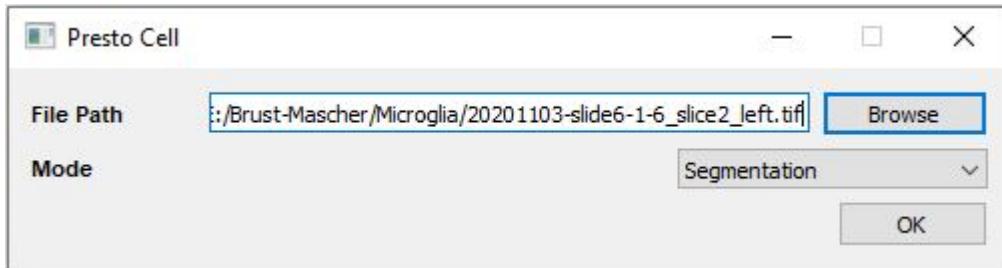


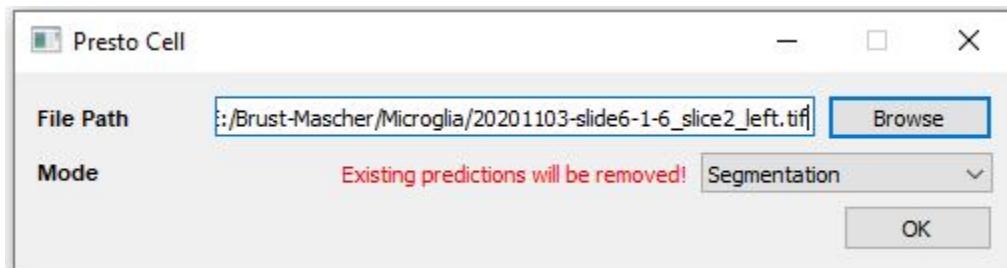
User guide for PrestoCell

Start PrestoCell (currently from command line)

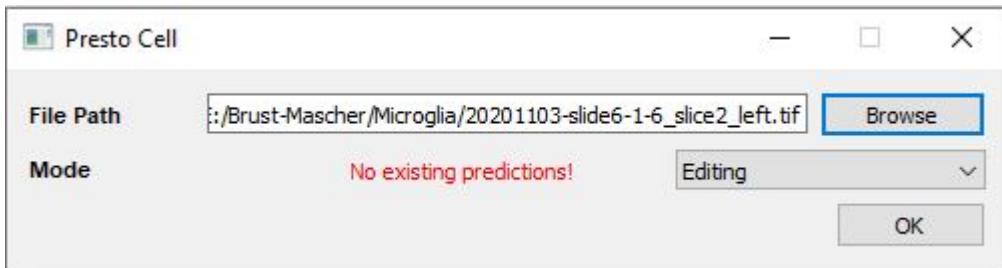
The first window allows you to choose the file and whether to perform segmentation or editing. The file should have two channels, one with nuclear data, one with marker to segment.



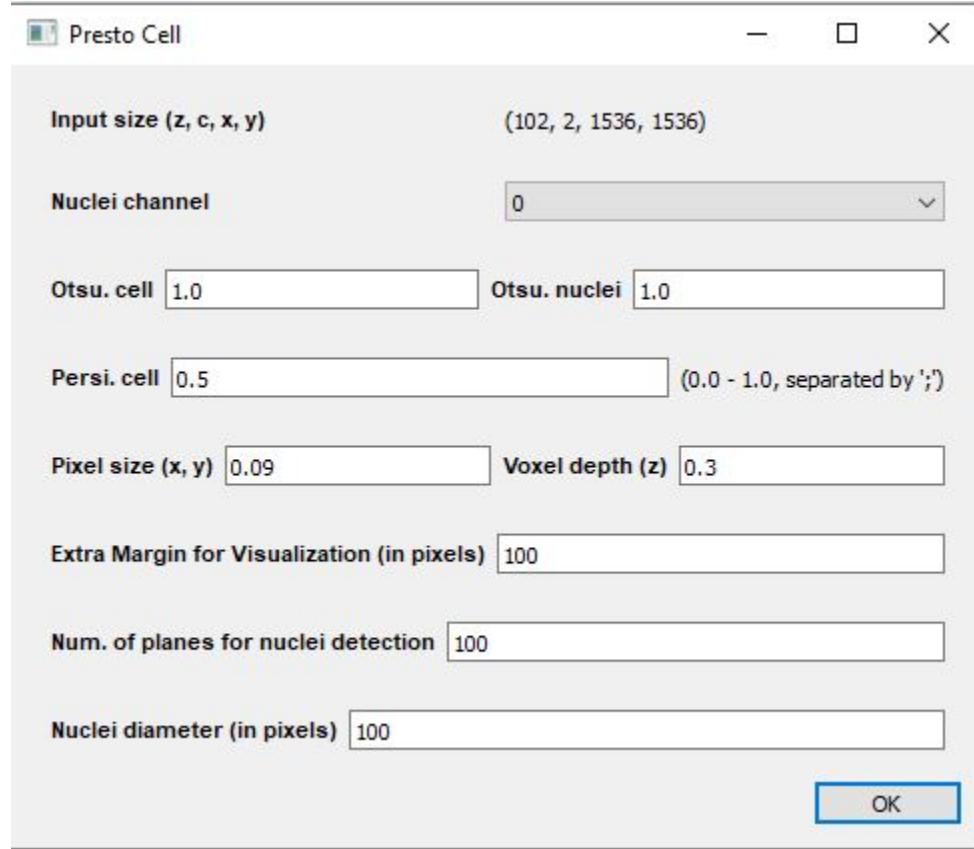
If you select segmentation and there is a previous segmentation result, it will warn that that result will be deleted:



If you select editing and there is no segmentation available it will give a warning:



After pressing ok, a new window opens for parameter selection. If this file had been analyzed before, it will remember the selected parameters, otherwise it will prepopulate with default parameters.



Input – size of file selected (number of planes, number of channels, x, y)

Nuclei channel – select 0 if nuclei data is in channel 1, 1 if nuclei data is in channel 2

Otsu. Cell – select starting Otsu threshold for marker of interest, e.g. microglia, this can be adjusted again.

Otsu. Nuclei – select starting Otsu threshold for nuclei channel, can also be adjusted during run

Persi. cell – select the persistence value(s) for clustering, values should be between 0 and 1. Higher values will keep more clusters for each cell, leading to larger cells, however, some cells will only be identified with low values, so it is best to run it with multiple values to obtain all cells and have the maximum extent of each.

Pixel size (x,y) – pixel size in microns

Voxel depth (z) – step size in microns

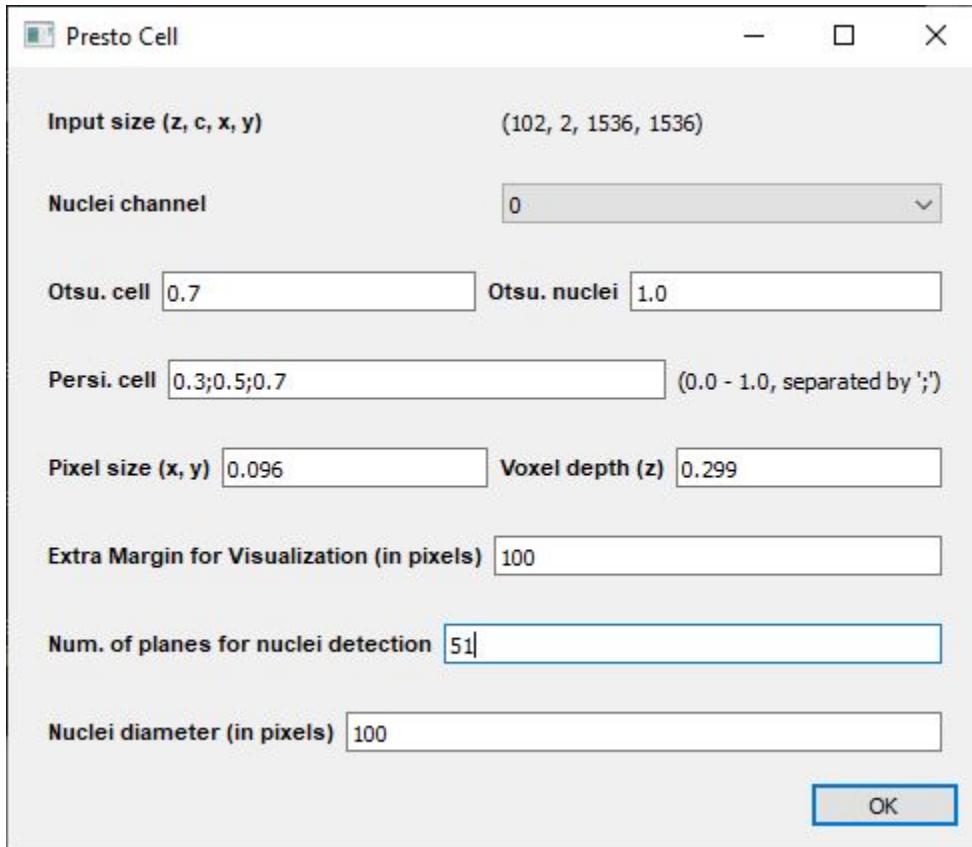
Extra margin for visualization (in pixels) – Margin added around each cell at the editing step, having an appropriate margin allows adding of missing branches.

Num. of planes for nuclei detection – Nuclei are detected in 2D using CellPose, if the stack is deep, it is best to split it for nuclei detection. This parameter defines number of planes to project, a multiple of

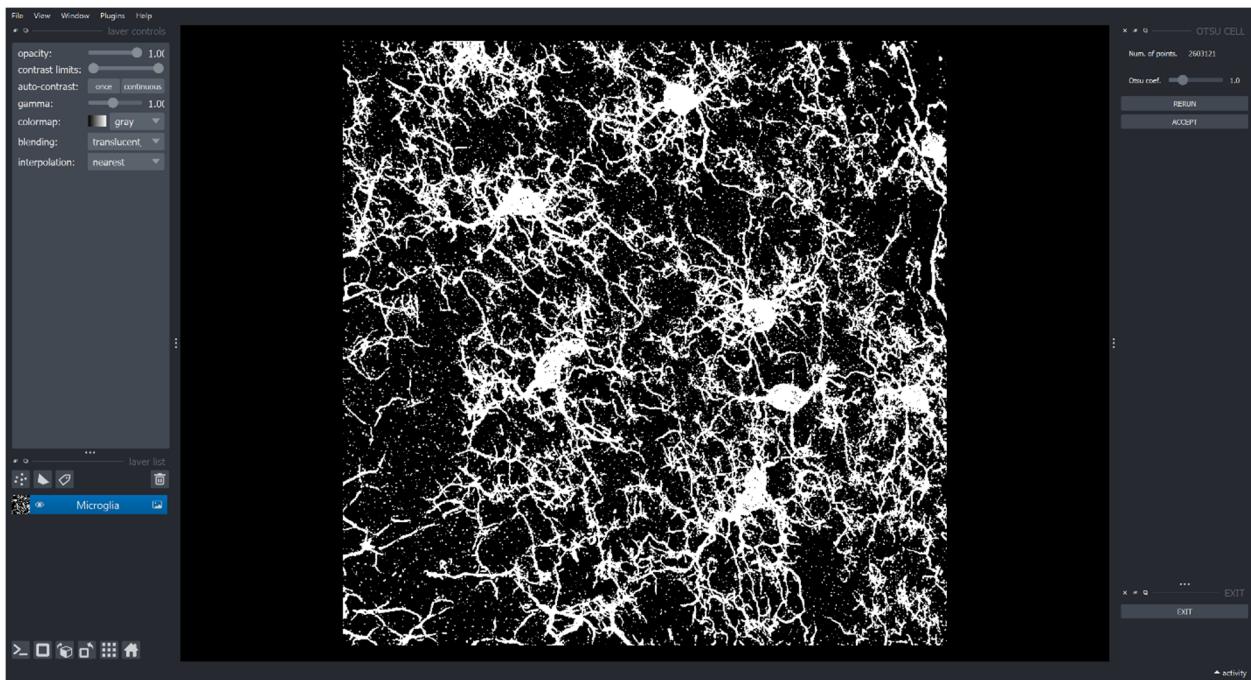
this number should cover most of the stack, for example if your complete stack is 101 planes and you want to divide it into two parts, choose 50, if you choose 51, it will take 51 planes and ignore the other 50.

Nuclei diameter (in pixels) – Diameter used in CellPose for initial nuclei detection, this can be adjusted during run.

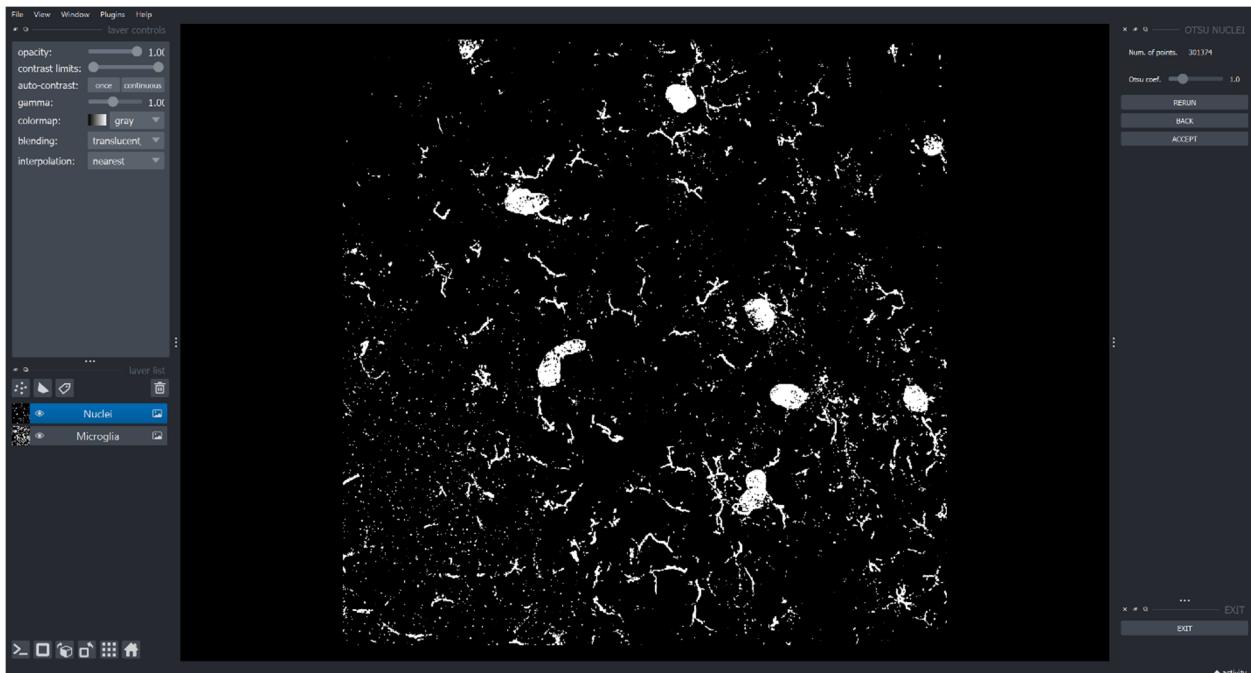
Example window with selected parameters:



The program will first run an OTSU threshold on the channel of interest and show the result in a projection. At this point, you have 3 choices, accept this, rerun with a different OTSU threshold (these choices are right top) or exit the program (right bottom).

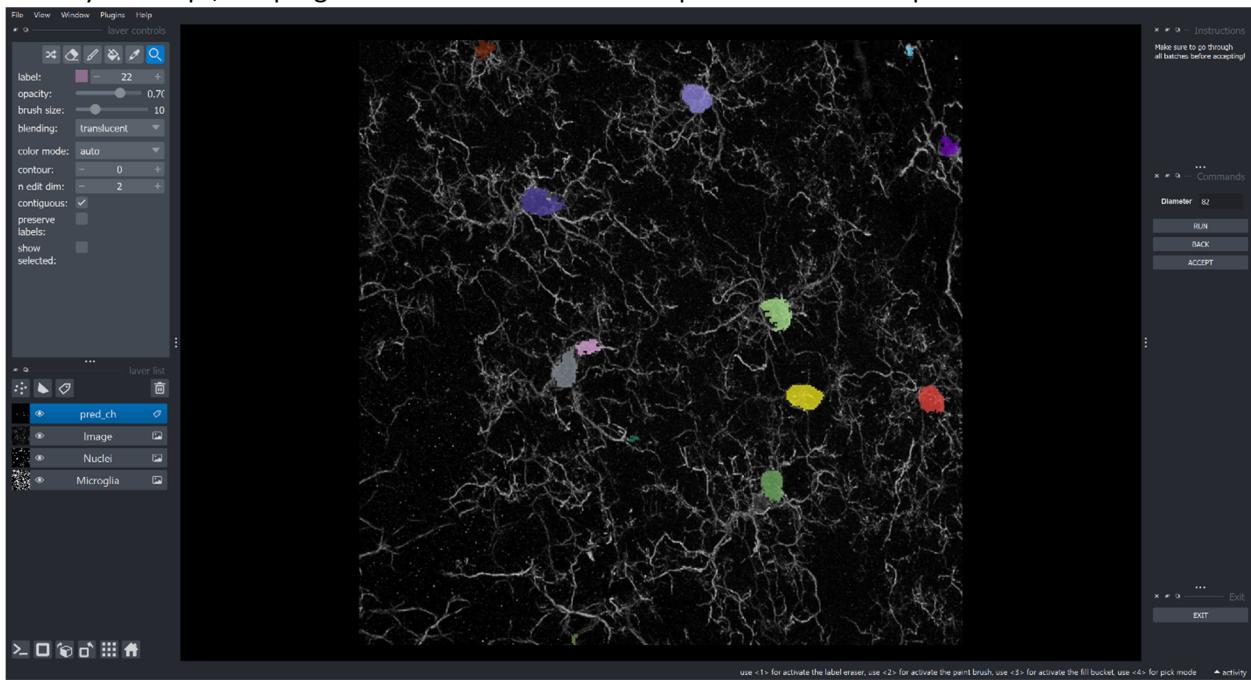


When you accept the threshold, the program will then combine the thresholded image with the nuclei image and OTSU threshold the new image to find the nuclei that overlap the marker of interest.

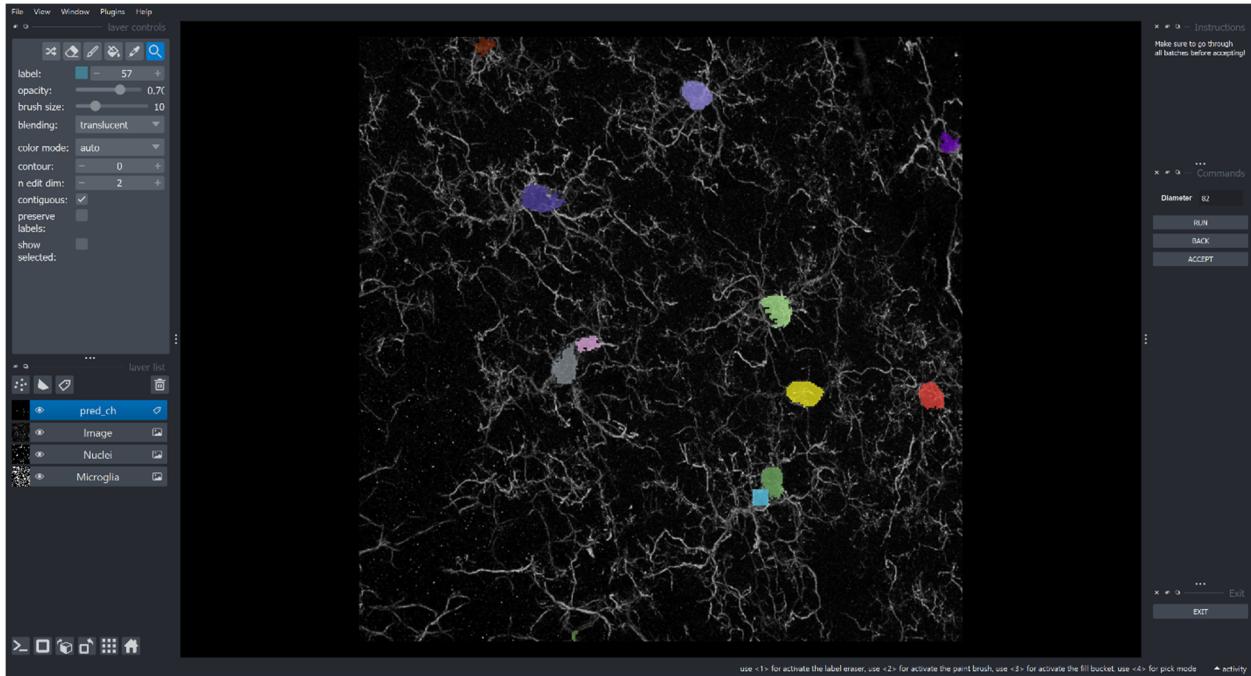


At this point you can rerun with a new OTSU threshold, go back to the cell OTSU threshold, accept or exit the program.

When you accept, the program will run CellPose on the specified number of planes.



Again you can rerun with a new diameter, go back, accept or exit. In addition you can remove or add nuclei. For the example shown above, there are extra detected nuclei and one missing nucleus, double clicking on an existing nucleus will remove it, double clicking on a missing nucleus will add it. You can also hide the image and see the nuclei threshold image.



If the data is divided into smaller number of slices, you need to use the arrow keys to move between the different options before accepting the results.

Once you accept the results, the program will save all the nuclei, run the pbc and then match the cells to the existing nuclei and split the cells if necessary. Once all the cells are matched, it will show each cell for editing.

The image contains 5 layers:

Pred_edit – saved predictions,

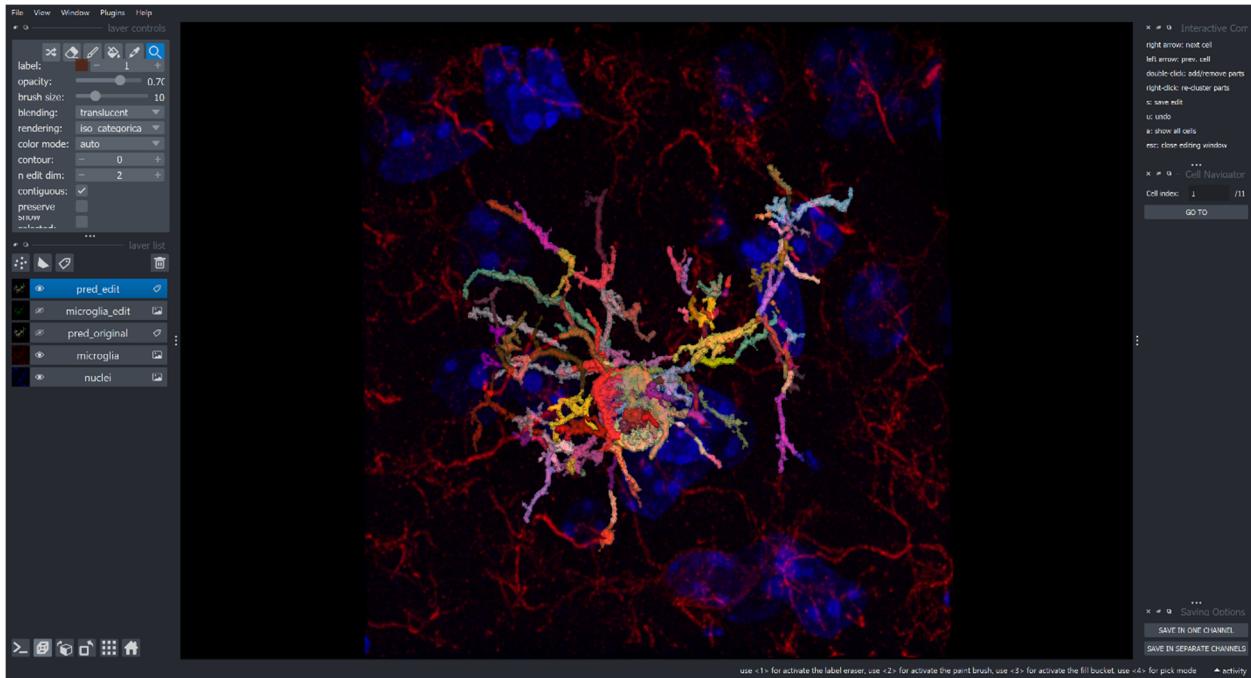
Microglia_edit – microglia in predicted clusters

Pred_original – original predictions

Microglia – original data

Nuclei – original data

Any of these layers can be “turned off” to see other layers.



Editing commands are shown on the right:

Double click on a shown cluster removes it, double click on a branch that should be present it adds the corresponding cluster – this can be done in 3D or 2D

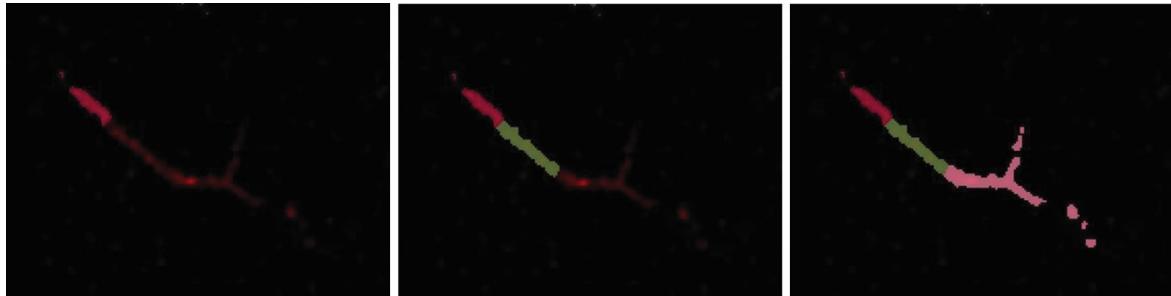
Right click on a cluster reclusters it into smaller clusters – useful if you want to remove part of that cluster

U - undo last command (addition or removal of cluster, or recluster)

S saves all the changes

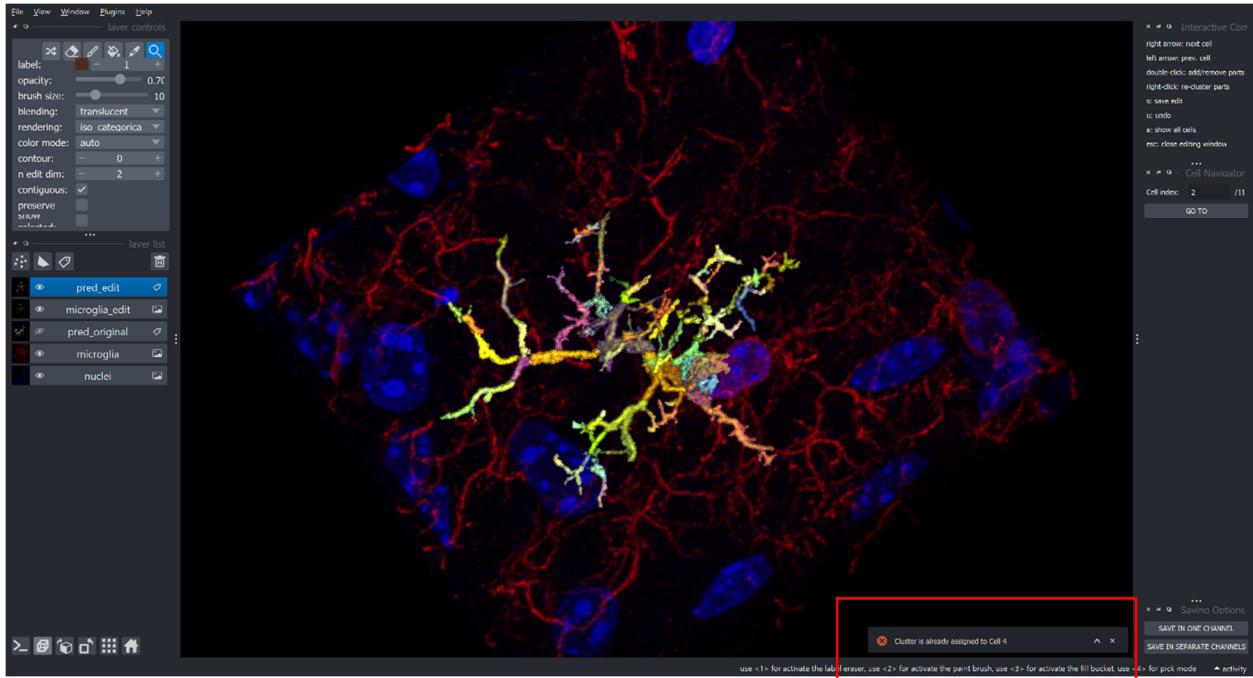
The left and right arrows move between cells without saving the changes

The following example shows addition of clusters in 2D. In the first image, the red cluster was predicted by PrestoCell, but the branch continues, clicking on the branch next to the red cluster added the green cluster, but the branch still continues, clicking adjacent to the green cluster, added the pink cluster.



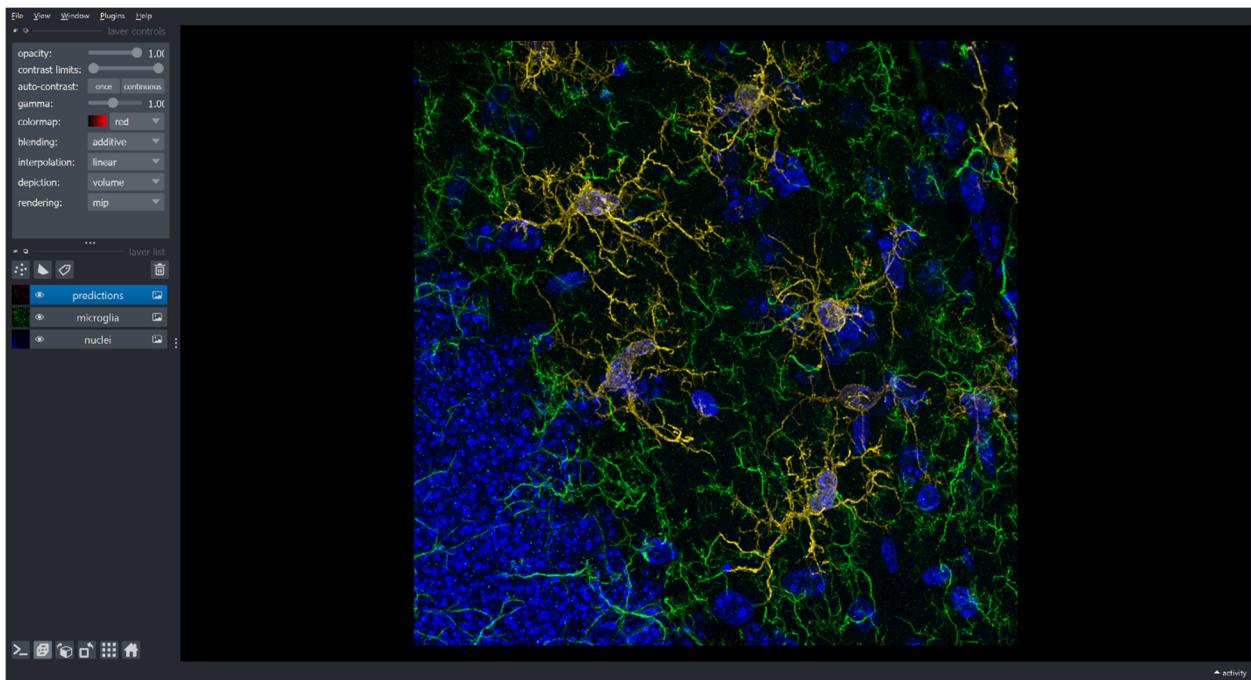
When you are satisfied with the predicted cell, you can save and move on to the next cell.

If you try to add a cluster that is already part of a different cell, a message saying so will pop up:

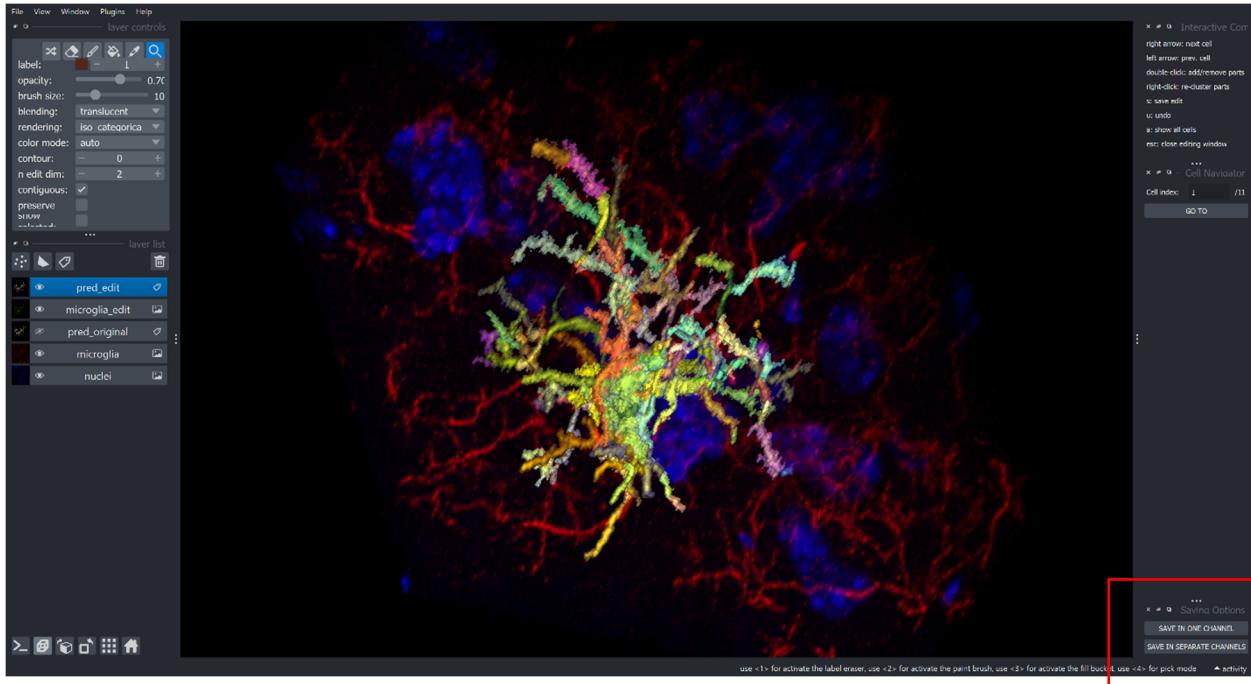


If the cluster should be assigned to the current cell, you need to go to the cell it is assigned to and remove it before you can add it to the current cell.

Once you have edited all the cells, you can see all cells by pressing the “a” key.



Press the “b” key to go back to the cell editing mode and make any necessary changes or save the results either to one channel or to separate channels (or both).



At any time during editing, the “esc” key will exit the program. You can restart it in editing mode and continue editing where you left off.