ImageJ (open, free image analysis program)

Google: NIH, ImageJ, download

main program
plus "plugins" (available special apps made by users)

(1) Image Sources

cameras: image = array of light intensities
(may be linear or non-linear representation of source light intensities)
(shapes also may be distorted)

AFM: height image converted to color image

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(2) form of original data: color, black/white } 8-bit, 16bit, 32bit per pixel

file formats: tiff, jpeg, bmp, png, avi, mp4

↳ compressed, some info. lost

others (specific to Leica, Zeiss, AFM etc....)

for averaging and adding images, best to work with 32-bit tiff to avoid number overflow

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Some examples of ImageJ features:

a) calibration – take picture of standard-sized object (lines, circles of known size)

use line tool on object to determine how many pixels are equivalent to known length

then Analyze, Set Scale
fill in parameters on menu
✓ on Global for using same scale on set of pictures
hit ok — image is now calibrated

b) add scale bar to picture
Analyze, Tools, Scale Bar
set parameters in menu, ok (now scale bar appears on picture and can be saved with picture)

c) with scale set, can click and draw lines to measure distances (hit M to save measurement to screen)

d) Line Profiles (intensity along a line):
draw line, Analyze, Plot Profile
— also, you may average over a set of parallel lines to improve statistics:
draw rectangular box, Analyze, Plot Profile
note: averages in vertical (y) direction, so you may need to rotate picture (Image, Transform, Rotate...)

e) Background Subtraction: Process, Subtract Background

f) Adding two images, subtracting, AND, OR operations
see notes below for details

Cropping (2nd page): 1) click on rectangular selection tool (below File)
0) open an image
2) on image, click and drag an area
3) hit Image, Crop

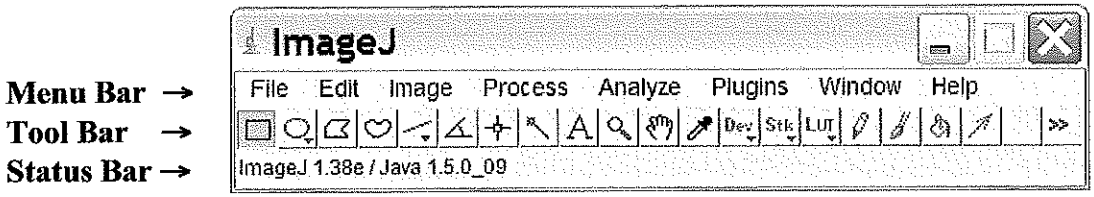
ImageJ Basics

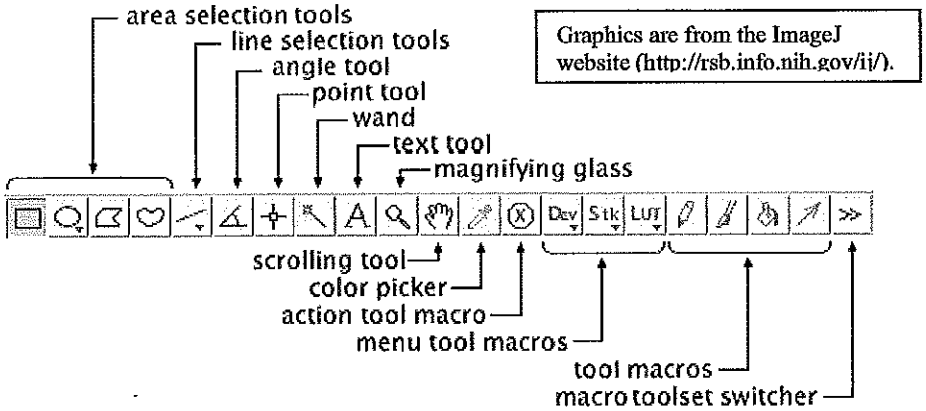
(Version 1.38)

ImageJ is a powerful image analysis program that was created at the National Institutes of Health. It is in the public domain, runs on a variety of operating systems and is updated frequently. You may download this program from the source (http://rsb.info.nih.gov/ij/) or copy the ImageJ folder from the C drive of your lab computer. The ImageJ website has instructions for use of the program and links to useful resources.

Installing ImageJ on your PC (Windows operating system): Copy the ImageJ folder and transfer it to the C drive of your personal computer. Open the ImageJ folder in the C drive and copy the shortcut (microscope with arrow) to your computer's desktop. Double click on this desktop shortcut to run ImageJ. See the ImageJ website for Macintosh instructions.

ImageJ Window: The ImageJ window will appear on the desktop; do not enlarge this window. Note that this window has a Menu Bar, a Tool Bar and a Status Bar.





Graphics are from the ImageJ website (http://rsb.info.nih.gov/ij/).

Adjusting Memory Allocation: Use the ***Edit → Options → Memory*** command to adjust the default memory allocation. Setting the maximum memory value to more than about 75% of real RAM may result in poor performance due to virtual memory "thrashing".

Opening an Image File: Select ***File → Open*** from the menu bar to open a stored image file.

Tool Bar: The various buttons on the tool bar allow you measure, draw, label, fill, etc. A right-click or a double left-click may expand your options with some of the tool buttons.

Area Selection Tools: The first four buttons on the tool bar allow you to surround an area on the image with a rectangle, oval, polygon or freehand shape. After selection, these areas may be

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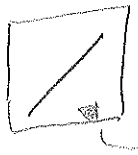
Length measurement in ImageJ

1) Open an image file
2) Identify an object and draw a line over object using line tool:

line drawing tool (5th from left on toolbar)



→ hit to adjust line width

Drawing lines

right-click in box, set choice

✓ Straight line
Segmented line
Freehand line
Arrow tool

(check one)

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a) click and drag a line on the picture
modify by clicking and dragging on squares on line

b) hit D key to leave black line after measurement of selected line, if desired

c) hit M key to measure length of line, results appear on Results screen

d) control + or − to zoom in or out

e) use  scrolling tool when zoomed in to go to desired region

Note: If the picture is not yet calibrated, the length will be in pixels. To calibrate, see following instructions.

calibration

Setting the scale in ImageJ pictures (so objects can be measured with the length tool):

1) open picture (either with know total size or some feature in the picture of known size as a calibration)
2) measure length of known object using line tool (if image is uncalibrated, this will give the length in pixels). Note the length. (under menu bar)
3) hit Analyze, Set Scale
4) Input "Distance in Pixels" of known object or full image width
5) Input "known Distance"
   (pixel aspect ratio – usually 1.0)
6) Input "Unit of length" – mm, cm, microns etc.
7) check "Global" if you will be opening more pictures with the same calibration

Now, the line tool measurements will be calibrated

Also, a scale bar with the calibration may be added to the picture (see instructions).

Measuring the area of an object in an image

1) Select a ROI (Region of Interest) with the rectangle tool, oval tool, polygon tool (clicks to mark, 2 clicks to complete object) or freehand tool (those are the 4 leftmost tools on toolbar)

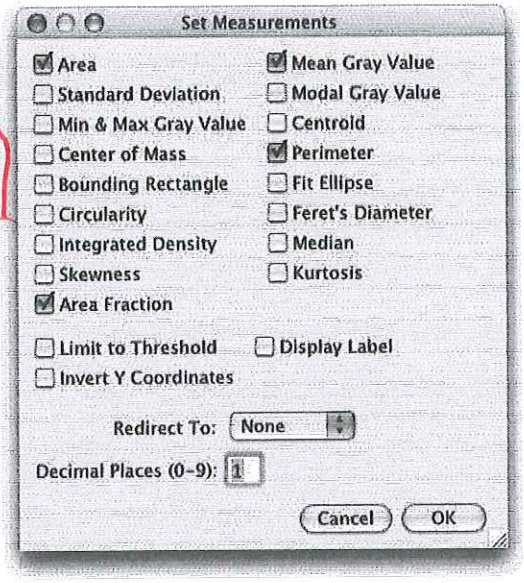
hit M to measure - output goes to Results screen
(Area is in (pixels)^2 if uncalibrated, if calibrated in microns, area is in (micron)^2, etc.)

hit D to preserve ROIs as black lines on image, if desired

2) ROIs can be saved by using the ROI manager:
   a) activate: Analyze, Tools, ROI manager
   b) hit t to add current ROI to memory (for any or all of your ROIs)
   c) you can recall a ROI by clicking in list on ROI manager screen
   d) you can hold Shift key down and click to select group of ROIs, then hit More, OR(combine) to restore all ROIs to picture

Analyze, Set Measurements

(controls what is output of meas. keystroke (M))



parameters output for measurement over ROI

→ *Area* - Area of selection in square pixels. Area is in calibrated units, such as square millimeters, if *Analyze>Set Scale* was used to spatially calibrate the image.

→ *Mean Gray Value* - Average gray value within the selection. This is the sum of the gray values of all the pixels in the selection divided by the number of pixels. Reported in calibrated units (e.g., optical density) if *Analyze>Calibrate* was used to calibrate the image. For RGB images, the mean is calulated by converting each pixel to grayscale using the formula *gray=0.299red+0.587green+0.114blue* or the formula *gray=(red+green+blue)/3* if "Unweighted RGB to Grayscale Conversion" is checked in *Edit>Options>Conversions*.

Standard Deviation- Standard deviation of the gray values used to generate the mean gray value.

Modal Gray Value - Most frequently occurring gray value within the selection. Corresponds to the highest peak in the histogram.

Min & Max Gray Level - Minimum and maximum gray values within the selection.

→ *Centroid* - The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the image or selection. Uses the *X* and *Y* Results table headings.

Center of Mass - This is the brightness-weighted average of the x and y coordinates all pixels in the image or selection. Uses the *XM* and *YM* headings. These coordinates are the first order spatial moments.

→ *Perimeter* - The length of the outside boundary of the selection.

Bounding Rectangle - The smallest rectangle enclosing the selection. Uses the headings *BX*, *BY*, *Width* and *Height*, where *BX* and *BY* are the coordinates of the upper left corner of the rectangle.

Fit Ellipse - Fit an ellipse to the selection. Uses the headings *Major*, *Minor* and *Angle*. *Major* and *Minor* are the primary and seconday axis of the best fitting ellipse. *Angle* is the angle between the primary axis and a line parallel to the x-axis of the image. Note that ImageJ cannot calculate the major and minor axis lengths if *Pixel Aspect Ratio* in the *Set Scale* dialog is not 1.0.

Circularity - 4pi(area/perimeter^2). A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated polygon. Values may not be valid for very small particles.

Feret's Diameter - The longest distance between any two points along the selection boundary. Also known as the caliper length. The Feret's Diameter macro will draw the Feret's Diameter of the current selection on the image.

→ *Integrated Density* - The sum of the values of the pixels in the image or selection. This is equavalent to the product of *Area* and *Mean Gray Value*. The Dot Blot Analysis example demonstrates how to use this option to analyze a dot blot assay.

total intensity in selected ROI

Median- The median value of the pixels in the image or selection.

Skewness- The third order moment about the mean. The documentation for the Moment Calculator plugin explains how to interpret spatial moments.

Kurtosis- The fourth order moment about the mean.

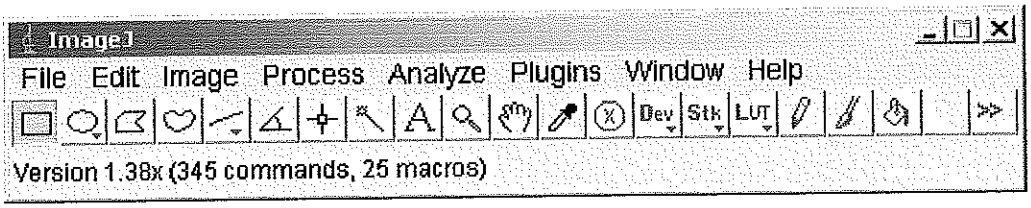
Area Fraction- The percentage of pixels in the image or selection that have been highlighted in red using *Image>Adjust>Threshold*. For non-thresholded images, the percentage of non-zero pixels.

Putting a labeled (or unlabeled) scale bar for sizing on an image

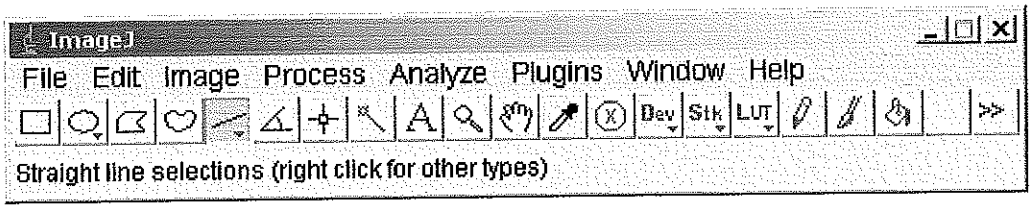
Adding scale bars to images using ImageJ

1) Install ImageJ on your computer if it is not installed already. You can download ImageJ from http://rsb.info.nih.gov/ij/download.html.

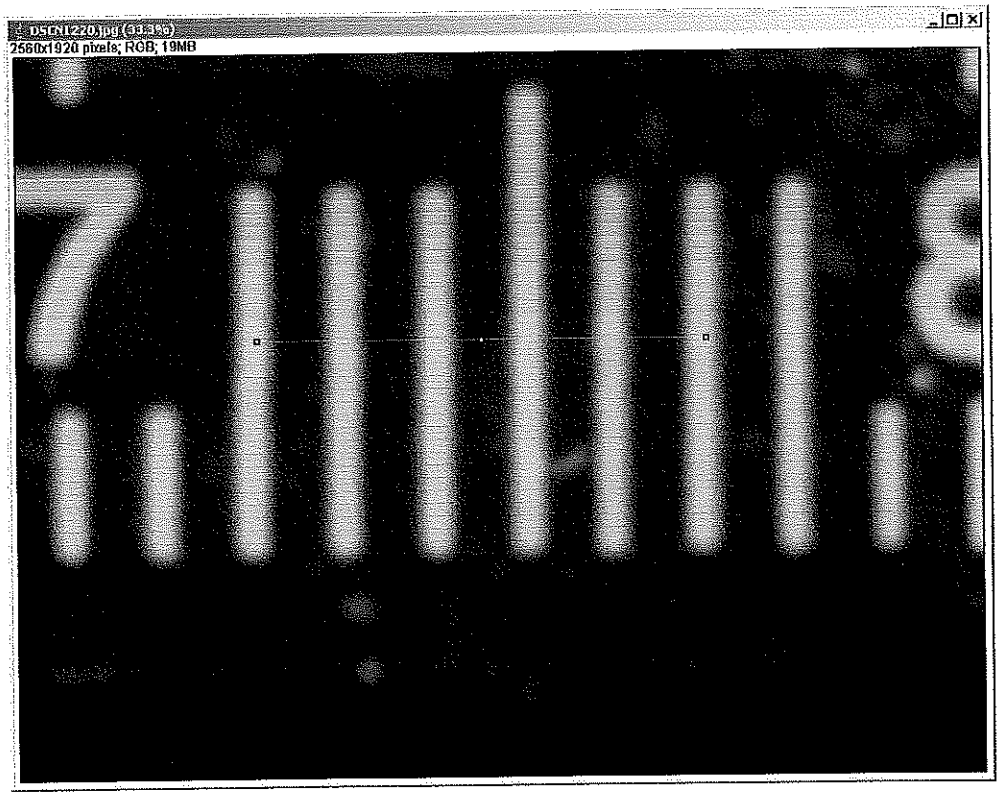
2) Start ImageJ:



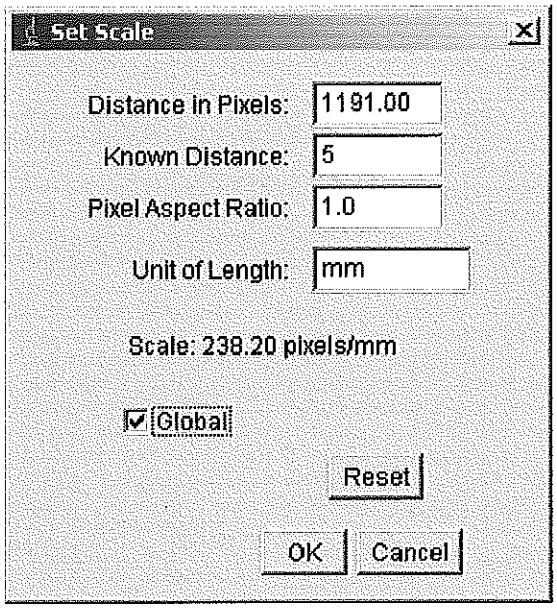
3) If you have an image with a calibration scale (ie a ruler or a stage micrometer) open the image. (If you don't go to step 4 and you can enter the scale information directly into the 'Set Scale' dialog.) Select the straight line selection tool:



Now draw a straight line that defines a known distance on your calibration image (5mm in this case):

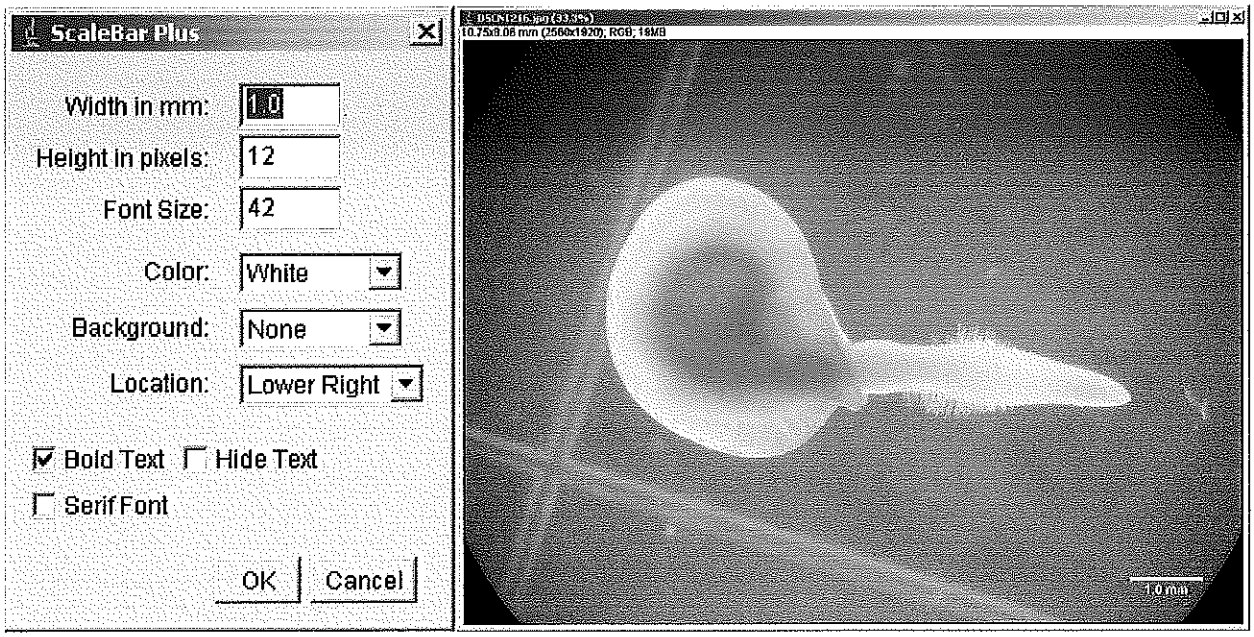


4) In the 'Analyze' menu select 'Set Scale'. The following dialog box will open:



The distance in pixels will be filled in for you based on the length of the line you drew in step 3. If you know your calibration values just enter the number of pixels in a known distance. Fill in the 'Known distance' (in this case 5mm) without units. Define the units of length in the 'Unit of Length' field. Click on 'Global' so that this calibration applies to all images that you open in this ImageJ session. Click 'OK'.

5) Now open the image you want to add a scale bar to. In the 'Analyze/Tools' menu select 'Scale Bar'. The scale bar dialog will open and a scale bar will appear on your image. You can adjust the size, color, and placement of your scale bar. Once you are finished click on 'OK", save your image, and you are done.

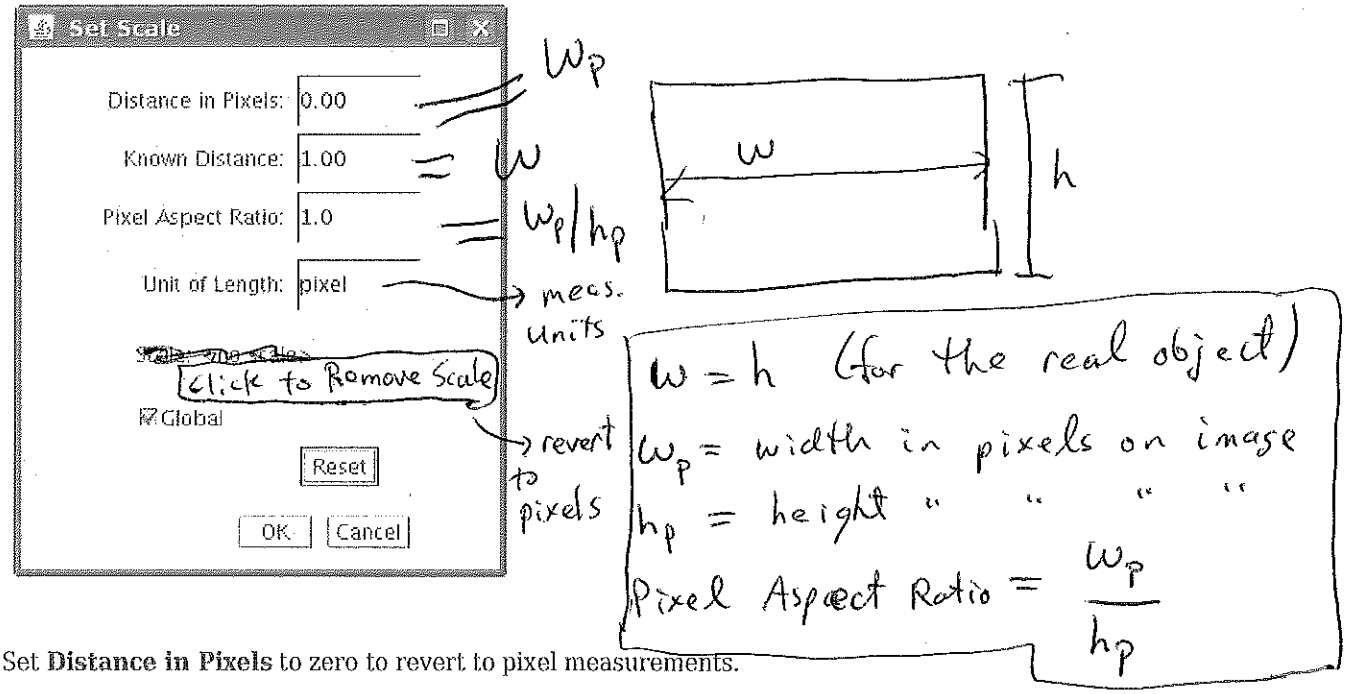


Set Scale...

Use this dialog to define the spatial scale of the active image.

Use this dialog to define the spatial scale of the active image so measurement results can be presented in calibrated units, such as millimeters.

Before using this command, use the straight line selection tool to make a line selection that corresponds to a known distance. Then, bring up the **Set Scale** dialog, enter the known distance and unit of measurement, then click OK. ImageJ will have automatically filled in the **Distance in Pixels** field based on the length of the line selection.



Set **Distance in Pixels** to zero to revert to pixel measurements.

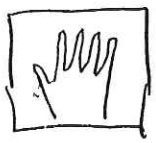
Setting **Pixel Aspect Ratio** to a value other than 1.0 enables support for different horizontal and vertical spatial scales, for example 100 pixels/cm horizontally and 95 pixels/cm vertically.

To set the pixel aspect ratio, measure the width and height (in pixels) of a digitized object with a known 1:1 aspect ratio. Enter the measured width (in pixels) in **Distance in Pixels**. Enter the known width in **Known Distance**. Then calculate the aspect ratio by dividing the width by the height and enter it in **Pixel Aspect Ratio**.

When **Global** is checked, the scale defined in this dialog is used for all images instead of just the active image.

Imagej

scrolling (when picture is zoomed (using + or − keys w/o shift!) and full picture is larger than window :

hit  button to activate scrolling

then left-click hold and drag to scroll

or on menu bar hit Image, Translate
set x, y offsets and Ok
(or preview)

Process, Image Calculation
can perform operation of 2 images :
Add, sub., mult., AND, OR, Average, Diff.

Invert : Edit, Invert

Adding a scale bar : (1st calibrate using Analyze, Set Scale)
hit Analyze, Tools, Scale Bar, fill in numbers and hit Ok

Edit / Paste (Strg+V) :

Inserts the contents of the clipboard into the active image. The pasted image *is automatically selected, allowing it to be dragged with the mouse*. Click outside the selection to terminate the paste. Select Edit/Undo to abort the paste operation.

Remark: ***With copy and paste you can arrange selected image areas and other results like histograms on the print-form.***

Setting of a ROI (region of interest)

The selection of a well defined section of an image (region of interest, ROI) is a basic operation for image analysis and is very important in our Image Processing course. It can be done in the following ways:

Edit / Selection / Select All (or better: Strg+A) :

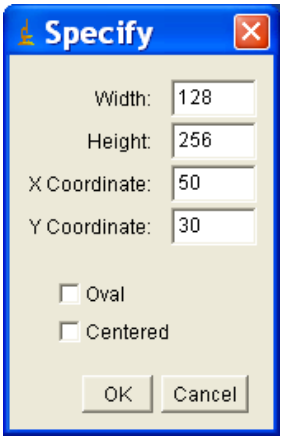
Creates a rectangular ROI that has the same size as the image.

Edit / Selection / Select None (or better: Strg+Shift+A) :

Deactivates the selection in the active image.

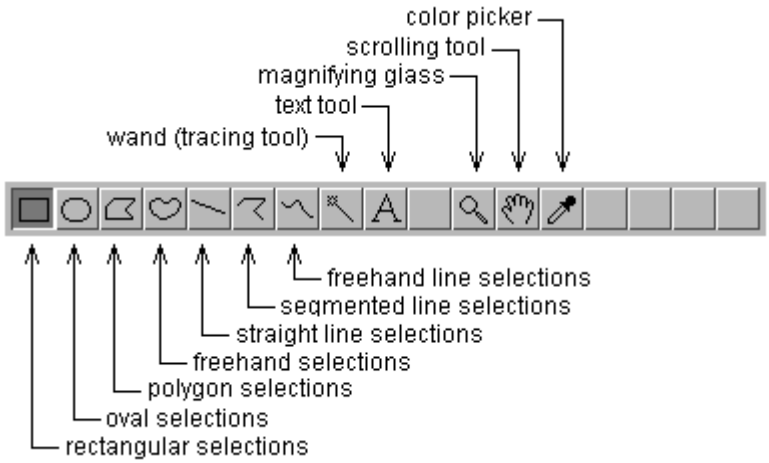
Edit / Selection / Specify … :

This command allows you to position a ROI precisely. The ROI is defined by specifying (in terms of pixels) the size of the ROI box (width and height) and the position of the upper left corner of the ROI (x- and y-coordinates). The values can be selected in the "Specify-window" shown below:



Manual ROI setting (with the button "rectangular selection" on the tool bar):

Manual ROI setting is performed with the „*rectangular selection*" tool button (left button in tool bar, see Fig. below).



Creating the selection is done by a mouse-drag. After a ROI selection use the points in the corners to resize. With the shift key held down during the drag the ROI is fixed to a square. As a selection is created or resized, its location, width and height are displayed in the status bar.

Using the arrow keys you can move the ROI box. With the arrow keys and the alt key held down you can change the width or the height of the ROI.

Text Annotations (with the button "text tool" (A) on the tool bar):

Use the text tool to add text to images. Left mouse click on the text-tool-button and double-click on the image where the text should be positioned creates a rectangular selection containing one or more lines of text. Use the keyboard to write the text and the backspace key to delete characters. The final position of the text can be selected by a mouse drag. *Use Edit/Draw (better: Strg+D) to permanently draw the text on the image.* Use Edit/Options/Fonts, or double-click on the text tool, to specify the typeface, size and style.

Remark: ***All images, histograms, LUT-boxes and other results should be labeled by text annotations on the print-form. So, the final print-form contains a clear description of all displayed components and a short specification of the applied analysis or evaluation.***

Imagej

can copy ROI from one image to another

get area of a ROI (in pixels or whatever units are used in calibration (if calibrated)):

1) select ROI, click inside ROI
2) hit Ctrl + M, Results list will give area

Find out in which menu (or .jar file) a certain command is

Hit **Ctrl+L** to start the command launcher. Type (part of) the name of the entry, then click on *Show full information*.

If *Edit>Options>Misc...>Require command key for shortcuts* is unchecked, typing **L** is sufficient.

Put the main window to the foreground

Pressing the Return key on any **image** will bring the main window to the foreground.

Close all images (without being asked whether to save them)

Plugins>Utilities>Close All Without Saving

Set the foreground color

Double-click on the pipette, or hit Ctrl+Shift+K (on Macs, it is Cmd+Shift+K), or select the menu item **Image**>*Color>Color Picker...*"

Set the line width

Line selections can have a width larger than **one**, which also has an effect on line profiles. You can set it by double clicking on the line selection tool, or by calling *Edit>Options>Line Width...*

copy ROI

Quickly **copy** a **ROI** from **one image** to **another**

Simply activate the **image** with the desired **ROI**, then the **image** you want to put that **ROI** into, and hit *Control+Shift+E*. This triggers the *Edit>Selection>Restore Selection* which "restores" the selection.

Save and recall a ROI:

1) open image
2) draw a ROI 
3) Analyze, Tools, ROI manager
4) hit letter t on keyboard (adds ROI to list)
5) hit Save in ROI manager, save as ~.ROI file

with a new file loaded (a new image):

1) open ROI manager (3) above)
2) hit open in ROI manager, hit desired ROI file
3) click on file name in ROI list - ROI now appears in current image

ImageJ

Rotating an image to have some feature be aligned precisely along the horizontal or vertical axis:

Method 1 1) open image, 2) click on 'select rectangle' box  on menu bar, 3) click and drag open a rectangle on the image, 4) hit Image, Transform, Rotate, preview (check box), input various angles and observe results (note: only area within rectangle is rotated) 5) note best angle, 6) hit Ok, reload full image and rotate (now, the whole image) by best angle and save file,

Note: can use Ctrl + or - to zoom in or out

Method 2 Use line tool 

1) Click and drag a horizontal (or vertical) line (you can read off angle and adjust to be 0° or 90°), 2) Rotate as above

Note: Can use Ctrl + or - for zooming
Can translate line segment by click and drag on middle box of segment

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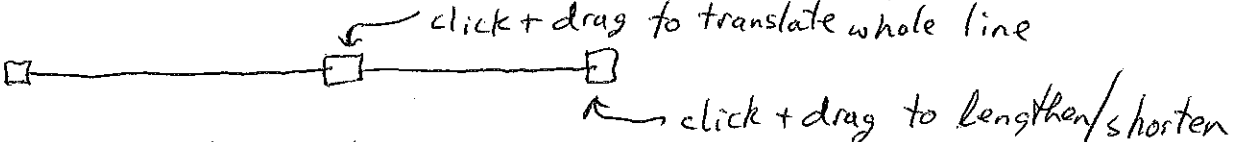
Precisely placing a line segment:

1) click on  line tool, 2) Place, by click and drag, the line approx.

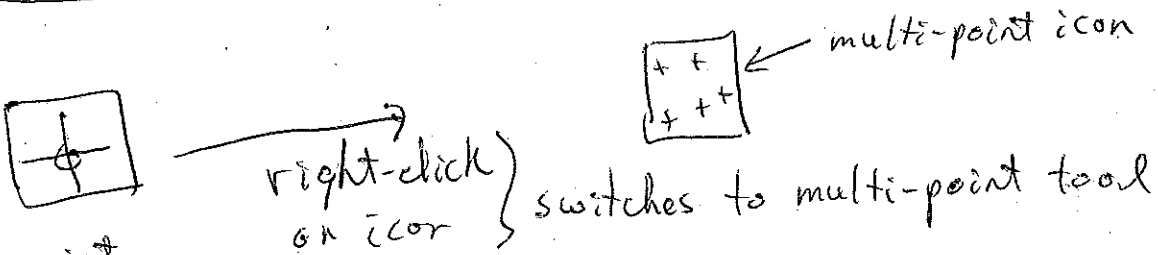
3) Blow up and lengthen/shorten or translate line segment



4) hit Ctrl M to output line segment parameters (length, etc.)

Note: Can use scroll tool  to click + drag through blown-up image

Marking one point or multiple points on an image



1) single points: drag cursor to location with mouse, left click
then hit Ctrl-M to output
(can do multiple times)

2) set of points: use multi-point icon
with mouse, locate and left-click a set of points
then hit Ctrl-M to output set of x-y values

Subtract background ("Rolling Ball")

ImageJ : Process, Subtract Background, set Rolling Ball Radius and options, ok

What does the subtract background command do?

(with preview option, can preview result)

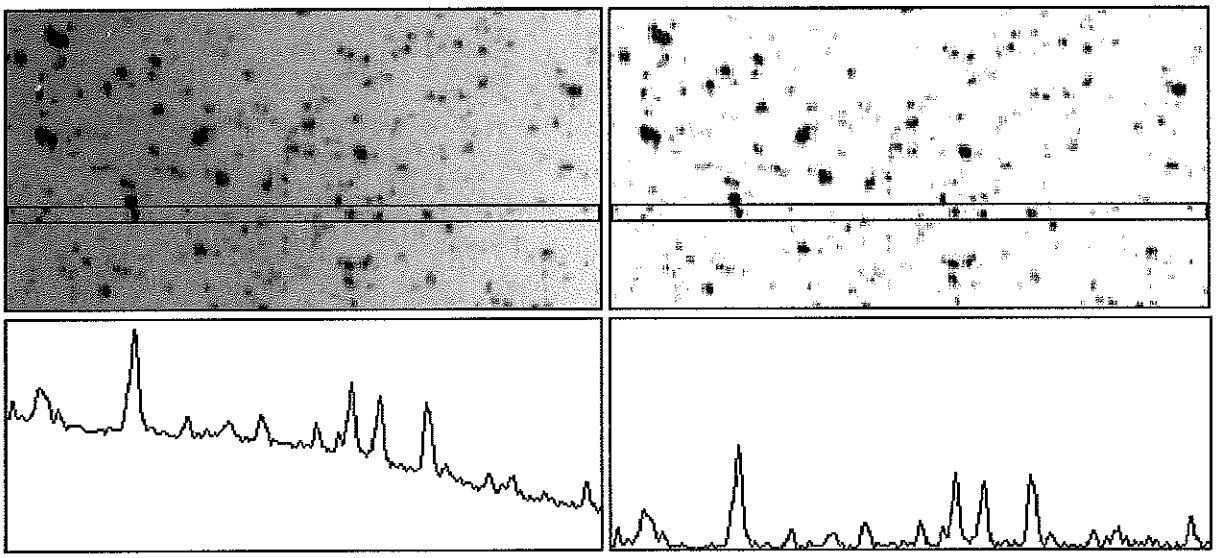
Removes smooth continuous backgrounds from gels and other images. Based on the a "rolling ball" algorithm described in Stanley Sternberg's article, "Biomedical Image Processing", IEEE Computer, January 1983. Imagine a 3D surface with the pixel values of the image being the height, then a ball rolling over the back side of the surface creates the background. The current algorithm (since version 1.39f) uses an approximation of a paraboloid of rotation instead of a ball.

- The **Rolling Ball Radius** is the radius of curvature of the paraboloid. As a rule of thumb, for 8-bit or RGB images it should be at least as large as the radius of the largest object in the image that is not part of the background. Larger values will also work unless the background of the image is too uneven. For images with pixel values having a very different range, note that the radius should be *inversely* proportional to the pixel value range. E.g., typical values of the radius are around 0.2 to 5 for 16-bit images (pixel values 0...65535).

- The **Light Background** option allows the processing of images with bright background and dark objects.

- With the **Create Background** option, the output is not the image with the background subtracted but rather the background itself. This option is useful for examining the background created (in conjunction with the **Preview** option). "Create Background" can be also used for custom background subtraction algorithms where the image is duplicated and filtered (e.g. removing "holes" in the background) before creating the background and finally subtracting it with **Process > Image Calculator**.

- For calculating the background ("rolling the ball"), images are normally smoothened to reduce noise (average over 3×3 pixels). With **Disable Smoothing**, the unmodified image data are used for creating the background. Check this option to make sure that the image data after subtraction will never be below the background.



From:
http://imagejdocu.tudor.lu/ - **ImageJ Documentation Wiki**

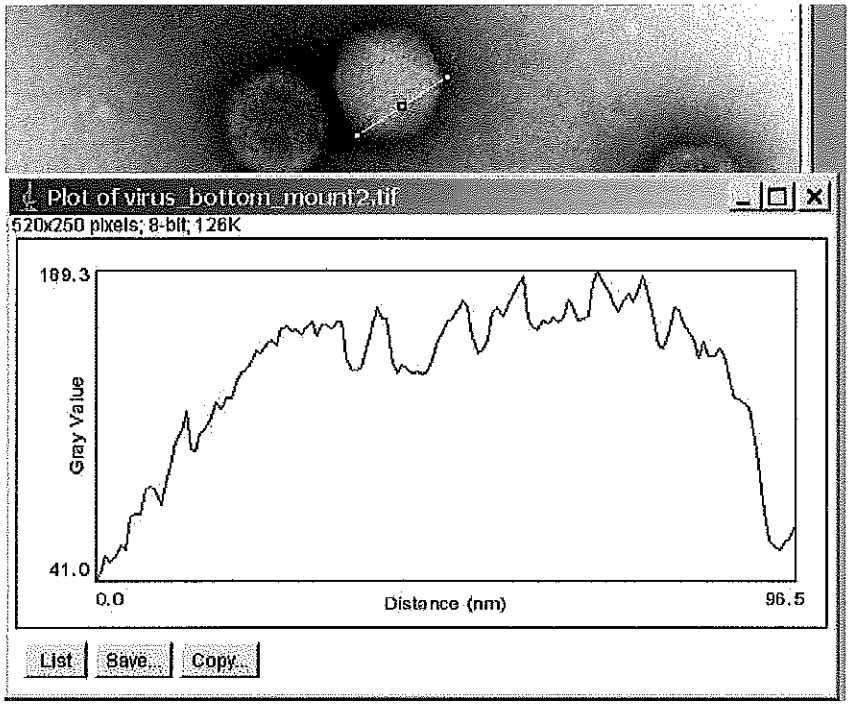
Permanent link:
http://imagejdocu.tudor.lu/doku.php?id=gui:process:subtract_background

Last update: **2008/09/09 13:33**

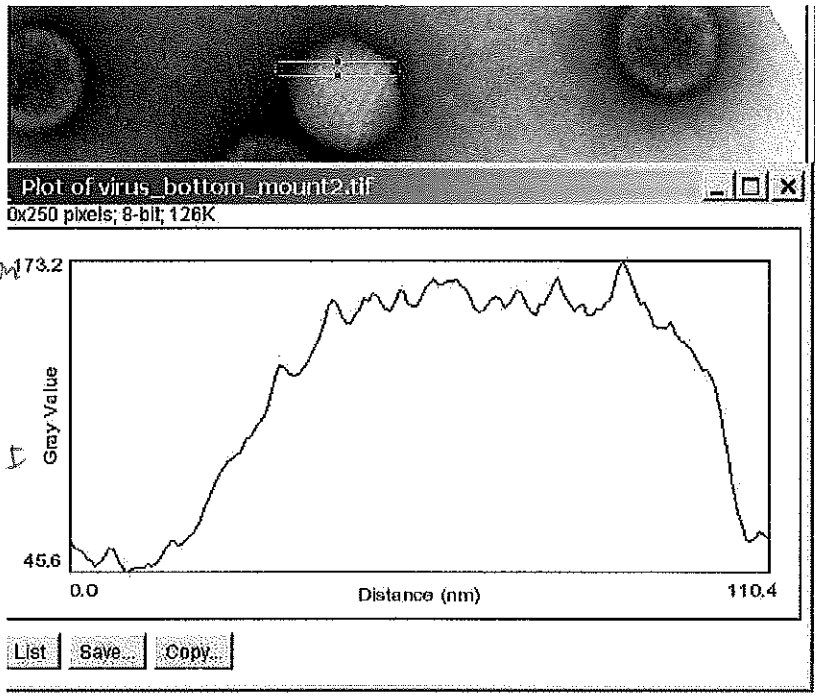
Intensity vs position

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

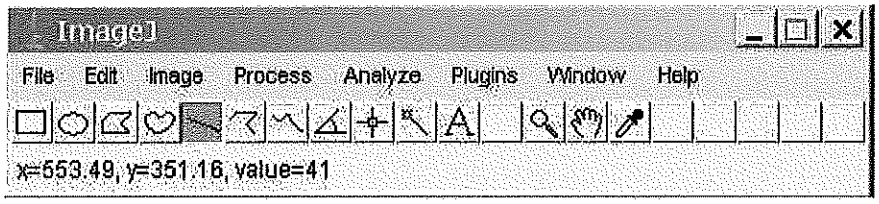


averaged in y-direction

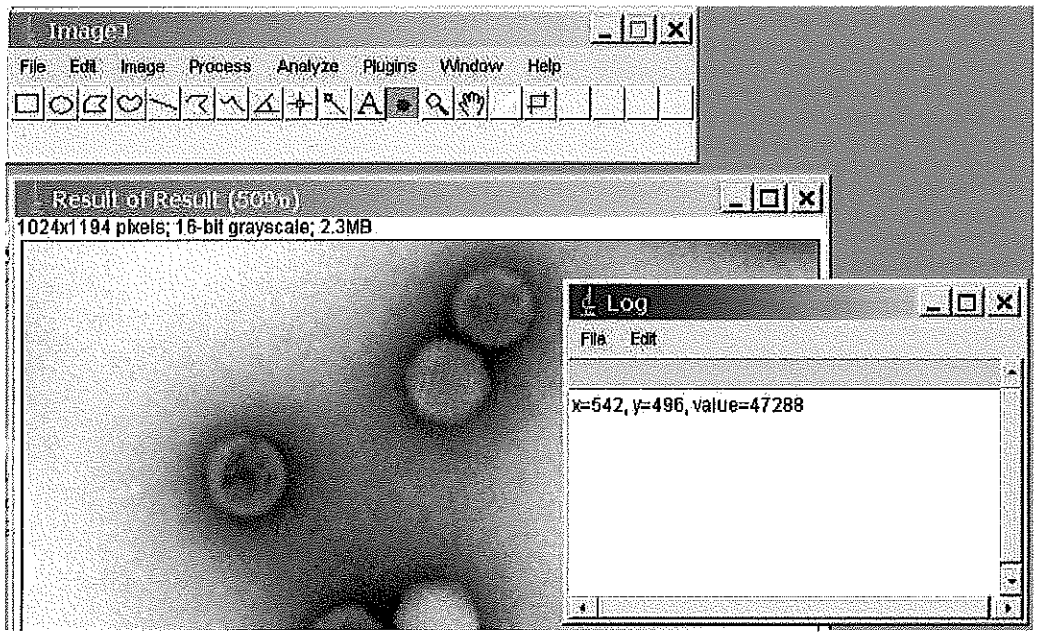
draw a rect. ROI and then Analyze, Plot Profile

Intensity at a point

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.



Plot Profile

Displays a two-dimensional graph of the intensities of pixels along a line within the image.

The x-axis represents distance along the line and the y-axis is the pixel intensity.

[insert image Plot]

For rectangular selections, the plot displays a "column average plot", where the x-axis represents the horizontal distance through the selection and the y-axis the vertically averaged pixel intensity.

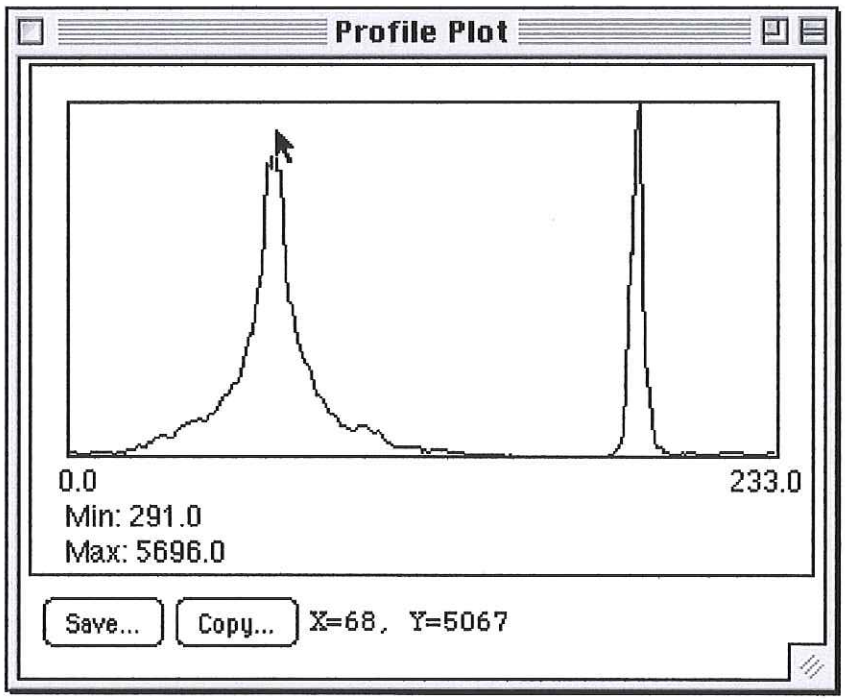
To average horizontally, hold down the **Alt** key.

Use the **Save** or **Copy** buttons to save the profile data, formatted as a sequence of lines each containing a single number.

1) open an image
2) hit line button [—], click and drag a line on the image
3) hit Analyze, Plot Profile

Plot Profile

Displays a two-dimensional graph of the intensities of pixels along a line within the image. The x-axis represents distance along the line and the y-axis is the pixel intensity.



For rectangular selections, displays a "column average plot", where the x-axis represents the horizontal distance through the selection and the y-axis the vertically averaged pixel intensity. To average horizontally, hold down the alt key. Use the *Save* or *Copy* buttons to save the profile data, formatted as a sequence of lines each containing a single number.

The Dynamic Profiler plugin creates a profile plot that is continuously updated as the selection is moved or the image is updated. The StackProfilePlot macro generates profile plots of all the images in a stack and saves them in another stack.

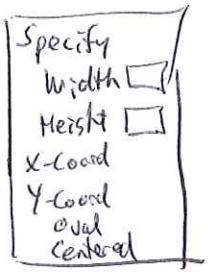
ImageJ

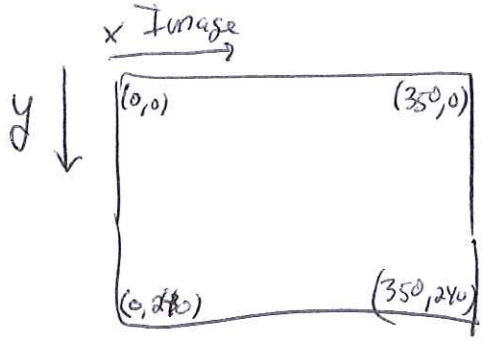
To blow up image to more precisely draw a line:

1) Open up image, click on full screen button 
2) ctrl + or ctrl − to blow up, or reduce

Edit, Options, Profile Plot Options (can change scales in profile plot)

Edit, Selection, Specify
(can make a line of width 1 and then can get the line profile)





Edit, Options, Conversions Conversion Options

- [x] Scale when converting
- [ ] weighted RGB Conversions

change width of line used in a tool — double click on the tool and set

Measuring DNA Contour Lengths

This is an example of how to measure the lengths of DNA contours on images acquired using an atomic force microscope (AFM). It requires ImageJ 1.30 or later.

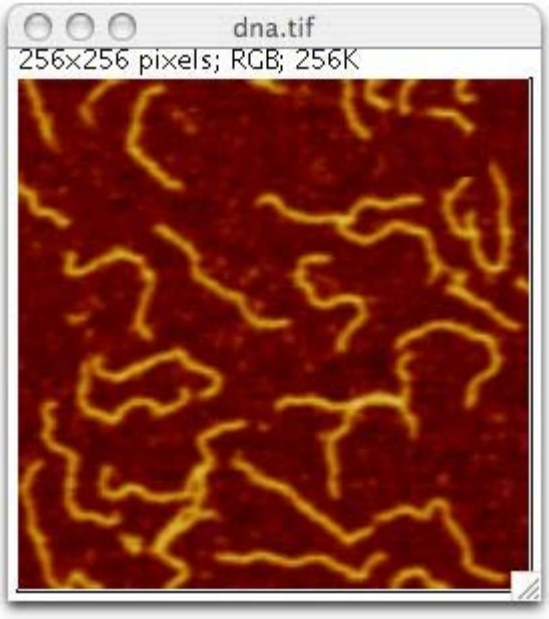


Figure 1. AFM image of 538 bp DNA fragment deposited on mica.

In this example, we measure the length of the DNA contour in the lower right corner. The field width of the image is 500nm. The image is from M. Lysetska, et al., "UV light-damaged DNA and its interaction with human replication protein A: an atomic force microscopy study", Nucleic Acids Research, 2002, Vol. 30, No. 12 2686-2691 (nar.oupjournals.org/cgi/content/full/30/12/2686), used with permission.

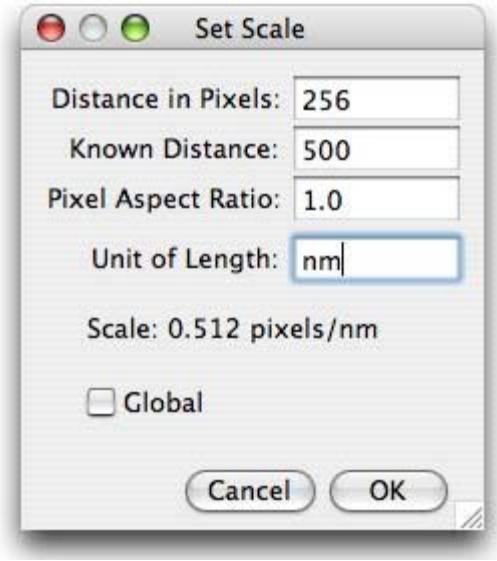


Figure 2. Set Scale Dialog Box.

Use the *Analyze/Set Scale* dialog to define the spatial scale. Enter the image width in the "Distance in Pixels" field, enter the field width in the "Known Distance" field, enter "nm" as the "Unit of Length", then click "OK".

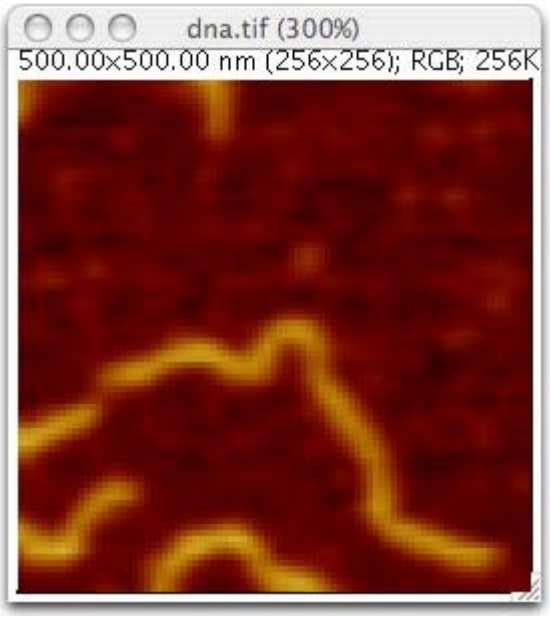


Figure 3. Zoomed and spatially calibrated image.

Use the magnifying glass tool to zoom in on the DNA contour to be measured, in this case, the one in the lower right corner of the image. To zoom out, right-click or alt-click with the magnifying glass tool.

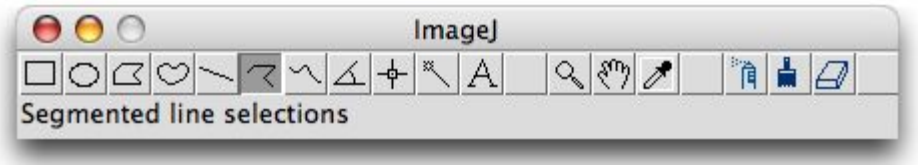


Figure 4. ImageJ toolbar with segmented line tool selected.

This is the ImageJ toolbar. Use the segmented line tool (sixth tool from the left) to outline the DNA contour. The three tools on the right end of the toolbar are tool macros.

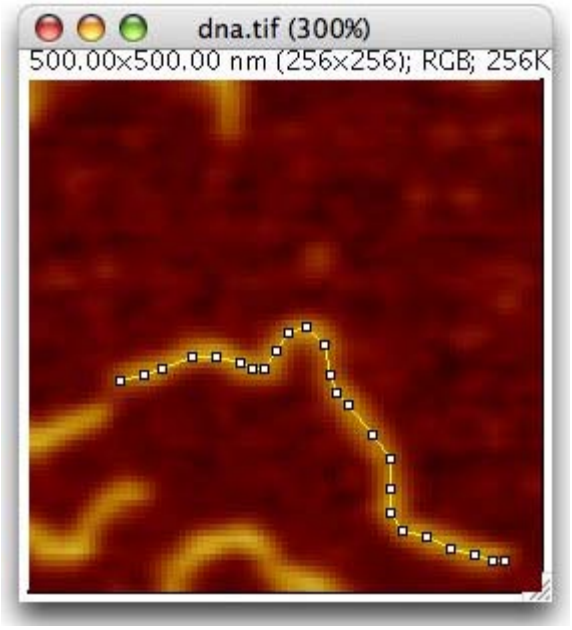


Figure 5. Selected DNA contour.

Use the segmented line tool to create a line selection that outlines the DNA contour. To finish outlining, right-click, double-click or click in the box at the starting point. The line selection can

be adjusted by clicking and dragging the the tiny black and white "handles" along the outline.

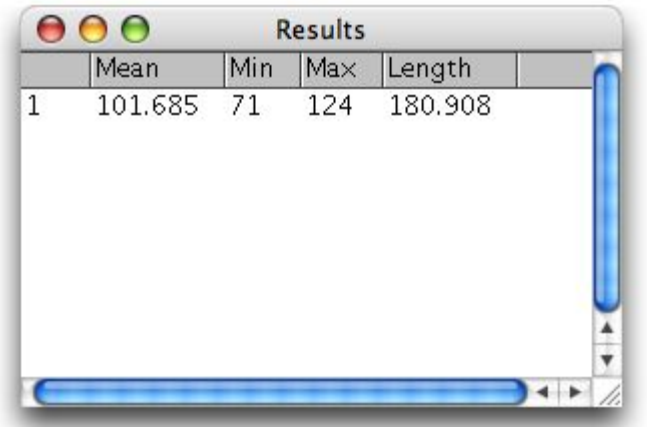


Figure 6. Results Table.

Finally, use the *Analyze/Measure* command to measure the length of the DNA contour, in this case 181nm. Measurements can be transferred to a spreadsheet by right-clicking in the "Results" window, selecting "Copy All" from the popup menu, switching to the spreadsheet program, and then pasting.

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cropping a precise area by x, y coordinates

1000
6

Select an area by numbers:

Edit, Selection, Specify

enter Width, Height, x, y (or start point (upper left is default)

then Image, Duplicate (then new image produced of ROI)

File, Save As, Text Image (for .txt of pixel #'s in rows x columns

ex. 250 x 4 image

504,0 218500.

0,511 226000

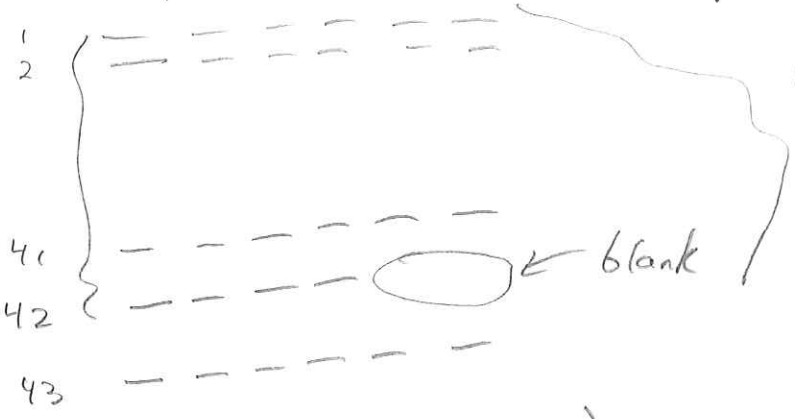
511,511 227500

save as Text Image

read in Notepad, do Format, Word Wrap

then output is 6 points per line of file

IV
↑
9



1st image line
250 pixels
41 x 6 = 246
plus 4 on 42nd line

(same for other 3 lines)

if save this file from Notepad, then read in Excel,

get 4 lines, 250 numbers each

for Notepad, can do this also for 512 x 512 image

* however, when I tried 512 x 512, for Excel got 512 rows but only ~230 columns!

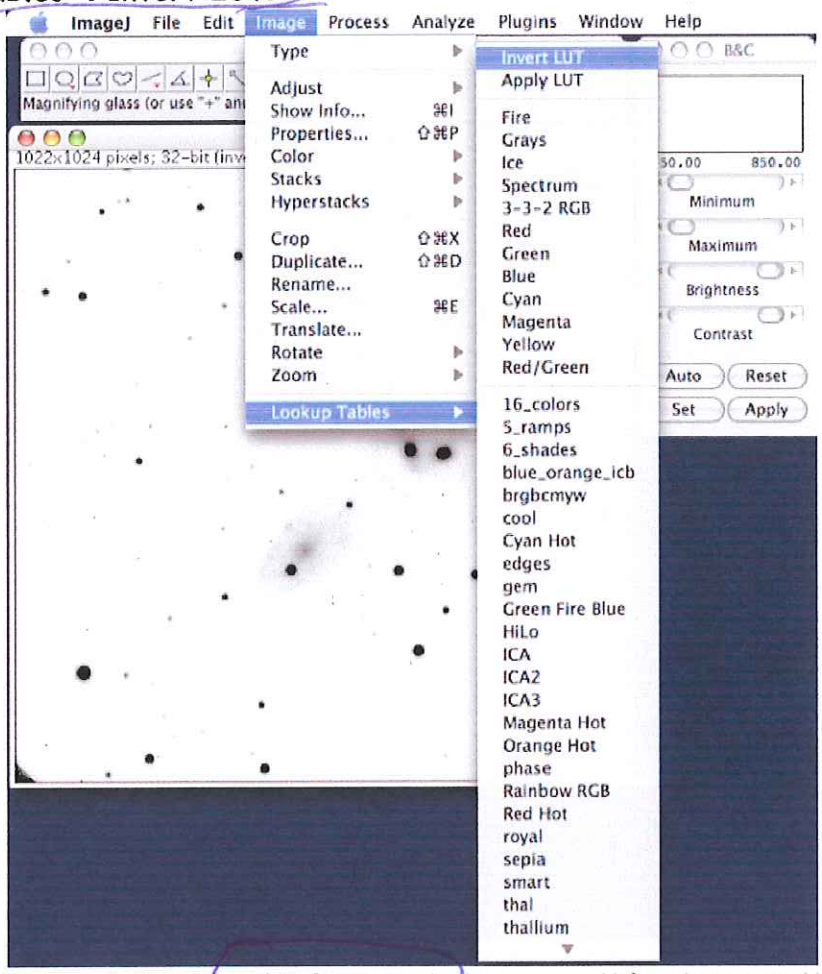
apparently max is 65536 rows x 256 columns } for Excel 2003

but 1M x 16K (Excel 2007)

Inverting an image in ImageJ (2 ways)

galaxies in an astronomy image. If you could make the sky white and the stars black, you could pick out fainter objects.

Although the Universe doesn't provide us this convenience, ImageJ does. To make your life easier, you need to invert ("flip") the image so that you are seeing black stars on a white background. To do this click: Image → Lookup Tables →Invert LUT. (1)



Important! Do *not* use Edit → Invert, because this changes the pixel values!! (2) → actually, is OK if you do not need precise intensity numbers

Another example of an inverted image is shown on the cover of this worksheet. Half the image is normal, and half is inverted. Note how much more detail you can see in the faint nebula in the inverted half!

6. **Play with the brightness and contrast some more to bring out faint features.** To get the best results from the inverting process, you will probably need to adjust the brightness and contrast some more. Personally, we like a light-gray background; we obtained best results by

6 Undo and Redo

Hide

Probably the first thing you will notice is that ImageJ does not have a large undo/redo buffer. Undo (Edit▹Undo [z]↓) is currently limited to the most recent image editing / filtering operation. With time you will appreciate that this is necessary to minimize memory overhead. Nevertheless, with IJ 1.45 and later, Undo [z]↓ is, in most cases, undoable and can be applied to multiple images if *Keep multiple undo buffers* is checked in Edit▹Options▹Memory & Threads…↓

If you cannot recover from a mistake, you can always use File▹Revert [r]↓ to reset the image lo its last saved state. For selections, Edit▹Selection▹Restore Selection [E]↓ can be used to recover any misdealt selection.

In ImageJ the equivalent to 'Redo' is the Process▹Repeat Command [R]↓, that re-runs the previous used command (skipping Edit▹Undo [z]↓ and File▹Open… [o]↓ commands).

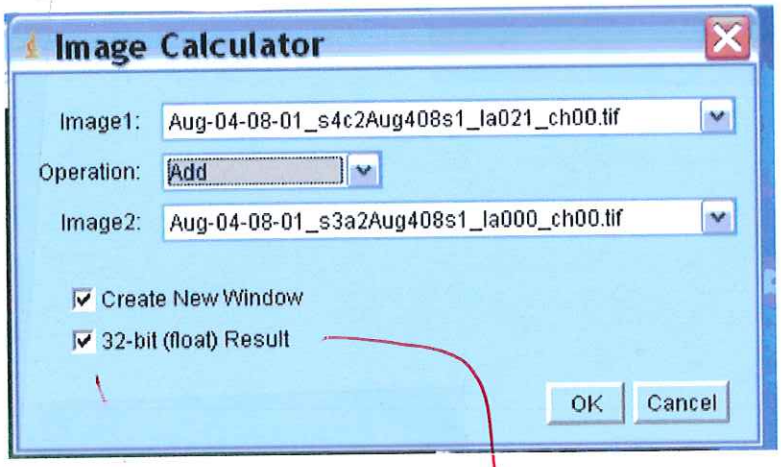
See Also: Plugins▹Utilities▹Reset…↓, Multi Undo plugin

ImageJ

1) Adding two images or subtracting one from another:

open two images (of same type and size)

Process, Image Calculator



pick images from open files

Check 32-bit for result to avoid overflow

operations: Add, Sub., Mult., Divide, AND, OR, XOR, Min, Max, Average, Difference, Copy, Transparent-zero

pick files and operation, OK, New window ("Result of ...")
close original files, then can save result file

2) to subtract a constant background:

load picture (may want to change to grayscale: Image, Type, 8-bit)

Process, Math, type intensity value to be subtracted (can preview result by checking Preview box (uncheck to revert), Ok

3) Rolling Ball background subtraction: Process, Subtract Background, set options
subtracts a smooth background (w/ rolling ball algorithm)

4) Calculator Plus plugin: can scale, add, sub. two images and subtract bg Constant background

can add, subtract two images and add/subtract constant bg. and can scale image

Calculator Plus

(Improved version of Process/Image Calculator which includes scaling so can avoid overflow with 8 bit images.)

Author: Wayne Rasband (wsr at nih.gov) and
Gabriel Landini (G.Landini at bham.ac.uk)

History: 2002/03/13: First version
2002/04/16: Added "Create Window" option; bug fixes
2003/06/09: Supports operations where *i1* is a stack and *i2* is not
2004/07/17: Does plane-wise calculations on RGB image 2009/06/09:
Fixed subtract image order bug

Requires: ImageJ 1.31q or later is required to run this plugin from a macro.

Source: Calculator_Plus.java

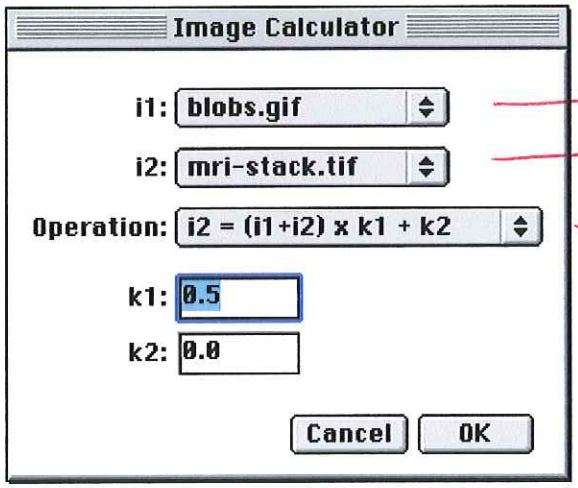
C:/Program Files/ImageJ/Plugins

Installation: Copy Calculator_Plus.class to the plugins folder and restart ImageJ.

then appears in plugins

Description: This plugin will add, subtract, multiply or divide two images or stacks. Unlike the built-in *Process/Image Calculator* command, it allows results to be scaled to prevent clipping when working with 8 and 16 bit images. Also, unlike the built-in calculator, it works with calibrated images and images do not have to be the same type. Note that if *i1* is a stack and *i2* is a single image then i2 will be applied to the entire stack.

Adapted to operate on the RGB planes independently by G. Landini 17/July/2004. Now colour images can be operated plane by plane. The result of the RGB operations are rounded and trimmed to the 8 bit space in each plane. This is useful to perform background uneven illumination correction in brightfield microscopy using colour images:
image/brightfield * 255
Before this plugin returned a greyscale result, now it returns an RGB result.



→ image 1
→ image 2
→ can
- scale
- add
- subtract
- multiply
- divide

set k2 = −() to subtract bg

Scale: i2 = i1 × k1 + k2
- i2: new image in window (not the original i2)
- k1: scale factor
- k2: add or subtract a bg

ImageJ notes

4) Process, Image Calculator,
can Add, Subtract, Mult, AND, OR two images

5) Image, Stacks, Make Montage
can make a tiled picture of all the images
in the current stack

6) Image, Stacks, ZProject
~~can~~ can average a group of ~~pict~~ pictures into
one output picture (also, min., max...)

7) Image, Rename, can rename stack

8) To save to AVI file from a stack (of video images), the images appear to be RGB (!) even though it is a B/W camera (check the Video capture settings – set to 8-bit grayscale?),

[Note: Image, Type (a check mark will be on the current type), click on another type to convert (not all are possible)]

so, convert to 8-bit, then File, Save As, AVI,

9) to load plugins, save to
c:\Program Files\ImageJ\plugins

(then do Run and Compile)

10) Edit, Options, Memory
↳ set max. mem.
(say to 512 MB?)

ImageJ

1) Multiplying intensity by a given factor
   a) Open image b) Process, Math, Multiply, enter mult. factor, click Preview, OK, File, Save As
   (note: can draw a ROI and only multiply that area, etc.)
2) Montage - gallery for a stack

3) convert to floating-point:
   Image > Type > 32-bit

4) Stack averages, sums, etc.:
   Image > Stacks > Z-Project
   set start slice, Stop slice and Projection Type
   (Avg. Int.
   Max "
   Min "
   → Sum slices
   Std. Dev.
   Median)

note: It seems that ImageJ will convert to 32-bit floating before doing the summing (to preserve accuracy) (check this out!).

5) making a stack from images in a folder:
   File > Import > Image Sequence

6) note: JPEG intensity range 0 → 255
   TIFF much larger - 32 bit

ImageJ

7) Multiply the intensity by a factor within a region-of-interest:
1) open image file, 2) pick shape for ROI from menu, rectangle, ellipse, polygon, freehand 3) click and drag open ROI
4) Process, Math, Multiply (etc.), set value, hit ok
5) File, Save AS, then save resulting file

Note: If picture is 8-bit ($0 \rightarrow 255$), multiplying can produce pixels with intensity > 255. These all end up as 255 and information is lost. So, first convert to 32 bit (Image, Type, 32-bit) and work with higher precision image ($2^{32} = 4.29 \times 10^9$)

Note: If no ROI is chosen, whole image is multiplied by same factor. The appearance on the display is unchanged, since it is normalized for display.

---

8) .bmp images from Printkey 200 are 8-bit color ($0 \rightarrow 255$ for R, G, B), can convert to 32-bit B/W, but not (it seems) to 32-bit color. RGB color is also 8-bit.

Note: When doing a Stack average Z-project with 8-bit images, the averaged image is also 8-bit (so precision is lost)

So, Better to first convert stack to 32-bit, then average.

↓

Image, Type, check 32-bit
(this will do entire stack)

Should check when summing and averaging and multiplying give the same result (although clearly you will get in trouble if summing goes over the maximum possible intensity)

* (Yes) - I did a check of summing (where automatically, it appears, images are converted to 32-bit) vs averaging (1st converting to 32-bit) followed by multiplying by the number of images in the stack

---

Note: RGB images (8 × 3 bit), if you convert to 32-bit, output file is 32-bit B/W where the 3 colors (RGB) are averaged

---

When displaying a 32-bit image, the display is normalized - so if you mult. by 10, displayed image has the same brightness. However, you can click and drag a ROI, then mult. and the ROI will be brighter.

www.csc.mrc.ac.uk/microscopy/links.htm

Free Software downloads

Leica LAS AF lite (Download): opens SP5 confocal LIF files
Leica LCS lite (Download): opens SP1 and SP2 confocal LEI files

Image J (website , Download): extremely useful tool for image analysis, can import all image formats produced in the facility in combination with Loci-Bioformats plugin.

Loci-Bioformats (Download): ImageJ plugin to import most commonly used microscopy file formats (eg. Leica LIF/LEI, Metamorph STK, Deltavision R3D).

Installation: download loci_tools.jar file to ImageJ/plugins folder and restart ImageJ.

Volocity LE (Download, Download Manual): Limited functionality version of Volocity

CellProfiler (Website, Download Manual): High content screening software, but generally useful for automated quantitative image analysis on a smaller scale.

→ plug-in for Imagej to read Leica files

then to use:
Plugins, LOCI, Bio-Formats Importer
set options, check Open all series, Specify range for each series
View stack with: Standard ImageJ

make ROI, image, stacks, Plot z-axis profile

Imagej

1) add 2 images : Process, Image Calculator

2) add images in a stack: Image > stacks > z-project, set slice #'s and operation

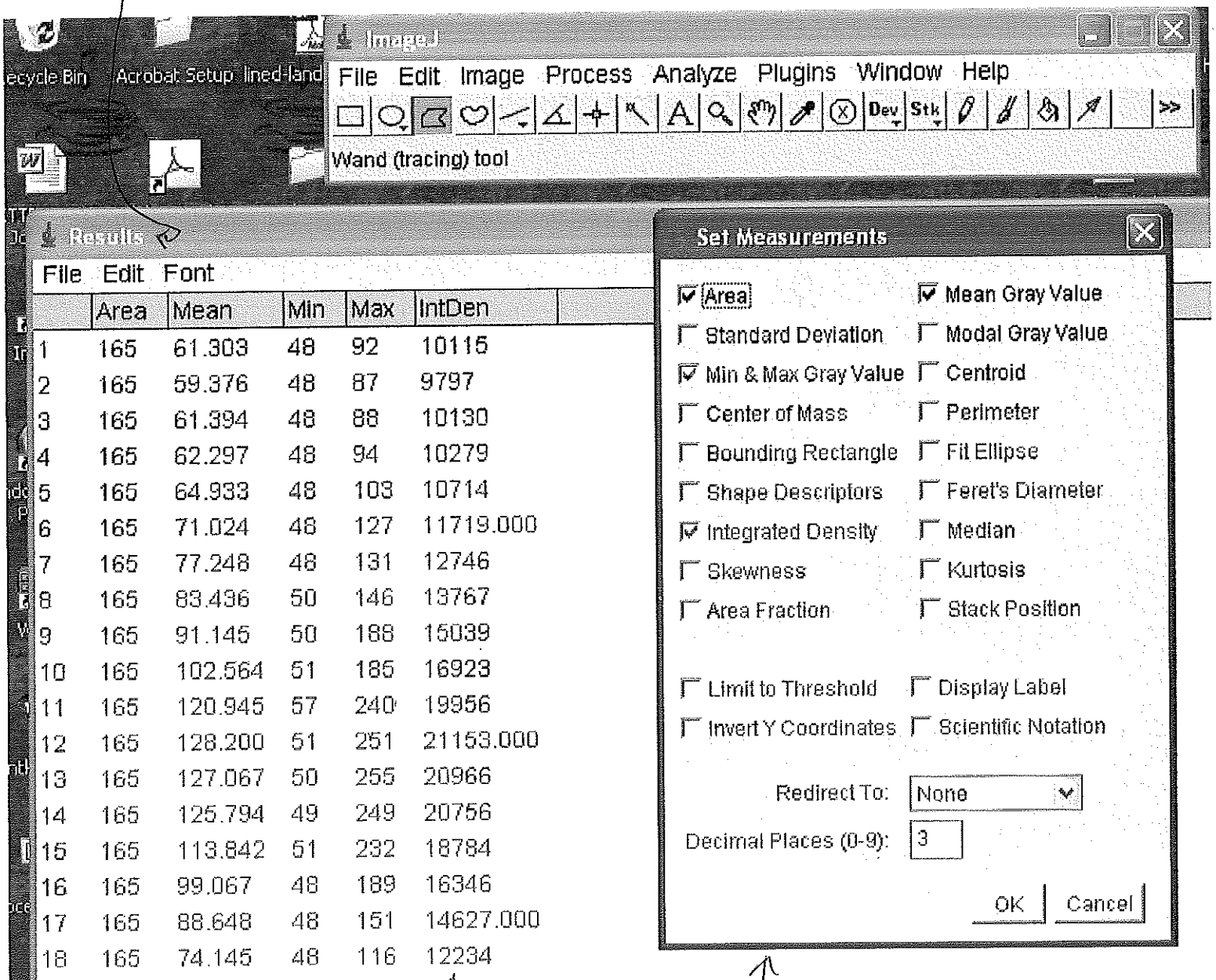
3) make a stack from images in folder: File, Import, Image Sequence
(pick one file in folder, click on it)
(Image, Type, check 32-bit → better accuracy for averaging)

4) Import uncompressed AVI : File, Import, AVI

5) Set to ROI Manager : Analyze, Tools, ROI Manger

0) input Image sequence (File, Import, Image sequence...)
1) draw a ROI
2) Image, Stacks, Plot Z-axis Profile
3) outputs plot of mean value of ROI vs slice number and a results file (ASCII), can then do File, Save As, give filename.txt to save as simple text file.

outputting and plotting all the mean values and sums of ROI intensities in a Z-stack



	Area	Mean	Min	Max	IntDen
1	165	61.303	48	92	10115
2	165	59.376	48	87	9797
3	165	61.394	48	88	10130
4	165	62.297	48	94	10279
5	165	64.933	48	103	10714
6	165	71.024	48	127	11719.000
7	165	77.248	48	131	12746
8	165	83.436	50	146	13767
9	165	91.145	50	188	15039
10	165	102.564	51	185	16923
11	165	120.945	57	240	19956
12	165	128.200	51	251	21153.000
13	165	127.067	50	255	20966
14	165	125.794	49	249	20756
15	165	113.842	51	232	18784
16	165	99.067	48	189	16346
17	165	88.648	48	151	14627.000
18	165	74.145	48	116	12234

# of pixels in ROI

= 165 * mean value

Analyze, Set Measurements

sets which parameters are output to Results window

Friday, Feb 25, 2011 12:17 PM

use of ROI manager to get all the integral of intensities in a ROI for a sequence of images (stack)

I do exactly what I believe you are considering. I measure the average intensity of many cells (neurons) over time in a **stack** of images. As Bob suggested do the following:

- open **ROI** Manager (Analyze, Tools, ROI Manager)
- you'll probably use the ellipitcal **ROI** so click on that on the main menu of **imageJ** and select your first cell
- press 't' (or click "Add" on the **ROI** Manager)
- then, and this is important, click Show All on the **ROI** Manager
- if you want the same size **ROI** on all cells its best to move the pointer to the centre of your first **ROI** and drag to a new cell - again press 't'. repeat until all cells selected. do not forget to press 't' after each cell
- in doing this you will see a list of all **ROIs** appear in the **ROI** Manager
- to save all of your **ROIs** click "Save" in the **ROI** Manager - be sure not to select any of the **ROIs** in the list because only those selected will be saved. If no **ROIs** are selected in the list then ALL will be saved in a zip file.
- I use Time series Analyzer to calculate all average intensities in the image **stack**
http://rsb.info.nih.gov/ij/plugins/time-series.html

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Tools

This submenu provides access to various image analysis plugins.

Save XY Coordinates...

Writes to a text file the XY coordinates and pixel value of all non-background pixels in the active image. For grayscale images, writes three values per line (x, y, and value), separated by spaces. For RGB images, writes five values per line (x, y, red, green and blue).

For grayscale images you can specify the background value in a dialog.

Note that the y coordinates are inverted with respect to those shown in the status bar (y=0 at the bottom) unless you select the appropriate checkbox (this unusual behavior is for compatibility with old versions).

Fractal Box Count...

This command is used to estimate the fractal dimension of a binary image. Counts the number of boxes of an increasing size needed to cover a one pixel binary object boundary.

The sequence of box sizes is defined in the Box Sizes field.

Check Black Background if the object is white [255] and the background is black [0].

The box size and the number of boxes necessary to cover the boundary are plotted on a log-log graph and the fractal dimension determined from the slope of the log-transformed data by linear regression, i.e.

```
D = - slope
```

For more information, see the “Fractal_Box_Counter.java” source file.

Analyze Line Graph

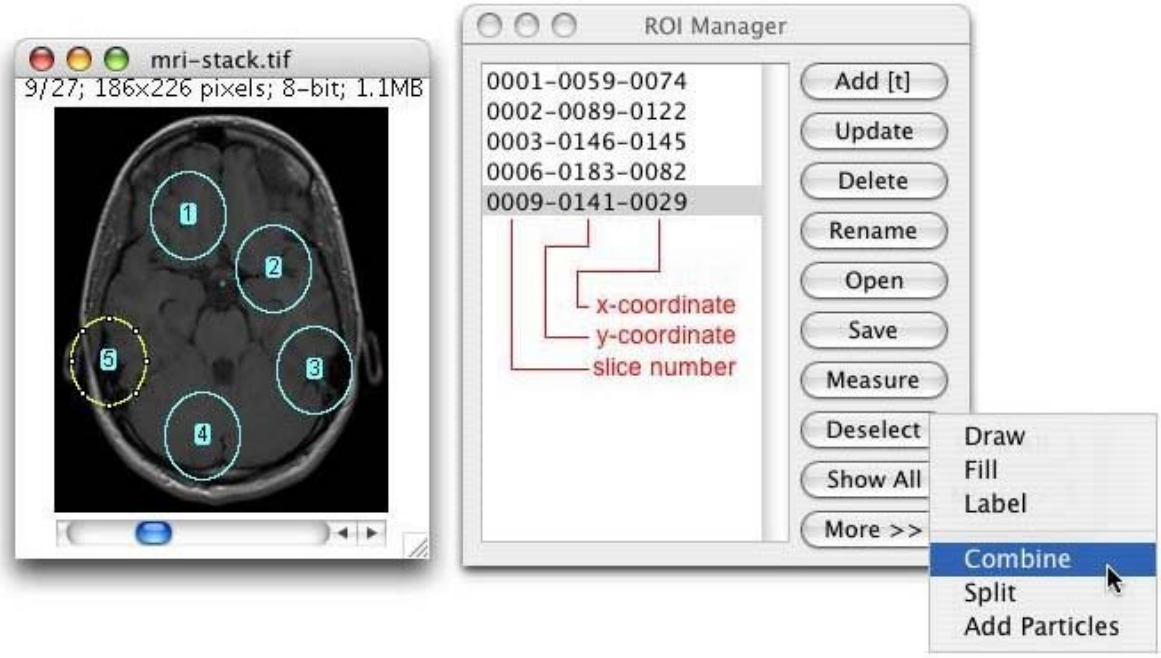
ImageJ can be used to recover numeric coordinate data from scanned line graphs using the following procedure. Steps 1-6 are not necessary for binary (black and white) graphs. For practice, use the File>Open Samples>Line Graph sample image.

1. Open the image containing the graph.
2. Open the thresolding tool (shift-t).
3. Adjust the threshold so the graph is highlighted in red.
4. Click on “Apply” (make sure foreground is black and background is white).
5. Close the thresholding tool.
6. Use the oval selection tool as an erasor (press backspace to erase) to isolate a single curve (note: background color must be white).
7. Select the curve by clicking to the left of it with the wand tool.
8. Use Edit>Clear Outside to erase everything but the curve.
9. Use Analyze>Tools>Analyze Line Graph get the XY coordinates.

ROI Manager

The ROI (Region of Interest) Manager is a tool for working with multiple selections. The selections can be from different locations on an image or from different slices of a stack. All selection types, including points

and lines, are supported.



Click Add to add the current selection to the list, or press “t”, the keyboard shortcut for the Edit>Selection>Add to Manager command. The Roi manager creates a three part label. The first part (stacks only) is the slice number, the second part is the X coordinate of the selection, and the third part is the Y coordinate. Click on a label to restore the associated selection to the current image. With stacks, the selection is restored to the slice it came from. Click on Show All to display all the selections on the list. Hold down the shift key while clicking Add to “add and draw” and the alt key to “add and rename”.

Install the ROIManagerMacros macro set and you will be able to add a selection by pressing the “1” key, add and name by pressing “2”, add and draw by pressing “3”, and add and advance to the next slice by pressing “4”.

Update replaces the selected ROI on the list with the current selection. This is usually a modified version of a selection from the ROI Manager list.

Delete deletes the selected ROIs from the list. Deletes all the ROIs if none are selected.

Use Rename to rename the selected ROI (requires v1.35c or later).

Open opens a ”.roi” file and adds it to the list or opens a ZIP archive (”.zip” file) and adds all the ROIs contained in it to the list. Use the Open All macro [insert link here] to add all the ”.roi” files in a folder to the list.

Save saves the selected ROI as an ”.roi” file. If none are selected, saves all the ROIs in a ZIP archive.

Measure measures all the ROIs on all the images in a stack as long as none of the ROIs are selected, and none are associated with a particular slice (all have names like “xxxx-yyyy”) or all are associated with the first slice (all have names like “0001-xxxx-yyyy”).

If this is not the case, use the More>Multi Measure function, added in ImageJ 1.38m. Multi Measure, based on a similar function in Bob Dougherty's Multi_Measure plugin, measures all the ROIs on all the images, creating a results table with either one row per image or one row per measurement.
With a stack, you will be given the option to measure all the slices if all items are associated with the first slice or all have labels in the form xxxx-yyyy. Use the Analyze/Set Measurements command to specify the measurement options.

Deselect deselects any selected items on the list. Delete, Save, Measure, Draw, Fill, Label and Combine

work with all items on the list when none are selected.

Show All causes all selections on the list to be non-destructively displayed on the current image. Click again to stop displaying the selections. Click on a label (selection number) in the image to activate the corresponding selection on the ROI Manager list.

More» displays a drop down menu with six additional commands:

- Draw draws the outline of the selected ROIs using the current foreground color and line width. Draws all the ROIs if none are selected. Click in the Image>Color>Color Picker window to set the foreground color. Use Edit>Options>Line Width to set the line width.
- Fill fills the ROI using the current foreground color .Fills all selections on the list if none are selected. Click in the Image>Color>Color Picker window to change the foreground color.
- Label labels and outlines the selected items using the current foreground color. Labels and outlines all selections on the list if none are selected. Unlike Show All, this changes the image contents.
- Combine uses the union operator on the selected items to create a composite selection. Combines all the items if none are selected.
- Split separates a composite ROI into simple ones and adds them to the ROI Manager.
- Add Particles adds objects segmented by the particle analyzer to the ROI Manager. Requires that "Record Starts" be checked in the Analyze>Analyze Particles dialog box. Particle analyzer objects can also be added to the ROI Manager by checking "Add to Manager" in the Analyze Particles dialog box.
- Multi Measure, (based on a similar function in Bob Dougherty's Multi_Measure plugin) measures all the ROIs on all the images, creating a results table with either one row per image or one row per measurement.
- Sort sorts the ROIs list in alphanumeric order.
- Specify lets you specify an ROI in the same way as Edit>Selection>Specify
- Remove Slice Info command removes the information in the ROI names that associates them with particular slices.
- Help opens the ROI help page (at IJ's site) in the default browser.
- Options shows a dialog to set the color used in Show All mode and to associate ROIs with slices.

Scale Bar

Draws a labeled calibration bar of the specified Width calibrated units and Height in pixels.

[insert image dialog]

Change Font Size to adjust the labels' font size.

Change Color to adjust the text color and the Background to increase the contrast, if needed.

Change Location to move the calibration bar. If there is a selection, the bar is initially drawn at the selection.

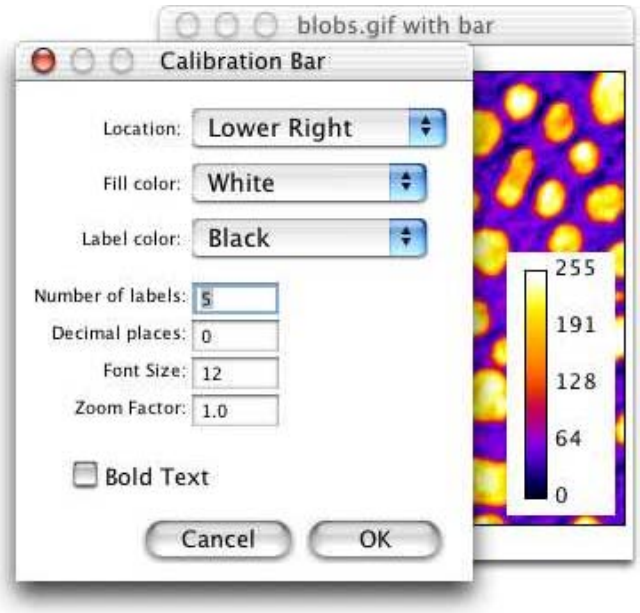
If Bold Text is checked, labels are drawn bold.

If Hide Text is checked, only the bar is drawn.

Check Serif Font to change the font accordingly.

Calibration Bar

Creates an RGB copy of the current image and displays a labeled calibration bar on it.



Change Location to move the calibration bar. If there is a selection, the bar is initially drawn at the selection.

Change Fill Color to adjust the bar's background color.

Change Label Color to adjust the text color.

Change Number of Labels to adjust the total number of values displayed.

Change Decimal Places to adjust the number of decimal places present in the labels.

Change Font Size to adjust the labels' font size.

Change Zoom Factor to scale the entire calibration bar.

If Bold Text is checked, labels are drawn bold.

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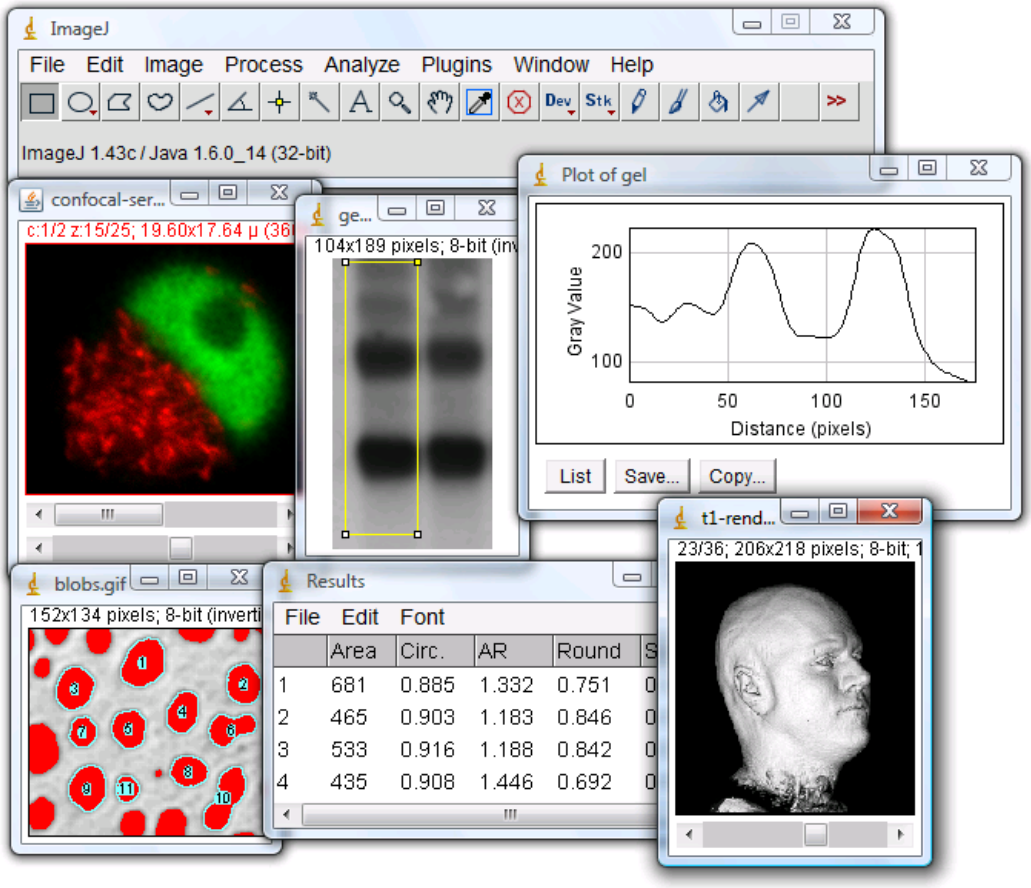
Basic Concepts

ImageJ is Free Software

ImageJ is public domain open source software. An ImageJ user has the four essential freedoms defined by the Richard Stallman in 1986:

1. The freedom to run the program, for any purpose.
2. The freedom to study how the program works, and change it to make it do what you wish.
3. The freedom to redistribute copies so you can help your neighbor.
4. The freedom to improve the program, and release your improvements to the public, so that the whole community benefits.



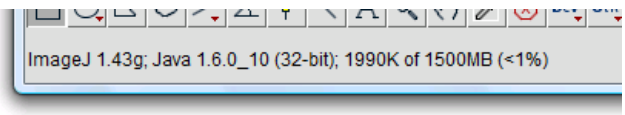
Windows

The "ImageJ" window contains a menu bar (at the top of the screen on the Mac), tool bar, status bar, and a progress bar. Images, histograms, line profile, etc. are displayed in additional windows. Measurement results are displayed in the "Results" window. Windows can be dragged around the screen and resized. Histograms and plots are ordinary image windows that can be copied to the clipboard, edited, printed and saved.

Toolbar

The toolbar contains tools for making selections, for zooming and scrolling images, and for changing the drawing color. Mouse over a tool and a description is displayed in the status bar. The tools and menus on the right side of the toolbar are created using macros defined in the file ImageJ/macros/StartupMacros.txt.

Status Bar



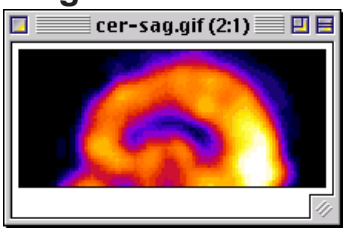
The status bar, when the cursor is over an image, displays pixel coordinates and values. After running a filter, it displays the elapsed time and processing rate in

pixels/second. Click on the status by and it will display (as shown above) the ImageJ version, the Java version, memory in use, memory available and percent memory used.

Progress Bar

The progress bar, located to the right of the status bar, shows the progress of time-consuming operations. It will not appear if the operation requires less then approximately one second.

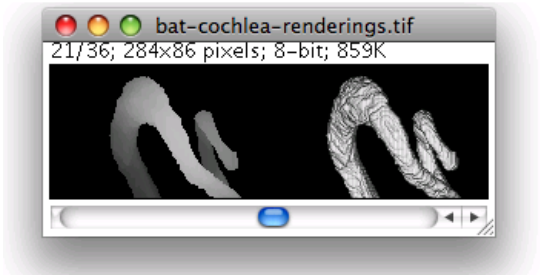
Images



ImageJ allows multiple images to be displayed on the screen at one time. The active window has its title bar highlighted. All operations will be performed on the active image. ImageJ supports 8-bit, 16-bit and 32-bit (real) grayscale images and 8-bit and 32-bit color images. 8-bit images are represented using unsigned integers in the range 0 to 255. 16-bit images use unsigned integers (0 to 65,535) and 32-bit grayscale images use floating-point numbers.

16-bit and 32-bit grayscale images are not directly displayable on computer monitors, which typically can show only 256 shades of gray. Therefore, the data are mapped to 8-bits by windowing. The window defines the range of gray values that are displayed: values below the window are made black, while values above the window are white. The window is defined by minimum and maximum values that can be modified using *Image>Adjust>Brightness/Contrast*.

Stacks



ImageJ can display multiple spatially or temporally related images in a single window. These image sets are called stacks. The images that make up a stack are called slices. All the slices in a stack must be the same size and bit depth. A scroll bar provides the ability to move through the slices. Most ImageJ filters will, as an option, process all the slices in a stack.

ImageJ opens multi-image TIFF files as a stack, and saves stacks as multi-image TIFFs. The *File>Import>Raw* command opens other multi-image, uncompressed files. *File>Import>Image Sequence* opens a folder of images as a stack. To create a new stack, simply choose *File>New>Image* and set the "Slices" field to a value greater than one. The *Image>Stacks* submenu contains commands for common stack operations.

Selections

Selections are user defined areas or lines within an image. Only one selection can be active at a time. Area selections are created using the rectangular, elliptical, polygonal and freehand selection tools. Area selections can be measured (*Analyze>Measure*), filtered, filled (*Edit>Fill*) or drawn (*Edit>Draw*). Line selections are created using the straight, segmented and freehand line selection tools. Use *Edit>Draw* to draw the line in the current color. The length of line selections can be measured using *Analyze>Measure*.

Selections can be moved by clicking and dragging. The status bar displays the coordinates of the upper left corner of the selection (or the bounding rectangle for

non-rectangular selections) as it is being moved. Notice that the cursor changes to an arrow when it is within the selection. To move the contents of a rectangular selection, rather than the selection itself, *Edit>Copy* (c), *Edit>Paste* (v), and then click within the selection and drag. Use the arrow keys to nudge selections one pixel at a time in any direction.

Rectangular and elliptical selections can be resized. As the selection is resized, the width and height are displayed in the status bar. Use the arrow keys with the alt key down to stretch rectangular or elliptical selections one pixel at a time.

To delete a selection, choose any of the selection tools and click outside the selection, or use *Edit>Selection>Select None* (shift-a). Use *Edit>Selection>Restore Selection* (shift-e) to restore a selection back after having deleted it.

A selection can be transferred from one image window to another by activating the destination window and using *Edit>Selection>Restore Selection*. Selections can be saved to disk using *File>Save As>Selection* and restored using *File>Open*. Use the ROI Manager to work with multiple selections.

File Formats

The *File>Open* command opens TIFF, GIF, JPEG, PNG, DICOM, BMP, PGM and FITS images. It also opens lookup tables and selections. In addition, the *File>Import* submenu provides access to plugins for reading "raw" files, images in ASCII format, and for loading images over the network using a URL. To import a raw file, you must know certain information about the layout, including the image size and the offset to the image data. Files can be saved in TIFF, GIF, JPEG, PNG, PGM, FITS, tab-delimited text, and raw formats. Add support for additional formats by downloading or writing plugins. The Bio-Formats plugin from the University of Wisconsin opens 69 different life sciences image file formats.

Plugins

ImageJ's functionality can be expanded through the use of plugins written in Java. Plugins can add support for new file formats or they can filter or analyze images. Plugins located in ImageJ's "plugins" folder are automatically installed in the Plugins menu or they can be installed in other menus using *Plugins/Hot Keys/Install Plugin*. Plugins can be created or modified using *Plugins/Edit*. More than 150 example plugins are available for download from the ImageJ website.

Lookup Tables

Grayscale images are displayed using a color lookup table which describes the color to be used for each of 256 possible displayed pixel values. Select alternative color palettes from the *Image/Lookup Tables* submenu. Use *Image/Adjust/Brightness/Contrast* to enhance images by dynamically changing the lookup table mapping and *Analyze/ShowLUT* to display the lookup table of the active image.