

Using Hardin Lab QuickTime and 4d Plugins – Fall, 2007

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1) Accessing the plugins:

The plugins can be accessed in one of two basic ways:

(a) Through the ImageJ plugins menu:

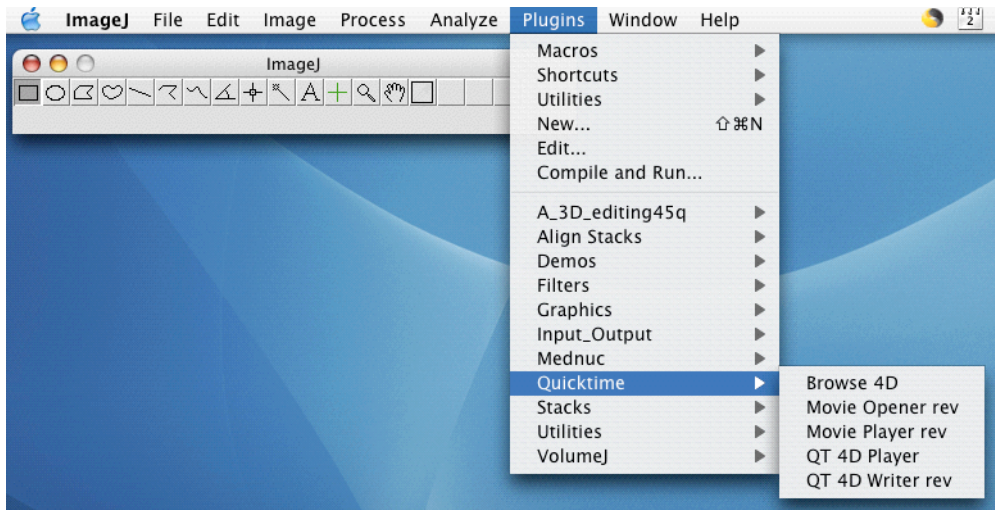


Figure 1 – Selecting QuickTime plugins using the ImageJ Plugins menu

If the plugins are installed manually, they can be accessed via the "Plugins" menu in ImageJ [note: most screen shots show ImageJ running under Mac OS X, but the interface is essentially identical under Windows, and, with limitations, under Mac OS 9].

(b) Through the 4d toolset:

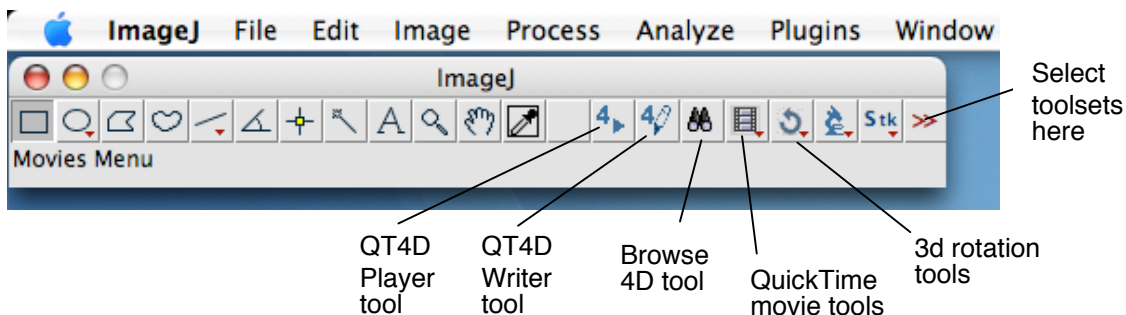


Figure 2 – Selecting QuickTime plugins using the ImageJ Plugins menu

Installing the "4d Tools.txt" toolset in the macros->toolsets directory within the main ImageJ directory allows access to a toolset that can be selected using the >> button at the right of the ImageJ main window. Selecting "4d Tools" loads the buttons shown in Figure 2. Note: this feature requires ImageJ 1.37t or later.

(2) QT4D Player

(a) Opening movies: When the plugin is invoked, you will be asked to open a movie. The plugin looks for a simple text file that contains information about the movie that has the same root name

as the movie itself (e.g., if the movie is called "foo.mov", then it will look for a file called "foo.mov.info" or "foo.mov.txt"). If it cannot find one, then it will ask you for the number of focal planes in the movie.

Advanced tip: The data structure of the info file is very simple. It is a text file with the following lines, separated by carriage returns:

Line 1: A text string with basic information about the movie

Line 2: The number of focal planes in the movie as an integer

Line 3: The spacing (assumed to be in microns) between focal planes

Line 4: The time interval between acquisition of Z series

Line 5: Blank [this deals with a quirk in the way Java under Mac OS 9 handled text files]

You can easily recreate an info file using your favorite vanilla text editor using this basic structure.

(b) Navigating through a movie: You can navigate through movies in several ways:

(i) Arrow keys: The arrows keys on the keyboard can be used to navigate up and down and backwards and forwards through a movie. Frames in the movie will be skipped based on the information in the associated info file, or based on the number of focal planes you entered when asked.

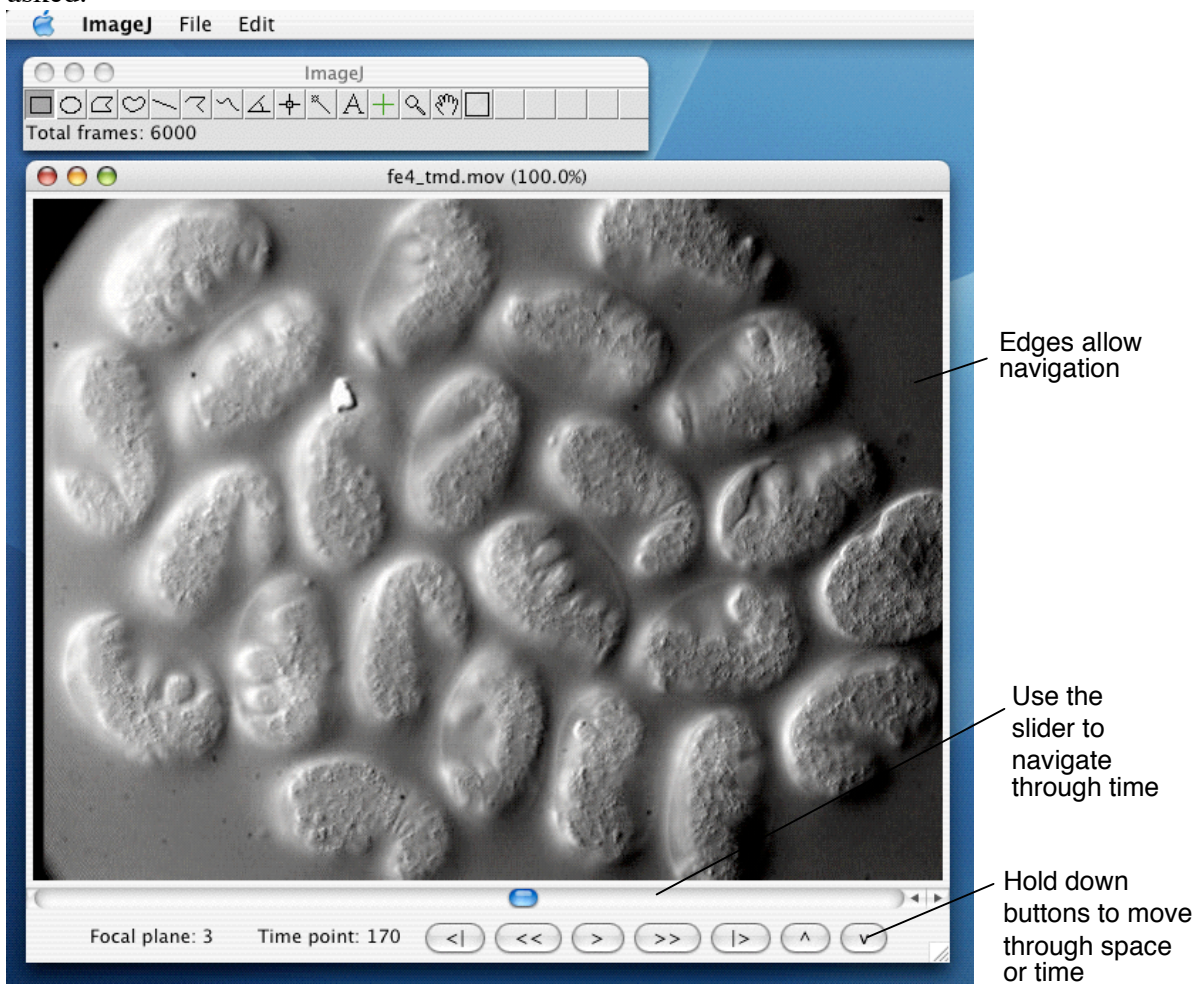


Figure 3 – The main QT4D Player window

ii) Buttons: Holding down the mouse on the stepper buttons (except under Mac OS 9) allows repeated steps forward, back, up, or down.

(iii) Mouse-based 4d navigation: At the edges of the movie window, the cursor will change to a forward/back symbol or up/down symbol. In these regions of the movie, holding down the mouse will likewise step in the appropriate directions (left edge = back; right edge = forward; top edge = up; bottom edge = down).

Figure 4 – Contextual menu options



(c) Other features of the main QT4D player window: There are several convenient – but non-obvious – features in the main movie player window. Right-clicking (Windows)/Clicking with the Control key pressed (Mac OS X): In addition, control-clicking (or right clicking) on the movie window brings up several options, including copying the data to standard ImageJ objects in various ways, getting information about the

movie sequences from its info file, etc. If the movie has been zoomed, the "Reset Window to Original Size" command allows reversion to the original movie/window dimensions.

(i) "Get info...": When this option is selected, information about the movie will be displayed:

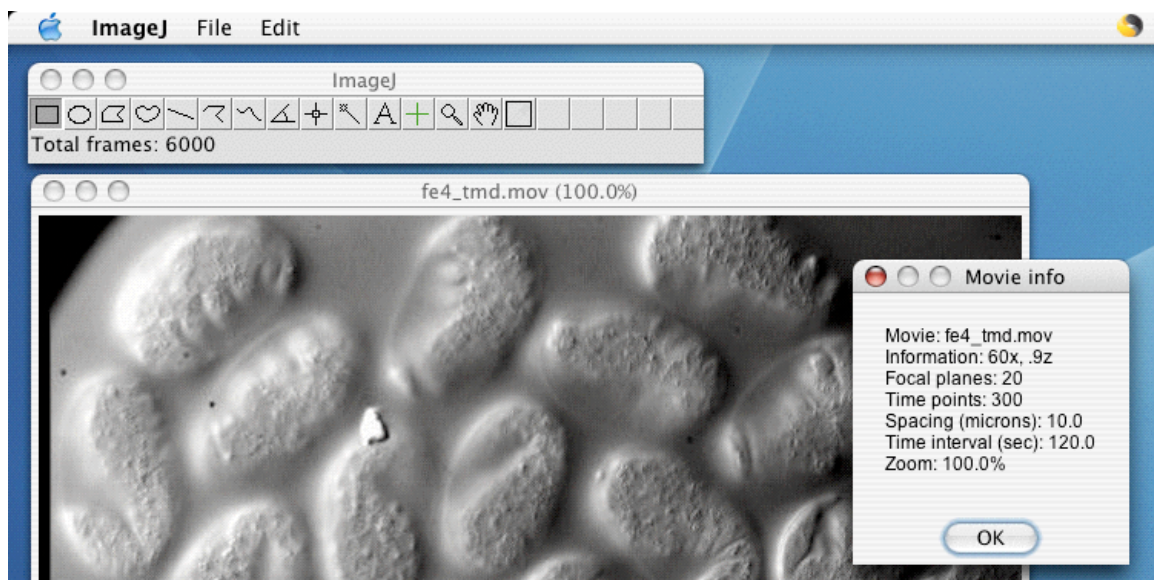


Figure 5 – The movie information window

(ii) Show/Hide Controls: When this option is selected, the visibility of the buttons at the bottom of the movie window will be toggled.

(iii) Apply Filter... : Experimental support is provided for applying temporary filters to a movie. This can be useful for applying brightness/contrast changes, sharpening filters, etc. This feature uses QuickTime filtering to add a temporary, non-destructive filter to a movie.

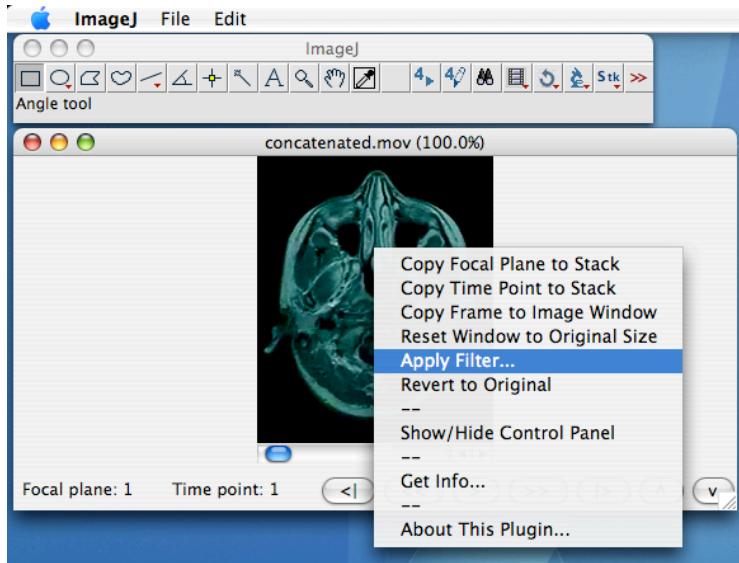


Figure 6 – Selecting the "Apply filter" option

When this option is selected, a list of filters provided by QuickTime will appear:

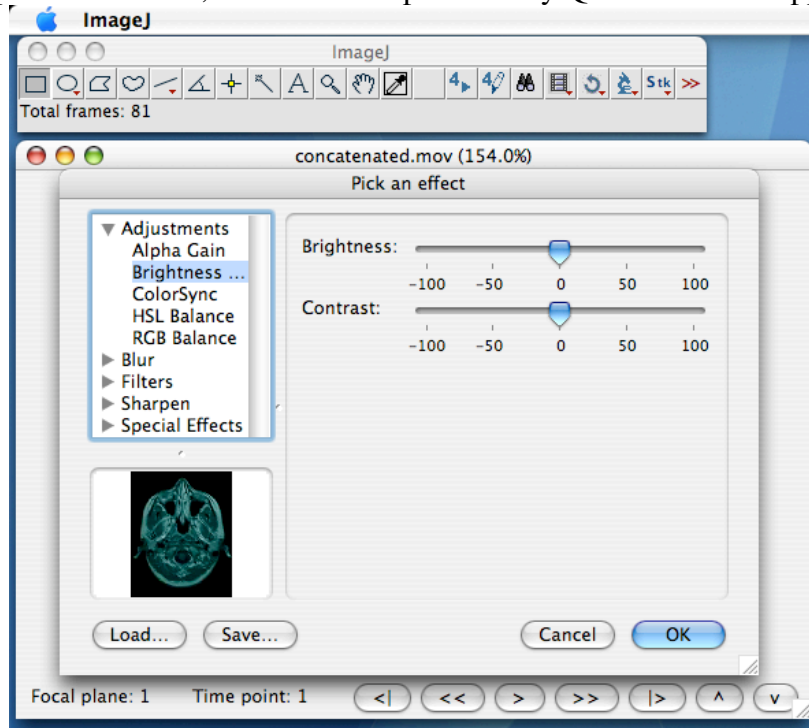


Figure 7 – Selecting a QuickTime filter

The contextual menu contains a "Revert to original..." option to remove the filter.

(4) **QT4D Writer:** This plugin is used to convert folders of single images arranged as captured in a 4d dataset into a QuickTime movie.

Running the plugin causes a file dialog to appear asking for the location of the raw files. Select the folder, and then one of the images:

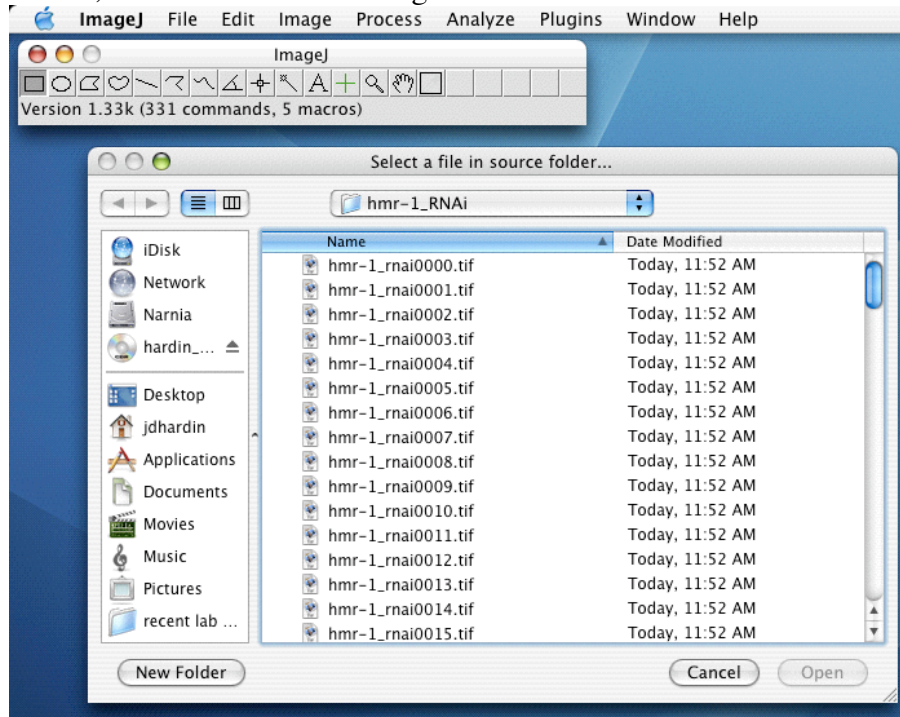


Figure 8 – Specifying a directory with images for QT4D Writer

Next, if there is no information file, you will be asked to supply information regarding the number of focal planes (important; 33 in this case) and other information in a series of dialogs:

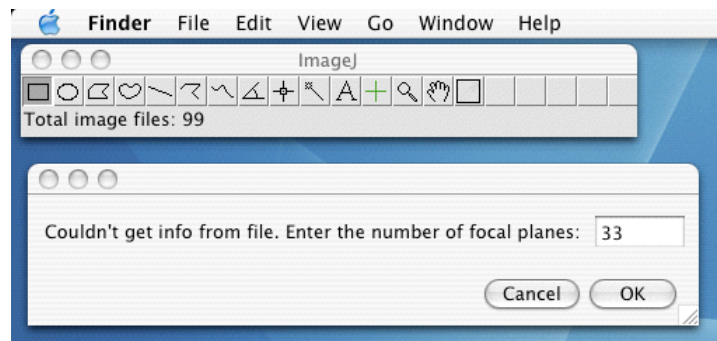


Figure 9 – Specifying the number of focal planes

Next, you will be presented with a standard compression dialog box [Note: due to a bug under OS X/1.4.1+ VM, the frame rate options and key frames will be in a separate dialog. Under other operating systems, they will appear as shown]:

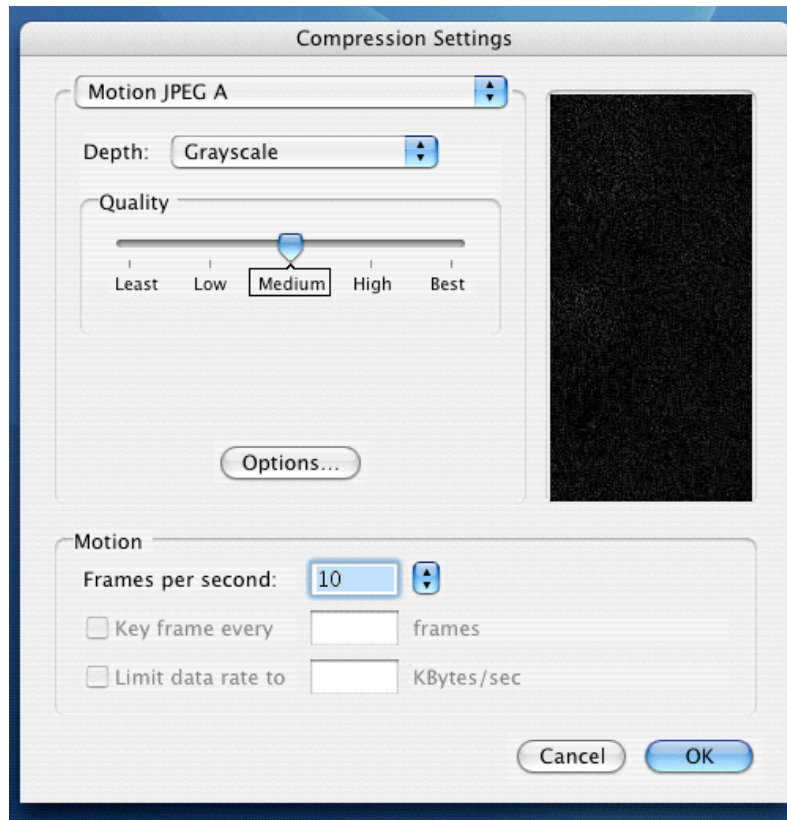


Figure 10 – Selecting compression parameters for the movie

Finally, you will be asked where to save the movie. After clicking "OK" to proceed, a progress indicator will appear during compression, which also allows cancellation of the compression:

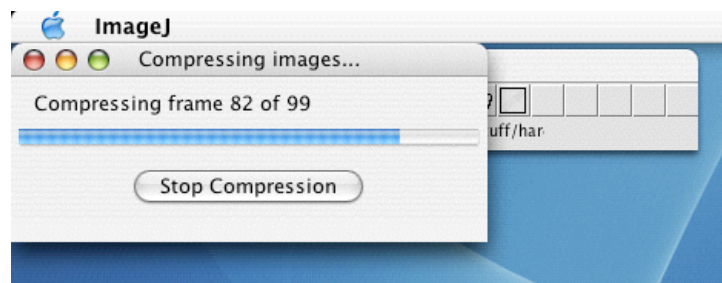
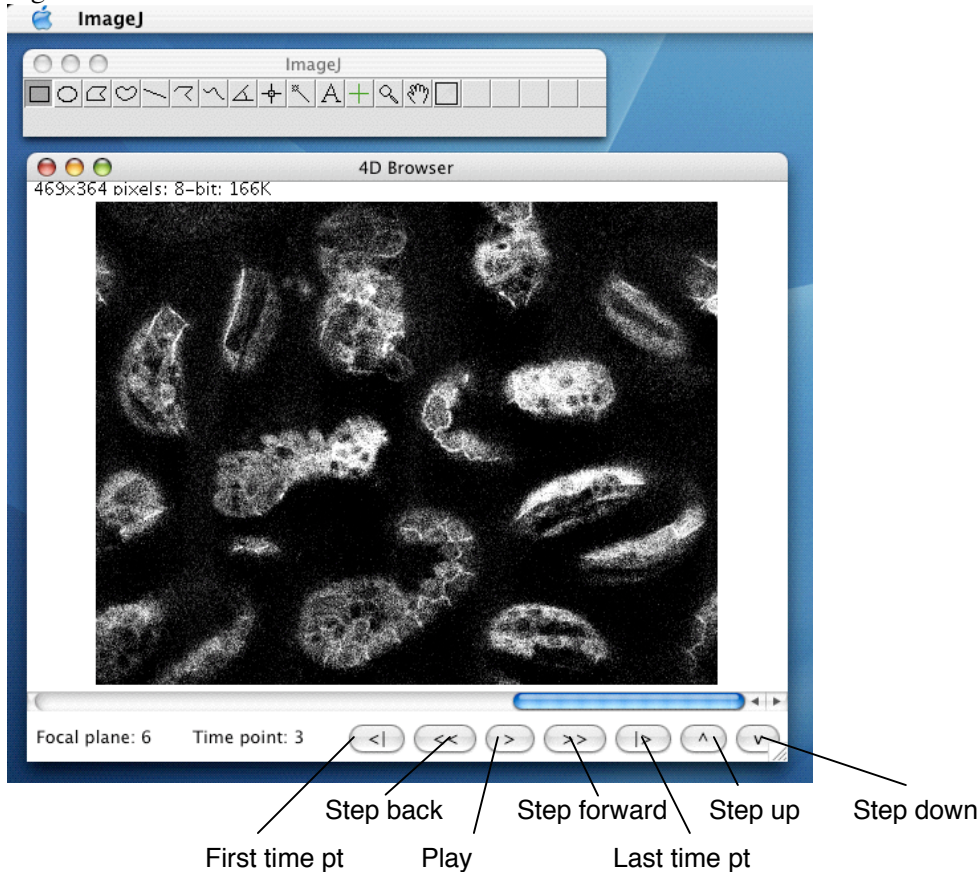


Figure 11 – The progress dialog during compression

(4) **Browse4D**: This plugin is used for browsing through a folder of single images that comprise a 4d dataset. The window behaves like a standard ImageJ image window in terms of edit commands, etc., but has an interface that is essentially identical to that of the QT4D Player plugin. It has an advantage over other plugins, such as the Image5D plugin, in that it does not load image data into RAM. This makes it less intensive in terms of computing resources, but slower for moving rapidly through a large number of large images.

Figure 12 –The Browse4D window



(5) **Acquire 4D:** This plugin is used in the Hardin Lab to acquire Nomarski 4D datasets using video cameras and Scion AG-5 framegrabbers. Although this digitization technology is old, the rest of the code could serve as a basis for using more modern Firewire cameras for data acquisition.

Invoking the plugin brings up a dialog in which parameters for the movie are entered:

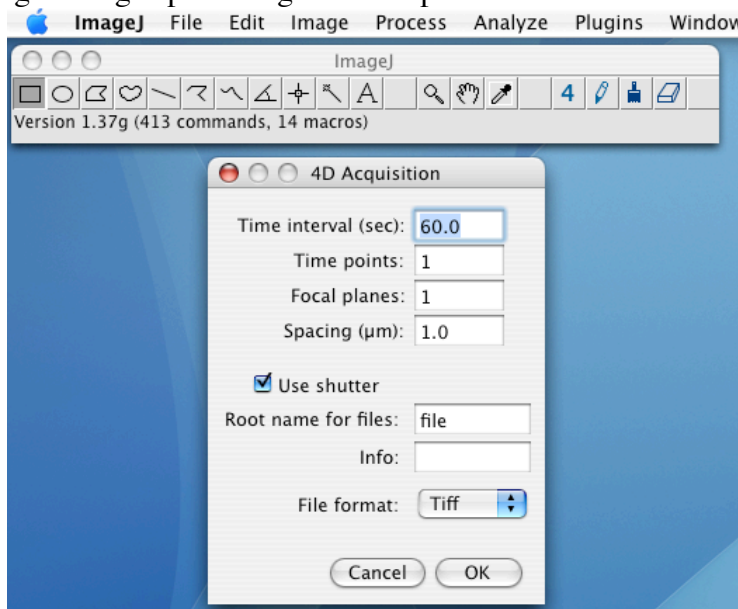


Figure 13 – Specifying parameters for a 4d movie

The "use shutter" option allows use of a shutter during acquisition. The root name for files is the basic name for files, to which numbers are appended. If the root name is "foo", files will be named "foo0001.tif", "foo0002.tif", etc.

Once parameters are entered, a confirmation dialog will appear:

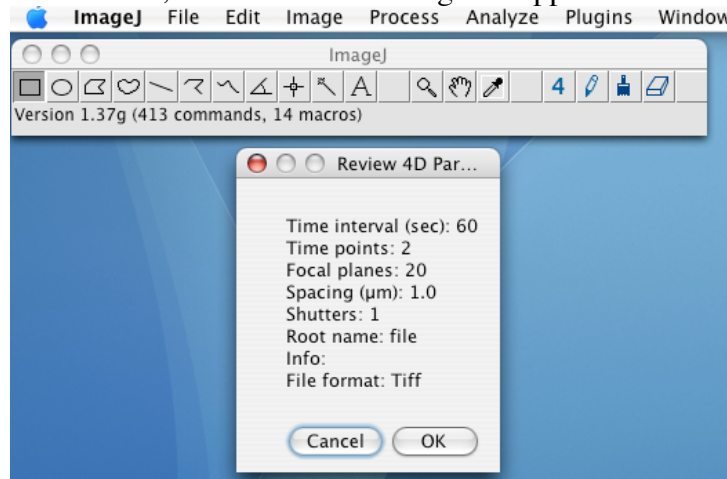


Figure 14 –Reviewing parameters

If you click "OK", an ImageJ window will open, the shutter will open (if this option was selected), and a standard "Save As..." dialog will appear asking where you wish to save the image files for the movie. Once you specify where files will be saved, the first Z series will be acquired, the shutter will close, and the standard control window will appear:

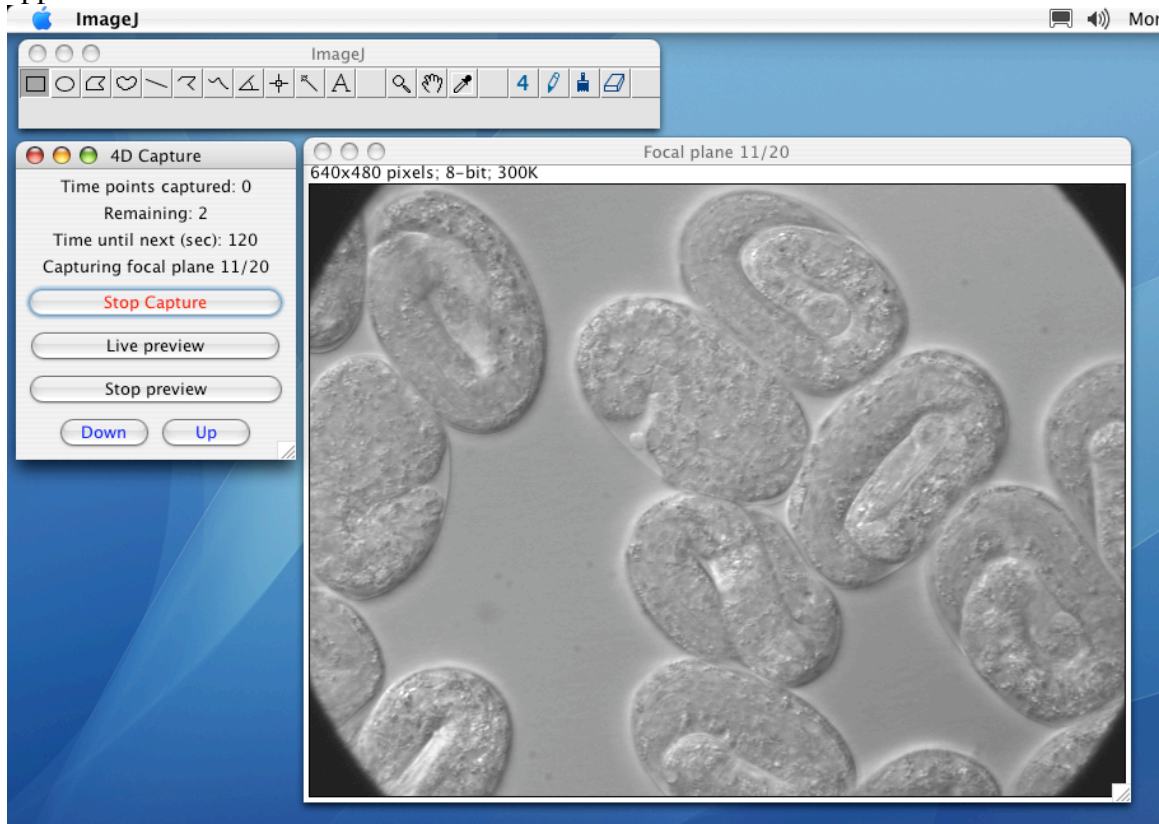


Figure 15 – The main 4D Capture windows

The "Stop capture" button does what it says. Live preview and cessation of live previewing are accomplished using the "Live preview" and "Stop preview" buttons. Clicking the "Live preview" button brings up a modified Scion live preview window:

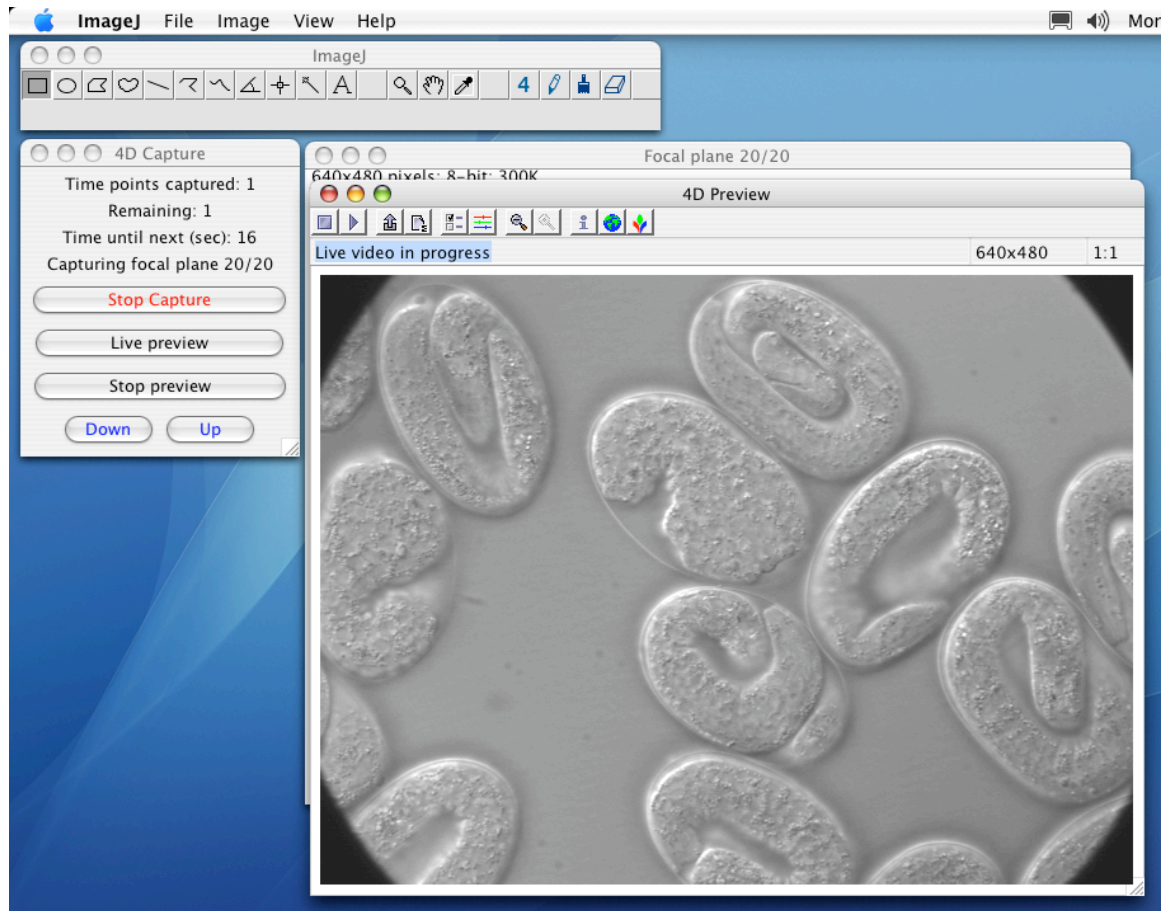


Figure 16 –The Live Preview window

Clicking the "Stop preview" button will stop the preview and hide the Preview window. Clicking its close box hides the window as well. A few seconds before a Z-series is acquired, if a Live Preview window is open, acquisition will stop to give processor time to the Z series being acquired. Once the Z series is acquired, live previewing resumes.

The "Down" and "Up" buttons will adjust the focal plane during acquisition.

Once a 4d dataset has been acquired, it can be viewed as raw images using the Browse4D plugin, compiled using the QT4D Writer plugin, and viewed using the QT4D Player plugin.

Good luck and happy 4d navigating!