

# Experimental and theoretical comparison of NIR spectroscopy measurements of cerebral hemoglobin changes

MICHAEL FIRBANK,<sup>1</sup> CLARE E. ELWELL,<sup>1</sup> CHRIS E. COOPER,<sup>2</sup> AND DAVID T. DELPY<sup>1</sup>

<sup>1</sup>Department of Medical Physics and Bioengineering, University College London, London WC1E 6JA; and <sup>2</sup>Department of Biological and Chemical Sciences, University of Essex, Essex, United Kingdom CO4 3SQ

**Firbank, Michael, Clare E. Elwell, Chris E. Cooper, and David T. Delpy.** Experimental and theoretical comparison of NIR spectroscopy measurements of cerebral hemoglobin changes. *J. Appl. Physiol.* 85(5): 1915–1921, 1998.—Two near-infrared spectroscopy (NIRS) methods are available for measuring changes ( $\Delta$ ) in total cerebral hemoglobin concentration (CHC): 1) a continuous measurement of the changes in total hemoglobin concentration ( $\Delta[\text{Hb}]_{\text{tot}}$ ) and 2) the difference between two absolute measurements of CHC, each derived from a small, controlled change in inspired  $\text{O}_2$  fraction. This paper investigates the internal consistency of these two methods by using an experimental and theoretical comparison. NIRS was used to measure  $[\text{Hb}]_{\text{tot}}$  in five newborn piglets before and after a change in arterial  $\text{PCO}_2$ .  $\Delta[\text{Hb}]_{\text{tot}}$  demonstrated a low coefficient of variation of  $2.8 \pm 2.8$  (SD) % which allowed changes in  $\text{CO}_2$ -cerebral blood volume reactivity to be clearly discriminated. However, a high coefficient of variation of  $22.8 \pm 3.5\%$  on the  $\Delta\text{CHC}$  measurements obscured any  $\text{CO}_2$  reactivity changes. A theoretical analysis demonstrates the effects of optical pathlength, background absorption, scatter, and blood vessel diameter on both methods. For more accurate monitoring of CHC, individual measurements of optical pathlength and more accurate pulse oximetry are required.

cerebral blood volume; near-infrared spectroscopy

OVER THE LAST DECADE, near-infrared spectroscopy (NIRS) has found widespread application for the monitoring of tissue hemodynamics and oxygenation (7, 11, 24). Because the technique allows the continuous and noninvasive measurement of changes in concentration of oxyhemoglobin ( $\Delta\text{HbO}_2$ ), deoxyhemoglobin ( $\Delta\text{Hb}$ ), and changes in the redox state of cytochrome oxidase, its simplest application is as a trend monitor of global tissue oxygenation status. If the  $\Delta\text{HbO}_2$  and  $\Delta\text{Hb}$  signals are summed, this provides a derived measurement of changes in total hemoglobin concentration ( $\Delta[\text{Hb}]_{\text{tot}}$ ), which can be used to reflect changes in blood volume.

An important step in the development of NIRS occurred when methods for the measurement of absolute hemodynamic parameters were devised. In 1988, Edwards et al. (6) described the measurement of absolute blood flow by using a small but rapid change in concentration of  $\text{HbO}_2$  as an intravascular near-infrared dye. The technique was first applied to the measurement of neonatal cerebral blood flow and, in a subsequent validation, was shown to correlate with the xenon-clearance method (20).

By 1990, a method for the measurement of absolute cerebral hemoglobin concentration (CHC) had been described (25). This method employs a small, but this

time slow, change in  $\text{HbO}_2$  concentration and, for this reason, is usually referred to as the  $\text{O}_2$  method for estimating cerebral blood volume (CBV). To date, independent validation of this method has not been performed, although the technique has been widely used after its initial application in neonatal medicine (15, 22, 26).

It is, therefore, possible to attempt an internal validation by comparing changes in CBV measured from the continuous  $\Delta[\text{Hb}]_{\text{tot}}$  measurement against the change calculated from two separate absolute concentration measurements ( $\Delta\text{CHC} = \text{CHC}_2 - \text{CHC}_1$ ). One study (2) attempted such a comparison by using  $\text{CO}_2$ -induced CBV changes in a small number of newborn infants and reported a significant difference in the values obtained by each method:  $0.89 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{kPa}^{-1}$  for the absolute  $\text{O}_2$  method vs.  $0.22 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{kPa}^{-1}$  for the continuous-change measurement ( $\Delta[\text{Hb}]_{\text{tot}}$ ).

The aim of the study reported here was to confirm these observations in a more controlled animal model, and to investigate, from a theoretical basis, the possible reasons for any differences. This paper will describe measurements in which a change in fraction of inspired  $\text{O}_2$  ( $\text{FI}_{\text{O}_2}$ ) was used to measure CBV (by using the  $\text{O}_2$  method) and a change in fraction of inspired  $\text{CO}_2$  ( $\text{FI}_{\text{CO}_2}$ ) was used to change CBV.

## NIRS MEASUREMENT THEORY

In a nonscattering medium, the attenuation  $A$  of light traveling a distance  $d$  is simply given by the equation

$$A = \mu_a^i C_i d \quad (1)$$

where each absorber  $i$  has a concentration  $C_i$  and a specific absorption coefficient  $\mu_a^i$ .

In a scattering medium, such as biological tissue, the scattering acts to increase the path traveled, and, as shown by the diffusion Eq. 1, attenuation is no longer a linear function of the  $\mu_a$ . For small changes in absorption, however, changes in attenuation can be approximated by

$$\Delta A = \Delta \mu_a^i C_i \beta d \quad (2)$$

which gives

$$\Delta C_i = \frac{\Delta A}{\mu_a^i \beta d} \quad (3)$$

where  $\beta$  is the factor by which the optical pathlength has been increased due to scattering [the so-called

differential pathlength factor (DPF) (4)]. Using Eq. 3, with the known specific  $\mu_a$  of hemoglobin and the other chromophores in tissue (water, cytochrome), it is possible to derive changes in the concentration of HbO<sub>2</sub> and Hb from the attenuation changes.

The O<sub>2</sub> method of measuring total CHC relies on using HbO<sub>2</sub> as a NIR "dye" which can be measured in both the peripheral and cerebral circulation. A small, slow change in F<sub>I</sub>O<sub>2</sub> is induced, the effects of which can be measured peripherally, by using a pulse oximeter, as a change in arterial O<sub>2</sub> saturation ( $\Delta$ Sa<sub>O<sub>2</sub></sub>) and in the brain by using NIRS as a change in cerebral HbO<sub>2</sub> concentration. If cerebral blood flow, CBV, and O<sub>2</sub> consumption remain constant during the maneuver, the overall  $\mu_a$  of the blood is affected without altering the [Hb]<sub>tot</sub>.  $\Delta$ HbO<sub>2</sub> is, therefore, equivalent to the product of the total CHC and  $\Delta$ Sa<sub>O<sub>2</sub></sub>. The total CHC can then be easily computed as

$$\text{CHC} = \frac{\Delta[\text{HbO}_2] - \Delta[\text{Hb}]}{2 \cdot \Delta f\text{Sa}_{\text{O}_2}} \quad (4)$$

where  $\Delta f\text{Sa}_{\text{O}_2}$  is the fractional change in Sa<sub>O<sub>2</sub></sub>. The term [Hb]<sub>diff</sub> is often used to describe the difference between the oxy- and deoxyhemoglobin concentration ( $\Delta$ HbO<sub>2</sub> -  $\Delta$ [Hb]). CHC can therefore be computed from the gradient of the plot of  $\Delta$ [Hb]<sub>diff</sub> and  $\Delta$ Sa<sub>O<sub>2</sub></sub>.

It is important to remember that NIRS measures changes in chromophore concentration in micromolar units. Estimates of blood volume are obtained from these measurements by converting the concentration data into the more conventional clinical units of milliliters/100 grams. This conversion requires knowledge of the concentration of red blood cells (the hematocrit). The hematocrit varies with vessel size and is lower in smaller vessels and capillaries. Lammertsma et al. (13) measured the cerebral-to-large vessel hematocrit ratio as 0.69, and Sakai et al. (19) found a value of 0.76. The value measured depends on the distribution of vessel sizes considered. Sakai et al. also found that the hematocrit may change with blood volume. For a 15% increase in blood volume (induced by 5% CO<sub>2</sub> inhalation), the hematocrit dropped by 5%. For these reasons, the present study considers only the measurement of hemoglobin concentration, rather than blood volume.

### Experimental Procedure

Five newborn piglets (age <24 h) were studied. Anesthesia was induced by using 5% isoflurane, mechanical ventilation was established, and the isoflurane level was reduced to 1.5%. Ventilation was maintained at F<sub>I</sub>O<sub>2</sub> of 40% (balance N<sub>2</sub>). The optodes from a Hamamatsu NIRO 500 spectrometer were placed on either side of the head at spacings between 3.8 and 4.5 cm (Table 1). By using pulsed laser diodes at four wavelengths (779, 821, 855 and 908 nm), the spectrometer was used to measure changes in the concentration of cerebral HbO<sub>2</sub> and Hb. For newborn piglets, a differential pathlength factor (4.57) was used to convert the data to micromolar units (R. Springett, personal communication). Sa<sub>O<sub>2</sub></sub> and heart rate were measured by using a pulse oximeter (Novamatrix 500) via a probe positioned on a skin flap on the right leg. Mean arterial blood pressure was recorded via the umbilical artery, and analog outputs from the oximeter and blood pressure transducer were linked directly to the spectrometer for display and storage alongside the NIRS data. All data were sampled every 2 s.

A computer-controlled gas blender (8) was used to supply a controlled, accurate mixture of O<sub>2</sub>, N<sub>2</sub>, and CO<sub>2</sub> to the time-cycled ventilator (Vickers model 77) with preset pressure. Initially, F<sub>I</sub>CO<sub>2</sub> was set to 0%, and at least six CHC measurements were made by slowly varying F<sub>I</sub>O<sub>2</sub> over several minutes. F<sub>I</sub>CO<sub>2</sub> was then increased to either 2.5 or 5%. Once a new stable baseline had been reached, the CHC measurements were repeated. Arterial blood samples were used to measure arterial PCO<sub>2</sub> throughout the study.

### Experimental Results

An example of the NIRS and Sa<sub>O<sub>2</sub></sub> data collected during a single CHC measurement in one piglet is shown in Fig. 1. In this example, Sa<sub>O<sub>2</sub></sub> was reduced to ~50%. However, only the initial portion of this change (in which Sa<sub>O<sub>2</sub></sub> is >90%) is used for the calculation of CHC. As previously described, CHC is calculated from the gradient of the  $\Delta$ [Hb]<sub>diff</sub> and  $\Delta$ Sa<sub>O<sub>2</sub></sub> plot shown in Fig. 2.  $\Delta$ [Hb]<sub>tot</sub> is included on this plot to demonstrate that, for small changes in Sa<sub>O<sub>2</sub></sub>, the cerebral circulation is not disturbed. At least three CHC measurements were made at each of the two levels of Pa<sub>CO<sub>2</sub></sub> for each

Table 1. Changes measured in total hemoglobin concentration by using continuous method and the O<sub>2</sub> method for each animal, as well as interoptode spacing and results of statistical power calculation of minimum detectable change in CHC

Piglet No.	IOS, cm	CHC <sub>1</sub> , $\mu$ M	CHC <sub>2</sub> , $\mu$ M	$\Delta$ CHC, $\mu$ M	$\Delta$ [Hb] <sub>tot</sub> , $\mu$ M	min [ $\Delta$ CHC] <sub>d</sub> , $\mu$ M
1	4.50	55.8 ± 10.3	70.9 ± 2.2	15.1 ± 10.5	14.7 ± 1.1	14.8
2	4.06	101.5 ± 25.1	113.1 ± 20.4	11.6 ± 32.3	23.4 ± 3.3	36.3
3	3.82	58.0 ± 15.2	69.0 ± 12.1	10.9 ± 19.4	17.1 ± 2.7	22.0
4	3.93	83.8 ± 21.8	85.2 ± 17.1	1.4 ± 27.7	12.3 ± 8.3	31.6
5	3.88	66.5 ± 12.3	70.6 ± 3.3	4.1 ± 12.7	15.0 ± 0.7	17.8

Values are means ± SD. IOS, interoptode spacing; CHC, cerebral hemoglobin concentration; CHC<sub>1</sub>, CHC<sub>2</sub>, absolute concentration measurements of CHC;  $\Delta$ , change in measurement; [Hb]<sub>tot</sub>, total hemoglobin concentration; min [ $\Delta$ CHC]<sub>d</sub>, minimum detectable change in CHC.

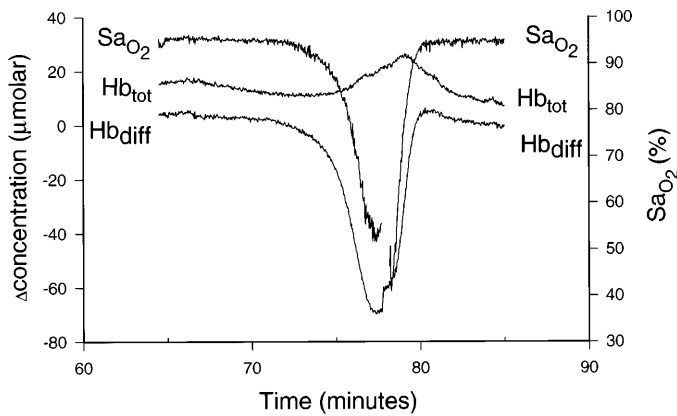


Fig. 1. Near-infrared spectroscopy (NIRS) and arterial  $O_2$  saturation ( $Sa_{O_2}$ ) data collected from single animal during reduction and increase in fraction of inspired  $O_2$  ( $F_{I_{O_2}}$ ) for measurement of absolute cerebral Hb concentration (CHC) by using the  $O_2$  method. An absolute value for CHC can be calculated from these data by using change ( $\Delta$ ) in difference in Hb concentration ( $[Hb]_{diff}$ ) and  $Sa_{O_2}$  during initial decrease in  $F_{I_{O_2}}$  while total Hb concentration ( $[Hb]_{tot}$ ) remains constant.

piglet. The summarized results of these measurements are shown in Fig. 3 and detailed in Table 1. The normocapnic  $Pa_{CO_2}$  levels for each piglet ranged between 3.8 and 6.8 kPa.

The coefficient of variation for the CHC method was  $22.8 \pm 3.5\%$  (mean  $\pm$  SD) compared with  $2.8 \pm 2.8\%$  for the  $[Hb]_{tot}$  method. A statistical calculation (assuming a power of 90% at the 95% significance level) was performed on the CHC data to determine the minimum change in CHC which could be detected ( $\min[\Delta CHC]_d$ ) under these experimental conditions. The values for this  $\min[\Delta CHC]_d$  are given in Table 1. In four of the five piglets,  $\min[\Delta CHC]_d$  was higher than the change in  $[Hb]_{tot}$  and CHC because of alteration of  $Pa_{CO_2}$ .

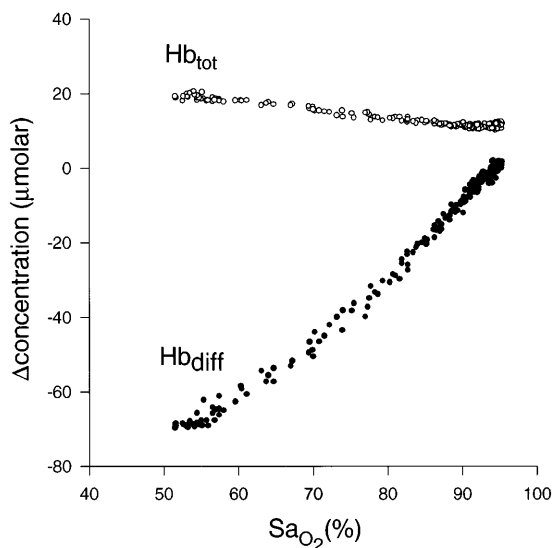


Fig. 2. Plot of  $\Delta[Hb]_{diff}$  vs.  $Sa_{O_2}$  for data shown in Fig. 1. Absolute CHC is calculated from initial slope of this line. Additional plot of  $\Delta[Hb]_{tot}$  vs.  $Sa_{O_2}$  indicates limits within which  $\Delta Sa_{O_2}$  does not produce a change in  $[Hb]_{tot}$ .

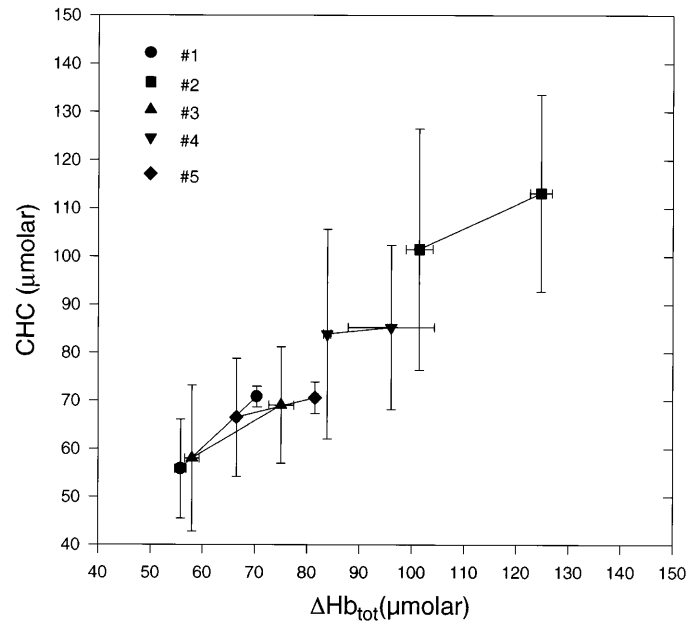


Fig. 3. Comparison of values of  $[Hb]_{tot}$  measurements made by using absolute method (CHC) and continuous method ( $\Delta[Hb]_{tot}$ ). Each symbol represents a different animal; bars, SD. For all piglets,  $\Delta[Hb]_{tot}$  values have been normalized to the lower CHC value.

#### THEORETICAL MODEL

To investigate this problem theoretically, we used diffusion theory (1, 18) to calculate the expected attenuation of light from known values of absorption and scattering of tissue and blood and also the source-detector separation. The tissue was assumed to be a uniform slab of 40-mm thickness, with an optode spacing of 40 mm (similar to that used in the experimental study). Data were calculated by using absorption and scattering properties at four wavelengths, chosen to be similar to those used by the NIRO 500. The values used for the  $\mu_a$  of hemoglobin and water are shown in Table 2. Data are taken from Matcher et al. (17) for blood and from Matcher et al. (16) for water and then converted to natural log base (ln). The  $\mu_a$  of blood was calculated by assuming a hemoglobin concentration in the blood of 1.68 mM (10.8 g/100 ml) and a mean saturation of 80%. To calculate blood volume, we used a saturation decrease of 3%.

The transport scattering coefficient,  $\mu'_s$ , is defined as  $\mu_s (1 - g)$ , where  $g$  is the anisotropy factor of blood). This was assumed to vary linearly with wavelength  $\lambda$  and was calculated from the following expression [approx-

Table 2. Absorption coefficient values for Hb,  $HbO_2$ , and  $H_2O$  used in theoretical calculations

Wavelength, nm	$\mu_a$ Hb, $mm^{-1}/mmolar$	$\mu_a$ $HbO_2$ , $mm^{-1}/mmolar$	$\mu_a$ $H_2O$ , $mm^{-1}$
770	0.331	0.156	0.00236
825	0.182	0.237	0.00269
847	0.182	0.266	0.00378
902	0.207	0.312	0.00625

$\mu_a$ , Absorption coefficient; Hb, deoxyhemoglobin;  $HbO_2$ , oxyhemoglobin.

mated from data measured on human neonatal brain (23)], with  $\lambda$  in nanometers

$$\mu'_s = 1.0 + \frac{0.3(900 - \lambda)}{(900 - 770)} \quad (5)$$

An average  $\mu_a$  for tissue was calculated by assuming a fraction of blood vessels ( $f_v$ ) per unit volume of tissue, using

$$\langle \mu_a \rangle = (1 - f_v)(\mu_a^w + k) + f_v \mu_a^b \quad (6)$$

where  $\mu_a^b$  is the  $\mu_a$  of blood,  $\mu_a^w$  is the absorption of water, and  $k$  is a wavelength-independent constant. For the study, calculations were made by assuming  $f_v = 0.02$  for the low blood volume and an increase of 15%, giving the higher blood volume fraction as  $f_v = 0.023$ . These correspond to CHC values of 33.6 and 38.64  $\mu\text{M}$ , respectively.

Recent papers (10, 14) have shown that, when the blood is confined to vessels, embedded in a high-scattering, low-absorbing background, the effective absorption of the tissue will vary inversely with the vessel diameter. To investigate the effect of blood vessel size, we used the expression derived by Liu et al. (14) for the effective  $\mu_a$

$$\langle \mu_a \rangle = \mu_a + f_v(\mu_a^b - \mu_a) \exp[-r(\mu_a^b - \mu_a)] \quad (7)$$

where  $r$  is the radius of the vessels, and the tissue absorption  $\mu_a = \mu_a^w + k$ .

It has been suggested that, in the neonate, because skull bones are not fused, the head could expand slightly with a blood volume increase, leading to a change in the optode spacing. To investigate the effect of optode movement, the source-detector separation used in the diffusion equation was varied by  $\pm 1$  mm.

## THEORETICAL RESULTS

### Optical Pathlength and Background Absorption

Both methods for measuring concentration change rely on knowledge of the differential pathlength factor  $\beta$ , because the hemoglobin concentrations calculated are inversely proportional to  $\beta$ . Thus, if  $\beta$  changes during the measurement, for whatever reason, the concentration measurement will be affected.

Changing the  $\mu_a$  of the medium will alter  $\beta$  by a small amount. On the basis of diffusion theory, it is possible to calculate that, for typical tissue optical properties of  $\mu_a = 0.02/\text{mm}$  and  $\mu_s = 1.0/\text{mm}$ , a 15% increase in absorption will lead to a 4% decrease in the pathlength  $\beta$ .

The continuous  $\Delta[\text{Hb}]_{\text{tot}}$  is calculated directly from changes in attenuation. Thus, this variation in pathlength will give rise to a 4% error in the measured change in concentration (i.e., a 15% change in concentration would be measured as a 14.4% change).

For the  $\Delta\text{CHC}$  method, the difference between two absolute concentrations is calculated, and the pathlength change will lead to an error in the absolute concentration (i.e., the second concentration will be 4% too small). If the difference is calculated for an initial

concentration of  $C$  and an increase of 15%

$$\Delta C = (C \cdot 0.96 \cdot 1.15) - C = 0.1C \quad (8)$$

In this case, a 10% difference has been measured instead of the actual 15%.

The change in pathlength with increased blood volume is dependent on the absorption properties of the bloodless tissue. If the background absorption of blood tissue is zero, then a 15% increase in blood volume results in a 15% increase in absorption. However, if the background absorption of the tissue is nonzero, then a change in the blood volume will have a lesser effect on the total absorption.

Figure 4 shows the theoretical estimate of the measured change in hemoglobin concentration for the two techniques as calculated for different background  $\mu_a$  (for differing values of  $k$  in Eq. 6). As expected from the above discussion, for low background absorption, the  $\Delta\text{CHC}$  method underestimates the real value. As the background absorption increases, the continuous  $\Delta[\text{Hb}]_{\text{tot}}$  method gives smaller values.

### Effect of $\mu_s$

As an example of the effect of a change in  $\mu_s$ , Fig. 4 also shows the effect on the blood volume measurements of a 1% drop in  $\mu_s$  simultaneous with an increase in blood volume.

### Blood Vessel Diameter

Figure 5 shows the effect on the  $[\text{Hb}]_{\text{tot}}$  measurements of restricting the change in volume to blood vessels of different diameters. As blood becomes concentrated in fewer, larger vessels, the observed concentration change, as measured by both the  $\Delta\text{CHC}$  and  $\Delta[\text{Hb}]_{\text{tot}}$  methods, decreases.

### Movement of Optodes

Figure 6 shows the effect of a  $\pm 1$ -mm optode movement on the measurement of  $\Delta[\text{Hb}]_{\text{tot}}$  and  $\Delta\text{CHC}$  for a

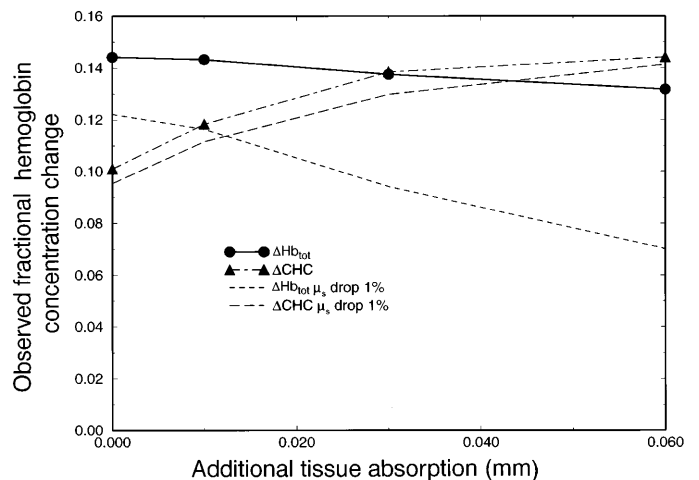


Fig. 4. Theoretical predictions for observed measured change in fractional hemoglobin concentration for the  $\Delta[\text{Hb}]_{\text{tot}}$  and  $\Delta\text{CHC}$  methods as a function of increase in absorption coefficient ( $\mu_a$ ) of tissue. Predictions are given for 2 levels of scattering coefficient ( $\mu_s$ ) of tissue.

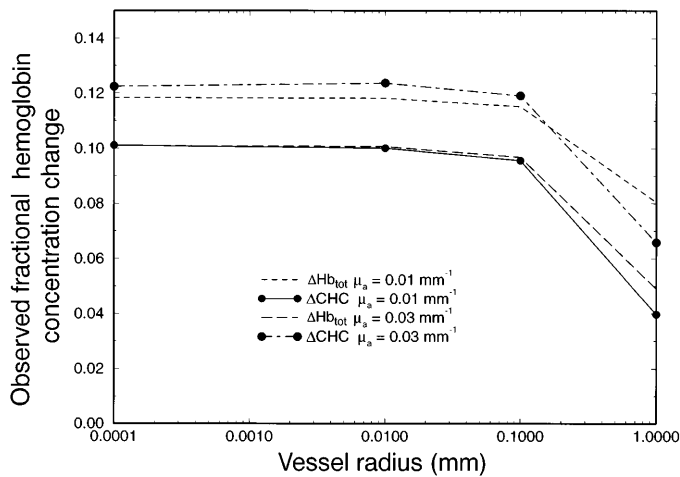


Fig. 5. Theoretical predictions for observed fractional change in hemoglobin concentration for  $\Delta[\text{Hb}]_{\text{tot}}$  and  $\Delta\text{CHC}$  methods resulting from fixed change in blood volume of 15% as function of blood vessel size. Predictions are given for 2 values (0.01 and 0.03/mm) of  $\mu_a$  of tissue.

fixed change in blood volume of 15%. This model predicts that optode movement will have a greater effect on the  $[\text{Hb}]_{\text{tot}}$  than on the CHC signal; i.e., with zero optode movement,  $\Delta[\text{Hb}]_{\text{tot}} = 15\%$  and  $\Delta\text{CHC} = 10\%$ ; however, in the presence of optode movement of 1 mm, the observed  $\Delta[\text{Hb}]_{\text{tot}} = 29\%$ , whereas the CHC signal will still show a change of 10%. These data have been calculated by assuming a movement of the optodes occurring between measurement of volumes but also assuming that the optode positioning is constant during any given CHC measurement.

## DISCUSSION

### Experimental Results

It can be seen clearly that the variability of the CHC values is far greater than the variability of the  $\Delta[\text{Hb}]_{\text{tot}}$  data. By using the CHC values obtained at the

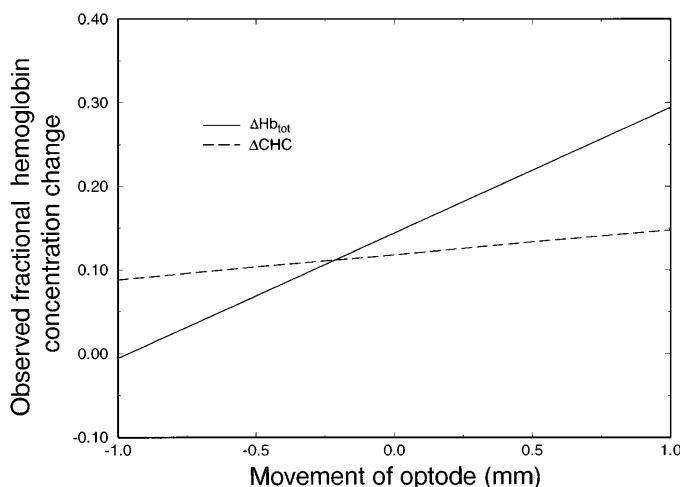


Fig. 6. Theoretical predictions of observed change in fractional hemoglobin concentration for  $\Delta[\text{Hb}]_{\text{tot}}$  and  $\Delta\text{CHC}$  methods resulting from 15% change in blood volume simultaneous with movement of optodes by  $\pm 1$  mm.

lower  $\text{CO}_2$  levels, the sensitivity of the measurement was calculated and was found to be inadequate to accurately resolve the changes in CHC caused by the increase in  $\text{F}_{\text{I}\text{CO}_2}$ . For the data presented, although  $\Delta[\text{Hb}]_{\text{tot}}$  always shows an increase with  $\text{F}_{\text{I}\text{CO}_2}$ , CHC does not show a significant change.

The calculation of CHC in Eq. 4 involves finding the regression between  $([\text{HbO}_2] - [\text{Hb}])$  and  $\Delta\text{fSaO}_2$ . To avoid changes in blood volume and flow, the changes of  $\Delta\text{SaO}_2$  are usually restricted to  $<10\%$ . These changes are measured by using pulse oximeters with typical accuracy of  $\pm 2\%$  (21). Therefore, it is likely that the error on the  $\text{SaO}_2$  reading may be responsible for a large degree of the noise in the CHC measurement. A further problem can be the limited analog-output step size of pulse oximeters, because some output data only in steps of 1%.

The noise in the measurement of  $[\text{Hb}]_{\text{diff}}$  is approximately the same as that for measurement of  $[\text{Hb}]_{\text{tot}}$ . However, in the conversion to an absolute concentration, the former is divided by a small factor ( $\Delta\text{fSaO}_2$ ) which acts to multiply the noise. This is further compounded by errors in the measurement of  $\text{SaO}_2$ .

Unfortunately, although greater  $\text{SaO}_2$  changes would potentially lead to more accurate estimations of blood volume, a large change in  $\text{SaO}_2$  might also result in physiological effects, such as changes in blood volume or flow, which would invalidate the basis of the measurement. Also, for clinical use of the technique, there are obvious limits on the change in  $\text{F}_{\text{I}\text{O}_2}$  which can be used without compromising the patient's health.

Intersubject variability in DPF has been shown to be on the order of 12–17% (5). However, in these studies, DPF acts purely as an equal scaling factor on both the  $\Delta\text{CHC}$  and  $\Delta[\text{Hb}]_{\text{tot}}$  data to convert the units from micromoles/centimeter to micromoles and, as such, will not contribute to the discrepancy shown between the two measurements.

### Theoretical Results

The effect of background tissue absorption can be understood by considering the fact that, when blood volume increases, the tissue volume sampled by the light decreases. Hence, any attenuation caused by tissue absorption will decrease. This acts to lessen the overall increase in attenuation with blood volume and will thus tend to lead to an underestimate of the hemoglobin concentration change. Increasing the number of wavelengths used would make the fitting to the Hb spectral shape more accurate and thus decrease this error (17).

The theoretical modeling of changes in  $\mu_s$  demonstrates a significant effect on the measurement of CHC. However, it is difficult to think of physiological circumstances under which such a change in  $\mu_s$  might be observed. It would seem unlikely that the  $\mu_s$  of tissue would change by more than a fraction of a percent under normal physiological circumstances. The overall  $\mu_s$  might vary as the blood volume changes, due to a change in the  $\mu_s$  of blood. Kienle et al. (12) measured

the anisotropy factor of blood  $g$  at 820 nm as 0.993 and its  $\mu_s$  as 5.5/mm for a hematocrit of 0.01 (which agrees well with Mie theory calculations for red blood cells). This corresponds to a  $\mu_s$  of 134/mm for a hematocrit of 0.41 and a  $\mu_s'$  of 0.94/mm. Because the transport  $\mu_s$  of neonatal brain tissue is  $\sim 1.0$ /mm (23), the change in  $\mu_s$  due to a small increase in blood volume will be negligible. Changes in scattering caused by the depolarization of neurons both in the normal brain during cortical activation and under pathological conditions of stroke are presently the subject of much investigation (3, 9). Typically the magnitude of any changes seen under these conditions is very small, particularly compared with changes resulting from a change in  $\mu_a$ ; hence, such changes would also have a negligible effect on the described model.

There is some uncertainty in the value of  $g$  for blood, and since it is so close to 1.0, uncertainty in  $g$  will lead to large uncertainty in  $\mu_s'$ . However, because a blood volume increase of 15% is only 0.3% of the total ( $\sim 2\%$ ) volume, the maximal decrease in scattering would be 0.3% if blood were nonscattering. A 1% increase in scattering could be caused only if blood had a  $\mu_s$  5 times that of the surrounding tissue, which is clearly not the case.

As expected, the modeling of the effect of vessel diameter demonstrates that the measured hemoglobin concentration change decreases as the blood becomes concentrated in fewer, larger vessels. However, because the majority of vessels inside the brain are  $<0.2$ -mm diameter, this is unlikely to have a major overall effect. It is possible, however, that a significant change in blood volume confined to the larger pial arteries and veins on the brain surface could lead to an underestimate of the hemoglobin concentration change.

The effect of optode movement is clearly more significant in the measurement of  $\Delta[\text{Hb}]_{\text{tot}}$  than  $\Delta\text{CHC}$ . In practice, it is probable that the optodes will be subject to continuous small random movements, which will give rise to greater noise and uncertainty in the measurement. This effect is likely to be larger for the CHC measurement, for the reasons previously stated, because  $\Delta\text{CHC}$  is derived from a difference of two measurements.

In conclusion, the measurement of absolute hemoglobin concentration has been shown to be inherently more liable to noise than measurements of changes in hemoglobin concentration.

The absolute value of CHC measured with this technique is dependent on the  $\mu_a$  of the tissue surrounding the blood vessels. The  $\Delta[\text{Hb}]_{\text{tot}}$  measurement is less sensitive to the background  $\mu_a$ , but it is affected more by any changes that occur in the  $\mu_s$  of the tissue. Measures which might lead to an improved understanding of the CHC data would be 1) to monitor the optical pathlength during the experiment, 2) to improve the accuracy of the pulse oximetry, and 3) to use more wavelengths to improve fitting to the hemoglobin absorption spectra.

We thank the Engineering and Physical Sciences Research Council, UK (Grant GR/K07386), the Medical Research Council, and Hamamatsu Photonics KK for financial support.

Address for reprint requests: C. Elwell, Dept. of Medical Physics and Bioengineering, Univ. College London, 1st Floor, Shropshire House, 11–20 Capper St., London WC1E 6JA, UK (E-mail: celwell@medphys.ucl.ac.uk).

Received 16 June 1997; accepted in final form 1 July 1998.

## REFERENCES

1. **Arridge, S. R., M. Cope, and D. T. Delpy.** The theoretical basis for the determination of optical pathlength in tissue: temporal and frequency analysis. *Phys. Med. Biol.* 37: 1531–1560, 1992.
2. **Brun, N. C., and G. Greisen.** Cerebrovascular responses to carbon dioxide as detected by near-infrared spectrophotometry: comparison of three different measures. *Pediatr. Res.* 36: 20–24, 1994.
3. **Chance, B., Q. Luo, S. Nioka, D. C. Alsop, and J. A. Detre.** Optical investigations of physiology: a study of intrinsic and extrinsic biomedical contrast. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 352: 707–716, 1997.
4. **Delpy, D. T., M. Cope, P. van der Zee, S. R. Arridge, S. Wray, and J. S. Wyatt.** Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys. Med. Biol.* 33: 1433–1442, 1988.
5. **Duncan, A., J. H. Meek, M. Clemence, C. E. Elwell, P. Fallon, L. Tyszczuk, M. Cope, and D. T. Delpy.** Measurement of cranial optical pathlength as a function of age using phase resolved near infrared spectroscopy. *Pediatr. Res.* 39: 1–7, 1995.
6. **Edwards, A. D., J. S. Wyatt, C. Richardson, D. T. Delpy, M. Cope, and E. O. R. Reynolds.** Cotside measurement of cerebral blood flow in ill newborn infants by near infrared spectroscopy. *Lancet* ii: 770–771, 1988.
7. **Elwell, C. E., M. Cope, A. D. Edwards, J. S. Wyatt, D. T. Delpy, and E. O. R. Reynolds.** Quantification of adult cerebral hemodynamics by near infrared spectroscopy. *J. Appl. Physiol.* 77: 2753–2760, 1994.
8. **Elwell, C. E., M. Cope, D. Kirkby, H. Owen-Reece, C. E. Cooper, E. O. R. Reynolds, and D. T. Delpy.** An automated system for the measurement of the response of cerebral blood volume and cerebral blood flow to changes in arterial carbon dioxide tension using near infrared spectroscopy. *Adv. Exp. Med. Biol.* 361: 143–155, 1995.
9. **Fabini, M., G. Gratton, and P. M. Corballis.** Non-invasive NIR optical imaging of human brain function with sub-second temporal resolution. *J. Biomed. Optics* 1: 387–398, 1996.
10. **Firbank, M., E. Okada, and D. T. Delpy.** Investigation of the effect of discrete absorbers upon the measurement of blood volume with near-infrared spectroscopy. *Phys. Med. Biol.* 42: 465–477, 1997.
11. **Hampson, N. B., E. M. Camporesi, B. W. Stolp, R. E. Moon, J. E. Shook, J. A. Greibel, and C. A. Piantadosi.** Cerebral oxygen availability by NIR spectroscopy during transient hypoxia in humans. *J. Appl. Physiol.* 69: 907–913, 1990.
12. **Kienle, A., M. S. Patterson, L. Ott, and R. Steiner.** Determination of the scattering coefficient and anisotropy factor from laser Doppler spectra of liquids including blood. *Appl. Opt.* 35: 3404–3412, 1996.
13. **Lammertsma, A. A., D. J. Brooks, R. P. Beaney, D. R. Turton, M. J. Kensett, J. D. Heather, J. Marshall, and T. Jones.** In vivo measurement of regional cerebral haematocrit using positron emission tomography. *J. Cereb. Blood Flow Metab.* 4: 317–322, 1984.
14. **Liu, H., B. Chance, A. H. Hielscher, S. L. Jacques, and F. K. Tittel.** Influence of blood vessels on the measurement of hemoglobin oxygenation as determined by time-resolved reflectance spectroscopy. *Med. Phys.* 22: 1209–1217, 1995.
15. **Livera, L. N., S. A. Spencer, M. Thorniley, Y. Wickramasinghe, and P. Rolfe.** Effects of hypoxaemia and bradycardia on neonatal cerebral haemodynamics. *Arch. Dis. Child.* 66: 376–380, 1991.
16. **Matcher, S. J., M. Cope, and D. T. Delpy.** Use of the water absorption spectrum to quantify tissue chromophore concentration changes in near-infrared spectroscopy. *Phys. Med. Biol.* 39: 177–196, 1994.
17. **Matcher, S. J., C. E. Elwell, C. E. Cooper, M. Cope, and D. T. Delpy.** Performance comparison of several published tissue near

- infrared spectroscopy algorithms. *Anal. Biochem.* 227: 54–68, 1995.
18. **Patterson, M. S., B. Chance, and B. C. Wilson.** Time resolved reflectance and transmittance for the noninvasive measurements of tissue optical properties. *Appl. Opt.* 28: 2331–2336, 1989.
  19. **Sakai, F., K. Nakazawa, Y. Tazaki, I. Katsumi, H. Hino, H. Igarashi, and T. Kanda.** Regional cerebral blood volume and haematocrit measured in normal human volunteers by single emission computed tomography. *J. Cereb. Blood Flow Metab.* 5: 207–213, 1985.
  20. **Skov, L., O. Pryds, and G. Griesen.** Estimating cerebral blood flow in newborn infants: comparison of near infrared spectroscopy and  $^{133}\text{Xe}$  clearance. *Pediatr. Res.* 30: 570–573, 1991.
  21. **Taylor, M. B., and J. G. Whitwam.** The accuracy of pulse oximeters. *Anaesthesia* 43: 229–232, 1988.
  22. **Van Bel, F., C. A. Dorrepaal, M. J. N. L. Benders, P. E. M. Zeeuwe, M. van de Bor, and H. M. Berger.** Changes in cerebral haemodynamics and oxygenation in the first 24 hours after birth asphyxia. *Pediatrics* 92: 365–372, 1993.
  23. **Van der Zee, P.** *Measurement and Modelling of the Optical Properties of Biological Tissues in the Near Infrared* (PhD thesis). University of London, 1993.
  24. **Wyatt, J. S., M. Cope, D. T. Delpy, A. D. Edwards, S. C. Wray, and E. O. R. Reynolds.** Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet* ii: 1063–1066, 1986.
  25. **Wyatt, J. S., M. Cope, D. T. Delpy, C. E. Richardson, A. D. Edwards, S. Wray, and E. O. R. Reynolds.** Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy. *J. Appl. Physiol.* 68: 1086–1091, 1990.
  26. **Wyatt, J. S., A. D. Edwards, M. Cope, D. T. Delpy, D. C. McCormick, L. A. Potter, and E. O. R. Reynolds.** Response of cerebral blood volume to changes in arterial carbon dioxide tension in preterm and term newborn infants. *Pediatr. Res.* 29: 553–557, 1991.

