Nutil: Neuroscience Image Processing and Analysis Utilities

Contents

Introduction

Nutil aims to both simplify and streamline the mechanism of pre-and-post processing 2D brain image data from mouse and rat. Nutil is developed as a stand-alone application that runs on all operating systems requiring little-to-no experience to execute. The user specifies the path to the input and output directories and the parameters for the pre-and post- processing operations in the Nutil GUI.

Pre-processing operations include conversion of images from JPEG/PNG format to tiled TIFF, 2D transformations of extremely large tiled TIFF files (rotation, flipping and scaling), in addition to renaming, copying and downsizing. Post-processing is based on analysis of segmented images in the context of brain regions defined by a reference atlas, such as the Allen Mouse Brain reference atlas or the Waxholm Space Atlas of the Sprague Dawley rat brain. All functions operate in batch mode, and operate in parallel on multiple CPUs.

Nutil enables four operations:

A. *TiffCreator*: convert JPEG/PNG images to tiled TIFF format
B. *Transform:* rename, rotate, resize and compile thumbnails of large tiled TIFF images
C. *Quantifier*: for the batch extraction, quantification and spatial analysis of labelling segmented from 2D mouse or rat brain section images (for example, immunohistochemical labelling).
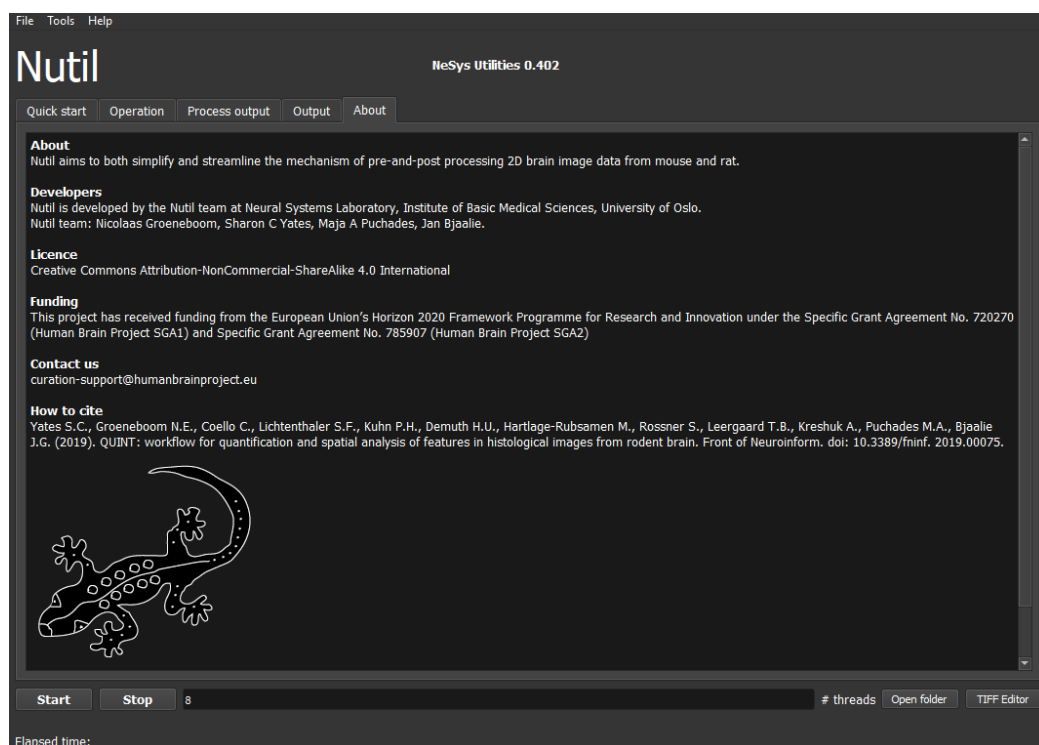D. *Resize:* for resizing JPEG/PNG images with output in PNG format.

Figure 1: Nutil GUI.

Installation and usage

Nutil is a stand-alone 64-bit Windows application with a simple GUI. There are no installation procedures, just extract the folder and double click on "Nutil.cmd". Nutil is an integral part of the QUINT workflow developed by the Nesys laboratory.

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

Licence: Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International

Funding: This work was supported by the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 720270 (Human Brain Project SGA1) and Specific Grant Agreement No. 785907 (Human Brain Project SGA2).

Cite: Yates SC, Groeneboom NE, Coello C, Lichtenthaler SF, Kuhn P-H, Demuth H-U, Hartlage-Rübsamen M, Roßner S, Leergaard T, Kreshuk A, Puchades MA and Bjaalie JG (2019) QUINT: Workflow for Quantification and Spatial Analysis of Features in Histological Images From Rodent Brain. Front. Neuroinform. 13:75. doi: 10.3389/fninf.2019.00075.
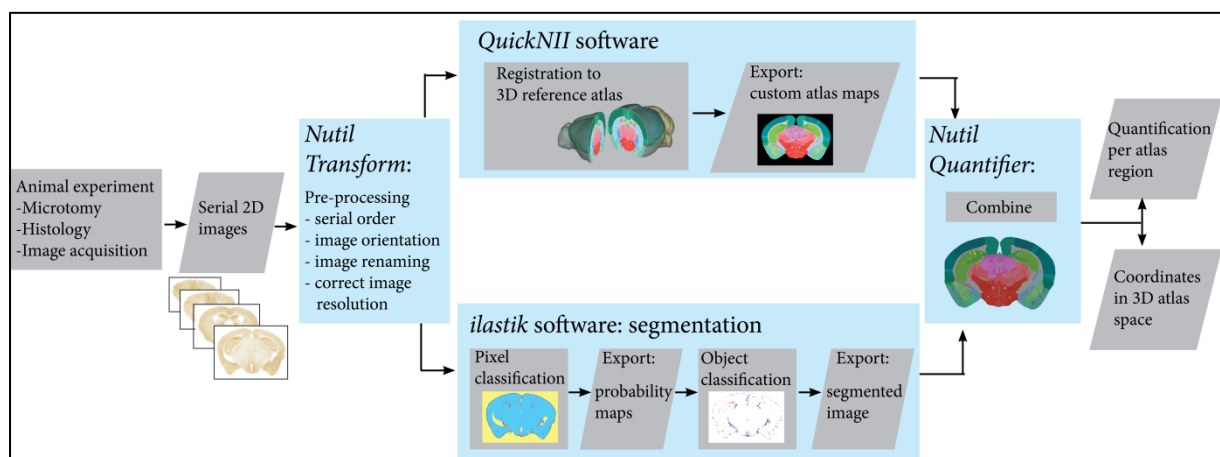

Figure 2: QUINT workflow

Operation: TiffCreator

TiffCreator converts JPEG, PNG, BMP and GIF images to the tiled TIFF format that is compatible with *Transform. TiffCreator* operates in batch, converting all the images in an input folder and saving them in the specified output directory.
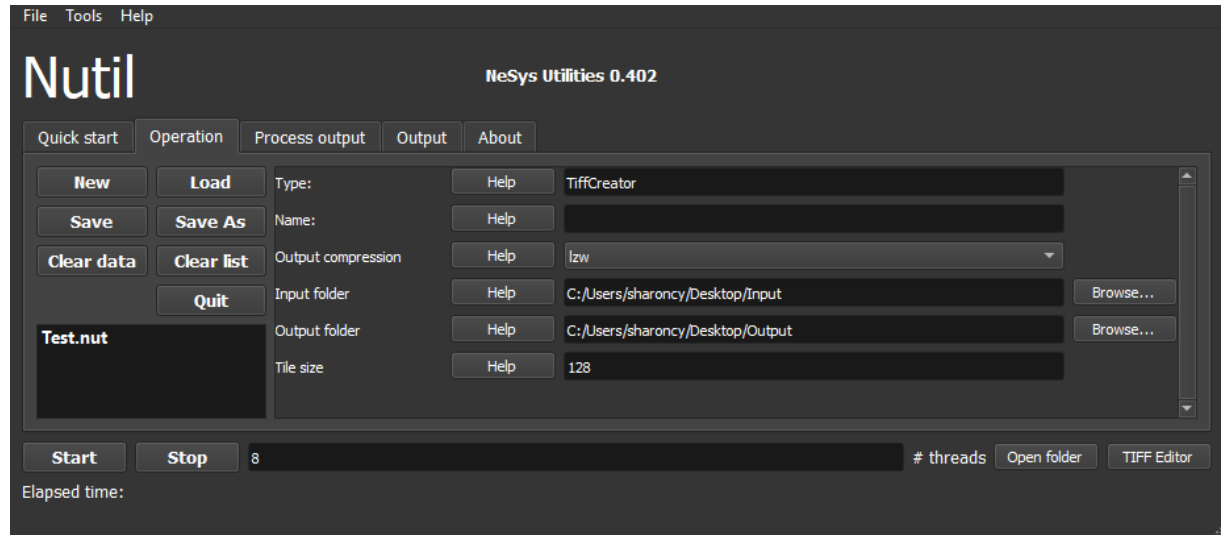

Figure 3: TiffCreator GUI

1. To begin, click "New". Select "TiffCreator".
2. Select the input folder (containing the images to be converted), output folder and enter the TiffCreator parameters in the Nutil GUI (e.g. desired output compression type).
3. Press "Save as". This saves a copy of the selected settings in a simple text file in .NUT format. The NUT file is for your own records but may also be reloaded into Nutil via the "load" button.
4. *Nutil* automatically detects the number of core processor available (8 in the example). Choose one less than the total available to ensure adequate processing power (6 or 7 here) and press "Start". Wait until the process is complete.
5. The tiled TIFFs are automatically saved in the specified output folder.

   The GUI includes an inbuilt user manual accessed via the "Help" buttons.

Operation: Transform

Transform allows the batch renaming, rotation, resizing and thumbnail compilation of large tiled TIFF images.
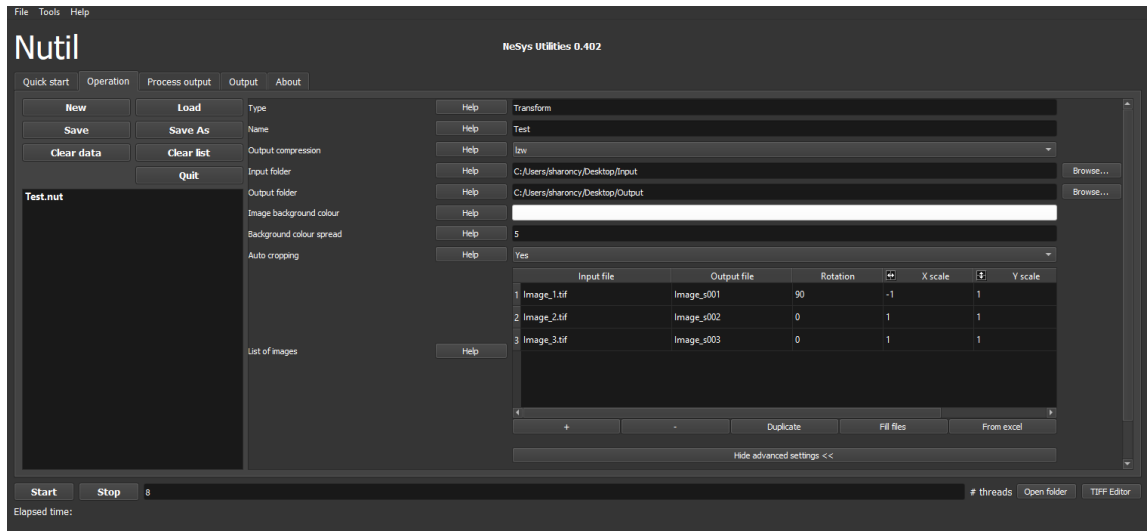

Figure 4: Transform GUI

1. Click "New". Select "Transform".
2. Select the input folder (containing the images to be transformed), output folder and enter the transformation parameters in the Nutil GUI. The GUI includes an inbuilt user manual accessed via the "Help" buttons.
3. Once everything is filled in press the "Save as" button. This saves a copy of the transformation parameters in a simple text file in .NUT format. This is for your own records, but may also be reloaded into Nutil via the "load" button.
4. *Nutil* automatically detects the number of core processor available (8 in the example). Choose one less than the total available to ensure adequate processing power (6 or 7 here).
5. Press "Start" and wait until the process is complete.

Operation: Resize

Resize enables rapid resizing of PNG and JPEG images by application of a resize factor or a fixed pixel width.
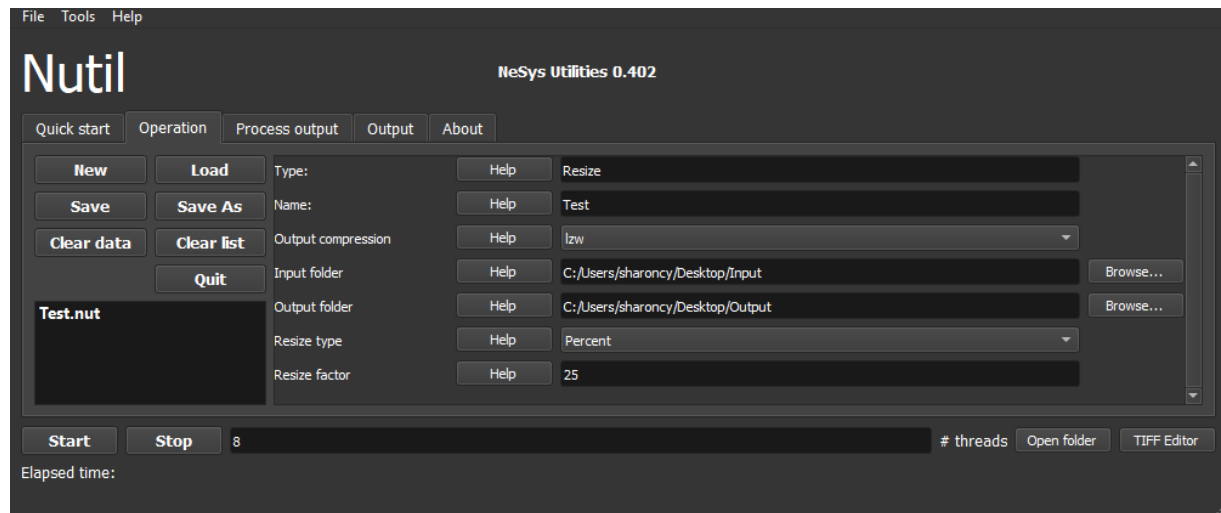
Figure 5: Resize GUI

1. To begin, click "New". Select "Resize".
2. Select the input folder, output folder and resize factor in the Nutil GUI (see the "help" buttons for more information).
3. Press "Save as". This saves a copy of the parameters in a simple text file in .NUT format. This is for your future records but may also be reloaded into Nutil via the "load" button.
4. *Nutil* automatically detects the number of core processor available (8 in the example). Choose one less than the total available to ensure adequate processing power (6 or 7 here) and press "Start". Wait until the process is complete.
5. The PNG files save automatically in the specified output folder.

Operation: Quantifier

Input files

1. **Segmentations**

   Any image analysis software may be used to generate the segmentations. We use the Pixel and Object Classification workflows in the *ilastik* software (ilastik.org).
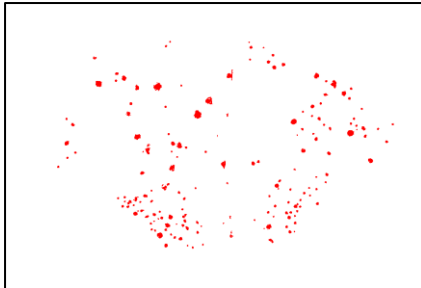
   Requirement:

   - must be 24-bit RGB images in PNG format
   - same proportions as the atlas maps (but not necessarily the same size as the images used in *QuickNII* to generate the atlas maps)
   - *Quantifier* is only able to extract one RGB (red green blue) colour at a time. Apply one RBG colour to all of the objects of interest, and specify this colour code in the GUI (e.g. objects shown in red above, which is RGB colour code: 255,0,0).

     See the ilastikUserManual (included in the Nutil package) for more information.

   Ilastik is a versatile image analysis tool specifically designed for the classification, segmentation and analysis of biological images based on supervised machine learning algorithms.

   A user manual that describes how to use ilastik in the context of the QUINT workflow is included as part of the Nutil package. See: ilastikUserManual_v0402

   Ilastik is available at: http://ilastik.org/download.html

2. **Customised brain atlas maps**

   Generate the atlas maps with the *QuickNII* software with nonlinear refinement with *VisuAlign* if necessary. See the *QuickNII* and *VisuAlign* manuals for more information. (www.nitrc.org/projects/quicknii) (https://www.nitrc.org/projects/visualign)

3. **Anchored XML** file generated with the *QuickNII* software.

   > **QuickNII** is a stand-alone tool for user guided affine spatial registration (anchoring) of sectional image data, typically high resolution microscopic images, to a 3D reference atlas space. A key feature in the tool is the capability to generate user defined cut planes through the atlas templates, matching the orientation of the cut plane of the 2D experimental image data (customised brain atlas maps). The reference atlas is transformed to match anatomical landmarks in the corresponding experimental images. In this way, the spatial relationship between experimental image and 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 to reduce the amount of manual steps required.
   >
   > *QuickNII is available at:* www.nitrc.org/projects/quicknii

   > **VisuAlign** is a stand-alone tool applying user-guided nonlinear refinements (inplane) to an existing, affine 2D-to-3D registration (compatible with the output of the QuickNII tool).
   >
   > *VisuAlign is available at:* https://www.nitrc.org/projects/visualign)

File name requirements

The file names of the <u>segmentation</u> and <u>atlas maps</u> that correspond to a particular section must contain a unique ID. The file names must also be correct in the anchored XML file (this happens automatically as long as the images that are anchored with QuickNII are named correctly).

Nutil Quantifier support*s* IDs in the format: s*XXX*.., with *XXX* representing the section number. 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). The IDs must match those in the .xml file.

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

Nutil Quantifier also supports user defined IDs (using regular expressions). For more information see the "help" button in the Nutil GUI.

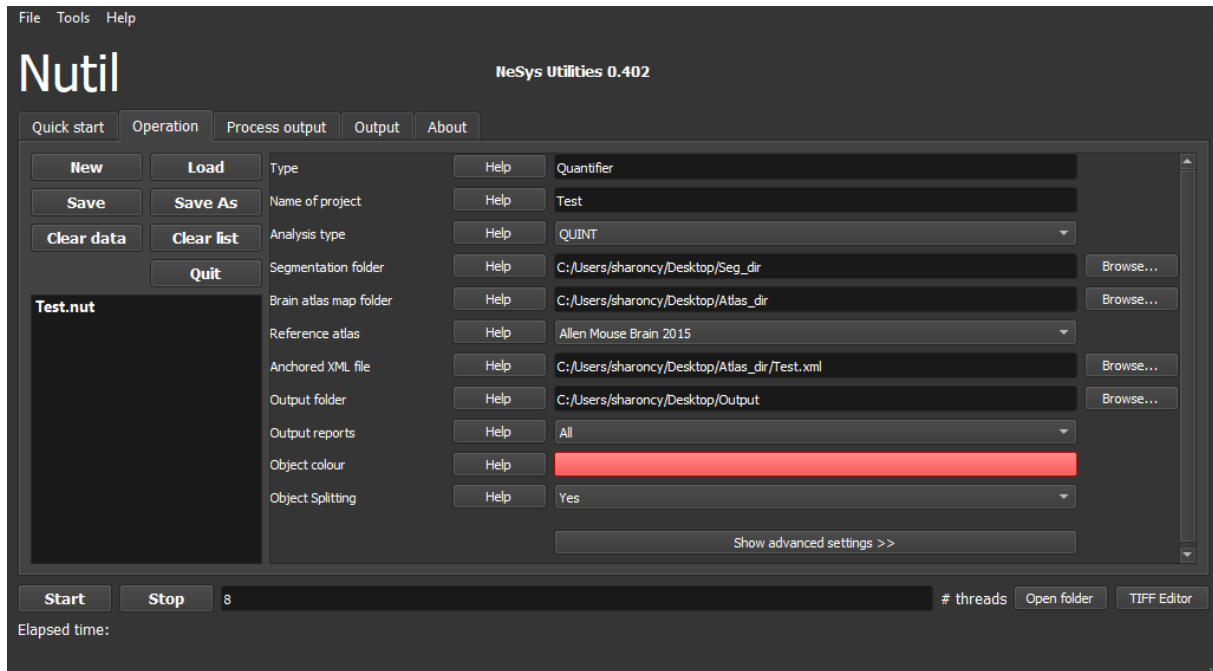|	
NOTE:	The sXXX naming convention applies to both *QuickNII* and *Nutil Quantifier*. We therefore recommend changing the file names as the first step in the workflow (with *Nutil Transform,* see Figure 1).

Running Quantifier

Figure 5: Quantifier GUI

1. To begin, click "New". Select "Quantifier" and analysis type "QUINT". Enter the name of your project.
2. Select the folder containing the segmentations, the atlas maps and the output folder, and navigate to the anchored XML file.
3. Select the reference brain atlas. This must match the version used to generate the brain atlas maps (Allen mouse brain 2015 or 2017, or Waxholm Space Atlas of the Sprague Dawley Rat v2 or v3).
4. Fill in the rest of the parameters. The Nutil GUI includes an inbuilt user manual accessible via the "Help" buttons, and gives an in depth description of each parameter/setting.
5. The "show advanced settings" button reveals more settings that may be altered by the user (e.g. max and min object size cut-off, option to generate customised reports, etc.). This gives more flexibility for customised analysis. If nothing is changed in the advanced settings, the default settings shown in the table below are applied.

Advanced Parameter	Default settings
Minimum object size	1 pixel
Maximum object size	100000 pixels
Pixel scale	1 pixel
Use custom masks?	No
Output report type	CSV
Apply customised regions?	Default
Coordinate extraction?	Yes, for whole series and per section (All)
Pixel density	1 coordinate per pixel
Nifti size	0 (no nifti file generated)
Unique ID format	_sXXXX…

6. Press "Save as". This saves a copy of the selected settings in a simple text file in .NUT format. This is for your future records, but may also be reloaded into Nutil via the "load" button (for example, to repeat the analysis on a new set of images, etc).
7. *Nutil* automatically detects the number of core processor available (8 in the example). Choose one less than the total available to ensure adequate processing power (6 or 7 here) and press "Start". Wait until the process is complete.
8. The output files are automatically saved in the specified output folder.
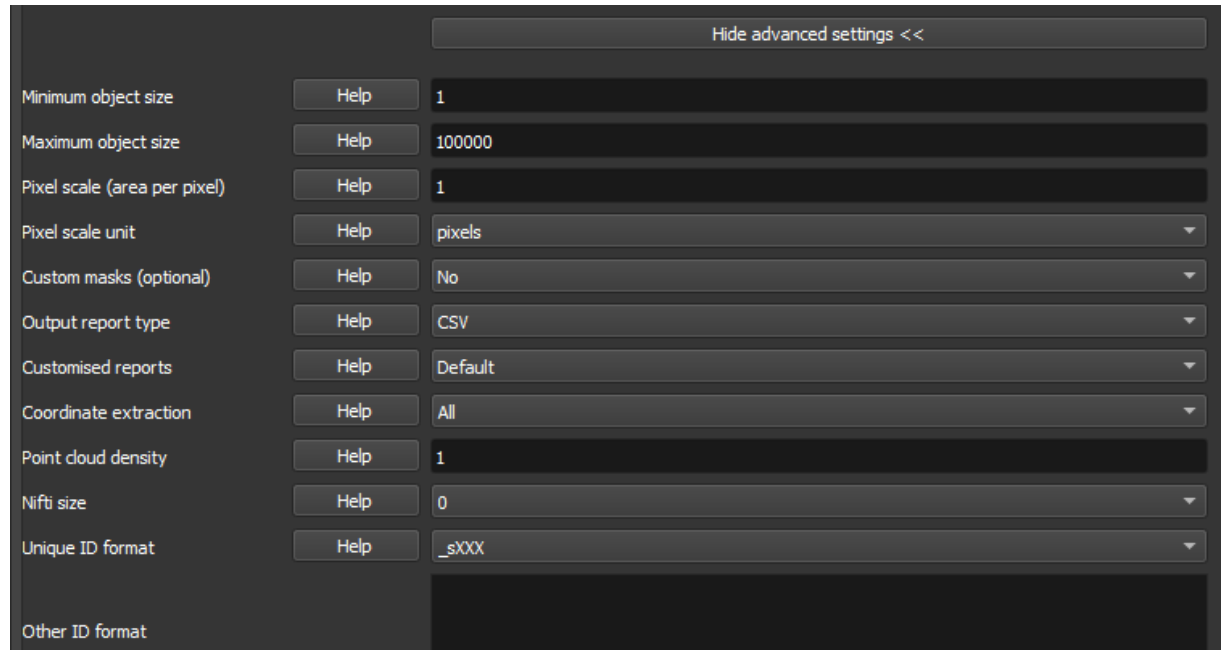
Figure 6: Quantifier advanced settings GUI

Object Splitting Explained

In Quantifier, users must specify whether to turn on or off "object splitting". Object splitting divides segmented objects that overlap atlas regions, with individual pixels assigned their precise location. This gives accurate load measurements (load is the percentage of the region occupied by objects), but invalidates the object counts.

Recommendation: Select <u>NO</u> for small objects to get accurate object counts (e.g. cells). Select <u>YES</u> for large objects that overlap atlas regions (e.g connectivity data). This gives precise load output. See the help button for more information.

How to generate customised reports with Quantifier

Nutil Quantifier always generates two sets of reports:

- *RefAtlasRegion* reports contain quantifications per atlas region based on the finest level of granularity of the selected atlas.
- *CustomRegion* reports contain quantifications for broader regions, such as cortex and hippocampus ("default"), or user defined regions ("custom"). The custom regions are compilations of reference atlas regions. Users have the option to either define their own using the CustomRegionsTemplate, or to use the default regions included in Nutil Quantifier. More info on the default regions are found in the CustomRegion files in the Nutil package (see folder titled "CustomRegion" and navigate to the xlsx file that corresponds to your atlas). For all the reference atlases, the default option includes all the reference atlas regions in the whole brain.

Customised reports	Help	Custom
Custom report file	Help	
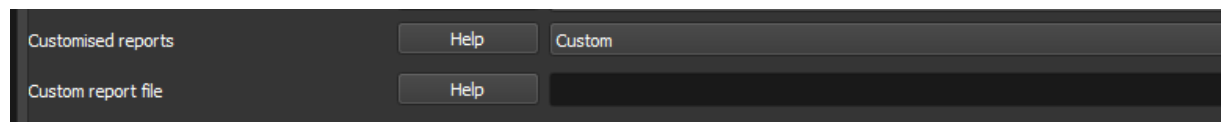
How to define your own regions

1. To define your own regions, use the *CustomRegionsTemplate.xlsx* that is included in the Nutil package, and populate as described below:

	A	B	C	D
1	Custom brain region	Cortex	Fibretracts	Hippo
2	RGB colour	255;0;0	255;0;255	255;255;0
3	Colour	red	fusia	yellow
4	Atlas Ids		8	1009	1089
5			567	967	1080
6			688	885	375
7			695	949	382
8			315	840	391
9			184	1016	399
10			68	21	407
11			667	665	415
12			500	538	423
13			107	459	431
14			219	900	438
15			299	848	446
16			644	876	454
17			947	916	463
18			985	336	471
19			320	117	479

ROW 1: assign your own names to the regions (e.g. Cortex).

ROW 2: assign colours to the regions. Do this by typing a RGB colour code in the following format: 255;0;0 (for red). This colour will be assigned to the objects located in the custom region for the purposes of the image and coordinate output (for display purposes only).

ROW 3: enter the colour name (this is for your information only).

ROW 4: define the region by listing the reference atlas IDs that you wish to include. The excel sheets in the AtlasHierarchy folder list all the regions and IDs for each atlas.

For mouse, see the *ABAHier2015.xlsx or ABAHier2017.xlsx* file for the full list of regions and IDs.

For rat, see the *WHS_rat_atlas_v2.xlsx or WHS_rat_atlas_v3.xlsx* file for the full list of regions and IDs.

NOTE: Use the default xlsx as a guide to filling in the template.

How to interpret the Quantifier output

1. **Reports** (CSV or HTML format)

Region ID	Region Name	Region pix	Region are	Area unit	Object cou	Object pix	Object are	Area unit	Load
0	Clear Label	0	0	um2	207	10028	20056	um2	0
997	root	85.765518	171.53103	um2	0	0	0	um2	0
8	Basic cell groups and regions	0	0	um2	0	0	0	um2	0
567	Cerebrum	0	0	um2	0	0	0	um2	0
688	Cerebral cortex	0	0	um2	0	0	0	um2	0
695	Cortical plate	0	0	um2	0	0	0	um2	0
315	Isocortex	0	0	um2	0	0	0	um2	0
184	Frontal pole, cerebral cortex	0	0	um2	0	0	0	um2	0
68	Frontal pole, layer 1	0	0	um2	0	0	0	um2	0
667	Frontal pole, layer 2/3	0	0	um2	0	0	0	um2	0
500	Somatomotor areas	0	0	um2	0	0	0	um2	0
107	Somatomotor areas, Layer 1	0	0	um2	0	0	0	um2	0
219	Somatomotor areas, Layer 2/3	0	0	um2	0	0	0	um2	0
299	Somatomotor areas, Layer 5	0	0	um2	0	0	0	um2	0
644	Somatomotor areas, Layer 6a	0	0	um2	0	0	0	um2	0
947	Somatomotor areas, Layer 6b	0	0	um2	0	0	0	um2	0
985	Primary motor area	85239.234	170478.46	um2	33	1767	3534	um2	0.020730
320	Primary motor area, Layer 1	345318.34	690636.68	um2	32	1871	3742	um2	0.005418

RefAtlasRegions

Report with output organised based on all the regions in the reference atlas: per section and for the whole series (all sections combined).

> **IMPORTANT**: The *Allen Mouse Brain Reference Atlas* includes a lot of regions that are not actually delineated. These regions are either big regions that have been delineated into smaller regions and so are not assigned to any pixels in the reference atlas, or are smaller regions that have not been delineated. In the reports, these regions have no results (zero for region pixels and for object pixels) and should be excluded from analysis.
>
> The *Clear Label ID* covers objects that fall outside of the atlas maps.

CustomRegions

Reports with the output organised based on the customised regions defined in the CustomRegionsTemplate.xlsx: per section and for the whole series.

Objects

List of all the objects in the whole series and per section.

In each report, interpret the results as follows:

|	
Region pixels	No. of pixels representing the region.
Region area	Area representing the region
Area unit	Region area unit
Object count	No. of objects located in the region. NOTE: Object counts are not generated if object splitting is switched "on".
Object pixels	No. of pixels representing objects in this region.
Object area	Area representing objects in this region.
Load	Ratio of Object pixels to Region pixels (Object pixels/Region pixels).

2. IMAGES

Segmentations superimposed on the atlas maps (PNG format). Object colours are assigned based on the customised regions. If no regions are specified, or object falls outside of the specified areas, the objects are shown in red by default.

3. COORDINATES

JSON files containing point clouds that can be visualised with the MeshView atlas viewer, which is available at www.nitrc.org/projects/meshview via the MediaWiki link.
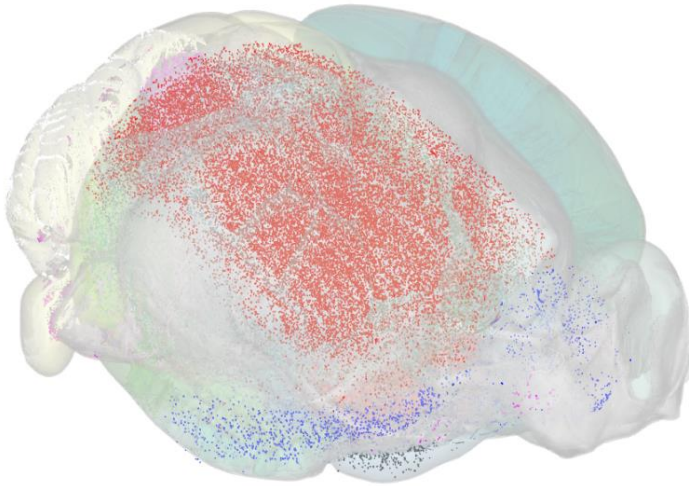
4. NUT file

The NUT file is a text file containing the analysis parameters. To view, open with *Notepad.*


```
02_12_19.nut - Notepad
File  Edit  Format  View  Help
type = Quantifier
name =
analysis_type = QuickNII
quantifier_input_dir = Z:/HBP_Analytic
quantifier_atlas_dir = Z:/HBP_Analytic
label_file = Allen Mouse Brain 2015
xml_anchor_file = Z:/HBP_Analytics/Nut
quantifier_output_dir = Z:/HBP_Analyti
output_report = All
extraction_color = 255,0,0,255
object_splitting = No
object_min_size = 1
object_max_size = 100000
global_pixel_scale = 2
quantifier_pixel_scale_unit = um2
use_custom_masks = No
custom_mask_color = 255,255,255,255
output_report_type = XLSX
custom_region_type = Yes
custom_region_file =
```

Technical information

Development platform

Nutil is written as a stand-alone windows 64-bit application written in Qt C++, which enables the full usage of both memory and processor cores. *Nutil* can be downloaded and compiled from the github page. When performing batch processes, *Nutil* will utilise all cores available on the system.

The external libraries that are used in *Nutil* are:

- Libtiff for fast and efficient TIFF file handling (http://www.libtiff.org/)
- LibXLNT for excel file IO (https://github.com/tfussell/xlnt/)

TiffCreator

TiffCreator produces tiled TIFF files from JPEG or PNG images, and employs the support of multiple CPUs for efficient, parallelised operations.

Transform

Transform enables rotations, scaling and thumbnail compilation of large tiff files (currently up to 4GB).

Quantifier

Quantifier identifies individual binary objects in a PNG file, while matching these to output from *QuickNII*. The method first finds and sorts areas by using a standard pixel filler routine. Afterwards, a random area pixel is chosen as the look-up in the binary QuickNII label slice for this particular image. When all areas have been assigned a label ID, multiple selections of pre-defined area IDs are assembled (ID list from the excel input file), and finally output reports are assembled and written to disk (in xlsl format). In addition, original ilastik .png files with colour/ID coding added to underlying atlas slice data are assembled and saved to the output directory.

Authors

Nicolaas E Groeneboom, Sharon C. Yates, Maja A. Puchades, Jan G. Bjaalie.

Licence

Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International

Cite

Yates SC, Groeneboom NE, Coello C, Lichtenthaler SF, Kuhn P-H, Demuth H-U, Hartlage-Rübsamen M, Roßner S, Leergaard T, Kreshuk A, Puchades MA and Bjaalie JG (2019)

QUINT: Workflow for Quantification and Spatial Analysis of Features in Histological Images From Rodent Brain. Front. Neuroinform. 13:75. doi: 10.3389/fninf.2019.00075.

Acknowledgements

Contact us

curation-support@humanbrainproject.eu