Case Study 3: Step by Step Image Analysis Guide
Image of Viral Particles

Analyzing a Single-channel image

1. Open MATLAB
2. From MATLAB, navigate to the 'image analysis' folder, select the 'interfaces' folder
3. Open 'virus_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
    a. Change the 'File Format' to Grayscale Files
    b. Load CaseStudy3_FITC by selecting the image and clicking 'Open'
6. The 'Channel Select' dialogue box will appear. Use drop-down menus to set colors for each channel. FITC = DNA and it will read No Image as LABELED. Select 'Done'.
7. The raw image will now be displayed in the main window and you can walk through the image processing steps by clicking 'Next Steps'
8. If you would like to change the output directory, click '…' on the second line below the images (the first line is the input directory).
9. The first step will be background subtraction. You can leave the default disk size at 10. Then click next.
10. The next step is thresholding. You can leave the default or adjust to the following:
    a. DNA = 0.004
11. The alignment step will not be enabled when you load one image.
12. The next step enables you to remove pixel artifacts and view the ROI statistics.
    a. You can remove pixel artifacts by setting the min and max pixel size. Set the min as 5 pixels and the max 75 pixels. The panel will update and have 716 DNA-based viral particles.
13. At this step, you can also add a conversion factor. Type in 0.1 in the µm/pixel conversion window and data will appear in the micrometer statistics panel.
    a. As noted in the manuscript and manual this is NOT absolute size, but the size of the fluorescence signal.
14. In the final panel, you can view the data in histograms and play with the settings. Choose micrometers from the drop-down menu instead of pixels to view the size of the particles in microns.
15. To save the data, go to the file menu and select 'Save Data'. This creates a .xlsx (or .csv) file of the data for each ROI.
    a. In this file, the second sheet provides statistics including abundance and size of the particles.

Analyzing a dual-channel image

1. Open MATLAB
2. From MATLAB, navigate to the 'image analysis' folder, select the 'interfaces' folder
3. Open 'virus_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

    a. Change the ‘File Format’ to Grayscale Files
    b. Load CaseStudy3_Cy3.tiff and CaseStudy3_FITC by selecting both images and clicking ‘Open’
6. The ‘Channel Select’ dialogue box will appear. Use drop-down menus to set colors for each channel. Cy3 = LABELED, FITC = DNA and Select ‘Done’.
7. The raw images will now be displayed in the main window and you can walk through the image processing steps by clicking ‘Next Steps’
8. If you would like to change the output directory, click ‘…’ on the second line below the images (the first line is the input directory).
9. The first step will be background subtraction. You can leave the default disk size at 10. Then click next.
10. The next step is thresholding. You can leave the default or adjust to the following:
    a. DNA = 0.004
    b. LABELED = 0.006
    c. Figure 1 provides an example of what the images will look like.
11. The next step will align the images. No user interaction is required. This step will provide you the x and y movement for these images. Then click next.
12. The next step enables you to remove pixel artifacts and view the ROI statistics.
    a. Set the min as 5 pixels and the max 75 pixels. The panel will update and have 716 DNA-based viral particles and 587 labeled particles.
13. At this step, you can also add a conversion factor. Type in 0.1 in the µm/pixel conversion window and data will appear in the micrometer statistics panel.
    a. As noted in the manuscript and manual this is NOT absolute size, but the size of the fluorescence signal.
    b. Figure 2 provides an example of the ROI statistics from this panel.
14. In the final panel, you can view the data in histograms and play with the settings. Choose micrometers from the drop-down menu instead of pixels to view the size of the particles in microns.
15. To view centroids on the images, go to the display menu and select ‘Display Centroids’.
    a. This will display the DNA-based ROIs on both images.
16. To save the data, go to the file menu and select ‘Save Data’. This creates a .xlsx (or .csv) file of the data for each ROI.
    a. In this file, the second sheet provides statistics including abundance and size of the particles.

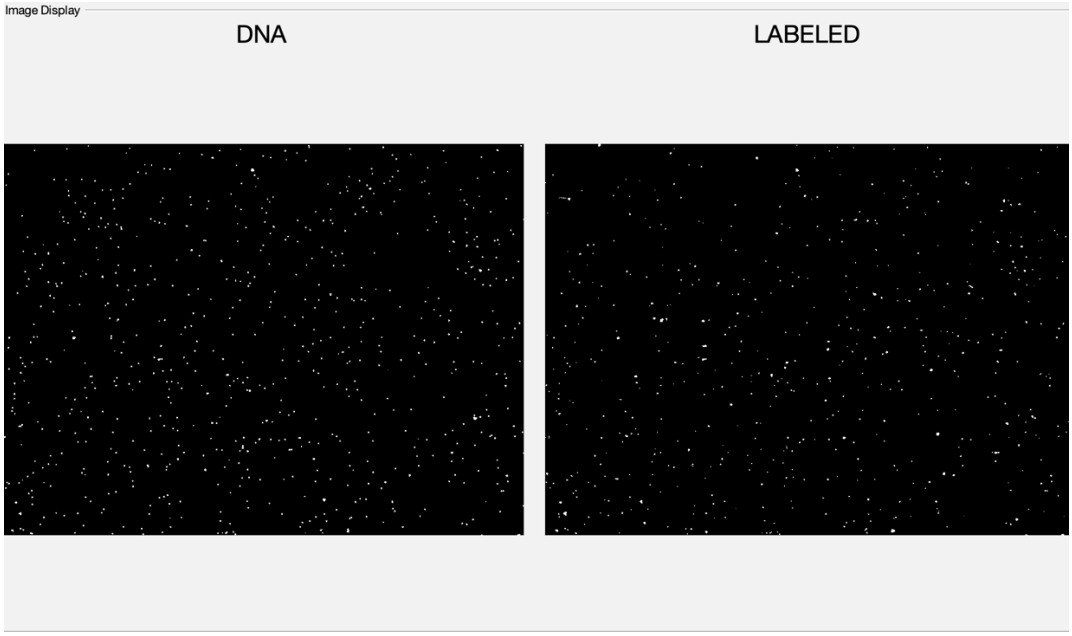

Figure 1. Example of thresholding step with dual-channel images

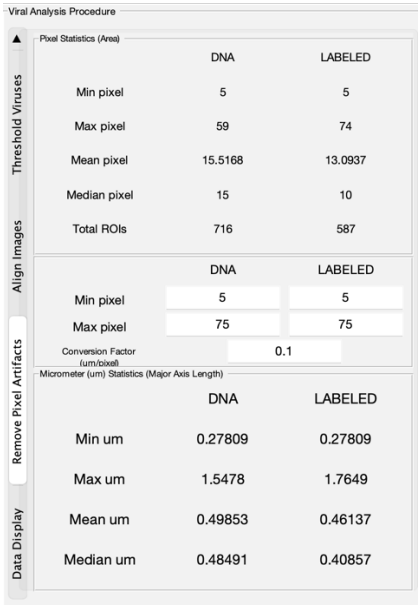

Figure 2. Example of ROI statistics from dual channel image in Figure 1.