

## ImageJ: Simple Tasks (updated June 2009)

### Installing ImageJ

Go to the ImageJ download site at

<http://rsb.info.nih.gov/ij/download.html>

Follow the instructions for your operating system. All of the instructions on this sheet are written for version 1.41 of ImageJ or later.

### Installing plugins

Plugins are additional programs that increase the functionality of ImageJ. Installing them always involves these two steps:

- Download the plugin file to your computer
- Move the plugin file (\*.jar, \*.class, or \*.java) to the Plugins folder inside your main ImageJ folder

Occasionally a plugin will need to be unzipped before it's moved to the Plugins folder. If your plugin is a \*.java file, it must be compiled before you can use it. After you move the downloaded \*.java file to the Plugins folder, start ImageJ. Go to Plugins → Compile and Run... , and select the \*.java file. You only need to do this the first time you use the plugin; after you compile, the plugin will show up in the Plugins menu every time you use ImageJ.

### Opening files

**Method 1: File → Open → choose file**

Note: In addition to .tif and .jpg files, ImageJ will open the following proprietary formats used in the CDB Microscopy Core:

- \*.ipl (IPLab, from spinning disk confocal before April 2009)
- \*.lsm (Zeiss LSM, from Zeiss confocal)
- \*.dv (Deltavision, from deconvolution microscope)

HOWEVER, ImageJ may not always understand the color or dimensional information stored with these files, so you may have to manually colorize or separate channels.

**Method 2:** The better way to open files, especially those from the Deltavision and Leica microscopes, is to use a plugin called the **LOCI Bio-Formats Importer**. This utility does a better job of understanding how image data are organized by the different software packages.

Go to this web site:

<http://www.loci.wisc.edu/ome/formats-download.html>

Download the latest stable version of loci\_tools.jar and move it to the Plugins folder in your main ImageJ folder.

Whenever you want to open a file, go to **Plugins → LOCI → Bio-Formats Importer**. Using LOCI, you will have a number of options for displaying your images.

- “Standard ImageJ” mode will display all the images in a single stack, with one slider to go through the slices. Select “Split Channels” if you choose this option.
- “Hyperstack” mode will display the images in a stack with two sliders, one for channel and one for z (if your data are a multi-channel z-stack).

**Leica confocal files:** choose the \*.lei file that is saved along with the images. A window will appear showing how the images are organized. Check the box(es) next to the image(s) or stack(s) you want to open. Another window will appear giving you options for displaying the image. Check the desired boxes and click “Ok”.

**Deltavision files:** select the single \*.dv file you want to open. Do not choose the options to colorize or merge channels, as it’s likely the wrong colors will be assigned.

**Adjusting contrast** (to make background look darker and cells look brighter, for example)

**Image → Adjust → Brightness/Contrast...** Move the top slider (“Minimum”) to the right to make the dark parts look darker. Move the next slider (“Maximum”) to the left to make the bright parts look brighter. Don’t click the “Apply” button unless you want to change the actual pixel values – it will overwrite the original data!!

**Assigning a color LUT to a black and white image**

**Image → Lookup Tables** → choose color scheme from the list. If you want save the file so that Adobe Photoshop can see the color information, you must first change the image to 8-bit or RGB format if you haven’t done so already (see below).

**Changing the data depth of an image**

To change to 8-bit: **Image → Type → 8-bit**

To change to 24-bit RGB: **Image → Type → RGB Color**

**Separating channels in a 24-bit RGB image**

**Image → Color → Split Channels**

This will return three 8-bit images representing the red, green, and blue components of the original image.

## Merging up to four separate 8- or 16-bit channels into a single RGB+gray image

**Image → Color → Merge Channels...** → select the desired channels for red, green, blue, and gray. Important: you should start with monochrome (not RGB) images and set your brightness & contrast before merging to get the desired result. When merging fluorescence images with a gray brightfield image, you may want to darken the gray image first.

## Cropping an image

Choose the rectangular selection tool, draw a box around the region you want to keep, and go to **Image → Crop**.

## Separating a stack into individual slices

**Image → Stacks → Stack to Images**

## Picking out a particular slice or range of slices from a stack

To do this you need to download a plugin called “Substack Maker” that is not included in the base version of ImageJ (it is included in the MBF ImageJ bundle).

Go to the following web page and follow the download instructions:

<http://rsb.info.nih.gov/ij/plugins/substack-maker.html>

## Removing noise

**Process → Smooth** replaces each pixel with the mean of it and its immediate neighbors.

**Process → Noise → Despeckle** replaces each pixel with the median of it and its immediate neighbors. This is the most common way to remove “salt-and-pepper” noise without blurring the data too much.

**Process → Filters → Mean...** or **Process → Filters → Median...** are similar to Smooth and Despeckle, but they allow you to set the number of pixels used in the average/median calculation. More pixels means more smoothing but also more blurring.

## Handling Hyperstacks

When you open a multi-channel z-stack \*.ism file, ImageJ automatically displays it as a “hyperstack”. This is a stack with two sliders, one for viewing the different colors, one for scrolling through the z slices. There are several ways to handle a hyperstack, depending on what you need.

- You can separate into individual images as described above. This will give you many open windows, which can be hard to organize.
- You can merge the channels: **Image → Hyperstacks → Channels Tool... → More → Make Composite**. This creates an RGB merged stack. To get rid of the extra channel slider, **Image → Hyperstacks → Channels Tool... → More → Convert to RGB**. Now you'll have a stack with just one slider, to scroll through Z.

- You can also split the hyperstack into separate stacks, one for each channel: Select **Image → Hyperstacks → Channels Tool → More → Split Channels**

### **Creating a maximum projection from a z-stack**

**Image → Stacks → Z Project...**

For Projection Type select “Max Intensity” (or try “Average Intensity” or “Sum slices”, to see how they look – sometimes they look nicer than a maximum projection)

### **Setting the correct pixel size (scaling) for an image**

ImageJ should be able to read pixel size information from the file header if you opened a .lsm, .dv, or .ipl file. However, if your image has incorrect or missing scaling information, you can set it manually using ImageJ.

Go to **Analyze → Set Scale...**

For Distance in Pixels type in 1

For Known Distance type in the length of one pixel. If you don't know this, you need to measure it with a stage micrometer on your microscope.

If you check the box next to “Global”, ImageJ will apply this scaling information to all open images and to any images you open during this session.

### **Adding a scale bar**

**Analyze → Tools → Scale Bar...**

Select the desired location and set your desired length and thickness. If you only want the bar (no text) check the box next to “Hide Text”. If you have a stack and want the bar on all slices, check the box next to “Label all Slices”.

### **Adding a time stamp**

**Plugins → Stacks → Time Stamper**

Fill in the blanks to set your desired time interval and units. The location must be specified in pixels, so you may need to experiment a bit to get it just right. Low numbers in x and y are near the top left corner; high numbers in x and y are near the bottom right corner.

### **Measurements – single image**

First, go to **Analyze → Set Measurements...** and check the boxes next to the quantities you want measured. Use a drawing tool to draw an ROI (Region Of Interest) or line segment on the image. Now go to **Analyze → Measure** (or type **M**) to perform the measurement. You can save the contents of the Results window as a text file to be opened later.

### **Measurements – stack of images**

First, go to **Analyze → Set Measurements...** and check the boxes next to the quantities you want measured. Use a drawing tool to draw an ROI (Region Of Interest) or line segment on the image. Examine all images in

the stack to make sure the object you want to measure stays within the ROI.

Next, save the ROI information in the ROI Manager using one of the following methods:

**Edit → Selection → Add to Manager**

Type ⌘T

**Analyze → Tools → ROI Manager; click the Add [t] button**

In the ROI Manager click the **More >>** button to see more options. From the list select Multi Measure and check both options in the small window that pops up. The Results window will contain measurements from the same region, performed on each slice.