NIH Image 1.41

Introduction

Image is a public domain program for the Macintosh for doing digital image processing and analysis. It can acquire, display, edit, enhance, analyze, print, and animate images. It reads and writes TIFF, PICT, and MacPaint files, providing compatibility with many other Macintosh 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 the area, average density, center of gravity, and angle of orientation of a user defined region 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. Results can be calibrated to provide real world values.

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.

It 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. It uses digital halftoning to print images on PostScript printers and Floyd-Steinberg dithering for printing on non-PostScript printers.

It supports the Data Translation QuickCapture card for digitizing images using a TV camera. Acquired images can be shading corrected and frame averaged.

Image is written using Think Pascal from Symantec Corporation, and the source code is available. The program can be easily ported to MPW Pascal.

System Requirements

Image requires a Macintosh with at least 2 megabytes of memory, but 4 megabytes or more are recommended for doing animation, for simultaneously displaying more than a handful of pictures, or for running under MultiFinder. It requires a monitor with the ability to display 256 colors or shades of gray. It also requires a floating-point coprocessor or the PseudoFPU Init, which can

emulate a missing coprocessor. *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 palette), 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 switch to alternate color palettes. *Image* currently supports two image file formats(TIFF and PICT) and four palette formats.

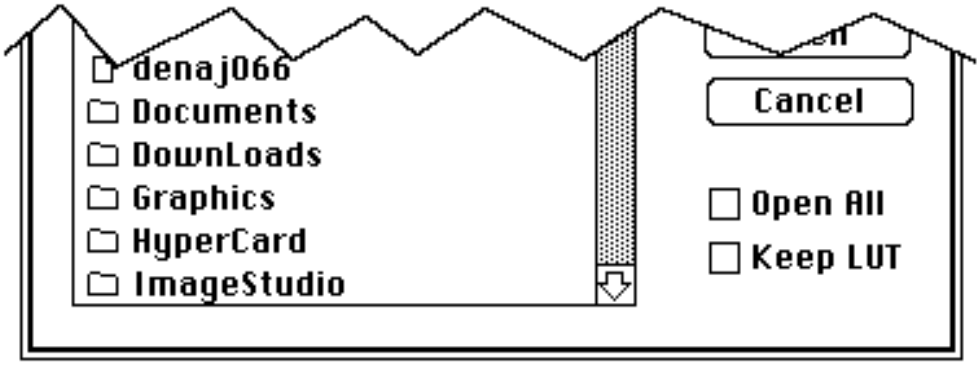
1) 8-bit grayscale and color TIFF files created by *Image* and many other Mac programs. Image will open as a stack a TIFF file containing multiple images, but the images must all have the same width and height and the width must be even. *Image* is unable to open bitmap(1-bit), 24-bit color, or compressed 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 many Mac programs, including MacDraw, Digital Darkroom, and PixelPaint. 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) *Image* pseudocolor palettes, which are currently limited to a maximum of 32 colors, and are unique to the *Image* program. Colors in these palettes can be edited using the eyedropper tool, and the number of colors can be changed using Set Number of Colors.

5) 256 color palettes created by PixelPaint, by Canvas , or by the Klutz DA. PixelPaint and Canvas have palette editors which can be used to create 256 color palettes for use by *Image* . The public domain PalEdit program from NCSA may also be used to create 256 color palettes. The Klutz DA can be used to “capture”palettes used by other programs.

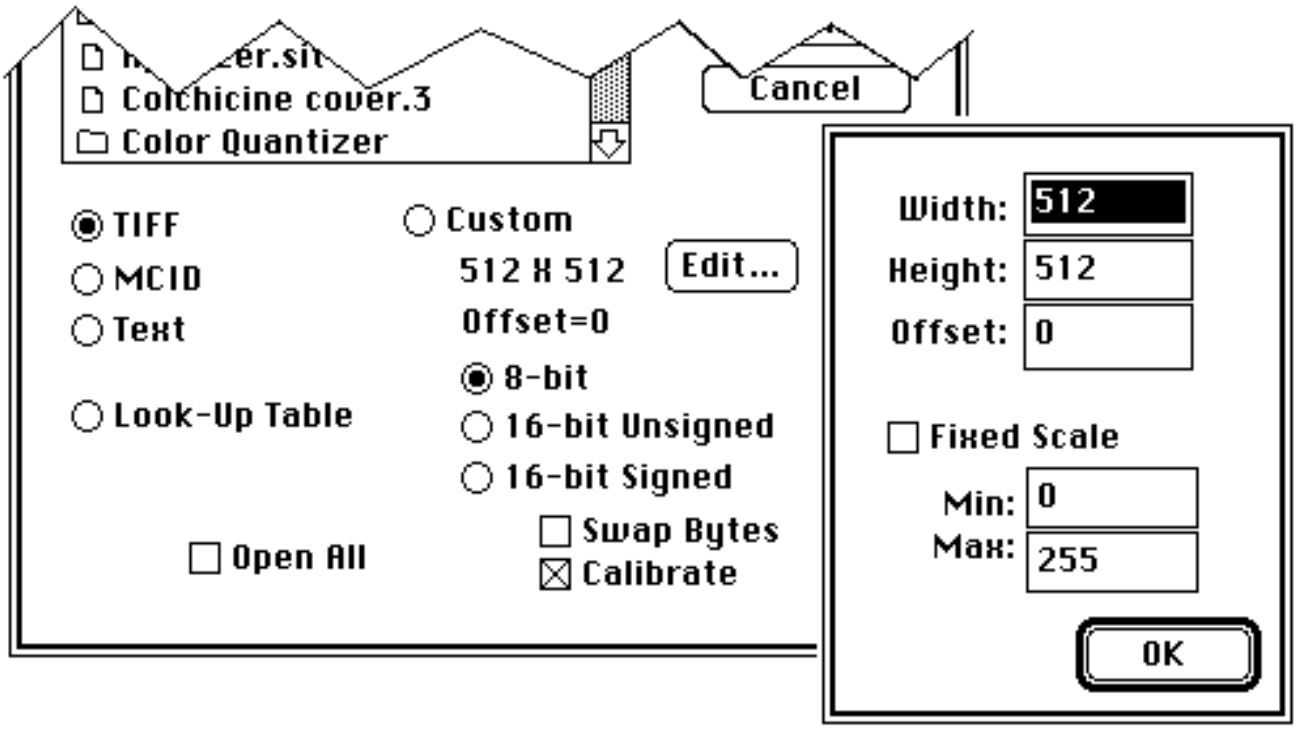


If you check *Open All*, select one image within a folder, and then click on the Open button, *Image* will open all the images in that folder, not just the one selected. This is a good way to load a series of images to be animated using the "Run Movie" command.

Check *Keep LUT* if you want the image being opened 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 a PICT file is drawn on the screen, and the image and screen LUTs are different, the pixel values are remapped to make the colors consistent.

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 palettes. It presents a dialog box that allows you to select the type of file to be imported. TIFF, MCID, Text, and Custom specify various image file formats.


er.sit
Colchicine cover.3
Color Quantizer
Cancel
TIFF
MCID
Text
Look-Up Table
Custom
512 X 512
Offset=0
Edit...
8-bit
16-bit Unsigned
16-bit Signed
Swap Bytes
Calibrate
Open All
Width:
512
Height:
512
Offset:
0
Fixed Scale
Min:
0
Max:
255
OK

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 real, but not 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 2048. *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 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 palettes 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. Custom 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.

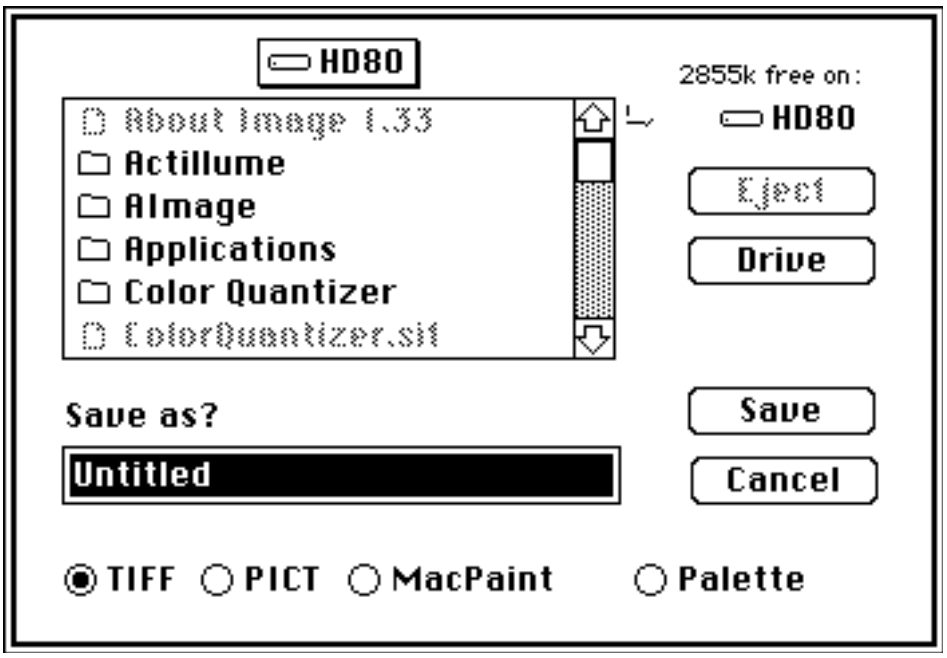
Save

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. This is a convenient way to save a set of images acquired using Make Movie.

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 to a file in 8-bit TIFF format. You will be asked to enter a name for the new file. Changes to Save Selection As TIFF if a rectangular selection is active, allowing you to save a subsection of the image. 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 Open and Save faster than PICT files.

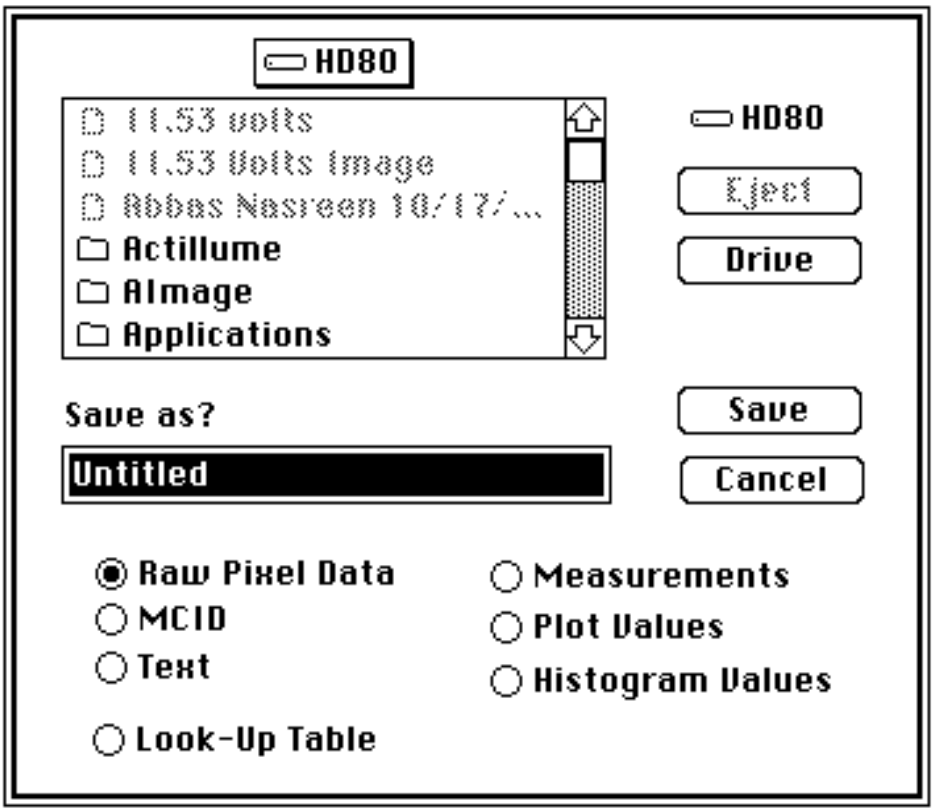
PICT
Saves the currently active image to a PICT file. PICT files can be exported to many other Mac programs, including PixelPaint, Digital Darkroom, SuperPaint, and MacDraw. Because they are compressed, PICT files also have the advantage of being more compact if the image contains large areas of uniform density, 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 PICT" if a rectangular selection is active. **Warning:** Don't save pseudocolored images in PICT format if you are using 32-bit QuickDraw, and if you plan to make future density measurements, since pixel values may get altered.

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

Palette
Saves the current pseudocolor palette.

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, stored 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 area and density measurements to a tab-delimited text file compatible with spreadsheet and statistical analysis programs. Additional measurements can be enabled using the Analyze/Options dialog box.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.

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 the Image application. 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

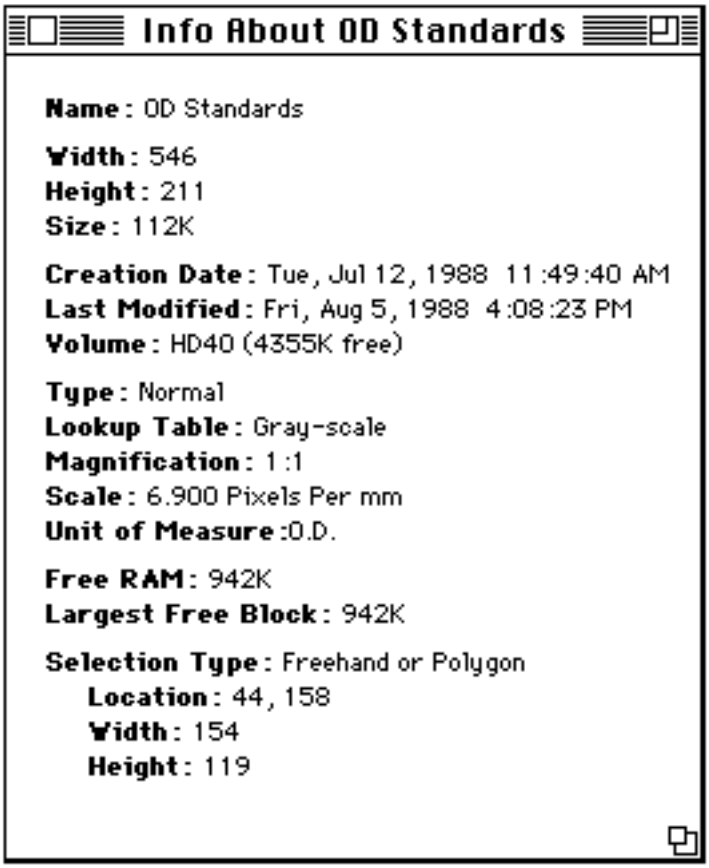
Reverts to the most recently saved version of the current image, effectively undoing all changes made since the last Save command. Reverting does not currently work with imported 16-bit images and with imported text files.

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 palette), 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. 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". 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 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 *Halftone* and *BitMap* buttons in the Print dialog box to specify what kind of printing to do.

Color printing is supported on the Tektronix 4693D, HP PaintJet, and similar printers. 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 Results or Measurements window is 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 copy colors from the LUT window to the Clipboard and then paste them back into another palette location. Copy and Paste of colors is limited to pseudocolor palettes, which have a maximum of 32 colors.

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 on it 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 in the Paste Control window, 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 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 the Text 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 Text 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 eye dropper tool in the LUT window or in the active image window. The color of the brush in the tool palette is an indicator of the current foreground color.

Invert

Inverts 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 picture, 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

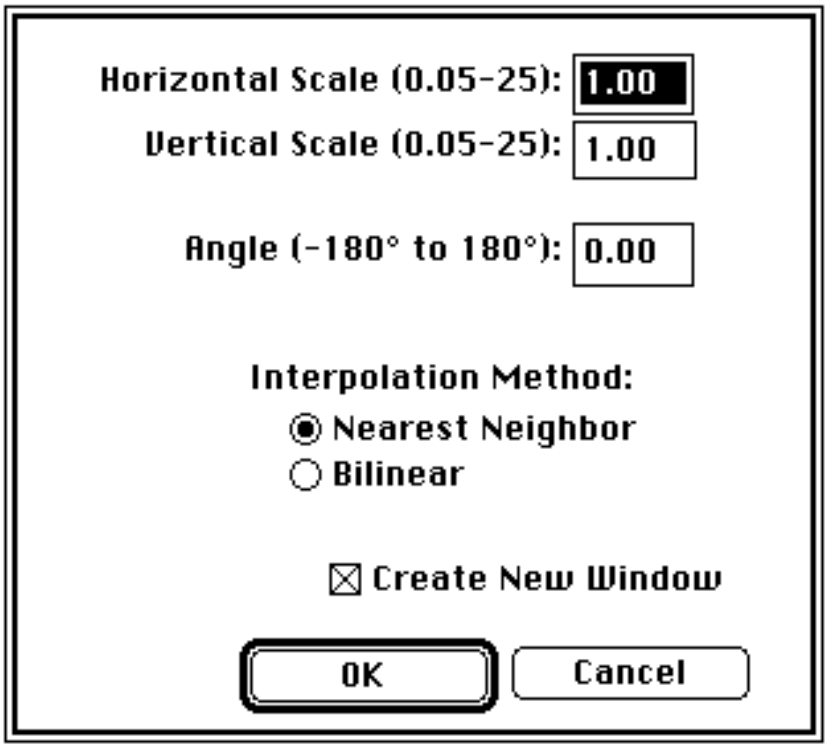
Flips the current rectangular selection horizontally around a vertical line through its center. The entire image is flipped horizontally if no selection is in effect.

Flip Vertical

Flips the current rectangular selection upside down. Flips the entire image if no selection is in effect.

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 lookup 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 of the look-up table, which are always set to white and black respectively, are not 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 pseudocolor palette. Any added colors will be set to shades of gray. Newly added colors can be edited by selecting the eyedropper tool and double-clicking in the LUT window on the color you want to change. Pseudocolor palettes are currently limited to 32 colors.

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. Extra colors are displayed at the bottom of the LUT window, allowing you to edit them by double-clicking with the eyedropper Tool.

Palettes

Image currently supports four different types of look-up tables:

1) **Grayscale** - Consists 256 shades of gray. Contrast and brightness can be altered by manipulating the transformation function displayed in the Gray Map window. This function can be applied to the pixel data in memory, thereby making any contrast and brightness changes permanent, by using the Apply Look-Up Table command.

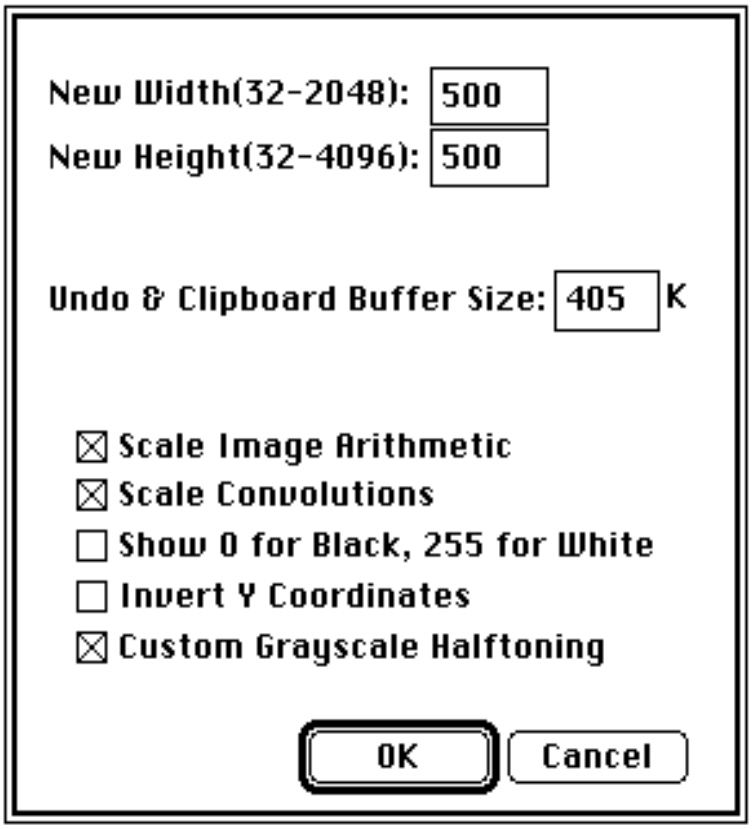
2) **Pseudocolor** - This is the default color mode in *Image* and was inherited from the PDP–11 version. In this mode your are limited to a maximum of 32 colors. You can use the LUT tool(the double headed arrow) to both stretch (changing contrast) and shift the colors within the LUT. Also, in this mode, you can edit colors by double clicking on them with the eyedropper tool.

3) **System** - This is Apple's standard 256-color palette. It is the same as the PixelPaint's System palette. You can use the LUT tool to rotate this palette.

4) **256 Color Spectrum** - This is a continuous color spectrum which you can rotate using the LUT tool.

Preferences

Displays the dialog box shown below, which allows you to change various *Image* parameters.



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 *Show Zero for Black, 255 for White* sets up an inverting linear density calibration function for the current image that results in gray level values displayed in the Results window, 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.

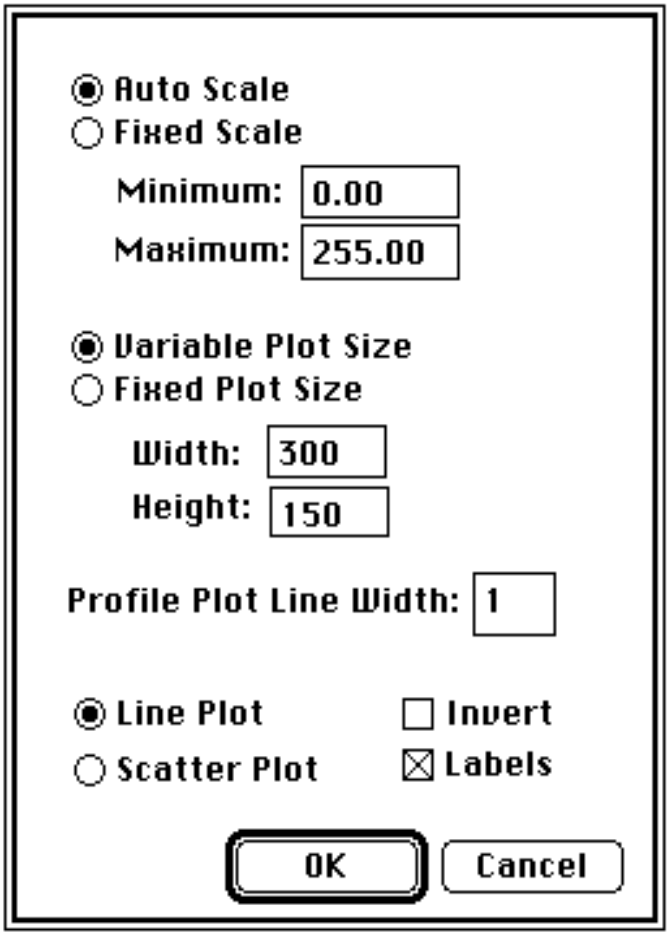
Check *Invert Y Coordinates* if you want the X-Y origin to be the lower left corner of the image window, otherwise it will be the upper left corner.

You can deselect *Custom Grayscale Halftoning* if you are using a LaserWriter driver with a version number of 6.0, or greater. This enables the grayscale and color printing capabilities of the driver and disables the Postscript halftoning built into *Image.* Changes made in the Halftone Options dialog box are only effective when this option is selected.

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 Column Average Plot command. In *Auto Scale* mode, profile plots will be scaled depending on the minimum and maximum density values. In *Fixed Scale* mode, the y-axis range is fixed and the minimum and maximum may be specified. *Fixed Plot Size* allows specify the size of the plot window. This is useful for pasting a series of plots into an image window. *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 Scale 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 density 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 Results window.

The Make Binary command can be used to set all thresholded pixels to black, and 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 Gray 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 density measurements. Use the LUT tool and click and drag near the top of the red region in the LUT window to vary the size of the density slice. Click and drag near the bottom of the red region to slide the density slice up and down. As you manipulate the density slice, its upper and lower limits are continuously displayed in the Results window.

Double click within the density slice in the LUT window with 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). The Make Binary command 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 of a set to all the other images in the set.

> **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.

Density Calibration - The density calibration associated with the currently active image is transferred to all other open images.

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 which use spatial convolution. The popularity of plus and minus one 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
```

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 Gray Map window before doing the dithering. True color images should be converted to grayscale using Apply LUT before being dithered.

Convolve - Does spatial convolutions using kernels, which are read from a text file, than can be up to 63 x 63 in size. Desk accessory text editors, such as MockWrite or MiniWriter, 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
```

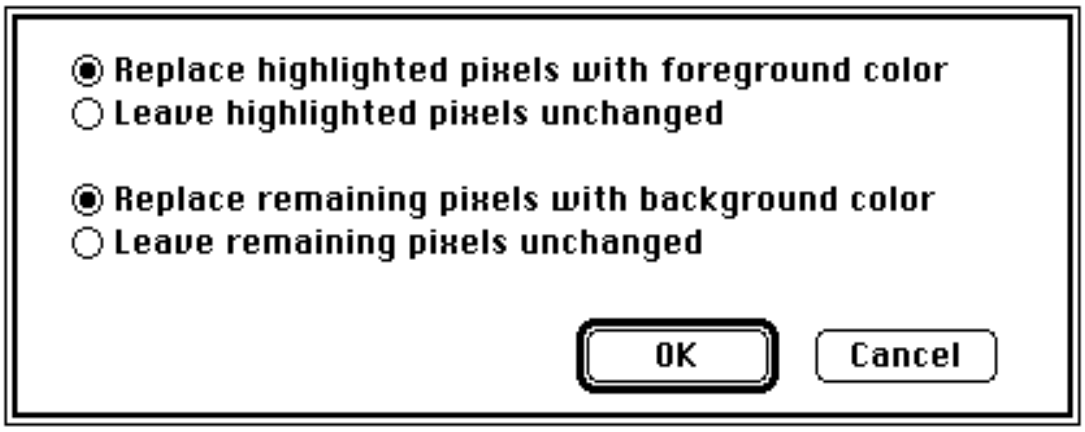
This is a 9 x 9 “Mexican hat” filter which will do both smoothing and edge detection in one operation. Each line should be terminated with a carriage return, and the coefficients should be separated by one or more spaces, or by a tab. Note that kernels such as this one can be opened and displayed as an image using the Import(Text) command, scaled to a reasonable size using Scale and Rotate, and plotted using 3D Plot.

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 Gray Map window. Use Apply LUT to make the contrast change permanent. Equalization only works with grayscale 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.

Binary

The commands in this submenu are used to convert grayscale images to binary(black and white only) and to process these binary images.

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 Iterations - Allows you to specify how many 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 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.

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 density of the current selection and displays the results in the Results 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. If either density slicing or thresholding are enabled only highlighted pixels are included in the computation. 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 a list of the current results, Print(with either the Results or

Measurement 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.

If Set Scale has been used to establish the spatial scale, then the area of the selection will be given in calibrated units, such as square millimeters. If Calibrate has been used to perform density calibration, then density 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.

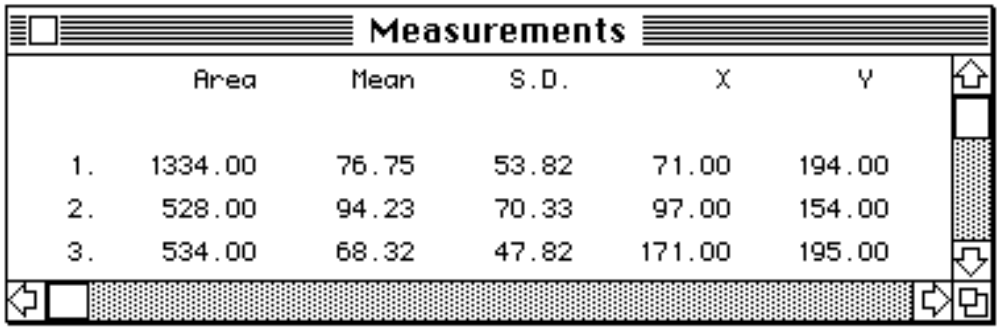
The most efficient way to record measurements is to use MultiFinder, and to have a spreadsheet on the screen at the same time you are using *Image*. Make a series of measurements(Command-1), Copy them to the Clipboard (Command-C), click on the spreadsheet to activate it, select the cell where you want the measurements stored, Paste the results(Command-V), click on the image window to reactivate *Image*, and finally, Reset the measurement counter(Command-3). If you use Microsoft Excel, make sure it's version 2.2(or later), since earlier versions do not work reliably under MultiFinder.

Analyze Particles

Analyze Particles automatically counts and measures objects in a binary image, or thresholded objects in a grayscale 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

Open a window named Measurements and displays the current results list in it. If the Measurements window is already open then it is activated.



Use *Export* (command-S when the Measurements 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 with the output. 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 Results 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 the Save As command.

Plot Profile

Generates a density profile plot from straight line or rectangular selections. For rectangular selections wider than they are tall(or 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 density 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. 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 control various aspects of these plots.

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.

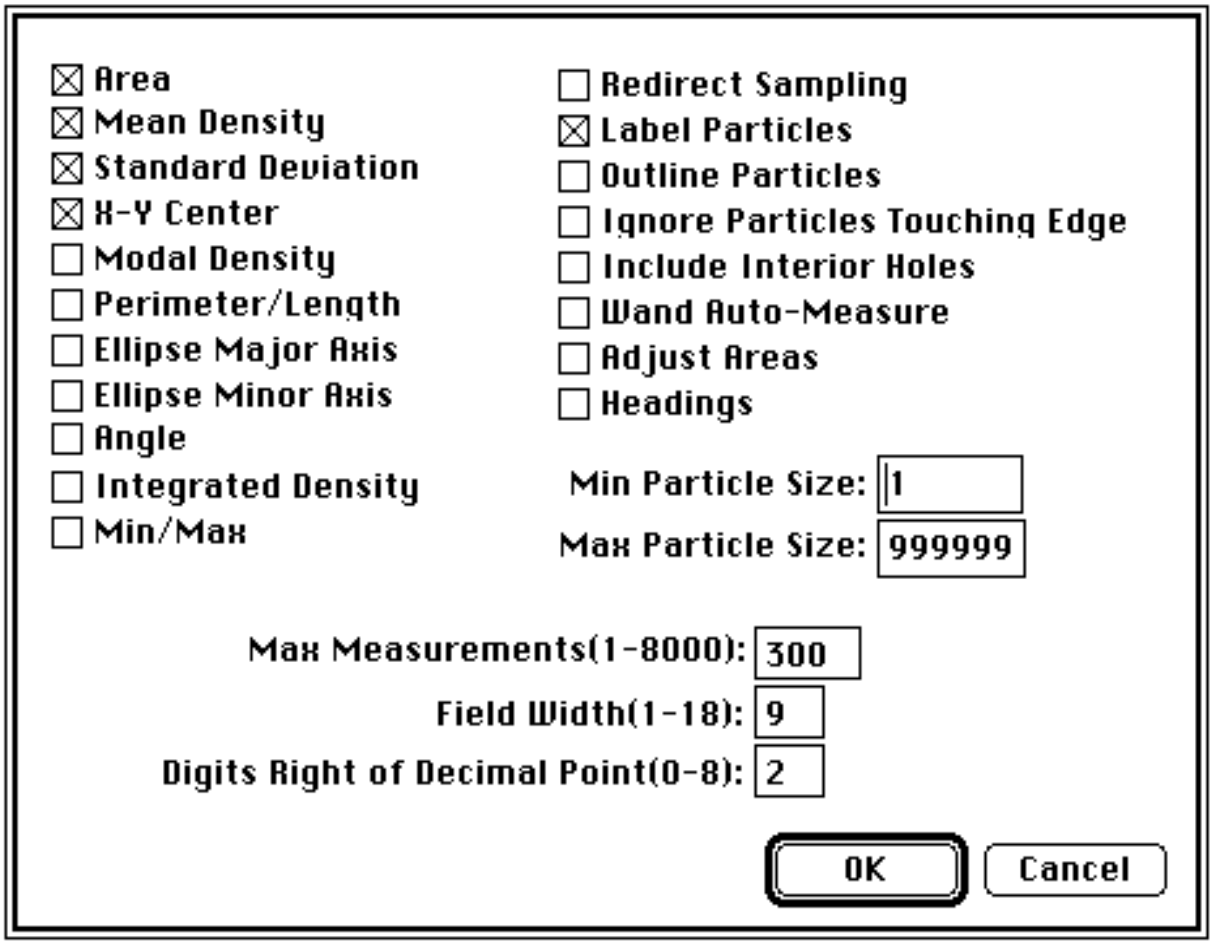
3D Plot

Generates a 3-dimensional plot of the current selection. If no selection is active the entire image is plotted. The 3-D plot will br 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 3D plots by increasing the contrast of the image before plotting using the controls in the Gray 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 the Show Results command. Double-clicking on the polygon tool also brings up this dialog box.



Area -Area of the 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 Density - Average gray level 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 pixel densities used to generate the mean density.

X-Y Center- Unweighted center of mass of the selection, 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 Density - Most frequently occurring gray level within the selection. Corresponds to the highest peak in the density histogram.

Perimeter/Length - Length around the outside of the selection, or line length for line selections. 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 * (MeanDensity - BackgroundDensity)

where N is number of pixels in the selection, and BackgroundLevel is the modal density(most common pixel value) after smoothing the density histogram. The background level may 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 pixel density values within the current selection.

When *Redirect Sampling* is checked, pixel data for calculating mean density 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. 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.

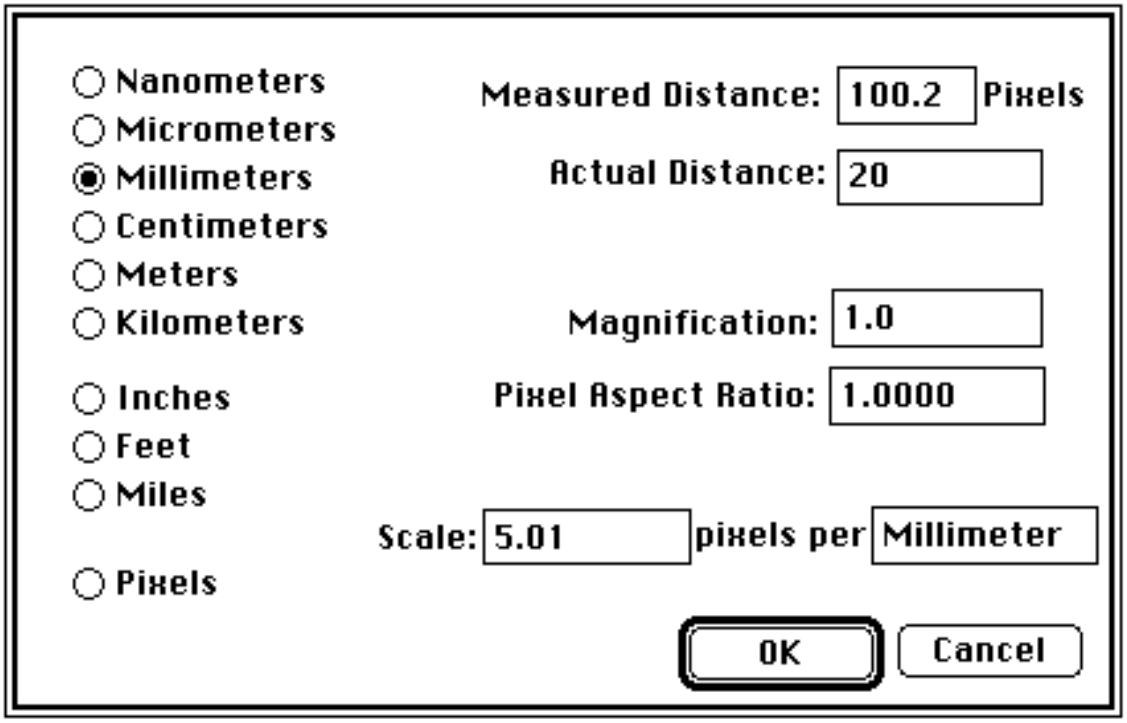
Column and row headings will be added to results Copied to the Clipboard or Exported to a text file if *Headings* is checked.

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 Measurements 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, you must first 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*.

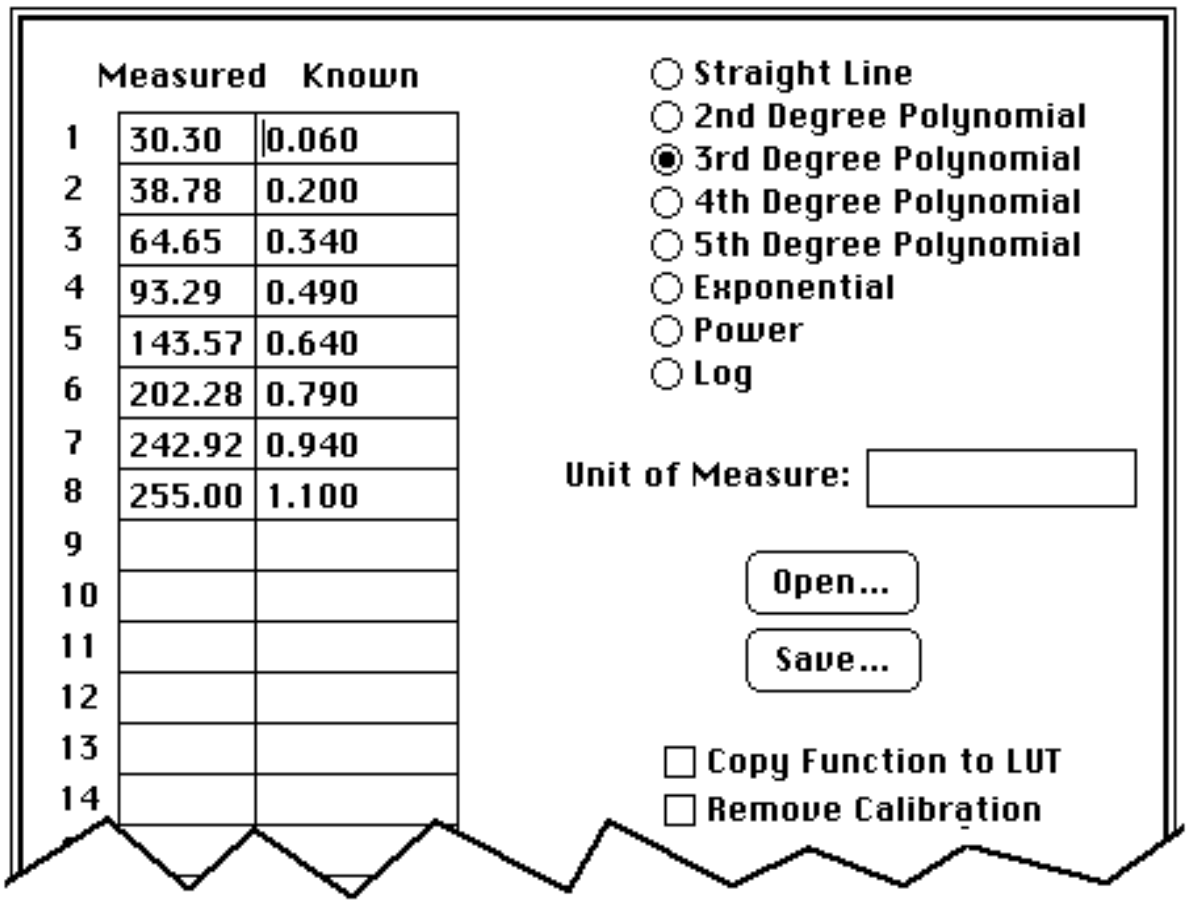


Setting *Pixel Aspect Ratio* to a value other than 1.0 enables support for different horizontal and vertical spatial scales, for example 100 pixels/cm horizontally and 95 pixels/cm vertically. Before setting the aspect ratio, calibrate to a known horizontal distance. Then enter the pixel aspect ratio into the Pixel Aspect Ratio field. 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, you can convert from inches to centimeters. If you know what the scale for an image is(e.g., 300 DPI), then, for the 300 DPI example, enter 300 in *Measured Distance*, and 1 in *Actual Distance*. Click on *Pixels* to disable spatial calibration. Double-clicking on the line selection tool will also present the Set Scale dialog box.

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 density of each of the standards. Note that the standards must be measured in order, starting with the lowest density(lightest) standard. When you have finished making the measurements, select Calibrate and enter the actual values into the *Known* column of the dialog box.



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 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.

Redo Measurement

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

Mark Selection

Outlines the current selection in the current foreground color 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 2 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 will 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. The number of frames to be averaged is specified using Preferences.

Hold down the option key 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.

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 Results window, is approximately 128.

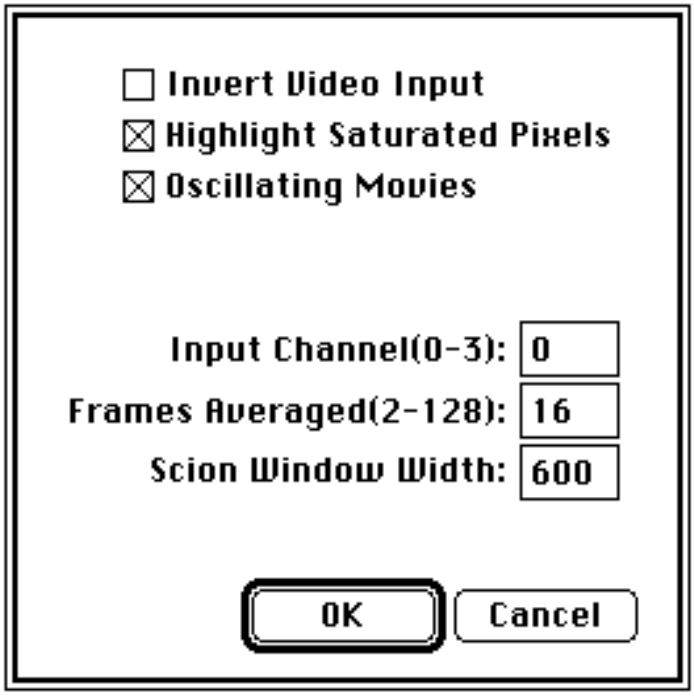
The Stop Capturing command will use the reference image previously acquired by Save Blank Field to correct shading errors in the newly acquired image. It does this by dividing each pixel in the newly acquired image by a correction factor computed for each of the 300,000 pixels in the blank field. This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

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.

Shading correction using division works best with CCD cameras. If you are using a tube camera, you may want to try the Subtract option in the Paste Control window to do shading correction.

Video Options

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



Images acquired using a 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).

Allows you to specify which input channel is used by the QuickCapture or Scion frame grabber cards. The input channels for both cards are numbered from 0 to 3.

Frames Averaged specifies the number of video frames that will be averaged by the Average

Frames command.

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.

Stacks

The commands in this submenu create or operate on stacks. Stacks 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. Most commands in *Image*, with the exception of Save, SaveAs, and Open, operate only on the current slice. Several example macros are available that operate on all the slices in a stack

Windows to Stack - Converts the set of images currently being displayed in separate windows to a stack. The images must all be the same size and must an even width.

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 big enough to hold the number of frames specified. You will then 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 .2. After *Image* has completed recording the movie it will compute the actual frame rate and display it in the Results window. If the computed rate is not the desired rate then the frames were probably captured at uneven intervals. If you need an even sampling rate, you should either specify a slower rate from the table, or make a smaller selection. Use command-period to abort a movie making.

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.

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 thickness(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.

Reslice Options - Allows you to change the slice thickness.

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, the macro named “Test Macro [T]” is assigned to the T key, and “Another Macro [F1]” is assigned to F1. 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 an arrow head; 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; 3-D reconstruction; Recovering X-Y coordinate data from line plots; and Generating an ASCII dump.

The macro programming language is documented in Appendix A.

Text Menu

Text handling in *Image* is similar to that in Macintosh painting programs such as MacPaint, SuperPaint, and PixelPaint, with the exception that you currently can only change 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.

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

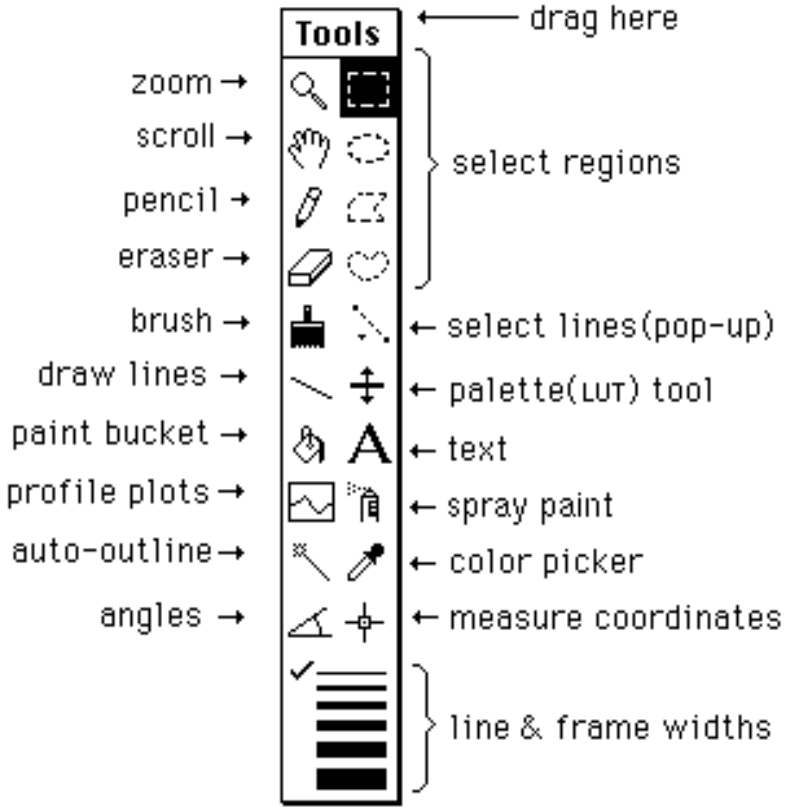
Expands all the image windows to their original size and redraws them slightly offset from each other. If you hold down the Option key, all windows will be drawn in the same location, at the upper left corner of the screen, next to the tool palette.

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 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 Results 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.

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 pseudocolor palettes 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.

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.

There is currently a bug which sometimes prevents paint from leaking near the right edge of images with widths that are not evenly divisible by eight.

Text Tool
When only words will do. Allows typing in the Font and Style chosen in the appropriate menus. 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. Using Show Results will allow you to automatically 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.

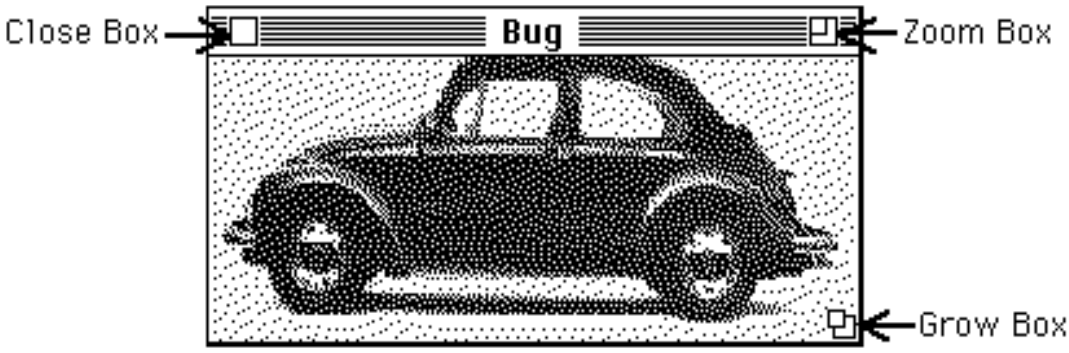
Angle Tool - Measures the angle formed by two lines drawn through a point by this tool. The angle is shown interactively in the results window. Undo can be used to delete the lines if they are not wanted. Use Show Results to display a set of angle measurements.

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 the commands in the Text menu to vary font, size, etc.
Use Show Results to display the X-Y coordinates.

Line Width - Allows the user 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,

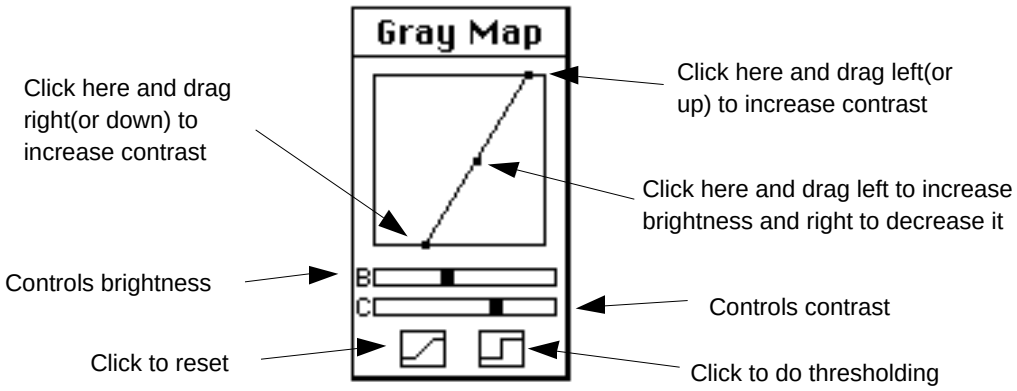
4, 6, or 8 pixels in width. Line widths greater than eight can be specified in the Profile Plot
dialog box.

Image Windows



Images are displayed within image windows, such as the one above, which are 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.

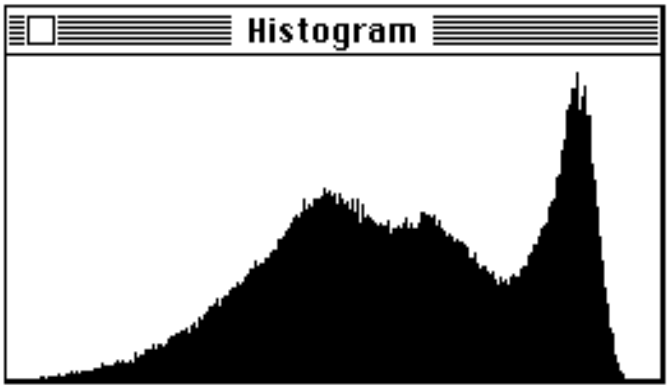
Gray Map



The Gray 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. If you are using a color palette, clicking in this

window switches to grayscale mode. Double-click to reset the look-up table to a 256 gray value ramp(an identity function).

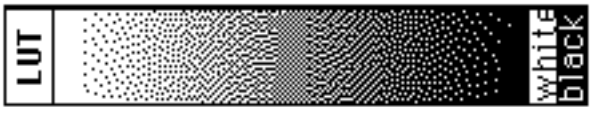
Histogram



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 Results 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



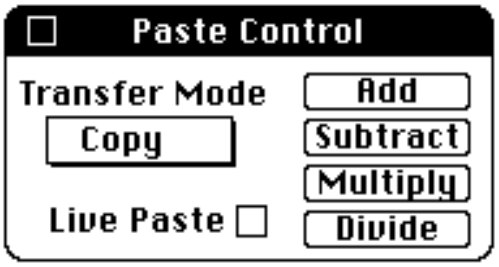
This window displays the contents of the video Look-Up Table(LUT), which is used

to transform each of the 256 possible pixels values into one of 16 million possible screen colors. Several types of look-up tables(also called palettes) are possible, including grayscale, pseudocolor, and custom.

Click in the LUT window with any of the drawing tools to pick up a new drawing (foreground) color. 256 color palettes, such as the one produced by the 256 Color Spectrum command can be “rotated”, but not stretched. Click in the LUT window with the eraser, or Option-click with any other tool, to pick up a new background color. The Save command saves modified palettes with the image. The Save as command can be used to save pseudocolor palettes separately.

The default pseudocolor palette consists of 20 colors. Click and drag near the bottom of the palette to move it within the LUT. Click and drag near the top of the palette to stretch it. Double-click on a color with the eyedropper tool to edit it.

Paste Control



The Paste Control window is only effective during paste operations. It allows you to control how image selections are pasted, or to do image arithmetic using pasted images. 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", and "Replace with Transparency" modes, respectively, allowing you to see both the object being pasted and the underlying image. This may be used to align two images, and works best for binary (black and white) images resulting from use of 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.

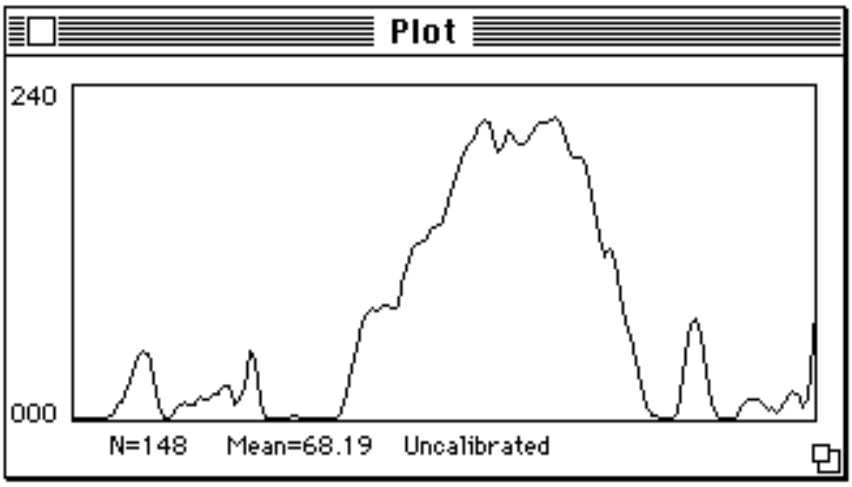
Arithmetic operations are normally done in two passes. In the first pass, *Image* determines the minimum and maximum gray levels that would result from performing the operation. In the second pass, the image arithmetic is actually performed, and the resulting pixel values are scaled to the range of 0 to 255. Scaling can be disabled in the Preferences dialog box, in which case results are clipped to 0 and 255. Arithmetic operations are always done on raw pixel values, ignoring any density calibration that my 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.

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 Column Average Plot 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

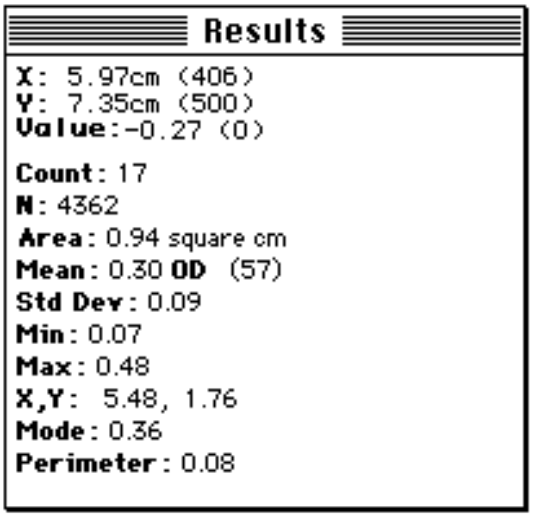
will be used if you paste the plot into a program that deals primarily with graphics, such as MacDraw. The text form(i.e., the *N* pixel values) will be used if you paste into a program that is text oriented, such as Excel.

N refers to the number of pixel values being plotted. For column average plots, *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 results window as the line is drawn) plus the line width. *Mean* is the mean density of the *N* pixel 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 results 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.

Results



The values displayed at top of the Results 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 or pseudocolor images, Value is the index used by the color look-up table.

LUT Window - Value is the grayscale value(look-up table index) pointed to by the eyedropper. You can use this information to set the foreground or background grayscale, or color, to a specific grayscale value or index.

Gray 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.

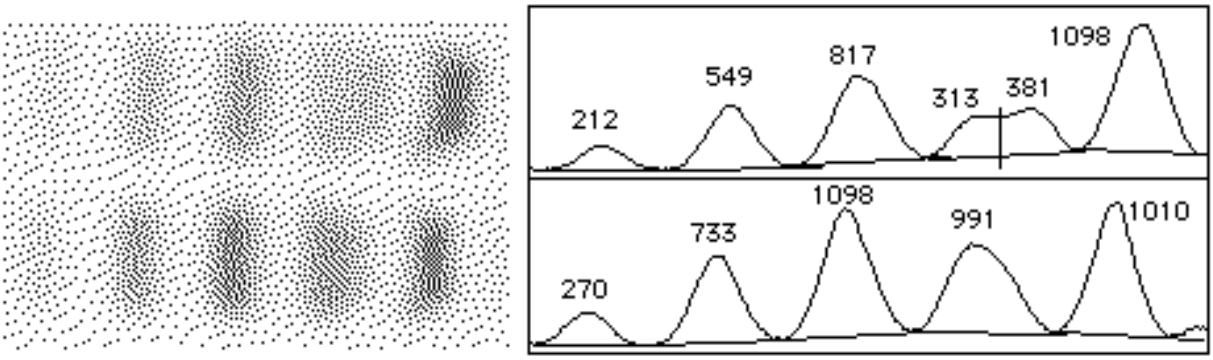
Histogram Window - X is the current X-axis(grayscale) value, Y is the number of pixels in the selection which have that value.

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.



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.

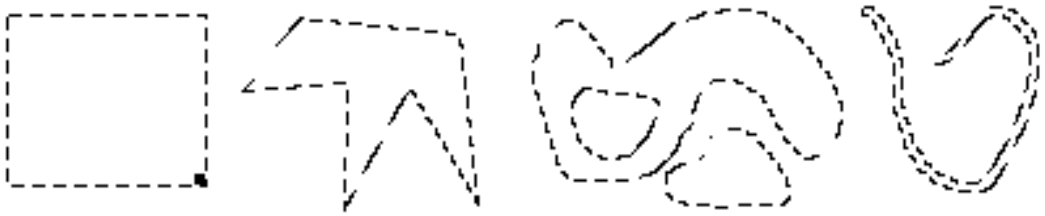
Setup to Plot Gel may fail if it tries to create a plot window that is larger than the Undo buffer. If this happens, you will need to increase *Undo & Clipboard Buffer Size* in Preferences, *Record Preferences*, and restart *Image*. The needed size is equal to (*New Height* * *New Width*) / 1024, where 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 Results 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 Results 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. This feature is implemented using QuickDraw's UnionRgn routine. 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 region 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. This feature is implemented using QuickDraw's DiffRgn routine.

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.

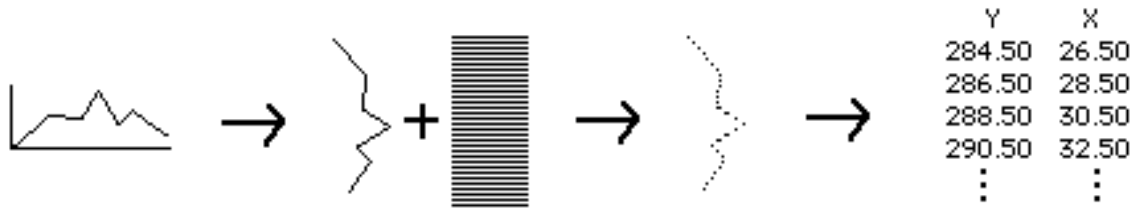
	75 DPI	150 DPI	300 DPI
13" Monitor	7.5" x 6"	3.75" x 3"	1.8" x 1.5"
19" Monitor	12" x 10"	6" x 5"	3" x 2.5"

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 of Gravity* 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 all windows to the "home" position in the upper left corner of the screen
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
Eye dropper - 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 *Use Zero for Black, 255 for White* 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. 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 for as little as $120. Two sources are Third Wave Computing(512-832-8282) and MacProducts USA (800-622-3475). 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.

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*, *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 set in the Options dialog box. Use the *rCount* function to get the value of the current measurement counter and SetCounter(n) to set it. *SetMajorLabel* and *SetMinorLabel* allow you to replace the labels used for two of the columns in the results table. Use *UpdateResults* to redisplay the last line in the results table and *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 derive and display new 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 log, square and square root 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.

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	
CopyResults	Equivalent to clicking on Copy in the Show Results window
Paste	
Clear	
Fill	
Invert	
DrawBoundary	
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', or '256 Color Spectrum'
SetNewSize(width,height)	Specifies *width* and *height* of new windows
ScaleArithmetic(b)	*b*=true or false
InvertY	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.

Enhance Menu

Smooth	Precede with SetOption for unweighted smoothing
Sharpen	Precede with SetOption for unweighted smoothing
Shadow	
TraceEdges	Precede with SetOption to prevent auto-thresholding
Dither	
ReduceNoise	
Convolve('Kernel file name')	

MakeBinary	
Erode	
Dilate	
Outline	
Skeletonize	
AddConstant(n)	-255 <= n <= 255
MultiplyByConstant(n)	0.0 <= n <= 255.0
ApplyLUT	
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 or ShowHistogram. Values returned are always uncalibrated. Use cValue function to calibrate them.
AnalyzeParticles
ShowResults
ShowHistogram
RestoreRoi
MarkSelection
MeasureArea(b) *b*=true or false
MeasureDensity(b)
MeasureStandardDeviation(b)
MeasureXY(b)
MeasureMode(b)
MeasurePerimeter(b)
MeasureMajorAxis(b)
MeasureMinorAxis(b)
MeasureAngle(b)
MeasureIntegratedDensity(b)
MeasureMinMax(b)
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

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

Text Menu

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'

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

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 left *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 Gray Map window

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)

SetMajorLabel('Label') Replaces label used for Major Axis column in Measurements window

SetMinorLabel('Label') Replaces label used for Minor Axis column in Measurements window

SetPicName(‘Name’)	Renames the currently active image window
SetOption	Equivalent to holding down Option key
ShowMessage('message') arguments	Displays 'message' in a Results window. Accepts multiple
	like the Write routine. Use a back-slash(‘\’) to start a new line.
UpdateResults windows	Redisplays last measurement in Results and Measurements
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*)
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
Divide

Precede DoCopy, DoAnd, etc. with SetOption to switch paste transfer modes, otherwise the operation is performed and the paste operation terminated.

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 will not work with the IIsi and IIci. 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
(508) 481-3700

2) **Scion Video Image 1000**

Price: $1195

Scion Corporation
152 West Patrick Street
Frederick, MD 21701
(301) 695-0035
AppleLink: D1887

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 camera is one camera that is well suited for quantitative densitometry. A less expensive camera that is also well suited for scientific imaging applications is the COHU Model 4815-5000. Both Sierra Scientific and COHU also sells lenses and filters.

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 the Sierra Scientific and COHU 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.
2010 North Forbes Boulevard
Tucson, AZ 85745
602-623-6992

Sierra Scientific
Sunnyvale, CA
408-773-5600

Sony XC77 (??)

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, 3D 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, scripts)
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, gel analysis)
NCSA, Champaign, IL
217-244-0072
Anonymous ftp: zaphod.ncsa.uiuc.edu

Personal Microscope Workstation
(Digital confocal microscopy, microscope control)
Scientific Imaging Solutions, Inc.
Knoxville, TN
615-675-1433

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

Transform, View, Format, and Dicer
(Scientific visualization, 3D Reconstruction)
Spyglass, Inc.
Champaign, IL
217-355-1665

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

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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 (128.231.128.251)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 that creates a picture
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.
```

Palette File Format

Pseudocolor palette files, which are of type ICOL, contain the number of colors in byte 0.

Byte 1 contains the starting location of the palette within the LUT and byte 2 specifies how many times each color is replicated. Bytes 3-31 are currently unused, and are set to zero. Bytes 32-63 are used for storing red, bytes 64-95 for storing green, and bytes 96-127 for storing blue. In other words, starting at byte 32, 32 unsigned bytes are used for storing the red intensity values, then 32 bytes are used for storing green intensity values, and 32 bytes are used for storing blue intensity values.

F. Program Limitations

The maximum number of pixels per line is 2048. 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 colors allowed in a pseudocolor palette is 32. This will probably be increased in some future version.

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.

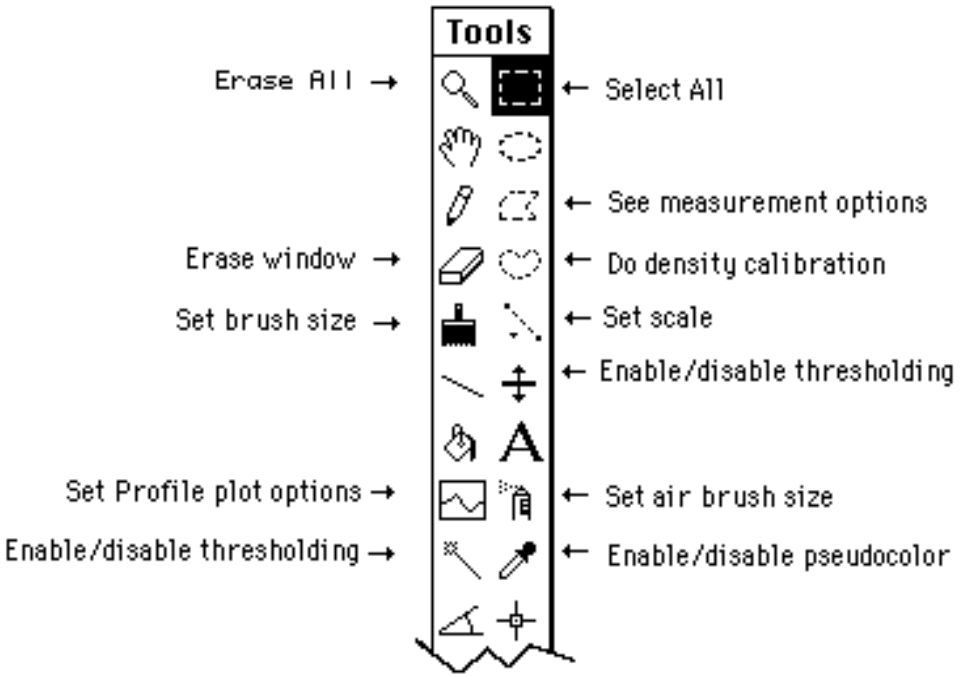
The default maximum number of measurements is 200. This can be changed in the Options dialog box. You must Save Preferences, Quit, and then restart *Image* before the change will take effect.

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.

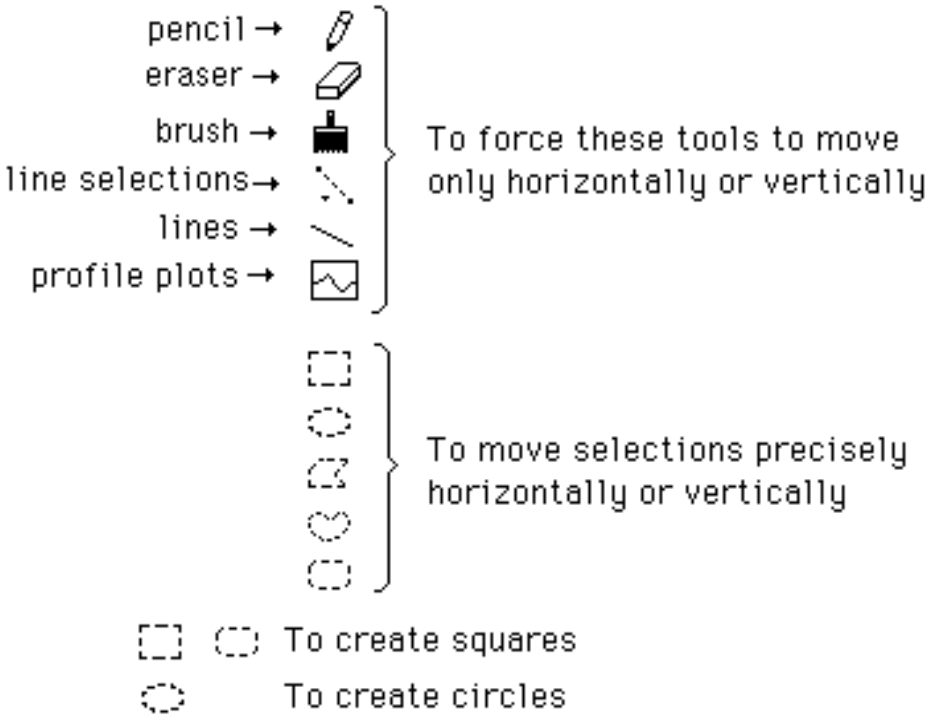
The maximum number of slices in a stack is 250.

G. Shortcuts

Double-Click on tool to:



To constrain tool functions hold down the shift key:



H. References

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

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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 alw.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 alw.nih.gov [128.231.128.251] in the directory /pub/image. Enter "anonymous" as the user name and anything you like as the password. The /image directory contains the latest version of Image, along with source code, HyperCard reference stack, and many sample images. It also contains versions of Image extended to do fractal analysis, to capture and analyze color images, and to support quantitative evaluation of cerebral blood flow, glucose metabolism, and protein synthesis.

3) Via anonymous FTP from sumex-aim.stanford.edu [36.44.0.6]. The application is in the directory /info-mac/app, and the source is in /info-mac/source/.

4) DL14 of MACSYS on CompuServe.

5) Twilight Clone BBS in Silver Spring, MD, 301-946-5032. *Image* is currently available at no charge from the Twilight Clone.

6) Send a request for an copy via email to wayne@helix.nih.gov. You will be sent three messages

containing the Image application(279K), the manual(206K), and the source code(404K), all in BinHex format. Use Stuffit 1.5.1(or later) to decode("Decode BinHex File...") and decompress("Open Archive...") the files.

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:

Internet, BitNet: wayne@helix.nih.gov
CompuServe: 76067,3454

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