Technique, *IEICE Trans. Inf. & Syst.*, Vol. E85-D, No. 1 (2002), pp. 133-142.
 - (13) Zhao, H., Gao, F., Tanikawa, Y., Onodera, Y., Ohmi, M., Haruna, M. and Yamada, Y., Imaging of *in vitro* Chicken Leg by Use of Time-resolved Near Infrared Optical Tomography, *Physics in Medicine and Biology*, Vol. 47, No. 11 (2002), pp. 1979-1993.
 - (14) Cheong, W.F., Prah, S.A. and Welch, A.J., A Review of the Optical Properties of Biological Tissues, *IEEE J. Quan. Elec.*, Vol. 26, No. 12 (1990), pp. 2166-2184.
 - (15) van Staveren, H.J., Moes, C.J.M., van Marie, J., Prah, S.A. and van Gemert, M.J.C., Light Scattering in Intralipid-10% in the Wavelength Range of 400-1100 nm, *Appl. Opt.*, Vol. 30, No. 31 (1991), pp. 4507-4514
 - (16) Matcher, S.J., Cope, M. and Delpy, D.T., *In vivo* Measurements of the Wavelength Dependence of Tissue-scattering Coefficients between 760 and 900 nm Measured with Time-resolved Spectroscopy, *Appl. Opt.*, Vol. 36, No. 1 (1997), pp. 386-396.
 - (17) Firbank, M., Hiraoka, M., Essenpreis, M. and Delpy, D.T., Measurement of the Optical Properties of the Skull in the Wavelength Range 650-950 nm, *Phys. Med. Biol.*, Vol. 38, No. 3 (1993), pp. 503-510
 - (18) Homma, S., Eda, H., Ogasawara, S. and Kagaya, A., Near Infrared Estimation of O₂ Supply and Consumption in Forearm Muscles Working at Varying Intensity, *J. Appl. Physiol.*, Vol. 80, No. 4 (1996), pp. 1279-1284
 - (19) Cope, M., The Application of near Infrared Spectroscopy to Non Invasive Monitoring of Cerebral Oxygenation in the Newborn Infant, PHD Thesis of University College London, (1991).