

Annotating Pan-Neuronal Fluorophore Volumes for ID'ing Neurons in *C. elegans*

Anson Sathaseevan, December 2019

(Adapted from Albert Lin's 'Worm Microfluidic Analysis Code' Manual)

This document outlines the procedure for utilizing the volume_viewer UI to create ID annotations for single- and multi- fluorophore marked neurons in the roundworm *Caenorhabditis elegans*. This methodology supports annotations of volumes containing multiple datasets (ex: DIC, fluorophores, etc.).

Please contact anson.sathaseevan@mail.utoronto.ca with any questions.

Programs required to use the annotator:

- 1) *Google Chrome*
- 2) *A Text Editor application*
- 3) *Web Server for Chrome* (<https://chrome.google.com/webstore/detail/web-server-for-chrome/ofhbbkphhbklhfoeikjpcbhemoegcigb?hl=en>)
- 4) *Fiji/ImageJ (Optional)*

Generating Volumes:

Pseudocolored volumes for annotation are typically generated *a priori* in relevant orientations (ex: XY, XZ and YZ views). If the user has their volumes loaded into ImageJ (or Fiji) in a suitable format (i.e. .TIF) they can generate images for all of these orientations using the following instructions:

- 1) Open Fiji/ImageJ
- 2) From the options menu, go to plugins>>new>>macro
- 3) Copy and paste the following code into the edit window

```
dim_z = 21;
dim_x = 512;
dim_y = 256

path1=getDirectory("Mydir");
file_name=getInfo("image.filename");

for (i=1; i<=dim_z; i+=1) {
    Stack.setSlice(i);
    saveAs("jpeg", path1+"Z_" + i + "_2_0.jpeg");
}
```

```

run("Reslice [/]...", "output=1.000 start=Left avoid");

for (i=1; i<=dim_x; i+=1) {
    Stack.setSlice(i);
    saveAs("jpeg", path1+"X_" + i + "_2_0.jpeg");
}

selectWindow(file_name);

run("Reslice [/]...", "output=1.000 start=Top avoid");
run("Rotate 90 Degrees Right");

for (i=1; i<=dim_y; i+=1) {
    Stack.setSlice(i);
    saveAs("jpeg", path1+"Y_" + i + "_2_0.jpeg");
}

close("*")

```

- 4) Change the value of `dim_z` variable to match the number of stacks in the volume. Change `dim_x` and `dim_y` to match the other dimensions of the volume.
- 5) With the .TIF stack (1 file) opened as the current window, hit “Run”
- 6) Choose the directory to where the generated .JPEG files should be saved (Create a separate folder with a meaningful name – ex: “muscle_contract_ATR_04”).
 - a. Elaboration: this short and simple macro generates annotatable .JPEG images of a volume for annotation in the volume_viewer.
- 7) Move the folder containing the stored .JPEG files to the directory: `volume_viewer/images`

Loading Volumes:

To create a handle to the newly generated folder containing the .JPEG files for annotation:

- 1) Open the ‘datasets.json’ file under `volume_viewer` using a preferred text editor application (ex: Sublime Text, and even TextEdit/Notepad will work):
- 2) Add the following lines for the new volume to be annotated:

```

"S_001": {
  "id": "S_001",
  "shape_x": 256,
  "shape_y": 128,

```

```

"shape_z": 21,
"shape_c": 2,
"shape_t": 0,
"pixel_size_x": 2,
"pixel_size_y": 2,
"pixel_size_z": 8
}

```

- 3) The values for `shape_x` and `shape_y` should match the dimensions of the generated .JPEG files. The value for `shape_z` should match the number of z-stack images for a given volume. The values for `shape_c` and `shape_t` are used to quickly query through the dataset volumes in the annotator. The value for `shape_t` specifies how many datasets a given volume has (for example: a loaded volume might contain both DIC and pseudocolor images, each of which represent a distinct dataset). Generally, these values can be left unaltered. Keep the values for binning the same.
- 4) Change the initial name and the “id” field (highlighted above) to match exactly the animal ID (i.e. the name of the folder containing the images for a given volume).
 - a. Elaboration: If no datasets show up in the dropdown, there is probably a mistake in the datasets.json file, either a missing comma or bracket.

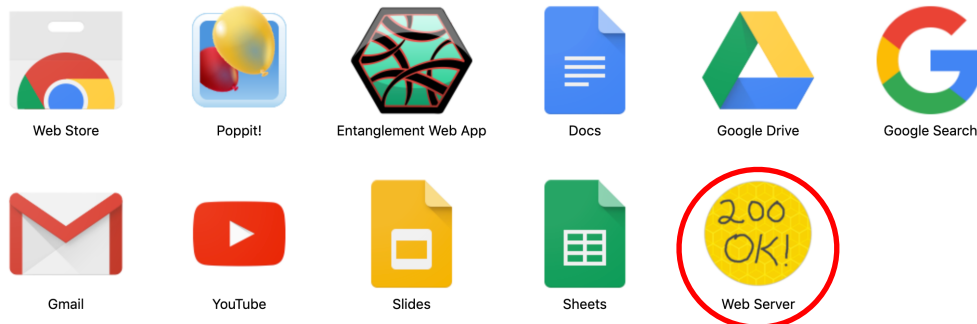
These steps only need to be performed once for a given volume.

Initializing the Annotator:

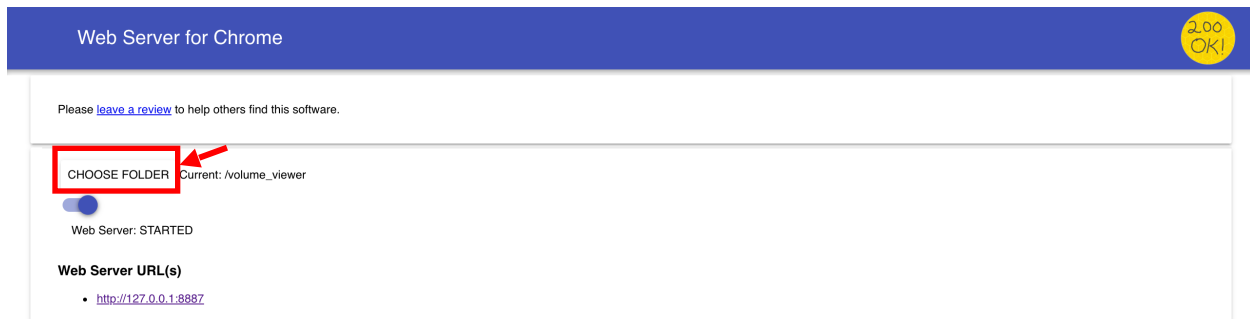
- 1) From Google Chrome, type the following into the search bar:

```
chrome://apps
```

- 2) Open ‘Web Server for Chrome’ by selecting its favicon

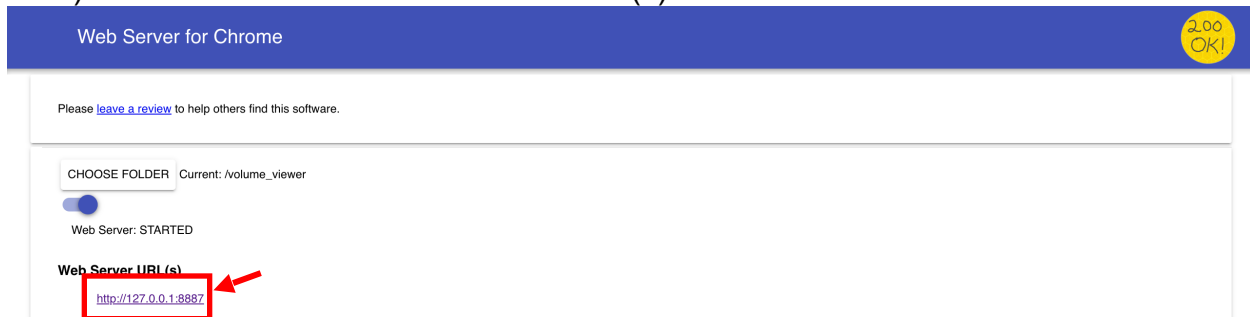


- 3) In the window which opens, select ‘Choose Folder’ and point to the location of the folder containing the stored volumes (i.e. `volume_viewer`).



- a. Elaboration: After the path to the folder containing the annotatable volumes (ex: 'volume_viewer') has been loaded, Step 3 can be skipped when re-initializing the annotator. The user will need to perform this step again if they change the location of this folder.

4) Click the link under 'Web Server URL(s)'



Annotating Volumes within the Annotator:

The neurons in the head volume may now be annotated. The annotator has three windows: an XY projection, XZ projection, and YZ projection. Each window has four options, Selection (Default), Pan, Zoom In, Zoom Out, and Fit to Viewer. Use these to move about, but stay in Selection mode when making annotations. The sliders will move through the volume in X, Y, and Z, with the gray lines indicating the planes of view. The hotkeys for the annotator (active when the XY projection window is selected) are:

C: up one frame in Z

V: down one frame in Z

E: green channel

R: red channel

D: previous dataset


F: next dataset

1) Use the dropdown menu to navigate to a desired volume for annotation.

- a. Elaboration: If you select a dataset and do not see any images appear in the annotation UI, select the XY projection with the cursor (the largest of the three visible windows) and hit 'D' to change the dataset until the volume comes into view.

- 2) To annotate a neuron, move in Z in the selected channel until a location is found that is thought to be close to the center of the nucleus of the neuron in X, Y, and Z. Double click to create an annotation. This should appear as a red dot. A red dot is an annotated but unidentified neuron.
- 3) To label the neuron, click the red dot, and a text box will appear. Write the best guess for the ID of the neuron (already used labels appear in grey in the dropdown, while unused neurons appear in white). To delete a bad annotation, hit the trash can button.
 - a. Elaboration #1: Low confidence annotations can be marked in yellow using the “?” symbol which appears from the dropdown menu.
 - b. Elaboration #2: Neurons can be marked without giving them annotations. These neurons will appear as red after the initial annotation spot is generated.

Saving annotations:

At any time, the current set of annotations can be saved by click the  icon adjacent to the dropdown menu. This generates an ‘annotations.json’ file containing all newly generated annotations (in addition to those which were generated in previous sessions). Transfer this file to the volume_viewer directory containing the old ‘annotations.json’ file and overwrite it.

Exiting and Re-opening the Annotator:

It is imperative that the user save any changes (see section above) before exiting the annotator. Unsaved annotations will not reload when the annotator is re-initialized!

The annotation window can be closed at any time by exiting the tab in which it is open or by exiting Chrome. The annotation window can be re-initialized at any time by following the relevant steps above.

- a. Elaboration: Multiple tabs containing multiple annotation windows can be opened simultaneously. This can be very useful when annotating many volumes collected in parallel. It is important to note that annotations made in one window do not transfer between other windows/tabs. Make all annotations in the SAME window/tab before saving and overwriting the old ‘annotations.json’ file!