

Group Comparisons and Longitudinal Studies – Voxel Based Analyses

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Data Analysis Approaches

There are many approaches to data analysis, with the most common techniques (for scalar values), falling into three main group - Region of Interest (ROI) based methods, histograms and group mapping methods. Each have pros and cons, and are more or less appropriate in different situations:

ROI	<ul style="list-style-type: none"> • potentially highly sensitive • hypothesis based • allow comparison of same anatomical structure across groups • easy to implement • very operator dependant • time consuming • useful when <ul style="list-style-type: none"> ○ study is focused on a particular part of the brain ○ area is easily defined ○ sensitivity is an issue <ul style="list-style-type: none"> ▪ (but multiple ROIs require correction for multiple comparisons) ○ processing time is not an issue
Histograms	<ul style="list-style-type: none"> • sensitive to global changes (but overall sensitivity may be low) • have little/no operator dependence • no positioning/repositioning of ROIs • relatively quick • avoid need for multiple statistical tests • give no information on location of abnormalities • useful when <ul style="list-style-type: none"> ○ no a priori hypothesis exists about the location of pathology / diffuse disease ○ operator-dependent bias may be an issue ○ time is an issue

Group Mapping	<ul style="list-style-type: none"> • sensitive to global and local changes <ul style="list-style-type: none"> ◦ but overall sensitivity may be lower than ROIs? • operator independent • computation may be slow, but little operator input required • deal with need for multiple statistical tests • give information on location of abnormalities • useful when <ul style="list-style-type: none"> ◦ no a priori hypothesis exists about the location of pathology / diffuse disease ◦ operator-dependent bias may be an issue
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Group Mapping Methods

The basic methodology consists of:

- warping images for each subject to a template in standard space.
- (Optionally) segmenting the images
- (Optionally) applying masks from the segmentation to restrict analysis to whole brain/white matter/grey/matter...
- (Optionally) smoothing the data
- Testing for significant differences in some parameter (eg grey matter concentration; MTR; FA ...) between groups
 - At a voxel and/or cluster level

Most group mapping techniques are derived from Voxel Based Morphometry (VBM), which uses 'structural' images (usually 3D-SPGR (Spoiled Gradient Echo or MP-RAGE (Magnetization Prepared Rapid Acquisition with Gradient Echoes)) to investigate local changes in grey & white matter volume, using methods derived from those used in the analysis of functional imaging data. Voxel-Based Morphometry is both a generic term, and also used by the Wellcome Department of Imaging Neuroscience (www.fil.ion.ucl.ac.uk) (the home of the commonly used SPM package) to describe their implementation.

What is now considered a 'conventional' VBM approach was first described by Wright et al and used to study schizophrenia⁽¹⁾, with more details given in Ashburner & Friston⁽²⁾. An 'optimized' methodology (see below) was later introduced by Good et al⁽³⁾⁽⁴⁾

'Standard' VBM

The basic VBM methodology (figure 1) consists of:

- 'normalisation' – the warping images for each subject to a template in standard space

- segmenting the images into white matter, grey matter and CSF
- (Optionally) smoothing the data
- (Optionally) 'modulating' the data
- Testing for significant differences in tissue concentration or volume between groups, at a voxel and/or cluster level

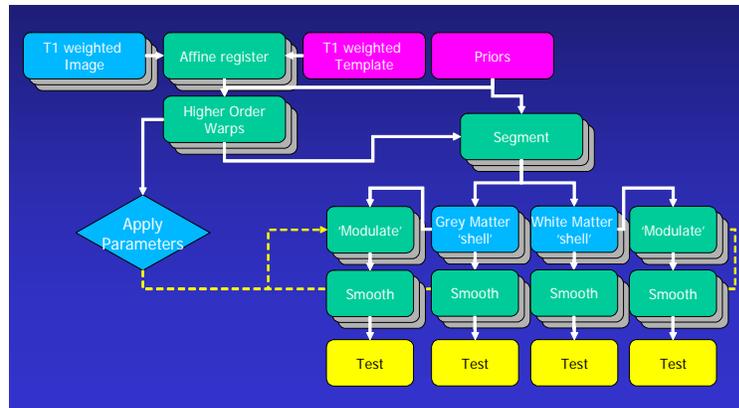


Figure 1

Spatial Normalisation

Normalisation aims to remove “uninteresting” anatomical variability leaving differences related to variable of interest. (Note that if two images are perfectly registered then there will be no voxel wise differences to be tested!). There is a continuum of registration algorithms; most common in VBM is the simple affine registration with 9 or 12 parameters (3 translations, 3 rotations, 3 zooms & 3 shears) which aligns images to the AC-PC axis & scales to same gross dimensions and the ‘Non-linear registration’ implemented in SPM, which follows an initial 12 parameter affine registration with a nonlinear registration using a set of cosine basis functions (default = 1176 parameters). The latter attempts to correct images for global shape differences, but does not attempt exact gyral matching; whether this is appropriate is controversial⁽⁵⁾⁽⁶⁾.

Note that:

- Non linear registration produces different volume changes throughout image
- Too much warping can be problematic ...
- Custom templates (see below) are often used, but the choice of template can make a huge difference to the final results – Figure 2 shows an example of two analyses of MTR data in epilepsy controls and patients which differ only in the template used.

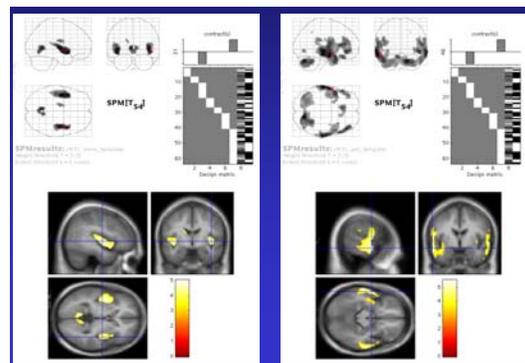


Figure 2 – left, control template; right, patient template

Segmentation

Segmentation is the process of partitioning images into constituent tissue types (grey & white matter & CSF, and also sometimes also other classes e.g. dura, blood vessels). Steps in the segmentation process include correction of spatial signal non-uniformity (often called bias field correction) and interpretation of 'partial volume' effects of voxels contain more than one tissue type, e.g. at the boundary of grey & white matter. The effect of the latter depends on algorithm; in a hard (binary) classification each voxel is assigned to one tissue class and assigned a value of 1, in soft, or fuzzy, classifications each voxel may be assigned probability 0-1 of being that tissue type (e.g. in SPM (www.fil.ion.ucl.ac.uk/spm) grey matter value = 0.8 means an 80% probability of being grey matter) or may be assigned a value 0-1 indicating proportion of that voxel comprised of that tissue type (eg in BAMB (<http://www-bmu.psychiatry.cam.ac.uk/software/>) & FAST (part of FSL <http://www.fmrib.ox.ac.uk/fsl>) grey matter value = 0.8 means 80% of that voxel contains grey matter).

'Modulation'

Nonlinear registration can result in variable shrinkage & volume increases across an image. The determinant of Jacobian at each voxel is measure of change in volume, so multiplying segmented voxel value by determinant of Jacobian retains original tissue volume at each voxel. Modulation, and the terminology surrounding it, can often be confusing – a good explanation for why modulation is used is:

"In effect, an analysis of modulated data tests for regional differences in the absolute amount (volume) of grey matter, whereas analysis of unmodulated data tests for regional differences in concentration of grey matter (per unit volume in native space)"

Good *et al* 2001

Smoothing

Smoothing involves weighting a voxel value by that of its neighbours, and is used for a number of reasons:

- To allow for imperfect registration
- To allow for intra-subject differences
- To make the data more closely follow the distribution assumed by the statistics used (ie to make the residuals Gaussian)

The need for smoothing, and degree to use, is controversial, particularly as applying smoothing is equivalent to applying a 'matched filter' - sensitivity is greatest if/when spatial extent of the tissue difference matches the filter size. Jones et al have looked at the effect in detail for Diffusion Tensor Imaging (DTI) data⁽⁷⁾.

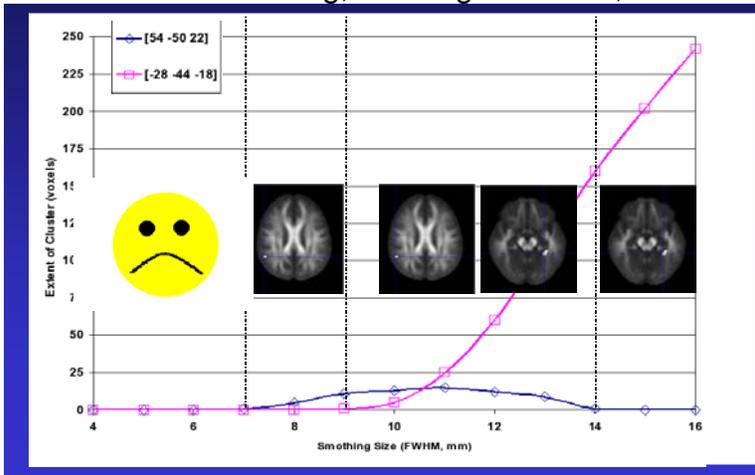


Figure 3

Modelling and Statistics

All major packages implement a 'General Linear Model' (GLM) (or Analysis of Covariance (ANCOVA)) which is regressed onto observed data at each voxel:

$$S = a_0 + a_1 X_1 + a_2 X_2 + \dots + a_n X_n + e$$

where S is vector of image value at a given voxel for each subject, X1 is a vector of the independent variable (eg group membership), other Xn's are vectors of other covariates (eg age, gender ...) and e represents random variation (ie an error term). The Xn are often referred to as the 'design matrix' and can be represented graphically (Figure 4).

There are two main approaches to the statistical testing of the model fit, parametric and non-parametric. In the former case maps of f or t statistics are computed, and compared to known distributions, and inferences are then corrected for multiple comparisons (in SPM) though 'Gaussian Random Fields' (GRF). In the latter, maps are computed of the 'voxelwise test statistic' ($a^* = a_1 / \text{StandardError}(a_1)$), and the value from the observed data is compared to a 'null distribution' created by randomly permuting group membership. In the non-parametric case, spatially contiguous supra-threshold voxels may also be 'clustered', and the sum of suprathreshold voxelwise test statistics ("mass") of each cluster can then be tested against corresponding null distribution⁽⁸⁾ (which may increase sensitivity).

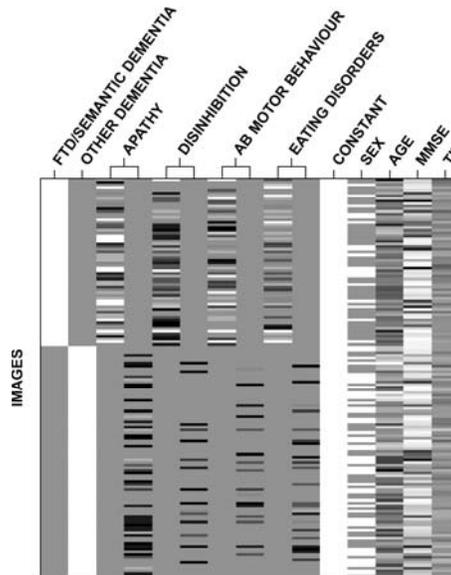


Figure 4 - Rosen, H. J. et al. Brain 2005 128:2612-2625

(NB, note that, unlike the case of fMRI⁽⁹⁾⁽¹⁰⁾, no parametric test exists for this value).

For a good overview of these issues (and others), see

<http://www.fil.ion.ucl.ac.uk/spm/doc/intro>

and <http://www-bmu.psychiatry.cam.ac.uk/software/docs/xbamm/index3.html>

Custom Template & Priors

When the subject group or groups are known to differ from control subjects, custom templates can help reduce the potential confound. Typically, a template based on both controls and patients is used, so that any registration

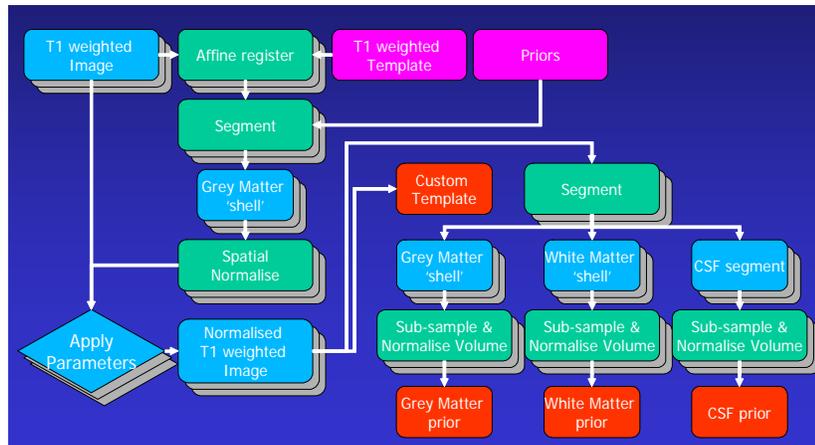


Figure 5

inaccuracies will be more similar between

the groups. Customised prior information can also be used in the segmentation stage, again allowing the groups to be treated more similarly. The template and priors are created by a process similar to the standard VBM methodology itself (Figure 5).

Optimised VBM

The optimised VBM method⁽³⁾⁽⁴⁾, uses custom templates and priors, and also

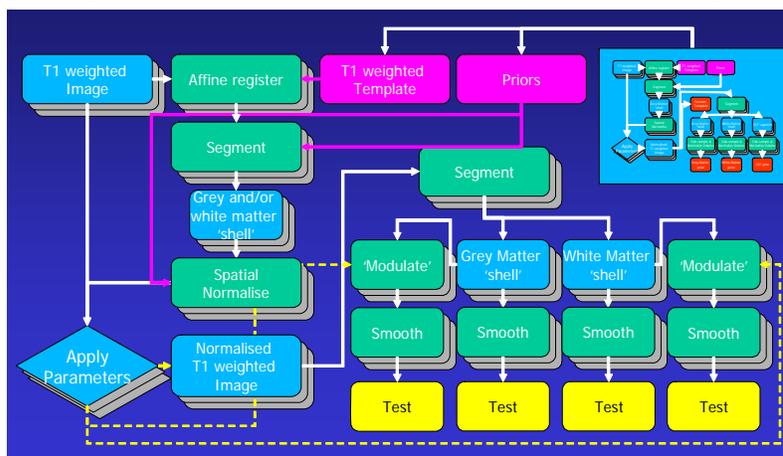


Figure 6

performs a second registration of the images to the template, using the image of the parameter to be tested (typically the 'grey matter shell'), to increase registration accuracy. The overall process is shown in Figure 6.

Validation of VBM

Changes seen by VBM are assumed to represent volumetric (or concentration) differences. A number of studies have attempted to validate this, for instance by comparing VBM results to those of ROI based studies. One early validation example is that of Wright et al (1999)⁽¹¹⁾, who reported artificially 'lesioning' T_1 weighted scans by putting "bullet holes" in various grey matter structures, which SPM (correctly) detected as grey matter loss. It must always be remembered, however, that some apparently volumetric differences may be due to displacement without volume change, and also that **any** factor which causes changes in voxel intensities in the original images (eg vascular changes, changes in hydration status leading to relaxation time changes, etc) may result in significant VBM differences.

Group Mapping Other Types of Data

Group mapping approaches have been applied to several other (quantitative) MR parameters, particularly Magnetisation Transfer Ratio (MTR) and Diffusion Tensor Imaging (DTI) measures. The basic methodology consists of:

MTR:

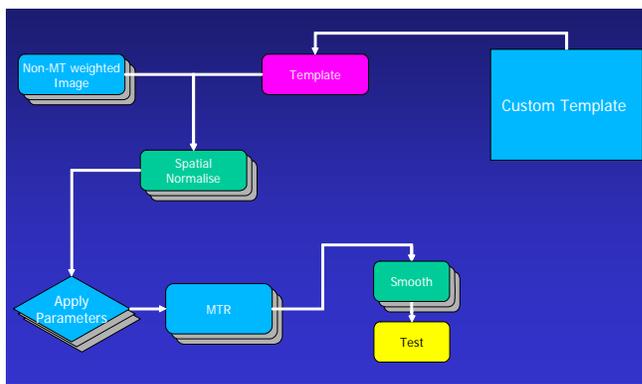


Figure 7

- warping images for each subject to a template in standard space
 - typically using non-MT weighted image.
- (Optionally) smoothing the data
- Testing for significant MTR differences between groups at voxel and/or cluster level

DTI:

- warping images for each subject to a template in standard space
 - typically using $b=0$ or FA map
- (Optionally) segmenting the images
- (Optionally) applying masks from the segmentation to restrict analysis to whole

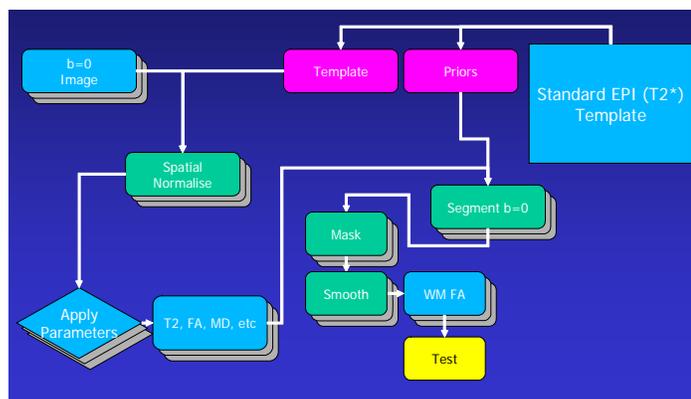


Figure 8

- brain/white matter/grey/matter...
- (Optionally) smoothing the data
- Testing for significant differences in FA/MD/... between groups
 - voxel and/or cluster level

Questions remain about validity, however, (particularly for DTI data):

- what is the appropriate registration/warping technique?
- which images to register (eg $b=0$, FA, tensor components?)
- what tissue(s) to test (eg white matter only, whole brain?)
- whether to smooth, and by how much
- what statistics to use (the residuals are NOT normally distributed)

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