Manual
Computational Anatomy Toolbox - CAT12

Quick start guide

VBM data

- **Segment** data using defaults (use **Segment Longitudinal Data** for longitudinal data).
- **Estimate Total Intracranial Volume** (TIV) to correct for different head size and volume.
- Check the data quality with **Check Sample Homogeneity** for VBM data (optionally consider TIV and age as nuisance variable).
- **Smooth** data (recommended start value 8mm[1]).
- **Specify 2nd-level** model and check for design orthogonality and sample homogeneity:
    - o Use "Full factorial" for cross-sectional data.
    - o Use "Flexible factorial" for longitudinal data.
    - o Use TIV as covariate (confound) to correct different brain sizes and select centering with overall mean.
    - o Select threshold masking with an absolute value of 0.1. This threshold can ultimately be increased to 0.2 or even 0.25.
    - o If you find a considerable correlation between TIV and any other parameter of interest it is advisable to use global scaling with TIV. For more information, refer to the section "Building the statistical model".
- **Estimate** model.
- Optionally, **Transform SPM-maps** to (log-scaled) p-maps or correlation maps and apply thresholds.
- Optionally, you can try **Threshold-Free Cluster Enhancement** (TFCE) with the SPM.mat file of a previously estimated statistical design.
- Optionally, **Overlay Selected Slices**. If you are using log-p scaled maps from **Transform SPM-maps** without thresholds, use the following values as the lower range for the colormap for the thresholding: 1.3 (P<0.05); 2 (P<0.01); 3 (P<0.001).
- Optionally, estimate results for ROI analysis using **Analyze ROIs**. Here, the SPM.mat file of a previously estimated statistical design is used. For more information, see the online help "Atlas creation and ROI based analysis".

Additional surface data

- **Segment** data and also select "Surface and thickness estimation" under "Writing options" (for longitudinal data use **Segment Longitudinal Data**).
- Optionally, **Extract Additional Surface Parameters** (e.g. sulcal depth, gyrification index, cortical complexity).
- **Resample & Smooth Surfaces** (recommended start value 15mm for cortical thickness and 20-25mm for folding measures[1], use the default merging of hemispheres).
- Check data quality using **Check Sample Homogeneity** for surface data.
- **Specify 2nd-level** model for (merged) hemispheres and check for design orthogonality and sample homogeneity:
    - o Use "Full factorial" for cross-sectional data.
    - o Use "Flexible factorial" for longitudinal data.

- It is not necessary to use TIV as a covariate (confound) because cortical thickness or other surface values are usually not dependent on TIV.
- It is not necessary to use threshold masking.
- If you find a considerable correlation between a nuisance parameter and any other parameter of interest it is advisable to use global scaling with that parameter. For more information, refer to the section "Building the statistical model".

- **Estimate Surface Model** for (merged) hemispheres.

- Optionally, **Transform SPM-maps** to (log-scaled) p-maps or correlation maps and apply thresholds.

- Optionally, you can try **Threshold-Free Cluster Enhancement** (TFCE) with the SPM.mat file of a previously estimated statistical design.

- Optionally, **Display Surface Results** for both hemispheres. Select the results (preferably saved as log-p maps with "Transform and threshold SPM-surfaces") to display rendering views of your results.

- Optionally **Extract ROI-based Surface Values** such as thickness, gyrification or fractal dimension to provide ROI analysis.

- Optionally, estimate results for ROI analysis using **Analyze ROIs**. Here, the SPM.mat file of a previously estimated statistical design is used. For more information, see the online help "Atlas creation and ROI based analysis".

Errors during preprocessing

Please use the **Report Error** function if any errors during preprocessing occurred. You first have to select the "err" directory, which is located in the folder of the failed record and finally the specified zip-file should be attached manually in the mail.

Additional options

Additional parameters and options are displayed in the CAT12 expert mode. Please note that this mode is for experienced users only.

[1]**Note to filter sizes for Gaussian smoothing**

Due to the high accuracy of the spatial registration approaches used in CAT12 you can also try to use smaller filter sizes of only a few millimeter. However, for very small filter sizes or even no filtering you have to apply a non-parametric permutation test such as the TFCE-statistics.

Please also note that for the analysis of cortical folding measures such as gyrification or cortical complexity the filter sizes have to be larger (i.e. in the range of 15-25mm). This is due to the underlying nature of this measure that reflects contributions from both sulci as well as gyri. Therefore, the filter size should exceed the distance between a gyral crown and a sulcal fundus.

Version information

Preprocessing should remain unaffected until the next minor version number. A new processing of your data is not necessary if the minor version number of CAT12 remains unchanged.

Changes in version CAT12.4 (1348)

- Changes in preprocessing pipeline (which affects your results compared to CAT12.3)
    - Detection of white matter hyperintensities (WMHs) is updated and again enabled by default.
- Important new features
    - CAT12 can now deal with lesions that have to be set to "0" in your image using the Stroke Lesion Correction (SLC) in expert mode. These lesion areas are not used for segmentation or spatial registration, thus these preprocessing steps should be almost unaffected.

Changes in version CAT12.3 (1310)

- Changes in preprocessing pipeline (which affects your results compared to CAT12.2)
    - Skull-stripping is again slightly changed and the SPM approach is now used as default. The SPM approach works quite stable for the majority of data. However, in some rare cases parts of GM (i.e. in frontal lobe) might be cut. If this happens the GCUT approach is a good alternative.
    - Spatial adaptive non-local mean (SANLM) filter is again called as very first step because noise estimation and de-noising works best for original (non-interpolated) data.
    - Detection of white matter hyperintensities (WMHs) is currently disabled by default, because of unreliable results for some data.
- Important new features
    - Cobra atlas has been largely extended and updated.

Changes in version CAT12.2 (1290)

- Changes in preprocessing pipeline (which affects your results compared to CAT12.1)
    - Skull-stripping now additionally uses SPM12 segmentations by default: The default gcut approach in CAT12.1 removed too much of the surrounding (extracranial) CSF, which led to a slight underestimation of TIV for atrophied brains. The skull-stripping approach based on the SPM12 segmentations prevents this through a more conservative approach. However, sometimes parts of the meninges (i.e. dura mater) or other non-brain parts still remain in the GM segmentation. By combining both approaches a more reliable skull-stripping is achieved.
    - More reliable estimation of TIV: The changed skull-stripping also affects estimation of TIV, which is now more reliable, especially for atrophied brains.
- Important new features
    - Automatic check for design orthogonality and sample homogeneity using SPM.mat in **Basic Models**
    - Added equi-volume model by Bok and multi-save option for mapping native volumes to individual surfaces.
    - Added Local-Global Intrinsic Functional Connectivity parcellation by Schaefer et al. for resting-state fMRI data.

Introduction and Overview

This manual is intended to help any user to perform a computational anatomy analysis using the CAT12 Toolbox. Although it mainly focuses on voxel-based morphometry (VBM) other variants of computational analysis such as deformation-based morphometry (DBM), surface-based morphometry (SBM), and region of interest (ROI) morphometric analysis are also presented and can be applied with few changes.

Basically, the manual can be divided into four main sections:

- Naturally, a quick guide of how to *get started* is given at the beginning. This section provides information about *downloading and installing* the software and *starting* the Toolbox. In addition, a brief *overview* of the steps of a VBM analysis is given.

- This is followed by a *detailed description of a basic VBM analysis* that guides the user step-by-step through the entire process – from preprocessing to contrast selection. This description should provide all the information necessary to successfully analyze most studies.

- There are some *specific cases* of VBM analyses, for which the basic analysis workflow needs to be adapted. These cases are *longitudinal studies* and studies in *children or special patient populations*. Relevant changes to a basic VBM analysis are described here and how to apply these changes. It is important that only the changes are described – steps such as quality control or smoothing are the same as those described in the basic analysis and not repeated a second time.

- The guide concludes with *information about native, normalized and modulated volumes* that determine how the results can be interpreted. In addition, an overview of the naming conventions used and technical information is given.

Getting Started

DOWNLOAD AND INSTALLATION

- The CAT12 Toolbox runs within SPM12. That is, SPM12 must be installed and added to your Matlab search path before the CAT12 Toolbox can be installed (see http://www.fil.ion.ucl.ac.uk/spm/ and http://en.wikibooks.org/wiki/SPM).

- Download (http://dbm.neuro.uni-jena.de/cat12/) and unzip the CAT12 Toolbox. You will get a folder named "cat12", which contains various Matlab files and compiled scripts. Copy the folder "cat12" into the SPM12 "toolbox" folder.

STARTING THE TOOLBOX

- Start Matlab

- Start SPM12 (i.e., type "spm fmri")

- Select "cat12" from the SPM menu (see Figure 1). You will find the drop-down menu between the "Display" and the "Help" button (you can also call the Toolbox directly by typing "cat12" on the Matlab command line). This will open the CAT12 Toolbox as additional window (Fig. 2).
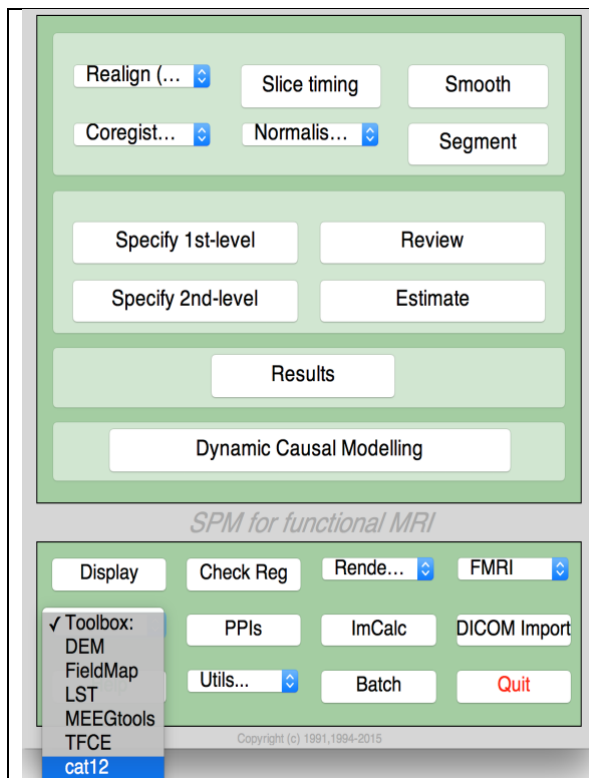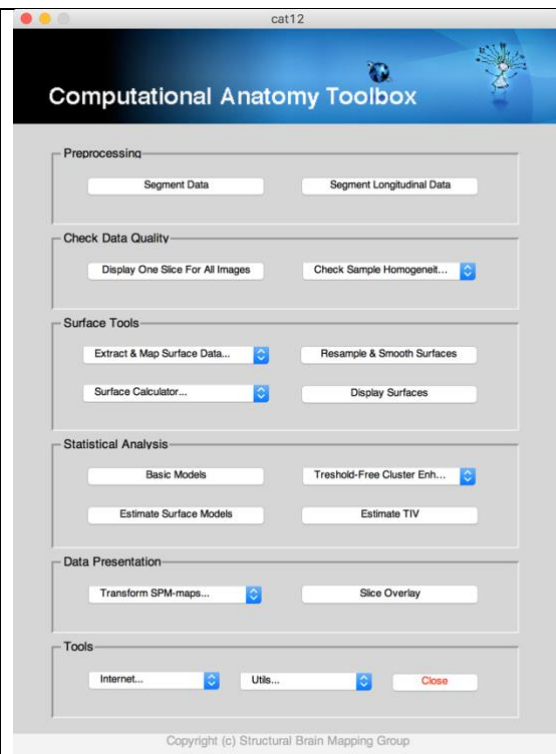
Figure 1: SPM menu	**Figure 2**: CAT12 Window

BASIC VBM ANALYSIS (OVERVIEW)

The CAT12 Toolbox comes with different modules, which may be used for an analysis. Usually, a VBM analysis comprises the following steps

(a) Preprocessing:

1. T1 images are **normalized** to a template space and **segmented** into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The preprocessing parameters can be adjusted via the module "Segment Data".

2. After the preprocessing is finished, a **quality check** is highly recommended. This can be achieved via the modules "Display one slice for all images" and "Check sample homogeneity". Both options are located in the CAT12 window under "Check Data Quality". Furthermore, quality parameters are estimated and saved in xml-files for each data set

during preprocessing. These quality parameters are also printed on the report PDF-page and can be additionally used in the module "Check sample homogeneity".

3. Before entering the GM images into a statistical model, image data need to be **smoothed**. Of note, this step is not implemented into the CAT12 Toolbox but achieved via the standard SPM module "Smooth".

(b) Statistical analysis:

4. The smoothed GM images are entered into a statistical analysis. This requires building a statistical model (e.g. T-Tests, ANOVAs, multiple regressions). This is done by the standard SPM modules "Specify 2nd Level" or "Basic Models" in the CAT12 window covering the same function.

5. The statistical model is estimated. This is done with the standard SPM module "Estimate" (except for surface-based data where the function "Estimate Surface Models" should be used instead).

6. If you have used total intracranial volume (TIV) as confound in your model to correct for different brain sizes it is necessary to check whether TIV reveals a considerable correlation with any other parameter of interest and rather use global scaling as alternative approach.

7. After estimating the statistical model, contrasts are defined to get the results of the analysis. This is done with the standard SPM module "Results".

The sequence of "preprocessing → quality check → smoothing → statistical analysis" remains the same for every VBM analysis, even when different steps are adapted (see "special cases").

A few words about the Batch Editor…

− As soon as you select a module from the CAT12 Toolbox menu, a new window (the Batch Editor) will open. The Batch Editor is the environment where you will set up your analysis (see **Figure 3**). For example, an "<-X" indicates where you need to select files (e.g. your image files, the template, etc.). Other parameters have either default settings (which can be modified) or require input (e.g. choosing between different options, providing text or numeric values, etc.).

− Once all missing parameters are set, a green arrow will appear on the top of the window (the current snapshots in **Figure 3** show the arrow still in gray). Click this arrow to run the module or select "File → Run Batch". It is very useful to save the settings before you run the batch (click on the disk symbol or select "File → Save Batch").

− Of note, you can always find helpful information and parameter-specific explanations at the bottom of the Batch Editor window.[1]

− All settings can be saved either as .mat file or as .m script file and reloaded for later use. The .m script file has the advantage to be editable with a text editor.

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[1] Additional CAT12-related information can be found by selecting "VBM Website" in the CAT12 window (Tools → Internet → VBM Website"). This will open a website. Here, look for "VBM subpages" on the right.
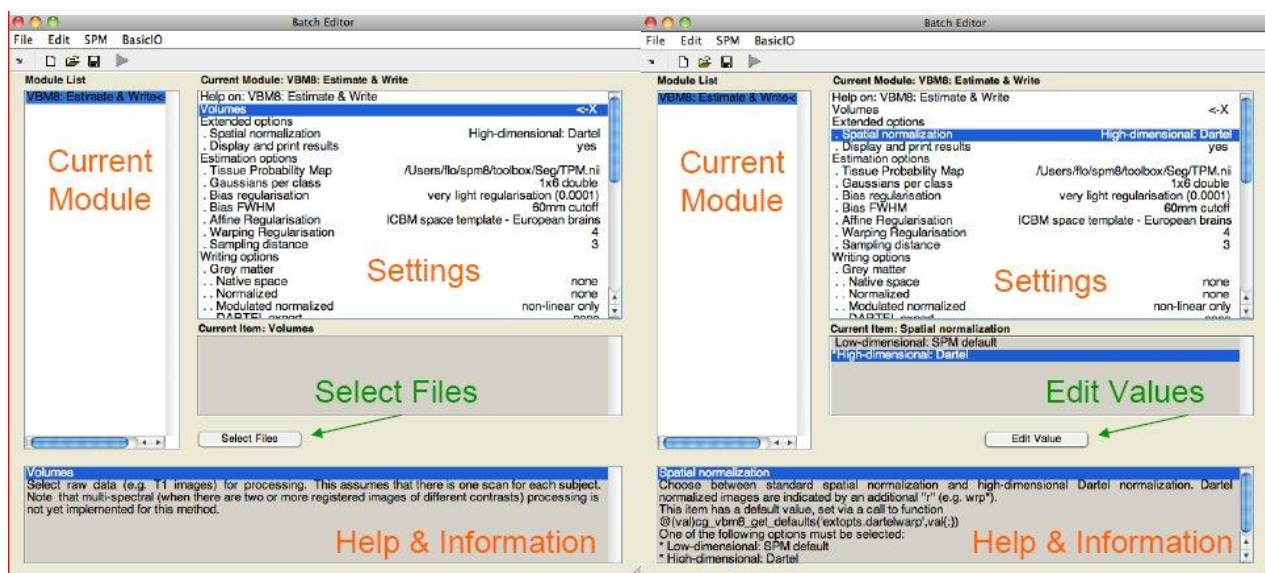
Figure 3: The Batch Editor is the environment where the analysis is set up. *Left*: For all settings marked with "<-X", files have to be selected ("Select Files"). *Right*: Parameters can be edited and adapted ("Edit Value").

Basic VBM Analysis (detailed description)

Preprocessing Data

FIRST MODULE: SEGMENT DATA

Please note that additional parameters for expert users are displayed in the GUI if you set the option cat.extopts.expertgui to "1" in cat_defaults.m or call cat12 by:

cat12('expert')

CAT12 → Preprocessing → Segment Data

Parameters:

- Volumes <-X → Select Files → *[select the new files]* → Done

  - Select one volume for each subject. As the Toolbox does not support multispectral data (i.e., different imaging methods for the same brain, such as T1-, T2-, diffusion-weighted or CT images), it is recommended to choose a T1-weighted image.
  - Importantly, the images need to be in the same orientation as the priors; you can double-check and correct via using "Display" in the SPM menu. The priors are located in your SPM folder "SPM12 → tpm → TPM.nii")

- Split job into separate processes →*[use defaults or modify]*
  - To use multi-threading the CAT12 segmentation job with multiple subjects can be split into separate processes that run in the background. You can even close Matlab, which will not affect the processes that will run in the background without GUI. If you don't want to run processes in the background then set this value to 0.
  - Keep in mind that each process needs about 1.5..2GB of RAM, which should be considered to choose the appropriate number of processes.
  - Please further note that no additional modules in the batch can be run except CAT12 segmentation. Any dependencies are broken for subsequent modules.

- Options for initial SPM12 affine registration → *[use defaults or modify]*
  - The defaults provide a solid starting point. The SPM12 tissue probability maps (TPMs) are used for the initial spatial registration and segmentation. Alternatively, customized TPMs can be chosen (e.g. for children data) that were created with the Template-O-Matic (TOM) Toolbox.

- Extended options for CAT12 segmentation → [*use defaults or modify*]
  - Again, the defaults provide a solid starting point. Using the extended options you can adjust specific parameters or the strength of different corrections ("0" means no correction and "0.5" is the default value that works best for a large variety of data).

- CAT12 provides a template for the high-dimensional DARTEL registration that should work for most data. However, a customized DARTEL template can be selected (e.g. for children data) that was created using the DARTEL toolbox. For more information on the necessary steps, see the section "Customized DARTEL Template".
  o Writing options → [*use defaults or modify*]
    - For **GM, and WM image volumes** see at the end of the document: "Additional information on native, normalized and modulated normalized volumes". *Note: The default option "Modulated normalized" results in an analysis of relative differences in regional GM volume, that have to be corrected for individual brain size in the statistical analysis using total intracranial volume (TIV).*
    - A **Bias, noise and globally intensity corrected T1 image**, in which MRI inhomogeneities and noise are removed and intensities are globally normalized, can be written in normalized space. This is useful for quality control and also to create an average image of all normalized T1 images to display / overlay the results. *Note: For a basic VBM analysis use the defaults.*
    - A **partial volume effect (PVE) label image volume** can also be written in normalized or native space or as a DARTEL export file. This is useful for quality control and also for future applications using this image to reconstruct surfaces. *Note: For a basic VBM analysis use the defaults.*
    - The **Jacobian determinant** for each voxel can be written in normalized space. This information can be used to do a Deformation-Based Morphometry (DBM) analysis. *Note: For a basic VBM analysis this is not needed.*
    - Finally, **deformation fields** can be written. This option is useful to re-apply normalization parameters to other co-registered images (e.g. fMRI or DTI data). *Note: For a basic VBM analysis this is not needed.*

Note: If the segmentation fails, this is often due to an unsuccessful initial spatial registration. In this case, you can try to set the origin (anterior commissure) in the Display tool. Place the cursor roughly on the anterior commissure and press "Set Origin" The now displayed correction in the coordinates can be applied to the image with the button "Reorient". This procedure must be repeated for each data set individually.

SECOND MODULE: DISPLAY ONE SLICE FOR ALL IMAGES

CAT12 → Check data quality → Display one slice for all images

Parameters:

- o Sample data <-X → Select Files → *[select the new files]* → Done

  - Select the newly written data [e.g. the "wm*" files, which are the normalized bias corrected volumes]. This tool will display one horizontal slice for each subject, thus giving a good overview if the segmentation and normalization procedures yielded reasonable results. For example, if the native volume had artifacts or if the native volumes had a wrong orientation, the results may look odd. Solutions: Use "Check Reg" from the SPM main menu to make sure that the native images have the same orientation like the MNI Template ("SPM → templates → T1"). Adjust if necessary using "Display" from the SPM main menu.

- o Proportional scaling →*[use defaults or modify]*
  - Check "yes", if you display T1 volumes.

- o Spatial orientation

- o Show slice in mm → [*use defaults or modify*]

  - This module displays horizontal slices. This default setting provides a good overview.

THIRD MODULE: CHECK SAMPLE HOMOGENEITY

CAT12 → Check data quality → Check sample homogeneity → VBM data

Parameters:

- o Data → New: Sample data <-X → Select Files → *[select gray matter volumes]* → Done
  - Select the newly written data [e.g. the "mwp1*" files, which are the modulated (m) normalized (w) GM segments (p1)]. It is recommended to use the unsmoothed segmentations that provide more anatomical details. This tool visualizes the correlation between the volumes using a boxplot and correlation matrices. Thus, it will help identifying outliers. Any outlier should be carefully inspected for artifacts or pre-processing errors using "Check worst data" in the GUI. If you specify different samples the mean correlation is displayed in separate boxplots for each sample.

- o Load quality measures (optional) → *[optionally select xml-files with quality measures]*
  - Optionally select the xml-files that are saved for each data set. These files contain useful information about some estimated quality measures that can be also used for checking sample homogeneity. Please note, that the order of the xml-files must be the same as the other data files.

- o Separation in mm → *[use defaults or modify]*
  - To speed up calculations you can define that correlation is estimated only every x voxel. Smaller values give slightly more accurate correlation, but are much slower.
- o Nuisance → *[enter nuisance variables if applicable]*
  - For each nuisance variable which you want to remove from the data prior to calculating the correlation, select "New: Nuisance" and enter a vector with the respective variable for each subject (e.g. age in years). All variables have to be entered in the same order as the respective volumes. You can also type "spm_load" to upload a *txt file with the covariates in the same order as the volumes. A potential nuisance parameter can be TIV if you check segmented data with the default modulation.

A window opens with a correlation matrix in which the correlation between the volumes is displayed. The correlation matrix shows the correlation between all volumes. High correlation values mean that your data are more similar to each other. If you click in the correlation matrix, the corresponding data pairs are displayed in the lower right corner and allow a closer look. The slider below the image changes the displayed slice. The pop-pup menus in the top right-hand corner provide more options. Here you can select other measures that are displayed in the boxplot (e.g. optional quality measures such as noise, bias, weighted overall image quality if these values were loaded), change the order of the correlation matrix (according to file name or mean correlation). Finally, the most deviating data can be displayed in the SPM graphics window to check the data more closely. For surfaces two additional measures are available (if you have loaded the xml-files) to utilize the quality check of the surface extraction. The Euler number gives you an idea about the number of topology defects, while the defect size indicates how many vertices of the surface are affected by topology defects. Because topology defects are mainly caused by noise and other image artifacts (e.g. motion) this gives you an impression about the potential quality of your extracted surface.

The boxplot in the SPM graphics window averages all correlation values for each subject and shows the homogeneity of your sample. A small overall correlation in the boxplot not always means that this volume is an outlier or contains an artifact. If there are no artifacts in the image and if the image quality is reasonable you don't have to exclude this volume from the sample. This tool is intended to utilize the process of quality checking and there is no clear criteria defined to exclude a volume only based on the overall correlation value. However, volumes with a noticeable lower overall correlation (e.g. below two standard deviations) are indicated and should be checked more carefully. The same holds true for all other measures. Data with deviating values have not necessarily to be excluded from the sample, but these data should be checked more carefully with the "Check most deviating data" option. The order of the most deviating data is changed for every measure separately. Thus, the check for the most deviating data will give you a different order for mean correlation and other measures which allows you to judge your data quality using different features.

If you have loaded quality measures, you can also display the Mahalanobis distance between two measures: mean correlation and weighted overall image quality. These two are the most important measures for assessing image quality. Mean correlation measures the homogeneity of your data used for statistical analysis and is therefore a measure of image quality **after** pre-processing. Data that deviate from your sample increase variance and therefore minimize effect size and statistical power. The weighted overall image quality, on the other hand, combines measurements of noise

and spatial resolution of the images **before** pre-processing. Although CAT12 uses effective noise-reduction approaches (e.g. spatial adaptive non-local means filter) pre-processed images are also affected and should be checked.

The Mahalanobis distance makes it possible to combine these two measures of image quality before and after pre-processing. In the Mahalanobis plot, the distance is color-coded and each point can be selected to get the filename and display the selected slice to check data more closely.

FOURTH MODULE: SMOOTH

SPM menu → Smooth

> Parameters:
>
> > o Images to Smooth <-X → Select Files → *[select grey matter volumes]* → Done
> >> - Select the newly written data [e.g. the "mwp1" files, which are the modulated (m) normalized (w) grey matter segments (p1)].
> >
> > o FWHM → *[use defaults or modify]*
> >> - 8-12mm kernels are widely used for VBM. To use this setting select "edit value" and type "8 8 8" (or "12 12 12", respectively) for a kernel with 8mm (with 12mm) FWHM.
> >
> > o Data Type → *[use defaults or modify]*
> > o Filename Prefix → *[use defaults or modify]*

FIFTH MODULE: ESTIMATE TOTAL INTRACRANIAL VOLUME (TIV)

CAT12 → Statistical Analysis → Estimate TIV

> Parameters:
>
> > o XML files <-X → Select Files → *[select xml-files]* → Done
> >> - Select the xml-files in the report-folder [e.g. the "cat_*.xml"].
> >
> > o Save values → *TIV only*
> >> - This option will save the TIV values for each data set in the same order as the selected xml-files. Optionally you can also save the global values for each tissue class, which might be interesting for further analysis, but is not recommended if you are interested in only using TIV as covariate.
> >
> > o Output file → *[use defaults or modify]*

Please note that TIV is strongly recommended as covariate for all VBM analysis to correct different brain sizes. This step is not necessary for deformation- or surface-based data. Please also make sure that TIV does not correlate too much with your parameters of interest (please make sure that you use "Centering" with "Overall mean", otherwise the check for orthogonality in SPM sometimes does not work correctly). In this case you should use global scaling with TIV.

Building the Statistical Model

Although many potential designs are offered in 2nd-level analysis I recommend using the "Full factorial" design as it covers most statistical designs. For cross-sectional VBM data you usually have 1..n samples and optionally covariates and nuisance parameters:

Number of factor levels	Number of covariates	Statistical Model
1	0	one-sample t-test
1	1	single regression
1	>1	multiple regression
2	0	two-sample t-test
>2	0	Anova
>1	>0	AnCova (for nuisance parameters) or Interaction (for covariates)

TWO-SAMPLE T-TEST

CAT12 → Statistical Analysis → Basic Models

 Parameters:

- o Directory <-X → Select Files → *[select the working directory for your analysis]* → Done
- o Design → "Two-sample t-test"
  - ▪ Group 1 scans → *Select Files* → *[select the smoothed grey matter volumes for group 1; following this script these are the "smwp1" files]* → Done
  - ▪ Group 2 scans → *Select Files* → *[select the smoothed grey matter volumes for group 2]* → Done
  - ▪ Independence → Yes
  - ▪ Variance → Equal or Unequal
  - ▪ Grand mean scaling → No
  - ▪ ANCOVA → No
- o **Covariates*** (see text box)
- o Masking
  - ▪ Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
  - ▪ Implicit Mask → Yes
  - ▪ Explicit Mask → <None>
- o Global Calculation → Omit
- o Global Normalization
  - ▪ Overall grand mean scaling → No
- o Normalization → None

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*You could specify one or more covariates (i.e., partial out the variance of specific factors when looking at group differences).

It is strongly recommended to always use total intracranial volume (TIV) as covariate for modulated data in VBM to correct different brain sizes. This is not necessary for surface analysis and DBM.

- ● Covariates → New Covariate
- ● Vector <-X → enter the values of the covariates (e.g. TIV and optionally age in years) in the same order as the respective file names or type "spm_load" to upload a *.txt file with the covariates in the same order as the volumes
- ● Name <-X → Specify Text (e.g. "age")
- ● Interactions → None
- ● Centering → Overall mean

FULL FACTORIAL MODEL (FOR A 2X2 ANOVA)

CAT12 → Statistical Analysis → Basic Models

Parameters:

- o Directory <-X → Select Files → *[select the working directory for your analysis]* → Done
- o Design → "Full Factorial"
    - ▪ Factors → *"New: Factor; New: Factor"*
      Factor
        - • Name → *[specify text (e.g. "sex")]*
        - • Levels → 2
        - • Independence → Yes
        - • Variance → Equal or Unequal
        - • Grand mean scaling → No
        - • ANCOVA → No
      Factor
        - • Name → *[specify text (e.g. "handedness")]*
        - • Levels → 2
        - • Independence → Yes
        - • Variance → Equal or Unequal
        - • Grand mean scaling → No
        - • ANCOVA → No
    - ▪ Specify Cells → *"New: Cell; New: Cell; New: Cell; New: Cell"*
      Cell
        - • Levels → *[specify text (e.g. "1 1")]*
        - • Scans → *[select files (e.g. the smoothed GM volumes of the left-handed males)]*
      Cell
        - • Levels → *[specify text (e.g. "1 2")]*
        - • Scans → *[select files (e.g. the smoothed GM volumes of the right-handed males)]*
      Cell
        - • Levels → *[specify text (e.g. "2 1")]*
        - • Scans → *[select files (e.g. the smoothed GM volumes of the left-handed females)]*
      Cell
        - • Levels → *[specify text (e.g. "2 2")]*
        - • Scans → *[select files e.g. the smoothed GM volumes of the right-handed females)]*
- o **Covariates*** (see text box in example for two-sample T-test)
- o Masking
    - ▪ Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
    - ▪ Implicit Mask → Yes
    - ▪ Explicit Mask → <None>
- o Global Calculation → Omit
- o Global Normalization
    - ▪ Overall grand mean scaling → No
- o Normalization → None

MULTIPLE REGRESSION (LINEAR)

CAT12 → Statistical Analysis → Basic Models

Parameters:

- o Directory <-X → Select Files → *[select the directory for your analysis]* → Done

- o Design → "Multiple Regression"
    - Scans → [select files (e.g. the smoothed GM volumes of all subjects)] → Done
    - Covariates → "New: Covariate"
      Covariate
        - Vector → *[enter the values in the same order as the respective file names of the smoothed GM images]*
        - Name → [specify test (e.g. "*age*")]
        - Centering → Overall mean
    - Intercept → Include Intercept
- o **Covariates*** (see text box in example for two-sample T-test)
- o Masking
    - Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
    - Implicit Mask → Yes
    - Explicit Mask → <None>
- o Global Calculation → Omit

- o Global Normalization
    - Overall grand mean scaling → No

- o Normalization → Non

MULTIPLE REGRESSION (POLYNOMIAL)

To use a polynomial model, you need to estimate the polynomial function of your parameter before analyzing it. To do this, use the function cat_stat_polynomial (included with CAT12 >r1140):

     y = cat_stat_polynomial(x,order)

where "x" is your parameter and "order" is the polynomial order (e.g. 2 for quadratic).

Example for polynomial order 2 (quadratic):

CAT12 → Statistical Analysis → Basic Models

    Parameters:

- Directory <-X → Select Files → *[select the directory for your analysis]* → Done

- Design → "Multiple Regression"
    - Scans → [select files (e.g. the smoothed GM volumes of all subjects)] → Done
    - Covariates → "New: Covariate"
      Covariate
        - Vector → *[specify linear term (e.g. "y(:,1)")]*
        - Name → [specify test (e.g. "*age linear*")]
        - Centering → Overall mean
    - Covariates → "New: Covariate"
      Covariate
        - Vector → *[specify quadratic term (e.g. "y(:21)")]*
        - Name → [specify test (e.g. "*age quadratic*")]
        - Centering → Overall mean
    - Intercept → Include Intercept
- **Covariates*** (see text box in example for two-sample T-test)
- Masking
    - Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
    - Implicit Mask → Yes
    - Explicit Mask → <None>

- Global Calculation → Omit

- Global Normalization
    - Overall grand mean scaling → No

- Normalization → None

FULL FACTORIAL MODEL (INTERACTION)

CAT12 → Statistical Analysis → Basic Models

Parameters:

- o Directory <-X → Select Files → *[select the working directory for your analysis]* → Done
- o Design → "Full Factorial"
    - ▪ Factors → *"New: Factor"*
      Factor
        - • Name → *[specify text (e.g. "sex")]*
        - • Levels → 2
        - • Independence → Yes
        - • Variance → Equal or Unequal
        - • Grand mean scaling → No
        - • ANCOVA → No
    - ▪ Specify Cells → *"New: Cell; New: Cell"*
      Cell
        - • Levels → *[specify text (e.g. "1")]*
        - • Scans → *[select files (e.g. the smoothed GM volumes of the males)]*
      Cell
        - • Levels → *[specify text (e.g. "2")]*
        - • Scans → *[select files (e.g. the smoothed GM volumes of the females)]*
- o Covariates → "New: Covariate"
    - ▪ Covariate
        - • Vector → *[enter the values in the same order as the respective file names of the smoothed GM images]*
        - • Name → [specify test (e.g. "*age*")]
        - • Interactions → With Factor 1
        - • Centering → Overall mean
- o **Covariates*** (see text box in example for two-sample T-test)
- o Masking
    - ▪ Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
    - ▪ Implicit Mask → Yes
    - ▪ Explicit Mask → <None>
- o Global Calculation → Omit
- o Global Normalization
    - ▪ Overall grand mean scaling → No
- o Normalization → None

FULL FACTORIAL MODEL (POLYNOMIAL INTERACTION)

To use a polynomial model you have to estimate the polynomial function of your parameter prior to the analysis. Use the function cat_stat_polynomial (provided with CAT12 >r1140) for that purpose:

y = cat_stat_polynomial(x,order)

where "x" is your parameter and "order" is the polynomial order (e.g. 2 for quadratic).

Example for polynomial order 2 (quadratic):

CAT12 → Statistical Analysis → Basic Models

   Parameters:

- Directory <-X → Select Files → *[select the working directory for your analysis]* → Done
- Design → "Full Factorial"
  - Factors → *"New: Factor"*
    Factor
    - Name → *[specify text (e.g. "sex")]*
    - Levels → 2
    - Independence → Yes
    - Variance → Equal or Unequal
    - Grand mean scaling → No
    - ANCOVA → No
  - Specify Cells → *"New: Cell; New: Cell"*
    Cell
    - Levels → *[specify text (e.g. "1")]*
    - Scans → *[select files (e.g. the smoothed GM volumes of the males)]*
    Cell
    - Levels → *[specify text (e.g. "2")]*
    - Scans → *[select files (e.g. the smoothed GM volumes of the females)]*
- Covariates → "New: Covariate"
  - Covariate
    - Vector → *[specify linear term (e.g. "y(:,1)")]*
    - Name → [specify test (e.g. "*age linear*")]
    - Interactions → With Factor 1
    - Centering → Overall mean
- Covariates → "New: Covariate"
  - Covariate
    - Vector → *[specify quadratic term (e.g. "y(:,2)")]*
    - Name → [specify test (e.g. "*age quadratic*")]
    - Interactions → With Factor 1
    - Centering → Overall mean
- **Covariates*** (see text box in example for two-sample T-test)
- Masking
  - Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
  - Implicit Mask → Yes
  - Explicit Mask → <None>
- Global Calculation → Omit
- Global Normalization

- Overall grand mean scaling → No
  - Normalization → None

ESTIMATING THE STATISTICAL MODEL

SPM menu → Estimate

Parameters:

- Select SPM.mat <-X → Select Files → *[select the SPM.mat which you just built]* → Done
- Method → "Classical"

CHECKING FOR DESIGN ORTHOGONALITY

If you have modeled TIV as confounding parameter, you must check whether TIV is orthogonal (in other words independent) to any other parameter of interest in your analysis (e.g. parameters you are testing for). This means that TIV should not correlate with any other parameter of interest, otherwise not only the variance explained by TIV is removed from your data, but also parts of the variance of your parameter of interest.

Please use "Overall mean" as "Centering" for the TIV covariate. Otherwise, the orthogonality check sometimes even indicates a meaningful co-linearity only because of scaling problems.

To check the design orthogonality, you can use the Review function in the SPM GUI:

SPM menu → Review

- Select SPM.mat <-X → Select Files → *[select the SPM.mat which you just built]* → Done
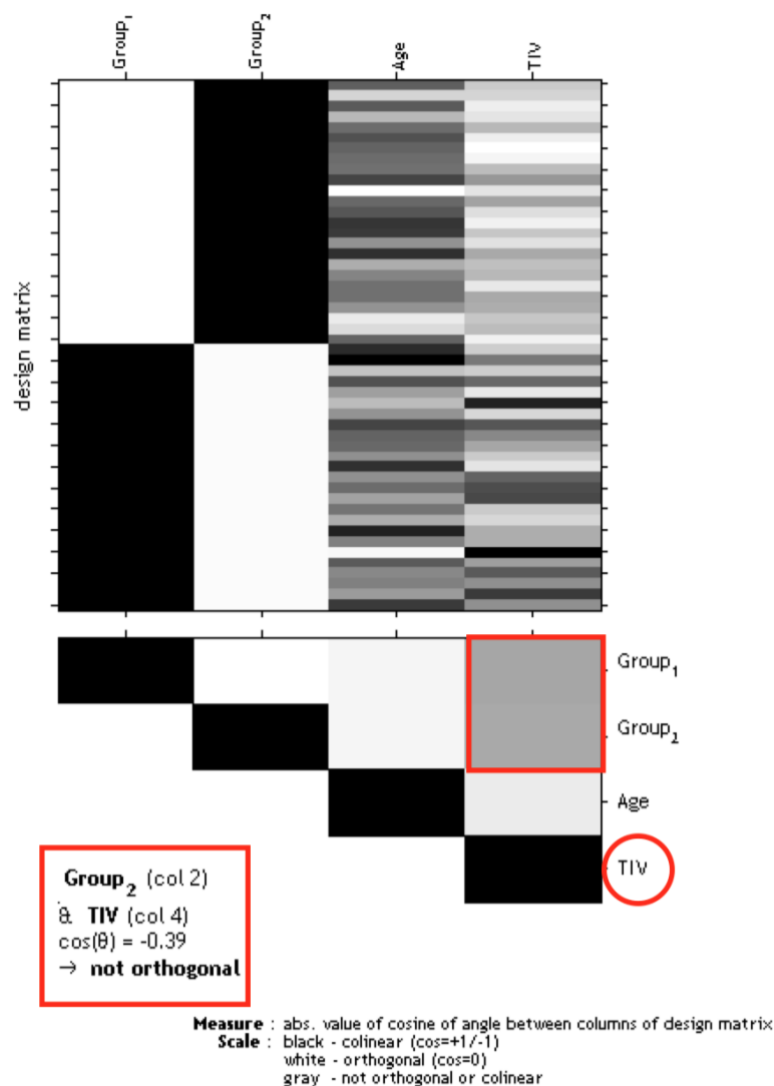- Design → Design orthogonality

Figure 4: Gray boxes between the parameters point to a co-linearity (correlation): the darker the box the larger the correlation (which also holds for inverse correlations). If you click in the box the co-linearity between the parameters is displayed.

The larger the correlation between TIV and any parameter of interest the more the need to not use TIV as nuisance parameter. In this case an alternative approach is to use global scaling with TIV. Use the following settings for this approach:

- ○ Global Calculation → User → Global Values <-X → Define the TIV values here
- ○ Global Normalisation → Overall grand mean scaling → Yes → Grand mean scaled value → Define here the mean TIV of your sample or (or as approximation a value of 1500 which might fit for the majority of data from adults)
- ○ Normalisation
  - Approach 1 (for all models): Normalization → Proportional
  - Approach 2 (for all models with more than one group): Normalization → ANCOVA

Approach 1 is (proportionally) scaling the data according to the individual TIV values. Instead of removing variance explained by TIV all data are scaled by their TIV values. This is also similar to the approach that was used in VBM8 ("modulate for non-linear effects only"), where global scaling was

internally used with an approximation of TIV (e.g. inverse of affine scaling factor). This approach can be used for all models including multiple regressions with one group.

Approach 2 is using TIV as nuisance parameter with separate columns for each group. The values for the nuisance parameters are here mean-centered for each group. As the mean values for TIV are corrected separately for each group, it is prevented that group differences in TIV have a negative impact on the group comparison of the image data in the GLM. Variance explained by TIV is here removed separately for each group. Please note that this approach is also equivalent to the use of TIV as nuisance parameter with interaction and mean centering with factor "group". The design also results in separate columns for TIV, which are mean-corrected for each group.

Unfortunately, a no clear advice for either of these two approaches can be given and the use of these approaches will also affect interpretation of your findings.

Please note that global normalization also affects the absolute masking threshold as your images are now scaled to the "Grand mean scaled value" of the Global Normalization option. If you have defined the mean TIV of your sample (or an approximate value of 1500) here, no change of the absolute threshold is required. Otherwise, you will have to correct the absolute threshold because your values are now globally scaled to the "Grand mean scaled value" of the Global Normalization option.

DEFINING CONTRASTS

SPM menu → Results → *[select the SPM.mat file]* → Done *(this opens the Contrast Manager)* → Define new contrast *(i.e., choose "t-contrast" or "F-contrast"; type the contrast name and specify the contrast by typing the respective numbers, as shown below):*

Please not that all zeros at the end of the contrast don't have to be defined, but are sometimes remained for didactic reasons.
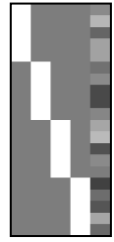

Contrasts:

Two-sample T-test

T-test

- For Group A > Group B   **1 -1**
- For Group A < Group B   **-1 1**

F-test

- For any differences between Group A and B   **1 -1**

2x2 ANOVA

T-test

- For left-handed males > right-handed males — **1 -1 0 0**
- For left-handed females > right-handed females — **0 0 1 -1**
- For left-handed males > left-handed females — **1 0 -1 0**
- For right-handed males > right-handed females — **0 1 0 -1**
- For males > females (main effect sex) — **1 1 -1 -1**
- For left-handers > right-handers (main effect handedness) — **1 -1 1 -1**
- For left-handers > right-handers & males > females — **1 -1 -1 1**
- For left-handers > right-handers & males < females — **-1 1 1 -1**

F-test

- For main effect handedness in males — **1 -1 0 0**
- For main effect handedness in females — **0 0 1 -1**
- For main effect sex in left-handers — **1 0 -1 0**
- For main effect sex in right-handers — **0 1 0 -1**
- For main effect sex — **1 1 -1 -1**
- For main effect handedness — **1 -1 1 -1**
- For interaction sex by handedness — **1 -1 -1 1**
- Effects of interest (any difference between the 4 groups) — eye(4)-1/4

Multiple Regression (Linear)
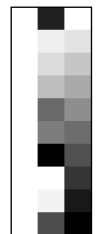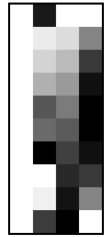

T-test

- For positive correlation — **0 0 1**
- For negative correlation — **0 0 -1**

F-test

- Any correlation — **0 0 1**

The two leading zeros in contrast indicate the constant (sample effect, 1st column in the design matrix) and TIV (2nd column in the design matrix). If no additional covariate such as TIV is defined you must skip one of the leading zeros (e.g. "0 1").

Multiple Regression (Polynomial)

T-test

- For positive linear effect   **0 0 1 0**
- For positive quadratic effect   **0 0 0 1**
- For negative linear effect   **0 0 -1 0**
- For negative quadratic effect   **0 0 0 -1**

F-test

- For any linear effect   **0 0 1 0**
- For any quadratic effect   **0 0 0 1**
- For any linear or quadratic effect   **0 0 1 0**
     0 0 0 1

The two leading zeros in the contrast indicate the constant (sample effect, 1st column in the design matrix) and TIV (2nd column in the design matrix). If no additional covariate such as TIV is defined you must skip one of the leading zeros (e.g. "0 1").

Interaction (Linear)
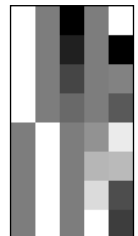
T-test

- For regression slope Group A > Group B   **0 0 1 -1 0**
- For regression slope Group A < Group B   **0 0 -1 1 0**

F-test

- For any difference in regression slope   **0 0 1 -1 0**

The two leading zeros in the contrast indicate the main effect "group".

Interaction (Polynomial)

T-test

- For linear regression slope Group A > Group B   **0 0 1 -1 0 0 0**
- For linear regression slope Group A < Group B   **0 0 -1 1 0 0 0**
- For quadratic regression Group A > Group B   **0 0 0 0 1 -1 0**
- For quadratic regression Group A < Group B   **0 0 0 0 -1 1 0**

F-test

- For any difference in linear regression slope   **0 0 1 -1 0 0 0**
- For any difference in quadratic regression   **0 0 0 0 1 -1 0**
- For any difference in regression   **0 0 1 -1 0 0 0**

                                                                    0 0 0 0 1 -1 0

The two leading zeros in the contrast indicate the main effect "group".

F-contrasts (effects of interest):

If you want to use the old SPM2 F-contrast "Effects of interest" the respective contrast vector is:

eye(n)-1/n

where *n* is the number of columns of interest.

For interaction and regression effects you need to add leading zeros for the constant term or sample effects:

[zeros(n,m) eye(n)-1/n]

where m is the number of columns of no interest.

This F-contrast is useful to check if there are any effects in your model (covariates of interest) and is needed to plot parameter estimates of effects of interest.

<u>**Getting Results:**</u>

SPM menu → Results → *[select a contrast from Contrast Manager]* → Done

- Mask with other contrasts → No

- Title for comparison: *[use the pre-defined name from the Contrast Manager or change it]*

- P value adjustment to:
  - None (uncorrected for multiple comparisons), set threshold to 0.001
  - FDR (false discovery rate), set threshold to 0.05, etc.
  - FWE (family-wise error), set threshold to 0.05, etc.

- Extent threshold: (either use "none" or specify the number of voxels[2])

---

[2] To determine the extent threshold *empirically* (instead of saying 100 voxels or 500 voxels, which is completely arbitrary), simply execute it first without specifying an extent threshold. This gives you an output (i.e., the standard SPM glass brain with significant effects). If you click on "Table" (SPM main menu) you will get a table with all relevant values (MNI coordinates, p-values, cluster size etc.). Below the table you will find additional information, such as "Expected Number of Voxels per Cluster". Remember this number (this is your empirically determined extent threshold). Re-run SPM → Results etc. and enter this number when prompted for the "Extent Threshold". There is also a hidden option in "CAT12 → Data presentation → Threshold and transform spmT-maps" to define the extent threshold as p-value or to use the "Expected Number of Voxels per Cluster".

Special Cases

CAT12 for longitudinal data

BACKGROUND

The majority of VBM studies are based on cross-sectional data in which one image is acquired for each subject. However, to be able to track learning effects over time, for example, longitudinal designs are necessary in which additional time-points are acquired for each subject. The analysis of these longitudinal data requires a customized processing, that takes into account the characteristics of the intra-subject analysis. While images can be processed independently for each subject for cross-sectional data, longitudinal data must be registered to the mean image for each subject by an inverse-consistent realignment. In addition, spatial normalization is only estimated for the mean image of all time points and applied to all images (Figure 4). Additional attention is then required for the setup of the statistical model. The following section therefore describes the data preprocessing and model setup for longitudinal data.
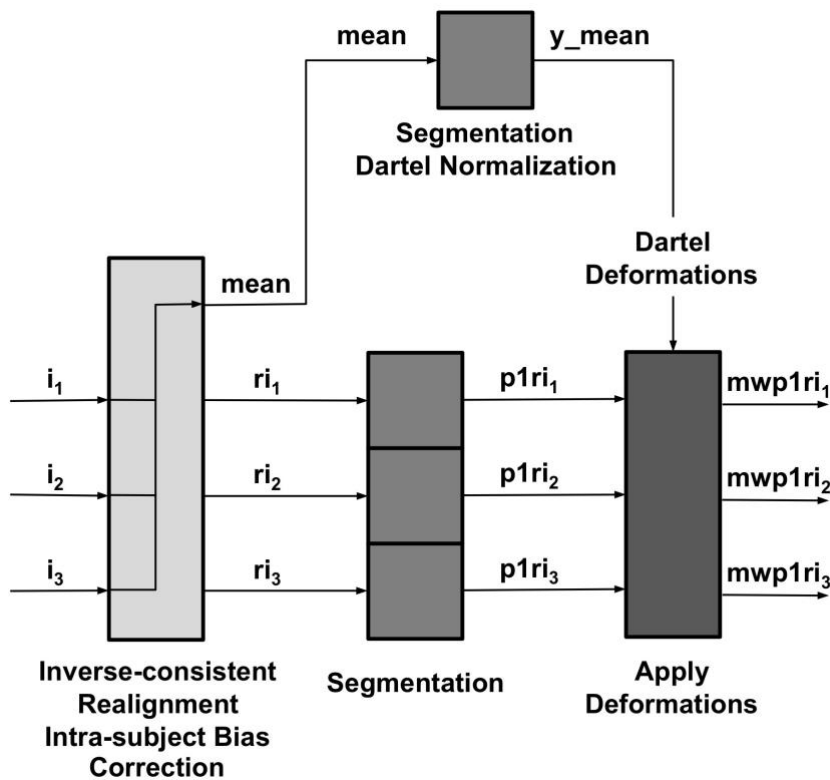
Fig 4.: Flowchart for processing longitudinal data with CAT12. This figure shows the steps for processing longitudinal data. After an initial inverse-consistent realignment, which also includes a bias correction between the different time points, the mean image of the realigned images is calculated (mean). Spatial normalization parameters with the help of a Dartel Normalization are estimated in the next step based on the segmentations of the mean image. These normalization parameters are applied to the segmentations of the images of all time points (p1ri$_x$) and are finally modulated (mwp1ri$_x$).

Preprocessing of longitudinal data - overview

The CAT12 Toolbox supplies a batch for longitudinal study design. Here, for each subject the respective images need to be selected. Intra-subject realignment, bias correction, segmentation, and normalization are calculated automatically. Preprocessed images are written as mwp1r* and mwp2r* for grey and white matter respectively. To define the segmentation and normalization parameters, the defaults in cat_defaults.m are used. Optionally surfaces can be extracted in the same way as in the cross-sectional pipeline and the realigned images are used for this step.

Comparison to SPM12 longitudinal registration

SPM12 also offers a batch for pairwise or serial longitudinal registration. Contrary to CAT12, this method relies on the deformations necessary to non-linearly register the images of all time points to the mean image. These deformations can then be used to calculate local volume changes, which are then multiplied (modulated) by the segmented mean image.

However, it is not only the underlying methods between SPM12 and the CAT12 longitudinal batch that differ, but also the focus of possible applications. SPM12 additionally regularizes the deformations with regard to the time differences between the images and has its strengths more in finding larger effects over longer periods of time (e.g. ageing effects or atrophy). The use of deformations between the time points makes it possible to estimate and detect larger changes, while subtle effects are more difficult to detect over shorter periods of time in the range of weeks or a few months. In contrast, the longitudinal preprocessing was developed and optimized in CAT12 to detect more subtle effects over shorter periods of time (e.g. brain plasticity or training effects after a few weeks or even shorter times).

Unfortunately, there is no clear recommendation for one method or another. However, one rule of thumb could be to use CAT12 the shorter the time periods and the smaller the expected changes are. If you are trying to find effects through learning or training or other intervention (e.g. medication, therapy) then CAT12 is the right choice. To find ageing effects or atrophy due to a (neurodegenerative) disease after longer period of time, I recommend trying the SPM12 longitudinal registration. For that type of data, the sensitivity of CAT12 is likely to be lower, as the DARTEL registration is based on the mean image and is applied to all time points. If the (structural) changes between the time points are large, this is likely to work less reliably, although this has not yet been systematically investigated.

Please note that the surface-based preprocessing is not affected by the potentially lower sensitivity for larger changes, as the realigned images are used independently to create the cortical surfaces and thickness. This could be also a good alternative to find larger changes.

OPTIONAL CHANGE OF PARAMETERS FOR PREPROCESSING

You can change the tissue probability map (TPM) via GUI or by changing the entry in the file cat_defaults.m (i.e. for children data). Any parameters that cannot be changed via the GUI must be set in the file cat_defaults.m:

Change your working directory to "/toolbox/CAT12" in your SPM directory:

→ select "Utilities → cd" in the SPM menu and change to the respective folder.

Then enter "open cat_defaults.m" in your matlab command window. The file is opened in the editor. If you are not sure how to change the values, open the module "Segment Data" in the batch editor as a reference.

PREPROCESSING OF LONGITUDINAL DATA

CAT12 → Segment longitudinal data

Parameters:

- ○ Data <-X → New: Subject → Subject → Longitudinal data for one subject → Select Files → *[select raw data]* → Done
  - Select all volumes for each subject. As the Toolbox does not support multispectral data yet (i.e., different imaging methods for the same brain, such as T1-, T2-, diffusion-weighted or CT images), it is recommended to choose a T1-weighted image.
  - Select "New: Subject" to add data for a new subject

    The data for each subject should be listed as one "subject" in the Batch Editor, i.e. there are as many subjects listed as included in the analysis.

- → For all other options you can follow the instructions for preprocessing of cross-sectional data as described before. Please note that not all writing options are available for longitudinal data.

For the naming conventions of all written files see "Naming convention of output files". The GM segments are mwp1r*, the WM segments are named mwp2r* if you have selected to option to modulate the data. Without modulation the leading "m" is omitted.

Statistical analysis of longitudinal data - overview

The main interest in longitudinal studies lies in the change of tissue volume over time in a group of subjects or the difference in these changes between two or more groups. The setup of the statistical model needed to evaluate these questions is described in two examples. First, the case of only one group of 4 subjects with 2 time points each (e.g. normal aging) is shown. Subsequently, the case of two groups of subjects is described, each with 4 time points per subject. These examples should cover most analyses – the number of time points / groups only needs to be adjusted. In contrast to the analysis of cross-sectional data as described above, we need to use the flexible factorial model, which takes into account that the time points for each subject are dependent data.

STATISTICAL ANALYSIS OF LONGITUDINAL DATA IN ONE GROUP

CAT12 → Statistical Analysis → Basic Models

  Parameters:

- Directory <-X → Select Files → *[select the working directory for your analysis]* → Done
- Design → "Flexible Factorial"
  - Factors → *"New: Factor; New: Factor"*
    Factor
    - Name → *[specify text (e.g. "subject")*]*
    - Independence → Yes
    - Variance → Equal or Unequal
    - Grand mean scaling → No
    - ANCOVA → No

    Factor
    - Name → *[specify text (e.g. "time")]*
    - Independence → No
    - Variance → Equal or Unequal
    - Grand mean scaling → No
    - ANCOVA → No

> *SPM is internally handling some keyword factors such as "subject" or "repl". If you use "subject" as keyword for the first factor the conditions can be easier defined by only labeling the time points as input (see below).

  - Specify Subjects or all Scans & Factors → *"Subjects"* → *"New: Subject; New: Subject; New: Subject; New: Subject;"*
    Subject
    - Scans → *[select files (the smoothed GM volumes of the first Subject)]*
    - Conditions → "1 2" *[for two time points]*
    Subject
    - Scans → *[select files (the smoothed GM volumes of the second Subject)]*
    - Conditions → "1 2" *[for two time points]*
    Subject
    - Scans → *[select files (the smoothed GM volumes of the third Subject)]*

- Conditions → "1 2" *[for two time points]*

  Subject
  - Scans → *[select files (the smoothed GM volumes of the fourth Subject)]*
  - Conditions → "1 2" *[for two time points]*

- Main effects & Interaction → *"New: Main effect"*

  Main effect
  - Factor number → 2

  Main effect
  - Factor number → 1

- **Covariates*** (see text box in example for two-sample T-test)

- Masking
  - Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
  - Implicit Mask → Yes
  - Explicit Mask → <None>

- Global Calculation → Omit

- Global Normalization
  - Overall grand mean scaling → No

- Normalization → None

STATISTICAL ANALYSIS OF LONGITUDINAL DATA IN TWO GROUPS

CAT12 → Statistical Analysis → Basic Models

Parameters:

- Directory <-X → Select Files → *[select the working directory for your analysis]* → Done

- Design → "Flexible Factorial"
  - Factors → *"New: Factor; New: Factor; New: Factor"*

    Factor
    - Name → *[specify text (e.g. "subject")*]*
    - Independence → Yes
    - Variance → Equal
    - Grand mean scaling → No
    - ANCOVA → No

    Factor
    - Name → *[specify text (e.g. "group")]*

*SPM is internally handling some keyword factors such as "subject" or "repl". If you use "subject" as keyword for the first factor the conditions can be easier defined by only labeling the time points as input (see below).

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- Independence → Yes
- Variance → Unequal
- Grand mean scaling → No
- ANCOVA → No

Factor

- Name → *[specify text (e.g. "time")]*
- Independence → No
- Variance → Equal
- Grand mean scaling → No
- ANCOVA → No

▪ Specify Subjects or all Scans & Factors → *"Subjects"* → *"New: Subject; New: Subject; New: Subject; New: Subject;"*

Subject

- Scans → [select files (the smoothed GM volumes of the 1st Subject of first group)]
- Conditions → " [1 1 1 1; 1 2 3 4]**'** " [*first group with four time points*]

    Do not forget the additional single quote! Otherwise you have to define the conditions as " [1 1; 1 2; 1 3; 1 4] "

Subject

- Scans → [select files (the smoothed GM volumes of the 2nd Subject of first group)]
- Conditions → " [1 1 1 1; 1 2 3 4]**'** " [*first group with four time points*]

Subject

- Scans → [select files (the smoothed GM volumes of the 3rd Subject of first group)]
- Conditions → " [1 1 1 1; 1 2 3 4]**'** " [*first group with four time points*]

Subject

- Scans → [select files (the smoothed GM volumes of the 4th Subject of second group)]
- Conditions → " [2 2 2 2; 1 2 3 4]**'** " [*second group with four time points*]

Subject

- Scans → [select files (the smoothed GM volumes of the 1st Subject of second group)]
- Conditions → " [2 2 2 2; 1 2 3 4]**'** " [*second group with four time points*]

Subject

- Scans → [select files (the smoothed GM volumes of the 2nd Subject of second group)]
- Conditions → " [2 2 2 2; 1 2 3 4]**'** " [*second group with four time points*]

Subject

- Scans → [select files (the smoothed GM volumes of the 3rd Subject of second group)

- - Conditions → " [2 2 2 2; 1 2 3 4]**'** " [*second group with four time points*]

    Subject

  - - Scans → [select files (the smoothed GM volumes of the 4th Subject of second group)]
    - Conditions → " [2 2 2 2; 1 2 3 4]**'** " [*second group with four time points*]
  - Main effects & Interaction → *"New: Interaction; New: Main effect"*

    Interaction

    - - Factor numbers → 2 3 *[Interaction between group and time]*

    Main effect

    - - Factor number → 1

- **Covariates*** (see text box in example for two-sample T-test)

- Masking
  - Threshold Masking → Absolute → *[specify value (e.g. "0.1")]*
  - Implicit Mask → Yes
  - Explicit Mask → <None>

- Global Calculation → Omit

- Global Normalization
  - Overall grand mean scaling → No

- Normalization → None

Contrasts

Longitudinal data in one group (example for two time points)
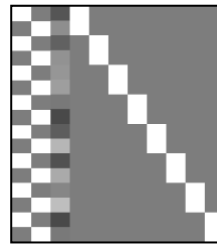
T-test

- For Time 1 > Time 2   **1 -1**

- For Time 1 < Time 2   **-1 1**
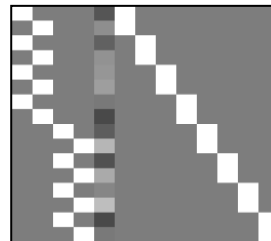
F-test

- For any differences in Time   **1 -1**

Longitudinal data in two groups (example for two time points)

T-test

- For Time 1 > Time 2 in Group A   **1 -1 0 0**

- For Time 1 > Time 2 in Group B   **0 0 -1 1**

- For Time 1 > Time 2 in both Groups   **1 -1 1 -1**

- For Time 1 > Time 2 & Group A > Group B   **1 -1 -1 1**

F-test

- For any differences in Time in Group A   **1 -1 0 0**

- For any differences in Time in Group B   **0 0 1 -1**

- For Main effect Time   **1 -1 1 -1**

- For Main effect Group   **ones(1,n)/n -ones(1,n)/n 0 ones(1,n1)/n1 -ones(1,n2)/n2**

- For Interaction Time by Group   **1 -1 -1 1**

Here n1 and n2 are the number of subjects in Group A and B respectively and n is the number of time points. Please note that the zero in the 5th column for the main effect Group indicates the covariate of no interest (e.g. TIV).

Adapting the workflows

Background

For most analyses, the VBM Toolbox provides all necessary tools. Since the new segmentation algorithm is no longer dependent on Tissue Probability Maps (TPMs) and pre-defined DARTEL templates for healthy adults exist, most questions can be evaluated using the default Toolbox settings. However, the Toolbox settings may not be optimal for some special cases, such as analysis in children or certain patient groups. Below we present strategies for dealing with these specific cases.

Customized tissue probability maps - overview

For data on children it is a good idea to create customized TPMs, which reflect age and gender of the population. The TOM8 Toolbox (available via: https://irc.cchmc.org/software/tom.php) provides the needed parameters to customize these TPMs. To learn more about the TOM toolbox, see also http://dbm.neuro.uni-jena.de/software/tom.

CUSTOMIZED TISSUE PROBABILITY MAPS

About the TOM8 Toolbox:

→ *select Module "create new template"*

    → *select "TOM.mat" (you will have to download this file together with the toolbox)*

    → *write priors/template as single file*

    → *for all others use default settings or modify. For "Age" either a vector or a mean*

    age (when using the average approach) must be specified.

Implementation into CAT12:

CAT12 → Segment Data

    Parameters:
- Options for initial SPM12 affine registration
  - Tissue Probability Map (→ Select your customized TPMs here)

Customized DARTEL-template - overview

An individual (customized) DARTEL template can be created for all cases involving at least 50-100 subjects. This means that an average template of the study sample is created from the tissue segments of the grey matter and white matter tissue segments of all subjects. Since the CAT12 toolbox writes all the files needed to create these templates ("DARTEL export"), only two additional steps are required. To be able to use these newly created DARTEL-Templates with the CAT12 Toolbox, an affine registration of the DARTEL export should be used. Customized DARTEL templates

can then be created from these affine registered segments and used with the CAT12 module "Segment Data".

CUSTOMIZED DARTEL-TEMPLATE

Please note that the use of your own DARTEL template results in deviations and unreliable results for ROI-based estimations as the atlases are different. Therefore, all ROI outputs in CAT12 are disabled if you use your own customized DARTEL template.

Several steps are required to create normalized tissue segments with customized DARTEL Templates. These steps can be bundled using dependencies in the Batch Editor. The last step can be repeated with the customized templates if additional output files are required. In the first step, the T1 images are segmented and the tissue segments are normalized to the Tissue Probability Maps by means of an affine transformation. Start by selecting the module "Segment Data".

CAT12 → Segment Data

> Parameters:
>
> → for all options except "writing options" use settings like for a "standard" VBM analysis.
>
> o Writing Options
>
> - "Grey Matter"→ "Modulated normalized" → "No"
> - "Grey Matter"→ "DARTEL export" → "affine"
> - "White Matter"→ "Modulated normalized" → "No"
> - "White Matter"→ "DARTEL export" → "affine"

These settings generate the volumes "rp1*-affine.nii" and "rp2*-affine.nii", i.e. the grey (rp1) and white (rp2) matter segments after affine registration. The following modules can be selected directly in the batch editor (SPM → Tools → DARTEL Tools → Run DARTEL (create Templates), and SPM → Tools → CAT12 → CAT12: Segment Data). It makes sense to add and specify these modules together with the "Segment Data" module within the Batch Editor and to set dependencies.

SPM → Tools → DARTEL Tools → Run DARTEL (create Templates)

> Parameters:
>
> Images → *select two times* "new: Images"
>
> ▪ Images: → select the "rp1*-affine.nii" files or create a dependency.
> ▪ Images: → select the "rp2*-affine.nii" files or create a dependency.
>
> → all other options: *use defaults or modify*

SPM → Tools → DARTEL Tools → Normalise to MNI space

Parameters:

DARTEL Template → select the final created template with the ending "_6".

- o Select according to → "Many Subjects"
    - ▪ Flow fields: → select the "u_*.nii" files or create a dependency.
        - • Images: → New: Images → *select the* "rp1*-affine.nii" *files or create a dependency.*
        - • Images: → New: Images → *select the* "rp2*-affine.nii" *files or create a dependency.*
- o Preserve: → "Preserve Amount"
- o Gaussian FWHM: → use defaults or modify

→ all other options: *use defaults or modify*

Please note, that a subsequent smoothing is not necessary before statistics if you have used the option "Normalise to MNI space" with a defined Gaussian FWHM.

Re-use of customized DARTEL-templates

Optionally you can also use the newly created, customized DARTEL templates in the CAT12 Toolbox. This can be helpful if new data is age-wise close to the data used to create the DARTEL template (e.g. you have an ongoing study and some more new subjects are added to the sample). In this case, it is not absolutely necessary to always create a new template and you can simply use the previously created DARTEL template. In this case, an additional registration to MNI (ICBM) space must be applied:

SPM → Tools → DARTEL Tools → Run DARTEL (Population to ICBM Registration)

Parameters:

DARTEL Template → select the final created template with the ending "_6".

SPM → Util → Deformations

Parameters:

Composition → "New: Deformation Field"

- ▪ Deformation Field: → *select the* "y_*2mni.nii" *file from step above.*
- o Output → "New: Pushforward"
    - ▪ Apply to → select all "Template" files with the ending "_0" to "_6".
    - ▪ Output destination → Output directory → *select directory for saving files.*
    - ▪ Field of View → Image Defined → *select final* "Template" *file with the ending "_6".*

- Preserve → Preserve Concentrations (no "modulation").

Finally, the new template can be used as default DARTEL template for any new data that are close to the data used for template creation:

CAT12 → Segment Data

   Parameters:
   - Volumes <-X → Select the original T1 images like in the first module "Segment Data".
   - Extended Options for CAT12 segmentation → "Spatial normalization Template" → *Select normalized DARTEL Template "wTemplate*_1.nii"*
     - For all other options use the same settings as in the first module, or modify.
   - Writing Options → *select the output files just like in any standard VBM analysis:*

Please note, that now the output files have to be smoothed as usual with before the statistical analysis.

How to proceed

All the steps described above are only an adaption of the CAT12 Toolbox module "Segment Data". As always, it is a good idea to store the modules and perform a quality control. The modules "Display one slice for all images" and "Check sample homogeneity" from the CAT12 Toolbox are helpful here.

Other variants of computational morphometry

Deformation-based morphometry (DBM)

Background

DBM is based on the application of non-linear registration procedures to spatially normalize one brain to another one. The simplest case of spatial normalization is to correct the orientation and size of the brain. In addition to these global changes, a non-linear normalization is necessary to minimize the remaining regional differences due to local deformations. If this local adaptation is possible, the deformations now provide information about the type and localization of the structural differences between the brains and can then be analyzed.

Differences between the two images are minimized and are now coded in the deformations. Finally, a map of local volume changes can be quantified by a mathematical property of these deformations – the Jacobian determinant. This parameter is well known from continuum mechanics and is usually used for the analysis of volume changes in flowing liquids or gases. The Jacobian determinant allows a direct estimation of the percentage change in volume in each voxel and can be analyzed statistically (Gaser et al. 2001). This approach is also known as tensor-based morphometry since the Jacobian determinant represents such a tensor.

A deformation-based analysis can be performed not only on the local volume changes, but also on the entire information of the deformations, which also includes the direction and strength of the local deformations (Gaser et al. 1999; 2016). Since each voxel contains three-dimensional information, a multivariate statistical test is required for analysis. For this type of analysis, a multivariate general linear model or Hotelling's T2 test is often used (Gaser et al. 1999; Thompson et al. 1997).

Additional Steps in CAT12

CAT12 → Segment Data

> Parameters:
>> o Writing options → Jacobian determinant → *Normalized* → Yes
>>> - To save the estimated volume changes change the writing option for the normalized Jacobian determinant to "yes".

Changes in statistical analysis

Follow the steps for the statistical analysis as described for VBM, select the smoothed Jacobian determinants (e.g. swj_*.nii) and change the following parameters:

CAT12 → Statistical Analysis → Basic Models

> Parameters:
>> o Covariates: **Don't use TIV as covariate**
>> o Masking
>>> ▪ Threshold Masking → **None**
>>> ▪ Implicit Mask → Yes
>>> ▪ Explicit Mask → **../spm12/tpm/mask_ICV.nii**

Surface-based morphometry (SBM)

Background

Surface-based morphometry has several advantages over the sole use of volumetric data. For example, is has been shown that using brain surface meshes for spatial registration increases the accuracy of brain registration compared to volume-based registration (Desai et al. 2005). Brain surface meshes also permit new forms of analyses, such as gyrification indices which measure surface complexity in 3D (Yotter et al. 2011b) or cortical thickness (Gaser et al. 2016). In addition, inflation or spherical mapping of the cortical surface mesh raises the buried sulci to the surface so that mapped functional activity in these regions can be made easily visible.

Local adaptive segmentation

Gray matter regions with high iron concentration, such as the motor cortex and occipital regions, often have an increased intensity leading to misclassifications. In addition to our adaptive MAP approach for partial volume segmentation, we use an approach that allows to adapt local intensity changes to deal with varying tissue contrasts (Dahnke et al. 2012a).

Cortical thickness and central surface estimation

We use a fully automated method that allows the measurement of cortical thickness and reconstruction of the central surface in one step. It uses a tissue segmentation to estimate the white matter (WM) distance and then projects the local maxima (which is equal to the cortical thickness) onto other gray matter voxels using a neighboring relationship described by the WM distance. This projection-based thickness (PBT) allows the handling of partial volume information, sulcal blurring, and sulcal asymmetries without explicit sulcus reconstruction (Dahnke et al. 2012b).

Topological correction

To repair topological defects, we use a novel method based on spherical harmonics (Yotter et al. 2011a). First of all, the original MRI intensity values are used to select either a "fill" or "cut" operation for each topological defect. We modify the spherical map of the uncorrected brain surface mesh in such a way that certain triangles are preferred when searching for the bounding triangle during reparameterization. Subsequently, a low-pass filtered alternative reconstruction based on spherical harmonics is patched into the reconstructed surface in areas where defects were previously present.

Spherical mapping

A spherical map of a cortical surface is usually necessary to reparameterize the surface mesh into a common coordinate system to enable inter-subject analysis. We use a fast algorithm to reduce area distortion, which leads to an improved reparameterization of the cortical surface mesh (Yotter et al. 2011c).

Spherical registration

We have adapted the volume-based diffeomorphic DARTEL algorithm to the surface (Ashburner, 2007) to work with spherical maps (Yotter et al. 2011d). We apply a multi-grid approach that uses reparameterized values of sulcal depth and shape index defined on the sphere to estimate a flow field that allows the deformation of a spherical grid.

Additional Steps in CAT12

CAT12 → Segment Data

Parameters:
- o Writing options → Surface and thickness estimation → Yes

  Use projection-based thickness to estimate cortical thickness and to create the central cortical surface for the left and right hemisphere.

CAT12 → Surface Tools → Resample & Smooth Surfaces

Parameters:
- o Surface Data <-X → Select the surface data (e.g. [lr]h.thickness.*)

- o Smoothing Filter Size in FWHM *[use defaults or modify]*
  - - 12-15mm kernels are widely used for SBM and I recommend to start with a value of 15mm for thickness data and 20mm for folding data (e.g. gyrification, complexity).
- o Split job into separate processes

  - - To use multi-threading the CAT12 segmentation job with multiple subjects can be split into separate processes that run in the background. You can even close Matlab, which will not affect the processes that will run in the background without GUI. If you don't not want to run processes in the background then set this value to 0.
  - - Keep in mind that each process needs about 1.5..2GB of RAM, which should be considered to choose the right number of processes.
  - - Please further note that no additional modules in the batch can be run except CAT12 segmentation. Any dependencies are broken for subsequent modules.

Extract optional surface parameters

You can also extract additional surface parameters that need to be resampled and smoothed with the above tool.

CAT12 → Surface Tools → Extract & Map Surface Data → Extract Additional Surface Parameters

Parameters:

- o Central Surfaces <-X → Select the central surface data (e.g. [lr]h.central.*)

- o Gyrification index
    - - Extract gyrification index (GI) based on absolute mean curvature. The method is described in Luders et al. NeuroImage, 29: 1224-1230, 2006.
- o Cortical complexity (fractal dimension)

Extract Cortical complexity (fractal dimension) which is described in Yotter et al. Neuroimage, 56(3): 961-973, 2011.

Warning: Estimation of cortical complexity is very slow!

- o Sulcus depth

Extract sqrt-transformed sulcus depth based on the Euclidean distance between the central surface and its convex hull.

Transformation with sqrt-function is used to render the data more normally distributed.

Changes in statistical analysis

For statistical analysis of surface measures you can use the common 2nd level models, which are also used for VBM. Follow the steps for the statistical analysis as described for VBM and change the following parameters:

CAT12 → Statistical Analysis → Basic Models

Parameters:

- o Covariates: **Don't use TIV as covariate**

- o Masking
    - ▪ Threshold Masking → **None**
    - ▪ Implicit Mask → Yes
    - ▪ Explicit Mask → <none>

As input you have to select the resampled and smoothed files (see above). A thickness file of the merged left and right hemispheres that was resampled and smoothed with the default FWHM of 15mm is named as:

S15.mesh.thickness.resampled.name.gii

where "name" if the original filename without extension. Please note that these files are saved in the surf-folder as default.

Do not use the "Estimate" function in the SPM window, but the corresponding function in CAT12. This allows you to automatically overlay the results on the Freesurfer average surface:

CAT12 → Statistical Analysis → Estimate Surface Models

Region of interest (ROI) analysis

CAT12 enables the estimation of tissue volumes (and additional surface parameters such as cortical thickness) for different volume and surface-based atlas maps (Gaser et al. 2016). All of these results are estimated in the native space before any spatial normalization. The results for each dataset are stored as XML files in the *label* directory. The XML file *catROI[s]_*.xml* contains information of all atlases as data structure for one dataset and the optional "s" indicates surface atlases. You can use the CAT function "cat_io_xml" to read the XML data as a structure.

Additional steps for surface data

While ROI-based values for VBM (volume) data are automatically stored in the *label* folder as XML file it is necessary to extract these values additionally for surface data. This must be done after preprocessing the data and creating cortical surfaces. You can extract ROI-based values for cortical thickness but also for any other surface parameter that extracted with the "Extract Additional Surface Parameters" function:

CAT12 → Extract ROI Data → Extract ROI-based surface values

> Parameters:
> - (Left) Surface Data Files <-X → Select surface data files such as lh.thickness.* for all subjects

Statistical analysis of ROI data

Finally, the XML files of several subjects can be analyzed using an existing SPM design with *CAT12 → Analyze ROIs*. Here, the SPM.mat file is used to get information about all corresponding label files, but also about your design (including all covariates/confounds you have modeled). In this way, the same statistical analysis saved in the SPM.mat file is applied to your ROI data. You can then select a contrast, a threshold value and a measurement type to be analyzed (e.g. Vgm, Vwm, thickness, gyrification...) and choose between different atlas maps. The results are printed and saved as thresholded log-p volume or surface map:

> logP*Threshold_NameOfContrast_NameOfAtlas_Measure*.nii
> [lr]h.logP*Threshold_NameOfContrast_NameOfAtlas_Measure*.gii

These maps can be optionally visualized using *CAT12 → Display Results → Slice Overlay* for volume maps or *CAT12 → Display Results → Display surface results* for surface maps.

To analyze different measures (e.g. Vgm/Vwm for volumes or thickness/gyrification for surfaces) you can use any existing volume-based analysis to extract different volume measures, or any existing surface-based analysis to extract different surface measures. To give you an example: An existing SPM.mat file with a VBM analysis of GM allows you to analyze ROI measures for both, GM as well as WM. Therefore, it is not necessary to have a SPM.mat file of a VBM analysis of WM. The same applies to surface-based analysis. If you already have an SPM.mat for the analysis of cortical thickness, you can also estimate the ROI analysis for gyrification or fractal dimension. For surfaces,

however, it is necessary to extract ROI-based measures for each subject using *CAT12 → Extract ROI Data → Extract ROI-based surface values* prior to this step.

For ROI analysis of surfaces you can select the SPM.mat file of the analysis, either for the left or right hemisphere, or for the merged hemispheres. The design should be the same and the ROI results are always estimated for both hemispheres.

Please note that if you have moved your data after estimating your original voxel- or surfaced-based statistics, the required ROI files cannot be found.

Optional extraction of ROI data

Finally, the XML files of several subjects can be combined and saved as CSV file using the "Estimate Mean Values inside ROI" function for further analysis:

CAT12 → Extract ROI Data → Estimate mean values inside ROI

> Parameters:
> - XML files <-X → Select xml files for each subject that are saved in the label folder.
> - Output file → Define output name for csv file. This name is extended by the atlas name and the name of the measure (e.g. "Vgm" for gray matter volume)

For each measurement (e.g. "Vgm" for gray matter volume) and each atlas a separate CSV file is written. This works for both volume and surface data, but volume and surface data has to be processed separately with this function (surface-based ROI values are indicated by an additional "s", e.g. ''catROIs_''). You can use external software such as Excel or SPSS to import the resulting CSV files for further analysis. Also pay attention to the different interpretations between "." and "," depending on your region and language settings on your system.

Use of atlas functions in SPM12

You can also use the volume-based atlases delivered with CAT as atlas maps with *SPM atlas functions*. This is particularly useful if you have used the default VBM processing pipeline, since the CAT12 atlas maps are then in the same DARTEL space as your data. If you have used the default VBM processing pipeline, it is recommended to use CAT12 atlases in the DARTEL space instead of the SPM Neuromorphometrics atlas. To use CAT12 atlases, you must call the *cat_install_atlases* function once , which copies the atlases to SPM. By default, only Neuromorphometics, LPBA40, and Hammers atlas maps are used, which are identified by a leading ''dartel_'' in the name.

Atlas maps for surfaces can be used with the function *CAT12 → Display Results → Display Surface Results*. You can use the data cursor function to display atlas regions under the cursor. In addition, you can use the ''Atlas labeling'' function to print a list of atlas regions of the resulting clusters or overlay the atlas borders on your rendered surface.

Additional information on native, normalized and modulated volumes

When preprocessing the images (see "First Module: Segment Data", on pages 5-6), the decision on the normalization parameters determines the interpretation of the analysis results. Please note that some of the output options are only available in the expert mode.

"**Native space**" creates tissue class images in spatial correspondence to the original data. Although this may be useful for estimating global tissue volumes (e.g. GM+WM+CSF=TIV) it is not suitable for

performing VBM analyses due to the lack of voxel-wise correspondence in the brain). If one is interested in these global tissue volumes in the native space ("raw values"), it is not necessary to actually output the tissue class images in native space. The "Segment Data"-function automatically generates an xml file for each subject (cat_*.xml), which contains the raw values for GM, WM, and CSF. The subject-specific values can be calculated (i.e., integrated into a single text file) using the function: CAT12 → Statistical Analysis → Estimate TIV.

"**Normalized**" creates tissue class images in spatial correspondence to the template. This is useful for VBM analyses (e.g. "concentration" of gray matter; Good et al. 2001; Neuroimage).

"**Modulated normalized**" creates tissue class images in alignment with the template, but multiplies ("modulates") the voxel values with the Jacobian determinant (i.e., linear and non-linear components) derived from the spatial normalization. This is useful for VBM analyses and allows the comparison of the *absolute amount of tissue* (e.g. "volume" of gray matter; Good et al. 2001; Neuroimage). Please note that in this type of modulation you must use TIV as covariate to correct different brain sizes. An alternative approach is to use global scaling with TIV if TIV is too strongly correlated with your covariates of interest. For more information, see the section "Checking for Design Orthogonality".

If you use the expert mode, you can optionally choose whether you want to modulate your data for non-linear terms only, which was the default for previous VBM-toolbox versions. This produces tissue class images in alignment with the template, but the voxel values are only multiplied by the non-linear components. This is useful for VBM analyses and allows comparison of the *absolute amount of tissue corrected for individual brain sizes*. This option is similar to using "Affine+non-linear" (see above) in combination with "global normalization" (when building the statistical model with the traditional PET design late one). Although this type of modulation was used in as default previous VBM-toolbox versions it is no longer recommended as the use of standard modulation in combination with TIV as covariate provides more reliable results (Malone et al. 2015; Neuroimage).

Naming convention of output files

Please note that the resulting files of CAT12 are organized in separate subfolders (e.g. mri, report, surf, label). If you don't want to use subfolders you can change the option "cat12.extopts.subfolders" in cat_default.m to "0".

Images (saved in subfolder "mri")

Segmented Images:		#p[0123]*	[m[0]w]p[0123]*[_affine].nii
Bias, noise and intensity corrected T1 image:	#m*		[w]m*.nii
Jacobian determinant		#j_	wj_*.nii
Deformation field (inverse field)		y_ (iy_)	y_*.nii (iy_*.nii)

*	filename
#	image space prefix

Image space prefix:

m	modulated
m0	modulated non-linear only (expert mode only)
w	warped (spatially normalized using DARTEL)

Image space extension:

_affine	affine registered only

Image data prefix:

p	partial volume (PV) segmentation
0	PV label
1	GM
2	WM
3	CSF

Surfaces in native space (saved in subfolder "surf")

SURF.TYPE.*.gii

SURF   left, right hemisphere [lh | rh]

TYPE   surface data file [central | sphere | thickness | gyrification | ...]

              central - coordinates and faces of the central surface

              sphere - coordinates and faces of the spherical projection of the central surface

              sphere.reg - coordinates and faces of the sphere after spherical registration

              thickness - thickness values of the surface

              sqrtsulc - sqrt-transformed values of sulcal depth based on the euclidean distance between the central surface and its convex hull

              gyrification - gyrification values based on absolute mean curvature

              fractaldimension - fractal dimension values (cortical complexity)

Surfaces in (normalized) template space (after resampling and smoothing; saved in subfolder "surf" by default)

FWHM.SURF.TYPE.RESAMP.*.gii

FWHM   filtersize in FWHM after smoothing (e.g. s15)

SURF   left, right, or both hemispheres [lh | rh | mesh]

TYPE   surface data file [thickness | gyrification | fractaldimension | ...]

              thickness - thickness values of the surface

              sqrtsulc - sqrt-transformed values of sulcul depth based on the euclidean distance between the central surface and its convex hull

              gyrification - gyrification values based on absolute mean curvature

              fractaldimension - fractal dimension values (cortical complexity)

RESAMP   resampling to 164k (Freesurfer) or 32k (HCP) mesh [resampled | resampled_32k]

Images and surface of longitudinal data

After processing longitudinal data, the filenames additionally contain an "r" between the original filename and the other prefixes to indicate the additional registration step. Please also note that only the bias, noise and intensity corrected average image of all time points for each subject is saved.

Reports (saved in subfolder "report")

Global morphometric and image quality measures are stored in the *cat_*.xml* file. This file also contains other useful information about software versions and options used to preprocess the data. You can use the cat_io_xml function to read data from xml-files. In addition, a report for each data set is saved as pdf-file *catreport_*.pdf*.

Regions of interest (ROI) data (saved in subfolder "label")

ROI data is optionally saved as xml-file *catROI [s]_*.xml*. The optional "s" indicates surface atlases.

Calling CAT from the UNIX command line

You can also call CAT as a shell script from the Unix command line. This makes it possible to run the process completely in the background without graphical output and distributes all given data to parallel jobs. This can be useful if you want to run CAT on a computer cluster.

You can use the shell scripts cat_batch_cat.sh or cat_batch_long.sh (for longitudinal data). Please call the scripts for more information on how to define optional defaults files, for example.

There is also a script distribute_to_server.sh that can be used to distribute jobs to different servers.

Technical information

This toolbox is an extension of the segmentation in SPM12, but uses a completely different segmentation approach.[3]

Interpolation

CAT12 uses an internal interpolation to provide more reliable results even with low resolution images and anisotropic spatial resolutions. Although interpolation cannot add more details to the images, some of the functions used benefit from the higher number of voxels and the usual strip artefacts in modulated images are greatly reduced.

Denoising

We also use two noise reduction methods. The first method is a spatial-adaptive Non-Local Means (SANLM) denoising filter and is applied after intensity normalization (Manjón et al. 2010). This filter removes noise while maintaining edges and is implemented as pre-processing step. The second method is a classical Markov Random Field (MRF) approach, which includes spatial information from adjacent voxels in the segmentation estimation (Rajapakse et al. 1997) and is part of the AMAP

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[3] The classic SPM12 segmentation is still used in addition, but only to initially remove non-brain tissue from the image and to get a starting estimate for the segmentation.

segmentation. The strength of the filters is automatically determined by estimating the residual noise in the image or can be set manually.

Affine Preprocessing (APP)

To improve the initial SPM segmentation, an initial affine registration is applied to a bias-corrected image and the intensity range is limited to avoid problems in special protocols. If the preprocessing fails a more aggressive version is available that applies a rough bias correction and removes non-brain parts the brain before the initial affine registration.

Local Adaptive Segmentation (LAS)

In addition WM-inhomogeneities, GM intensity can vary for different regions such as the motor cortex, the basal ganglia, or the occipital lobe. These changes have an anatomical background (e.g. iron content, myelenization), but are dependent on the MR-protocol and often lead to GM-underestimations at higher intensities and CSF-overestimations at lower intensities. Therefore, a local intensity transformation of all tissue classes is used to reduce these effects in the image before the final AMAP segmentation. The strength of the changes is controlled by the LASstr parameter, with 0 for no LAS, small values (0.01-0.5) for small adjustments, 0.5 for medium adjustments (default), and higher values (0.5-1) for strong adjustments.

AMAP Segmentation

The segmentation approach is based on an Adaptive Maximum A Posterior (AMAP) technique without the need for *a priori* information on the tissue probabilities. This means that the Tissue Probability Maps (TPM) are not constantly used in the sense of the classical Unified Segmentation approach (Ashburner et. al. 2005), but only for spatial normalization, initial skull-stripping, and as initial segmentation estimate. The subsequent AMAP estimation is adaptive in the sense that local variations of the parameters (i.e., means and variance) are modelled as slowly varying spatial functions (Rajapakse et al. 1997). This accounts not only for intensity inhomogeneities, but also for other local intensity variations.

Partial Volume Segmentation

In addition, the segmentation approach uses a Partial Volume Estimation (PVE) with a simplified mixed model of a maximum of two tissue types (Tohka et al. 2004). We begin with an initial segmentation into three pure classes: gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) based on the AMAP estimation described above. The initial segmentation is followed by a PVE consisting of two additional mixed classes: GM-WM and GM-CSF. This results in an estimate of the amount (or fraction) of each pure tissue type that is present in each voxel (since single voxels - given their size - probably contain more than one tissue type) and thus allows for more precise segmentation.

Skull-Stripping

CAT12 contains a revised graph-cut based skull-stripping, with 0 for a more liberal and wider brain masks and 1 for a more aggressive skull-stripping. The default setting is 0.5 and has been successfully tested on a variety of different images.

The strength parameter affects several internal parameters:

- Intensity thresholds for the treatment of blood-vessels and meninges
- Distance and growth parameters for the graph-cut/region-growing
- Closing parameters that fill the sulci
- Smoothing parameters that allow sharper or wider results

If your segmentations still contain skull and other non-brain tissue (e.g. dura) you can try to increase the strength. If parts of the brain are missing in the segmentations, the strength can be decreased.

Cleanup

CAT12 includes a new cleanup routine that uses morphological, distance and smoothing operations to remove the remaining meninges after the final segmentation. The strength of the cleanup is controlled by the cleanupstr parameter, with 0 for no cleanup, low values <0.5 for light cleanup, 0.5 for medium cleanup (default), and 1 for strong cleanup.

Spatial Normalization

Another important extension of the SPM12 segmentation is the integration of the DARTEL (Ashburner 2007) and Geodesic Shooting (Ashburner & Friston 2011) normalization into the toolbox by already existing DARTEL and Geodesic Shooting templates in MNI space. These templates were derived from 555 healthy control subjects of the IXI-database (http://www.brain-development.org) and are available in the MNI space[4] for six different iteration steps of the DARTEL and Geodesic Shooting normalization. Therefore, the creation of sample-specific DARTEL and Geodesic Shooting templates is no longer necessary for most studies [5].

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[4] Therefore, no additional MNI normalization is necessary.

[5] For studies investigating children data, I still recommend creating a customized DARTEL or Geodesic Shooting template. However, it should be noted that this option requires a representative sample with a sufficient number of subjects.

References

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