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Title: WalkThrough Example Procedures for MAMA

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WalkThrough Example Procedures for MAMA

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SEM Images from Angelique Wall, MST-16, LANL.

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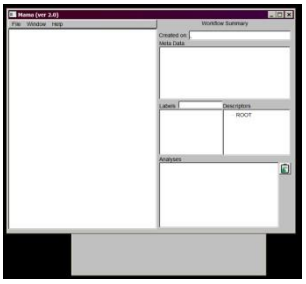
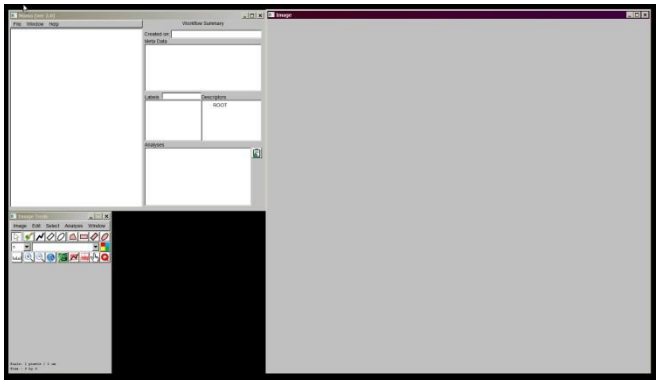
1.0 Introduction

1.1 Scope

This documentation is a growing set of walk through examples of analyses using the MAMA V2.0 software. It does not cover all the features or possibilities with the MAMA software, but will address using many of the basic analysis tools to quantify particle size and shape in an image. This document will continue to evolve as additional procedures and examples are added. The starting assumption is that the MAMA software has been successfully installed.

2.0 MAMA ANALYSIS EXAMPLE PROCEDURES

2.1 PROCEDURE Q1: Basic Quantification & Report

STEPS	IMAGES
1. Start MAMA. The MAMA windows will be stacked on top of each other. Arrange them to fit your screen and preferences. Resize the image window to make it bigger.	  

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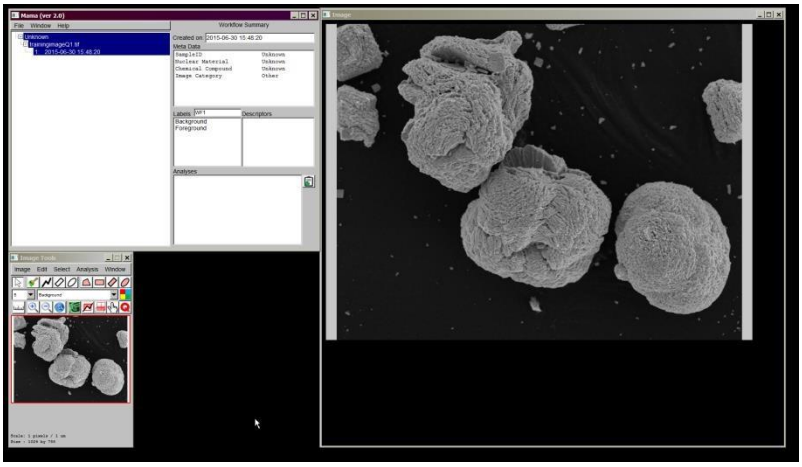
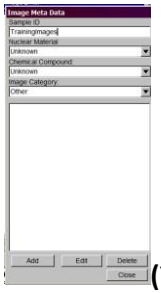
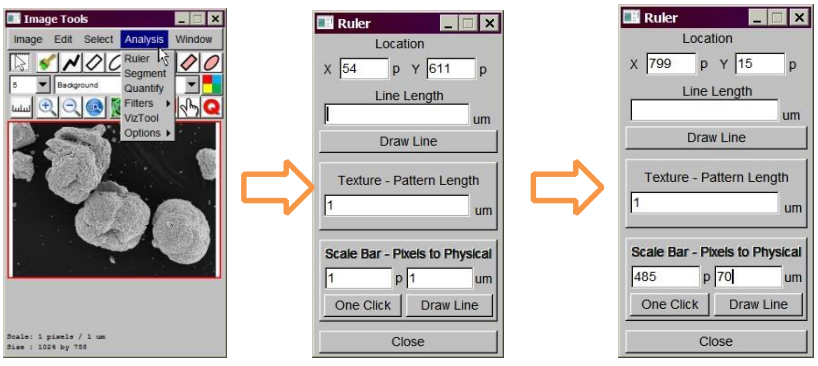
2. In the main MAMA window, click File→New image→ Find and select image "trainingimageQ 1.tif" (should be a training data folder that was installed with the MAMA software)	
3. In the MAMA window, click Window→ Show Metadata. Enter a Sample ID into the metadata top field.	 (You can add other info as well.)
4. In the Image Tools window, Click Analysis→Ruler **Enter 485 pixel and 70 □m into the bottom fields, then click close.**	 Note: in this image, the scale was in additional metadata, rather than in a displayed scale bar, so we are just entering it here.

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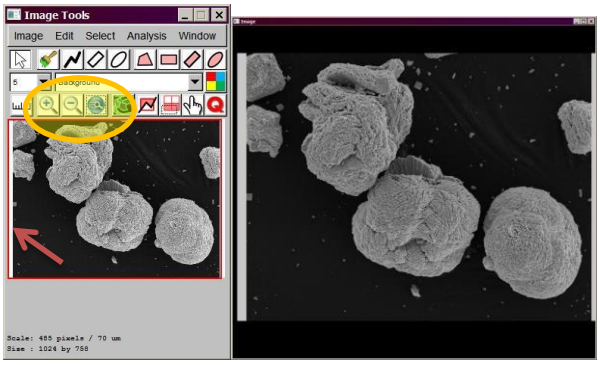
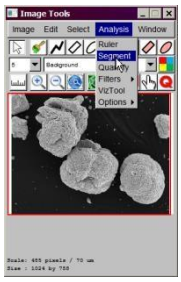
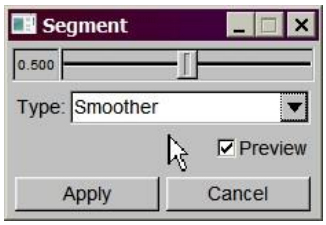
5. Use the magnifying glass icons and red slider window to make sure the image is fully displayed in the image window.



6. Segment the particles:

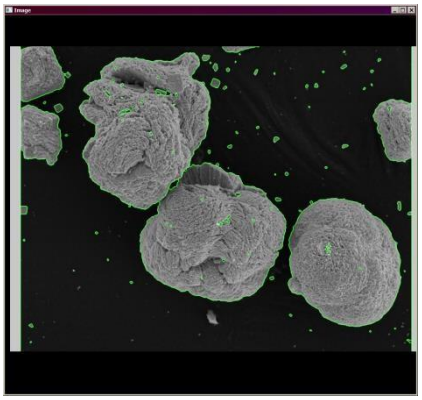
The goal here is to segment out and the three large particles for analysis. We are not going to worry about the little ones in this example.





Click analysis→ segment.
This will pop up the segmentation slider window.

Leave the setting on Smoother and adjust the slider to a setting of 0.880. Click Apply.



The particles will be slightly oversegmented, but separated.

At first, the image will be covered in red segments (it will be over-segmented— this is not shown here.)

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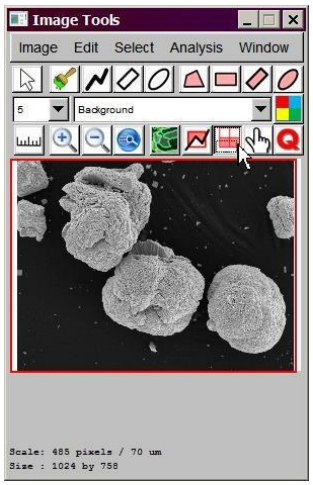
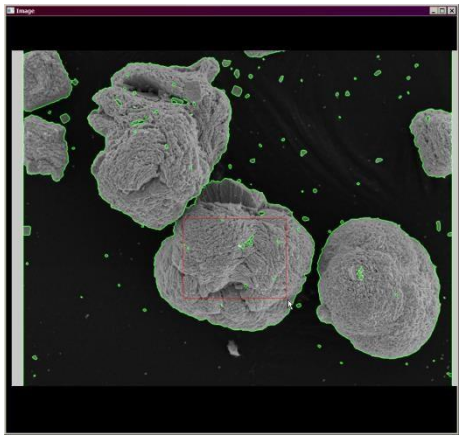
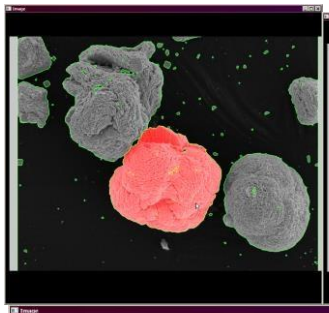
7. Clean up segmentation of particles.

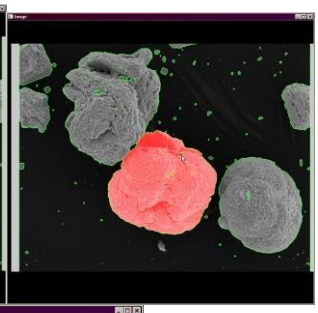
Select the bounding box tool and drag a bounding box around the center of the middle particle. This will select most of the small inner segments.



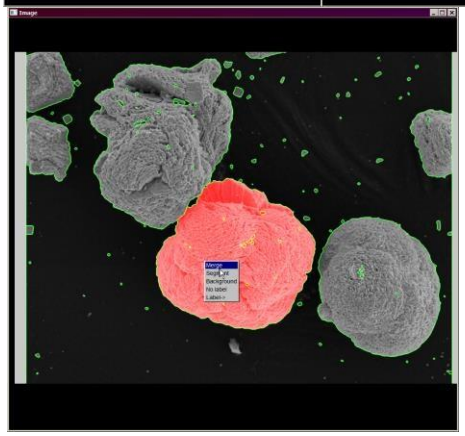


Click on any additional segments in this particle that are not selected (red highlight).





(note: if you accidently select or click something you don't want as part of the particle, select the hand tool to click select/unselect. Example in next step.)



The RIGHT CLICK in the particle that is highlighted and select MERGE. (You can repeat

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the merge as often as needed.)	

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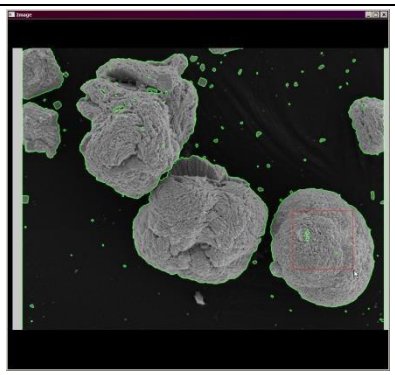
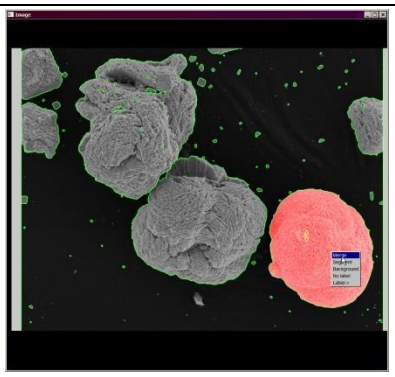
Repeat with the other 2 particles. **In lower particle, box the small segments in the particle and right-click merge.**	 

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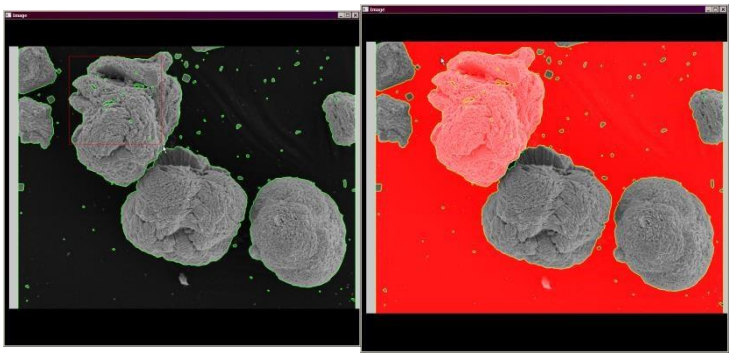
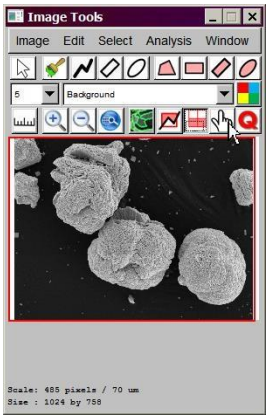
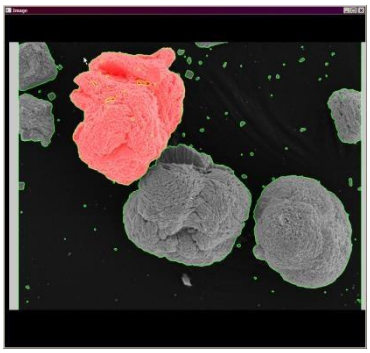
In the upper particle,

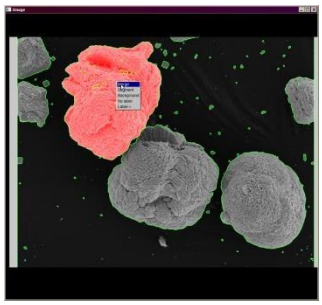
Select bounding box tool, and box a region that goes outside the particle in the upper left. (This gets all those little segments at once!) However, it also selects the background.



Select the hand 'Drag Select' tool. This allows you to click and select on any segment to turn it on & off **Click in the background to unselect it.**







If the particle looks complete, right click→ merge.

Note: if you make a mistake, you can click EDIT→ undo (XXXX) in image tools, where XXXX will be the name of the last step. If you have done a lot of clicking, it's all saved as edits, so it might take a while to undo back to a critical step like a merge. You can see the steps that are saved in the workflow window (window→show workflow) to see how many steps you'd need to undo, but you can only undo one step at a time.)

(You could have used the drag-select tool instead of bounding boxes to select all the segments to merge, but that takes a lot longer…)

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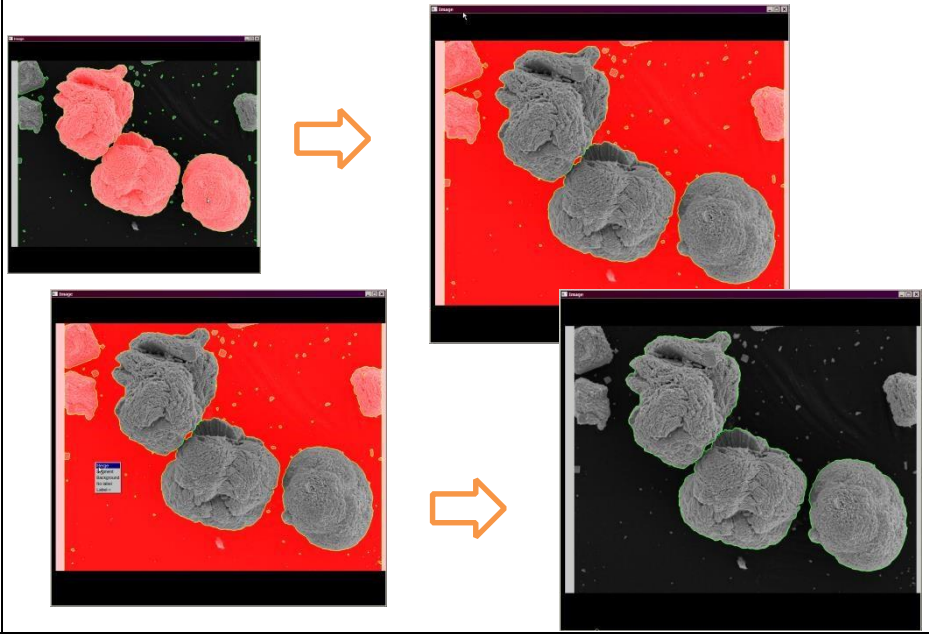
8. Clean up segments on everything else:

Click the hand drag-select tool, and click on the three particles to highlight them.

In Image tools, click Select→select inverse set. This will select inverse set for highlighting.

Right click somewhere in red highlighted region and merge.



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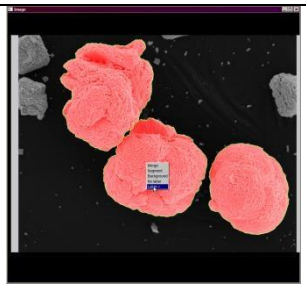
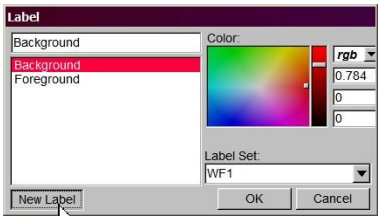
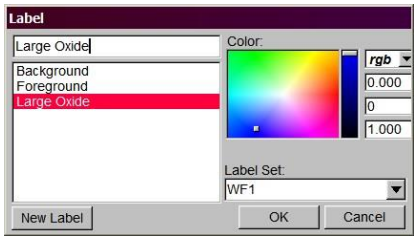
9. Label the particles

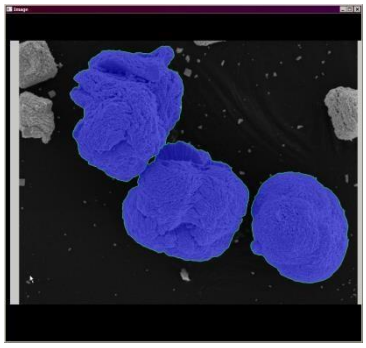
Use the handdrag select tool to select the 3 particles.

Right click → Label
This brings up the label window.
Select New Label. Enter a name in the top field, then click ok.
(use the color box to select a color for the particles)









Note: you don't have to label the particles…sometimes it is easier to label everything else, and just use the particles as "unlabeled."

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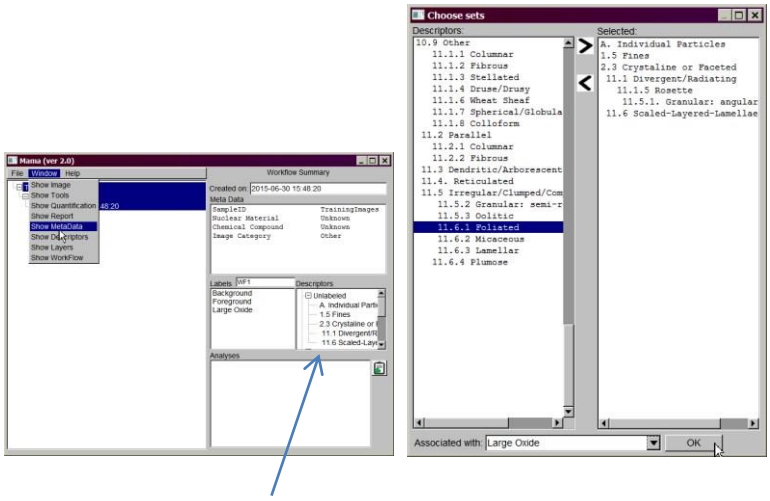
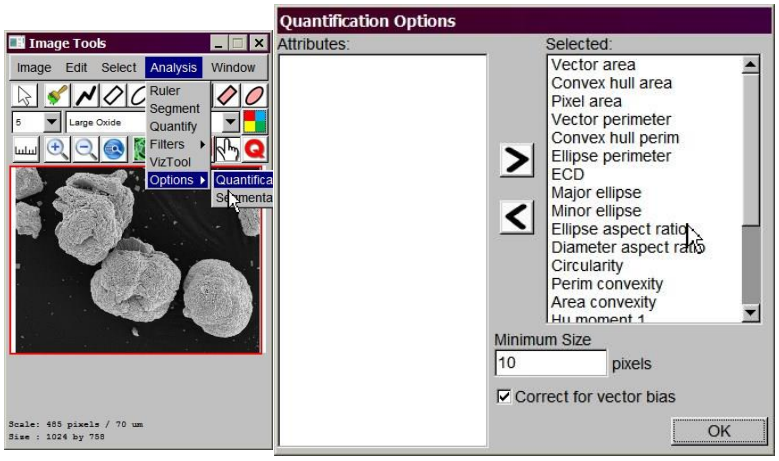
10. (Optional) Add Descriptors. **MAMA Window→ Show Descriptors.** **Use the term list at bottom to assign them to a labeled set of particles, then use the arrows to add terms to selected list.** **Click OK.**	 Note: Selected terms will now show up in the middle right hand window of the main MAMA window frame. Full definitions of terms are in separate document.
11. Prepare to Quantify **Select Analysis→ Options→ Quantification options. Use the arrows to remove attributes, or add them back in.**	 The default is ALL attributes, bias correction on, but you can change to other settings. It is probably best practice to always check since these settings will change quantification results.

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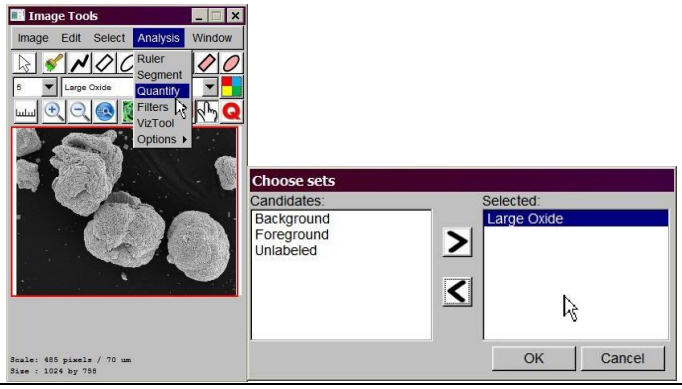
12 Quantify

In Image tools, select Analysis→ Quantify.

In the Choose Sets Window, select the name you chose for the particles and **remove 'unlabeled'** from the selected sets. **Click OK.**

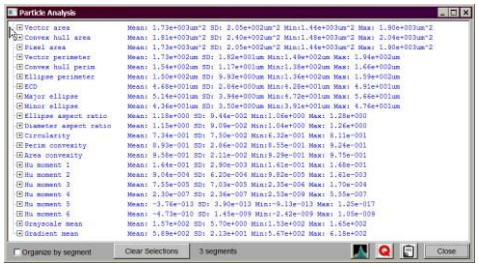


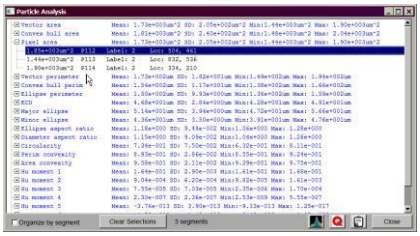
The list of attributes will pop up in the Particle Analysis window.

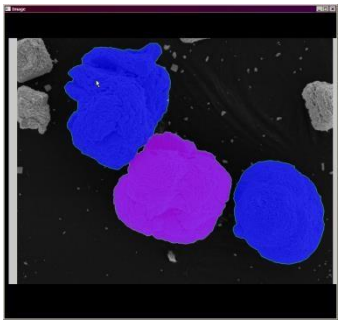
Click on any attribute to see the particles individually.

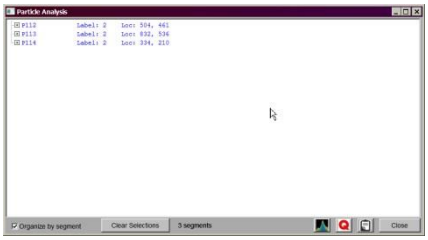
Click on a particle to highlight it in an image.

You can sort these by particle (segment) rather than by attribute.









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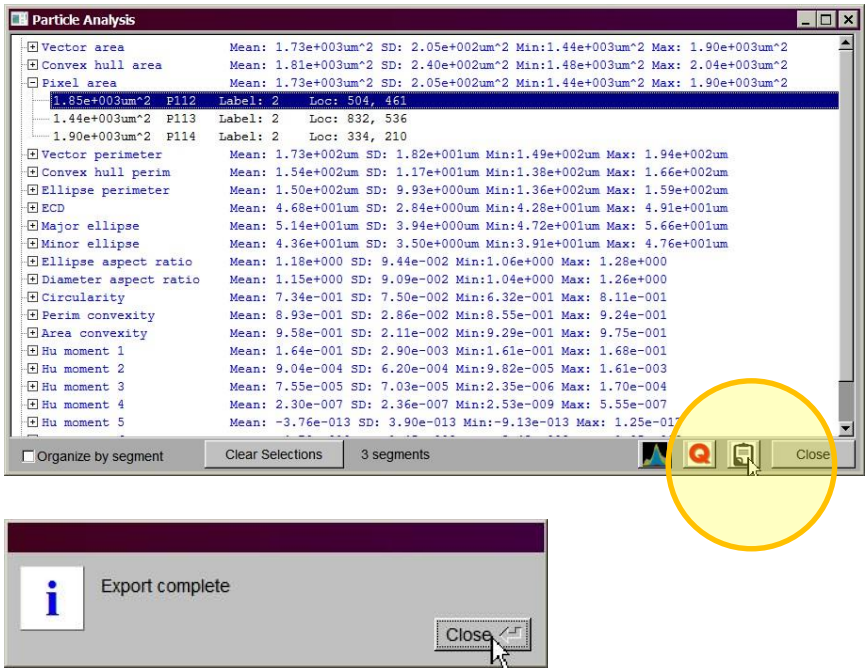
Click on the Report symbol to export your report. **Type in a file name and location**. Add a .txt extension so that it is easier for other programs to use it. **Click export.** A data file and a File with the meta data will be created.	
The files we generated are called: trainingimageQ1gold.txt & trainingimageQ1gold.txt.meta. Import these into excel for comparison. Please note that the particle order can change due to difference in the exact steps taken during segmentation. Use the Particle analysis interface to be sure which particle belongs to which quantification result when comparing.	
Additional Analysis steps examples:	

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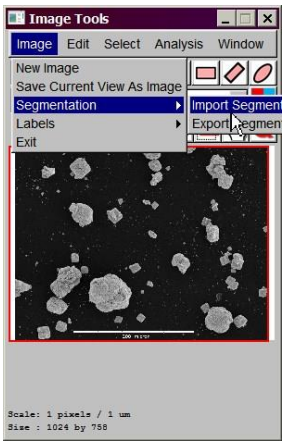
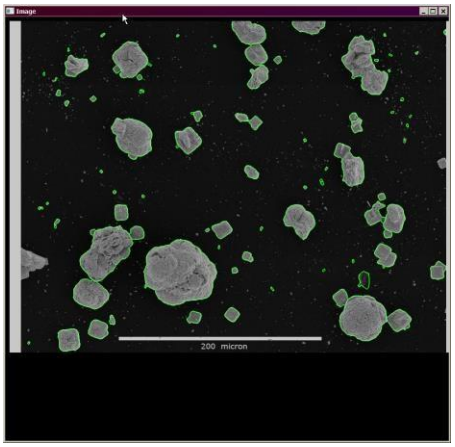
1. Import image TrainingimageQ2 .tif.
Then import a segmentation file for it: traingingimageQ 2.fits



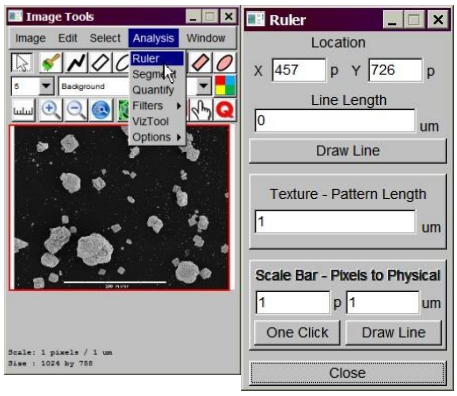


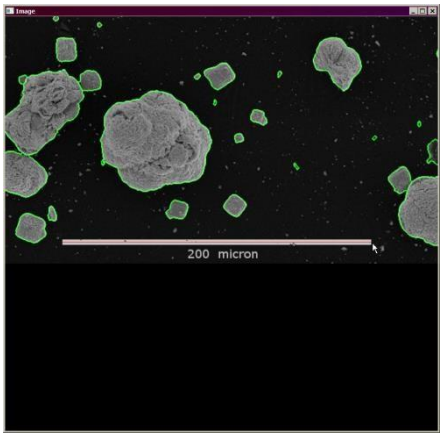
Note: you can segment it yourself; we provided it to allow this to focus on other steps in the analysis. TrainingimageQ1 also has a saved segmentation file you can import for that image. You can export segmentations and save them for performance testing or records.

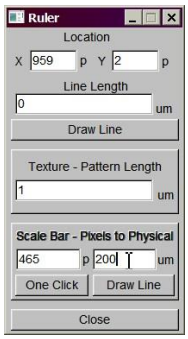
2. Set the ruler:
Image tools→ analysis→ ruler

Use the magnifiers in the image tool to zoom in on the scale bar in the image window

Click Draw line and draw a straight line the length of the scale bar. Set the micrometers to 200.







Make sure your
looking at image window and not image tool window. Your mouse wheel may also work for zoom.

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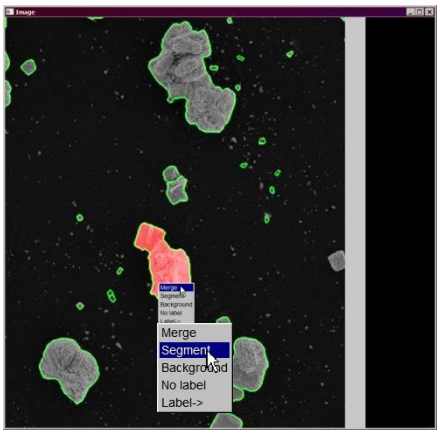
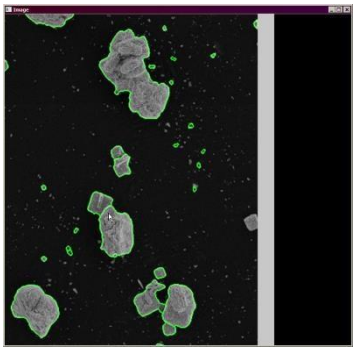
3. Clean up segmentation:

Look around image for segments that look wrong.

Click the particle, right click, then select segment. Use the slider to get the particle split into two (and not more than two.) Click Apply

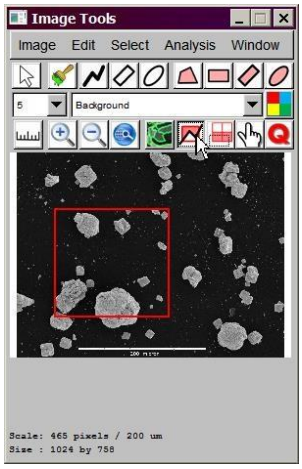


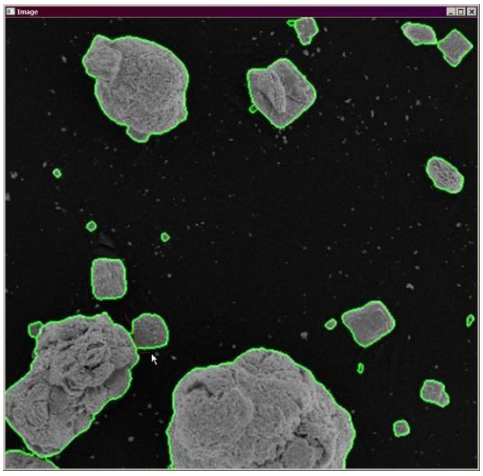


For example, in center right of image, there is a particle that appears to be two overlapping particles.

Alternately, manually split the particles.
This can be more effective in some cases where segmentation algorithms do not achieve the desired split.

Click the line split button, and draw a line passing through outer edges of the segments you want to split.





** Note: IF you think we missed too many small particles, for this training image we generated the segmentation, quantified everything (>1000 particles) and then selected all the particles that were less than 20 pixels. These were then merged back

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	into the background for this example. You can segment them out, and you can quantify them if needed, although uncertainty in size will become large.

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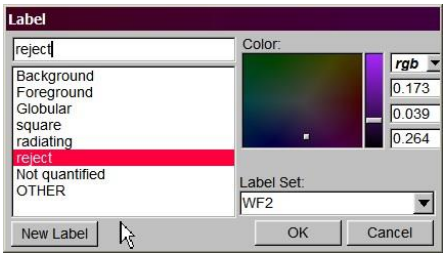
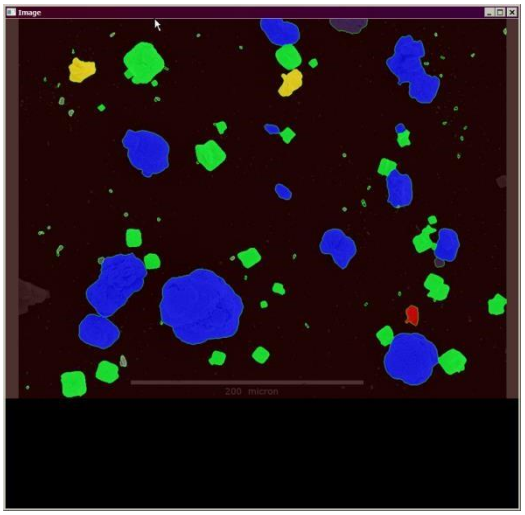
4, Label particles and background.

Use drag/click hand tool to select, and then right click→ label.



In this case, we labeled the particles by gross morphology: Globular, square, radiating.

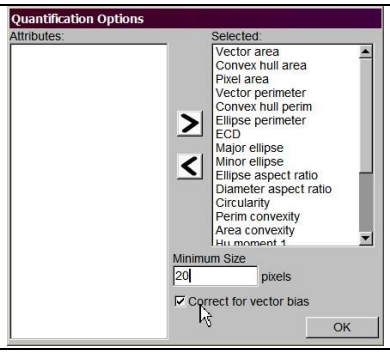
We labeled reject category for edge particles or particles too overlapped.



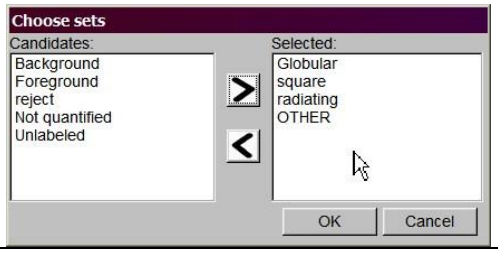
And we added a label for a "other" particle that looked different.

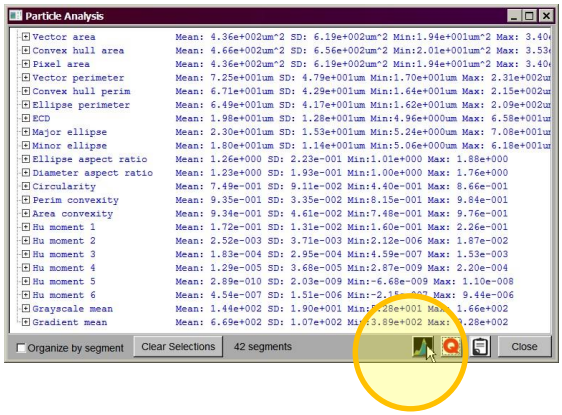
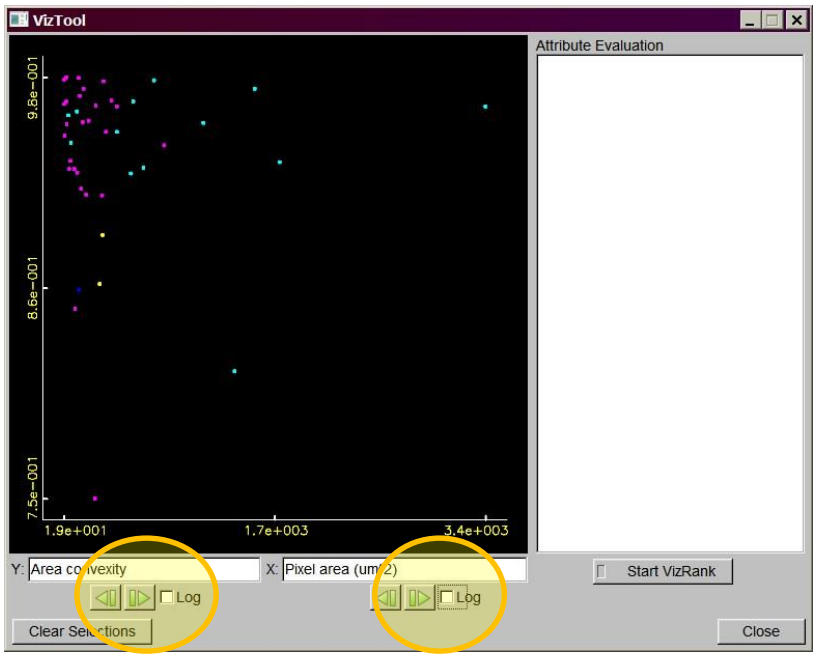
5. Set quantification options:

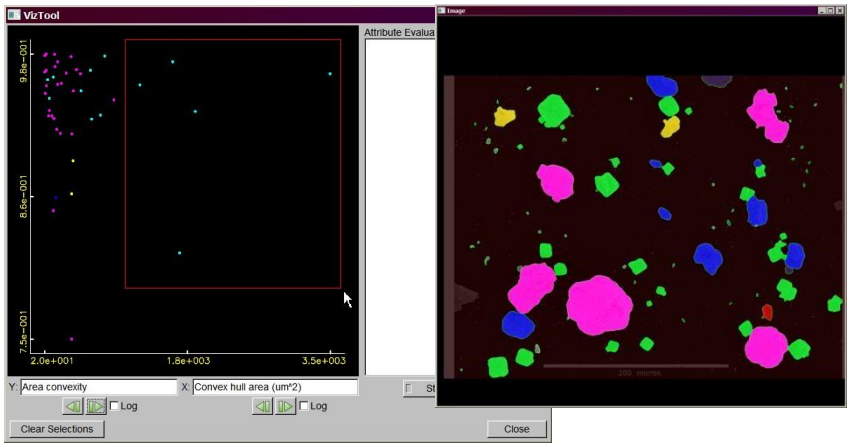
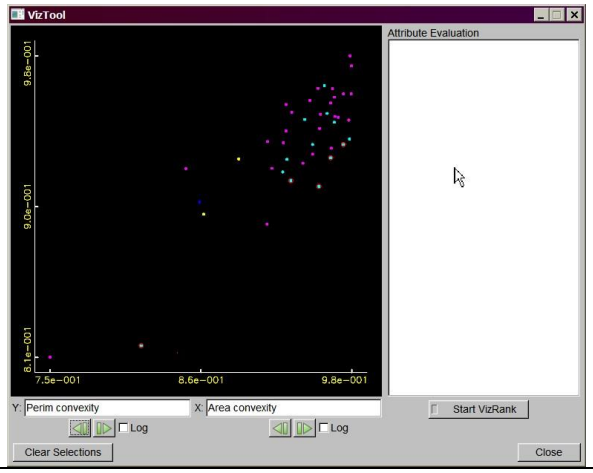
Correct for vector bias, 20 pixel minimum, all attribute.



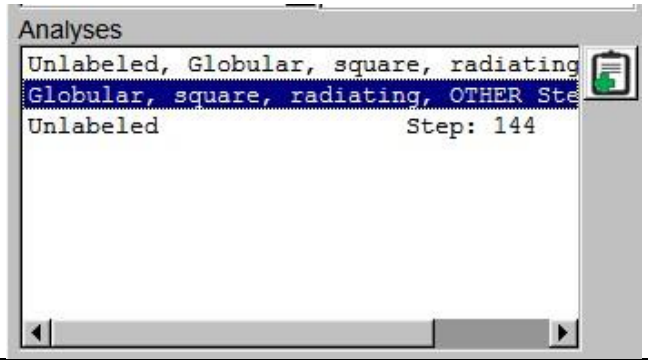
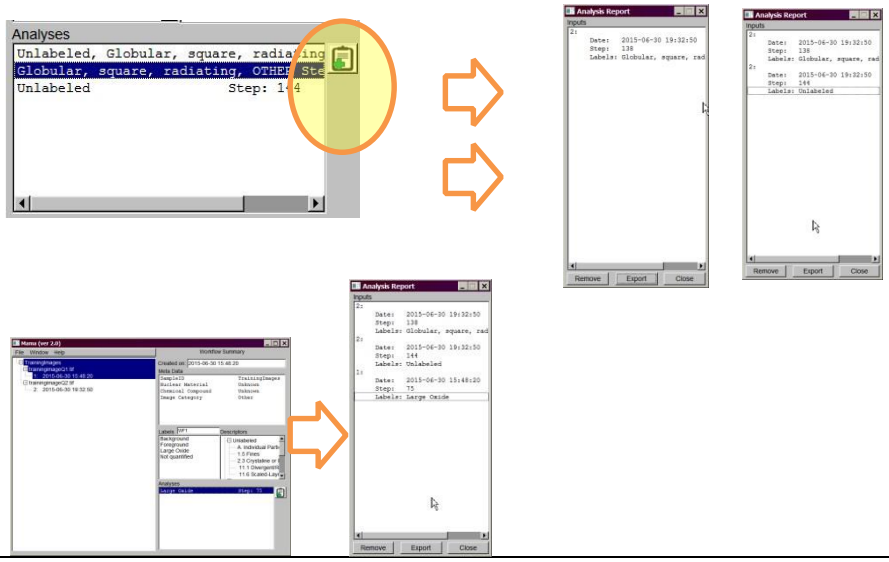
6. Select particles to quantify:



7. See the quantification results graphically. **Click the plot icon.**	
Select Y an X axis features with green arrows, select log or linear scale. The dots are colored in groupings based on particle labels (but may not match colors you selected…they are standard order set.)	

You can select plot points, and they will be highlighted in MAMA image window. Click or select by click and drag a box. (Note: if you drag off the graph, the no points will be selected.	
You can change the attributes displayed, and they will stay highlighted in other plots.	

DHS Funding Statement: The Department of Homeland Security sponsored the production of this material under the Department of Energy contract for the management and operation of Los Alamos National Laboratory.

You can regroup, relabel particles as needed. You can quantify on any grouping. All analyses will show up in the main window in the Analyses zone. Click on an analysis to open it (opens particle analysis window)	
8. Make combined analysis reports: Click on an analysis and click the "+report" button to add it to a combined report. Add as many analyses as you want. Add analysis from	

multiple images. **Export to a TXT file** and import into excel for pretty plots. A combined meta data file is also generated.	WFID | AnalysisID | PartID | Label | Output file has Workflow ID, Analysis ID, part ID, and a numeric label ID to allow you to sort/group as needed.

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