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Effects of vasodilation on intrinsic optical signals in the mammalian brain: a phantom study

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Abstract. Using a broadband spectral technique, we recently showed [*J. Biomed. Opt.* **10**, 064009 (2005)] that during visual stimulation of the cat brain there were not only changes in oxy- and deoxyhemoglobin levels, reminiscent of the optical blood oxygenation level dependence (BOLD) effect reported in humans, but also the apparent water content of the tissue and the optical scattering contribution decreased during stimulation. These relatively fast changes (in seconds) in water tissue content are difficult to explain in physiological terms. We developed a simple model to explain how local vasodilation, which occurs as a result of the stimulation, could cause this apparent change in water content. We show that in a phantom model we can obtain spectral effects similar to those observed in the cat brain such as the apparent decrease of the water spectral component without changing the water content of the bath in which the phantom measurements were performed. Furthermore, using the phantom model, we show that the relative apparent changes in the spectral components due to vasodilation during stimulation are roughly comparable in magnitude to the changes in tissue chromophores due to the optical equivalent of the BOLD effect reported in the literature. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2398920]

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1 Introduction

Neurovascular coupling or functional hyperemia in the mammalian brain relates the vascular response to an increase in metabolic rate.¹ In simple terms, in response to local neuronal activity, vasodilation occurs providing an increased supply of nutrient rich blood to the activated region. Physiological processes result in the exchange of the oxyhemoglobin with (O₂Hb) deoxyhemoglobin (HHb) such that there is an increase in the O₂Hb and a quasi-simultaneous decrease in HHb during stimulation: the classic blood oxygenation level dependence (BOLD) effect as seen by many authors.² using functional magnetic resonance imaging and optical techniques. Recently, the optical equivalent of the BOLD effect was reported by Tanner et al. in an animal model.³ In this animal study, we employed a range of wavelengths in the near ir (650 to 990 nm) to examine the hemodynamic response in the cat due to visual stimulation.³ Measurement in this broadband spectral range provided information about simultaneous changes of the relative concentration of tissue chromophores such as O₂Hb, HHb, and water. It was assumed that the spectral changes during stimulation can be described as a linear combination of spectral components due to the relatively small changes (1 to 2%). Scattering was modeled as a discrete spectral component described mathematically by λ^{-n} and by

allowing the n coefficient to vary. In regions of the cat visual cortex, we found that as a direct result of visual stimulation, an optical BOLD effect (increase of O₂Hb concentration and decrease of the HHb concentration) was observed. Exploiting the broad spectral range of the measurement, we determined that there was an apparent decrease of water concentration and scattering concomitant with the optical BOLD effect. However, the change in water concentration and decrease of the scattering contribution were unexpected in the cat model. Previous measurements of brain activation in humans did not have access to measurements in a broad spectral range. That paper reported for the first time that there was an apparent change in water concentration in tissue that occurred during the course of brain stimulation that lasted for several seconds. Physiologically, this rapid change (in seconds) of water content cannot be readily explained. It is highly improbable that there would be such a large change (1 to 2%) in water concentration on the timescale considered in our experiments. In our previous paper,³ we proposed that this apparent water change could be due to an optical artifact. In the current paper, we focus on this surprising result. We reason that vasodilation is associated with brain stimulation and that vasodilation could be the origin of this optical artifact. We simulated vasodilation with phantoms, where the water content was kept constant. We were able to reproduce qualitatively the ob-

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Fig. 1 Schematic of vasodilation and its effect on the observed optical signal of the transparent tissue. (a) Normal blood vessel (dark) in the tissue and transparent tissue (light gray). The blood vessel is opaque to light while the tissue is partially transparent. (b) When vasodilation occurs, the volume of the opaque compartment increases and the relative volume of partially transparent tissue decreases. The rest of the tissue stays unchanged.

served effects without requiring that the actual water concentration changes.

The basic idea of the phantom model is that there are at least two optical compartments in the brain tissue; the first one is opaque, which is composed of the large (larger than a millimeter) blood vessels, and the second is the brain tissue, which includes the small capillaries (less than $100\ \mu\text{m}$) (Fig. 1). We define the opaque compartment as the contribution of those structures that appear “black” at all wavelengths. Due to the physiological response to external stimuli, the opaque compartment increases in volume as the result of vasodilation, thereby increasing the total absorption (at all wavelengths). At the same time, the apparent amount of the chromophores in the tissue decrease as measured by the spectral amplitude, which decreases at all wavelengths. Consequently, vasodilation causes an apparent decrease in the absorption of all spec-

tral components, including O_2Hb , HHb, water, and scattering by the same relative amount, as shown schematically in Fig. 2. In the brain tissue, the increased blood flow due to vasodilation causes the exchange of HHb with O_2Hb . Therefore, we should observe an increase for the O_2Hb spectral component.

Our goal with the phantom studies is to demonstrate that simulated vasodilation increases the total absorption of the sample but decreases all spectral components of a mixture. We also studied the dependence of this effect on the separation between the source and detector from the opaque compartment (black spoke) to prove that vasodilation can affect the recovered spectrum even in the regime in which diffusion is presumably not reached. Because we observe a reduction of the spectral amplitude, an obvious question is whether or not this reduction is due to the onset of nonlinear effects in the diffusion regime. Therefore, we verified that the nonlinear effects can indeed be measured but that the changes in tissue chromophores or in scattering needed to reach this nonlinear regime are much larger than the changes we obtained in the cat experiments. Another purpose of this model is to estimate the amount of spectral changes due to vasodilation when compared to the true changes in absorption of the tissue chromophores and scattering.

In our dynamic phantom model, we mimic vasodilation by using a pinwheel of spokes of different diameters that are optically opaque and at different depths with respect to the source-detector configuration. The entire spoke assembly is rotated in a uniform scattering medium such that the spokes intercept the “light bundle” generated by the source-detector pair. In our phantom, we mimic localized spectral changes in absorption and scattering by inserting spokes that contain different concentrations of absorbers and scatterers. We show that we can accurately recover the spectrum (which is measured independently using a spectrophotometer) of the absorber and the spectral component due to scattering as a function of concentration (of absorber and scatterer). It is expected that in the diluted absorber–small scattering changes regime,

Fig. 2 Schematic representation of the effect of the introduction of a black (opaque) object in the light path. Lower spectrum (gray): normal spectrum of milk. Upper spectrum (black): the sample is the same as in the lower spectrum but a black object is now inserted in the optical path between source and detector. The overall absorption due to the insertion of the black object increases but the relative absorption of the milk decreases. The introduction of the opaque object is used to simulate vasodilation.

Fig. 3 Experimental setup. Light is brought to the sample and collected through fiber optics. A tank contains the scattering media (bath) at different dilutions. A holder rotates a series of spokes containing materials of different absorption and scattering properties in such a way as to intercept the optical path between source and detector.

the relative changes and concentration of absorbers or scatterer as recovered by the measurement method will follow a linear relationship. However, it is also expected that, when the concentration of the absorbers and scattering centers increases, the optical changes are no longer proportional to the concentration of the absorber or scatterer and the changes start to saturate the measured absorption. Hence, as the theory for spectroscopy of turbid media predicts, apparent saturation of the absorption could occur in real samples and that a simplified linear model cannot be used to determine changes in concentrations (of arbitrary size) of individual components. However, the changes in concentration needed to achieve this nonlinear regime for localized absorption and scattering are much larger than the changes observed in the physiological model. Our results can have implications on the way one interprets changes in chromophore components in the presence of vasodilation, or any other physiological condition that changes the relative contribution of the opaque compartment.

2 Experimental Procedure

The phantom model was set up as shown in Fig. 3, where the source-detector distance was 4.5 mm. The spoke assembly and the source-detector pair were immersed in a bath of milk (store-bought 2% milk). The scattering properties of 2% milk where measured with the frequency-domain multidistance method⁴ and are comparable to that of the mammalian brain, where the scattering coefficient μ_s of milk is 4.6 cm^{-1} (at 810 nm) and the scattering coefficient μ_s of the brain is 5 to 10 cm^{-1} .^{5,6} The light source was a tungsten light source at a nominal temperature of 3100 K (LS-1 Tungsten Halogen Light Source, Ocean Optics, Dunedin, Florida). The spec-

trometer employed was the model S2000, also from Ocean Optics. Both the light source and the spectrometer were coupled with a $1000\text{-}\mu\text{m}$ core diameter optical fiber. The spectrum of the light source was recorded prior to each measurement using a white reflector in place of the milk solution. The basic phantom design uses a pinwheel comprising spokes that were rotated by a stepper motor at a rate of 0.167 rev/s in the path of the “light bundle.” We examined three specific conditions: (i) where the size and position of the opaque objects was varied, (ii) where the scattering coefficient was varied, and (iii) where the concentration of absorbers was varied. In each case, one parameter corresponding to the test of interest was changed while keeping the geometry of the optical setup fixed. In each of the cases, a reference spectrum was taken of the “bath”: namely, the milk solution in which the pinwheel rotated. Spectra were taken at a rate of 50 spectra/s while the spokes were rotated, which was comparable to the timing of the spectra acquisition (200 spectra/s) used in the cat experiments as reported.³ For each of the cases examined, the spokes were interchanged to reflect the desired properties as described below.

2.1 Case I—Black Spokes of Varying Diameters

The pinwheel comprises four solid black spokes of diameters: 0.9, 1.2, 2.3, and 3.5 mm. These sizes were chosen to be comparable to that of blood vessels. Of course, there are also blood vessels smaller than 0.9 mm. Because the observed effect is linear for small spoke diameters, the effect of smaller spokes can be extrapolated from the effect of the larger spokes.⁷ The entire system was placed at a depth of 1 mm with respect to the source-detector configuration. The depth of

the pinwheel was then varied using a robotic arm that allowed the entire system to be moved in highly accurate incremental steps of 0.625 mm and the entire procedure repeated.

2.2 Case II—Spokes with Varying Scattering Coefficients

Six spokes were attached to the pinwheel and placed at a depth of 1 mm with respect to the source-detector configuration. The pinwheel was made of black rubber in which holes were made in a radial manner to secure test tubes (wall thickness 0.4 mm) filled with different scattering solutions. Each test tube was secured using Parafilm to ensure that there was no leakage of the solutions in the test tubes into the bath. The solutions consisted of different ratios of milk to water. The notation used refers to the fraction of 2% milk with respect to the total solution (milk and water): 0.05 (same as that of the bath), 0.1, 0.2, 0.333, 0.5, and 1 (undiluted milk). For the case II measurements, the bath was made of milk diluted 1/20.

2.3 Case III—Spokes with Varying Absorbing Materials (Low Absorbance Range)

For this series of measurements, the test tubes were filled with a mixture of the bath solution (milk diluted 1/5) and blue gel food coloring Betty Crocker gel food colors. The initial dye mixture was diluted by adding the milk solution (at a dilution equal to that of the bath) in appropriate amounts to get the desired concentrations. The concentration used is shown as a percent concentration of the blue dye in the overall mixture as follows: 3.125, 6.25, 12.5, 25, 50, and 100%. The absorbance of the 100% mixture was determined using a spectrophotometer (Perkin-Elmer Lambda 5, Shelton, Connecticut USA) with a standard 1-cm cuvette and it gave an absorbance of 1.44 optical density units at 650 nm. The absorption coefficient μ_a could not be calculated for this dye as the molecular weight was not known. However, the dye was purely absorbing with no scattering.

2.4 Case IV—Spokes with Varying Absorbing Materials (High Absorbance Range)

For these measurements, four spokes were used and the concentrations were 75, 80, 90, and 100% of a solution of the dye with an absorbance of 6.0 at 650 nm. This absorbance value was measured with a 1-mm cuvette.

2.5 Data Analysis

Data acquisition and analysis were performed by ELANTEST software created by E. Gratton.⁸ First, a reference spectrum of the bath was taken. All measurements were calculated with respect to this initial spectrum, that is, only the changes with respect to the spectrum of the milk bath are reported. Absorbance units were used for all spectral calculations. The overall spectral changes were calculated by averaging the changes at all wavelengths. The method of spectral deconvolution and data manipulation was previously discussed in the animal study in detail.³ In short, following established methods in the field (see for example Ref. 5), the apparent absorbance of the sample at a specific wavelength, μ' can be inferred from the measured light transmitted through the sample (I), making use of the modified Beer-Lambert law.

$$R = (I/I_0) = R_0 e^{-l\mu'}, \quad (1)$$

where the apparent absorption μ' is given by $[3\mu_a(\mu_a + \mu_s')]^{1/2}$, l is the effective path length, and μ_a and μ_s' are the absorption and reduced scattering coefficient, respectively. I is the intensity at a given wavelength measured by the spectrometer and I_0 is the intensity of the light source at the same wavelength. For spectral deconvolution purposes, it is assumed that at each wavelength, the absorption coefficient μ_a is due to the sum of the absorption of each chromophore in the sample, as shown in Eq. (2).

$$\mu_a = \sum_i c_i \mu_a^i, \quad (2)$$

where c_i is the contribution to the total absorption of the i 'th chromophore. The wavelength dependence of μ_a is that of the individual chromophores present in the sample and it is assumed to be known. The wavelength dependence of the scattering coefficient μ_s was approximated by the following relationship:

$$\mu_s = \mu_s^0 \lambda^{-n}, \quad (3)$$

where μ_s^0 is the relative scattering contribution, and the scattering power n is a variable quantity. Hence, the unknown numbers following this treatment are the relative spectral contributions c_i , the relative scattering contribution μ_s^0 , and n . The apparent absorption as given by the modified Beer-Lambert law is fit to Eqs. (2) and (3) for the absorption and scattering coefficient to obtain the unknowns using a nonlinear fitting algorithm. We have not calibrated c_i and μ_s^0 to determine the absolute values of the scattering and absorption coefficients at each wavelength, as we are interested only in the relative changes (in particular small changes on the order of 1 to 2% akin to physiological changes). For tissue and our phantom materials (i.e., milk, which is chosen to have similar bulk optical properties of the human brain), $\mu_s \gg \mu_a$, hence, the apparent absorption μ' in Eq. (1) reduces to

$$\mu' = [3\mu_a\mu_s']^{1/2}. \quad (4)$$

For small changes of the optical parameters, the resulting small changes in the apparent reflectance or absorption can be expressed using the following linear expansion of Eq. (4):

$$-\Delta R \approx \Delta\mu' \approx (k_a \Delta\mu_a + k_s \Delta\mu_s'), \quad (5)$$

where k_a and k_s are constants. Equation (5) is obtained by Taylor expansion of Eq. (4).

We note that the mathematical form of Eq. (5) (not the values of k_a and k_s) is the same as that for changes in absorbance of dilute samples in which the diffusion regime is not reached, that is, the form of this equation is independent on the regime of light transport. In our experiments, for small changes (1 to 2% of the total R value), data analysis performed to recover the changes in the spectral components using Eq. (5) or Eqs. (1)–(4) gives the same quality of the fit.

In regard to data analysis for the opaque compartment, we have not assumed a specific mathematical expression to describe it. Instead, we have deduced its behavior directly from the experimental data. We observed (Fig. 2) that as we in-

crease the amount of the black absorber in the medium (the black spokes), the entire spectrum is shifted up and decreases in amplitude. For small spoke diameters, this effect can be approximated by the following equation:

$$\mu'(\text{measured}) = A + B\mu', \quad (6)$$

where A and B are positive constants ($B < 1$). For small changes in the size of the opaque compartment, we can then treat the measured reflectance R as that due to the transparent tissue but with a reduced apparent lamp intensity and a reduced effective pathlength.

$$R_{\text{measured}} = R_0 \exp[-l(A + B\mu')] = R'_0 \exp[-l'\mu'], \quad (7)$$

where $l' = Bl$ and $R'_0 = R_0 e^{-lA}$. This equation is formally identical to Eq. (1). Therefore, unless specific experiments are done to reveal the opaque compartment, it is difficult to determine its existence. However there is a hidden difference between Eqs. (1) and (7). In Eq. (7), the values of R'_0 and l' depend on the size of the opaque compartment, which in our experiments on the cat model and on the phantoms is time dependent. The apparent changes in absorption and scattering that arise from the changes in the size of the opaque compartment are not due to changes of absorption or scattering of the transparent compartment. According to Eq. (7), the characteristic signature of the change of the opaque compartment is the simultaneous decrease of all spectral components, including scattering and the increase of the overall absorption background. Note that the changes due to vasodilation [Eq. (6)] occur even for small changes in the spoke diameters, while the changes (reduction of the apparent spectrum) due to the nonlinearity in the modified Beer-Lambert law occur only for relatively large changes of the concentration of the chromophores. These changes due to vasodilation are easily recognized in the raw data if the size of the opaque compartment can be modulated or if a broad spectral range can be measured.

There are two aims of our phantoms studies. First, we want to recognize the effect of the opaque compartment and experimentally determine the form of Eq. (6). This is done by modulating in time the size of this compartment. The second aim is to show that the size of the changes in absorption and scattering observed in our experiments with the cat are compatible with the linear approximation of Eq. (5). According to Eq. (5), we determined the changes of the tissue chromophores, including scattering, by forming a linear combination of the spectrum of each individual tissue chromophore.

Experimentally, when the size of the opaque compartment is changed, the entire spectrum is changed in amplitude. Therefore for cases I and II, a spectrum of the milk solution was also loaded as a basis spectrum to be used in spectral deconvolution. This is done (for case I) to prove that the measured spectrum after introduction of the black absorber is identical to the spectrum without the absorber, but reduced in amplitude. For cases III and IV, in addition to this milk solution spectrum, the spectrum of the blue dye obtained using a spectrophotometer was also added to the library of basis spectra. Relative changes in spectral components are reported in terms of average contribution to the total (average) absorption. Principle component analysis was used to determine the

minimum number of spectra required to fit the differential spectrum. In case I, the milk spectrum was sufficient. Case II required, in addition to the 0.2 milk solution spectrum, scattering, and cases III and IV, required the spectrum of the blue dye. The spectrum was then separated into the weighted contributions of these individual species and their changes observed as a function of time as the spokes rotated under the source-detector pair. The relative changes of these components as a function of time were plotted. In addition, the values of the relative contribution to the absorbance were then plotted to show the relationship between the relative absorption and spoke diameter (case I), and as a function of concentration of the scattering and the dye for cases II to IV.

3 Results

3.1 Case I—Black Spokes of Varying Diameters

As the opaque spoke intercepts the light bundle, there is an increase of the total light absorption [Fig. 4(a)]. The absorption increase depends on the diameter of the spoke. The absorption change is uniform at all wavelengths. This is proved by the fit of the spectrum obtained when spokes of different diameters intercept the light bundle. This fit only requires a multiplicative factor (less than 1) and a shift of the spectrum [Eq. (6)]. However, when spectral deconvolution is performed for the milk component and the total spectral changes are reported as a function of time, there is a relative decrease of the amount of milk measured when the spoke is in the light path [Figure 4(b)]. In other words, the apparent spectral coefficient of the milk component decreases although the overall absorption increases due to the common background term. This effect is linear for small spoke diameters but then increases in a nonlinear fashion as the spoke diameter becomes large [Figure 5(a)]. As the depth of the pinwheel was increased with respect to the source-detector position, one sees that the effect of the opaque object decreases as the depth is increased, but the effect is still appreciable up to a depth of 6 mm [Figure 5(b)].

The consequence of this experiment is that if we do not know that the opaque component is increasing as the spokes pass under the light bundle, we will erroneously attribute the spectral changes to a decrease of the concentration of the different components, while the concentrations of the components in the transparent part of the sample is clearly not changing in our experiments. A second consequence of this experiment is that the effect of the opaque compartment is visible at all depths and source-detector separations explored, which include the nondiffusing regime at small depths and small source-detector separations.

3.2 Case II—Spokes with Varying Scattering Coefficients

The aim of these experiments is to establish the range of validity of the approximation in Eq. (5), which is the linear expansion of Eq. (4). This equation tells us that the changes in absorption and scattering can be determined independently in Eq. (5), which is of course an approximation. As the scattering of each spoke increased, we measured an increase in the scattering spectral component when spectral deconvolution was performed [Fig. 6(a)]. In the case where the spoke contained the same solution as the bath (0.05 milk solution), only

Fig. 4 (a) Raw data showing the effect of rotating black spokes of varying diameters on the average absorption (black). Average spectrum of milk (gray). The overall absorption increases when the opaque objects intercept the optical path. (b) Effect of spectral deconvolution when one of the spectral components is the spectrum of the milk itself. The relative amount of the milk spectral component decreases when the opaque spokes intercept the optical path. The spectral coefficient represents the fractional change of one of the components of the basis set used to fit the data. Because the spectrum of the milk has an absorbance of about 1 [part (a)], it happens that a value of 1 of the spectral coefficient also corresponds to an absorbance of 1.

a very small change in the scattering was observed, probably due to thickness of the walls of the spoke [Fig. 6(a)]. Furthermore, the changes in the milk spectrum (the absorption part) were extremely small (two orders of magnitude less) in comparison to the changes in the scattering signal [Fig. 6(a)]. Hence, we conclude that the changes due to the mismatch of the refractive indices between the urfaces of the milk solution and the cuvette produced a negligible effect. The plot of the recovered magnitude of the scattering spectral component as a function of scatterer concentration shows a linear relationship at low scatterer concentration and then displays saturation as the scatterer concentration increases [Fig. 7(a)]. With these experiments, we showed that for small changes of the scattering coefficient in the spoke, the scattering changes do not affect the absorption part.

3.3 Cases III and IV—Spokes with Varying Absorbing Materials

When the absorbing material is added in the spoke, there are changes in absorption that correspond exactly to the spectrum

of the dye obtained in a clear (nonscattering) solution [Fig. 6(b)]. At a low dye concentration, a plot of the recovered amount of the blue dye spectral component as a function of the dye concentration shows a linear relationship ($r^2 = 0.9976$) [Figs. 6(b) and 7(b)]. Under this condition, the scattering coefficient as recovered from the spectral deconvolution method is not affected by the presence of increasing amount of the dye. Instead in the case of the highly absorbing dye solution, a saturation of the recovered amount of the spectral component is seen [Figs. 6(c) and 7(b)]. Taken together, cases II, III, and IV show that the relative changes of scattering and absorption can be determined using the approximation of Eq. (5) when the changes are small.

4 Discussion

The resolution of spectral components in a mixture is a classical problem in spectroscopy. A common approach is the establishment of the number of independent components using methods such as principal component analysis.⁹ When the

Fig. 5 (a) Plot of spectral coefficient of milk component versus diameter of the spokes. (b) Three-dimensional plot of spectral coefficients of the milk component versus diameter and distance between the spoke and the fiber optic tips.

components of the mixture are known *a priori*, linear spectral deconvolution is sufficient to determine their relative contributions based on the Beer-Lambert law. In the case of tissue spectroscopy, there is the additional issue in regard to the validity of the Beer-Lambert law. In general, in the presence of strong scattering, the Beer-Lambert law must be modified to account for the changes in optical path introduced by multiple scattering. In our work, we are mainly interested in relative changes of the amount of spectral components due to physiological activity. We assume that we know the spectral components and we want to determine their relative changes. What is unique about our work is that we want to determine fast changes in the brain following a stimulus: from a few milliseconds corresponding to neuronal activity to several seconds due to metabolic processes. During this process vasodilation occurs. In our system, we have a time-dependent compartment, which is generally absent in classical spectroscopy of turbid media. Furthermore, we need to interpret changes occurring in the brain of a relatively small animal (the cat). Due to the small size of the cat brain, the total optical path of the light in our configuration is not sufficient for achieving the conditions of the diffusion approximation.

Also, we believe that superficial effects have a much stronger influence on our results than for the case of human studies. Spectroscopy of the open brain in small animals and in phantoms has been studied using reflected light.^{10–15} In our case, we use fiber optics to illuminate a point on the skull and light is collected after traveling several millimeters in the brain. In our experiments, light travels some distance in the

tissue before being collected by the detector fiber. In this research, we study light propagation in a phantom system that closely mimics the situation of the cat brain. The source-detector distances as well as the distance from the surface where we put our scatterers or absorbers is similar to the known geometry of the cat brain. Several groups have addressed the inhomogeneous nature of tissue in the diffusive approximation and in the cases of small (a few millimeters) optoelectrode separations, and these groups explored the second-order corrections that must be made to existing models of tissue dynamics.^{16–25} Our results from the dynamic phantom made of the various diameters of opaque spokes show that vasodilation per se, as simulated by changing the diameter of the spokes, could cause an apparent decrease in all spectral components, including water and scattering. Therefore in the presence of vasodilation of the large blood vessels, all spectral components should decrease proportionally to their contribution to the overall spectrum. We propose that the apparent decrease in water content (and partially scattering) observed in the cat brain following visual stimulation is in fact due to vasodilation.

We also demonstrated that the relationship between apparent absorption changes and black spoke diameter is linear for small diameters so that the effect of changing the spoke diameter can be extrapolated to very small vessels. However, when the diameter of the blood vessel is small (smaller than 1 mm), the vessel becomes partially transparent, in contrast to the spokes in the phantom, which are always opaque at all diameters. Our phantom studies also show that there is an apparent decrease of the scattering spectral component when vasodilation occurs (the size of the spoke increases). However, in the brain, there is a possibility that there are additional “true” scattering changes due to variation of the size or number of scattering centers.

With respect to the true spectral changes (addition of absorbing material), we demonstrated that the relative absorption changes are linear with the dye concentration only at low absorbances. At high absorbances, the apparent changes saturate, due to the approximation used in Eq. (5). However, this saturation occurs at values (of the changes) much larger than the changes measured in the physiological model.

Our phantom model is a simplification of the real brain structure, as we only consider two compartments; while in the real brain, we presumably have a continuum of vessel sizes. However, the expected response due to intermediate vessel size will be a mixture of the two extreme cases (opaque versus transparent). Even if the real brain situation is more complex, our conclusion that the vasodilation artifact will affect the relative contribution of all tissue chromophores is still valid.

In the real physiological system, we have changes in vasodilation and true changes in chromophore concentration. We should estimate the relative effect of these two physiological effects. It could be that the vasodilation artifact is a small part of the true concentration changes, that is the coefficients *A* and *B* in Eq. (6) could be small compared to the true changes in chromophore concentration. In the following discussion, we estimate the size of the changes in spectral components due to vasodilation for comparison with the changes in chromophore concentration reported in the literature during the optical BOLD effect.^{3,26} Typical changes in the concentration

Fig. 6 Result of spectral deconvolution using two components. The changes are expressed in terms of spectral coefficients. These coefficient values are used to multiply the spectrum of the database to obtain the best fit. (a) Coefficient of the scattering component. Because in the database the scattering component is not normalized to an absorbance value, the coefficient of the scattering component can be larger than 1. (b) Coefficient of the blue dye spectral component when the dye is relatively diluted. (c) Coefficient of the blue dye spectral component when the dye is at high concentration. Note that the difference in spectral coefficient when the different spokes that pass under the source-detector pair are now reduced.

of tissue chromophore (O_2Hb and HHb) during stimulation are on the order of 1 to 2% both for humans and for the cat model.^{3,26} Similar values are found for the changes in the scattering coefficient. It has been estimated that vasodilation

changes the diameter of the blood vessels by about 20%.²⁷ To use our data to estimate the effect of vasodilation in the human brain, we should estimate the average diameter of the large blood vessels and also their depth with respect to the

Fig. 7 (a) Spectral coefficient of scattering versus concentration of scattering centers. (b) Spectral coefficient of the blue dye spectral component as a function of the dye absorbance (as measured in the clear solution in the cuvette). For the large absorbance values, a cuvette with 1-mm optical path was used.

surface. From Fig. 5(a), we can estimate that for a vessel diameter of about 2 mm, a change of 20% of the diameter will give a change of about 0.04 absorbance units. In Fig. 5, the y axis is the spectral coefficient. The spectrum of milk in the data set used for the fit is normalized to one unit of absorbance. Therefore the y axis coincides with changes in absorbance. However, this change will depend on the depth of the vessels. At a depth of 6 mm or more, the changes will be much smaller [Fig. 5(b)]. Because the average apparent absorbance in tissue is about 1 to 2 units, for source-detector distances of a few millimeters, the relative changes due to vasodilation should be about 4% for superficial blood vessels but less than 0.4% for vessels at 6 mm or more from the surface. In the case of the cat brain, the changes due to vasodilation could be more significant due to the small size of the brain with respect to humans.

Note that this artifact in the estimation of spectral components due to vasodilation would not have been recognized if we had only used a few wavelengths. In fact, using a broadband spectral analysis allowed us to distinguish changes that affect equally all spectral components from specific chromophore changes affecting only one spectral component. In fact, if we had used a wavelength range where mainly one component was present (e.g., O_2Hb), the changes due to vasodilation or the changes due to decrease (or increase) of the hemoglobin would have been indistinguishable. In fact, va-

sodilation decreases the total light transmission (offset in Fig. 2) and reduces the spectral amplitude. Therefore, if we only measure relative spectral changes (without considering the changes in baseline) in the presence of vasodilation, we will measure an apparent reduction of the spectral amplitude at all wavelengths. This is exactly what we reported for the cat experiment in our previous paper.

There is nothing special about vasodilation: other mechanisms that broaden the relatively large blood vessels should produce a similar artifact. For example, the pulse could have a similar effect. However, following the discussion of the effect of vasodilation with vessel depth, the pulse due to relatively deep blood vessels should have a minor effect on the overall spectrum offset. Only pulsating arteries close to the skin surface should produce an appreciable effect in reducing all spectral components. This observation could explain a common yet seldom reported observation that the pulse is barely visible when measurements are obtained from deep layers when using, for example, the frequency-domain multidistance method. Instead, any method based on steady state continuous illumination (cw measurements) at one point should be affected by the vasodilation artifact, which is more prominent at the surface.

Note that in our experiments, we are using spokes of different diameters to simulate the opaque compartment, and one could think that by proper image reconstruction, we could tell that there is a “black object” in the medium. However, if the opaque object has been distributed in volume, for example, the opaque object could have been a mesh or web of black wires, of size below the resolution of the reconstruction method, the result of increasing the mesh diameter will be that of increasing the overall absorption background and decreasing the amplitude of the apparent absorption spectrum. Therefore the reconstruction method will not show the black object.

There is a conceptual point to discuss in relation to the “linear effect” observed. We show that small changes of absorption and scattering in the spokes are linear with concentration (of the absorber and scatterer). We also show that a small increase in the spoke diameter gives a linear decrease of the overall spectrum (both absorption and scattering components). Although all the above small changes give linear effects, the increase in the size of the opaque compartment generates an opposite effect with respect to true increase in absorption or scattering of one component. We believe that this is due to the highly nonlinear effect intrinsic to the opaque compartment. The apparent contradictory finding is that by increasing the total sample absorption by increasing the size of the opaque compartment, we decrease the amplitude of the spectral part (or of its components). This should not be surprising because we have a sample that is not homogeneous at some length scale, and the nonhomogeneity is due to extreme differences in optical parameters (opaque versus transparent).

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