

## Case Study 2: Step by Step Image Analysis Guide

Image of grazer *Oxyrrhis marina* and phytoplankton *Dunaliella tertiolecta* in culture

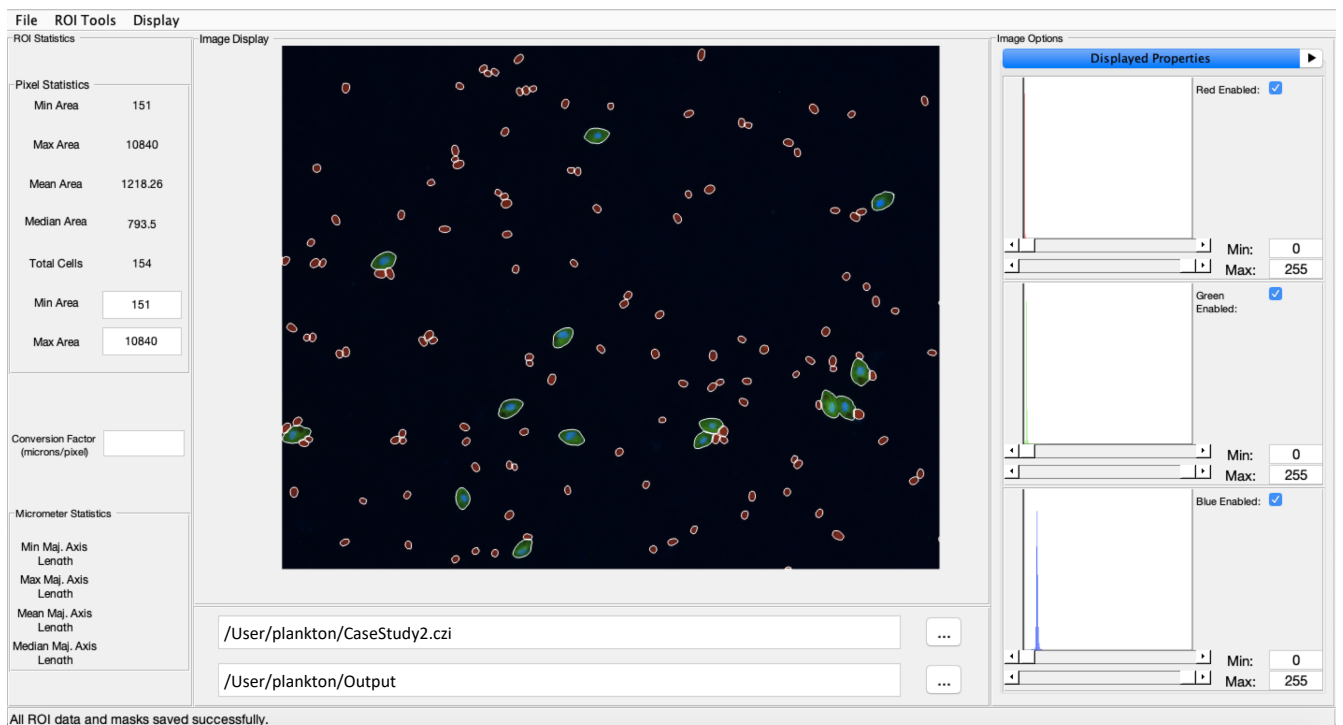
### Analyzing Example Image

1. Open MATLAB
2. From MATLAB, navigate to the 'image analysis' folder, select the 'interfaces' folder
3. Open 'image\_analysis.m' file
4. With the script file open, select 'Run' to launch the program
5. From the File menu, select 'load images', navigate to and select the image file "CaseStudy2.czi"
  - a. You can check if the file path is correct in the text box below the image space.
6. The 'Channel Select' dialogue box will appear. Use drop-down menus to set colors for each channel. DAPI = Blue, FITC = Green, and Chlorophyll A = Red. Select 'Done'.
7. From the ROI Tools menu, set the 'Threshold Channel' to 'All'
8. From the ROI Tools menu, select 'Manual Threshold All ROIs'
  - a. Toggle threshold level to achieve best value (for this image the auto-select at 0.10588 works well).
  - b. Set minimum pixel cutoff size to 50.
  - c. Select 'Confirm Outlines'
9. From the 'Display' menu, select Zoom (ctrl + z) to magnify and move around the image.
  - a. Use this to examine how the ROIs turned out from the automatic threshold.
  - b. You'll notice some of the cells that were in close proximity to each other did not get individually separated.
10. To fix this, From the ROI Tools menu, select 'Split ROI' (ctrl + x)
  - a. draw a line between the ROI to split and double click to confirm the split.
  - b. Repeat this step until all of the cells have their own ROI (154 cells for this image; see Figure 1,2 below for visual comparison).
11. With the image analysis complete, you can save the data and masks.
12. Set the outgoing directory to the destination of your choice for the files to save to.
13. From the File menu, there are a number of saving options for data and masks.
  - a. Select 'Save ROI Data & Masks'. This creates a .xlsx (or .csv) file of the data containing each ROI and a .mat file of the ROIs masks created.
14. You can use this or the previously provided data file with the R code to produce figures from this data.

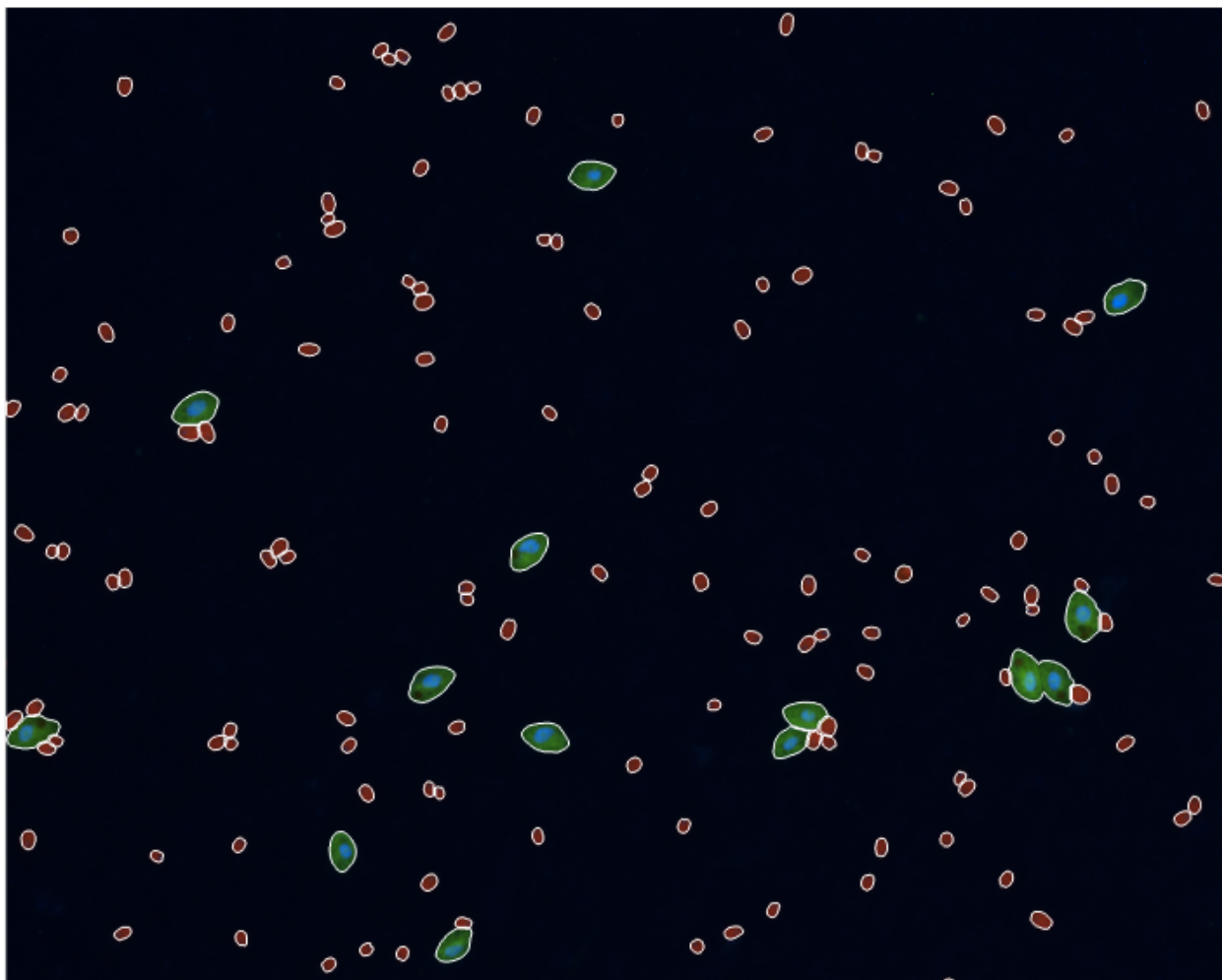
### Loading Example Masks onto Image

1. Open MATLAB
2. From MATLAB, navigate to the 'image analysis' folder, select the 'interfaces' folder
3. Open 'image\_analysis.m' file
4. With the script file open, select 'Run' to launch the program
5. From the File menu, select 'load images', navigate to and select the image file "CaseStudy2.czi"
  - a. You can check if the file path is correct in the text box below the image space.

6. The 'Channel Select' dialogue box will appear. Use drop-down menus to set colors for each channel. DAPI = Blue, FITC = Green, and Chlorophyll A = Red. Select 'Done'.
7. From the ROI Tools menu, set the 'Load Mask'
8. Navigate to the "CaseStudy2\_mask" and click okay
9. This will load the example mask onto your image.
10. If you would like to save the data Select 'Save ROI Data & Masks'.
  - a. A version of this data is also provided as part of the case study.
11. You can use this or the previously provided data file with the R code to produce figures from this data.



**Figure 1.** Screen shot of Case Study 2 image in the MiA Image Analysis program after Regions of Interest (ROIs) have been selected based on the steps outlined in this guide.



**Figure 2.** Zoomed in screen shot of Case Study 2 image in the MiA Image Analysis program after Regions of Interest (ROIs) have been selected.