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A. Jackson PhD MB ChB MRCP FRCR,  
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Imaging Science and Biomedical Engineering,  
School of Cancer and Imaging Sciences,  
University of Manchester, Stopford Building,  
Oxford Road, Manchester M13 9PT, U.K.

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N. A. Thacker PhD, M. L. J. Scott MSc  
Imaging Science and Biomedical Engineering,  
School of Cancer and Imaging Sciences,  
University of Manchester, Stopford Building,  
Oxford Road, Manchester M13 9PT, U.K.  
`neil.thacker@manchester.ac.uk`

## Abstract

This paper reviews the analysis techniques currently in common use for the calculation of cerebral blood flow (CBF) from dynamic susceptibility contrast enhanced magnetic resonance imaging (DSC-MRI). The paper highlights and explains a series of requirements of the data which must be met in order to accurately derive CBF measurements. These data characteristics are then examined and tested using experimental approaches. We conclude that the characteristics of DSC-MRI data breach the requirements of the standard analysis approaches, resulting in significant errors in the estimation of CBF. We present an alternative approach to the measurement of CBF from DSC-MRI data based on an alternative physiological model which assumes that a measurable component of directional flow is present in all areas of the brain.

## 1 Introduction

Abnormalities of the cerebral vasculature are one of the commonest features of brain disease and enormous effort has been expended to develop methods for their identification and quantitation [2, 32]. One of the most important descriptive parameters is cerebral blood flow (CBF) which allows identification of altered blood flow states such as those resulting from haemodynamic restriction, vascular stenosis or occlusion, or from decreased regional demand as is seen in Alzheimer's disease [15, 4]. Clinically we need to be able to answer the question of whether cerebral blood flow is normal and if not, then where and how severe is the abnormality? At the present time the answer to these questions is not easily obtained in routine clinical practice.

Physiologists have developed a number of methods to estimate whole brain cerebral blood flow (CBF), cerebral blood volume (CBV) and mean cerebral transit time (MTT) without the use of imaging techniques. These methods are effective in monitoring total cerebral blood flow resulting from injury, pathology or therapeutic interventions but do not provide any indication of the spatial distribution of abnormalities within the brain [9]. Two main groups of analysis methods have been described. The first, commonly used in intensive care monitoring systems to measure changes in CBF, uses a bolus injection of an intravascular marker (usually a dye) which stays within the blood stream. CBF measurements are derived from changes in bolus width and height which occur during passage through the brain [9]. The second group of techniques use a marker which will penetrate freely into brain tissue (ie nitrous oxide) and estimate CBF by measuring the extraction of marker from the circulation [9, 14]. Measurements based on imaging techniques use similar basic principles but have major potential advantages since they are able to identify and quantify regional changes in blood flow parameters. Isotope techniques including hexamethyl propyleneamine oxime single photon emission computed tomography (HMPAO-SPECT), [ $^{15}\text{O}$ ]- $\text{H}_2\text{O}$  positron emission tomography (PET) and Xenon perfusion computed tomography use markers which freely pass from the blood into the brain and allow measurement of regional CBF from images of the distribution of the marker within the brain. Each has inherent advantages and disadvantages which are beyond the scope of this article.

There are two commonly used methods for measuring cerebral blood flow (CBF) using magnetic resonance imaging (MRI). Arterial spin labelling uses magnetic labelling of protons in blood to provide an endogenous tracer of flow. This technique is attractive but not yet sufficiently robust for routine clinical use [2, 7]. Dynamic susceptibility contrast enhanced MRI (DSC-MRI) uses the rapid measurement of MR signal change following the injection of a bolus of a paramagnetic MRI contrast agent [7, 3, 25]. The resulting signal loss from passage of the contrast agent bolus on T2\* or T2 weighted images can be used to calculate estimates of cerebral blood volume (CBV), mean transit time (MTT) and cerebral blood flow (CBF).

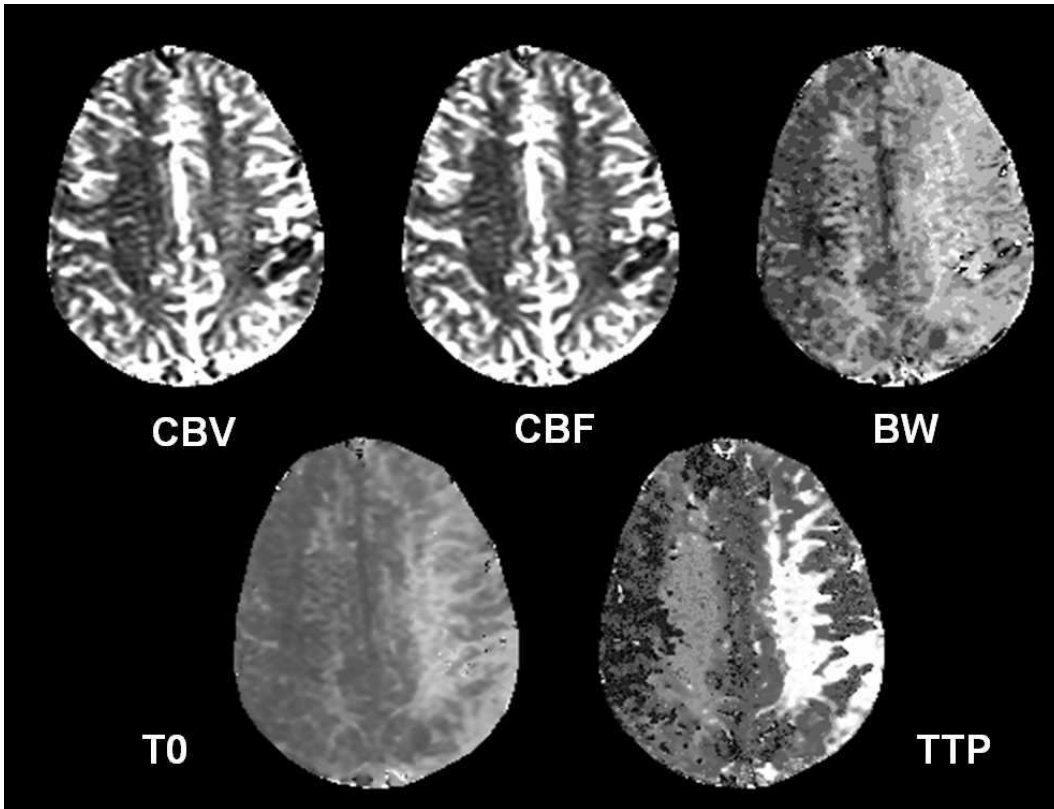


Figure 1: Typical parametric maps of rCBV, rCBF, rMTT, T0 and TTM in a patient with unilateral carotid stenosis. Images demonstrate the expected lengthening of T0, TTM, rMTT, increase in rCBV and decrease in rCBF in the stenosed hemisphere.

The analysis of DSC-MRI data is described in detail below. In basic terms the data is used to calculate contrast concentration changes from signal change. The area under the contrast concentration time course curve is used as an estimate of the CBV. The relative width of the curve is used to derive an estimate of the MTT, and the CBF is calculated using the central volume theorem which defines CBF as the ratio of CBV/MTT [2, 7, 5]. Although there is no doubt that DSC-MRI provides clinically valuable data (see Figure 1) it has become clear that the calculation of CBF using the standard techniques is subject to very significant errors [2, 32, 7, 5]. One source of error is the presence of variable bolus broadening which occurs before the bolus reaches the head due to individual variations in physiology and injection technique [23, 24]. This can be minimised by the identification of an arterial input function (AIF) from a large basal artery which can be used to deconvolve the observed tissue residence signals from individual voxels [24, 19]. More problematic is that the passage of the contrast bolus through the brain is complicated by the existence of multiple parallel vascular pathways with varying lengths and transit times. As a consequence of these multiple pathways the arrival times of the contrast bolus within the brain gradually de-phase giving rise to progressive broadening and delay of the contrast bolus observed in individual voxels [5, 19, 18]. These changes make the identification of an appropriate AIF impossible since an individual AIF would need to be identified for each voxel to provide the appropriate correction for the bolus broadening in the vascular pathway proximal to the measurement point. Attempts to identify local AIFs have been described [19] but are complex and essentially unsatisfactory.

This paper will review the standard approach to the analysis of DSC-MRI data. In particular we will explicitly define the characteristics of the DSC-MRI data which are required for the calculation of CBF and describe a series of experiments which have been performed to investigate them [33, 31, 32]. The results of these experiments clearly demonstrate discrepancies between the idealised behaviour of the cerebral circulation, which is assumed by the analysis technique, and the observed behaviour of the data. These discrepancies are significant and can be expected to adversely affect CBF estimates. We also describe a novel analysis technique based on an alternative physiological model of blood flow and which requires a different set of assumptions.

## 2 Conventional Theory

The conventional approach to modelling cerebral blood flow from DSC-MRI involves computation of relative cerebral blood volume (rCBV) [1, 11] which is a well-defined and easily calculated parameter. The equation describing formation of T2\* weighted image intensity values  $I_i$  for voxel  $i$  ignores flow and partial volume measurement artifacts and is written:

$$R_i(t) = - \frac{\ln(I_i(t)/I_i(0))}{\alpha T_E} \quad (1)$$

Where  $R_i(t)$  is the relaxivity,  $\alpha$  is a constant and TE is the echo time. The assumption of a linear relationship between relaxivity and concentration of the contrast agent has been shown to be valid both by experiment and simulation for blood volume fractions in the physiological and pathological range [3]. For a bolus with time varying contrast concentration we can thus write:

$$\int_0^\infty R_i(t)dt = \beta \oint_{V_i} \int_0^\infty C(\mathbf{p}, t)dt d\mathbf{p} \quad (2)$$

Where  $\beta$  is a constant and  $C(\mathbf{p}, t)$  is the spatio-temporal contrast density. This quantity is generally referred to as rCBV, implying that the integrated time varying contrast concentration must have the same value at all locations  $\mathbf{p}$  such that;

$$\int_0^\infty R_i(t)dt = \beta V_i \int_0^\infty C_i(t)dt \quad (3)$$

where  $C_i$  is the concentration of contrast in the fractional volume  $V_i$  of the voxel. This amounts to an assumption of zero dispersion in an incompressible fluid, in which changes in cross-sectional volume are accompanied by compensating changes in velocity. If these assumptions are correct then the right hand side integral is a constant for all locations ( $i$ ) within the volume and can now be re-written as

$$\int_0^\infty R_i(t)dt = \beta V_i \langle C_a \rangle \langle T_a \rangle \quad (4)$$

where  $\langle T_a \rangle$  is the time taken for some proportion of the bolus to pass through an arterial voxel. This equation then defines the effective arterial inflow concentration  $\langle C_a \rangle$ . As a consequence rCBV can be estimated from a sum over the appropriate measured image data although a non-linear correction for variation in the haematocrit may be required. With real data we have the additional problem that the shape of the observed R(t) curve is modified by the voxel residence time. However, as this can be modelled as a convolution the area under the curve is theoretically unaffected.

MTT can be estimated from the temporal width of the measured bolus by assuming that the net shape of the bolus can be modelled as a convolution of the arterial bolus shape with the within-voxel molecular transit time distribution [2, 7, 6, 8, 24, 22].

Division of the calculated rCBV by the mean transit time (MTT) recovers relative cerebral blood flow measurements (rCBF):

$$RCBF = RCBV/MTT \quad (5)$$

It should be noted that these values for CBV and CBF are expressed as relative values (ie; rCBV and rCBF) since the analysis does not support the calculation of absolute values for CBV. Therefore, unless some calibration stage is included in the analysis then the values obtained must be considered only as valid ratios of flow and blood volume in an individual case and not as absolute values. The consequence of this is that the technique as described supports comparisons of areas within a single study but not comparison between individuals or between sequential scans.

## 3 Requirements of the Standard Analysis Techniques

The use of the standard analysis method is based on a number of specific assumptions concerning the physiology of cerebral blood flow and the consequent characteristics of the imaging data. These must be appreciated if potential

shortcomings of the standard analysis approach are to be understood. In this section we will identify a number of specific characteristics of the data which are required by the standard analysis approach in order to provide accurate measurements of CBF.

### **1. The Data Must Allow Accurate Estimates Of Absolute Fractional Blood Volume In Each Voxel**

Measurement of absolute CBV is essential if absolute CBF is to be calculated. Relative measurement of CBV (rCBV) is easily estimated from the area under the first pass component of the contrast concentration time course curve. However, it cannot be easily transformed into an absolute measurement since the contrast concentration calculated from the observed signal intensity changes is in arbitrary units. Measurements of rCBV can be calibrated by comparison with values in voxels which are 100% blood to provide approximations of absolute CBV. This assumption appears to be valid to within a few percent and the calculation of CBV provides estimates for white and grey matter in keeping with known values.

### **2. Broadening Of The Contrast Bolus Must Represent The Mean Transit Time Of Contrast Through The Voxel**

Changes in MTT will directly affect the local width of the contrast bolus and so early workers used the width of the contrast concentration residue function directly as an MTT surrogate. Weisskoff et al. [34] have shown that although the MTT computed directly in this way is not equal to  $CBV/CBF$ , it can still give a reasonably correct relative measurement of rCBF between regions, provided however, that they feature a similar vascular physiology. This can also be used to continuously monitor the effect of a perturbation on a given region [2]. However, the use of bolus broadening as a measurement of MTT also requires that bolus width is unaffected by other factors.

### **3. Variation In Contrast Bolus Width Must Not Result From Variations In Injection Technique Or Bolus Passage Through The Systemic Circulation**

In practice it was soon realised that the width of the contrast bolus is strongly affected by individual variations in injection technique, contrast dose and cardiovascular function. Direct comparison of CBF estimates between individuals requires that these sources of variation have been minimised or removed [24]. As mentioned above, one approach to solving this problem is to deconvolve data from each voxel with an input response function that reduces such variability and this has been used as the basis of quantitative techniques for the absolute measurement of CBF [2, 7, 24, 22]. The use of deconvolution approaches requires the choice of an appropriate arterial input function (AIF) which is used as a base function for the deconvolution. This is usually one of the large arteries at the base of the brain and the middle cerebral artery is most commonly used. The use of a single measurement for the AIF relies on the assumption that a single AIF is appropriate for all measurement voxels.

### **4. The Surrogate AIF Must Be Identical To The True AIF For Every Measurement Voxel In The Brain**

The use of a surrogate AIF requires that no additional broadening of the contrast bolus occurs (between the artery in which the AIF is measured and the measurement voxel) which would give rise to spurious increases in the measured AIF following deconvolution [2, 7, 20].

### **5. The Contrast Bolus Must Arrive Simultaneously In All Measurement Voxels And All Flow Within Measurement Voxels Must Be Random In Direction**

These assumptions are logical conclusions of the way in which the central volume theorem has been applied to the data. This may be a little hard to appreciate but can be explained by reviewing the basic central volume theorem which states that  $CBF=CBV/MTT$ . Consider a large grey matter area with homogeneous flow. The CBF, measured in  $mls/min/100g$  of tissue and the CBV measured in  $mls/100g$  of tissue are constant throughout the tissue. The MTT is therefore a constant value in units of seconds. This means that the MTT is independent of the voxel dimensions and is not affected by the distance over which it is measured. If we sub-sample the tissue and measure the MTT from one half or some other fraction of the volume these arguments still apply; the MTT remains constant and independent of tissue volume. This clearly could not be true if there were directional flow in the tissue. As a model of directional flow imagine a large vessel flowing through the sample. The time the contrast

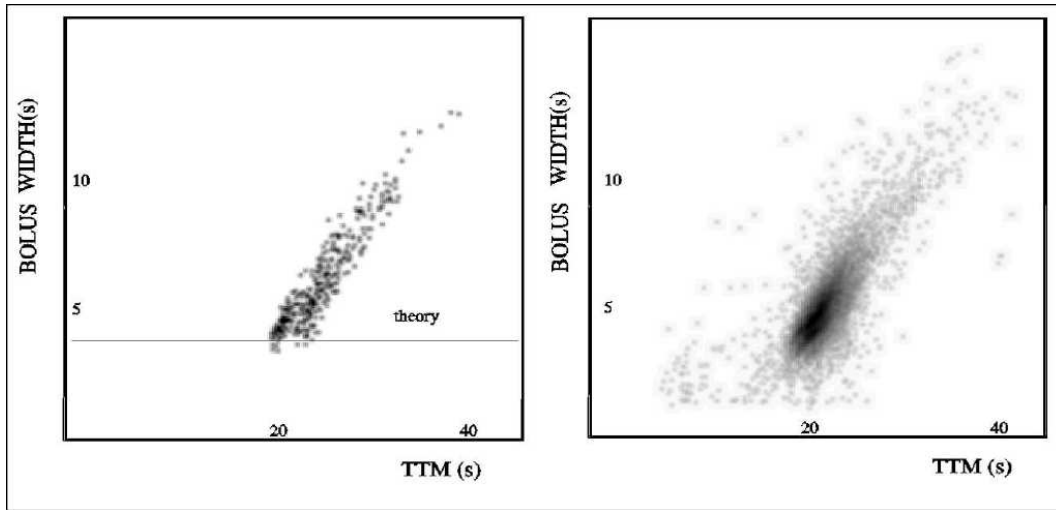


Figure 2: Plots illustrating the extent of bolus broadening in a normal subject from a gradient echo data set. The width of the bolus is plotted against the time to the middle of the contrast bolus or time to mean (TTM). Arteries will be represented by short TTM, veins by long TTM and capillaries by intermediate values. (a) shows the plot for pixels with  $CBV < 0.3$  and therefore excludes data from capillary beds ( $CBV$  typically 2.5-5%). Arteries and veins should exhibit very short transit times, but in fact the measured bolus width demonstrates a linear relationship with bolus age (TTM). In standard perfusion studies the use spin echo sequences should minimise the contribution from these high  $CBV$  voxels. (b) shows the same plot including data from all voxels. A clear linear relationship can be seen and it is evident the many capillary bed areas display bolus widths far shorter than those seen in the venous system in (a) despite the fact that capillary flow is clearly far less than that in large veins.

takes to transit the vessel is clearly dependent on the vessel length and therefore on the voxel size and shape. The use of arbitrary scanning geometry thus corresponds to an assumption that there is no directional flow in the data.

The use of an arbitrary geometry also requires that the contrast bolus should arrive in all voxels simultaneously. Consider two adjacent grey matter voxels between which there is a delay in the arrival of the contrast bolus. The MTT in each of the voxels will be the same and can be calculated from the width of the contrast bolus. However, if imaging were performed using a larger voxel which encompassed both of the original ones, then the width of the bolus would now reflect both the MTT and delay in bolus arrival between the voxels. In practice this argument will apply within all voxels, so, the standard technique is dependent on the absence of bolus delay.

## 6. The MRI Data Must Result Purely From Contrast Within The Capillary Beds

This is essential since signal from large vessels will introduce the effects of directional flow into the data. Many workers have stressed the importance of spin echo acquisition sequences for perfusion experiments since these are less sensitive to the effects of contrast in large vessels [26, 29] However more recently it has been shown that the use of gradient echo techniques with their improved signal to noise ratio make little actual difference to the calculated MTT and CBF despite the dominance of signal from large vessels in the data sets [27, 30].

## 4 Testing the Data

In this section we will describe a series of experiments to test the identified required characteristics of the data. As discussed above the requirements listed under 1, 2 and 3 appear to be appropriate assuming that the voxel residence data is deconvolved using the surrogate AIF. The remaining requirements can be directly tested by analysis of the dynamic MRI data.

### Requirement 4. The Surrogate AIF Must Be Identical To The True AIF For Every Measurement Voxel In The Brain

Both delay and dispersion of the bolus do occur even in normal subjects [2, 33, 20]. Recent simulation studies [5] have suggested that this error introduces significant underestimation of CBF and overestimation of MTT so that

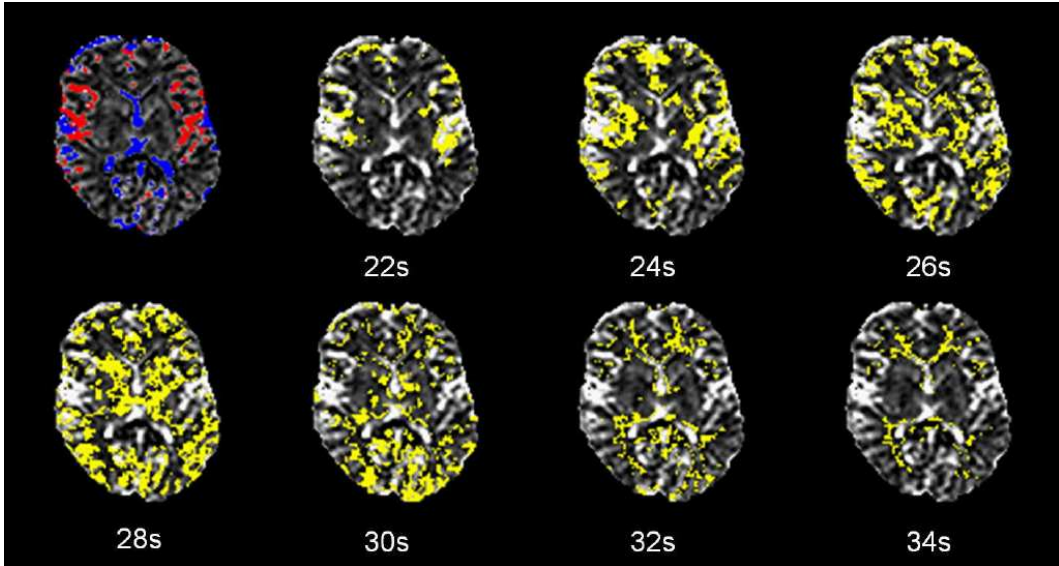


Figure 3: Temporal images illustrating the spatial distributions of a given time of arrival of contrast for a single axial slice through the brain. Arteries and veins are shown in the first image and are identified by the presence of high CBV. The yellow areas on subsequent images show the centre of the contrast bolus in voxels with low CBV (capillaries). The clear pattern to the passage of the bolus through the brain is in keeping with the known vascular anatomy and illustrates a wave of contrast passing through superficial areas to reach the deep white matter and periventricular regions.

additional broadening of the bolus by 2.5 seconds or more overestimates MTT by 200% and underestimates CBF by 50%. This underestimation increases further as bolus broadening increases [5]. Figure 2 illustrates the extent of bolus broadening in a normal subject from a gradient echo data set. The width of the bolus is plotted against the mean time of arrival (TTM) of the contrast bolus in the voxel. In this plot we would expect to see arteries represented by short TTM, veins by long TTM and capillaries by intermediate values. Figure 2a shows the plot for pixels with  $CBV > 0.3$  and therefore excludes data from capillary beds ( $CBV$  typically 2.5-5%). We would expect to see very short transit times, and therefore bolus widths, in both arteries and veins. In fact the measured bolus width demonstrates an approximately linear relationship with bolus age (TTM). In standard perfusion studies the use of a spin echo sequence should minimise the contribution from these high  $CBV$  voxels. However figure 2b shows the same plot including data from all voxels. Once again a clear linear relationship can be seen and it is evident that the many capillary bed areas display bolus widths far shorter than those seen in the venous system in figure 2a despite the fact that capillary flow is clearly far less than that in large veins. This data demonstrates the presence of a significant degree of bolus broadening which occurs independently of regional flow rates.

**Requirement 5. The Contrast Bolus Must Arrive Simultaneously In All Measurement Voxels And All Flow Within Measurement Voxels Must Be Random In Direction**

The requirement that the contrast bolus must arrive simultaneously in all voxels can be tested by visualising the temporal distribution of the bolus passage through the capillary beds [32]. Figure 3 shows a series of time resolved images illustrating the position of the centre point of the contrast arrival time distribution in a single axial slice through the brain. Arteries and veins are shown in the first image and are identified by the presence of high  $CBV$ . The yellow areas on subsequent images show the centre of the contrast bolus in voxels with low  $CBV$  (capillaries). There is a clear pattern to the passage of the bolus through the brain. The frontal and insular cortices show the first enhancement and from here the bolus passes into the deep white matter. The occipital cortex enhances later than the frontal and parietal cortices and the very deep periventricular white matter enhances last of all. These patterned delays in the arrival of the contrast bolus are in keeping with the known vascular anatomy and illustrate a wave of contrast passing through superficial areas to reach the deep white matter and periventricular regions. This does not support the hypothesis that the contrast bolus arrives simultaneously in all voxels. Examination of the data suggests that calculation of MTT from a large white matter region of interest would be affected by bolus delays in excess of 4 seconds whilst smaller regions of interest would be affected to a proportionately lesser extent.

The assumption that no directional flow occurs within the measurement voxels can again be directly tested. We have previously described a method for measuring the apparent velocity of directional flow in any voxel [33, 32].

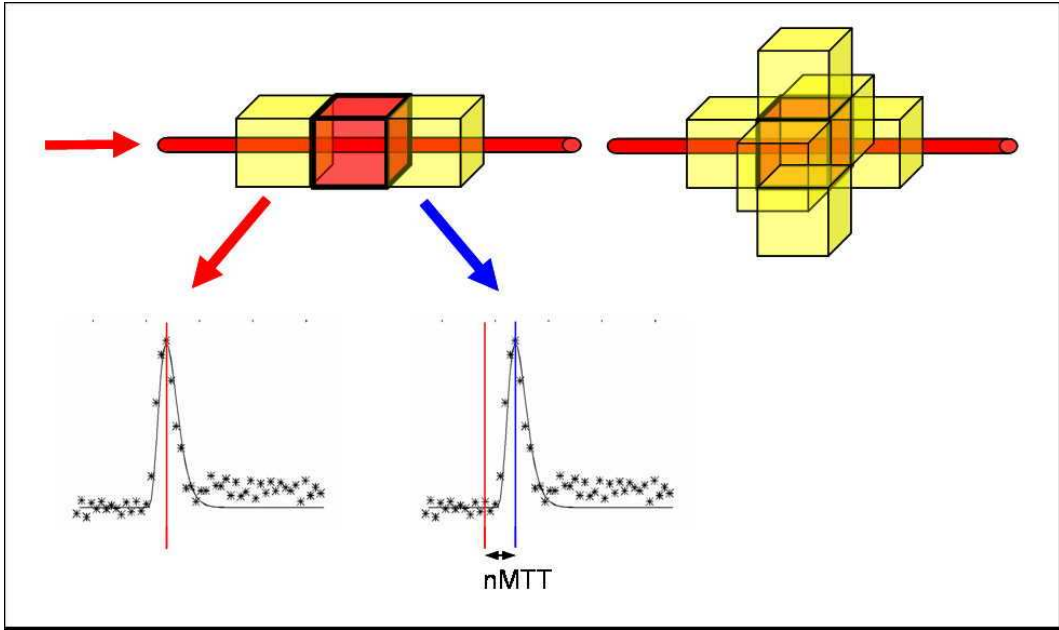


Figure 4:

This technique uses accurate measures of TTM from each of the six adjacent voxels surrounding the measurement voxel (Figure 4). The spatial derivative of these values will represent the mean time taken for a contrast molecule to transit the measurement voxel assuming that the flow is directional in nature. If the flow in a voxel is truly random then the measurement will be consistent with zero except for the effects of noise in the data. We have called this measurement the net mean transit time (nMTT) to distinguish it from MTT estimates derived from measurements of bolus broadening. In practice measurable values of nMTT have been observed in all grey and white matter voxels of the brains of normal volunteers. The mean apparent velocities of blood flow in grey and white matter in 12 normal volunteers were  $0.25 \pm 0.013$  cm/s and  $0.21 \pm 0.014$  cm/s respectively [32]. The nMTT can be calculated from these velocities and will vary linearly with the voxel size.

**Requirement 6. The MRI Data Must Result Purely From Contrast Within The Capillary Beds**

In practice many workers now routinely use, and recommend the use of gradient echo images where there is no reason to believe that the MRI signal does result purely from contrast in the capillary beds [27, 30]. Even in spin echo images the technique relies on the routine identification of an AIF from a large vessel illustrating the fact that large vessel signals are present even if they contribute proportionally less to signal than voxels containing only capillaries. Similarly it can be seen from parametric maps obtained using standard techniques that areas with large vessels are not represented by fit failures or data voids as would be expected but rather are indistinguishable from the surrounding brain [27, 30].

A scatterplot of the pixel-by-pixel values of obtained using T1 and T2\* weighted methods is given in figure 5, enabling the comparison of the two sequences. The square-root has been taken, as this measure renders the error on the uniform and also expands the dynamic range of the data. The data from the two volume acquisitions have been coregistered using an affine linear transformation, in order to give equivalent voxel positions. The susceptibility effect of the contrast when using T2\* weighted sequences leads to a degree of distortion in the resulting images and there is also some non-rigidity between the two volumes. In order to obtain the best match between the two sets of data, we apply a pixel shuffle technique: we take the most similar value from the T1 map to the T2\* map corresponding to a half pixel linear interpolation in one of four directions (up, down, left, right). The scatterplot shows several important points. Except at the very lowest CBV, there is a linear relationship between the T1 and T2\* values. The T2\* weighted acquisition is more sensitive to data at lower CBV than the T1, hence the tailing off at low CBV for the T1. The most important point is that the large vessels (grey and white matter are typically less than 0.27) do contribute to signal in both T1 and T2\* images, indicating again that the MR data does not arise from purely capillary-based contrast. The data also illustrates the validity of the CBV estimation process for both T1 and T2\* based methods.

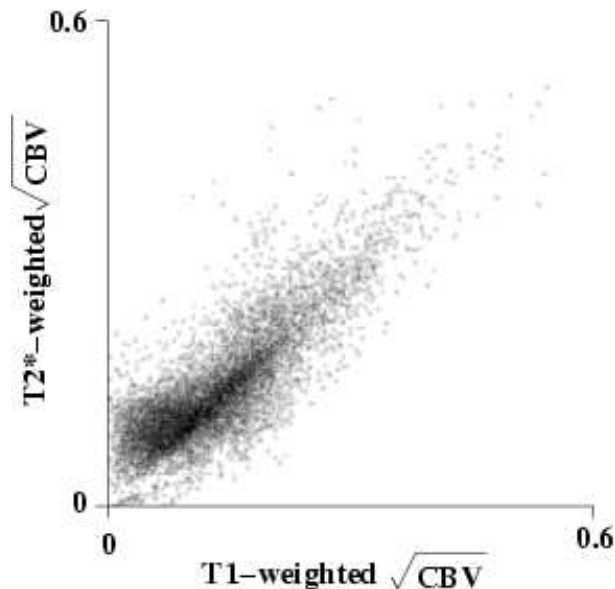


Figure 5: Scatterplot of  $T2^*$  vs  $T1$  for an identical coregistered slice from each dataset. The  $T1$  data has undergone a pixel shuffle technique (see main text) in order to best match the two datasets, as the  $T2^*$  susceptibility effect will result in some distortion in the  $T2^*$  data.

## 5 An Alternative Physiological Model

The inherent problems in the standard approach to the analysis of DSC-MRI arise from the underlying assumption that all flow in capillary beds is entirely random in direction. This constrains us to using analysis of the voxel residence time to characterise the flow rate across the voxel. In fact as we have demonstrated there is a significant measurable directional flow present in voxels composed entirely of grey and white matter. We postulate an alternative physiological model in which flow through capillary beds can be considered as a mixture of directional and random flow. Using the method described above we can estimate  $nMTT$  in any voxel (Fig 4). Since we can also estimate  $CBV$  we can directly calculate the directional component of the blood flow in any voxel ( $nCBF$ ). For the reasons described above this technique will leave us unable to estimate the true random component of flow and may therefore represent a variable component of the overall flow through the voxel. In order to assess the size of the directional flow component we have previously derived predicted values for flow velocity and  $nMTT$  in the normal brain based on an assumption that all flow in the voxel is directional [32]. The purpose of this model is to provide an estimate of the maximum values of flow velocity which might be expected if the random flow component were negligible [33, 32]. This model, based on parameters derived from non-MRI based studies produces estimated maximal values of blood flow velocity of  $0.25\text{cm/s}$  and  $0.18\text{cm/s}$  in grey and white matter. These compare to measured values of  $0.25 \pm 0.013\text{ cm/s}$  and  $0.21 \pm 0.014\text{ cm/s}$  seen using our direct measurement technique in the brains of 12 normal volunteers. These values give a measured  $nMTT$  for a  $1\text{mm}$  isotropic voxel of grey and white matter of  $0.45 \pm 0.12\text{ s}$  and  $0.52 \pm 0.11\text{ s}$  respectively compared to predicted model values of  $0.47\text{s}$  and  $0.55\text{s}$  [32].

## 6 Measuring Blood Flow Using the New Model

The ability to directly measure  $nMTT$  and  $CBV$  provides us with the information required to directly measure  $nCBF$  using direct application of the central volume theorem (vide supra). These estimates will be free from errors due to bolus delay and dispersion but will reflect only the directional component of flow. Any significant contribution from non-directional flow will not be seen. Figure 6 shows measurements taken from a series of 26 normal elderly volunteers. The  $nCBF$  calculated separately in  $\text{mls}/100\text{g}/\text{min}$  is plotted against the flow of blood into the head measured by phase contrast angiography in the carotid and basilar arteries and normalised to head size. There is a close correlation between these independent estimates of  $CBF$ . Values of grey matter  $nCBF$  are approximately twice those observed in the white matter in each individual. The values of  $nCBF$  in grey and white matter calculated using the new technique are consistent with those obtained using other modalities. Table 1 gives grey and white matter  $CBF$  values using ASL, PET and this new method. As can be seen, our data is comparable with the quite variable literature values. All these observations support the use of analysis techniques based on

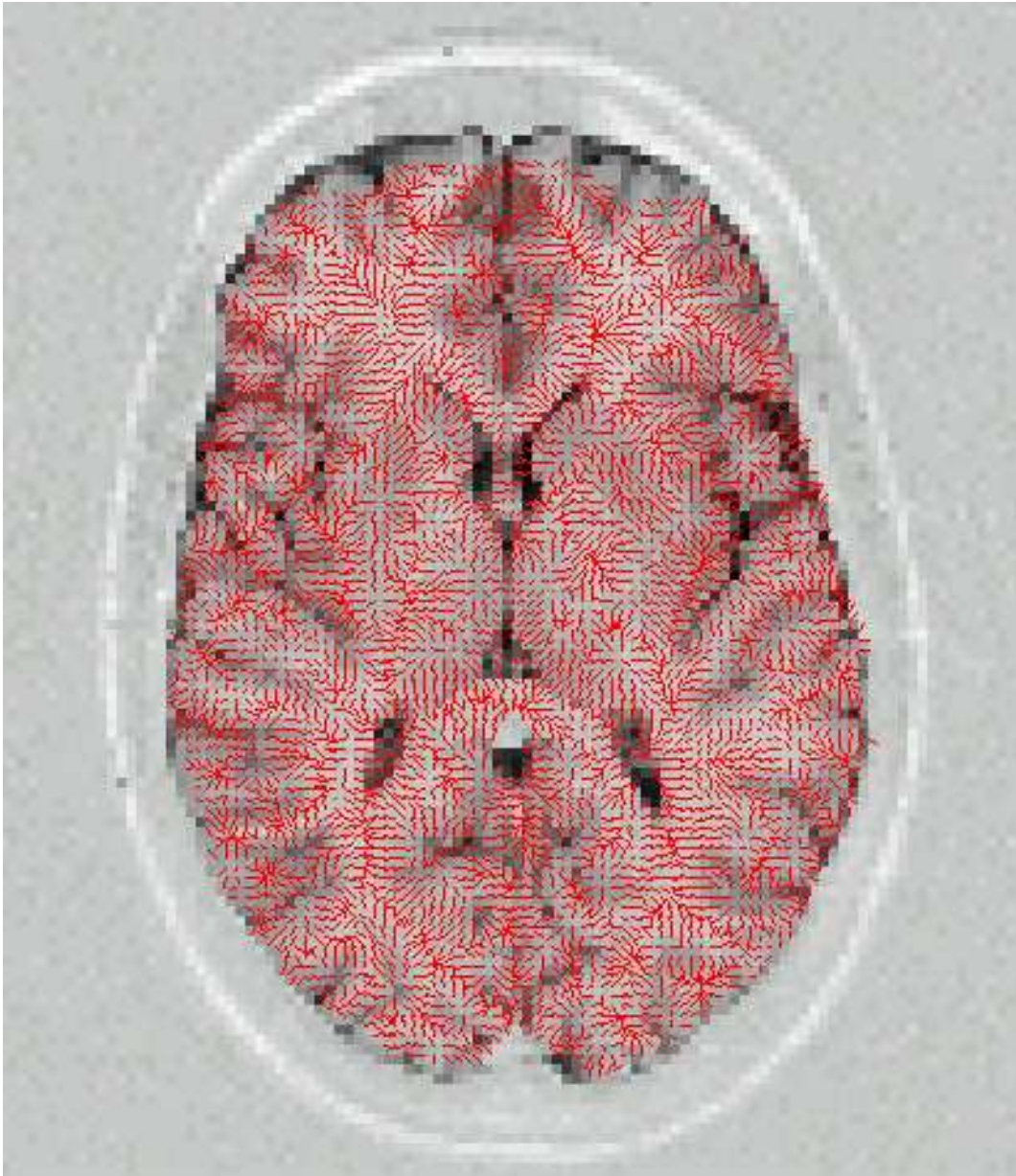


Figure 6: Section through the brain of a normal volunteer with a coloured overlay showing the predominant flow direction in the plane of the section for each voxel. It can be seen that the flow directions form patterns which conform to the known anatomical distribution of small vessels in the grey and white matter.

Study	Modality	Cerebral Blood Flow (ml/100g/min)	
		Grey Matter	White Matter
Watabe et al. 1996	PET	22.5-37.8	
Ostergaard et al. 1998	PET	33-50	10-30
Ye et al. 2000	PET	67±13	33±4
	ASL	64±12	23±8
Francis et al. 1999	ASL	87±9	23±5
Kim & Tsekos 1997	ASL	71±15	
Present Study	MRI	45.3±15.2	22.8±8.1

Table 1: Typical CBF values obtained using PET and ASL in Normal volunteers. Data is given either as a mean standard deviation, or as a range.

the assumption of directional blood flow as an alternative to traditional bolus width based techniques.

Another feature of this analysis approach is that the technique provides estimates of both flow velocity and flow

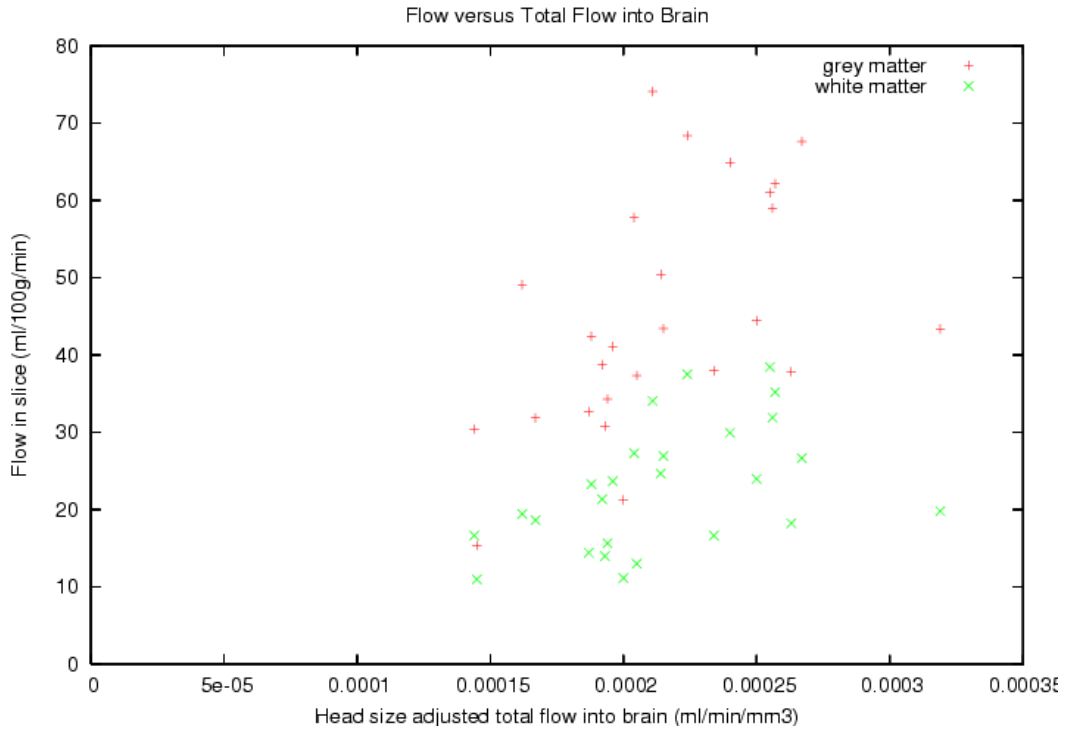


Figure 7: Plot of nCBF (mls/100g/min) for grey and white matter for 26 elderly normals, against the flow of blood into the head measured by phase contrast angiography in the carotid and basilar arteries and adjusted for head size. There is a close correlation between these independent estimates of CBF and values of grey matter nCBF are approximately twice those observed in the white matter in each individual.

direction from each voxel [32]. Figure 7 shows a section through the brain of a normal volunteer with a coloured overlay showing the net flow direction for each voxel in the plane of the section. It can be seen that the flow directions form patterns which conform to the known anatomical distribution of small vessels in the grey and white matter. In particular, the estimated flow vectors appear to be perpendicular to the grey-white matter interface.

## 7 Practicalities of Measuring nCBF

The use of direct local measurements of nMTT and calculation of nCBF appears to provide an attractive alternative analysis approach for DSC-MRI data. However, it must be appreciated that the technique places significant additional demands on both the image acquisition and analysis technique. Since the measurement of blood flow in the voxel is a vector it is essential that the voxels should be as near isotropic (ie; cubic) as possible in order to minimise statistical errors, but a scale factor correction can be made to give the equivalent nMTT that would be seen in an isotropic voxel. The typical data acquisition used for DSC-MRI, which will most often be an echo planar single slice technique, is far from isotropic, with typical voxel dimensions of 3mm x 3mm in plane and up to 10mm through plane. The reduction in voxel volume will of course have an inevitable detrimental effect on signal to noise ratio which will affect the accuracy of curve fitting used for analysis. In addition the technique requires imaging the six adjacent voxels of every measurement voxel. This means that slices must be contiguous and that data from the pixels at the edges of the volume will not be analysable. More worryingly the technique relies upon accurate estimates of TTM from all of the surrounding voxels so that a single inaccurate fit will adversely affect estimates of nCBF in all six of the voxels on its orthogonal boundaries. In addition to these constraints the imaging sequence must be heavily T2 or T2\* weighted, requiring a long TE, but must have a sufficiently short temporal resolution (< 2 seconds) to capture the behaviour of the short lived contrast bolus as it passes through the tissues. We have addressed this with the use of an echo shifted, gradient echo, echo planar volume acquisition (PRESTO [16, 17]). This technique gives coverage of the whole forebrain with 3mm isotropic voxels and adequate signal to noise ratio for analysis [33, 31, 32].

Another potential difficulty with this analysis approach is the required precision of the TTM estimates. The nMTT through a typical 3mm<sup>3</sup> voxel of grey matter using the PRESTO sequence is approximately 1.5 seconds and if

we wish to accurately detect changes of 10-20% then we need a fitting accuracy for TTM of the order of 0.15-0.3 seconds. This is demanding and needs careful optimisation of the fitting procedures used for TTM estimation. However, if we consider a large artery with a flow rate of 60 cm/s then the nMTT in a single voxel will be in the order of 0.005 seconds and would need a fitting accuracy of 0.0005-0.001 seconds. This is clearly impossible and so the effective errors in the estimates of nCBF will be acceptable in areas of slow flow (capillaries) but will invalidate measurements made in large vessels. Fortunately the effective statistical error in each voxel can be estimated and measurements limited to grey and white matter voxels where accuracy is acceptable. These limitations highlight the need for improved data quality for this technique. In the future it is expected that the use of high field systems, high osmolarity contrast agents and parallel coil imaging techniques such as SENSE will all support appropriate improvements in data quality.

## 8 Discussion

This paper focuses on the selection of an appropriate physiological model of cerebral blood flow to underpin the analysis of DSC-MRI data. There is little doubt that DSC-MRI can provide clinically valuable information in a number of disorders. Measurements of CBV provide valuable information concerning angiogenesis and microvascular structure in neoplastic and inflammatory disease. Measurements of arrival time parameters and bolus width are sensitive indicators of change in regional perfusion pressure and the presence of local vascular occlusions or compensatory vasodilatation. Despite these clear clinical benefits significant problems relating to the quantification of CBF remain [2, 33, 32, 5, 6, 8]. The currently used method was designed to cope with a flow process which was perceived to be primarily random in nature necessitating analysis of the contrast residue function to derive estimates of MTT [25, 34]. This model requires acceptance of a number of assumptions concerning the patterns of cerebral blood flow which can be shown by modelling and observational studies to be incorrect and to significantly impact the accuracy of the resulting estimates of CBF. In particular, bolus width appears not to convey the information necessary for the estimation of local velocity via deconvolution. The consideration of an alternative physiological model based on directional flow is not new and was the basis of early MR perfusion techniques designed to measure intravoxel coherent motion [36, 35, 37].

The suggestion that a local net flow process is visible in the brain goes against the most strongly held assumption that has been made in this field for the last ten years. The experiments which we have reviewed here clearly demonstrate that a measurable directional flow component exists and can be measured not only in large vessels but also in the capillary beds of grey and white matter. A directional flow based model is supported by observations from intra-vital capillaroscopy where passage of blood through the cerebral microvasculature is observed directly using confocal microscopic techniques. These studies show that the capillary bed in grey matter consists of arteriolar capillaries that feed into approximately twice the number of true capillaries, which are drained in turn by a reduced number of venous capillaries. True capillaries have heterogeneous flow lengths between 150 and 500 microns and red blood cell transit times of 100-300ms [13]. Flows in arteriolar and venous capillaries are at least twice this speed [13] and “non-autoregulating thoroughfare capillaries”, which represent non-exchange thoroughfare routes, have also been described [12]. This structure supports the rapid delivery of blood to the exchange capillaries and is entirely in keeping with the behaviour of the contrast bolus observed using DSC-MRI.

Based on the observations we have made the pattern of cerebral blood flow appears to be best modelled as a series of voxels representing capillary beds of grey and white matter. Each of these will contain arteriolar, venous and transit capillaries which exhibit directional flow. They will also contain true transfer capillaries whose flow pattern is truly random. Each of these voxels is fed by an arterial input whose timing will be governed principally by anatomical location and specifically by the length of the inflow path. The contrast arrival times of these voxels is tremendously variable even in normal individuals and the bolus width will vary linearly with the age of the bolus. Some capillary voxels (ie frontal cortex) will have given rise to venous outflow whilst others have still not received arterial input (ie deep periventricular white matter). The need to identify an AIF for deconvolutional analysis of DSC-MRI forces the use of inappropriate surrogate AIFs which invalidate the measurements of CBF on a voxel by voxel basis.

The adoption of a directional flow model allows the development of novel approaches for the measurement of nCBF. Direct estimation of local derivatives allows the measurement of the flow rate and direction of flow in each voxel as an independent assessment. This frees the analysis from all of the problems associated with the need to identify an appropriate AIF and the presence of bolus dispersion and delay. This technique will not reflect any random flow component in the data. However close agreement is seen between the measurements of nMTT obtained in normal subjects and those derived from a physiological model based on directional flow. This suggests that the random flow component is small at the spatial scale of the individual voxel and that the flow process is dominated by directional flow contributions. This is in agreement with the capillaroscopy data described above which shows

that a true transfer capillary has a typical pathlength far smaller than the dimensions of a typical voxel and is supplied and drained by vessels with a directional flow. Thus the contribution of truly random flow will be limited to those transfer capillaries which cross the boundaries of the voxel. Furthermore, assuming the path of these transfer capillaries is truly random then the contributions to individual voxel flow will cancel out, as inflow due to random flow will equal outflow due to random flow.

The major advantage of the new technique is the ability to derive measurements based entirely on local spatial derivatives. This means that the method should be immune to variations in the vascular tree produced by pathological processes such as Moyamoya syndrome where conventional methods are unable to provide estimates of CBF [6].

The findings of these studies are also surprising since the currently accepted methods for DSC-MRI imaging have been shown to produce estimates of CBF that correlate as expected in different tissues and in pathological conditions [20, 21, 28]. To some extent this may be explained by a combination of factors including the correlations between rCBF and rCBV that exist in normal tissues [10]. In addition, a model of cerebral perfusion with a net flow component would actually predict that bolus broadening would correlate with local flow velocity thus producing qualitative changes in estimated flow with the correct sign. The local dephasing of contrast arrival which increases bolus width might also be expected to be proportional to flow velocity. However, this is very different to saying that the method is quantitative and we would expect that it would be inappropriate to make direct comparisons between flow values obtained over different local areas and between equivalent areas in different subjects. It would seem sensible to us however, to monitor changes in bolus width over time, in a fixed region of a single subject .

Although deconvolution based flow estimates made over large regions may correlated with alternative methods, the MR data clearly displays features in individual voxels which contradict the characteristics required for reliable CBF estimation. If such estimates of CBF truly depend on such coincidental relationships then this must cause considerable concern about their application in diseases where these relationships might no longer hold true. It is particularly worrying that software implementing the currently accepted MR methods is now routinely packaged and sold with CT scanners and used clinically. We would expect that the characteristics of the CT data will also suffer the same problems, detailed here, as the MR data.

Although the approach described here appears extremely promising as a routine method for the quantification of CBF from DSC-MRI, it is not without difficulties. The need for (near) isotropic voxels, large imaging volumes, high temporal resolution, heavy T2 or T2\* weighting and good signal to noise ratio place considerable demands on the imaging sequence. The sensitivity of the analysis technique to errors in estimates of TTM and to inappropriate or failed fits makes designing the analysis algorithms equally challenging. The extreme shortening of nMTT in large vessels makes measurement of nCBF impractical except in capillary beds. Despite these problems we feel that the use of a directional flow model represents one of the most exciting developments in cerebral DSC-MRI since its original description 15 years ago [26].

## 9 Acknowledgements

The Authors would like to thank Hamied Haroon for Figure 5 and the data used in its creation.

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## NeuroImage Reviewers Comments

This section contains the reviewers comments received when this paper was submitted to NeuroImage (in italics), together with the authors' responses (in normal text).

### Letter to the Editor

Dr. N.A.Thacker  
Tel: 0161 275 5147.

1/6/2004

RE: Measuring Cerebral Blood Flow using Dynamic Susceptibility Contrast Enhanced MRI, M.J.Scott et al., NIMG-04-268.

Dear Sirs,

Thank you very much for reviewing our paper for Neuroimage Comments and Controversies. **We accept entirely your decision to reject our paper and this letter is not intended to suggest that you change it.** The reviewers comments received were not entirely unexpected as we have had similar reactions previously, indeed it has been a history of such responses which had led us to submit the work to you.

Invariably, we have found that our contemporaries are very familiar with the standard approach to DSC-MRI perfusion analysis and long ago accepted all of the assumptions involved and conclusions which follow. They then have difficulty making the switch to a new biological model. As is the case with the review comments here, our work is continually implicitly evaluated on the basis of the existing assumptions, including not only the biological data (which such techniques are intended to investigate), but how the calculation should proceed and the interpretation of analysis results. Any deviation from the accepted conclusions arising from these assumptions is criticised and even when these assumptions are pointed out they are refuted. In fact if you change the basic assumptions even slightly all currently accepted results inevitably unravel. Good science needs to continually re-assess the assumptions made, and this must start by understanding what they are, this was the main motivation for our article.

**Clearly, we do not expect you to change your decision.** In the interest of science, however, we would very much appreciate it if you would forward the following responses to the reviewers. We feel that there are many issues which need explaining to them in order to minimise the possibility of future misrepresentation of our work. I hope you do not consider this request to be too unreasonable and think as we do, that the reviewers may even be interested in how we would respond to their critique. We are aware however, that they may not be interested in taking this debate further and do not expect a response.

If you are unprepared to pass these comments on we would fully understand but then alternatively we would like to ask your advice regarding what steps we should take (regarding permissions) in order to make these comments (any any future correspondence) public on our web site. We intend to make further efforts to stimulate what we see as an essential need for a debate in the area. These review comments provide quite a concrete start for what we see as the problem.

Yours faithfully

Dr. Neil Thacker (Senior Lecturer in Neuro-Imaging Physics)  
Ms. Marietta Scott (Research Associate)

# Response to Reviewers Comments

We would like to start by thanking the reviewers for taking the time to review this paper and will address all of the technical points individually. We assume that both reviewers are happy to share their comments (in italics below) with each other.

## Reviewer 1

*Synopsis: The prevailing theory for interpretation of DSC MRI is critically assessed and found inadequate and an alternative approach to quantification of such data based on a bulk flow model is proposed. Results, mostly from a prior publication, are presented in support of this new analysis framework.*

*Critique:*

*Major concerns*

*1. Much of this work is a restatement and reproduction of text and figures in the paper Thacker et al. JMRI 17:241-255(2003). This certainly raises copyright issues that must be addressed but also brings into question the originality of much of the work. As the Comments and Controversies article type is not clearly defined in terms of originality, I'll leave this issue to the editors.*

It is true that sections of this paper are identical to our previous publication and that the data we included had not been changed. This was because we expected Comments and Controversies to be a forum for presenting difficult issues, not a standard section with the usual restrictions on content. We thought the existing sections of the previous paper were most appropriate and acceptable and did not see the need to rewrite them. **With hindsight we can see this was a mistake and would accept rejection entirely on this issue.**

The novel sections of the current article are the discussion of assumptions which had been specifically left out of our previous publication in order to avoid overmuch controversy. We are well aware that in order for people to understand our approach they would need to be prepared to consider an alternative set of assumptions and to completely re-think the problem. It was our observation that this largely had not happened and that our work was being assessed on the basis of the standard set of assumptions (which we believe potentially flawed) which led us to consider sending this version of the paper to Neuroimage in order to stimulate what we see as a required debate.

*2. I disagree with a number of the criticisms of central volume DSC analysis raised by the authors.*

*a. I don't interpret the TTM vs. bolus width in figure 2 as the authors do. Arteries should have short arrival times and more narrow boluses, veins should have the longest arrival times and the broadest boluses because the bolus has been broadened by passage through the tissue and the tissue should have intermediate arrival time and bolus width more like veins. The use of TTM is confusing since broadening of the bolus increases TTM for constant arrival time and hence the correlation between TTM and bolus width is not at all unexpected just for that reason.*

There are two main points here one concerns how we measure an arrival time point the other the assumed biological model. The use of Time to Mean (TTM) and the reasons for using it are fundamental to understanding our approach to characterising cerebral flow. Although it is often quoted that T0 is unaffected by bolus broadening, this is measuring only the arrival of the fastest contrast molecule at the voxel. In order to characterise the entire distribution the mean is the appropriate quantity to use. The analysis you provide for the interpretation of correlation between bolus broadening and arrival suggests that that you do not think that the contrast agent reflects the distribution of delay times in blood constituents on arrival at the voxel.

Redefining the arrival time variable does not remove the observed correlation with bolus width. As the marginal distribution of the bolus width distribution is fixed, changing the arrival variable to a correlated one produces a correlation only of different slope. It does not remove the observed dependency. Try it.

Otherwise, we agree entirely with your description of the way that delay times are generated by biological structure and how this affect observed bolus width and do not think that we had said anything to suggest differently. We have only tried to characterise what others are (or appear to be) assuming when they apply the standard analysis techniques.

*b. I completely disagree with requirement 5. Delay is not a problem for central volume analysis and only weaknesses in certain deconvolution algorithms have suggested it can be. Only dispersion is a problem. There is also no requirement on the directionality of flow in the central volume theorem. What the central volume theorem does produce is a measure of all flow through the voxel so if the flow is directional in large vessels, this flow is not perfusion as can be seen by the bright intensity of arteries in central volume theorem type analyses.*

It is the implicit analysis of regions that we were attempting to describe in this section, and in particular that the observed bolus width will change if the region is composed of multiple regions of different arrival time. The numerical problems associated with specific forms of analysis approaches is a separate issue with which we are familiar. The assumption regarding geometry is at the heart of the computation process in the standard technique and is described in more detail below in response to the second reviewer, who has also implicitly made this assumption while assessing the validity of our work.

*c. Requirement 6 is also incorrect. The only requirement for measuring true perfusion, instead of flow through the voxel, is that that all flow that enters the voxel then passes on down through to the capillaries.*

It is all a matter of how the MR measurement is affected by contrast. If the measurement is sensitive to contrast, regardless of the size of the vessel it is in, then the contrast molecule will have an effect on the voxel measurement as long as it is within the voxel. This is the voxel residence time. The measurement of “true perfusion” would seem to us to necessitate measurement only in capillaries. This can only be achieved if the signal is only modified while the contrast is within the capillaries, and is consistent with biological definitions of mean transit time. This has been directly stated by other authors in this area. When larger vessels are observed it is therefore common to make attempts to remove them. We expect that the reviewers would be entirely familiar with this practice.

*The presence of large vessels in DSC studies is clearly recognised and the bulk flow based analysis method does not appear to address this problem.*

At no time have we claimed that our new technique is expected to remove larger vessels, only that by accepting an alternative definition, such as net flow, the new method simply does not claim to do what it cannot. Indeed, we are happy to observe all blood volumes in order to characterise the entire flow process. A definition of net flow does not place restrictions on what types of vessels the blood is flowing through, in fact it requires that the technique is uniformly sensitive to the entire volume distribution. As it happens, latter statistical analysis stages have the effect of reducing the significance of large blood vessels for us when results are compared, which will be explained in a paper currently undergoing review.

*3. While central volume theorem analysis of DSC is not perfect, it attempts to extract a tissue specific indicator of perfusion or blood flow. The proposed analysis method really abandons all attempts at measuring tissue specific perfusion and instead defines a characteristic of bulk flow transport with an uncertain relationship to perfusion or any solid theory of flow transport. Because DSC is not a diffusible tracer, it is sensitive to effects throughout the vascular tree. Central volume theorem approaches may err by ignoring the larger vessels but the proposed approach really attempts to ignore perfusion and extract out bulk flow phenomena.*

The assertion that we have abandoned true perfusion is somewhat harsh but largely accurate. Having arrived at the conclusion that the standard method was too flawed to give quantitatively reliable results, we set about constructing a technique which would allow us to extract at least something of value from this potentially valuable source of data. We decided that quantitative measures which related directly to flow patterns around the brain would be of use. Of course it is true that we need to do a lot of research to interpret the data, but don't understand why this merits criticism. This would seem to imply that only measurements of “true perfusion” could be of any value. If we had trusted the standard approach we would have certainly used the available software or made use of our own (which we had already written and tested when we still thought the method might work). We would not have taken the time to develop another technique purely for the sake of it.

*Because the flow measure is based on magnitudes of spatial derivatives it is also likely to have major contributions from the presence of larger vessels, tissue heterogeneity, and even rectified noise. How would this measure relate to perfusion?*

Yes, we have systematically investigated these effects and characterised the resulting distributions. We have concluded that the data is quantitatively useful. Whatever the difficulty with the interpretation of what we are measuring in comparison to “true perfusion” we hope that you can see that quantitative differential time of arrival will contain useful spatial information regarding the delivery of blood across a given brain provided that a measurable signal is present.

*Why would it be reflective of tissue ischaemia in the presence of vascular pathology perhaps with collateral flow?*

Collateral flow within a region does complicate a simple understanding of the approach. The TTM measurement will be potentially drastically altered by changing the proportions of blood delivery. However, the use of a differential MTT for the calculation of local flow velocity reduces the significance of the problem in our approach to a second order effect. Firstly, you should be able to see that provided two adjacent voxels in the direction of net flow are fed from the same direction gross contributions from collateral flow cancel in this calculation to give an appropriate flow result. For blood arriving in a region from completely different directions (and therefore reducing the observed net flow by definition), a fixed proportional change in flow velocity across the brain will still be observed as the correct proportional change in our measurement, because of the use of mean arrival times in this calculation. This

is exactly the trade off between net-flow vs random flow described in the Thacker et al. 03 paper.

If anything, we believe that under these circumstances conventional approaches will be less quantitatively useful, as the AIF needed will be very different from that expected. In this case proportional changes in flow velocity are directly affected by the distribution of bolus arrival times and will not even reproduce proportional changes in measured flow. This was one of many motivations for developing the new technique.

*Critique summary: There are serious flaws in the arguments against central volume theorem analysis and in the "theory" underlying the bulk flow based analysis advocated in this manuscript.*

We hope you can understand our approach and interpretation now based upon the above clarifications, but some of the responses given to the second reviewer may also help with these points.

*Because the ideas and even many of the figures of this work have been previously published, the primary effect of publication of this additional manuscript would be to provide additional attention and exposure that I do not feel the work merits. I recommend against publication.*

Many people working in the MR field will not work in the area of perfusion measurement because they have also concluded that current techniques do not work. When asked, these people identify, among other things, subsets of the problems we are attempting to highlight. Our approach is much more in line with ideas of those people developing new techniques in the area of spin inflow labelling. As such people will not write any papers in the DSCE MRI area, as they feel they have nothing constructive to say, there is already a very strong bias towards the status-quo.

## Reviewer 2

*The manuscript by Scott et al presents a review of the standard approach to quantify dynamic susceptibility contrast enhanced MRI data (i.e. by deconvolution of the arterial input function). The manuscript reviews the various assumptions and requirements of the standard approach, and discusses their validity. The authors discuss the alternative physiological model they introduced in a previous article (Thacker et al 2003), and discuss the use of this approach to quantify the directional component of CBF. The manuscript has been submitted under the category of "Comments and Controversies".*

*Although the manuscript discusses an interesting subject, the current format and content does not seem appropriate. The manuscript includes a long review of the standard approach, and a description of the alternative method previously proposed by the authors. However, only a small proportion of the manuscript introduces or discusses issues that have not been previously presented and discussed by the authors (in particular, in the reference "Thacker et al 2003" in the manuscript) or by others. In fact, large parts of the manuscript (in the Introduction, as well as in the Discussion) are copied word for word from their previous article, and several figures are reproduced from that article (without even including acknowledgment of this fact). I do not feel that the manuscript, in its current form, adds sufficient information to their previous article. Therefore, the manuscript could be significantly shortened (to approximately 30% of the current length), without including unnecessary repetition of the issues presented and discussed in previous articles, and with a clear discussion of the key and novel issues that are brought in this manuscript.*

Fair opinion. See above. We did feel however, that spreading all of the required information across multiple papers would just lead to more confusion.

*Alternatively, and more interestingly, the authors should include in vivo data for a study or application in which their alternative approach provides improved (or at least complementary) information compared with the standard approach. Otherwise, the manuscript does not seem to provide enough further discussion to that included in their previous work.*

We have recently submitted an in-vivo paper to MRM and received very similar reviewers comments regarding "theory" to those here, blocking publication. This is why we decided we needed to try to get people to re-examine their assumptions.

As the two theories are based upon contradictory assumptions they cannot be expected to produce either equivalent or complementary results. If they agreed at all it could be argued this was chance and you cannot use disagreement to refute either method. If people had published sufficient details regarding the geometries they use, a comparison may have been possible (see below).

*Furthermore, two main points are included in the manuscript to support the directional CBF approach: the results from figure 7 (comparison with phase contrast angiography measurements) and table 1 (comparison with previously published CBF values from other methodologies). Although the authors claimed a close correlation for the data in figure 7, there seems to be a lot of scatter. The agreement of the nCBF measurements with those quoted in the table*

*is not surprising considering the large range of 20-90 ml/100g/min for gray matter CBF in table 1. On the other hand, if the nMTT is 0.5 sec (i.e. approximately 8 times shorter than the MTT calculated using the standard model), how is it possible that the standard CBV measurement gives an nCBF of 45 ml/100g/min (table 1) using the central volume theorem? (i.e. why isn't the nCBF 8 times larger than the common CBF measured using the standard model?).*

The question of why two methods giving different values at one stage of the calculation for apparently equivalent measurements can later give similar results is at the heart of the issue and a common misunderstanding of our work. With a net flow model, in order to convert a flow measurement from a voxel to one of a mass of tissue of 100g with equivalent flow we must scale the measured voxel net flow by the conventional  $v^{2/3}$  dependency. This approach gives the results we have published without any need for further scaling of the data. For the standard approaches which assume no net flow, MTT is assumed to be volume independent (as is stated in our paper but refuted by the first reviewer) and, as RCBV is a dimensionless quantity the calculation of perfusion flow proceeds without any apparent need for volumetric scaling. If the standard model assumptions regarding the biological flow process are correct then our method would measure nothing but sensor noise. On the other hand, if our biological model is correct then there will be a distribution of arrival times within a region and the standard approach will show equivalent measures of perfusion for regions corresponding to approximately 100g of tissue. The fact that we observe a measurable net flow process means that these delay distributions are present at the required level. Unfortunately, as the standard approach in this area implicitly assumes there is no volumetric dependency, researchers do not feel it necessary to publish the details of the volume analysed. It is therefore impossible for us to carry the comparison further. Only someone who currently publishes using these techniques could tell us specifically what they have done regarding geometry. We can say however, that it is also common in the standard analysis to rescale the measurements to a known tissue, thereby ensuring that computed values must lie in the range of those expected.

Although this may look like we can justify the existing approaches using our biological model and fix things by making a few simple modifications to the existing calculation we do not think so. In fact we see the problems presented by lack of knowledge of an appropriate input function for each region as an insurmountable barrier to quantitative analysis.

*Although I have many comments regarding the manuscript, I do not include a list in this report since, as mentioned before, I do not consider the current content and format of the manuscript appropriate.*

As we have said in the covering letter, once the assumptions are changed many conclusions that people are familiar with are affected. I'm sure that the many additional comments you have on the theory are unlikely to be ones we have not considered and discussed with others, and we have generally had reasonable success (given enough time) explaining these matters. However, there is a limit to the number of people that can be influenced when the ideas are not published and we have had a particular problem with researchers who already use the existing techniques.

Some guidelines as to what you would consider appropriate for this kind of article would have been gratefully received.