

## QUANTITATION OF PATHLENGTH IN OPTICAL SPECTROSCOPY.

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### Introduction

The relative transparency of tissues to near infrared light means that it is possible to transilluminate intact organs. In the infrared region, oxygen dependent absorptions due to haemoglobin and cytochrome  $aa_3$ , can be observed, and it is therefore possible to monitor changes in both the blood and tissue oxygenation of the organ.<sup>1</sup> This monitoring technique is particularly applicable to the study of the brain since there is no interfering absorption from myoglobin, and recent technical developments of the instrumentation have made it possible to transilluminate 8-9 cm of brain tissue.<sup>2</sup> However, once measurements of absorption change at several wavelengths are available, there are still considerable problems in converting this data into quantitative changes in the concentration of oxy and deoxy haemoglobin and of oxidised cytochrome  $aa_3$ .

These problems arise because of the scattering of light as it traverses the tissues. It has been shown previously that when transilluminating a thick section of highly scattering material, a simple "Beer Lambert" formula can to a first approximation be applied to calculate concentration changes, although the optical pathlength used must be increased to take into account the effects of multiple scattering.<sup>3</sup> This increased pathlength can be measured indirectly by using additional data from other physiological monitors, or directly by measuring the absorption due to water whose concentration in brain tissue is known.<sup>4</sup> It is however obvious that this pathlength is not a constant, but will vary slightly with changes in tissue absorption or scattering coefficient. In this paper this variation in pathlength is considered theoretically using a Monte Carlo model of light transport in tissue, and measured experimentally by timing the passage of ultrashort light pulses through tissue.

## Background

Light travels through tissue at a speed of approximately 0.2 mm/picosecond. Using a synchronously pumped dye laser as a source of ultrashort light pulses, and a synchroscan streak camera as a detector it is now possible to measure the time of flight of light pulses through tissue with a resolution of a few picoseconds, and hence measure pathlength to a few millimetres. However, the multiple scattering that occurs in tissue means that the emerging photons are dispersed in time, and have not travelled a single unique pathlength. This leads to a problem when trying to choose a single transit time (and hence pathlength) to use in the "Beer Lambert" calculation of concentration. In a previous study<sup>5</sup> this problem has been addressed using a Monte Carlo model of light transport in tissue. The model predicted that the mean value of the time dispersed pulse (the Temporal Point Spread Function) correlated best with the pathlength in the "Beer Lambert" calculation. This prediction was verified experimentally using a phantom of known optical characteristics. Finally, transit time was measured experimentally across the rat head, and a mean pathlength of  $5.3 \pm 0.3$  times the head diameter calculated. This work has now been extended, to study the way in which this pathlength will change with absorption.

## Model predictions and verification

A Monte Carlo model of light transport in tissue was used. The scattering phase function in the model was the phase function calculated from Mie theory for the phantom used for

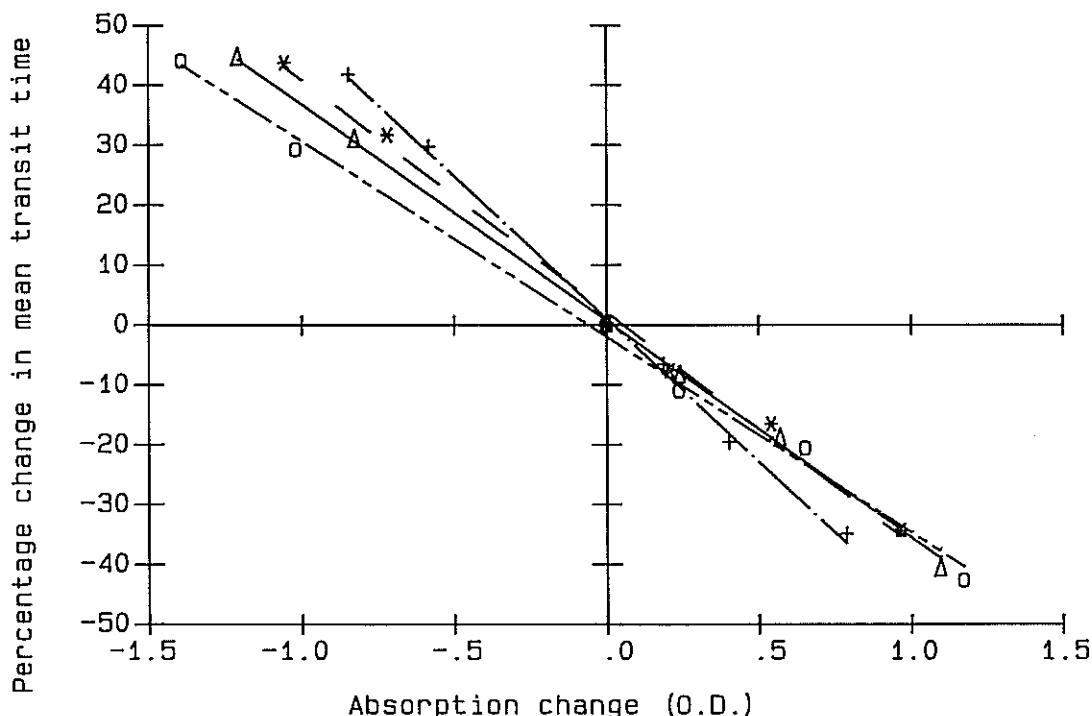


Figure 1. Monte Carlo predictions of the relationship between percentage change in mean optical pathlength and variation in optical density. Data is shown for four different scattering coefficients (+)  $\mu_s = 40 \text{ cm}^{-1}$ ; (\*)  $\mu_s = 60 \text{ cm}^{-1}$ ; ( $\Delta$ )  $\mu_s = 80 \text{ cm}^{-1}$ ; (O)  $\mu_s = 100 \text{ cm}^{-1}$ . The slope for the regression line for  $\mu_s = 100$  is 33%.

the experimental verification. Specular reflections at the slab boundaries due to differences in refractive index were taken into account. The time dispersion of a spatial and temporal delta function transmitted through a 1 cm slab was modelled for a range of absorption and scattering coefficients. Absorption coefficients (base 10) of 0.456, 0.334, 0.263, 0.217, 0.0867 and 0.0434  $\text{cm}^{-1}$  and scattering coefficients (base e) of 100, 80, 60, 40, and 20  $\text{cm}^{-1}$  were simulated encompassing the range quoted in the literature for brain tissue. The mean value of each Temporal Point Spread Function (TPSF) was then calculated. The percentage change in this value, referenced to the value at an absorption coefficient of 0.217, was then plotted against the calculated change in optical density. Figure 1 shows this relationship for scattering coefficients of 40, 60, 80 and 100  $\text{cm}^{-1}$ . It can be seen that a linear relationship is obtained over a wide range in optical density. The slope of the relationship is dependent upon the scattering coefficients, an increase in scattering coefficient causing a decrease in slope.

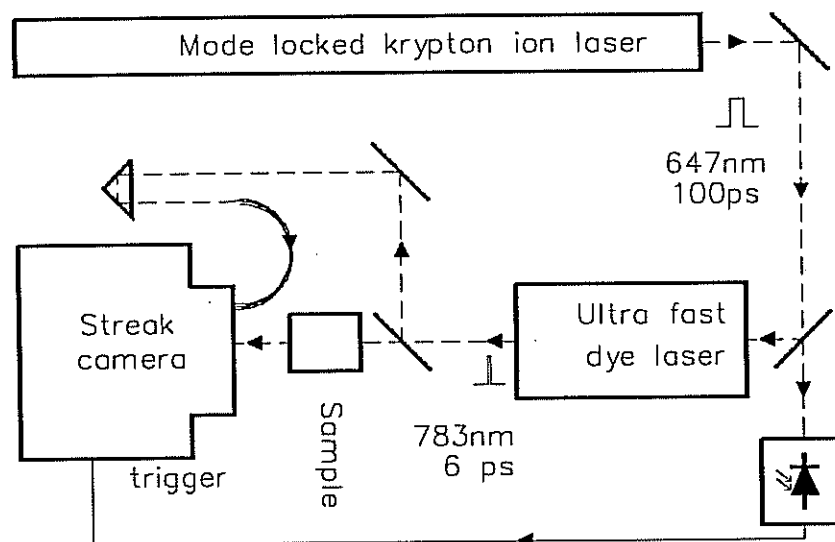


Figure 2. Experimental apparatus for the verification of model predictions and in-vivo time of flight measurements.

The model predictions were verified using a phantom consisting of polystyrene latex microspheres in water containing an infrared absorbing dye.<sup>5</sup> Two sizes of polystyrene latex microspheres were used in the phantom in an attempt to reproduce the scattering phase function of brain tissue, measured previously.<sup>6</sup> The solution contained 2.5% by volume of 1.0  $\mu\text{m}$  diameter particles and 6.7% by volume of particles of 0.05  $\mu\text{m}$  in diameter. Changes in scattering coefficient were obtained by volumetric dilution of this mixture. Absorption coefficient changes were obtained by adding known quantities of infrared absorbing dye (ICI S109564). Measurements were made

using the experimental system shown in figure 2. with a sample cell 6 cm wide, 6 cm high and 1 cm thick. The absorption coefficient was varied over a range of 0.05 to 0.42  $\text{cm}^{-1}$ . Scattering coefficients were 80, 40 and 20  $\text{mm}^{-1}$ . The percentage change in mean was again referenced to the value at an absorption of 0.21  $\text{cm}^{-1}$ . The results in figure 3 show a similar trend to those from the model predictions.

### Pathlength measurement in brain

Experimental measurements have been made of the optical pathlength in the transilluminated rat brain. Nine adult wistar rats were studied. Following anaesthesia (urethane 36% w/v, intraperitoneal, 5ml/Kg), the temporoparietal muscles were reflected, the skull exposed and cleared of residual tissue. The animals were ventilated via a tracheal tube and the femoral artery was cannulated for blood sampling. The animal was placed in front of the streak camera in the sample position shown in figure 2, the head being immobilised in a stereotactic frame. Light pulses from the dye laser were incident upon the skull diametrically opposite the streak camera entry slit. Some of the light emerging from the far side of the head was sampled via a 1 mm diameter optical fibre coupled to a photomultiplier tube (Hamamatsu R928). Measurements were made with the animals breathing 100%, 21% and 12% oxygen (balance  $\text{N}_2$ ) and 90% oxygen with 10% carbon dioxide. Measurements were also made immediately upon death by  $\text{N}_2$  inspiration.

Figure 4 shows the data points for all animals, the reference pathlength being that obtained with 100%  $\text{O}_2$  inspiration. Several points can be noted from this figure. Firstly, the overall

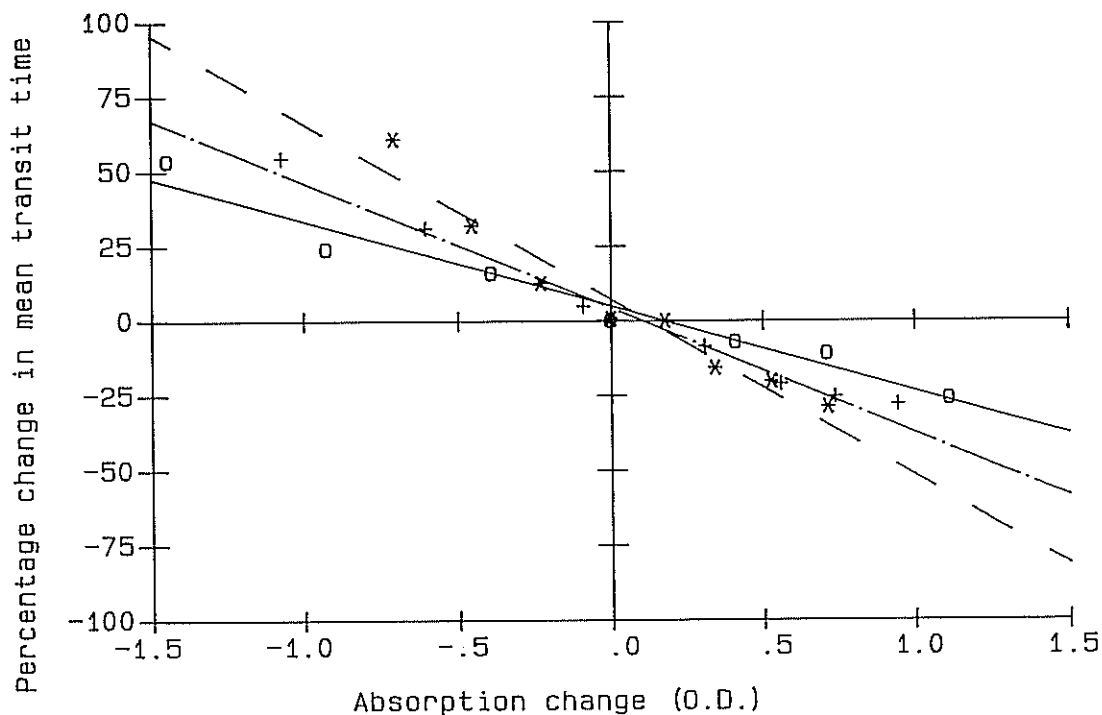


Figure 3. Measured percentage change in the mean optical path as a function of changing optical density for the phantom solution. Data is shown for three different values of scattering coefficients. (\*)  $\mu_s = 20 \text{ mm}^{-1}$ ; (+)  $\mu_s = 40 \text{ mm}^{-1}$ ; (o)  $\mu_s = 80 \text{ mm}^{-1}$ .

relationship is very similar to that predicted by the Monte Carlo model, and verified in the phantom studies. Secondly, the relationship appears to be linear over a total change in optical density of 0.5 OD induced both by a variation in blood oxygenation ( $F_{I}O_2 = 100 \rightarrow 12\%$ ) and blood volume ( $F_{I}CO_2 = 10\%$ ). The slope of the regression line gives a value for the change in pathlength per OD of 30% for the data on live rats. Finally, it can be seen that the data obtained during immediate death does not follow the same relationship. The reasons for this are as yet unclear, but may be due to changes in tissue scattering coefficient post mortem<sup>7</sup>.

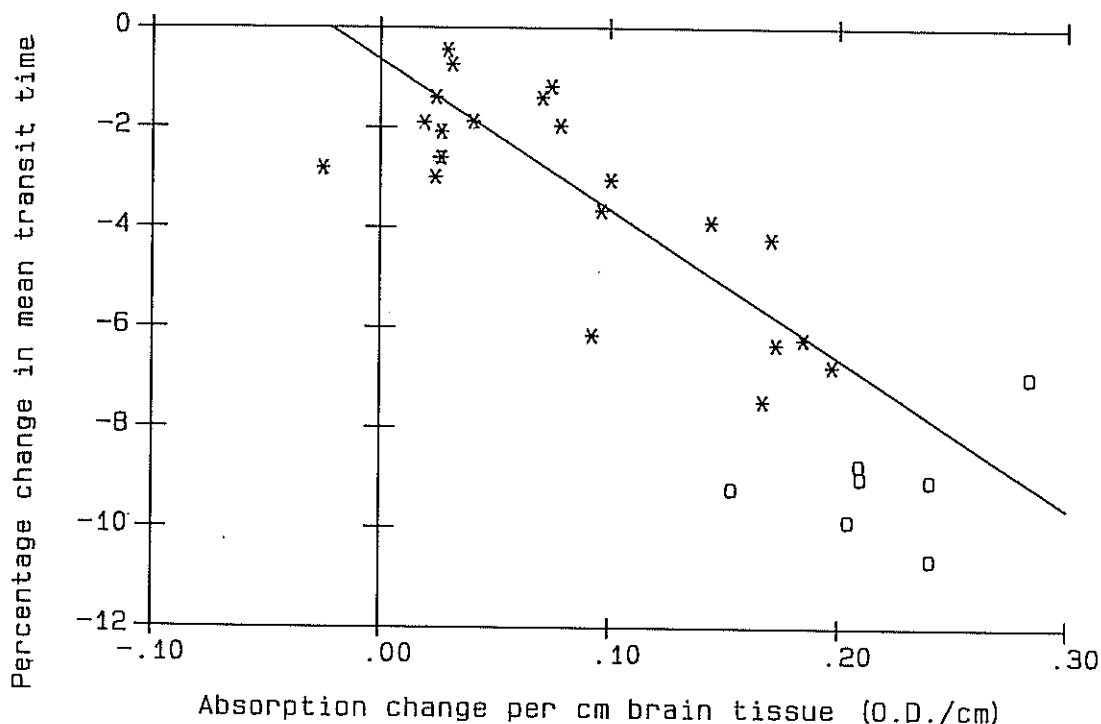


Figure 4. Percentage change in the mean optical path across the rat head as a function of change in optical density. (\*) Data obtained on the live animal; (o) data obtained during death. The slope of the regression line (excluding 'o' data points) is 30% /OD.

### Conclusions

The optical pathlength through the transilluminated rat head has been experimentally measured, and changes in the pathlength have been shown to follow the predictions of a Monte Carlo model of light transport in tissue. The mean pathlength increased by approximately 3% for each 0.1 OD increase in transmitted intensity. Incorporation of this pathlength change into the Beer Lambert equation should significantly improve the accuracy of estimation of chromophores at low concentration.

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