Exploring Source Data and Models behind NeuroSC

Intro: Viewing/Exploration software, CytoSHOW.

CytoSHOW is a custom-modified version of the open-source, community-developed program ImageJ. As such, it has many menu commands and features in common with popular versions ImageJv1, ImageJv2, and Fiji. All of the images, image annotations, and 3D models used to create NeuroSC were processed in CytoSHOW. Installing and using CytoSHOW to interact with these images will enhance your understanding of data behind the models.

If you ***have ever*** used ImageJ before, you may find much in CytoSHOW to be arranged much as you already know it. However, please do browse this guide now, to learn the several unique ways in which image data, rendered models, and metadata (all ImageJ compatible in general) may be optimally handled and understood using functionality that is unique to CytoSHOW.

If you ***have never*** used ImageJ before, please understand that CytoSHOW is designed so novices *can* immersively experience the full scope of C. elegans connectomic analyses by learning a few intuitive steps, without a deep understanding of image processing theory or the many other special capabilities that CytoSHOW provides. Beyond those key functions, you can certainly begin to familiarize yourself with all the basics and many capabilities of the ImageJ platforms, via the excellent Introductory and Tutorial web content online at ImageJ.net.

And, in either case above, please record/submit your questions, observations, and requests regarding CytoSHOW to support@cytoshow.org. The code is under constant active development. We are eager to hear your suggestions.

Final Note: We use CytoSHOW for a number of experimental/specialized tasks in many forms of imaging…There are loads of menu commands you can/should ignore while exploring the NeuroSC data set. If we don't mention them in this guide, you won't need them (but will be happy to explain them if you ask).

Step 1. Get CytoSHOW installed on your computer.
Take your pick, or try both.

OptionA: Stand-Alone Versions of CytoSHOW

Download an all-in-one .zip file (Mac OS) or .msi installer (Windows) for the app. Manually copy the application folder to the Applications folder on Mac, or to the “Program Files” folder on Windows.

OptionB: Light-Weight, Self-Updating Versions of CytoSHOW, if Your System Has Java 8 Already Installed

Download and save a **.jnlp** file as your shortcut to launch the latest version of the CytoSHOW app, delivered on demand from the CytoSHOW web server via Java Web Start. Since we have not generated standalone Linux versions, OptionB is the choice for anyone running CytoSHOW on Linux. The required (for OptionB) one-time pre-installation of Java Runtime Environment JRE 8 can be handled for any OS with a specific matching installer from this page:
https://www.openlogic.com/openjdk-downloads
or this page:
https://www.oracle.com/java/technologies/javase/javase8u211-later-archive-downloads.html

For either OptionA or OptionB, since the program is being obtained somewhere other than the official App Store for your OS, you may need to allow/accept security adjustments to permit CytoSHOW to be opened and run on your computer. Rest assured, the app files that we have posted for download are certified by a chain of trust from an internationally accredited code-signing authority (Sectigo) through the University of Connecticut.

Please contact us for advice if you have trouble getting CytoSHOW to install and run.

Step 2. 3D Neuroanatomy Models shown in NeuroSC, here available in OBJ-MTL and:or GLTF Format/

The 3D models of cell morphologies, cell-cell contact patches, and putative synapses shown in the NeuroSC viewer are all encoded as GLTFs, a new losslessly-compressed web-optimized format, which is unfortunately not yet generally supported by all available viewing software. In our construction of these model surface meshes from the annotated coordinates within vEM image stacks, we used the Wavefront format to save vertices, faces, and shading in OBJ and MTL file pairs. This 30-year-old, universally adopted system uses human-readable, batch-editable text-encoded files that contain the exact same content as GLTFs…and are readily translated to compressed GLTFs. Every .gltf file displayable in NeurSC has a "parental" .obj/.mtl file pair in the collections posted at this link. **NOTE:** It is critical when working with Wavefront format files that ***each .obj file and its matching .mtl file must be saved in the same folder.*** Otherwise, shapes lose their specific colors and transparencies that can be critical in building complex comprehensible scenes in a 3D viewer.

CytoSHOW provides a custom-tailored 3D viewer, based on adaptation of the excellent IJ3DViewer codebase provided in a now classic plugin to ImageJ. In addition to spatially intuitive, informatically rich user interaction with the objects in the window, CytoSHOW's viewer manages the roster of elements in a complex scene in powerful ways that can speed exploration and understanding. Similarly facile control of scene objects should be coming to the NeuroSC web interface soon, but at this time there are some distinct advantages to working with CytoSHOW with locally-saved .obj/.mtl files to build an ideal 3D view of many component parts.

Click any part's **.obj** link to download it. Same for the matching .**mtl** file. Drag and drop the **.obj** file icon onto the purple-shaded bottom left portion of the CytoSHOW toolbar window. The object will appear in a new IJ3DViewer window. Dragging multiple selected **.obj** icons will open them all in the same new window. And dragging additional **.obj** icons onto an already-open IJ3DV window will add them to the scene. All objects are color-coded, name-aware, and spatially targeted to appear in their correct anatomical position within the viewer.

_Step3_Calibrated_vEM_TIFF_Stacks/

Click the link for any specimen's multi-slice TIFF stack file to download it. Find the downloaded file on your computer, then drag and drop the icon for the **.tif** file onto the green-shaded bottom right portion of the CytoSHOW toolbar window. The stack of slices will be opened "virtually" in an image window that demands minimal system memory and gives you instant access to any slices in the volume without needing to wait for the full multi-gigabyte stack to be read.

The 3D image space represented by the stack has been calibrated into the metadata for each of these TIFF stack files. The nm-to-pixel ratio in x, y, and z dimensions is encoded in any of the measurement tools or functions you might select from the upper toolbar. Any copied subvolumes, and resliced or rendered 3D views from the image data will be likewise calibrated, if you use the function buttons on the left of the window frame to generate them.

These "front-loaded" TIFF stacks are yet another benefit of the ImageJ codebase. They are universally compatible with the full suite of ImageJ projects. However, many "everyday" image browsers are not multislice capable or calibration-aware.

_Step4_Image_Annotations_of Cells,Contacts,and Putative Synapses,_Saved as_CytoSHOW_ROI_tags/

Each image stack is associated with 3 distinct **.zip** archives – cell morpholgies, cell-cell contact patches, and putative synapses – which contain ImageJ ROI tags for each feature annotated by the groups originally reporting these data sets.

To display the tags on the image stack, simply drag and drop the **.zip** file of interest onto the image window and wait for them all to be loaded into the Tag Manager. The loading process occurs with all tags hidden, to speed up the process of many thousands of complex objects being created. When the messages about loading have disappeared from the image window, click twice on the “eye” icon to activate the visibility of the entire tag set on the vEM stack.

Once in the manager, tags can be searched for, sorted, shown in custom groupings, recolored, renamed…in short you can modify them all you like and save a local version of tags that is unique to you. And each image tag set can be used to generate custom 3D .obj models, using the SketchVolumeViewer function linked to the  button on the Tags toolbar in an image window.

All 3 sets of tags – cell morphologies, putative synapses, and cell-cell contact regions – are based on published coordinates from papers by labs that did the annotations.

_Step 5_Why is there no step 5?? Onto step 6!!

_Step6_DC-CPHATE_Models_of_Cell_Adjacency_Clustering_and_Emerging_Strata/

Diffusion Condensation CPHATE plots are viewable from single .obj files that each contain all of the several thousand clustering iteration groups during the course of an experiment on a given brain specimen. Drag-Drop a CPHATE .obj file onto the CytoSHOW window purple region to load the model. Please wait until all of the cluster objects have loaded before interacting with the window.

_Step7_Optional_Custom_ColorLegends_For_ROIs_And_OBJs/

Two examples of ColorLegend.lgd files are given. When such a file is dropped onto an image window or 3D Viewer window, the colors defined for each tagged entity in the window are redefined. The process is rapid, savable, reversible, and fully customizable using either the Tag Manager or a simple text editor to same your own ColorLegend.lgd files.

_Step8_Optional--_All_of_the_Above--NeuroSC_EntireSourceDataBundle.zip

You can download all programs and data files from this web archive within a single .zip file and work with it entirely locally on your own machine, if you like.