

## **Using the MaskObjects Function With A Manually Drawn or Imported Region Of Interest**

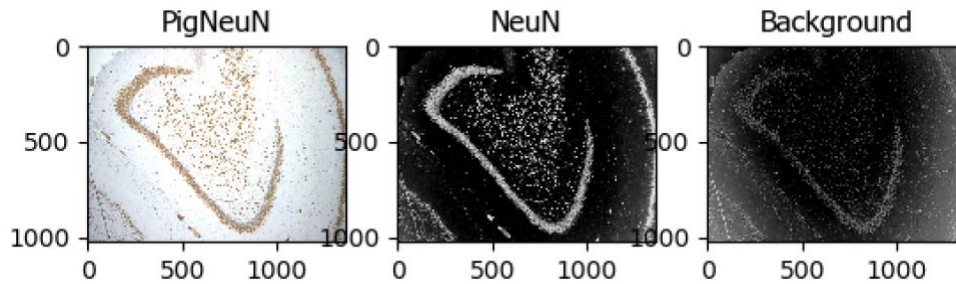
**Goal:** The goal is to identify objects within a region of interest that is either manually drawn within CellProfiler or has been pre-saved as a mask.

**Images:** One multi-colored neuronal image and a binary image of a pre-defined mask representing the Region Of Interest (ROI).

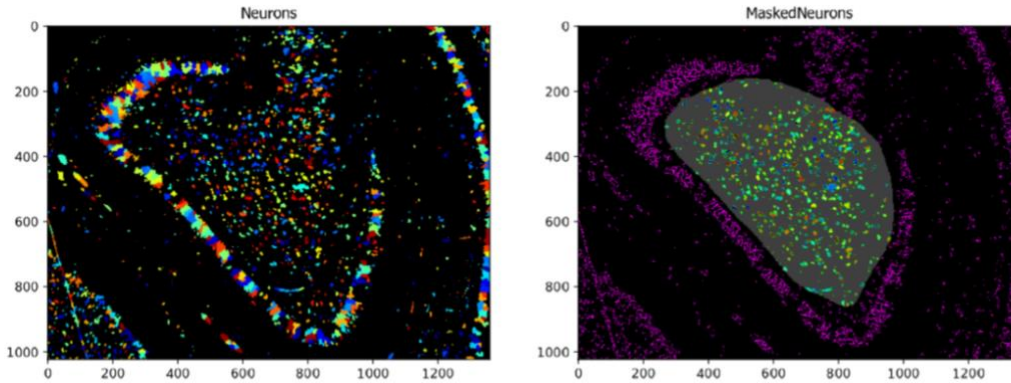
**Pipeline:** There are two pipelines in this example. When using the Neuron\_ROI\_manually.cpproj pipeline, the ROI is defined manually within CellProfiler. When using the Neuron\_ROI\_automatic.cpproj pipeline, the ROI has already been created and saved as a binary image (mask). For both pipelines, measurements will be saved only for objects inside of the ROI (or predefined mask). The workflow is as follows:

1. Open **CellProfiler**.
2. Click on **Images**. Highlight the images listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the images into the appropriate CellProfiler window. For the Neuron\_ROI\_manually.cpproj pipeline, only 865\_HL\_1\_new.tiff is required. For the Neuron\_ROI\_automatic.cpproj pipeline, the 865\_HL\_Mask.tif is also required. The original images maintain the folder structure of the original computer used to create the pipeline. If the images are not reloaded from your computer an error will occur.
3. Ignore the **Metadata** step. It is not used in this pipeline.
4. **NamesAndTypes** For the Neuron\_ROI\_automatic.cpproj pipeline, one rule is set to identify the neuronal image and labelled as “PigNeuN” and **Color** image is selected for **Select the image type**. A second rule is set to identify the image containing the mask and **Binary** mask is selected for **Select the image type**. For the Neuron\_ROI\_manually.cpproj pipeline, since the mask image is not imported, all images are labelled “PigNeuN” and **Color** image is selected for **Select the image type**.
5. **UnmixColors** can automatically recognize popular stains used in histology and separate them by color into new images. In this example, the stain DAB will be identified as the foreground image and labelled “NeuN”, while Fast blue will be identified as the

“background” image.



6. **IdentifyObjectsManually** (Neuron\_ROI\_manually.cpproj pipeline only) enables you to identify ROI's manually using your mouse. In this case the “NeuN” foreground image is used and the ROI object that is created is labelled “mask”
7. **ConvertObjectsToImage** (Neuron\_ROI\_manually.cpproj pipeline only) is used to convert the “mask” object created above into a Binary (black & white) mask image to be used below. It will be used to identify the ROI from which measurements will be output by CellProfiler. The Neuron\_ROI\_automatic.cpproj pipeline uses a mask image containing the ROI that was previously created.
8. **IdentifyPrimaryObjects** Neurons are identified from the image in this module.  
**Automatically calculate size of smoothing filter for declumping** was turned off and set to 2. Since there are many small objects that are clumped and need to be separated, the value is set to lower than the normal because the size of the objects are also small.  
**Automatically calculate minimum allowed distance between local maxima** was turned off and set to 10. This size is set based on the smallest sized object of interest from the image. Since this image has very small objects, the rough estimate is calculated using the “Measure length” tool located in the “Tools” menu.
9. **MaskObjects** The manually created ROI is used as a mask on the identified Neurons (from the PrimaryObject module). Only objects that fall within the masked area will be identified as Neurons. In the case of the automatic pipeline, the mask that is loaded in the input module is used as the mask. In automated pipeline, “yes” is selected for **invert the mask**, so that the black portion of the image is used as the mask.



10. **MeasureObjectSizeShape** saves size and shape values for the masked Neurons objects.
11. **OverlayOutline** was used to create an image of the masked neurons outlined in green. In the Neuron\_ROI\_manually.cpproj pipeline, an image of the manually created mask is also created and outlined in red.
12. **SaveImages** saves an image of the identified neurons and the result of the Overlayoutline module.
13. **SaveImages** (Neuron\_ROI\_manually.cpproj pipeline only) the manually created ROI or mask is also saved.
14. **ExportToSpreadheet** exports all calculated values for each object as separate .csv files.