Image Processing GUI Help File

[UPDATED 2023-02-22]

>> Please note that any aesthetic changes made to the application may not be accurately reflected in the reference images in this help file. While general functionality will remain the same, please contact Steven Summey if you find issues or errors. <<

TABLE OF CONTENTS:

[Prerequisites]:

IT ADMINISTRATIVE ACCESS IS NEEDED FOR MANY OF THESE ITEMS.

→ **MATLAB 2022b** (or latest release)

The Sphere Image Analysis is an application that runs via MATLAB. While the design and code can be edited through the AppDesigner space within MATLAB, the operation of the application can be called from the command line (see: Accessing and Setting *Sphere Image Processing* path from SharePoint).

TOOLBOXES (selected when installing or added via Add-Ons in MATLAB):

-Image Processing Toolbox

-Parallel Processing Toolbox (optional)

→ **ArunaNAS Access (for images uploaded from the Collins Microscope Computer):**

The specific drive assigned will be addressed, but, for ease of use, it is recommended to have the ArunaNAS mounted to the (Z:) drive.

[Accessing and Setting *Sphere Image Processing* path from SharePoint]:

https://arunabiomedical.sharepoint.com/:f:/r/sites/ProcessDevelopment/P121004/Sphere%20Image%20Processing?csf=1&web=1&e=Z0Rwvq

- ➔ Folder titled Sphere Image Processing houses all functions, MATLAB Apps, and data files.
- ➔ Click on folder and select [Sync]
  - **(1)** Process Development – Sphere Image Processing should now appear in ArunaBio folder under C:\User\
    - **Notice:** If P1-21004 is already syncing on your computer, then all subdirectories should also be syncing.

Setting MATLAB Path	(1) When opening MATLAB, you will be able to set the path two ways:	(1a) Reference the highlighted section in **Figure A**. The arrow following your name will take you to the Aruna Bio folder. (1b) Use the icons on the side get to the desired folder (**Figure B**).
	(2) The Sphere Image Processing app orients itself based on the present working directory (pwd). The current folder panel on the left should look similar to **Figure C**.	

Figure A:

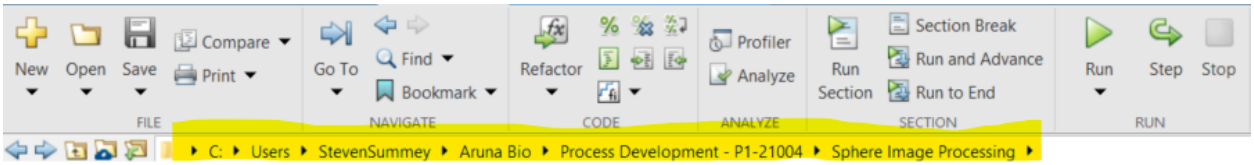


Figure B:

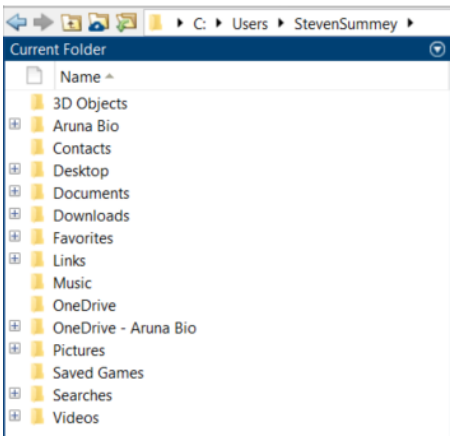
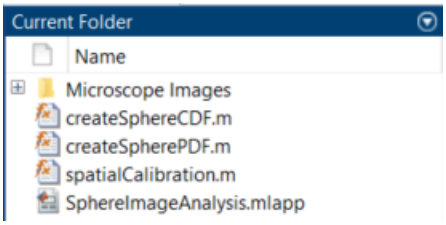


Figure C:



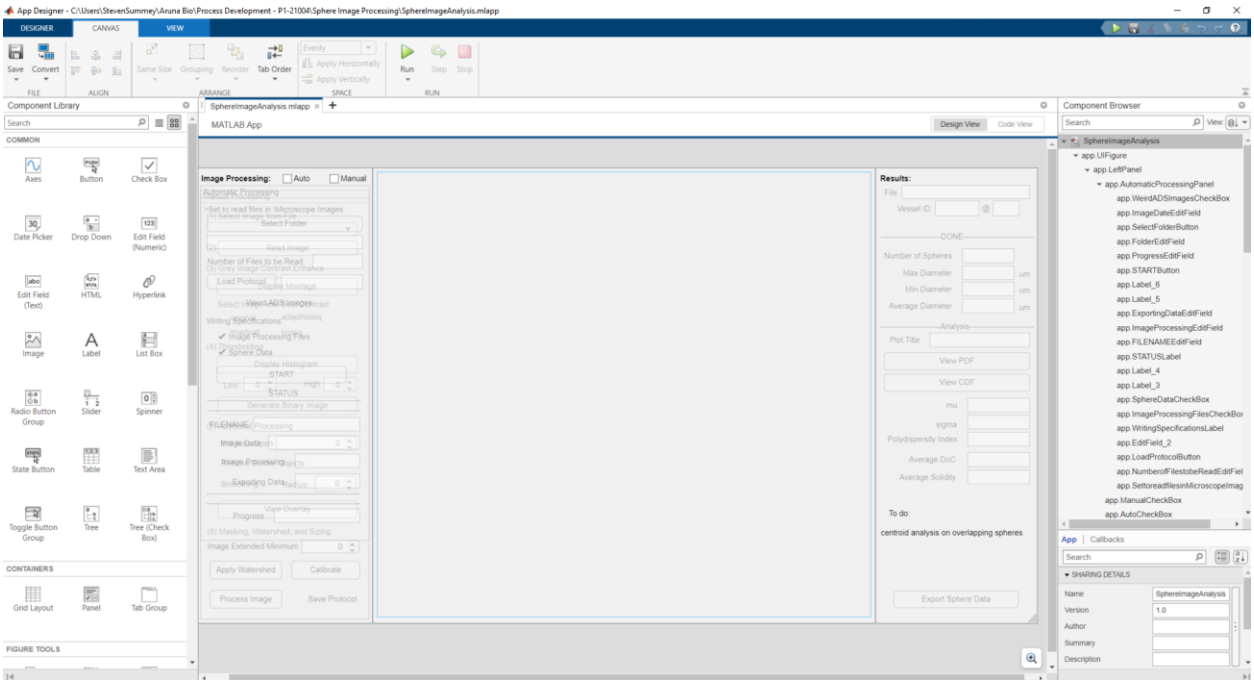
[Starting the Application]:

- **(1)** Since the bulk of the image processing will occur as an intermediate between NAS and SharePoint, it is important to monitor the CPU utilization as the program is running.
  - **a.** [Ctrl + Alt + Delete] and select Task Manager to monitor CPU status and close extraneous applications that are not currently being used.
  - **b.** This application can be used in the background while performing other tasks; however, be mindful of the CPU status and only proceed to the next step when under ~20%.

(2) There are two ways to access the application:

- **a.** Type “SphereImageAnalysis” into the command window.
- **b.** Click on the SphereImageAnalysis.mlapp file to open the AppDesigner Suite.
  - **i.** When enabled along the top banner, click [RUN]. **Figure D.**

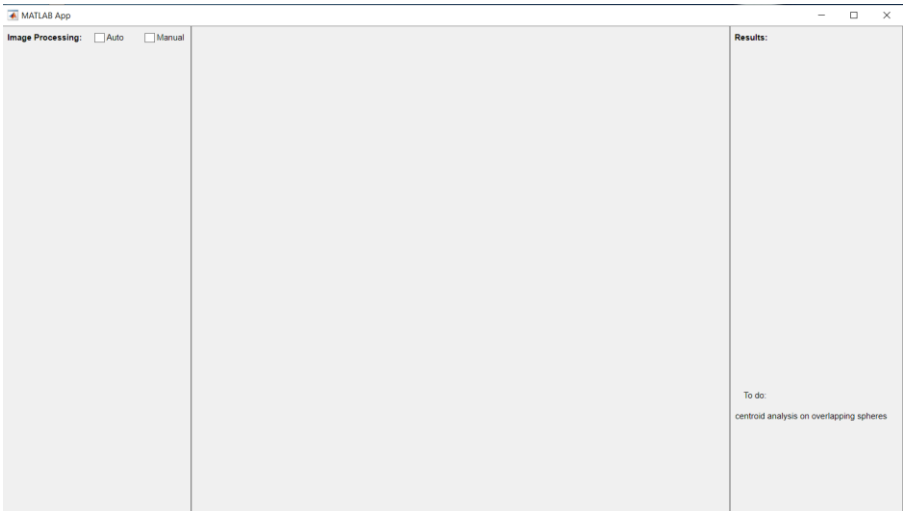
Figure D:



(3) The initial window is sparse, with options on the left-most panel to select [AUTO] or [MANUAL] processing. **Figure E.**

- **a.** [AUTO] will be used for much of the processing workload.
- **b.** [MANUAL] (still under development) will be used to train, revisit flagged files, and reprocess files in bulk using logged methods from [AUTO].

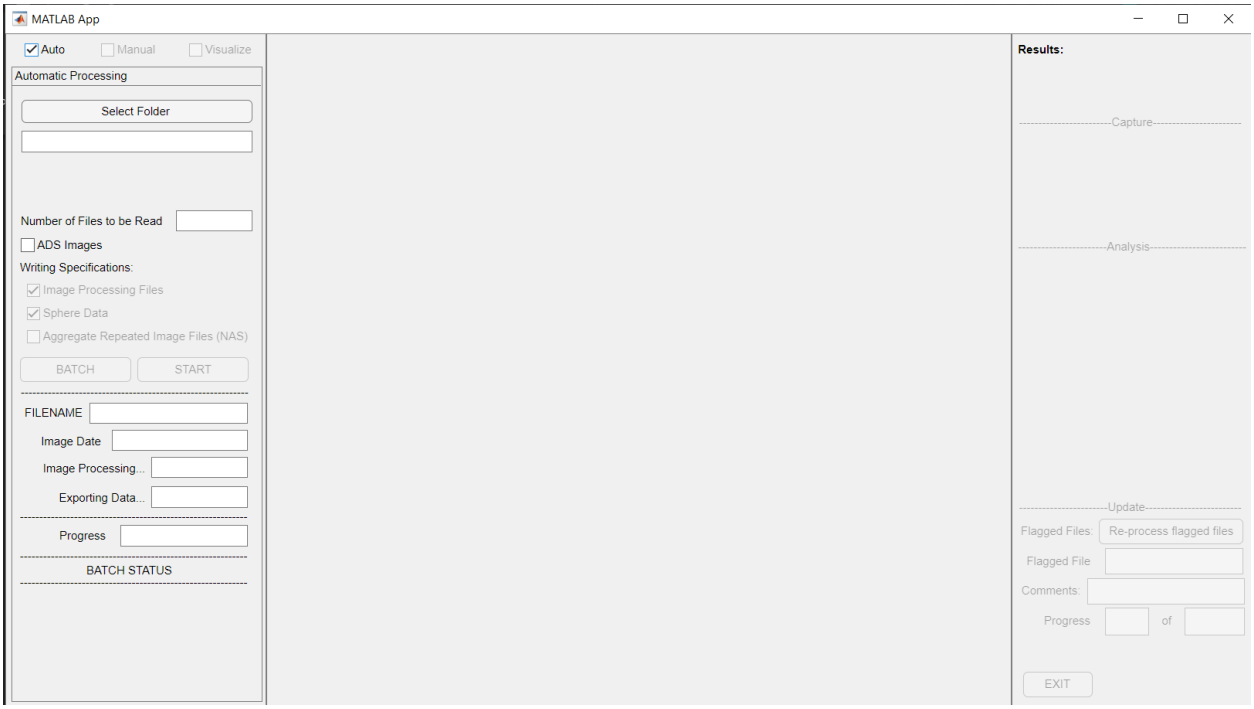
Figure E:



[Running AUTO]:

(1) With the [AUTO] checkbox selected, choose the [SELECT FOLDER] button. **Figure F.**

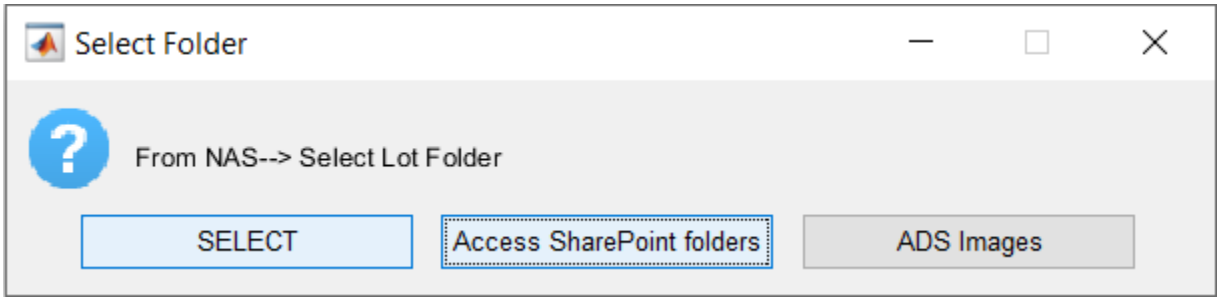
Figure F:



(2) A message box will appear prompting the user to [SELECT] folder from the NAS. **Figure G.**

- **a.** [SELECT] is what will used most often in this application.
- **b.** [Access SharePoint folder] is obsolete now that images are no longer stored on SharePoint.
- **c.** [ADS Images] parses through sphere images taken at ADS.
  - **i.** These files are in a separate PD folder (P1-22006) that will also need to be synced prior to running the application.

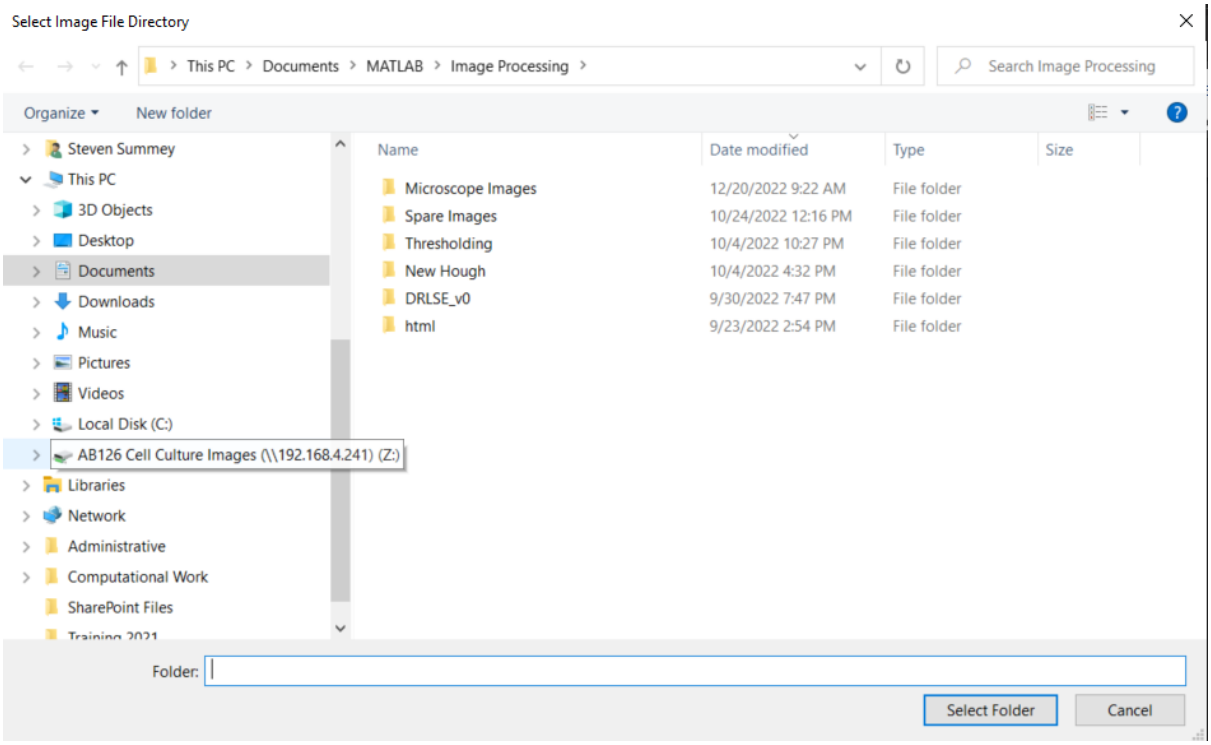
Figure G:



(3) **Figure H.** After pressing [SELECT], find the mapped NAS drive (Z:) and follow the path below to the desired vessel lot:

PO SUSPENSION CULTURES → [suspension culture lot] → [STOP]

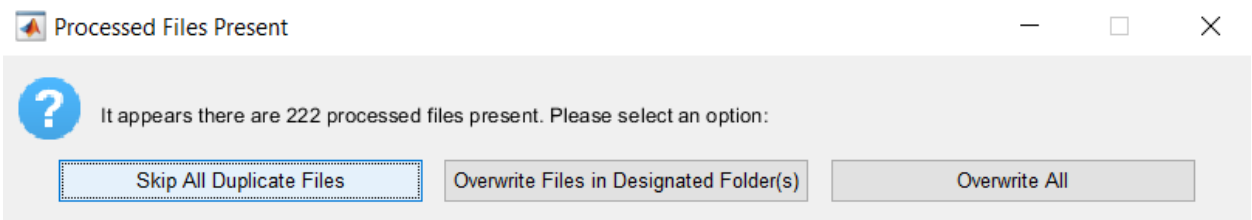
Figure H:



(4) **Figure I.** Since there are processed files present, a window will appear prompting the user to select an option prior to processing: **Figure**

- **a.** [Skip All Duplicate Files] will, naturally, skip all files previously processed.
- **b.** [Overwrite Files in Designated Folders(s)] will allow the user to select which files they prefer to process again.
  - **i.** This option will be better addressed via flagged file processing in [Manual] mode, so there is no need to use this unless there are entire folders that have been significantly changed when uploading from the microscope computer.
- **c.** [Overwrite All] not assigned functionality, but, if necessary, will remove all processed files.

Figure I:



(5) **Figure J,K.** Bulk processing of the entire file list parsed from the NAS system takes a heavy computational toll. If the are multiple vessels in cell lot, the user will be prompted to select the desired vessel ID to process.

Figure J:

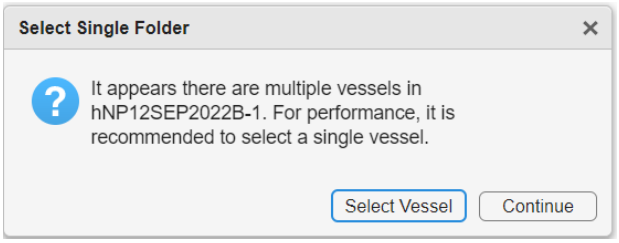
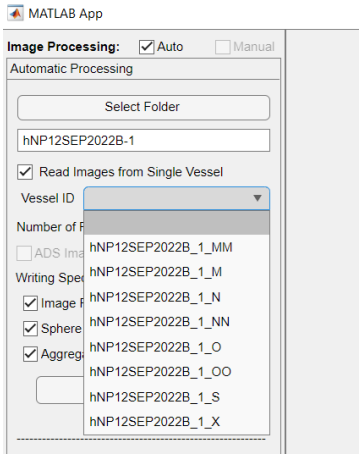


Figure K:

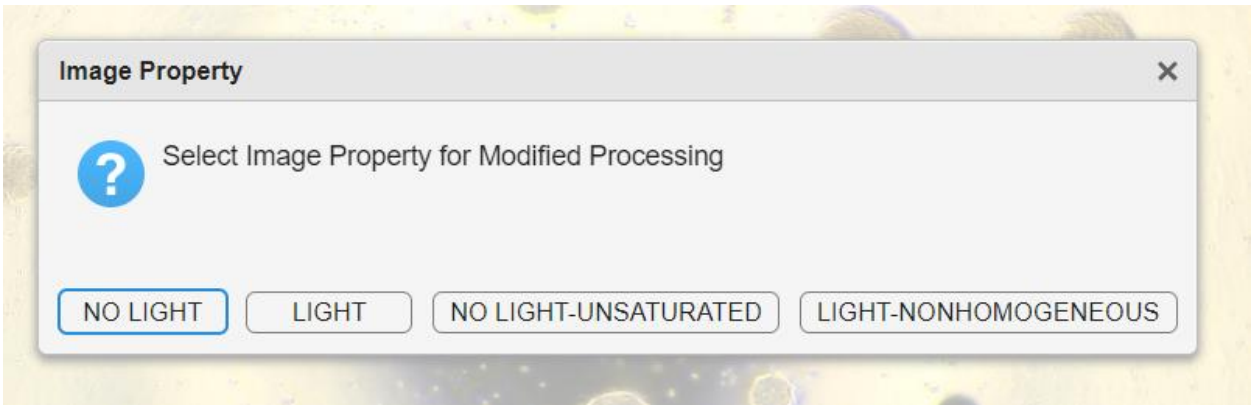


(6) The processing specification fields will be populated (e.g. Vessel ID, Number of Files, and Writing Specifications).

a. Click [Start] to begin processing!

(7) **Figure L.** The current image to be processed will appear with four (4) image properties. This step is essential as the strategies following the selection are optimized (with little manual input) for each image property. **→SEE APPENDIX B for examples and the strategy outline for each image property.**

Figure L:

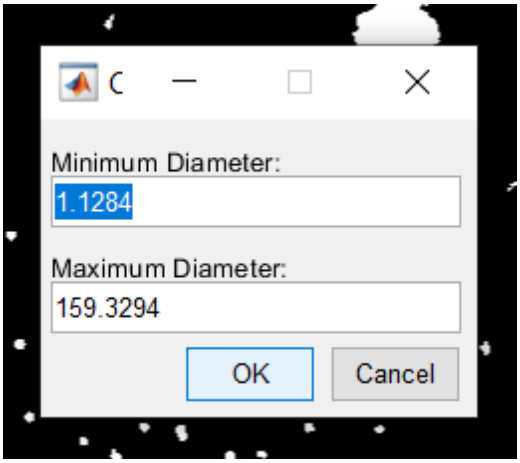


(8) Following the conclusion of the image property processing, a final size filter will be applied to address extraneous sizing or segmentation faults. **Figure M.**

a. The [Minimum Diameter] and [Maximum Diameter] fields are automatically populated with the minimum and maximum diameter detected from the region properties.

i. Since the minimum diameter is normally the detection of small pixel neighborhoods created after erosion, it is recommended—depending on the maximum diameter observed—to begin the minimum filter at 25-30.
ii. If there are aggregates or poorly segmented spheres that are larger than the expected distribution of sizes, the user can scale down the maximum diameter.

Figure M:

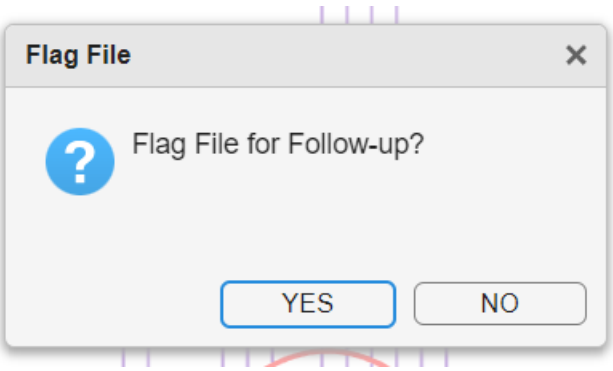


(9) **Figure N.** The filtered, normally distributed sphere diameters will be displayed. A window will appear asking the user if they want to flag the processed file for follow-up.

a. [YES] will open a dialogue box for the user to enter notes appended to the file data. **For tracking purposes as multiple users begin processing images, please append the flag note with user initials and date (e.g. SS24JAN2023).**
   i. Segmentation was poor and re-processing is recommended.
   ii. Comment on the quality of the image
   iii. Leave a note to revisit in [Manual] mode.

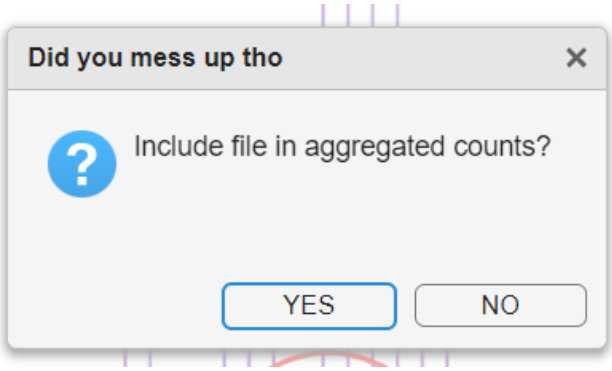
b. [NO] if processing was satisfactory.

Figure N:



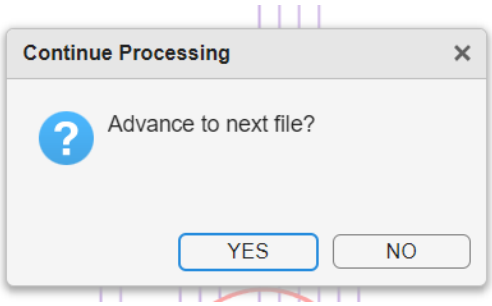
(10) Figure O. User will be prompted to include file in aggregate counts. If the processing was satisfactory, then select [YES]. Otherwise, selecting [NO] will omit the sphere data from further aggregated processing.

Figure O:



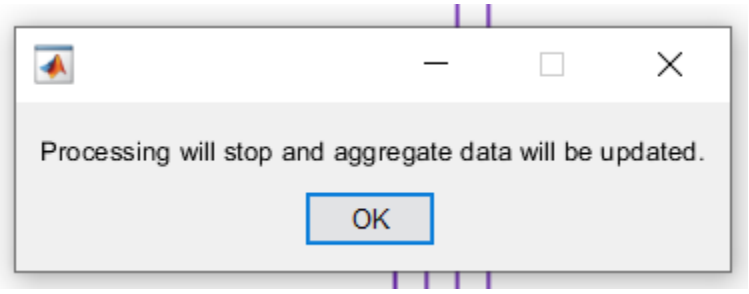
(11) Select [YES] to continue to the next file and cycle back to step (7). Selecting [NO] will end the processing step and prompt a function to detect novel file data. **Figure P.**

Figure P:



(12) Select [OK] to create/update an aggregate file. **Figure Q.**

Figure Q:



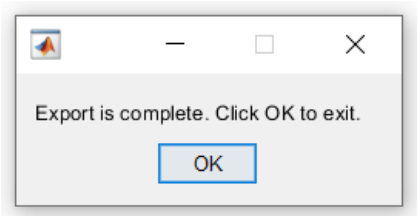
(13) Observe the command window. If an aggregate file was present during the start of processing, the user will see a display message detailing that data was cleared and updated in the file path of the aggregate file. **Figure R.** A window will appear indicating the export is complete. Clicking [OK] will close the application. **Figure S.**

Figure R:

Sphere #	Mean Intensity	Area	Perimeter	Centroid		Diameter
#56	204.8	935.0	143.2	288.1	659.9	34.5
#87	210.5	767.0	174.3	422.5	40.8	31.3
#109	160.3	15150.0	533.5	559.8	563.3	138.9
#230	132.2	19938.0	637.1	1284.0	1538.8	159.3
#255	176.6	2151.0	217.0	1364.8	1390.1	52.3
#257	168.5	11866.0	508.5	1398.1	293.7	122.9
#259	174.5	723.0	149.3	1371.7	1847.9	30.3
#262	159.1	13318.0	495.3	1428.1	611.2	130.2
#342	186.0	730.0	127.6	1858.4	1478.1	30.5
#348	160.6	9956.0	438.2	1926.3	1016.3	112.6
#425	156.1	1652.0	258.8	2192.0	1579.0	45.9

Data cleared from C:\Users\StevenSummey\Documents\MATLAB\Image Processing\Microscope Images\hNP12SEP2022B-1 MM-PROCESSED\MM-Aggregated Data.xlsx

Figure S:



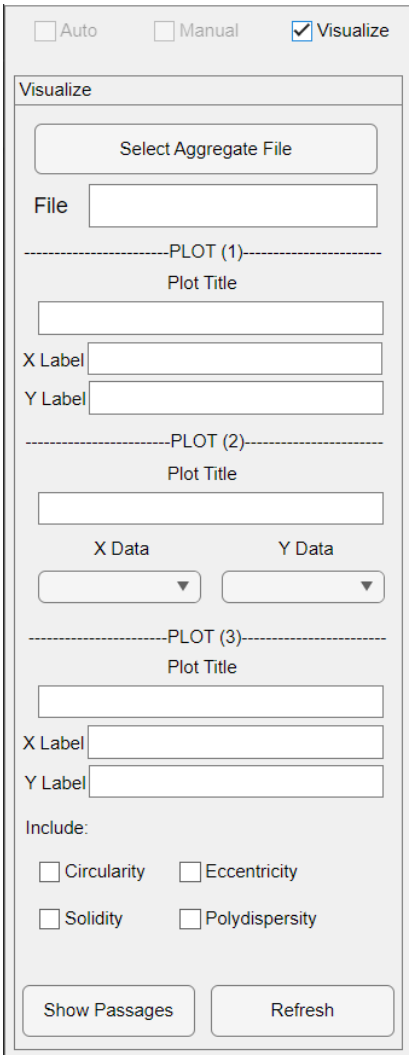
[Analyzing/Visualizing Data]:

➔ Two Excel files are created:

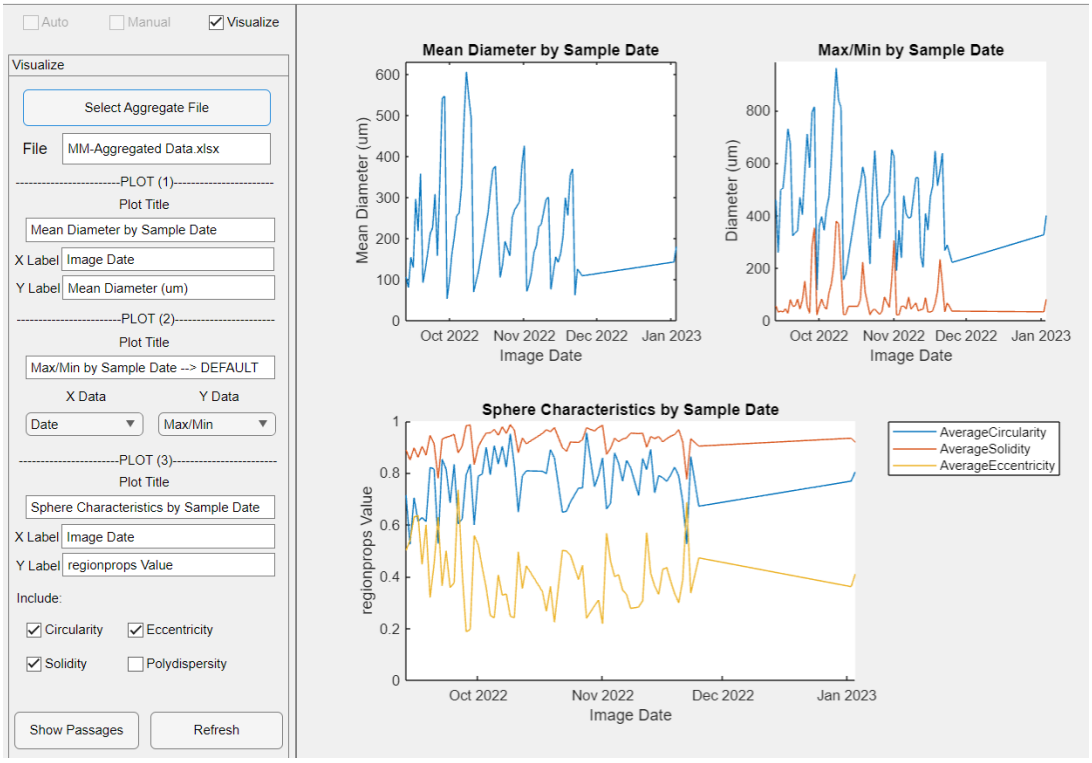
(1) [Vessel ID].xlsx is the stored processed data from which aggregated data and manual processing data is pulled.

(2) [Vessel ID]-Aggregated Data.xlsx is the aggregated data by day. Parameters stored here are used for trending and visualization.

(1) [Select Aggregate File] to visualize processed aggregate files (below).



(2) When selected, the default plots will be populated. Plot (1) will always display the mean diameter by sample date. The user can customize the plot title and axis labels. **Figure BB:**



(3) Plot (2)—top right—and Plot (3)—bottom—are customizable.

a. PLOT (2) will display the variables selected in the drop down menus.

b. PLOT (3) can toggle between measured sphere characteristics.

(4) Selecting [Refresh] will update the plots with any change made in the left panel.

(5) Selecting [Show Passages] will display the dates at which passages occurred.

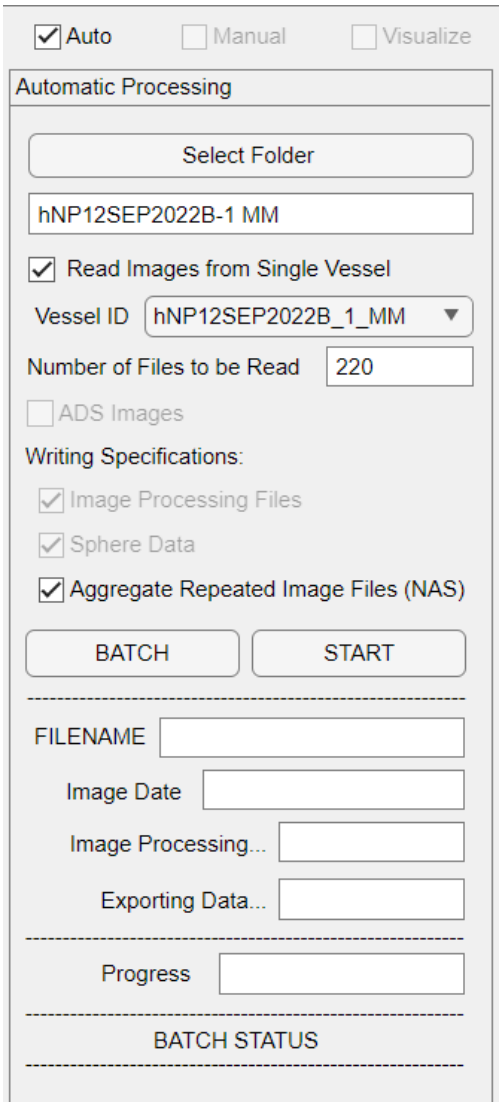
[Batch Processing]:

This section outlines the use of established segmentation settings to rapidly iterate through batches of images taken on the same day. The algorithm makes use of a reference mean either acquired through a single iteration of AUTO or from aggregated statistics should there be files previously processed on that date.

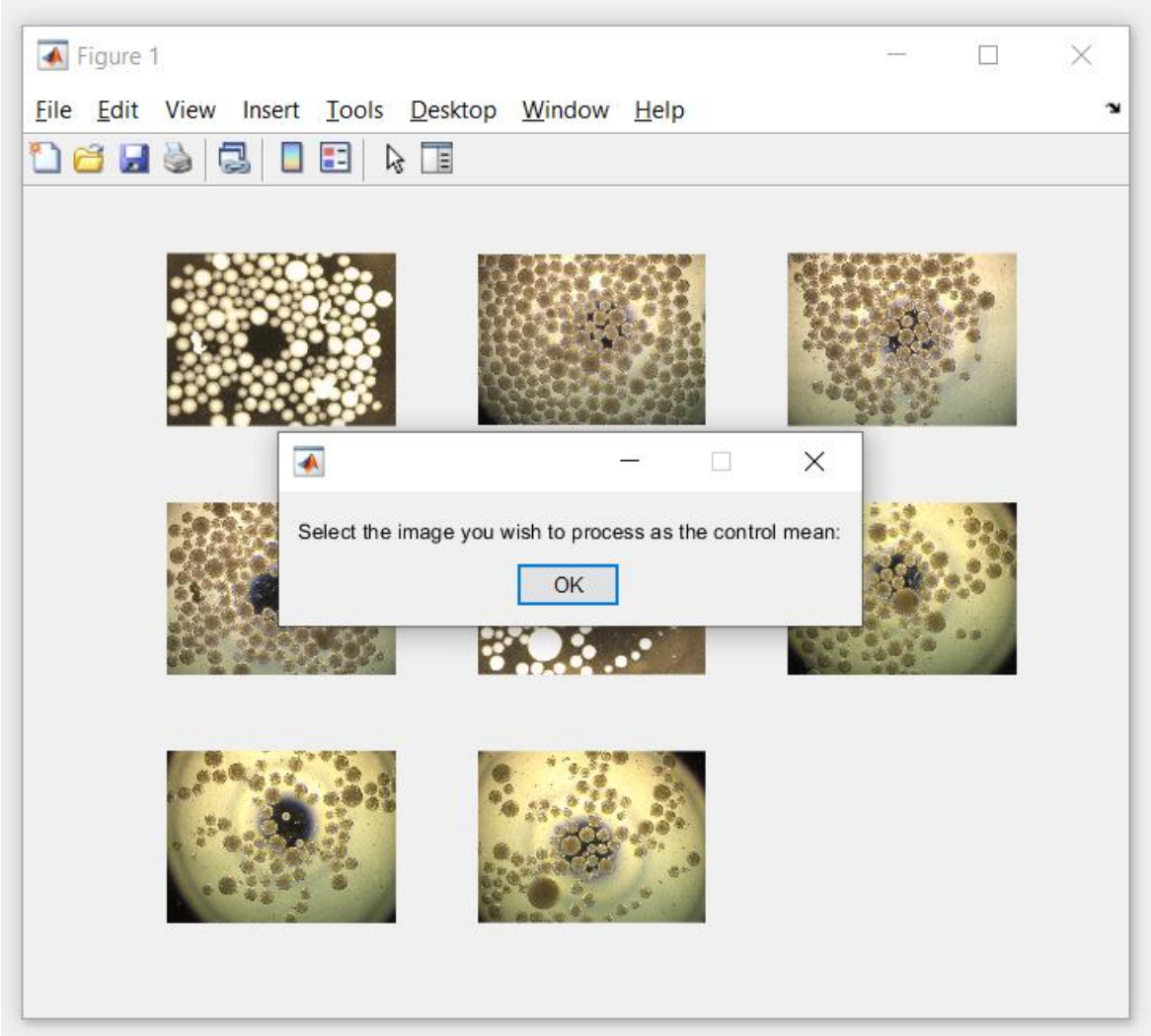
For now, the batch process is limited to single vessel lots until naming errors become less frequent.

(1) The user can begin a batch processing step after completing Step (5) in the [Running AUTO] section. In short, the user will need to select the cell culture lot folder and, subsequently, a single vessel from which to process.

(2) Select [BATCH]. **Figure AA:**



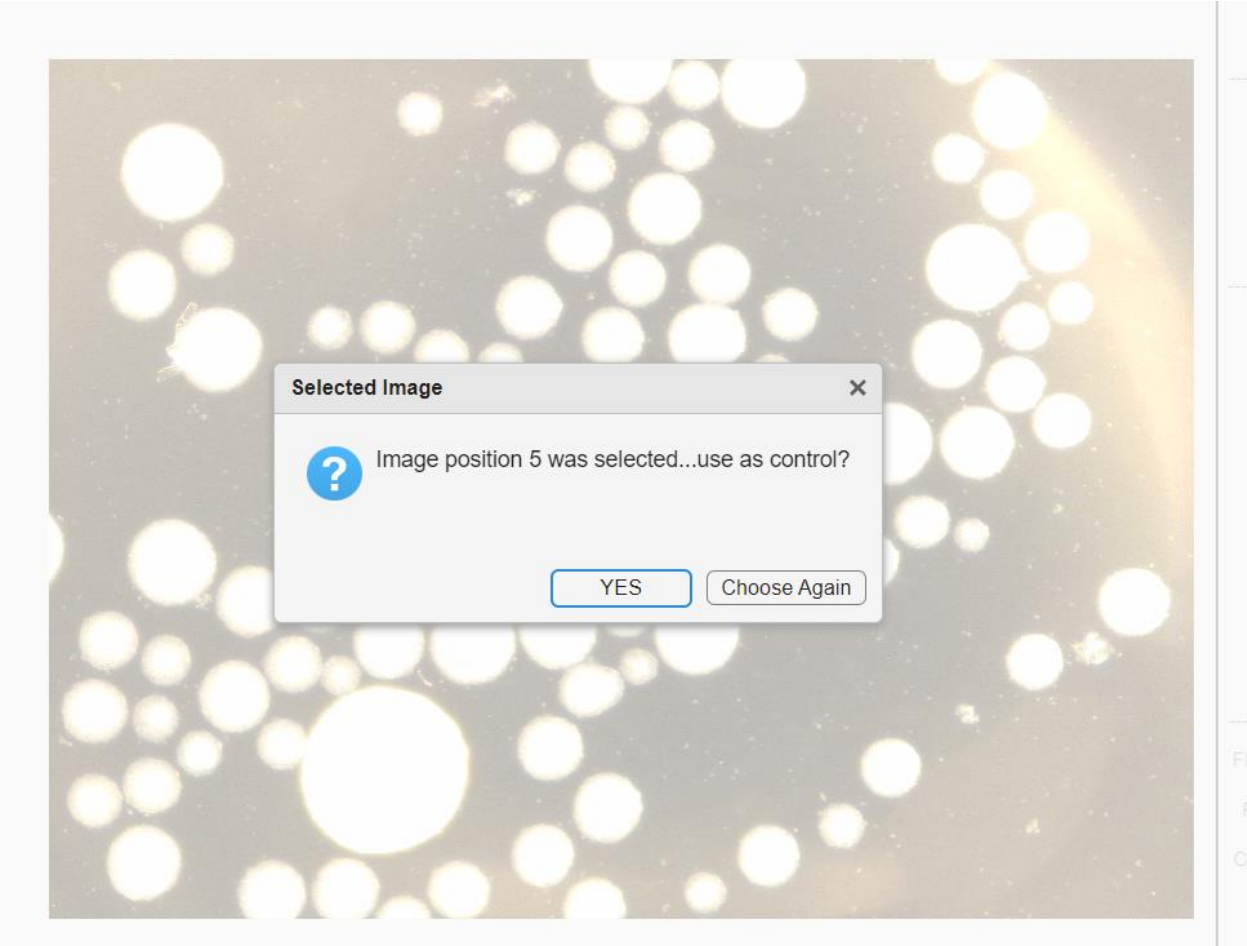
(3) As describe in the introduction to this section, if there are aggregate statistics present for the same date and vessel ID, then the user will be prompted to use these data for reference calculations. Otherwise, a figure will appear prompting the user to select the image they wish to use in reference calculations. **Figure AB:**



(4) After selecting [OK], the user will then select the image of their choosing.

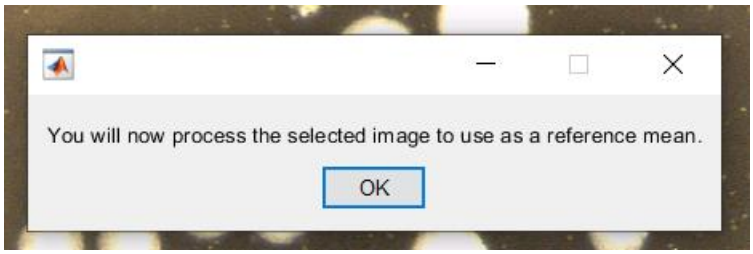
a. It is recommended to select a NO LIGHT image with high contrast. If none are available, the next best option would be any image with evenly dispersed spheres against a background with level lighting.

(5) When an image is clicked, it will then appear in the Sphere Image Analysis center panel with the following prompt, **Figure AC:**



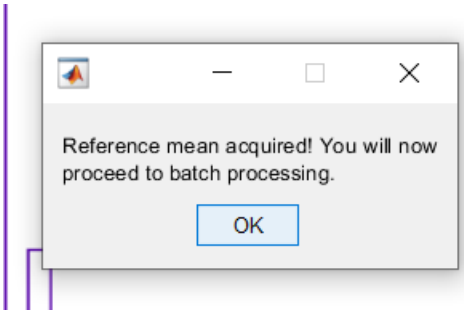
(6) Selecting [YES] will begin a single iteration of AUTO processing, **Figure AD**. Selecting [Choose Again] will return **Figure AB** and the user will need to select the desired reference image again.

Figure AD:



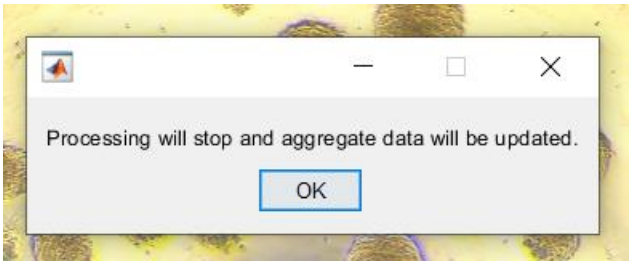
(7) When AUTO processing is of the reference batch image is complete, **Figure AE**, the batch processing loop will begin. Each image will be displayed on the center panel with the file name as the title.

a. Iterative run data will be displayed in the command window while the batch group is processed.

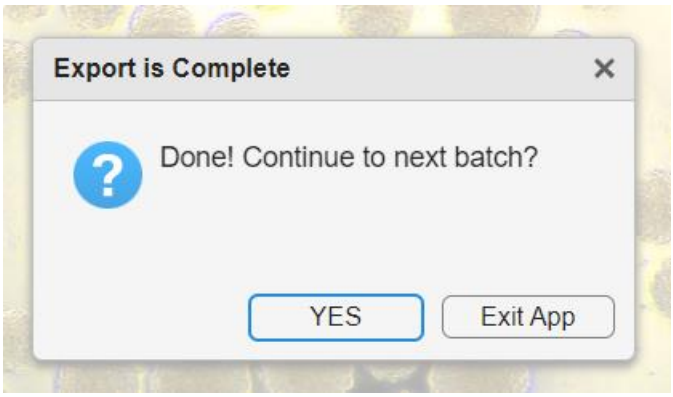


(8) The user will be prompted when the batch processing loop is complete, **Figure AF**. Selecting [OK] will aggregate the image data and update the corresponding aggregate data file (see: Analyzing Data).

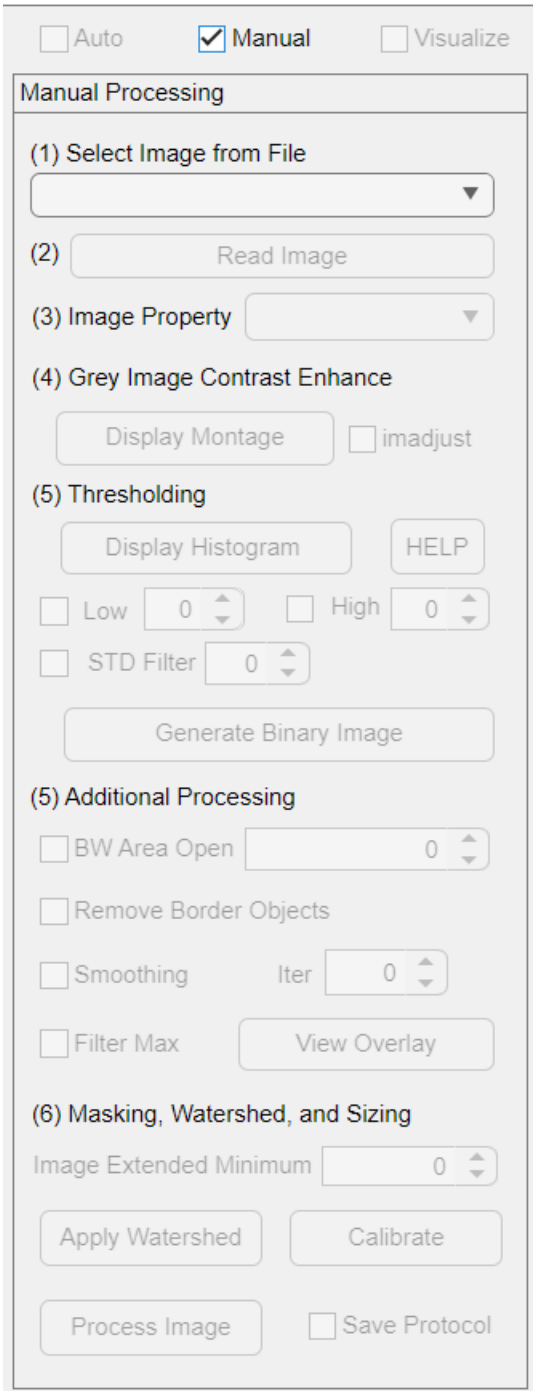
Figure AF:



(9) When complete, the user will be prompted to either continue to the next batch or exit the application, **Figure AG:**

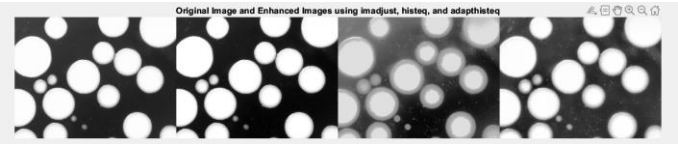


[Manual Mode]:



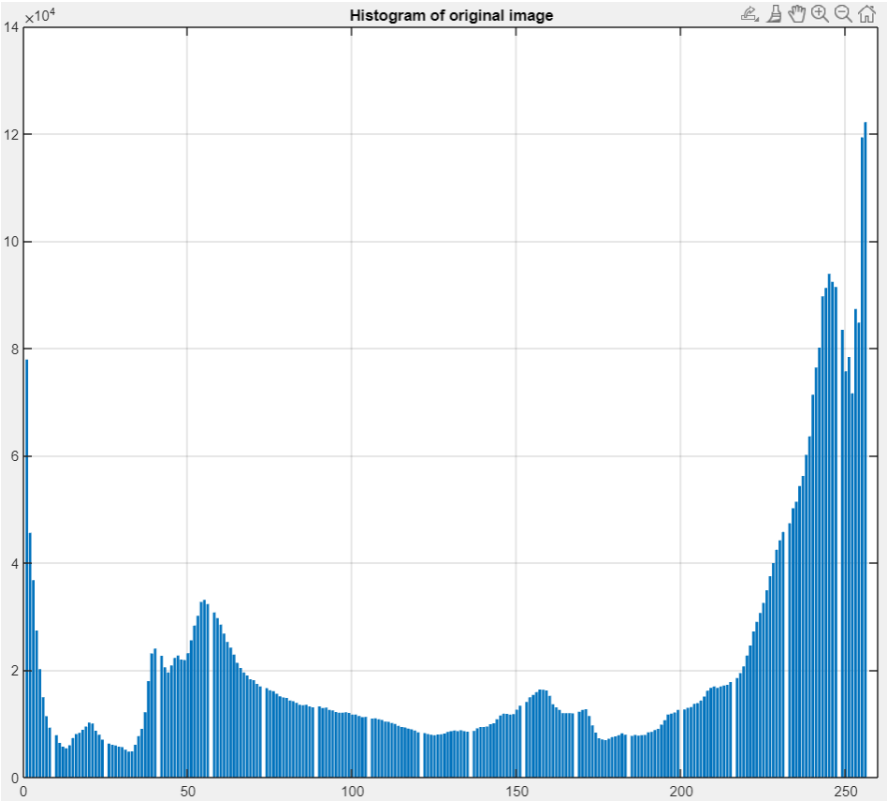
→ The manual processing panel is primarily used for training and, as addressed later, processing flagged images.

- **(1)** Select an image from the drop-down menu. These images are read from the "Training" folder in Microscope Images.
- **(2)** Read image will display the RGB image, and then ask to convert the image to grayscale. **Take note of the image properties for the next step.
- **(3)** Based on the original image, select the image property that best describes the image being processed. This is define the strategy used to best capture the region properties.
- **(4)** A montage of different gray-enhancement functions will be displayed. For our images, the most robust process is imadjust.



- **(5)** Either select [Display Histogram] **Figure T**, [HELP], or input an STD Filter.
  - **a.** For images with more ambiguous threshold values, an STD ridgeline threshold might be more effective.
  - **b.** [HELP] will open a thresholding tutorial that will guide you in selecting threshold values and update the spinner values before selecting [Generate Binary Image]. For protocol, see **APPENDIX C.**

Figure T:



(6) Select [Generate Binary Image] to advance to Additional Processing.

- **a.** BW Area Open: Start with a **minimum value of 200** to filter out areas of pixel groups counted as background noise.
- **b.** Remove Border Objects: If there are non-essential spheres or sphere segments (partial spheres on the edge of image will not be filled), check the box to remove these objects.
- **c.** Smoothing: Iteratively erode the image to close rough pixel neighborhoods. A **minimum of 5** is recommended since the erosion element radius is quite small.
- **d.** Filter Max**:** If "NO LIGHT-UNSATURATED" is selected, this option will be enabled. Select the check box to run through the process (generally requires a single iteration)
- **e.** When finished, select [View Overlay] to visualize the performance of the image processing.

(7) Input a value for the [Image Extended Minimum] **(a good default value is 2)**.

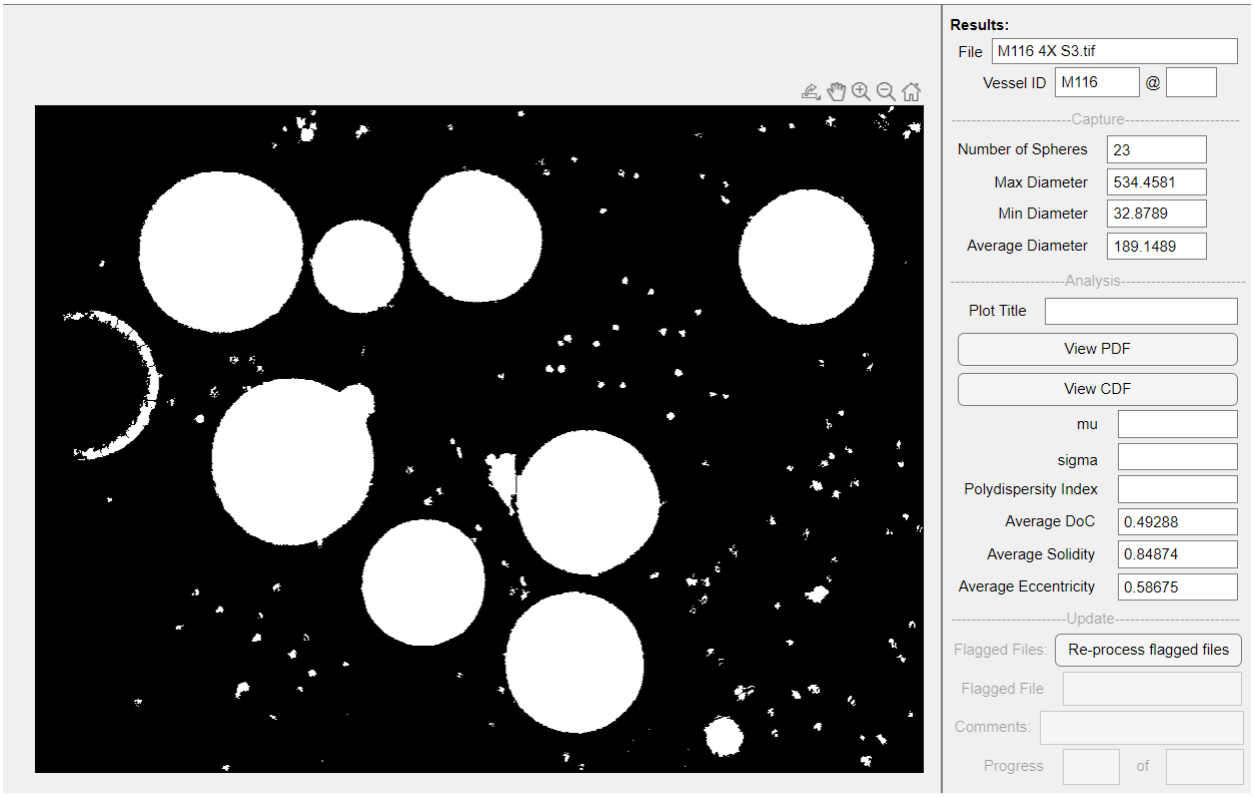
(8) Select [Apply Watershed] to run through the watershed protocol.

(9) If pixel/micron conversion calibration of a new image size is necessary (historical image sizes are analyzed during processing), select [Calibrate] to open a GUI that will run you through the calibration process.

- **a.** Images with the scale bar are mandatory for this process. These images are found in the "Spatial Calibration" folder. Any new images needed for this procedure will need to be uploaded there.

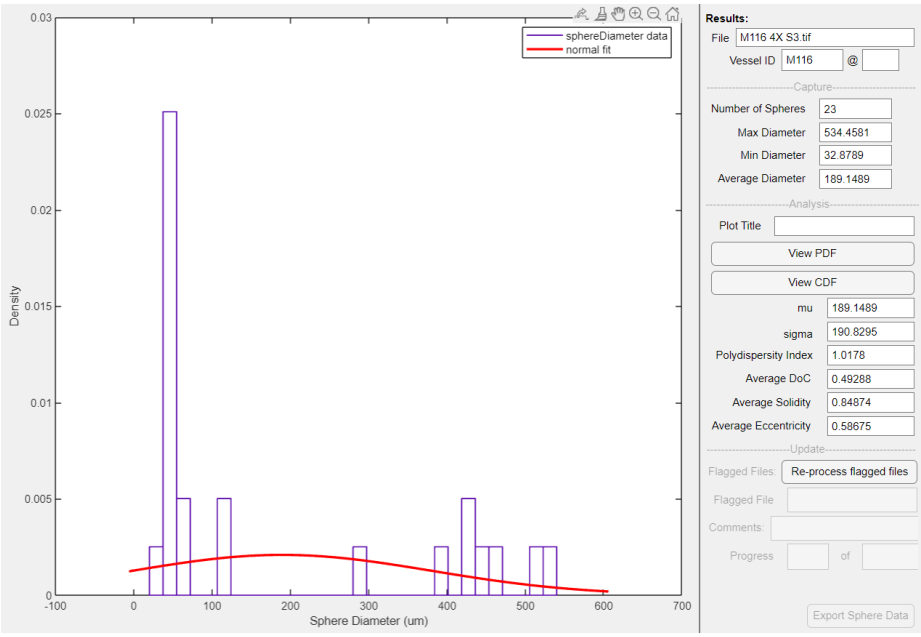
(10) When all pre-processing is complete, select [Process Image] button to acquire the region properties and view the results. **Figure U.**

Figure U:



(11) Select [View PDF] to display the bin probability density function of the spheres captured in the image. **Figure V.**

Figure V:



(12) Select [View CDF] to display cumulative density function across the bin sizes analyzed.

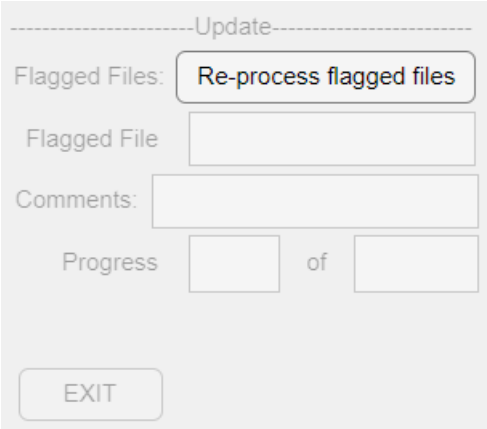
(13) Training data is not exported into any aggregate files…Manual Processing Complete!

[Flagged File Re-processing]:

➔ This section will address the flagged files that may have been poorly segmented in auto-processing. Keep in mind that re-processing may also be unsuccessful due to excessive crowding or poor image quality (thus, simply comment the issue and do not include in aggregate counts).

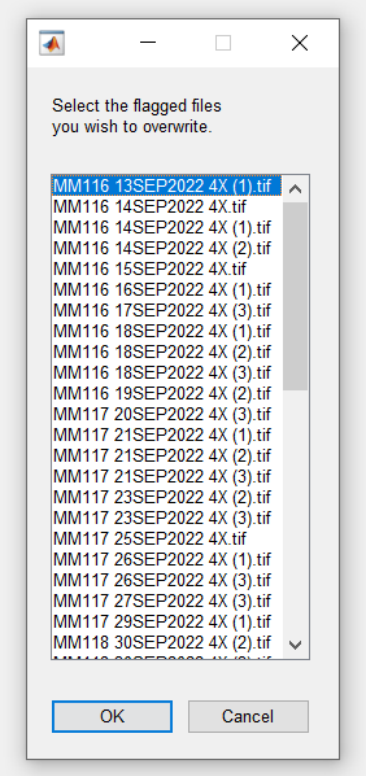
(1) With the [Manual] check box in the left panel selected, press [Re-process flagged files] in the bottom right panel. **Figure W.**

Figure W:



(2) A list box will appear with all flagged files present in each directory of processed images. Select a single file or multiple. **Figure X:**

(3) Advance to [Read Image] on the left panel to begin processing. See [Manual Mode] for additional information on processing in this mode.



(4) When processing is complete, advancing past the user prompt to export sphere data will display the old/new analyses for determining which values to accept. **Figure YA, Figure YB, Figure YC.**

Figure YA:

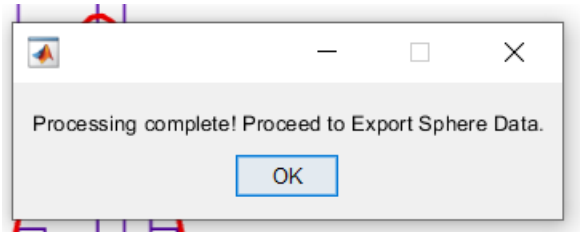


Figure YB:

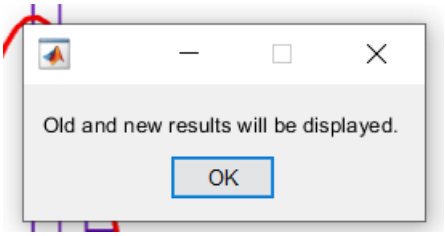
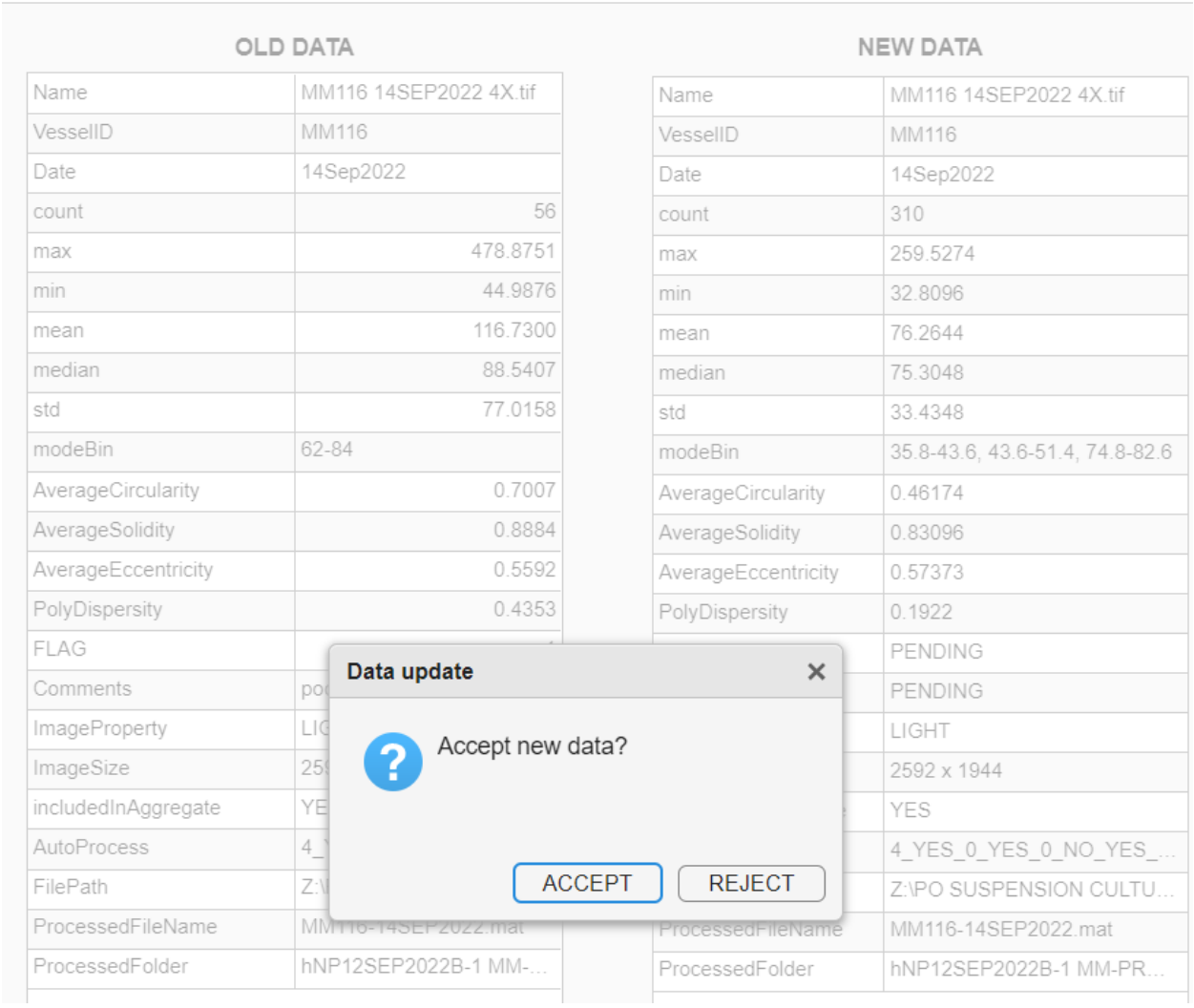


Figure YC:



(5) Accepting the new data will overwrite the individual sphere characteristics and excel file data.

(6) Rejecting the new data will keep the file as is.

- The flag file setting will be set to 0 and the comments will note that re-processing was unsuccessful. Don't worry, some images are far too poor to segment properly.

[APPENDIX A] FUNCTIONS AND SCRIPTS

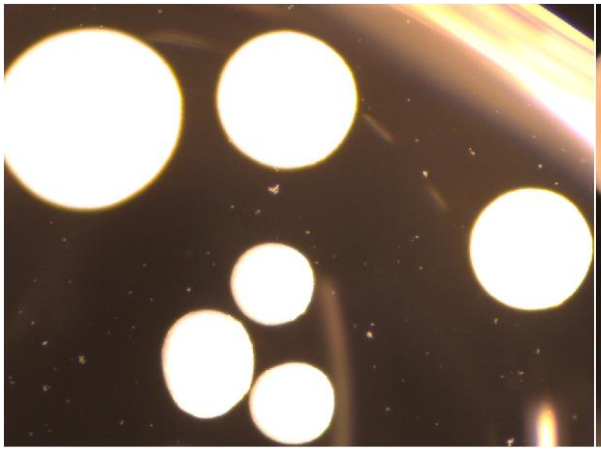
(descriptions to be added)

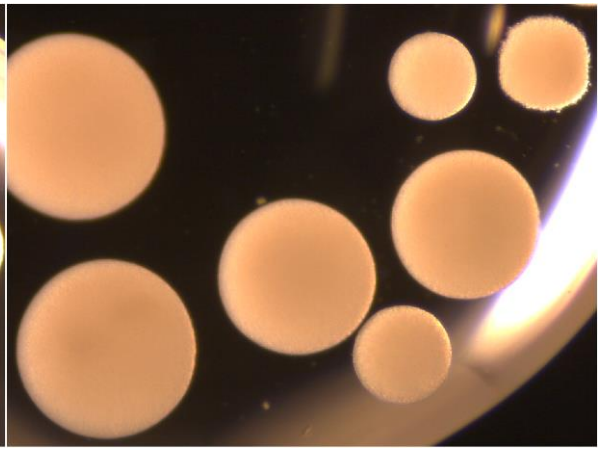
- aggregateData
- batchProcess
- circle_hough
- circle_houghpeaks
- clearExcelFile
- createSphereCDF
- createSpherePDF
- detectChange
- fileListParse
- fileSearch
- imagePropertyAnalysis
- imagePropertyProcessing
- individualImageDataSearch
- lightNonHomogeneousProcessing
- lightProcessing
- noLightProcessing
- savistzky_golay_filter_smooth_outline
- spatialCalibration
- threshold
- thresholdHELP

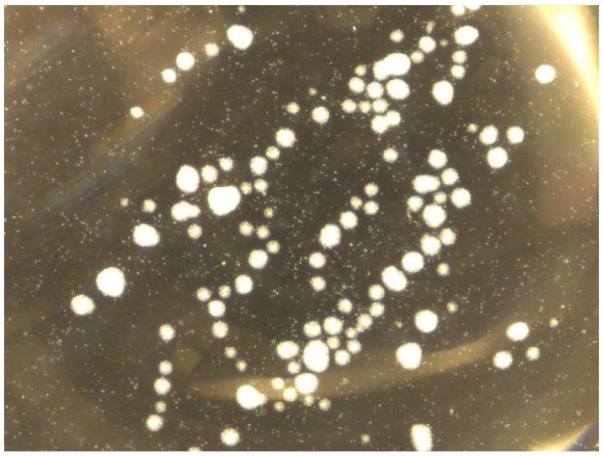
[APPENDIX B] IMAGE PROPERTY OUTLINE

==========NO LIGHT==========

Example Images:







Top right image can be processed using NO LIGHT and NO LIGHT-UNSATURATED.

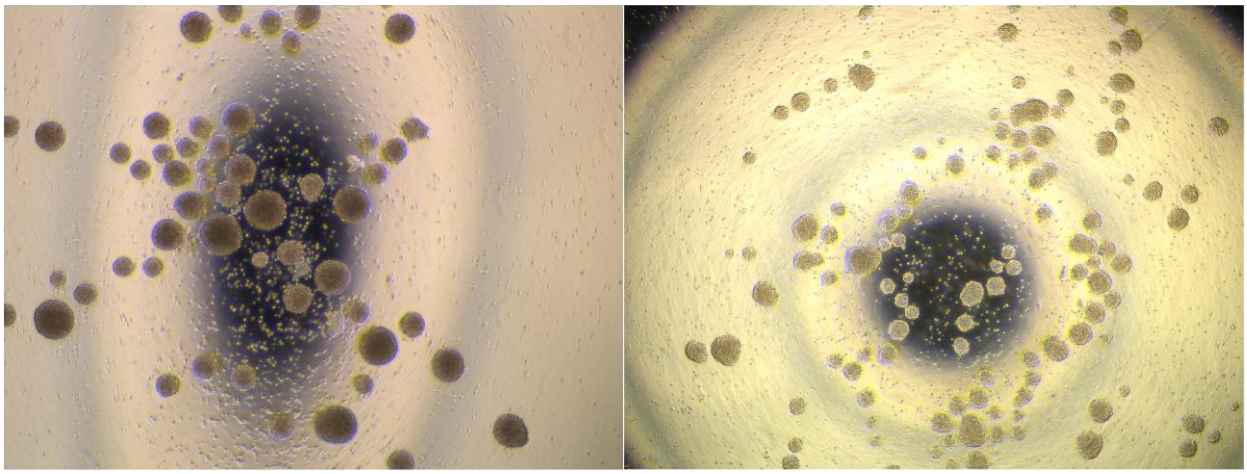
***Highlighted sections require user input. These values are stored for learned batch-processing.

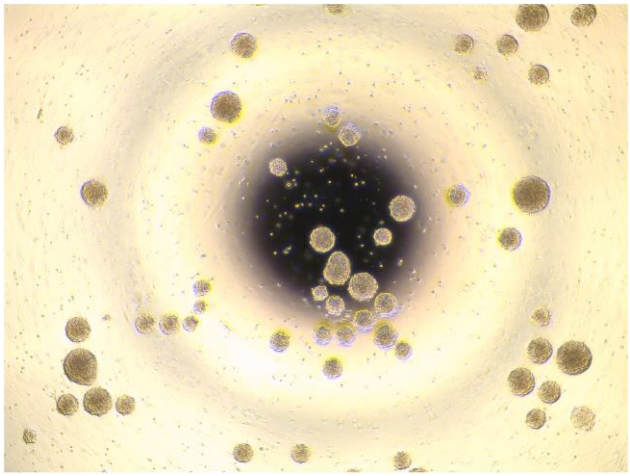
(1) Image converted using rgb2gray
(2) Enhanced grayscale contrast with imadjust
(3) Display histogram of grey values (0-255)
    a. Set low threshold (darkest value) → depending on exposure, this value will sit between 225 and 245 (both left images above were set using 245)
    b. Set high threshold (lightest value) → set at 255 when spheres are clearly the brightest objects in the foreground (for images like the top right example, the histogram will show a peak around which the user should threshold to capture the sphere grey values—omitting cases of glare or poor image quality)
(4) Binary image is displayed.
(5) Clear border objects if they are not connected to groups of spheres in frame. YES or NO prompt.
(6) Eliminate binarized areas below area of interest with bwareaopen.
(7) Automatic dilation of sharp edges with bwmorph, 'close.'
(8) Display newly masked image over the original image.

(9) Watershed transform to isolate crowded spheres and optimize edge-detection of binary image.
(10) Final sizing filter:
    a. Set minimum diameter.
    b. Set maximum diameter.

==========LIGHT==========

Example Images:





Used for smaller, uniformly intense sphere images.

(1) Image converted using rgb2gray
(2) Enhanced grayscale contrast with imadjust
(3) Standard Deviation ridge-line filter
    a. Choose ridge-line value
    b. Pixel neighborhoods with a standard deviation below the threshold value will be filtered out. Smaller spheres tend to require a value of 8-10.
(4) Binary image is displayed.
(5) Clear border objects if they are not connected to groups of spheres in frame. YES or NO prompt.
(6) Eliminate binarized areas below area of interest with bwareaopen.
(7) Connect edges by dilating image for imfill.

a. This function will iteratively run dilation steps of a set thickness based on the user input. YES/NO.
    i. Look for the edges of spheres to be completely connected to ensure the filling process is successful. If the sample is polydisperse and the edges are inconsistently connected, aim to connect an operable majority of the sphere samples.
b. Fill dilated image with imfill.

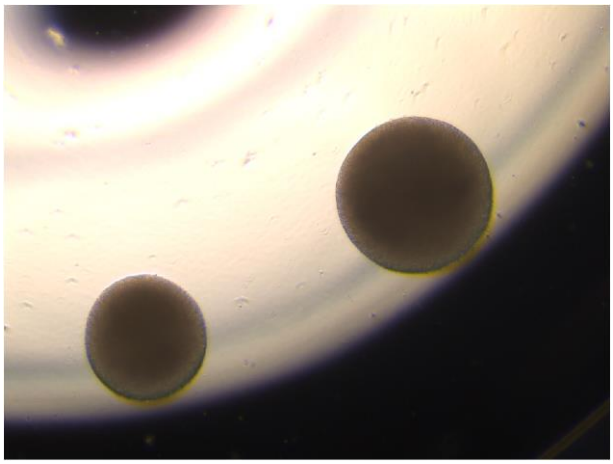
(8) Erode filled image until the overlay fits the original image.
a. If multiple dilation steps were taken to fill the image, then an image erosion might be necessary.
b. Cycle through YES/NO until the masked image accurately captures the original image.

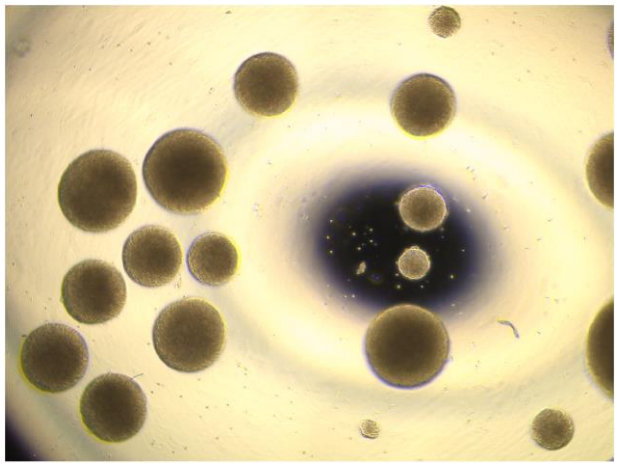
(9) Watershed transform to isolate crowded spheres and optimize edge-detection of binary image.

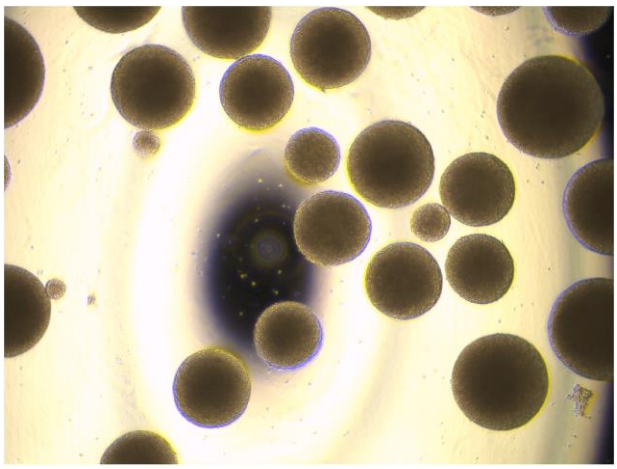
(10) Final sizing filter:
a. Set minimum diameter.
b. Set maximum diameter.

==========LIGHT-NONHOMOGENEOUS==========

Example Images:







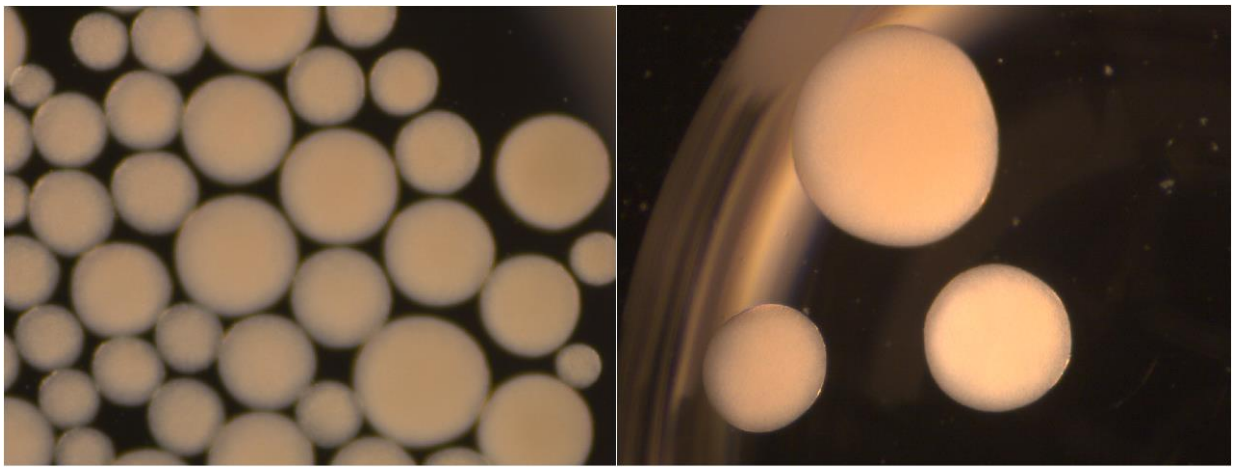
Ideal for large, sparsely populated, and polydisperse samples.

(1) Image converted using rgb2gray

(2) Enhanced grayscale contrast with imadjust
(3) Standard Deviation ridge-line filter
    a. Choose ridge-line value
    b. Pixel neighborhoods with a standard deviation below the threshold value will be filtered out. Medium to large spheres tend to require a value of 4-5.
(4) Binary image is displayed.
(5) Eliminate binarized areas below area of interest with bwareaopen.
(6) Erosion of overly saturated binary image
    a. Often unused, but if spheres display significant erosion to separate or shave detected debris, cycle through the YES/NO prompt until the image is properly eroded.
(7) Connect edges by dilating image for imfill.
    a. This function will iteratively run dilation steps of a set thickness based on the user input. YES/NO.
        i. Look for the edges of spheres to be completely connected to ensure the filling process is successful. If the sample is polydisperse and the edges are inconsistently connected, aim to connect an operable majority of the sphere samples.
    b. Fill dilated image with imfill.
(8) Clear border objects if they are not connected to groups of spheres in frame. YES or NO prompt.
(9) Erode filled image until the overlay fits the original image.
    a. If multiple dilation steps were taken to fill the image, then an image erosion might be necessary.
    b. Cycle through YES/NO until the masked image accurately captures the original image.
(10) Watershed transform to isolate crowded spheres and optimize edge-detection of binary image.
(11) Final sizing filter:
    a. Set minimum diameter.
    b. Set maximum diameter.

==========NO LIGHT-UNSATURATED==========

Example Images:

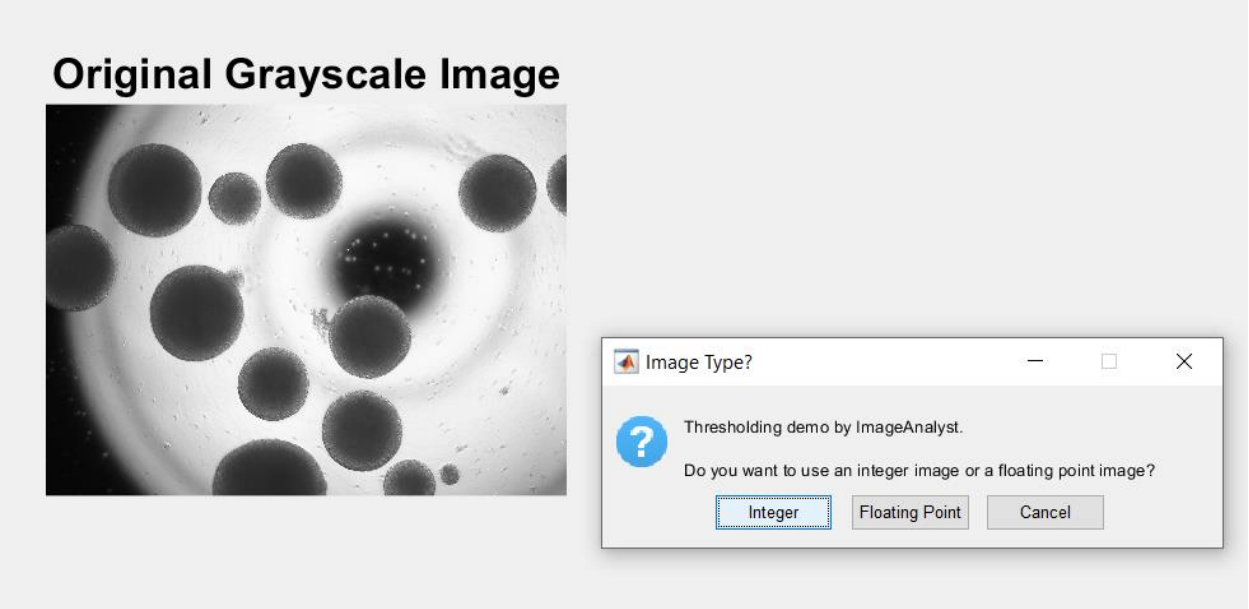


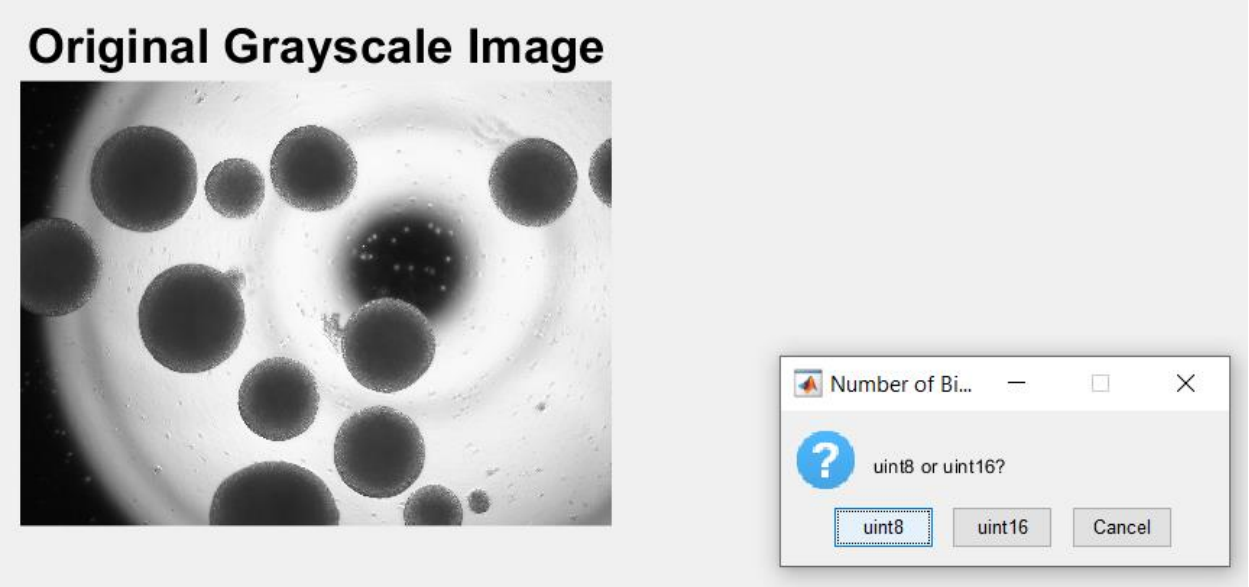
**A key feature of this image property strategy is filter out the maximum area of negative space in the binary image created by an inverse std filter step. Keep this in mind, as the user may need to erode the image to ensure the background will be the largest area filtered. When in doubt, use the NO LIGHT setting or flag the file for follow-up.

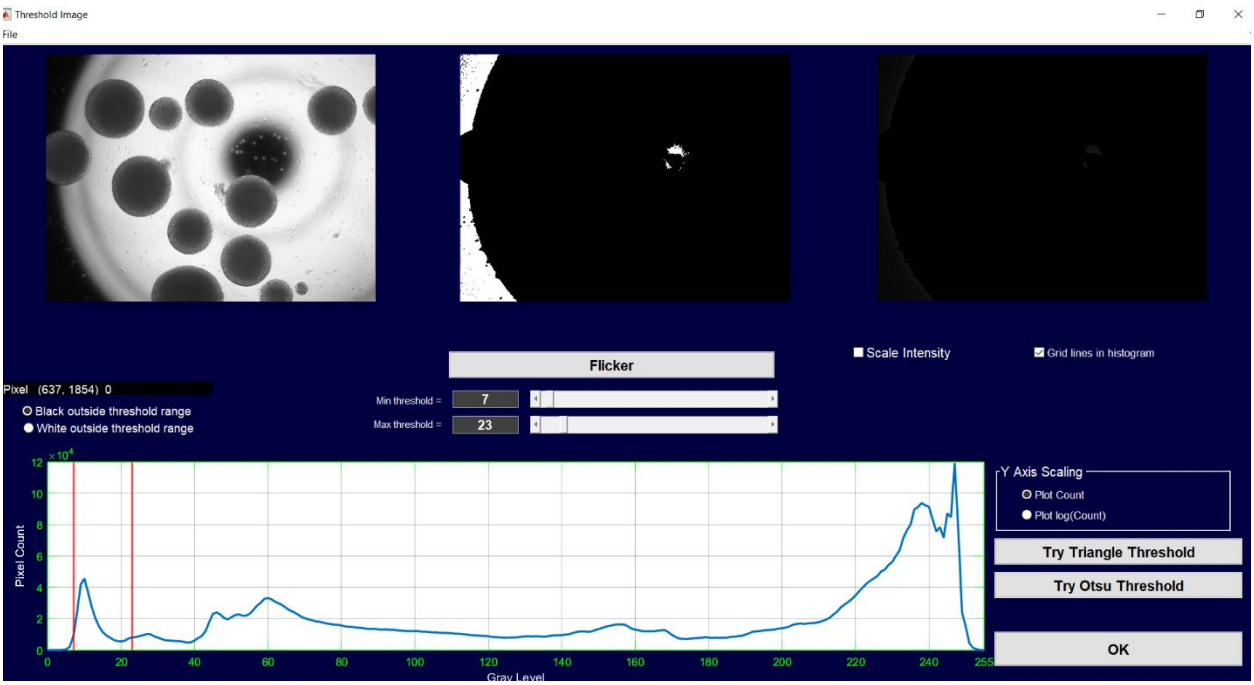
(1) Image converted using rgb2gray
(2) Enhanced grayscale contrast with imadjust
(3) Standard Deviation ridge-line filter
    a. Choose ridge-line value
    b. Pixel neighborhoods with a standard deviation below the threshold value will be filtered out. Medium to large spheres tend to require a value of 4-5.
(4) Erosion of overly saturated binary image
    a. Cycle through the YES/NO prompt until the image is properly eroded and spheres are disconnected from the background area to be filtered out.
(5) Filter out the negative space. YES or NO.
    a. Usually requires a single iteration if erosion was not too harsh.
(6) Clear border objects if they are not connected to groups of spheres in frame. YES or NO prompt.
(7) Eliminate binarized areas below area of interest with bwareaopen.
(8) Connect edges by dilating image for imfill.
    a. This function will iteratively run dilation steps of a set thickness based on the user input. YES/NO.
        i. Look for the edges of spheres to be completely connected to ensure the filling process is successful. If the sample is polydisperse and the edges are inconsistently connected, aim to connect an operable majority of the sphere samples.
    b. Fill dilated image with imfill.
(9) Erode filled image until the overlay fits the original image.
    a. If multiple dilation steps were taken to fill the image, then an image erosion might be necessary.
    b. Cycle through YES/NO until the masked image accurately captures the original image.
(10) Watershed transform to isolate crowded spheres and optimize edge-detection of binary image.
(11) Final sizing filter:
    a. Set minimum diameter.
    b. Set maximum diameter.

[APPENDIX C] Manual Thresholding

(1) When selecting [HELP] at Step (5) of Manual Processing, a separate GUI figure will appear to guide you on incrementally thresholding the image. Select [Integer] and [unit8] to proceed to the GUI.





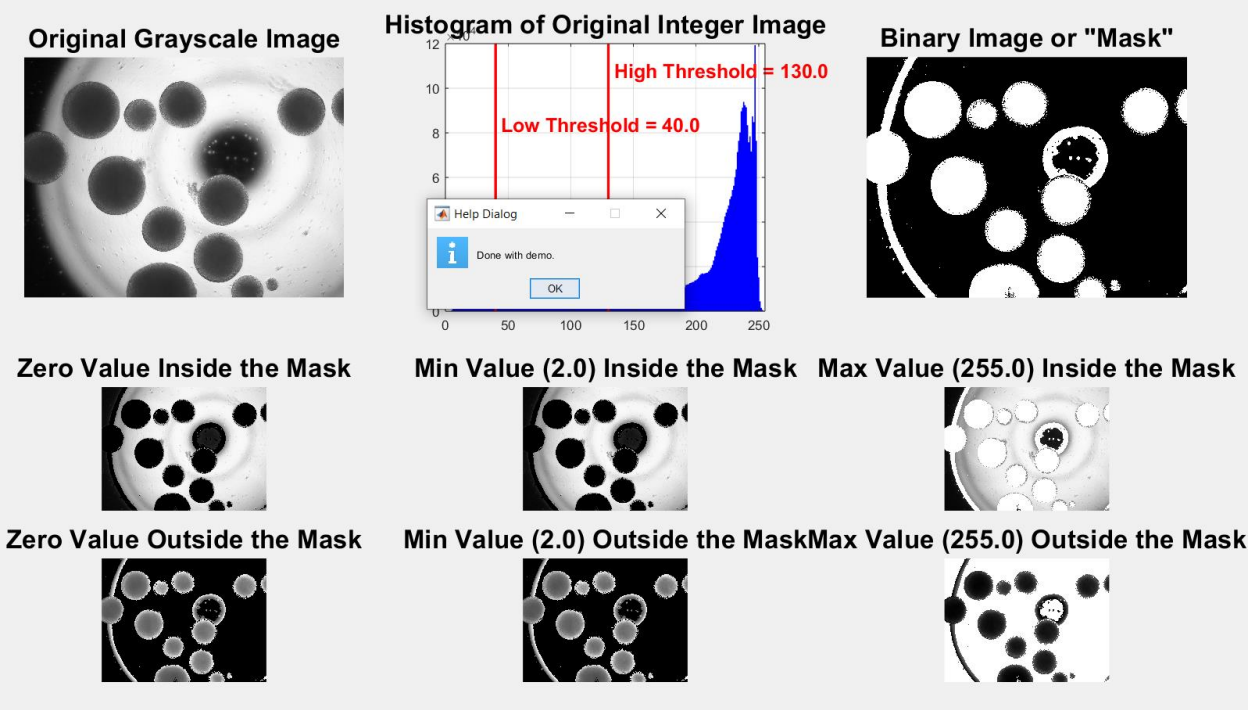


(2) The bottom pane will display the grayscale values. The middle sliders will adjust the threshold intensities.

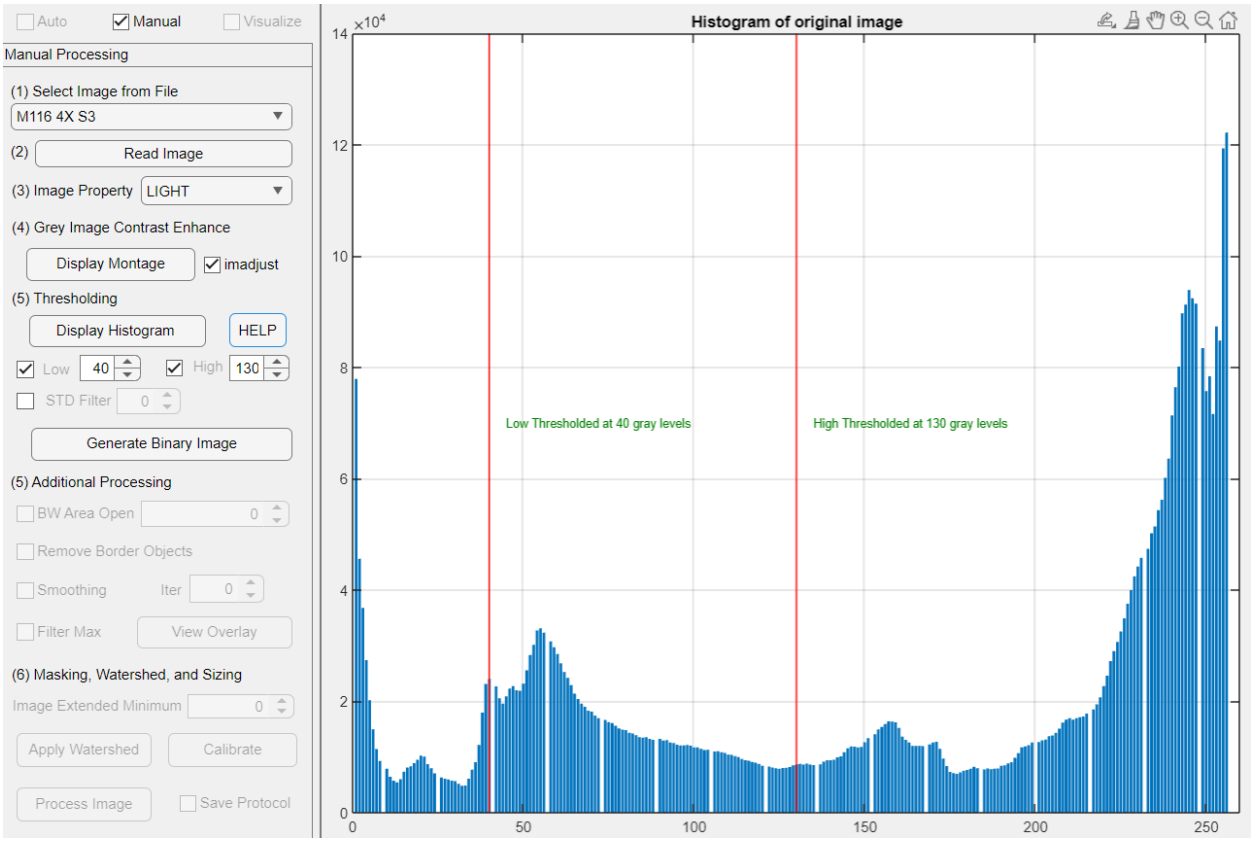
a. Start with maximum threshold and then minimum threshold.

(3) When image thresholding is satisfactory, select [OK] in the bottom right corner.

(4) A final figure will be displayed when thresholding is complete. It is helpful to observe the boundary characteristics captured by this approach.



(5) The manual threshold values will then be displayed on the histogram in SphereImageAnalysis. Additional adjustments can be made if needed. Select [Generate Binary Image] to proceed to Step (6).



[APPENDIX D] Microscope Computer to NAS

➔ **MIGRATING MICROSCOPE IMAGES TO ArunaNAS:** This section will be updated when tutorial screenshots and processes are more consistently outlined.

➔ **NAMING:** Please double check file names before copying images over to the ArunaNAS as these files cannot be re-written from non-administrative computers.

(1) Keep naming consistent. {VESSEL ID + SAMPLE (if necessary) + MAGNIFICATION (4X)}

- Vessel ID should include a differentiating identifier (normally a letter(s)) along with the passage number e.g. B52

(2) Pay attention to file names and magnifications when acquiring images.

EXAMPLES/GRIEVANCES:

fileSearch.m has turned from a parsing function to the preliminary strike in addressing naming errors with conditional specificities. For the sake of performance, avoiding run-time errors, and, worse yet, misrepresentation of data, please be mindful of the way your images are named and acquired

MM19 S1 4X.tif … NO

MM1221 S2 4X.tif … NO

MM1.14X S2.tif … NO

MM14.3 S1 4X.tif … NO

MM114. S3 4X.tif … NO

4X STR50 [Date] … NO

wrong passage numbers

missing letter from vessel ID

zeros instead of 'O'

[APPENDIX E] References and Additional Exploratory Literature

Notable Additions/Under Development