

## Voxel Segmentation-Based Partial Volume Correction using FSL: Theory and Implementation

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### Abstract

The use of a relatively large and straight-edged voxel to acquire spectra from rather small and curved brain structures leads to data contamination in magnetic resonance spectroscopy (MRS), known as partial volume effect (PVE). PVE arises due to the inclusion of cerebrospinal fluid (CSF) in the voxel, leading to underestimation of metabolite concentrations in quantitative MRS. Among the PVE correction techniques available, voxel segmentation to remove the CSF fraction appears to be the most reproducible and reliable method. However, there has not been a comprehensive documented procedure of this technique to guide its implementation, particularly among scientists who may be interested in brain research using quantitative MRS, but lack the skill in the implementation of PVE correction, which is one of the most important corrections of the MR spectra in quantitative MRS. This paper details the procedure for PVE correction, using an example *in vivo* acquired spectra. A method to deal with tilted voxel placements is also suggested. Thus, this paper may serve as a step-by-step guide to the implementation of PVE correction in single-voxel MRS, by the voxel segmentation technique.

**Keywords:** MRS; FSL; Partial Volume Effect; FAST; Single-Voxel; Brain

### Abbreviations

3D: 3-Dimensional; AP: Anterior-Posterior Direction; BET: Brain Extraction Tool; CSF: Cerebrospinal Fluid; FAST: FMRIB's Automated Segmentation Tool; FMRIB: Functional MRI of the Brain Analysis Group; FSL: FMRIB's Software Library; GE: General Electric Company Ltd; GM: Grey Matter Tissue; MR: Magnetic Resonance; MRI: Magnetic Resonance Imaging; MRS: Magnetic Resonance Spectroscopy; PVE: Partial Volume Effect; RF: Radiofrequency; RL: Right-Left Direction; ROI: Region of Interest; SI: Superior-Inferior Direction;  $T_1$ : Longitudinal Relaxation Time Constant;  $T_2$ : Transverse Relaxation Time Constant; VOI: Volume of Interest; WM: White Matter Tissue

### Introduction

Magnetic Resonance Spectroscopy (MRS) voxels with straight edges are used to acquire spectra from brain regions that are usually small and curved. The usual practice therefore is to use relatively large voxel sizes which would, in most cases, inevitably encompass some fraction of cerebrospinal fluid (CSF); this is known as the partial volume (PV) effect. A method of removing the CSF fraction from the MRS voxel (i.e. PV correction) is thus described and implemented in this report.

In addition to metabolite signals normally acquired from a brain tissue mix (i.e., grey and white matter), the MR spectra may also include some CSF signal [1,2]. Therefore, if this CSF fraction is not removed, the metabolite concentrations will be underestimated [2]. For instance, for the same voxel size, metabolite signal intensities from a voxel containing 50% tissue and 50% CSF would be half of that acquired from a voxel containing 100% tissue [3].

Three PV correction techniques are reported in the literature: correction with grey matter fraction; bi-exponential fitting using multi-echo dataset; and segmentation of a structural MR image.

In the grey matter fraction technique [4-6], signals are acquired using a series of voxels with known, varying grey matter fractions. In a plot of signal intensity versus grey matter fraction, intensity values corresponding to grey matter fractions of 0 and 1 give the amounts of signal arising from white matter (WM) and grey matter (GM) respectively. The disadvantage of this technique is that it prolongs the acquisition time, and also the estimation of the fraction of grey matter in the voxel is likely to be subjective. Reproducibility of the measurements may therefore not be good.

The bi-exponential fitting technique distinguishes between signal from CSF and that from tissue water [2,7]. In its implementation, a multiecho spectral acquisition is performed and a bi-exponential fit of the  $T_2$ -decay can reveal long- $T_2$  and short- $T_2$  components. The long- $T_2$  decay is attributable to CSF and thus gives a measure of the amount of CSF in the voxel; the short- $T_2$  component is attributable to water trapped between myelin sheaths in brain tissue [7,8]. However, the  $T_2$ -decay in healthy brain is reported to exhibit three distinct pools of water: CSF with  $T_2 > 1000$  ms, intra/extra-cellular water with  $80 \text{ ms} < T_2 < 200 \text{ ms}$ , and myelin water with  $10 \text{ ms} < T_2 < 50 \text{ ms}$  [9]. The number of water pools could even be increased in pathology [10,11]. To correctly measure the  $T_2$ -decay of water, considerations to these 'pool effects' will have to be taken into account; a full water  $T_2$ -decay will also have to be acquired and fitted accurately. A non-negative least squares fitting method is usually applied [9,12,13], the accuracy of which depends on the number of plotted points. At least twelve time points yield reasonable goodness of fits [14]; this therefore means more acquisitions. Consequently, the measurement time may be clinically unacceptable [8], and could be affected by motion artefacts.

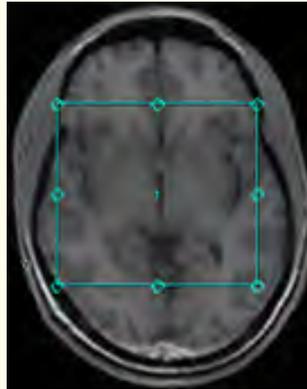
In the imaging-based CSF correction technique, a high resolution structural 3D MR image (usually  $T_1$ -weighted) is used to determine the proportions of CSF, grey and white matter in the voxel. The  $T_1$ -weighting gives CSF a low pixel intensity value relative to brain tissue. An image segmentation routine is then performed on the 3D image to segment it into CSF, grey and white matter fractions [2]. The CSF fraction can then be excluded from the voxel composition. The advantage of this technique is that it is implemented as a post-processing correction technique, and does not impact significantly on the MRS data acquisition time. It is thus not associated with subject tolerance issues. Moreover, movement of the subject between the MRS and 3D MRI acquisitions can be corrected for by co-registration of the 3D MRI to the structural MRI used for planning the MRS voxel [15]. The technique has fewer potential sources of error, and is therefore more reproducible and reliable.

Despite the advantages and relevance of the image segmentation-based PV correction technique in quantitative MR spectroscopy, there has not been a comprehensive documentation on the implementation procedure of the technique in the literature to guide MRS researchers who are unfamiliar with the PV correction technique. This paper therefore aims to provide a detailed and comprehensive procedure for single voxel localisation in high-resolution 3D MRI, and the subsequent segmentation of the voxel into CSF, grey and white matter fractions. This will then be followed by a method of elimination of the CSF fraction. Finally, a technique of dealing with tilted voxels is also suggested.

## Materials and Methods

MRS acquisition was conducted on one healthy male volunteer by prescribing a large voxel ( $AP \times RL \times SI = 92.0 \times 103.7 \times 20.0 \text{ mm}^3$ ) on an axial MRI of the volunteer's brain (Figure 1). This made it possible for the 3.0 T GE MR scanner to store a retrievable MRS header file containing the voxel coordinates. A high-resolution  $T_1$ -weighted 3D brain MRI was also acquired for segmentation.

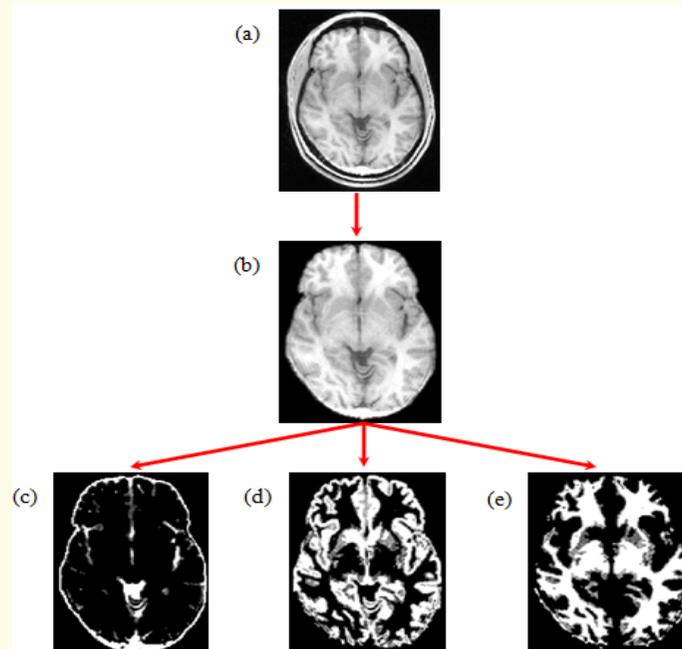
PV correction was then performed post-MRS acquisition, using the FMRIB's Software Library (FSL, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl) [16,17]) software (version v5.0). The PV correction technique involved four steps: segmentation of the 3D MRI, voxel localisation in each image segment, estimation of CSF, GM and WM fractions in the voxel, and elimination of the CSF fraction. Each step is described as follows.



**Figure 1:** Voxel placement in brain MRI during MRS acquisition.

### Segmentation of the 3D MRI

Brain tissue is extracted from the skull (Figure 2a-b) using the Brain Extraction Tool (BET) in FSL. The brain is then segmented into three tissue classes (Figure 2c-e) using the FMRIB's Automated Segmentation Tool (FAST) of FSL [18]. The segmentation routine also corrects the image for spatial intensity variations, also known as bias field or RF inhomogeneities. The FAST algorithm assigns intensity values (between 0 and 1) to all voxels of the image and then classifies these intensities as high, intermediate and low. Tissue classes 0, 1 and 2 have low, intermediate and high intensities, and are thus assigned to CSF (Figure 2c), grey matter (Figure 2d) and white matter (Figure 2e), respectively. The output images of the tissue classes are known as partial volume maps, whose intensity values represent the proportion of each tissue class in the voxels of the 3D MRI [18].



**Figure 2:** Implementation of the BET and FAST algorithms on the high-resolution  $T_1$ -weighted 3D brain MRI.

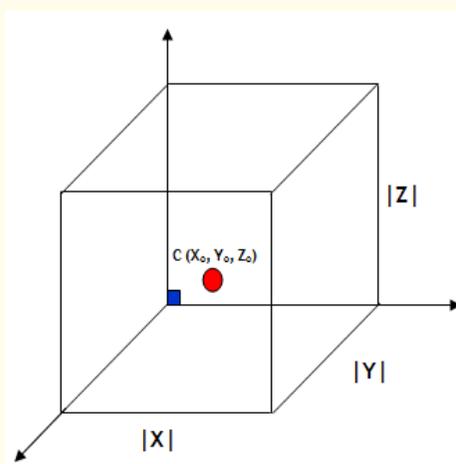
The brain extraction tool (BET) removes the skull (Figure 2a-b), while the FMRIB's Automated Segmentation Tool (FAST) segments the extracted brain into three tissue classes: CSF (Figure 2c), GM (Figure 2d) and WM (Figure 2e).

### Localisation of the voxel in the segmented brain MR images

This procedure involves two steps: determination of the coordinates at the vertices of the VOI, and then using these coordinates to generate the precise volume of the voxel. While the coordinates are calculated manually, the voxel volume is determined automatically by FSL when the coordinates are used as input values. These steps are detailed as follows.

### Calculation of the VOI coordinates

The MRS voxel is a region of interest defined in three-dimensional space. Thus, a three-coordinate space can be used to represent the three sides, LR x AP x SI of the voxel, where these sides correspond to the x, y and z axes, respectively (Figure 3). A voxel is normally specified in three (xyz) dimensions by three quantities: “starting values”, “dimensions” and “centre coordinates”. The starting values represent the coordinates of each corner of the voxel and the dimensions are the lengths of each side of the voxel; the centre coordinates define the centre of the voxel. These three quantities are related such that when any two of them are known, the third can be calculated.



**Figure 3:** A three-dimensional representation of the MRS voxel.

The small blue square represents the starting point for the RL side (along the x-axis) of the voxel; the small red circle is the centre, C of the voxel with coordinates  $X_o, Y_o, Z_o$ ; the dimensions of the three sides, RL, AP and SI of the voxel are respectively represented by  $|X|, |Y|$  and  $|Z|$ .

The dimensions of the voxel and their corresponding centre coordinates are usually stored in the MRS header file after every spectral acquisition. Therefore, the starting values for each side of the voxel can be calculated using the following equations for each side of the voxel:

$$X_{start} = X_o - (|X|/2); Y_{start} = Y_o - (|Y|/2); Z_{start} = Z_o - (|Z|/2) \quad \text{(Equation 1)}$$

These calculated starting values are usually predefined by the proprietary software of the GE MR scanner (SAGE) in scanner anatomical space (SAS). By this convention, a voxel side lies along, say, the R or L axis but not along the RL axis. Therefore, the voxel dimensions will normally be presented in SAGE, for example, as 20R x 20A x 20I and not as 20RL x 20AP x 20SI. It should be noted, however, that certain sign conventions apply to the relationships stated in equation 1:  $X_{start}$  and  $Y_o$  should be negated when the SAS labels are L and P, respectively. In this case, the negation of  $Y_o$  should be done before calculating  $Y_{start}$ . This is necessary due to the reversal of image orientation (and hence the voxel) when loading the MR image/voxel in the FSL program. The Z-axis serves as the mirror line for the reorientation and so its values are not negated.

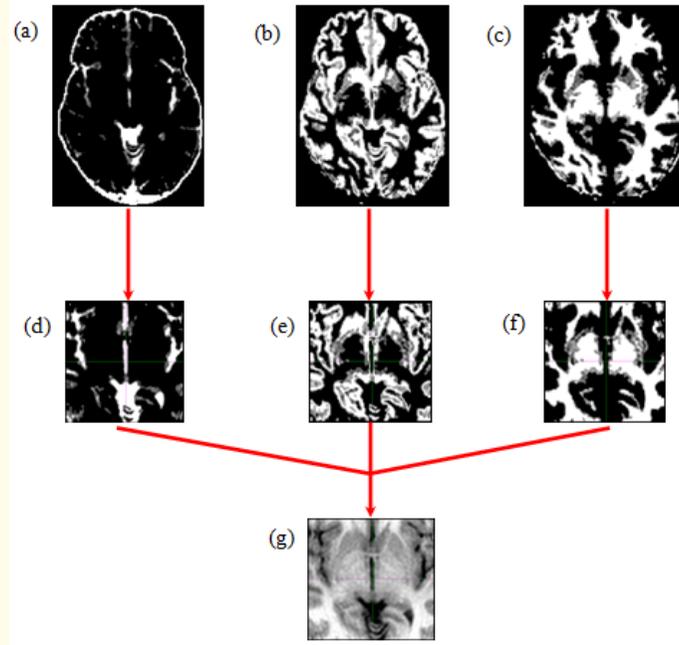
### Localisation of the 3-dimensional voxel in the 3D MRI

In order to localise the MRS voxel in its corresponding 3D MR image, the FSL program requires the starting values (determined previously) in the coordinate space (CS). The CS starting values are generated automatically by FSL when the calculated SAS starting values are entered in the program. Note should however be taken of the above sign conventions when entering the SAS starting values.

The voxel is then localised in, and ‘cut out’ from, the 3D MR image (Figure 4g) by running the following command on the image file of each tissue class in the command line:

`fslroi <input tissue class filename> <output voxel image filename> X_start |X| Y_start |Y| Z_start |Z|`

where  $X_{start}$ ,  $Y_{start}$  and  $Z_{start}$  are the coordinates of one of the vertices, and  $|X|$ ,  $|Y|$  and  $|Z|$  are respectively the dimensions in the RL, AP and SI axes of the voxel. Output voxel images are then generated and saved to a directory for each one of the CSF (Figure 4d), grey matter (Figure 4e), and white matter (Figure 4f) fractions.



**Figure 4:** Procedure for localisation of the voxel in the high-resolution  $T_1$ -weighted 3D MRI.

The whole brain MRI is segmented into CSF (Figure 4a), GM (Figure 4b), and WM (Figure 4c) fractions using the FAST algorithm of FSL, followed by the localisation of the voxel in each tissue segment (Figure 4d-f). Figure 4g results when the three voxel segments (Figure 4d-f) are put together; note how Figure 4g compares with the brain region encompassed by the large voxel in figure 1.

#### Estimation of fractions of CSF, GM and WM in the voxel

The volume of CSF, grey matter and white matter of each voxel image (in voxel units) is calculated as the product of the mean pixel intensity value of the voxel image and the volume of that tissue class in the voxel image. Estimation of the voxel content in this way is known as partial volume estimation, executed in the command line of FSL by the following lines of code on each one of the CSF, grey matter and white matter voxel images [18]:

```
vol=`$FSLDIR/bin/fslstats voxel image filename -V | awk '{print $1}'`
mean=`$FSLDIR/bin/fslstats voxel image filename -M`
tissuevol=`echo "$mean * $vol" | bc -l`
echo $tissuevol
```

This returns the tissue volume in voxel units, stored in the variable ‘tissuevol’. To get the volume in units of  $\text{mm}^3$ , {print \$1} is replaced with {print \$2} in the first line.

### Elimination of the CSF fraction

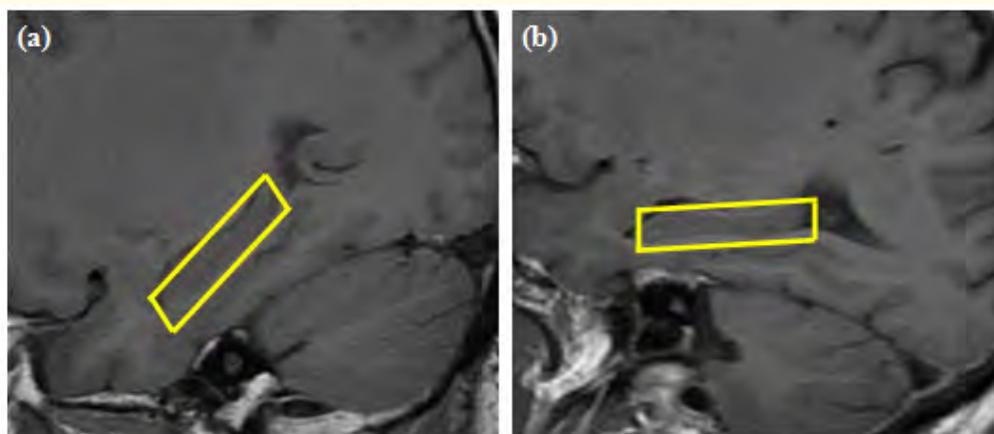
If  $v_{CSF}$ ,  $v_{GM}$  and  $v_{WM}$  are the FSL estimated voxel volumes of CSF, grey and white matter, respectively, then the tissue content of the voxel will be  $(v_{GM} + v_{WM})$  and the fractions of grey ( $f_{GM}$ ) and white ( $f_{WM}$ ) matter will be  $v_{GM}/(v_{GM} + v_{WM})$  and  $1 - f_{GM}$ , respectively. This calculation excludes  $v_{CSF}$  leaving behind only solid brain tissue (i.e. grey and white matter fractions).

### Results and Discussion

For the large voxel prescribed in the single volunteer shown in figure 1, the partial volume estimates in voxel units of CSF (Figure 4d), GM (Figure 4e) and WM (Figure 4f) were 16744 mm<sup>3</sup>, 90986 mm<sup>3</sup> and 127662 mm<sup>3</sup>, respectively. These represented 7%, 39% and 54% of CSF, GM and WM, respectively, in the voxel.

Excluding the CSF content of the voxel to correct for the partial volume effect, the voxel tissue content then had a total partial volume estimate of 218648 mm<sup>3</sup>, representing 93% (or a fraction of 0.93) of the voxel. This tissue fraction of the voxel was then made up of 42% (or 0.42) and 58% (or 0.58) of GM and WM, respectively.

The voxel localisation technique presented in this paper is accurate for non-oblique voxel placements. For oblique or tilted voxel placements, the orientation angle will have to be taken into account. In this case, a rotation matrix involving the voxel starting values and rotation angle would have to be used. However, if the scanner does not generate information on the orientation angle of the voxel in the MRS header files; and there is no straightforward way of obtaining such information, then the slices should be planned along the angle of the structure of interest prior to structural MRI for voxel localisation (Figure 5a-b). By doing this, the hippocampi, for instance, in both brain hemispheres can be reasonably horizontal so that there will be no need to tilt the MRS voxel to cover most of the length of the hippocampus. The partial volume correction technique presented in this paper can then be directly applied in such a case.



**Figure 5:** Sagittal MRI scans showing voxel placements on the hippocampus in a tilted orientation (a) and in a horizontal orientation (b).

### Conclusion

A method for partial volume correction using MR image segmentation of the MRS voxel has been presented. Though the voxel localisation technique described is not suitable for cases where oblique voxels are used, a method of dealing with this has been suggested in this paper. It was recommended that for brain structures like the hippocampus that may sometimes be tilted, the structural MRI for voxel localisation should be acquired with slices tilted to the region of interest so that the voxel can be prescribed in that region in a non-oblique orientation.

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## Conflict of Interest

The authors have no conflict of interest to declare.

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