**Importing Images and Cell Morphology into the Database**

Starting in June 2024, we will be importing all scientific images of retinas, cells, and brains into the datajoint database. We are starting with images of retinal cells. This also includes their morphology data from the tracing and flattening software.

Doing this sucessfully requires care and attention to detail with file naming and data entry!

In general these are the steps:

1. Make a folder for your import and check that all the files in there are named correctly and have the same settings. What I mean by the same settings is that the number of channels of the images and their meaning (which is a cell fill, which is ChAT, etc.) must be the same for everything in the folder. It’s totally fine if you have taken different images with different settings, but you need to make a separate loading folder for each batch.

2. Export the metadata from the .tif files for the ScanImage .tif files.

3. Load the raw images. Depending on how many and how big they are, this may take a while. Please do this on a machine connected via ethernet. The office PC and rig machines should all work for this.

4. Load the morphology data from the arborData.mat files that are created by RGCMorphologyAnalyzer. This step also requires careful folder structure, but it is very quick and can be done from your own machine.

A few notes before we get started:

1. Any cell recorded in Symphony for which you have an image must be curated BEFORE starting this process. When the Curator runs, it makes an entry in the table sln\_cell.Cell (and also in sln\_cell.RetinalCell). These are required for correctly assigning images to cells. If you forget this step, the importer will assume that a matching cell does not exist, and it will create a new cell. This will be a mess to untangle, so please try to avoid this error!

2. As impied above, there is a distinction between a cell entry (sln\_cell.Cell and sln\_cell.RetinalCell) and a cell recorded in Symphony (which has an entry in sln\_symphony.ExperimentCell). The importers are built to handle cells for which there is no corresponding symphony data. This is totally fine. They will be created as new sln\_cell.Cell entries, each with a with unique cell\_unid. If you do things correctly in the importer, they will also be associated with the correct animal (sln\_animal.Animal) and eye (sln\_animal.Eye).

3. The mapping between cells and images does not need to be 1:1 in either direction. A single image can be linked to multiple cells, since we often take images with more than one cell in them, and a single cell can be mapped to multiple images (e.g. if we took a 2P and a confocal image of the same cell).

4. Stitching needs to be done in FIJI before the import process. You will be importing the stitched image as a single image, not the parts. However, as you will see below, you will need to keep the parts around during the import process.

5. The importer should handle 3 kinds of files: (A) .nd2 files from a Nikon scope, (B) .tif files from ScanImage recorded on the rigs, (C) .tif composite files from FIJI that are made by stitching (whether initially from .nd2 of .tif).

**Import Steps**

**1. Decide which kind of batch import you want to do: by tissue or by cell.**

By tissue: Your folder will be for a single eye. This makes the most sense for eyes in which you imaged a lot of cells, individually, on a confocal.

By cell: Your folder will have a subfolder for each cell named by its Symphony name (e.g. 050224Ac3). Of course, this only works if every cell in the folder has a corresponding (CURATED!) Symphony recording.

Of course, you can mix and match. For example, if you did an experiment on a rig and recorded 5 cells in Symphony but then imaged 6 cells because the last one died before you could get any Symphony data, I would suggest the following. First, do a “by cell” import for the first 5 cells. Then do a “by tissue” import for the last cell by putting it in its own folder.

**2. Set up your import folder correctly. Examples are on the server in the SampleImages folder.**

By tissue:

The code simply searches recursively for all .nd2 or .tif (or both, as you specify) files in the folder. You will specify the DJID of the animal and which eye in a dialog box. From there, it will try to import each file and look at the filename to try to assign it to a recorded cell from that eye. Thus, if there are cells that match recorded cells, their filenames must include the full Symphony name of the cell. Be careful not to have extra files of the .nd2 or .tif extension in this folder.

By cell:

The first level folders must each be named exactly by their Symphony names. Once inside the folder, the code will again look for .nd2 or .tif files. The matching is done by the folder name, not by the file name.

**3. Save the ScanImage metadata.**

For ScanImage files (from the rigs), we need to first export the metadata from the .tif. Currenty, the code to do this is only compiled for Windows. Trung is working on a version for M1 macs. It is called like this: extractScanimageTiffMetadata. This function will prompt you to point at the file for which you want the metadata extracted. It will save a file with the same name but with “\_meta” appended to the end and with a .mat extension instead of .tif. It may also create another file that you can ignore. Leave the metadata file where it is and don’t change its name.

**4. Call the loader:**

By tissue: sln\_image.loadFolder

By cell: sln\_image.loadFolderOfCuratedRigImages

The loader function will prompt you to pick the folder. It will also open a GUI in which you have to fill out the pertinent information. Please do this carefully!

Notes on the channels:

1. You cannot skip channels. The channels are listed in the order of the image. For example, if you took an image on the rig using channels 1 (cell fill) and 3 (DIC), then you should list channel 1 as cell fill and **channel 2** as DIC.

2. For color-merged composite images from FIJI (e.g. ones you have stiched), the order of channels is Red, Green, Blue, Grey, Cyan, Magenta, Yellow (as listed in the MergeColors dialog box). So if you save an image with ChAT red and a cell fill green then channel 1 should be listed as ChAT and channel 2 should be listed as cell fill.

The channel order must be consistent for all cells in the batch load.

3. The z-scale is read in automatically from ScanImage .tif files (from the rigs), but it is NOT read in automatically for .nd2 files. Therefore, you will need to specify it at import. Since you can only specify it once during the batch import, all your images need to have the same z scale. The x-y scale is read automatically from both types of images.

**5. Call the morphology loader.**

Finally, to load the cell morphology data (in the arborData.mat) files, you need to call sln\_image.loadMorphologyFromFolder on EITHER type of batch imported folder. For this function to work, the images must first be loaded and a single file, named arborData.mat, must be in the same folder as the corresponding image file.

**Stitched images:**

For stitched images, there are special instructions.

1. Your file must be named ending in “\_stitched.tif”.

2. The original files for the parts of the image should NOT be in the folder (so they don’t also get imported incorrectly), EXCEPT…

3. One of the parts needs to be saved with the same name but replacing “stitched” with “\_part”. This will allow the code to read in the x,y (and for ScanImage the z) scaling from the part file and then everything else from the stitched file. If this is done for a ScanImage file, you need to extract the metadata as well (see step 3), so the filename will end in “\_part\_meta.mat”.