

Measurement of changes in optical pathlength through human muscle during cuff occlusion on the arm

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Concentration changes of haemoglobin and cytochromes indicative of tissue perfusion and oxygenation can be quantitatively evaluated from near-infrared (NIR) measurements of absorption and optical pathlength through tissue. The accuracy of such measurements is limited as current bedside instrumentation cannot measure optical pathlength. Using a recently developed phase-resolved spectroscopic technique we can produce continuous and simultaneous measurements of changes in both attenuation and optical pathlength at four wavelengths in the NIR in a bedside instrument. The change in optical pathlength through arm muscle during a cuff occlusion is compared with the absolute pathlength estimated from time resolved spectroscopy measurements in the laboratory. These show that the pathlength varies by 4–10% during the occlusion, demonstrating the need for continuous measurements of optical pathlength.

KEYWORDS: near-infrared, phase resolved spectroscopy, optical pathlength, haemoglobin, cytochrome

Introduction

Near-infrared spectroscopy (NIRS) has been demonstrated to be a viable bedside technique for monitoring cerebral oxygenation and haemodynamics in the newborn infant^{1–4}. Additionally, it is valid for investigating muscle oxygenation and metabolism in adults^{5–8} and shows great promise as a method for observing brain oxygenation in the foetus during birth^{9,10}. The technique relies firstly on: the absorption of near-infrared light caused by the oxygen-dependent chromophores present in tissue, notably haemoglobin and cytochrome oxidase; and, secondly, on the optical pathlength of the light that has traversed the tissue. From these parameters, attenuation and pathlength, and a knowledge of the absorption spectra for the chromophores present, changes in concentration of haemoglobin, oxyhaemoglobin and cytochrome oxidase can be determined in the tissue^{11,12}.

Current technology in pulsed laser diode sources and gallium arsenide photomultiplier tube detectors has allowed the non-invasive measurement of attenuation

through up to 8 cm of tissue with an instrument small enough to be used at the bedside^{1,3}. Current commercially available NIRS instruments (NIRO 500, Hamamatsu Photonics KK) can produce concentration change data in 'real-time' with near continuous sample times of 0.5 s. Using only attenuation data, qualitative results for the concentration changes can be evaluated. In order to quantify these changes in concentration measurements on tissue, the optical pathlength must be determined. Owing to the high scattering and absorption of light in tissue the optical pathlength is not only much greater than the physical distance between the optodes (the connections for light input and detection from tissue) but will vary with scattering coefficient, absorption coefficient (and consequently wavelength), tissue geometry and, for continuous measurements, the changes in the optode spacing. To make accurate quantitative measurements of the changes in concentration of the chromophores, an absolute and continuous measurement of optical pathlength through the tissue is required.

Optical pathlength measurement in tissue

Three methods currently exist for the measurement of optical pathlength through tissue. The first two involve measurement in either the time or frequency domain of

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the relationship between the input and output light. These two measurements are analogues of each other and their results have been shown to be theoretically identical¹³. The third method involves only measurement of attenuation as a function of wavelength of the strong water absorption peaks visible in the near-infrared combined with knowledge of the known water concentration in tissue. The principles and advantages of each approach are discussed below.

Time-resolved spectroscopy

It has been shown that the optical pathlength can be evaluated from the mean time of flight for an ultrashort pulse of light to travel through tissue. The light pulse is typically delivered from a picosecond laser, and its impulse response is detected via a streak camera¹². The optical pathlength is the mean time derived from the time point spread function multiplied by the speed of light through the tissue (the refractive index of tissue is normally taken as 1.4—see Ref. 14). Experiments performed on a variety of tissue sections have demonstrated that the Beer Lambert expression, used to calculate concentration in spectroscopy, can be used in a modified form by replacing the distance with a product of the inter-optode spacing and a constant, the differential pathlength factor (DPF)¹⁵. DPF is dependent on the type of tissue; but for all tissues, its value becomes approximately constant for inter-optode spacings greater than 2.5 cm. Typical values range from 3.59 ± 0.32 in the adult arm to 5.93 ± 0.42 in the adult brain¹⁶. Although the standard deviation is quite high, researchers have attributed the greatest percentage of this variation to inaccuracies in measuring the inter-optode spacing. Further experiments have demonstrated a wavelength dependence of the DPF, showing maximum standard deviations of normalized DPF spectra between 700 and 800 nm to be 5.5% in muscle and 5.0% in brain¹⁷.

For clinical applications, the inaccuracies involved in the use of the DPF and the measurement of the optode spacing with callipers to calculate the chromophore concentration changes are currently accepted. This is because the picosecond laser/streak camera system for accurate measurements is too bulky and expensive to be incorporated into a bedside monitor. Current advances in the development of picosecond laser diodes may yet make this a viable option¹⁸.

Laboratory experiments using the picosecond laser/streak camera equipment have recently been carried out to examine the variability in DPF and consequently pathlength during experimental procedures. Preliminary tests at 760 nm on an adult forehead during graded hypoxic hypoxia showed a 7% decrease in DPF¹⁹. More detailed measurements carried out during muscle ischaemia and a graded exercise on the adult forearm showed both an initial spread of DPF between subjects and a decrease in DPF of 5–10% during a 10 minute period of venous outflow occlusion²⁰. These results were gathered at 5 s intervals and analysed after completion of the experiment. Unfortunately, the time to scan and process data makes this system unsuitable for continuous real-time measurement.

Phase-resolved spectroscopy

Measurement of time response using a picosecond light pulse is analogous to measurement in the frequency domain with an effective bandwidth of 250 GHz. In fact, if the tissue sections are greater than 3 cm the frequency content of the signal only extends up to 1 GHz and so equivalent data can be obtained by employing frequency domain measurements extending to 1 GHz. The attenuation, modulation depth and phase shift of the modulated signal can be obtained by heterodyning the detected signal from the photomultiplier tube. The phase shift experienced by light as it travels through tissue can be equated to the pathlength by consideration of the modulation frequency. Theoretical results show that, in scattering media like tissue, a linear relationship between mean time of flight and phase shift exists to 200 MHz, after which the skewness of the time point spread function causes the phase to under-read the mean time¹³. So, by modulating the source at a single frequency below 200 MHz, continuous measurements of both attenuation and pathlength are possible, via the measured DC intensity and the phase shift in the signal.

The first frequency-based measurements in tissue were made by Lackowicz *et al.*²¹, and a relatively simple measurement system was demonstrated by Chance *et al.* in 1990²². Using a single laser diode (760 nm) modulated at a fixed frequency of 220 MHz, measurements of up to 2° phase shifts were observed for a 2 minute cuff ischaemia on an adult arm and on the head of a hyperventilating adult. A dual wavelength system (754 nm and 840 nm) was subsequently developed that allowed evaluation of the concentration changes in haemoglobin and deoxyhaemoglobin²³. To quantify changes of three chromophores in tissue, especially the rather weakly absorbing cytochrome oxidase signal, we have found it advantageous to use a four-wavelength system. This facilitates the use of multilinear regression to fit the component spectra and residual analysis for the determination of systematic errors. We have developed a four wavelength system for continuous monitoring of quantitative changes in the concentration of the three chromophores²⁴. The instrument will be described in the experimental section, but its main features are time multiplexing and integration of the four laser signals to produce a sampling time of 0.5 s, the use of variable frequency synthesizers between 0–500 MHz coupled with a fast photomultiplier tube for detection of the signal and complete control via a PC, so that online calculations of attenuation and pathlength can be displayed.

Recently, Gratton *et al.*²⁵ have used a phase-resolved system to produce images through the human hand based on raw data. These images showed the increased information available by using phase data over the normal intensity profiles.

Water absorption

This technique was first suggested by Wray *et al.* in 1988¹¹ and has recently been developed by Matcher *et al.*^{26,27}. The method relies solely on attenuation

measurements and is therefore an attractive option for incorporation into existing NIRS instruments. The method utilizes the strong water absorption peak in the near-infrared at 975 nm and its weaker overtone at 820 nm together with a knowledge of the water concentration in the tissue. Since the optical pathlength in a tissue typically exceeds the inter-optode spacing by up to six times, the amplitude of any spectral features, such as the absorption peak at 975 nm, should be enhanced by the same amount. If the apparent concentration of water calculated from the absorption peak is divided by the known concentration of water in the tissue, an estimate of the pathlength is obtained. Work has shown that, in measurements on the adult forearm and the neonatal head, the estimates of pathlength match those recorded using a time resolved system, both for the 975 nm absorption peak and the weaker 820 nm absorption peak. At present these results are recorded using a CCD spectrometer taking recordings at wavelengths in 1.1 nm steps. The data are then fitted to the water spectra using second differentials of the signals to eliminate the effects of the unknown background tissue attenuation. Work is currently under way to investigate if this measurement can be done using a discrete number of wavelengths, enabling its easy incorporation into existing NIR spectrometers, and hence the continuous calculation of the pathlength.

Considering the three techniques described above, measurement of optical pathlength can obviously be done accurately within the laboratory using time-resolved systems. However, there is a need for a system that can operate at the bedside and measure both intensity changes and pathlength in real time. At present, instruments based on phase-resolved spectroscopy look the most promising solution since the instrumentation is relatively inexpensive and adaptable for incorporation into a bedside unit.

In this paper we shall describe a four-wavelength intensity modulated optical spectrometer and provide results obtained with this system on the continuous measurements of both attenuation and phase shift, recorded across an adult forearm during cuff ischaemia. Changes in the optical pathlength will be derived and compared with those obtained at one wavelength by Ferrari *et al.* using the picosecond laser/streak camera equipment, measuring in the time domain²⁰.

Experimental method

The intensity modulated optical spectrometer (IMOS)

A full description and operating characteristics of the spectrometer have been published elsewhere²⁴. Here we will give a summary to show how the phase shift and attenuation signals are obtained. Figure 1 shows a schematic of the instrument. The four CW laser diodes (690, 740, 804, 829 nm) are modulated from a frequency synthesizer at any frequency in the range 1–500 MHz. This synthesizer is phase-locked to another, which provides a second modulation

frequency offset from the first, typically by 10 kHz, which is fed to the two mixers. One mixer provides the pure offset frequency as a reference for the dual phase lock-in amplifier. The second forms part of the heterodyning system on the output of the photomultiplier tube. Light is delivered to the tissue and the detected light transmitted to the photomultiplier tube along optical fibre bundles. Prisms at the end of the bundles redirect the light through 90° on to the tissue surface. The detected signal from the photomultiplier tube is amplified and the DC component filtered off. The AC component is mixed with the frequency plus offset to produce an amplitude modulated signal at the offset frequency for the dual phase lock-in amplifier. The X and Y outputs from the lock-in, together with the DC signal and the input laser diode power, obtained from photodiodes in the laser diode packages, are read into a controlling computer via an ADC card. A counter card provides the multiplexing for the laser diodes together with a period when there are none firing so that a 'dark' background signal can be measured for subsequent subtraction to improve the signal-to-noise ratio of the individual laser signals summed over 0.5 s intervals. The PC is used for the remainder of the signal processing and continuous display of the data.

The attenuation is calculated by division of the transmitted DC signal with the input light intensity from the laser diodes. It is measured in units of optical density. Phase shift is calculated from the X and Y components obtained by integration of the lock-in amplifier outputs. Phase shift, ϕ , is the inverse tangent of Y/X . For any given tissue geometry the pathlength¹³ is $d = c\phi/n\omega$, where c is the speed of light, n the refractive index of the medium through which the light is transmitted, and ω , the modulation frequency of that light. For absolute quantitation of the concentration of chromophores in tissue a knowledge of both the attenuation and absolute pathlength is required. Since no bedside instrument can record absolute values at present, the parameters are generally normalized at the beginning of the procedure, thus we are generating quantitative changes from an arbitrary starting point.

Measurement procedure

For the example data given here, the subject used was a healthy 30 year old male volunteer. The optodes were placed 3 cm (± 1 mm) apart over the muscles on the medial aspect of the arm away from any palpable bone. The optodes were attached using double-sided adhesive

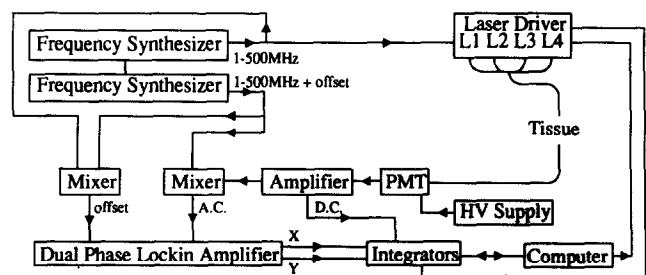


Fig. 1 Schematic diagram of a four-wavelength intensity modulated optical spectrometer

tape and the fibre bundles were loosely held in retort stands so that they provided no variable pressure on the skin. This was done to try and reduce optode movement. The arm was wrapped in black cloth to decrease the amount of background light and placed in a comfortable resting position on a flat surface. In this position a pneumatic cuff was placed loosely around the upper arm.

The IMOS was set at a modulation frequency of 200 MHz with an offset of 10 kHz. After a 30 min warm-up period the optodes were placed on the arm. Once on the arm, the high voltage supply to the photomultiplier tube was increased to a point where signals from all the laser diodes were detected (1317 V on a maximum scale of 1500 V). The system was left for 80 s to settle on the arm. Next the cuff was rapidly inflated to 260 mm Hg to provide an abrupt arterial occlusion. The occlusion was maintained at a cuff pressure of 240–260 mm Hg for a 160 s period, then the cuff was released and the arm rested for 210 s. At this time (450 s, 7.5 min), the cuff was inflated in the same manner and for the same duration as before. Finally, the cuff was released and the optodes removed after a short relaxation period (210 s, 3.5 min).

Results

Figure 2 shows the change in attenuation during the cuff occlusion at the four wavelengths, 690 nm, 740 nm, 804 nm and 829 nm. For the first 80 s while the arm is at rest, negligible variations are seen. In the few seconds it takes to inflate the cuff, attenuation increases at all wavelengths demonstrating the increase in blood volume that occurs as the venous return is momentarily occluded before the arterial flow. When the cuff is fully inflated, blood volume should remain approximately constant while the arm becomes deoxygenated. For those wavelengths below 800 nm, i.e. 690 nm and 740 nm, attenuation increases as the haemoglobin becomes deoxygenated, since the absorption coefficient for deoxygenated haemoglobin is much greater than that for oxygenated haemoglobin below 800 nm (at 800 nm their absorption coefficients are the same and this is called the isobestic point). Beyond 800 nm the absorption coefficient of oxygenated haemoglobin is greater than that of deoxygenated haemoglobin. For 804 nm, attenuation varies little as this wavelength is close to the isobestic point. With the longer wavelength, 829 nm, the signal decreases slightly before levelling off. If the cuff remained inflated for a longer length of time the attenuation would become constant for all wavelengths as oxygen became almost totally depleted. On release of the cuff the arm rapidly becomes oxygenated and attenuation reduces.

Those wavelengths greater than 800 nm show an increase first as the blood volume increases rapidly. The increase is predominantly from oxygenated arterial blood which accounts for the reverse in the attenuation signal. After rapid oxygenation there is a return to equilibrium. Once the signals level off the entire process is repeated. As previously stated, the measurements are taken from an arbitrary starting point. In general, one optical density is equivalent to one centimetre of tissue. In the adult arm, attenuation is slightly higher due to

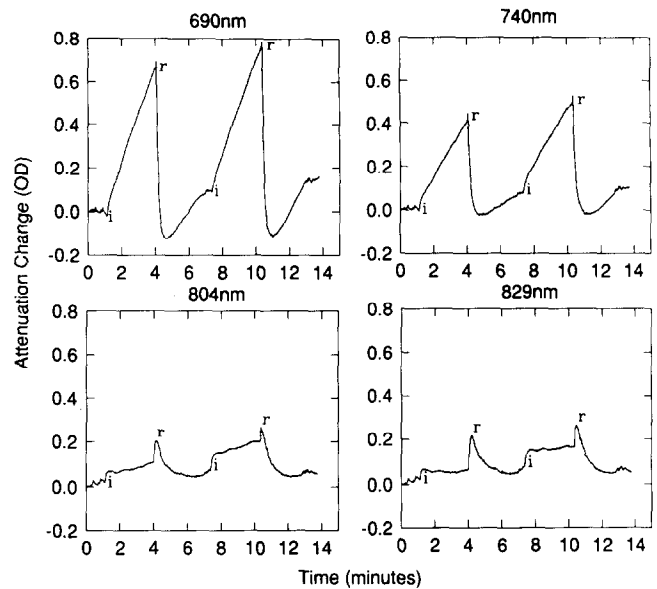


Fig. 2 Attenuation changes at four wavelengths measured during a repeated cuff occlusion on the forearm. The cuff was inflated twice (80 and 450 s) and released after 240 s occlusions. Inflation is indicated by i, and release by r

the higher blood volume and increased absorption from the myoglobin found in muscle tissue. For an inter-optode spacing of 3 cm we can estimate the initial attenuation as around 5.6 OD. This suggests that attenuation varies by almost 20% when measured at a wavelength of 690 nm compared with only 5% at a wavelength of 804 nm for a cuff occlusion on the arm. Following both cuff occlusions, Fig. 2 shows the attenuation remaining above its initial value. This is, in part, due to a drift in baseline attenuation, previously measured as 0.003 OD over a similar period²⁴, as the instrument warms up. In this case the higher attenuation values recorded reflect a different blood volume in the arm following occlusion.

A change in optical pathlength is shown in Fig. 3. In general, the pathlength changes mirror the attenuation changes, i.e. the optical pathlength decreases as the attenuation increases. This is in agreement with predictions based on modelling of light transport in tissue²⁸. At the long wavelengths, 804 nm and 829 nm, there is little change in pathlength, but for the shorter wavelengths, 690 nm and 740 nm, where the attenuation change is greatest, the pathlength change is fairly significant, with values of 2–3 cm. During deoxygenation of the arm, when the cuff is inflated, the pathlength drops steadily. On releasing the cuff, the pathlength rapidly increases as the blood in the arm reoxygenates. However, the attenuation change seen with increased blood volume as the cuff is initially inflated, is not visible in the pathlength data. Any increase in pathlength is lost in the noise of the signal. For the graphs in Fig. 3, the 0.5 s samples have been averaged up to 2 s in order to reduce the noise.

Phase shift measurement appears to be very sensitive to electromagnetic and instrumental noise and this suggests instrumentation must be well constructed to reduce any possible phase errors, and consideration must be given to the noise on the pathlength data if it is

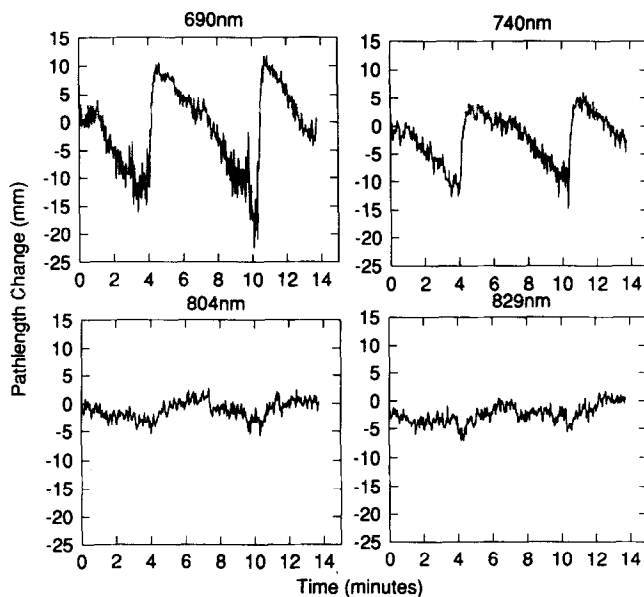


Fig. 3 Changes in optical pathlength measured at four wavelengths during a repeated cuff occlusion on the forearm

to be incorporated into the algorithms that are used to calculate the chromophore concentration change.

In order to judge the significance of these variations in the optical pathlength, we require a value for the absolute optical pathlength across the arm at the start of the procedure. At present, a frequency-resolved instrument has yet to measure this value accurately. Using the method suggested by time-resolved experiments we shall use the product of the DPF and inter-optode spacing to obtain an estimate of the absolute optical pathlength across the forearm. The DPF for an adult arm has been variously reported as 3.59 ± 0.32 at 783 nm (Ref. 16), 3.81 ± 0.53 at 760 nm (Ref. 29) and 4.48 ± 0.41 at 800 nm (Ref. 17). The latter reference also revealed the wavelength dependence of DPF. Unfortunately no results are available for the 690 nm wavelength, but using the latter reference the DPF for 740 nm, 804 nm and 829 nm can be given as 4.75 ± 0.08 , 4.48 ± 0.03 and 4.33 ± 0.06 . These values of DPF produce absolute values for the optical pathlengths of $14.25 \text{ cm} \pm 0.24 \text{ cm}$, $13.44 \text{ cm} \pm 0.09 \text{ cm}$ and $12.99 \text{ cm} \pm 0.09 \text{ cm}$ for 740, 804 and 829 nm respectively. For the 690 nm wavelength, comparisons of the attenuation and pathlength changes measured here, with those occurring at the longer wavelengths, suggest an absolute pathlength similar to the value at 740 nm.

To determine the variation in DPF, and hence the likely changes in chromophore concentrations, we have evaluated the change in absolute DPF and the percentage change in DPF based on the absolute values presented above and the pathlength changes given in Fig. 3. Figure 4 shows the variations with cuff occlusion on the arm for the three wavelengths for which a DPF is available. As expected, the variation directly maps the pathlength change. Considering the magnitudes of the changes, the highest DPF is recorded when blood volume is at its highest value for the shortest wavelength, 740 nm. At the longer

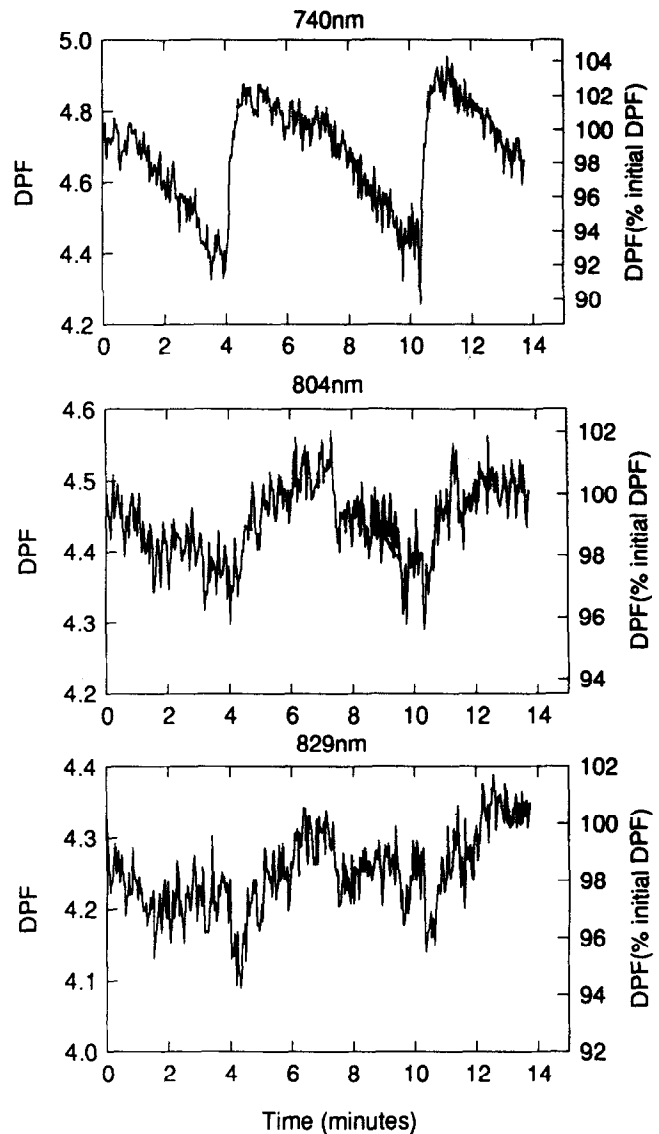


Fig. 4 Changes in the absolute and percentage DPF at three wavelengths during a repeated cuff occlusion on the forearm. Initial values for DPF were obtained from Ref. 20

wavelengths, DPF is at a maximum just before the cuff is inflated. Throughout the experiment the DPF for each wavelength changes by 14%, 6% and 7% respectively. Ferrari *et al.* have measured variations in DPF over 10 and 15 min cuff occlusions on the forearm, at a single wavelength from time resolved measurements²⁰. For a wavelength of 760 nm, they recorded 7–10% decreases in the DPF. These lie within the percentage changes seen using the frequency-resolved system, showing a correlation between the time and frequency based measurements. No measurement of pathlength variation during experimental procedures have yet been made using the water absorption technique. However, estimates of absolute pathlength at 820 nm across the forearm have been shown to be equivalent to those obtained using a time-resolved system²⁷.

Conclusions

Frequency resolved spectroscopy shows promise as a method for continuous monitoring of optical pathlength in real time at the bedside. In clinical

techniques where optode movement is known to exist, such as foetal monitoring and exercising muscle studies, an online measurement of pathlength will improve the accuracy of calculations of changes in chromophore concentrations. In particular, questions have been raised over predictions of the cytochrome oxidase signal, which is around ten times weaker than that of the haemoglobin signal. The results presented here show variations in pathlength comparable with the changes in attenuation, suggesting a need for both to be considered as variable throughout any measurements.

The measurements of pathlength variation with forearm cuff occlusion presented here highlight the shortfalls in the current use of a constant value of pathlength for clinical measurements of concentration changes. The DPF has already been shown to vary greatly between individuals, reflected in the different DPF values available. These differences in DPF are shown here to be comparable to the changes in DPF measured during a cuff occlusion. In the arm, determination of DPF is made more difficult by the different muscle/fat/bone ratios present, both across the arm and between individuals. Using an initial absolute pathlength measurement followed by continuous measurement of changes in the optical pathlength will provide the most accurate determination of cytochrome concentration changes across all tissue. We have shown that an intensity modulated optical spectrometer has the potential to achieve this.

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