

SectionToVolume Version 1.0

User Guide

SectionToVolume is used to assess the volume of different brain regions using area measurements in serial sections. It facilitates this work by arranging all image files in a tree structure, measuring some areas automatically and performing all required calculations. The final result can be pasted into a spreadsheet software like Excel or OpenOffice Calc.

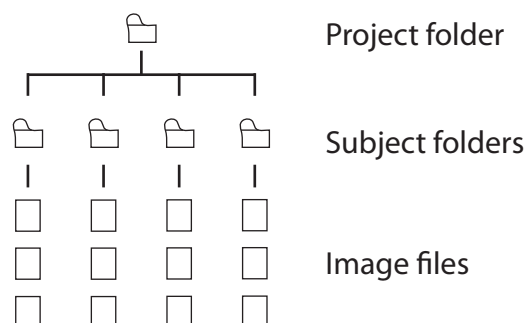
Creating a new project

Before creating a new project you have to make sure that your image files are organized in the proper way. All subjects should have their own folder where their images are stored (subject folder) and all the subject folders have to be placed in a single folder (project folder). The names of the subject folders will be used as subject names and the file names as section names in the project.

The files will be added in alphabetical order to the project. It is important that they end up in the intended order since this will affect the assigned levels and the calculated volumes. Especially note that "Section11.jpg" will be added before "Section2.jpg". By naming the file "Section02.jpg" instead this problem is avoided.

When you have organized and named your files properly you can start SectionToVolume and click the "Create new project" button. You will first be asked to locate the folder containing your source images. When you locate the project folder the program will scan for usable image files and present the number of subject and sections.

In the next dialog you will be asked to locate a folder where the project data should be saved (save folder). Three files will be created for each section, one that holds the original image, one that holds any modifications done to the image and one file which stores data about the section. The program checks that the folder is empty before proceeding to make sure that no files are overwritten and to avoid mixing up project files with other files. The save folder will require approximately twice as much storage space as the size of the source images.



After this you will have to choose the image which will be used to convert pixels to mm². This image has to be acquired using the same microscope settings as the section images and contain something of a known size, preferably a scale bar. If the microscope can't include scale bars an image of a grid or some other object of known size will work as well. The program will check to make sure that it is a readable image file.

The next step is to assign bregma levels. The program will check for the highest number of sections in a subject and you will be prompted to enter that many numbers. This number will be used as the bregma level for that section in each subject. If the sections come from different bregma levels the bregma level can be assigned individually for each section once the project is created.

The program will automatically detect tissue and ventricles but if you wish to measure any other brain region this can be done using "User defined areas". For each area you will be asked for a name and to specify whether the area should include background, tissue or both.

Open an existing project

To open an existing project simply click the "Open project button" and locate the file "ProjectData.ser".

Section Results and Subject Results

The section results panel presents the result from each section and contains all data generated in the project. The Subject results panel contains results from each subject with calculated volumes. The data in the table is rounded to 2 decimal points for clarity. Use the "Copy to clipboard" button to export the result and paste into Excel or other spreadsheet software. These data are not rounded off to preserve accuracy.

Detect tissue panel

This panel allows you to adjust the threshold value used to separate tissue from background. Use the “Apply to all” button to use the setting on all sections. The values can also be adjusted individually for each section in the adjust image panel. Each pixel in the image has one value for red, one for green and one for blue that can range from 0 to 255. The stained tissue will have higher values for red and blue but low for green. The program calculates two values, the color value and the intensity value:

$$\text{Color} = 100 * (\text{Red} + \text{Blue}) / (\text{Red} + \text{Blue} + \text{Green})$$

$$\text{Intensity} = \text{Red} + \text{Blue} + \text{Green}$$

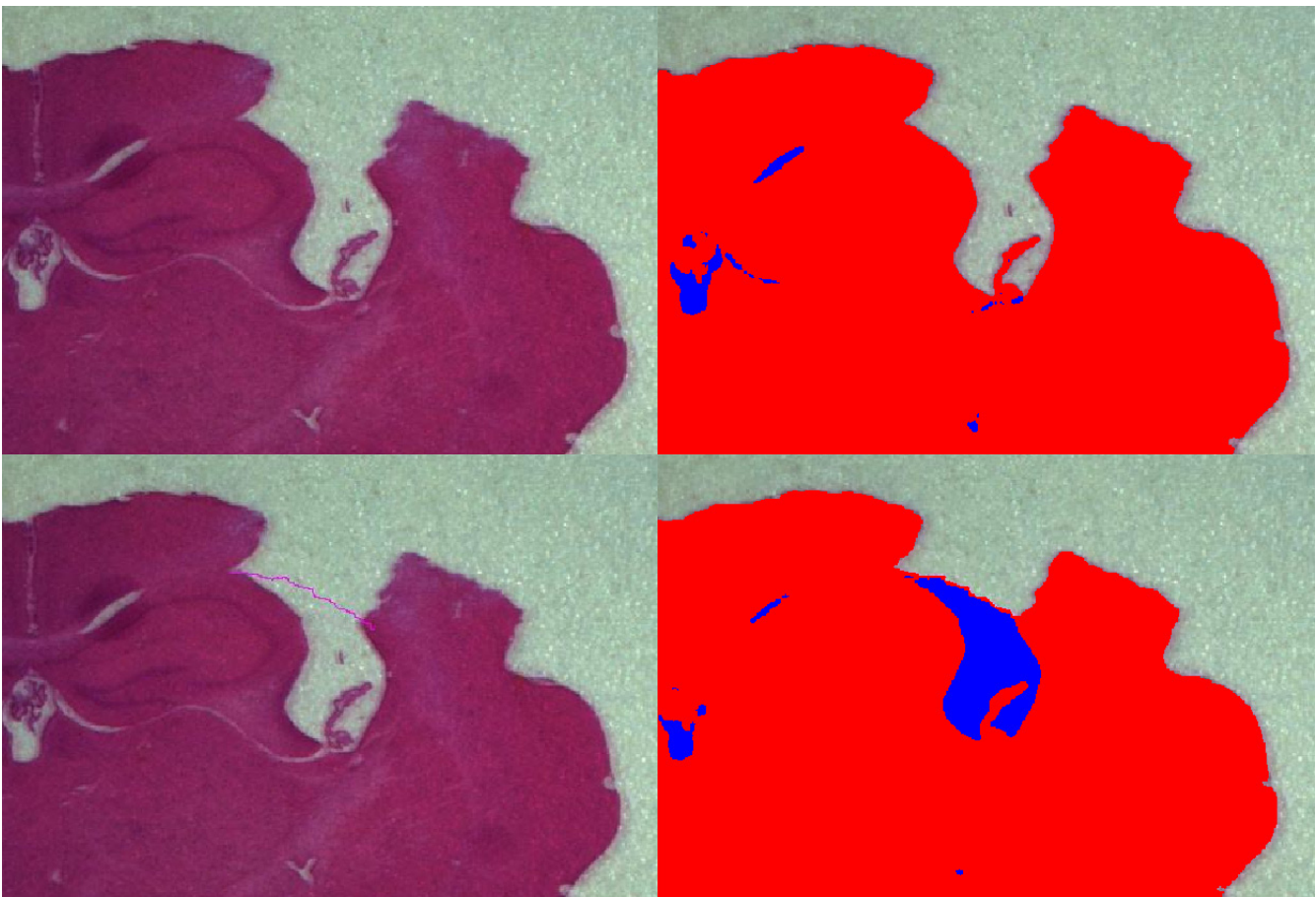
If both values are above the set thresholds the pixel will be considered as tissue. Move the mouse over the image to see values for red, green, blue, the color, the intensity and whether a pixel is considered to be tissue or background. The intensity value is used to filter out dark pixels which have high color value, for example (R=1;G=0;B=0) will appear black but have a color value of 100.

Conversion panel

When the images are analyzed the number of pixels in each region is counted but has to be converted to mm² and mm³ before presenting the result. To do this a distance in the conversion image has to be measured in both pixels and mm. The number of pixels is measured by drawing a line in the image, left click for the start point and right click for the end point. The program will calculate the distance between the points in pixels. Enter this distance in millimeters in the text box on the right side panel. Press the update conversion button when the distance is both pixels and millimeters has been entered. The program will now recalculate all areas and volumes using these settings and the number of pixels per square millimeter is reported.

Adjust image panel

Normally at least some sections in a project will contain folds or tears or other errors from the sectioning and staining. This can be corrected for by adding or removing tissue. It is also possible to change the setting for tissue detection. If your image contains a large cortical cavity which has fused with the lateral ventricle the program will not be able to distinguish the ventricle from the background. Draw a thin line of tissue to that separate the ventricle from the cavity to solve this problem. The extra area of tissue will be small a likely negligible for most applications.

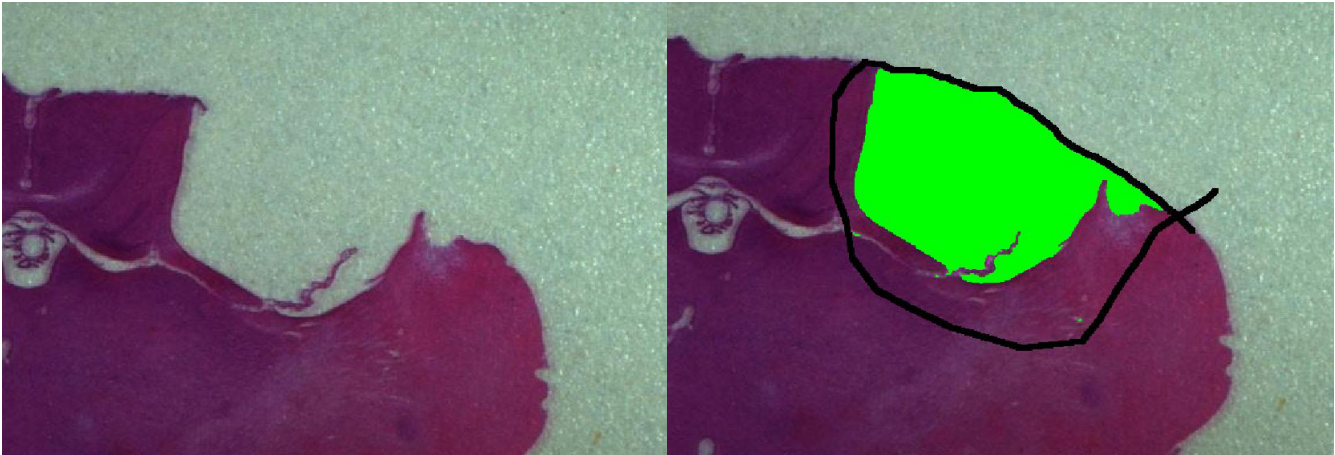


Left-Right panel

To obtain separate values for the two hemispheres the midline has to be manually drawn. Left click the image to add points and click the “Update image” button to see the result. It is also possible to hold down the left button and move the mouse over the image to draw the line. Right clicking the image has the same effect as the “Update image” button. In case you make a mistake use the “Clear lines” button and start over.

User defined areas

Every section will have a separate panel for each User defined area. It is named after the name provided when creating the project. Left click or drag to add points to draw the outline of the area. Right click in the area when the outline is complete. If the entire image is filled after the right click there might have been an opening in the outline. Use the “Clear lines button” and try again if this happens. The user defined area displayed below is set to measure only background which removes the need to trace the border between tissue and background.



Starting the program

Double click the file SectionToVolume.jar to start the program. If it doesn't work make sure that Java is installed on the computer. Java is used in web browsers and is already installed on most computers. If not it can be downloaded from:

<http://www.java.com>

Supported image formats

Images has to be in the formats jpg or png.

Navigating in the program

Use the tree panel on the left side of the window to bring up new panels. The keyboard letters A and D can also be used to move around in the program. A will take you to the previous panel and D to the next.

Zooming in and out

Use the buttons in the upper panel to zoom in and out. You can also use the keys S and W to do this.

Line width

The lines drawn by the user can be difficult to see if the image is zoomed out to much. Increase the line width to avoid this. The setting for the line width doesn't change the measured areas, it only alter the displayed image.

How the calculations are made

The measurement of areas start by counting the number of pixels. All measured areas are colored to give a visual hint of what is being measured. To convert the number of pixels to mm the number of pixels per mm² has to be determined. This is done by measuring a distance in the Conversion panel. The distance in pixels is calculated as the distance between two points, (x₁;y₁) and (x₂;y₂).

$$\text{Distance} = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

The same distance in mm is entered in a text box. The number of pixels/mm² is then calculated as:

$$\text{Pixels/mm}^2 = (\text{Distance}_{\text{pixels}})^2 / (\text{Distance}_{\text{mm}})^2$$

The area in mm² is then calculated as:

$$\text{Area}_{\text{mm}} = \text{Number of pixels in area} / \text{Number of pixels per mm}^2$$

The volume between two sections with the measured areas (A₁) and (A₂) separated by a distance d measured in mm is calculated as:

$$\text{Volume} = d * (A_1 + A_2) / 2$$

These volumes are then added to get the total volume.

A note on number format

When exporting the result using the “Copy to clipboard” button all numbers use “.” as the decimal separator rather than “,” used in some countries. For example will SectionToVolume write “3.14” and not “3,14”. If your spreadsheet software is using “,” as the decimal separator the numbers will be interpreted as text. To solve this use the search-and-replace function to switch all “.” to “,”.

Running the example project

Follow these steps to measure volumes in the example project:

- Start SectionToVolume and press the button “New Project” in the top panel.
- Locate the folder “Example Project”. The program should detect 2 subjects and 12 sections. Then press the “Next” button to move to the next screen.
- Locate a folder where your project should be saved. The folder has to be empty and space available for approximately 60 Mb.
- To select a conversion image locate the file “ConversionImage.jpg” included in the example project.
- The bregma levels in the example project range from -0.5 to -3.0. The easiest way to enter these numbers is to enter “-0.5” in the box First level and “-0.5” in the box distance and then pressing the button “Calculate levels”. The values can also be entered manually.
- To measure the cavity a User Defined Area has to be specified. Click the “Decrease” button until the number of User Defined Areas is one. Replace the text “Area1” with “Cavity”. Click the radio-button “Include background”.
- Click the button “Create Project”. The program should start analyzing sections and when finished you should see an image of a ruler.
- The image of the ruler is used to determine the number of pixels/mm². To do this left click at the 3 cm mark and then right click at the 4 cm mark. If you can’t see a line try clicking the “White lines” button and the “+ Line Width” button. The distance should be approximately 900 pixels. Enter “10” in the “Distance (mm)” box and click the “Update Conversion” button. The program should now recalculate the volumes according to this setting and report the number of pixels/mm² as approximately 8000.
- The values for total tissue volume and total ventricle volume should now be ready. Click on “Subjects Results” in the tree structure in the left side panel to view them.

- To determine volumes in the left and right hemisphere and the volume of the cavity go through each section and specify the midline and the cavity.
- When finished go to the “Subjects results” and click the button “Copy to clipboard”. The results should now be possible to paste into Excel or similar programs.