

Case Study 1: Step by Step Image Analysis Guide

Image of natural phytoplankton community

Analyzing Example Image – Using mask with dinoflagellate and diatom Identifications

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 "CaseStudy1.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. Adjust color contrasts in the 'Displayed Properties' Tab to better visualize cells. This will enable you to see the faint cells. You can adjust the properties more to explore the image. *Note – this does not change the data, just the visualization.*
 - a. Red: Min=0, Max=120
 - b. Green: Min=15, Max=150
 - c. Blue: Min=20, Max=140
8. From the ROI Tools menu, set the 'Load Mask'
9. Navigate to the "CaseStudy1_mask1" and click okay
 - a. This mask has a total of 7 ROIs outlined which have already been identified.
 - b. To view identifications, go to the Display Menu and select "Enable hover ROI ID Display" from the drop-down menu. Then use your mouse to hover over the outline cells and it will display the cells ID.
10. To explore the ROI ID feature, go to 'ROI Tools' → 'Identify ROIs' → 'From Start'
 - a. This will open up the ROI ID window
 - b. Set the "Zoom out (pixels)" window to 20 and press enter
 - c. Using the next ROI button, you can navigate through the ROIs and you'll see the ROI ID pop up in the "Select ROI Type" window for each ROI.
 - i. If you wanted to change the identification of an ROI, you can simply click on this window and drop-down another choice.
 - d. To add another ROI designation, you can navigate to the top left of the window and click on "Add". This will enable you type and add another ROI designation to the list.
 - e. When you are done, you can leave the window by clicking "Save and Finish".
11. To explore the data output, select 'Save ROI Data & Masks'. This creates a .xlsx (or .csv) file of the data containing the ROIs and a .mat file of the ROIs masks created. Examples of these were provided as part of the case study as well.
 - a. Because cells were given ID designations, when you export data a CSV file of ROI IDs is also created. The ROI ID information is also contained in the main data file.

Loading an example of a complete mask onto the image

1. From the File menu, select 'load images', navigate to and select the image file "CaseStudy1.czi"
 - a. You can check if the file path is correct in the text box below the image space.
2. 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'.
3. Adjust color contrasts in the 'Displayed Properties' Tab to better visualize cells. This will enable you to see the faint cells. You can adjust the properties more to explore the image. *Note – this does not change the data, just the visualization.*
 - a. Red: Min=0, Max=120
 - b. Green: Min=15, Max=150
 - c. Blue: Min=20, Max=140
4. From the ROI Tools menu, set the 'Load Mask'
5. Navigate to the "CaseStudy1_mask2" and click okay
 - a. This mask is a more complete mask with a variety of cells outlined (Figure 1 below).
6. Use the zoom feature to zoom in and explore the image and ROIs.
7. In order to create this mask, thresholding all ROIs at once does not work due to the debris on the image. Therefore, a number of ROI Tools were used including regional ROI thresholding options and freehand drawing. Images like this require more manual image analysis.
8. This image and mask are meant to provide an example of a more challenging analysis that benefits from the flexibility of ROI selection options in MiA.
 - a. You can use this mask as a guide and play with the different ROI tools to re-create it.

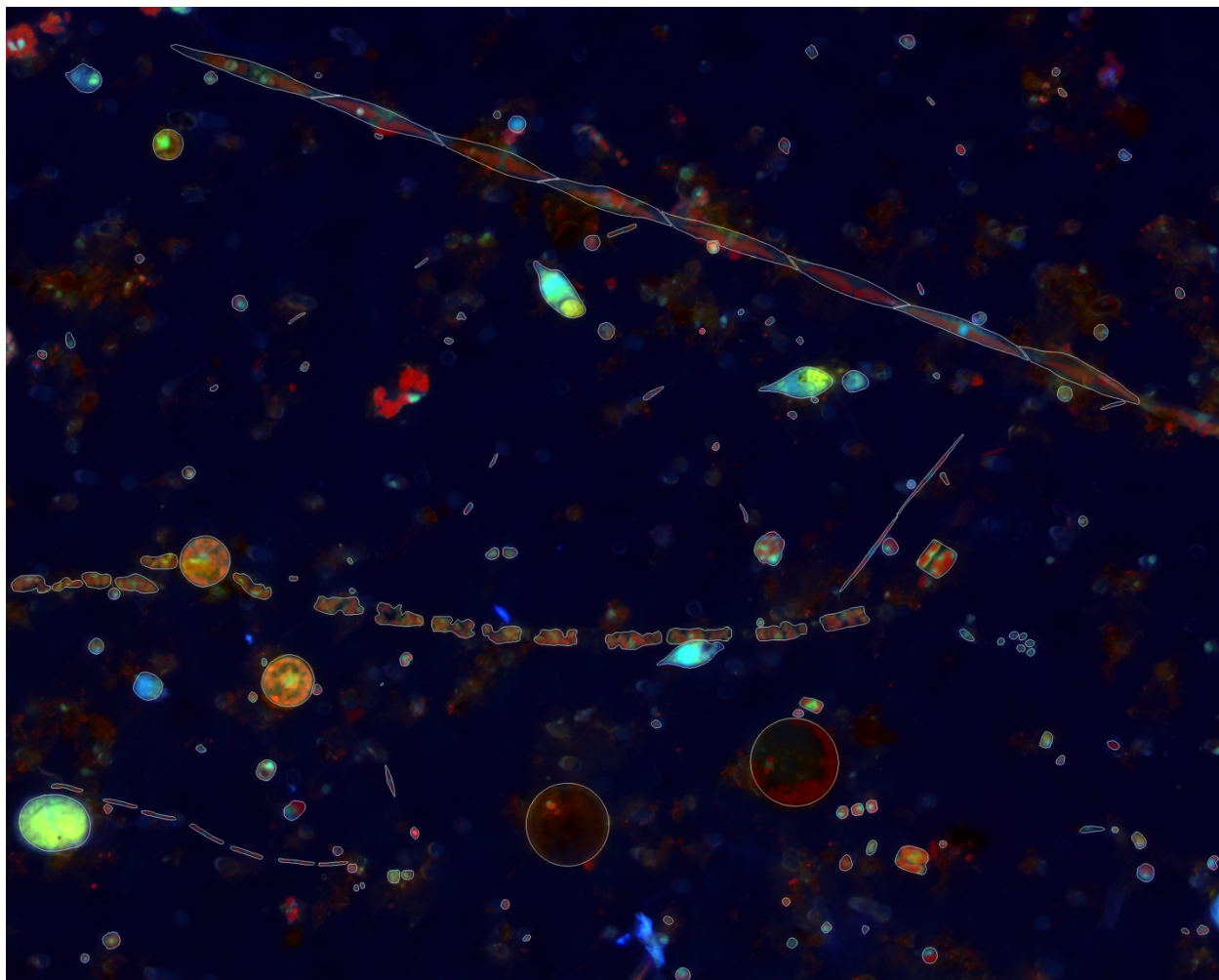


Figure 1. Screen shot of Case Study 1 image with Regions of Interest (ROIs) from Mask2.