

# Allen ROI Extraction Toolbox - Instruction Manual

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## 1 Introduction and Overview

This manual summarizes the two main functionalities of the ARET toolbox and explains its use. These two functionalities are ROI Extraction and Cluster Labelling. ROI Extraction involves creation of masks of user-specified brain regions from the Allen Brain Atlas, using the Allen annotation template (ANO.nii) file. Cluster Labelling involves stating the proportion of brain regions of each cluster blob in the cluster image, which is obtained from a SPM analysis. For example, a voxel-based morphometry (VBM) pipeline generates F- or T-images representing statistical effects that have been observed in a group comparison or other type of GLM statistics. Thresholded versions of these images are used as input for Cluster Labelling. Moreover, the specific source of the result image (also referred to as cluster image) of such an analysis is not relevant, as cluster images may also stem from other imaging techniques such as diffusion tensor imaging or functional imaging.

## 2 Getting Started

### 2.1 Download and Installation

- This toolbox has been tested on Matlab R2019a.
- This toolbox has been tested on SPM12 version 7771. Download and install SPM12 and add it to your Matlab search path using the `addpath(genpath())` command from Matlab. SPM can be downloaded from the following link:<https://www.fil.ion.ucl.ac.uk/spm/software/download/>.

- Download the Allen ROI Extraction Tool from the following link: <https://github.com/tanchaud/ARET.git>. Copy the toolbox to a preferred location in your file system and add its path to Matlab, using `addpath(genpath)` command from Matlab. This can be done in the following way - `addpath(genpath(*fullpath to ARET in your filesystem*))`.

### 3 Toolbox Functionalities

#### 3.1 Implementation Instructions

- Open the script named `Start_ARET.m` in Matlab's editor window, by typing out the following command in Matlab's command window - `Start_ARET.m`.
- Change the global variables in this script. These global variables are `DataDir` and `ToolboxDir`.
- `DataDir` is the fullpath to the folder that contains the cluster image or several cluster images. Hence, for example, if the cluster images are located in `/home/data/cluster_images`, the value of the `DataDir` input argument will be given as, `DataDir = '/home/data/cluster_images/'`.
- `ToolboxDir` is the fullpath to the Allen ROI Extraction tool. Enter that as a string as the script is executed. Hence, for example, if the ARET toolbox is located in `/home/toolboxes/Allen ROI Extraction`, the value of the `ToolboxDir` input argument will be given as, `ToolboxDir = '/home/toolboxes/Allen ROI Extraction/'`.
- Save and run the script.
- Choose an option for performing transformation of the annotation template (ANO.nii file) to the group template (obtained from the same SPM analysis that generated the cluster image) as the script is executed. As per the display messages in Matlab's command window, the user can type 'Y' for Yes and 'N' for No for this step. All the results of this toolbox (ROIs and cluster labels) apply to the group template space. This step was carried out using Dartel Registration.
- Next, choose an option to execute a toolbox functionality of choice, as per the display messages that appear on Matlab's command window. Type in '1' for ROI Extraction and '2' for Cluster Labelling. As per the user's choice, a toolbox functionality is executed.

#### 3.2 ROI Extraction: Implementation Instructions

- Selection of '1' as a toolbox functionality option, executes ROI extraction step.
- For the ROI Extraction functionality, the following points are to be kept in mind:
- Refer to the Allen table excel file named `ANO.xlsx` to enter the brain region name exactly as stated in the 'Region' column of the table. This needs to be done because Matlab is case-sensitive, and there will be no recognition of the user-entered brain region by the toolbox, if the name entered by the user and the one written in the table are not identical.
- `brain_region` is the brain region of which an ROI should be generated, as per the user's requirements. The Allen table, `ANO.xlsx` is located in  `'/ARET/templates/ mouse.Allen2017HikishimaLR'`. To view this, load the Allen table in the Matlab workspace using the following command: `Allen_table = readtable(strcat(TemplatesDir,'/ANO.xlsx'))` or open it in Excel to view it.
- The result image named as "brain\_region+ROI" can be viewed by doing a check registration between the mean image and the result image. The mean image is the one that was generated in the same SPM analysis that also resulted in the cluster image.

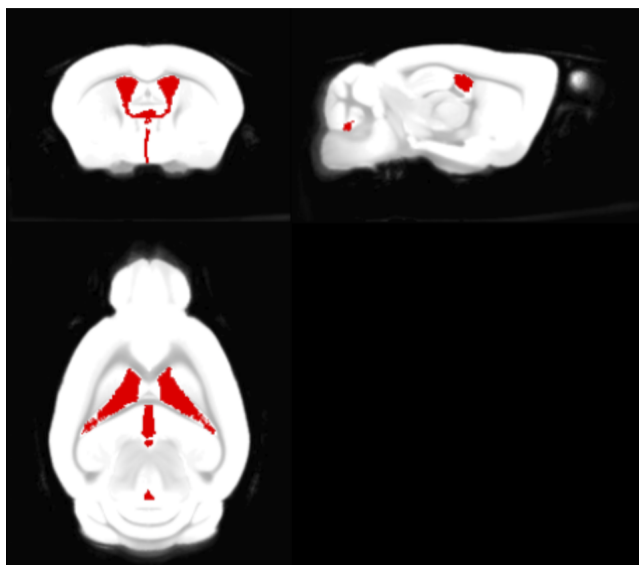


Figure 1: Ventricular systems as ROI (marked in red) with the mean image in the background.

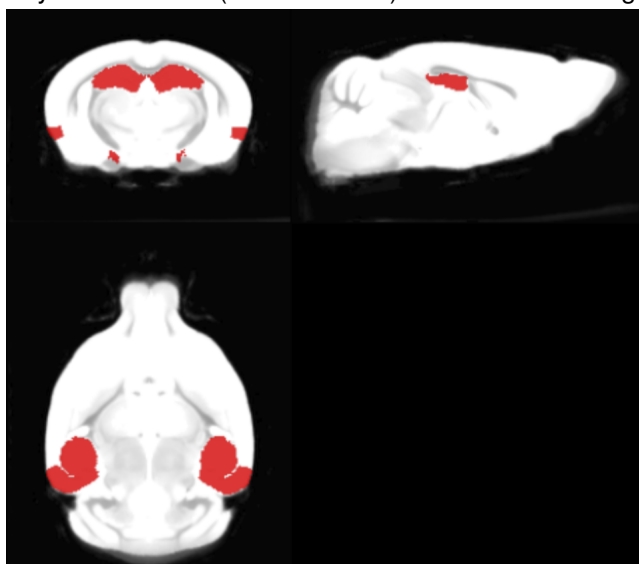


Figure 2: Hippocampal Formation as ROI (marked in red) with the mean image in the background.

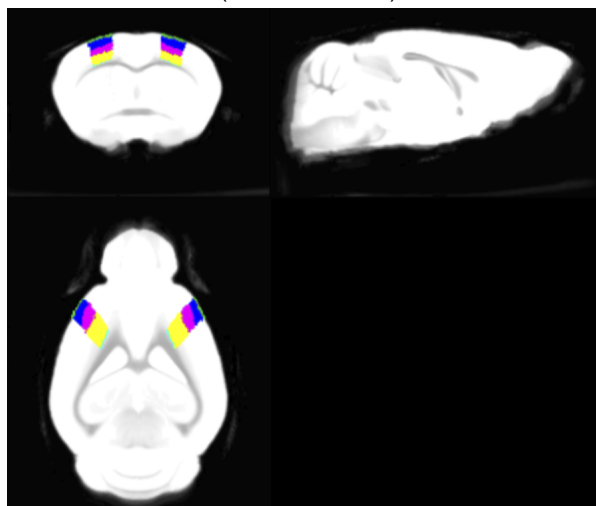


Figure 3: Primary Motor Area (with sub-regions) as ROI with the mean image in the background.

## 4 Background Information

### 4.1 ROI Extraction

- The Allen table (ANO.xlsx file) as shown in Figure 3, contains five columns, namely **Region**, **colHex**, **colRGB**, **ID** and **Children**. **Region** column contains the names of different brain regions, that the user wishes to generate a ROI of. The **colHex** column contains the hexadecimal color code for each region, which is used for displaying the color of the brain region on the Allen webpage [1]. The column **colRGB** contains the equivalent RGB values as decimal color codes. The column **ID** contains the ID number of each brain region. The ID number represents the voxel intensity value of a brain region in the annotation template (ANO.nii file). The **Children** column consists of the IDs (intensity codes in ANO file) of the respective subordinated children regions.
- For ROI extraction, the ID numbers of all the subregions (children) residing within the brain region is used for generating the ROI of the user-specified brain region. This corresponds to using the values associated with the user-specified brain region in the **Children** column. Whereas, to generate the ROI of a subregion, which does not have any children regions, only the number in the **ID** column is used. The lowest level in the anatomical tree hierarchy represents these children regions or 'leaves'. With respect to Figure 3, for Somatosensory areas in row 31 of the Allen table, all the values of the 'Children' are used for extracting Somatosensory areas as a ROI file. Furthermore, for extracting the ROI of one of its children regions, namely Somatosensory areas layer 1 in row 32, only the ID number in row 32 is used.
- Following this, the annotation file is then employed to create a single binary ROI file (NIFTI-image) of the Allen brain region requested by the user.

	Region	colHex	colRGB	ID	Children
27	'Secondary motor area layer 2/3'	'1F9D5A'	'31 157 90'	962	"
28	'Secondary motor area layer 5'	'1F9D5A'	'31 157 90'	767	"
29	'Secondary motor area layer 6a'	'1F9D5A'	'31 157 90'	1021	"
30	'Secondary motor area layer 6b'	'1F9D5A'	'31 157 90'	1085	"
31	'Somatosensory areas'	'188064'	'24 128 100'	453	'12993;1299...
32	'Somatosensory areas layer 1'	'188064'	'24 128 100'	12993	"
33	'Somatosensory areas layer 2/3'	'188064'	'24 128 100'	12994	"
34	'Somatosensory areas layer 4'	'188064'	'24 128 100'	12995	"
35	'Somatosensory areas layer 5'	'188064'	'24 128 100'	12996	"
36	'Somatosensory areas layer 6a'	'188064'	'24 128 100'	12997	"
37	'Somatosensory areas layer 6b'	'188064'	'24 128 100'	12998	"
38	'Primary somatosensory area'	'188064'	'24 128 100'	322	'793;346;86...
39	'Primary somatosensory area la...	'188064'	'24 128 100'	793	"
40	'Primary somatosensory area la...	'188064'	'24 128 100'	346	"

Figure 4: Allen Anatomy Table, highlighting Somatosensory areas (Parent) and its subregions (Children).

### 4.2 Cluster Labelling

Labelling using Voxel intensities

- Cluster labelling involves using the voxel intensity values of brain regions, instead of their hierarchical information to label and give proportion of the cluster regions in percentages for each cluster blob detected in one cluster image.
- The voxel intensity values correspond to the column ID of the Allen table, as shown in Figure 4.
- Labelling is done by searching for the presence of a particular voxel intensity value in the 'ID' column, and assigning a label by taking the region corresponding to the found voxel intensity value, which is the ID number in the Allen table.
- Cluster labelling functionality of the toolbox labels the regions up to the finest annotated sub-region of a user-provided brain region, along with information about its immediate parent brain region.
- The immediate parent brain region is found by detecting the first occurrence of the ID value of the cluster label in the Children column, while going upwards from the index of the cluster label in the Allen table.

## 5 Additional Information

- [TemplatesDir](#) is the fullpath to the folder that contains the latest version of the ANTx templates. This directory is included as a subfolder 'templates' in the Allen\_ROI\_extraction folder. For further information about the contents of this 'templates' directory, please refer to the section on Contact Information in this manual.

## 6 Contact Information

1. For information about the templates and the Allen table, please contact the ANTx toolbox creators, Stefan Koch [stefan.koch@charite.de](mailto:stefan.koch@charite.de) and Philipp Boehm-Sturm [philipp.boehm-sturm@charite.de](mailto:philipp.boehm-sturm@charite.de).
2. For information about ARET toolbox functionalities, please contact Tanusree Chaudhuri [tanchaud1990@gmail.com](mailto:tanchaud1990@gmail.com) and Michael Czisch [textcolorblueczisch@psych.mpg.de](mailto:textcolorblueczisch@psych.mpg.de).

## 7 References

1. Templates: <https://drive.google.com/drive/folders/1q5XOOVLvUYLqYsQJLqNRF7O-K8fNwYhI9>.
2. Allen brain online reference atlas: <https://mouse.brain-map.org/static/atlas>.
3. SPM's GitHub repository: <https://github.com/spm/spm12>.