NIH Image 1.44

Introduction

NIH *Image* is a public domain image processing and analysis program for the Macintosh. It can acquire, display, edit, enhance, analyze, print and animate images. It reads and writes TIFF, PICT, PICS and MacPaint files, providing compatibility with many other applications, including programs for scanning, processing, editing, publishing and analyzing images. It supports many standard image processing functions, including histogram equalization, contrast enhancement, density profiling,

smoothing, sharpening, edge detection, median filtering, and spatial convolution with user defined kernels up to 63x63. *Image* also incorporates a Pascal-like macro programming language, providing the ability to automate complex, and frequently repetitive, processing tasks.

Image can be used to measure area, average gray value, center, and angle of orientation of a user defined regions of interest. It also performs automated particle analysis and can be used to

measure path lengths and angles. Measurement results can be printed, exported to text files, or copied to the Clipboard.

Spatial calibration is supported to provide real world area and length measurements. Density calibration can be done against radiation or optical density standards using any user specified units. The user can select from any of eight different curve fitting methods for generating calibration curves.

Image provides MacPaint-like

editing of color and grayscale images, including the ability to draw lines, rectangles, ovals and text. It can flip, rotate, invert and scale selections. It supports multiple windows and 8 levels of magnification. All editing, filtering, and measurement functions operate at any level of magnification and are undoable. Digital halftoning is used to print images on PostScript printers and Floyd-Steinberg dithering for printing on non-PostScript printers.

Image supports the Data

Translation QuickCapture card for digitizing single images or movie sequences using a TV camera. Acquired images can be shading corrected and frame averaged.

Image is written using Think Pascal from Symantec Corporation, and the complete source code is freely available.

System Requirements

Image requires a Macintosh with at least 4MB of memory, but 8MB or more are recommended for working with 3D images or animation sequences. It requires a monitor with the ability to display 256 colors or shades of gray. It normally requires a floating-point coprocessor(FPU) or the PseudoFPU Init, which can emulate a missing coprocessor, but a special version is available

that does not require an FPU. *Image* directly supports, or is compatible with, large monitors, flatbed scanners, film recorders, graphics tablets, PostScript laser printers, photo typesetters and color printers.

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Menus

File Menu

New

Creates a new image window which is filled with the current background color. The default size is 600 pixels wide by 512 pixels high, but this can be changed in the Preferences dialog box. The new window inherits its gray map (or color look-up table), as well as any spatial or density calibration, from the currently active image window.

Open…

Use the Open command to load and display images or to load color look-up tables(LUT). *Image* currently supports four image file formats(TIFF, PICT, PICS and MacPaint) and four look-up table formats.

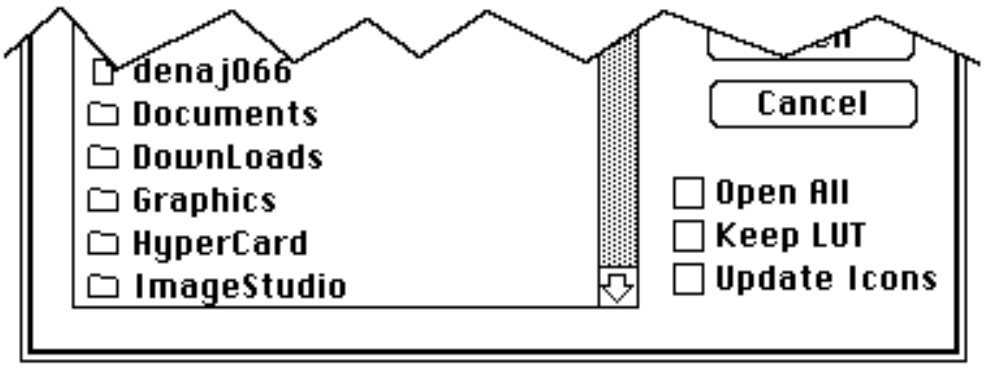
1) 8-bit grayscale and color TIFF files created by *Image* and as well as many other programs. Image will open(as a stack) a TIFF file containing multiple images, but the images must all have the same width and height. *Image* is unable to open bitmap(1-bit), 24-bit color, or compressed TIFF files. TIFF files that were created on other systems, such as an IBM-PC, usually need to be opened using the Import command.

2) PICT files created by *Image* and numerious other Macintosh programs. Check *Keep LUT* in the Open dialog box(shown below) if you want to display the image using the current palette, instead of the one contained in the PICT file.

3) MacPaint documents. *Image* will trim white space from the right edge and bottom of MacPaint documents to reduce memory requirements. You will want to set *Undo Buffer Size* in Preferences to 405K if you regularly open large MacPaint drawings, since this is the memory required for a full size(576 x 720) MacPaint document.

4) PICS files created by *Image* and by many Macintosh animation programs.

5) Look-up tables created by *Image* and by several other Macintosh programs, including PixelPaint, Canvas, UltraPaint, and the Klutz DA. PixelPaint, Canvas and UltraPaint have palette editors that can be used to create color tables for use by *Image* . The public domain PalEdit program from NCSA may also be used to create color tables that can be opened by *Image*. The Klutz DA can be used to “capture”palettes used by other programs.



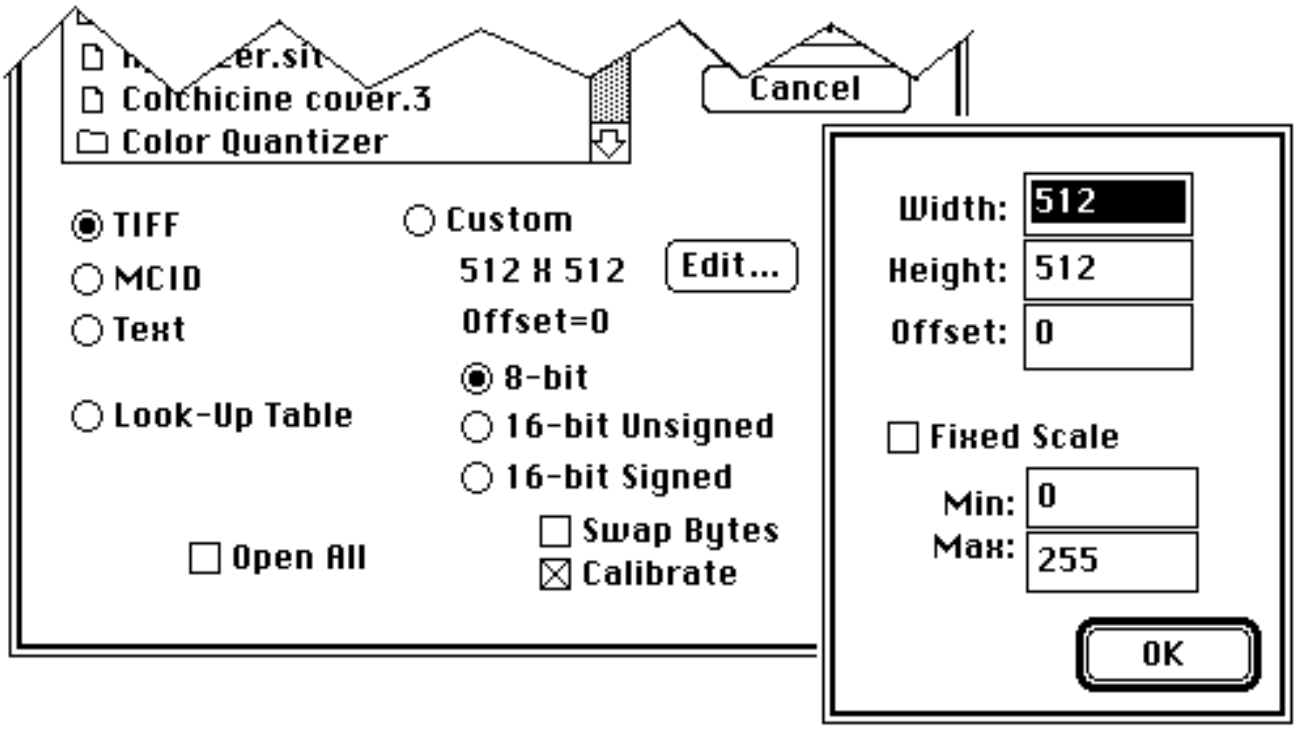
If you check *Open All*, select an image, and then click on the Open button, *Image* will open all the images in that folder, not just the one selected.

Check *Keep LUT* if you want the image to be displayed using the current video look-up table, rather than the one saved with the image. This feature can be used to combine PICT files with different palettes into a montage. When the PICT file is opened, and the image and screen LUTs are different, the pixel values will be remapped to make the colors consistent.

The *Update Icons* check box is used for updating documents created by older versions of Image. No files are opened if this option is selected. Instead, all files in the currently selected folder with a creator type of 'IMAG' well have their creator type changed to 'Imag'. 'IMAG' is the creator type used by versions of image earlier than 1.41. Files with the new 'Imag' creator type have icons with a white background and launch the latest version of Image when you double-clicking on them.

Import…

Use the Import command to read image files created by non-Macintosh based systems, to display spreadsheet data(text) as an image, or to import raw color look-up tables. It presents a dialog box that allows you to select the type of file to be imported.



Select *TIFF* to open 4-bit and 8-bit grayscale TIFF files imported from other systems, such as an IBM PC.

Select *MCID* to opens files compatible with the IBM-PC based MCID image analysis system from Imaging Research. The MCID format, which is very simple, is described in Appendix E.

Select *Text* to import a two-dimensional array of numbers stored in tab-delimeted text format, for example, a spreadsheet saved as “Text Only”. Values do not have to be in the range 0-255, and may be in decimal or scientific format. By reading in the text file twice, image is able to determine the number of rows and columns, and to scale to 8-bits(1-254). Blank cells are assumed to have a value of zero. If *Fixed Scale* is checked then automatic scaling is disabled, values are scaled to the range *Min-Max,* values less than *Min* are set to *Min,* and values greater

than *Max* are set to *Max*.

Select *Custom* to open other types of 8-bit and 16-bit binary images. *Width* is the width of the image in pixels and *Height* is the number of lines in the image. The maximum value that can be entered for *Width* is 4096. *Offset* specifies the number of bytes *Image* will skip before it starts reading the pixel data. For example, use an offset of 512 to skip over a 512 byte file header.

Image computes the minimum and maximum pixel values of 16-bit images and uses this information to linearly scale to 8-bits(1-254). Check *Swap Bytes* if you are importing 16-bit images from "little-endian" systems, such as the IBM-PC, PDP-11 or VAX. If *Calibrate* is checked *Image* automatically sets up a linear density calibration function to provide an approximation of the original 16-bit pixel values. If *Fixed Scale* is checked then automatic scaling is disabled and values are scaled to the range *Min-Max*. As an example, to import MRI scans from a GE SIGNA scanner, set *width* and *height* to 256, select *16-bit Signed*, and set *Offset* to 14336.

Image expects imported look-up tables to be 768 byte binary files consisting of 256 consecutive red values, 256 consecutive green values, and 256 consecutive blue values. The 768 color values should be in the range 0-255.

Check *Open All* to import all of the images in a folder. Images must all have the same width, height, etc. *Open All* does not work with imported text files and palettes.

Close

Closes the currently active image, Plot, or Histogram window, i.e., the one with the highlighted title bar. Hold down the Option key to close all currently open image windows. Typing Option-Command-W or Option-Clicking in the close box of an image window will also close all the image windows. When closing many windows, pressing Command-Period will bypass intermediate screen updates, causing the windows to close quicker.

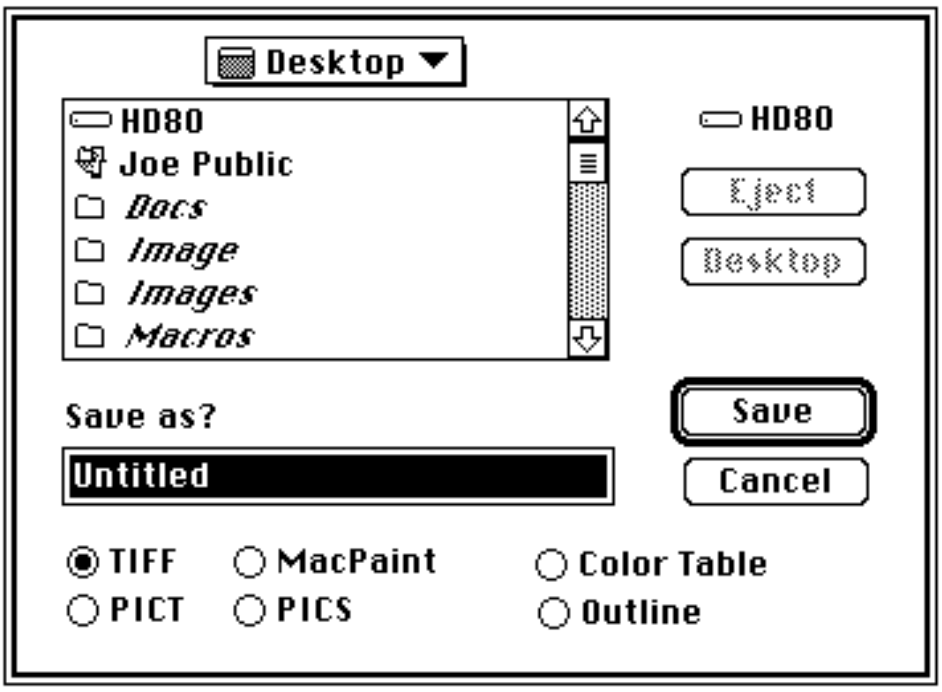
Save

Resaves the currently active image to the disk. In the case of a window that was newly created using New, Duplicate or Start Capturing, you will be prompted for a name. Files are resaved in their original format. In other words, TIFF files are resaved in TIFF format, and PICT files are resaved in PICT format.

Hold down the Option key(notice how Save changes to Save All) to save all currently open image windows.

Save As…

Allows you to saves the currently active image(or rectangular selection) in one of three formats, or to save the current pseudocolor palette.



TIFF

Saves the currently active image or stack to a file in uncompressed 8-bit TIFF format. You will be asked to enter a name for the new file. The menu command changes to *Save Selection As* if a rectangular selection is active, allowing you to save a subsection of the image. Note that the ability to save selections does not work for stacks, but there are macros available to get around this limitation. TIFF is the default file format in *Image,* and is usually the best format for saving digitized or scanned images. Because TIFF files are not compressed, they usually Open and Save faster than PICT files.

PICT

Saves the currently active image to a PICT file. PICT files can be exported to many other Mac programs, including PixelPaint, Digital Darkroom, SuperPaint, and MacDraw. Because they are compressed, PICT files also have the advantage of being more compact if the image contains large homogeneous regions, which is typical of computer generated images. Unfortunately, the run length compression scheme used with PICT files is ineffective with most scanned images. Changes to *Save Selection As* if a rectangular selection is active.
Warning: Don't save pseudocolored images in PICT format if you plan to make future gray value measurements, since pixel values may get altered.

MacPaint
Saves the currently active image as a MacPaint document. Since MacPaint images are binary(black and white only), you will probably want use Dither or Make Binary to convert grayscale images to binary before saving them.

PICS
Saves the currently active stack to disk in PICS format, a file format used by most Mac animation programs. PICS files are compressed using the same run length encoding scheme used for PICT files. Stacks containing areas of uniform color or gray value saved

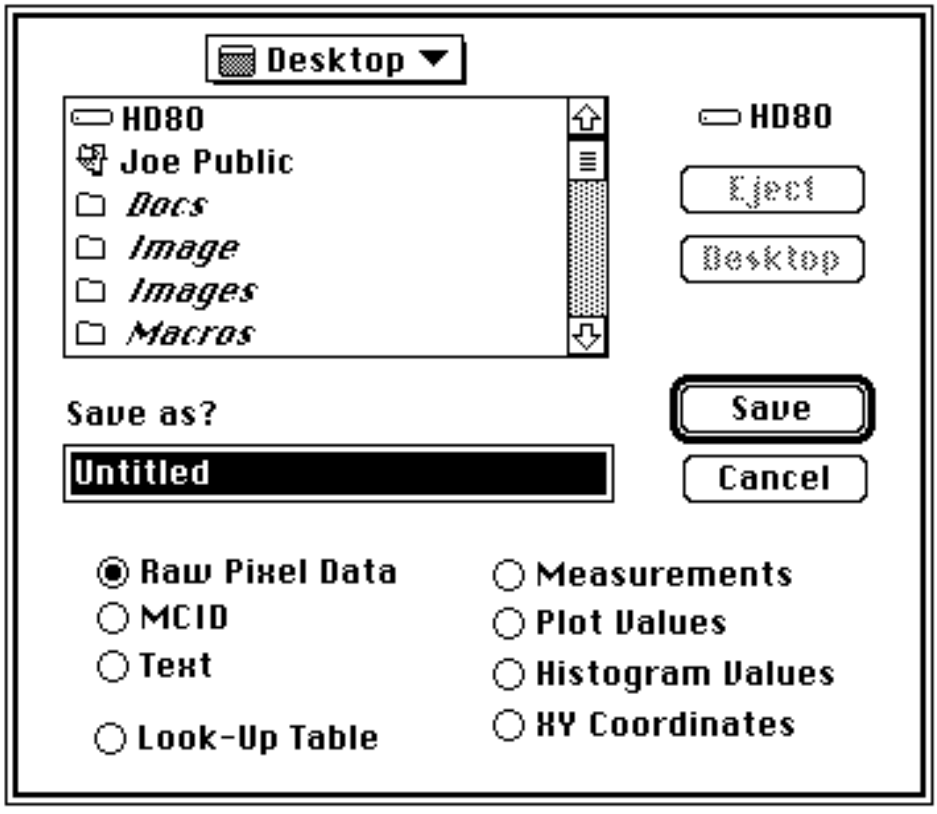
in PICS format will be smaller than stacks saved in TIFF format.

Color Table
Saves the current look-up table.

Outline
Saves the XY coordinates of the current selection outline. XY coordinates of curved line selections, and selections that have been edited, can not be saved.

Export…

Exports images, look-up tables, measurement results, density profile plot values, or histogram values to disk for use with other programs.



Raw Pixel Data
Exports the current image(or selection) as binary pixel data, in row order, one byte per pixel, and without a header.

MCID
Exports the current image(or selection) as an MCID file. MCID files have the same format as raw pixel data files, but are preceded by a four byte header which contains the width(minus one) and height(minus one), stored as two 16-bit numbers in Intel byte order.

Text
Exports the current image(or selection) as a tab-delimited, spreadsheet compatible text file.

Look-Up Table
Saves the current video Look-Up Table(LUT) as a 768 byte file consisting of 256 red values, 256 green values and 256 blue values.

Measurements
Exports the current measurements to a tab-delimited text file compatible with spreadsheet and statistical analysis programs. Use the Analyze/Options dialog box to specify which measurements are recorded and/or exported.This option is also used to export angle measurements made with the angle tool, and X-Y coordinate measurements made with the cross hair tool. Measurements can also be exported by copying them to the Clipboard.

Plot Values
Exports the data values representing the most recent density profile to a text file. If you have just used the Calibrate command to do density calibration, and are viewing the density calibration plot, then this option saves the 256 Y-values of the current calibration plot to a text file.

Histogram Values
Exports the 256 gray level counts from the most recent histogram to a text file.

XY Coordinates
Exports the XY coordinates of the current selection outline as a two column, tab-delimited text file. XY coordinates of curved line selections, and selections that have been edited, can not be exported.

Save Screen

Saves the contents of the entire screen, including menu bar, trash can, etc., to a PICT file named *Screen* in the same folder as *Image*. Any existing file with that name will be replaced without warning.

Record Preferences

Saves the current state of the following *Image* program parameters. Settings are saved in a file named *Image Prefs* in the System Folder. You can revert to the default settings be deleting this file.

- Foreground color
- Background color

- Brush size
- Airbrush diameter
- Color Look-Up Table mode
- Color palette position and width
- Text attributes, including font, size, style and justification
- Extra colors
- Invert Video switch
- Measurement Options
- Profile Plot Options
- Frames averaged by *Average Frames* command
- Size of image created by New command
- Undo(and Clipboard) buffer size
- *Apple Look Table* options for images with density slicing enabled

- Frame grabber input channel
- Import command parameters
- Most options in Preferences dialog box

Revert to Saved

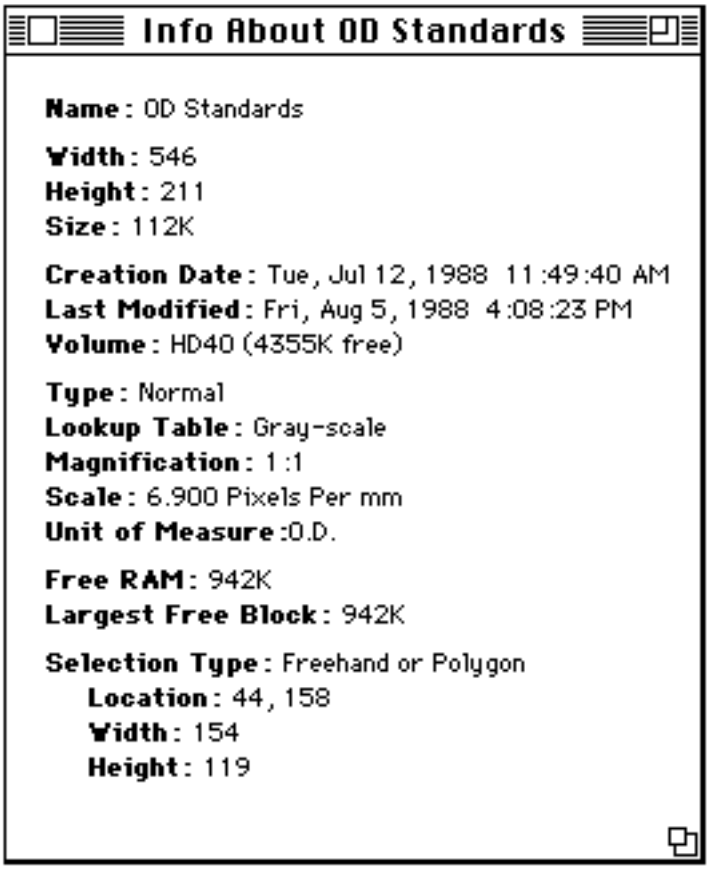
Reverts to the most recently saved version of the current image, effectively undoing all changes made since the last Save command. Reverting does not currently work with stacks, imported 16-bit images, or imported text files.

Duplicate

Creates a new window containing a copy of the current image window, or of the current selection if only part of the image is selected. The newly created window will inherit the gray map(or color table), as well as any spatial or density calibration, of the window being duplicated.

Get Info

Displays a window, such as the one shown below, giving information about the currently active image window and the status of the system.



Halftone Options…

Allows you to specify how halftoning is done on the LaserWriter or other PostScript

printers. Halftoning is a technique which allows grayscale images to printed on a device whose pixels can only be black or white. This is accomplished by dividing the printer pixels into small groups(or cells) according to some pattern(called the halftone screen). Within each cell, a dot is

drawn whose size is proportional to the gray value of that part of the image. A screen frequency of about 50 halftone cells per inch, providing a cell size of 6 x 6 pixels, is optimum for a 300 dots per inch laser printer. A frequency of 106 is optimum for the LaserWriter IIf(with expanded memory) and LaserWriter IIg printers. Custom halftoning options can be saved using Record Preferences. Note that these settings have no effect if *Custom Grayscale Halftoning* is not selected in the Preferences dialog box.

Page Setup…

Lets you specify printing orientation (portrait or landscape) and scale (25% to 400%). The quality of density profile and calibration plots will be better if you leave the scale set at 100% and enlarge the plot window before printing the plot. To take advantage of special effects, such as Graphics Smoothing, hold down the option key(or deselect *Custom Grayscale Halftoning* in Preferences) when selecting the Print command.

Print…

This command lets you print images, plots, histograms, and the results of measurements. What gets printed is determined by which window is currently active. The print command changes (e.g. **Print Image**, **Print Plot**, etc.) depending on which window is active.

> **Print Image** - Prints the currently active image on PostScript printers using digital halftoning to simulate shades of gray. Pixels in color images are converted to grayscale prior to printing using the equivalent of *Convert to Grayscale*, a variation of the *Apple LUT* command. If you have made a rectangular selection, only the selection will be printed.
>
> Hold down the Option key at the start of printing to get higher quality text and line drawings on PostScript printers. In this case, printing is done using bitmap smoothing instead of halftoning. Holding the Option key down is equivalent to temporarily deselecting *Custom Grayscale Halftoning* in Preferences.
>
> When using version 6.0 (or later) of the LaserWriter driver, deselecting *Custom Grayscale Halftoning* in Preferences allows *Image* to take advantage of the grayscale and color printing capabilities of the newer print divers. In this case, use the *Black & White* and *Color/Halftone* buttons in the Print dialog box to specify how printing is to be done.
>
> On color printers and film recorders, the output will be centered on the page (or slide). You must deselect *Custom Grayscale Halftoning* in Preferences (or hold the Option key down) when printing on Color PostScript printers. Use the Dither command to simulate shades of gray when printing on non-PostScript printers, such as the ImageWriter.

Print Plot - Prints the current density profile or calibration plot. The size of the printed plot is proportional to the size of the plot window.

Print Histogram - Prints the contents of the histogram window.

Print Measurements - Prints the results of measurements made with the Measure command, or with the angle or the cross hair tools. This command is enabled when you have recorded one or more measurements and either the Values or Results window are active.

Quit

Closes all image windows after asking, in each case, whether changes made during the session should be saved, then quits to the Finder. If measurements have been made but not saved a

dialog box will be displayed notifying you of that fact. The *Save Changes?* dialog boxes will be not be displayed if you hold down the Command and Period keys while quitting, and any changes will not be saved. Pressing Command-Period also bypasses intermediate screen updates, causing windows to close more quickly.

Edit Menu

Undo

Reverses the effect of the last editing or filtering operation. When using the Measure command, Undo deletes the most recent measurements.

Cut

Copies the contents of the current selection to the Clipboard and fills it with the current background color.

Copy

Places a copy of the contents of the current selection on the Clipboard.

Copy will also copy the entire contents of the LUT, Plot or Histogram window to the Clipboard if one of these is the active window. If the Plot or Histogram window is active then both the data values and the graphics will be copied to the Clipboard, allowing you to transfer plot and histogram data to other programs for plotting or statistical analysis.

You can use the eyedropper tool to select colors to be copied from the LUT window to the Clipboard. These colors can then be pasted back into another palette location.

After using the Measure command, Copy changes to Copy Measurement, allowing you to use the Command-C keyboard shortcut to copy measurements to the clipboard.

Paste

Displays the contents of the Clipboard in the currently active image window. The pasted object is automatically selected, allowing you to move it by clicking and dragging. With rectangular objects, you can click and drag on the resize handle in the lower left corner to expand or contract the selection.

You can also use the paste command to paste colors copied with the eyedropper tool back into the LUT window.

If you make a rectangular selection in the Camera window, copy it to the Clipboard, paste into another window(without deleting the Camera window), and select *Live*

Paste in the Paste Control window, then pasting will be “live” from the camera. This feature, along with the ability to select different transfer modes, allows the image currently being digitized to be aligned with a previously digitized image. “Live Paste” is not available when using the Scion frame grabber.

Text as well as images can be pasted into Image windows. For example, you can copy measurement results to the Clipboard, then paste them into an image window. The text will be displayed inside the current rectangular selection, or, if there is no selection, into a default selection. Use submenus in the Option menu to select font, size, style, etc. If the text doesn't fit within the selection, Undo, switch to a different font or size(or make a different selection), and try again. Text background is always erased to white, the “No Background” option in the Style menu is ignored. This is not a problem, however, since you can select the Or transfer mode in the Paste Control window to paste text transparently. When pasting text in the Or mode, you can click in the LUT window to change color or gray level.

Clear

Erases the current selection to the current background color. The Delete key is a handy shortcut for this command. The background color is set by clicking with the eraser tool in the LUT window. The color of the eraser indicates the current background color.

Fill

Fills the current region or line selection with the current foreground color. You can change the color after the selection has been filled by clicking in the LUT window. The entire image window is filled if no selection is active. The foreground color is set by clicking with the eyedropper tool in the LUT window or in the active image window. The color of the brush in the tool palette indicates the current foreground color.

Invert

Inverts the contents of the current selection, or the entire image if no selection is active.

Draw Boundary

Outlines the current region or line selection using the current foreground color and line width. Both the color and line width can be dynamically changed as long as the selection is still active.

Draw Scale

Draws a grayscale or color ramp within the current rectangular selection. Use this command to create density calibration scales. You can use Flip Vertical or Flip Horizontal to invert a newly created scale. Look at the file “Demo Macro” for an example of how to use a macro to label a scale created using Draw Scale.

Select All

This command is equivalent to using the rectangular selection tool to select the entire

image, including portions that may be offscreen. Once the selection has been made, Select All changes to Deselect All, allowing you to deactivate the selection. You can also deactivate the selection by clicking on any tool other than one of the selection tools, the hand tool, or the magnifying glass.

Rotate Left

Rotates the contents of the current rectangular selection counter-clockwise 90˚. Holding the Option key down causes the original object to be erased before being replaced by the rotated version. The entire window is rotated if there is no selection.

Rotate Right

Rotates the contents of the current rectangular selection clockwise 90˚. Hold the Option key down to erase before rotating. The entire window is rotated if there is no selection.

Flip Horizontal

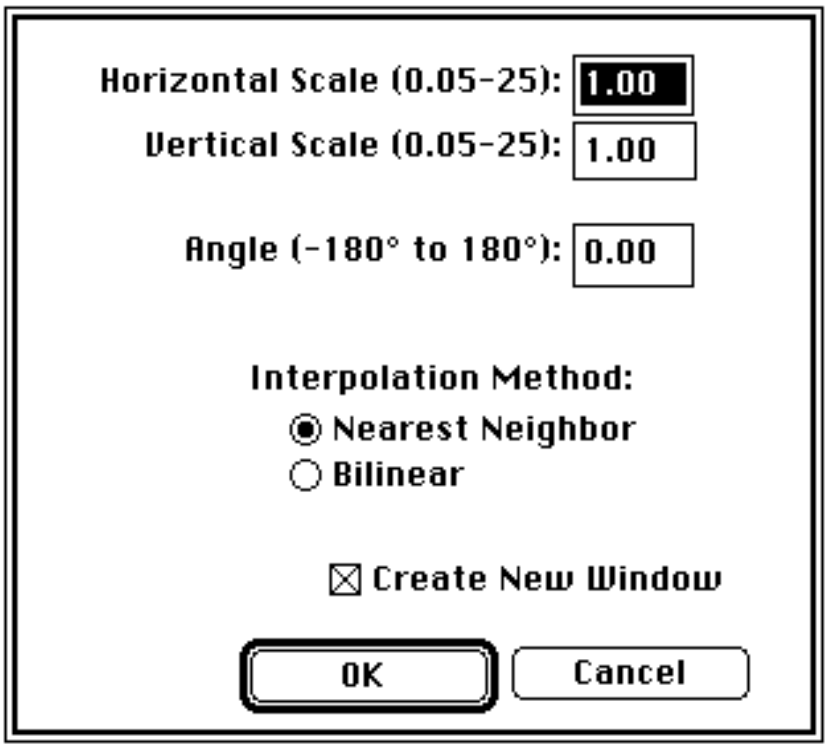
Flips the current rectangular selection horizontally around a vertical line through its center. The entire image is flipped horizontally if no selection is in effect.

Flip Vertical

Flips the current rectangular selection upside down. Flips entire image if there iz no selection.

Scale and Rotate…

Reduces or enlarges the contents of the current rectangular selection. If *Nearest Neighbor* is checked, scaling is done quickly using pixel replication, but the resulting image my not be smooth. Check *Bilinear* for smoother, but slower, interpolated magnification. If *Angle* is non-zero, the contents of the selection are also rotated an arbitrary number of degrees. Rotation will be clockwise for positive angles and counter-clockwise for negative.



Show Clipboard

Creates a new window the size of the image currently on the clipboard, and then displays it. This window, which will have the title Clipboard, will not be updated if the Clipboard later changes.

Options Menu

Invert LUT

Flips the current video look-up table. Unlike the Invert command, *Invert LUT* does not alter pixel values, only the way the image is displayed on the screen. The first(0) and last(255) entries in the look-up table, which are always white and black respectively, are never inverted since these entries are used for drawing the menu bar, title bars, dialog boxes, etc.

Set Number of Colors…

Allows you to change the number of colors used in the current color look-up table. New colors are created by interpolating between existing colors. For example, if the current color table consists of the two colors, white and black, then setting the number of colors to 64 creates a grayscale palette with 64 shades of gray.

Set Extra Colors…

Allows you to reserve up to six entries in the look-up table for colors that will not be altered by routines that manipulate the look-up table. For example, you could use an extra color to add color annotation to a grayscale picture. Any extra colors are displayed at the bottom of the LUT window, allowing you to edit them by double-clicking with the eyedropper Tool.

Grayscale

Enables the default grayscale look-up table. To convert a true color image to grayscale, use the Convert to Grayscale variation of the Apply LUT command. Once you have switched to grayscale, contrast and brightness can be altered by manipulating the transformation function displayed in the Map window. The transformation function can be applied to the pixel data in memory, thereby making any contrast and brightness changes permanent, by using *Apply LUT*.

Color Tables

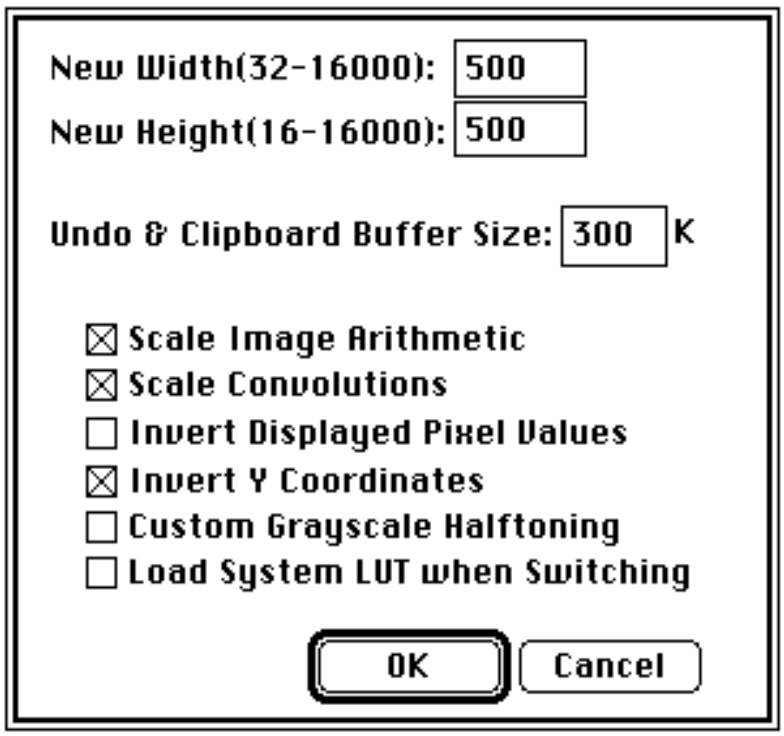
Image currently supports nine built-in color look-up tables. In addition, the Open command can be used to load color tables stored in the Palettes folder distributed with *Image*. You can use the LUT tool(the double headed arrow) to both stretch(changing contrast) and shift the colors within the color table. The colors in a color table can be edited by double clicking on them in the LUT window using the eyedropper tool, although this works best with tables containing only a few colors, around 20 or less.

Font, Size, Style

Text handling in *Image* is similar to that in Macintosh painting programs such as MacPaint, SuperPaint, and PixelPaint, with the exception that you can only change the characteristics, such as size and style, of the last line entered. You are allowed to change the color(by clicking in the LUT window), size, typeface, style and justification of the current line of text after the text has been entered. The background color can be changed by option-clicking in the LUT window.

Preferences…

Displays the dialog box shown below, allowing you to change various parameters used by *Image* .



New Width and *New Height* specify the size of images created using the New command.

Undo & Clipboard Buffer Size allows you to change the size of the two internal image buffers used by *Image*. You must *Record Preferences*, Quit, and then restart *Image* before any buffer size change will take effect.

Pixel values resulting from Add, Subtract, Multiply, or Divide operations(Paste Control) will be scaled to the range of 0 to 255 if *Scale Image Arithmetic* is checked, otherwise, results are clipped to 0 and 255.

Pixel values resulting from filtering operations done using the Convolve command will be scaled to 8-bits if *Scale Convolutions* is checked, otherwise, they are clipped at 0 and 255.

Checking *Invert Displayed Pixel Values* sets up an inverting linear density calibration function for the current image that causes gray values displayed in the Values and Results windows, in density profile plots, and in histograms, to be inverted. Subsequent image windows created using New, Start Capturing, and the Import of 8-bit images, will inherit this calibration function.

Check *Invert Y Coordinates* if you want the X-Y origin to be the lower left corner of the image window, otherwise it will be the upper left corner.

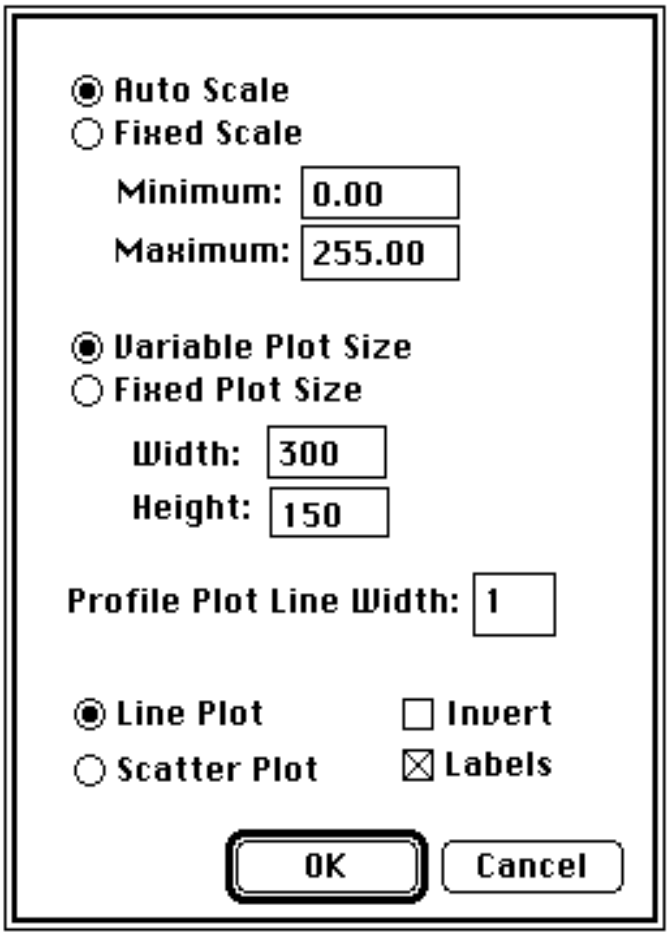
Check *Custom Grayscale Halftoning* to enable the Postscript halftoning capability that is built into *Image.* Changes made in the *Halftone Options* dialog box are only effective when this option is selected.Deselect this option to use the grayscale and color printing capabilities of Laserwiter driver 6.0 or later. You must deselect this option to print on color Postscript printers.

Checking *Load System LUT When Switching* causes *Image* to load the Apple default color palette before switching to other application. This may correct color display problems experienced with other applications, such as Excel 3.0.

The status of settings in the Preferences dialog box can be made permanent using *Record Preferences*.

Profile Plot Options…

Displays a dialog box that allows you to set various options relating to the density profile plots generated by the profile plot tool and the *Plot Profile* command. In *Auto Scale* mode, profile plots are scaled based on the minimum and maximum gray values. In *Fixed Scale* mode, the y-axis range is fixed and the minimum and maximum may be specified. *Fixed Plot Size* allows you to specify the size of the plot window. This is useful when pasting a series of plots into an image window that you want to all be the same size. *Profile Plot Line Width* specifies the width of the line which controls the amount of pixel averaging done by the profile plotting tool. All of these settings can be saved using *Record Preferences.*



Scale to Fit Window

Switches the currently active image window in and out of *Scale to Fit* mode. If checked, the window is in the *Scale To Fit* mode, and you can use the size box, located in the lower right corner, to rescale the image. Clicking in the zoom box of an image window(upper right

corner) will also switch that window to *Scale to Fit* mode, and then scale the window(and image) to fit the screen. In *Scale to Fit* mode you are not allowed to change the aspect ratio of an image and the magnifying glass and grabber tools do not work. When a window is in S*cale to Fit* mode the current magnification level is shown in the title bar.

Thresholding

Thresholding is used to segment an image into objects of interest and background on the bases of gray level. When thresholding is enabled, objects are displayed in black and background is white. Background pixels will be ignored when making area and gray value measurements using the Measure and *Analyze Particles* commands. To vary the threshold, use the LUT tool(the one with the double headed arrow) and click and drag near the black/white boundary in the LUT window. As you vary the threshold, its value is continuously displayed in the Values window.

The Make Binary command can be used to set all thresholded pixels to black, and all background pixels to white. The wand tool can be used to outline thresholded objects, and *Analyze Particles* can be used to automatically measure objects segmented by thresholding. As a shortcut, you can enable thresholding by double clicking on the stair step icon in the Map window.

Density Slice

Like thresholding, density slicing allows objects to segmented on the bases of gray level. When density slicing is enabled, objects are highlighted in red and background pixels are left unchanged. As in thresholding, background pixels are ignored when making area and gray value measurements. Click and drag in the LUT window using the LUT tool to vary the size and location of the density slice. As you manipulate the density slice, its upper and lower limits are continuously displayed in the Values window.

In the LUT window, you can double click within the density slice using the eyedropper tool to change the highlighting color to something other than the default red. As a shortcut, you can enable density slicing by double clicking on either the wand or LUT tools.

To prevent desktop items, such as the menu bar, from changing color, white(0) and black(255) entries in the LUT are never included within the density slice. Therefore, thresholding usually works better for discriminating objects containing black pixels.

The *Apply LUT* command can be used to set all pixels which are within the density slice to the foreground color (or leave then unchanged) and all other pixels to the background color(or leave them unchanged). *Make Binary* will set pixels within the density slice to black, and all other pixels to white.

Propagate

The commands in this submenu allow you to propagate various attributes that have been set in one image to all other currently open images.

Look-Up Table - Copies the current video lookup table to all open image windows. This provides a way to transfer contrast, brightness or pseudocolor changes made to one image in a set(e.g., a series of MRI scans), to all other images in the set.

Spatial Calibration - The spatial calibration(e.g., Pixels/centimeter) and unit of measurement associated with the currently active image are transferred to all other open images. Spatially calibrated images have a black diamond in the title bar following the name.

Density Calibration - The density calibration associated with the currently active

image is transferred to all other open images. Density calibrated images have a white diamond in the title bar following the name.

EnhanceMenu

Filtering Functions(Smooth, Sharpen, etc.)

These functions, with the exception of *Reduce Noise* and Dither, are implemented using 3 x 3 spatial convolutions, where the value of each pixel in the selection is replaced with the weighted average of its 3 x 3 neighborhood. For correct operation, they require a grayscale image, or a pseudocolored image that started out as a grayscale image. Filtering is not limited to rectangular selections. The entire image will be filtered if no selection is active. Filtering operations can be aborted by typing Command-Period.

The 3 x 3 tables shown below are the coefficients for the filters. The popularity of plus and minus in these tables is due to the fact that multiplication by one is very efficient, i.e, it is unnecessary.

Smooth - This filter blurs(softens) the selection area. It can be used to reduce noise in an image. Hold the Option key down for increased blurring.

```
1  1  1          1  1  1  (If Option key down)
1  4  1          1  1  1
1  1  1          1  1  1
```

Sharpen - Increases contrast and accentuates detail in the selection, but may also accentuate noise. To minimize this problem, you can Smooth and/or *Reduce Noise* before using Sharpen. Hold the Option key down for increased sharpening.

```
-1 -1  -1              -1  -1  -1  (If Option key down)
-1  9  -1-1 12  -1
-1 -1  -1-1  -1  -1
```

Shadow - Produces a shadow effect, with the light appearing to come from the lower right.

```
-2  -1   0
-1   1   1
 0   1   2
```

Trace Edges - Produces a binary image with a white background and black outlines representing edges in the original image. Two convolutions are done, generating vertical and horizontal derivatives and the larger of the two result is used. If the resulting pixel is above a threshold value it is set to black, otherwise it is set to white. You can increase or decrease the number of edges by preprocessing the image. Increasing contrast and Sharpening will increase the number of edges found, decreasing contrast and smoothing do the opposite. If you hold down the Option key the thresholding step will not be done, allowing you to do it yourself.

1	1	1		-1	0	1 (If Option key down)
0	0	0		-1	0	1
-1	-1	-1		-1	0	1

Reduce Noise - This is a “median filter”, where each pixel is replaced with the median value of its 3 x 3 neighborhood. This is a time consuming operation because, for each pixel in the selection, the nine pixels in the 3x3 neighborhood must be sorted and the center pixel replaced with the median value(the fifth). To demonstrate the effectiveness of median filtering, try removing random spot noise generated using an air brush with a diameter of around 50.

Dither - Uses the Floyd-Steinberg error diffusion algorithm to convert the current selection to a binary(black and white only) image. Dithering can be useful for exporting pictures to applications such as MacPaint or MacDraw that can only accept binary images. Dithering can also be used for printing on the ImageWriter, or other non-PostScript printers. The quality of dithered images can frequently be improved by increasing contrast using the controls in the Map window before doing the dithering. True color images should be converted to grayscale by using the *Convert to Grayscale* variation of the *Apply LUT* command before being dithered.

Convolve - Does spatial convolutions using kernels, read from a text file, that can be up to 63 x 63 in size. Desk accessory text editors, such as MockWrite, are particularly convenient for creating or examining these kernels.

For an example, use a text editor to create the following file and then use the Convolve command to try it out.

```
 0  0  0 -1 -1 -1  0  0  0
 0 -1 -1 -3 -3 -3 -1 -1  0
 0 -1 -3 -3 -1 -3 -3 -1  0
-1 -3 -3  6 13  6 -3 -3 -1
-1 -3 -1 13 24 13 -1 -3 -1
-1 -3 -3  6 13  6 -3 -3 -1
 0 -1 -3 -3 -1 -3 -3 -1  0
 0 -1 -1 -3 -3 -3 -1 -1  0
 0  0  0 -1 -1 -1  0  0  0
```

This is a 9 x 9 “Mexican hat” filter which will do both smoothing and edge detection in one operation. Each line should be terminated with a carriage return, and the coefficients should be separated by one or more spaces, or a tab. Note that kernels such as this one can be opened and displayed as an image using the Import(Text) command, scaled to a reasonable size using Scale and Rotate, and plotted using *Surface Plot*.

Binary

The commands in this submenu are used to convert grayscale images to binary(i.e. black and white only) and to process these binary images. Note that the *Erode* and *Dilate* tranformations in *Image* do not perform erosion and dilation with structuring elements as described in the literature of classical mathematical morphological.

However, several of the Mac image processing programs listed in Appendix D, such as DIP Station, do support mathematical morphology.

Make Binary - Converts the current grayscale image to binary by setting pixels that have been highlighted by either density slicing or by thresholding to black (255), and all other pixels to white (0).

Erode - Removes pixels from the edges of objects in a binary images, where contiguous black areas in the image are considered objects, and background is assumed to be white. A pixel is removed(set to white) if four or more of its eight neighbors are white. Erosion

separates objects that are touching and removes isolated pixels.

Dilate - Adds pixels to the edges of objects in a binary images. A pixel is added(set to black) if four or more of its eight neighbors are black. Dilation connects discontinuous objects and fills in holes.

Open - Performs an erosion operation, followed by dilation, which smooths objects and remove isolated pixels.

Close - Performs a dilation operation, followed by erosion, which smooths objects and fill in small holes.

Set Count… **-** Allows you to specify the number of adjacent background or foreground pixels necessary before a pixel is removed from or added to the edge of objects during erosion or dilation operations. The default is four.

Set Iterations… **-** Allows you to specify the number of times erosion, dilation, opening, and closing are performed. The default is one.

Outline - Generates a one pixel wide outline of objects in a binary image.

Skeletonize - Repeatable removes pixels from the edges of objects in a binary image until they are reduced to single pixel wide skeletons. Command-period can be used to abort the thinning process.

Arithmetic

The commands in this submenu add(subtract, multiply, etc.) a constant to each pixel in the current rectangular selection, or if there is no selection, the entire image. Results are rounded to the nearest integer.

Add - Adds a constant to each pixel in the selection. Results greater than 255 are set to 255.

Subtract - Subtracts a constant from each pixel in the selection. Results less than 0 are set to 0.

Multiply - Multiplies each pixel in the selection by a constant. Results greater than 255 are set to 255.

Divide - Divides each pixel in the selection by a constant.

Log - Each pixel(V) in the selection is replaced with ln(V) * 255.0 / ln(255.0), where ln(V) is the natural logarithm($\log_e$) of V. The result is set to 0 if V is equal

to 0.

Subtract Background

The commands in this submenu remove smooth continuous backgrounds from gels and other images. The rolling ball and rolling disk algorithms used by these routines were inspired by Stanley Sternberg's article, "Biomedical Image Processing", IEEE Computer, January 1983. The routines were written by Michael Castle and Janice Keller of the University of Michigan Mental Health Research Institute.

1D Horizontal - Rolls an arc(rolling arc) horizontally under each row(shrunk 2 or 4

times) of the image in order to remove the background.

1D Vertical - Rolls an arc(rolling arc) vertically under each column(shrunk 2 or 4 times) of the image in order to remove the background.

2D Rolling Ball - Rolls a patch from the top of a sphere(rolling ball) under every point in the image(shrunk 4 or 8 times) in order to find the background.

2D Remove Streaks - Gets rid of horizontal and vertical streaks as it removes background by calling 1D Horizontal and 1D Vertical consecutively.

Faster - When checked, the image is shrunk 8 times(instead than 4) for 2D rolling ball subtraction, and lines or columns are shrunk 4 times(instead of 2) when doing 1D subtraction.

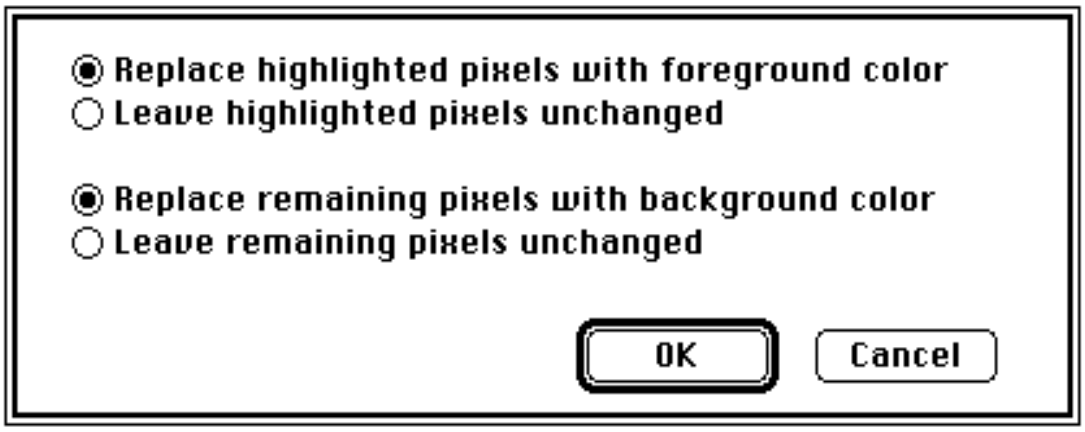
Set Radius - Offers a dialog box to set the rolling ball or disk radius. Generally, the disk/ball radius should be at least as large as the width/diameter of the largest object in the image that is not part of the background.

Equalize

Performs histogram equalization based on the density histogram of the current selection. A new look-up table function is generated that more evenly distributes the gray values of the image. The newly created function is displayed in the Map window. Use *Apply LUT* to make the contrast change permanent. Equalization does not work with true color images.

Apply LUT

Applies the current look-up table to each pixel in the current selection (or of the entire image if there is no current selection) and then restores the default look-up table(the identity function). This modifies the gray values so that when the image is viewed using the default look-up table it will look the same as it did before. This command provides a way of making brightness and contrast changes permanent. It can be used to posterize(reduce the number of gray values) an image by loading and Applying, for example, a palette consisting of four shades of gray. It can also be used to convert color images to grayscale, and to convert a thresholded image to binary.



If density slicing is in effect, the above dialog box is displayed, allowing you to process highlighted pixels in one way, and all other pixels another way.

Change Values…

Changes the value of all pixels in the selection(or the entire image if no selection is active) that are in the current foreground color to the current background color. For example, to change a

solid white background to black you would set the foreground color to white, the background color to black and then use Change Color.

To be more precise, Change Values changes all pixels using the current foreground index value (a number in the range 0 to 255) to the current background color index value. It is possible that a given color on the screen could be represented by several different index values. In this case, Change Values may not behave as expected.

Analyze Menu

Measure

Computes the area and mean gray value of the current selection and displays the results in the the Values window. In addition, you can use the Options dialog box to enable other measurements, such as perimeter length. Use Undo to delete the last measurement or *Delete Measurement* to delete an arbitrary measurement. Only highlighted pixels are included in the computation if either density slicing or thresholding are enabled. The measurement counter is incremented by one each time you use Measure. The maximum number of measurements is 200, but this can be increased in the Options dialog box.

Use *Show Results* to display the list of measurement results, *Print*(with either the Values or Results window active) to print the results, *Copy* to copy results to the Clipboard, *Export* to export results to a tab-delimeted(spreadsheet compatible) text file, and *Reset* to reset the measurement counter to zero. There is no limit to the number of measurements that can be exported, but there is a 32K limit when displaying, printing, or copying results.

The area of the selection will be given in calibrated units, such as square millimeters if *Set Scale* has been used to establish the spatial scale. If Calibrate has been used to perform density calibration, then gray value readings will be converted, using the standard curve generated by Calibrate, and the results reported in calibrated units, such as optical density or isotope concentration.

The most efficient way to record measurements is to use System 7(or MultiFinder), and to have a spreadsheet on the screen at the same time you are using *Image*. Make a series of measurements(Command-1), Copy them to the Clipboard (Command-C), click on the spreadsheet to activate it, select the cell where you want the measurements stored, Paste the results(Command-V), click on the image window to reactivate *Image*, and finally, Reset the measurement counter(Command-3).

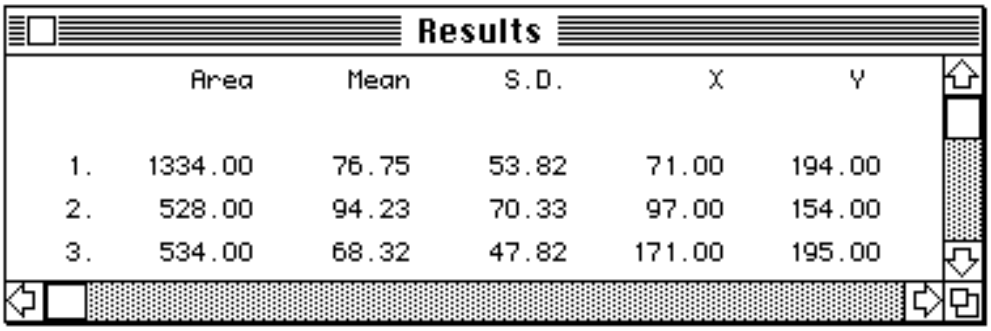
Analyze Particles

Analyze Particles automatically counts and measures objects in a binary or

thresholded image. It does this by scanning across the image until it finds the boundary of an object, outlines the object using the same outlining routine used by the wand tool, measures the object using the equivalent of the Measure command, and then redraws the object in a different gray level so that it becomes invisible to the scanning process. The Options dialog box allows you to specify the measurement parameters to be recorded, to enable labeling and outlining of particles, to ignore particles touching the edge, and to specify minimum and maximum particles sizes. Use *Show Results* to display the recorded measurements. Up to 32767 particles can be counted, but measurement recording is limited to the first *Max Measurements*(set in Options) particles. Command-Period aborts particle analysis.

Show Results

Opens a window, named Results, and displays the current results list in it. If the Results window is already open, then it is activated.



Use *Export* (command-S when the Results window is the active) to save the measurements as a tab delimited text file that can be Opened by many Mac programs, including Excel, StatView, and KaleidaGraph. For correct display, it may be necessary to adjust tab settings when opening an exported file with a word processing program such as Microsoft Word. Hold down the Option key(or check *Headings* in Options) when Exporting(or Copying) results to include column and row headers. Use Print(command-P) to print the measurements, and Copy(command-C) to copy them to the Clipboard.

Show Histogram

Displays a plot showing the distribution of gray values within the selection. The plot shows, for each of the possible 256 gray values, the number of pixels in the selection that have that gray value. Histograms are not limited to rectangular areas. When the cursor is over the Histogram window, X(the gray value) and Y(the number of pixel with that gray value) are displayed dynamically in the Values window.

Using the Copy command when the histogram window is the active window will copy both the histogram plot(as a PICT) and the histogram data values(as a single column of text) to the clipboard. Histogram data values can also be saved to a text file using *Save As*.

Plot Profile

Generates a density profile plot from straight line or rectangular selections. For rectangular selections wider than they are tall(or if the Option key is down), it produces a “column average plot”, where the width of the plot is equal to the width of the selection and each point in the plot represents the average gray value of the pixels in the corresponding column in the selection. For rectangular selections higher than they are wide, it does a top to bottom “row average plot”, where the width of the plot is equal to the height of the selection and each point in the plot is the average of the corresponding row.

For straight line selections, it displays a plot of the gray values along the selected line. Pixel averaging will be performed if the line selection width is greater than one. For example, assuming the maximum line width is selected by clicking on the thickest line at the bottom of the tool palette, then each data point plotted is the average of eight pixels.

The *Profile Plot Options* dialog box can be used to vary the way profile plots are displayed.

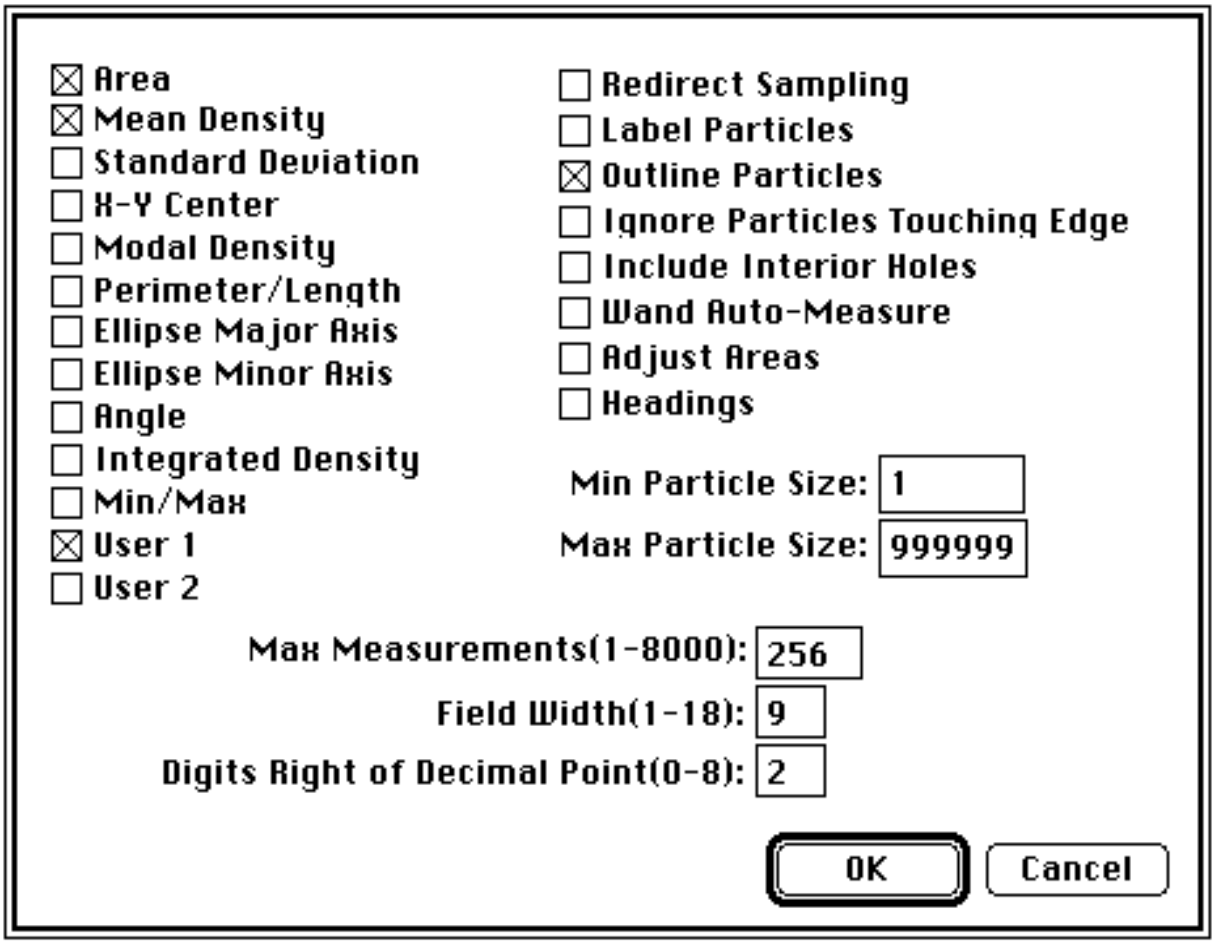
Using the Copy command when the Plot window is the active window will copy both the profile plot(as a PICT) and the data values(as a single column of text) to the clipboard. Plot values can also be saved to a text file using the Export command.

Surface Plot

Generates a surface plotof the current selection. The entire image is plotted if there is no selection. The plot will be drawn in a new window whose size is determined by *New Width* and *New Height* in the Preferences dialog box. Use Command-Period to abort plotting. You can exaggerate the vertical scale in the plot by increasing the contrast of the image before plotting using the controls in the Map window. To improve the quality of plots of small selections, use *Scale and Rotate* to enlarge the selection before plotting, which will increase the number of lines in the plot.

Options…

The Options dialog box allows you to specify the measurements that will be recorded by the Measure and *Analyze Particles* commands and displayed by *Show Results*. Double-clicking on the polygon tool also brings up this dialog box.



Area - Area of selection in pixels. Area will be given in calibrated units, such as square millimeters, if you have used *Set Scale* to do spatial calibration.

Mean - Average gray value of the pixels within the selection. Given in calibrated units if you have used the Calibrate command to generate a standard curve.

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

X-Y Center - Center of the best fitting ellipse, measured from either the upper left or lower right corner of the image, depending on the status of the *Invert Y Coordinates* check box in the Preferences dialog box. This option is automatically checked when X-Y coordinate values are recorded using the cross hair tool.

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

Perimeter/Length - Length around the outside of the selection, or line length for line selections. The perimeter is only computed for freehand and polygon selections, or when using Analyze Particles. Note that the perimeter is computed at the time the outline is created, not when you use the Measure command. This option is automatically checked when Measuring a line selection.

Major/Minor Axis - Lengths of the major and minor axes of the best fitting ellipse.

Angle - This is the angle between the major axis and a line parallel to the x-axis of the image, or it is an angle measured using the angle tool. This option is automatically checked when measuring angles using the angle tool.

Integrated Density - Used to measure the size(volume) of spots(such as protein spots on a two-dimensional electrophoresis gel). Computed using the following formula:

IntegratedDensity=N * (Mean - Background)

where N is number of pixels in the selection, and Background is the modal gray value(most common pixel value) after smoothing the histogram. Note that this formula assumes that the background is lighter(has lower pixel values) than the object being measured. The background level may be computed incorrectly if there isn't a well defined peak in the histogram. This can happen if not enough background is included within the selection or the background is not very uniform.

Min/Max - Minimum and maximum gray values within the current selection.

User 1, User 2 - Add new columns to the results table that can be used by user written macros to record and display derived results. Several example macros in the file “Measurement Macros” display results using *User 1* an *User 2*.

When *Redirect Sampling* is checked, pixel data for calculating the mean gray value is taken from a second image. Requires that exactly two image windows be open, and that these two images be the same size.

Checking *Label Particles* causes Analyze Particles to draw a number, corresponding to the measurement number, on each particle found. Check *Outline Particles* if you want particles to be outlined(this currently only works for density sliced images). Checking *Ignore Particles Touching Edge* causes Analyze Particles to ignore particles touching the edge of the image. The Wand tool and the Analyze Particles command will include interior holes in area computations if *Include Interior Holes* is checked. Set *Min/Max Particle Size* to get Analyze Particles to discriminate particles by size.

If *Wand Auto-measure* is checked, the Measure command is automatically invoked whenever an object is outlined using the wand tool. In addition, if *Label Particles* is checked, the object will be numbered. If *Adjust Areas* is checked, and the Wand tool is in auto-measure mode, the perimeter is computed and added to the area. This is useful when measuring the area under peaks, since adding the perimeter corrects for the tendency of the Wand tool to underestimate

the size of small peaks by not counting pixels under the boundary.

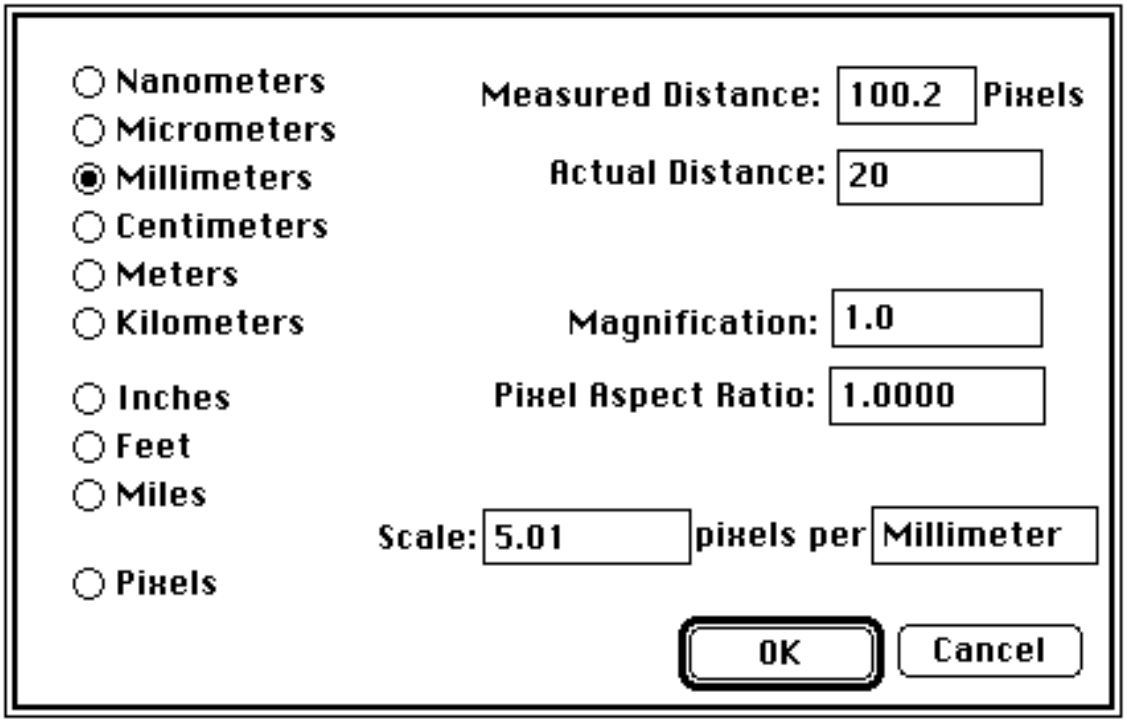
If *Headings* is checked, column and row headings will be added to results Copied to the Clipboard or Exported to a text file.

Max Measurements specifies the size of the arrays used to hold results produced by the Measurement command, by Analyze Particles, and by the angle and cross hair tools. You must Record Preferences, Quit, and then restart *Image* before any change you make will take effect.

Field Width specifies the column width in characters for results displayed in the Results window, Printed, Copied to the Clipboard, or Exported. *Digits to the Right of Decimal Point* specifies the number of digits that follow the decimal point.

Set Scale…

Performs spatial calibration so that results from length and area measurements are presented in calibrated units, such as millimeters. Before setting the scale, use the line tool to make a straight line selection that corresponds to known distance. Then, bring up the Set Scale dialog box, select a unit of measurement, and enter the known distance in *Actual Distance*.



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. Before setting the aspect ratio, calibrate to a known horizontal

distance. Then enter the pixel aspect ratio into *Pixel Aspect Ratio*. To find the pixel aspect ratio, measure the width and height(in pixels) of a digitized object with a known 1:1 aspect ratio. The pixel aspect ratio is computed by dividing the width by the height. Note that the dialog box only shows the horizontal scale. Divide the horizontal scale by the aspect ratio to compute the vertical scale.

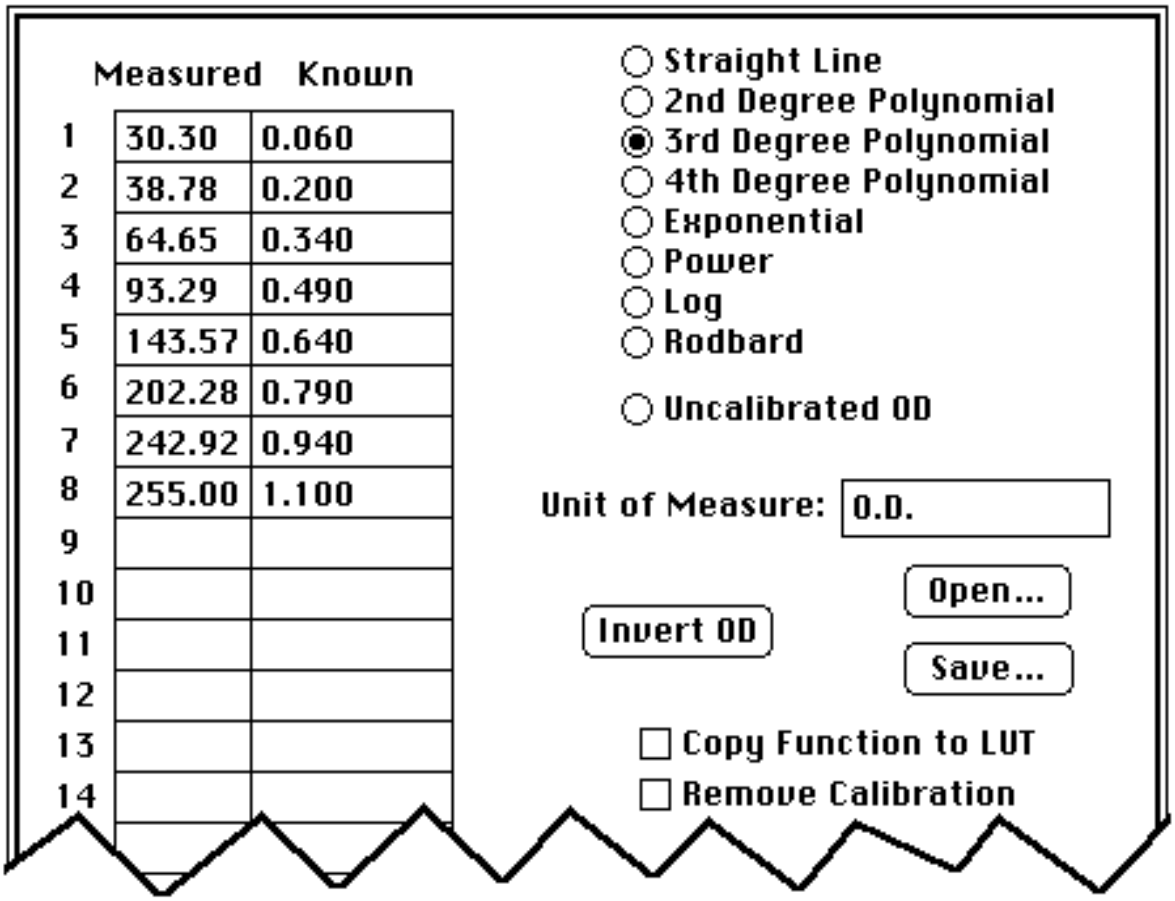
Once the scale has been set you can switch to other units of measurements, for example from inches to centimeters. If you know what the scale for an image is(e.g., 300 DPI), then that value can be directly entered into *Scale* . Click on *Pixels* to disable spatial calibration. Double-

clicking on the line selection tool will also present the Set Scale dialog box.

Note that *Image* displays a black diamond in the title bar of images that are spatially calibrated.

Calibrate…

Allows you to calibrate to a set of density standards, such as a calibrated optical density step tablet, or radioactive isotope standards. Before calibrating, you must measure the standards. To do this, first use Reset to set the measurement counter to zero. Then use the Measure command to record the mean gray value of each of the standards. Note that the standards must be measured in order, starting with the lowest gray value(lightest) standard. When you have finished making the measurements, select Calibrate and enter the actual values into the *Known* column of the dialog box.



Rodbard is a four parameter general curve fit function proposed by David Rodbard at NIH and implemented by Cary Mariash at the University of Minnesota. The form of the equation is: y = (D) + (A - D)/(1 + (x/C)^B).

Selecting *Uncalibrated OD* causes *Image* to convert gray values to uncalibrated optical density values using the following function:

Uncalibrated OD = log10(255 / (255 - Gray Value))

You do not need to measure OD standards or enter known OD values to enable this transformation.

Pressing *Invert OD* performs the following function on the entered(known) standards:

Inverted OD = -log10(1.000 - 10^(-Entered OD))

Optical densities are converted to transmission, subtracted from perfect transmission and converted back to OD yielding the reciprocal density. This function is of use to those who have a positive set of optical density standards but a photographic or other negative image. It is also of use to those who quantitate reflected light images but wish to specify results in terms of transmitted light. This is often the case in anatomical imaging. Entered optical densities must range from 0.00001 to 4.62.

The *Save* button allows you to save both measured and known values to a text file so they can be restored later using the *Open* button. If there are no measured values(count=0), *Open* restores both the measured and known values, otherwise, only the known values are restored.

If *Copy Function to LUT* is checked then the current calibration function will be copied to the video look-up table when you exit this dialog box. You can then use Apply LUT to apply the calibration function the pixel values. If *Remove Calibration* is checked then the spatial calibration, if any, associated with the currently active image will be removed.

If you hold down the option key when invoking Calibrate you will be allowed to change the measured values. This feature allows you to enter measurements acquired previously or acquired from another system.

Note that *Image* displays a white diamond in the title bar of images that are density calibrated.

Redo Measurement

Allows you to repeat a previous measurement. Simply enter the number for the measurement you want to repeat and it will be replaced the next time you use the Measure command. Redo only works for measurements made using the Measure command, not for angle or XY coordinate measurements.

Delete Measurement

Deletes the specified measurement and renumbers any subsequent measurements.

Reset

Resets the measurement counter to zero.

Restore Selection

Returns the previous region or line selection to its original position immediately after an operation that has removed it. If there is a selection currently active, this command restores the previous one. Can also be used to transfer a selection from one window to

another.

Label Selection

Outlines the current selection in white and labels it with the measurement count. Can be used to keep track of which objects have been measured when doing redirected sampling using the Measure Redirected macro.

Special Menu

Start Capturing

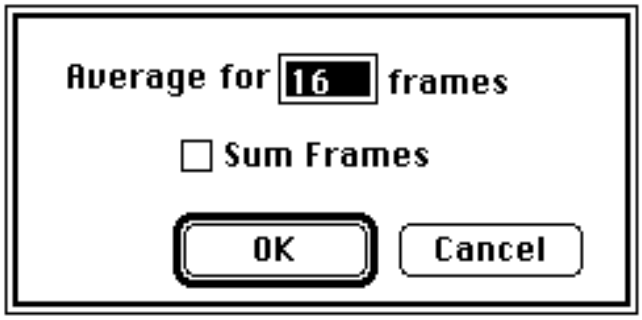
Continuously captures TV camera images using either the Data Translation DT2255

QuickCapture or the Scion Image Capture 1000 frame grabber cards. The command changes to *Stop Capturing* while digitizing is in progress. Clicking in the Camera window with any tool except the magnifying glass or the grabber will also cause digitizing to stop. Newly created Camera windows inherit the gray map(or color palette), as well as any spatial or density calibration, of the currently active window, if there is one.

When digitizing using the Data Translation card you are allowed to change contrast, alter color schemes, zoom, or pan. Also, when using the QuickCapture, histograms are continuously computed and displayed when you use the Histogram command. Hold down the option key to digitize using the whole screen with the Scion card.

Average Frames

Averages two or more video frames to reduce time-varying random noise. Averaging will be done only for the current rectangular selection, or for the entire image if there is no selection.



Check *Sum Frames* to do frame summation, rather than frame averaging. Frame summing increases image quality and contrast in low light situations. Frames are summed using a 16-bit buffer and the resulting 16-bit pixel values are linearly scaled to a range of 0 to 255. Note that there is a possibility of overflow when averaging more than 128 frames since frames are summed in a signed integer buffer.

Save Blank Field

Use this command to save a median brightness blank field which will be used to correct for nonuniform illumination and for the nonuniform response of the video camera. Before using this command, you must *Start Capturing* and adjust the lens diaphragm and/or lightbox intensity so that the average pixel value, as shown by the dynamic pixel value displayed in the Values window, is approximately 128.

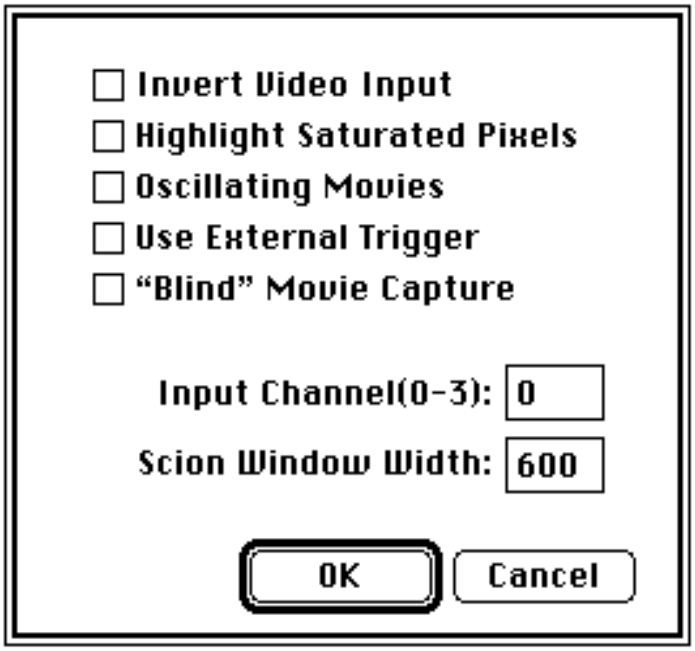
The *Stop Capturing* command will use the reference image previously acquired by *Save Blank Field* to correct shading errors in the newly acquired image. It does this by dividing each pixel in the newly acquired image by a correction factor computed for each of the 300,000 pixels in the blank field. This correction factor is generated by

dividing each gray value in the blank field by the mean gray value.

You can digitize a single frame without shading correction by holding down the Option key while selecting *Stop Capturing*. Close the “Blank Field” window if you want to stop doing shading correction.

Video Options…

Displays a dialog box, shown below, that allows you to alter the behavior of the *Start Capturing*, *Average Frames*, *Make Movie* and *Animate* commands.



Images acquired using a frame grabber card will be inverted if *Invert Video Input* is checked.

Checking *Highlight Saturated Pixels* enables a feature that, during live capture, causes all pixels that over saturate the camera(are too bright) to be displayed in green and all pixels that under saturate the camera(are too dark) to be displayed in red.

Check *Oscillating Movies* if you want movies to run in a reversing mode(1234321234), as opposed to the default wrap-around mode(123412341234).

Use External Trigger enables support for the external trigger feature of the Data Translation QuickCapture card when using the Start Capturing, Average Frames or Make Movie commands. External trigger events are signaled by a 5 volt falling TTL edge on input line 7 of the QuickCapture cable.

Checking *"Blind" Movie Capture* disables screen updates when using the Make Movie command, allowing faster frame capture rates.

Input Channel allows you to specify which of four input channels is used by the QuickCapture or Scion frame grabber cards. The input channels for both cards are numbered from 0 to 3.

Scion Window Width allows you to change the width of the window used for acquiring images when using the Scion frame grabber. This value may need to be reduced to get

the Scion card to work properly with some video cards.

Photo Mode

Erases the entire screen to the current background color and then redraws the current image window without the menu bar, title bar, tool palette, etc. You can change the background color by selecting the eraser tool and clicking in the LUT window. Holding down the Option key while selection this command will cause the image to be drawn starting at the top of the screen using the space formally occupied by the Menu Bar.

Load Macros…

Opens a text file, which should contain a set of macros written in Image's Pascal-like macro programming language. Each macro in the text file causes a new command to be inserted at the bottom of the Special Menu. Macros may be assigned to any key, including the function keys on the extended keyboard. For example, a macro named "Test Macro [T]" would be assigned to the T key, and one named "Another Macro [F1]" would be assigned to the F1 function key. In both cases, it is not necessary to hold down the command key. Macros can usually be aborted by pressing command-period. When launched, *Image* automatically loads the macros contained in the file named "Image Macros", as long as this file is in the same folder as *Image*, or in the System Folder.

Example macros for performing the following functions, and many others, are distributed with Image: plotting gel lanes; drawing arrows; generating a sine wave; creating a grayscale step function; creating a montage; printing all currently open images; generating bas-reliefs; clearing outside of the current selection; plotting XYZ coordinate data; Importing FITS files; recovering X-Y coordinate data from line plots; and generating an ASCII dump.

The macro programming language is documented in Appendix A.

Stacks Menu

The commands in this menu create or operate on stacks, which are three dimensional images consisting of two or more "slices". The slices in a stack can be consecutive serial sections in a 3D data set, frames in a movie loop, or any related set of images. The < and > keys are used to move through the slices in a stack. The current slice and total number of slices are displayed in parentheses in the title bar. Stacks are displayed in a single window and can be saved to a single disk file using the Save or Save As commands. Most commands in *Image*, with the exception of Save, Save As, and Open, operate only on the current slice. Several example macros(in the file "Stacks") are available that operate on all the slices in a stack

Window to Stack

Converts the set of images currently being displayed in separate windows to a stack.

The images must all be the same size. A macro, also called “Window to Stack, is available that works with windows that are not all the same size.

Stack to Windows

Converts the currently active stack to windows. A stack with 20 slices would be converted to 20 normal image windows.

Add Slice

Adds a new blank slice to the stack following the currently displayed slice.

Delete Slice

Deletes the currently displayed slice. Undo will restore the last slice deleted.

Next Slice

Displays the slice following the currently displayed slice. Note that is not necessary to hold down the command key to use the keyboard shortcut, i.e., you only need to press the > key. You can also use the Page Down key.

Last Slice

Displays the slice before the currently displayed slice. As a shortcut, use the < or Page Up keys.

Make Movie…

Captures a series of video frames into a stack. *Image* will create a stack the same width and height as the current selection and large enough to hold the number of frames specified. You will be asked to enter the time to delay between frames. Entering zero will result in the fastest possible sampling rate. As shown in the table below, the delay time is the inverse of frames per second. For example, if you want to capture at the rate of two frames per second you would enter a delay of .5. After *Image* has completed recording the movie it will compute the actual frame rate and display it in the Values window. If the computed rate is not the same as the desired rate then the frames were probably captured at uneven intervals. If an even sampling rate is required then you should either specify a slower rate, or make a smaller selection. Use command-period to abort the movie making process.

Time Delay	Frames/Sec	Maximum Size
.067	15	128 X 128
.1	10	200 X 200
.133	7.5	300 X 300
.2	5	400 X 400
.333	3	640 X 480
.5	2	640 X 480

Capture Frames

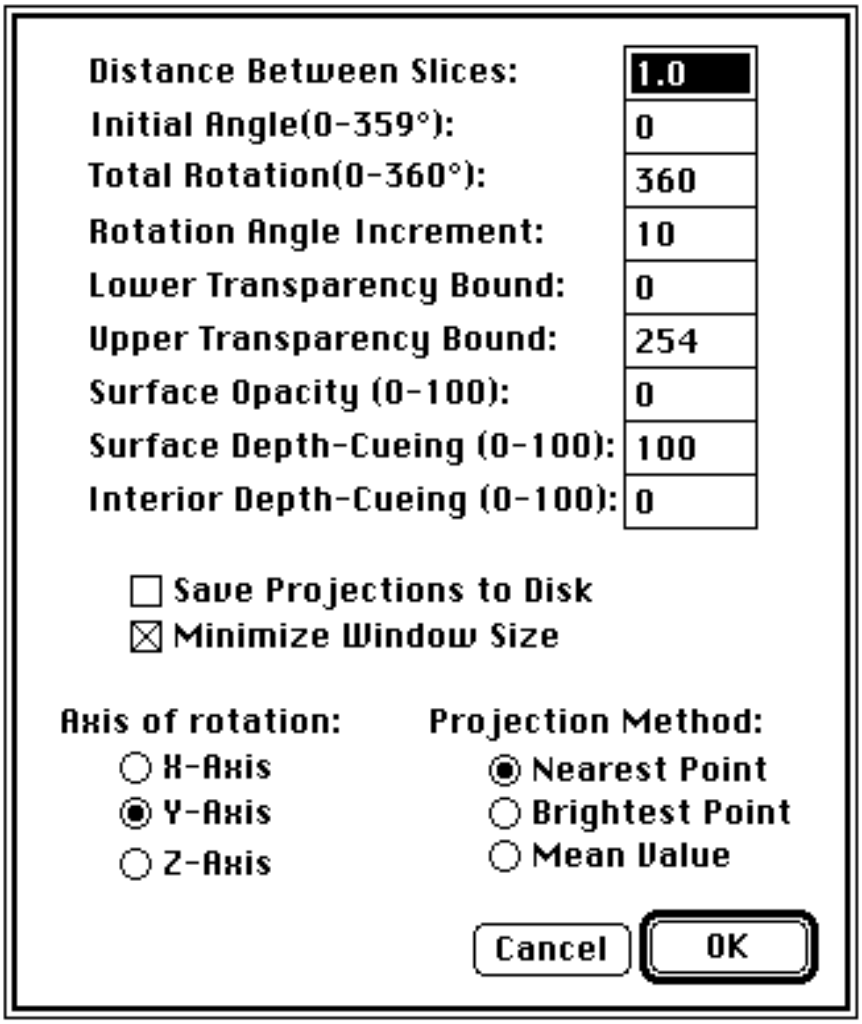
Captures a frame and adds it to a stack each time the mouse button is pressed. Press any key to stop capturing frames.

Animate

Animates the current stack by repeatedly displaying its slices(frames) in sequence. If you hold the Option key down, the screen will be erased to the current background color before the animation starts. The animation speed can be controlled by pressing keys '1' through '9'. The right and left arrow keys can be used to single step through the slices. Press the mouse button to stop the animation. Check *Oscillating Movies* in the Preferences dialog box to get *Image* to automatically reverse direction at the beginning end of the frame sequence.

Project…

Generates an animation sequence by projecting a rotating 3D data set onto a plane. Each frame in the animation sequence is the result of projecting from a different viewing angle. To visualize this, imagine a field of parallel rays passing through a volume containing one or more solid objects and striking a screen oriented normal to the directions of the rays. Each ray projects a value onto the screen, or projection plane, based on the values of points along its path. Three methods are available for calculating the projections onto this plane: nearest-point, brightest-point, and mean-value. The choice of projection method and the settings of various visualization parameters determine how both surface and interior structures will appear. This routine was written by Michael Castle (email: mike.castle@med.umich.edu) and Janice Keller of the University of Michigan Mental Health Research Institute (MHRI).



Distance Between Slices is the interval, in pixels, between the slices that make up the volume. *Image* projects the volume onto the viewing plane at each *Rotation Angle Increment*, beginning with the volume rotated by *Initial Angle* and ending once the volume has been rotated by *Total Rotation.*

The *Lower and Upper Transparency Bound* parameters determine the transparency of

structures in the volume. Projection calculations disregard points having values less than the lower threshold or greater than the upper threshold. Setting these thresholds permits making background points (those not belonging to any structure) invisible. Be setting appropriate thresholds, you can strip away layers having reasonably uniform and unique intensity values and highlight (or make invisible) inner structures. Note that you can use density slicing to set

the transparency bounds.

Sometimes, the location of structures with respect to other structures in a volume is not clear. The *Surface Opacity* parameter permits the display of weighted combinations of nearest-point projection with either of the other two methods, often giving the observer the ability to view inner structures through translucent outer surfaces. To enable this feature, set *Surface Opacity* to a value greater than zero and select either *Mean Value* or *Brightest Point* projection.

Depth cues can contribute to the three-dimensional quality of projection images by giving perspective to projected structures.The depth-cueing parameters determine whether projected points originating near the viewer appear brightest, while points further away are dimmed linearly with distance. The trade-off for this increased realism is that data points shown in a depth-cued image no longer possess accurate densitometric values. Two kinds of depth-cueing are available: *Surface Depth-Cueing* and *Interior Depth-Cueing*. *Surface Depth-Cueing* works only on nearest-point projections and the nearest-point component of other projections with opacity turned on. *Interior Depth-Cueing* works only on brightest-point projections. For both kinds, depth-cueing is turned off when set to zero (i.e. 100% of intensity in back to 100% of intensity in front) and is on when set at $0<n\leq100$ (i.e. (100-n)% of intensity in back to 100% intensity in front). Having independent depth-cueing for surface (nearest-point) and interior (brightest-point) allows for more visualization possibilities.

The *Minimize Window Size* option allows you to save memory by making projection windows as small as possible given the size of the volume and the axis of rotation. If this box is not checked, then projection windows for a given set of slices will be the same size regardless of the axis of rotation (convenient for animations using multiple axes of rotation).

Select *Nearest Point* projection to produce an image of the surfaces visible from the current viewing angle. At each point in the projection plane, a ray passes normal to the plane through the volume. The value of the nearest nontransparent point which the ray encounters is stored in the projection image. *Brightest Point* projection examines points along the rays, projecting the brightest point encountered along each ray. This will display the brightest objects, such as bone in a CT (computed tomographic) study. *Mean Value* projection, a modification of brightest-point projection, sums the values of all transparent points along each ray and projects their mean value. It produces images with softer edges and lower contrast, but can be useful when attempting to visualize objects contained within a structure of greater brightness(e.g. a skull).

Several macros(in the file “Stacks”) are available for performing various operations on the slices making up a volume prior to using the Project command. Use “Smooth” to reduce noise in a volume. Since the projection routine assumes volumes contain one or more objects within a black background, use “Invert” to invert volumes with a

white background. For low contrast volumes, increase the contrast using the controls in the Map window and then apply the look-up table to the volume using the “Apply LUT” macro. The “Remove 0 and 255” macro changes pixel values of 0 and 255 to 1 and 254 respectively. This is sometimes useful when pseudocoloring projections. Use “Replicate Slices” to eliminate the gaps seen in projections of volumes with a slice interval greater than one by increasing the number of slices. The “Make Cone” macro creates a 64^3 volume containing a cone with two rods inside that is very useful for understanding the effect of various projection parameters.

Reslice

Reconstructs a 2D image from the image volume contained in the current stack. Use the straight line selection tool to select were the reconstruction will be done. You will be prompted for the slice spacing(displacement between slices in the stack) in pixels if this information has not been previously entered. To try out the Reslice command, a sample MRI volume consisting of 27 5mm slices is available via anonymous ftp from alw.nih.gov, in the directory /pub/image/image. Two macros are available(in the file "Stacks") for repetitive reslicing of image volumes, for example, to generate a set of coronal slices from an MRI volume consisting of sagittal slices.

Options

Allows you to change the slice spacing parameter, which is used by the Project and Reslice commands.

Windows Menu

Next Window

Deactivates the current image window and activates the next window in the list of window shown in the Windows Menu.

Stack Windows

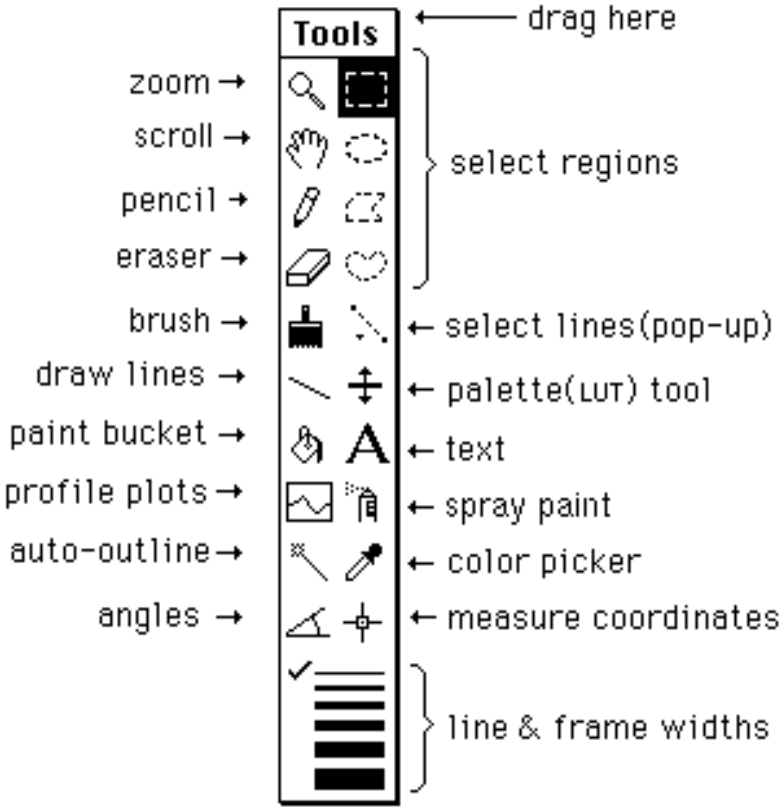
Expands all the image windows to their original size and redraws them slightly offset from each other. If you hold down the Option key, all windows will be drawn in the same location, at the upper left corner of the screen, next to the tool palette.

Tile Windows

Contracts all the images windows and repositions them so that they will fit on the screen without overlapping. If you hold the Option key down the tiled windows will be drawn using "Scale to Fit" mode. Click on the Zoom Box of a tiled window to expand it to fill the screen.

Tools

The window containing the tool palette, as well as all the other windows displayed by *Image(*with the exception of the Scion Camera window), can be freely moved around the screen. All the tools work with images at any of the eight available levels of magnification.



Magnifying Glass - Click within the active image window to zoom. Double-click on the magnifying glass to unzoom. Option-click(or use Undo) within the image window to unzoom in steps. Notice how the plus sign changes to a minus sign when you hold the option key down. Eight magnification levels are available: 1:1, 2:1, 3:1, 4:1, 8:1, 16:1, 32:1, and 64:1.

Scrolling Tool(Grabber) - Moves images within windows. When

using other tools, with the exception of the text tool, you can temporarily switch to the grabber by holding down the space bar.

Selection Rectangle - Use this tool to specify a rectangular subregion for use by commands in the Edit, Functions and Analysis menus. Rectangular selections can be Saved, Copied, Cleared, Filled, Duplicated, Scaled, Flipped, Rotated, Inverted, filtered, or Measured. Hold down the Shift key to constrain the selection to be square. Double-click to select the entire image. As the rectangle is being drawn, its width and height are shown in the Values window.

Oval, Polygon, Freehand - These are outlining tools for defining non-rectangular subregions for use by various commands in the Edit, Functions and Analysis menus. Regions defined by these tools, along with the rectangle tool, can be Copied, Filled, Cleared, Inverted, Framed, filtered, or Measured. The Fill command allows you to change colors after a region has been filled by clicking in the LUT window. Similarly, Draw Boundary allows you to change the line width by clicking on the lines in the Tools window. Double-click on the polygon tool to bring up the Measurement Options dialog box. Use the arrow keys to "nudge" selections one pixel at a time.

Selections can be edited by holding down the Control key to add a new selection to an existing selection or the Option key to subtract a new selection from the existing selection.When using the polygon or freehand tools you can use the shift key to add to the current selection.

Line Selection Tools - Use these tools to create straight, freehand or segmented line selections. A pop-up menu(note the tiny arrow) is used to specify the line type. The pop-up menu is accessed by clicking on the tool icon and holding the mouse button down for at least 1/2 second. Once you have created a line selection, you can measure its length using the Measure command, draw the line using the Fill command, and outline the line(assuming line width is greater than one) using the Draw Boundary command. With straight lines only, you can also generate density profile plots using the Plot Profile command, and dynamically vary the line width by clicking on the lines at the bottom of the tool palette.

Freehand line selections are created in the same way as freehand region selections, except they are not required to be closed.

Segmented line selections are created using a technique similar to the way polygon region selection are made, using a double-click to terminate the line.

Line width is specified by clicking on the lines at the bottom of the Tools window. Straight lines can be constrained to be either vertical or horizontal by holding down the Shift key. Line lengths recorded using the Measure command are given in pixels unless Set Scale has been used to perform spatial calibration. Use Command-F(Fill) and Command-1(Measure) to both draw and measure the length of a line selection. Use Show Results to display length measurements. Option-click with the text tool to label lines with the measured length. Double-click on the line selection tool to bring up the Set Scale dialog box.

Line selection can be moved in the same way as region of interest selections by clicking inside the "marching ants" and dragging, although this has to be done carefully for one pixel wide lines. Lines selections can also be moved a pixel at a time using the arrow keys. Straight line selections have three handles drawn as small squares. The two at either end swing and stretch the line and the one in the center can be used to move the selection without changing its orientation.

Line selections can be edited in the same way as region selections by holding down the Control key to add a new selection or the Option key to subtract a new selection. Line selections, however, cannot be added or subtracted from previous selections. Once they have been edited, line selections revert to ordinary region of interest selections.

Pencil - Draws thin lines using the current foreground color. Option-click to pick up the color under the pencil. It is not

necessary to hold down the Option key to pick up colors from the LUT window. Holding down the Shift key causes pencil movements to be constrained to be either horizontal or vertical.

Eraser - Erases to the current background color. Option-click to pick up background colors from within the image window. The eraser can also pick up background colors from the LUT window. Holding down the Shift key causes eraser movements to be constrained to be either horizontal or vertical. The color of the eraser indicates the current background color. The background color is used by the Cut and Clear commands and as the background color for text. Double-click on the eraser to erase the active image window.

Paint Brush - Draws in the current foreground color. Option-click to pick up the color under the brush. It is not necessary to hold down the Option key to pick up colors from the LUT window. The color of the brush indicates the current foreground color. Double-click on the brush to change its size. Holding down the Shift key causes brush movements to be constrained to be either horizontal or vertical.

LUT Tool - This tool is used to dynamically modify color look-up tables by clicking and dragging in the LUT window. It is also used to manipulate the density slice when density slicing is enabled. Double-click on this tool to enable/disable density slicing.

Airbrush Tool - Double-click to change the brush diameter.

Line Drawing Tool
Draws straight lines using the current foreground color. Line width is specified by clicking on the lines at the bottom of the Tools window. Lines can be constrained to be either vertical or horizontal

by holding down the Shift key.

Automatic Outlining Tool (Wand)
Traces the edge of a binary, density sliced, or thresholded object and, optionally, measures and/or numbers it, producing a standard *Image* outline in the process. To outline an object, click inside near the right edge, or outside to the left of the object. Imagine a turtle that starts moving to the right from where you click looking for a binary edge. Once it finds an edge, it will trace it in a counter clock-wise direction until it returns to the point where it first found the edge.

Selections created with the wand tool are added to any existing selection if either the Shift or Control keys are held down and subtracted from any existing selection if the Option key is held down.

You can specify that the objects be automatically measured by checking *Wand Auto-Measure* in the Analyze/Options dialog box. Objects that have been measured will also be numbered if *Label Particles* is checked. The numbers correspond to measurement numbers. Undo can be used to remove the most recent number, along with the corresponding measurement.

Density Profile Tool
Displays a plot of the gray values along an arbitrary line. You generate this line in the same way you use the line drawing tool to draw lines. Hold down the option key if you want the line drawn for reference purposes. Averaging will be performed if the line width is greater

than one. For example, assume the maximum line width is selected by clicking on the thickest line at the bottom of the tool palette, then each data point plotted is the average of eight pixels. The Plot window, unlike the Histogram window, my be resized. Lines can be constrained to horizontal or vertical be holding down the Shift key.

Plots can be copied to the Clipboard and then pasted into a picture window. In addition to the plot, the Copy command also copies the plot data to the Clipboard as a single column of numbers, where they can be pasted into analysis and plotting programs, such as KaleidaGraph. Profile plot data values can also be saved to a file using the Export command.

Various aspects of the plots produced by this tool can be altered using the Profile Plot Options dialog box. For a shortcut, double-click on this tool to bring up the dialog box.

Paint Bucket

This is a MacPaint-like Paint Bucket. It causes all pixels located where paint can leak from the starting point(the end of the paint coming from the bucket) to be changed to the foreground color. In conjunction with density slicing, it can be used for measuring areas under profile plots. Profile plots must first be pasted into an image window before they can be filled using the Paint Bucket.

Text Tool

This tool allows text to be added to an image in a Font and Style chosen from submenus in the Options menu. Various attributes of the text, such as font, size and color, can be changed after the text has been entered, but once another tool has been chosen, or you have typed Return, the text becomes part of the image's bitmap.

Hold down the option key and the text tool will automatically type for you the most recent length, angle, or area measurement. Repeated option-clicking will enter previous readings, starting with the most recent one. Selecting Show Results resets the pointer, allowing you to type a set of measurements a second time.

Eyedropper - Picks up colors from the active picture window and from the LUT window. Option-click to pick up background colors. If you are using pseudocolor, double-clicking on a color in the LUT window causes the Color Picker dialog box to be displayed, allowing you to modify that color. Double-clicking in the LUT window also allows you to change the density slice color when *Image* is in the density slicing mode. Pixel values are displayed as RGB components when the eyedropper tool is in use.

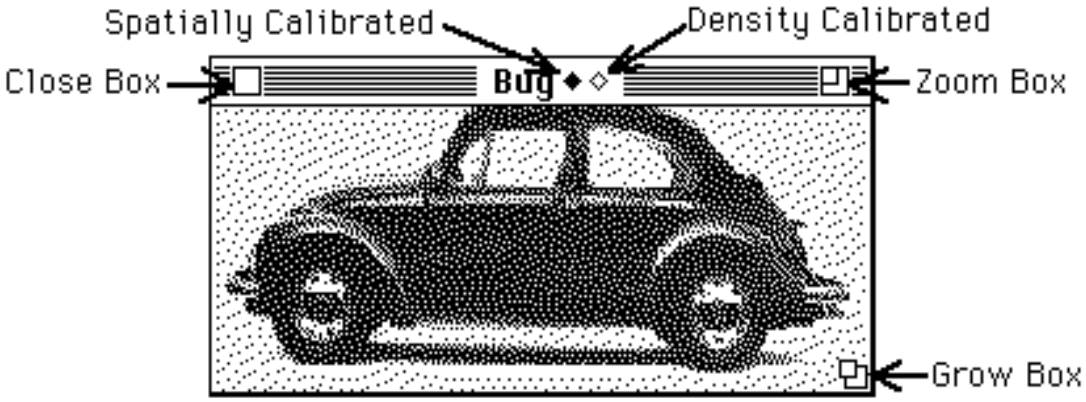
Angle Tool - Measures the angle formed by two lines drawn through a point by this tool. The angle is shown interactively in the Values window. Undo can be used to delete the lines if they are not wanted. Use Show Results to display a set of angle measurements.

Cross Hair Tool - Counts objects and records their X-Y coordinates, leaving markers so that objects are not counted twice. Markers are drawn in the current foreground color and their size is related to the current line width. Hold the Option key down to label using the current measurement count instead of round marker. Alternately, hold the command key down to displays the X-Y coordinates. Use submenus in the Options menu to vary font, size, etc.
Use Show Results to display the X-Y coordinates.

Line Width - Allows you to choose the line width used by the line selection tool, the line drawing tool, the profile ploting tool, or the Draw Boundary command. The lines are 1, 2, 3, 4, 6, or 8 pixels in width. Line widths greater than eight can be specified in the Profile Plot
dialog box.

Windows

Image Windows



Images are displayed within image windows, such as the one above, created using New, Open, Import, or Duplicate. The size of windows created using the New command can be specified in the Preferences dialog box. The Close Box closes the window and frees the memory used to store the image. Hold down the option key when clicking in the close box to close all image windows.

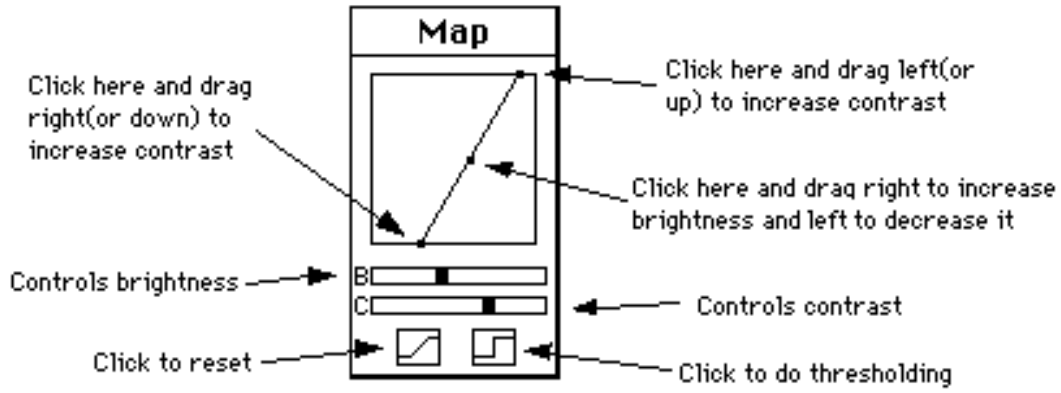
The Zoom Box switches the window to “Scale to Fit Window” mode and resizes the window to make it as large as possible, while still maintaining the same aspect ratio. You can restore the window to its original size by clicking again in the Zoom Box, or by using Unzoom. Note that the magnifying Glass and the Grabber tools do not work in Scale to Fit mode.

The Grow Box is used for resizing the window. If you are in the Scale to Fit mode the image will be rescaled to fit any resized window. You can use the Grow Box to make

the window larger than its original size if you have zoomed in using the magnifying glass.
Map.

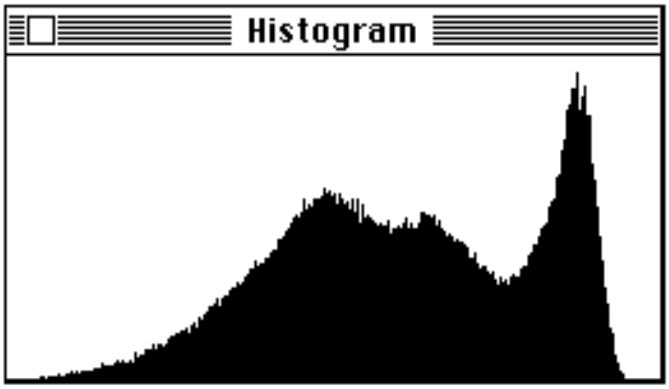
A black diamond is displayed in the title bar of spatially calibrated images and a white diamond is displayed if the image is density calibrated.

Map



The Map window is used to vary brightness and contrast by loading different functions into the video look-up table. To vary brightness, click and drag inside the frame, which moves the plot(a piece-wise linear density transformation function) horizontally. This changes the Y-intercept of the function while maintaining the slope fixed. To change contrast, click and drag in the margin to move the two points which define the function. A good strategy for improving the contrast of grayscale image is to click(outside the frame) in the lower left hand corner, than drag horizontally to the right until the image starts to saturate. Similarly, click in the upper right hand corner, and drag horizontally to the left.

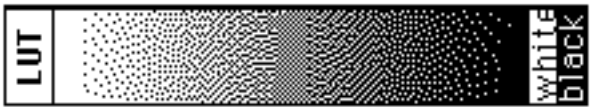
Histogram



This window displays the gray value histogram produced by the Show Histogram command. This function shows, for each of the 256 possible gray values, the number of pixels within the selection that have that gray value. The actual histogram values are dynamically displayed in the Values window whenever the cursor is over the histogram window. Both the histogram plot and the 256 data values can be copied to the clipboard (using Copy) whenever the histogram window is active. The data values can also be saved to a text file using the Export command.

Another way to display histograms is to use the macro distributed with *Image* that displays histograms in grayscale or color, where each of the 256 columns is drawn in the grayscale or color that that column represents.

LUT

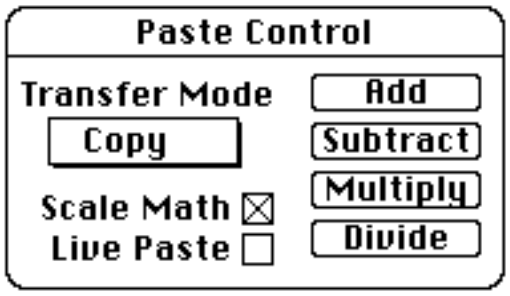


This window displays the contents of the video Look-Up Table(LUT), which is used to transform each of the 256 possible pixels values into one of 16 million possible screen colors.

Click in the LUT window with any of the drawing tools to pick up a new drawing (foreground) color. Click in the LUT window with the eraser, or Option-click with any other tool, to pick up a new background color. The Save As command can be used to save look-up tables separately from images.

With the LUT tool(the one with double-headed arrow), click and drag near the top or bottom of a color table to stretch it. Click and drag near the center to move it up or down. To edit a color in a color table, double-click on it with the eyedropper tool. All 256 entries in the LUT can be rotated by clicking and dragging with the option key held down.

Paste Control



The Paste Control window allows you to control how image selections are pasted, or to do image arithmetic on pasted images. It is only effective during paste operations. The pop-up menu is used to select the transfer mode(Copy, And, Or, Xor, Replace, or Blend) used for the paste operation. The Paste Control window is activated by selecting Paste Control from the Windows menu, or by typing Command-Y.

In the Copy mode, pasting occurs normally. When And, Or, or Replace are selected, the selection is copied to the screen using

“And”, “Or”, or “Replace with Transparency” modes, respectively, allowing you to see both the object being pasted and the underlying image. This can be used to align two images, but works best if one of the image is first converted to binary using the Make Binary, Trace Edges, or Dither commands.

The foreground color is initially set to black and the background color to white whenever And, Or, or Replace are selected, but you are can vary the foreground and background colors during the paste operation by clicking(or Option-clicking) in the LUT window. This will produce some interesting, and possibly useful, effects. Or mode can be used to color objects in binary images created by *Image* or programs such as by SuperPaint and MacDraw. Simply select the object you want to color, Copy, switch to Or mode, then select a color by clicking in the LUT window.

Replace mode replaces the destination pixel with the source pixel if the source pixel isn't equal to white. Replace mode is useful for overlaying colored objects with white backgrounds(e.g.

text or plots) onto another image.

In the Blend mode, destination pixels are replaced with the arithmetic average of source and destination pixels. It is similar to an Add operation with Scale Image Arithmetic checked in Preferences. Blend mode, however, works with true color images, and does not terminate the paste operation. Color images to be blended must have the same color palette.

Clicking on *Add*, *Subtract*, *Multiply*, or *Divide* causes the specified image arithmetic operation to be performed and terminates the paste operation. For example, clicking *Subtract* will subtract the selection being pasted from the current window. Arithmetic operations are done in two passes if *Scale Math* is checked. In the first pass, *Image* determines the minimum and maximum gray levels that would result from performing the operation. In the second pass, the image arithmetic is actually performed and the resulting pixel values scaled to the range of 0 to 255. Results are clipped to 0 and 255 if *Scale Math* is not checked. Arithmetic operations are always done on raw pixel values, ignoring any density calibration that may be in effect.

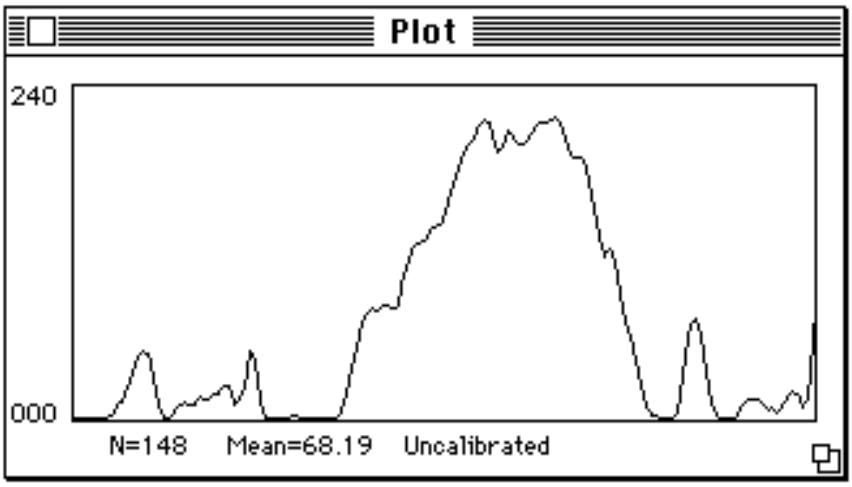
Subtraction is probably the most useful arithmetic operation. It can be used to subtract the background(the image with features of interest removed from view) from an image. It can also be used to detect differences between two images or for motion analysis.

Selecting *Live Paste* causes the currently pasted selection to use "live" TV camera input. The selection must have been copied from the Camera window and pasted into some other window. *Live Paste* currently only works with the Data Translation frame grabber card.

The arrow keys can be used to nudge the selection currently being

pasted in any of four direction by a single pixel. You can get interesting effects, including an embossed look similar to a bas-relief, by using the arrow keys to offset an image by one pixel, and then subtracting it from itself. To see how this works, try Select All(Command-A), Copy(Command-C), Paste(Command-V), Down Arrow, Right Arrow, and Subtract.

Plot



The Plot window is created, or updated, by the density profile tool, by the Plot Profile command, or by the Calibrate command. The plot can be made larger(or smaller) by resizing the Plot window using the Grow Box in the lower right corner. The Plot window must be the currently active window in order to Print or Copy the current plot . To activate it, click on it or select Plot from the Windows menu. When active, the Plot window's title bar will be highlighted(as show above), and Plot will be checked in the Windows menu.

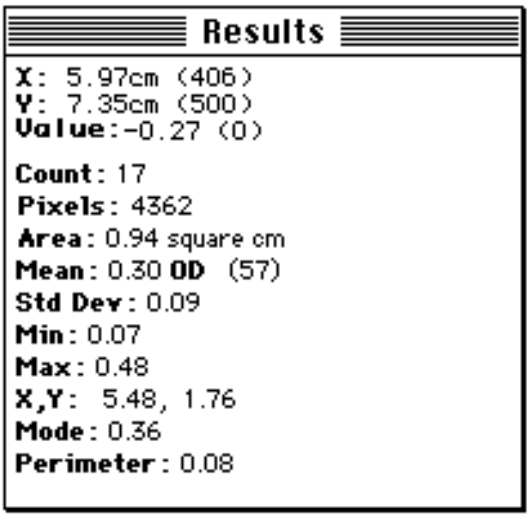
The current plot can be copied to the Clipboard using the Copy command. Once on the Clipboard, the plot can be pasted into an image window, or into another application. The plot is copied to the Clipboard in both graphic and text formats. The Graphic form will be used if you paste the plot into a program that deals primarily with graphics, such as MacDraw. The text form(i.e., the *N* pixel values) will be used if you paste into a program that is text oriented, such as Excel.

N refers to the number of pixel values being plotted. For plots created using the Plot Profile command, *N* is equal to the width of the selection. For plots generated by the density profile tool, *N* is the greater of DX and DY(as displayed in the Values window as the line is drawn) plus the line width. *Mean* is the mean of the *N* gray values. The word *Calibrated,* with the unit of measurement in parenthesis, is displayed if the image has been calibrated to density standards using the Calibrate command, otherwise *Uncalibrated* is displayed.

Whenever the cursor is over the Plot window, X(the pixel number) and Y(the pixel value) coordinate values are continuously displayed in the Values window. In the case of calibration plots, X is the uncalibrated pixel value(in the range 0-255) and Y is the calibrated value.

Several aspects of profile plots, such as y-axis scaling and plot size, can be changed using the Profile Plot Options command.

Values



The values displayed at top of the Values window are dynamically updated variables that have different meanings depending on which window the cursor is in.

Image Window - X and **Y** are the current cursor coordinates, where the origin is at the lower left-hand corner and positive X is to the right and positive Y is up. (Note, you can change the origin to the upper left in Preferences.) Value is the gray value of the pixel at the current X-Y coordinates. The range of values is from 0(white) to 255(black). In the case of color images, Value is the index used by the color look-up table.

LUT Window - Index is the look-up table index at the cursor location. RGB are the associated red, green, and blue brightness values in the look-up table. You can use this information to set the foreground or background grayscale, or color, to a specific grayscale value or index.

Map Window - X and Y display the X-Y coordinates of the gray map control point you are currently changing by dragging the mouse. To change one of the two control points you click and drag near the border of the gray map.

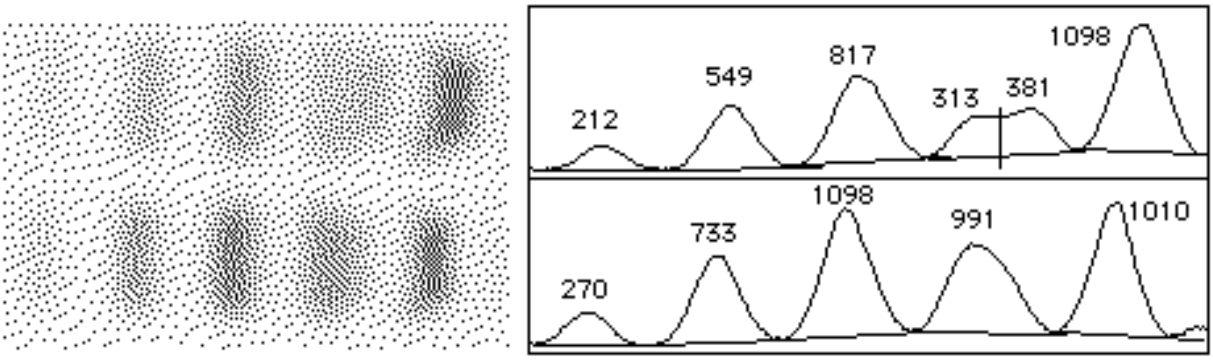
Histogram Window - X is the current X-axis(grayscale) value, Y is the number of pixels in the selection which have that value.

Plot Window - X is the cursor x-coordinate. Y is the corresponding plot y value.

Techniques

Analyzing Electrophoretic Gels

Here is one possible procedure for using *Image* to analyze a one dimensional electrophoretic gel such as the one shown below. This procedure also demonstrates some of the less obvious features in *Image,* and also illustrates a few shortcuts. Note that to make precise measurements it is usually necessary to calibrate to standards included in the gel or to calibrate using a calibrated optical density step tablet.



1) If the *Setup to Plot Gel* and *Plot Lane* commands are not shown in the Special menu then use *Load Macros* to open the macros contained in the file *Gel Plotting Macros*.

2) Close all image windows except for the gel to be analyzed.

3) Use the rectangular selection tool to outline the first lane.

4) Select *Setup to Plot Gel* in the Special menu.

5) Move the rectangular selection(by clicking inside it and dragging) and plot(using *Plot Lane*) each of the lanes in succession.

6) Use the line drawing tool to draw base lines and drop lines

so that each peak defines a closed area as shown above. Note that you can hold the Shift key down to constrain lines to be vertical.

7) Measure the areas of the peaks by clicking inside each one in succession with the wand tool.

8) Option-click with the text tool to automatically label the peaks, in reverse order, with the area measurements. The measurements are also recorded in tabular form, and can, using *Show Results,* be printed or exported to a spreadsheet.

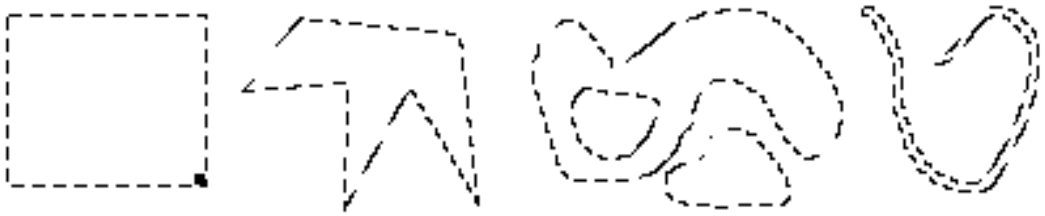
Setup to Plot Gel may fail if it tries to create a plot window that is larger than the Undo buffer. If this happens, you will need to increase *Undo & Clipboard Buffer Size* in Preferences, *Record Preferences*, and restart *Image*. The needed size is equal to (*New Height* * *New Width*) / 1024, where the values for *New Height* and *New Width* are shown at the top of the Preferences dialog box.

Note that *Setup to Plot Gel* changes several of the settings in the Analysis/Options dialog box. It enables *Wand Auto-Measure.* It disables *Label Particles;* otherwise peaks would be automatically numbered. It enables *Include Interior Holes*. If this were not done, the Wand tool

would measure zero area. It enables *Adjust Areas*. This compensates for the tendency of the Wand tool to underestimate the size of small peaks by computing the perimeter and adding it to the area. The size of small peaks is underestimated because some of the actual peak area is represented on the screen by the pixels which define the boundary, and, on small peaks, the ratio of boundary pixels to interior pixels is higher.

Using Selections

A selection is a user defined region or line of interest created using one or more of *Image's* five selection tools. Selections are used for defining areas or lines to be measured or to be operated on using various filtering or editing commands. Selections, such as those shown below, are indicated by the selection marquee, sometimes referred to as “marching ants”.



Moving a Selection. Selections can be moved by clicking within the selection and dragging. The Values window 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. If you want to move the *contents* of a selection, rather than the selection itself, first do a Copy(Command-C) and a Paste(Command-V), then click within the selection and drag. Use the shift key to constrain movement to be horizontal or vertical. The arrow keys can be used to nudge the selection one pixel in any direction.

Stretching a Selection. Rectangular selections can be stretched

using the handle in lower right corner. The contents of the selection will also be stretched if the selection is the result of a paste operation. The width and height are displayed in the Values window as the selection is stretched. Use the arrow keys with the option key down to stretch a selection one pixel at a time. With the Option key down, the arrow keys can be used to stretch the selection one pixel at a time.

Adding to a Selection. If you hold down the Control key(notice the little plus sign in the cursor) while making a selection, then the new selection you create will be added to the current selection. This feature allow you to edit existing selections, or to create discontinuous selections, such as the one above. The Shift key can also be used to extend selections when using either the polygon or freehand tool. Note that it is not possible to measure the perimeter of outlines that have been edited in this way except by doing a Draw Boundary and then using the wand tool too recreate the selection. Also note that line selections cannot be added to or subtracted from existing selections, but freehand and polygon selections can added to and subtracted from line selections.

Subtracting from a Selection. If you hold down the Option key(notice the little minus sign) while making a selection, then the new selection you create will be subtracted from the current selection. This feature allow you to edit existing selections, or to create selections with holes in them, like the one above.

Deleting a Selection. To delete the current selection, choose any of the selection tools and click anywhere outside the selection. Or, alternately, choose a tool other than one of the selection tools, the magnifying glass, or the grabber hand. You can use Restore Selection to bring the selection back after you have deleted it.

Transferring a Selection. A selection can be transferred from one image window to another by using the Restore Outline command. Simple activate the window you want to transfer the selection to by clicking on it, and use Restore Outline. You can also use the Next Window command to activate another image window.

Saving and Restoring Selections.
A selection can be saved to disk by transferring it to a blank(white) window, using the Draw Boundary command(make sure it's drawn black), and saving the resulting binary image as a PICT file. To restore the selection, open the PICT file and click to the left of the drawn outline with the wand tool.

Using *Image* with Flatbed Scanners

Several potential problems can occur when you use a flatbed scanner to digitize images for use with *Image*. For example, scanners which only support 4-bit (16 gray level) scanning are a poor choice for use with *Image,* since at least 32 gray levels are needed for realistic looking images, and 256 gray levels are desirable for quantitative analysis. You should also avoid scanners with software that is unable to save in uncompressed 8-bit TIFF format, since PICT files digitized at greater than 75 dots per inch(DPI) will probably not be displayed correctly by *Image*.

You also have to be careful not to generate files that are two large for *Image* to handle. *Image* was optimized to handle the 640 x 480 (300K) images produced by frame grabber cards, whereas an 8 x 10 inch page scanned at 300 DPI is 2400 x 3000 pixels , or 7.2MB, which is much larger than *Image* was designed to handle. The following table gives suggested maximum scan areas for different scanning resolutions for use with the standard Apple 13 inch monitor(640 x 480) and for 19 inch monitors(typically 1024 x

768). *Image* can handle scans somewhat larger than these, but you will probably run into problems if you greatly exceed these recommended sizes.

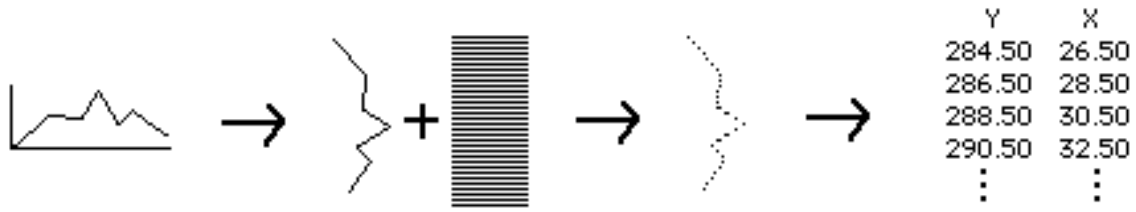
	75 DPI	**150 DPI**	**300 DPI**
13" Monitor	7.5" x 6"	3.75" x 3"	1.8" x 1.5"
19" Monitor	12" x 10"	6" x 5"	3" x 2.5"

The Undo buffer should be set to 700K, or larger, when working with scans the size of the ones suggested for 19 inch monitors.

You should probably stick to 75 DPI unless you are scanning small selections. 75 DPI also has the advantage of producing images that are near actual size when displayed on the screen, since nominal screen resolution is 72 DPI.

Recovering Data from Line Plots

Image can be used to recover numeric coordinate data from line plots using the following procedure.



1) Digitize the plot using a TV camera or flat bed scanner.

2) Edit the plot to remove x-axis, y-axis, and labels.

3) Rotate the plot 90° clock-wise.

4) Create a line mask similar to the one above using the line drawing tool and repeated use of Copy and Paste.

5) Convert the line plot to a scatter plot by ANDing the plot with the mask using Paste Control.

6) Select *X-Y Center* and deselect *Label Particles* in the Analyze/Options dialog box.

7) Deselect *Invert Y-Coordinates* in the Preferences dialog box.

8) Use Analyze Particles to record the X-Y coordinates.

Alternately, and much easier, you can use the macros in "Line Plots->Data", which automate most of these steps. Use the cross hair tool to outline the plot(click to the left of the curve), then use the Clear Outside macro to erase everything except the plot. Next, use Convert Line Plot to Points, which will perform steps 3-8 above. It assumes the active window contains a binary image and you have selected a single isolated line plot drawn on a white background. You can use the Plot Points macro to display the resulting coordinate data.

Using the Option Key

Holding the option key down when using many of the commands and tools in *Image* causes alternative functions to be performed. You only need to hold the option key down when you first invoke a menu command.

Menu Command Options

Starting *Image* - Use QuickCapture card instead of Scion card, assuming both are installed

Close - Changes to Close All
Save - Changes to Save All
Export(Measurements) - Exports measurement results with column and row headers
Print - Do bitmap printing (which is better for text and line art), rather than halftoning.

Copy(Measurements) - Measurement results copied with column and row headers
Rotate Left, Rotate Right - Erase before rotating

Smooth - More (unweighted) smoothing
Sharpen - Increased sharpening
Trace Edges - Result is not thresholded, allowing you to do it yourself

Calibrate - Allows you to edit the measured values

Start Capturing - Digitize with Scion frame grabber using whole screen
- With QuickCapture card, wait for external trigger
Stop Capturing - Omit shading correction
Average Frames - Sum frames instead of averaging
Make Movie - With QuickCapture, for each frame, wait for external trigger
Animate - Erase screen to background color before doing animation
Photo Mode - Move window to top of screen before erasing screen

Stack Windows - Move windows to the "home" position in the upper left corner
Tile Windows - Use "Scale to Fit" mode to draw windows

Tool Options

Magnifying Glass - Zoom out instead of zooming in
Text Tool - Draw results of area or length measurements

Eraser - Pick up background color from image window
Brush - Pict up foreground color from image window
Profile Plot Tool - Draw reference line
Eyedropper - Select background color rather than foreground color

Questions and Answers

Q. I am used to black having a value of zero, and white a value of 255, but in *Image* black is 255 and white is zero. How can I change this?

A. Choose one of the selection tools and use the Measure command twice (it doesn't matter what you measure). Hold down the option key and bring up the Calibrate dialog box. Enter 0 and 255 in the first column(replacing what was there) and 255 and 0 in the second column. Click the button labeled Straight Line and then click OK. Or, check I*nvert Displayed Pixel Values* in Preferences, that does the equivalent of all of the above for you.

Q. How can I create a new window of a particular size, for example 1024x1024?

A. Bring up the Preferences dialog box(its in the Options menu). Set New Height to 1024 and New Width to 1024 and click on OK. Any windows created with the New command during this session should now be 1024x1024. You will need to set the size of the Undo and Clipboard buffers so that they are at least as large as the new window. In the case of a 1024x1024 window, you would need to set the Undo buffer to 1024K.

Q. Sometimes, after I have copied an image to the clipboard, the Paste command becomes dim. Why is this?

A. Images copied to the clipboard are stored in a buffer(called the Clipboard buffer), which is the same size as the Undo buffer. In some situations, such as filtering, *Image* has to use the Clipboard buffer for internal operations. When this is necessary, the Paste command gets dimmed out.

Q. Why do the Finder's icons change color when I run *Image*?

A. *Image* uses all but two of the 256 available screen colors. The two "colors" that *Image* never changes are white(0) and black(255). Objects on the screen that are not black and white are likely to change color when in *Image* is being used. Colored objects are redrawn in their original color when you quit *Image*.

Q. Why don't the Halftone and BitMap buttons in the print dialog box do anything?

A. You are probably using version 6.0, or later, of the LaserWriter driver. This version supports halftone printing for all Mac applications. This feature is normally not used by *Image* since *Image* does its own halftoning by sending PostScript commands directly to the printer. You can disable *Image*'s built-in halftoning by deselecting *Custom Grayscale Halftoning* in the Preferences dialog box, in which case, you can take advantage of the buttons in the Print dialog box.

Q. I want to combine two color images to produce a montage, but when I Copy from one window and Paste into the other the colors are wrong.

A. You can't normally combine images that have different LUTs, but if you check *Keep LUT* when you open the second image(which must be stored as a PICT file) its pixel values will be remapped to conform to the LUT of the first image. You will now be able to successfully Copy and Paste, since both images have the same LUT.

Q. Why won't *Image* run on my LC?

A. *Image* normally requires a floating-point coprocessor, which is optional on the LC. However, there are three things you can do to get it to run on an LC: 1) Add a floating-point coprocessor. They are available by mail order for as little as $100. 2) Download the Non-FPU version of *Image* from alw.nih.gov using anonymous ftp. 3) Use the normal version of *Image* along with the free PseudoFPU Init, which is also available from alw.nih.gov.

Q. Is there a version of *Image* that runs on PC compatibles or on UNIX workstations?

A. *Image* has not been ported to the PC or to UNIX and no ports are planned at NIH. However, the complete Pascal source code(~32,000 lines) is available for anyone willing to attempt a port.

Q. Is there some way to more rapidly switch between the 256 color mode required by *Image* and the "millions" of colors mode required by a few other programs?

A. There is a free Fkey called Switch-A-Roo that will allow you to rapidly switch between any two monitor depths settings by pressing a few keys. Switch-A-Roo is available by anonymous ftp from alw.nih.gov, in the directory /pub/image.

A. Macro Programming Language

The Load Macros command requires a text file containing one or macros. A macro is similar in format to a Pascal procedure. A macro file may also contain procedures.

Here is an example macro file:

```
Procedure add(a,b:integer);
Begin
  result:=a+b;
End;

Macro 'Test [T]';
Var
  i,j,result:integer;
Begin
  MoveTo(100,100);
  add(2,2);
  DrawNumber(result);
End;

Macro 'Another Macro [F5]';
begin
  beep;
End;
```

The text in quotes following the keyword Macro is passed unchanged to the Menu Manager, becoming a new command at the bottom of the Special menu. Macros may be assigned to keys by enclosing the key character in brackets. Macros may also be

assigned to function keys as shown in the example above. A few characters(';', '^', '!','<', '/', '(') have special meaning to the Menu Manager, and should normally be avoided. You can use / to assign a command key equivalent to a macro, e.g., 'Test Macro/6'. Use '(-' as the macro name to create a dividing line in the menu.

Procedures may not be nested inside other procedures, or inside macros. However, unlike Pascal, variables declared in a calling procedure or macro are made available to a called procedure. This feature can be used to get around the lack of VAR procedure parameters, as shown in the example above. Procedure names are required to be unique in the first twelve characters.

Key Words

MACRO PROCEDURE BEGIN END VAR FOR TO DO
IF THEN ELSE WHILE REPEAT UNTIL

Operators

\+ - * / DIV MOD :=
= < > <> <= >= AND OR NOT

Types

INTEGER REAL BOOLEAN

Both integer and real variables are stored internally in extended precision real format, which has a range of 1.9×10^{-495} to 1.1×10^{4932} and 19-20 digits precision. Real numbers are automatically converted (by rounding) to integer without warning as needed. Variable names must be unique within the first twelve characters.

Arrays

Although user defined arrays are not supported, most measurement results can be accessed using predefined one-dimensional arrays. These arrays are named *rArea, rMean, rStdDev, rX*, *rY*, *rMin*, *rMax*, *rLength*, *rMajor*, *rMinor*, and *rAngle*. These arrays use indexes ranging from 1 to *Max Measurements*, where the value of *Max Measurements* can can be changed in the Options dialog box. Use the *rCount* function to get the value of the current measurement counter and SetCounter(n) to set it.

Two predefined arrays(*rUser1* and *rUser2*) can be used to record and display derived results. Unlike other results arrays, *rUser1* and *rUser2* are reserved for use by macros, and are never written to by any of the commands in Image. The column headings used for User1 and User2 can be set from within macros using the routines *SetUser1Label*('Label') and *SetUser2Label*('Label). After computing a derived result, use *UpdateResults* to redisplay the last line in the results table or use *ShowResults* to redisplay the entire table. Use *SetCounter* to control the number of lines displayed by *ShowResults*. Several example macros distributed with *Image*(in "Measurement Macros") use these arrays to display derived results.

Histogram values are available using the read-only array *Histogram*, which accepts indexes in the range 0 to 255. For example, after using Measure, histogram[0] returns the number of white pixels.

Three built-in read/write arrays(*RedLUT*, *GreenLUT*, and *BlueLut*) provide access to the video lookup table(LUT) associated with each open image. These arrays use indexes in the range 0-255 and return intensity values in the range 0-255. Several macros(in "LUT Macros") that use these arrays are distributed with *Image* , including a macro to to export the current LUT as a text file, macros to load various functions into the LUT, a macro to plot the current LUT, a macro to display RGB pixel values, and a macro to load a grayscale step function("Posterize") into the LUT.

The built-in array *LineBuffer* provides access to the internal line buffer used by GetRow, PutRow, GetColumn and PutColumn. *LineBuffer* uses indexes in the range of 0-4095 and returns pixel values in the range 0-255. Using GetRow, LineBuffer[x] and PutRow to do pixel-by-pixel processing is up to twice as fast as using GetPixel and PutPixel, but is still at least 100 time slower than using compiled code.

Built-in Procedures and Functions

File Menu

MakeNewWindow('Name')	Use SetNewSize to specify the size of the new window
Open('File Name')	
SetImport('string')	Where *string* contains some combination of: 'TIFF', 'MCID', 'Palette', 'Text', 'Custom', '8-bits', '16-bits Unsigned', '16-bits Signed', 'Swap Bytes', 'Auto-Scale',Fixed Scale', and 'Open All'
SetCustom(width,height,offset)	Specifies the *width*, *height*, and *offset* of imported files
SetImportMinMax(min,max)	Disables auto-scaling and fixes the range of Imported text files

Import('File Name')	
Close	Closes the active image window
Dispose	Similar to Close, but user is never prompted to save changes
DisposeAll	
Save	Use SetPicName(‘Name’) to specify file name
SaveAll	
SaveAs	Use SetPicName(‘Name’) to specify file name
Export	Use SetPicName(‘Name’) to specify file name
RevertToSaved	
Duplicate('Window Title')	
GetInfo	
Print	Precede with a call to SetOption for bitmap smoothing

Edit Menu

Undo	
Copy	Copies contents of current ROI to Clipboard
CopyResults	Equivalent to clicking on Copy in the Results window
Paste	Pastes into current ROI if Clipboard object and ROI have the same dimensions, otherwise, pastes into center of image.
Clear	Erases current ROI to background color
Fill	Fills current ROI with foreground color
Invert	Inverts current ROI
DrawBoundary	Outlines current ROI using foreground color. Use SetLineWidth to control width of outline.
DrawScale	
SelectAll	
SetScaling(‘string’)	Where *string* contains some combination of: 'Nearest', 'Bilinear', 'New Window', 'Same Window' or 'Interactive'
ScaleAndRotate(xscale,yscale,angle)	
ScaleSelection(xscale,yscale)	0.05 <= xscale,yscale <= 25.0
RotateLeft(b)	Create new window if *b* true
RotateRight(b)	Create new window if *b* true
FlipVertical	
FlipHorizontal	

Options Menu

SetPalette(string)	Where *string* is one of: 'Grayscale', 'PseudoColor', 'System Palette', ‘Rainbow’ or 'Spectrum'
SetFont('Font name')	
SetFontSize(size)	
SetText(string)	Where *string* contains any combination of: 'Bold', 'Italic', 'Underline', 'Outline', 'Shadow', 'Left Justified', 'Right Justified', 'Centered', 'No Background', or 'With Background'

SetNewSize(width,height)	Specifies *width* and *height* of new windows
ScaleMath(b)	*b*=true or false. Sets or resets *Scale Image Arithmetic* flag.
InvertY(b)	Invert Y-coordinates if *b* is true
SetPlotLabels(b)	*b*=true or false
SetPlotScale(min,max)	Set *min* and *max* to zero for auto-scaling
SetPlotSize(width,height)	Set *width* and *height* to zero for auto-sizing of plots
SetThreshold(level)	0 <= level <= 255. SetThreshold(-1) disables thresolding.
SetDensitySlice(lower,upper)	1 <= *lower*,*upper* <= 254. SetDensitySlice(0,0) disables density slicing, SetDensitySlice(255,255) enables it.
PropagateLUT	Propagates current LUT to all other open images
PropagateSpatial	Propagates current spatial calibration to all other open images
PropagateDensity	Propagates current density calibration to all other open images

Enhance Menu

Smooth	Precede with SetOption for unweighted smoothing
Sharpen	Precede with SetOption for greater sharpening
Shadow	
TraceEdges	Precede with SetOption to prevent auto-thresholding
Dither	
ReduceNoise	
Convolve('Kernel file name')	
MakeBinary	
Erode	
Dilate	
SetBinaryCount(n)	1 <= *n* <= 8
Outline	
Skeletonize	
AddConstant(n)	-255 <= *n* <= 255
MultiplyByConstant(n)	0.0 <= *n* <= 255.0
ApplyLUT	Transforms the pixel data using the current look-up table
EnhanceContrast	
ChangeValues(v1,v2,v3)	Changes pixels with a value in the range *v1-v2* to a value of *v3*

Analyze Menu

Measure	
GetResults(n,mean,mode,min,max)	Use after Measure. Values returned are always uncalibrated. Use cValue function to calibrate them.
AnalyzeParticles	
ShowResults	
ShowHistogram	
RestoreRoi	
MarkSelection	
SetOptions(‘string’)	Where *string* contains some combination of ‘Area’, ‘Mean’, ‘Std. Dev.’, ‘X-Y Center’, ‘Mode’, ‘Perimeter’(or ‘Length’), ‘Major’, ‘Minor’, ‘Angle’, ’Int. Den.’, ‘Min/Max’, ‘User1’, or ‘User2’. Any variable not listed is disabled.
Redirect(b)	Enable/disable redirected sampling. *b*=true or false.
LabelParticles(b)	*b*=true or false
OutlineParticles(b)	
IgnoreParticlesTouchingEdge(b)	
IncludeInteriorHoles(b)	
WandAutoMeasure(b)	
AdjustAreas(b)	
SetParticleSize(min,max)	
SetPrecision((digits)	Were *digits* is the number of digits to the right of the decimal poi
SetScale(scale,’units’)	*Scale* is the number of pixels per unit of measurement. ‘*Units’* is

one	
	of the following: ‘nm’, 'µm', ‘mm’, ‘cm’, ‘me’, ‘km’, ‘in’, ‘ft’,
‘mi’,	
	or ‘pixels’. Use SetScale(0,’pixels’) to disable spatial calibration
and	
	SetScale(0,’’) to activate the Set Scale dialog box.
PlotProfile	Replaces Column Average Plot
ResetCounter	Sets the measurement counter to zero

Special Menu

StartCapturing	Start live video
StopCapturing	Stop live video
Capture	Grabs one frame. Currently only works with DT2255.
AverageFrames	Precede with SetOption to sum frames

SetChannel(channel)	Where channel=0, 1, 2, or 3

Stacks Menu

AddSlice	Adds a slice following the current slice
DeleteSlice	Deletes the current slice
Reslice	

Windows Menu

NextWindow	
TileWindows	
ShowPasteControl	Activates the Paste Control window

Miscellaneous (Note: ROI means Region of Interest)

Beep	
ChoosePic(n)	Selects Nth image window without activating it
ChooseSlice(n)	Selects Nth slice in a stack without displaying it
DrawNumber(n)	Displays *n* at the current location(set with MoveTo)
DrawText('text')	Displays text at the current location
Exit	Terminate execution of the macro
GetColumn(x,y,length)	Copies a column of pixels from the active image to the line buffe
GetLine(x1,y1,x2,y2,LineWidth)	Returns coordinates and width of the current straight line selection
GetMouse(x,y)	Returns location of cursor in local coordinates
GetPicSize(width,height)	Returns *width* and *height* of active image
GetRoi(left,top,width,height)	Returns ROI location and size. Returns *width*=0 if no ROI.
GetRow(x,y,length)	Copies a row of pixels from the active image to the line buffer
GetTime(year, month,day, hour, minute, second, dayofweek)	
InsetRoi(delta)	Shrinks or expands(if *delta*<0) ROI by *delta*
KillRoi	
LineTo(x,y)	Draws a line from current location to (x,y)
MakeLineRoi(x1,y1,x2,y2)	Origin(0,0) is at upper left
MakeNewStack(‘name’)	Creates a new 1-slice stack. Use SetNewSize to specify size.
MakeRoi(left,top,width,height)	Origin(0,0) is at upper left
MakeOvalRoi(left,top,width,height)	
MoveRoi(dx,dy)	Moves ROI right *dx* pixels and down *dy* pixels
MoveTo(x,y)	Origin is always at upper left corner
PlotXYZ	Plots XYZ coordinate data stored in a text file
PutColumn(x,y,length)	Copies *length* pixels from the line buffer to a column in active image
PutMessage('message')	Displays 'message' in a dialog box. Accepts multiple arguments like the Write routine.
PutPixel(x,y,value)	Displays *value* at location (*x*,*y*)
PutRow(x,y,length)	Copies *length* pixels from the line buffer to a row in the active

image	
ResetGrayMap	Equivalent to clicking on reset icon in Map window
SaveState	Saves foreground and background color, new window width and height, status of invert Y flag, and various ScaleAndRotate and SetScaling parameters
RequiresVersion(n)	Aborts macro if Image version number is less than the one specified
RestoreState	Restore settings saved by SaveState
SelectPic(n)	Activates Nth image window
SelectSlice(n)	Display Nth slice in a stack
SetCounter(n)	Sets the measurement counter to *n*
SetForegroundColor(c)	0 <= c <= 255, were 0=white and 255=black
SetBackgroundColor(c)	0 <= c <= 255
SetLineWidth(width)	
SetUser1Label('Label')	Replaces label used for User 1 column in Results window
SetUser2Label('Label')	Replaces label used for User 2 column in Results window

SetPicName(‘Name’)	Renames the currently active image window
SetOption	Equivalent to holding down Option key
SetSliceSpacing(n)	
ShowMessage('message')	Displays 'message' in the Values window. Accepts multiple arguments
	like the Write routine. Use a back-slash(‘\’) to start a new line.
UpdateResults	Redisplays last measurement in Values and Results windows
Wait(seconds)	Fractions of a second are allowed, e.g., wait(1.5)
WaitForTrigger	Waits for QuickCapture external trigger(or mouse button)
Write(e1,e2,...)	Draws text, variables, or constants at the current location. Like th
	Writeln procedure in Pascal, expressions may have optional field width specifications in the form e:f1:f2(e.g., write(‘M= ‘,mean:8:3),
	where f1 is the field width, and f2 specifies the number of digits t he right of the decimal point.
Writeln(e1,e2,...)	Similar to Write, but does the equivalent of a line feed and carriag return after displaying the specified values.

Miscellaneous Functions

AllSameSize	Returns true if all open images have the same dimensions
Button	TRUE if mouse button down
Calibrated	True if current image is density calibrated
cValue(PixelValue)	Converts raw pixel values(0-255) to density calibrated values
GetNumber(‘Prompt’,default)	Displays a dialog box and returns with number entered
GetPixel(x,y)	Returns the pixel value at (*x*,*y*)
GetSliceSpacing	Returns slice spacing(in pixels) of current stack
nPics	Returns number of Image Windows
nSlices	Returns number of slices in current stack
PicNumber	Returns number(used by SelectPic) of active window
rCount	Returns current measurement counter value
Round(n)	Converts a real value to integer with rounding
SliceNumber	Returns number of current slice in a stack
Trunc(n)	Converts a real value to integer with truncation

Math Functions

abs(n)	Returns absolute value of *n*
arctan(n)	Returns arctangent of *n*
cos(n)	Returns cosine of *n* (radians)
exp(n)	Returns exponential of *n*
ln(n)	Returns natural logarithm of *n*
odd(n)	TRUE if integer *n* odd
Random	Generates a random number between 0 and 1
sin(n)	Returns sine of *n*(radians)
sqr(n)	Returns square of *n*

sqrt(n)	Returns sqrt of *n*

Paste Control Options (Use immediately after a paste command)

DoCopy DoAnd DoOr DoXor DoReplace DoBlend Add Subtract Multiply	Precede DoCopy, DoAnd, etc. with SetOption to switch paste transfer modes, otherwise the operation is performed and the paste operation terminated.

Divide

Note that routines that require a file name or window title(MakeNewWindow, Open, SaveAs, Import, Duplicate, and SetPicName) accept multiple arguments similar to Writeln, except that numeric field are left filled with zeros rather than spaces. As an example, SetPicName(‘PIC’,n:2) result in window titles in the form ‘PIC01’, ‘PIC02’, ‘PIC03’, etc.

B. Frame Grabber Cards

Image supports two frame grabber cards for acquiring images from video sources, such as TV cameras and VCRs: the Data Translation QuickCapture and the Scion Image 1000.

Both cards have advantages and disadvantages. When using the QuickCapture, *Image* allows you to adjust brightness, contrast, and pseudocoloring while digitizing. The continuous histogram and live paste features are also currently only available with the QuickCapture. *Image* also supports the full 768 x 512 resolution available with 50Hz(PAL) version of the QuickCapture card. On the other hand, when capturing using the QuickCapture, the screen is redrawn only about four frames a second. Also, the A/D converter in the QuickCapture seems to favor odd pixels values, which results in strange looking histograms. This does not, however, seem to be a problem in normal use, and Data Translation claims to have a fix.

The Scion card, in addition to being less expensive, has the ability to capture and displaying on the screen at 30 frames per second. One disadvantage of this card is that, since it copies video data directly to the video card over the NuBus at video rates(about 9 MB/sec.), it doesn't work with some third party video cards, or is unable to capture the entire 640x480 frame. On the 640x480 Apple video card, it is able to capture 600x480 frames. A major disadvantage of the Scion is that it does not work with the built-in video available on newer Macintosh's, such as the IIsi, IIci, Quadra 700, and Quadra 900. The Scion card also has a 4-8% vertical shading problem and is also considerably noisier than the QuickCapture card.

Both Data Translation and Scion offer optional software for their respective cards for digitizing in color using an RGB color camera, and then reducing the resulting 24-bit color image to an 8-bit color

PICT file that can be opened in *Image*.

1) **Data Translation QuickCapture**

   Part Number: DT2255
   Price: $1295
   Requires EP205 Cable Assembly: $125

   Data Translation, Inc.
   100 Locke Drive
   Marlboro, MA 01752
   Phone: 508-481-3700
   Fax: 508-481-8620

2) **Scion Video Image 1000**

   Price: $895

   Scion Corporation
   152 West Patrick Street
   Frederick, MD 21701
   Phone: 301-695-7870
   Fax: 301-695-0035
   D1887@applelink.apple.com

C. Recommended Camera and Light Box

Quantitative image analysis using *Image* requires the use of a high quality TV camera and light source. You should select a high resolution solid-state(CCD) monochrome camera designed for imaging applications. There should be a way to disable any automatic gain control(AGC) or automatic black level features. The light source should provide uniform illumination, as well as stable output with normal line voltage variation.

The Sierra Scientific MS-4030 solid state(CCD) camera is one camera that is well suited for quantitative densitometry. A less expensive camera that is also suited for scientific imaging applications is the COHU Model 4815-5000. Both Sierra Scientific and COHU also sells lenses and filters. Another highly rated CCD camera is the Sony XC-77. When ordering a CCD camera, particularly the Sony XC-77, make sure it comes with all necessary components, such as power supply, tripod attachment(if needed), and documentation.

Imaging Research, creator of the highly regarded MCID biomedical image analysis system for the IBM-PC, makes a stabilized fluorescent illuminator that provides the uniform illumination needed for autoradiographic densitometry. They also sell several needed accessories, including the Nikon Micro-Nikkor f2.8/55 mm. lens, the F-C adapter needed to connect the lens to a TV camera, and the Kaiser RS-1 copy stand. In addition, they sell Sierra Scientific, COHU, and Sony cameras.

For specialized applications, Perceptics has a Nubus frame grabber card that supports the 1340X1037 resolution Videk MegaPlus camera, and can supply a version of Image that supports the MegaPlus. Photometrics has a cooled CCD imaging system for the Mac II that supports CCDs with a resolution of up to 2K X 2K. Dage-MTI has a real-time video processor that does real-time

averaging and integration to enhance noisy, low contrast images. They also have an image intensifier for CCD cameras.

COHU Inc.
5755 Kearny Villa Road
San Diego, CA 92138
619-277-6700

Dage-MTI, Inc.
701 N. Roeske Ave.
Michigan City, Indiana 46360
219-872-5514

Imaging Research Inc.
Brock University
St. Catharines, Ontario
Canada L2S 3A1
416-688-2040

Perceptics Corp.
725 Pellisippi Parkway
Knoxville, TN 37933
615-966-9200

Photometrics Ltd.
3440 East Britannia Drive
Tucson, AZ 85706
602-889-9933

Sierra Scientific
Sunnyvale, CA
408-773-5600

Sony Corporation of America
Component Products Company
10833 Valley View St.
Cypress, CA 90630
714-229-4197

D. Alternative Macintosh Analysis Systems

BRAIN
(2-DG and receptor autoradiography)
Image and Computer Vision Center, Drexel University
Philadelphia, PA
215-895-1381

DIP Station
(Medical image analysis, 16–bits, surface rendering)
Hayden Image Processing Cleveland, Ohio
216-721-2388 (whc@po.cwru.edu)

Enhance
Micro Frontier
Des Moines, Iowa
515-270-8109

The Explorer
(Medical image analysis)
UCLA
Los Angeles, CA
213-825-0757

Image Analyst
Automatix
Billerica, MA
508-667-7900

ImageSet
Dapple Systems, Inc.
Sunnyvale, CA
408-733-3283

IPLab, IPLab/Spectrum
(FFT, >8-bits grayscale, 24-bit color, scripting)
Signal Analytics
Vienna, VA
703-281-3277

MedVision
(Medical image analysis, multi-modality studies, 16-bits, extendible)
Evergreen Technologies, Inc.
Gaithersburg, MD
301-948-1800
(evergreen@applelink.apple.com)

NCSA Image, PalEdit, Layout, and Gel Reader (all public domain)
(Scientific visualization, palette editing, gels)
NCSA, Champaign, IL
217-244-0072
Anonymous ftp: zaphod.ncsa.uiuc.edu

Photoshop
(Color image processing, 24-bit to 8-bit color conversion, file conversion, plug-in modules)
Adobe Systems
Mountain View, CA
415-961-4400

PixelTools, TCL-Image
(Video rate processing, 1340x1035 frame grabber)
Perceptics Corporation
Knoxville, TN
615-966-9200

Transform, View, Format, and Dicer
(Scientific visualization, presentation graphics, volume visualization)
Spyglass, Inc.
Champaign, IL
217-355-1665

VoxBlast
(Volume rendering)
VayTek,Inc
Fairfield, Iowa
515-472-2227

VoxelView/Mac
(Volume rendering)
Vital Images
Fairfield, Iowa
515-472-7726

Ultimage
GTFS Inc.
Santa Rosa, CA
707-579-1733

E. File Formats

TIFF Files Created by *Image*

Bytes 0-7 contain the TIFF header. Bytes 8-129 contain the TIFF Image File Directory(IFD). Within the IFD, bytes 30-31 contain the image width, and bytes 42-43 contain the height. Bytes 130-255 are currently unused and are set to zero. Bytes 256-767 contain a 512 byte header unique to the *Image* program. Image data starts at byte 768, stored in row order, one byte per pixel.In the case of a stack, there are two or more consecutive images starting at byte 768 and bytes 516-517 contain the number of images(slices). The TIFF color table, if any, follows the image data. It consists of 256 16-bit red values, 256 16-bit green values, and 256 16-bit blue values. Any additional TIFF IFDs(only needed for stacks) follow the color table. Note that the 16-bit integers containing the width, height, and number of images have the opposite byte order as compared to IBM PC and VAX systems.

The information provided here should be adequate for writing a program to read a TIFF file created by *Image*. It is not sufficient for creating TIFF files that *Image*, or any other program, can read. More detailed information on TIFF is available via anonymous FTP from alw.nih.gov in the directory /pub/image, or from the Developers Desk, Aldus Corporation, 411 First Avenue South, Seattle, WA 98104, (206) 622-5500.

MCID File Format

The easiest way to create variable sized image files that can be read by *Image* is to duplicate the MCID format accepted by the Import command. These files contain the line width less one in bytes 0-1, the number of lines less one in bytes 2-3, followed by the 8-bit pixel data in row order. The 2 bytes (one 16-bit word)used to store the width and height are stored in Intel/VAX byte order, where the low order byte is first. The following Pascal program is an example of how to create a file that can be Imported into *Image*.

```
PROGRAM MakeRamp;
{
This example Pascal program creates an image
file that can be opened using Image's Import command.
}
VAR
 f:text;
 i,line,pixel,PixelsPerLine,nLines:integer;
BEGIN
 write('Pixels Per Line: '); readln(PixelsPerLine);
 write('Number of Lines: '); readln(nlines);
```

```
 writeln('Creating image. This may take a few minutes.');
 rewrite(f,'Ramp');
 write(f,chr((PixelsPerLine-1) MOD 256));
 write(f,chr((PixelsPerLine-1) DIV 256));
 write(f,chr((nlines-1) MOD 256));
 write(f,chr((nlines-1) DIV 256));
 FOR line:=1 TO nlines DO
   FOR pixel:=0 TO PixelsPerLine-1 DO write(f,chr(pixel));
 close(f);
END.
```

F. Program Limitations

The maximum number of pixels per line is 4096. There is no arbitrary limit(other than memory requirements) on the maximum number of lines in an image. *Image* uses two 300K buffers as Undo and Clipboard buffers. Many functions will not work with images larger than these buffers. The size of these buffers can be changed in the Preferences dialog box. You must Save Preferences, Quit, and then restart *Image* before the buffer size change will take effect.

The maximum number of characters that can be displayed, printed, or copied to the clipboard is 32,700. There is no limit to the number of measurements that can be exported to a text file.

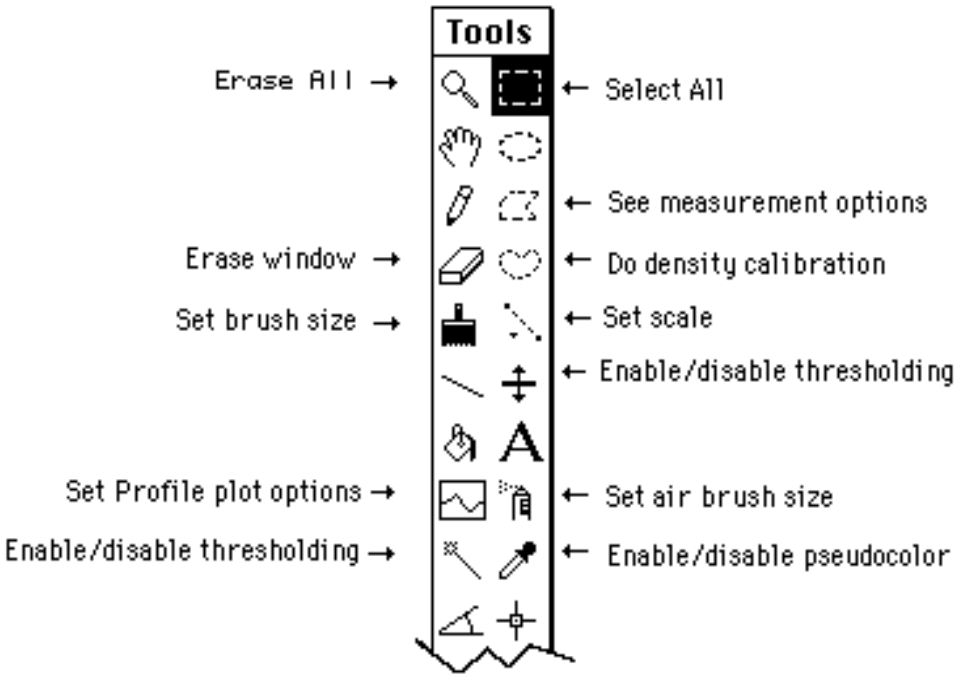
The default maximum number of measurements is 200. This can be changed in the Options dialog box. You must Save Preferences, Quit, and then restart *Image* before the change will take effect.

The maximum size of a macro file is 15,000 bytes. A macro file can contain up to 50 macros and 50 procedures. The push-down stack used by the macro language holds up to 100 variables.

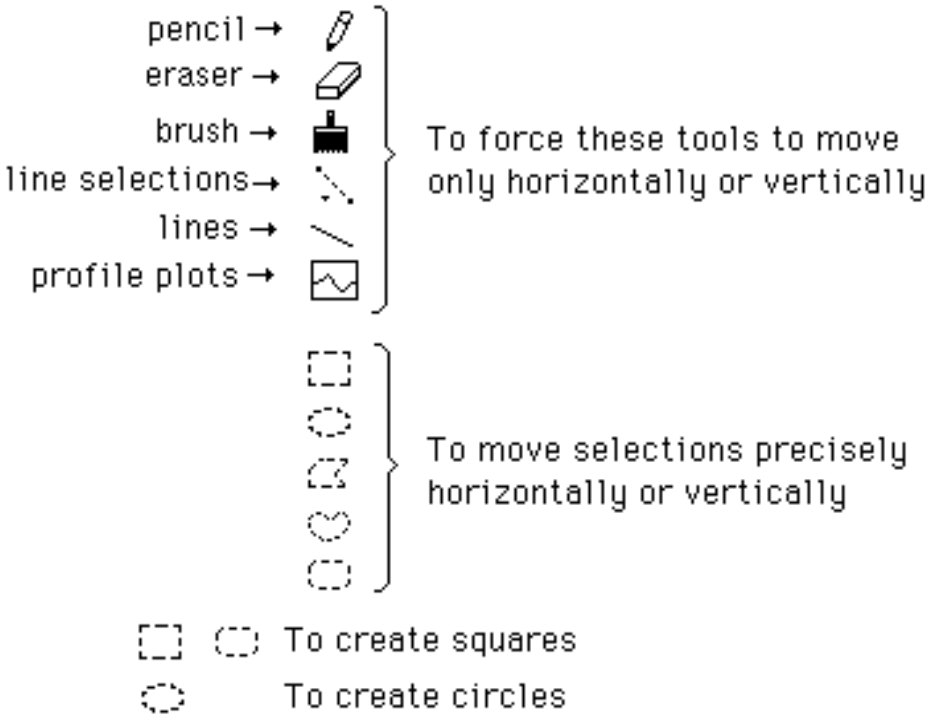
The maximum number of slices in a stack is 256.

G. Shortcuts

Double-Click on tool to:



To constrain tool functions hold down the shift key:



H. References

Color, Motion Analysis and Biological Imaging, Joseph Ayers and Garth Fletcher, *Advanced Imaging*, November 1990, pages 39-42.

Computer-Aided Cell Colony Counting, Robert Parry, et. al., *BioTechniques*, Vol. 10, No 6, 1991, pages 772-774.

Digital Image Processing, Kenneth Castleman, Prentice-Hall, 1979.

Digital Image Processing: A Practical Primer, Gregory Baxes, Prentice-Hall, 1984.

Image Analysis for All, Paul Lennard, Nature, Vol 347, 6 September 1990, pages 103-104.

Image Processing on the Macintosh, Robert Morris, *IEEE Computer*, August 1990, pages 103-106.

Microcomputer-based Three-dimensional Reconstruction of in situ Hybridization Autoradiographs, Thomas Ford-Holevinski et al., Journal of Chemical Neuroanatomy, Vol 4, 1991, pages 373-385.

PCs Invade Processing of Biomedical Images, *Diagnostic Imaging*, February 1990, pages 139-148.

A Six-Pack for the Mac, Steven Hollinger, *ESD: The Electronic System Design Magazine*, June 1989, pages 26-36.

Use of Image Analysis to Quantitate Changes in Form of Mitochondrial DNA After X-irradiation, Raymond O'Neill et al., *Applied and Theoretical Electrophoresis*, 1989-1, pages 163-167.

Video Microscopy, Shinya Inoue', Plenum Press, 1986.

I. Updated Versions and Bug Reports

Updates to *Image* are available to Internet users via anonymous ftp from zippy.nimh.nih.gov. Those without Internet access can get updates from many Macintosh bulletin boards and user group libraries. A reasonably current version, including Pascal source code and example images, should be available from one of the following:

1) From a friend. The *Image* program, including source code and documentation, is public domain and may be freely copied, distributed, and modified. However, if you modify *Image*, please update the about box before distributing your version of the program.

2) Via anonymous FTP from zippy.nimh.nih.gov[128.231.98.32]. Enter "anonymous" as the user name and anything you like as the password. The /pub/image directory contains the latest version of *Image*(image144.hqx), a version of *Image* that runs on Macs without a floating-point coprocessor (image144NonFPU.hqx), documentation in Word format(image144_manual.hqx), and complete Think Pascal source code(image144_source.hqx). The directory /pub/image/images contains sample TIFF and PICT images. The directory /pub/image/image_spinoffs contains versions of *Image* extended to do FFTs, fractal analysis, to capture and analyze color images, and to support quantitative evaluation of cerebral blood flow, glucose metabolism, and protein synthesis. Most of the same files are also available from alw.nih.gov [128.231.128.7] in the directory /pub/image. There is a README file(0README.txt) with information on the file formats used.

3) DL14 of MACSYS on CompuServe.

4) Twilight Clone BBS in Silver Spring, MD. The Clone has 16 lines on sequential rollover, starting with 301-946-8677. To guarantee a V.32 connection, call 946-5034. *Image* is currently available at no charge from the Twilight Clone.

5) NTIS(National Technical Information Service), phone 703-487-4650, order number PB90-500687 ($100 check, VISA, or Mastercard). Both the zippy.nimh.nih.gov FTP site and the Twilight Clone BBS are likely to have newer versions of *Image* than NTIS.

Bug reports and suggestions are welcome, as are corrections or additions to this manual. The author (Wayne Rasband) can be reached at the following electronic mail addresses:

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AppleLink: wayne@helix.nih.gov@internet#
CompuServe: >INTERNET: wayne@helix.nih.gov

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