GUIDE

Ion Channel Cluster Counter

The Kowalski Edition

Open file

Importing Files

Files have to be imported as **.czi** files and can only contain 3 channels.

Defining Channels

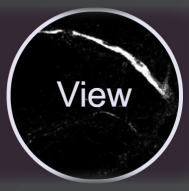
Select the channel that you want to analyze as the 'cluster channel'. Optionally, you can define a ROI channel. In the example below, a marker staining the axon initial segment was imaged, which will, later on, serve to define the region of interest.

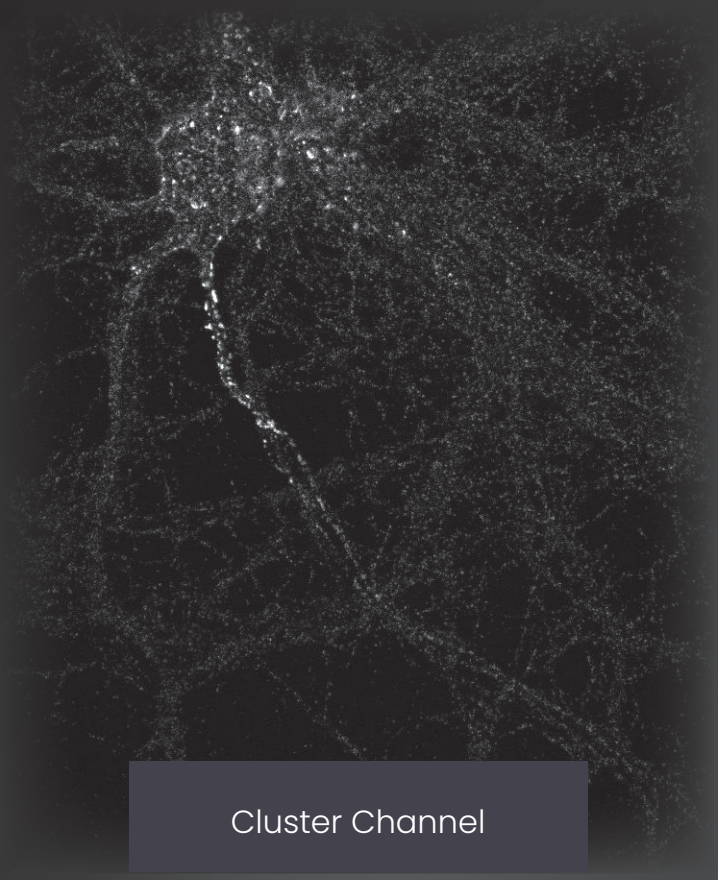
Select cluster channel:

One | Two | Three

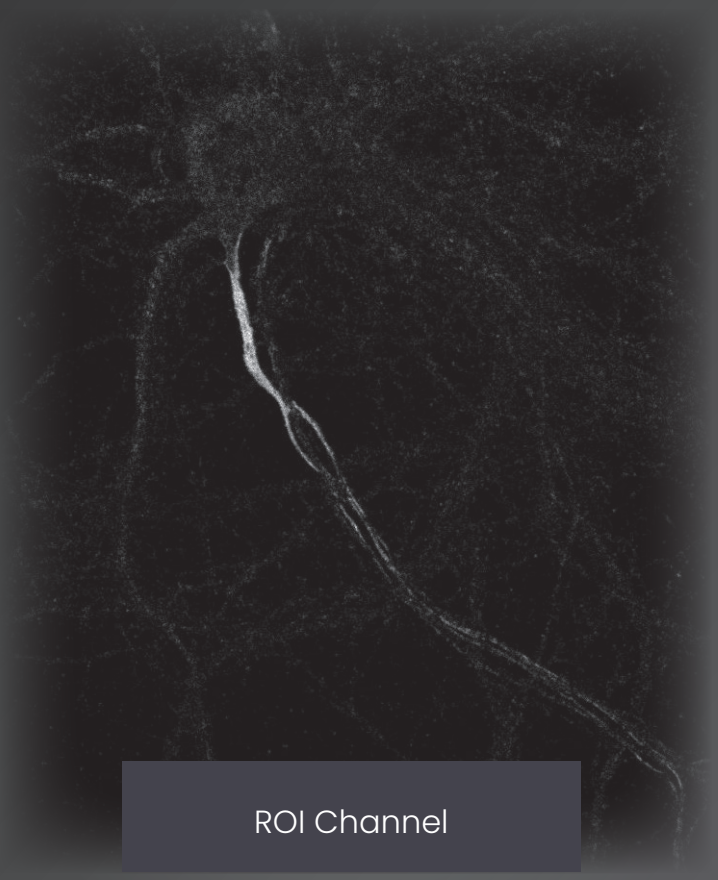
Select ROI channel:

One | Two | Three

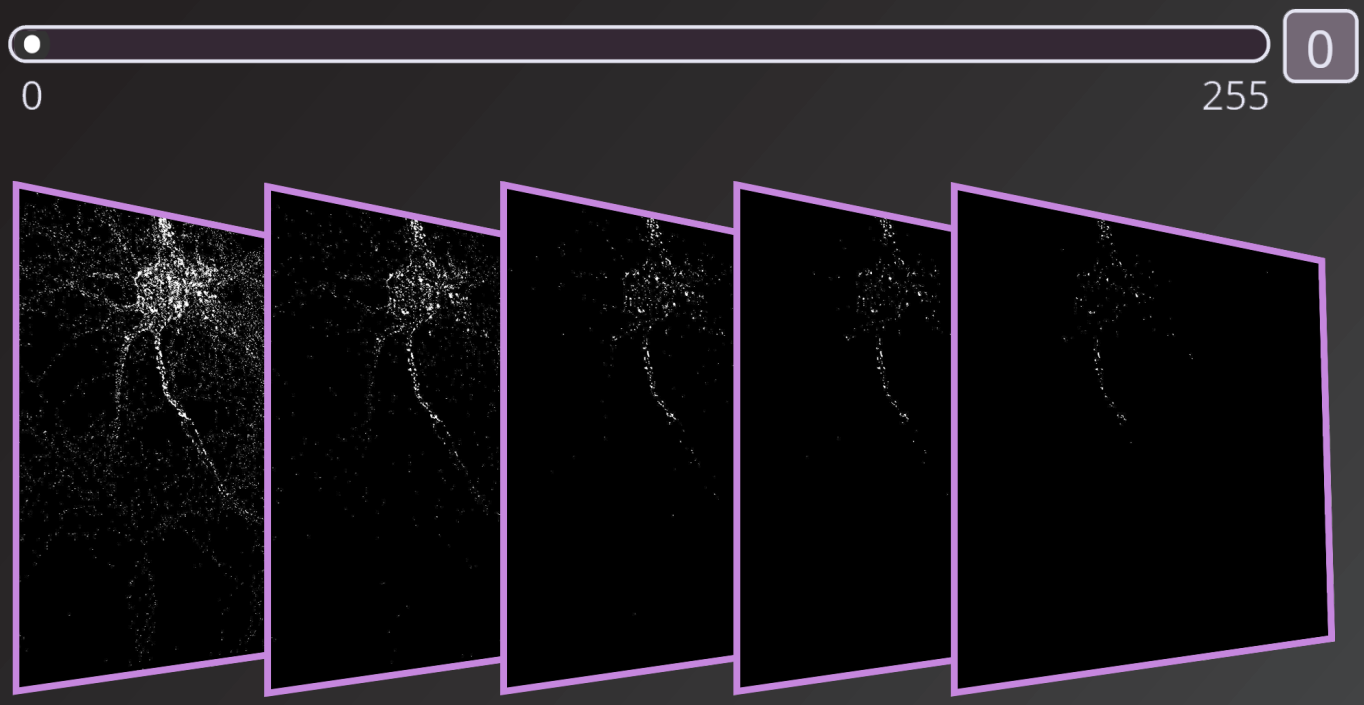




Cluster Channel



ROI Channel

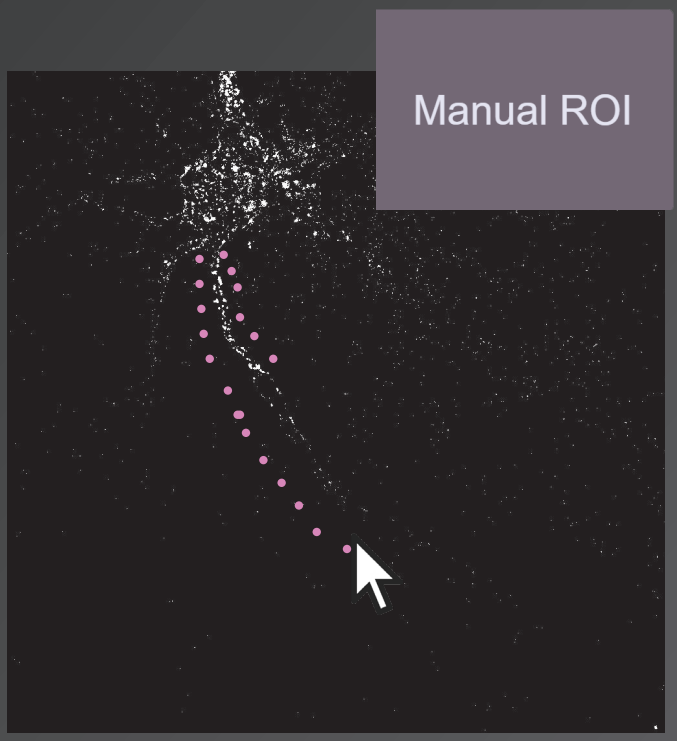


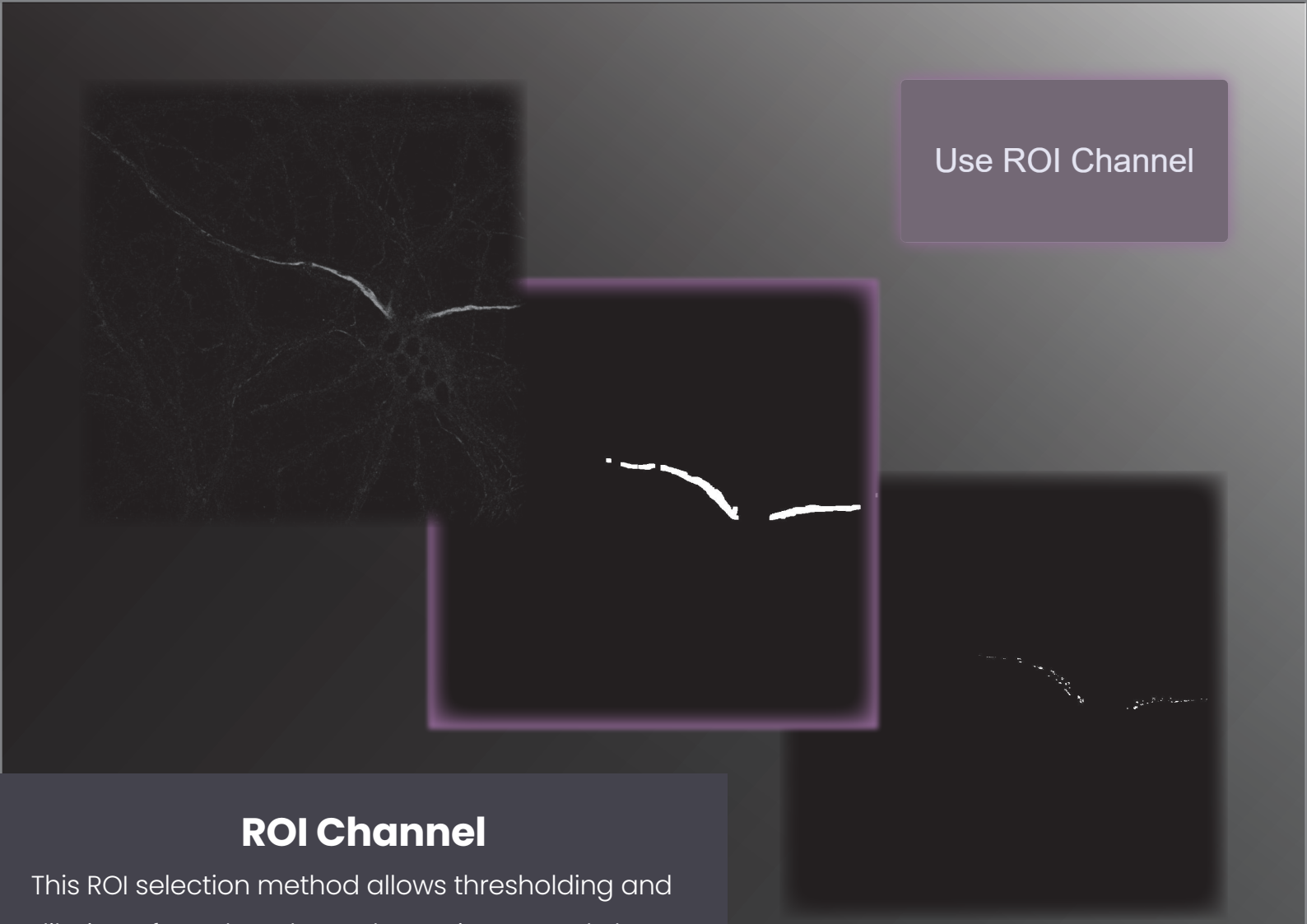
Thresholding

The cluster channel needs to be thresholded to a value at which all clusters you want to include in the analysis are clearly visible. This heavily depends on the overall intensity of your clusters. Images are always converted automatically to 8-bit images, which is required to perform the watershedding later on. Therefore the thresholding value will always be between 0 and 255, where a pixel intensity of 0 is pure black, and a pixel intensity of 255 is pure white.

Manual ROI

The analysis can be restricted to a specific area of the image. Clicking on the 'Manual Roi' button allows you to define coordinates on the image viewer window that will serve as the outline of your region of interest.



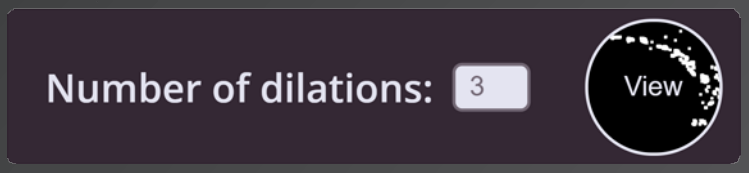


ROI Channel

This ROI selection method allows thresholding and dilating of another channel, creating a mask that will serve as the ROI in the cluster analysis. The ROI channel can be used in combination with manual ROI selection.

Sure Background

The watershedding method requires an area around each cluster that includes some background. This can be achieved by dilating every white pixel in the thresholded image. The number of dilations needs to be modified to receive the best watershedding results. This background image is essential when using the distance transformation method but less important when using the local max peaks method (see next page).



Sure Foreground

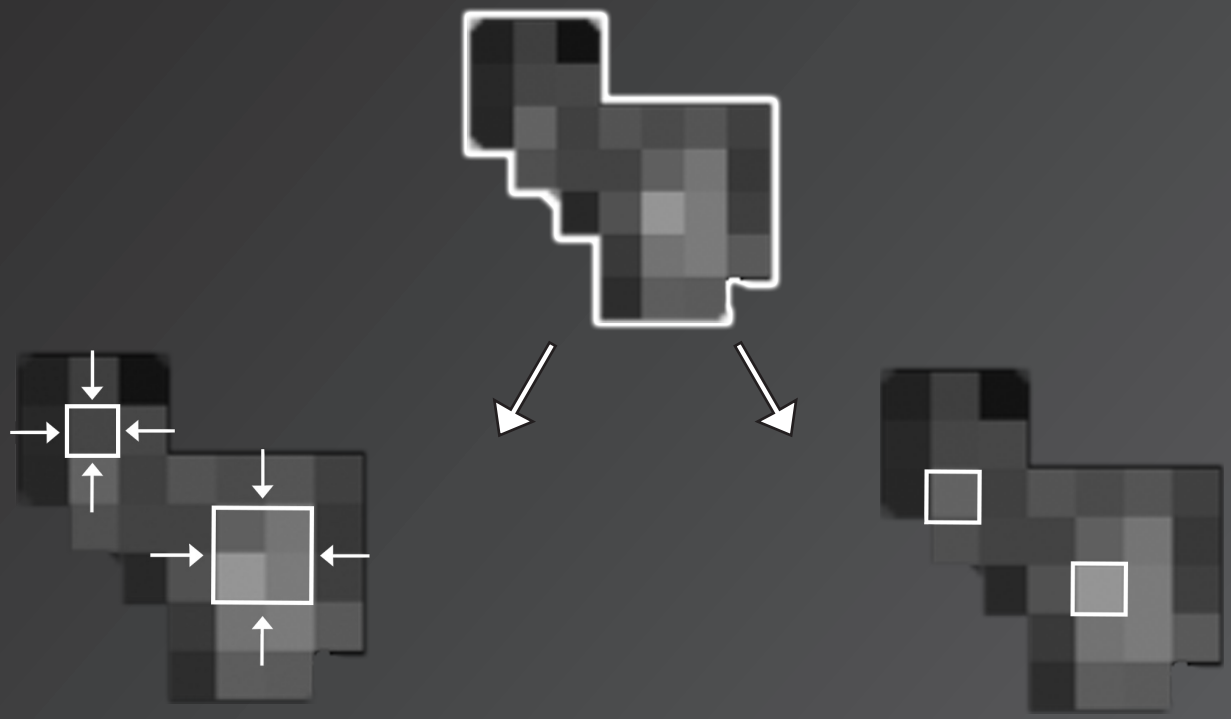
Because spots in the thresholded image can contain multiple clusters, there is a need for a method that separates and defines the different clusters in the image. Two of these methods have been implemented..

Use distance tranformation

Use local max peaks

Distance transformation transforms every spot in the thresholded image to a new image where each pixel's intensity is determined by the distance between the pixel and the outer edge of the cluster. This new image can then be thresholded to isolate the most central pixels and optionally eroded to separate the different clusters.

In contrast to distance transformation this method searches for intensity peaks in your image. Asuming that every cluster has one pixel that is the most intense, this method is able to successfully seperate different clusters. This method is better compared to the distance transformation method when your image contains mutiple clusters that cover and area of only a few pixels.

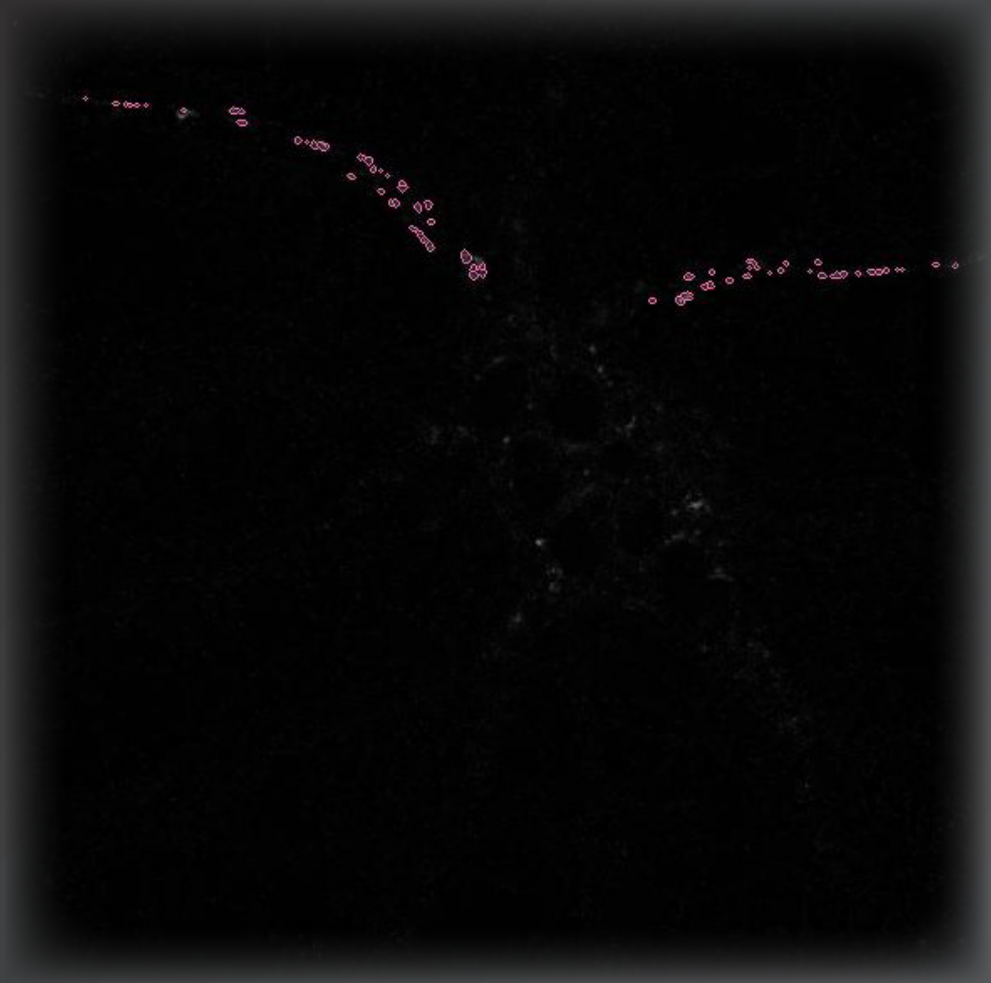


Analyze & Add

With all preparations in order, you are now ready to start watershedding. The analyze button will perform the analysis and display the result in the image viewer. To extract data, click ‘Analyze & Add’. This will perform the watershedding and save information like average cluster intensity, cluster diameter, and cluster perimeter in an internal data frame. Do not forget to set the correct pixel per micrometer ratio in the options menu. This value is needed to calculate the cluster area and diameter in microns.

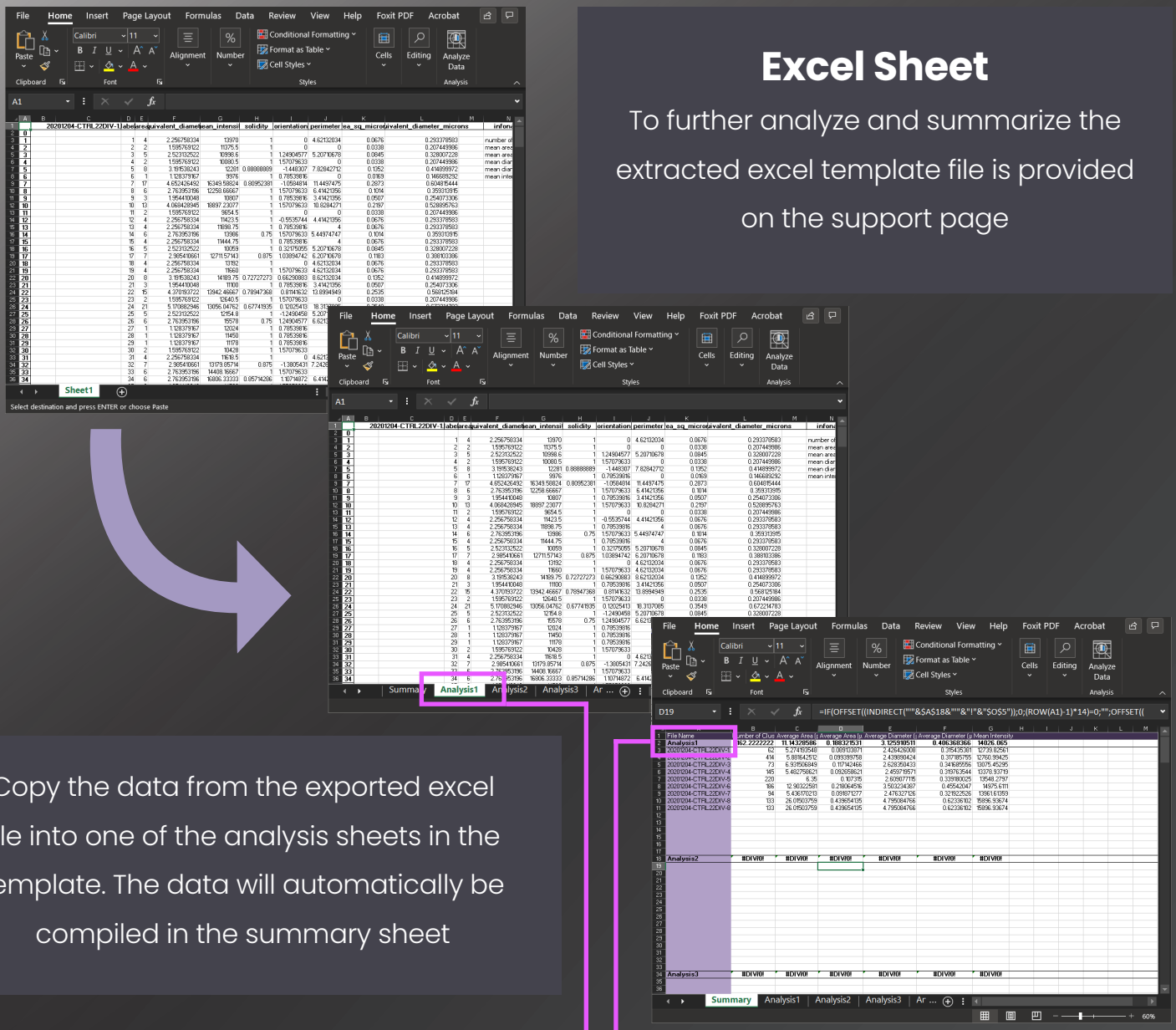
Options

In the options menu, the results can be downloaded in an excel file format. Additionally, all the generated images can be downloaded for safekeeping and later use in publications. Here you can also set the pixel per micrometer ratio of your images. When all data has been extracted, the internal excel file can be cleared by clicking the ‘clear results’ button.



Excel Sheet

To further analyze and summarize the extracted excel template file is provided on the support page



Copy the data from the exported excel file into one of the analysis sheets in the template. The data will automatically be compiled in the summary sheet

Ensure the sheet name in which you copy the data is exactly the same as the sheet name in the summary box. Beware the sheet name is case sensitive.