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Last revised Mar 27, 2022

WormPsyQi User Guide

How to run PsyQi GUI

Before running the pipeline, make sure the pipeline is correctly installed as the installation guideline describes.

1. Run a command prompt, go into the pipeline directory
cd <your_pipeline_directory>

2. Activate the virtual environment

Windows: .***venv\Scripts\activate***

Linux/OSX: ***source venv/bin/activate***

* '(venv)' should appear in front of the command line

3. Run the pipeline

python -m gui.main

* The GUI window shown in Figure 1 should show up

Basic UI (see *Figure 1*)

1. Control panel
    - Set parameters and start processing in this panel
    - Consist of multiple tabs on the top (see page 3 for detail)
        i. Settings
            - Set parameters.
            - Run all steps with one-button-click.
        ii. Mask ~ Quantify
            - Check the parameters.
            - Run a single step.
2. Log panel
    - Logs will appear in this panel
    - Format: [<log level>] <timestamp> - <message>

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Figure 1: Basic UI

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Settings tab

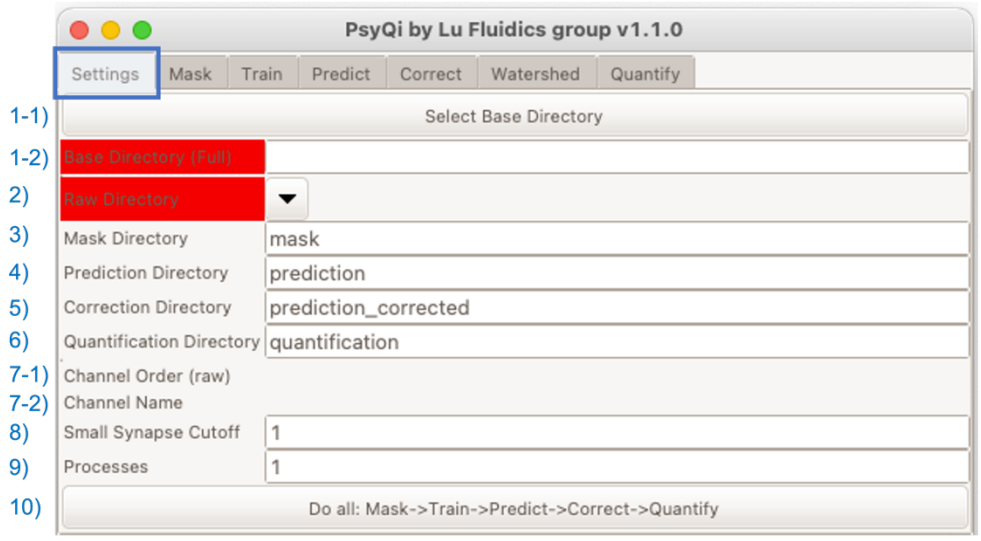

Figure 2: **Settings tab before selecting a base directory**. Note that 'Base Directory (Full)' and 'Raw Directory' menus are highlighted in red, meaning that input is required. Once you put any value, they turn normal. Also, 'Channel Order (raw)' and 'Channel Name' menus are void until 'Base Directory (Full)' menu is filled.

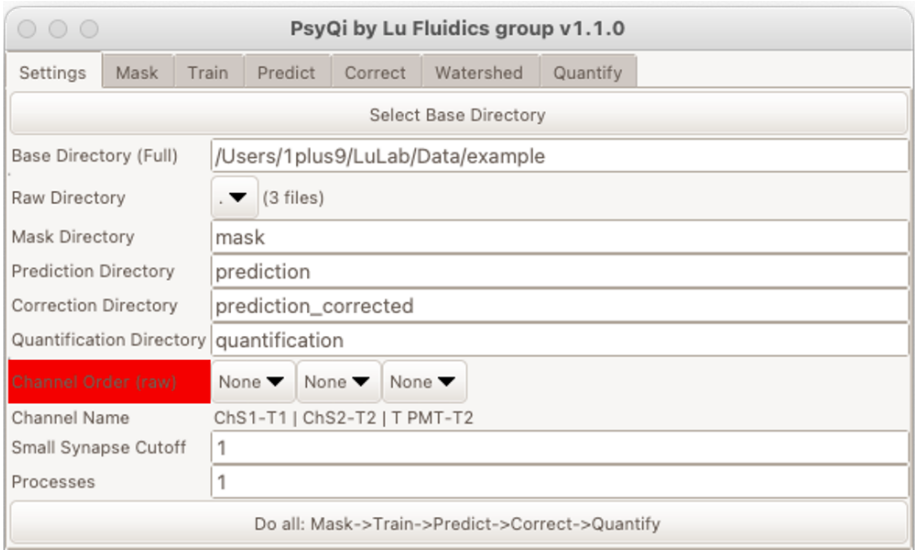

Figure 3: **Settings tab after selecting a base directory**. Note that the dropdown lists and texts have appeared next to 'Channel Order (raw)' and 'Channel Name' menus.

1. Base Directory
   - A base directory is where the user has input data and PsyQi will save output data.
     1) Select Base Directory: Open a base directory select dialog (see *Figure 4*) that enables users to select a base directory.
     2) Base Directory (Full): After selecting a base directory in the dialog, the full path of the selected directory appears in the text box. Users can also directly edit the text.
2. Raw Directory
   - Raw directory is a subdirectory that contains the input microscopy images.

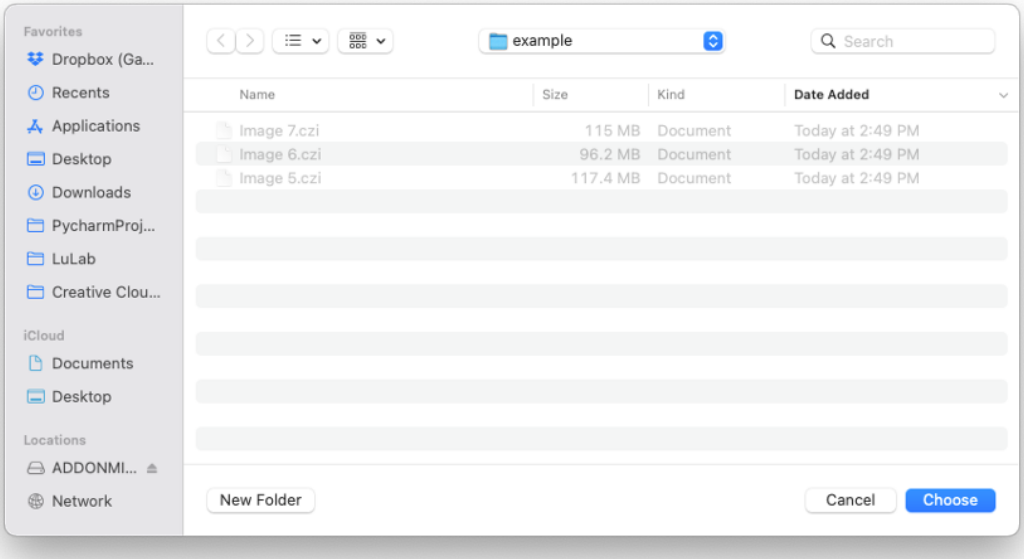

Figure 4: **Base directory select dialog**

- After the base directory is selected, PsyQi automatically surveys all the directories in the base directory including '.', the base directory itself, and make them as a drop-down list. Select the folder where you have the raw images in the list.
- The text next to the drop-down list displays the number of image files in the selected directory.

3. Mask Directory
   - The 'Mask' process writes output in this directory. (See **Mask tab** for details)
   - The default value is 'mask'.
4. Prediction Directory
   - The 'Predict' process writes output in this directory. (See **Predict tab** for details)
   - The default value is 'prediction'.
5. Correction Directory
   - The 'Correct' process writes output in this directory. (See **Correct tab** for details)
   - The default value is 'prediction_corrected'.
6. Quantification Directory
   - The 'Quantify' process writes output in this directory. (See **Quantify tab** for details)
   - The default value is 'quantification'.
7. Channel information
   - In this menu, users must provide correct information about the channel order of the raw images.
     1) Channel Order (raw)
        - Users must specify the channel order of the raw images here. Once a raw directory is selected, PsyQi reads the metadata of the first image file in the raw directory and make the same number of drop-down lists to the number of color channels

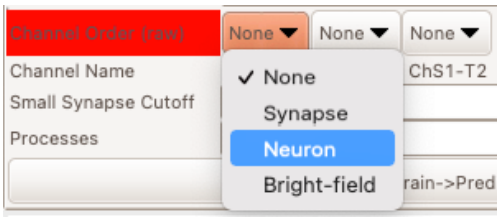

Figure 5: **Channel Order dropdown menu**

in the image. For each channel, users can pick an option among 'None', 'Synapse', 'Neuron', and 'Bright-field' (see *Figure 5*.) Choosing the right options is necessary for the following processes.

- For example, if the first channel is showing neuronal marker, the second channel is showing synapses, and the third channel is showing bright-field, then 'Neuron', 'Synapse', and 'Bright-field' should be selected in that particular order.

2) Channel Name
   - This menu provides additional information about the channels by reading the metadata from the first image file in the raw directory.

8. Small Synapse Cutoff
   - In the prediction process, segmented synapses of equal or less volume to this cutoff value will be eliminated.
   - The default value is 1, which will eliminate single-voxel synapses. If you do not want to eliminate any synapses, set this value as 0.
9. Processes
   - This menu indicates the number of processes to use. To maximize performance, check the number of logical processors your machine has (Task Manager->Performance->CPU->Logical Processors) and put that number in the text box.
   - The default value is 1.
10. 'Do all…' button
    - Perform all steps sequentially. However, each step can be performed separately in the other tabs.

Mask tab

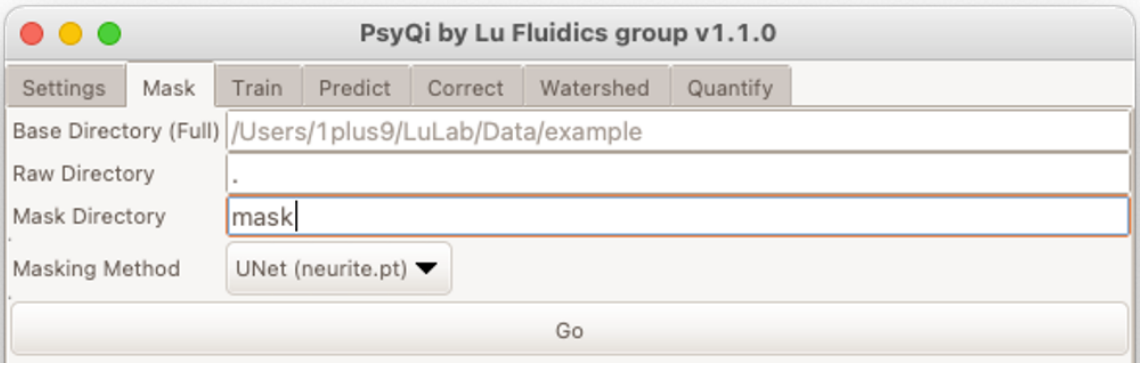

Figure 6: **Mask tab**

In the mask step, the pipeline takes the raw images from the raw directory and segment out neurite, generating binary mask images into mask directory. If you already have mask images in the mask folder, PsyQi will bypass them, instead of reprocessing them.

Additional Parameters

1. Masking Method
   - This pipeline provides three different methods for masking. The default is 'UNet (neurite.pt)' method.

1) UNet (neurite.pt)
   - Using a deep-learning network called U-Net designed for biomedical image segmentation. Slow but accurate. You can install CUDA 10.2 to speed up this method.
2) UNet (multi_scale.pt)
   - Similar to the previous method, but this method segments out the neurite and synapses simultaneously. Note that this is less accurate in the neurite segmentation part compared to the previous one.
3) Edge detection
   - Using a conventional edge-detection based method. Relatively fast but less accurate.

Train tab

In the train step, the pipeline takes synapse label images and corresponding original images from the label directory to train a pixel classifier. A label image should be named after the original image with the prefix '_label' (ex: 'Image 1.czi' for original & 'Image 1_label.tif' for label). After training, it serializes and saves the classifier in the train directory. You can reuse those files for skipping the labeling and training steps later. You can choose a model for your classifier in 'Classifier Model' dropdown menu. The default value is 'Kernel SVM', which is generally more robust but relatively slow. This step can be skipped if the user is going to use the built-in classifiers for the synapse segmentation.

Additional Parameters

1. Label Directory
   - 'Train' process takes input files from this directory. (See **Label tab** for details) One or more hand-labeled synapse images and corresponding raw images are required to do 'Train' process. If you are not familiar with the hand-labeling, see this document.
   - The default value is 'label'.
2. Classifier Directory
   - 'Train' process writes the output in this directory.
   - The default value is 'classifier'.
3. Classifier Model
   - Choose the type of classifier. The default value is 'Kernel SVM', which is generally more robust but relatively slow. 'AdaBoost' is not fully optimized yet, but very fast compared to SVM.

Predict tab

In the prediction, step, the pipeline takes 1) raw images from the raw directory, 2) neurite masks from the mask directory and 3) a pixel classifier either selected from the built-in classifiers or a custom classifier from the classifier directory as input. It generates binary synapse prediction images as output into the prediction directory.

Additional Parameters

1. Classifier
   - Choose to use either one of the built-in classifiers or a custom classifier.
2. Classifier option

- If 'Built-in' option is selected, users further need to select from the radio buttons (see *Figure 7*.) If 'Custom' option is selected, users need to specify the directory where the serialized classifier is located (see *Figure 8*.)
  1) Built-in Type
     - PsyQi provides four different types of built-in classifiers. 'GRASP (sparse)' is optimized to segment sparsely expressed GRASP puncta. 'GRASP (dense)' is optimized to segment densely expressed GRASP puncta. Expected radius of the synaptic puncta for these classifiers is 1-5 pixels. 'CLA-1' is optimized to segment CLA-1 GFP puncta, which usually have larger radius than GRASP. 'RAB-3' is optimized to segment more diffused fluorescent signals.
  2) Custom Classifier Dir.
     - Put the directory name which contains the serialized classifier made in the train step.

3. Use the neurite mask
   - If checked, synaptic puncta outside of the neurite mask from the mask step are removed.

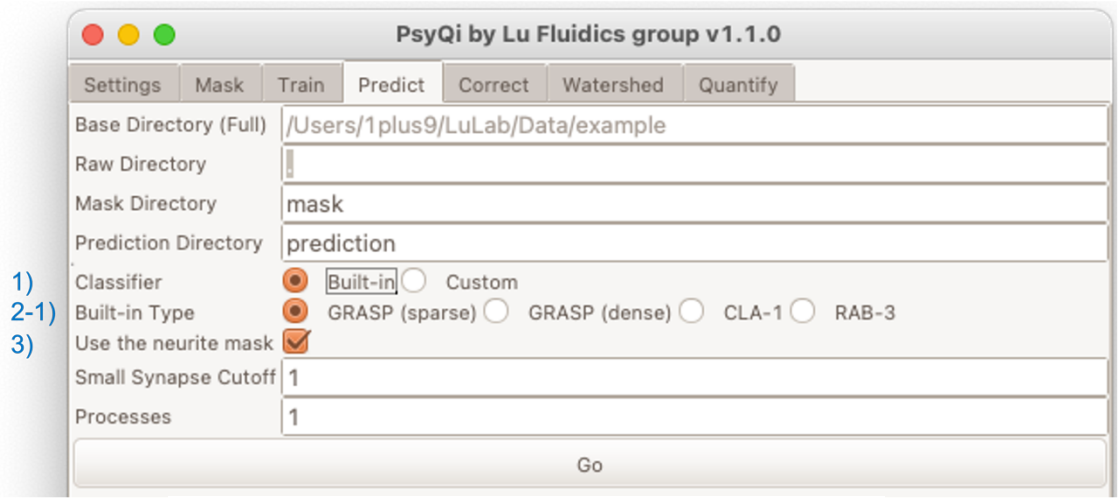

Figure 7: **Predict tab with 'Built-in' is selected in the Classifier menu.**

Figure 8: **Predict tab with 'Custom' is selected in the Classifier menu.**

Correct tab

In the correction step, the pipeline takes the predicted synapse images from the prediction directory and corrects them using separate GUI Synapse Corrector. Synapse Corrector offers a convenient visualization of segmentation result and synapse-wise rejection/regional rejection functionality. The corrected result is saved in the correction directory, but the user can change the save directory in Synapse Corrector.

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Quantify tab

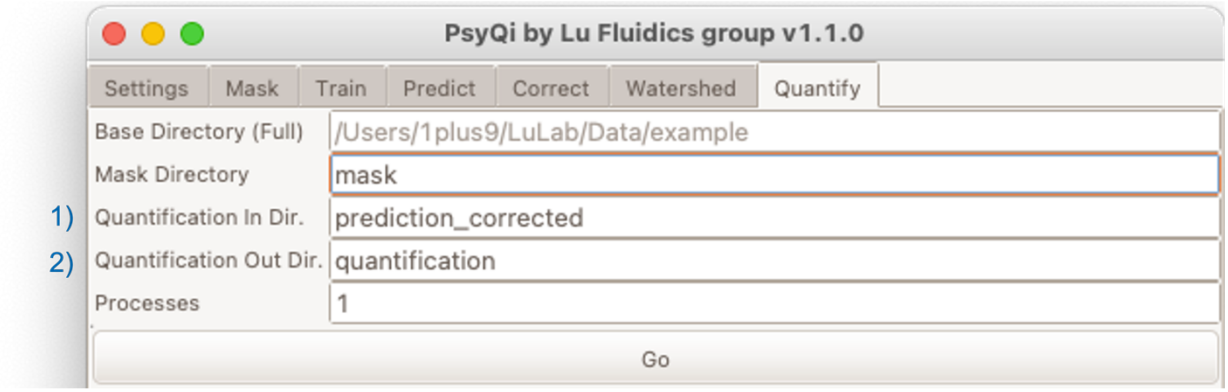

Figure 9: **Quantify tab**

In the quantification step, the pipeline takes the segmented synapse images from the "Quantification In Dir." directory and generates the quantification result into the "Quantification Out Dir." directory. The quantification result includes the position and size of each synapse and some basic statistics such as the average number of synapses.

Additional Parameters

1. Quantification In Dir.
   - Designate the directory where the segmented images you want to quantify are located.
2. Quantification Out Dir.
   - Designate the directory where the quantification result will be placed.