

**ANALYSIS OF SEGMENTATION METHODS FOR PARTIAL VOLUME
CORRECTION IN MAGNETIC RESONANCE SPECTROSCOPY VOXELS**

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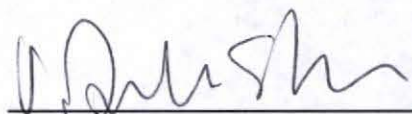
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LIST OF ABBREVIATIONS

CSF: Cerebral Spinal Fluid
GM: Gray Matter
WM: White Matter
SPM: Statistical Parametric Mapping
MT: Manual Trace
FgM: Frontal Gray Matter Location
FwR: Frontal White Matter Location
SPM_SS_DF: Images segmented by SPM, skull stripped template and images
FSLXX: Images segmented by FSL with the following options: N = no probability maps, I = initial a-prior, B = both a-priori, H = hard partial volume data, S = probability data output
DFXX: Images segmented by FSL with pre-processing (same options as FSL apply)
Cr: Creatine
Glu: Glutamate
ml: MyoInositol
Cho: Choline
NAA: N-acetyl Aspartate
Glx: Glutamate + Glutamine

CHAPTER 1

INTRODUCTION

1.1 MR Spectroscopy

MR spectroscopy is a method by which the interaction of matter with electromagnetic radiation is used to detect and study the chemical make-up of different types of materials and / or organisms. Using this interaction for the study of the brain, MR spectroscopy spectra can be used to determine metabolite concentrations in a certain targeted area. This volume is considered an MR spectroscopy voxel, which differ in locations depending on which part of the brain or other anatomic area is targeted. Figure 1.1.1 show a typical MR image with the frontal gray matter region (FgM) voxel region marked by a white 20 x 20 x 20 mm cube.

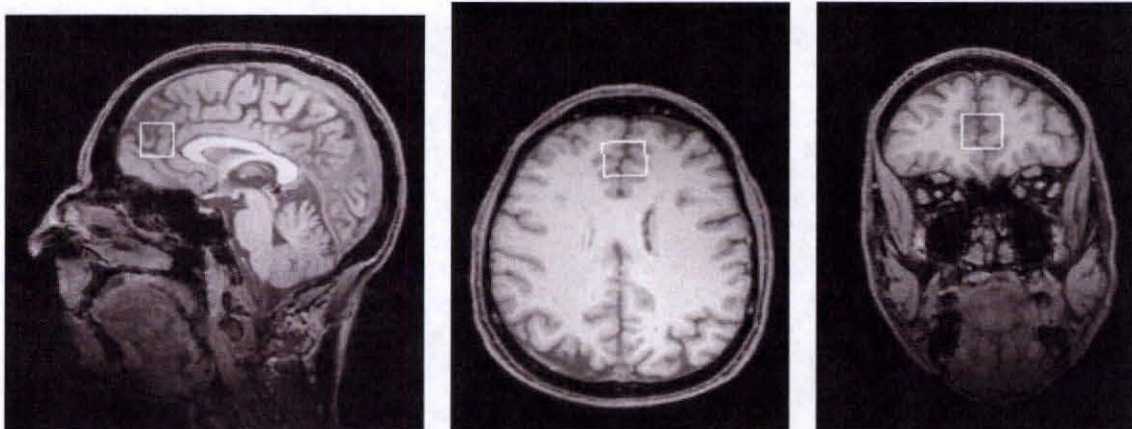


Figure 1.1.1: Typical FgM Voxel Location

In an MR spectroscopy spectrum, certain sequences of peaks and valleys may represent a certain metabolite, which concentrations may be calculated. Metabolites that are analyzed include N-acetyl acetate (NAA), creatine (Cr), choline (Clo),

glutamate (Glu), and myoinositol (mI). Figures 1.1.2 and 1.1.3 show metabolite peak locations on a spectrum. The brain is considered to consist of three major tissue types: cerebral spinal fluid (CSF), white matter (WM) and gray matter (GM). Since a typical MR spectroscopy voxel is 2 mL, all three tissues are included in a typical voxel. Certain locations in the brain may contain more of one type over another, for instance the FgM voxel shown in Figure 1.1.1 contains mostly GM. It is important to accurately determine the partial volume of each tissue type since concentration levels of metabolites may differ between tissue types. Figures 1.1.3 and 1.1.4 show some of the differences in metabolite concentrations based on the amount of GM or WM. Please note that figure 1.1.2 is from an FgM voxel (predominately GM) and 1.1.3 from an FwR voxel (predominately WM).

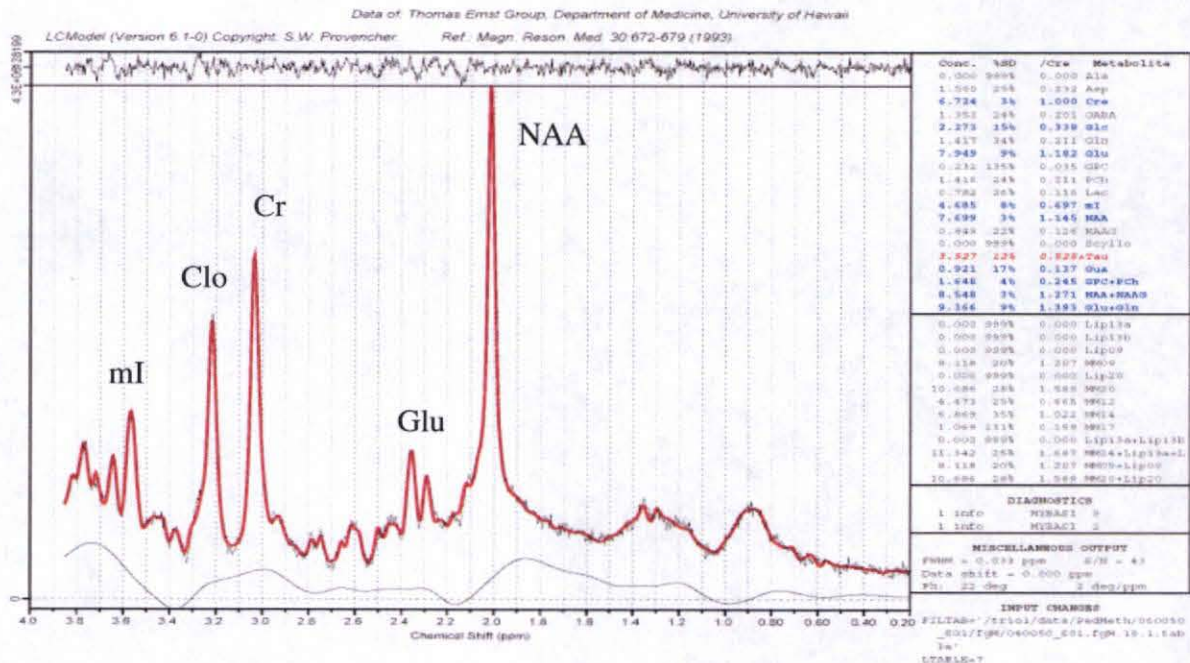


Figure 1.1.2: FgM Spectrum

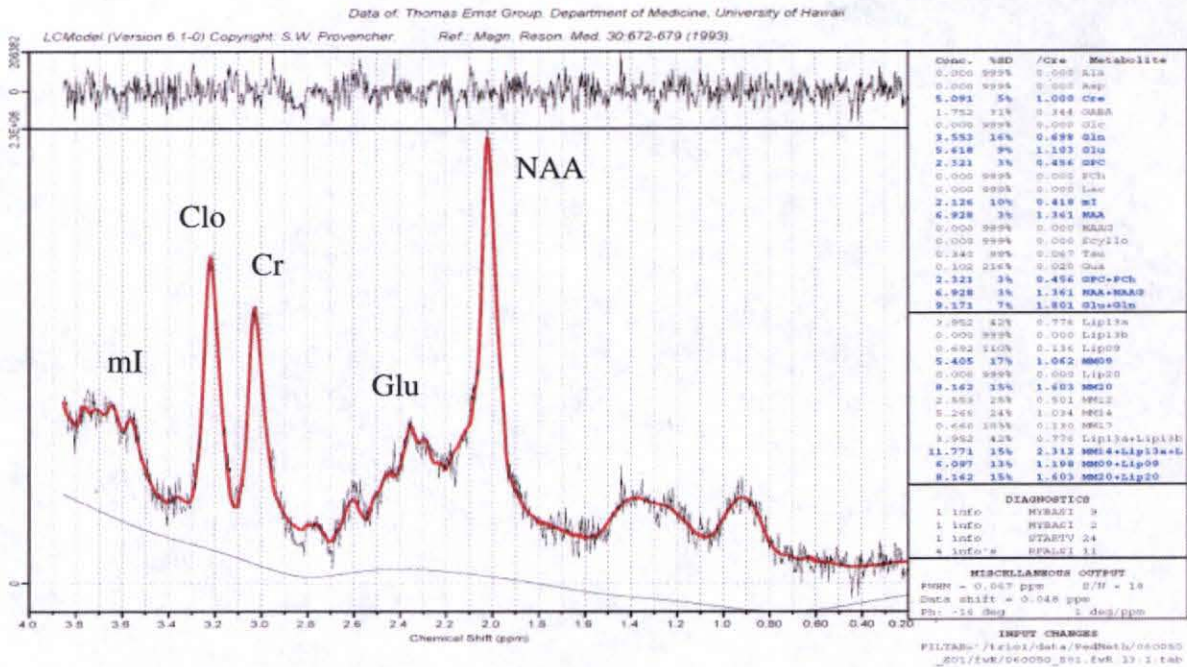


Figure 1.1.3: FwR Spectrum

Due to the differences in metabolite concentrations between GM and WM, it may be important to correct measured metabolite concentrations for the percentage of GM and WM. Furthermore, the ability to interpolate the concentration of either pure GM or WM would be desirable.

1.2 Partial Volume

Partial volume (a fractional value) is defined as the sum of one type of brain tissue over the total volume of a selected region (localized voxel or entire brain). There are two popular formats of partial volume data, soft (or fuzzy) and hard data. Soft data is the actual percentage of that tissue type in the voxel or pixel. Consider a voxel, which its partial volume for CSF is 23.4%. This is stating that the actual amount of CSF in that voxel is 0.234. This is different than a probability output. Probabilistic data for partial volume is only stating the probability of that

voxel being a certain tissue type. If the probability was 15.7% for CSF, then there is a 15.7% chance that that voxel is CSF. Partial volumes can be calculated using probability maps by assuming that their probabilities represent the percentage of partial volume for each tissue type. Hard data output is a logical or integer number, stating what tissue type the voxel actually represents (100%). With hard data, any voxel or pixel is considered to contain a single tissue type only. Using these three data types, segmentation methods provide a partial volume for each pixel or voxel for images, ranging from tissue specific probability maps to combined hard data images. Partial volumes are normally calculated using image-processing software such as MATLAB or IDL however, custom programming or code in C or C++ may be used. Packages used for the current work included FSL and SPM2 (9,14).

1.3 T2 method to Correct for % CSF

The T2 method is a means to determine compartmentation of a localized region: utilizing the signal strengths of CSF and tissue water to quantify partial volumes for both CSF and brain tissue (1). T2 is the time for decay of the transverse magnetization, which for this study, T2 decay time would be dependent upon the H₂O (water) molecule population (¹H proton spectroscopy) (12,20). T2 data points are detected by measuring the T2 amplitude at multiple time echo (TE – time between the 90° pulse and the maximum amplitude of the echo) (20). Since the T2 values are very different (approximately 75 msec for brain tissue and 1000-3000 msec for CSF), measuring the T2 decay times makes it possible to separate CSF from the brain tissue, by fitting both a fast and slowly decaying component. This

results in a double-exponential T_2 decay of the water signal, which is then used to determine different amplitudes for CSF and tissue water, interpolated to $TE=0$ (1).

Figure 1.1.4 show T_2 decay of CSF, tissue water and pure water.

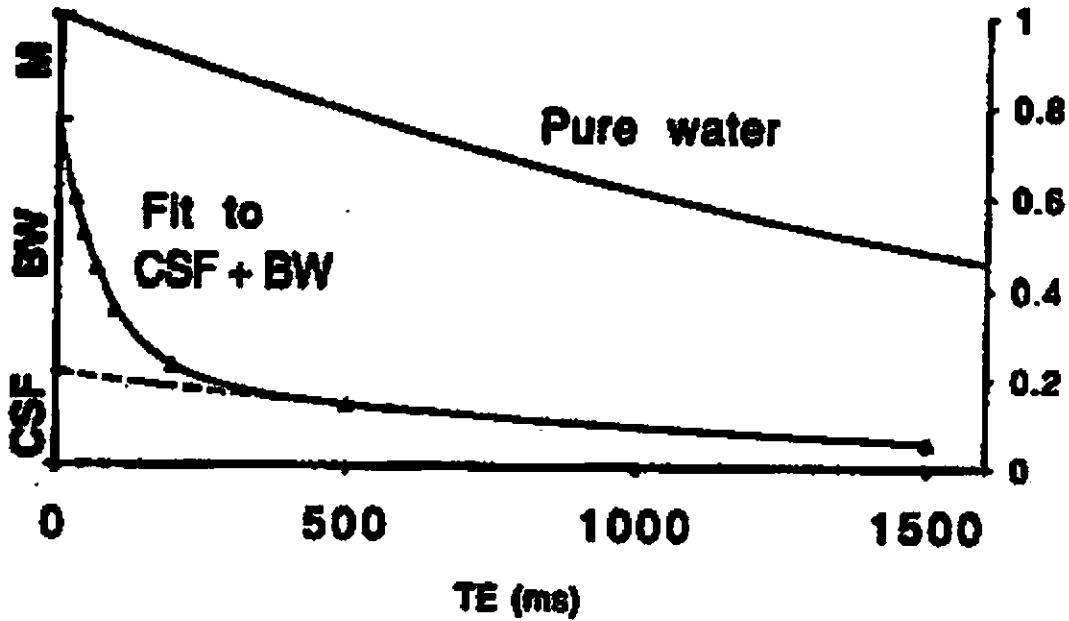


Figure 1.1.4: T_2 Decay and Fit

These signal amplitudes reflect the partial volumes of CSF and brain tissue. Signal amplitude from tissue water accounts for approximately 75% of the actual tissue volume, since brain tissue make up is $\frac{3}{4}$ water. From the different signal amplitudes, calculation of partial volumes was conducted using the following equations, where PV is partial volume, A is the signal amplitude and k is a constant:

$$A_{CSF} = kPV_{CSF}, \quad A_{TissueWater} = 0.75kPV_{Tissue}, \quad PV_{CSF} + PV_{Tissue} = 1$$

$$1 = \left[PV_{CSF}A_{CSF} + PV_{Tissue}A_{TissueWater} \left(\frac{1}{0.75} \right) \right] \quad (1.1)$$

$$1 - PV_{CSF}A_{CSF} = PV_{Tissue}A_{TissueWater} \left(\frac{1}{0.75} \right) \quad (1.2)$$

$$PV_{CSF} = \frac{A_{CSF}}{A_{CSF} + \frac{A_{TissueWater}}{0.75}} \quad PV_{BrainTissue} = \frac{\frac{A_{TissueWater}}{0.75}}{A_{CSF} + \frac{A_{TissueWater}}{0.75}} \quad (1.3, 1.4)$$

The T2 method has been used for CSF partial volume measurement for over a decade. Since GM and WM are seen as a combined signal output from the T2 method, due to their similar T2 values, GM and WM cannot be distinguished from each other. The need for partial volume standards for both WM and GM causes a need for a standard that can incorporate all three tissue types.

CHAPTER 2 PROBLEM

2.1 CSF Partial Volume Discrepancies

In many MR spectroscopy related research projects, partial volume of the targeted voxel, for all three-tissue classes (CSF, WM and GM) is required for correct adjustment of measured metabolite concentration and correlation between partial volume and metabolite concentrations. It was discovered, during a normal review of processed data, that a significant discrepancy in the calculated partial volume of CSF in the frontal gray matter region (FgM) voxel existed between the T2 method and SPM in all subjects. The CSF partial volume was calculated by the T2 method using the normal means described in section 1.3. Partial volume for CSF using SPM was calculated via custom code programmed using MATLAB. The method for extracting specific spectroscopy voxel data and partial volume calculations from SPM segmented images will be discussed, with detail, in Chapter 3. Table 2.1 and Figure 2.1 shows the discrepancy noted in partial volume for CSF in the FgM region. Please note that SPM CSF partial volume essentially remained

Subject	SPM_CSF	T2
1	0.24645	0.0510
2	0.26940	0.1091
3	0.28681	0.1398
4	0.29901	0.1101
5	0.34506	0.0877

Table 2.1: CSF Partial Volume Discrepancies

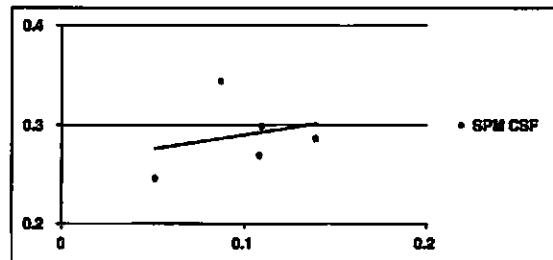


Figure 2.1: CSF Partial Volume Discrepancies

between 25-35%. A review of the structural images for artifacts or any image distortions that could contribute to the discrepancies revealed no such errors. A review of the segmented images also revealed no obvious segmentation problems.

2.2 Integrity of SPM Segmentation

For the purpose of this thesis, the T2 method of determining CSF partial volume in a spectroscopy voxel is considered the standard and the “true” partial volume. Due to the discrepancy between CSF partial volumes, the WM and GM partial volume values from SPM are also in question. Since the brain tissue (combination of GM and WM) is equal to $(1 - CSF_{pv})$, an inaccurate measurement of CSF suggests incorrect partial volumes for brain tissue (GM and / or WM) as well.

2.3 Determination of Methods

The question of “which segmentation method is correct” is difficult to answer. Not only does the question of “which”, but “why” is one segmentation method correct over another. Although all segmentation methods can be used in determining partial volume in a MR spectroscopy voxel, all will have to be validated by a standard or true partial volume. Determining the true partial volume for CSF can be achieved by using the T2 method, however WM and GM partial volumes will need to be addressed. A comparison of all segmentation methods, with a common group of subjects, was conducted to validate the different methods and determining the most accurate segmentation method. Correction for CSF is accomplished by the following model:

$$C_{measured} = PV_{CSF} C_{CSF} + PV_{GM} C_{GM} + PV_{WM} C_{WM} \quad (2.3.1)$$

With $PV_{CSF} + PV_{GM} + PV_{WM} = 1$. Since CSF is known to have negligible concentrations of any of the metabolites of interest, a new model is derived:

$$C_{measured} = PV_{GM} C_{GM} + PV_{WM} C_{WM} \quad (2.3.2)$$

Solving for either GM or WM concentration, actual concentration of a metabolite can be determined for a tissue type.

$$C_{GM} = \frac{C_{measured} - PV_{WM} C_{WM}}{PV_{GM}} \quad C_{WM} = \frac{C_{measured} - PV_{GM} C_{GM}}{PV_{WM}} \quad (2.3.3, 2.3.4)$$

These equations are not particularly useful since calculation of the GM concentration requires knowledge of the WM concentration and vice versa. However, equation 2.3.3 and 2.3.4 can be used to calculate “pure” concentrations in GM or WM in a group of subjects, using interpolation from a correlation graph of concentrations versus partial volume.

CHAPTER 3 METHODS

3.1 Determination of Partial Volume Standards and Segmentation Validation

A population of 17 subjects was used for comparison and analysis. These subjects were acquired using a 3T Siemens scanner, T1 weighted, MP-RAGE, $1 \times 1 \times 1 \text{ mm}^3$, sagittal, TR / TE / TI = 2200, 4.91, 1000 msec, and a flip angle of 12° . The 17 subjects were in good health and had a mean age of 44; 16 males and 1 female. MR spectroscopy voxel size of $20 \times 20 \times 20 \text{ mm}^3$ was used in the FgM and FwR regions.

3.1.1 Validation of Manual Tracing (MT) as a Partial Volume Standard for CSF

The use of MT as a standard for validating segmentation has been used in many research areas over the years. Since brain partial volumes are unique for each individual, it is impossible to determine actual partial volume without using a segmentation method, which does not allow validation of the manual tracing method. Due to the conception of the T2 method, there is a quantitative method to determine the partial volume of CSF in a MR spectroscopy voxel, based solely on the T2 relaxation times. This gives an advantage when determining partial volumes in MR spectroscopy, since it gives the ability to validate segmentation methods. Using the partial volume of CSF calculated from the T2 decay curves, manual tracing was validated by comparing partial volumes of 17 subjects (acquisition methods described earlier in section 3.1). MR spectroscopy voxels ($20 \times 20 \times 20 \text{ mm}^3$) were extracted from MP-RAGE structural image with custom MATLAB programming.

Each 1 mm slice was saved as a 20 x 20 pixel BMP file. Due to the extremely small size of the voxel slices, bi-cubic interpolation was performed to enlarge the images to 634 x 634 pixels. Figure 3.1.2.1 represents three extracted and interpolated slices (not to scale).



Figure 3.1.2.1: Voxel Slices

Tracing was performed using Photoshop and a pressure sensitive Wacom graphics tablet. This allowed free-handed smooth tracing of segmented slices. Each tissue type was assigned a different RGB color: CSF 0/0/0 (black), GM 255/0/0 (red) and WM 0/255/0 (green). Figure 3.1.2.2 shows slices after manual tracing (not to scale).



Figure 3.1.2.2: Traced Voxel Slices

Calculation of CSF partial volume was determined by the following equation:

$$PV_{CSF} = \frac{\sum_{CSF} Pixels}{\sum Pixels}$$

Table 3.1.2 show the calculated CSF partial volumes in 17 subjects, using both the T2 method and MT.

Figure 3.1.2.3 show the correlation between the partial volumes calculated with an R value of 0.871.

Due to the excellent correlation between the T2 method and manual tracing, it was determined and proved that the manual tracing is an accurate

segmentation method for a MR spectroscopy voxel. Therefore, manual tracing is considered an equal standard to the T2 method and was used in determining accuracy in segmentation methods.

Subject #	M	T2
M008	0.1188	0.1411
M009	0.1141	0.0841
M012	0.0998	0.0972
M010	0.1534	0.1476
M007	0.1310	0.1681
M011	0.0851	0.0472
M013	0.0956	0.1114
M002	0.1794	0.1490
M014	0.1446	0.1655
M015	0.1681	0.1869
M016	0.1260	0.1070
M017	0.2187	0.2207
M005	0.1892	0.1957
M001	0.0916	0.0706
M006	0.2002	0.1649
M004	0.1676	0.1616
M003	0.2697	0.2150

Table 3.1.2.1: CSF Partial Volumes

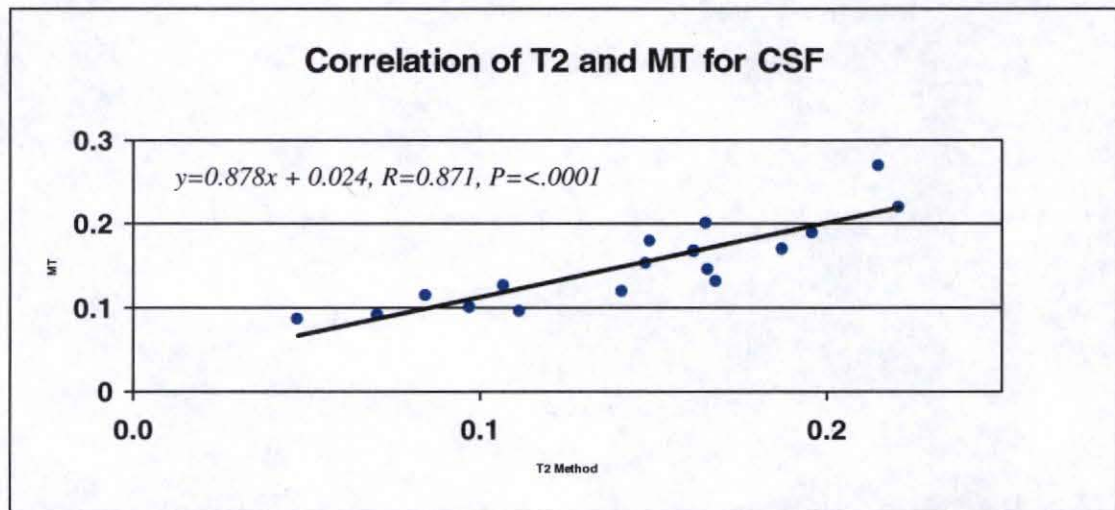


Figure 3.1.2.3: Correlation for CSF

3.1.3 WM and GM Partial Volume Standards

Standards for both WM and GM are required to determine the accuracy of different segmentation methods. Due to the limitation of the T2 method, which can only determine partial volume of CSF, a standard must be determined. MT will be selected as the standard for all three tissue types. Due to the good correlation of CSF partial volumes of MT versus the T2 method, an assumption has been made that the MT method is equally good at segmenting GM and WM. GM and WM partial volumes were determined using the following equations:

$$PV_{GM} = \frac{\sum_{GM} Pixels}{\sum Pixels} \quad PV_{WM} = \frac{\sum_{WM} Pixels}{\sum Pixels}$$

Partial Volume Standards

Compartment	CSF	Gray Matter	White Matter
Standard	Manual Tracing	Manual Tracing	Manual Tracing

3.2 Validation of Segmentation Methods for a FgM MR Spectroscopy Voxel

Using the determined manual tracing standards for CSF, WM, and GM, a comparison against the calculated partial volumes from different segmentation methods was performed therefore, validation of their accuracy can be determined. Segmentation was performed on all 17 subjects, using SPM2, FSL using Brain Extraction Tool (BET – separates the brain from the skull, output is the brain only) and various segmentation output options, manual tracing and the T2 method.

3.2.1 Statistical Parametric Mapping (SPM)

SPM2 segmentation was performed using two different templates: a normal T_1 weighted template and a custom skull stripped (skull manually deleted) T_1 weighted template (2). Figure 3.2.1.1 show the normal T_1 weighted template while Figure 3.2.1.2 how the skull stripped T_1 weighted template.

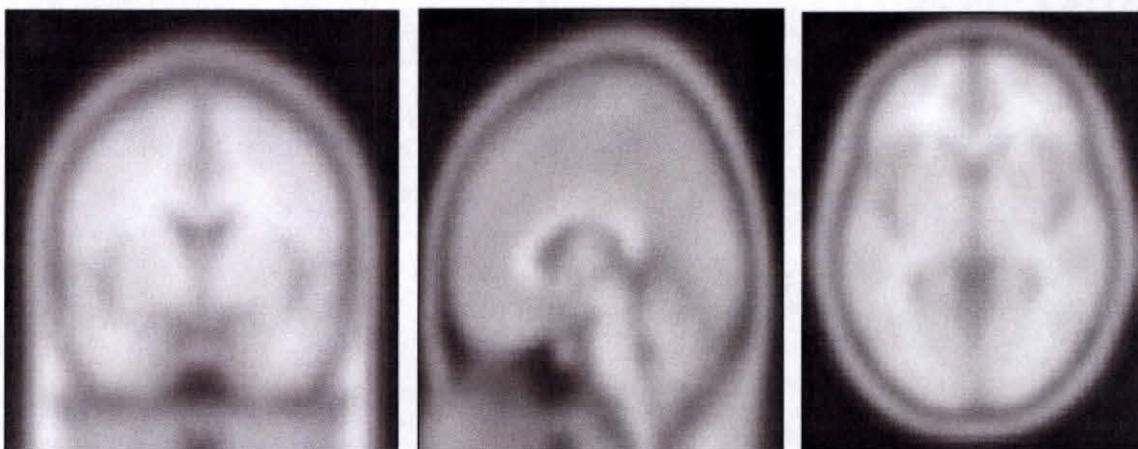


Figure 3.2.1.1: SPM2 Template

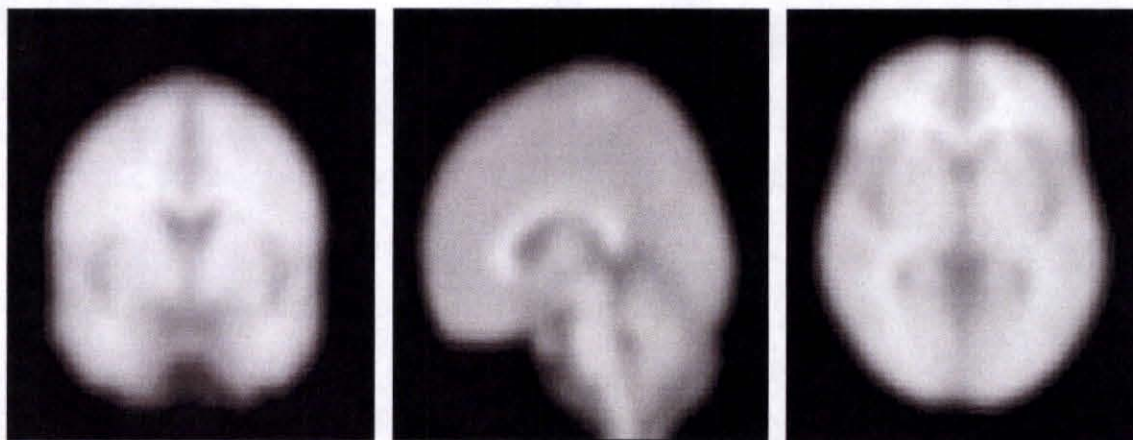


Figure 3.2.1.2: Modified Template

Segmentation resulted in three probability maps, one for each tissue type, for both templates. Structural MP-RAGE images from the 17 subjects were pre-processed using a custom Java application, allowing manual erasing of skull and non-brain

structures missed by the use of BET (2). These images were only used with the skull stripped template. Figures 3.2.1.3 show segmented probability maps, transversal, for CSF, GM and WM, for normal T₁ weighted template respectively.

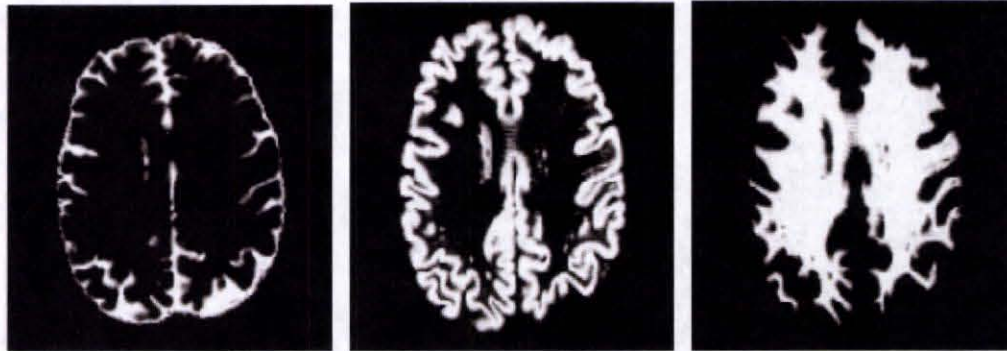


Figure 3.2.1.3: Segmented Probability Maps

Segmentation results from the skull stripped template showed similar structural images. Since the data in each segmented image is soft data (i.e. probability that this pixel is the selected tissue type), partial volume will be assumed to be synonymic with probability. Probabilities for the spectroscopy voxel location were extracted and partial volumes calculated using the following equations, where x,y and z are probabilities:

$$PV_{CSF} = \sum_{CSF} x \quad PV_{GM} = \sum_{GM} y \quad PV_{WM} = \sum_{WM} z$$

3.2.2 FSL

Similar to SPM2, FSL is a software program that allows a user to analyze fMRI images and perform segmentation. FSL has many advantages over SPM2 allowing the user more options for segmentation. For instance, the Brain Extraction Tool allows skull stripping of the image. Figure 3.2.2.1 show sagittal views of the image prior and after brain extraction.

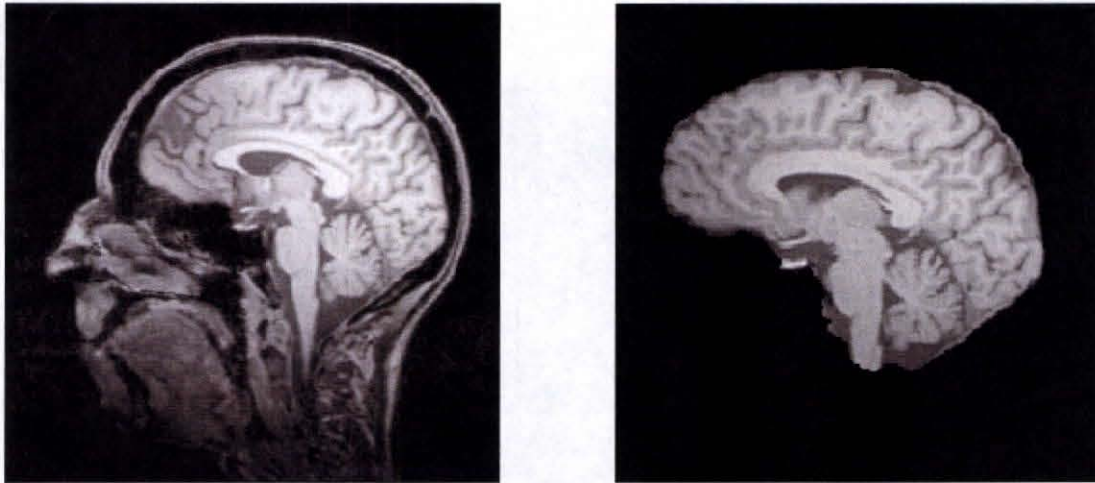


Figure 3.2.2.1: Skull Stripped Image

FSL segmentation uses K-means clustering (pixel based segmentation of multi-band images) however, the use of a-priori (initial and posterior) probability maps are available output options. FSL also has the ability to output segmented images either as three separate probability maps (one for each tissue type, similar to SPM2) or has one combined hard data map. Hard data maps are coded into four integer numbers to define which tissue type the pixel represents; Non-image = 0, CSF = 1, GM = 2 and WM = 3. Figure 3.2.2.2 show examples of hard data maps.



Figure 3.2.2.2: Hard Data Maps

Extraction of the data located in the MR spectroscopy voxel was performed using the same programming as for SPM2. Calculation of hard data partial volumes used the following equations:

$$PV_{CSF} = \frac{\sum_{CSF} Pixels}{\sum Pixels} \quad PV_{GM} = \frac{\sum_{GM} Pixels}{\sum Pixels} \quad PV_{WM} = \frac{\sum_{WM} Pixels}{\sum Pixels}$$

Probability maps are similar to those produced by SPM2. Each pixel for each tissue map provides the probability of that pixel actually being the tissue type. Figure 3.2.2.3 show transversal images of each tissue probability map, CSF, GM and WM respectively.

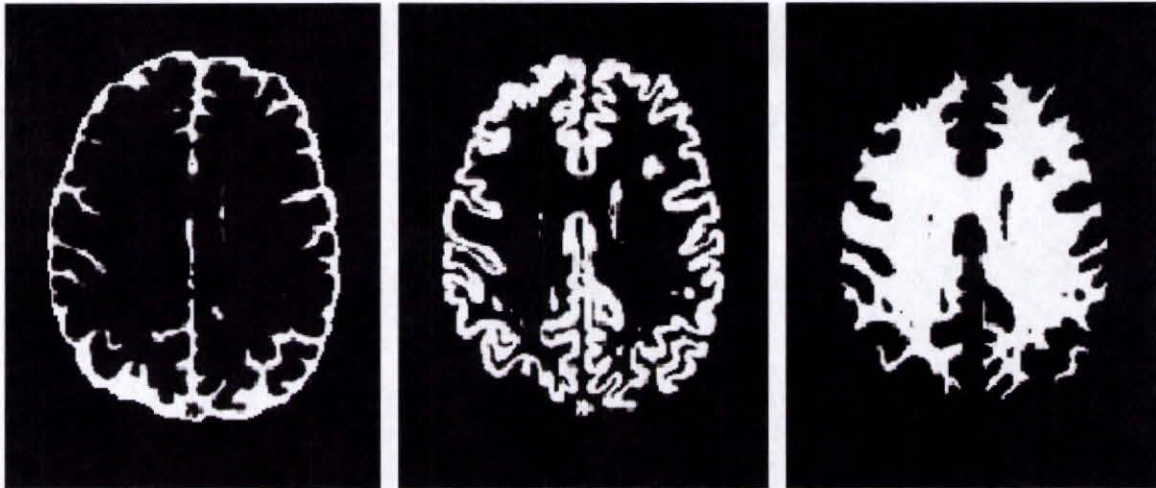


Figure 3.2.2.3: Probability Maps

Partial volumes were calculated using the following equations where x , y and z are probabilities:

$$PV_{CSF} = \sum_{CSF} x \quad PV_{GM} = \sum_{GM} y \quad PV_{WM} = \sum_{WM} z$$

3.2.3 Manual Tracing (MT)

Manual tracing of all subjects in the MR spectroscopy voxel (in the FgM region) was performed as described in section 3.1.2. Interpolation of the voxel slices was performed for ease in manual tracing. Manual tracing was performed once per subject. An initial trial was performed on 5 subjects, using a separate population, using 3 manual tracing runs for each subject. Comparison between the 3 runs shows a minor difference in traced tissue locations and an average difference of 2% in partial volumes. This supported the use of only one trace per subject. Initial runs included a comparison of MT from sagittal, transversal and coronal views of the voxel. Calculated partial volumes were within 3% of each other for all subjects.

CHAPTER 4 RESULTS

4.1 Partial Volume Results After Segmentation

After segmentation, partial volumes for the MR spectroscopy voxel, located in the FgM region, for each subject was calculated. Table 4.1.1, 4.1.2 and 4.1.3 show results of all partial volume calculations for WM, GM and CSF respectively.

Subject	SPM2	SPM2 SS DF	FSLNH	FSLIH	FSLBH	FSLNS	FSLIS	LFSSS	DFNH	DFIH	DFBH	DFNS	DFIS	DFBS	M
M008	0.0456	0.0725	0.1360	0.1256	0.1058	0.1338	0.1242	0.0827	0.1256	0.1173	0.1018	0.1279	0.1154	0.0931	0.145
M009	0.0209	0.0378	0.0714	0.0850	0.0644	0.0703	0.0632	0.0483	0.0631	0.0728	0.0520	0.0637	0.0723	0.0451	0.109
M012	0.0621	0.0788	0.1178	0.1064	0.0883	0.1188	0.1082	0.0632	0.1128	0.0978	0.0911	0.1113	0.0981	0.0828	0.129
M010	0.0378	0.0618	0.0883	0.0828	0.0741	0.0868	0.0920	0.0528	0.0821	0.0851	0.0753	0.0807	0.0834	0.0663	0.1288
M007	0.0548	0.0716	0.1385	0.1120	0.1002	0.1343	0.1105	0.0856	0.1269	0.1033	0.0991	0.1197	0.1021	0.0930	0.1288
M011	0.0333	0.0508	0.1123	0.0999	0.0750	0.1106	0.0978	0.0588	0.1014	0.0884	0.0704	0.0884	0.0852	0.0637	0.1480
M013	0.0627	0.0886	0.1620	0.1303	0.1136	0.1136	0.1805	0.1287	0.0970	0.1371	0.1211	0.1113	0.1388	0.1160	0.1801
M002	0.0479	0.0695	0.1608	0.1714	0.1143	0.1489	0.1698	0.0790	0.1228	0.1385	0.1028	0.1208	0.1368	0.0912	0.1588
M014	0.0469	0.0557	0.0931	0.0775	0.0630	0.0926	0.0783	0.0832	0.0904	0.0723	0.0684	0.0893	0.0718	0.0601	0.1078
M015	0.0335	0.0607	0.1184	0.1215	0.1143	0.1141	0.1209	0.0857	0.0884	0.0894	0.0805	0.0979	0.0889	0.0748	0.1190
M016	0.0428	0.0618	0.1218	0.1118	0.0840	0.1206	0.1101	0.0637	0.1168	0.0968	0.0870	0.1181	0.0953	0.0739	0.1397
M017	0.0459	0.0685	0.1120	0.0986	0.0833	0.1107	0.0968	0.0680	0.1033	0.0883	0.0825	0.1022	0.0889	0.0745	0.138
M005	0.0765	0.1010	0.1301	0.1338	0.1284	0.1288	0.1323	0.1003	0.1286	0.1378	0.1278	0.1273	0.1360	0.1178	0.1642
M001	0.0418	0.0606	0.1065	0.0943	0.0711	0.1058	0.0932	0.0468	0.0945	0.0773	0.0673	0.0934	0.0782	0.0590	0.1445
M006	0.0700	0.0887	0.1483	0.1801	0.1334	0.1444	0.1474	0.1127	0.1381	0.1406	0.1178	0.1342	0.1387	0.1114	0.1291
M004	0.0360	0.0544	0.0886	0.0748	0.0624	0.0973	0.0737	0.0480	0.0963	0.0725	0.0643	0.0947	0.0713	0.0677	0.1289
M003	0.0383	0.0472	0.0830	0.0889	0.0878	0.0821	0.0948	0.0708	0.0789	0.0885	0.0713	0.0755	0.0873	0.0631	0.1012

Table 4.1.1: WM Partial Volumes

Subject	SPM2	SPM2 SS DF	FSLNH	FSLIH	FSLBH	FSLNS	FSLIS	LFSSS	DFNH	DFIH	DFBH	DFNS	DFIS	DFBS	M
M008	0.6895	0.7180	0.8470	0.8558	0.7815	0.8457	0.8548	0.7804	0.8505	0.8641	0.7318	0.8490	0.8620	0.7878	0.738
M009	0.7135	0.7867	0.7883	0.7833	0.8250	0.7843	0.7823	0.8595	0.7883	0.7860	0.8298	0.7834	0.7832	0.8492	0.777
M012	0.7480	0.7827	0.7768	0.7875	0.8383	0.7745	0.7853	0.8669	0.7878	0.7824	0.8133	0.7868	0.7891	0.8305	0.771
M010	0.6821	0.6983	0.6811	0.6780	0.7408	0.6794	0.6758	0.7774	0.6825	0.6788	0.7169	0.6800	0.6788	0.7418	0.7170
M007	0.6912	0.7118	0.6230	0.6431	0.7838	0.6193	0.6395	0.7843	0.6228	0.6385	0.7113	0.6182	0.6325	0.7407	0.7402
M011	0.7302	0.8077	0.8100	0.8234	0.8630	0.8086	0.8224	0.8956	0.8103	0.8239	0.8568	0.8087	0.8222	0.8722	0.7698
M013	0.6889	0.7390	0.6993	0.6781	0.7813	0.6816	0.6764	0.7822	0.6869	0.6818	0.7330	0.6633	0.6788	0.7822	0.7844
M002	0.8234	0.7233	0.6819	0.6705	0.8176	0.6838	0.6722	0.8589	0.6988	0.6854	0.7784	0.6988	0.6884	0.8068	0.6621
M014	0.8478	0.6914	0.6398	0.6489	0.7385	0.6349	0.6455	0.7739	0.6284	0.6389	0.7038	0.6237	0.6389	0.7333	0.7478
M015	0.6309	0.6852	0.8169	0.8111	0.7800	0.8158	0.6090	0.8073	0.8184	0.8151	0.6874	0.8135	0.8105	0.7231	0.7128
M016	0.7140	0.7322	0.6724	0.6814	0.7888	0.6895	0.6793	0.7839	0.6728	0.6888	0.7394	0.6702	0.6865	0.7677	0.7342
M017	0.6228	0.6710	0.5908	0.5985	0.7091	0.5895	0.6993	0.7549	0.6995	0.8104	0.6789	0.5971	0.6078	0.7088	0.6435
M005	0.6320	0.6712	0.5908	0.5834	0.6987	0.5887	0.5824	0.7457	0.5868	0.5778	0.6595	0.5825	0.5734	0.6905	0.6486
M001	0.6828	0.7498	0.7779	0.7881	0.8439	0.7762	0.7887	0.8768	0.7478	0.7889	0.8073	0.7455	0.7870	0.8289	0.7838
M006	0.6158	0.6574	0.5783	0.5738	0.6843	0.5738	0.5714	0.6934	0.5815	0.5763	0.6395	0.5780	0.5725	0.6660	0.6707
M004	0.6827	0.7123	0.6311	0.6439	0.7838	0.6282	0.6413	0.6032	0.8180	0.6360	0.7088	0.6141	0.6322	0.7378	0.7065
M003	0.6832	0.6983	0.6643	0.6588	0.6735	0.6628	0.6567	0.7213	0.5718	0.5891	0.6528	0.5678	0.5582	0.6845	0.6291

Table 4.1.2: GM Partial Volumes

Subject	SPM2	SPM2_SS_DF	FSLNH	FSLIH	FSLBH	FSLNS	FSLIS	LFSSS	DFNH	DFIH	DFBH	DFNS	DFIS	DFBS	M
M008	0.2848	0.2078	0.2170	0.2188	0.1428	0.2205	0.2212	0.1288	0.2198	0.2188	0.1687	0.2330	0.2228	0.1494	0.119
M009	0.2857	0.1843	0.1424	0.1418	0.1108	0.1434	0.1448	0.0832	0.1398	0.1413	0.1183	0.1428	0.1448	0.1057	0.114
M012	0.1918	0.1367	0.1058	0.1061	0.0765	0.1080	0.1095	0.0639	0.1198	0.1201	0.0958	0.1219	0.1228	0.0869	0.1
M010	0.3100	0.2420	0.2306	0.2283	0.1851	0.2339	0.2322	0.1638	0.2334	0.2351	0.2079	0.2394	0.2380	0.1819	0.1534
M007	0.2538	0.2152	0.2415	0.2449	0.1480	0.2484	0.2500	0.1201	0.2584	0.2613	0.1898	0.2811	0.2854	0.1683	0.1310
M011	0.2385	0.1409	0.0778	0.0768	0.0558	0.0807	0.0800	0.0480	0.0884	0.0898	0.0729	0.0914	0.0967	0.0841	0.0881
M013	0.2485	0.1754	0.1828	0.1905	0.1350	0.1878	0.1858	0.1138	0.1969	0.1971	0.1558	0.2011	0.2014	0.1387	0.0588
M002	0.3287	0.2061	0.1874	0.1881	0.0681	0.1873	0.1890	0.0621	0.1785	0.1781	0.1190	0.1785	0.1770	0.1020	0.179
M014	0.3054	0.2818	0.2871	0.2728	0.2005	0.2725	0.2780	0.1729	0.2813	0.2879	0.2310	0.2869	0.2823	0.2068	0.1448
M016	0.3338	0.2530	0.2678	0.2674	0.1288	0.2702	0.2701	0.1070	0.2833	0.2855	0.2221	0.2888	0.3008	0.2023	0.1881
M018	0.2431	0.2049	0.2058	0.2069	0.1573	0.2099	0.2108	0.1424	0.2105	0.2145	0.1738	0.2147	0.2180	0.1884	0.1280
M017	0.3310	0.2581	0.2874	0.3019	0.2076	0.2888	0.3041	0.1771	0.2873	0.3014	0.2418	0.3008	0.3052	0.2169	0.219
M005	0.2814	0.2287	0.2794	0.2809	0.1770	0.2845	0.2853	0.1540	0.2848	0.2845	0.2129	0.2802	0.2807	0.1919	0.1892
M001	0.3044	0.1887	0.1158	0.1178	0.0880	0.1183	0.1202	0.0787	0.1580	0.1639	0.1255	0.1811	0.1888	0.1121	0.0916
M006	0.3141	0.2560	0.2773	0.2783	0.2124	0.2820	0.2812	0.1839	0.2824	0.2831	0.2428	0.2878	0.2887	0.2225	0.2002
M004	0.3012	0.2318	0.2703	0.2814	0.1739	0.2745	0.2851	0.1488	0.2838	0.2815	0.2270	0.2813	0.2868	0.2047	0.1878
M003	0.3005	0.2548	0.3528	0.3454	0.2386	0.3882	0.3488	0.2081	0.3524	0.3814	0.2760	0.3568	0.3548	0.2824	0.2897

Table 4.1.3: CSF Partial Volume

4.2 Correlation Results

An analysis of correlation between MT and all segmentation methods was conducted using StatView. Table 4.2.1 is a summary of all R values.

Segmentation Method vs. MT	CSF	GM	WM
SPM2	0.633	0.715	0.492
SPM2 Skull Stripped, manual edit	0.789	0.748	0.650
FSL Hard Data, no a-priori	0.844	0.774	0.705
FSL Hard Data, initial a-priori	0.823	0.816	0.581
FSL Hard Data, both a-priori	0.727	0.713	0.460
FSL Soft Data, no a-priori	0.836	0.765	0.704
FSL Soft Data, initial a-priori	0.818	0.809	0.580
FSL Soft Data, both a-priori	0.725	0.681	0.369
FSL Hard Data, no a-priori, manual edit	0.823	0.744	0.711
FSL Hard Data, initial a-priori, manual edit	0.811	0.798	0.628
FSL Hard Data, both a-priori, manual edit	0.800	0.744	0.642
FSL Soft Data, no a-priori, manual edit	0.813	0.738	0.675
FSL Soft Data, initial a-priori, manual edit	0.807	0.790	0.623
FSL Soft Data, both a-priori, manual edit	0.799	0.738	0.602
T2 Method	0.871	n/a	n/a

Table 4.2.1: Summary of R Values

More detailed graphs, located in Appendix A, show the correlations between segmentation methods and MT for each tissue type. R values for each comparison

are shown on each graph. Figure 4.2.1 show the difference in correlation between WM for SPM2 and SPM2_SS (skull stripped images and template).

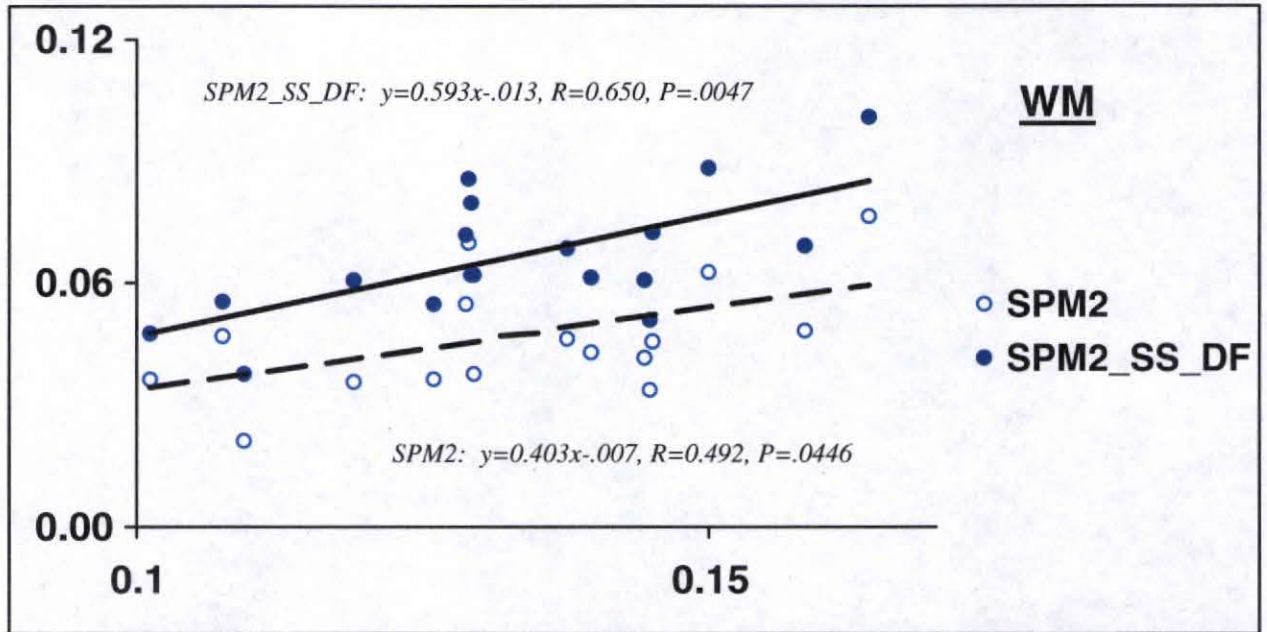


Figure 4.2.1: Correlation of WM and SPM2

4.3 Skull Stripping for Partial Volume Calculations of MR Spectroscopy Voxels

From the results obtained, skull stripping is an important part of obtaining accurate partial volumes for a MR spectroscopy voxels located in the FgM region. Comparing SPM2 and SPM2 using a skull stripped temple and subjects, correlations for all three tissue types increased. WM partial volume showed a low R value, from 0.369 to 0.711, for every segmentation method however, compared to SPM2, skull stripping made the greatest improvement in R value. Figures 5.1.1, 5.1.2 and 5.1.3 show the improvement of correlation in tissue types between the two SPM2 methods.

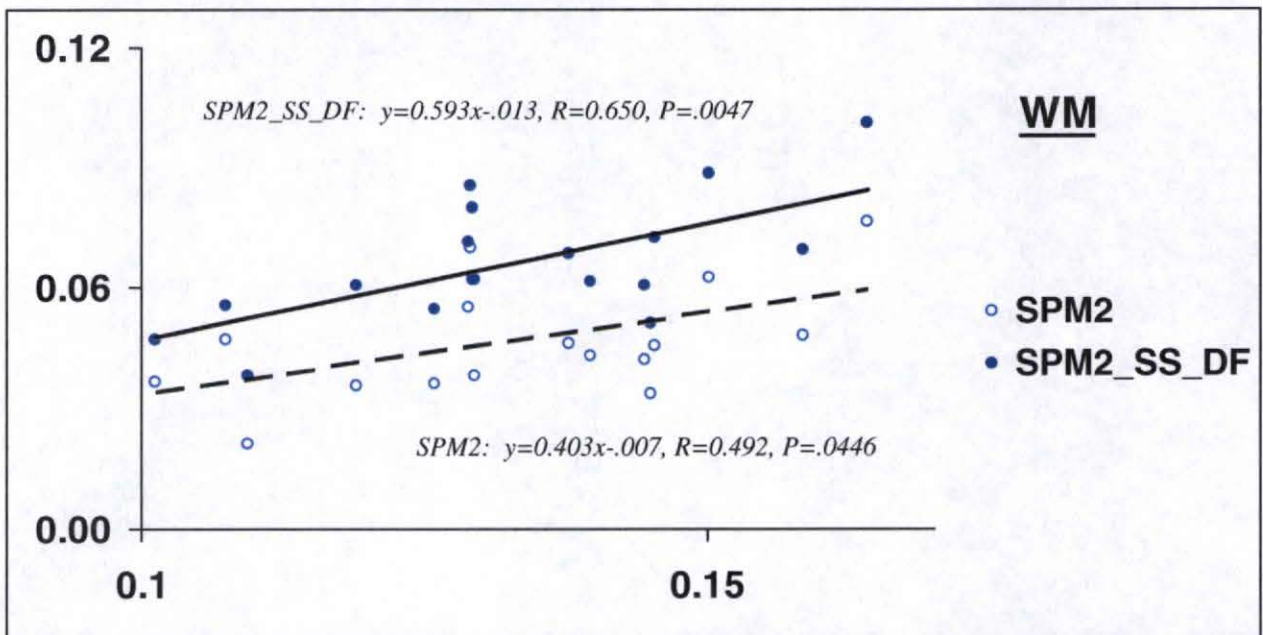


Figure 5.1.1: Partial Volume of WM Vs. MT

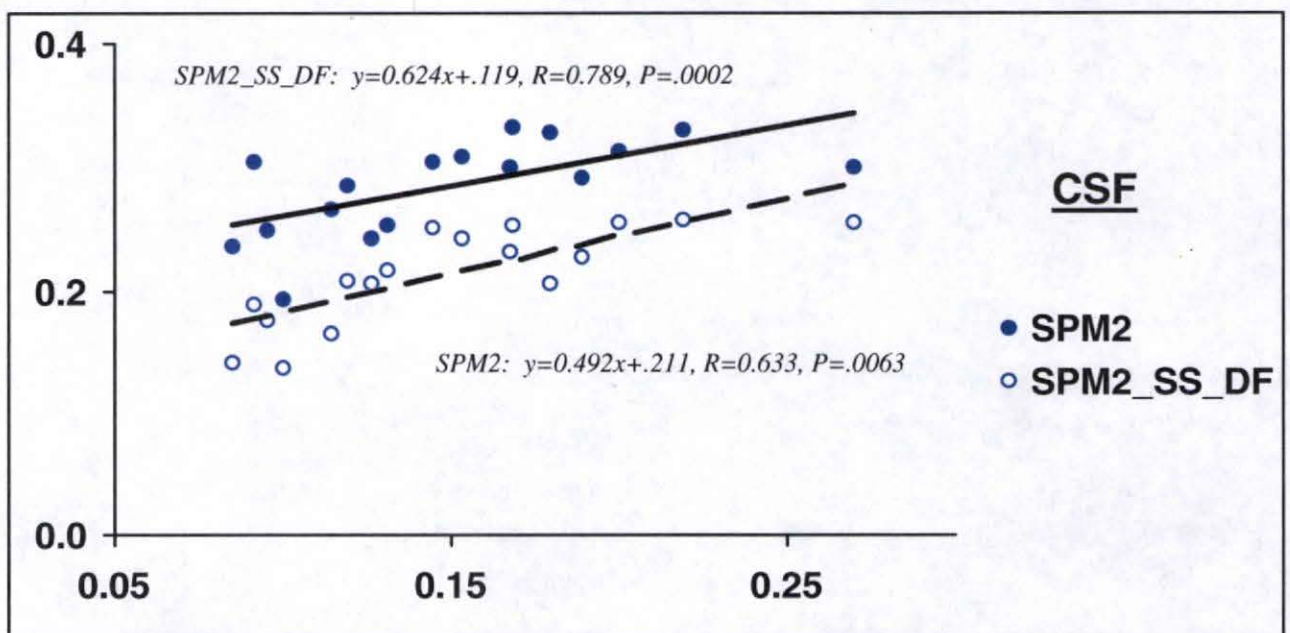


Figure 5.1.2: Partial Volume of CSF Vs. MT

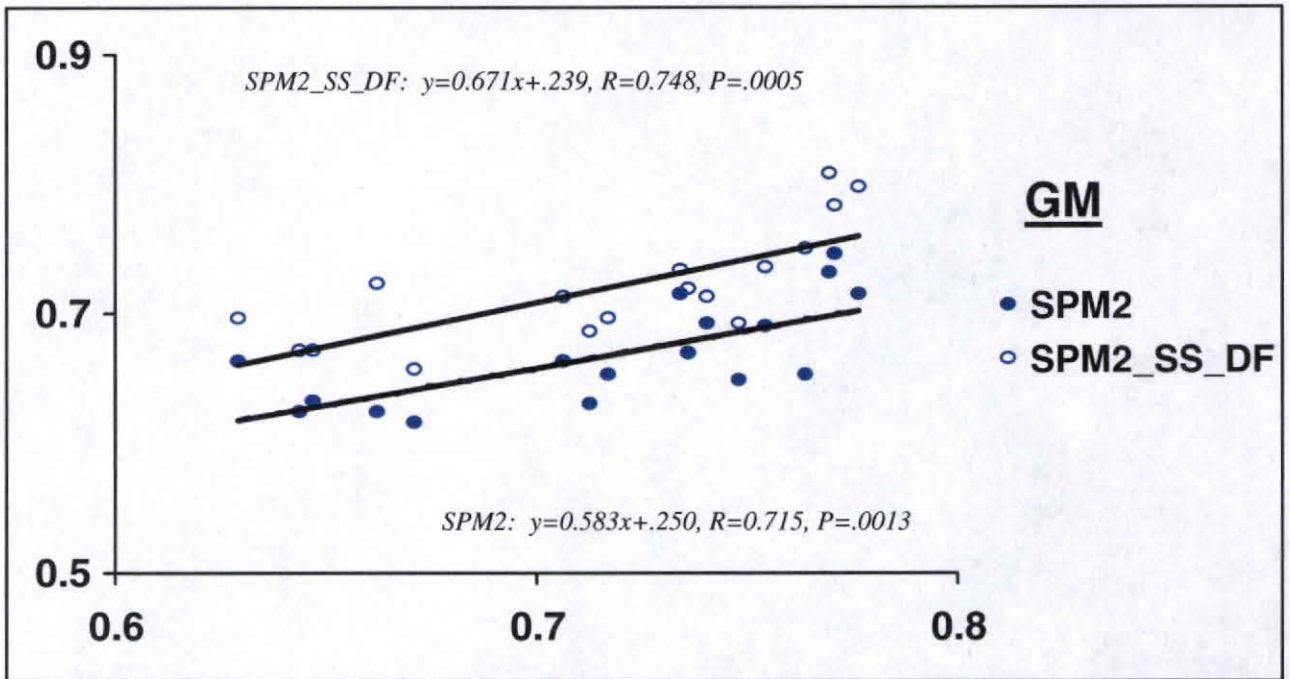


Figure 5.1.3: Partial Volume of GM Vs. MT

Figures 5.1.4 and 5.1.5, in 3-D, shows the profile of one slice directly through the center of the FgM voxel of subject M002. This profile shows the different in tissue distribution between SPM2 and SPM2 with skull stripped image and template. There is an obvious difference between distributions, which account for the difference in partial volumes.

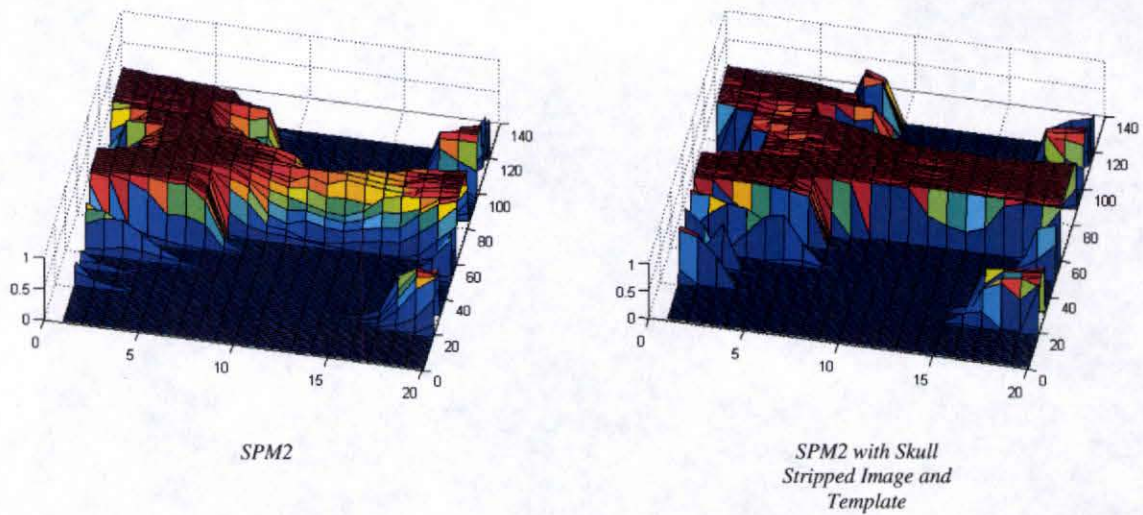


Figure 5.1.4: Subject M008 Slice WM Profile Vs. MT

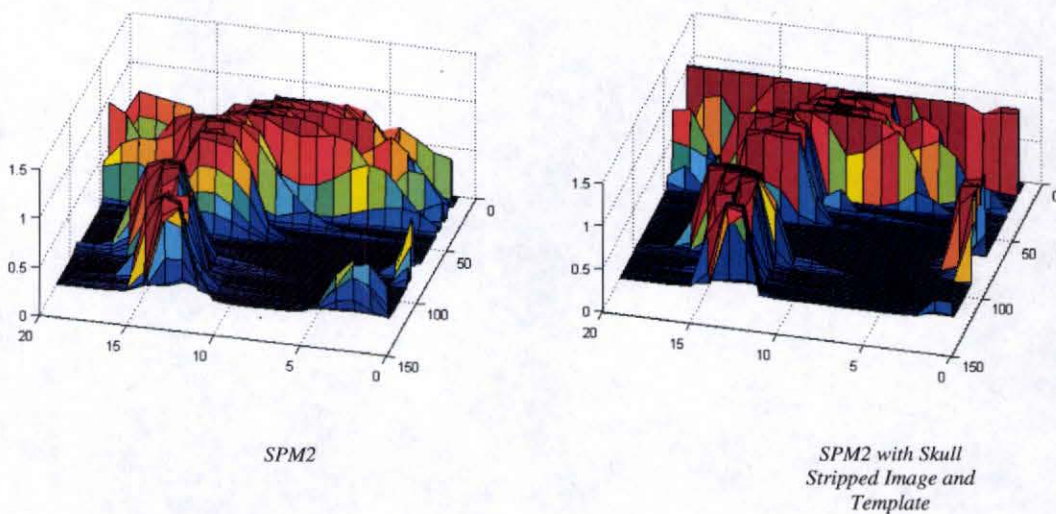


Figure 5.1.5: Subject M008 Slice CSF Profile Vs. MT

It is concluded that due to errors during registration (3), significant partial volume inaccuracies occur in SPM2 without skull stripping. Skull stripping (or brain extraction) causes a significant increase in accuracy of voxel segmentation and is recommended for all segmentation processing.

CHAPTER 5 DISCUSSION

5.1 FSL for MR Spectroscopy Voxel Segmentation

Results show that FSL, using no a-priori probability map output options, with hard data output map, gave the best overall R values (considering each tissue type). FSL with no a-priori probability map output options, soft data output, was second best for correlation with MT. There is little difference between correlations of manually edited images, after BET tool was used, for all tissue types. Figures 5.2.1 and 5.2.2 show correlation graphs of FSL using no a-priori probability maps and hard data output versus SPM2 for CSF and WM.

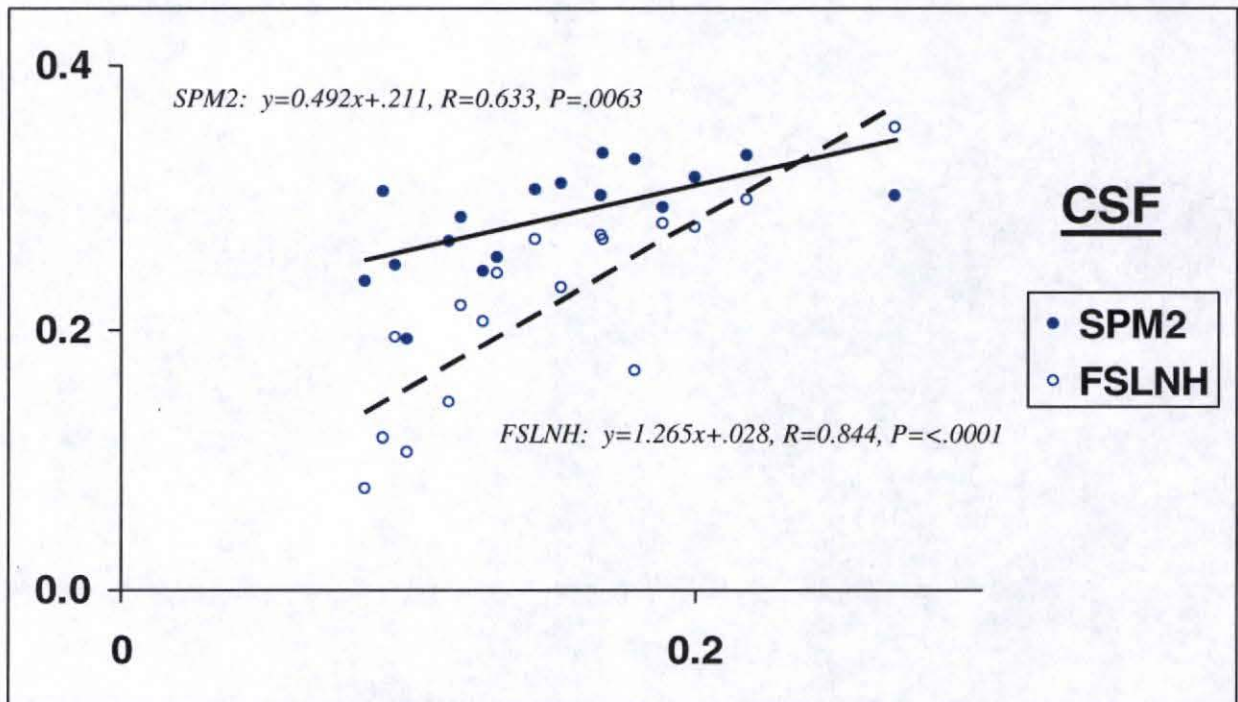


Figure 5.2.1: Correlation of CSF

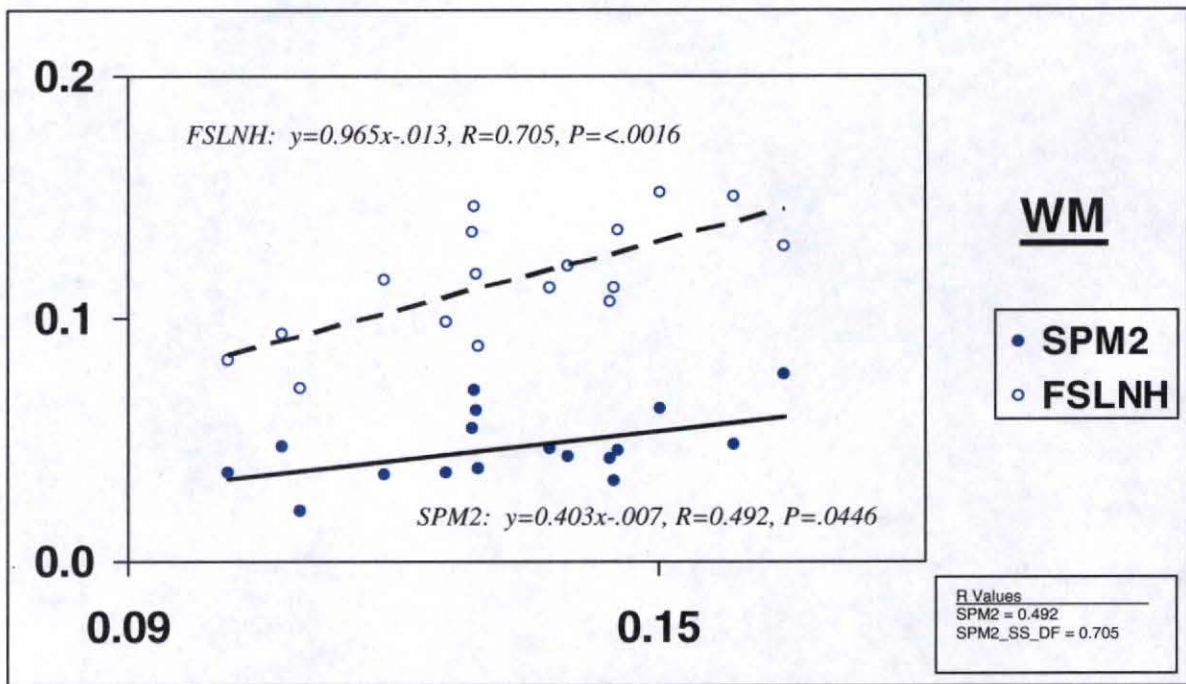


Figure 5.2.2: Correlation of WM

WM regressions were poor compared to the other tissue types. This is due to the relatively narrow range of WM partial volumes (approximately 5 – 15%). It is concluded and recommended that FSL, using BET prior to segmentation, no a-priori probability map options used, hard data selected as output option, is the best overall segmentation method and should be used for all partial volume calculations of MR spectroscopy voxels.

5.2 Cross Validation of the T2 Method

Most studies reference MT as the standard for segmentation, which they use to validate their own segmentation methods. In this study, the T2 method was used to validate MT. In section 3, figure 3.1.2.3, show the correlation between T2 method and MT, resulting in an R value of 0.871. Since FSL is the recommend segmentation method, Figure 5.3.1 shows the correlation between the recommended FSL method and SPM2 versus the T2 method.

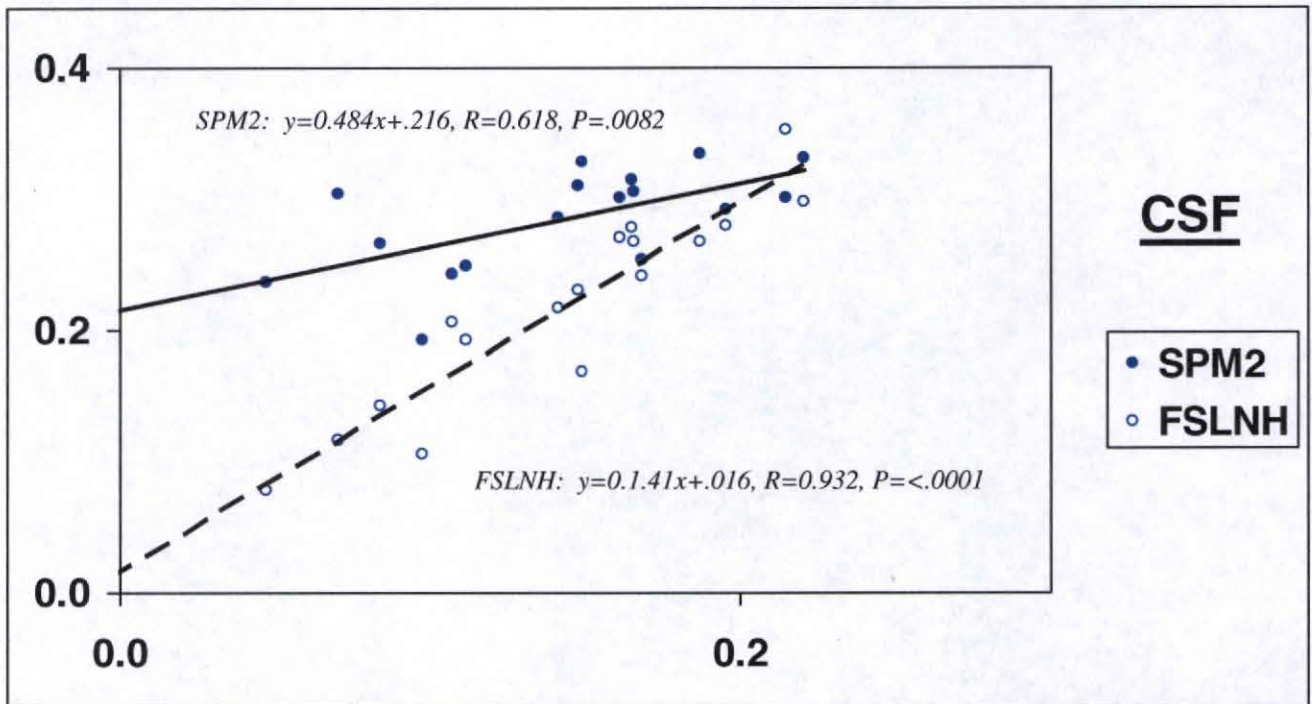


Figure 5.3.1: Correlation of CSF vs. T2 Method

Skull stripping also increased the R value of SPM2 when using the skull stripped template. It is concluded that manual tracing is an accurate means of segmentation and can be used as a standard in studies. The T2 method is also validated as the standard for calculating CSF in MR spectroscopy voxels.

5.3 Correlation with Metabolite Concentrations

Regressions were performed for partial volumes of the FgM, FwR, and a combined partial volume of FgM and FwR versus GM index, using data from all 42 subjects (16). GM index is the ratio of GM and the combination of GM and WM. Figure 5.4.1 show the regression for Cr in the FgM region. The P value of .0818 is considered significant due to the positive slope of the regression, which is expected from previous research.

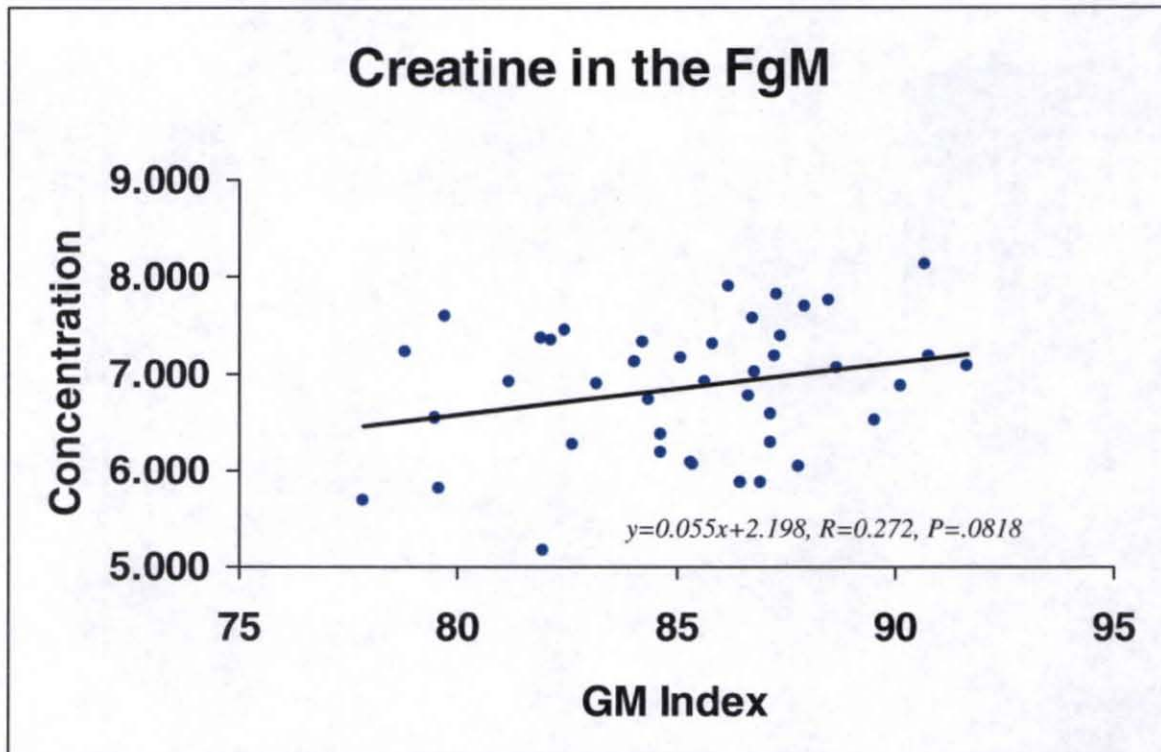


Figure 5.4.1: Creatine in the FgM Region.

In the FwR region, Glu showed significance with GM index (Figure 5.4.2) having a P value of .0104. Both the FgM and FwR regressions are limited to the relatively narrow range of GM in the voxel. However, the graphs show that correct placement of the voxel during scanning, both FgM and FwR, is consistent for different operators and over the range of subjects, and that typical voxels have a relatively small admixture of the “unwanted” tissue type.

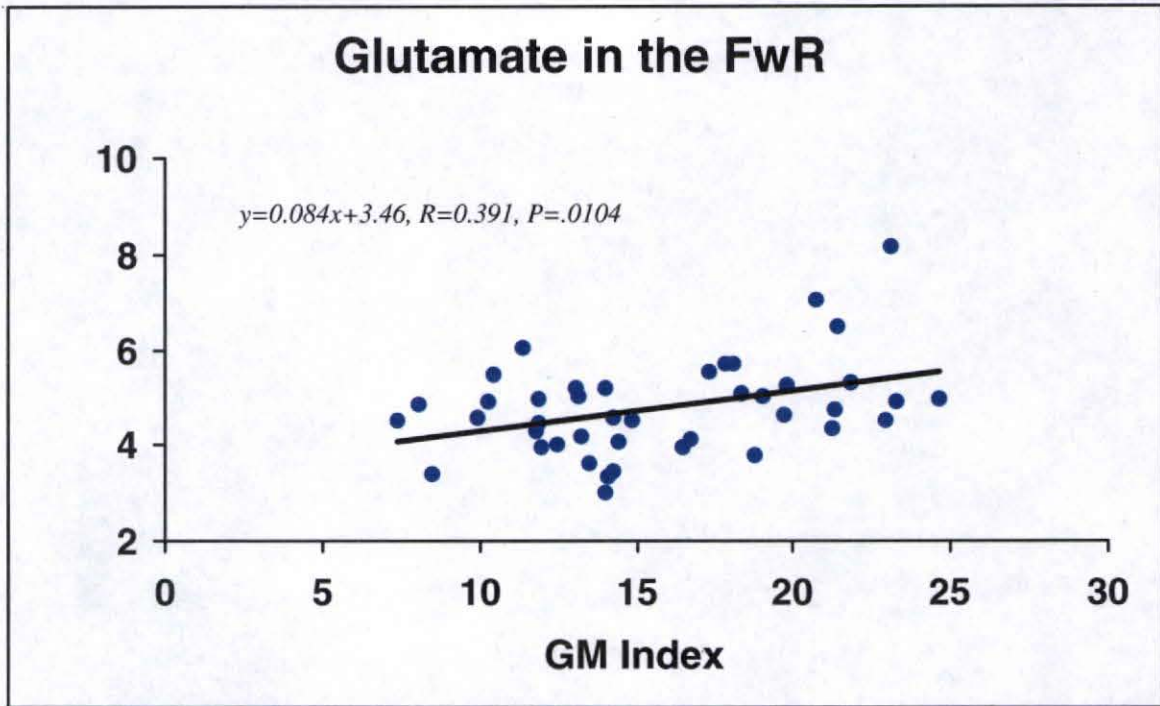


Figure 5.4.2: Glutamate in the FwR Region

Combined FgM and FwR partial volumes extend the range of the GM index therefore, allowing a greater range of data points to be used for correlation.

Regressions of combined partial volumes gave excellent results (figure 5.4.3).

Using the y intercept and slope of the regressions, a percent increase or decrease of

GM at 100% versus WM at 100% was calculated: $\Delta\%C = \frac{mx}{y_{incpt}}$ The slope for Cr

shows a 28% higher concentration in GM than WM. This contradicts Hetherington, 1994, who showed lower Cr concentrations of 20% in GM over WM (18). However,

Schuff, 2001, found an increase in Cr with GM of 11% (19), which is consistent with our results. Cho showed a negative slope (lower concentrations of 19% in GM than

WM), which was consistent in direction with both Hetherington's and Schuff's

findings (18 & 19). NAA showed an

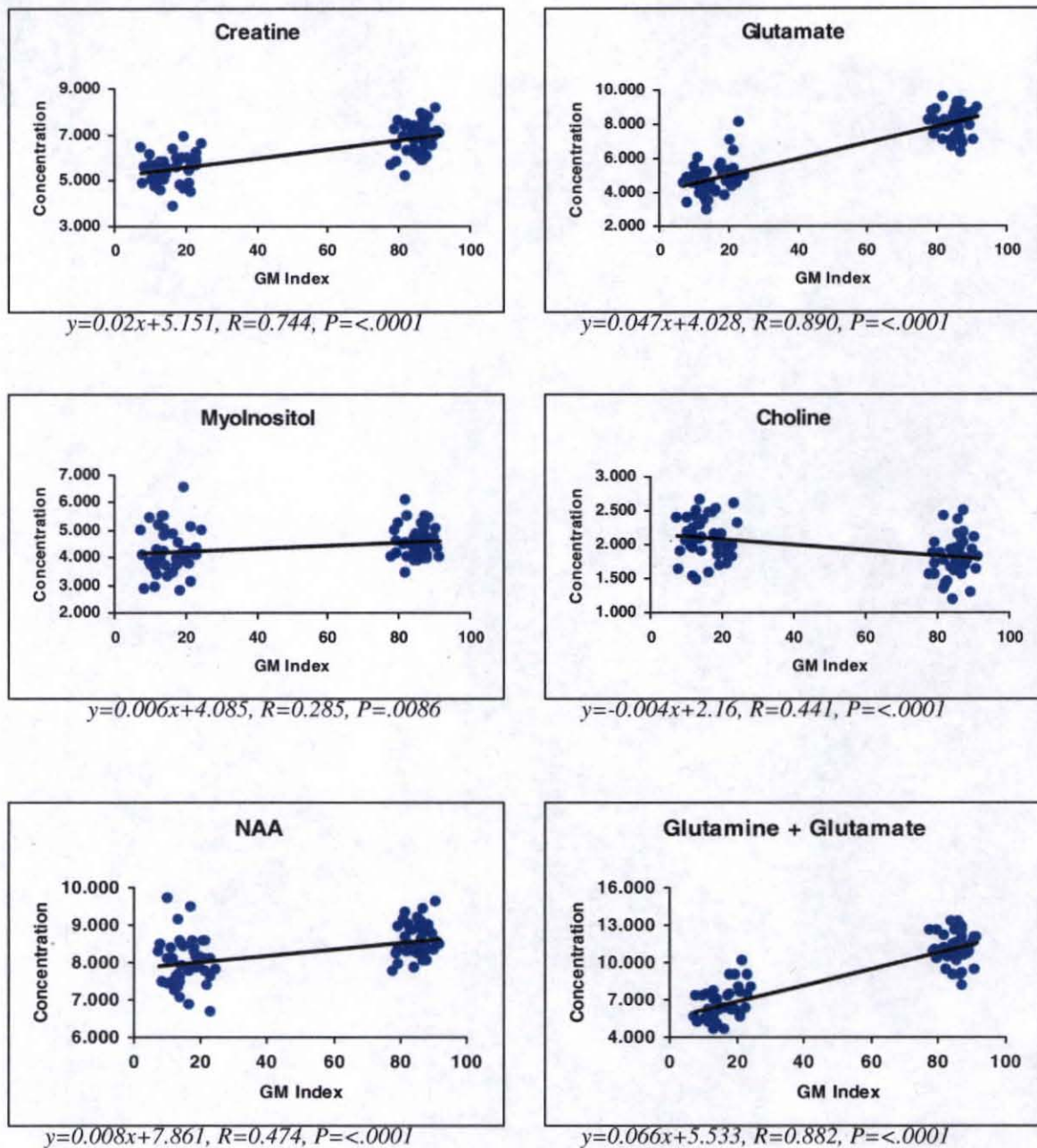


Figure 5.4.3: Regression of Metabolites

increase with GM index of 10%. This is consistent with Schuff's findings of 13% however contradicts Hetherington (18 & 19). Glu and Glx showed an increase of 117% and 119% respectively, and ml of 15%. Of note, neither Hetherington nor

Schuff were able to report on Glu/Glx or ml since they used a long echo time for spectrum acquisition, which suppresses these resonances.

CHAPTER 6 CONCLUSION

6.1 Segmentation

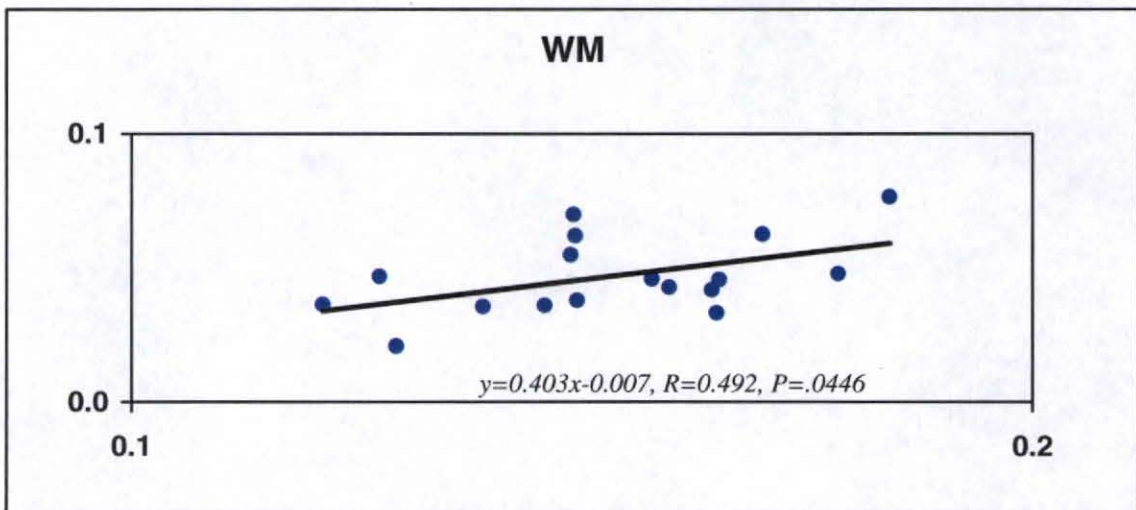
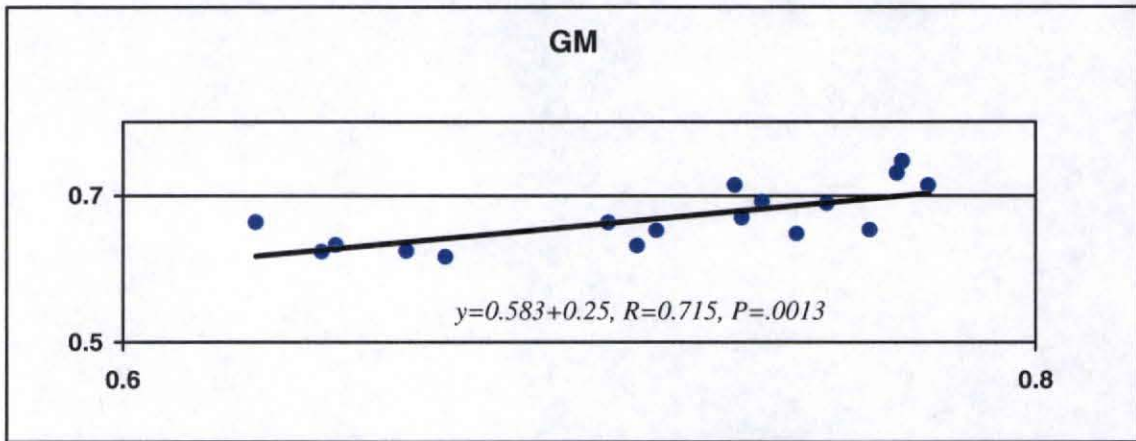
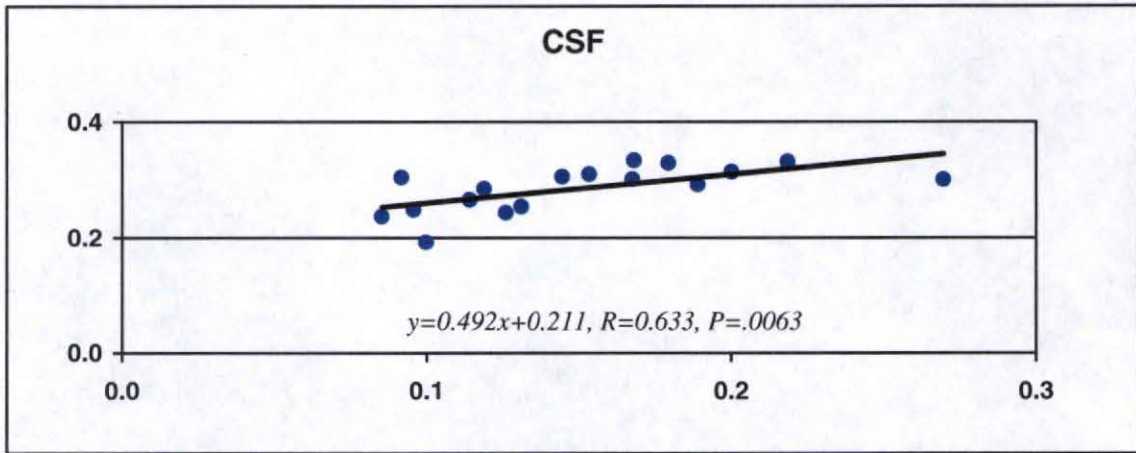
Due to registration errors during segmentation, SPM2 should be used in conjunction with skull stripped images and template to reduce the errors in partial volume calculations for MR spectroscopy voxels. FSL, without using any output options and hard output data, is recommended for the segmentation of images that will be used for partial volume calculations of MR spectroscopy voxels and for correct correlation of metabolite concentrations versus partial volumes.

6.2 Metabolite Concentrations

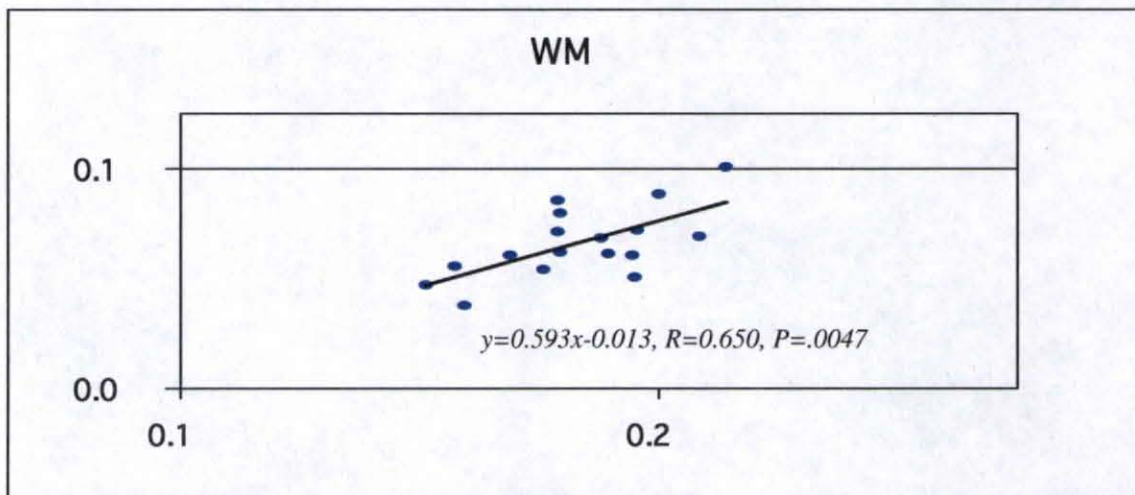
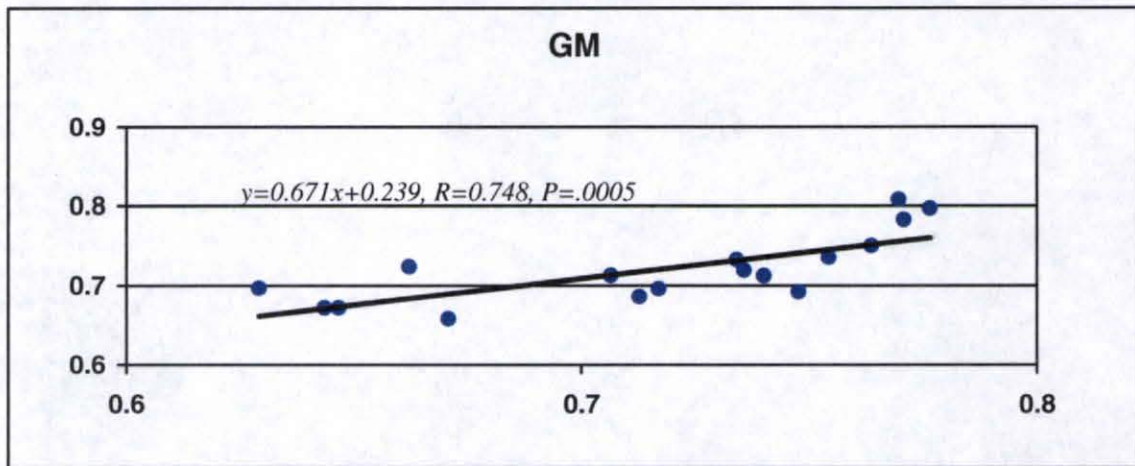
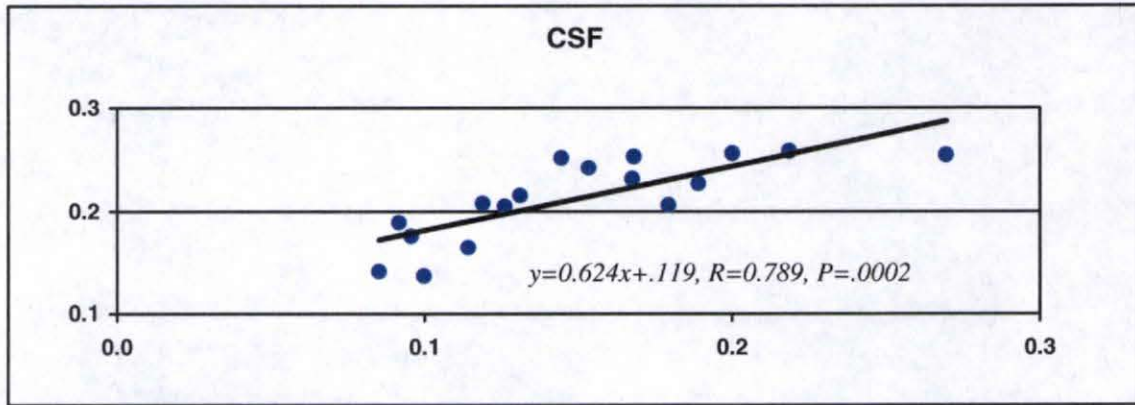
Due to the increase in accuracy in partial volume calculations, correct interpolation and correlations for metabolites versus GM index was accomplished. There is a 28% increase in Cr, 117% increase in Glu, 15% increase in ml, 19% decrease in Cho and a 13% increase in Glx from 100% WM to 100% GM. Most of these findings are in agreement with those of prior studies.

**APPENDIX A
CORRELATION GRAPHS**

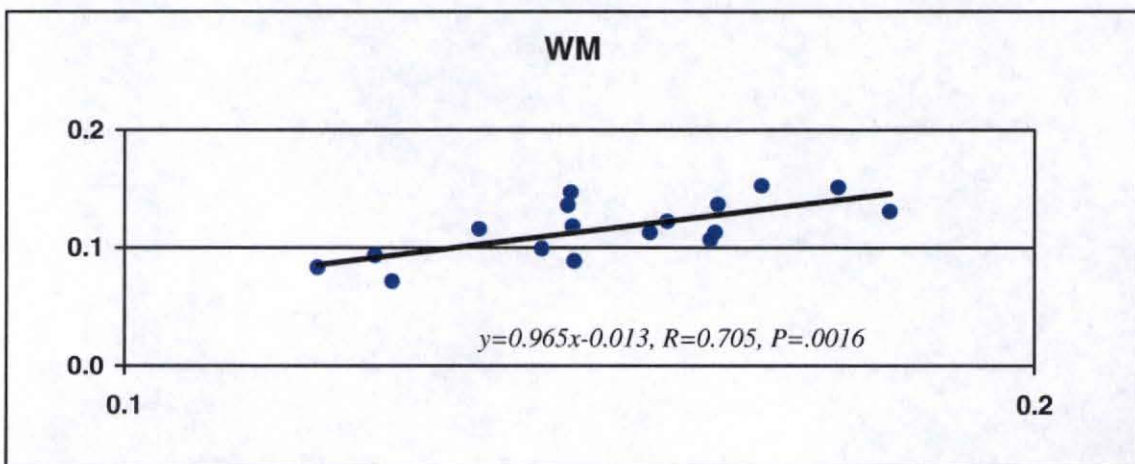
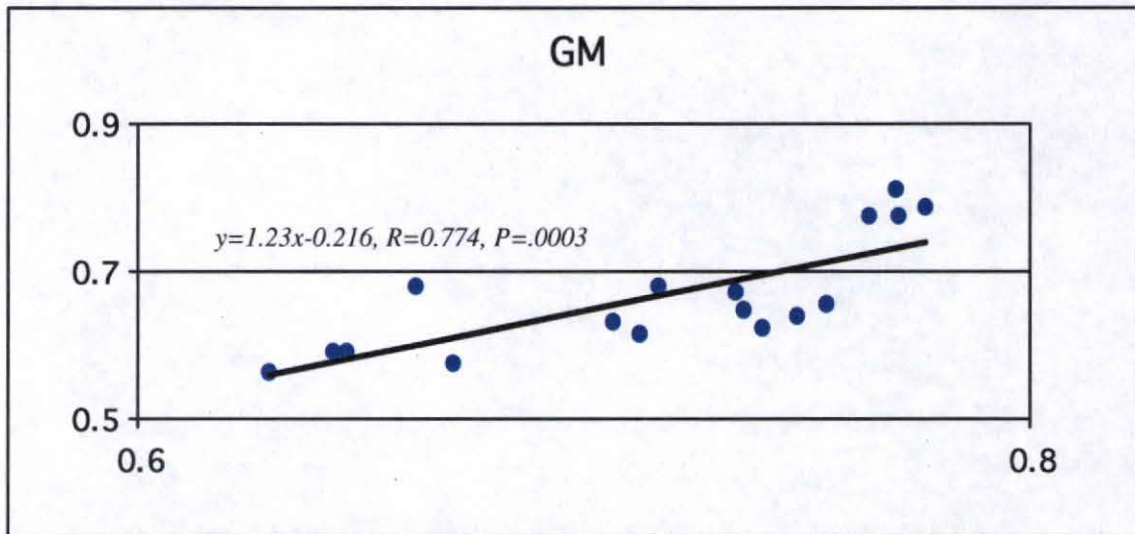
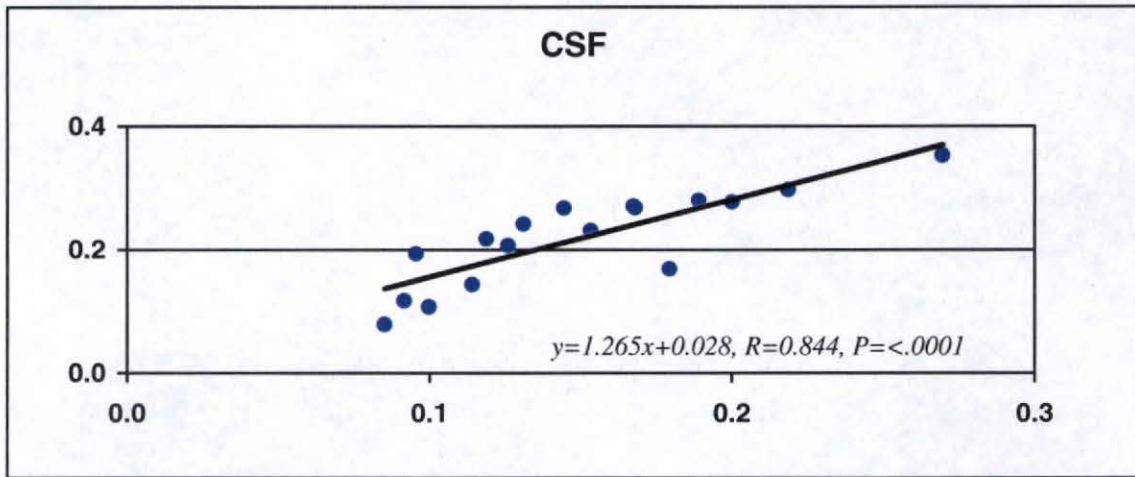
SPM2



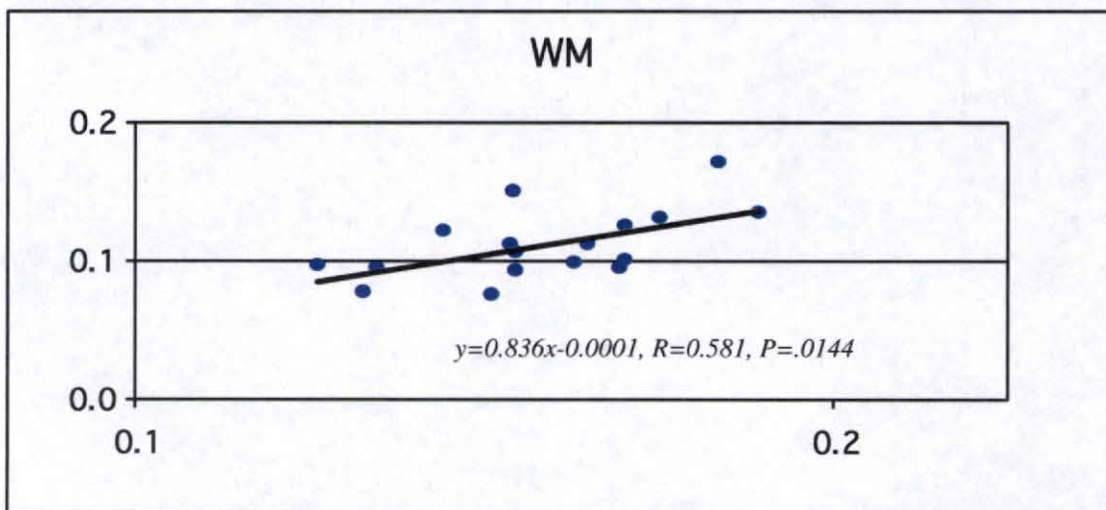
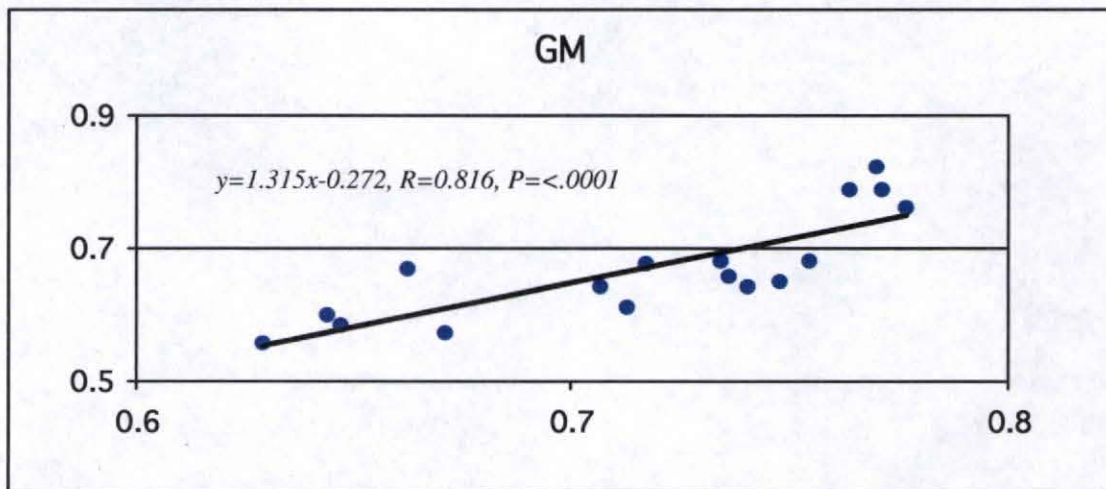
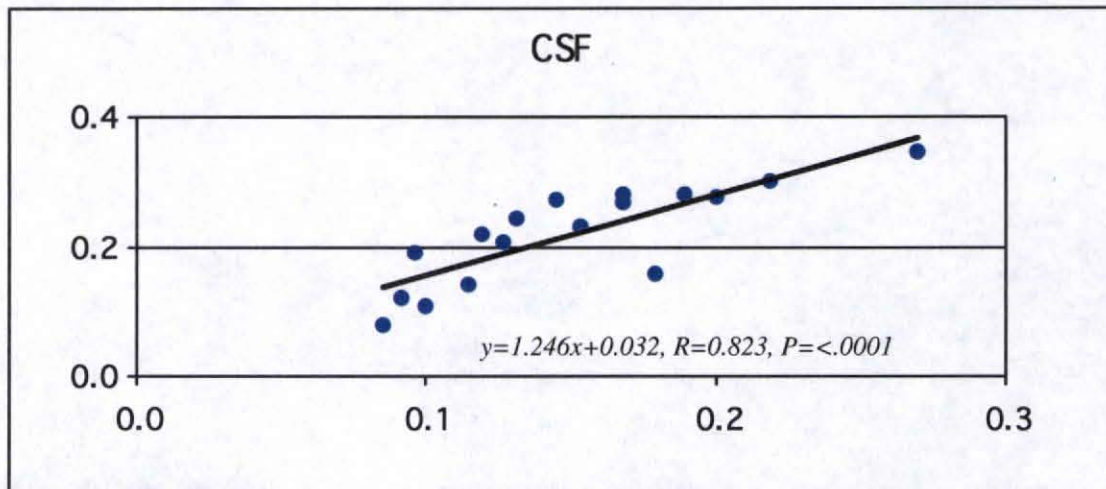
SPM2 WITH SKULL STRIPPED IMAGES AND TEMPLATE



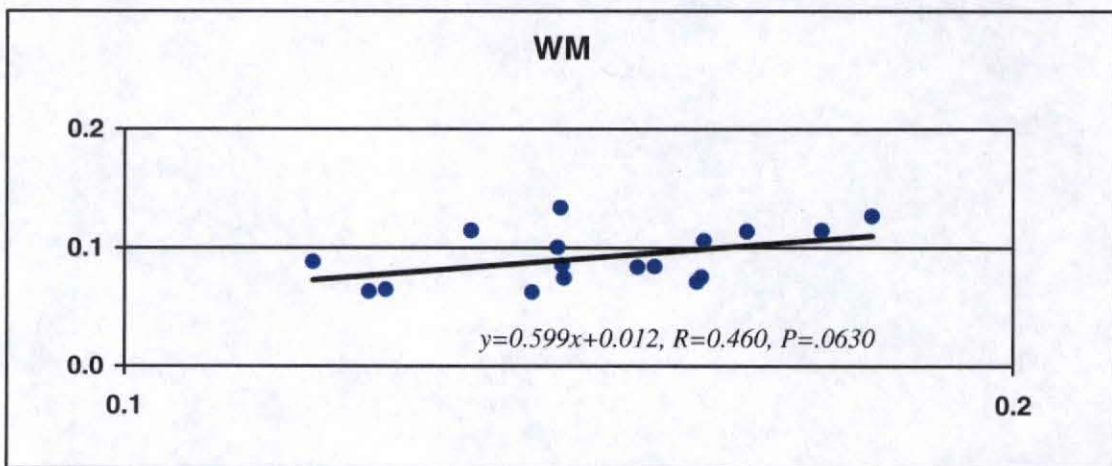
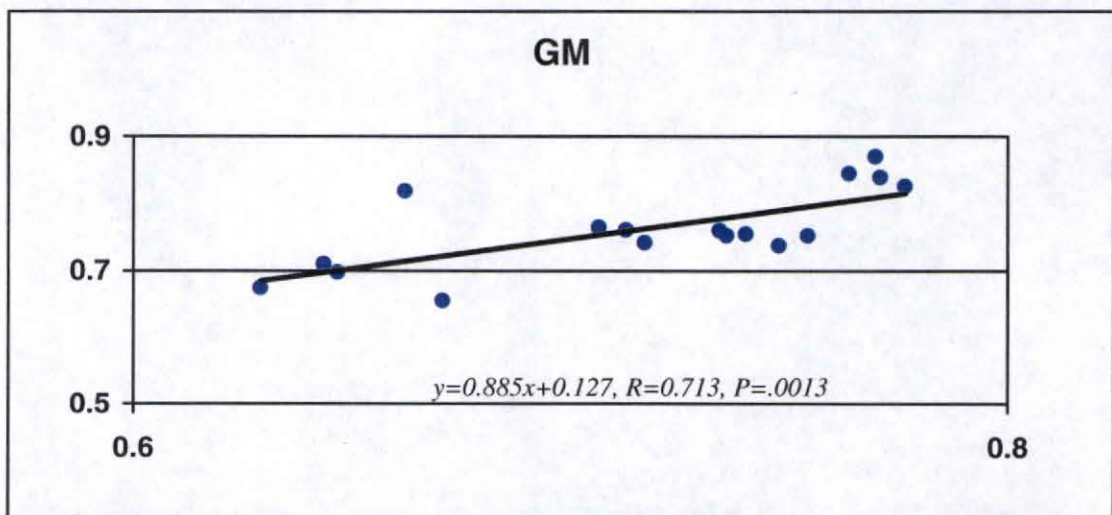
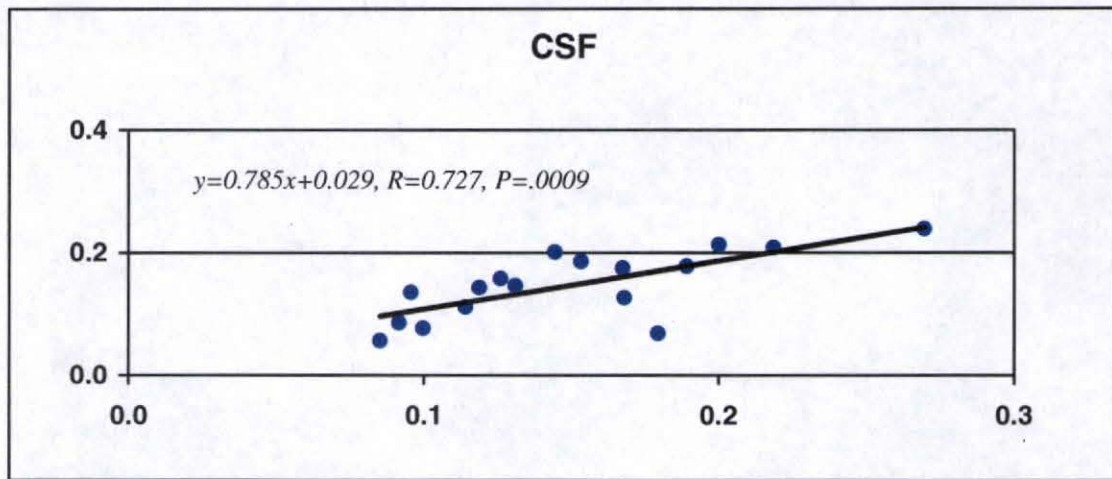
FSL, NO A-PRIORI, HARD DATA OUTPUT



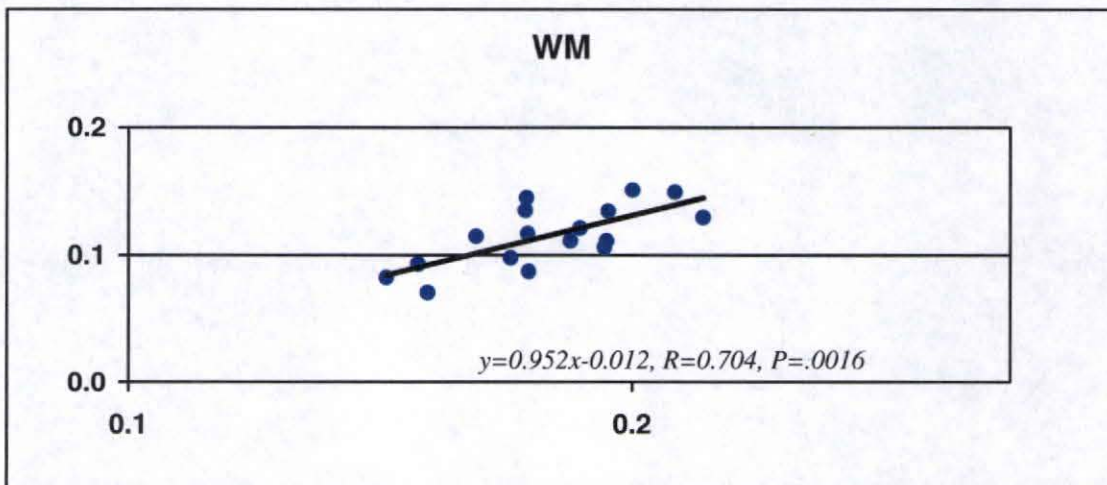
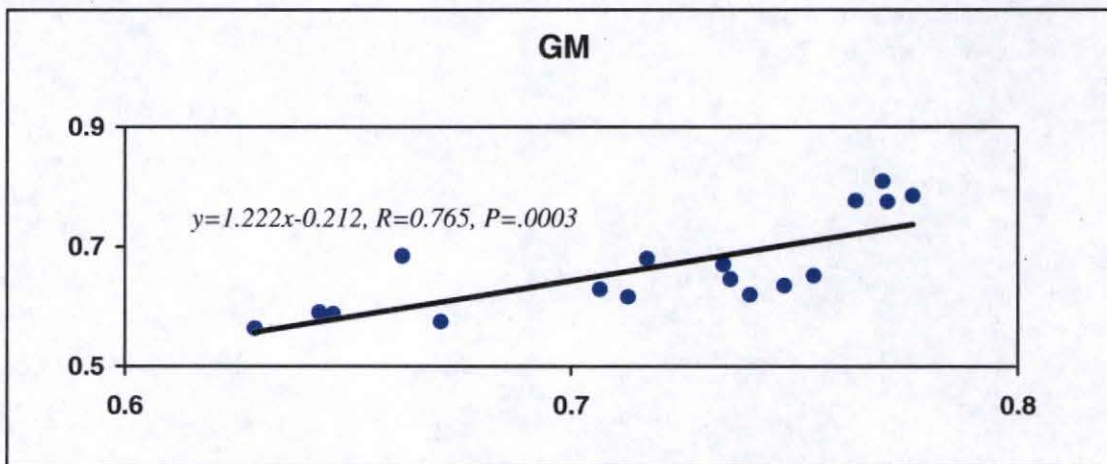
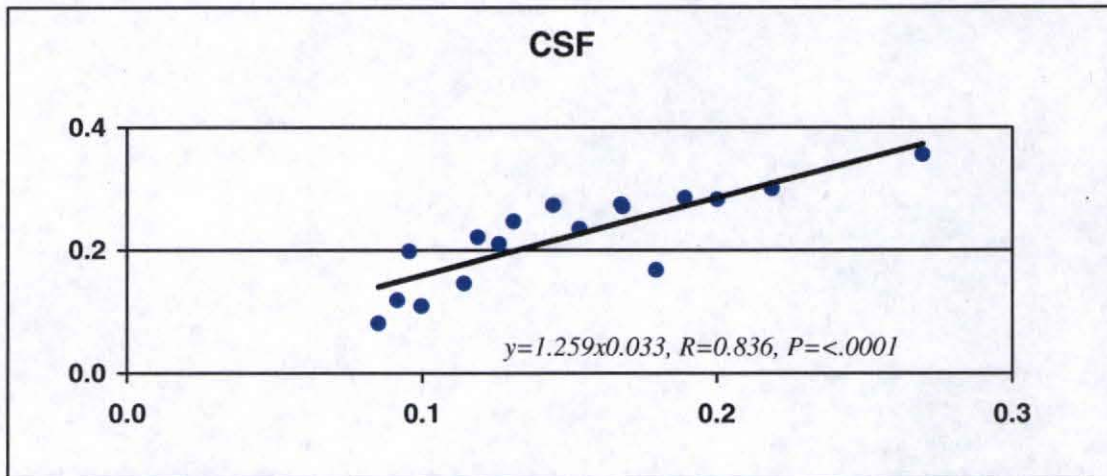
FSL, INITIAL A-PRIORI, HARD DATA OUTPUT



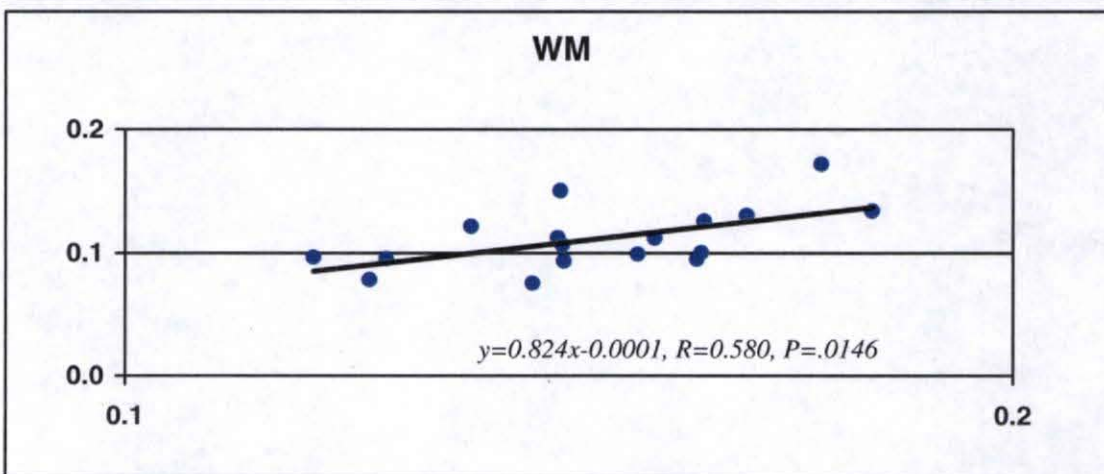
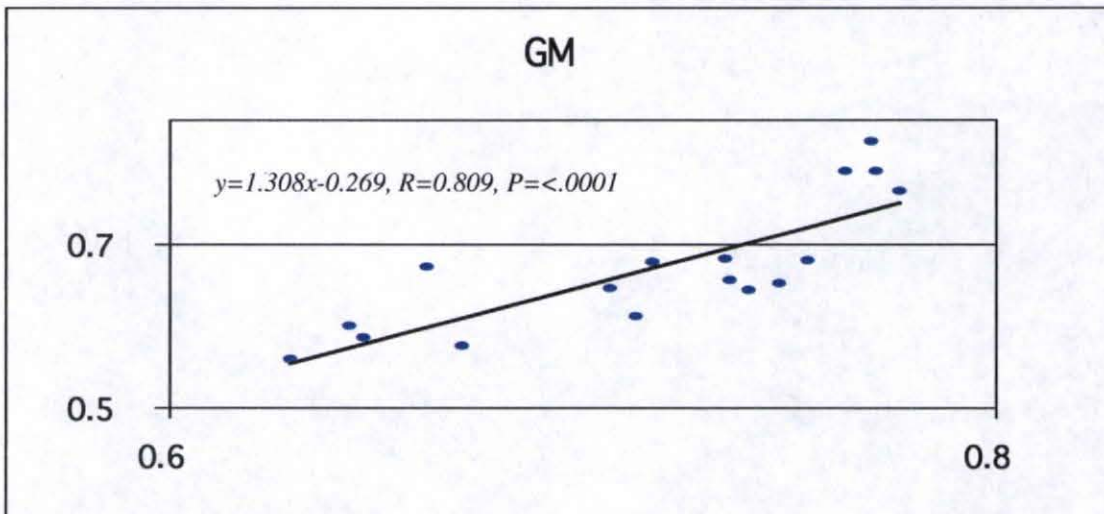
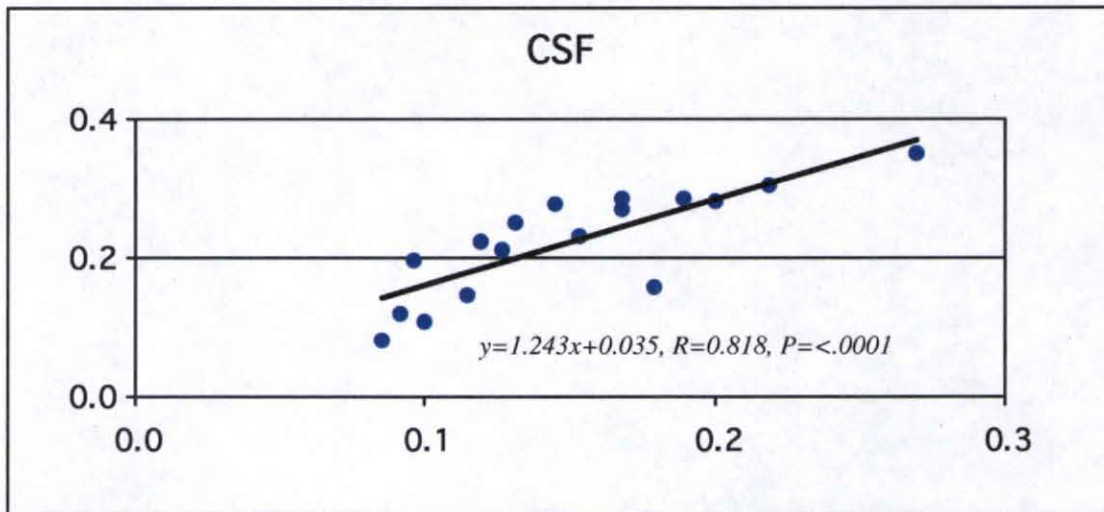
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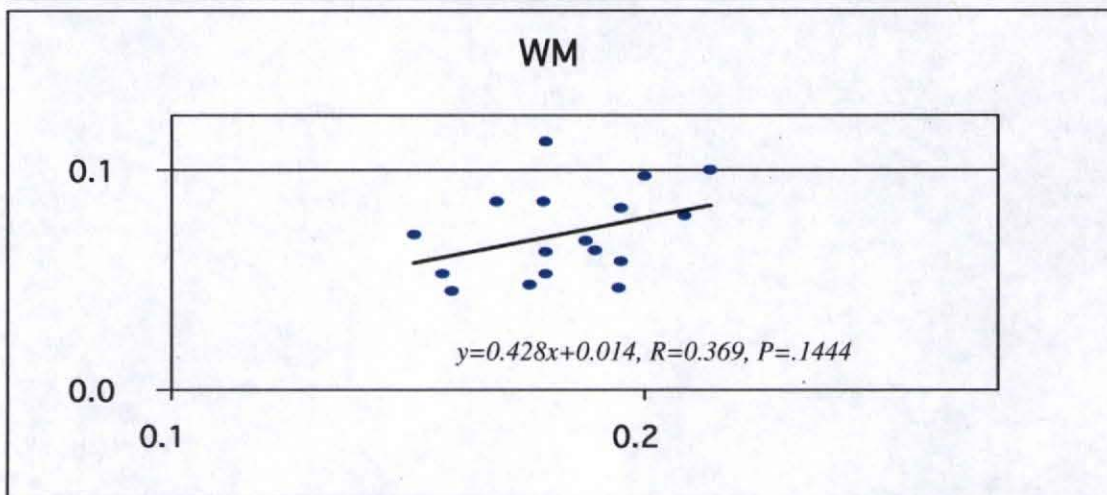
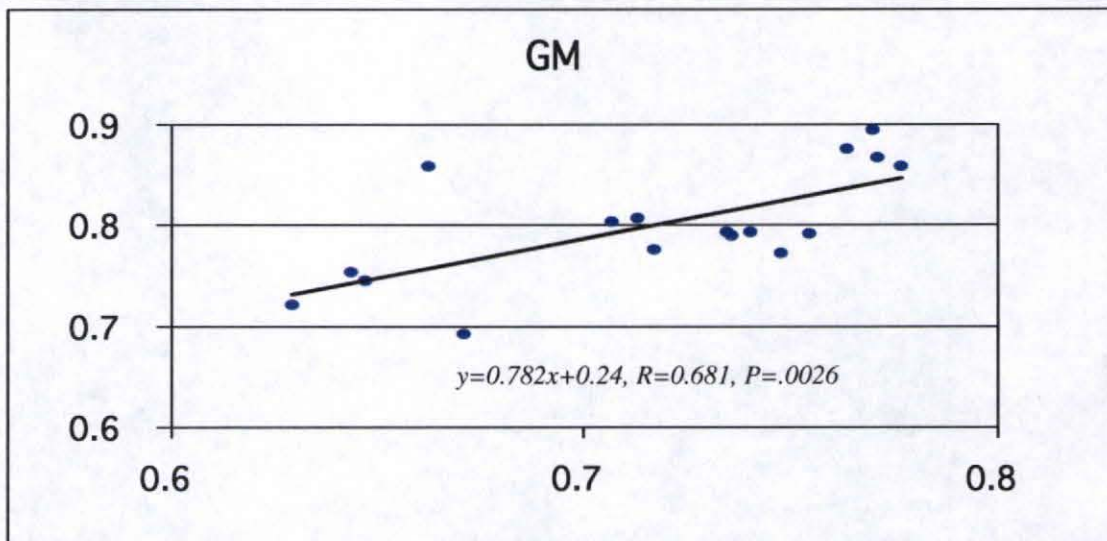
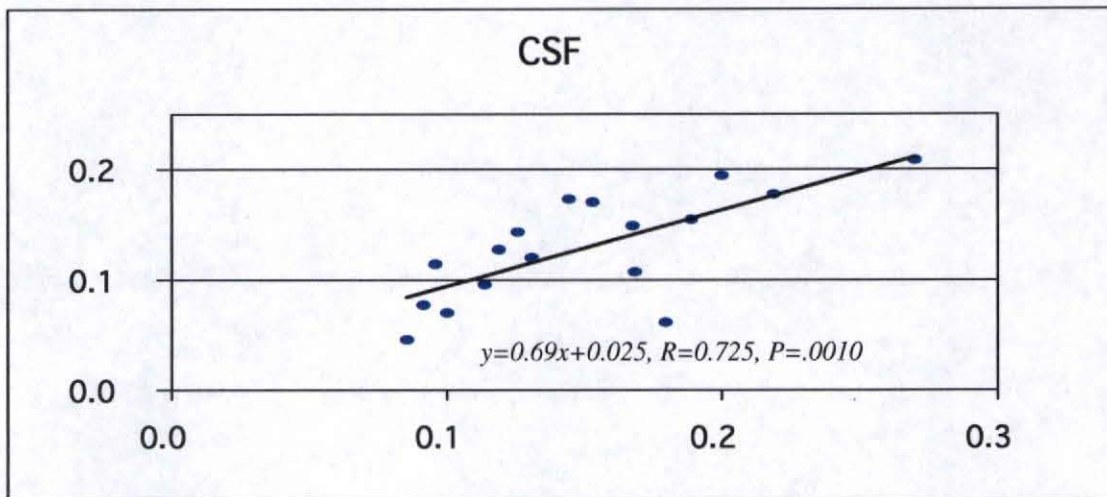
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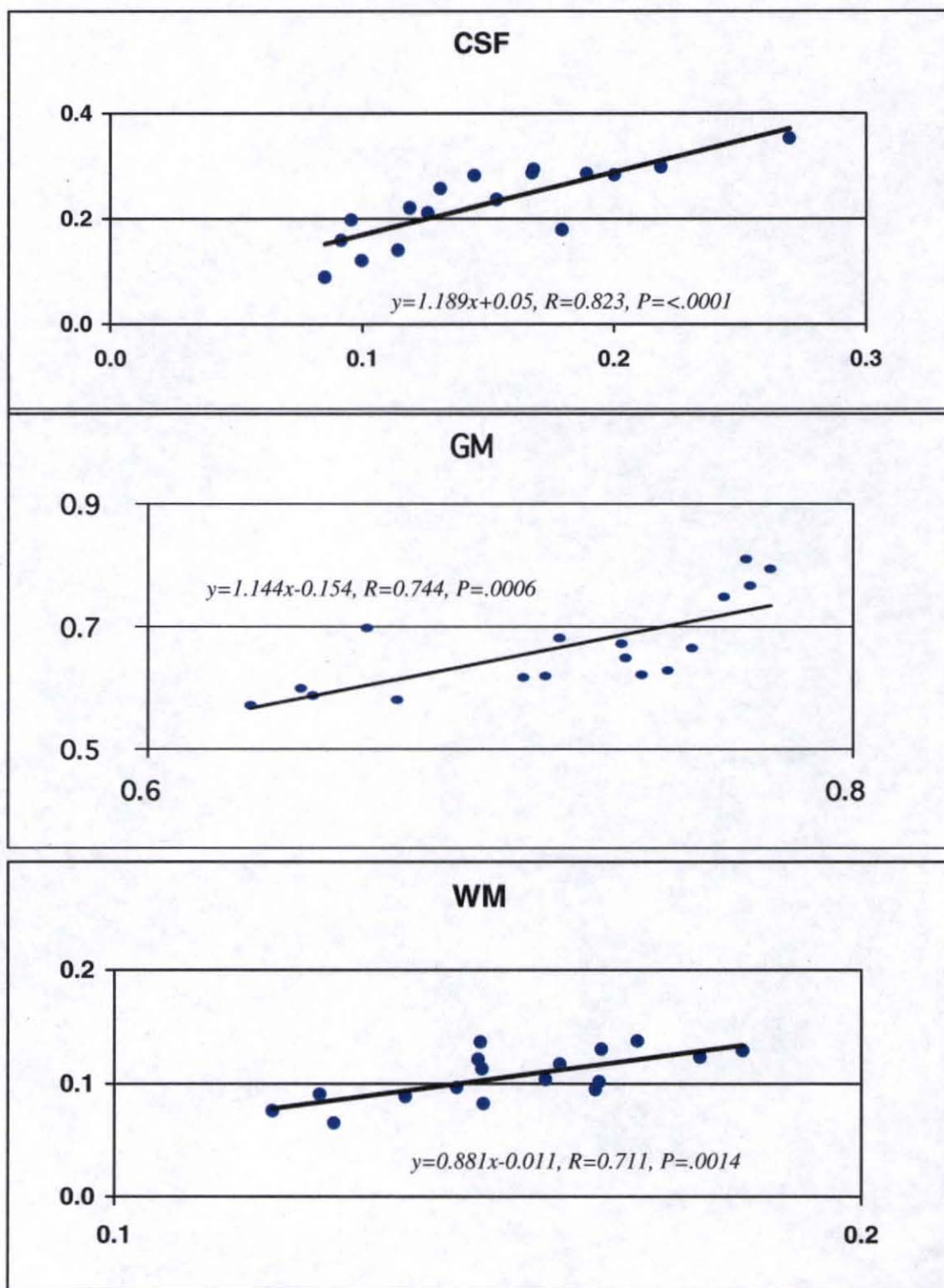
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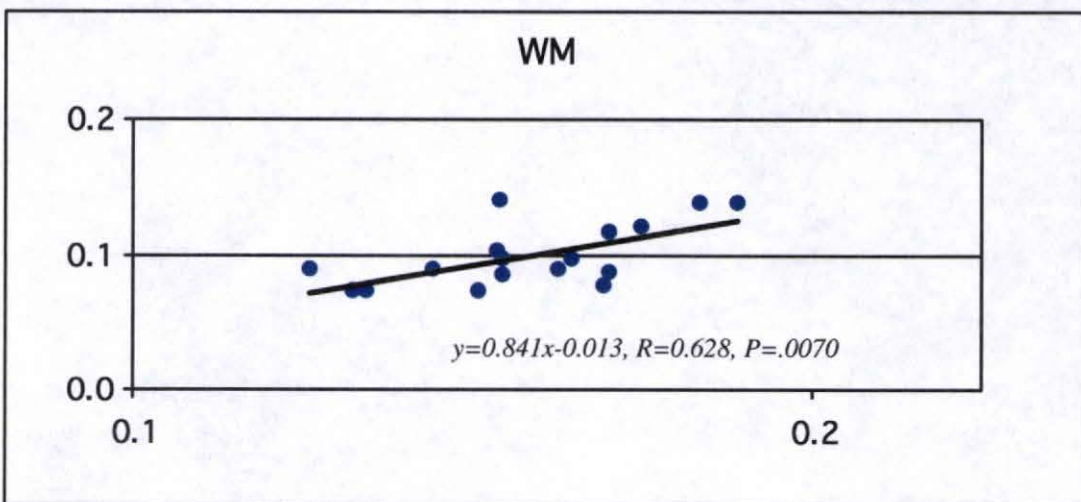
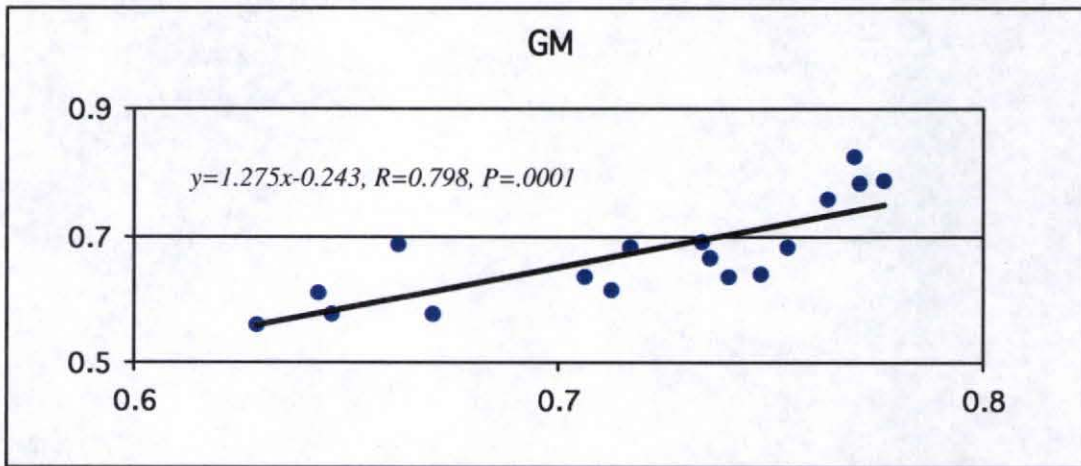
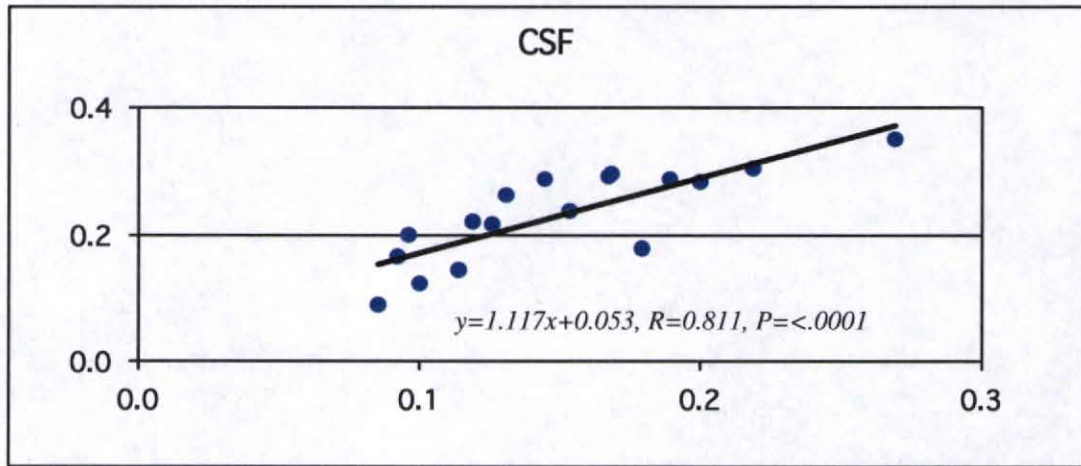
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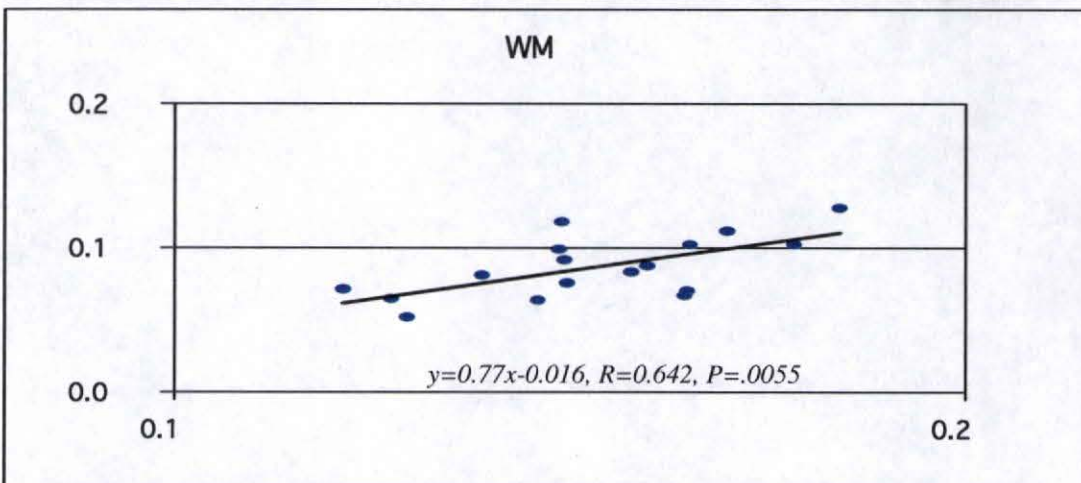
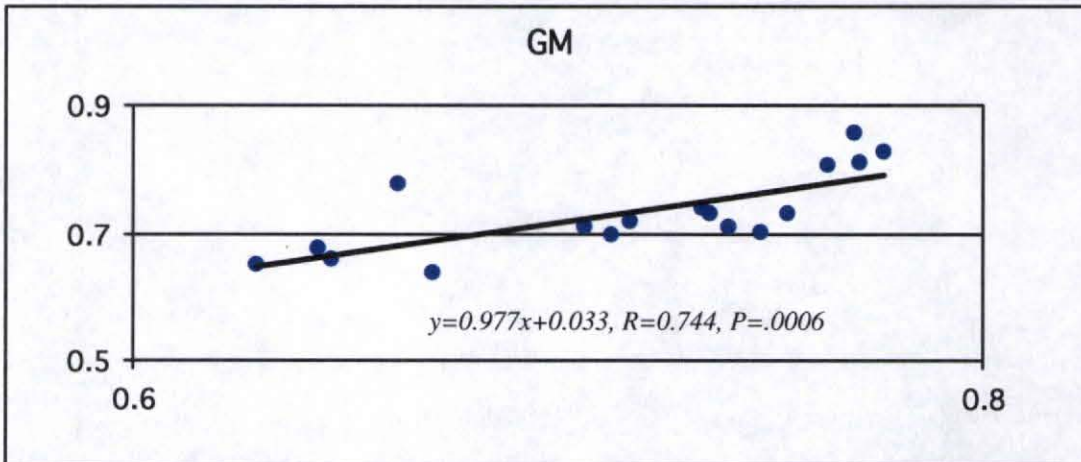
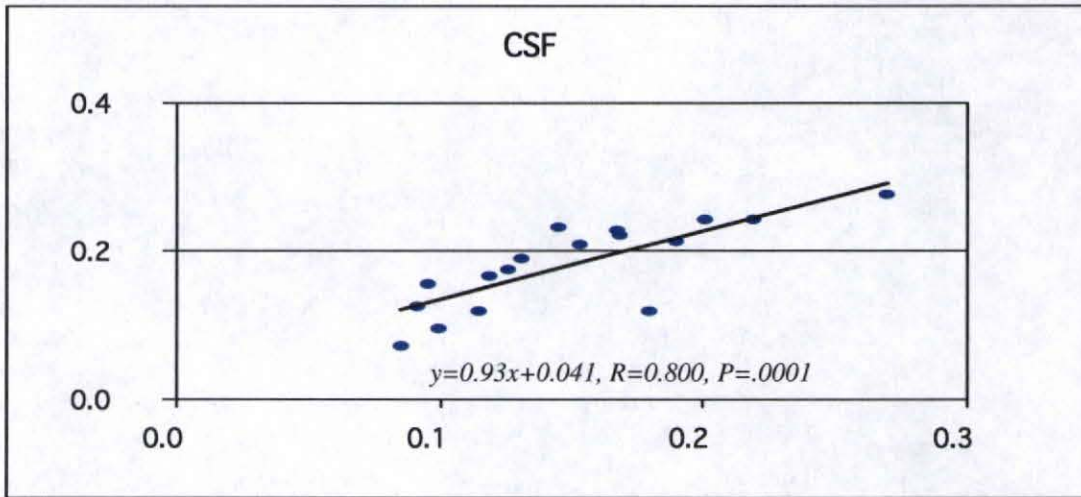
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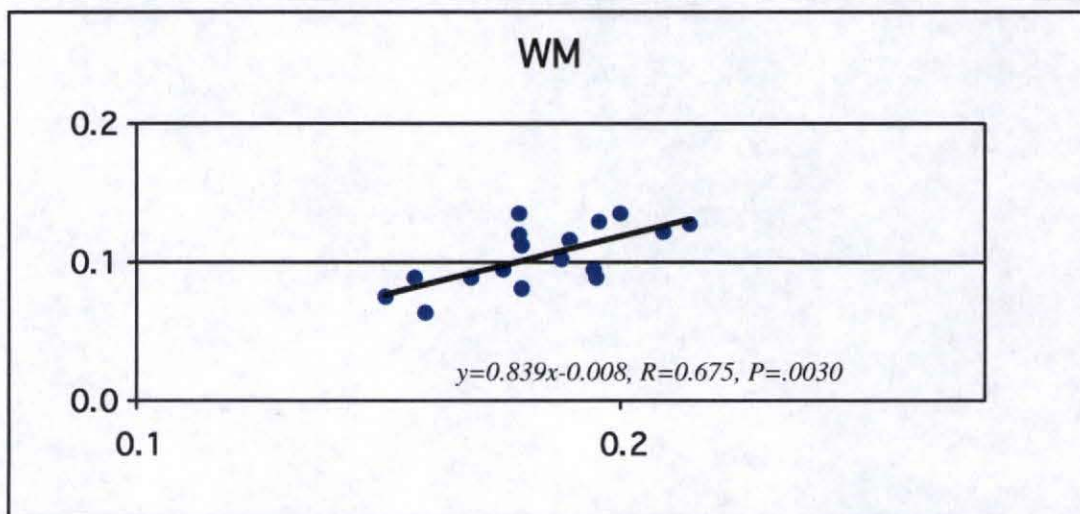
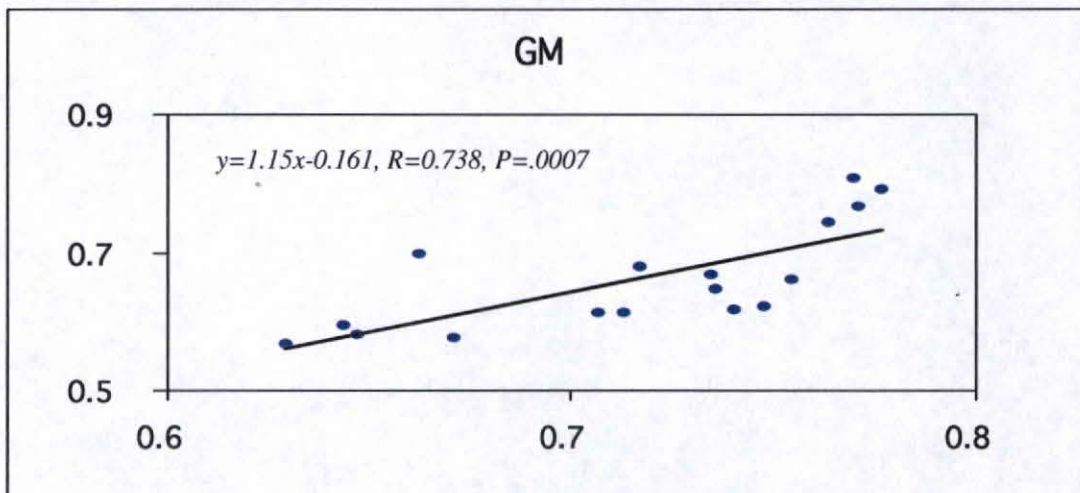
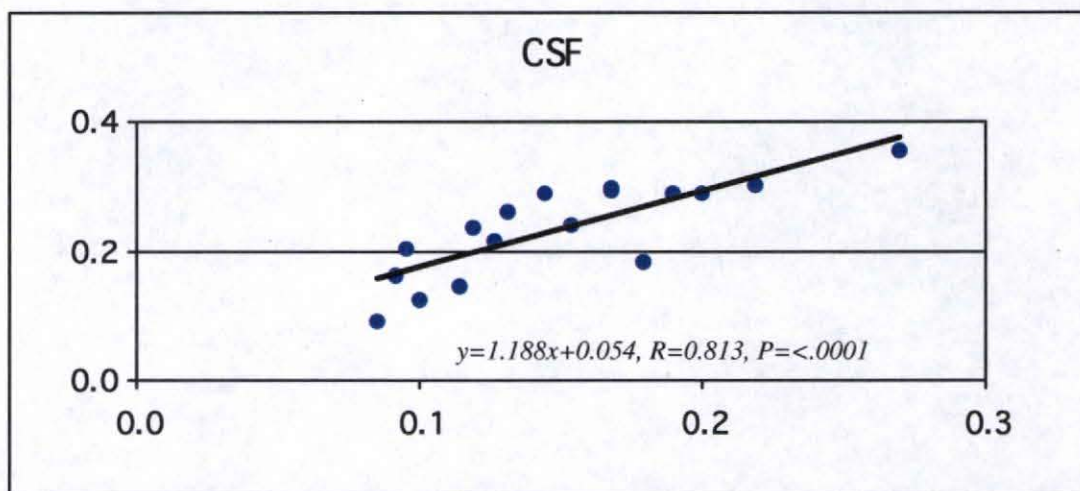
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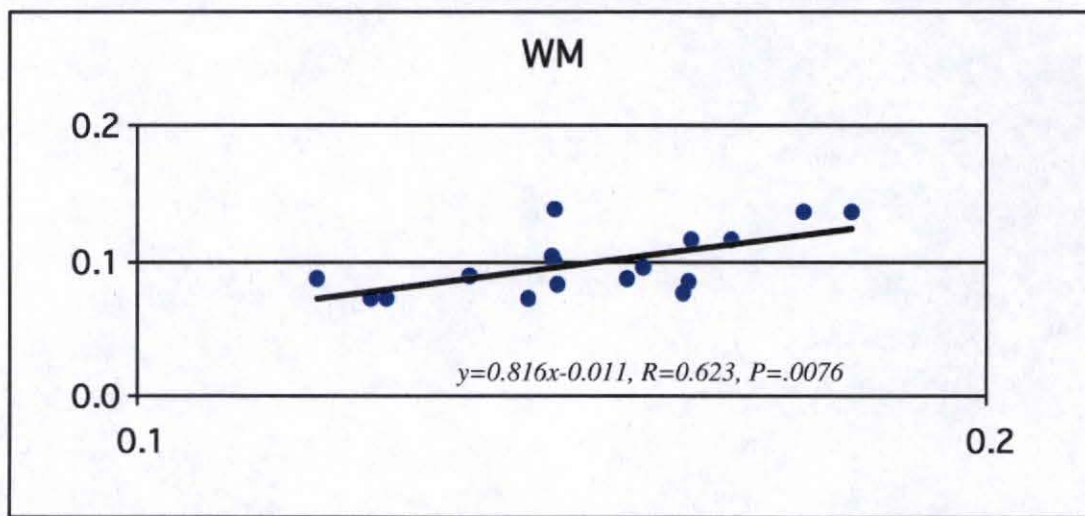
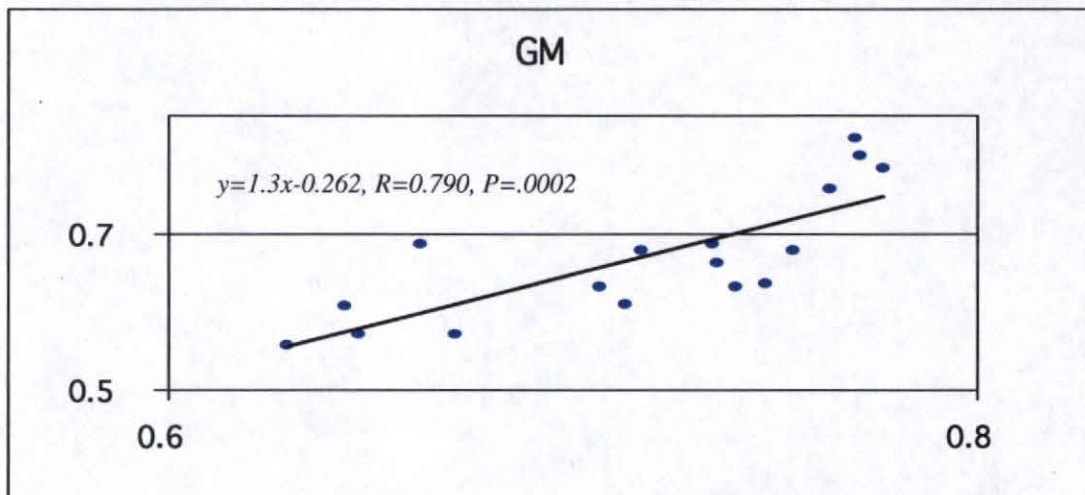
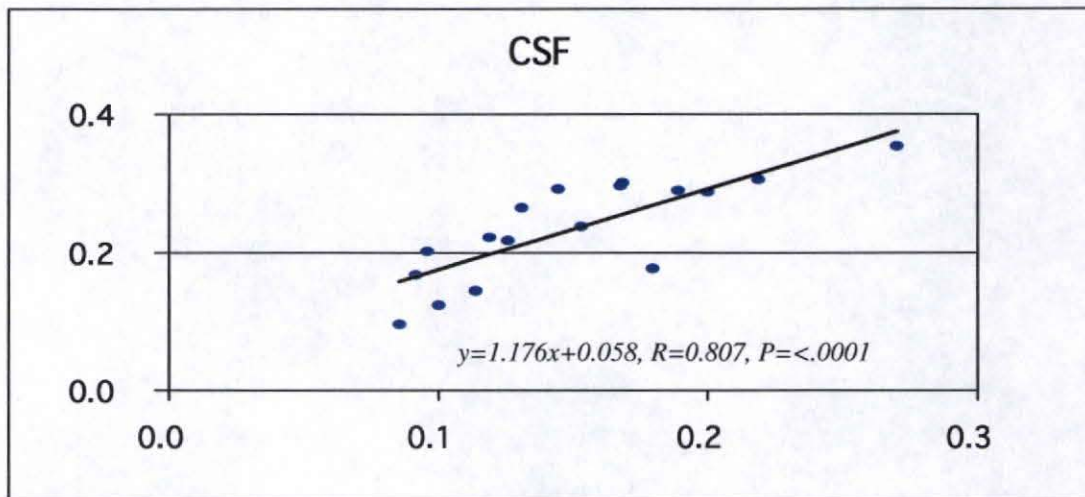
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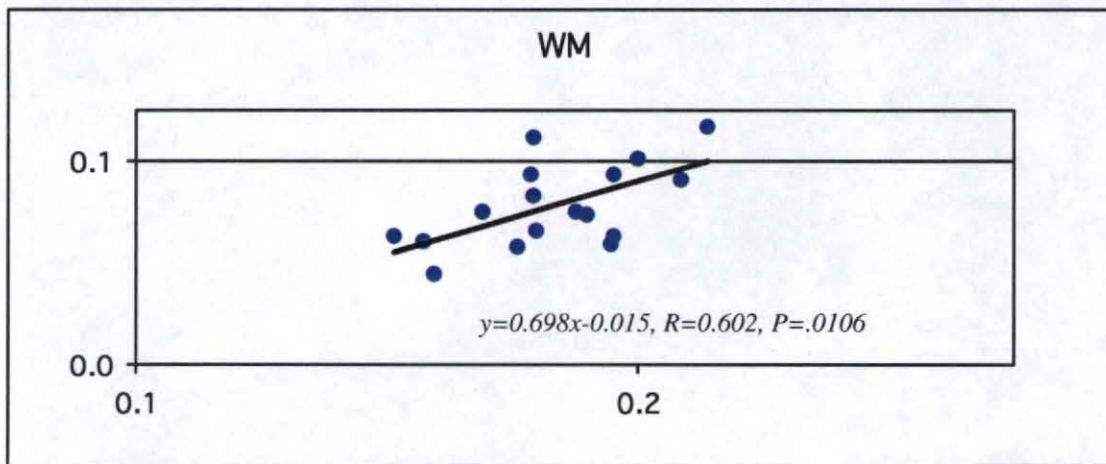
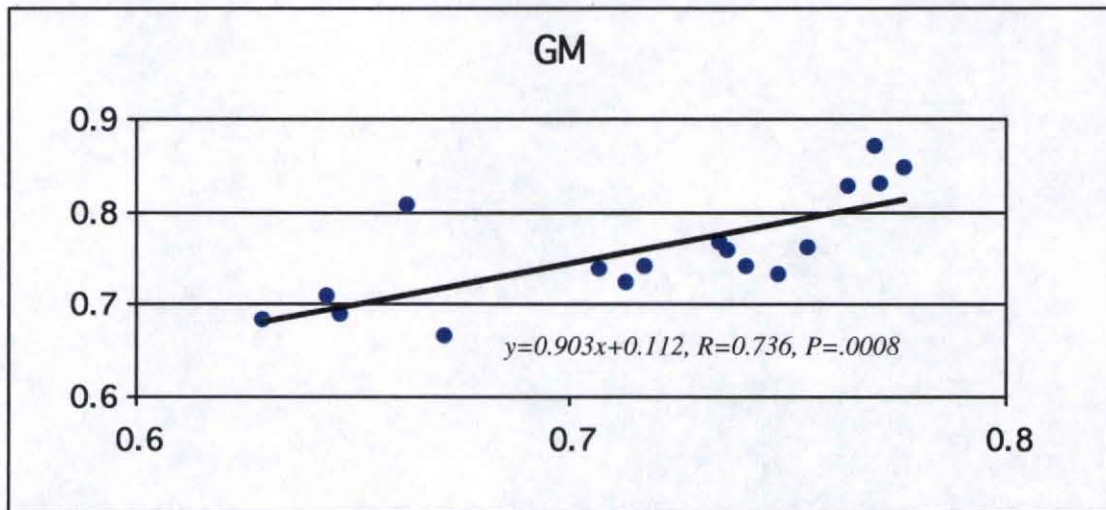
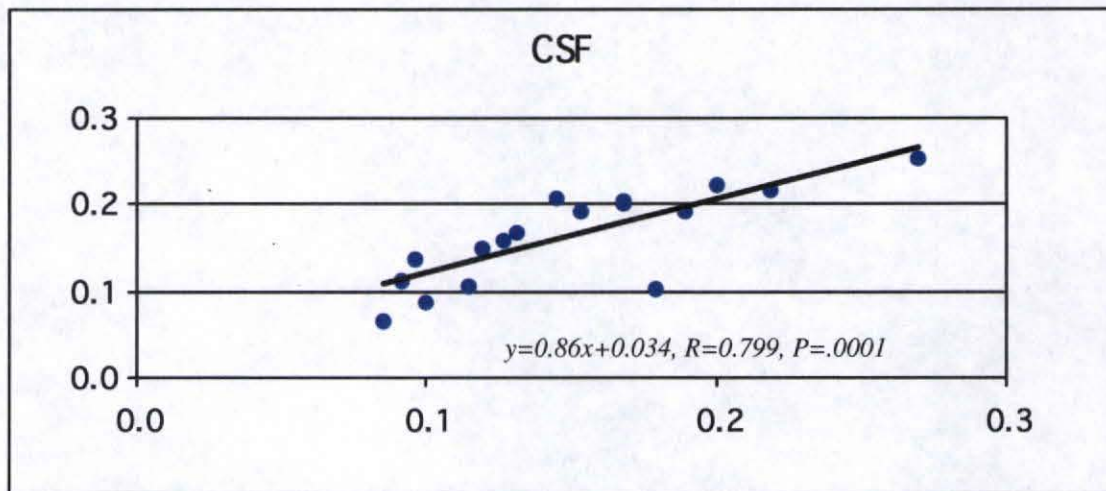
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FSL, INITIAL A-PRIORI, PRE-PROCESSED IMAGES, SOFT DATA OUTPUT



FSL, BOTH A-PRIORI, PRE-PROCESSED IMAGES, SOFT DATA OUTPUT



**APPENDIX B
METABOLITE CONCENTRATIONS**

FgM	CSF	GM	WM	GM Index	Cr	Glu	ml	Cho	NAA	Glx
40005	0.2170	0.6470	0.1360	82.63	6.258	7.958	3.996	1.409	9.006	9.393
40008	0.1424	0.7863	0.0714	91.68	7.082	8.985	4.043	1.828	8.488	11.965
40009	0.1056	0.7765	0.1179	86.82	7.004	9.076	3.954	2.082	8.382	13.015
40010	0.2306	0.6811	0.0883	88.52	7.754	8.282	5.451	1.787	9.003	11.216
50001	0.2415	0.6230	0.1355	82.14	7.338	9.595	6.079	1.770	8.309	12.120
50002	0.0778	0.8100	0.1123	87.82	6.032	8.431	4.083	1.777	8.499	10.606
50007	0.1926	0.6553	0.1520	81.17	6.914	7.819	4.450	1.872	9.158	10.709
50012	0.1674	0.6819	0.1508	81.89	7.362	8.145	4.546	2.431	8.689	11.154
50014	0.2671	0.6398	0.0931	87.30	7.810	9.212	4.813	2.493	8.741	12.070
50016	0.2678	0.6169	0.1154	84.24	7.327	8.111	4.697	1.853	8.282	13.338
50017	0.2058	0.6724	0.1219	84.65	6.176	8.829	4.324	1.197	8.313	9.004
50019	0.2974	0.5906	0.1120	84.06	7.111	7.978	4.458	1.913	8.856	13.221
50026	0.2794	0.5905	0.1301	81.95	5.158	7.013	3.399	1.344	9.337	10.705
50061	0.1156	0.7779	0.1065	87.96	7.702	8.597	3.941	1.684	8.042	10.777
50062	0.2773	0.5763	0.1465	79.73	7.601	8.859	5.275	2.035	8.316	12.588
50130	0.1551	0.7344	0.1104	86.93	5.865	6.328	4.917	1.562	8.041	9.043
50135	0.2703	0.6311	0.0986	86.49	5.872	7.484	4.626	1.953	8.294	11.235
50144	0.1611	0.6675	0.1714	79.57	5.811	7.339	4.135	1.543	7.968	10.321
50164	0.1610	0.7165	0.1225	85.40	6.058	7.268	3.874	1.757	8.236	10.433
50204	0.2130	0.6863	0.1008	87.19	6.582	7.121	5.505	1.732	8.200	8.180
50209	0.1241	0.7845	0.0914	89.57	6.509	7.879	4.643	1.290	8.783	10.872
50211	0.0686	0.7926	0.1388	85.10	7.149	8.500	4.825	1.601	8.274	11.137
60001	0.1681	0.6860	0.1459	82.46	7.436	8.191	5.488	1.395	8.531	10.942
60002	0.1512	0.7285	0.1203	85.83	7.299	9.213	4.427	2.152	9.110	11.572
60003	0.2580	0.6263	0.1158	84.40	6.711	6.636	3.857	1.661	8.328	11.172
60004	0.3703	0.5586	0.0711	88.70	7.049	8.521	4.520	1.753	8.891	10.656
60007	0.1070	0.7804	0.1126	87.39	7.389	6.832	4.292	2.039	8.298	9.199
60012	0.2108	0.6736	0.1156	85.35	6.069	8.891	4.588	1.613	9.197	12.502
60014	0.3528	0.5643	0.0830	87.18	6.291	8.483	4.879	1.748	8.077	12.520
60023	0.1676	0.7046	0.1278	84.65	6.371	7.034	4.138	1.850	7.852	11.472
60043	0.4574	0.4921	0.0505	90.69	8.135	8.594	5.048	2.104	9.620	12.060
60047	0.2370	0.6620	0.1010	86.76	7.582	7.673	4.810	2.163	9.072	10.520
60048	0.1913	0.6974	0.1114	86.23	7.909	8.822	5.259	2.365	8.938	13.254
60063	0.1316	0.6844	0.1840	78.81	7.220	8.731	4.858	1.862	8.227	10.826
60064	0.2439	0.5885	0.1676	77.83	5.680	8.208	3.993	1.543	7.784	12.600
60065	0.0791	0.7318	0.1891	79.47	6.525	8.263	4.601	1.719	8.970	10.937
60072	0.1895	0.6743	0.1363	83.19	6.893	7.787	4.463	1.438	8.839	10.461
60076	0.2575	0.6743	0.0683	90.80	7.168	7.050	4.357	1.632	8.560	9.476
60082	0.2053	0.6890	0.1058	86.69	6.770	8.123	4.209	1.831	8.598	11.577
60096	0.1899	0.7070	0.1030	87.28	7.168	8.381	5.149	2.195	9.409	12.820
60097	0.1373	0.7779	0.0849	90.16	6.858	8.603	4.713	1.928	8.322	11.599
60099	0.1965	0.6883	0.1153	85.65	6.914	8.233	4.240	1.948	8.367	10.213

FwR	CSF	GM	WM	GM Index	Cr	Glu	ml	Cho	NAA	Glx
40005	0.0023	0.1438	0.8540	14.41	5.266	4.062	3.660	2.108	8.494	5.025
40008	0.0049	0.1300	0.8651	13.06	4.575	5.199	3.984	1.483	7.638	7.756
40009	0.0089	0.1036	0.8875	10.45	5.026	5.490	3.707	2.397	8.073	5.490
40010	0.0093	0.1394	0.8514	14.07	5.605	3.344	4.197	2.313	8.558	
50001	0.0204	0.1944	0.7853	19.84	6.889	5.234	6.561	2.153	8.007	9.018
50002	0.0016	0.1138	0.8846	11.40	4.751	6.039	2.893	1.978	8.388	7.434
50007	0.0359	0.1148	0.8494	11.91	5.081	4.889	4.005	2.243	8.034	5.589
50012	0.0021	0.1421	0.8558	14.24	5.605	3.433	4.202	2.671	7.499	6.962
50014	0.0103	0.1386	0.8511	14.00	5.737	2.989	5.516	2.292	7.041	4.789
50016	0.0419	0.2219	0.7363	23.16	5.622	8.137	4.172	1.825	6.685	9.014
50017	0.0009	0.0743	0.9249	7.44	6.422	4.489	5.013	2.397	8.269	5.532
50019	0.0316	0.2389	0.7295	24.67	6.579	4.860	4.883	2.321	7.798	7.939
50026	0.0139	0.2155	0.7706	21.85	5.539	5.327	5.084	1.831	7.373	10.187
50061	0.0001	0.0846	0.9153	8.46	5.557	3.396	2.834	1.893	7.484	5.141
50062	0.0171	0.1779	0.8050	18.10	5.770	5.894	4.518	2.164	8.398	8.993
50130	0.0060	0.1315	0.8625	13.23	5.325	4.180	3.891	2.497	7.326	4.599
50135	0.0350	0.1208	0.8443	12.52	4.973	4.002	5.161	2.412	7.228	5.669
50144	0.0013	0.1904	0.8084	19.06	5.936	5.040	3.899	1.961	8.157	6.149
50164	0.0091	0.0986	0.8923	9.95	5.641	4.552	3.805	2.063	7.450	7.295
50204	0.0006	0.1354	0.8640	13.55	5.267	3.608	5.530	2.364	7.654	5.511
50209	0.0226	0.1636	0.8138	16.74	3.885	4.128	3.453	1.573	6.861	4.591
50211	0.0005	0.1781	0.8214	17.82	5.926	5.690	3.928	2.096	8.595	7.346
60001	0.0168	0.2040	0.7793	20.75	5.940	7.061	4.215	2.031	8.559	8.018
60002	0.0025	0.2138	0.7838	21.43	4.458	6.448	3.117	1.705	7.766	7.577
60003	0.0054	0.2318	0.7629	23.30	5.892	4.930	4.316	2.613	8.099	7.420
60004	0.0116	0.1386	0.8498	14.02	5.041	5.167	4.821	2.080	7.608	
60007	0.0124	0.1625	0.8251	16.45	6.367	3.980	4.831	2.482	7.748	6.327
60012	0.0246	0.0785	0.8969	8.05	4.856	4.846	4.052	1.634	8.489	7.274
60014	0.0394	0.1895	0.7711	19.73	4.632	4.632	4.187	1.695	8.157	6.132
60023	0.0141	0.1465	0.8394	14.86	5.505	4.528	3.274	2.376	8.427	6.122
60043	0.1496	0.0876	0.7628	10.30	6.108	4.824	5.435	2.174	9.718	
60047	0.0156	0.1160	0.8684	11.78	5.621	4.280	3.536	2.121	8.081	5.102
60048	0.0099	0.2269	0.7633	22.91	6.196	4.521	4.060	1.974	7.625	6.333
60063	0.0108	0.1716	0.8176	17.35	5.658	5.514	3.714	2.461	9.473	6.227
60064	0.0071	0.1869	0.8060	18.82	4.798	3.809	2.802	1.849	7.870	6.158
60065	0.0019	0.1151	0.8470	11.96	4.773	3.943	3.335	1.529	7.484	6.199
60072	0.0131	0.1408	0.8460	14.27	5.775	4.563	5.038	1.892	7.896	6.580
60076	0.0210	0.2080	0.7710	21.25	4.880	4.343	3.732	1.883	8.115	5.641
60082	0.0094	0.1306	0.8600	13.18	5.773	5.013	3.886	2.066	9.130	6.964
60096	0.0160	0.1174	0.8666	11.93	5.585	4.475	4.250	1.955	7.416	6.015
60097	0.0040	0.1825	0.8135	18.32	5.768	5.085	3.732	2.518	8.292	7.387
60099	0.0099	0.2110	0.7791	21.31	5.413	4.739	4.144	1.855	8.585	5.718

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