

BrainRAT user guide

BrainRAT - Brain Reconstruction and Analysis Toolbox

A freely available toolbox for the 3D reconstruction of
anatomo-functional brain sections in rodents



BrainRAT-Team
brainrat@cea.fr
<http://brainvisa.info>

Version 1.0
November 17, 2008

Contents

1	Description of BrainRAT	3
1.1	Contributors	3
1.2	Objectives	3
1.3	History	4
1.4	References	4
1.5	Ongoing work	8
2	BrainVISA/Anatomist	9
2.1	Presentation	9
2.2	Installation of BrainVISA/Anatomist	9
3	BrainVISA user interface	15
3.1	General user interface	15
3.2	User interface of a process	17
4	Anatomist visualization software	24
4.1	Presentation	24
4.2	Windows	25
4.3	Objects	26
4.4	ROI manual drawing toolbox	31
5	General support	33
6	Description and use of BrainRAT process interfaces	35
6.1	Presentation	35

6.2	01. post mortem imaging rebuilt 2D \Leftrightarrow 3D	36
6.3	02. post mortem imaging analysis	45
6.4	Toolbox	52

Chapter 1

Description of BrainRAT

1.1 Contributors

BrainRAT results from collaborative work of image processing methodologists and biologists of [MIRCent](#), a new CEA facility headed by Philippe Hantraye and dedicated to preclinical research.

BrainRAT is mainly developed by the MIRCent image processing team of Thierry Delzescaux, PhD (Julien Dauguet, PhD, Jessica Lebenberg, PhD student, N. Souedet, software engineer and Didier Thenadey, IT specialist) as well as by some close collaborators (Albertine Dubois, PhD, Inserm U803, Vincent Frouin, CEA/SCSR, Denis Rivière, PhD and Yann Cointepas, PhD, CEA/NeuroSpin).

Expert biologists have been associated with this project, from the beginning, to guarantee the relevance of developed methods and the compatibility/performances of processing times with biological experiments (Anne-Sophie Hérard, PhD, Gilles Bonvento, PhD, and Françoise Condé, PhD).

All the above-mentioned people can be contacted by e-mail at brainrat@cea.fr.

1.2 Objectives

BrainRAT's goal is to provide an automated and generic toolbox for the processing of histological and autoradiographic sections based on a two-step approach, which involves:

1. an optimized digitization including an automated individualization, section number assignment, stacking, and dealing with hundreds of autoradiographic and histological sections (series of individual sections as well as multiple sections digitized in a single acquisition);
2. a robust reconstruction algorithm of the volumes based on a reliable registration method (block-matching) enabling 3D reconstruction of a single volume from a series of slices as well as the co-alignment of slices with a reference volume.

1.3 History

At the beginning, these methods have been developed to analyse autoradiographic data in 3D using complementary histological information. The 3D reconstruction of biological volumes arising from several histological and/or autoradiographic sections enables to take advantage of the high resolution of *ex vivo* autoradiography as well as of the diversity of available histological stainings.

The analysis of autoradiographic data, 3D reconstructed or not, remains traditionally limited to conventional region of interest (ROI)-based analysis. Although the users have the corresponding histological stained sections available to consider anatomical information, the delineation of ROI(s) to be analyzed is usually directly performed on the autoradiographic sections, which may be inaccurate and subject to observer bias. To overcome this limitation, BrainRAT enables to perform multimodal co-registration between slices or 3D reconstructed volumes. Recent studies have highlighted the ability of voxel-wise statistical analysis to deal with 3D reconstructed autoradiographic volumes for group comparisons. This automated, exploratory, whole-brain approach can be used to analyze local functional differences between population groups without the need for prior assumptions. BrainRAT provides useful tools to carry out such studies in optimal conditions (optimized digitization, 3D reconstruction algorithms, activity conversion and intensity normalization procedures).

These methods have been applied to Nissl stained (cresyl violet) sections and [^{14}C]-2DG autoradiography, and intensively validated both in rat and mouse brain. They have been also successfully tested on other *post mortem* imaging modalities such as immunohistochemistry, fluorescence, etc. They could now be extended to various biological applications (activation models, morphology, lesion extension, development, etc.) and to domains of application including pathological and therapeutic studies involving animal models of various brain diseases (neurodegenerative diseases, ischemia, and cancer).

1.4 References

For the time being, the only reference to BrainRAT is related to proceedings of the SFN 2008:

BrainRAT: Brain Reconstruction and Analysis Toolbox. A freely available toolbox for the 3D reconstruction of anatomo-functional brain sections in rodents.

A. Dubois, J. Daguette, N. Souedet, A.-S. Hérard, D. Rivière, Y. Cointepas, G. Bonvento, P. Hantraye, V. Frouin, T. Delzescaux. In Proc. **38th annual meeting of the Society for Neuroscience**, Washington, USA, 2008.

The main reference relating to the computerized treatments and procedures gathered in BrainRAT to be mentioned is:

Automated three-dimensional analysis of histological and autoradiographic rat brain sections: application to an activation study.

A. Dubois, J. Dauguet, A-S. Hérard, L. Besret, E. Duchesnay, V. Frouin, P. Hantraye, G. Bonvento and T. Delzescaux. **Journal of Cerebral Blood Flow and Metabolism**, 2007, 27(10), 1742-1755. [Abstract](#).

Yet, these developments were the basis of other works published by our team:

They enabled to provide evidence that the metabolic response to synaptic activation (*i.e.* glucose uptake) is decreased in the superior colliculus during visual stimulation in young adult mice deficient for the glial glutamate transporter GLT-1 (Figure 1.1).

Decreased metabolic response to visual stimulation in the superior colliculus of mice lacking the glial glutamate transporter GLT-1.

A-S. Herard, A. Dubois, C. Escartin, K. Tanaka, T. Delzescaux, P. Hantraye and G. Bonvento. **European Journal of Neuroscience**, 2005, 22(7):1807-11. [Abstract](#).

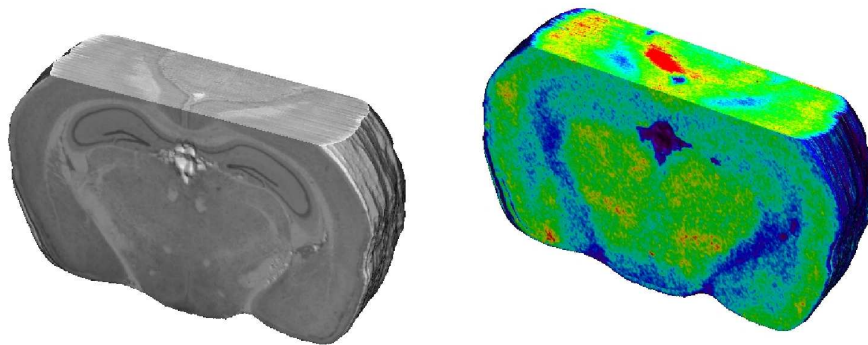


Figure 1.1: (Left) 3-D reconstruction of cresyl violet-stained sections clearly outlines the superior colliculus. (Right) The corresponding 3-D reconstruction of autoradiographic images indicates the extent of the metabolic activation during checkerboard stimulation in a control C57BL/6 mouse with one eye shut and one eye open.

They enabled to accurately study stimulus-driven synaptic activity *in vivo* in a restricted rat brain region, the superior colliculus (Figure 1.2).

siRNA targeted against amyloid precursor protein impairs synaptic activity in vivo.

A-S. Hérard, L. Besret, A. Dubois, J. Dauguet, T. Delzescaux, P. Hantraye, G. Bonvento and K.L. Moya. **Neurobiology of Aging**, 2006, 27(12):1740-50. [Abstract](#).

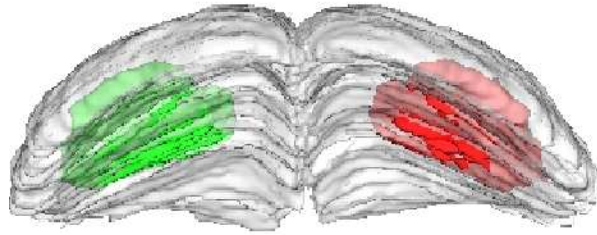


Figure 1.2: 3-D reconstruction of the left and right manually segmented superior colliculus. The automatic extraction of the "activated" volume using the threshold method is represented in red and the symmetric volume in green.

They also enabled the investigation of new methods of analysis of 3D biologic data in groups of rodents, based on voxel-wise statistical approaches without the need for prior assumptions (Figure 1.3).

Quantitative validation of voxel-wise statistical analyses of autoradiographic rat brain volumes: application to unilateral visual stimulation.

A. Dubois, A-S. Hérard, E. Duchesnay, G. Flandin, L. Besret, P. Hantraye, G. Bonvento and T. Delzescaux. **NeuroImage**, 2008, 40(2), 482-494. [Abstract](#).

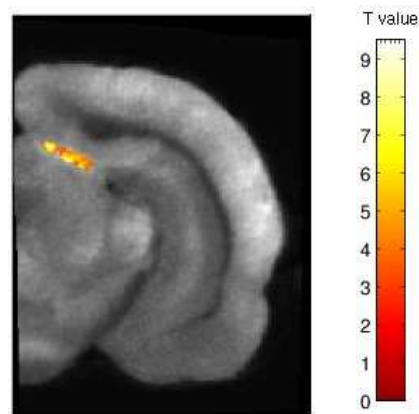


Figure 1.3: Brain area (the superior colliculus) in which CMRGlut was significantly higher or lower in visually stimulated hemibrains than in the corresponding control hemibrains automatically detected by a voxel-wise statistical analysis. Significance is indicated with t statistic color scales, corresponding to the level of significance at voxel level.

Lastly, these developments were also the basis of works performed in collaboration with other teams:

Metabolic and vascular changes induced in mice olfactory glomeruli by odor presentation (Figure 1.4).

Gurden et al. IMNC, UMR8165 CNRS-University of Paris 7 and Paris 11, Orsay, France. 5th **Forum of European Neuroscience (FENS)**, Vienna, Austria, 2006.

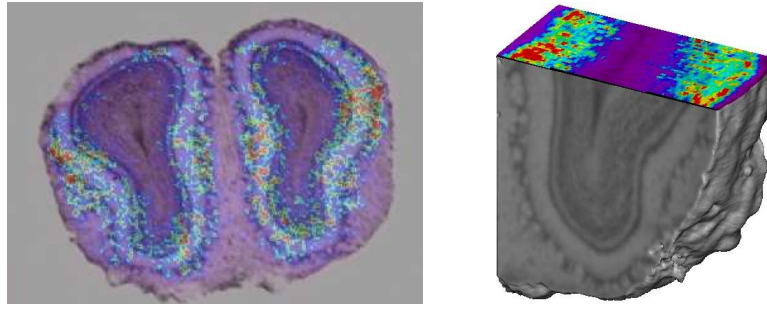


Figure 1.4: Anatomofunctional superimposition and 3D reconstruction of Nissl-stained histological sections and $[^{14}\text{C}]$ -2DG autoradiographic coronal olfactory bulb sections.

The potential of the radiosensitive β -microprobe to monitor the $[^{18}\text{F}]$ MPPF binding in the mouse hippocampus in vivo (Figure 1.5).

Desbrée et al. IMNC, UMR8165 CNRS-University of Paris 7 and Paris 11, Orsay, France. *Journal of Nuclear Medicine*, 2008, 49(7):1155-61. [Abstract](#).

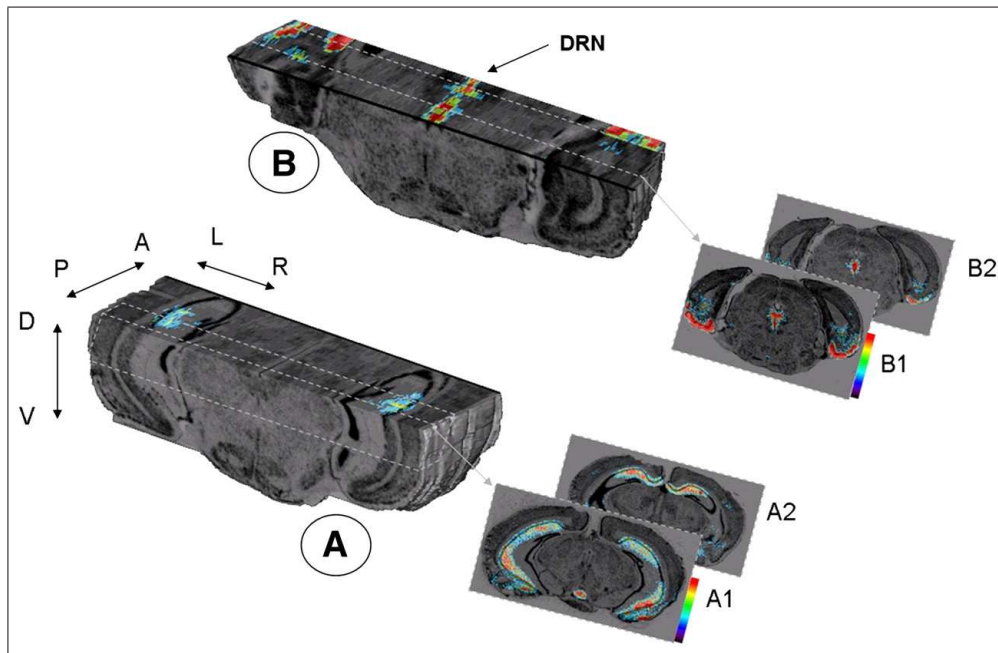


Figure 1.5: 3D postmortem imaging of radioligand distribution in mouse brain after injection of $[^{18}\text{F}]$ -MPPF. Background black and white anatomic volumetric reconstruction of cresyl violet-stained sections shows bilateral location of 5-HT_{1A} binding in mouse hippocampus. Pseudocolored digitized autoradiographs illustrate level of $[^{18}\text{F}]$ -MPPF binding and are color-coded from low (blue) to high (red) binding.

1.5 Ongoing work

The next releases of BrainRAT will contain new processes allowing to:

1. Speed up of registration processes (optimization, parallelization);
2. Use blockface images (photographs of the brain face taken during the sectioning process) as a consistent 3D geometric reference for reconstruction of *post mortem* volumes;
3. Bring *in vivo* data such as MR images into registration with 3D reconstructed *post mortem* volumes;
4. Use a MRI-based 3D digital atlas as a template for fully-automated brain structure segmentation of *in vivo* and *post mortem* data;
5. Configure dedicated databases by defining a reliable organization description (ontology).

Chapter 2

BrainVISA/Anatomist

2.1 Presentation

BrainRAT is a BrainVISA add-on module package providing additional processes and functionalities relating to 3D reconstruction and analysis of *post mortem* brain sections.

BrainVISA, in-house image processing software, is already available for free download on the Internet (<http://brainvisa.info>) (Figure 2.1). Since then, the core of BrainVISA is mainly developed in CEA/NeuroSpin by the LNAO lead by Jean-François Mangin, PhD (Yann Cointepas, PhD and D. Rivière, PhD) and by software engineers of the IFR 49 (Isabelle DENGHIEN and Dominique GEFFROY). The first prototype was developed by Yann Cointepas during years 2000/2001. BrainVISA also relies on Anatomist software visualization capacities.

Anatomist has been designed in the [Service Hospitalier Frederic Joliot](#) of CEA and is now mainly developed by Denis Rivière, PhD. The current version stems from ten years of work on several prototypes. One of its main original features is the generic module dedicated to structural data, namely sets of objects linked one another into a graph structure. It also provides a toolbox for ROI manual drawing.

2.2 Installation of BrainVISA/Anatomist

2.2.1 Download

The use of BrainRAT requires you to download and install a BrainVISA/Anatomist binary package (from version 3.1.4).

The list of operating systems and versions compatible with binary packages is available using the following link: <http://www.brainvisa.info/download.html> (Figure 2.2). You can download a package using the following link: <http://www.brainvisa.info/downloadpage.html> (Figure 2.3).

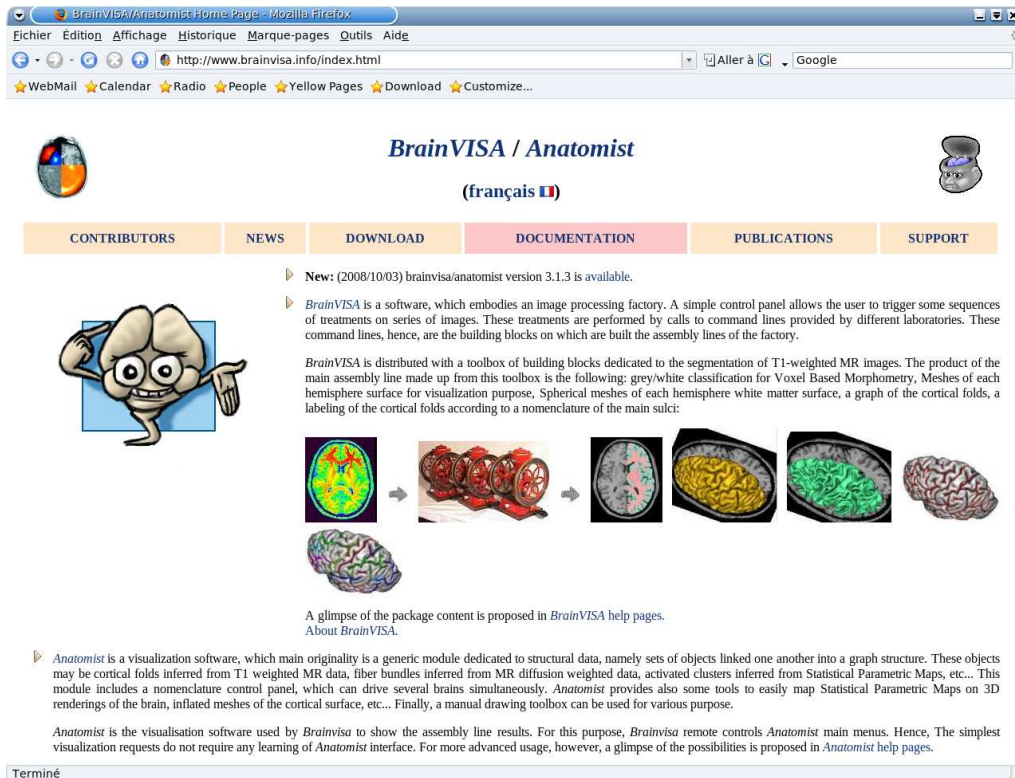


Figure 2.1: Home page of BrainVISA web site.

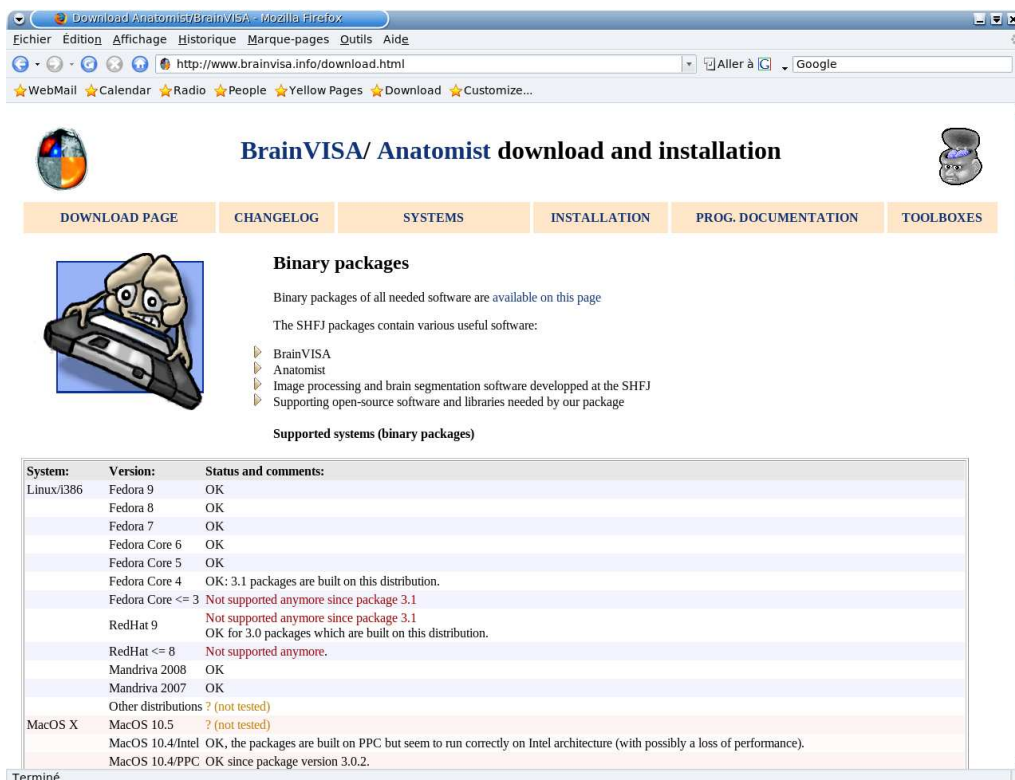


Figure 2.2: List of operating systems and versions compatible with binary packages.

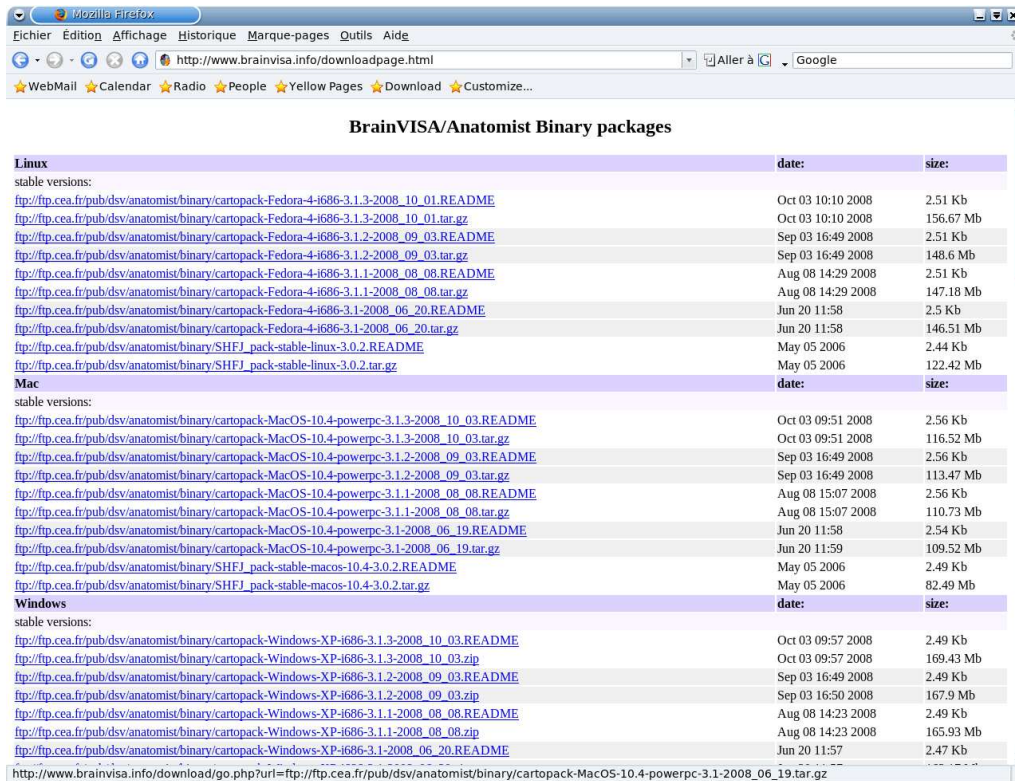


Figure 2.3: Download page of BrainVISA web site.

2.2.2 Content of the package

BrainVISA is packaged together with many softwares. The distribution contains all the core open-source software of BrainVISA itself and of image analysis libraries. It also includes all the algorithms which are closed-source, but binaries are provided for free. Additionally, several third-party software programs and libraries are packed in the bundle (especially the Python language interpreter and several other modules).

- BrainVISA (graphical process interfaces)
- Anatomist (image visualization software)
- Freeware libraries from our lab, with their sets of commandlines: cartobase, carto-data, graph, aimsdata and IO formats plugins
- Some closed-source libraries and programs from our lab
- Freeware libraries: libsigc++, libxml2, DcmTk, Ecat library, Minc and netcdf libraries
- Qt, used for all GUI
- Qwt: additional widgets for Qt (mainly for curve plotting)
- The Python interpreter
- Various modules for Python

2.2.3 Licence

All programs and software libraries present in the binary distributions of BrainVISA/Anatomist do not have the same licence. External softwares come with their own licence, which may depend on the system. All other included softwares are open-source and under GPL, LGPL or BSD-like licences. Our open-source softwares are governed by the CeCILL licence (version 2) under French law and abiding by the rules of distribution of free software. You can use, modify and/or redistribute the software under the terms of the CeCILL licence version 2 as circulated by CEA, CNRS and INRIA at the following URL: <http://www.cecill.info>.

But we also have closed-source programs. They can be freely copied and redistributed as binaries. But they are not released under the CeCill licence, and are not using CeCill nor GPL software.

Detailed information about download and installation of BrainVISA is available in [the handbook of BrainVISA](#) (Figure 2.4).

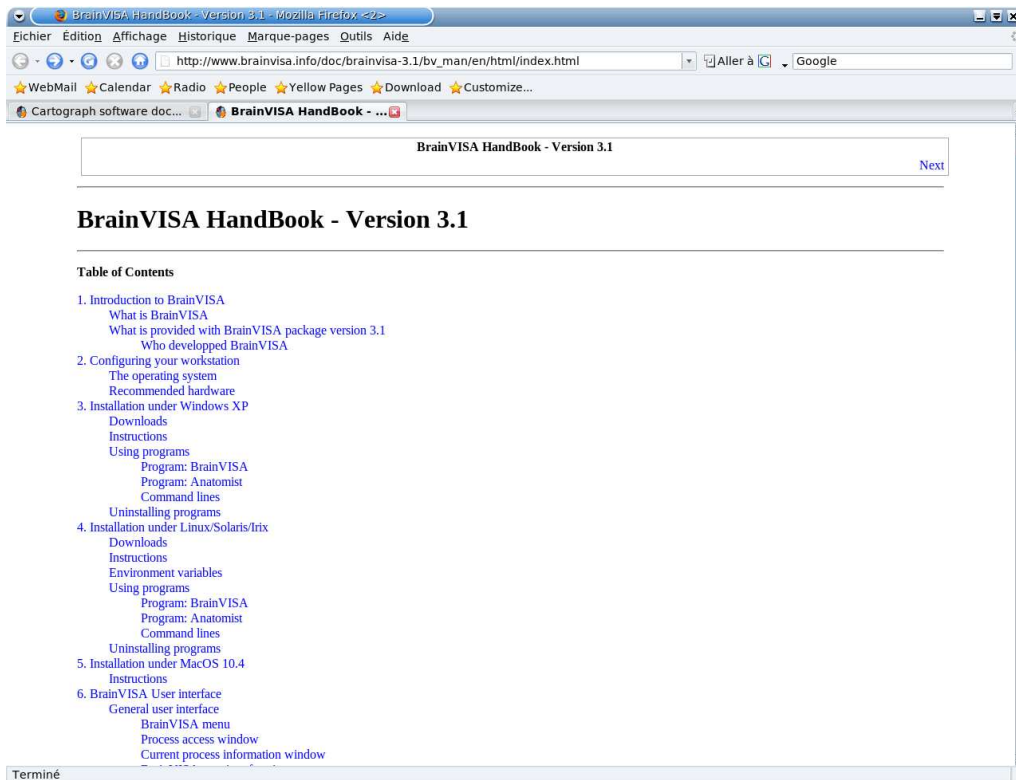


Figure 2.4: Home page of the handbook of BrainVISA.

2.2.4 Using BrainVISA/Anatomist under Windows XP

Several programs are in fact available within a BrainVISA/Anatomist binary package: two pieces of software (BrainVISA and Anatomist), along with command lines from the Aims and Vip libraries. All the programs are independent of each other. They are in the *bin* subfolder of the binary package.

Program: BrainVISA

To use BrainVISA, run the *brainvisa.bat* file by double clicking it using either a shortcut on the desktop or the *bin* folder.

Refer to this [appendix](#) to see the brainvisa command options.

Program: Anatomist

The Anatomist application is independent of BrainVISA. You can run Anatomist without running BrainVISA.

To use Anatomist, run the *anatomist.bat* file by double clicking it using either a shortcut on the desktop or the *bin* folder.

2.2.5 Using programs under Linux or MacOS

All the programs are in the */bin* sub-folder of the package. The most frequently used programs are *brainvisa*, *anatomist* and 2 scripts for configuring your environment variables (*cartopack.csh* or *cartopack.sh*, depending on your shell).

You are not advised to add this folder to your PATH variable, as this could create conflicts with other programs in your system (for example: python, KDE, etc.). We can suggest three possible ways of initiating these programs (remember that *~* means that you are at the root of your home directory and that *folder_name* is the name of the folder containing the uncompressed package, for example *brainvisa*).

Program: BrainVISA

To launch BrainVISA, use the following command:

```
brainvisa
```

Go to the appendix to see the options for the brainvisa command, or enter one of the following command lines:

```
brainvisa -h
```

or

```
brainvisa --help
```

Program: Anatomist

To launch Anatomist, enter the absolute path name for the program:

```
~/folder_name/bin/anatomist
```

You also can create symbolic links from the root of your directory to start up these programs. The commands are as follows:

```
cd ~  
ln -s ~/folder_name/bin/anatomist anatomist
```

Chapter 3

BrainVISA user interface

NOTE: For a complete and more detailed description of BrainVISA software, please refer to [the handbook of BrainVISA](#).

3.1 General user interface

The general user interface of BrainVISA (Figure 3.1) consists in three parts:

- A menu (1)
- A window providing access to processes, including a toolbox/bookmark panel (2.a) and a process list (2.b)
- A window providing information on the selected process (3)

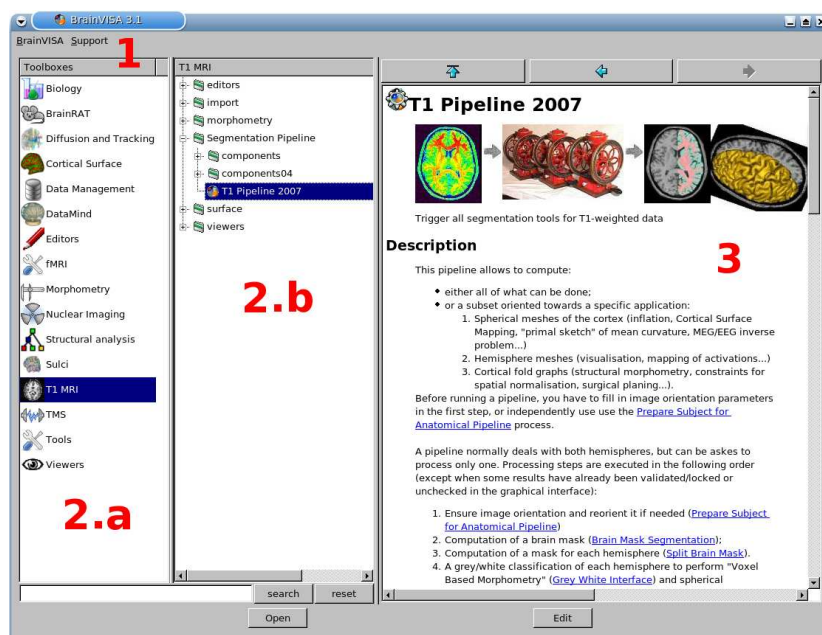


Figure 3.1: General user interface of BrainVISA.

3.1.1 BrainVISA menu: (1)

The BrainVISA menu gives you the following choices:

- *Help*: opens the help window, which provides access to a manual, a tutorial, etc.
- *About BrainVISA*: opens a window that identifies your version of BrainVISA and introduces the people who helped to create it.
- *See log*: opens a window displaying execution parameters, to any errors generated during processes, and to the list of processes used.
- *Preferences*: opens the configuration window.
- *Exit*: closes the application.

The Support menu gives you the following choices:

- *Bug report*: to automatically send a bug report, either with or without the log file.

NOTE: Preferably, do not use this automatic log file transfer function but send an e-mail to brainrat@cea.fr with the log file enclosed.

3.1.2 Process access window: (2a and 2b)

The process access window contains the menus for all the available processes, which are organized into categories. These process categories are identified by a figure, which denotes the user level:



This icon represents user level 0 processes, accessible to all users.



This icon represents user level 1 processes, accessible to advanced users.



This icon represents user level 2 processes, for which no help is available. These processes are accessible to very advanced users or evaluation purposes. In fact, these processes consist in internal BrainVISA processes (started up using level 0 or level 1 process) or processes that are still being developed and do not, therefore, give guaranteed results.

T1 MRI \Rightarrow Segmentation pipeline (Figure 3.1) provides access to the process T1 Pipeline 2007. To access this process, either double click the process name in the access window, or select it and click the Open button at the bottom centre of the BrainVISA general interface.

3.1.3 Current process information window: (3)

This window displays information to help you to use either BrainVISA in general or a specific process. When BrainVISA is opened, this window automatically displays the main BrainVISA help page. When a process is selected, the related documentation is shown.

3.2 User interface of a process

3.2.1 Presentation

When you get access to a process, its own interface is displayed (Figure 3.2).

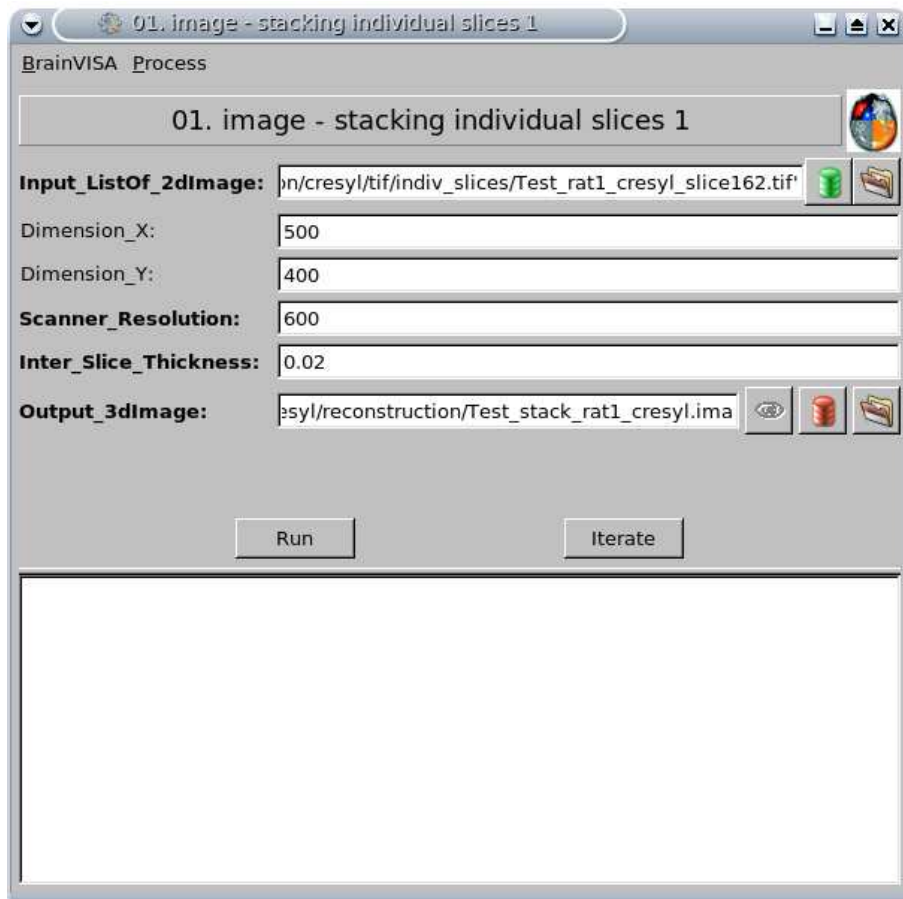


Figure 3.2: Example of a process interface.

Processes are always configured in the same way, *i.e.* they always have the same interface and use the same operating mode. Processes differ in the way that the command lines applied to data are assembled.

For example, there is a process for the 3D reconstruction of the anatomical volume arising from histological slices, another one for the 3D reconstruction of the functional volume

arising from autoradiographic slices, etc. To run a process, values have to be entered by the user, which will be called "parameters".

There are mandatory parameters (shown in bold type) and optional parameters.

The parameters are entered in various ways: selection of a file or a list item, entry of a value, multiple-choice items, etc.

NOTES:






- 1) A default value can be assigned to a parameter field. This value is naturally modifiable.
- 2) If parameters you have entered are incorrect, you will be notified by an informative error message. For instance, if you have entered a negative value in the field of a necessarily positive parameter, the following error message will display on screen:



- 3) According to parameters chosen among multiple-choice items, new mandatory parameters may appear and be required.

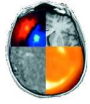
The Table 3.1 describes some process interface icons:

Table 3.1: Description of process window icons.

Icon	Description
	This icon provides access to your files . It enables you to select an input or output file without using the BrainVISA database system.
	This icon represents an input parameter. It provides access to existing data stored in a BrainVISA database. It shows all the data corresponding to the parameter type and allows selection by attribute value.
	This icon represents an output parameter. It provides access to existing or non existing data in a BrainVISA database. It shows all the data corresponding to the parameter type and allows selection by attribute value.
	This icon represents a link with Anatomist . It is mainly used for parameters that are, in fact, 3D points. When this icon is clicked, the current location of the 3D cursor (<i>i.e.</i> the red cross) in Anatomist is taken as the parameter value. It allows the user to select a value by clicking it in Anatomist.
	This icon tells you whether a data item can be visualized or not. If the icon exists and is active, it means that BrainVISA has a viewer capable of displaying that type of data with Anatomist. If the icon is disabled (<i>i.e.</i> not clickable), it means that the corresponding file is not readable (usually because the file does not exist yet).

3.2.2 Running and interrupting a process

When you have completed all the mandatory fields (in bold), you can launch the process by clicking the Run button in the middle left of the process window (Figure 3.2). You can then watch the progress of the process in the lower half of the process window. You will be told explicitly when the process starts and ends.



While the process is running, the opposite icon will be constantly animated at the top right corner.

The duration of a process varies according to the algorithms used and the performances of your workstation. In fact, a process such as a volume image flip is almost instantaneous, whereas a process such as the 3D reconstruction of a biological volume arising from several dozens of sections may take anything from 1 to 2 hours depending on your workstation.

When the process begins, the Run button is replaced by an Interrupt button, which enables to stop the process if needed.

3.2.3 Monitoring processes

The log file

The log file enables the user to monitor all the actions performed by BrainVISA. This type of file is used to store configuration data, information on the processes used during a session, and process parameters (Figure 3.3 and Table 3.2).

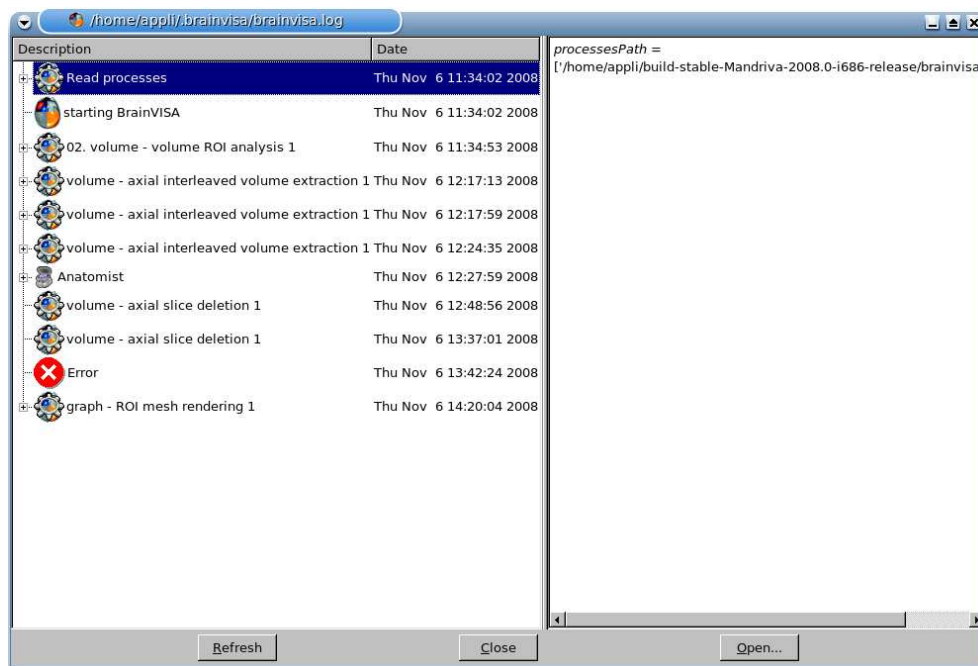








Figure 3.3: Log file interface.


Table 3.2: Description of BrainVISA user interface log file.

Icon	Description
	This icon represents a BrainVISA process in the log interface.
	Icon in the log interface, denoting communication with Anatomist .
	Icon in the log file, representing a warning . When BrainVISA is loaded, all the processes are analyzed to make sure that all the external programs required to run them are available. If not, the process is not loaded, and a warning message is displayed in the log interface. For example, if you wish to use a process that requires Matlab, and Matlab is not installed on your system or your object program path is not properly configured, a warning message will be displayed.
	This icon represents a process .
	This icon represents a command line called by a process.
	This icon represents an error .

The log file may include the specific parameters used for a process, or the errors occurring during a session. You should therefore keep this file if you wish to submit an execution error to BrainVISA support. In many cases, if this file is not available, the information submitted is not specific enough to enable the error to be understood or reproduced.

The log file can be accessed using BrainVISA \Rightarrow Show log. If you open it just after starting the session (*i.e.* before running a process) you can view the list of analyzed processes before they are loaded, and check that all the components required to run them are installed on your workstation. If one of the components is missing, you will receive a warning message.

3.2.4 Example of log file

You can view this interface at all times. In the example below (Figure 3.4), we can see the list of processes run, and the associated parameter values. Here, we are looking at the Axial interleaved volume extraction process, but we can also see that the Anatomist Show Volume process has been run, and view the communication between BrainVISA and Anatomist via  .

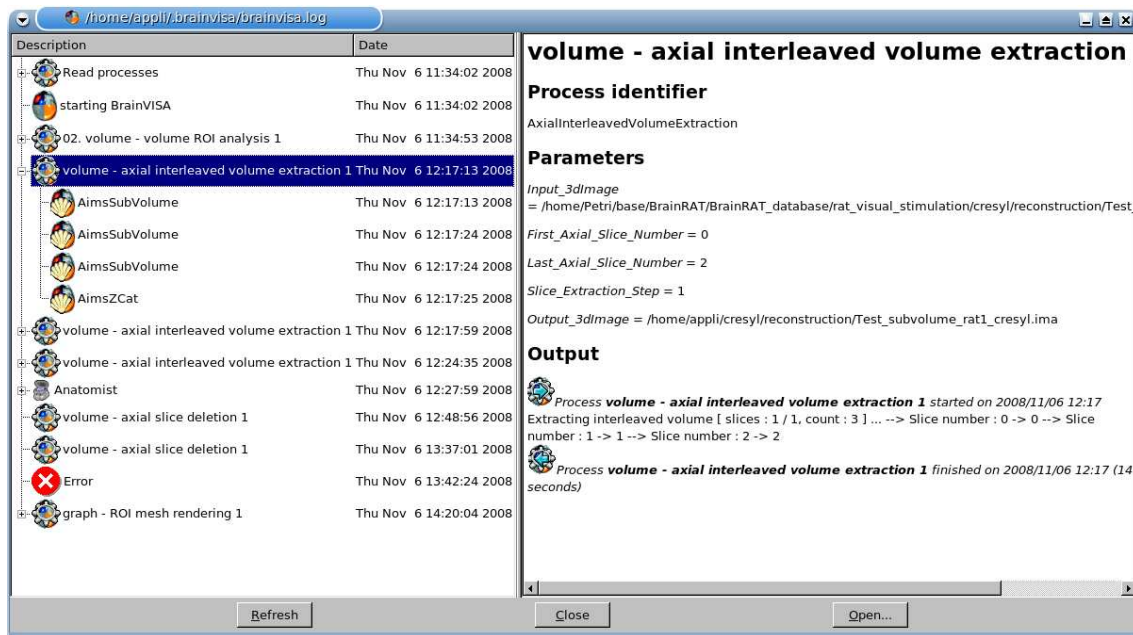


Figure 3.4: Viewing the execution of a process via the log file interface.

3.2.5 Saving the log file

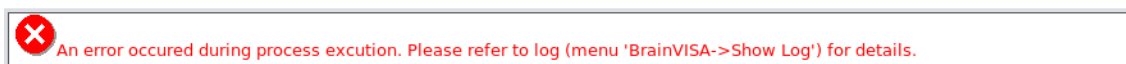
The log file is re-edited each time a new session is opened. So, if you want to save it, you must do so before opening a new session, otherwise the data will be lost.

To save the log file, follow the instructions below:

1. Exit BrainVISA (and don't start it up again until you have saved the log file).
2. In Linux, go to the following folder:
/home/user/.brainvisa
3. In Windows, go to the following folder:
C:\Documents and Settings\user\.brainvisa
4. Save the *brainvisa.log* file of this folder.

3.2.6 Managing errors

When you work with BrainVISA, do not be afraid by obtaining errors. The log file of BrainVISA can be used to view the errors generated during processes (Figure 3.5). In such a case, the following message will invite you to see the log file for more details:



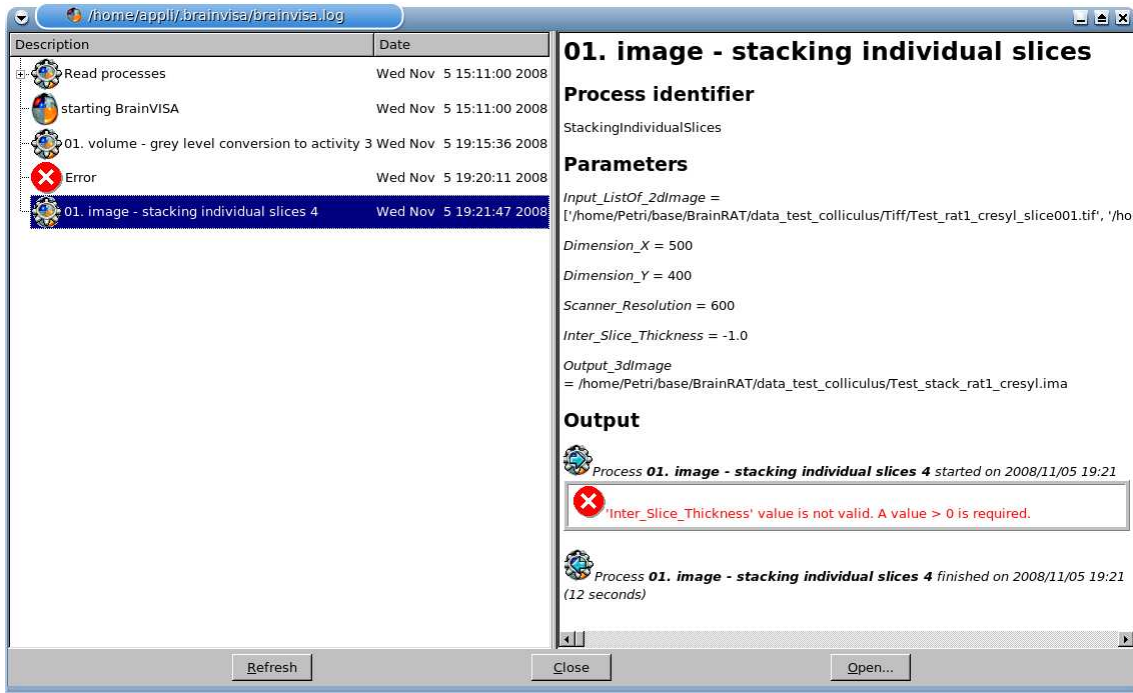


Figure 3.5: Error screen in log file.

The causes are mentioned below.

Error screens are divided in 2 parts (Figure 3.6): the first (1) which indicates the error cause to user and the second (2) which indicates an explanation to experts (where is located the error in source files or algorithm).

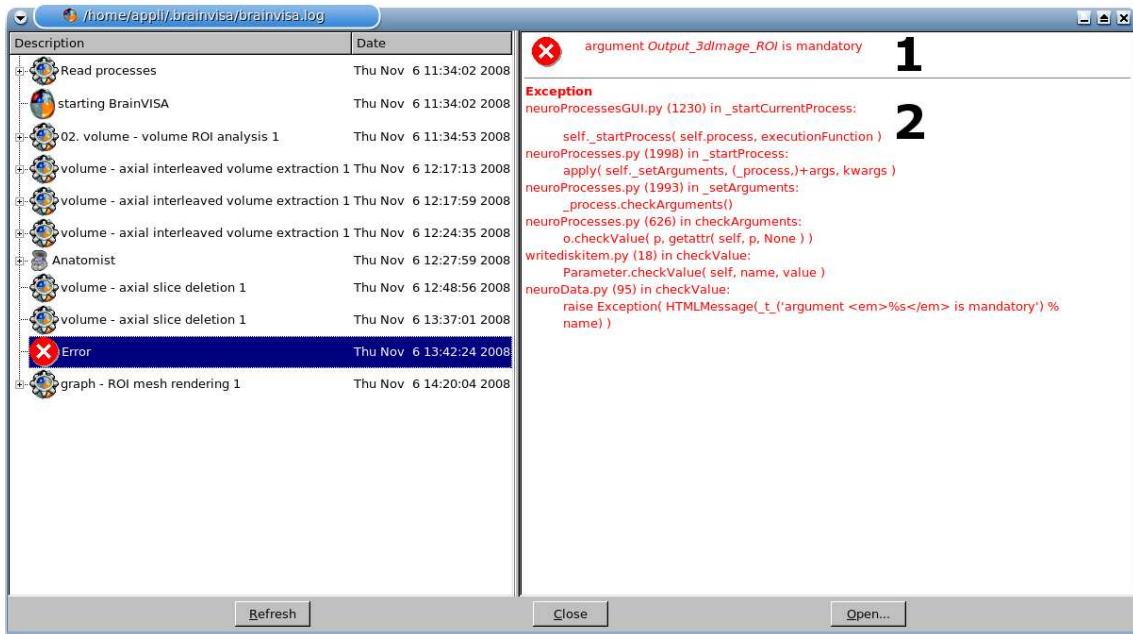


Figure 3.6: Error message: the Output_3dImage_ROI has not been completed.

Current errors

The error messages generated during the running of a process may be caused by several different things:

- Mandatory field not completed → if a mandatory field (in bold type) has not been completed, the following error is systematically generated: *argument Output_3dImage_ROI is mandatory*.
- Problems related to input data → the data selected are incompatible with the running of the process.
- Other errors may be generated during the running of a process (information on these errors will be displayed in the window).

What to do in the event of an error?

Here is a little advice on what to do if an error occurs while a process is running:

- Make sure that the error is not related to the type of data selected (wrong type of data, inexistent data, etc.).
- Consult the process log using BrainVISA ⇒ Show log.
- Sign up to and consult the forum at <http://brainvisa.info/forum> (for more information, see chapter General support).
- Report the error to the BrainRAT-Team by sending an e-mail to brainrat@cea.fr with the log file enclosed .

Chapter 4

Anatomist visualization software

NOTE: For a complete and more detailed description of BrainVISA software, please refer to [the Anatomist user manual](#).

4.1 Presentation

General purpose of Anatomist software is to load objects, change their referential if needed, modify their characteristics, handling them together, and put them in windows in order to visualize them.

Original functionalities of Anatomist are based on:

- Handling of different objects: structured (ROI graphs,...) or not (2D, 3D, 4D volumes, meshes/surfaces,...)
- Handling of different coordinate systems and transformations: transformation matrix can be loaded to change the object coordinate systems; it is possible to merge a non normalized anatomical volume and a normalized activation map (functional data), and to have a coherent display if we load the transformation from the normalized image to the non normalized anatomical image or the reverse
- Building complex 3D scenes with several objects of any type: merging, superimposing, etc.
- A lot of tools: color palettes, ROI module, mathematic morphology, manual registration (transformation control), etc.

Anatomist control window (main window) enables to have a general view of loaded objects and opened windows, to choose actions for handling objects or controls to use on windows regarding to contained objects (Figure 4.1).

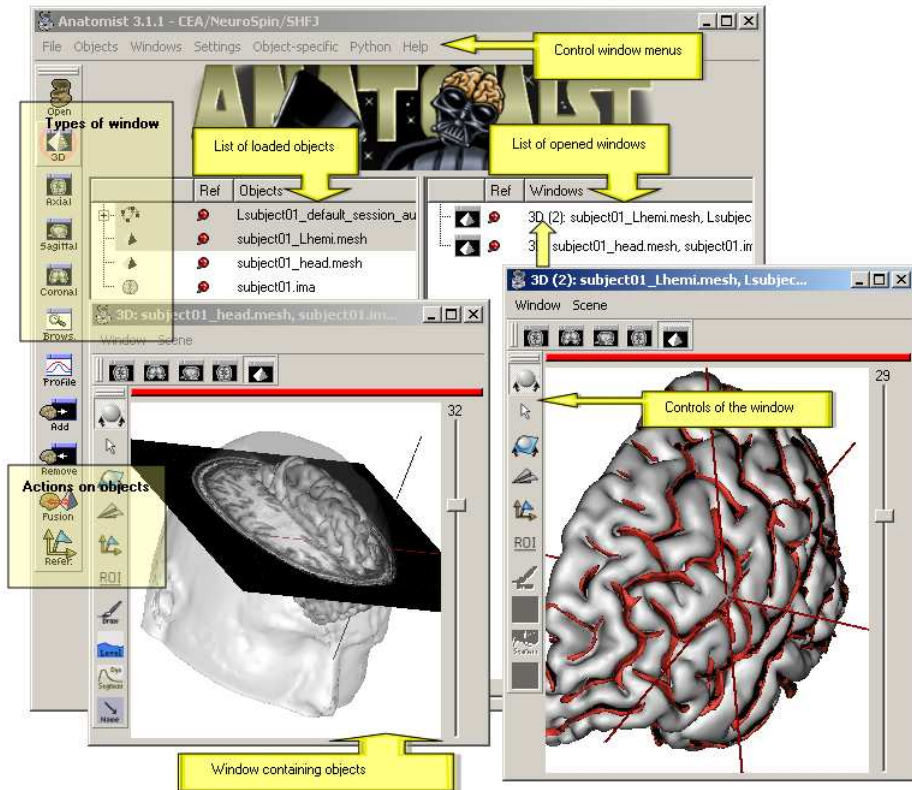


Figure 4.1: Anatomist control window.

So, here are some basic principles for using Anatomist:

1. Load objects that represent 2D, 3D volumes, meshes, etc.
2. These objects are put in windows for visualization. At a more advanced level of use, it is possible to put several objects in the same windows, for example to superimpose a mesh and a volume.
3. It is also possible to handle objects characteristics as referential (coordinate system) or colors, for example by modifying a volume's palette.
4. A window offers different control modes according to the type of its contained objects. For example, if a window contains a ROI graph, the selection control will be available and will enable to select regions in the graph.

4.2 Windows

A window enables to visualize one or several objects of the same type (2 meshes for the brain hemispheres) or not (a mesh and a volume).








Windows have a name, for example A(2): subject01.ima. This name means that the window is the second axial window and contains the volume subject01.ima. It is possible to handle windows individually or in groups.

There are several ways to open a window:

- Menu Windows \Rightarrow Choose window's type
- Click on window type icon

Windows enable to visualize objects after their loading. Note that visualization is different from loading. Indeed, loading gives raw data that can be visualized in various ways. For example, you can change the display convention without modifying the data. Table 4.1 shows the different window types. You can switch from one type to another by clicking on the icons on window's top bar.

Table 4.1: Anatomist windows.

Icon	Description
	2D Axial window - Visualization of volumes
	2D Coronal window - Visualization of volumes
	2D Sagittal window - Visualization of volumes
	2D Oblique window
	3D window - Visualization of 2D objects and 3D objects (meshes)
	Browser - Visualization of object attributes, window content or structured objects
	Profile - Visualization of grey levels range along an axis










4.3 Objects

4.3.1 Objects handled by Anatomist

Object stands for all types of data that Anatomist can handle. This software manages 2D and 3D volumes (slice images, T1, fMRI, PET...), meshes, textures (to stick on other objects), structured objects like sulci graphs or ROI graphs. All these objects can be visualized and combined (merging, superimposing...). Some data types and formats are peculiar to Anatomist, like for example meshes (.mesh or .tri), or nomenclature (.hie).

Main objects handled by Anatomist are listed in Table 4.2 (the list is not exhaustive because there are also merged objects, volume slices,...).

Table 4.2: Formats managed by Anatomist (non exhaustive list).

Icon	Format-Extension-Anatomist data type	Description
	2D, 3D and 4D volumes	<p>Format list according to the operating system (Linux, Windows...):</p> <ul style="list-style-type: none"> • GIS/tivoli: .ima (volume data), .dim (header file) and .minf (attributes list) • Analyze (SPM): .img (volume data) and .hdr (header file) • NIFTI: .nii (only one file) • Genesis: .adv • CAPP/ECAT • DICOM: .* or .dcm • VIDA: .vimg (volume data), .vdhr (header file) and .vinfo (attributes list) • MINC: .mnc • JPEG, GIF, PNG: .jpg, .jpeg, .gif, .png
	Bucket : .bck	A bucket is a set of points corresponding for example to a ROI, <i>i.e.</i> a voxel list (it is not possible to see the coordinates, only display is managed).
	Mesh : .tri ou .mesh	Files storing meshes (3D mesh of hemispheres, brain, ...)
	FUSION2D Object	Object created by merging objects with Fusion2D method (for example : merging two volumes).
	FUSION3D Object	Object created by merging objects with Fusion3D method (for example : merging a volume and a mesh).
	CutMesh object	Object created by merging objects with CutMesh method (for example : merging a volume and a mesh).
	PlanarFusion3D object	Object (textured plan) obtained by merging a mesh slice plan (Planar mesh) and a volume. For example, in a FusionCutMeshMethod fusion between a volume and a mesh, PlanarFusion3D object will be the textured plan of the volume according to the slice plan of the mesh.
	Graph : .arg et .data	.arg file is associated to a .data directory, and enables to organize data as a graph (ROI graph).
	Nomenclature : .hie	File containing a hierarchy of names or labels. It enables to do hierarchical selections in a graph: you can select using the nomenclature file .hie the graph nodes that have the same name or label. It is also possible in a nomenclature to associate a color to name and label graph attributes.

4.3.2 Load an object

Click on File ⇒ Open or on icon  of the left menu bar.

Select your object (2D volume, 3D volume, etc.) with the file browser, for instance Test_rat1_section080.ima (2D volume) or Test_vlm_rat1_cresyl.ima (3D volume).

This object appears in the left list of the control window (Figure 4.2).

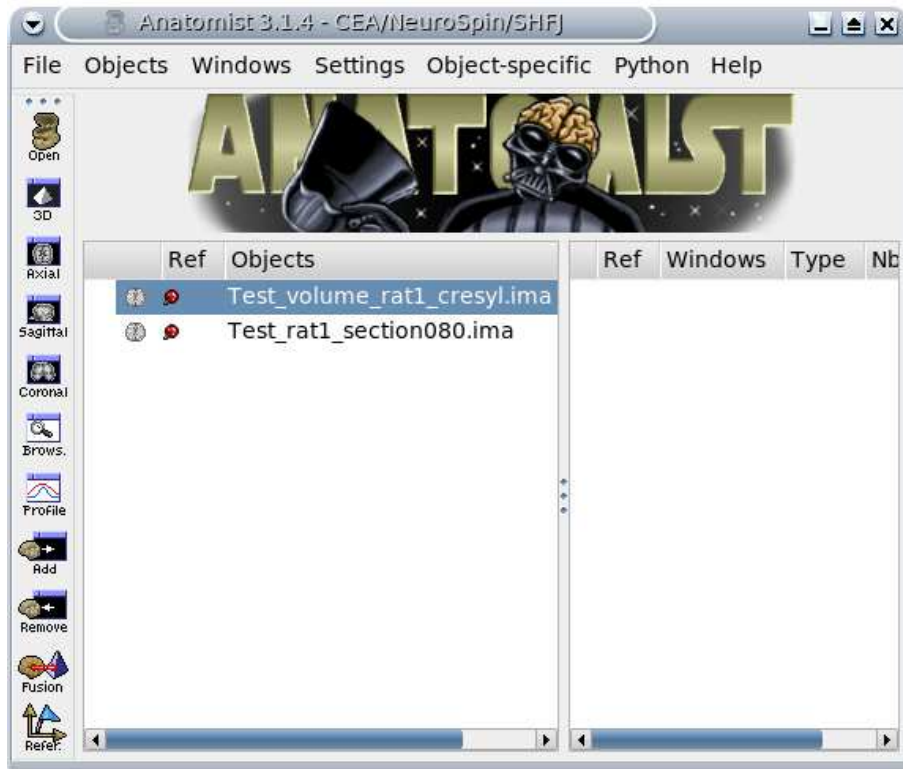


Figure 4.2: Load an object with Anatomist.

4.3.3 Visualize an object

Load any volume, for instance Test_rat1_section080.ima (2D volume; Figure 4.3 (a)) or Test_vlm_rat1.cresyl.ima (3D volume; Figure 4.3 (b)).

Drag and drop the selected object (highlighted in blue) on an axial, sagittal or coronal window icon of the left menu bar (it will open a new window containing this object).

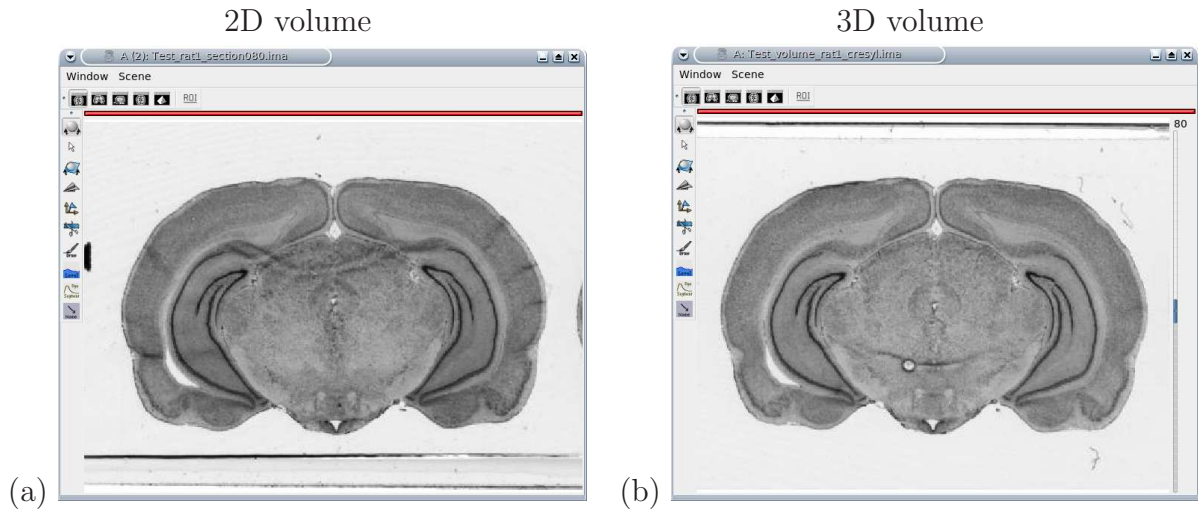


Figure 4.3: View of Test_rat1_section080.ima (a) and Test_vlm_rat1_cresyl.ima (b) in axial windows. See the cursor on the right side of the (b) window. It enables you to move along the antero-posterior axis (*i.e.* the direction orthogonal to the cutting-plane).

NOTE: Axis orientation and origin of volumes.

Axis in Anatomist are oriented like this (Figure 4.4):

- X axis : right \Rightarrow left
- Y axis : anterior \Rightarrow posterior
- Z axis : top \Rightarrow bottom
- T axis : 4th dimension to visualize a volume with an adding cursor to move from volume to volume; This axis can stand for the time in functional volumes visualization or directions for a diffusion sequence.

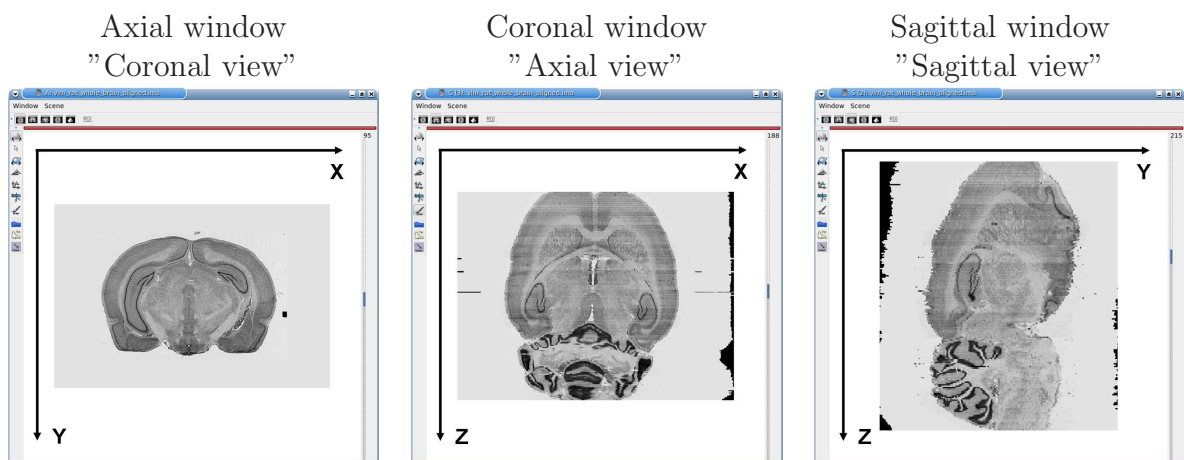


Figure 4.4: Orientation of axis in Anatomist.

The origin of volumes is the voxel whose coordinates are (0, 0, 0). This voxel is located forward, on top and on the right of the volume. So, in an axial Anatomist window, this point will be at the top left corner if you keep the radiological display mode. If the origin is in mm, the origin of the volume is the centre of the voxel located at the origin.

4.3.4 Object color

Colors can be changed for objects of type : volume, texture, mesh and graph nodes. According to the object type, colors are changed by different methods:

- *Mesh* : Material modification.
- *Texture* : Texturing and Palette modification.
- *Volume* : Palette, Texturing and Material modification. Palette is the most commonly used. But a volume is also considered as a geometric object (mesh) with values (texture) associated to each point. So by extension, volumes have access to mesh (Material) and texture (Texturing) menus.
- *Textured object* (obtained by mesh and texture fusion): Texturing modification.

Each point (x,y,z) of a volume has a value which is associated to a color via a palette (Figure 4.5). A palette is a table that associates a color to voxels values. Anatomist palettes can be modified using the values Min (minimum value for the voxels of the volume) and Max (maximum value for the voxels of the volume). Changing these boundaries modifies the field of voxels values where the palette applies. So you can focus more on a part of the histogram.

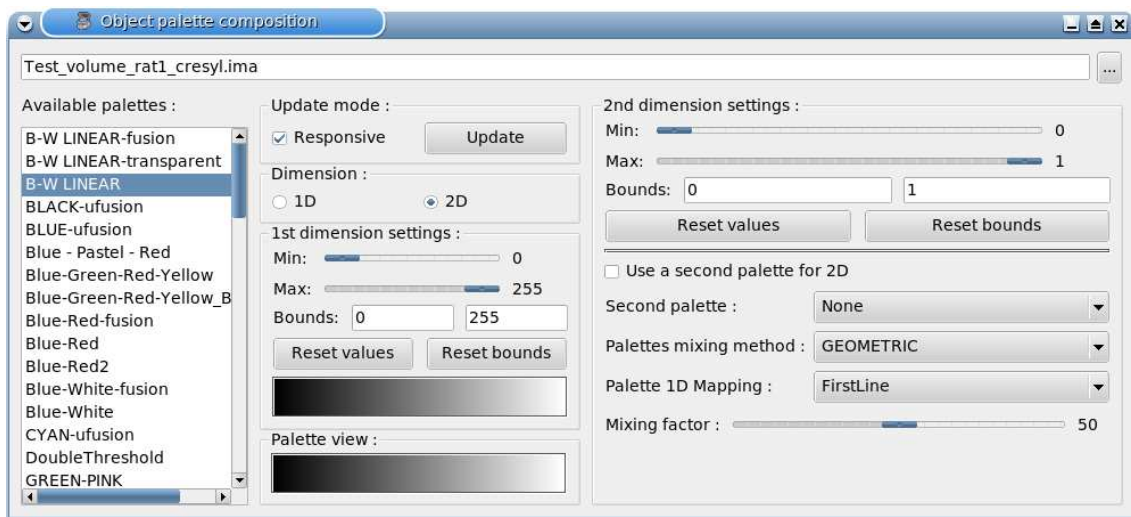


Figure 4.5: Palette window.

This menu applies to texture part of objects. Choose Color \Rightarrow Palette in Object-specific menu or by right-click on the object. Palette modification enables to change colors table. A lot of palettes are available and you can add your own palettes.

NOTE: Default palette for volumes and textures is B-W LINEAR. Min and Max bounds are set so that they contain 99% of histogram values. Sometimes volumes can appear with a "white translucent film" effect, due to palette bounds incorrect values. To correct this effect, you can modify Min and Max bounds values.

4.4 ROI manual drawing toolbox

Regions of interest are stored in a graph where each node is a ROI. Some attributes are associated to the graph and its nodes : the voxels size, name of each region.

The file format is a couple .arg/.data. The .data is a directory and contains data of each region. The .arg is a file and contains the graph's structure, that is to say nodes organisation.

Load any volume, for instance Test_vlm_rat1_cresyl.ima.

Place it into a window.

Click on icon  of this window.

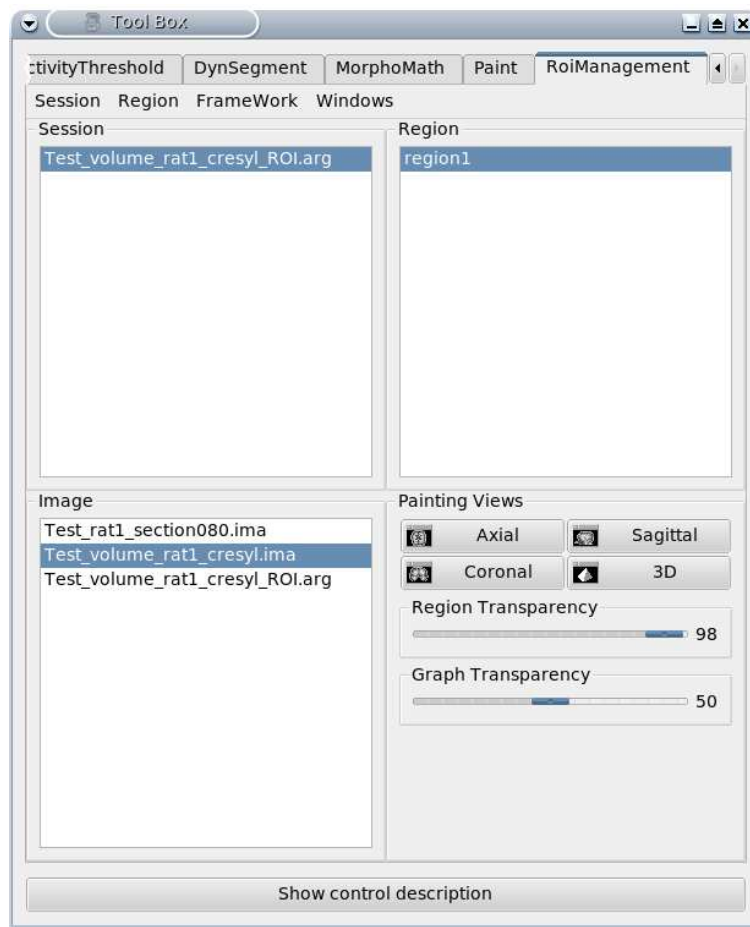


Figure 4.6: Anatomist ROI drawing toolbox.

Click on Session \Rightarrow New (Figure 4.6).

A session is actually a ROI graph. A default name is created according to the name of the selected volume in Image panel by adding suffix `_ROI`, for instance `Test_vlm_rat1_cresyl_ROI.arg`. You can change the name of the graph using Save as option. The current session appears in the Session panel of the ROI toolbox.

If you want to choose your own region names, verify that the Free framework is selected by default by clicking on `FrameWork`.

Click on Region \Rightarrow New and provide a name, for instance `region1`. This region appears in the Region panel of the ROI drawing toolbox.

Change the brush: click on `Paint` \Rightarrow `Disk`, `Brush Radius = 2` or `3`.

Draw your region on the window of the selected volume in Image panel (Figure 4.7 (a)).

Save the current ROI drawing session: click on `Session` \Rightarrow `Save As`.

You can keep the default name, `Test_vlm_rat1_cresyl_ROI.arg`, or provide a new name to the graph.

Click `Ok`.

It is also possible to export a ROI drawing session as a mask (a binary volume) using the option `Region` \Rightarrow `Export as mask` in ROI toolbox menu (Figure 4.7 (b)).

For instance, select `region1`. Click on `Region` \Rightarrow `Export as mask`. Enter the name under which you want to save the selected region, for instance `Test_rat1_region1.ima`.

Click `Ok`.

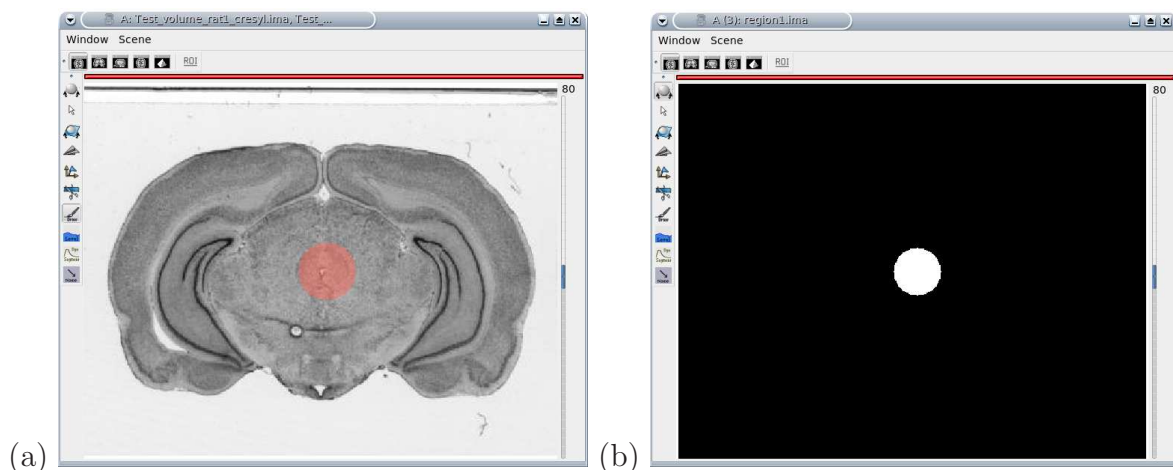


Figure 4.7: Manual drawing of a region of interest. (b) Binary mask of `region1`.

Chapter 5

General support

If you have any problems or queries relating to BrainRAT, BrainVISA or Anatomist, please contact us through the BrainVISA forum (Figure 5.1) at: <http://brainvisa.info/forum>.

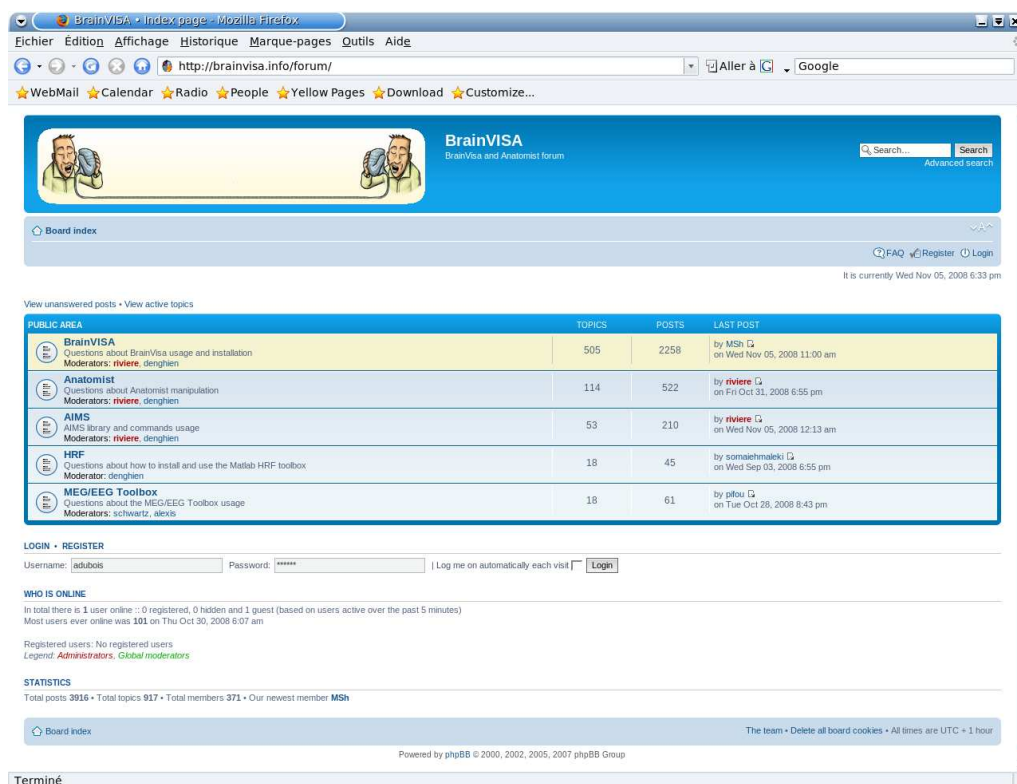


Figure 5.1: Access page to BrainVISA/Anatomist forum.

Yet, before asking questions or reporting problems, please make sure your query is not solved in the [FAQ](#) (Figure 5.2).

Thank you to consider that we have very few resources for technical support at the moment.

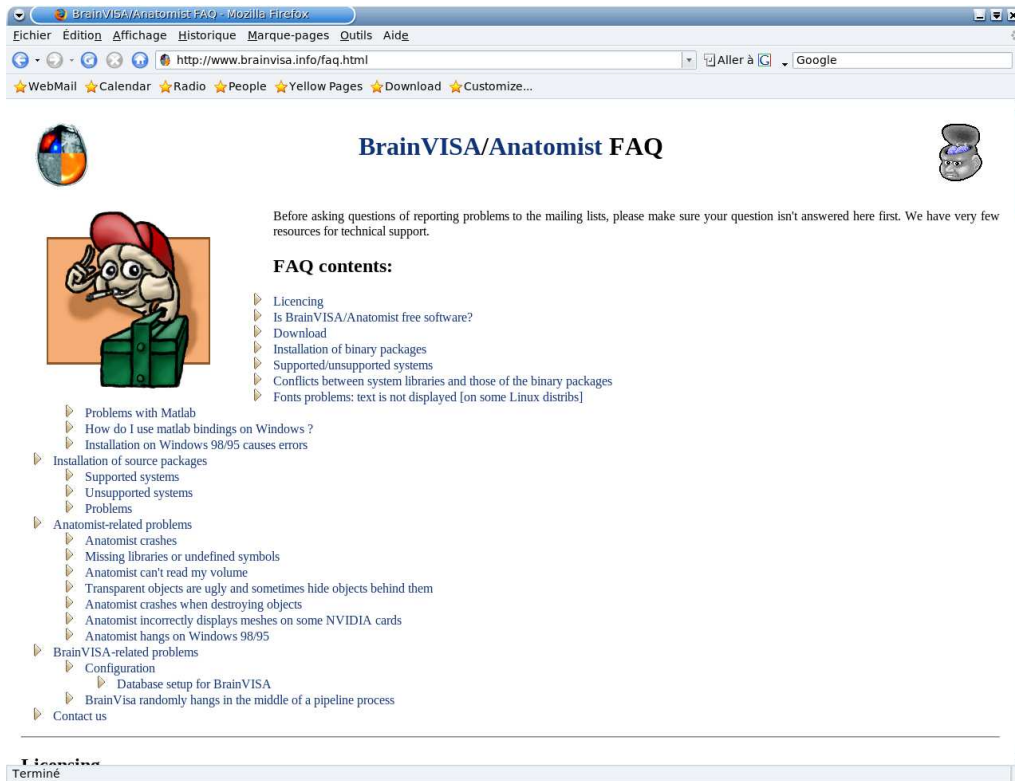


Figure 5.2: Access page to BrainVISA/Anatomist FAQ.

If you have any problems with your data, please check the procedure described in the attached document entitled "Optimized high throughput digitization procedure of autoradiographic and histological sections ([How To Scan](#))".

Chapter 6

Description and use of BrainRAT process interfaces

IMPORTANT: In order to work through the following sections, you need to download demonstration data. A request for access to these data can be submitted by sending an e-mail to the following address: brainrat@cea.fr. Please mention your institution, lab, research area, e-mail address and operating system (Windows, MacOS or Linux).

Demonstration data enables to test all the functionalities of BrainRAT through two applications:

- ♣ A unilateral stimulation of the rat visual system ($[^{14}\text{C}]$ -2DG autoradiography, Nissl staining $\rightarrow \sim 150$ adjacent slices)
- ♣ A whole rat brain anatomical dataset (Nissl staining $\rightarrow \sim 200$ slices)

Demonstration data will be made available electronically via anonymous FTP from the CEA FTP server as .zip or .tar.gz files according to our operating systems.

Remember that they are CEA property and made available for educational purposes only. The use of these data for other purposes are submitted to CEA authorization. In case of use of BrainRAT for academic researches, please mention Dubois et al. (2007).

Before processing your own data, it is strongly recommended to read carefully and follow the instructions of the companion guide [How To Scan](#).

6.1 Presentation

All the processes available in BrainRAT module package are organized into three directories (Figure 6.1). Each directory realizes a specific task.

01. post mortem rebuilt 2D \Leftrightarrow 3D allows the 3D reconstruction of biological volumes arising from several histological and/or autoradiographic sections, beforehand individually digitized or acquired as overall scans (*Cf.* [How To Scan](#)).

02. post mortem imaging analysis allows the conversion of grey level values in 3D reconstructed $[^{14}\text{C}]$ -2DG autoradiographic volumes to activity (nCi/g) or CMRGlu ($\mu\text{mol}/100\text{g}/\text{min}$). A module enabling to perform a conventional ROI-based analysis of these volumes is also proposed.

03. toolbox contains plugged-in modules allowing basic image processing operations such as deletion, permutation or replacement of damaged sections, flip of a slice/volume either in a mirror way or swapping coordinates, etc.

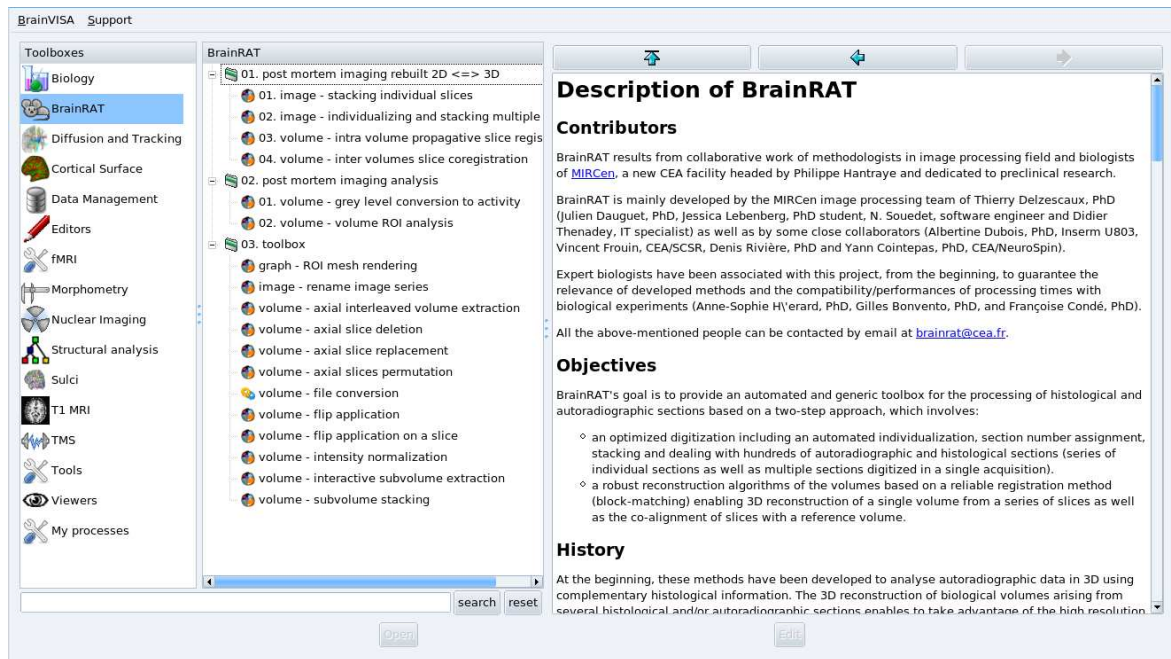


Figure 6.1: Description of the BrainRAT module package.

6.2 01. post mortem imaging rebuilt 2D \Leftrightarrow 3D

6.2.1 01. image - stacking individual slices (Figure 6.2)

This process enables the stacking of individually digitized slice images in the direction orthogonal to the cutting-plane. Slices are arranged in the order in which they were selected (see comments on parameter Input_ListOf_2dImage below).

This process also initializes the following 3D reconstruction by stacking these slices according to their gravity centre parameters (centering procedure).

The process operates with individual images having different horizontal and vertical dimensions. However, make sure that all the slice images were digitized with the same in-plane digitization resolution.

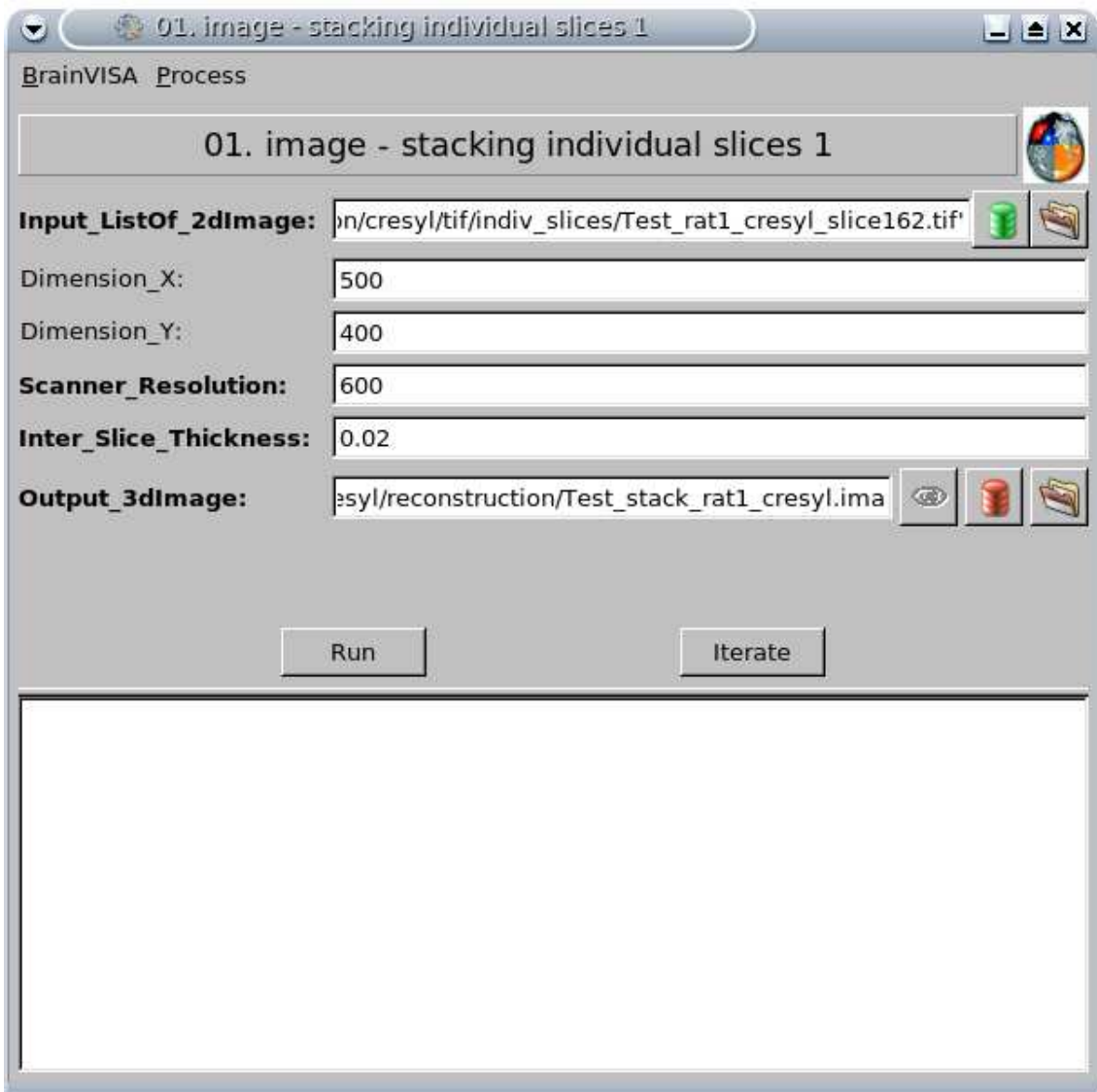


Figure 6.2: Process interface: 01. image - stacking individual slices.

Parameters of 01. image - stacking individual slices are:

Input_ListOf_2dImage

Click on .

Select the multiple slice images to be stacked by holding down on the control button on your keyboard (Ctrl), for instance Test_rat1_cresyl_slice001.tif, Test_rat1_cresyl_slice002.tif, Test_rat1_cresyl_slice003.tif ... Test_rat1_cresyl_slice162.tif.

Dimension_X

Enter the horizontal dimension (in number of pixels) of the final stacked volume.

This parameter is optional, if you do not complete it, the final horizontal dimension of the stacked volume will be based on the horizontal dimension of the largest image in the

series.

Dimension_Y

Enter the vertical dimension (in number of pixels) of the final stacked volume.

This parameter is optional, if you do not complete it, the final vertical dimension of the stacked volume will be based on the vertical dimension of the largest image in the series.

Scanner_Resolution

Enter the in-plane resolution in dpi (dot per inch) of your digitizing system (flatbed scanner, CCD camera,...).

A default value of 600 dpi, corresponding to $\sim 42 \mu\text{m}$, is automatically assigned.

Inter_Slice_Thickness

Enter the interslice interval, *i.e.* the distance in mm between two consecutive slices in the final stack, for instance 0.02 (0.02 mm = 20 μm).

NOTE: if you have acquired one out three 20 μm -thick sections, the interstice interval will be $0.02 \times 3 = 0.06$ mm.

Output_3dImage

Click on .

Specify the output directory where the stack volume will be written and enter the output file name, for instance Test_stack_rat1_cresyl.ima.

Click on RUN to launch the process.

6.2.2 02. image - individualizing and stacking multiple slices (Figure 6.3)

This process provides a fully automated procedure for extraction and individualization of histological or autoradiographic slices from overall scans, using histogram analysis, threshold and iterative mathematical morphology operations. The individualized slices are then stacked in the direction orthogonal to the cutting-plane. Slices are arranged in the sectioning process order, hence in a continuous antero-posterior relationship.

This process also initializes the following 3D reconstruction by stacking these slices according to their gravity centre parameters (centering procedure).

NOTE: Make sure to process an animal after another as well as a *post mortem* imaging modality after another. For instance, start by processing all overall scans of histological sections of rat n 1. Resume processing on all overall scans of autoradiographic sections of the same animal. Then, move to rat n 2 and repeat these two steps.

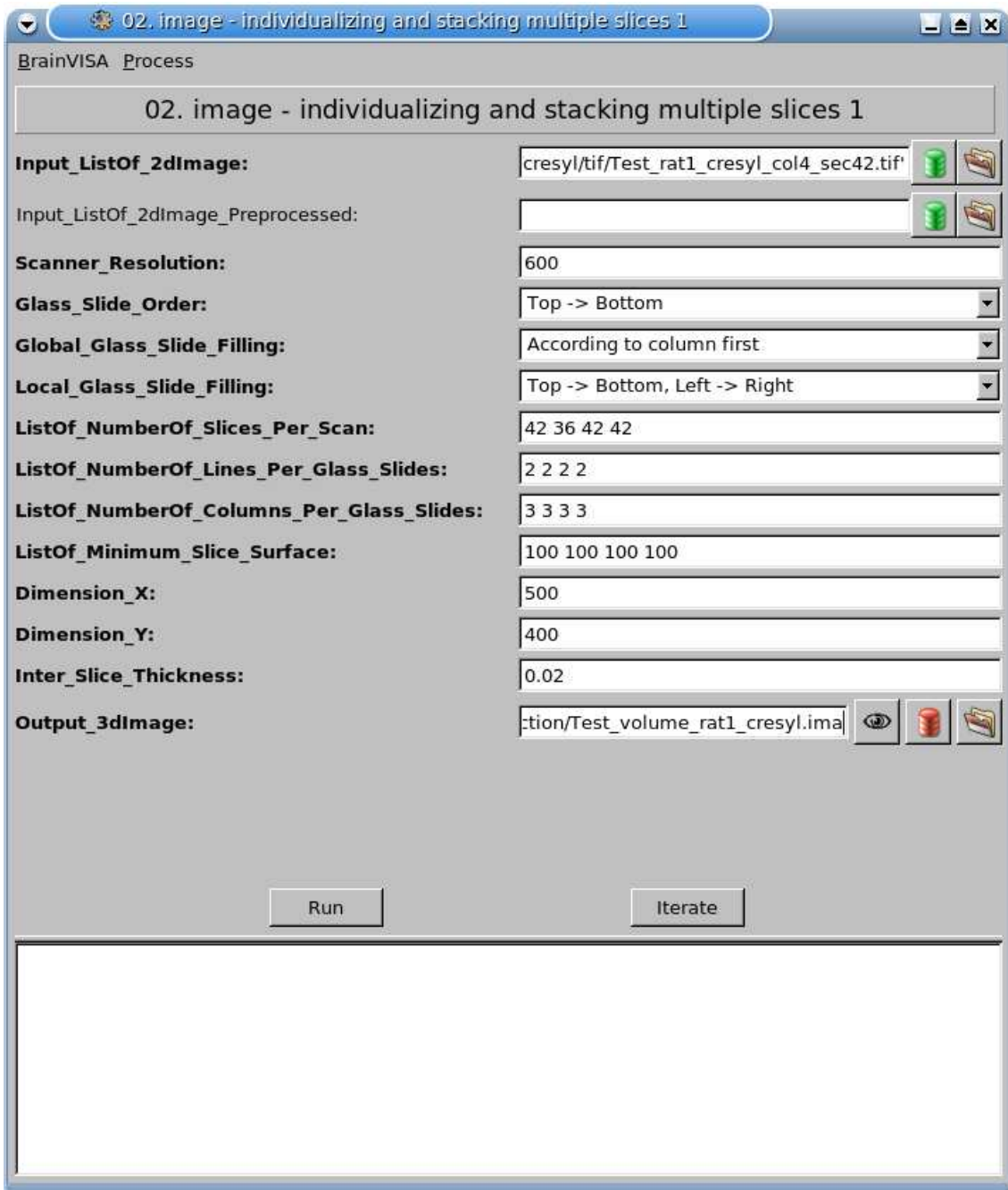


Figure 6.3: Process interface: 02. image - individualizing and stacking multiple slices.

Parameters of 02. image - individualizing and stacking multiple slices are:

Input_ListOf_2dImage

Click on .

Select all overall scans to be processed by holding down on the control button on your keyboard (Ctrl), for instance Test_rat1_cresyl_col1_sec42.tif, Test_rat1_cresyl_col2_sec36.tif, Test_rat1_cresyl_col3_sec42.tif and Test_rat1_cresyl_col4_sec42.tif.

Input_ListOf_2dImage_Preprocessed

Click on .

Select all overall scans which have been needed to be pre-processed (*Cf.* [How To Scan](#)) by holding down on the control button on your keyboard (Ctrl).

Scanner_Resolution

Enter the in-plane digitization resolution in dpi (dot per inch) of your flatbed scanner.

Glass_Slide_Order

Choose the arrangement in which the glass slides have been exposed to an autoradiographic film or laid down on the surface of the scanner: top-bottom or bottom-top.

Global_Glass_Slide_Filling

Choose the global arrangement slices on glass slides: according to line or to column first (*Cf.* [How To Scan](#)).

Local_Glass_Slide_Filling

Choose the local arrangement slices on glass slides (*Cf.* [How To Scan](#)).

ListOf_NumberOf_Slices_Per_Scan

Enter the number of slices contained in each overall scan. Separate them from each other by pressing the space bar.

ListOf_NumberOf_Lines_Per_Glass_Slides

Enter the number of lines according which the slices have been arranged on glass slides in each overall scan. Separate them from each other by pressing the space bar.

ListOf_NumberOf_Columns_Per_Glass_Slides

Enter the number of columns according which the slices have been arranged on glass slides in each overall scan. Separate them from each other by pressing the space bar.

ListOf_Minimum_Slice_Surface

Enter the surface (in mm²) of the shortest slices in each overall scan. Separate them from each other by pressing the space bar.

In rat, this surface is of about 5 mm² for olfactory bulb sections, 10-20 mm² for frontal brain sections (frontal cortex, etc.), 50-100 mm² for midbrain sections (striatum, hippocampus, thalamus, colliculus, etc.) and 20 mm² for cerebellum sections.

Dimension_X

Enter the horizontal dimension (in number of pixels) of the output volume.

Dimension_Y

Enter the vertical dimension (in number of pixels) of the output volume.

Inter_Slice_Thickness

Enter the interslice interval, *i.e.* the distance in mm between two consecutive slices in the final stack, for instance 0.02 (0.02 mm = 20 μm).

NOTE: If you have acquired one out three 20 μm-thick sections, the interstice interval will be $0.02 \times 3 = 0.06$ mm.

Output_3dImage

Click on .

Specify the output directory where the stack volume will be written and enter the output file name, for instance Test_stack_rat1_cresyl.ima.

Click on RUN to launch the process.

6.2.3 03. volume - intra volume propagative slice registration (Figure 6.4)

This process can be used after the histological slices have been stacked in the direction orthogonal to the cutting-plane, by running one of the two following processes: Stacking individual slices or Individualizing and Stacking multiple slices. It enables you to align 2D histological slices into a 3D anatomical volume using the block-matching registration method (Ourselin et al., 2001) in a propagative scheme.

The anatomical volume is reconstructed by registering each histological slice with the following one in the stack. Then, by composition of the previously assessed transformations, each slice is aligned to a reference slice so as to obtain a consistent 3D anatomical volume.

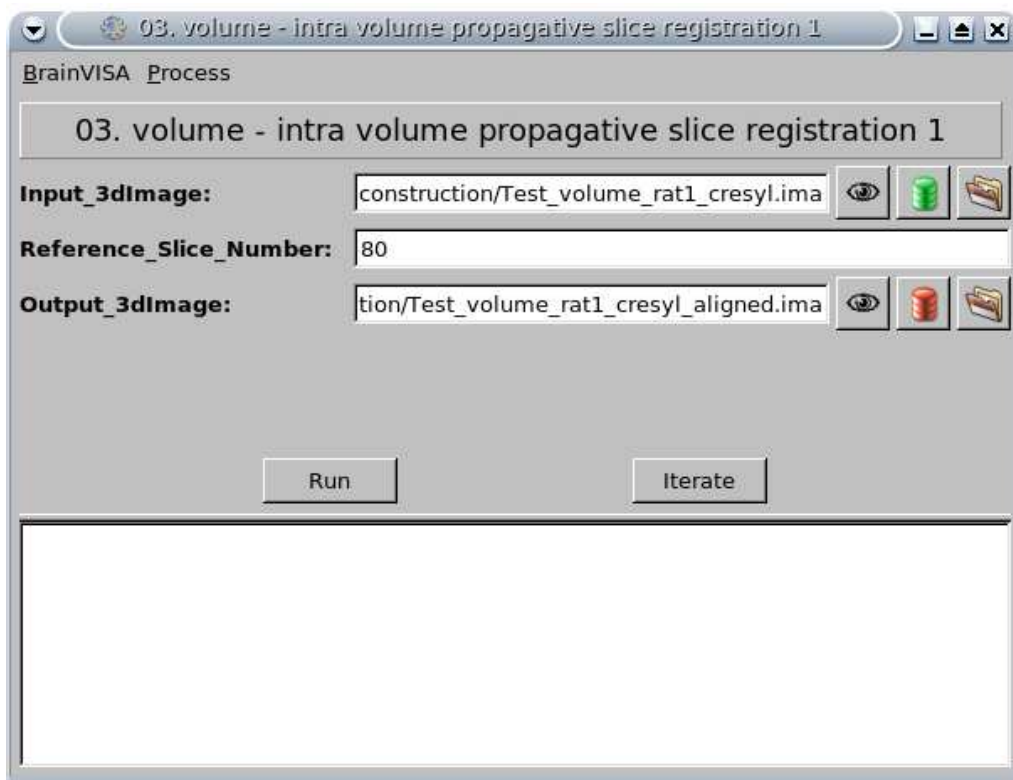


Figure 6.4: Process interface: 03. volume - intra volume propagative slice registration.

Parameters of 03. volume - intra volume propagative slice registration are:

Input_3dImage

Click on .

Select the initial reconstructed volume you have obtained by stacking the individualized coronal histological slices in the direction orthogonal to the cutting-plane (output file from the process Stacking individual slice or Individualizing and stacking multiple slices), for instance Test_volume_rat1_cresyl.ima.


Reference_Slice_Number

Enter the number of the slice chosen as reference.

The reference slice has to be selected because it carries few artefacts such as folds or tears and to be located in the middle of the volume to limit error propagation. A default value is automatically assigned according to the total number of sections in the volume to be reconstructed. This is the result of the following calculation: $\text{ceil}(\frac{\text{Total number of slices}}{2}) - 1$.

There are 162 slices in Test_volume_rat1_cresyl.ima, its corresponding Reference_Slice_Number value is hence 80.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_volume_rat1_cresyl_aligned.ima

NOTE: Name choice is important to identify the content of an image and distinguish it from others.

Click on RUN to launch the process.

6.2.4 04. volume - inter volumes slice coregistration (Figure 6.5)

This process can be used after the anatomical volume has been made spatially consistent in 3D by running the Intra volume propagative slice registration process. It enables you to obtain a 3D consistent functional volume by using the previously registered anatomical volume as a reference.

Each 2D autoradiographic slice in the stacked functional volume is directly co-registered with its corresponding registered histological slice from the anatomical volume using the same block-matching method as in the process Intra volume propagative slice registration.

NOTE: To perform such an anatomo-functional co-registration, anatomical and functional volumes must have exactly the same number of slices. However, the two volumes can have different resolutions (voxel sizes).

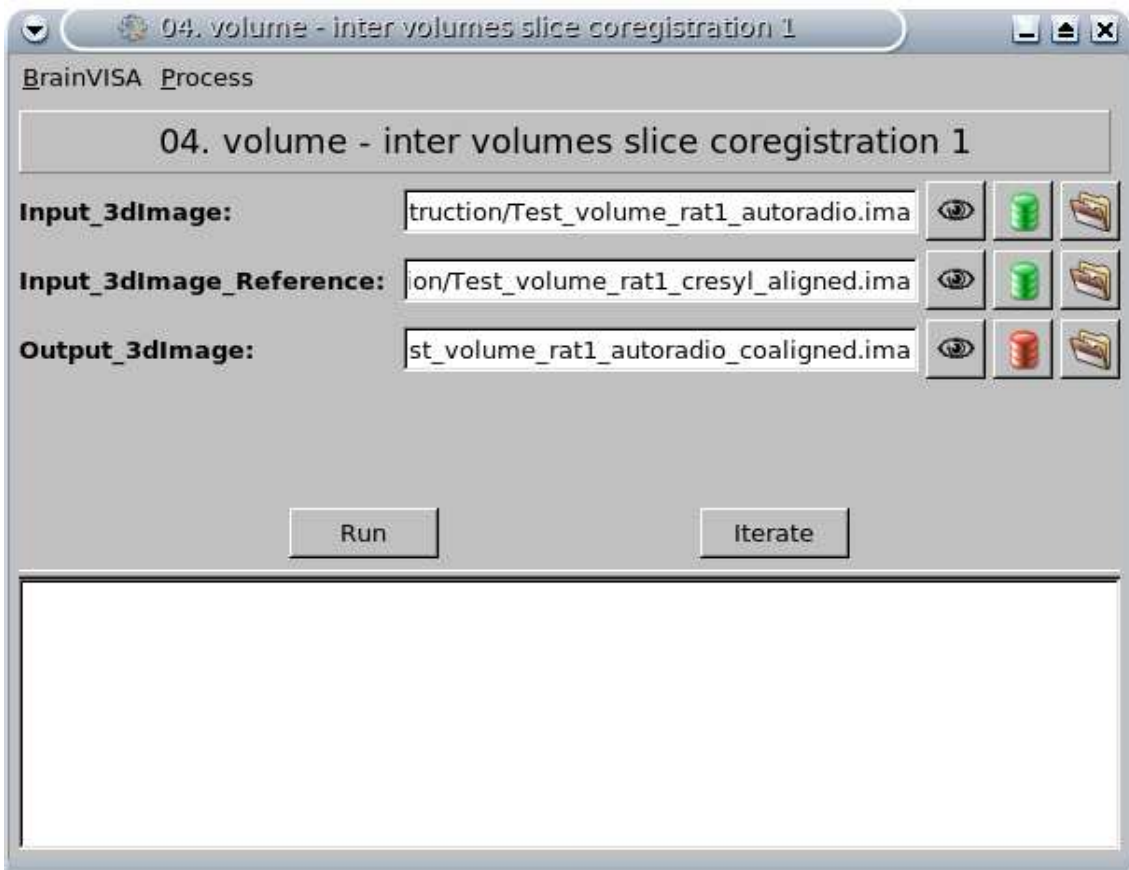


Figure 6.5: Process interface: 04. volume - inter volumes slice coregistration.

Parameters of 04. volume - inter volumes slice coregistration are:

Input_3dImage

Click on .


Select the initial reconstructed volume you have obtained by stacking the individualized coronal autoradiographic slices in the direction orthogonal to the cutting-plane (output file from the process Stacking individual slice or Individualizing and stacking multiple slices), for instance Test_volume_rat1_autoradio.ima.

Input_3dImage_Reference

Click on .

Select the 3D reconstructed anatomical volume used as a reference (output file from the Intra volume propagative slice registration process), for instance Test_volume_rat1_cresyl_aligned.ima.


Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_volume_rat1_autoradio_coaligned.ima.

Click on RUN to launch the process.

6.2.5 Quality check

After running one of the previous processes, you will be able to view the output files and assess the quality of the registrations and 3D reconstruction procedures in different orthogonal incidences by clicking on  in Output_3dImage and visualizing volumes in Anatomist (Figures 6.6 and 6.7).

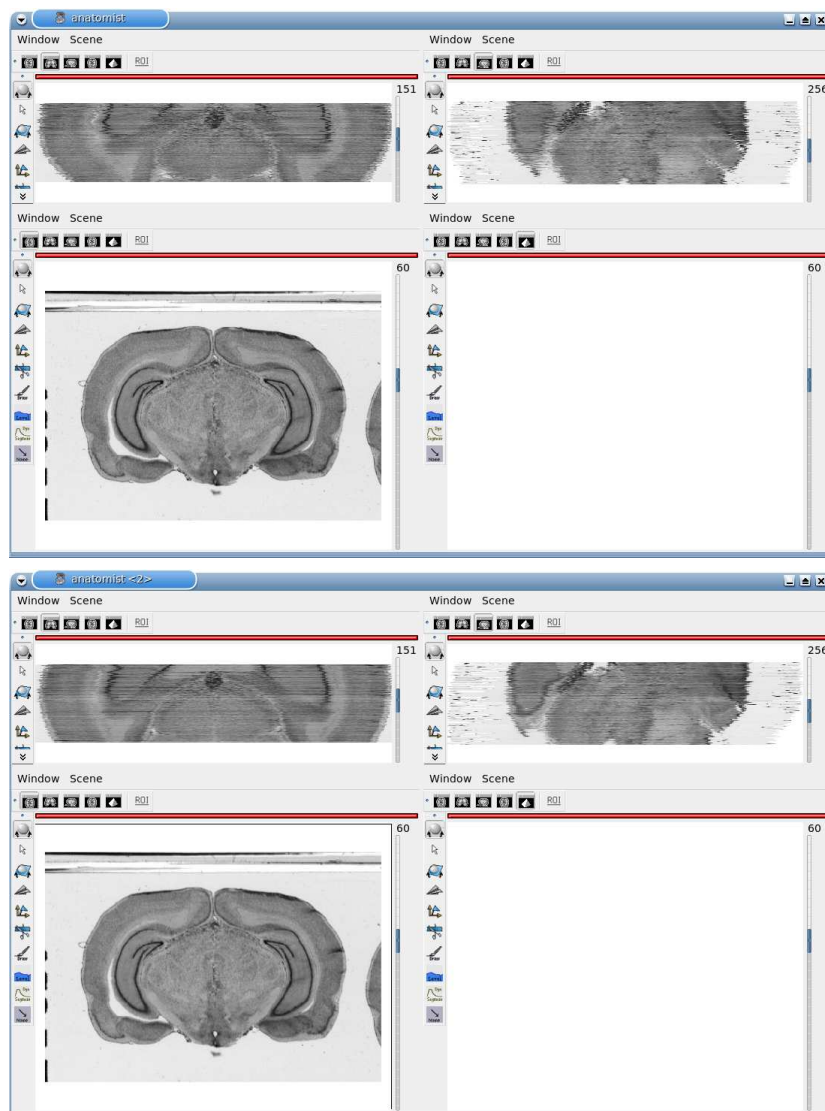


Figure 6.6: 3D reconstruction of the histological volume (Nissl stain), encompassing a large part of the visual system (between bregma -5.2 mm and bregma -8.2 mm), in coronal, axial and sagittal views before (top) and after (bottom) registration.

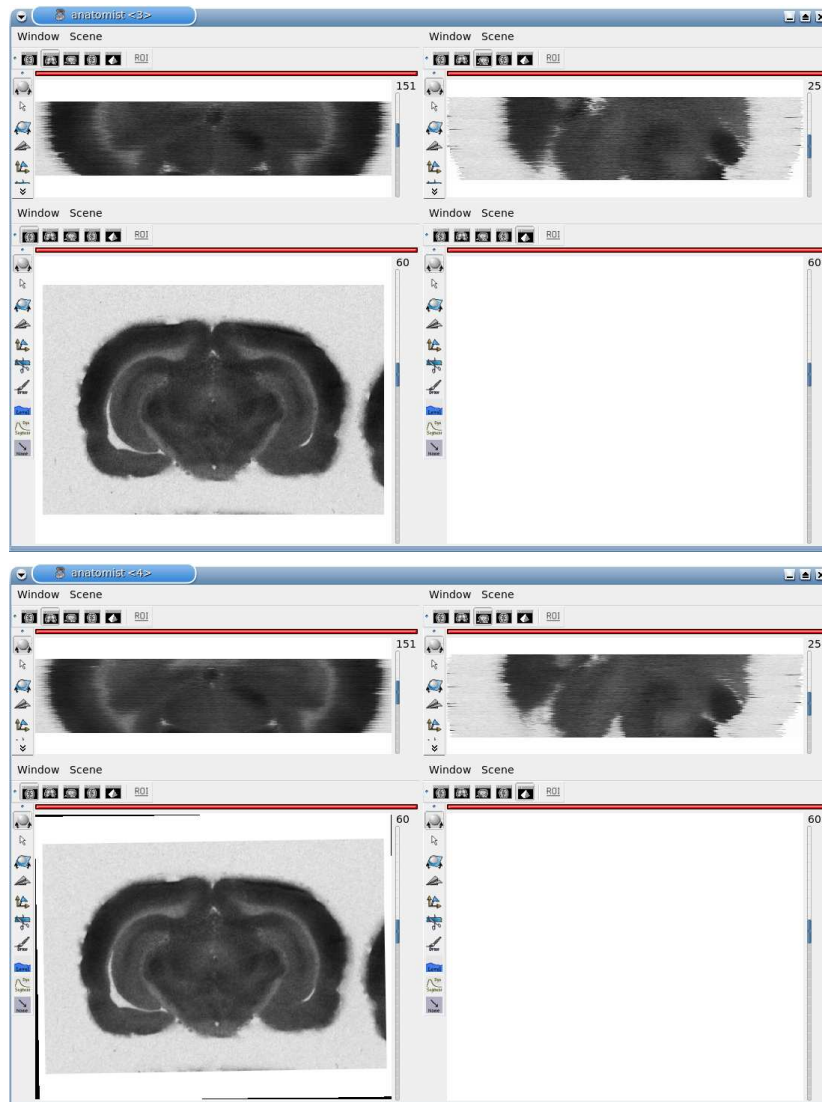


Figure 6.7: 3D reconstruction of the $[^{14}\text{C}]$ -2DG autoradiographic volume, encompassing a large part of the visual system (between bregma -5.2 mm and bregma -8.2 mm), in coronal, axial and sagittal views before (top) and after (bottom) registration.

6.3 02. post mortem imaging analysis

6.3.1 01. volume - grey level conversion to activity (Figure 6.10)

This process requires that the grey level values in the autoradiographic images have been previously calibrated using the calibration scale available with the scanner (*Cf. [How To Scan](#)*).

It enables you to convert the grey level values in the 3D reconstructed $[^{14}\text{C}]$ -2DG autoradiographic volume to activity values (nCi/g of tissue) using the co-exposed $[^{14}\text{C}]$ standard scale. A polynomial fourth degree fit method, identified by the radioactive $[^{14}\text{C}]$ standard curve, ensures the conversion.

Additionally, if blood samples were taken from the animals during the experiment, they can be used to compute the parameters of the modified operational equation of Sokoloff (Sokoloff, 1979) and to convert activity to CMR_{Glu} ($\mu\text{mol}/100\text{ g}/\text{min}$).

Before using this process, you have to compute the mean grey level value of each standard to associate one known amount of [¹⁴C] activity to a specific grey level value. For the time being, we propose you to manually draw rectangular or circular ROIs on each of these standards with Anatomist, as follows:

Open Anatomist.

Load the image of radioactive [¹⁴C] standards which has been exposed and then digitized together with autoradiographs, for instance Test_rat1_standards.tif.

Place it into an axial window.

Click on icon  of this window.

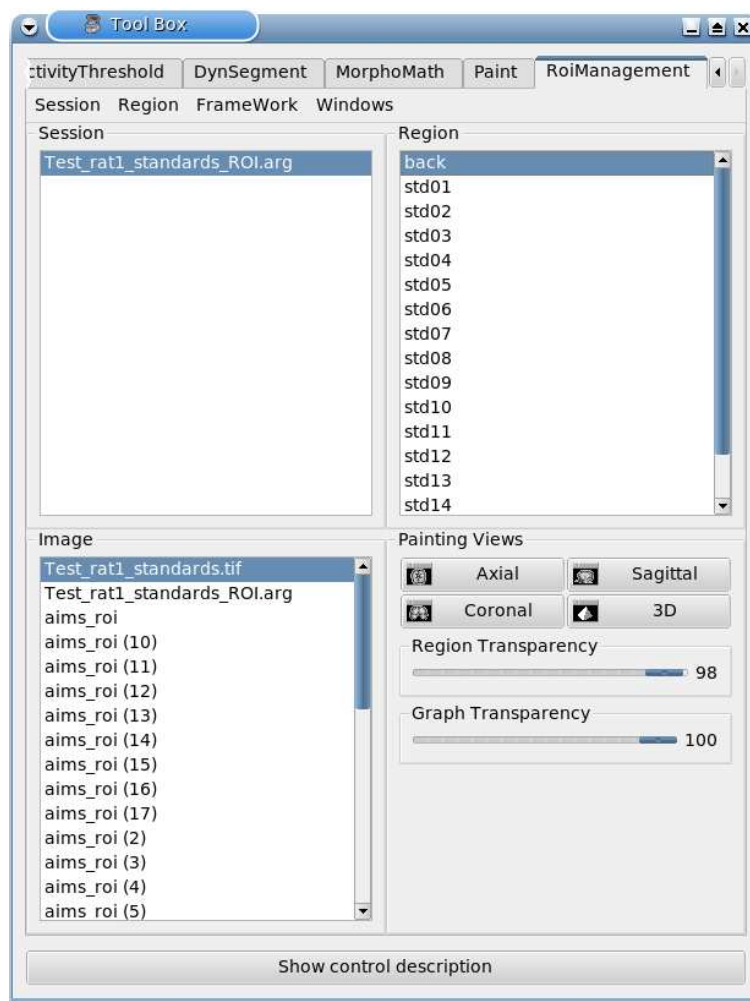


Figure 6.8: Use of ROI drawing toolbox.

Click on Session ⇒ New (Figure 6.8).

A default session name is created, for instance Test_rat1_standards_ROI.arg. You can change the name using Save as option.

Click on Region ⇒ New and provide a name.

Repeat this last step as many times as there are standards in the image and one more time for the background. In the present example, 17 different regions (16 standards + 1 background) have to be created. To facilitate subsequent processing, provide the name back to the region corresponding to the background of the image and the names std01, std02 ... std16 to regions which will respectively correspond to the 16 standards.

IMPORTANT: This nomenclature has to be strictly respected for a proper operation of the algorithm.

Change the brush: Paint ⇒ Disk, Brush Radius = 3.

NOTE: Adapt the Brush Radius to the digitization resolution.

Go back to the previous panel by clicking on the RoiManagement menu.

Select back region. Draw a rectangle within the background of the image of standards. Then, hold down on the shift button on your keyboard (⇧) and press the left mouse button to fulfil the rectangle.

Repeat the same operation with the 16 remaining regions and corresponding standards from the brightest (std01) to the darkest (std16) (Figure 6.9).

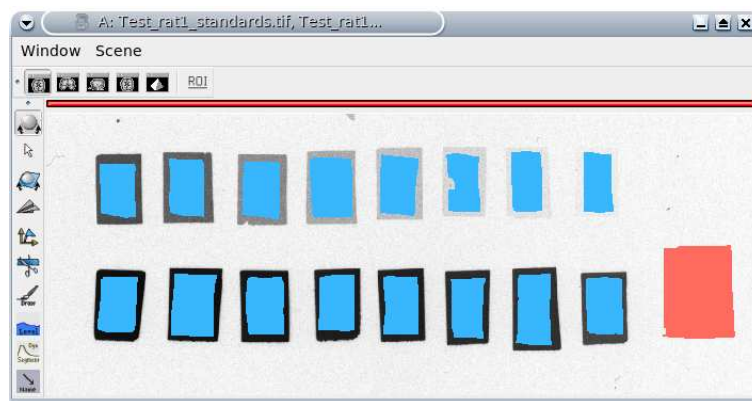


Figure 6.9: ROIs manually segmented on each standard.

Save the ROI session: click on Session ⇒ Save As.

Click Ok.

NOTE: If possible, avoid including artefacts within the ROIs (darker or empty impression areas).

Now, process 01. volume - grey level conversion to activity can be run.

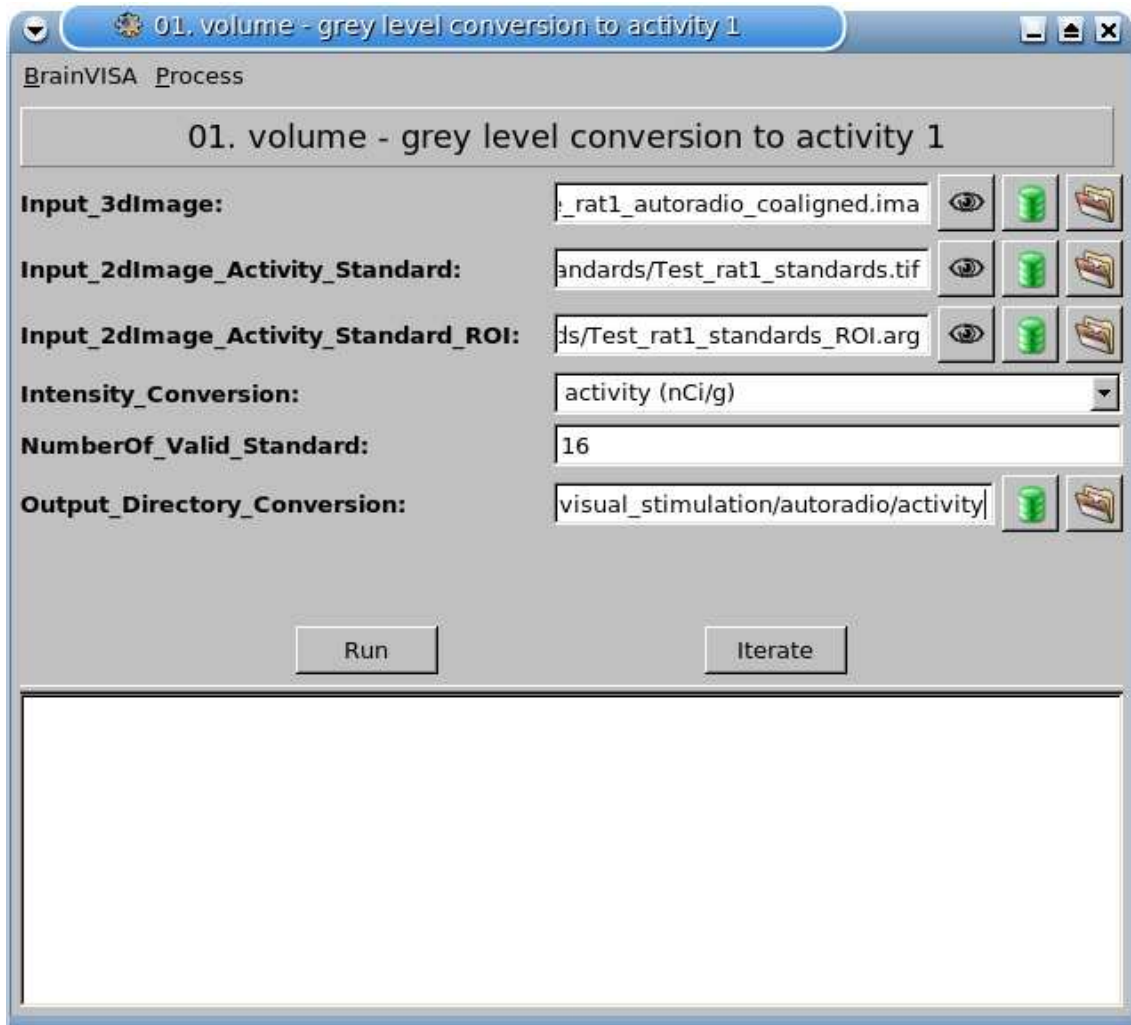


Figure 6.10: Process interface: 01. volume - grey level conversion to activity.

Parameters of 01. volume - grey level conversion to activity are:

Input_3dImage

Click on .

Select the 3D reconstructed autoradiographic volume to be converted, for instance Test_volume_rat1_autoradio_coaligned.ima.

Input_2dImage_Activity_Standard

Click on .

Select the image of radioactive [^{14}C] standards, for instance Test_rat1_standards.tif.

Input_2dImage_Activity_Standard_ROI

Click on .

Select the above-mentioned ROI drawing session, for instance Test_rat1_standards_ROI.arg.

Intensity_Conversion

Choose between two following types of conversion.

- Conversion to activity only, choose activity (nCi/g).
- Conversion to activity and to CMRGlu, choose CMRGlu ($\mu\text{mol}/100\text{g}/\text{min}$).

Sokoloff_Alpha_Parameter

Enter the alpha parameter derived from the modified operational equation of Sokoloff. This parameter is required only if you have chosen CMRGlu in Intensity_Conversion field.

Sokoloff_Beta_Parameter

Enter the beta parameter derived from the modified operational equation of Sokoloff. This parameter is required only if you have chosen CMRGlu in Intensity_Conversion field.

NumberOf_Valid_Standard

Enter the number of valid standards to be taken into account when fitting values with a polynomial fourth degree fit method (*i.e.* without any type of saturation).

A default value of 16 (*i.e.* the total number of standards) is automatically assigned.

Output_Directory_Conversion

Click on .

Specify the output directory in which the output files will be stored.

The output file names will be automatically assigned according to the name of the 3D autoradiographic volume to be converted.

For instance Test_volume_rat1_autoradio_coaligned_activity.ima after volume obtained after conversion to activity values (Figures 6.11 (b) and (c)) and Test_volume_rat1_autoradio_coaligned_CMRGlu.ima for that obtained after conversion to CMRGlu values.

Click on RUN to launch the process.

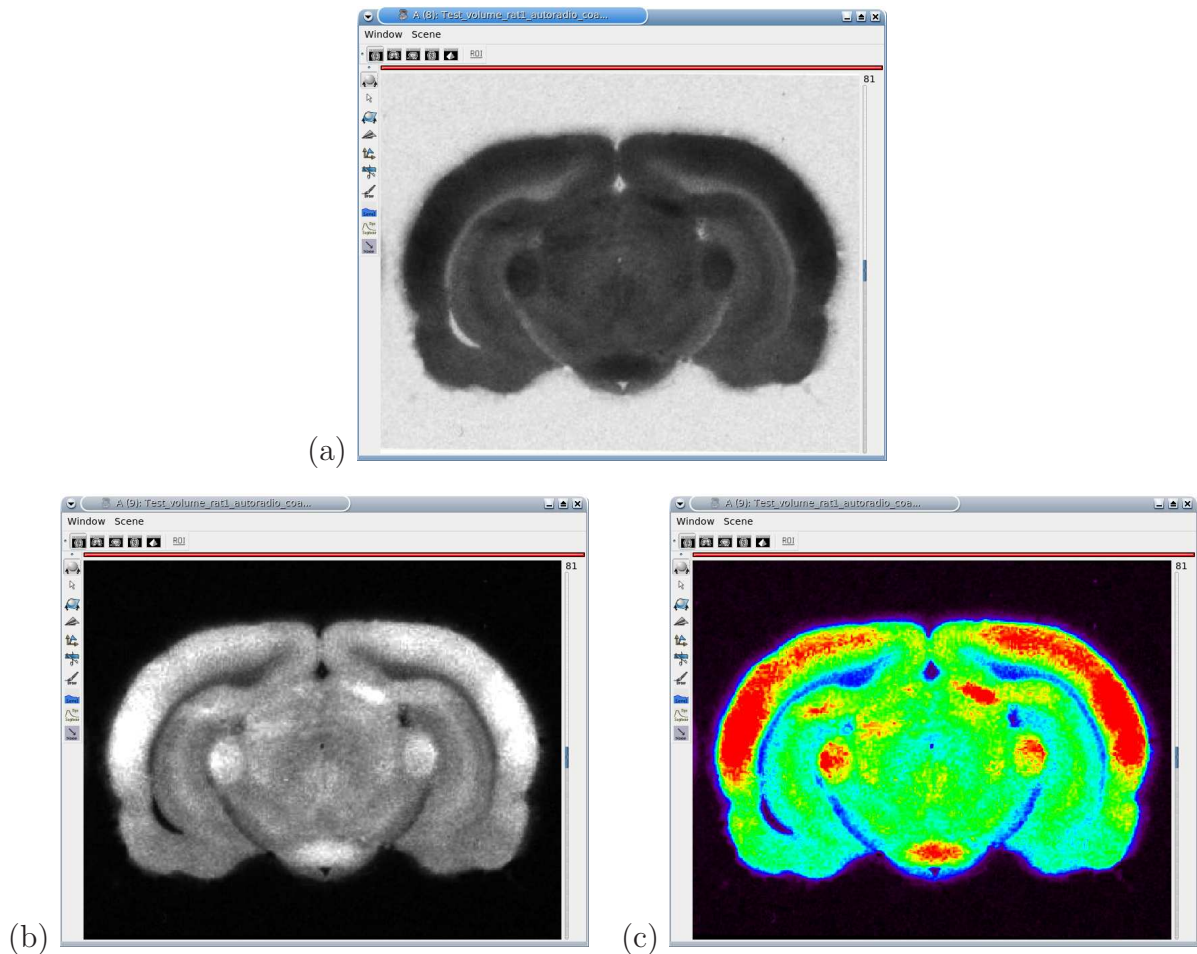


Figure 6.11: 3D reconstruction of $[^{14}\text{C}]$ -2DG autoradiographic volume in coronal view before (a) and after (b and c) conversion to activity (nCi/g). In (c) signal intensities are color-coded according to the quantitative radioactive $[^{14}\text{C}]$ scale (Anatomist palette Rainbow).

6.3.2 02. volume - volume ROI analysis (Figure 6.12)

This process enables you to perform a conventional ROI-based analysis of 3D reconstructed autoradiographic volumes.

The information is extracted from specified ROI(s) and then used to compute ordinary statistics (volume in mm^3 , mean, standard deviation, min, max).

NOTE: In the demonstration data set, the region of interest was the superior colliculus. It has been manually segmented on each section of the 3D rigidly aligned histological volume (Test_volume_rat_cresyl_aligned.ima) yielding a volume of interest (VOI) and allowing assessment of its 3D shape.

As the histological (reference) and autoradiographic volumes have been co-registered, this VOI can be directly mapped on the autoradiographic volume.

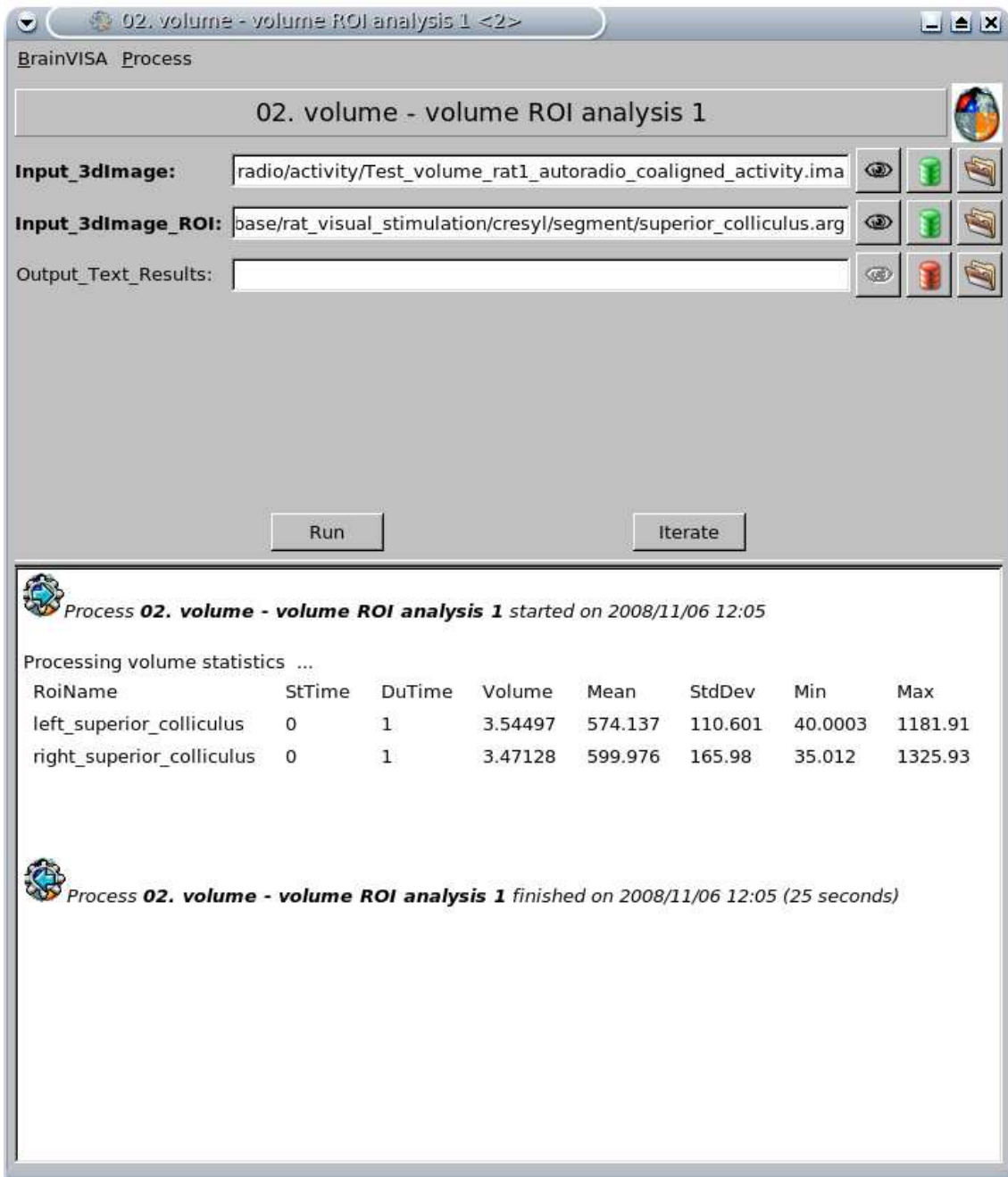


Figure 6.12: Process interface: 02. volume - volume ROI analysis. The computed statistics are displayed in the lower half of the process window.

Parameters of 02. volume - volume ROI analysis are:

Input_3dImage

Click on .

Select the 3D reconstructed autoradiographic volume to be analyzed. Make sure the grey level values of this volume have been previously converted to activity or CMRGlu.

Input_3dImage_ROI

Click on .

Select the ROI drawing session associated to the manual segmentation of the superior colliculus (superior_colliculus.arg) or the binary image of one of its region (right_superior_colliculus.ima or left_superior_colliculus.ima). Note that both operate.

Output_Text_Results

Click on .

Specify the output directory and the output text file that will contain the statistics computed from the specified ROI, for instance Test_rat1_superior_colliculus.txt.

This parameter is optional. Computed statistics are also displayed in the lower half of the process window.

Click on RUN to launch the process.

After running the process, you will be able to retrieve results by opening the output text file with a common text editor such as gedit, kate, kwrite under Linux or with a spreadsheet.

6.4 Toolbox

6.4.1 Image - rename image series (Figure 6.13)

This process automatically renames a series of 2D images in order to harmonize the names.

Parameters of image - rename image series are:

Input_ListOf_2dImage

Click on .

Select the multiple images to be renamed by holding down on the control button on your keyboard (Ctrl), for instance Test_rat1_cresyl_slice001.tif, Test_rat1_cresyl_slice002.tif, Test_rat1_cresyl_slice003.tif ... Test_rat1_cresyl_slice162.tif.

Series_Name_Prefix

Enter the prefix that will be used to create the output image names, for instance Test_rat2_cresyl_slice.

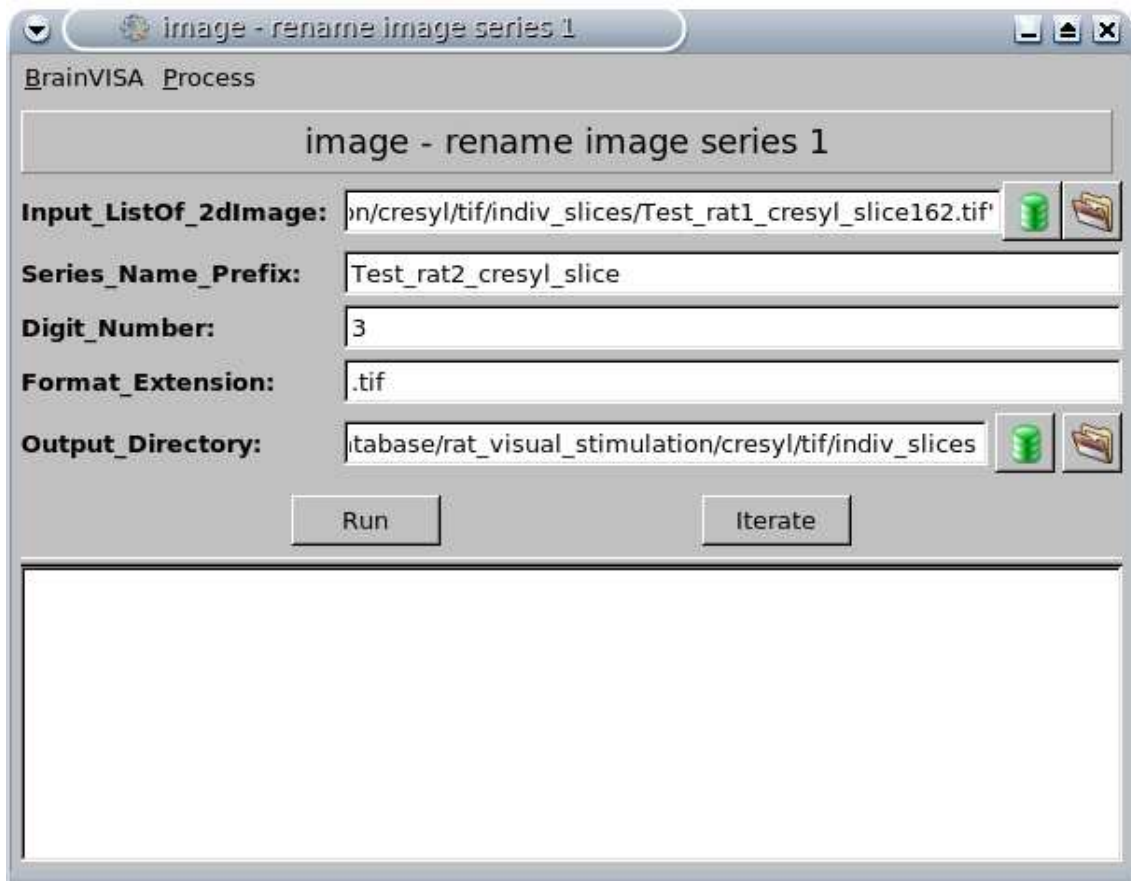


Figure 6.13: Process interface: image - rename image series.

Digit_Number

Enter the digit type of the total number of images to be renamed. A double-digit number is between 10 and 99, a third-digit number is between 100 and 999, etc. This number hence defines the total number of images which will be able to be successively renamed. For instance, if you want to rename 167 images, you have to enter 3.


A default value of 3 is automatically assigned.

Format_Extension

Enter the extension format of each output image.

By default, the same extension format as the first file entered in Input_ListOf_2dImage is automatically assigned, for instance .tif.

Output_Directory

Click on .

Specify the output directory in which the renamed images will be stored. Note that the original input images are not deleted.

Click on RUN to launch the process.

6.4.2 Volume - axial interleaved volume extraction (Figure 6.14)

This process extracts an interleaved subvolume from the original volume.

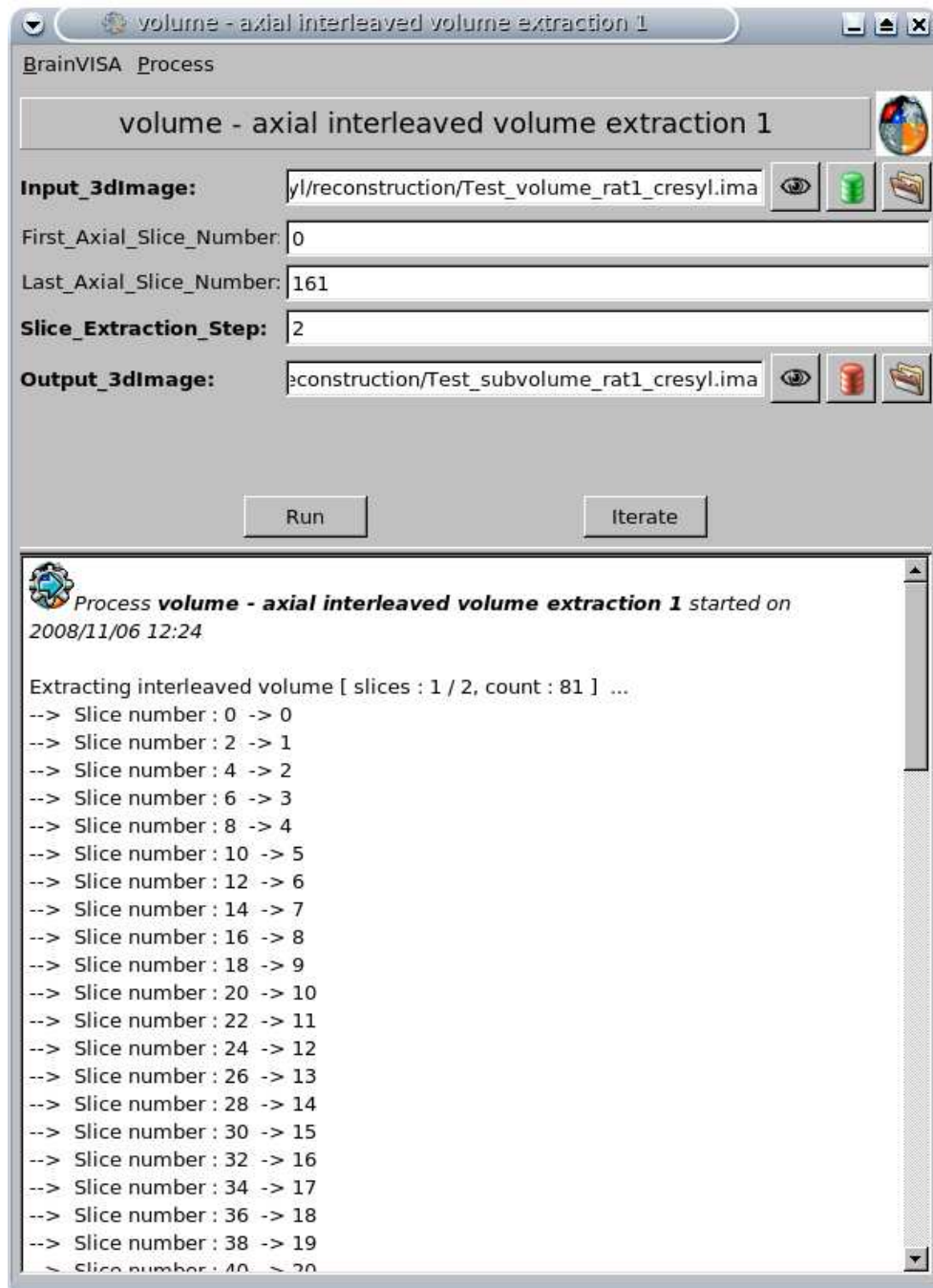


Figure 6.14: Process interface: volume - axial interleaved volume extraction.

Parameters of volume - axial interleaved volume extraction are:

Input_3dImage

Click on .

Select the original volume, for instance Test_volume_rat1_cresyl.ima.

First_Axial_Slice_Number

Enter the number of the slice in the original volume that will represent the first slice of the interleaved volume.

This parameter is optional, by default this parameter corresponds the first slice of the original volume.

Last_Axial_Slice_Number

Enter the number of the slice in the original volume that will represent the last slice of the interleaved volume.

This parameter is optional, by default this parameter corresponds the last slice of the original volume.

Slice_Extraction_Step

Enter the number of slices specifying the interslice interval in the interleaved volume, *i.e.* the distance between two consecutive slices. For instance, enter 2 if you want to retain one original slice out of two in the interleaved volume.

By default this parameter is set to 1 (*i.e.* all the original slices are retained in the interleaved volume).

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_subvolume_rat1_cresyl.ima.

Click on RUN to launch the process.

6.4.3 Volume - axial slice deletion (Figure 6.15)

This process deletes a too much distorted or damaged slice from a volume.

Parameters of volume - axial slice deletion are:

Input_3dImage

Click on .

Select the volume to be processed, for instance Test_volume_rat1_cresyl.ima.

Deletion_Slice_Number

Enter the number of the slice to be deleted, for instance 63.

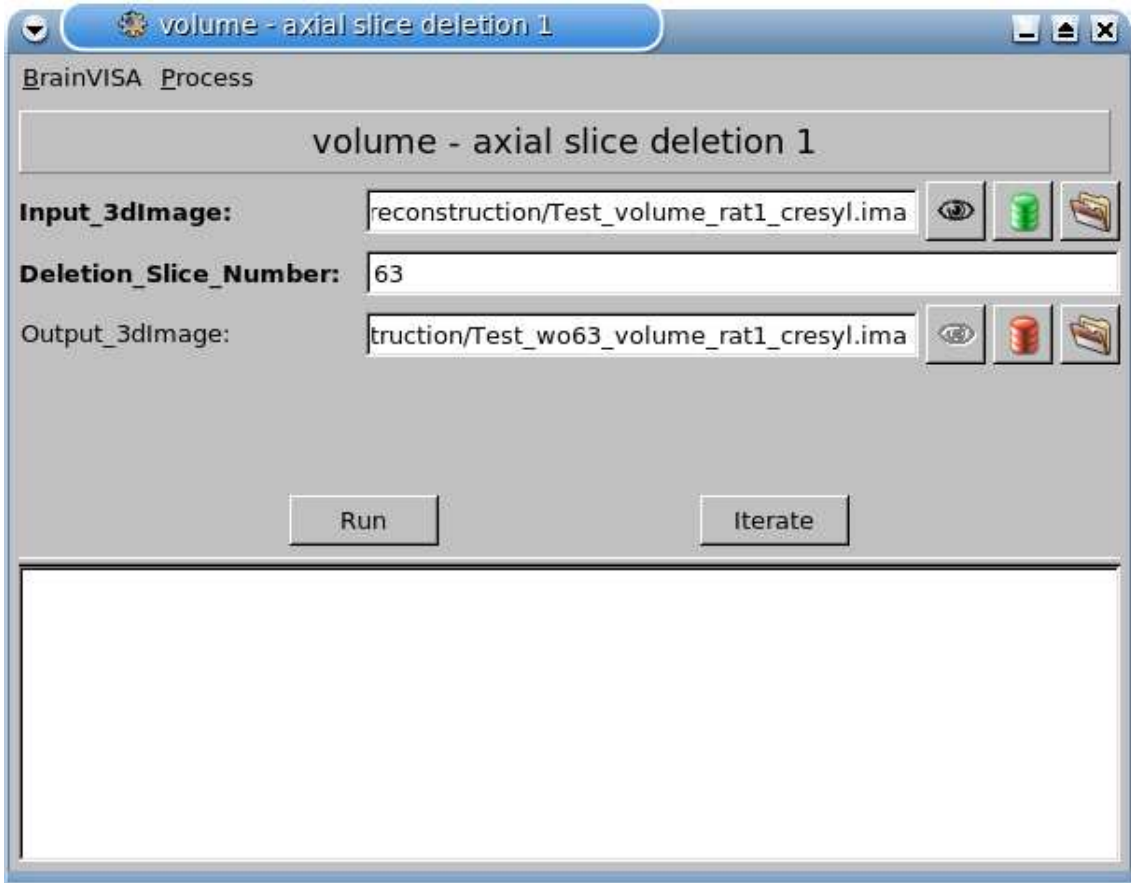



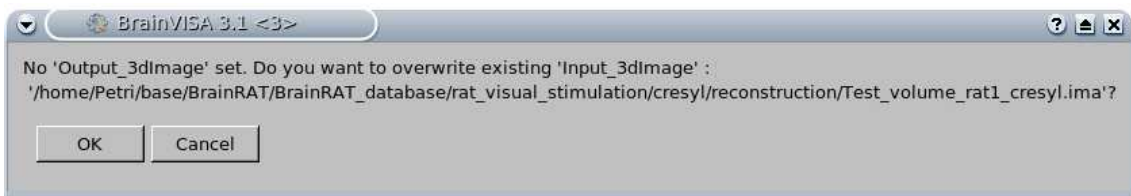
Figure 6.15: Process interface: volume - axial slice deletion.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_wo63.volume_rat1.cresyl.ima (Figure 6.16).

This parameter is optional. If you do not complete it, you will be notified by a warning message that the process will be going to overwrite the input volume.



Click on RUN to launch the process.

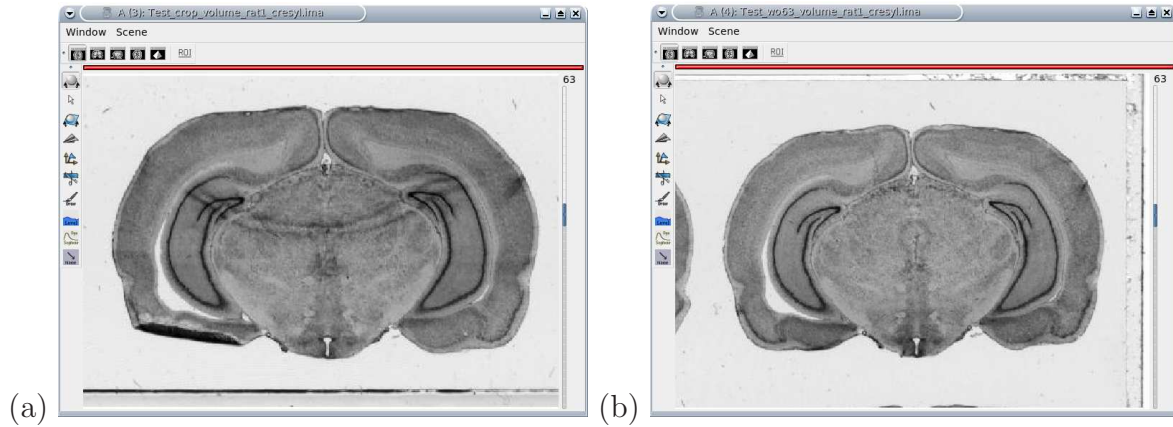


Figure 6.16: (a) The slice 63 to be removed from Test_volume_rat1_cresyl.ima. (b) The new slice 63 in Test_wo63_volume_rat1_cresyl.ima.

6.4.4 Volume - axial slice replacement (Figure 6.17)

This process replaces a too much distorted or damaged slice by a copy of the previous or the next slice from the original volume.

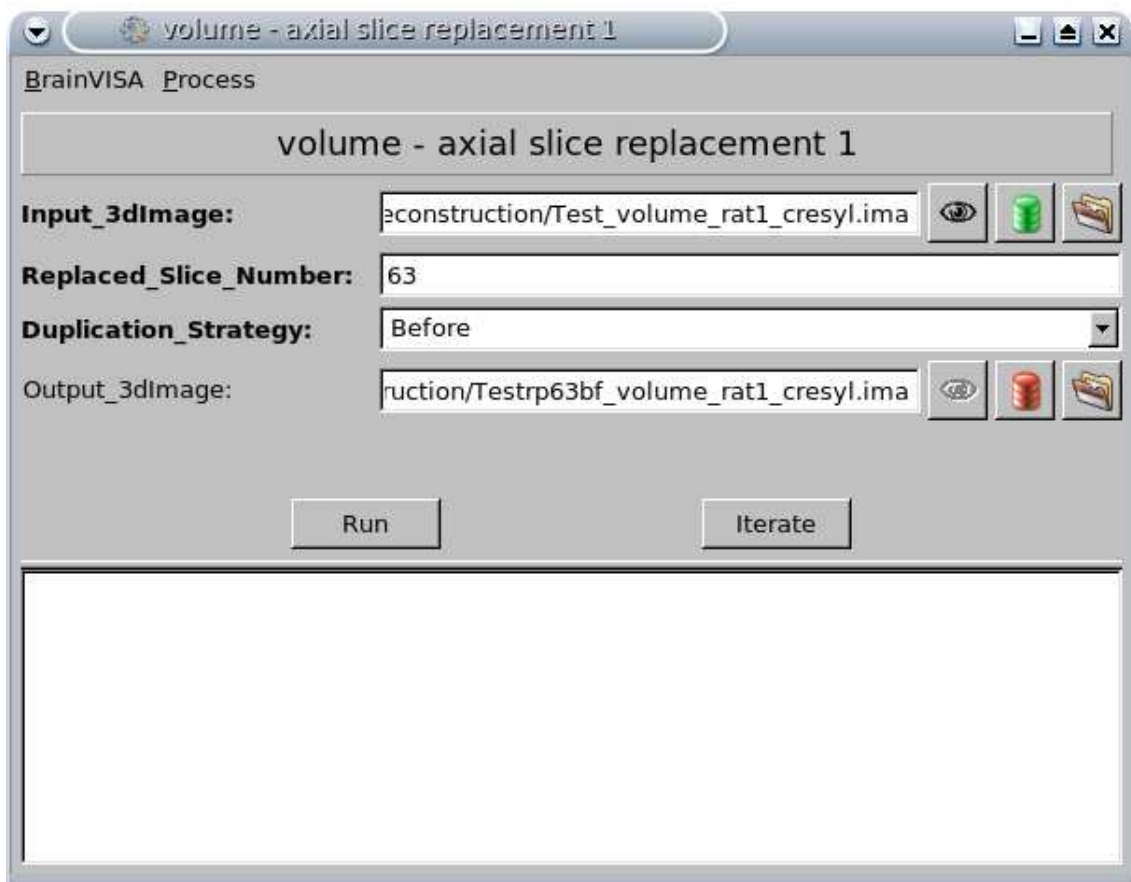


Figure 6.17: Process interface: volume - axial slice replacement.

Parameters of volume - axial slice replacement are:

Input_3dImage

Click on .

Select the volume to be processed, for instance Test_volume_rat1_cresyl.ima.

Replaced_Slice_Number

Enter the number of the slice to be replaced, for instance 63.

Duplication_Strategy

Choose if you want to replace this slice by a copy of the previous (Before) or the next (After) slice from the original volume.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_rp63bf_volume_rat1_cresyl.ima (Figure 6.18).

This parameter is optional. If you do not complete it, you will be notified by a warning message that the process will be going to overwrite the input volume.

Click on RUN to launch the process.

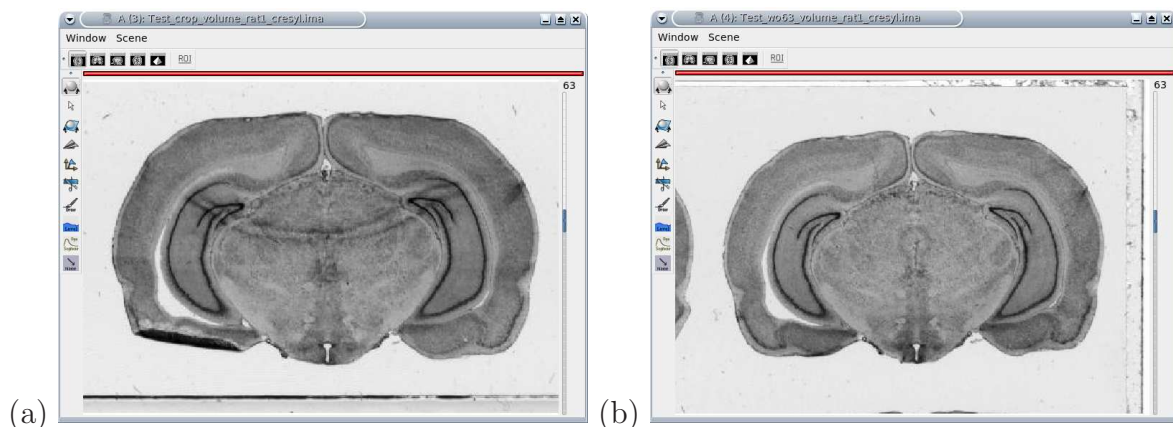


Figure 6.18: (a) The slice 63 to be removed from Test_volume_rat1_cresyl.ima. (b) The new slice 63 in Test_rp63bf_volume_rat1_cresyl.ima.

6.4.5 Volume - axial slices permutation (Figure 6.19)

This process inverts the order of two slices which have been incorrectly disposed on the glass slide during the cryostat sectioning process.

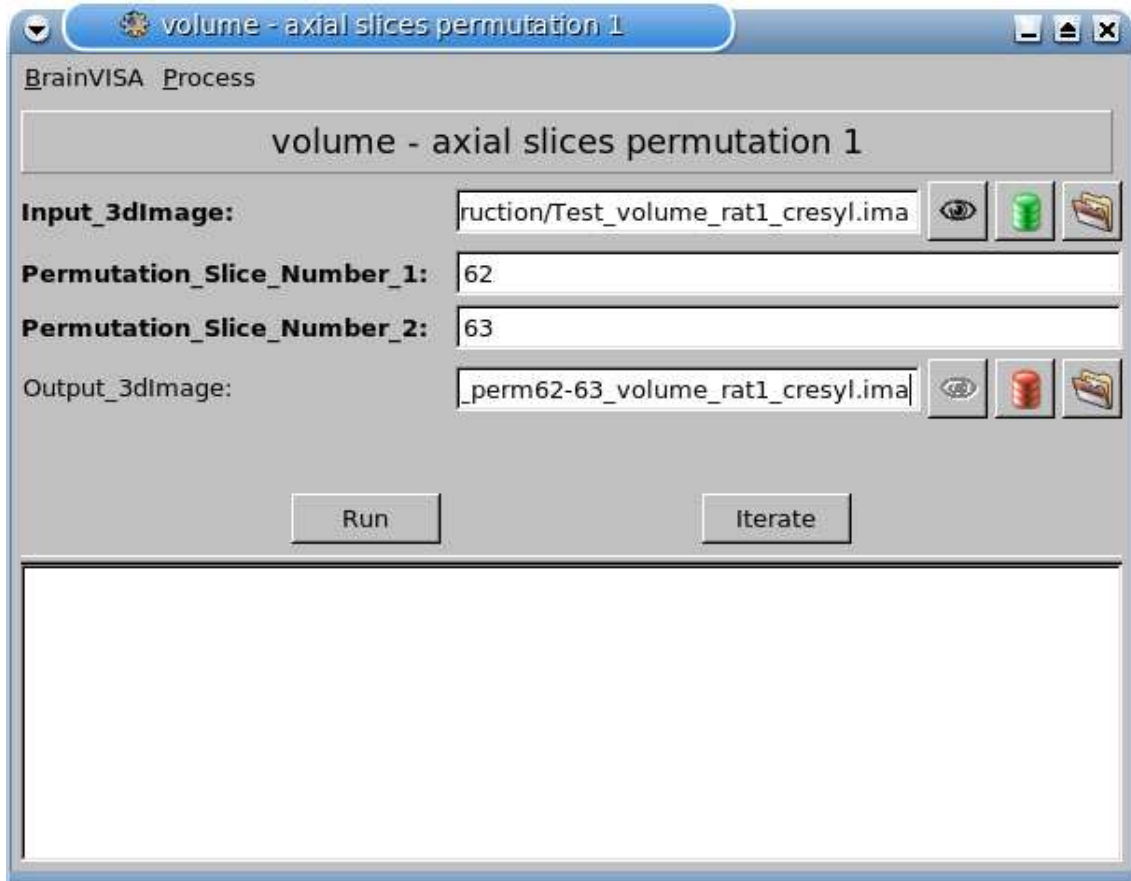


Figure 6.19: Process interface: volume - axial slices permutation.

Parameters of volume - axial slices permutation are:

Input_3dImage

Click on .

Select the volume to be processed, for instance Test_volume_rat1_cresyl.ima.

Permutation_Slice_Number_1

Enter the number of the first slice to be inverted, for instance 62.

Permutation_Slice_Number_2

Enter the number of the second slice to be inverted, for instance 63.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_perm62-63_volume_rat1_cresyl.ima (Figure 6.20).

This parameter is optional. If you do not complete it, you will be notified by a warning message that the process will be going to overwrite the input volume.

Click on RUN to launch the process.

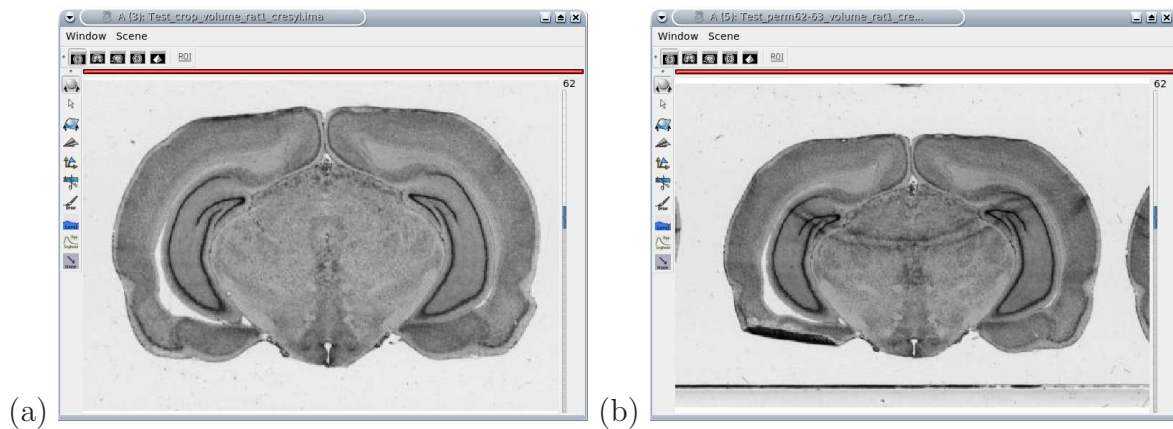


Figure 6.20: The initial slice 62 (a) has been replaced by the slice 63 (b) and vice versa.

6.4.6 Volume - file conversion (Figure 6.21)

This process performs file format and data conversion.

Parameters of volume - file conversion are:

read

Click on .

Select the file (2D, 3D volume, etc.) to be converted.

write

Click on .

Specify the output directory and enter the output file name.

preferredFormat

Choose a specific output format (GIS, VIDA, Analyze).

This parameter is optional. By default, it is guessed by the output filename extension.

removeSource

Choose if you want to delete (True) or not (False) the original input files.

ascii

This parameter allows to write in ASCII mode if the output format supports it.

voxelType

Choose the voxel type of the output files. By default, it is the same as input.

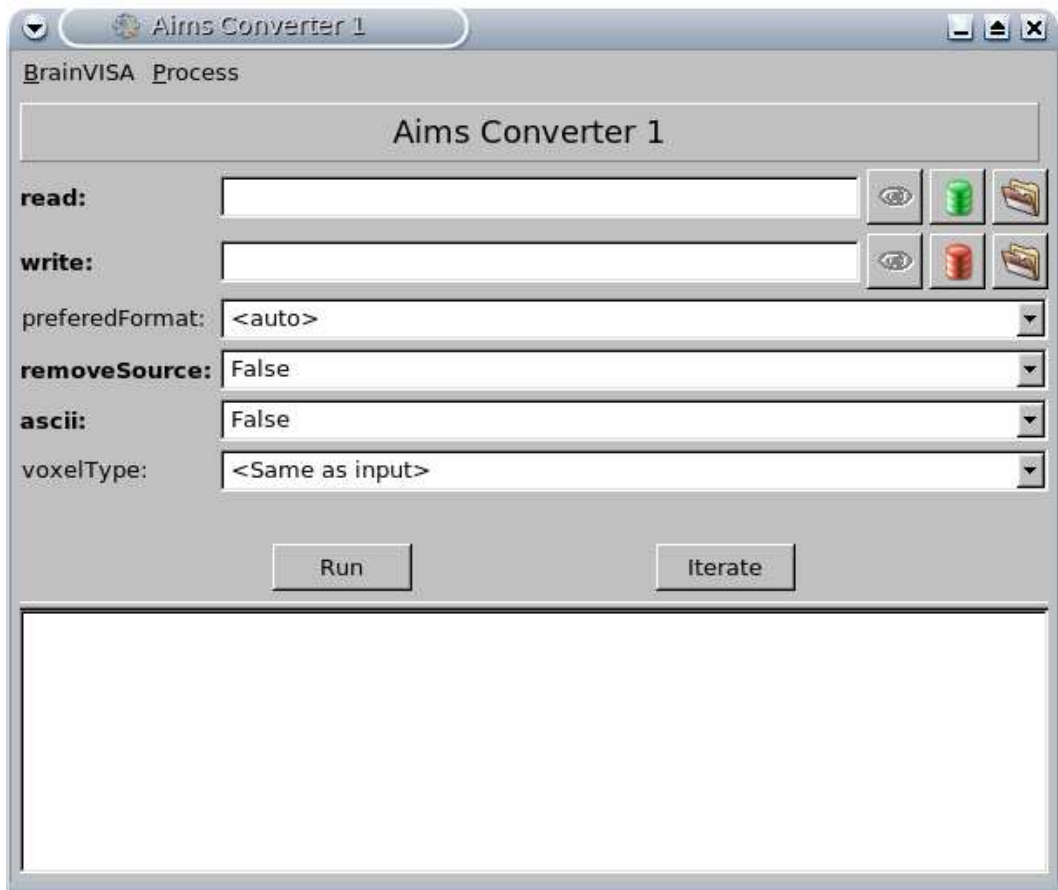


Figure 6.21: Process interface: Aims Converter.

This parameter is optional because it is only applicable to volumes or textures of scalars.

Click on RUN to launch the process.

6.4.7 Volume - flip application (Figure 6.22)

This process enables to flip a volume either in a mirror way or swapping coordinates.

Parameters of volume - flip application are:

Input_3dImage

Click on .

Select the volume to be flipped, for instance Test_volume_rat1_cresyl.ima.

Flip_Parameter_1

Choose the first flip mode among the list, for instance zz (swap the Z-axis *i.e.* reverse the Z-axis coordinates).

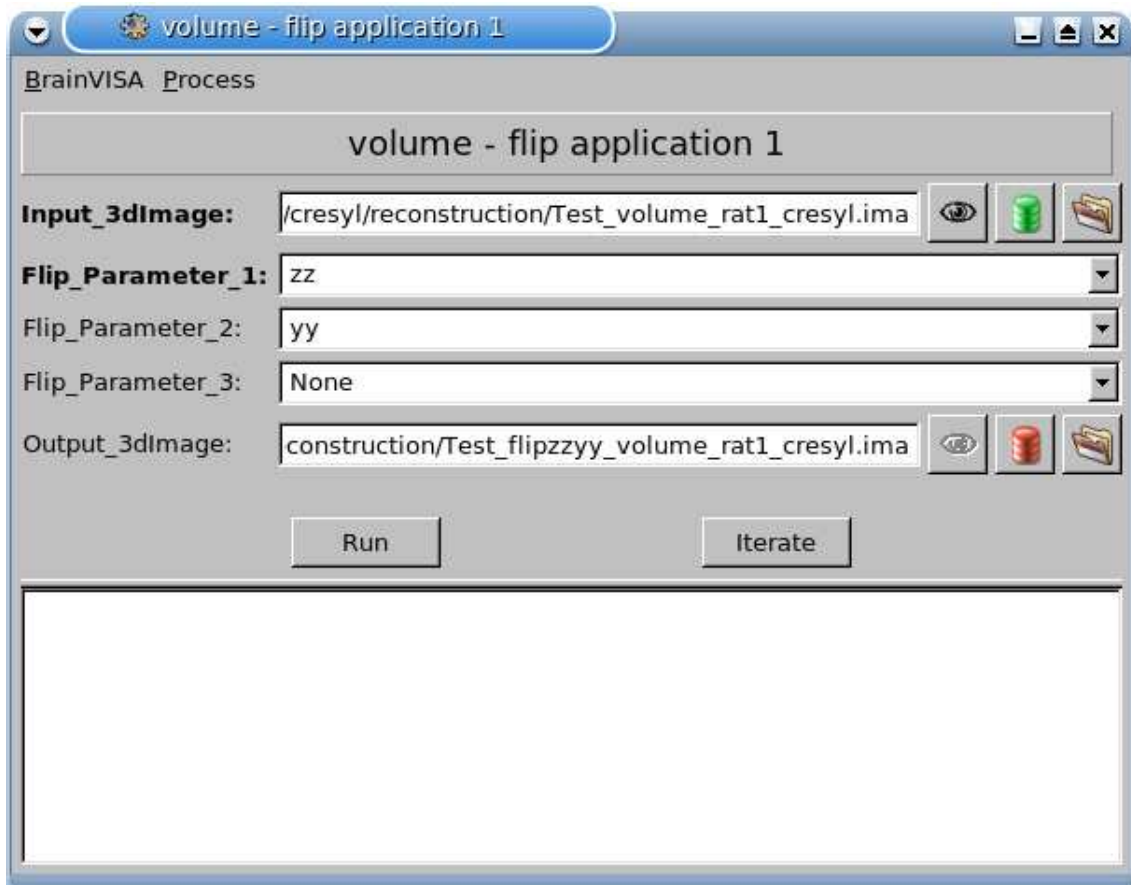


Figure 6.22: Process interface: volume - flip application.

Concerning our demonstration data, it reverses the stack order of slices (from antero-posterior to postero-anterior relationship).

Flip_Parameter_2

Choose the second flip mode among the list, for instance yy (swap the Y-axis *i.e.* reverse the Y-axis coordinates).

Concerning our demonstration data, it flips the volume in a horizontal mirror way.

This parameter is optional and by default set to None.

Flip_Parameter_3

Choose the third flip mode among the list, for instance None.

This parameter is optional and by default set to None.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_flipzzyy_volume_rat1_cresyl.ima (Figure 6.23).

This parameter is optional. If you do not complete it, you will be notified by a warning

message that the process will be going to overwrite the input volume.

Click on RUN to launch the process.

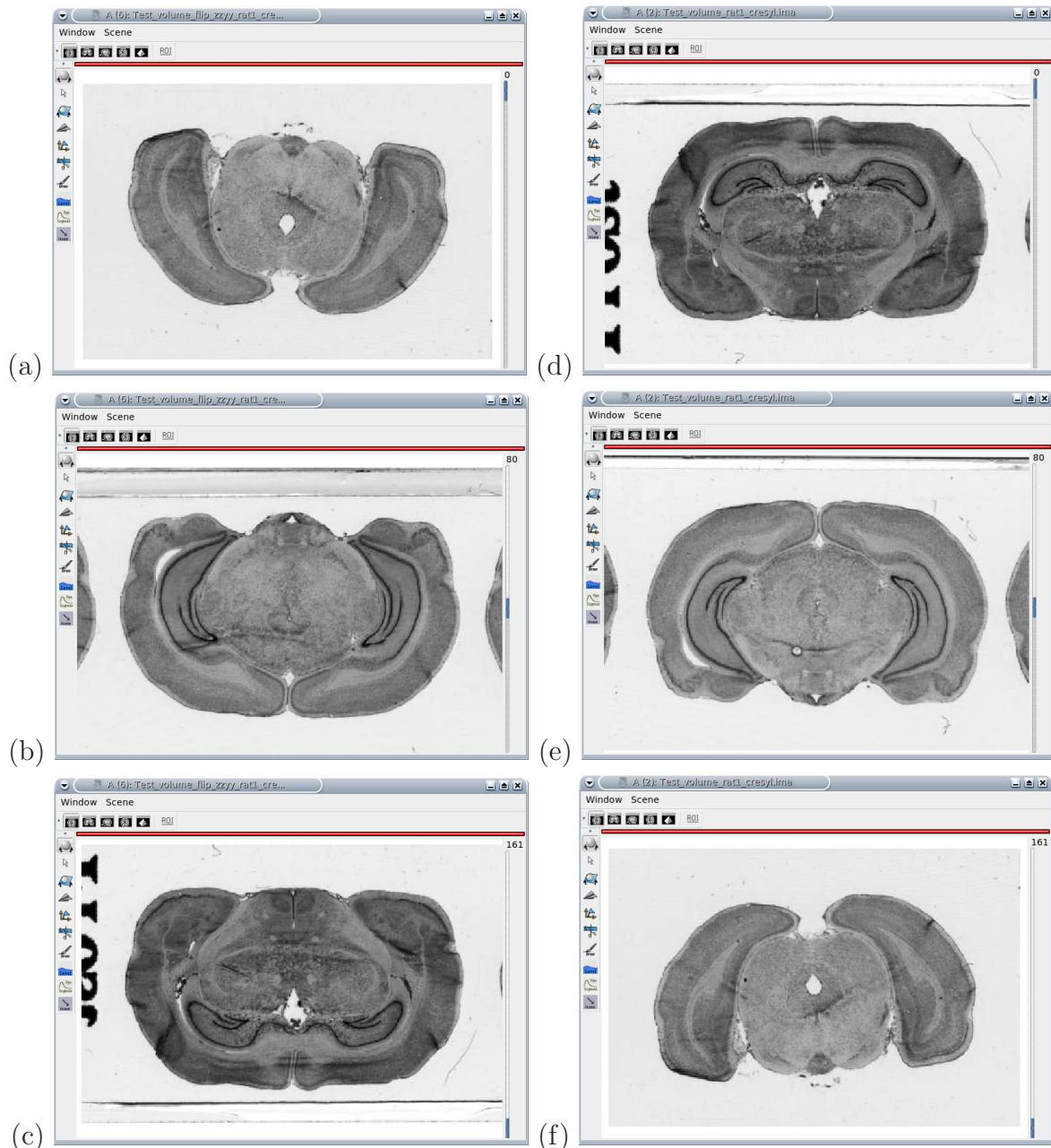


Figure 6.23: The slices numbers 0, 80 and 161 before (a, b and c) and after (d, e and f) having successively swapped the Z- and Y-axis (flips zz and yy).

6.4.8 Volume - flip application on a slice (Figure 6.24)

This process enables to flip one slice from a volume either in a mirror way or swapping coordinates.

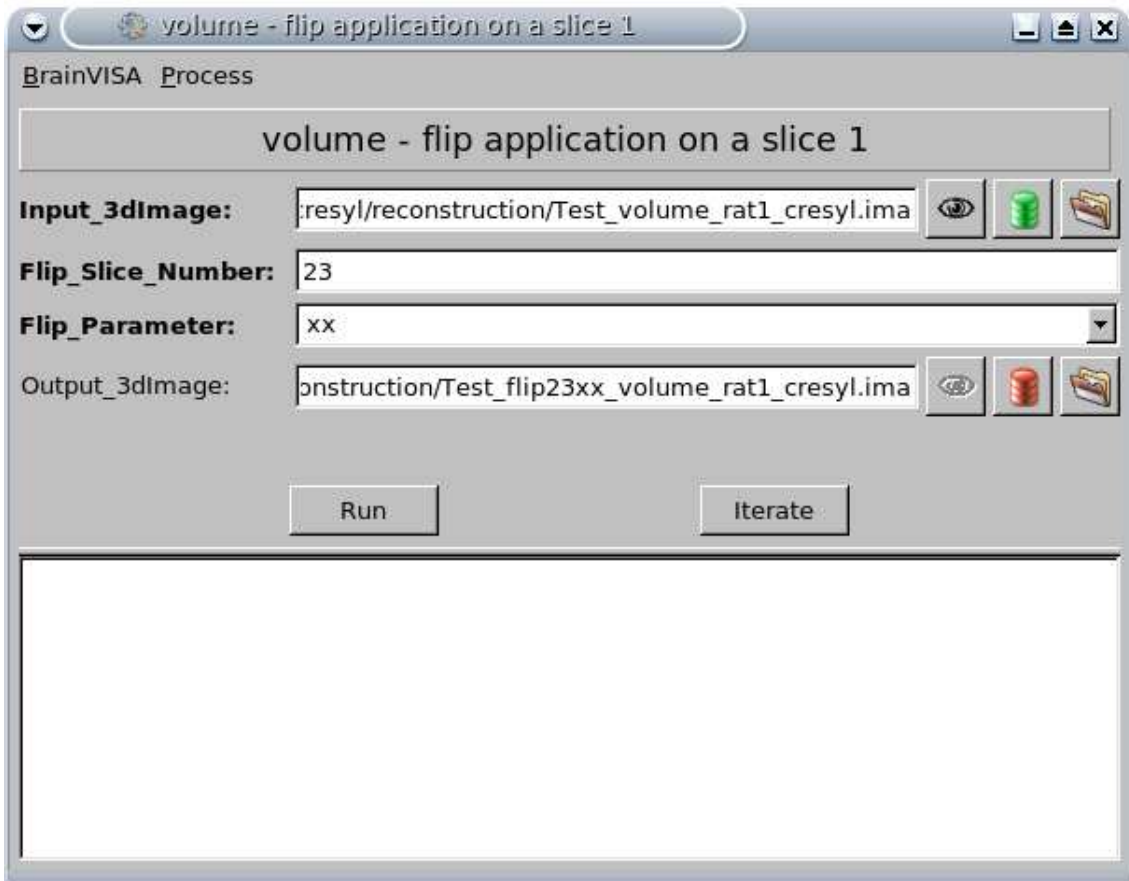


Figure 6.24: Process interface: volume - flip application on a slice.

Parameters of volume - flip application on a slice are:

Input_3dImage

Click on .

Select the volume containing the slice to be flipped, Test_volume_rat1_cresyl.ima.

Flip_Slice_Number

Enter the number of the slice to be flipped, for instance 23.


Flip_Parameter

Choose the flip mode among the list, for instance xx (swap the X-axis *i.e.* reverse the X-axis coordinates).

Concerning our demonstration data, it flips the slice number 23 in a vertical mirror way.

By default, this parameter is set to xx.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_flip23xx_volume_rat1_cresyl.ima (Figure 6.25).

This parameter is optional. If you do not complete it, you will be notified by a warning message that the process will be going to overwrite the input volume.

Click on RUN to launch the process.

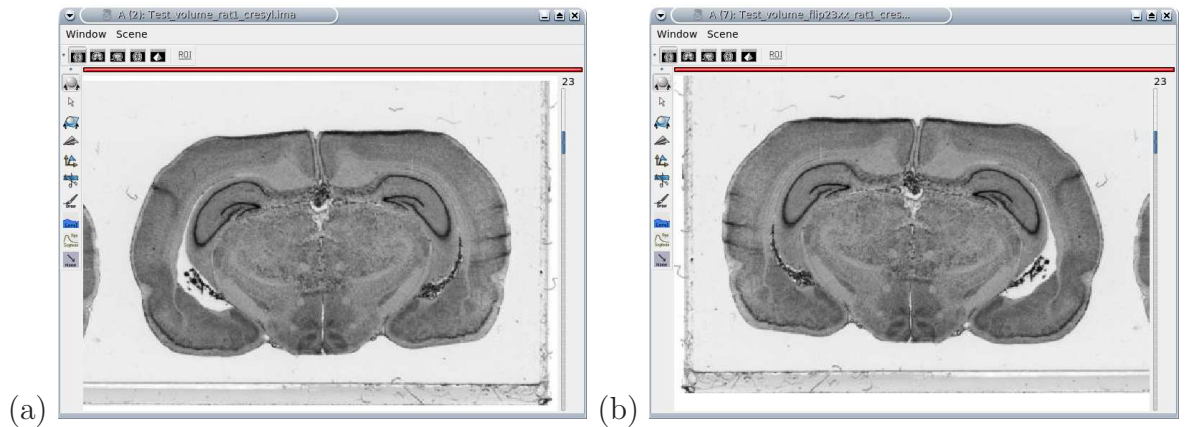


Figure 6.25: *The slice 23 before (a) and after (b) having swapped the X-axis (flip xx).*

6.4.9 Volume - intensity normalization (Figure 6.26)

This process enables to investigate regional differences in cerebral glucose uptake between groups of animals independent of global differences in the absolute amount of radiotracer delivered to the brain.

The voxel values of each 3D reconstructed autoradiographic volume are normalized to a reference value by applying a multiplicative or additive coefficient so that the mean intensity for each brain is the same.

For example, this reference value can be calculated as the mean of the mean activity values measured in each respective brain.

Parameters of volume - intensity normalization are:

Input_3dImage

Click on .

Select the 3D reconstructed autoradiographic volume to be normalized. Make sure the grey level values of this volume have been previously converted to activity or CMRglu.

Multiplicative_coefficient

Enter the multiplicative coefficient.

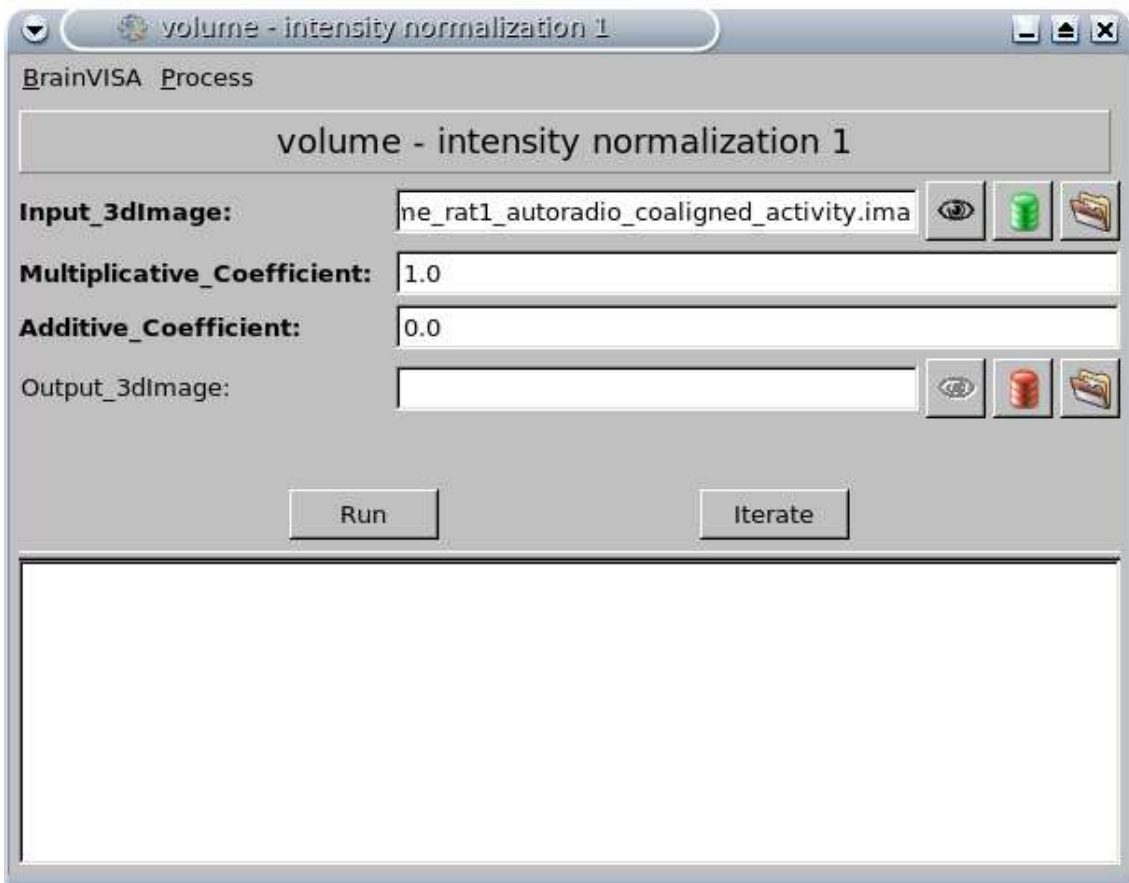


Figure 6.26: Process interface: volume - intensity normalization.


A default value of 1.0 is automatically assigned.

Additive_coefficient

Enter the additive coefficient.

A default value of 0.0 is automatically assigned.

Output_3dImage

Click on .

Specify the output directory and enter the output file name.

This parameter is optional. If you do not complete it, you will be notified by a warning message that the process will be going to overwrite the input volume.

Click on Run to launch the process.

6.4.10 Volume - interactive subvolume extraction (Figure 6.27)

This process carves a subvolume in the original volume.

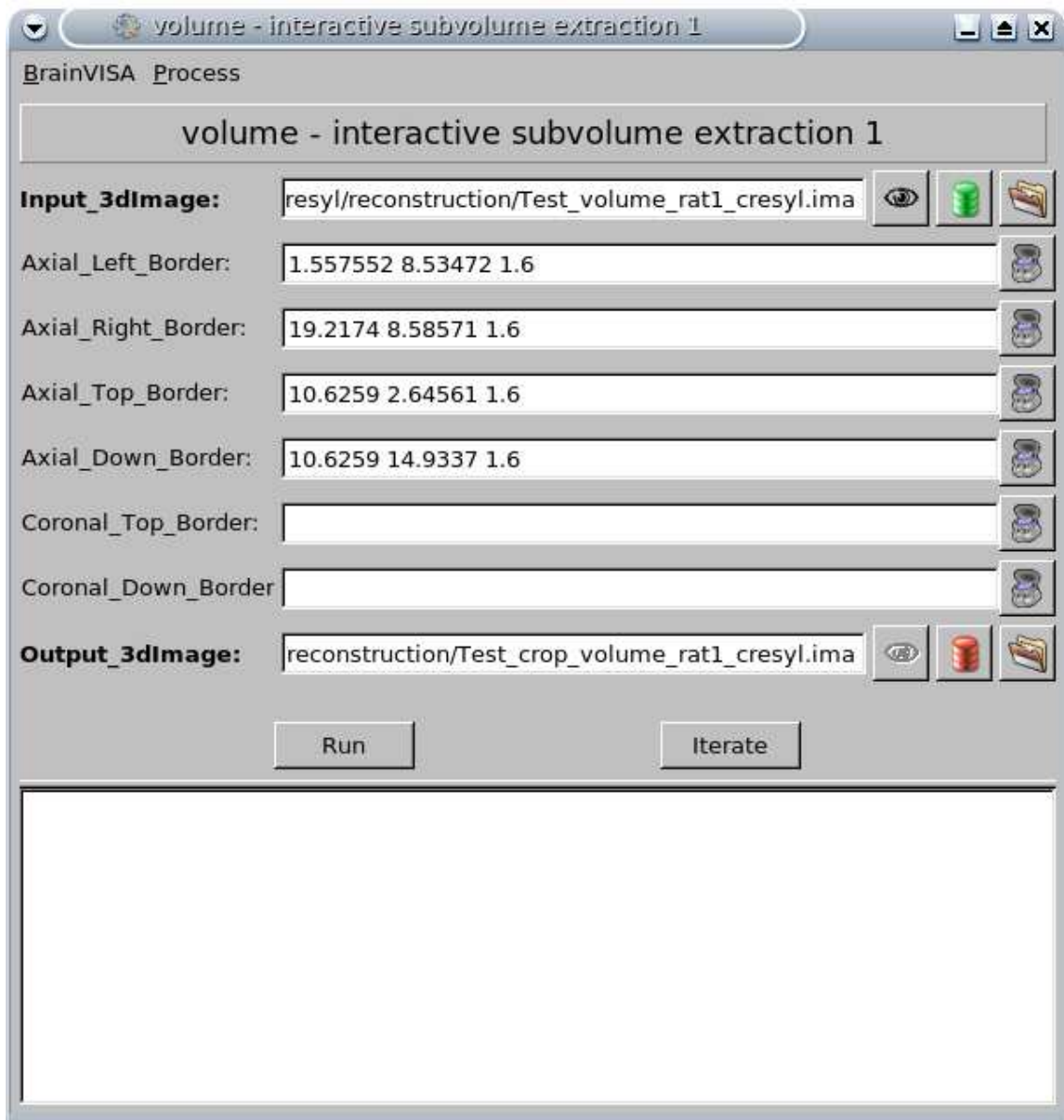


Figure 6.27: Process interface: volume - interactive subvolume extraction.


Parameters of volume - interactive subvolume extraction are:

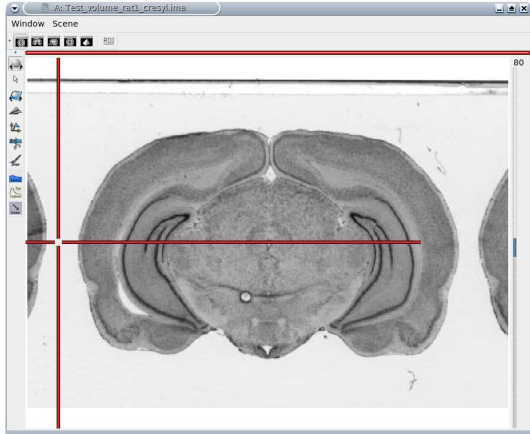
Input_3dImage

Click on .

Select the original volume to be carved, for instance Test_volume_rat1_cresyl.ima.

Axial_Left_Border

Click on  to access the volume to be carved and to select the coordinates of the axial left border. When you click the first time, a new Anatomist session is opened. Make sure your volume appears in an axial window. Then, if the cursor is correctly located on the volume, click again to display the coordinates in the field.

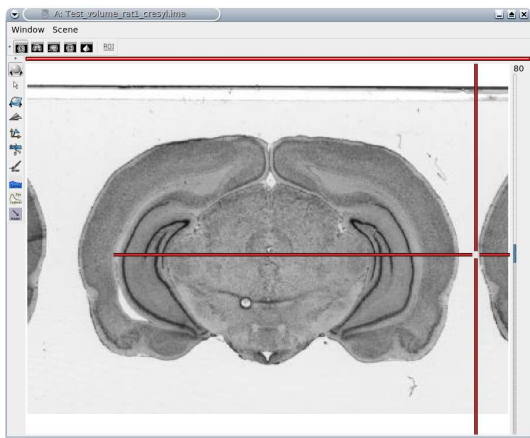


Selection of the axial left border coordinates.

This parameter is optional as well as the following ones.

Axial_Right_Border

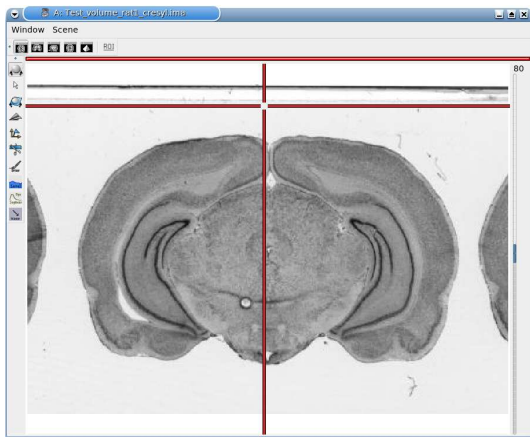
Cf. Axial_Left_Border



Selection of the axial right border coordinates.

Axial_Top_Border

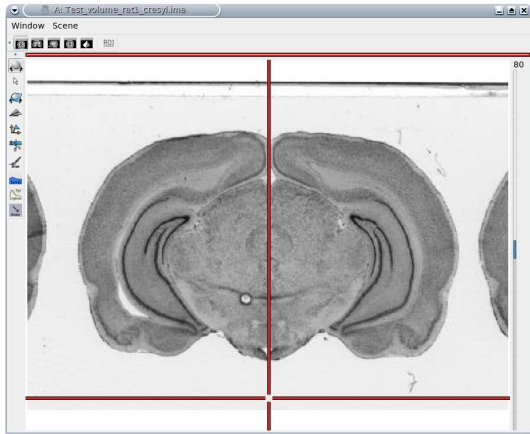
Cf. Axial_Left_Border



Selection of the axial top border coordinates.

Axial_Down_Border

Cf. Axial_Left_Border



Selection of the axial down border coordinates.

Coronal_Top_Border

Cf. Axial_Left_Border.

Make sure your volume appears in a coronal window.

Coronal_Down_Border

Cf. Axial_Left_Border.

Make sure your volume appears in a coronal window.

Output_3dImage

Click on .

Specify the output directory and enter the output file name, for instance Test_crop_volume_rat1_cresyl.ima (Figure 6.28).

Click on RUN to launch the process.

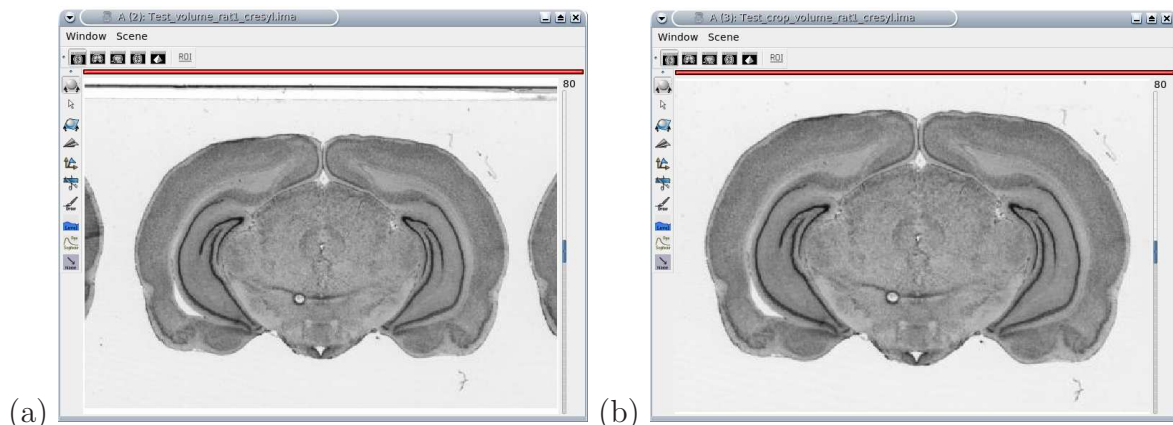


Figure 6.28: (a) Test_volume_rat1_cresyl.ima. (b) Test_crop_volume_rat1_cresyl.ima.

6.4.11 Volume - subvolume stacking (Figure 6.29)

This process enables to concatenate volumes (along Z-axis) that have been individually stacked by running the process 02. image - individualizing and stacking multiple slices.

NOTE: Make sure the volumes have all the same horizontal and vertical dimensions.

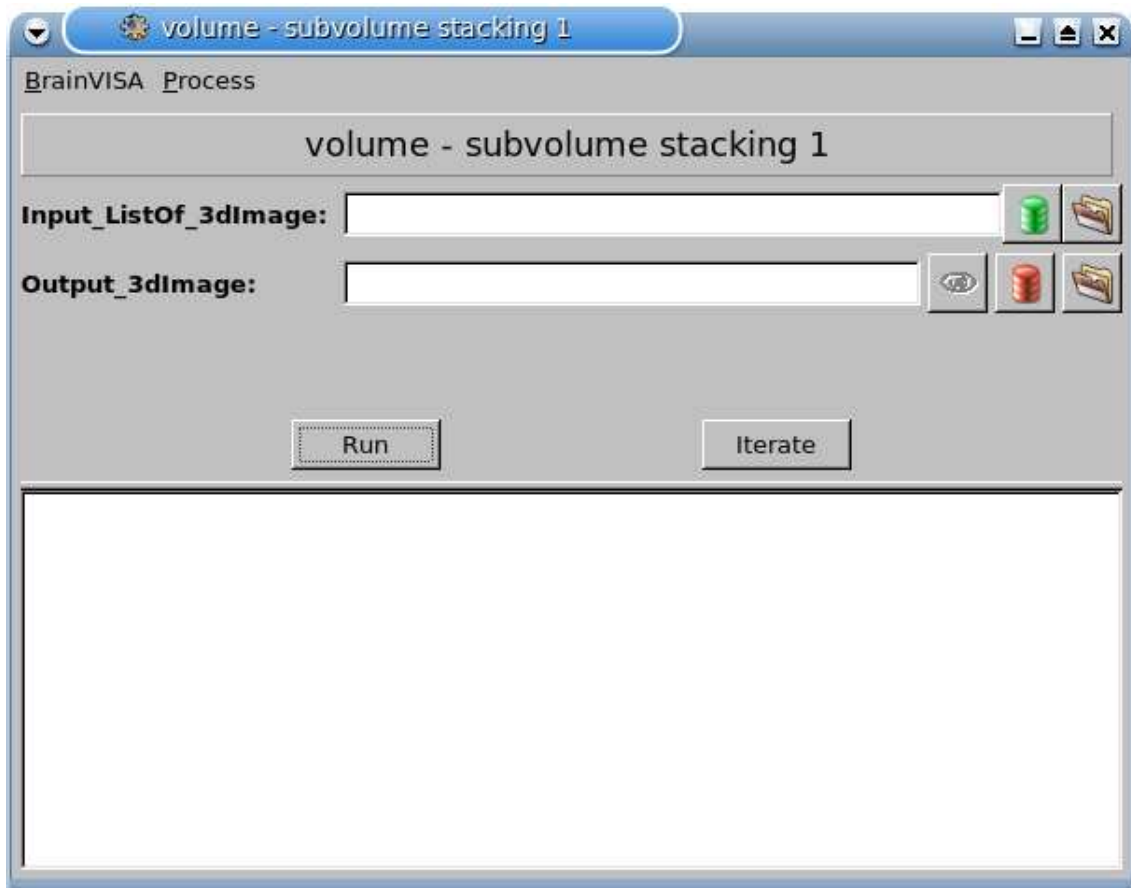


Figure 6.29: Process interface: volume - subvolume stacking.

Parameters of volume - subvolume stacking are:

Input_ListOf_3dImage

Click on .

Select the volumes to be concatenated by holding down on the control button on your keyboard (Ctrl).

Output_3dImage

Click on .

Specify the output directory and enter the output file name.

Click on RUN to launch the process.

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