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Version 2

QUINT Workflow Appendix V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol contains additional details for the modified QUINT workflow.

Attachments



[812-2118.pdf](#)

4.6MB

QuPath: Setting Stain Vectors

- 1 Draw a small rectangle over a characteristic DAB or hematoxylin stain.
- 2 On Image tab, double-click the Stain you would like to set. Click Yes on the prompt.
- 3 Give the new settings a unique name (Project ID). Click OK.

Note

***Note:** You could also use "Estimate Stain Vectors."
- 4 This has only saved the parameters for this particular image. To change all images in a project, go to the Workflow tab. This script can also be added to an analysis script and run at the same time.
- 5 "Set color deconvolution stains" should be the last Command. Click on Command, verify that the parameters are correct, and click "Create script."
- 6 In the Script Editor, click Run > Run for Project.
- 7 Move all images from Available to Selected, and click "OK." Modified setting from Aperio Scanner:

Hematoxylin: 0.667, 0.658, 0.349
DAB:0.324, 0.547, 0.772
DAB:0.485, 0.616, 0.620

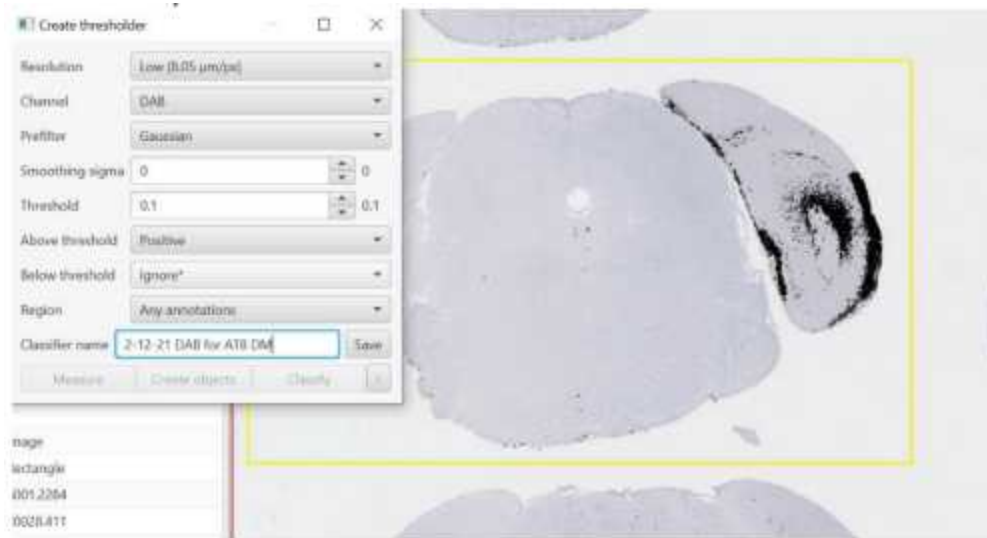
QuPath: Pixel Classifier Generation

- 8 To create a new pixel classifier for segmentation:

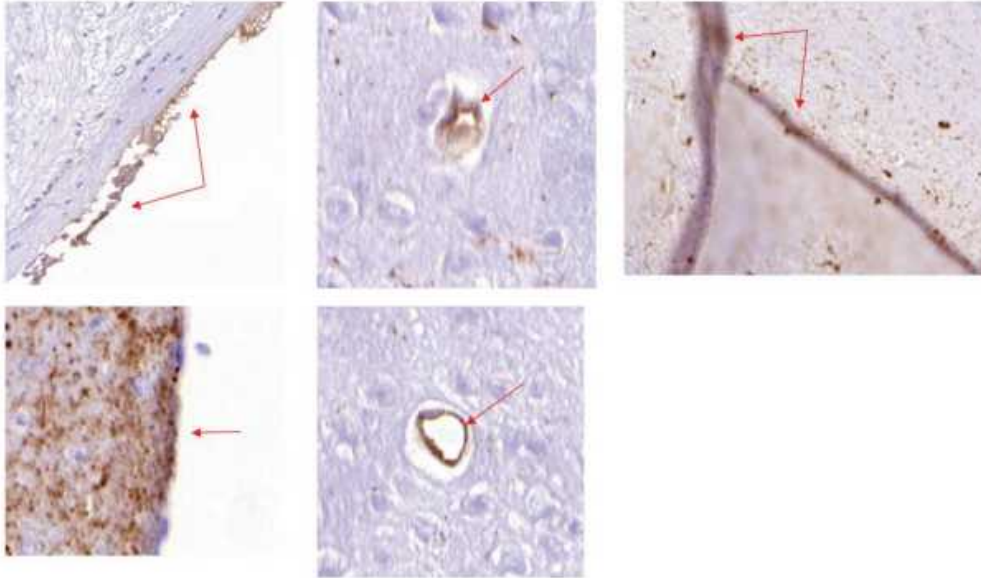
It is easiest if the output segmentation from QuPath is black. Click on the "Annotations" tab,
double click on "Positive" and change the color to black. Click "OK".
- 9 Use the rectangle tool to box an area you can use to test the classifier.

10 Classify > Pixel classification > Create thresholder

11 Example settings are below, but can be optimized by project. Generally higher resolution is better, but slower to run and will create larger files. We generally use "Very high (1.01 um/px)" resolution. Give the classifier a name and click "Save". The classifier will be in your project folder under classifiers and can be moved to another qupath project folder to share with another set of images for consistency.

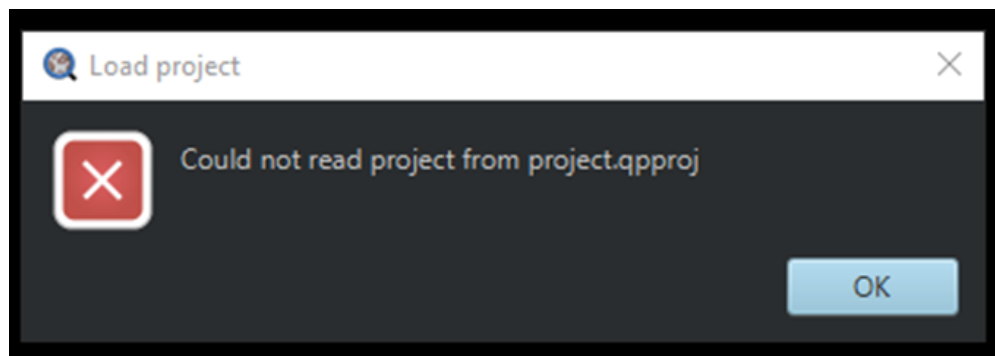


12 Examples of Staining Artifacts:



QUINT Tips & Tricks: QuPath

13 QuPath Project Won't Open: error pops up when attempting to open project



The project.qpproj is essentially just a txt file formatted for the specifications of qupath, telling it how to organize and read the data—all the images, annotations, and classifiers are located in the other data/classifier folders so it's not the end of the world if the qupath project wont open

13.1 Fix = 1. *RENAME BACKUP TO PRIMARY QUPATH PROJECT:*

a.store original faulty project.qpproj in a new folder temporarily to avoid duplicates in the same file space

b. rename *project.qpproj.backup* > *project.qpproj*, open new project. Should change file type from BACKUP File to QPPROJ File

classifiers	11/21/2023 10:40 AM	File folder	
data	11/21/2023 10:40 AM	File folder	
project	2/23/2024 2:39 PM	QPPROJ File	3 KB
project.qpproj.backup	11/21/2023 10:41 AM	BACKUP File	3 KB

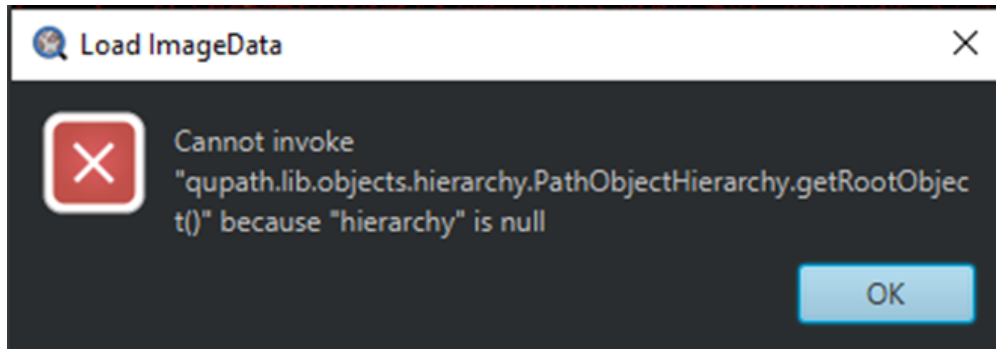
Name	Date modified	Type	Size
classifiers	5/8/2024 10:25 AM	File folder	
data	5/8/2024 10:11 AM	File folder	
project	6/3/2024 4:26 PM	QPPROJ File	15 KB
faulty qupath project	6/6/2024 9:35 AM	File folder	

13.2 Fix 2 = if tmp file present, rename tmp file to project.qpproj – should change file type from TMP File to QPPROJ

classifiers	12/1/2022 10:44 AM	File folder	
data	12/2/2022 1:36 PM	File folder	
export	12/1/2022 11:21 AM	File folder	
project.qpproj.backup	12/2/2022 1:36 PM	BACKUP File	5 KB
project.tmp	3/21/2024 4:14 PM	TMP File	226 KB

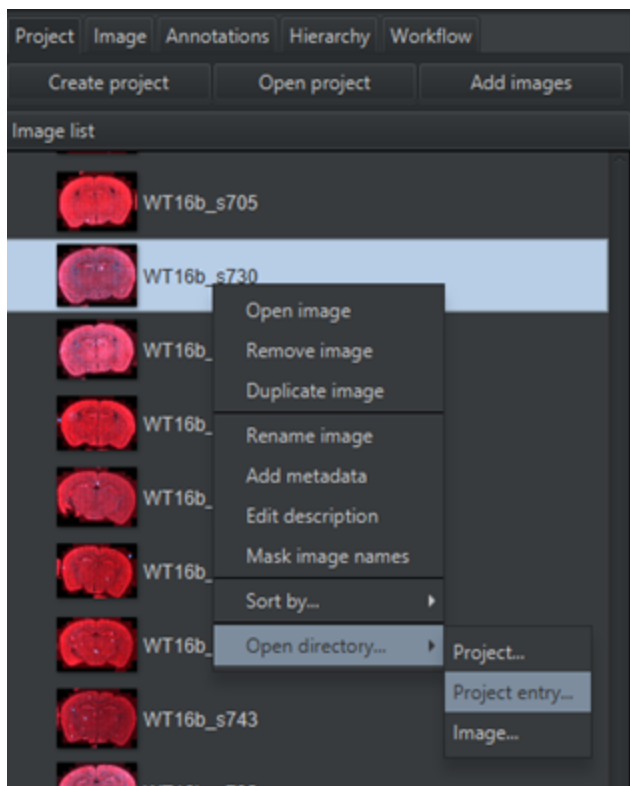
Name	Date modified	Type	Size
classifiers	12/1/2022 10:44 AM	File folder	
data	12/2/2022 1:36 PM	File folder	
export	3/21/2024 4:36 PM	File folder	
project.qpproj.backup	12/2/2022 1:36 PM	BACKUP File	5 KB
project.qpproj	3/21/2024 11:22 AM	QPPROJ File	5 KB

14 **QuPath Image Won't Open:** error below comes when trying to open an image



14.1 Fix: Recover Backup data file

a. Open data folder associated with image: Right click over problem image > *Open directory* > *Project entry*



Name	Date modified	Type	Size
data.qpdata	10/19/2022 10:11 AM	QPDATA File	2 KB
data.qpdata.backup	10/19/2022 10:11 AM	BACKUP File	2 KB
server	10/19/2022 10:11 AM	JSON Source File	1 KB
summary	10/19/2022 10:11 AM	JSON Source File	1 KB
thumbnail	10/19/2022 10:10 AM	JPG File	119 KB

b. Temporarily store *data.qpdata* file in a new folder (can delete later if restored)

c. Remove the *.backup* in the *data.qpdata.backup*

Reattempting to open the image in the QuPath project should allow it to open. If your data folder does not have a *.bkp* file and the image is still not opening, your best bet may be to add the image again and run the analysis from the start as there is no current fix aside from this.

15 **Transferring Data Across Projects**

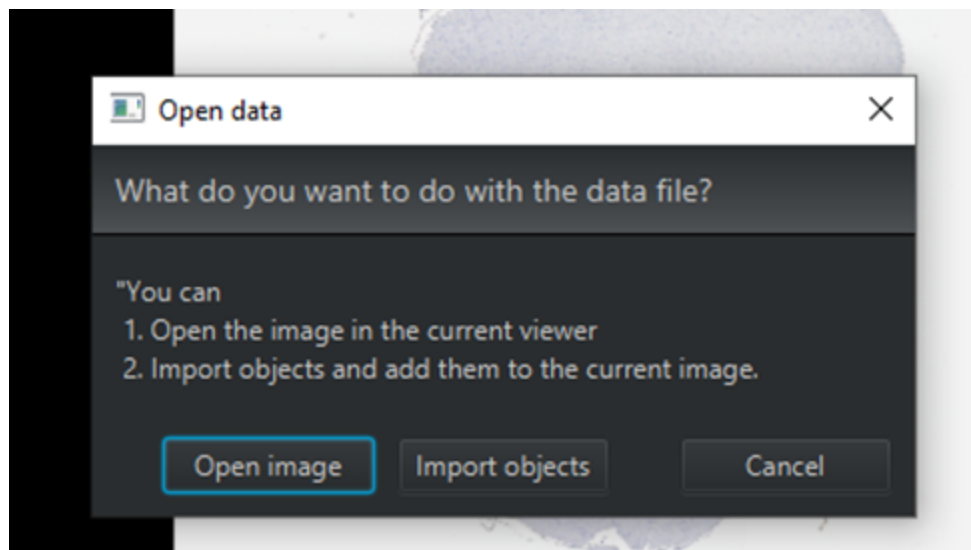
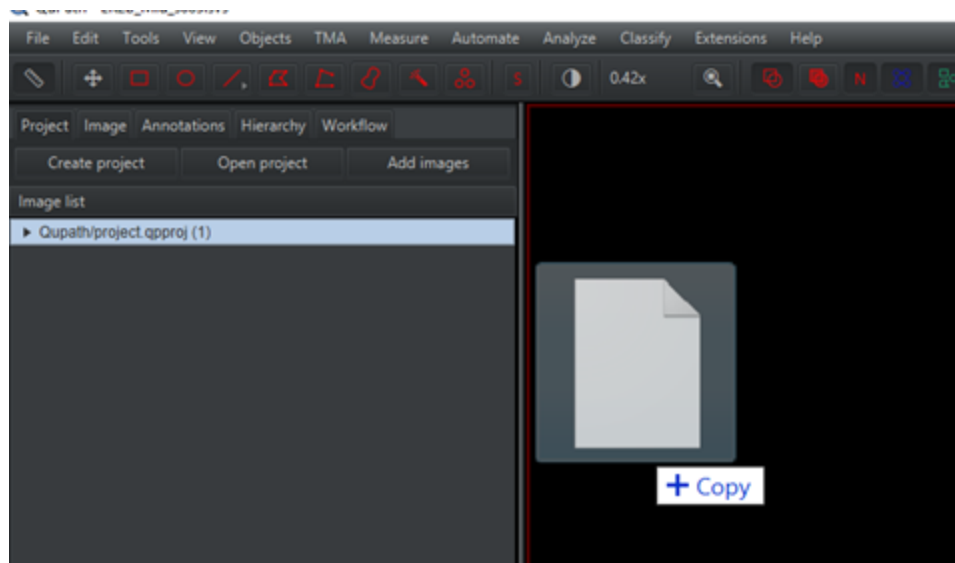
Transferring data like images, annotations, classifiers and even display settings across projects is possible and quite straightforward.

15.1 **Data**

To transfer a specific image from one project to another, drag the associated *data.qpdata* to an open QuPath project window.

svai.org > projects_secondary > henderson > KEVIN > QUINT > QUINT Practice > DAB > data > 1

Name	Date modified	Type	Size
data.qpdata	11/21/2023 10:40 AM	QPDATA File	4 KB
server	11/21/2023 10:40 AM	JSON Source File	1 KB
summary	11/21/2023 10:40 AM	JSON Source File	1 KB
thumbnail	11/21/2023 10:40 AM	JPG File	119 KB



Select *Open Image* to import the image with its included annotations/detections to your project. (Selecting *Import Objects* will only import annotations/detections to the current open image in the QuPath window).

15.2 **Classifiers**

Your QuPath folder should include a “classifiers” folder that includes all object/pixel classifiers you’ve generated throughout the project. Copy individual object/pixel classifier JSON files or whole object/pixel classifier folders and paste them into the appropriate classifier folder for the project you would like to import them to.

ai.org > projects_secondary > henderson > KEVIN > ASAP WT Time Point Project > QUINT > WT6 (3MPI) > classifiers >

Name	Date modified	Type	Size
 object_classifiers	11/22/2023 11:26 AM	File folder	
 pixel_classifiers	11/15/2023 1:43 PM	File folder	
 classes	5/28/2024 10:29 AM	JSON Source File	1 KB

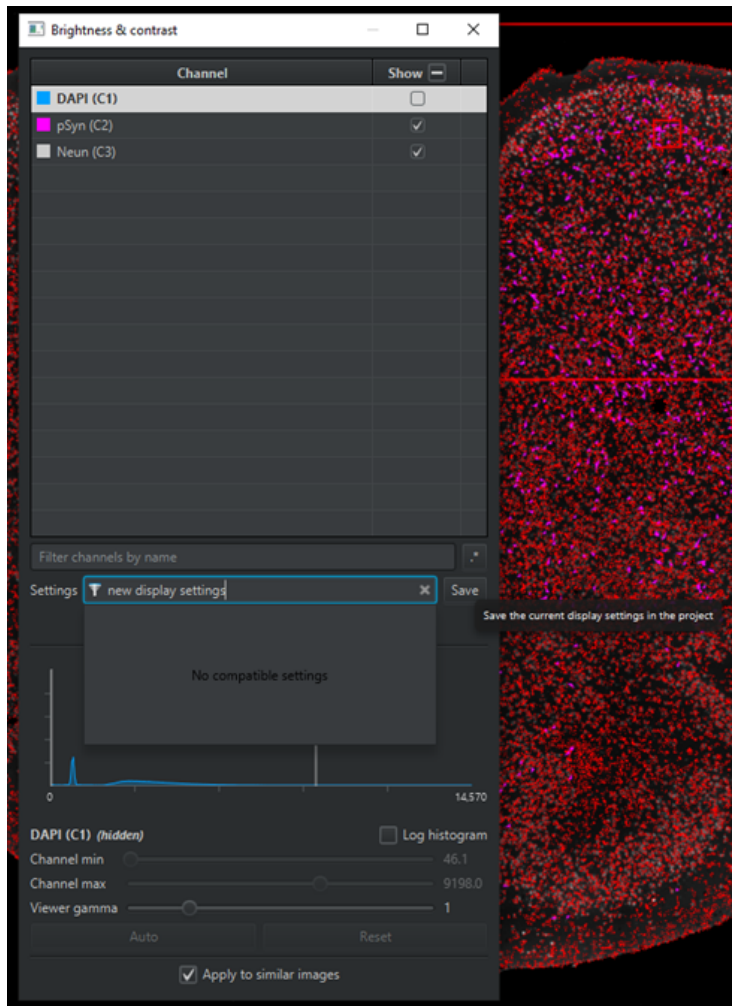
pn.vai.org > projects_secondary > henderson > KEVIN > ASAP WT Time Point Project > QUINT > WT6 (3MPI) > classifiers > pixel_classifiers

Name	Date modified	Type	Size
 pSyn 3000	8/24/2023 2:46 PM	JSON Source File	1 KB
 psyn 4000	8/22/2023 1:32 PM	JSON Source File	2 KB
 psyn 5000	9/13/2023 2:34 PM	JSON Source File	2 KB
 psyn 6000	10/10/2023 10:19 AM	JSON Source File	2 KB
 psyn 7000	9/26/2023 1:54 PM	JSON Source File	2 KB
 psyn 9000	9/29/2023 1:41 PM	JSON Source File	2 KB
 psyn 12000	10/2/2023 11:23 AM	JSON Source File	2 KB

15.3 **Display settings (versions>0.5.0)**

Newer versions of QuPath allow you to save display settings to streamline visualizing fluorescent stains across multiple images/projects.

You can manipulate channel color by right-clicking the colored box next to channel name. After setting to the optimal min/max settings, you can save this display by typing a name in the "Settings" box and selecting **Save**.



This will generate a *resources* folder in your QuPath folder. In it there is a *display* folder that contains all the settings you've generated in that project. To transfer settings over to other projects, copy the display folder's JSON files and place them into your new projects display folder. If there is no *resource/display* folder in the new project, paste the entire resource folder from the old one in the new QuPath project folder.

n.vai.org > projects_secondary > henderson > KEVIN > ASAP WT Time Point Project > QUINT > WT16c (0.3MPI) > resources > display		
Name	Date modified	Type
figure	4/16/2024 10:05 AM	JSON Source File
psyn scrub	2/13/2024 2:33 PM	JSON Source File
red	3/29/2024 1:42 PM	JSON Source File
regis	3/15/2024 11:04 AM	JSON Source File
regis2	3/11/2024 10:45 AM	JSON Source File

QUINT Tips & Tricks: Registration

16 **Merging/Transferring QuickNII/Visualalign Images**

In the event you forgot to add an image to your QuickNII project, or you want to make edits to the plane of registration but are already in VisuAlign and you don't want to redo it all, utilize the XML/JSON files to insert/edit/remove desired images from your project.

16.1 **Adding QuickNII images**

You can add images to your QuickN project by inserting the file data from a QuickN project containing just the image(s) you want to add.

- a. Create a new QuickNII project with only the images you want to add to your original project.
- b. After you are satisfied with the section(s) alignment, export the QuickNII XML
- c. Open your QuickNXML with Notepad
- d. Each image will be listed as <slice filename='name'....atlas plane details>

```
<?xml version='1.0' encoding='UTF-8'?>
<series name='Filebuilder XML' target='ABA_House_CCFv3_2017_25um_cutlas' target-resolution='456 528 320'>
  <slice filename='WT12_s004.png' nr='4' width='1593' height='998' anchoring='ox=381.65873868839793&oy=388.8224143429237&oz=280.88288550289053&ux=
</series>
```

- e. Copy the desired images. Be sure to include <brackets>.

- f. To insert into original project, paste copied file data into original QuickN XML in Notepad

```
*QuickN XML - Notepad
File Edit Format View Help
<?xml version='1.0' encoding='UTF-8'?>
<series name='Filebuilder XML' target='ABA_House_CCFv3_2017_25um_cutlas' target-resolution='456 528 320'>
  <slice filename='WT12_s001.png' nr='1' width='1465' height='1031' anchoring='ox=364.5605365517395&oy=404.4375635863232&oz=269.86862132384545&ux=
  <slice filename='WT12_s002.png' nr='2' width='1542' height='1043' anchoring='ox=371.0693653388204&oy=400.1082335811191&oz=271.4208510901433&ux=
  <slice filename='WT12_s003.png' nr='3' width='1456' height='1065' anchoring='ox=381.206279338031&oy=394.0282387068221&oz=268.553614929588&ux=
  <slice filename='WT12_s004.png' nr='4' width='1593' height='998' anchoring='ox=381.65873868839793&oy=388.8224143429237&oz=280.88288550289053&ux=
</series>
```



g. Your added images should now appear when you open the original QuickNXML. Make any edits needed, export and carry on with registration as normal.

Note

Image Names can be tweaked within the XML as well.

16.2 ***Adding Visualign images***

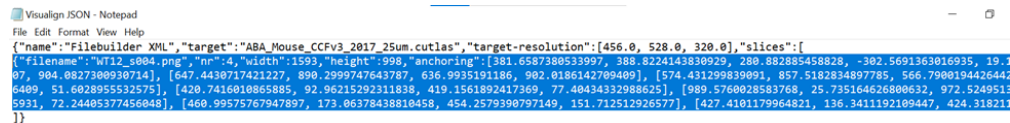
The same principle applies to Visualign JSONs.

a. Create new Visualign project with the image(s) you want to add

b. Export the Visualign JSON

c. Open the Visualign JSON with Notepad

- Each image will be listed within curly brackets, the start/end should look like this
`{"filename": "name.png"...}]}`



```
Visualign JSON - Notepad
File Edit Format View Help
{"name": "Filebuilder XML", "target": "ABA_Mouse_CCFv3_2017_25um.cutlas", "target-resolution": [456.0, 528.0, 320.0], "slices": [
  {"filename": "MT12_s004.png", "nr": 4, "width": 1593, "height": 998, "anchoring": [381.6587380533997, 388.8224143830929, 280.882885458828, -302.5691363016935, 19.7
07, 904.0827300930714], [647.4430717421227, 890.2999747643787, 636.9935191186, 902.0186142709409], [574.431299839091, 857.5182834897785, 566.7900194426442
6409, 51.602895532575], [420.7416010865885, 92.96215292311838, 419.1561892417369, 77.40434332988625], [989.5760028583768, 25.735164626800632, 972.5249513
5931, 72.24405377456048], [460.99575767947897, 173.06378438810458, 454.2579390797149, 151.712512926577], [427.4101179964821, 136.3411192109447, 424.318211
]]}
```

Note

The end of each image's file data is right after the last anchor coordinate value. The "}" at the very end of the project should not be included in the filedata you copy.

Visualign JSON - Notepad

File Edit Format View Help

```
4994514118034, 1221.3344134802335, 850.4157187176836], [1089.8475898120807, 862.1.3210742633355, 1396.843162670123, 132.10341261633923], [1503.8816766252935, 31.25214865217, 1310.1211924821778, 133.13547052740435], [1115.981774409169, 94.422.378.20715140484964, 873.4141283214518, 362.25232678386766]]}
```

d. Copy the desired image filedata.

e. Open the original Visualign JSON in Notepad and paste into it with proper formatting

*Visualign JSON - Notepad

File Edit Format View Help

```
{ "name": "Filebuilder XML", "target": "ABA_Mouse_CCFv3_2017_25um.cutlas", "target-resolution": [456.0, 528.0, 320.0], "slices": [
  { "filename": "WT12_s001.png", "nr": 1, "width": 1465, "height": 1031, "anchoring": [364.50853655780545, 404.4375635864291, 269.86862132373955, -270.7079564466813, -4.86970010341], [1290.540824757678, 721.7567343889133, 1316.79403202329, 702.6153050672182], [1287.9287192327898, 754.7963116458105, 1332.7074818049493, 735.61.1406411582129], [158.9336791809181, 179.7823388851002, 178.0604075691412, 199.37642192347465], [61.91445836990353, 294.6390311024773, 88.49708879584861, 58662, 164.19234746639088], [885.5179911970412, 90.61270077034115, 889.235807860262, 121.54498448810754], [1015.269404356949, 102.1392783681939, 1015.050946],
  { "filename": "WT12_s002.png", "nr": 2, "width": 1542, "height": 1043, "anchoring": [371.0693653388204, 400.1082335811191, 271.4208510901433, -285.02310689525183, 5.36, 957.791106514995], [584.2732666905024, 932.4917315923844, 510.403079076277, 966.4198552223371], [359.2043812783754, 835.9603167157245, 308.61581525542334, 5, 117.56670113753879], [406.14603348118436, 81.28249964008472, 396.02099370188944, 76.58014477766287], [520.1164561616797, 39.404388692296116, 521.19384184, .8817354793562, 198.46122026887284], [1250.9916999758755, 393.4987245397573, 1262.5192442267319, 390.4508790072389], [817.8818020398444, 269.730993208912, 86.044, 182.3638908327502, 314.9493278179938], [222.94446135906847, 572.0004626471207, 201.78726362085375, 568.4188210961737], [312.5632405962027, 154.85810614],
  { "filename": "WT12_s003.png", "nr": 3, "width": 1456, "height": 1065, "anchoring": [381.2062803248773, 394.02823867332177, 268.55361487723495, -278.119327818736, 9.44, 951.561505067216], [535.8093385588898, 980.8583055353024, 514.3358547655068, 1003.3247156153051], [212.85010186866418, 810.3727767633493, 193.839636913767, 520.9358841778699], [93.14820460819202, 323.36135727315434, 92.5143721633888, 325.99793174767325], [420.84216738875875, 91.44005448680745, 414.1119515885023, 733736762481, 462.5646328852119], [818.4366936989763, 380.1098546307711, 820.5143721633889, 343.6194415718718], [819.0653428419414, 442.34099571294314, 826.6],
  { "filename": "WT12_s004.png", "nr": 4, "width": 1593, "height": 998, "anchoring": [381.6587380533997, 388.8224143830929, 280.882885458828, -302.5691363016935, 19.140, 97, 904.8827300930714], [647.4430717421227, 890.2999747643787, 636.9935191186, 902.0186142709409], [574.431299839091, 857.5182834897785, 566.7900194426442, 6409, 51.002895532575], [420.7410010806885, 92.96215292311838, 419.1561892417369, 77.40434332988625], [989.5760028583768, 25.735164626800632, 972.524951393], [5931, 72.24405377456048], [460.99575767947897, 173.06378438810458, 454.2579300797149, 151.712512026577], [427.4101179964821, 136.3411192109447, 424.31821127, ] }
```

f. It is easiest to paste after right before the end brackets, but note commas separate each image file



Note

be sure to add comma to the file data before the one you've added

```
97001034], [420.44087278315914, 934.6606137398061, 381.07110  
838677], [71.70379268212778, 529.8299485138632, 60.57488653!  
252.20785935884177], [1354.6219441219812, 454.248721425482!  
4190620271, 278.6401240951395]]},  
43019648399], [792.6120472205162, 908.7441136063766, 779.46  
6670113753878], [567.5727660373328, 45.83730621828785, 571.9  
6928645294726], [644.9251250872659, 76.15468897142787, 648.:
```

g. Your images should now appear when you open Visualign

QUINT Tips & Tricks: Nutil

17 *Nutil Settings and Image Naming Nomenclature*

Nutil can sometimes make a fuss if settings are wrong or the names given to images are improper. Some guidelines to follow when working with Nutil are below:

17.1 **Choosing the right Atlas:**

Be sure to select Allen Mouse Brain 2017 for Reference Atlas (this is the edition Visualign uses)

- Sometimes Nutil will throw an error message directing you to this error

```
Tue Jun 4 16:37:04 2024 : Warning: Atlas map specified in the anchor file not the same as specified in nutil. Overriding with the specified type: ('Allen Mouse Brain 2017' from the xml anchor file vs 'Allen Mouse Brain 2015' in Nutil)  
Tue Jun 4 16:37:04 2024 : Error: Could not find FLAT files that contains: _s801.
```

```
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
Tue Jun 4 16:56:35 2024 : Trying to access atlas index : 1294 which is above 1288.
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
Tue Jun 4 16:56:35 2024 : Trying to access atlas index : 1301 which is above 1288.
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
Tue Jun 4 16:56:35 2024 : Trying to access atlas index : 1301 which is above 1288.
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
Tue Jun 4 16:56:35 2024 : Trying to access atlas index : 1301 which is above 1288.
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
Tue Jun 4 16:56:35 2024 : Trying to access atlas index : 1301 which is above 1288.
Please make sure that you are using the correct atlas label file (i.e. the same version as was used with QuickNii).
```

- Other times it will put out an error along the lines of “these points are out of range”, be sure to double-check atlas year as Nutil defaults to 2015 while QUINT apps use 2017.

17.2 Naming Sections:

a. Name each section with a unique 3-digit (`_s###`) code. Nutil will not run if two images have the same 3-digit number at the end.

- When analyzing multiple mice with the same figure, opt to use sequential numbers above and below figure of interest
- For example, if I have two replicates from each of 8 mice for figure 82, I may name my sections `s074-s081` and `s083-s090`
- Alternatively, you can just name your images `_s001-s0016` so long as you have a well-kept log denoting which section refers to what.

b. Avoid including `_s` in other parts of image names

- Names like `project_slide_s###` will result in Nutil error as it has trouble recognizing the unique 3-digit number when the `_s` in `_slide` is present
- There is flexibility in what you can put before the `_s###`. Whatever is put there is for the sake of organization and does not influence analysis at all