

Analysis and Processing of AMT Images Using *ImageJ**

****ImageJ* is a work of the United States Government, is in the public domain and is open source. Please refer to the Preface for more details.**

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Preface

ImageJ is an image processing application written by Wayne Rasband (wayne@codon.nih.gov). It is a work of the United States Government, is in the public domain and is open source. It is available from the National Institutes of Health (<http://rsb.info.nih.gov/ij/>). All modifications, source code, applications and additions to this program by AMT are also freely available from AMT.

This is a supplement to the existing documentation for *ImageJ*. Its main purpose is to highlight operations typically for CCD images obtained by transmission electron microscopy. This orientation also describes customizations made by AMT for TEM imaging.

Installation

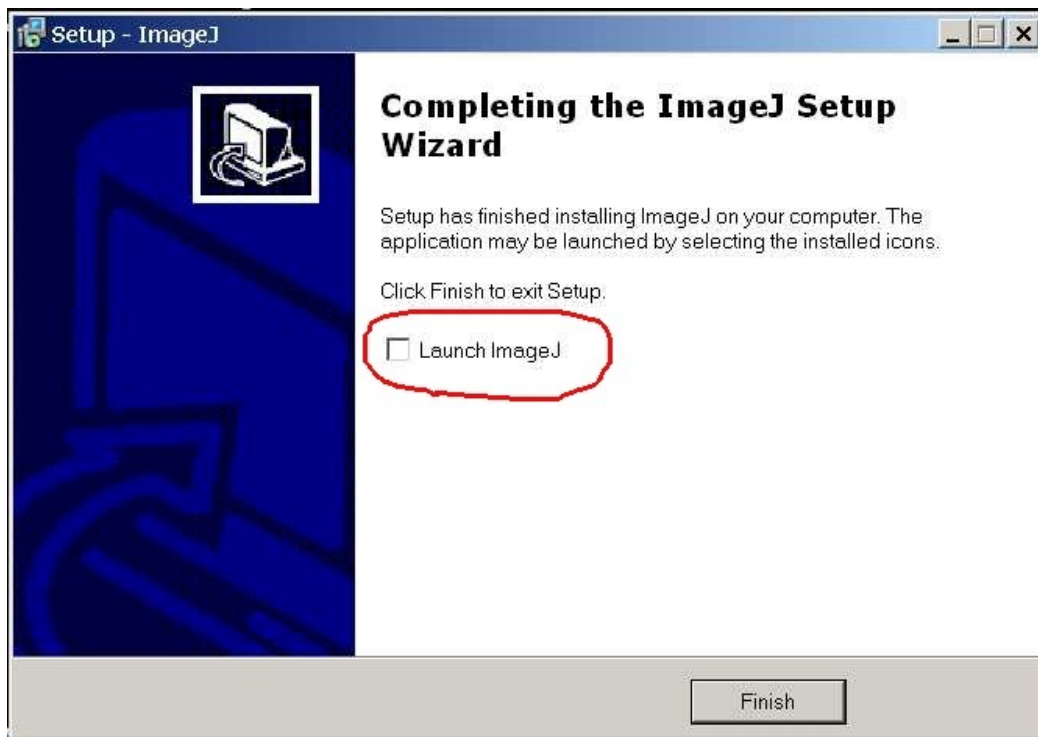
Installation On The Image Capture PC

ImageJ will be installed and customized when the system is delivered. If it becomes necessary to re-install follow the instructions below for Independent work stations.

Installation On An Independent Work Station

The installer for the customized version of ImageJ is found in the folder C:\AMTCommon\ImageProcessing. To install a customized version of ImageJ on a separate work station:

- 1) Copy the above folder and contents to the PC you wish to install ImageJ.
- 2) Double click on InstallAndCustomizeImageJ.bat.
- 3) Accept default locations. At the final window of the standard ImageJ installer **uncheck** the Launch ImageJ checkbox.



- 4) Click on "Finish." Then press any key to continue.

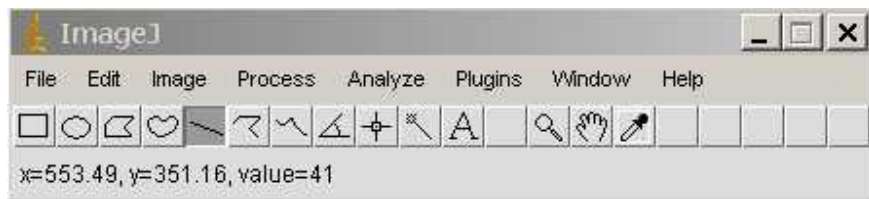
Analysis

Measurement

Spatial calibration information is incorporated into the tiff file header when AMT's Capture Engine saves a file. The customized version of *ImageJ* will read the calibration parameters for an image when it opens a tiff file. If the calibration information is not present, an alerted box will remind you to manually calibrate the image. Calibration information may be lost if you open an image in another image processor and then overwrite the original image.

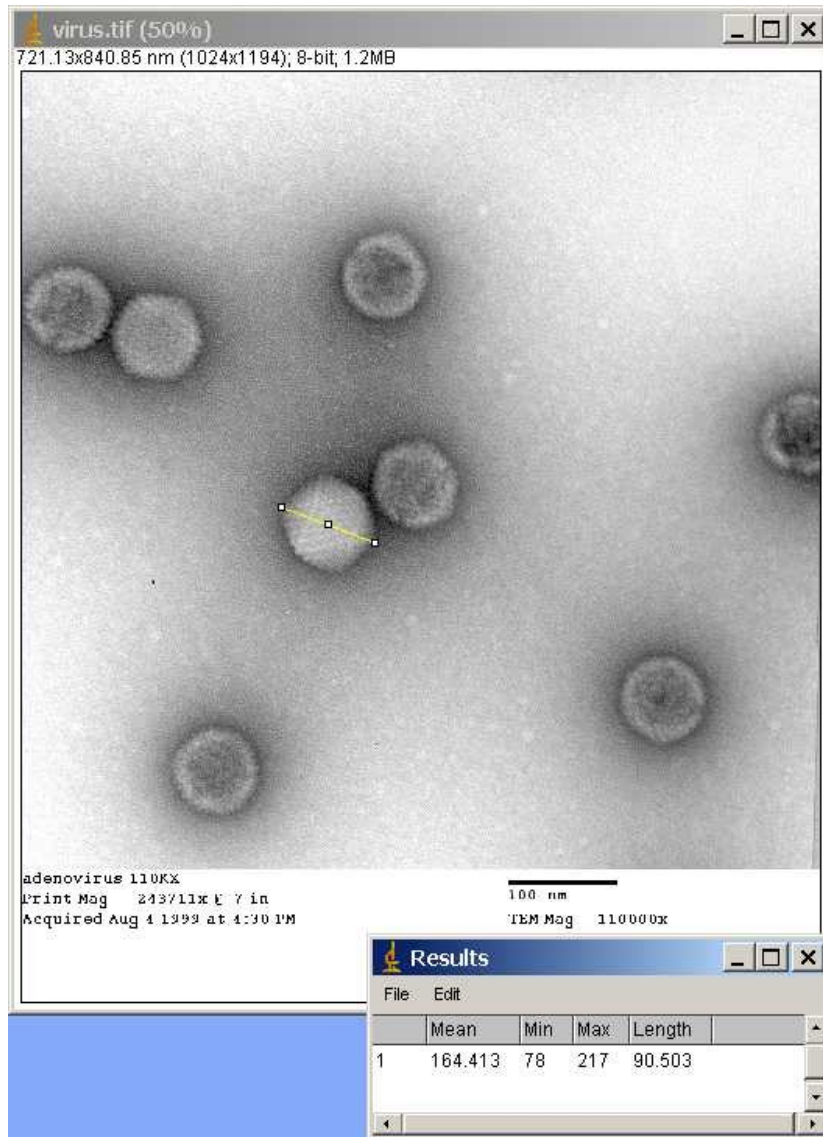
Length Measurements

Open a Tiff Image in *ImageJ* and select the line tool from the *ImageJ* toolbar.

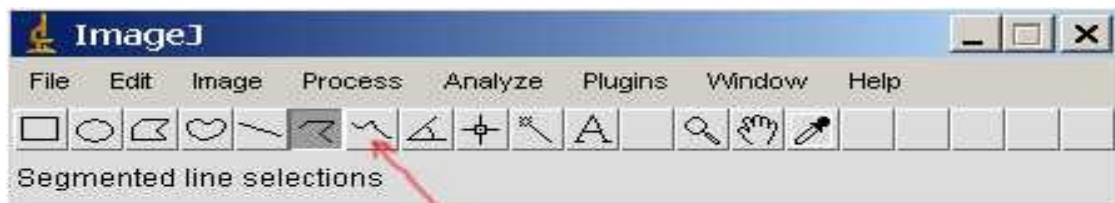


Position the cursor at one end of the object to be measured. Click and drag to the other end of the object. Type Ctrl-M to enter the length of the line drawn into the measurement list. Repeat the process for other objects that you wish to measure. For difficult or small objects you can use the zoom tool and then make measurements. More accurate measurements, of course, are obtained by collecting a higher magnification image.

Convenient shortcuts have been defined. F1 will draw a line on the image between the end points label the image and enter the measurement into a list with a header which shows the units. F2 is the same as Ctrl-M.



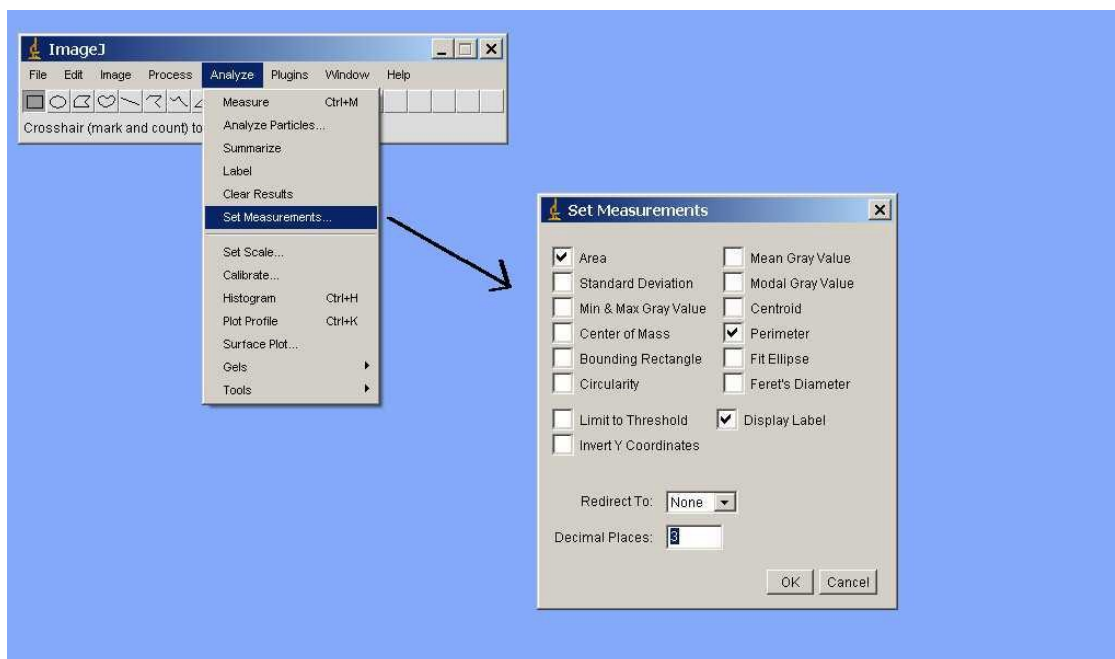
Distance along an irregular path can be measured by selecting the Segmented Line Selection or the Freehand Line Selection tools in the *ImageJ* toolbar.



Freehand line selections

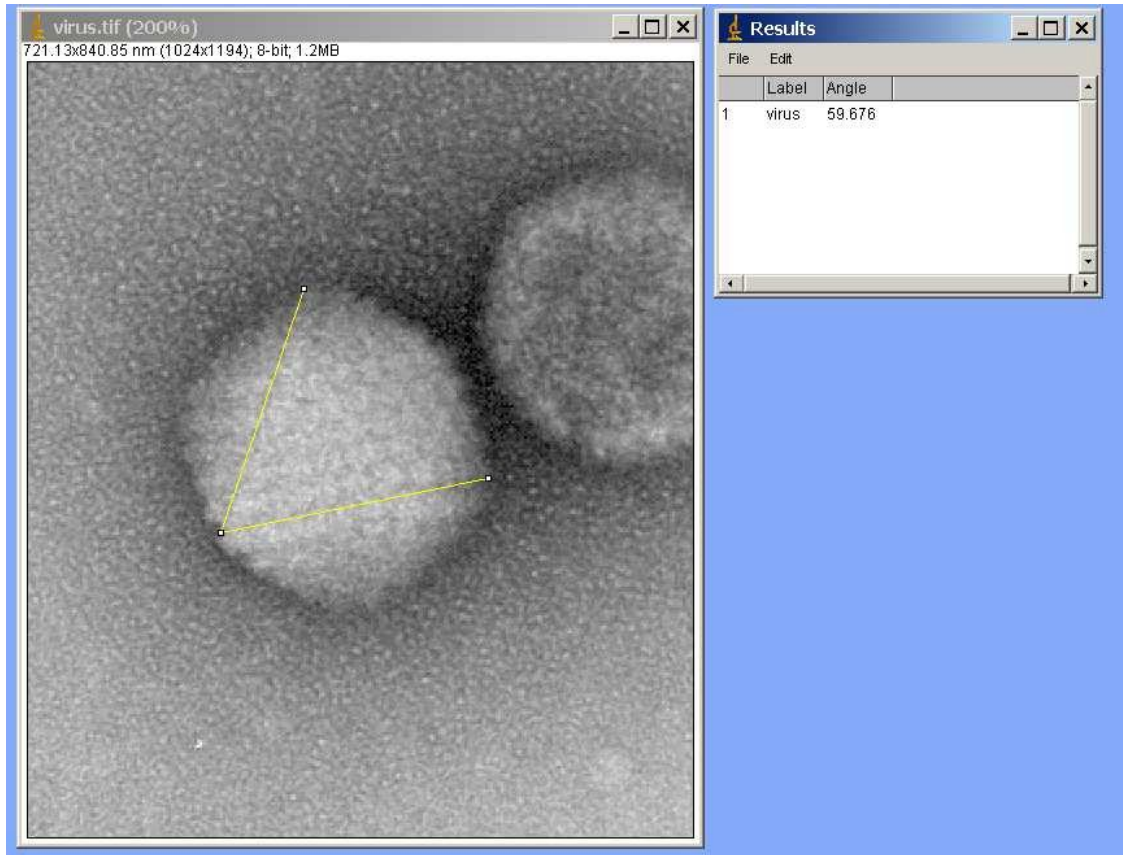
Perimeter and Area Measurements

Perimeter can be measured by using one of the length measurement tools as described above or by using one of the area selecting tools. In the Analyze|Set Measurements Menu item you can select the measurements that are displayed in the Results window when ctrl-M is entered. In the figure both Area and Perimeter options have been selected.



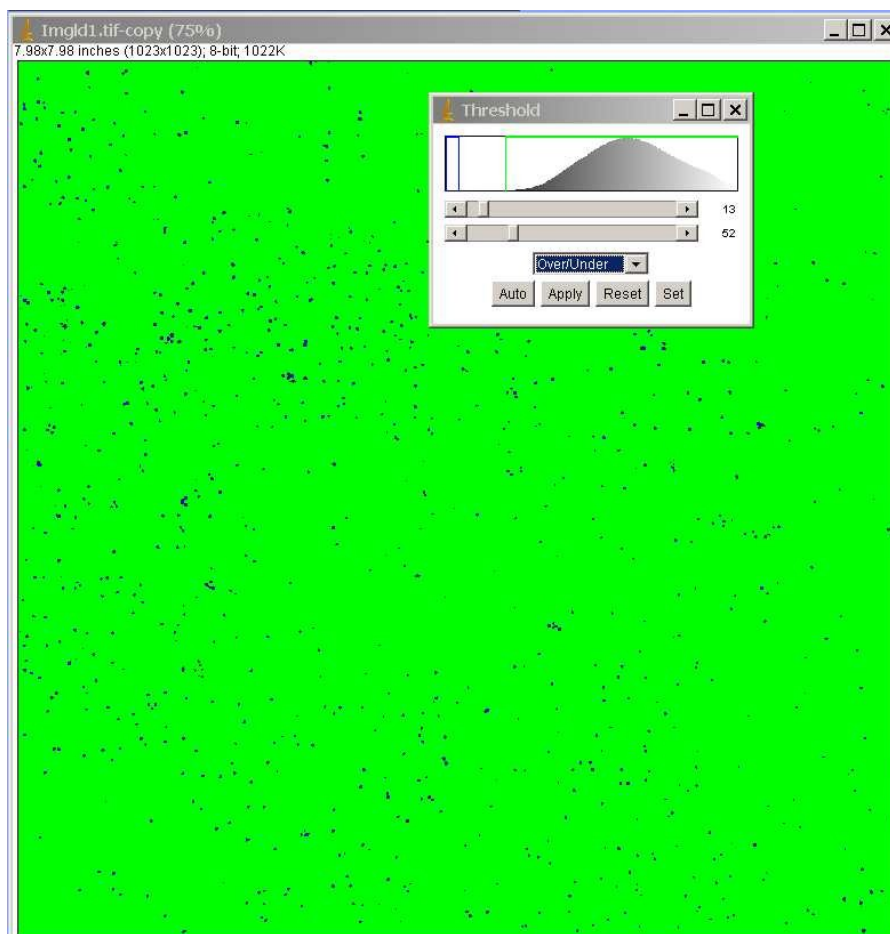
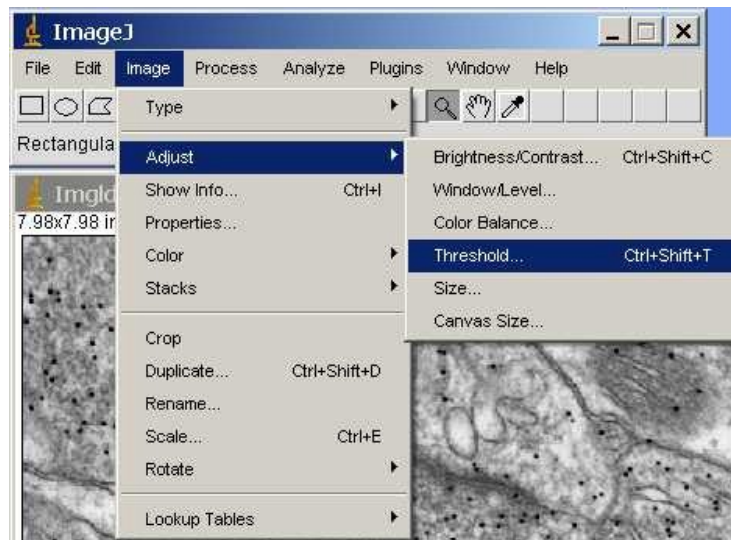
Angle Measurements

Angles can be measured by selecting the angle measurement tool, double clicking on the first point, single clicking on the vertex, and clicking once more at the terminus of the final ray.

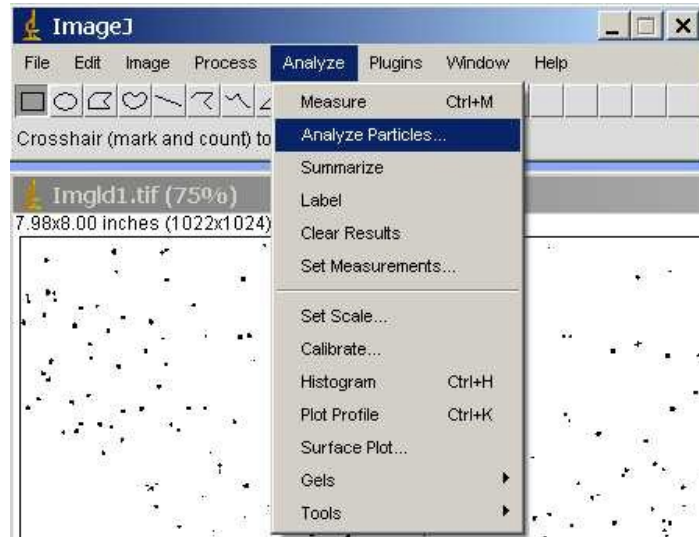


Particle Counting

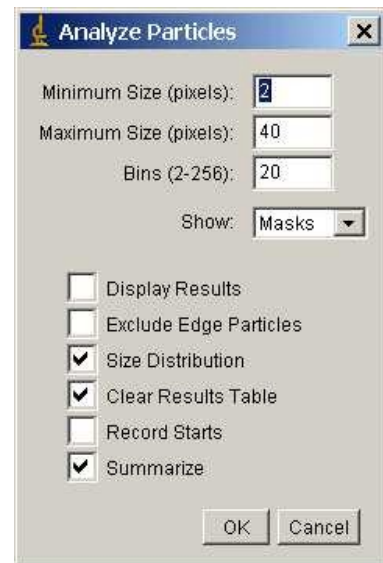
ImageJ supports particle counting. The essential steps in particle counting are:
1) **Thresholding** Click the menu item Image|Adjust|Threshold. Set thresholds to suppress image detail and enhance the particles. Use the control box displayed to select threshold levels that emphasize the particles.



2) **Counting** Click the menu item Analyze|Analyze Particles.



This will display the following dialog box. Set the minimum particle size in pixels, the maximum particle size, and choose which results you would like to display.



Particle Counting Macro

It is often the case that the contrast in an image makes it difficult to locate the particles with thresholding alone. In those cases it may be necessary to reduce the contrast of the image background without reducing the contrast of the particles. AMT supplies a simple macro (AMTparticleCount.txt) which automates the reduction in contrast as well as the thresholding and particle counting. The

macro can be edited to suit the user's particular situation. On startup the macro is assigned a keyboard shortcut (F2). Run the macro by opening an image and then pressing the F2 key.

Histogram and Intensity Measurement

Image intensities and gray scale measurements can also be made. It is important here to remember that the Capture Engine normally applies a contrast enhancement to images as they are acquired and displayed. This improves the appearance but will, of course, modify grayscale information. If quantitative measures are to be applied to images that are collected, the contrast enhancement of the Capture Engine should be turned off and images should be saved as 16 bit tiff files.

The screenshot shows the 'Camera Settings Control' dialog box. The 'Display and Contrast Settings' section is circled in red, and a red arrow points to it from the left. A red text annotation 'Set these values to zero' is placed above the circled area. The dialog box includes sections for 'Camera Setup Procedures', 'Display and Contrast Settings', 'Intensifier Controls', and 'Exit Control'.

Modes	Exposures	Gains
Survey	56	1
Focus	112	2.0
Super Pix	14	1.0
Record	222	1.0

Record Integrations: 8

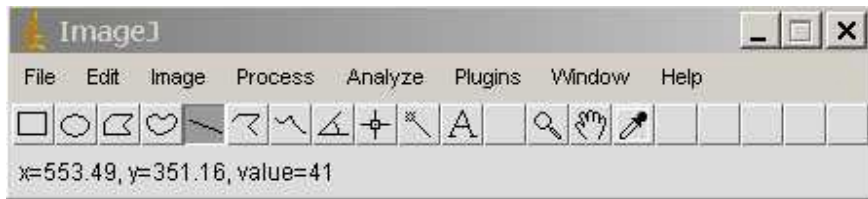
Display and Contrast Settings:

- Quality Setting (Frame Average): 4
- Sample Region: 80
- Display Size: 100
- Black Threshold: 0
- Black Level Tail %: 0
- White Threshold: 0
- White Level Tail %: 0

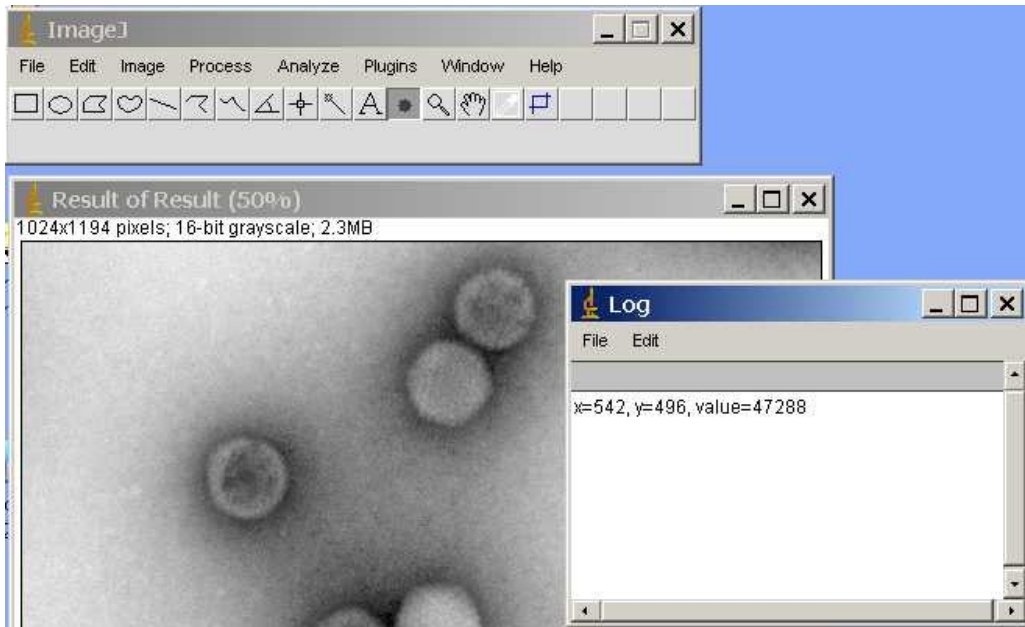
Exit Control:

- Save and Apply
- Apply For Now Only

To measure the gray scale value at a point simple position the cursor at that point the gray scale intensity is displayed in the status bar just below the *ImageJ* tools.

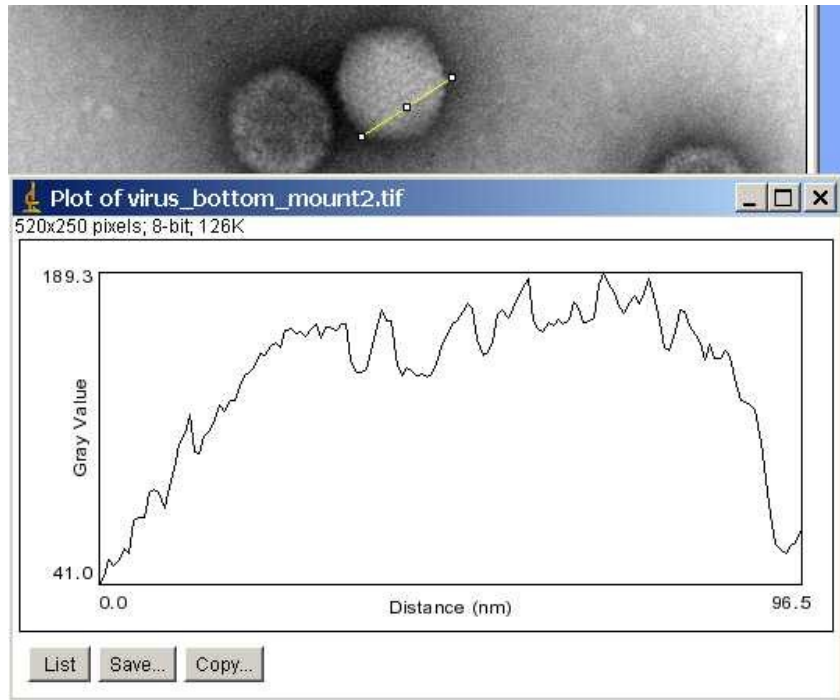


Alternatively, the Pixel Picker macro can be used. This tool enters the value at the selected pixel into the Log window.



Line Profiles

Line profiles can be generated by drawing a line through the features and then selecting Analyze|Plot Profile or pressing the ctrl-K key.



To improve the statistics of the plot one can select a rectangle and request a column profile.

average

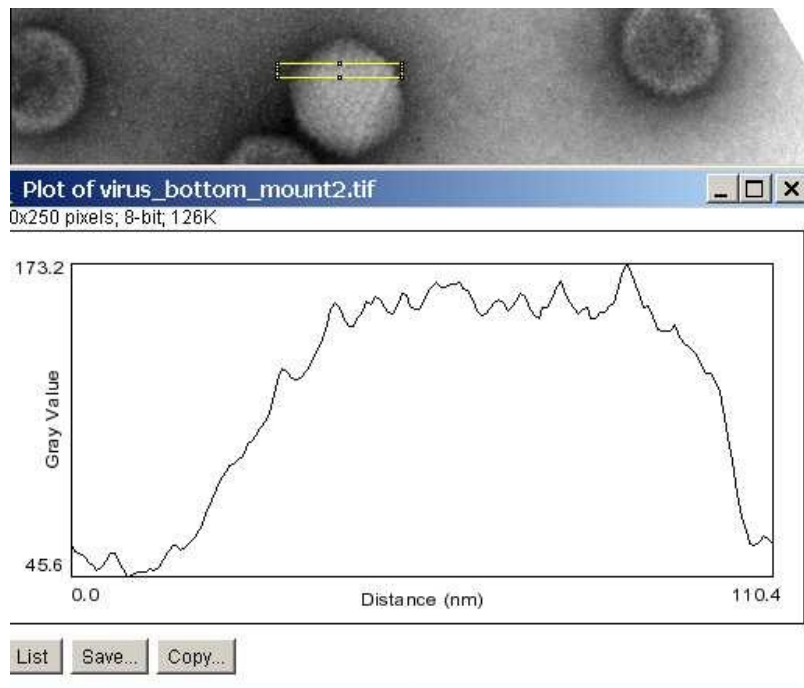


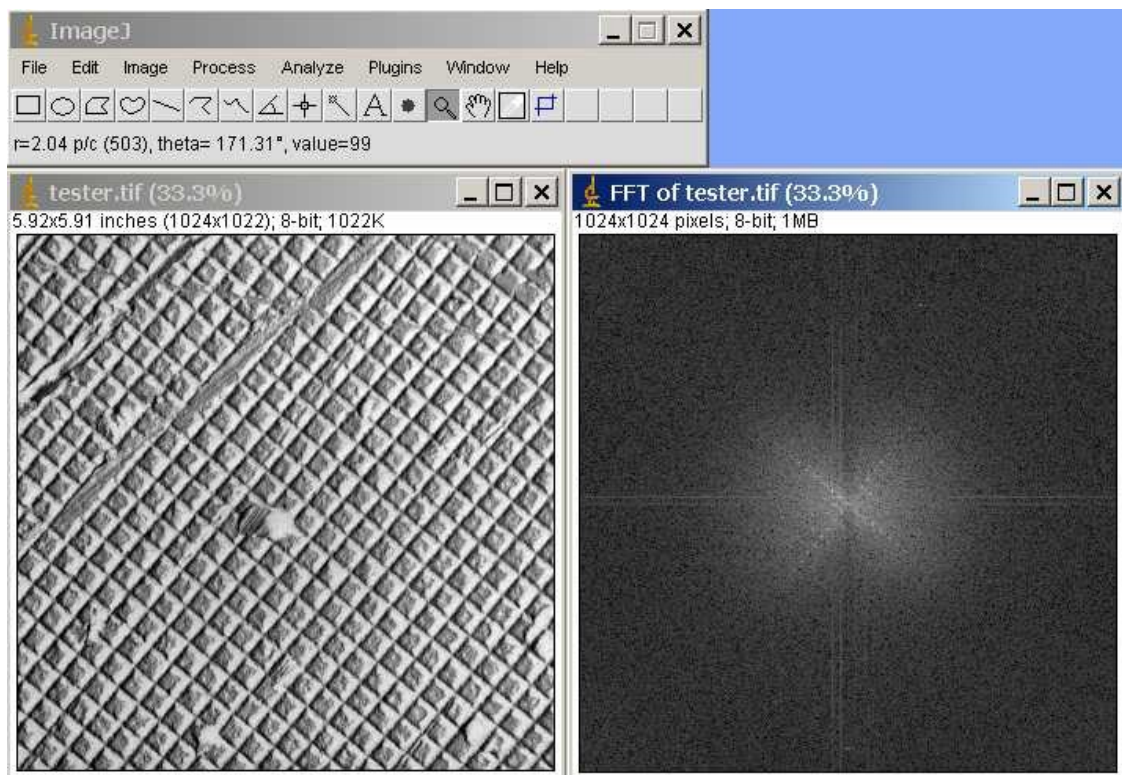
Image Math

Extraction of interesting features from a complex image can be a challenging aspect of image analysis. It is very useful to be able to perform simple mathematical functions on whole images and between two images. Once a sequence of operations is found to be useful a macro can be created to automate that sequence. Embedded in the particle counting macro discussed above, for example, is an algorithm to extract small dense particles from a complex image that includes both tissue and gold particles. This algorithm relies on some simple math. The image is first blurred and then a portion of it is subtracted from the original image. This tends to enhance small particles and suppress the background. The Image Calculator in the Process menu permits one to add, subtract, multiply, divide, and apply several other operators to pairs of images.

Fourier Transform

The Fourier transform expresses an image in terms of the spatial frequencies in the image. A sharply focused image will be rich in high spatial frequencies. Large uniform areas are represented by low frequencies. This can be used to select various parts of an image and can be very useful in removing artifacts, or improving image sharpness. *ImageJ* has a built in FFT and in addition there are several plugins posted on the NIH web site that implement their own specialized versions of the algorithm. There are also plugins that rely on the FFT as an integral part of the algorithm they implement. These include convolution, filtering, and deconvolution plugins. Many of these plugins are provided with references to the literature.

Doing the FFT of an image is straightforward. Select the region of interest and then in Process menu select FFT. The nearest power of two sized region will be used in the FFT.



Macros

Macros are sequences of commands, which can be executed as a single operation. We discussed the use of the macro AMTparticleCount.txt above. An existing macro can be run by selecting the Plugins|Macros|Run and then navigating to the location of the macro. Macros are usually kept in

C:\Program Files\ImageJ\macros



and can also be installed as a menu item as well as assigned a keyboard shortcut.

ImageJ has over ninety standard macros that are supplied as part of the initial installation. These macros range from tools through macros that apply functions to every image pixel, to measurement of image parameters and drawing tools. More information is available on the NIH web site.

<http://rsb.info.nih.gov/ij/docs/menus/plugins.html>

The macro StartupMacros.txt is a special macro that runs when *ImageJ* is launched. It can be used to define shortcuts to macros and place macros in menus and tools. We have set this up to run a useful sample set. It can be modified by the user as desired.

The shortcuts we have defined are:

Shortcut	Name	Description
F1	Draw and Measure	Draws a line between points defined by the line selection tool. The distance between the points is calculated, entered into the results table and drawn on the image.
F2	Measure	The same as ctrl M just a single key needs to be depressed.
F3	Particle Counting	Particle discrimination and counting macro.
F4	Threshold	A shortcut to the standard thresholding tool.
	Pixel Picker Tool	IJ standard macro which lists gray scale values in the Results Window.
	AMT Crop Tool	Automatically crops an AMT image. The caption band is separated from the image data.

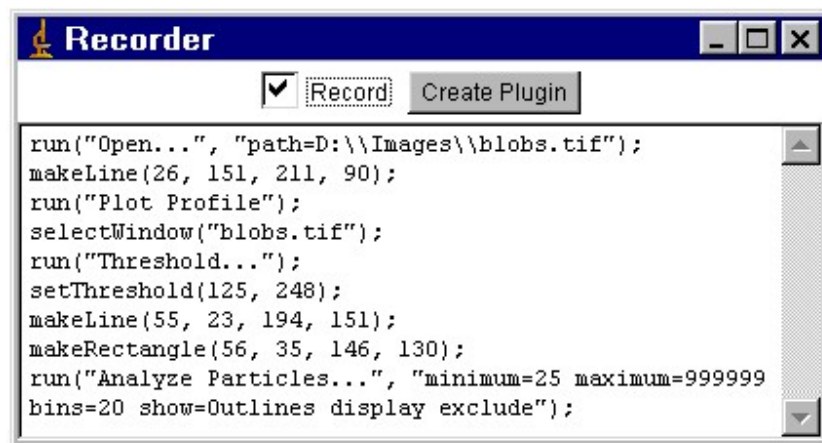
Recording Macros:

(From NIH web site <http://rsb.info.nih.gov/ij/docs/shortcuts.html>, which is reproduced and modified here for your convenience)

The easiest way to create a macro is to open the command recorder under the menu item

Plugins/Macros/Record

Then record a series of commands, and then click *Create*. In record mode, each menu command you use generates a call to ImageJ's run() method. This method has one or two string arguments. The first is the command name. The optional second argument contains dialog box parameters.



```
run("Open...", "path=D:\\Images\\blobs.tif");
makeLine(26, 151, 211, 90);
run("Plot Profile");
selectWindow("blobs.tif");
run("Threshold...");
setThreshold(125, 248);
makeLine(55, 23, 194, 151);
makeRectangle(56, 35, 146, 130);
run("Analyze Particles...", "minimum=25 maximum=999999
bins=20 show=Outlines display exclude");
```

Create a rectangular, oval or line selection and the recorder will generate a makeRectangle(), makeOval() or makeLine() method call. Click on "Auto" or "Set" in the *Image/Adjust/Threshold* window to generate a setThreshold() call, and on "Reset" to generate a resetThreshold() call. Select an image from the Window menu to generate a selectWindow() call. Click in the *Image/Colors* window to generate a setForegroundColor() call and alt-click to generate a setBackgroundColor() call.

Plugins

Plugins are another form of extension available to the user of *ImageJ*. Plugins are very powerful and very fast. More information as well as over 100 plugins are available on the *ImageJ* web site

<http://rsb.info.nih.gov/ij/docs/menus/plugins.html>

Both plugins and macros are often contributed by a large body of users.

Appendix A

Many keyboard shortcuts are already defined in *ImageJ*. These are listed on the NIH web site <http://rsb.info.nih.gov/ij/docs/shortcuts.html> which is reproduced here for your convenience.

Keyboard Shortcuts

Command	Shortcut	Description
New	N	Creates a new image or stack
Open	O	Opens a TIFF, GIF, JPEG, BMP, DICOM or FITS
Open Samples	Shift-B	Opens "Blobs" example image
Close	W	Closes the active window
Save	S	Saves active image in Tiff format
Revert	R	Revert to saved version of image
Print	P	Print active image
Undo	Z	Reverses the effect of the last operation
Cut	X	Copy image to clipboard and clear selection
Copy	C	Copy image to clipboard
Paste	V	Paste clipboard selection into active image
Clear	backspace	Erases selection to background color
Select All	A	Select entire image
Select None	Shift-A	Delete selection
Restore Selection	Shift-E	Restores ROI
Fill	F	Fills selection in foreground color
Draw	D	Draw selection
Invert	Shift-I	Invert image or selection
Adjust Contrast	Shift-C	Adjust brightness and contrast
Adjust Threshold	Shift-T	Adjust threshold levels
Show Info	I	Displays information about active image
Next Slice	>	Advance to next stack slice
Previous Slice	<	Backup up to previous stack slice
Start Animation	=	Starts/stops stack animation

Duplicate	Shift-D	Duplicates active image or selection
Scale	E	Scale image or selection
Smooth	Shift-S	3x3 unweighted smoothing
Find Edges	Shift-F	Performs Sobel edge detection
Repeat Command	Shift-R	Repeats previous command
Measure	M	Displays statistics about active image or selection
Histogram	H	Displays a histogram of the active window or selection
Plot Profile	K	Displays density profile plot of current selection
ImageJ	enter	Brings ImageJ window to front
Put Behind	tab	Switches to next image window

Alt Key Modifications

- Image/Adjust/Threshold:* Adjusting **Min** also adjusts **Max**
- Image/Stacks/Add Slice:* Insert before current slice
- Image/Stacks/Next Slice:* Skip nine slices
- Image/Stacks/Previous Slice:* Skip nine slices
- Image/Duplicate:* Don't show dialog
- Image/Colors:* Alt-click to "pick up" background color
- Process/Equalize:* Do classic histogram equalization
- Process/Subtract Background:* Show background image
- Analyze/Plot Profile:* For rectangular selections, generate row average plots
- Analyze/Tools/Analyze Line Graph:* Show intermediate image
- Plugins/Utilities/ImageJ Properties:* List all Java properties
- Plugins/Utilities/Monitor Memory:* Simulate 640x480 frame grabber
- Any User Plugin:* Load using new class loader

Space Bar Modifications

- Any Tool:* Temporarily switch to the "hand" (scrolling) tool
- Moving through a stack:* Automatically adjust min/max display values

Arrow Keys

Use the arrow keys to move selection outlines one pixel at a time. Resize rectangular and oval selections by holding down the alt (option) and while using the arrow keys.