QuickNII Workflow/User guide

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1. Introduction

1.1 Background

For example of use, tutorials and demo video, Please visit:

https://ebrains.eu/service/quicknii-and-visualign

and related pages.

QuickNII is one of several tools developed by the Human Brain Project (HBP) with the aim of facilitating brain atlas based analysis and integration of experimental data and knowledge about the human and rodent brain. QuickNII is a stand-alone tool for user guided affine spatial registration (anchoring) of sectional image data, typically high resolution histological images, to a 3D reference atlas space. A key feature of the tool is its ability to generate user defined cut planes through the atlas templates that match the orientation of the cutting plane of the 2D experimental images (atlas maps). The reference atlas is transformed to match anatomical landmarks in the corresponding experimental images. In this way, the spatial relationship between the experimental image and the atlas is defined, without introducing transformations in the original experimental images. Following anchoring of a limited number of sections containing key landmarks, transformations are propagated across the entire series of images. These propagations must be validated and saved by the user for each section, with application of fine positional adjustments as required. We recommend the use of VisuAlign to perform nonlinear adjustments after the QuickNII registration for an optimal fit (https://ebrains.eu/service/quicknii-and-visualign).

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1.2 Condition of use

QuickNII v1.0 is developed by the Neural Systems Laboratory, Institute of Basic Medical Sciences, University of Oslo, Norway, with support from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 604102 (HumanBrain Project).

QuickNII v2.0 is developed by the Neural Systems Laboratory, Institute of Basic Medical Sciences, University of Oslo, Norway, with support from the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Framework Partnership Agreement No. 650003 (HBP FPA).

Citations:

-RRID on SciCrunch: (QuickNII, RRID:SCR_016854)

-Puchades MA, Csucs G, Ledergerber D, Leergaard TB, Bjaalie JG (2019) Spatial registration of serial microscopic brain images to three-dimensional reference atlases with the QuickNII tool. PLOS ONE 14(5): e0216796. https://doi.org/10.1371/journal.pone.0216796

Download: https://www.nitrc.org/projects/quicknii

1.3 Contact

For technical inquiries and user support: https://ebrains.eu/support

2. Image requirements and pre-processing steps

QuickNII v1.0 and v2.0 supports standard web-compatible image formats, 24-bit PNG and JPEG. Images can be loaded up to the resolution of 16 megapixels (e.g. 4000x4000 or 5000x3000 pixels); however, QuickNII does not benefit from image resolutions exceeding the resolution of the monitor in use. For a standard FullHD or WUXGA display (1920x1080 or 1920x1200 pixels) the useful image area is approximately 1500x1000 pixels: using a similar resolution ensures optimal image-loading performance and eliminates excess storage size.

2.1 File naming requirements

Serial section images should be assigned consecutive serial numbers, preferably indicated by three-digit numbers at the end of the file name, e.g. Sample_ID_s001.tif.

The section number should reflect the serial order and spacing of the sections (e.g. s002, s006, s010 for every 4th section starting with section 2).

As fulfilling the size and naming requirements typically requires preprocessing of the images (converting to PNG or JPEG and downscaling to screen-like size), QuickNII keeps track of original image dimensions as part of its series descriptor. Preprocessing of images (downsampling, rotation, renaming) can be achieved with open access software tools (e.g. Nutil Transform, ImageMagick, Matlab scripts) or python scripts found in many open source libraries (e.g. PIL). Generate your images descriptor file with FileBuider found in the QuickNII folder.

NOTE: if you plan to analyse your images with the QUINT workflow, both the segmentation file and the atlas map that correspond to a particular section must contain a unique ID that meets the file naming requirement described above. These unique IDs must also be present in the XML/JSON file containing the anchoring information: this happens automatically as long as the images that are anchored with QuickNII contain the unique IDs.

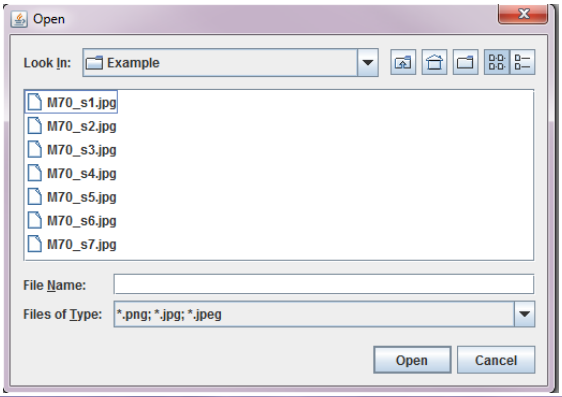
Nutil Quantifier supports IDs in the format: sXXX.., with XXX.. representing the section number, as well as formats defined by regular expressions.

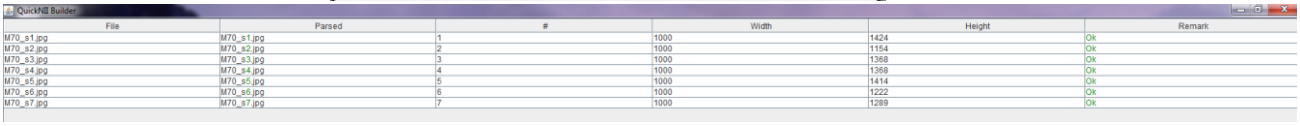
Example: tg2345_MMSH_s001_segmentation.png (It is fine to include a string of letters and numbers followed by the unique ID).

As Nutil Quantifier scans and detects the _s part of the name, the file name should not contain additional _s. Example that would not work: tg2345_MMSH_ss_s001.png

3. Generate XML descriptor file

- Assign serial section images consecutive serial numbers, preferably indicated by three-digit numbers at the end of the file name, e.g. Sample_ID_s001.tif. The section sampling is defined by the serial numbers.
- Collect the section images in a folder.
- Use the small program “FileBuilder.bat” provided with QuickNII to generate the XML descriptor file. A new window will open, and ask for the folder where your images are located. Point to the correct folder, mark all image files, and click ok.





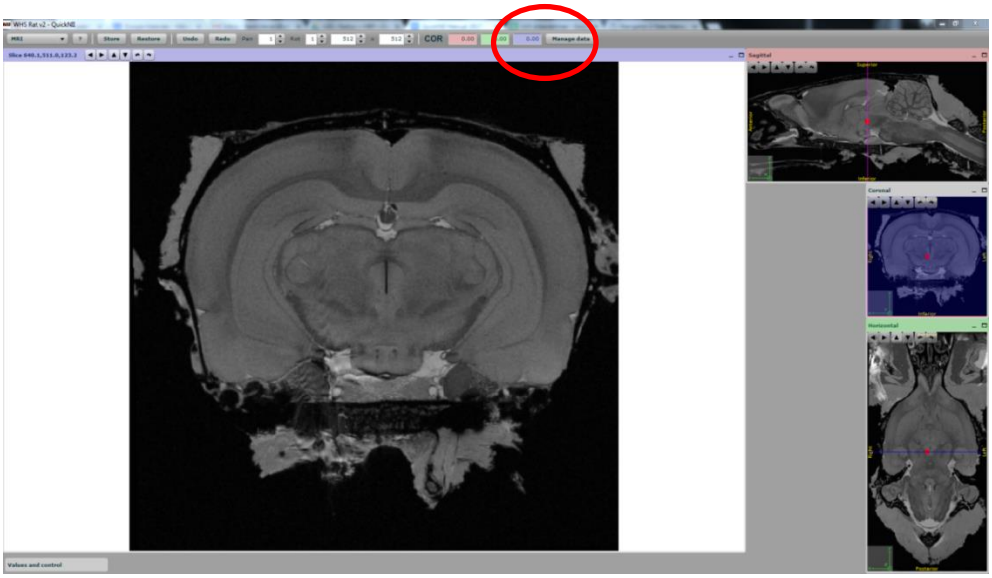
- Files will be reviewed, and an xml file will be generated. Click “Save xml”. You can now open the xml in QuickNII. If the section number is not recognized, you have the option to number the images in file builder.

4. Open QuickNII and load the data

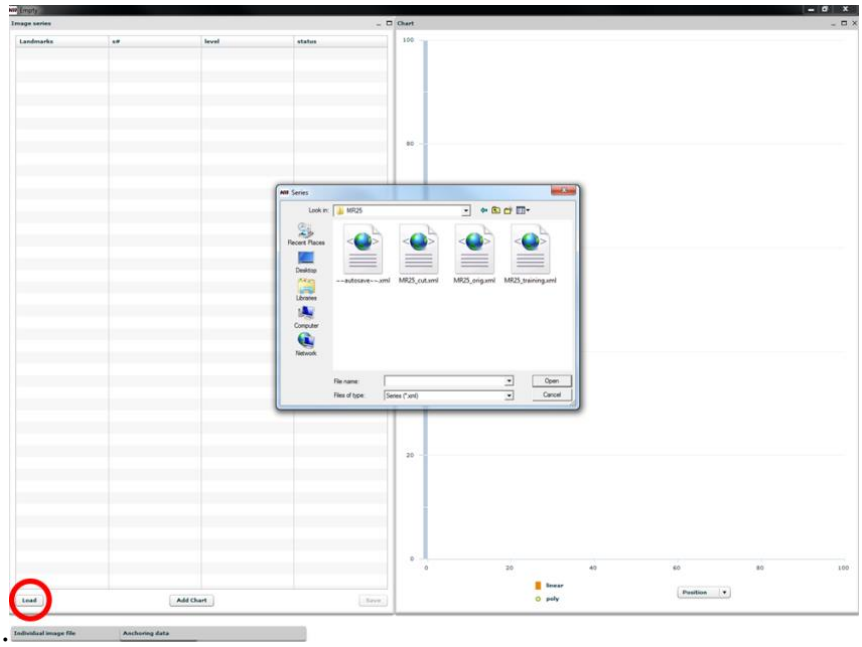
On https://www.nitrc.org/projects/quicknii , you can find Mac and Windows versions of QuickNII for mouse and rat brains.

- For the mouse brain, two versions: "QuickNII-ABAv3-2015" and "QuickNII-ABAv3-2017" contain the Mouse reference atlas from the Allen Institute version 3[1,2,].
- For the rat brain, two versions "QuickNII-WHS-v2" and "QuickNII-WHS-v3" contains the Waxholm rat reference atlas version 2[3,4].

Download the version of QuickNII containing the required reference atlas, and open the QuickNII program by clicking on the .exe file. Once the program opens, click the **Manage data button**.



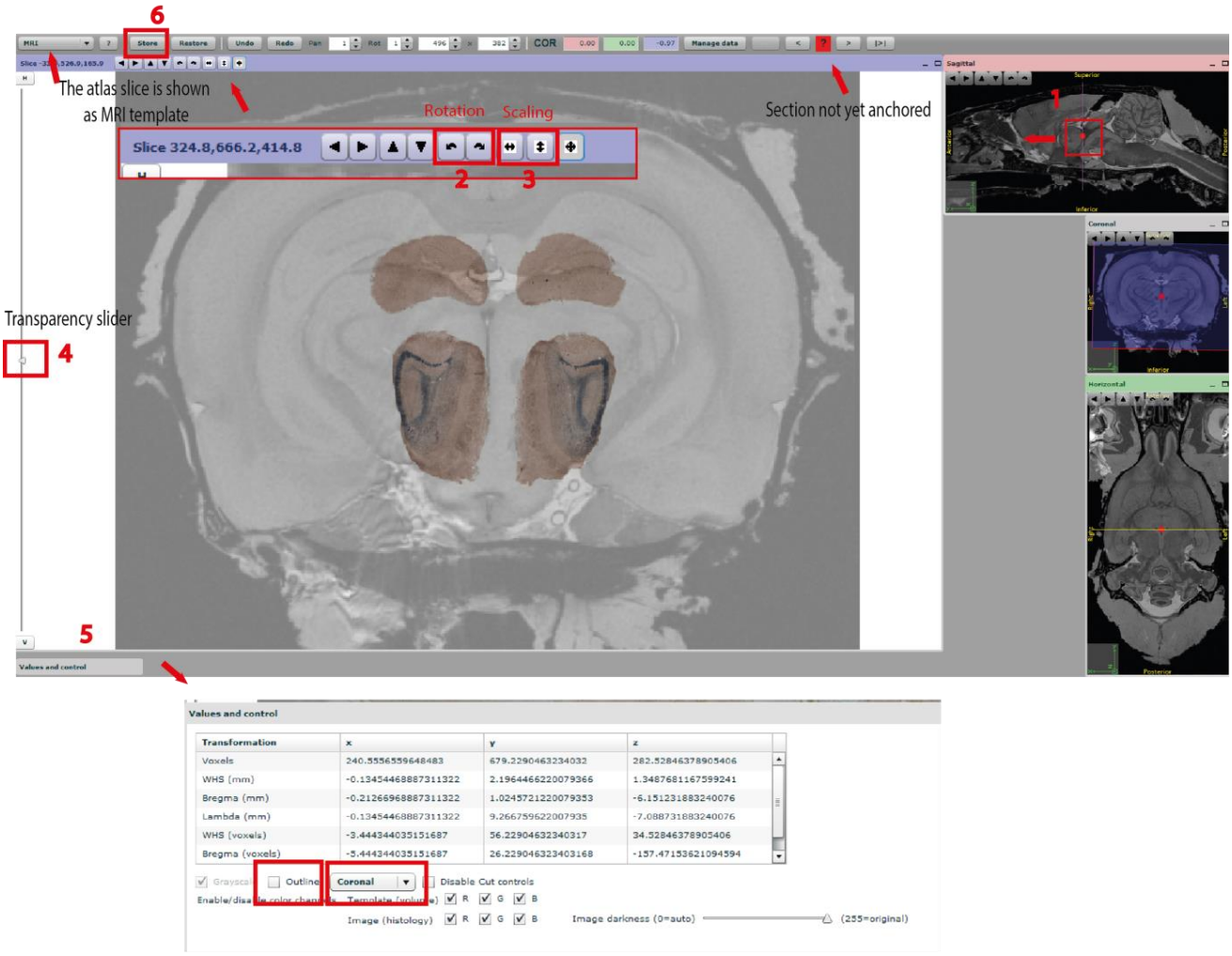
A second window, the data management window, will open. Here you can load your data by clicking the Load button and choosing the .xml (or .JSON) file related to your images, choose the "orig file" the first time.



NB! Low resolution .png images must be in the same folder as the .xml files for QuickNII to be able to open the data set. Navigate between the two interfaces by clicking the **Manage data button**. Select sections to work on by using the arrows in the upper right panel or by double-clicking the section number in the data management window.

5. Use landmarks in the images to find their approximate anteroposterior position

The first step in a successful anchoring is to find the approximate anteroposterior position of the slices (Y position for coronal sections). Do this first for the first and last section of the series (or first and last sections with clear landmarks). Select sections to work on by using the arrows in the upper right panel or by double-clicking the section number in the data management window.



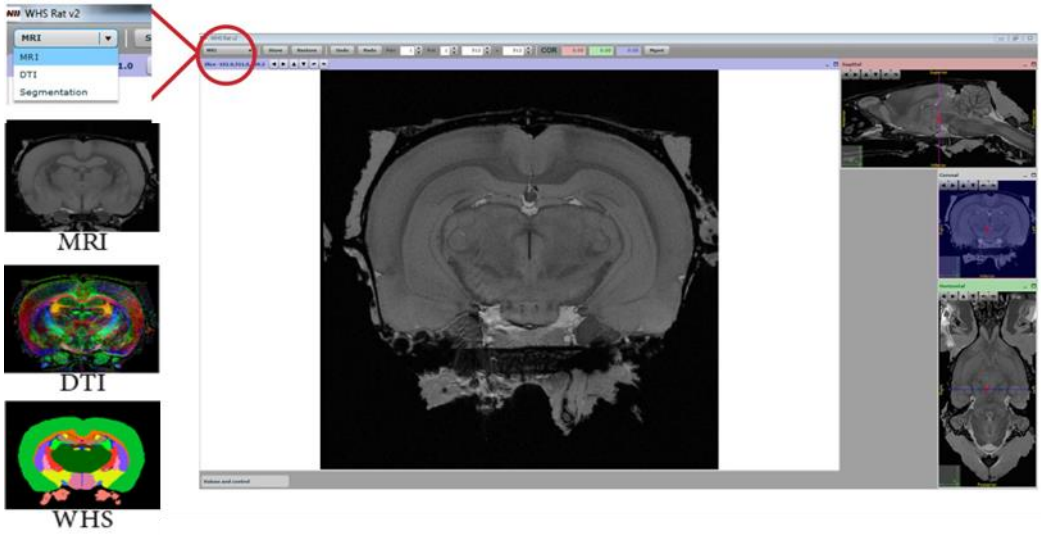
The anteroposterior position is adjusted by clicking and sliding the red circle in the sagittal navigation window (1). After finding the approximate position of your section, determine whether

the midline of the section is completely vertical. If not, the rotation of the template can be adjusted using the rotate left/right-buttons (2). The atlas proportions might need adjustment to fit the section. This is done separately for the horizontal and vertical direction by using the scaling buttons (3). **In order to scale** your atlas, press the **space bar** while holding the mouse pointer over the place you want the reference point for scaling. A small cross will appear. Usually it is easier to choose a side and not place the cross in the middle of the section.

Then, click on the **scaling button**: a double arrow will appear. Place your **mouse pointer** at the opposite side of the double cross, and press the left button of your mouse. While keeping the left button of the mouse pressed you can now gently drag the atlas in the direction indicated by the double arrow.

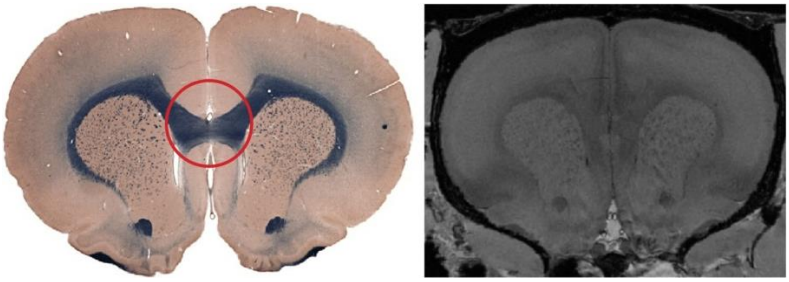
To drag in the other direction, choose the other arrow. **The transparency slider** (4) can be used continuously to determine how well the atlas fits the section. By clicking on the **"Values and control"** (5) button in the bottom left corner, choose the section orientation (coronal, sagittal or horizontal). The "outline" button allows you to shift between an outline view and a color view of the atlas segmentations. Save the anchoring by clicking the **Store button** (6) in the upper left panel: a green exclamation mark appears in the upper right panel.

By clicking the roll-down bar in the top left corner, MRI, DTI (for WHS atlas only) or atlas templates can be chosen. It is useful to use these different atlases actively as they give different information that can be used when anchoring. Detailed description of the UI can be found at https://www.nitrc.org/projects/quicknii.

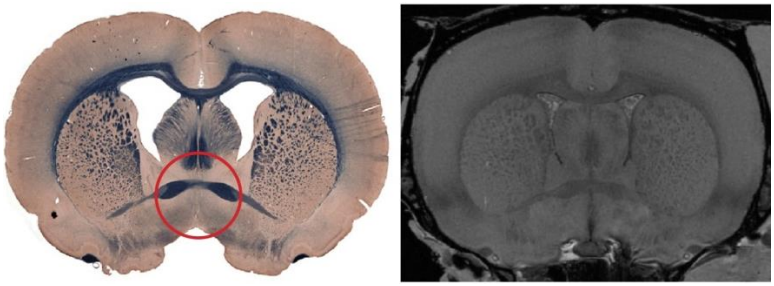


It is easiest to establish the anteroposterior position of sections that containing key anatomical landmarks. Some examples are shown below:

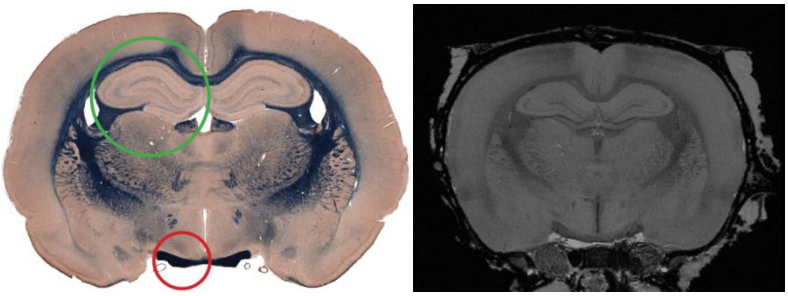
❖ ***Genu of the corpus callosum***



❖ ***Decussation of the anterior commissure***



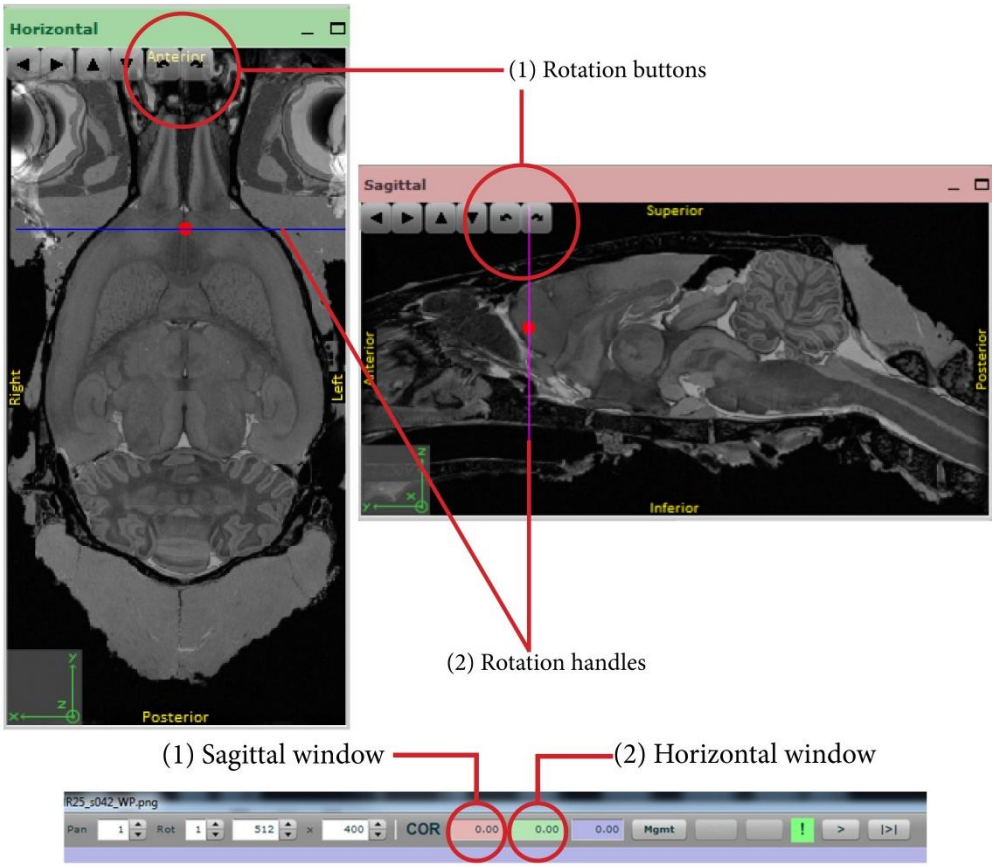
❖ ***Optic tract in red, mid-level of the anterior hippocampus in green***



6. Determine the sectioning angles

Next, adjust the angles of the atlas slice to match the angles of sectioning. Even the best sectioning routines can induce small deviations from the vertical and horizontal planes. Furthermore, those

angles can vary in a whole series, especially if the tissue was cut into two separate blocks. The cutting angles of the atlas should be adjusted to match the mediolateral and dorsoventral angles of the sections. This is done in the horizontal and sagittal navigation windows, respectively. Use either the rotation buttons (1) or rotation handles (2) to tilt the MRI template in the direction needed. Adjust the anteroposterior position to compensate for the rotation.



The angles of the current atlas slice relative to the default atlas plane can be read out in the boxes shown above, corresponding to the sagittal (1) and horizontal (2) navigation windows.

In coronal sections, the dorsoventral angle can be determined by examining the relationship between landmarks in dorsal and ventral parts of a section, e.g. between the corpus callosum and anterior commissure, between the dorsal and ventral hippocampus, or between the pons and inferior colliculus. The mediolateral angle can be determined by comparing landmark structures across hemispheres. It is most easily found by examining the development of the corpus callosum, anterior striatum, anterior commissure, anterior hippocampus, or size differences of the cortex in the posterior part of the brain.

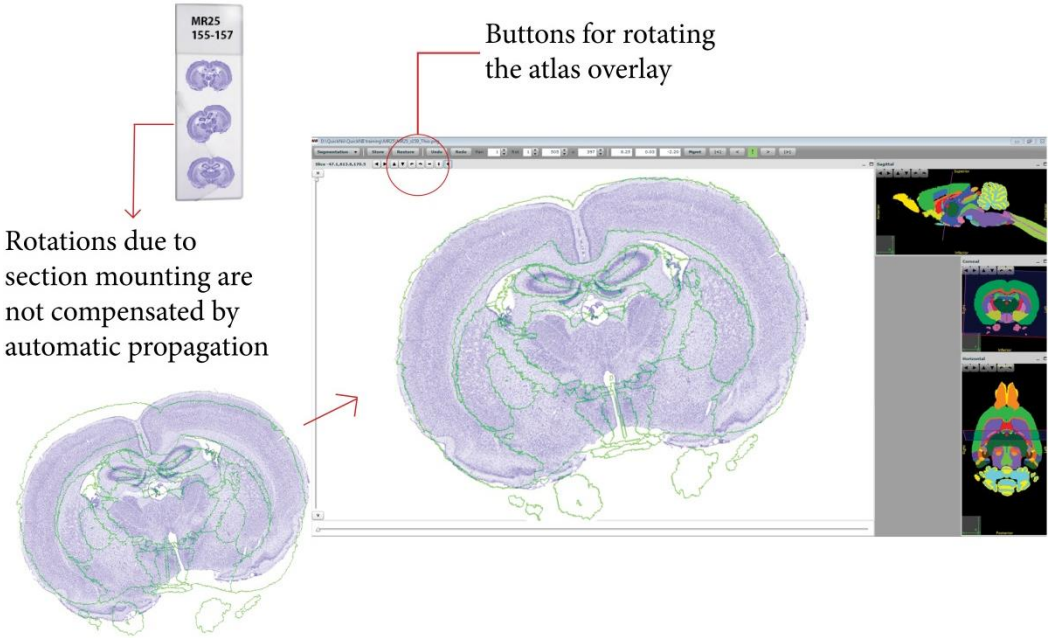
Note: the results might look similar with angles that deviate 180 degrees (corresponding to looking at the animal from the back or from the front). To ease the curation process, however, we recommend using the smallest angle possible.

7. Final adjustments of in plane positions

Go to another image located at the end of the series and repeat the anchoring procedure. Once the second image is stored, QuickNII will automatically calculate the anteroposterior positions of the images, as well as propagating the registered angles and scaling to the sections between the anchored sections. This accelerates the anchoring procedure, and ensures the section spacing and serial order are respected. However, there might be cases where the automatically propagated parameters do not fit the section, for example if a section has been tilted during the mounting procedure. It is therefore essential to validate the positions by visual inspection, and to correct any mismatch by fine adjustment of the anteroposterior position, and scaling and rotation of the atlas maps to match the position of the sections.

Once defined, apply the same angles to all the sections in the series and review each section!

The **"export propagation"** button allows you to validate all the sections at once. However, caution is recommended in the use of this feature, as some sections might not have the correct position. We strongly recommend reviewing all the sections in order to validate the anchoring. Perform in plane rotations using buttons in the main window. Rotations in the small coronal window will result in a rotation around the anteroposterior axis.

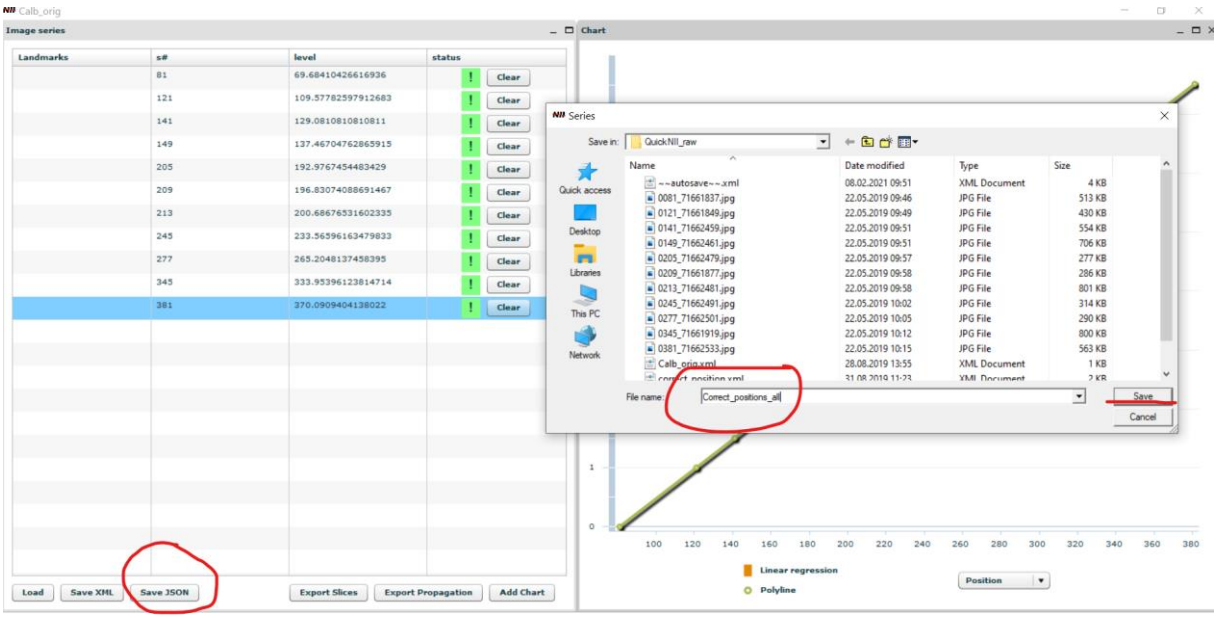


Note: Adjustments made with QuickNII are linear. If considerable mismatch remains between the atlas maps and the sections, despite fine linear adjustments, further nonlinear adjustments can be applied using VisuAlign (https://ebrains.eu/service/quicknii-and-visualign).

8. Saving results and validation

Remember to save the anchoring result by clicking **"store"**. Export the anchoring vector data by clicking **"Save XML"** or **"save JSON"**. The two are identical; however, the JSON format is required to proceed to nonlinear registration in VisuAlign.

A new window will open and you will be able to export results into a new file. Type a new name, e.g. initials and date.

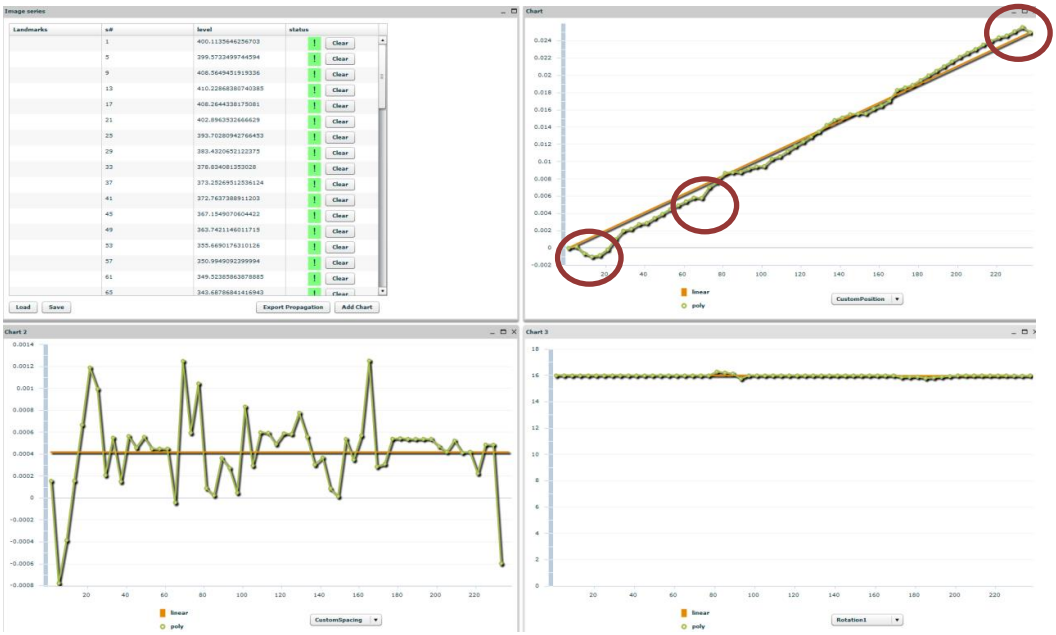


You will also be able to export custom atlas slices corresponding to your series. Press the **"export Slices"** button and select the destination folder.

Note: The .flat files are used for the QUINT workflow.

0081_71661837-Nissl_2015.png	01.09.2019 19:34	PNG File	107 KB
0081_71661837-pMRI_2015.png	01.09.2019 19:34	PNG File	77 KB
0081_71661837-Rainbow_2015.flat	01.09.2019 19:34	FLAT File	1 677 KB
0081_71661837-Rainbow_2015.png	01.09.2019 19:34	PNG File	10 KB
0121_71661849-Nissl_2015.png	01.09.2019 19:34	PNG File	96 KB
0121_71661849-pMRI_2015.png	01.09.2019 19:34	PNG File	68 KB
0121_71661849-Rainbow_2015.flat	01.09.2019 19:34	FLAT File	977 KB
0121_71661849-Rainbow_2015.png	01.09.2019 19:34	PNG File	10 KB
0141_71662459-Nissl_2015.png	01.09.2019 19:34	PNG File	118 KB
0141_71662459-pMRI_2015.png	01.09.2019 19:34	PNG File	87 KB
0141_71662459-Rainbow_2015.flat	01.09.2019 19:34	FLAT File	1 248 KB
0141_71662459-Rainbow_2015.png	01.09.2019 19:34	PNG File	14 KB
0149_71662461-Nissl_2015.png	01.09.2019 19:34	PNG File	92 KB
0149_71662461-pMRI_2015.png	01.09.2019 19:34	PNG File	69 KB
0149_71662461-Rainbow_2015.flat	01.09.2019 19:34	FLAT File	1 269 KB
0149_71662461-Rainbow_2015.png	01.09.2019 19:34	PNG File	12 KB

Graphs provide an initial indication of registration accuracy. If deviations from the linear regression line are present, a revision of the anchoring should be done. Independent validation by a curator is recommended.



9. Atlas References

1) Lein et al. (2007) Nature 445(7124):168-76
2) Oh, S. W. et al. (2014).A mesoscale connectome of the mouse brain. Nature 508, 207-214, doi:10.1038/nature13186
3) Papp EA et al. (2014). NeuroImage, 97:374-86
4) Kjonigsen et al. (2015) NeuroImage, 108 :441-449