NIH Image

Version 1.57

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(Version 1.57)

INTRODUCTION

NIH *Image* is a public domain image processing and analysis program for the Macintosh. It can acquire, display, edit, enhance, analyze and animate images. It reads and writes TIFF, PICT, PICS and MacPaint files, providing compatibility with many other applications, including programs for scanning, processing, editing, publishing and analyzing images. It supports many standard image processing functions, including contrast enhancement, density profiling, smoothing, sharpening, edge detection, median filtering, and spatial convolution with user defined kernels.

Image can be used to measure area, mean, centroid, perimeter, etc. of user defined regions of interest. It also performs automated particle analysis and provides tools for measuring path lengths and angles. Spatial calibration is supported to provide real world area and length measurements. Density calibration can be done against radiation or optical density standards using user specified units. Results can be printed, exported to text files, or copied to the Clipboard.

A tool palette supports editing of color and gray scale images, including the ability to draw lines, rectangles 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.

Image directly supports Data Translation and Scion frame grabber cards for capturing images or movie sequences using a TV camera. Acquired images can be shading corrected and frame averaged. Other frame grabbers are supported via plug-in modules.

Image can be customized in three ways: via a built-in Pascal-like macro language, via externally compiled plug-in modules and on the Pascal source code level. Example macros, plug-ins and complete source code are available from the Internet by FTP (File Transfer Protocol) from zippy.nimh.nih.gov.

System Requirements

Image requires a color capable Macintosh and at least 2MB of free RAM. A Macintosh with 16MB or more of RAM is recommended for working with 3D images, 24-bit color or animation sequences. System 7.0 or later is required. *Image* directly supports, or is compatible with, large monitors, flatbed scanners, film recorders, graphics tablets, PostScript laser printers, photo typesetters and color printers.

Acknowledgments

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OVERVIEW
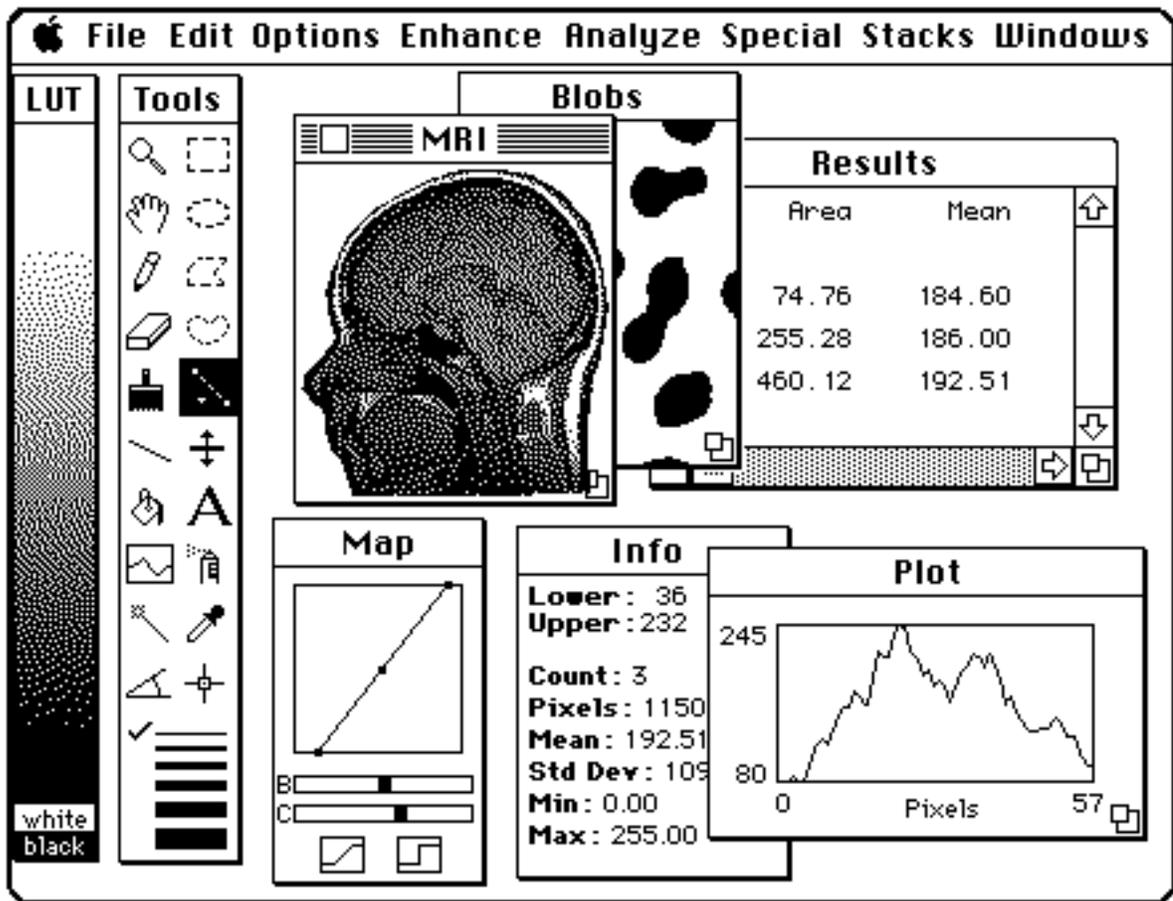
Windows

Image displays images, measurement results, profile plots, etc. in windows. Windows can be dragged around the screen by their title bars. In addition, some windows can be resized (e.g. the Plot window) using the "drag box" in their lower right corner. A window buried underneath other windows can be activated by selecting it in the Windows menu.

The LUT window displays the current video look-up table. The Tools window contains a palette of tools for making selections, editing images, drawing text, and making measurements. The Map window is used for adjusting the contrast and brightness of images and for enabling and disabling thresholding. The Info window displays status information, such as cursor position and value, and the most recent measurement results. The Results window displays the current table of measurement results. The Plot window displays density profile and calibration plots. The currently active window (e.g., the MRI window above) has a highlighted menu bar.

Images

Image manipulates, displays and analyses images. Images are two dimensional arrays of pixels (picture elements). Pixels are represented by 8-bit unsigned integers, ranging in value from 0 to 255. *Image* follows the Macintosh convention and displays zero pixels as white and those with a value of 255 as black. 16-bit images, however, can be imported and scaled to 8-bits. The 16-bit (up to 65,00 gray levels) to 8-bit (256 gray levels) scaling can

be controlled by the user or performed automatically based on the minimum and maximum gray values in the 16-bit image. The Rescale command (in the File menu) allows the user to redo the 16-bit to 8-bit scaling at a later time.
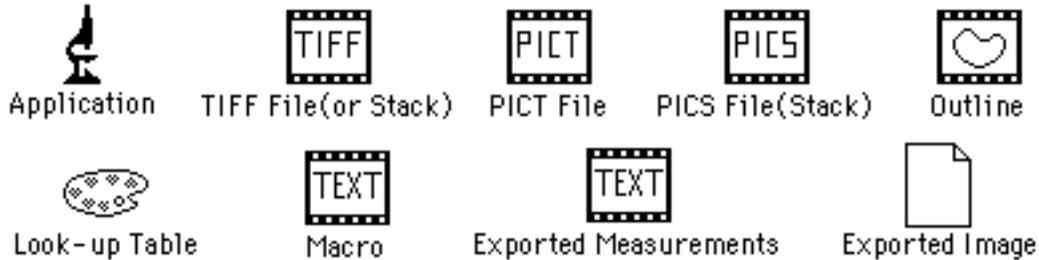
Stacks

Image supports the organization and manipulation of a series of 2D images as a 3D array called a stack. A stack contains set of related 2D images, such as a movie loop or serial sections from a volume. The 2D images that make up a stack are called slices. You can step through the slices using the ">" and "<" keys. The number of the current slice and the total number of slices are displayed in the title bar. The Stacks menu contains commands that work with stacks. Windows to Stack converts a set of 2D images into a stack. Animate animates the images in a stack at a rates up to 100 frames per second. Reslice reconstructs a new 2D image perpendicular to the plane of the slices in a stack. Project does volume rendering, useful for visualizing the internal structures of 3D images. Commands not in the Stacks menu, except for Open, Save and Export, have no knowledge of stacks. For example, the Invert command only inverts the current slice, not the entire stack. Macros are available, however, for performing various operations on all the slices in a stack.

Memory Requirements

Opened images are stored entirely in the Mac's internal memory, or RAM (Random Access Memory). An image requires one byte of RAM for each pixel plus a few thousand additional bytes for data structures containing information about the image, such as color tables and calibration. For example, a 512 X 512 image requires 262,144 bytes, or 262K, where 1K equals 1024 bytes. A 256 X 256 X 256 stack would require 16,777,216 bytes or 16MB. To see how much RAM an image uses, use the Get Info command, or pull down the Windows menu, which shows the size of all open images.

The number and size of images that *Image* can work with depends on *Image*'s RAM allocation. To check or change the allocation, select *Image*'s icon (a microscope) and use the Finder's Get Info command. The default allocation is 2500K. To use all of *Image*'s features requires a RAM allocation at least three times the size of the largest image, plus the size of the program (about 300K). The extra RAM is used for a buffer that supports one level of Undo (the Undo Buffer) and a Clipboard buffer that supports copy and paste functions. The default size of these buffers is 300K. This can be changed using the Preferences command in the Options menu. You can find out how much memory is currently available for opening images by selecting "About NIH Image" from the Apple menu.

File Formats

Application TIFF File(or Stack) PICT File PICS File(Stack) Outline

Look-up Table Macro Exported Measurements Exported Image

Image supports TIFF, PICT, PICS and MacPaint file formats. Stacks can be saved as either multiple image TIFF files or as a PICS file. The Import and Export commands allow images with arbitrary binary and tab-delimited text (spreadsheet) formats to be read and written. The Open All option of the Open and Import commands allows a series of images to be read automatically. Measurement results can be exported to a text file compatible with spreadsheet and statistical analysis programs.

Macros

Image can be extended using a Pascal-like macro programming language that allows users to customize and automate repetitive and frequent tasks. The easiest way to develop a custom macro is to modify one of the macros in the Macros folder distributed with *Image*. Macros are stored in text files that can be edited using *Image*'s built-in text editor. Using the Load Macros command causes the macros in the file to appear as commands at the bottom of the Special menu. The macro language is described in Appendix A. The description assumes some familiarity with the Pascal programming language. Additional help on macro writing, and other ways to extend *Image*, can be found in a separate document named "Inside Image".

Plug-ins

Image can also be extended using Photoshop compatible plug-ins. Acquisition plug-ins are used to support scanners or frame grabbers, or to read images in file formats that *Image* does not normally support. They are accessed using the Acquire sub-menu. Export plug-ins are used to output to printers that do not have a Chooser selectable driver or to save images in file formats not normally supported by *Image*. They are accessed using the Export sub-menu. Filter plug-ins perform filtering operations on images. They are accessed using the PlugIn Filters sub-menu. *Image* expects to find plug-ins in a folder named Plug-ins located in either the same folder as *Image* or in the System folder.

Video Capture

Using a frame grabber card, *Image* can digitize images from TV cameras, VCRs or video disks. Captured frames can be averaged or integrated. Integration can be done in RAM (using software), on-chip at video rates using Cohu or Dage CCD cameras, or on-board at video rates using the Scion AG-5. The Histogram command will display a continuously updated density histogram during "live" capture. The Make Movie captures a sequence of frames into a stack at rates up to 15 frames per second. The Capture Color command digitizes 24-bit color into a three slice stack. For alignment purposes, Show Paste Control (in the Windows menu) allows "Live" video to be superimposed over a previously captured image. Information about the three frame grabber cards directly supported by *Image* (the Scion LG-3, the Scion AG-5, and the Data Translation Quick Capture) is given in Appendix B.

Image Enhancement

The Process menu provides filters for smoothing, sharpening, finding edges and reducing noise in images. The Convolve command uses user-defined filters that operate on up to 63 X 63 neighborhoods. The Binary sub-menu's Make Binary command converts grayscale images to images consisting of only black and white pixels and includes commands to process such images. Use the Arithmetic sub-menu to add or multiply an image (or selection) by a constant. Arithmetic operations between two images are done using the Image Math command or by using the Paste Control dialog box during paste operations.

Making Measurements

To make a manual area measurement, first outline a region of interest using the rectangular, elliptical, polygonal, or freehand selection tool. Then select the Measure command, which will compute the area, mean gray value, and the minimum and maximum gray value. Other measurements, such as perimeter, can be enabled using the Options command in the Analysis menu. Measure distances by making a straight, freehand or segmented line selection, and then using the measure command. (Note that the line selection tool uses a pop-up menu for selecting different line types.) Use the angle tool to measure angles. The cross hair tool counts objects, marks them, and records their X-Y coordinates. The wand tools automatically outlines structures isolated using thresholding. Results from the most recent measurement are displayed in the Info window. Use Show Results to display a table of results since the last time the Reset command was used.

The Analyze Particles command automatically counts and measures features of interest. This requires thresholding to discriminate objects of interest from surrounding background based on their gray values. *Image* has two thresholding methods. In thresholding mode (Threshold is checked in the Options menu), all pixels equal to or greater than a single threshold level are displayed in black, and all other pixels (the background) are displayed in white. In Density Slicing mode (Density Slice is checked), all pixels between a lower and upper threshold are highlighted in red. For both modes, you adjust threshold levels by dragging the LUT tool (the one with the double-headed arrow) in the LUT window. For successful thresholding, it may be necessary to use the Subtract Background command to remove the effects of uneven illumination.

Images can be calibrated to either spatial or density standards using the Set Scale and Calibrate commands respectively. To calibrate spatially, use the line selection tool to measure a known distance, then use Set Scale to specify a unit of measurement (e.g. millimeters) and enter the known distance. To calibrate to a set of density standards (e.g. an optical density step tablet), measure the mean gray level of each of the standards, then use the Calibrate command to enter the known values and specify the type of curve fit.

Editing

Image's tool palette contains several tools used for image editing and annotation. The pencil, brush, line, paint bucket and spray draw in the current foreground color (the color of the brush in the Toolbox window). The text tool draws text using the current font, size, style, and foreground color. Click on a color with the eyedropper tool to select a new foreground color. The eraser tool erases to the current background color (the color of the eraser in the Toolbox window). Click on a color in the LUT window with the eraser to select a new background color.

Commands in the Edit menu operate on the current selection. Cut, Copy and Paste perform the standard Macintosh Clipboard operations. Fill fills the selection in the foreground color. Clear erases it to the background color. Draw Boundary draws a line around the selection. Scale and Rotate enlarges, shrinks and/or rotates the current rectangular selection. Commands are also available for flipping and rotating by 90°.

Text Windows

Image has a built-in text editor that can open and edit text files up to 32K in size. The following command know how to deal with text windows: New, Open, Close, Save, Save As, Print, Cut, Copy, Paste, Clear, Select All, Font, Size, Convolve (uses the kernel in the current text window), and Load Macros (loads the macros contained in the current text window). Also note that the Fill command changes to Find when a text window is active.

Look-up Tables

Each open image has a look-up table (LUT) associated with it. The LUT window displays the contents of the look-up table for the currently active image. The LUT is used to map, at video rates, pixels (in the range 0-255) to screen colors. Click and drag with the LUT tool in the LUT window to adjust brightness and contrast. Measured density values will not change since you are manipulating the look-up table, not the image's pixel values.

Various commands at the top of the Options menu allow you to invert the current LUT, to specify the number of gray levels or colors it uses, or to switch to one of several built-in color tables. LUTs are automatically stored with an image when it is saved to disk, or they can also be saved separately.

On-line Help

Balloon help is available in the Tools, Map and LUT windows, in menu commands, and in most dialog boxes. Balloon help is enabled by selecting Show Balloons from the Help (?) menu. The Tools, Map and LUT windows must be activated (by clicking on them) to get the help messages to appear.

NIH Image Mailing List

No telephone support is available for *Image*. The NIH *Image* mailing list, however, provides a way to get questions answered. It can also be used to contact other users, to keep abreast of new developments, and to get updates via e-mail. To subscribe, send an e-mail message containing the single line

    subscribe nih-image <first name> <last name>

to listproc@soils.umn.edu. If you get tired of being bombarded with a dozen e-mail messages a day, you can remove yourself from the list by sending a message containing the line "unsubscribe nih-image" to listproc@soils.umn.edu. There is also an option to receive messages as a once per day digest. All past messages are archived and available via e-mail, FTP and Gopher. Instructions on how to retrieve archived messages is in the welcome message sent at subscription time. The mailing list, which currently has about 1000 subscribers, is run by the Soil Science Department at the University of Minnesota.

Information on other ways to get updates, and on how to report bugs, is given in Appendix I.

Citing NIH Image

Published research assisted by NIH *Image* should use a statement similar to the following in the materials and methods section "... analysis performed on a Macintosh <model> computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GEI)".

MENUS

File Menu

New…

Creates a new image or text window. Image windows are filled with the current background color. New image windows inherit the look-up table, as well as any spatial or density calibration, of the currently active window. The size of newly created images is limited by the amount of free RAM, which is displayed in the About NIH Image dialog box.

Text windows can be used to create macros accessed using the Load Macros command or convolution kernels to be used by the Convolve command. Text windows have a maximum size of 32K.
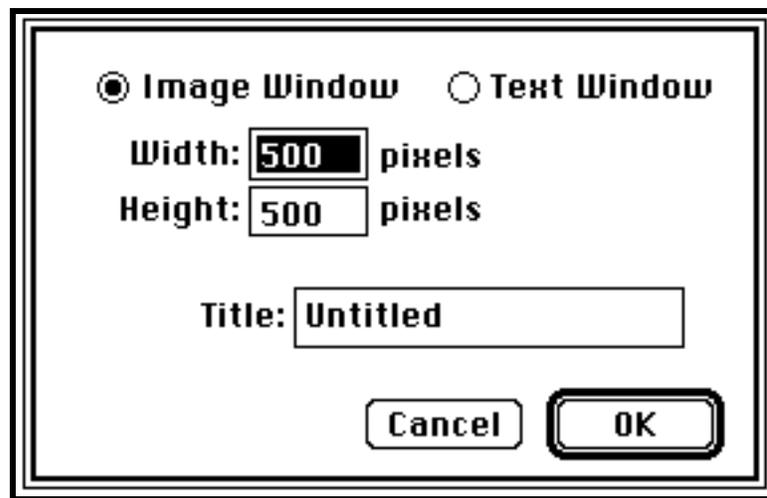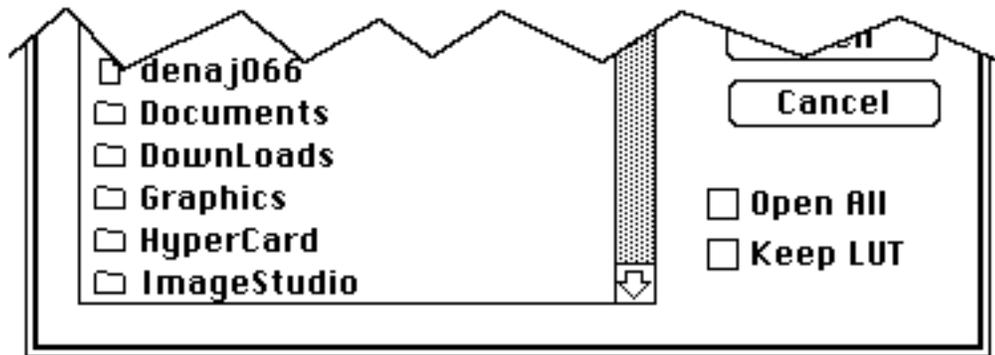

Open…

Use the Open command to load and display images (in TIFF, PICT, PICS and MacPaint format), text files, color look-up tables (LUTs) or region of interest outlines. The following file types are supported:

1) TIFF files created by *Image* and many other programs. *Image* will open (as a stack) a TIFF file containing multiple images, but the images must all have the same width and height. *Image* is unable to open bitmap (1-bit) 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. 16-bit TIFF files can be opened using the Import command. 24-bit (RGB) TIFF files are opened into a 3-slice (red, green and blue) stack.

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

3) MacPaint documents. You will want to set *Undo Buffer Size* in Preferences to 405K if you regularly open large MacPaint drawings, since this is the memory required for a full size (576 x 720) MacPaint document.

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

5) Look-up tables created by *Image* and by several other Macintosh programs, including PixelPaint, Canvas and UltraPaint. These programs have palette editors that can be used to create color tables for use by *Image*. The public domain PalEdit program from NCSA (ftp://zippy.nimh.nih.gov/pub/nih-image/programs/ncsa/PalEdit.hqx) can also be used to create color tables that can be opened by *Image*.

6) Selection outlines created using the Save As command in *Image*.

6) Text files, such as macros and convolution kernels, up to 32K in size.

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

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

Import…

Use this command to read image files created by non-Macintosh based systems, to display spreadsheet data (text) as an image, or to import raw color look-up tables. It presents the following dialog box that allows you to select the type of file to be imported.
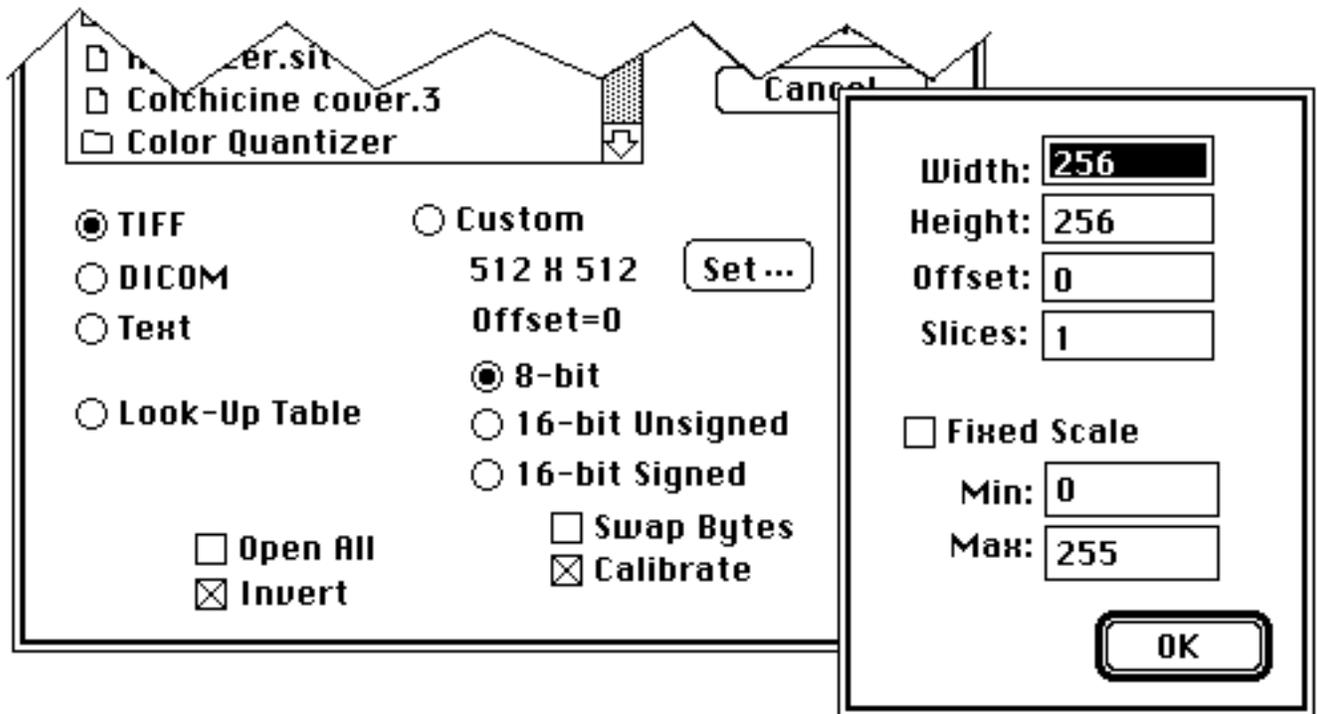
Select *TIFF* to open 8 and 16-bit TIFF files imported from other systems, such as an IBM PC. Once a 16-bit TIFF file has been imported, you can use Rescale (a k a Revert to Saved) to find the optimum 16-bit to 8-bit mapping. 16-bit TIFF files that represent white as zero open inverted.

Select *DICOM* to import 16-bit files in the DICOM-3 (Digital Imaging and Communications in Medicine) and ACR-NEMA formats used in radiology. A DICOM dictionary is required to decode the DICOM or ACR-NEMA header. This dictionary is available from zippy.nimh.nih.gov, in the /pub/nih-image/documents directory. It must be located in the same folder as NIH Image or in the System folder. When opening a DICOM file, hold the option key down to get a full header dump. When using the "Open All" option, hold the shift key down to fix 16-bit to 8-bit scaling.

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

Select *Look-Up Table* to import a 768 byte binary LUT consisting of 256 consecutive red values, 256 consecutive green values, and 256 consecutive blue values. The values should be in the range 0-255.

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. For 16-bit images,

the maximum value that can be entered for *Width* is 4096. *Offset* specifies the number of bytes *Image* will skip before it starts reading the pixel data. For example, use an offset of 512 to skip over a 512 byte file header. Set *Slices* to more than one to import 3D data sets such as multi-slice volumes from medical scanners

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* when importing 16-bit images from "little-endian" systems, such as an IBM-PC 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. The approximated values should not vary from the original 16-bit values by more than (Max-Min)/254, where Min and Max are the minimum and maximum 16-bit values. If *Fixed Scale* is checked then automatic scaling is disabled and the 16-bit data is linearly scaled from *Min-Max* to 1-254. You can redo the 16-bit to 8-bit scaling at any time using the Rescale command.

Use the Import command to scale 16-bit images from medical scanners to the 8–bit format required by *Image*. For example, to import raw MRI scans from a GE SIGNA scanner, set *width* and *height* to 256, select *16-bit Signed*, and set *Offset* to 14336. The macro "Import GE Signa Files" (in the file Stacks) will automatically import a series of raw GE SIGNA files.

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

Check *Invert* to invert imported 8-bit images.

Close

Closes the currently active image, text, 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.

Save

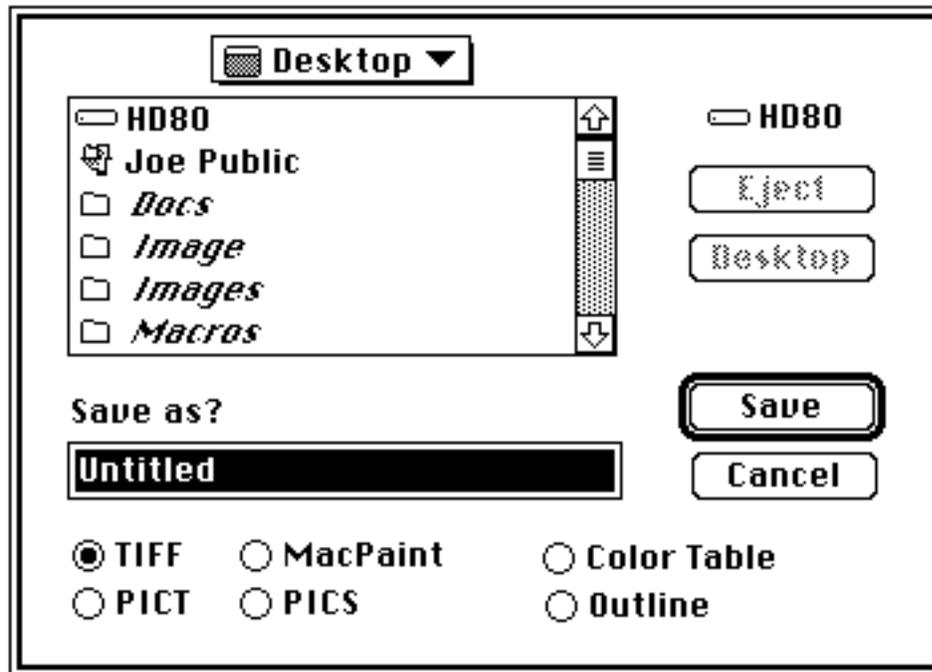Resaves the currently active image or text window to disk. You will be prompted for a name in the case of an image newly created using New, Duplicate or Start Capturing. Files are resaved in their original format. In other words, TIFF files are resaved in TIFF format, and PICT files are resaved in PICT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

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

Save As…

Allows you to saves the currently active image (or rectangular selection) in TIFF, PICT or MacPaint) format, to save a stack in TIFF or PICS format, or to save the current look-up table, selection outline, or text window.

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

PICT
Saves the currently active image to a PICT file. PICT files can be exported to many other Mac programs, including Canvas, Photoshop, SuperPaint and MacDraw. Because they are compressed, PICT files also have the advantage of being more compact if the image contains large homogeneous regions, which is typical of computer generated images. Unfortunately, the run length compression scheme used with PICT files is ineffective with most scanned images.

You should avoid PICT files if you are doing quantitative image analysis. The Mac imaging model is most concerned with the color of each pixel, and does not care so much about the actual pixel value. Thus PICT files cannot guarantee that the pixel values remain fixed. TIFF is a machine and system independent image standard that avoids this problem.

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

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PICS
Saves the currently active stack to disk in PICS format, a file format supported by many Mac animation programs. PICS files are compressed using the same run length encoding scheme used for PICT files. Stacks containing areas of uniform color or gray value saved in PICS format will be smaller than stacks saved in TIFF format. Use the "Movie Player" utility that comes with QuickTime to convert PICS files into QuickTime movies.

Color Table
Saves the current look-up table (LUT) as a separate file.

Outline
Saves the X-Y coordinates of the current selection outline. X-Y coordinates of curved line selections, and selections that have been edited, cannot be saved.

Export…

Exports images, look-up tables, measurement results, density profile plot values, or histogram values to disk for use with programs other than *Image*.
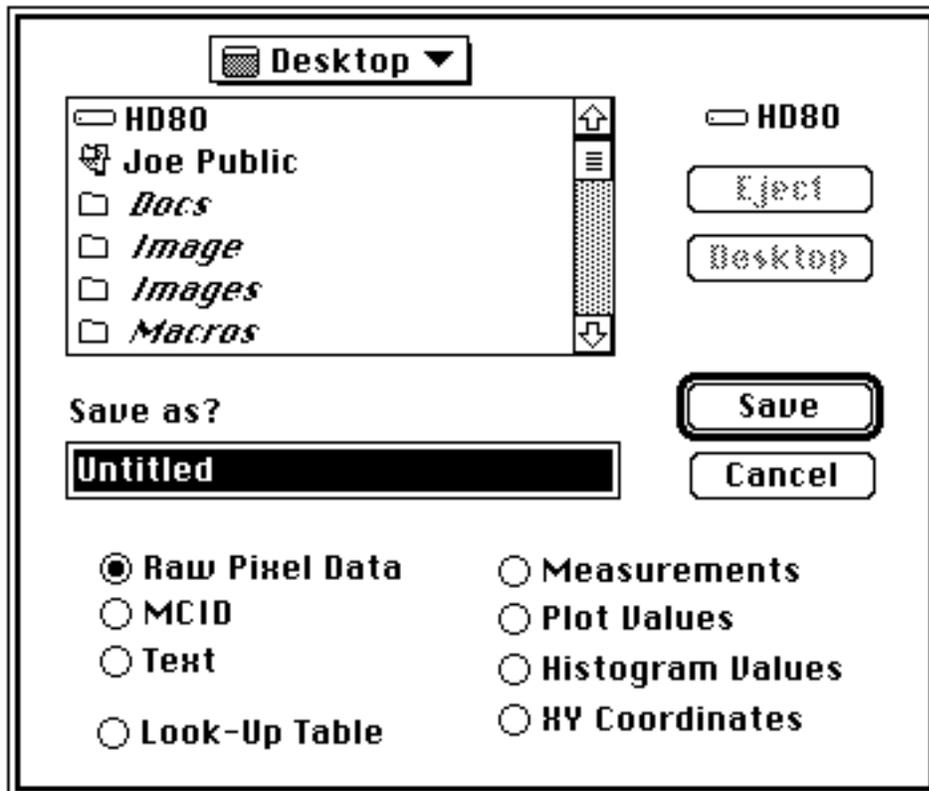
Raw Pixel Data
Exports the current image (or selection) as binary pixel data, in row order, one byte per pixel, without a header.

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 rectangular selection) as a tab-delimited, spreadsheet compatible text file consisting of uncalibrated pixel values.

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

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

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

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

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

Record Preferences

Saves the current state of the following *Image* program parameters. Settings are saved in a file named *Image Prefs* in the Preferences folder inside the System folder. You can revert to the default settings be deleting copies of this file in either the System or Preferences folders and restarting *Image*.

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

Revert to Saved

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

Rescale

Replaces Revert to Saved whenever an imported 16-bit image or imported 16-bit TIFF file is active. This command linearly scales the 16-bit data (on disk) from the range *Min-Max* to 1-254, where *Min* and *Max* are determined by using the controls in the Map window or by using the LUT tool. 16-bit pixels with values less than *Min* are set to 1 and 16-bit pixels with values greater than *Max* are set to 254. The image's absolute minimum and maximum pixel values are used if the LUT has not been altered.

The *Highlight Saturated Pixels* option in the Preferences dialog box can be used determine locations within the image where density measurements may be invalid do to clipping at *Min* or *Max*. It causes pixels that were less than or equal to *Min* to be displayed in yellow and pixels that were greater than or equal to *Max* to be displayed in red.

Duplicate

Creates a new window displaying a copy of the current image, or of the current selection if only part of the image is selected. The newly created image inherits the look-up table, plus any spatial or density calibration, of the image being duplicated.

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Get Info

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

```
┌──────────────────────────────────────────┐
│ ▤□▤  Info About OD Standards ▤▤◳▤ │
├──────────────────────────────────────────┤
│                                          │
│  Name: OD Standards                      │
│                                          │
│  Width: 546                              │
│  Height: 211                             │
│  Size: 112K                              │
│                                          │
│  Creation Date: Tue, Jul 12, 1988  11:49:40 AM │
│  Last Modified: Fri, Aug 5, 1988  4:08:23 PM   │
│  Volume: HD40 (4355K free)               │
│                                          │
│  Type: Normal                            │
│  Lookup Table: Gray-scale                │
│  Magnification: 1:1                      │
│  Scale: 6.900 Pixels Per mm              │
│  Unit of Measure: O.D.                   │
│                                          │
│  Free RAM: 942K                          │
│  Largest Free Block: 942K                │
│                                          │
│  Selection Type: Freehand or Polygon     │
│     Location: 44, 158                    │
│        Width: 154                        │
│        Height: 119                       │
│                                          │
│                                      ▵   │
└──────────────────────────────────────────┘
```

Acquire

Use this sub-menu to access Photoshop compatible acquisition plug-ins. The plug-ins must be stored in a folder named "Plug-ins" located either in the same folder as *Image* or in the System Folder. Using plug-ins, *Image* can acquire 8-bit grayscale, 8-bit indexed color, and 24-bit color images. 24-bit color images are stored in three slice stacks and displayed using 8-bit indexed color, similar to the way the Capture Color command works. Several of the plug-in listed below, including "Plug-in Digitizer" for use with the AV Macs, are available by anonymous FTP from zippy.nimh.nih.gov, in the /pub/nih-image/plug-ins directory.

The following plug-ins have been reported to work with *Image*:

    Agfa Arcus plus color scanner
    Agfa PhotoScan
    Canon CLC 500 Color Laser Copier with Electronics for Imaging interface
    Computer Eyes /RT Pro
    ComputerEyes SCSI frame grabber
    Datacopy 730GS scanner
    Ektron 1400 series camera (Eikonix 4096x4096 CCD
    Epson GT-6000 scanner
    Graphics Unlimited Kingfisher frame grabber (grayscale only)
    Howtek II slide scanner
    La Cie SilverScanner (8-bit grayscale, 8-bit color, 24-bit color)

Kodak DCS-100 and DCS-200 digital cameras (grayscale and 24-bit color)
Kodak Photo CD Image Browser
Kodak RFS2035 scanner
MacVision video capture
MIC systems DVA 4000 frame grabber
Microtek Scanmaker II flatbed scanner
Microtek Scanmaker 35t slide scanner
MirrorScan 600 Plus
Neotech Image Grabber
Perceptics PixelBuffer, MegaGrabber and PixelHR-24 frame grabbers
Plug-in Digitizer (supports AV Macs and other QuickTime digitizers)
QuickImage 24 plug-in
Radius VideoVision video grabber
RasterOps Video Capture V1.51 with 24STV and 24XLTV cards
RealTech Scan 800 (Tamarack) flatbed scanner
Scantastic plug-in with Apple Color One scanner
Scion LG-3 frame grabber
Sharp JX-325 scanner
Truvel scanner
Umax UC630 scanner
VideoSpigot card with "Plug-in Digitizer" and SpigotVDIG 1.0ß3
Voyager decompression plug-in

Export

Use this sub-menu to access Photoshop compatible export plug-ins. Export plug-ins must be stored in a folder named "Plug-ins" located either in the same folder as *Image* or in the System Folder. Two export plug-ins have been successfully tested with *Image*: a plug-in that supports the Scion TV-3 video output card (used to drive a video printer) and the PhaserPrint plug-in for the Tektronix Phaser IIsd dye sublimation printer.

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. Hold down the option key while selecting Page Setup to display a dialog box offering several custom grayscale halftoning options.

Print…

Print images, text, plots, histograms, and measurement results. What gets printed is determined by which window is currently active. The print command changes (e.g., *Print Image*, *Print Plot*, etc.) depending on what kind of window is currently active.

Print Image - Prints the currently active image. Images larger than the printer page (normally 552 x 730 pixels) are automatically scaled to fit the page.

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, which can be made larger using the "grow box" in the lower right corner.

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 option is enabled whenever

you have recorded one or more measurements and either the Info or Results window is active.

Print Text - Prints the contents of the current text window. Text is always printed using 9-point Monaco.

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.

Edit Menu

Undo

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

Cut

Copies the contents of the current image selection to the Clipboard and fills it with the current background color. For text windows, the currently selected text is copied to the Clipboard and the selection is deleted.

Copy

Places a copy of the contents of the current image 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 graphics will be copied to the Clipboard, allowing you to transfer plot and histogram data to other programs for plotting or statistical analysis. For text windows, the currently selected text is copied to the Clipboard.

Use the eyedropper tool to select a color to be copied from the LUT window to the Clipboard. This color can then be pasted back into another location in the LUT window. Copy and paste of LUT colors works best with look-up tables that have 32 or fewer 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 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 or to paste text into a text 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". This feature, along with the ability to select different transfer modes, allows the image currently being digitized to be aligned with a previously digitized image.

Text as well as images can be pasted into Image windows. For example, you can copy measurement results to the Clipboard, then paste them into an image window. The text will be displayed inside the current rectangular selection, or, if there is no selection, into a default selection. Use sub-menus in the Options 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 pasting again. Text background is always erased to white, the "No Background" option in the Style menu is ignored. This is not a problem, however, since you can select the Or transfer mode in the Paste Control window to paste text transparently. When pasting text in the Or mode, you can click in the LUT window to change color or gray level.

Clear

Erases the current image 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. Deletes the current selection if the current window is a text window.

Fill (or Find)

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

The Fill command changes to "Find" whenever a text window is active. Type option-command-f to repeatedly search for the same string. You can move the cursor to a specified line by entering a search string in the form '#n', where n in a line number. As an example, to go to line 100, enter "#100".

Invert

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

Draw Boundary

Outlines the current selection (including line selections) 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. Use Flip Vertical or Flip Horizontal to invert a newly created scale. The "Draw Calibration Bar" macro in "Plotting Macros" will draw labeled calibration scales.

Select All

This command is equivalent to using the rectangular selection tool to select the entire image, including portions that may be offscreen. Once the selection has been made, Select All changes to Deselect All, allowing you to deactivate the selection. You can also deactivate the selection by clicking on any tool other than one of the selection tools, the hand tool, or the magnifying glass. For text windows, selects all the text.

Rotate Left

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

Rotate Right

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

Flip Horizontal

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

Flip Vertical

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

Scale and Rotate…

Reduces or enlarges the contents of the current rectangular selection. If *Nearest Neighbor* is checked, scaling is done quickly using pixel replication, but the resulting image my not be smooth. Check *Bilinear* for smoother, but slower, interpolated magnification. Note that bilinear interpolation will probably not work correctly with true color images because of discontinuities in the LUT. If *Angle* is non-zero, the contents of the selection are also rotated the specified number of degrees. Rotation will be clockwise for positive angles and counter-clockwise for negative.
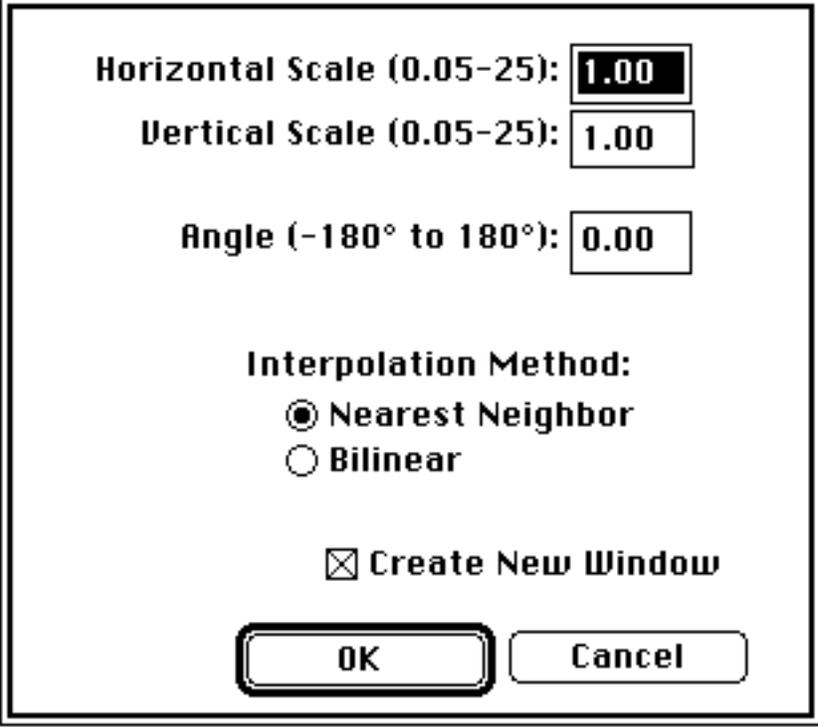
```
┌──────────────────────────────────────────────┐
│                                                │
│   Horizontal Scale (0.05–25): [ 1.00 ]         │
│     Vertical Scale (0.05–25): [ 1.00 ]         │
│                                                │
│     Angle (–180° to 180°):    [ 0.00 ]         │
│                                                │
│         Interpolation Method:                  │
│            ◉ Nearest Neighbor                  │
│            ○ Bilinear                          │
│                                                │
│                    ☒ Create New Window         │
│              (   OK   )   ( Cancel )            │
│                                                │
└──────────────────────────────────────────────┘
```

Show Clipboard

Opens a new window and displays the image or text currently on the Clipboard. This window, which has the title "Clipboard", will not be updated if the Clipboard later changes.

Options Menu

Grayscale

Loads the default grayscale look-up table. The contrast and brightness of grayscale images can be adjusted by direct manipulation of the transformation function displayed in the Map window. This function can be applied to the pixel data in memory, thereby making contrast and brightness changes permanent, using the Apply LUT command. The Grayscale command will not convert 8-bit true color (as opposed to pseudocolor) images to grayscale. Instead, use the Convert to Grayscale variation of the Apply LUT command.

Color Tables

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

LUT Options…

Allows you to specify the number of distinct colors or shades of gray in the current look-up table, to reserve up to six entries for annotation and graphics, or to invert the look-up table.
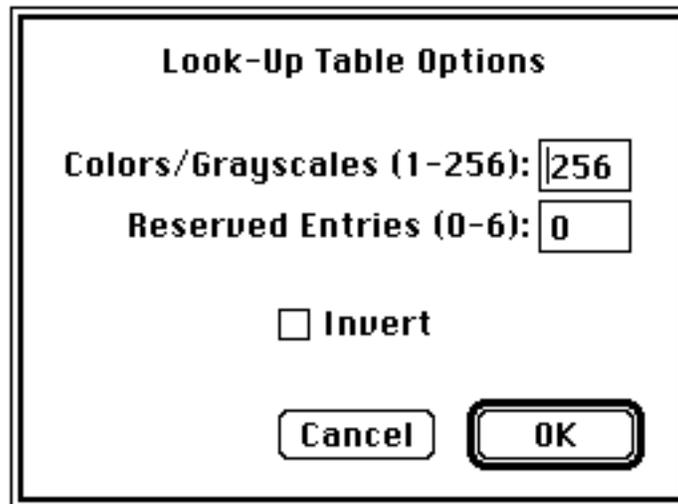
Colors/Grayscales is the number of colors or shades of gray used in the current look-up table. When increasing the number of colors, the required new colors are created by interpolating between existing colors. For example, if the current color table consists of the two colors, white and black, setting the number of colors to 64 creates a grayscale palette with 64 shades of gray.

Reserved Entries allows you to allocate up to six entries in the look-up table for colors that are never altered. For example, you could reserve one entry to add color annotation to a grayscale picture. Any extra colors are displayed at the bottom of the LUT window, allowing you to edit them by double-clicking with the eyedropper tool.

Check *Invert* and the current look-table will be inverted when you click on *OK*. Unlike the Invert command in the File Menu, pixels values are not altered, only the way the image is
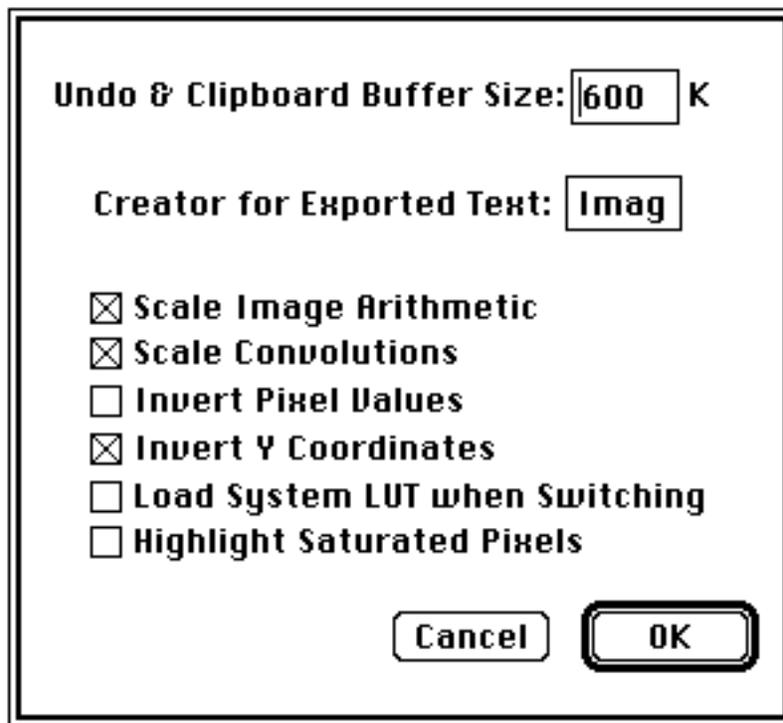
displayed on the screen. The first (0) and last (255) entries in the LUT, which are always white and black respectively, are never inverted since these entries are used for drawing the menu bar, title bars, dialog boxes, etc.

Font, Size, Style

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

Preferences…

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

```
┌─────────────────────────────────────────┐
│  ┌───────────────────────────────────┐  │
│  │                                   │  │
│  │  Undo & Clipboard Buffer Size: │600│ K│  │
│  │                                   │  │
│  │  Creator for Exported Text: │Imag││  │
│  │                                   │  │
│  │  ☒ Scale Image Arithmetic         │  │
│  │  ☒ Scale Convolutions             │  │
│  │  ☐ Invert Pixel Values            │  │
│  │  ☒ Invert Y Coordinates           │  │
│  │  ☐ Load System LUT when Switching │  │
│  │  ☐ Highlight Saturated Pixels     │  │
│  │                                   │  │
│  │           ( Cancel )  ╔══════╗    │  │
│  │                       ║  OK  ║    │  │
│  │                       ╚══════╝    │  │
│  └───────────────────────────────────┘  │
└─────────────────────────────────────────┘
```

Undo & Clipboard Buffer Size allows you to change the size of the two internal image buffers used by *Image*. You must quit, and restart *Image* before buffer size changes take effect. Note that increasing the size of these buffers decreases the amount of RAM available for opened images. For example, increasing the size from 300K to 1000K decreases the RAM available for images by 1400K. You can get around this problem, assuming your computer has enough RAM, by increasing *Image*'s memory allocation using the Finder's Get Info command.

Creator for Exported Text specifies the four character creator code Image uses for exported text files. This code determines which application is launched when you double-click on an exported text file (e.g., measurements, profile plot data, XY coordinates). Use 'Imag' for Image, 'XCEL' for Excel and 'QKPT' for KaleidaGraph.

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

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

Checking *Invert Pixel Values* sets up an inverting linear density calibration function for all non-calibrated open images. This causes gray values displayed in the Info and Results windows, in density profile plots, and in histograms, to be inverted. Subsequent image windows created using New, Start Capturing, and the Import of 8-bit images, will inherit this inverting 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 in the upper left corner.

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

Checking *Highlight Saturated Pixels* causes pixels that are less than or equal to 1 to be displayed as yellow and pixels that are greater than or equal to 254 to be displayed in red.

The status of these settings can be made permanent using the Record Preferences command in the File menu.

Profile Plot Options…

Displays a dialog box that allows you to set various options relating to the density profile plots generated by the profile plot tool and the Plot Profile command. If *Fixed Y-Axis Scale* is checked, the y-axis range is fixed and the specified minimum and maximum are used, otherwise, plots are scaled based on the minimum and maximum gray values. *Fixed Plot Size* allows you to specify the size of the plot window. This is useful when pasting a series of plots that you want to all be the same size into an image window. *Profile Plot Line Width* specifies the line selection width. This controls the amount of pixel averaging done when using the profile plotting tool or the Plot Profile command when used with a line selection. All of these settings can be saved using the Record Preferences command.
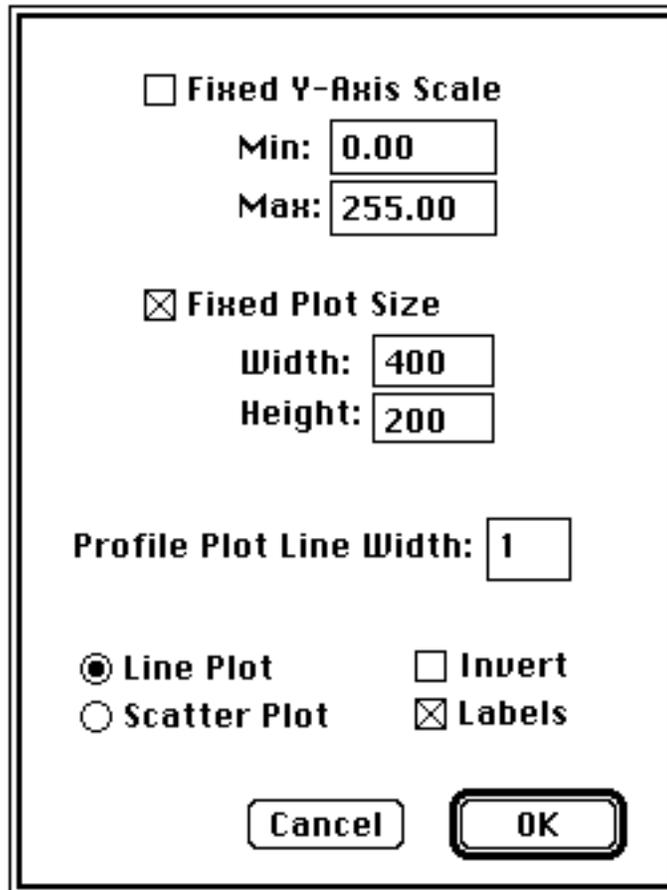
```
☐ Fixed Y-Axis Scale
     Min: 0.00
    Max: 255.00

☒ Fixed Plot Size
   Width: 400
  Height: 200

Profile Plot Line Width: 1

◉ Line Plot      ☐ Invert
○ Scatter Plot   ☒ Labels

        Cancel      OK
```

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 "grow box", in the lower right corner, to rescale the image. Clicking in the "zoom box" at the right end of the title bar will switch that window to *Scale to Fit* mode and 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 in *Scale to Fit* mode, the current magnification is shown in the title bar.

Threshold

Enables and disables thresholding mode. 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. The threshold is automatically set based on an analysis of the histogram of the current selection, or of the

entire image if there is no selection. Background pixels will be ignored when making area and gray value measurements using the Measure and Analyze Particles commands. To vary the threshold, use the LUT tool 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 Info window.

Use the Make Binary command to set all thresholded pixels to black and all background pixels to white. Use the wand tool to outline thresholded objects, and Analyze Particles to automatically measure objects segmented by thresholding. As a shortcut, you can enable thresholding by clicking on the stair step icon in the Map window. This shortcut, however, always sets the threshold to 128.

Density Slice

Enables and disables density slicing. 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. Click and drag in the LUT window using the LUT tool to vary the size and location of the density slice. As you manipulate the density slice, its upper and lower limits are continuously displayed in the Info window.

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

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

Use the Apply LUT command 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 sub-menu allow you to copy various attributes of one image to all other currently open images.

> **Look-Up Table -** Copies the current video look-up 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. Note that spatially calibrated images have a black diamond in the title bar following the name.

> **Density Calibration -** The density calibration associated with the currently active image is transferred to all other open images. Note that density calibrated images have a white diamond in the title bar following the name.

Process Menu

Filtering Functions (Smooth, Sharpen, etc.)

These filters, 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. They are not limited to rectangular selections. The entire image is filtered if no selection is active. They do not work correctly with 8-bit true color images. They can be aborted by typing command-period.

The 3 x 3 tables shown below are the coefficients (weighting factors) for the filters. 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 - Blurs (softens) the selected 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  12  -1                -1  9  -1
-1  -1  -1                -1 -1  -1
```

Shadow - Produces a shadow effect, with the light appearing to come from a direction specified in a dialog box. Three of the kernels used are shown below.
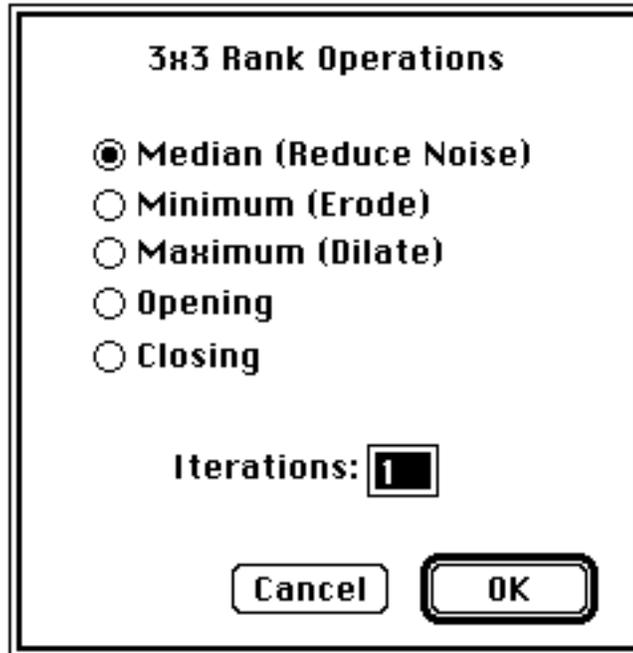
```
      West            North West            North

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

Find Edges - Performs a Sobel edge detection operation. Two convolutions are done using the kernels shown below, generating vertical and horizontal derivatives. The results are then combined by using the square root of the sum of the squares of the two derivatives.

```
   1   2   1                1   0 -1
   0   0   0                2   0 -2
  -1  -2 -11  0  -1
```

Rank Filters

These filters rank (sort) the nine pixels in each 3 x 3 neighborhood and replace the center pixel with the median, minimum (lightest), or maximum (darkest) value. Use the Median filter to reduces noise. The Minimum filter erodes (shrinks) objects in grayscale images similar to the way binary erosion shrinks objects in binary images. The Maximum filter dilates (expands) objects in grayscale images similar to the way binary dilation expands objects in binary images.

```
┌─────────────────────────────────┐
│                                 │
│        3x3 Rank Operations      │
│                                 │
│      ⦿ Median (Reduce Noise)    │
│      ○ Minimum (Erode)          │
│      ○ Maximum (Dilate)         │
│      ○ Opening                  │
│      ○ Closing                  │
│                                 │
│                                 │
│         Iterations: [1]         │
│                                 │
│                                 │
│        ( Cancel )  (( OK ))     │
│                                 │
└─────────────────────────────────┘
```

Select *Median* and each pixel will be replaced with the median value in 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 the spray can tool with the diameter set to 50 or greater.

Select *Minimum* and each pixel is replaced with the minimum value in the 3 x 3 neighborhood. With binary images, this produces the same result as the Binary/Erode filter with count set to one.

Select *Maximum* and each pixel is replaced with the maximum value in the 3 x 3 neighborhood. With binary images, this produces the same result as the Binary/Dilate filter with count set to one.

Select *Opening* and *Iterations* erosion operations are performed, followed by *Iterations* dilations. Select *Closing* and *Iterations* dilation operations are performed, followed by *Iterations* erosions.

Dither

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

Convolve

Does spatial convolutions using kernels read from a text file or text window. Kernels can be up to 63 x 63 in size. Output pixel values are clipped to 8-bits unless *Scale Convolutions* is checked in the Preferences dialog box. Use *Image*'s built-in text editor to create or examine these kernels.

As an example, use the New command to open a blank text window, enter (or paste) the following kernel, then use the Convolve command to try it out.

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

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

Plug-in Filters

Use this sub-menu to run Photoshop compatible filter plug-ins. The plug-ins must be stored in a folder named "Plug-ins" located either in the same folder as *Image* or in the System Folder.

Binary

The commands in this sub-menu are used to convert grayscale images to binary (i.e. black and white only), and to process these binary images. Note that Erode and Dilate do not perform erosion and dilation with structuring elements as described in the literature of classical mathematical morphological. However, several of the Mac image processing programs listed in Appendix D, such as "Alice", do support mathematical morphology.

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

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

neighbors are white. Erosion separates objects that are touching and removes isolated pixels.

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

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

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

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

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

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

Skeletonize - Repeatable removes pixels from the edges of objects in a binary image until they are reduced to single pixel wide skeletons. Type command-period to abort.

Arithmetic

The commands in this sub-menu 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 - Replaces each pixel (V) in the selection 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.

Image Math

Performs arithmetic and logical operations between two images selected from pop-up menus. The operation to be performed is also selected from a pop-up menu.

```
        Image Calculator

         [ Image #2      ▼ ]

  [ – ▼ ] [ Image #1      ▼ ]

         X  [0.5000    ]
         +  [128       ]

         =  [Result    ]

              ☐ Calibrate

         [ Cancel ]  [ OK ]
```
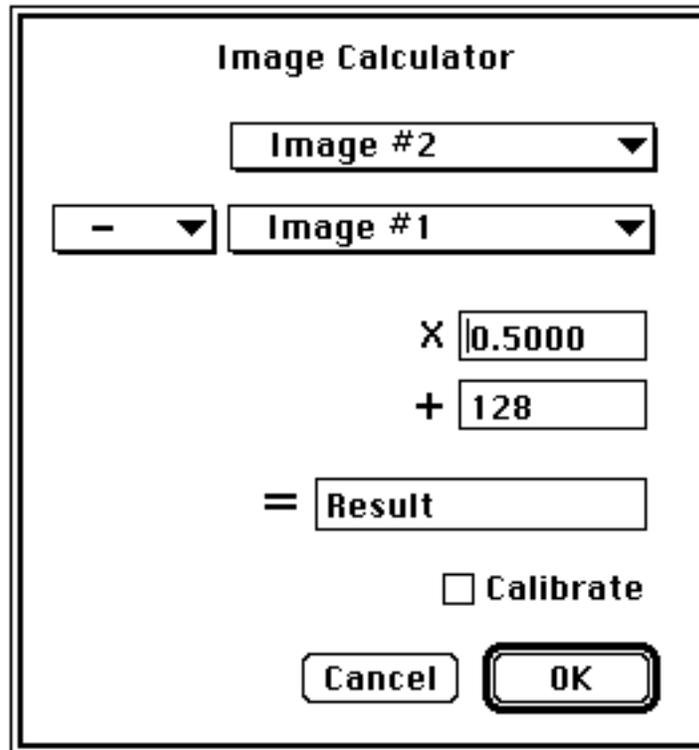
For each pixel, the arithmetic operation is performed, the result is multiplied by the scale factor, the offset value is added, and the final result is clipped to 8-bits (0-255). The operations are carried out in the upper left corner of each image using the largest common rectangle. If *Calibrate* is checked, the calibrated pixel values for the two input images are used and the output image is calibrated using a linear calibration function. For example, adding two imported 16-bit MRI images with calibrated pixels values in the range 0-1000 would result in an image with calibrated pixel values in the range 0-2000.

Subtract Background

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

> **1D Horizontal -** Rolls an arc (rolling arc) horizontally under each row (shrunk 2 or 4 times) of the image in order to remove the background.

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

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

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

Faster - When checked, the image is shrunk 8 times (instead of 4) for 2D rolling ball subtraction. When the ball radius is less than 15 the image is shrunk 4 times (instead of 2).

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

Apply LUT

Applies the current look-up table function to each pixel in the current selection (or the entire image if there is no current selection) and restores the default look-up table (the identity function). This modifies the gray values so that when the image is viewed using the default grayscale look-up table it will look the same as it did before. It also makes any 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 LUT 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.
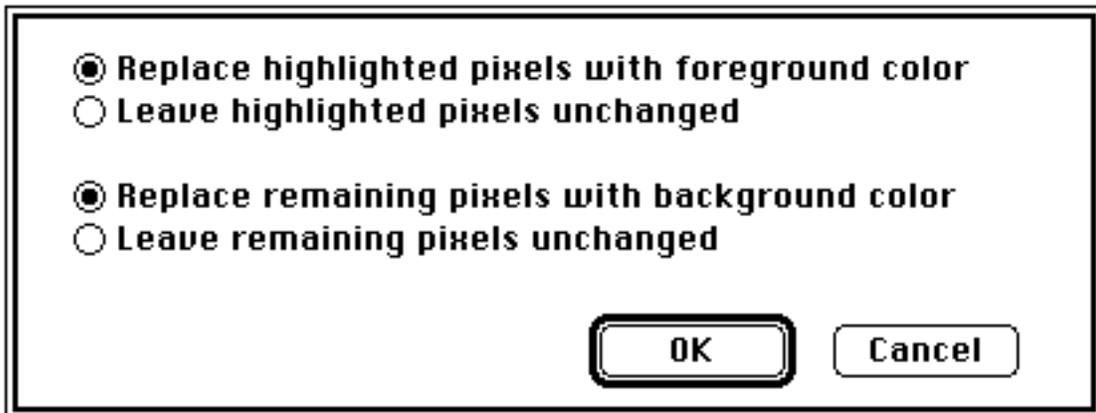
If density slicing is in effect, the above dialog box is displayed, allowing you to process highlighted pixels in one way, and all other pixels another way.

Enhance Contrast

Enhances the contrast of the currently active image. A new look-up table function is generated by doing a linear histogram stretch of the current selection. Use the Apply LUT command to make the contrast change permanent. Enhance Contrast does not work with true color images.

Equalize

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

Fix Colors

Attempts to correct color anomalies by mapping pixels with values of either 0 or 255 to the nearest matching color in the other 254 LUT entries. This usually corrects pseudo-

color problems due to the way *Image* "hard wires" entries 0 and 255 to white and black respectively.

Analyze Menu

Measure

Computes the area and mean gray value of the current selection (or length if it's a line selection) and displays the results in the Info and Results windows. Use the Options dialog box to enable other measurements, such as perimeter. Use Undo to delete the last measurement or Delete Measurement to delete an arbitrary measurement. Only highlighted pixels are included in the computation if either density slicing or thresholding are enabled. The measurement counter is incremented by one each time you use Measure. Use Reset to reset it to zero. The default maximum number of measurements is 200, but this can be increased in the Options dialog box.

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

Calculated areas are displayed in calibrated units, such as square millimeters, if the Set Scale command has been used to establish the spatial scale. Density readings are displayed in calibrated units, such as optical density or isotope concentration, if the Calibrate command has been used to perform density calibration.

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

Analyze Particles

Analyze Particles automatically counts and measures objects in binary or thresholded images. 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. 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 the Options dialog box) particles. Hold down the option key to bypass the dialog box. Use command-period to abort particle analysis.
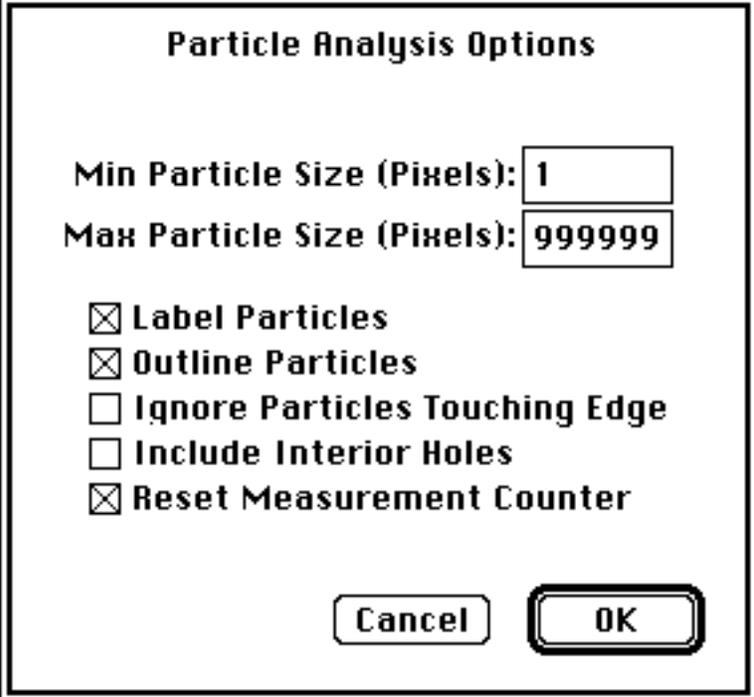
```
┌─────────────────────────────────────────┐
│         Particle Analysis Options        │
│                                          │
│                                          │
│   Min Particle Size (Pixels): │1        │ │
│   Max Particle Size (Pixels): │999999   │ │
│                                          │
│        ⊠ Label Particles                 │
│        ⊠ Outline Particles               │
│        ☐ Ignore Particles Touching Edge  │
│        ☐ Include Interior Holes          │
│        ⊠ Reset Measurement Counter       │
│                                          │
│              ( Cancel )   ⟦  OK  ⟧        │
└─────────────────────────────────────────┘
```

Particles smaller than *Min Particles Size* or larger than *Max Particle Size* will be ignored. Note that the sizes must be specified in pixels. 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. Interior holes in area and mean density computations are included if *Include Interior Holes* is checked.

Show Results

Opens a window and displays the current results table in it. If the Results window is already open, then it is activated. No more than 32K characters can be displayed in the Results window, but there is no limit to the number of measurements that can be exported to a text file.
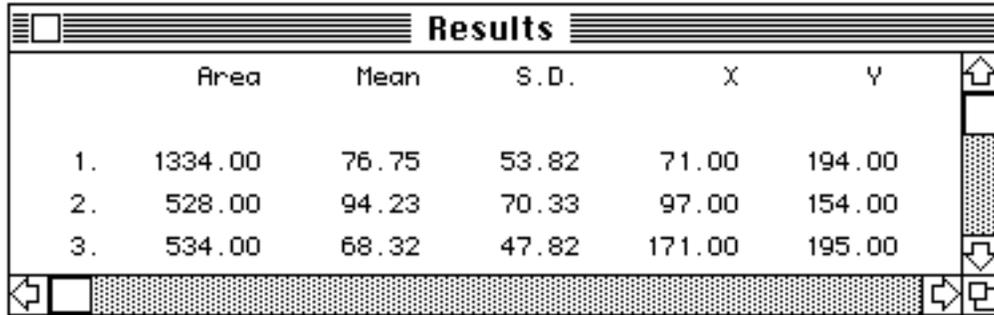
```
┌─────────────────────────────────────────────────────────────┐
│▤□▤▤▤▤▤▤▤▤▤▤ Results ▤▤▤▤▤▤▤▤▤▤▤▤│
├─────────────────────────────────────────────────────────────┤
│      Area     Mean     S.D.       X        Y          ⬆       │
│                                                              │
│  1.  1334.00   76.75    53.82    71.00   194.00              │
│  2.   528.00   94.23    70.33    97.00   154.00              │
│  3.   534.00   68.32    47.82   171.00   195.00      ⬇       │
├─────────────────────────────────────────────────────────────┤
│ ◁ □ ▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒▒ ▷ ▣          │
└─────────────────────────────────────────────────────────────┘
```

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

Show Histogram

Displays a plot showing the distribution of gray values within the selection. The plot shows, for each of the possible 256 gray values, the number of pixels in the selection that have that gray value. 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 Info window. Note that the Histogram command works with non-rectangular selections.

The Copy command, when the histogram window is the active window, copies 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 Export command.

Plot Profile

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

For line selections, it displays a plot of the gray values along the selected line. Pixel averaging is performed when using straight line selections and the line 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, each data point plotted is the average of eight pixels.
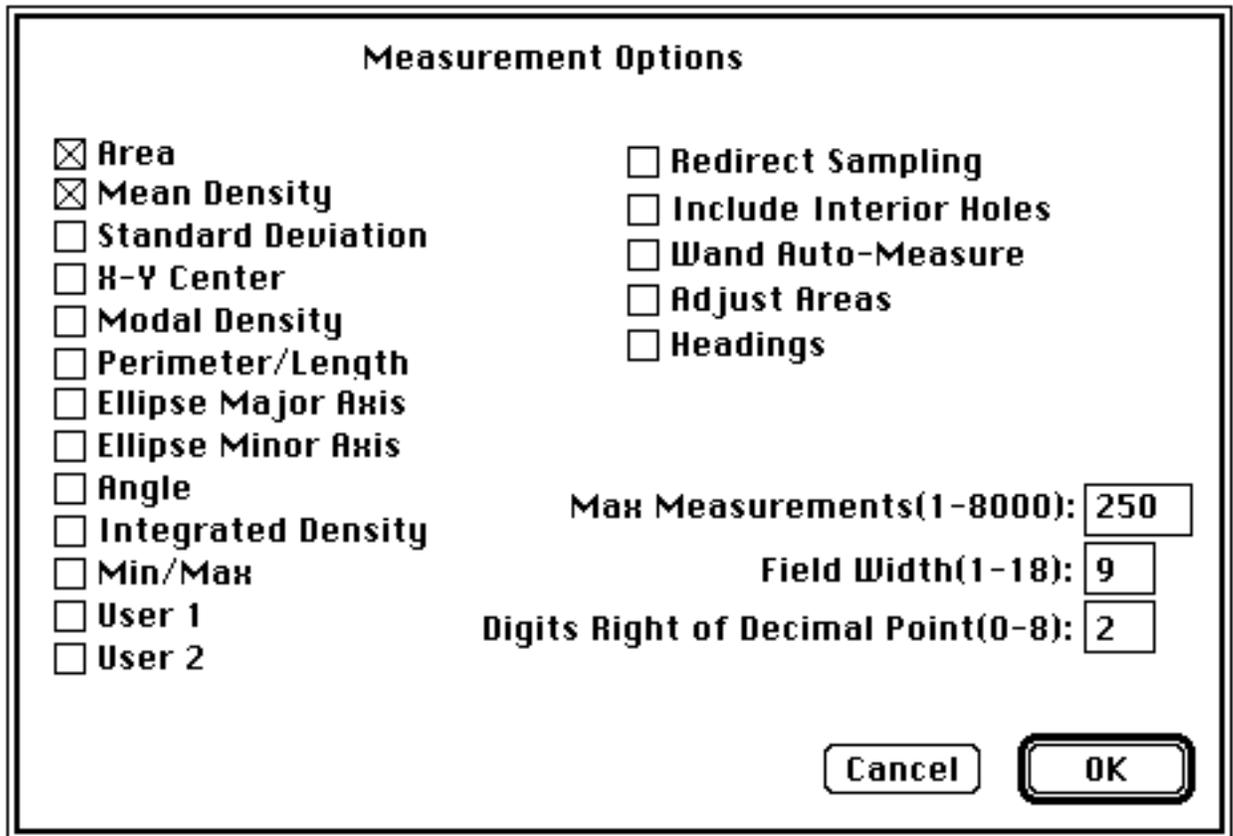
36

The Profile Plot Options command can be used to vary the way profile plots are displayed. Use the Copy command to copy both the profile plot (as a PICT) and the data values (as a single column of text) to the clipboard. Plot values can also be saved to a text file using the Export command.

Surface Plot…

Generates a surface plot of the current selection. The entire image is plotted if there is no selection. Type command-period to abort plotting. You can exaggerate the vertical scale in the plot by increasing the contrast of the image before plotting using the controls in the Map window. To improve the quality of plots of small selections, use the Scale and Rotate command to enlarge the selection before plotting.

Options…

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

```
                    Measurement Options

  ☒ Area                          ☐ Redirect Sampling
  ☒ Mean Density                  ☐ Include Interior Holes
  ☐ Standard Deviation            ☐ Wand Auto-Measure
  ☐ X-Y Center                    ☐ Adjust Areas
  ☐ Modal Density                 ☐ Headings
  ☐ Perimeter/Length
  ☐ Ellipse Major Axis
  ☐ Ellipse Minor Axis
  ☐ Angle
  ☐ Integrated Density        Max Measurements(1-8000): 250
  ☐ Min/Max                         Field Width(1-18): 9
  ☐ User 1             Digits Right of Decimal Point(0-8): 2
  ☐ User 2

                                      [ Cancel ]   [ OK ]
```

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

> **Mean -** Average gray value within the selection. This is the sum of the gray values of all the pixels in the selection divided by the number of pixels. Reported in calibrated units (e.g., optical density) if the Calibrate command has been used to calibrate the image.

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

X-Y Center - Center of the best fitting ellipse, measured from either the upper left or lower right corner of the image, depending on the status of the *Invert Y Coordinates* check box in the Preferences dialog box. This is the geometric center of the selection, not the density weighted center. This option is automatically enabled when the cross hair tool is used.

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

Perimeter/Length - Length around the outside of the selection, or line length for line selections. The perimeter is not computed for composite selections created using the control and option keys. This option is automatically enabled when Measuring a line selection.

Major/Minor Axis - Lengths of the major and minor axes of the best fitting ellipse. These values are set to zero for images with a pixel aspect ratio other than one because the fitting routine currently does not work with non-square pixels.

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

Integrated Density - Sum of the gray values in the selection, with background subtracted. Use to measure the size (volume) of spots or bands in electrophoresis gels. It is computed using the following formula:

$$IntegratedDensity = N * (Mean - Background)$$

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

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

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

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

The wand tool includes interior holes in area and mean density computations if *Include Interior Holes* is checked. If *Wand Auto-measure* is checked, the Measure command is automatically invoked whenever an object is outlined using the wand tool. *Adjust Areas* is used by the Gel Plotting Macros to attempt to correct for the tendency of the wand tool to underestimate the size of small peaks by not counting pixels under the peak boundary. When this option is selected, the area is increased by 0.5*Perimeter + 1.5*AspectRatio, where AspectRatio is the width to height ratio of the selection's bounding rectangle.

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

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

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

Set Scale…

Performs spatial calibration so that results from length and area measurements are presented in calibrated units, such as millimeters. Before setting the scale, use the line selection 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 from the *Units* pop-up menu, and enter the known distance in *Known Distance*.
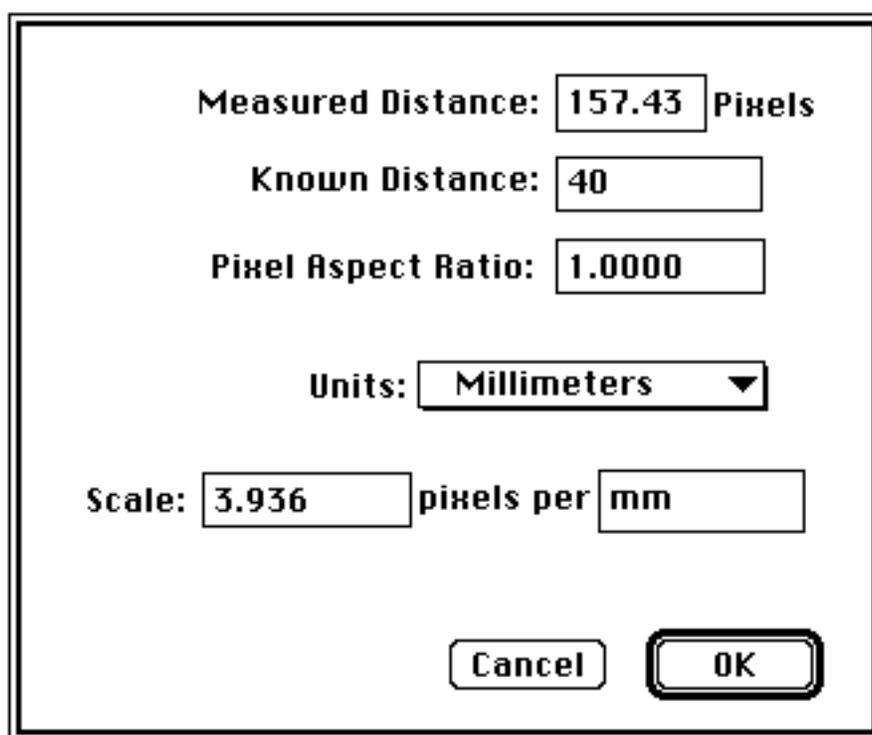
Setting *Pixel Aspect Ratio* to a value other than 1.0 enables support for different horizontal and vertical spatial scales, for example 100 pixels/cm horizontally and 95 pixels/cm vertically. Before setting the aspect ratio, calibrate to a known horizontal distance. Then enter the pixel aspect ratio into *Pixel Aspect Ratio*. To find the pixel aspect ratio, measure the width and height (in pixels) of a digitized object with a known 1:1 aspect ratio. The pixel aspect ratio is computed by dividing the width by the height. Note that only the horizontal scale is shown. Divide the horizontal scale by the aspect ratio to compute the vertical scale. Note that the Measure command sets the ellipse major and minor axis lengths to zero when the pixel aspect ratio is not one.

Once the scale is set, you can switch to other units of measurements, for example from inches to centimeters. If you know what the scale for an image is (e.g., 300 dpi), then that value can be directly entered into *Scale*. Select *Pixels* from the pop-up menu to disable spatial calibration. To use a unit of measurement that is not predefined, select *Arbitrary Units* and manually type in the unit. As a shortcut, double-click on the line selection tool

to bring up the Set Scale dialog box. Note that *Image* displays a black diamond in the title bar of spatially calibrated images.

Calibrate…

Use this dialog box to calibrates to a set of density standards, such radioactive isotope standards or a calibrated optical density step tablet (available from Kodak). Before calibrating, you must record the mean gray value of each of the digitized standards. First, use the Reset command to set the measurement counter to zero. Then use one of the selection tools and the Measure command to record the mean gray value of each of the standards. Note that the standards must be measured in order, starting with the lowest gray value (lightest) standard.

	Measured	Known		
1	30.30	0.060	○ Straight Line	
2	38.78	0.200	○ 2nd Degree Polynomial	
3	64.65	0.340	● 3rd Degree Polynomial	
4	93.29	0.490	○ 4th Degree Polynomial	
5	143.57	0.640	○ Exponential	
6	202.28	0.790	○ Power	
7	242.92	0.940	○ Log	
8	255.00	1.100	○ Rodbard	
9			○ Uncalibrated OD	
10			○ Uncalibrated	
11				
12			Unit of Measure: 0.D.	
13			Save… Open…	
14			Invert OD	

When you have finished making the measurements, select Calibrate to display the calibration dialog box. To calibrate the image, enter the actual values in the *Known* column, select a curve fitting method, enter the unit of measurement, and click OK. If the calibration curve is not satisfactory, bring up the Calibrate dialog box again and select a different curve fitting method.

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

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

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

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

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

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

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

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

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

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

Redo Measurement

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

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. If a selection is currently active, this command restores the previous one. Can also be used to transfer a selection from one window to another.

Label Selection

Outlines (in white) the current selection and labels it with the measurement count. Can be used to keep track of which objects have been measured. Hold the option key down to draw (also in white) the best-fit ellipse. To draw the ellipse, you must have previously used the Measure command and have selected X-Y Center, Ellipse Major Axis or Ellipse Minor Axis in Options.

Special Menu

Start Capturing

Continuously captures and displays video using a Data Translation QuickCapture, Scion LG-3 or Scion AG-5 frame grabber card. The capture rate varies from four to eight frames per second, with 68040 and PowerPC based Macintoshesusing built-in video providing the fastest rate. Notice how, during continuous capture, the name of this command changes to "Stop Capturing" and "(Live)" appears in the title bar. To "freeze" the image, select "Stop Capturing", type command-g, or click in the Camera window with any tool except the magnifying glass or the grabber. Newly created Camera windows inherit the look-up table, as well as any spatial or density calibration, of the currently active window.

While capturing, you are allowed to change contrast, alter color schemes, and zoom or pan the image. Histograms are continuously calculated and displayed if you select Show Histogram while the Camera window is in "Live" mode.

Average Frames

Averages or integrates 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.

Number of Frames is the number of frames to be averaged or integrated. Note that there is a possibility of overflow if this value is set to more than 128 since frames are summed in a signed integer buffer with a maximum pixel value of 32,767.

Check *Integrate* to sum the frames, rather than averaging them. Integration 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. Check *Calibrate* to set up a calibration function so that approximations of the 16-bit values are displayed in the Info and Results windows.

Check *Integrate On-chip* to do on-chip integration using the Scion LG-3 and Cohu 4910 series cameras or modified versions of the Dage-MTI CCD72. The camera will integrate on-chip for the specified number of frames and then the LG-3 will capture the integrated frame. On-chip integration requires a special cable, available from Scion, for connecting the integration input of the camera to the LG-3.

Check *Video Rate* to average or integrate at video rates using the Scion AG-5. With the Scion LG-3, *Image* will use the LG-3's on-board memory to capture frames to be averaged or integrated at video rates (25 or 30 frames per second). For example, when averaging 32 frames, the frames are captured in about one second instead of 15-25 seconds. It requires 15-25 seconds, however, to compute the average because the LG-3 does not have the AG-5's ability to do video rate arithmetic. The number of frames that can be captured depends on how much memory is installed. A 4MB LG-3 can capture 8 frames at video rates and one with 16MB can capture 32.

Macros are available in the "Video" macro file to continuously integrate and display frames either off-chip, using the Scion AG-5, or on-clip, using the Scion LG-3 and a Cohu or Dage camera. Instructions for using these macros are in the macro file.

If *Fix Scale* is checked, the values in *Min* and *Max* control how integrated images are linearly scaled from 16-bits to 8-bits. If it is not checked, the computed minimum and maximum values are used. The actual minimum and maximum values are always displayed in the Info window.

You can bypass the Average Frames dialog box by holding down the option key. The Average Frames macro (in the "Video" macro file) also bypasses the dialog box, and, in addition, allows you to do averaging or integration by simply pressing the "A" key.

Save Blank Field

Saves (in a window named "Blank Field") a background image that will be used to correct for uneven illumination. The Stop Capturing command will use this reference image to correct shading errors in newly acquired images. It does this by dividing each pixel in the newly acquired image by a correction factor computed for each pixel in the blank field. This correction factor is generated by dividing each gray value in the blank field by the mean gray value.

You can capture a single frame without shading correction by holding down the option key while selecting Stop Capturing. Close the "Blank Field" window to stop doing shading correction.

Video Control…

Displays a modeless dialog box that allows you to interactively control video capturing and to alter the behavior of the Animate and Make Movie commands.

Images acquired using a frame grabber card are 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 red and all pixels that under saturate the camera (are too dark) to be displayed in yellow.

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

Use External Trigger enables support for the external trigger feature of the Data Translation QuickCapture and Scion LG-3 when using the Start Capturing, Average Frames or Make Movie commands. External trigger events are signaled by a 5 volt falling TTL edge on input line 7 of the QuickCapture cable.

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

Check *Separate Sync* when using the Scion LG-3 frame grabber and an RGB color camera with separate composite sync output.

Channel allows you to specify which of four input channels is used by the QuickCapture or Scion frame grabber cards. Note that with the QuickCapture card, the input cable labeled "0" is used for channel 1.

Offset and *Gain* allow you to adjust the A/D offset (brightness) and gain (contrast) of the Scion LG-3 and AG-5 frame grabber cards. Changes are interactively displayed in live video mode. The objective when using these controls is to optimize the image brightness and contrast. One method for doing this is to enable *Highlight Saturated Pixels* and interactively adjust offset and gain to just eliminate the saturated pixels within the region

of interest. Another method is to display the histogram and interactively adjust offset and gain to spread out the histogram as much as possible.

Several command key shortcuts are useful in conjunction with this dialog box. Command-w closes the Video Control dialog box. Command-g enables or disables live video capture. Command-h displays a histogram, continuously updated during live video. And, if it is active, Command-w closes the histogram window.

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. Before selecting Photo Mode, you can change the background color by clicking in the LUT window with the eraser tool. Holding down the option key while selecting Photo Mode causes 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 containing one or more macros written in *Image'*s Pascal-like macro programming language. Each macro in the file causes a new command to be inserted at the bottom of the Special Menu. Macros may be assigned to any key, including function keys on the extended keyboard. For example, a macro named "Test Macro [T]" would be assigned to the 'T' key, and one named "Another Macro [F1]" would be assigned to the F1 function key. In both cases, it is not necessary to hold down the command key. Macros can be aborted by typing command-period. When launched, *Image* automatically loads the macros contained in the file "Image Macros", as long as this file is in the same folder as *Image*, or in the System Folder.

Load Macros may display an error message referring to a line number in the macro file. If this happens, you can find the offending line by opening the macro file using the Open command and using the Find command's ability to move the cursor to a specified line number.

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

The macro programming language is documented in Appendix A.

Stacks Menu

Use the commands in this menu to create or process stacks. Stacks are three dimensional images consisting of two or more "slices". The slices can be consecutive serial sections in a 3D data set, frames in a movie loop, RGB (red, green and blue) channels in a 24-bit color image, or any related set of images. Use the '<' and '>' keys to move through the slices in a stack. The current slice and total number of slices are displayed in parentheses in the title bar. Stacks are displayed in a single window and can be saved to a single disk file using the Save, Save As or Export commands. Most commands in *Image*, with the exception of Save, Save As, Export and Open, operate only on the current slice. There are, however, macros are available (in the file "Stacks") 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. A macro, also called "Window to Stack", is available that works with windows that are not the same size.

Stack to Windows

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

Add Slice

Adds a new blank slice to the stack following the currently displayed slice. The maximum number of slices in a stack is 1000.

Delete Slice

Deletes the currently displayed slice. You can use Undo to restore it.

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 on extended keyboards.

Last Slice

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

Make Movie…

Captures a series of video frames into a stack. *Image* will create a stack the same width and height as the current selection and large enough to hold the number of frames specified. You will be asked to enter the time to delay between frames. Entering zero results in the fastest possible sampling rate. As shown in the table below, the delay time is the inverse of the capture rate in frames per second (fps). For example, to capture at two fps, you would enter a delay of .5. After *Image* has completed recording the movie it will compute the actual frame rate and display it in the Info window. The frames were probably captured at uneven intervals if the computed and desired rates are not the same. In the case, you should specify a slower rate, use a smaller selection, or try enabling "Blind" Capture in the Video Control dialog box. Type command-period to abort the movie making process.

The following table assumes a 60Hz frame grabber card that captures at a maximum rate of 30 fps, or .033 seconds per frame. A 50Hz (PAL) card captures at a maximum rate of 25 fps, or 0.04 seconds per frame. For 50Hz cards, the time delay should be a multiple of .04, the time needed to capture one frame. For example, specify a delay of .08 to capture 12.5 fps (every other frame) or .2 to capture 5 fps (every fifth frame). *Maximum Size* is the maximum possible frame size for the specified rate when capturing on a Quadra 700. Faster Macs should be able to capture larger frames.

Time Delay	FPS	Maximum Size
.067	15	160 X 160
.1	10	280 X 280
.133	7.5	480 X 480
.2	5	640 X 480
.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.

Animate

Animates the current stack by repeatedly displaying its slices (frames) in sequence. Holding the option key down causes the screen to be erased to the current background color before the animation starts (or type command-option-'=' as a shortcut). The animation speed is controlled by pressing keys '1' through '9'. 68040 and Power PC based Macintoshes using built-in video provide the fastest and smoothest animation. The right and left arrow keys can be used to single step through the slices. Press the mouse button to stop. Check *Oscillating Movies* in the Video Control dialog box to get *Image* to automatically reverse direction at the beginning end of the frame sequence.

Average

Creates an new image that is the arithmetic average of all the slices in the current stack.

Make Montage…

Creates a new composite image from the slices in the current stack.
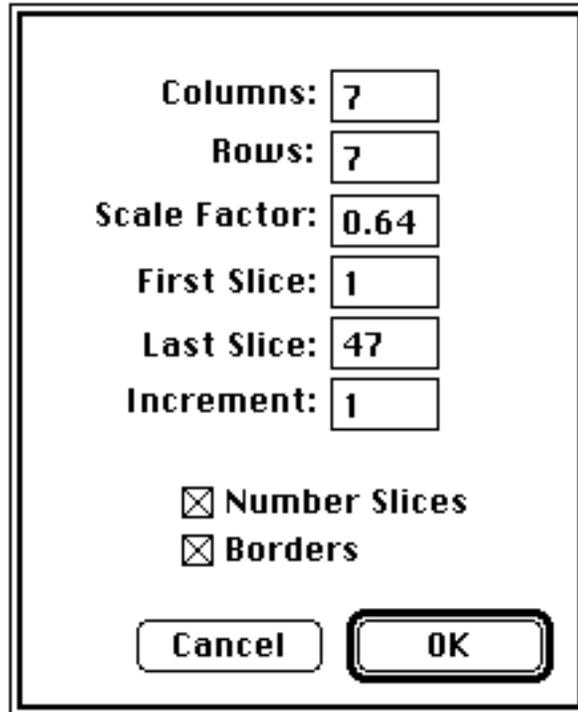
```
          Columns: 7
             Rows: 7
     Scale Factor: 0.64
      First Slice: 1
       Last Slice: 47
        Increment: 1

        ⊠ Number Slices
        ⊠ Borders

        ( Cancel )    [ OK ]
```

Columns and *Rows* control the number of columns and rows used in the composite image. *Scale Factor* determines how the slices in the stack are scaled when they are copied to the composite image. Decreasing the scale factor makes the composite image smaller and reduces the amount of memory required. *First Slice* and *Last Slice* specify which slices in the stack get copied to the composite image. Every slice (starting at *First Slice*) is copied if *Increment* is set to one, every other slice if it is set to two, every third slice if it is set to three, etc. Check *Number Slices* to have the slices in the composite image labeled with the slice number. Use the Font, Size and Style commands in the Options menu (before using Make Montage) to vary the format of the labels. Check *Borders* to draws borders around the slices. The width of the borders can be varied by clicking on the lines at the bottom of the Tools window. Make Montage normally draws labels and borders in white. You can force it to use black by setting the pixel in the upper left hand corner of the first slice to white (value = 0).

Capture Color

Captures and displays a 24-bit RGB color image. Requires a Scion or Data Translation frame grabber card and an RGB color camera with separate composite sync output. With the Scion LG-3 and AG-5, an optional four channel cable is required and *Separate Sync* must be checked in the Video Control dialog box. With the Data Translation QuickCapture, the W5 jumper must be installed and the red, green, blue and sync camera outputs connected to cables 1, 0, 2 and 4 respectively. Color capture is very memory intensive.

RGB to 8-bit Color…

Converts an RGB color image (in the form of a three slice stack) to 8-bit indexed color. You have a choice of three 256 color look-up tables to use with the indexed color image. Select *Existing* to use the current look-up table. Select *System* to use the Apple default 8-bit color table. Select *Custom* to create a custom 256 color table based on the colors in the RGB image.

```
Palette:
    ○ Existing
    ○ System          ⊠ Dither
    ● Custom

              [ Cancel ]  [[ OK ]]
```
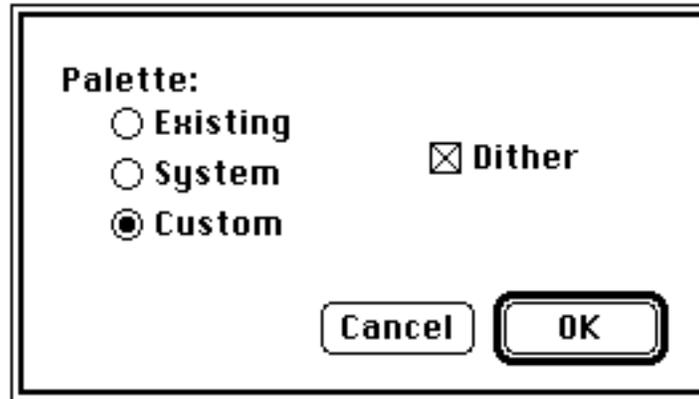
Checking *Dither* causes the 8-bit color image to be drawn using error diffusion dithering, a technique that mixes existing colors to create the illusion of additional colors. Dithering will usually eliminate color banding in the 8-bit image.

8-bit Color to RGB

Converts an 8-bit indexed color image into a three slice (red, green and blue) stack, a format that allows filter operations such as smoothing and sharpening to be performed. Macros are available in the file "Stacks" for automating filtering operations on RGB stacks.

RGB to HSV

Converts an RGB color image (in the form of a three slice stack) to HSV (Hue, Saturation and Value).

Register…

Translates and rotates the slices in the current stack into alignment based on fiducial points.

There are two interfaces. The more intuitive of the two requires the user to click on visible landmarks in a reference slice, then click on corresponding fiducial points in the other slices in the stack. The code then lines up the slices based on these points. The user may easily throw out unreliable points in non-reference slices and correct input errors. This registration interface may only be used in cases where all fiducial points are visible on screen in the captured slices.

The second interface is more difficult to use, but it works regardless of whether or not fiducial marks are included within the borders of the captured slices. Here, the user provides fiducial data in a text file. These data consists of relative coordinates of fiducials from each slice as read from a microscope digitizer (or similar device) as well as the necessary information to map the coordinate system of the microscope digitizer to the coordinate system of windows in NIH Image.

The text file method uses relative coordinates of fiducial marks as recorded with some device such as a microscope digitizer. Let us call these coordinates "stage" coordinates. It also uses screen coordinates of fiducials in Image. Before registration may take place, stage coordinates must be mapped to the NIH Image screen coordinate system. To do this, NIH Image reads the following, tab-delimited information from a text file. In every case, x-coordinates are followed by y-coordinates.

> **line 1:** the screen coordinates of a point which is fixed for every image captured. In the sample fiducial file, this is the point which corresponds to the cross hairs on the microscope at capture time. This is the "image center" point.

> **line 2:** the location, in screen coordinates, of two fixed points in an image.

> **line 3:** the location, in stage coordinates, of these same two fixed points.

> **line 4:** (and each subsequent line) the locations, in stage coordinates, of the "image center" and at least two, but nor more than 12, fiducial points for an image to be registered.

Obviously, the stage coordinate system may not change in rotation, translation, or scale in the middle of a set of images being captured, so it is advisable to record stage coordinates at the time of image capture (without changing camera settings or the position of the object whose image is being captured).

Lines 4-n must entered in the same order as the slices in the stack. It doesn't matter which one is the reference image. Where fiducial coordinates are unavailable or unreliable, use the number 10000 for both the x and y stage coordinate in the file. Each slice must be represented by the same number of fiducial coordinates in the data file.

As you can see, using the text file method introduces many possible sources of errors, so results are not usually as good with this method as with the on-screen method.

An example stack with text file fiducial points ('RegistrationExample.bin') is available by FTP from zippy.nimh.nih.gov, in the /pub/nih-image/stacks directory.

This routine was written by Michael Castle (mike.castle@med.umich.edu) of the University of Michigan Mental Health Research Institute (MHRI).

Project…

Generates an animation sequence by projecting through a rotating 3D data set onto a plane. Each frame in the animation sequence is the result of projecting from a different viewing angle. To visualize this, imagine a field of parallel rays passing through a volume containing one or more solid objects and striking a screen oriented normal to the directions of the rays. Each ray projects a value onto the screen, or projection plane, based on the values of points along its path. Three methods are available for calculating the projections onto this plane: nearest-point, brightest-point, and mean-value. The choice of projection method and the settings of various visualization parameters determine how both surface and interior structures will appear.

This routine was written by Michael Castle (e-mail: mike.castle@med.umich.edu) and Janice Keller of the University of Michigan Mental Health Research Institute (MHRI).

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

The *Lower and Upper Transparency Bound* parameters determine the transparency of structures in the volume. Projection calculations disregard points having values less than the lower threshold or greater than the upper threshold. Setting these thresholds permits making background points (those not belonging to any structure) invisible. By setting

appropriate thresholds, you can strip away layers having reasonably uniform and unique intensity values and highlight (or make invisible) inner structures. Note that you can use density slicing to set the transparency bounds.

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

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

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

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

Several macros (in the file "Stacks") are available for performing various operations on the slices making up a volume prior to using the Project command. Use "Smooth" to reduce noise in a volume. Since the projection routine assumes volumes contain one or more objects within a black background, use "Invert" to invert volumes with a white background. For low contrast volumes, increase the contrast using the controls in the Map window and then apply the look-up table to the volume using the "Apply LUT" macro. The "Remove 0 and 255" macro changes pixel values of 0 and 255 to 1 and 254 respectively. This is sometimes useful when pseudocoloring projections. Use "Replicate Slices" to eliminate the gaps seen in projections of volumes with a slice interval greater than one by increasing the number of slices. The "Make Cone" macro creates a 64^3 volume containing a cone with two rods inside that is very useful for understanding the effect of various projection parameters.

Reslice

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

Options

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

Windows Menu

Next Window

Deactivates the current image window and activates the next image in the image list, as shown at the bottom of the Windows Menu.

Cascade 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

Shrinks all the images windows and repositions them so that they will fit on the screen without overlapping. Hold down the option key to draw the tiled windows in "Scale to Fit" mode. Click on the "Zoom Box" of a tiled window to expand it to fill the screen.

Image supports "synchronized scrolling" of tiled windows. To try this out, open two or more windows (they must be the same size), tile them using Tile Windows, zoom in on one of them, then pan one of the images with the option key down. Notice how all the other images zoom and pan to the same relative location.

TOOLS

The window containing the tool palette, as well as all other windows displayed by *Image* can be freely moved around the screen. All the tools work with images at any of the eight available levels of magnification. One of the tools (the line selection tool) has three variations selected using a pop-up menu.
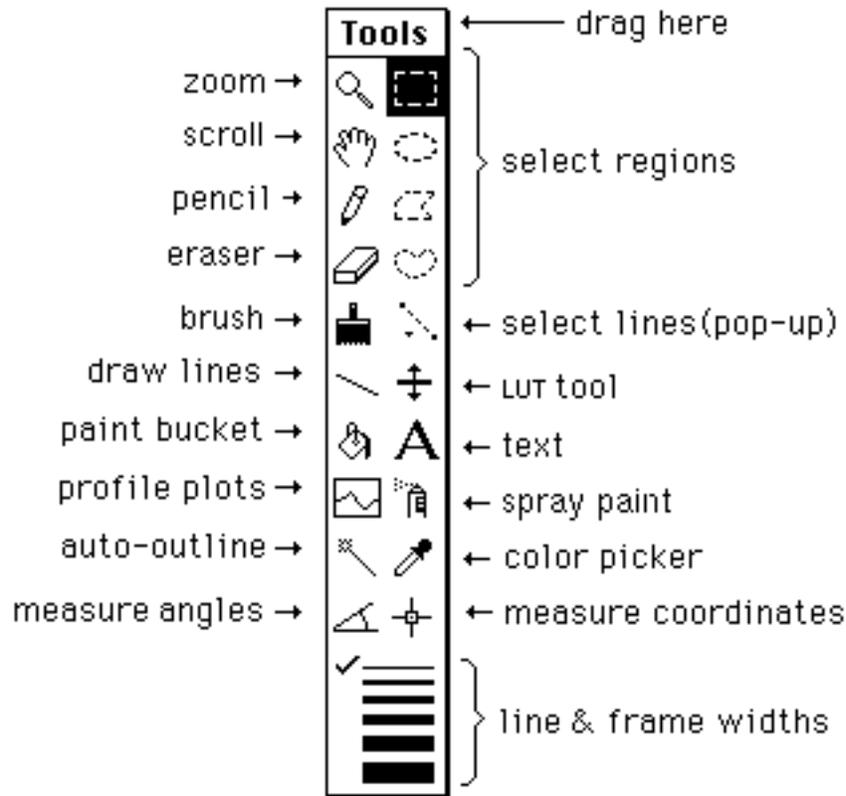
Magnifying Glass - Click within the active image window to zoom. Double-click on the magnifying glass icon to revert to 1:1 magnification. Option-click (or use Undo) within the image to reduce magnification in steps. Notice how the plus sign changes to a minus sign when you hold the option key down. 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) - Allows you to scroll through an image that is larger than its window. When using other tools (except the text tool), you can temporarily switch to the grabber by holding down the space bar.

Rectangular Selection Tool - Use this tool to specify a rectangular subregion to be used by commands in the Edit, Process and Analysis menus. The contents of a rectangular selection 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 on this tool's icon to select the entire image. As the rectangle is being drawn, its width and height are shown in the Info window.

Elliptical Selection Tool - Creates elliptical subregions for use by various commands in the Edit, Process and Analyze menus. Hold down the shift key to constrain the selection to a perfect circle. As the ellipse is being drawn, its width and height are shown in the Info window.

Polygonal Selection Tool - Creates polygon-shaped subregions for use by various commands in the Edit, Process and Analyze menus. Click in the small box at the starting point to end drawing. (Double-clicking also terminates drawing.) Double-click on this tool's icon to bring up the measurement options dialog box.

Freehand Selection Tool - Creates non-rectangular subregions for use by various commands in the Edit, Process and Analysis menus. Double-click on this tool's icon to bring up the Calibrate dialog box.

Image regions defined by the four outlining tools can be Copied, Filled, Cleared, Inverted, Framed, filtered, or Measured. Immediately after using the Fill command, you can change colors by clicking in the LUT window. Similarly, after using Draw Boundary, you can change line widths by clicking on the lines in the Tools window.

Selections can be edited by holding down the control key to add a new selection to an existing selection or the option key to subtract a new selection from the existing selection. When using the polygon or freehand tools, you can also use the shift key to add to selections. 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 little triangle) is used to select one of three line types. The pop-up menu is accessed by clicking on the line selection tool's 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 it using the Fill command, generate a density profile plot using the Plot Profile command, and outline the line (assuming line width is greater than one) using the Draw Boundary command. With straight lines only, you can 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 selections are made, using a double-click to terminate the line.

Line width is specified by clicking on the lines at the bottom of the tool palette. 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. Type command-f (Fill) and command-1 (Measure) to quickly draw and measure the length of a line selection. Use Show Results to display the list of 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 selections 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 "nudged" a pixel at a time using the arrow

keys. Straight line selections have three "handles" (small black squares). The handles at either end swing and stretch the line and the one in the center is 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 one pixel wide lines in the current foreground color. Click in the LUT window, or option-click in the image, to change the foreground color. Hold down the shift key to force pencil movements to be either horizontal or vertical.

Eraser - Erases to the current background color. Click in the LUT window, or option-click in the image, to change the background color. The color of the eraser indicates the current background color. The background color is used by the Cut, Clear and Photo Mode commands, and as the background color for text. Hold down the shift key to force eraser movements to be either horizontal or vertical. As a shortcut, you can double-click on the eraser to erase the entire active image.

Paint Brush - Paints in the current foreground color. Hold down the shift key to force brush movements to be either horizontal or vertical. Click in the LUT window, or option-click in the image, to change the foreground color. The color of the brush indicates the current foreground color. Double-click on the brush to change its size.

LUT Tool - Click and drag in the LUT window with this tool to dynamically modify the current color look-up table. Hold the option key down to rotate all 256 entries in the LUT. The LUT tool is also used to manipulate the density slice when density slicing is enabled. As a shortcut, you can double-click on this tool to enable/disable density slicing.

Spray Can Tool - Draws random pixels in the current foreground color. Double-click on the tool to change the brush diameter.

Line Drawing Tool
Draws straight lines using the current foreground color and line width. The line width is specified by clicking on the lines at the bottom of the tool palette. 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 selection outline in the process. To outline an object, either click inside near the right edge, or outside to the left of the object. To visualize what's happening, imagine a turtle that starts moving to the right from where you click looking for a binary edge. Once it finds an edge, it follows it in a counter clock-wise direction until it returns to the starting point.

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

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

Density Profile Tool
Creates a straight line selection and uses it to generate a density profile plot. Pixel averaging is done if the current line width is greater than one. Change the line width by clicking on the lines at the bottom of the Tools window. The line selection can be constrained to horizontal or vertical be holding down the shift key. Use the Draw Boundary command (command-B) to draw a reference line in the current foreground color. To highlight the pixels used to generate the profile plot, hold down the mouse button and drag in the Plot window. The Plot window, unlike the Histogram window, can be resized.

Plots can be copied to the Clipboard and then pasted into an image window. In addition to the plot, the Copy command also copies the plot data values as a single column of numbers. These numbers can then be pasted into analysis and plotting programs, such as Excel and KaleidaGraph. Profile plot data values can also be exported to a text file using the Export command.

Various aspects of the plots produced by the profile plotting tool can be altered using the Profile Plot Options dialog box. As a shortcut, you can bring up this dialog box by double-clicking on the density profile tool.

Paint Bucket
Flood fills, using the current foreground color, the area with the same color as the point you click on. Note that the "hot spot" is at the end of the paint coming from the bucket.

Text Tool
Adds text to images using a typeface and style chosen from the Font, Size and Style sub-menus in the Options menu. Various attributes, such as typeface, size and color, can be changed after the text has been entered. Once you choose another tool, however, or type return, the text becomes part of the image's bitmap.

Hold down the option key to get the text tool to automatically type in the most recent length, angle, or area measurement. Clicking repeatedly with the option key down enters multiple readings, starting with the most recent one.

Eyedropper - Sets the foreground color by "picking up" colors from the image or from the LUT window. Option-click to change the background color. 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. In density slicing mode, double-clicking in the LUT window allows you to change the density slice color. Note

that, when using this tool, pixel values are displayed in the Info window as RGB components.

Angle Tool - Measures the angle formed by two lines drawn through a point. The angle is displayed interactively in the Info window as the lines are drawn. Use Show Results to display the complete list of angle measurements. The angle tool creates a line selection that can be used to draw the angle using the Draw Boundary command. The value of the angle can be permanently displayed in the image by option-clicking with the text tool.

Cross Hair Tool - Counts objects and records their X-Y coordinates, leaving markers so objects are not counted twice. The markers are drawn in the current foreground color and their size is related to the current line width. Holding down various modifier keys alters the behavior of the cross hair tool. Use the option key to label using the current measurement count. Use the control key to display X-Y coordinates. Use the shift key to decrement the count and leave a marker in the background color. Use the Font, Size and Style sub-menus in the Options menu to vary typeface, size, etc. Use Show Results to display the list of X-Y coordinates. The cross hair tool alters the image pixel data. To avoid this, duplicate the image and work on a copy.

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

WINDOWS

Image Windows
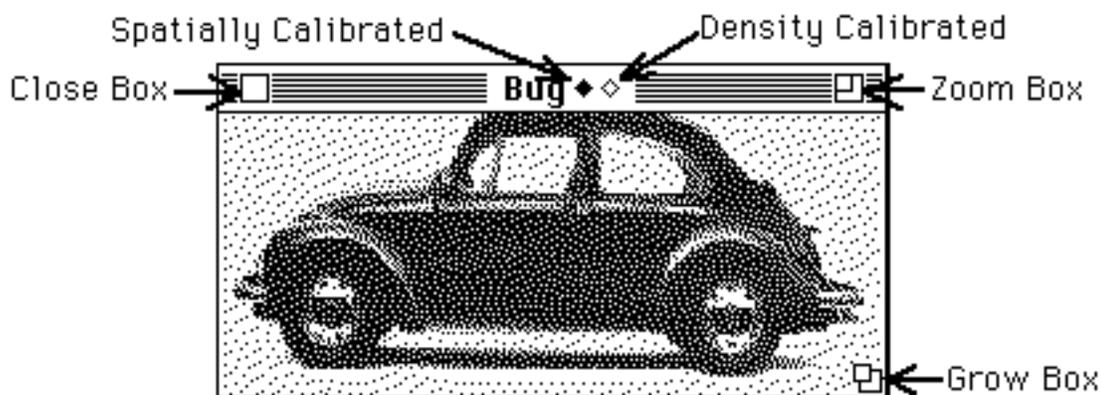
Images are displayed in windows such as the one above. Image windows are created when you use the New, Open, Import or Duplicate commands. Click in the Close Box to close the window and free the memory used to store the image. Hold down the option key when clicking in the Close Box to close all image windows.

Click in the Zoom Box to switch the window to "Scale to Fit Window" mode and make it as large as possible, while still maintaining the same aspect ratio. Restore the window to its original size by clicking again in the Zoom Box, or by using the Unzoom command. 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. In Scale to Fit mode, the image will be rescaled to fit any resized window. For magnified images, you can use the Grow Box to make the window larger than its original size.

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

Map
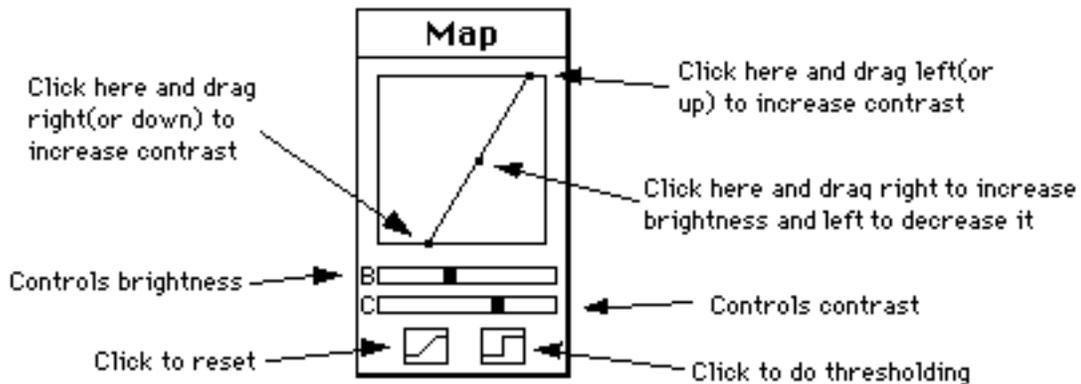
Use the Map window to optimize the brightness and contrast of images by modifying the video look-up table. To vary brightness, click and drag inside the frame. This moves the plot (a piece-wise linear transformation function) horizontally, changing the Y-intercept while maintaining a fixed slope. Brightness can also be altered using the 'B' slide control. To change contrast, click and drag in the margin to move the two end-points that define the function, or use the 'C' slide control. A good strategy for optimizing the contrast of an image is to click (outside the frame) in the lower left hand corner, than drag horizontally to the right until the image starts to saturate. Similarly, click in the upper right hand corner, and drag horizontally to the left.

Histogram
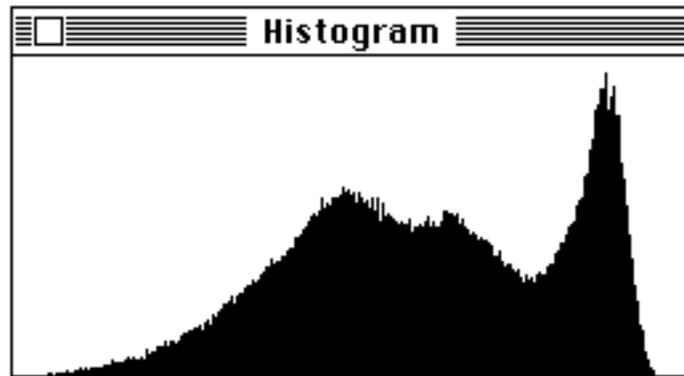
The Histogram window displays the gray value histograms produced by the Show Histogram command. This function shows, for each of the 256 possible gray values, the number of pixels within the selection that have that value. The histogram values are dynamically displayed in the Info window whenever the 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* (in "Plotting Macros") 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

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

Click in the LUT window with any of the drawing tools to "pick up" a new drawing (foreground) color. Click with the eraser, or option-click with any other tool, to pick up a new background color. Select "LUT" in the Save As dialog box to save the look-up table of the current image.

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

Paste Control
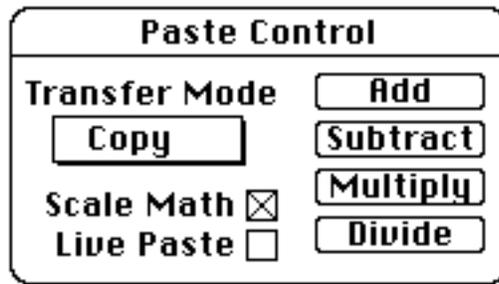
The Paste Control window allows you to control how image selections are pasted, or to do image arithmetic on pasted images. It is only effective during paste operations. Use the *Transfer Mode* pop-up menu to specify the method (*Copy*, *And*, *Or*, *Xor*, *Replace* or *Blend*) used to copy the image during a paste operation. The Paste Control window is activated by selecting Paste Control from the Windows menu, or by typing command-y.

In the default *Copy* mode, pasting occurs normally. When *And*, *Or*, or *Replace* are selected, the selection is copied to the screen using "And", "Or", or "Replace with Transparency" modes, respectively, allowing you to see both the object being pasted and the underlying image. When attempting to align two images, it is usually easier if one of the images is first converted to binary using Make Binary, Find Edges, or Dither. Macros are available in the file "More Macros" for rapidly switching among the different transfer modes.

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 *Blend* mode, destination pixels are replaced with the arithmetic average of the source and destination pixels. It is similar to an *Add* operation with *Scale Math* checked. *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 on *Subtract* subtracts the selection being pasted from the current window. If *Scale Math* is checked, arithmetic operations are 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 scaled to the range of 0 to 255. Results are clipped to 0 and 255 if *Scale Math* is not checked. Arithmetic operations are always done on raw pixel values, ignoring any density calibration that may be in effect. *Add*, *Subtract*, *Multiply* and *Divide* only work with rectangular selections.

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

Selecting *Live Paste* causes the currently pasted selection to use "live" TV camera input. The selection must have been copied from the Camera window and pasted into some other window.

Use the arrow keys 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
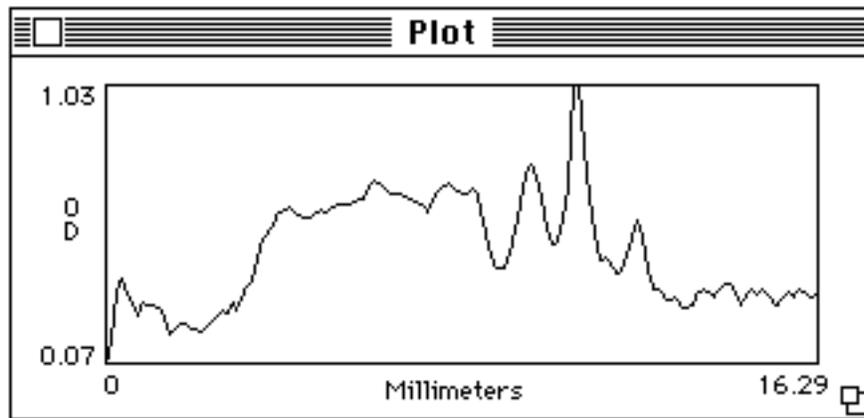
The Plot window is created, or updated, by the density profile tool, the Plot Profile command, or the Calibrate command. Use the Grow Box in the lower right corner of the window to make the plot larger (or smaller). The Plot window must be the currently active window in order to Print or Copy the current plot. To activate the Plot window, 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 into a program that deals primarily with graphics, such as MacDraw. The text form (i.e. the pixel values) will be used if you paste into a program that is text oriented, such as Excel.

Whenever the cursor is over the Plot window, X (the pixel location) and Y (the pixel value) coordinate values are continuously displayed in the Info window. In the case of calibration plots, X is the uncalibrated pixel value (in the range 0-255) and Y is the calibrated value. For straight line selections, holding down the mouse button in the plot window causes a cross hair cursor to appear in the corresponding part of the image.

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

Info

```
╔═══════════════════════════════════╗
║ ≡≡≡≡≡≡≡≡  Results  ≡≡≡≡≡≡≡≡ ║
╟───────────────────────────────────╢
║ X:  5.97cm  (406)                 ║
║ Y:  7.35cm  (500)                 ║
║ Value:-0.27  (0)                  ║
║                                   ║
║ Count: 17                         ║
║ Pixels: 4362                      ║
║ Area: 0.94 square cm              ║
║ Mean: 0.30 OD  (57)               ║
║ Std Dev: 0.09                     ║
║ Min: 0.07                         ║
║ Max: 0.48                         ║
║ X,Y:  5.48, 1.76                  ║
║ Mode: 0.36                        ║
║ Perimeter: 0.08                   ║
╚═══════════════════════════════════╝
```

The Info window displays the current cursor position and value, measurement results, and status messages. The values displayed at top of the window (e.g. *X*, *Y* and *Value*) are dynamically updated and 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 using the Preferences command.) *Value* is the gray value of the pixel at the current X-Y coordinates. Gray values range from 0 (white) to 255 (black). For density calibrated images, *Value* is the calibrated pixel value and the gray value is shown in parenthesis. In the case of color images, *Value* is the index used by the color look-up table.

> **LUT Window -** *Index* is the look-up table index at the cursor location. *RGB* are the associated red, green, and blue brightness values in the look-up table. You can use this information to help you set the foreground or background grayscale, or color, to a specific grayscale value or index. When using the LUT tool, *Lower* and *Upper* are the lower and upper LUT indexes of the current grayscale or color look-up table.

> **Map Window -** When manipulating the Map, *Lower* and *Upper* are the lower and upper LUT indexes of the current grayscale or color look-up table.

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

> **Plot Window -** *X* is the pixel location and Y is the corresponding plot y value. For calibration plots, *X* is the uncalibrated pixel value (in the range 0-255) and *Y* is the calibrated value.

TECHNIQUES

Analyzing Electrophoretic Gels

The following is one possible procedure for using *Image* to analyze a one-dimensional electrophoretic gel. It also demonstrates some of the less obvious features in *Image,* and also a few shortcuts. Note that this technique cannot be used to compare bands on different gels unless the gels are calibrated to known standards. Any results obtained using this procedure should not be trusted without testing using standards with known concentrations or by comparing with results obtained using a densitometer.

A 23 page manual ("gel_macros_doc.hqx") that describes this procedure in much greater detail is available by anonymous FTP from zippy.nimh.nih.gov, in the /pub/nih-image/documents directory.
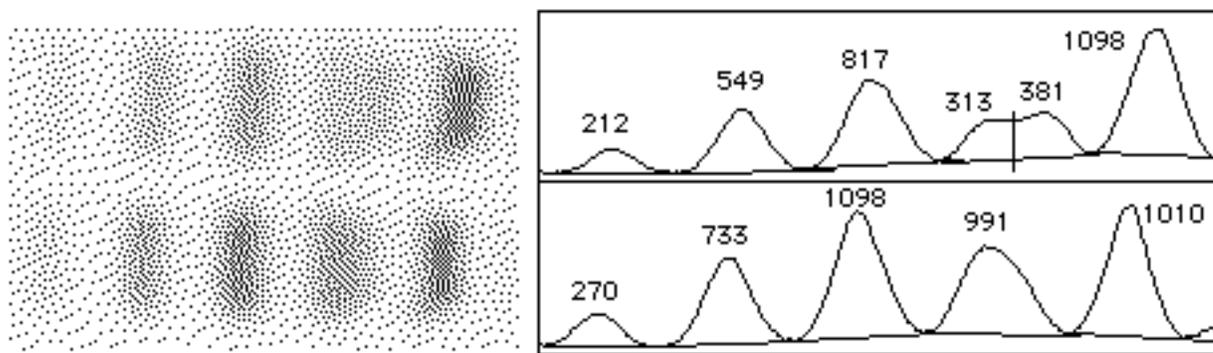
1) Use the Calibrate command to calibrate the image to a calibrated optical density step tablet. Failure to do this could result in incorrect and misleading measurements.

2) If the commands *Mark First Lane*, *Mark Next Lane* and *Plot Lanes* are not shown in the Special menu then use *Load Macros* to open the file "Gel Plotting Macros" in the Macros folder.

3) Use the rectangular selection tool to outline the first lane. This is the left most lane for vertically oriented lanes and the top lane for horizontal lanes.

4) Select *Mark First Lane* in the Special menu. A copy of the image will be displayed with the first lane outlined.

5) Move the rectangular selection (by clicking inside it and dragging) and outline (using *Mark Next Lane*) each of the other lanes in succession.

6) Use *Plot Lanes* to generate the lane profile plots.

7) 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.

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

9) Option-click with the text tool to automatically label the peaks, in reverse order, with the area measurements. The area measurements are also recorded in tabular

form, and can be displayed (Show Results) printed (Print) or exported (Export) to a spreadsheet.

Plot Lanes 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 the Preferences dialog box, Record Preferences, and restart *Image*.

Note that this macro package changes several of the settings in the Analysis/Options dialog box. It enables *Wand Auto-Measure* so that the area is automatically measured when you click with the wand tool under a peak. It disables *Label Particles* so that peaks are not automatically numbered by the wand tool. It enables *Include Interior Holes*. If this were not done, the wand tool would measure zero area. It enables *Adjust Areas* to compensates for the tendency of the wand tool to underestimate the size of small peaks. 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

Selections are user defined regions or lines within an image that can be measured, filtered or edited. Region selections are created using the rectangular, elliptical, polygonal or freehand selection tools. Line selections are created using the line selection tool, which has three forms selected from a pop-up menu. Line selections can be straight, freehand or segmented. Selections are outlined by a moving marquee, sometimes referred to as the "marching ants". Only one selection can be active at a time.
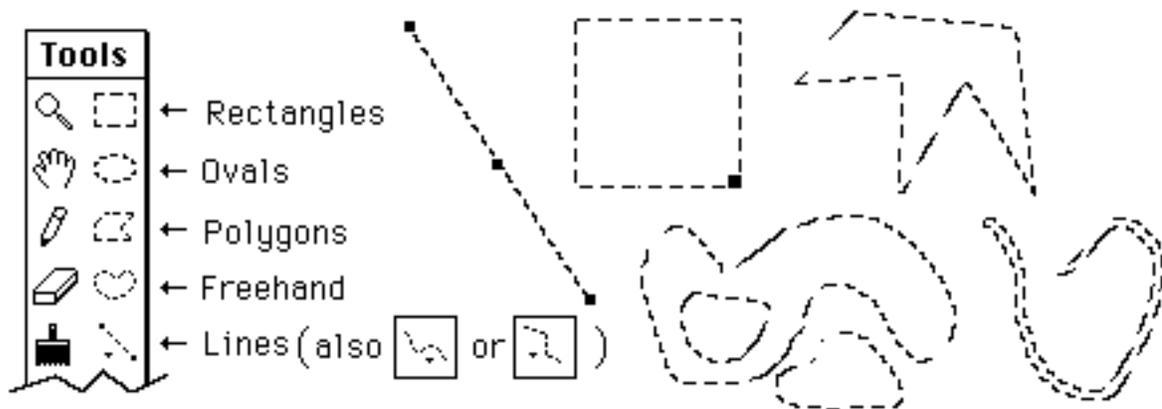
Moving a Selection. Selections can be moved by clicking inside them and dragging. The Info window displays the coordinates of the upper left corner of the selection (or the bounding rectangle for non-rectangular selections) as it is being moved. Notice that the cursor changes to an arrow when it is within the selection. Straight line selections can be moved using the handle (small black box) in the center of the line. If you want to move the *contents* of a selection, rather than the selection itself, do a Copy (command-c), a Paste (command-v), then click within the selection and drag. Use the shift key to constrain movement to be horizontal or vertical. Use the arrow keys to nudge the selection one pixel at a time in any direction.

Stretching a Selection. Rectangular selections can be stretched using the handle (small black box) in the lower right corner. The contents of the selection will also be stretched if the selection is the result of a paste operation. The width and height are displayed in the Info window as the selection is stretched. Use the arrow keys with the option key down to

stretch a rectangular selection one pixel at a time. Straight line selections can be stretched and repositioned using the handles at the ends of the line.

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

Subtracting from a Selection. Hold down the option key (notice the little minus sign) while making a selection and any new selection you create will be subtracted from the current selection. This feature allows you to edit existing selections, or to create selections with holes in them.

Deleting a Selection. To delete a selection, choose any of the selection tools and click outside the selection. Alternately, choose any tool other than one of the selection tools, the magnifying glass, or the grabber hand. 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 using the Restore Selection command. Simply activate the destination window and use the Restore Selection command. The Next Window command (command-`'`) can be used to activate a series of windows in sequence.

Saving and Restoring Selections.
Selections can be saved to disk using the Save As command (with the Outline option) and restored using Open. Multiple selections can be saved by transferring them to a blank (white) window, using the Draw Boundary command (make sure the boundary is black) for each selection, and saving the resulting binary image as a PICT file. To restore a selection, open the PICT file and click to the left of one of the drawn outlines with the wand tool.

USING *IMAGE* WITH FLATBED SCANNERS

The Acquire command provides direct support for most scanners that have Photoshop plug-ins. *Image* supports three scanning modes: 8-bit grayscale, 8-bit indexed color and 24-bit color. The plug-in that comes with the La Cie Silver scanner is one that works well with *Image*.

You also have to be careful not to generate images that are too large for *Image* to handle. *Image* was optimized for 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 (7.2MB), much larger than *Image* was designed to handle. The following table gives suggested maximum scan areas for different scanning resolutions and monitor sizes. *Image* can handle scans somewhat larger than these, but you will probably run into problems if you greatly exceed these recommended sizes.

Monitor	75 DPI	150 DPI	300 DPI
14" (640x480)	8" x 6"	4" x 3"	2" x 1.5"
17" (832x624)	11" x 8"	5.5" x 4"	3" x 2"
20" (1152x870)	14" x 11"	7" x 5.5"	3.5" x 2.75"

Use the Preferences command to increase the size of the Undo and Clipboard buffers to 600K when working with scans of the size suggested for 17" monitors, and to 1000K for 20" monitors. Note that making the Undo and Clipboard buffers larger then necessary can require a lot of extra memory. For example, increasing the buffer sizes from 1000K to 1500K increases *Image's* memory requirements by 1MB.

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

RECOVERING DATA FROM LINE PLOTS

Image can be used to recover numeric coordinate data from printed line plots using the following procedure.
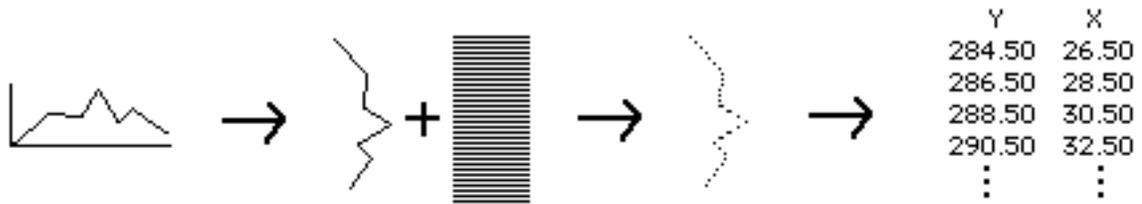
1) Digitize the plot using a TV camera or flat bed scanner.

2) Edit the plot to remove the 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 the Image Math command.

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

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

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

Alternately, and much easier, use the macros in the file "Line Plots->Data", which automates most of these steps. Use the wand 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, using the rectangular selection tool, a single isolated line plot drawn on a white background. 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. When using menu commands, you only need to hold the option key down when you first select the command.

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

Menu Command Options

Close - Changes to Close All
Save - Changes to Save All
Export (Measurements) - Exports measurement results with column and row headers

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

Smooth - More (unweighted) smoothing
Sharpen - Increased sharpening

Analyze Particles - Omit dialog box
Calibrate - Allows you to edit the measured values

Stop Capturing - Omit shading correction
Average Frames - Omit dialog box
Animate - Erase screen to background color before doing animation
Photo Mode - Move window to top of screen before erasing screen

Cascade Images - Move all image windows to the "home" position (upper left corner)
Tile Images- Use "Scale to Fit" mode to draw image windows

Tool Options

Magnifying Glass - Zoom out instead of zooming in
Grabber - Scroll all other tiled windows to the same position
Text Tool - Draw results of area or length measurements
Eraser - Pick up background color from image window
LUT Tool - Rotate the LUT
Brush - Pick up foreground color from image window
Eyedropper - Select background color rather than foreground color

QUESTIONS AND ANSWERS

Q. I just upgraded my Mac to 20MB, but when I run *Image* I still don't have enough memory. What gives?

A. You need to tell the Finder to give *Image* more memory. Applications can only use as much memory as they are allocated. To increase the memory allocation, click (once) on the NIH Image icon (a microscope) and select the Finder's Get Info command. In the example below, *Image*'s allocation has been increased to 12MB.
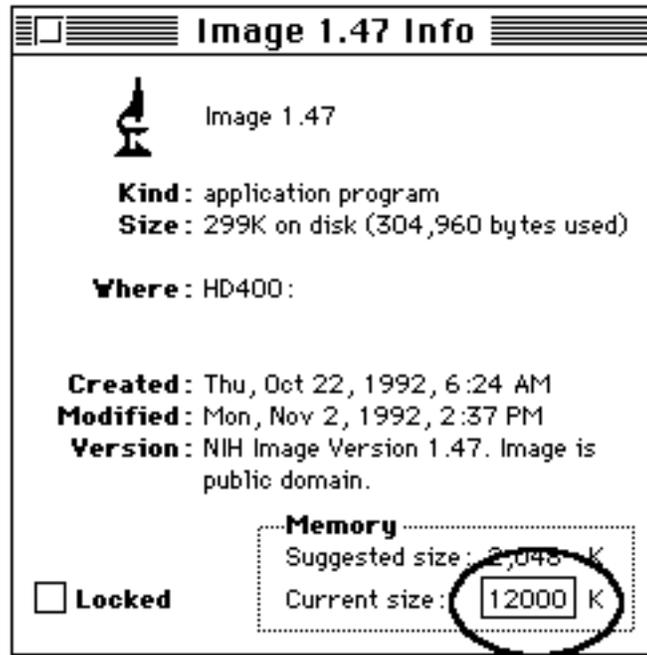
Q. I am used to black having a value of zero, and white a value of 255, but in *Image* black is 255 and white is zero. How can I change this?

A. Check *Invert Pixel Values* in Preferences, which sets up an inverting density calibration function as shown below. This causes pixels values displayed in the Info and Results windows to be inverted.
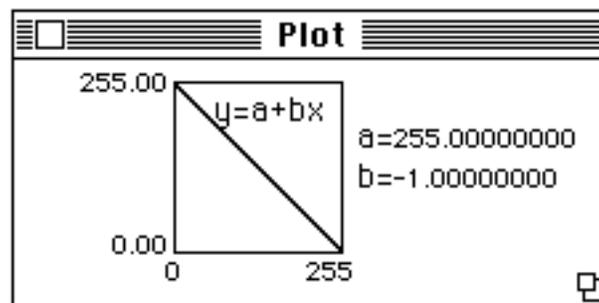
Q. Sometimes, after I have copied an image to the clipboard, the Paste command becomes dim. Why is this?

A. Images copied to the clipboard are stored in *Image*'s Clipboard buffer. In some situations, such as filtering, *Image* has to use the Clipboard buffer for internal operations. When this is happens, the Paste command gets dimmed out.

Q. Why does the desktop 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 *Image* is being used. To minimize this problem, use the General Controls Control panel to create a gray desktop consisting of a pattern of black and white dots.
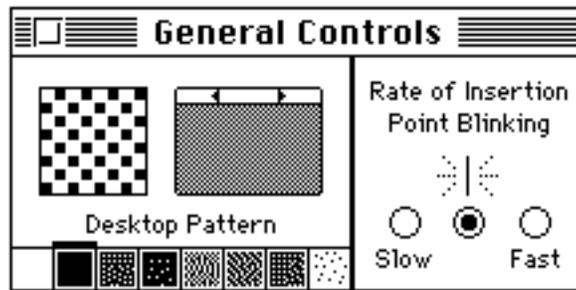
Q. I want to combine two color images to produce a montage, but the colors get corrupted when I Copy from one window and Paste into the other.

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 then be able to successfully Copy and Paste, since both images have the same LUT.

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

A. *Image* has not been ported to the PC or UNIX and no ports are planned at NIH. However, *Image* does run on SUN and HP workstations using Apple's Mac emulator for UNIX called Macintosh Application Environment (MAE). In addition,, the complete Pascal source code (~40,000 lines) is available for anyone willing to attempt a PC or UNIX port.

Q. Text looks very ragged when I print an image on my laser printer. Why is that?

A. *Image* treats text as if it were part of the bit mapped image. To create high quality text for laser printer output and slide makers you need to export the image to a multi-layer, object-oriented drawing program, such as Canvas.

A. Macro Programming Language

Image's built-in macro programming language can be used for automating complex or repetitive tasks. Macros are similar to Pascal procedures, except the *procedure* keyword is replaced by *macro* and the procedure heading is a text string that becomes a command in the Special menu when the macro is loaded. Macros are stored in text files that may also contain Pascal-like procedures. Macros files are created and edited using *Image*'s built-in

text editor. The best way to learn how to write your own macros is by studying and customizing the example macros distributed with *Image* in the Macros folder.

The documentation provided here assumes the reader has some Pascal programming experience. If this is not the case, you will probably need to read an introductory Pascal text book such as "Programming in Pascal" by Peter Grogono, or "Oh! Pascal" by Doug Cooper and Michael Clancy. There is also an "alternative" macro reference manual available from ftp://zippy.nimh.nih.gov/pub/nih-image/documents/macro_notes.hqx.

```
{Example macro file. Comments, such as
this one, go between curly braces.}

Var
  x,y,z:real; {Global variables}

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

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

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

Global variables (optional) are defined at the beginning of the macro file before any procedures or macros. Text within curly braces ({ }) is treated as a comment. 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 name of an empty macro to create a dividing line in the Special 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   STRING

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. Strings have a maximum length of 255 characters and string comparisons are case insensitive. Variable names must be unique within the first twelve characters.

Built-in Arrays

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

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

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

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

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

The X-Y coordinates of the current selection are available from within macros using the built-in arrays xCoordinates[n] and yCoordinates[n]. The number of coordinates can be obtained using the macro function nCoordinates. The coordinates are relative to the upper

left corner of the selection's bounding rectangle. For an example, look at the macro "Plot X-Y Coordinates" in "Plotting Macros".

A four element built-in array (Scion) allows access to the Scion LG-3's I/O registers. The four elements are defined as follows:

Scion[1]	8-bit DAC(A)	read/write
Scion[2]	8-bit DAC(B)	read/write
Scion[3]	8-bit Control Reg 1	read only
Scion[4]	4 digital out bits	read/write

Example macros for setting and reading the LG-3 I/O registers are available in the file "Video".

The data values generated by the PlotProfile and GetPlotData macro commands are stored in a built-in real array named PlotData, which uses indexes in the range 0-4095. The macro "Plot Profile" in "Plotting Macros" illustrates how to use GetPlotData and PlotData.

Built-in Commands and Functions

The macro language has more than 200 built-in commands and functions. Macro commands corresponding to menu commands are listed below under the heading for the appropriate menu.

Note that ROI means Region of Interest.

File Menu

MakeNewWindow('Name')	Use SetNewSize to specify the size of the new window
NewTextWindow('Name',w,h)	Creates a new text window with the title *'Name'. W* and *h* (optional) specify the width and height of the new window.
Open('File Name')	
SetImport('string')	Where *string* contains some combination of: 'TIFF', 'DICOM', 'MCID', 'Palette', 'Text', 'Custom', '8-bits', '16-bits Unsigned', '16-bits Signed', 'Swap Bytes', 'Auto-Scale', Fixed Scale', 'Calibrate' and 'Open All'
SetCustom(width,height,offset,slices)	Specifies the *width*, *height*, *offset*, and number of *slices* for imported files. The *slices* argument is optional.
SetImportMinMax(min,max)	Disables auto-scaling and fixes the range of imported 16-bit images and images in text format
Import('File Name')	
Close	Closes the active window
Dispose	Similar to Close, but user is never prompted to save changes
DisposeAll	
Save	Resaves the contents of the current image or text window
SaveAll	
SetSaveAs('mode')	Where *'mode'* is one of: 'TIFF', 'PICT', 'MacPaint', 'PICS', 'LUT' or 'Outline'
SaveAs('name')	Saves the current image using the specified file name. Uses the window title as the file name when saving a text file or if SaveAs is used with no argument. When saving images, the dialog box should only be displayed the first time SaveAs is called within a macro. For both image and text windows, 'name' can be a full folder path (e.g., 'HD400:Images:My Image'). In this case, no dialog box is displayed.
SetExport('mode')	Where *mode* is one of: 'Raw', 'MCID', 'Text', 'LUT',

	'Measurements', 'Plot Values', Histogram Values' or 'XY Coordinates'
Export('name')	Use SetExport to specify what to export. Similar to SaveAs, the dialog box is not displayed more than once per macro and full folder paths are allowed.
RevertToSaved	
Duplicate('Window Title')	
GetInfo	
Acquire('Plug-in Name')	Runs a Photoshop compatible acquisition plug-in. The plug-in is assumed to be in the Plug-ins folder.
CallExport('Plug-in Name')	Runs the specified export plug-in.
Print	Prints the active image

Edit Menu

Undo	
Copy	Copies contents of current ROI to Clipboard
CopyResults	Copies measurement results to the Clipboard
Paste	Pastes into current ROI if Clipboard object and ROI have the same dimensions, otherwise, pastes into center of image.
PasteLive	Pastes "live" from video (frame grabber) source into a selection. Note that the destination window cannot be larger that the Camera window.
Clear	Erases current ROI to background color
Fill	Fills current ROI with foreground color
Invert	Linearly inverts image or ROI
DrawBoundary	Outlines current ROI using foreground color. Use SetLineWidth to control width of outline.
DrawScale	
SelectAll	
SetScaling('string')	Sets ScaleAndRotate options, where *string* contains some combination of: 'Nearest', 'Bilinear', 'New Window', 'Same Window' or 'Interactive'
ScaleAndRotate(xscale,yscale,angle)	$0.05 <= xscale, yscale <= 25.0$ and $-180 <= angle <= 180$
RotateLeft(b)	Create new window if *b* true
RotateRight(b)	Create new window if *b* true
FlipVertical	
FlipHorizontal	

Options Menu

InvertLUT	Inverts video look-up table
SetPalette(string)	Where *string* is one of: 'Grayscale', 'PseudoColor', 'System Palette', 'Rainbow' or 'Spectrum'
SetFont('Font name')	Where '*Font Name*' is 'Geneva', 'Monoco', Helvetica', 'Times', etc.
SetFontSize(size)	Set font size in points, where $6<=size<=720$
SetText(string)	Where *string* contains any combination of: 'Bold', 'Italic', 'Underline', 'Outline', 'Shadow', 'Left Justified', 'Right Justified', 'Centered', 'No Background', or 'With Background'
SetNewSize(width,height)	Specifies *width* and *height* of new image windows
ScaleConvolutions(b)	*b*=true or false. Sets or resets *Scale Convolutions* flag.
ScaleMath(b)	*b*=true or false. Sets or resets *Scale Image Arithmetic* flag.
InvertY(b)	Invert Y-coordinates if *b* is true
SetPlotLabels(b)	*b*=true or false
SetPlotScale(min,max)	Set *min* and *max* to zero for auto-scaling

SetPlotSize(width,height)	Set *width* and *height* to zero for auto-sizing of plots
SetThreshold(level)	0 <= level <= 255. SetThreshold(-1) disables thresolding.
AutoThreshold	Set threshold to automatically determined value
SetDensitySlice(lower,upper)	1 <= *lower*,*upper* <= 254. SetDensitySlice(0,0) disables density slicing, SetDensitySlice(255,255) enables it.
GetThresholds(lower,upper)	In density slicing mode, returns the lower and upper thresholds. In thresholding mode, lower is set to the threshold and upper is set to 255. Otherwise, both are set to zero
PropagateLUT	Propagates current LUT to all other open images
PropagateSpatial	Propagates current spatial calibration to all other open images
PropagateDensity	Propagates current density calibration to all other open images

Process Menu

Filter('name')	Runs the specified filter, where *'name'* is one of the following: 'smooth', 'smooth more', 'sharpen', 'sharpen more', 'find edges' (or 'sobel'), 'median', 'max', 'min' or 'dither'.
Shadow('direction')	Set *'direction'* (optional) to 'N', 'NE', 'E', 'SE', 'S', 'SW', 'W' or 'NW'.
Convolve('Kernel file name')	Note that the name can be a directory path such as 'HD80:Image:Kernels:Smooth'
CallFilter('name')	Runs the specified filter plug-in
MakeBinary	
Erode	
Dilate	
SetBinaryCount(n)	1 <= *n* <= 8
Outline	
Skeletonize	
AddConstant(n)	-255 <= *n* <= 255
MultiplyByConstant(n)	0.0 <= *n* <= 255.0
ImageMath('op',pic1,pic2,scale,offset,result)	Where *'op'* is one of 'add', 'sub', 'mul', 'div', 'and', 'or', 'xor', 'min', 'max' or 'copy'. Add the keyword 'calibrate' to do calibrated math (e.g. 'add calibrated'). *Pic1* and *pic2* are pic numbers or pid numbers. The specified operation is performed, the result is multiplied by *scale*, *offset* is added and, if calibrate wasn't specified, the final result is clipped to 8-bits. The arithmetic operation is performed in the upper left corner of each image using the largest common rectangle. *Result* can be either a string or a pid number. If it's a string, a window with that name is created to store the result, otherwise the result is stored in the image specified by the pid number.
SubtractBackground('str',radius)	Where *'str'* is one of: '1D Horizontal', '1D Vertical', '2D Rolling Ball' or '2D Remove Streaks'. Add 'faster' to 'str' (e.g. '2D Rolling Ball(faster)') for faster operation.
ApplyLUT	Transforms the pixel data using the current look-up table
EnhanceContrast	
ChangeValues(v1,v2,v3)	Changes pixels with a value in the range *v1-v2* to *v3*

Analyze Menu

Measure	Results are stored in the results arrays rArea[], rMean[], etc. and in the Histogram[] array
GetResults(n,mean,mode,min,max)	Use after Measure. Values returned are always uncalibrated. Use cValue function to calibrate them.
AnalyzeParticles	Dialog box is not displayed

LabelParticles(b)	*b*=true or false
OutlineParticles(b)	
IgnoreParticlesTouchingEdge(b)	
IncludeInteriorHoles(b)	
SetParticleSize(min,max)	
ShowResults	
ShowHistogram	
RestoreRoi	Same as the Restore Selection menu command
MarkSelection	Same as the Label Selection menu command
SetOptions('string')	Where *'string'* contains some combination of 'Area', 'Mean', 'Std. Dev.', 'X-Y Center', 'Mode', 'Perimeter' (or 'Length'), 'Major', 'Minor', 'Angle', 'Int. Den.', 'Min/Max', 'User1', or 'User2'. Any variable not listed is disabled.
Redirect(b)	Enable/disable redirected sampling. *b*=true or false.
WandAutoMeasure(b)	
AdjustAreas(b)	
SetPrecision(digits,fwidth)	Were *digits* is the number of digits to the right of the decimal point and *fwidth* (optional) is the field width
GetScale(scale,unit,AspectRatio)	Returns the number of pixels per unit of measurement in the real variable *scale*, the unit of measurement in the string variable *unit*, and (optional) the pixel aspect ratio in the real variable *AspectRatio*. For uncalibrated images, *scale* and *AspectRatio* are set to 1.0 and *unit* to 'pixel'.
SetScale(scale,'unit',AspectRatio)	*Scale* is the number of pixels per unit of measurement. Set *'Unit'* to 'nm', 'µm', 'mm', 'cm', 'meter', 'km', inch', 'ft', 'mile' or 'pixel' or use an arbitrary unit up to 11 characters in length. *AspectRatio* (optional) is the x/y pixel aspect ratio. Us SetScale(0,'pixel') to disable spatial calibration and SetScale(0,'') to activate the Set Scale dialog box.
Calibrate('fit', 'unit', m1, k1, m2, k2, ...)	*'fit'* is one of 'straight', 'poly2', 'poly3', 'poly4', 'exp', 'power', 'log', 'rodbard', 'uncalibrated' or 'uncalibrated od', *'unit'* is the unit of measurement, *m1*, *m2*, etc. are the measured values and *k1*, *k2*, etc. are the known values. For example, "Calibrate('Straight', 'Invert', 0, 255, 255, 0)" sets up a simple inverting function. Use "Calibrate('Uncalibrated OD')" to enable uncalibrated OD and "Calibrate('Uncalibrated')" to disable calibration.
PlotProfile	Generates a gray scale profile plot of the current rectangular selection or line selection.
GetPlotData(count,ppv,min,max)	Where *count* is the number of values, *ppv* is the number of pixels averaged for each value, and *min* and *max* are the minimum and maximum values. The plot values are returned in the built-in PlotData array. It is not necessary to call PlotProfile before calling GetPlotData.
SurfacePlot	Creates a surface plot of the current image. Use SetNewSize to specify the size of the plot.
ResetCounter	Sets the measurement counter to zero

Special Menu

StartCapturing	Start live video capture
StopCapturing	Stop live video capture
Capture	Captures and displays a single video frame
AverageFrames('str',frames)	*'str'* is some combination of 'Average', 'Integrate', 'Video Rate Capture' and 'On-chip' and *frames* is the number of frames. Any option not specified is disabled. Set *frames* to zero to display the Average Frames dialog box.

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SetVideo('string')	Where 'string' is some combination of 'Invert, 'Highlight', 'Oscillating', 'Trigger', 'Blind' or 'Separate'. Any option not specified is disabled.
SetChannel(channel)	Sets the video input channel, where channel=1, 2, 3, or 4
PhotoMode(b)	Set *b* true to enable "Photo Mode" and false to disable it

Stacks Menu

AddSlice	Adds a slice following the current slice
DeleteSlice	Deletes the current slice
AverageSlices	Averages all the slices in the current stack
Capture Color	Capures a 24-bit RGB color image
RGBToIndexed('string')	Converts 24-bit RGB to 8-bits with LUT. S*tring* contains some combination of 'System LUT', 'Existing LUT', 'Custom LUT' and 'Dither'. Custom LUT and Dither are the defaults.
IndexedToRGB	Converts 8-bit color images to RGB (V1.58 and later)
Project	Dialog box is only displayed the first time you call Project
Reslice	Requires a line selection

Windows Menu

NextWindow	
TileWindows	
ShowPasteControl	Activates the Paste Control window

Miscellaneous

AutoOutline(x,y)	Equivalent to clicking with the wand tool at location (x,y). The outline was successfully created if GetRoi returns a width greater than zero.
Beep	
ChangeValues(v1,v2,v3)	Changes pixels with a value in the range *v1-v2* to *v3*
ChoosePic(n)	Selects then Nth image window without activating it. Faster than SelectPic but changes are not displayed. Also accepts PidNumbers (see PidNumber function).
ChooseSlice(n)	Selects the Nth slice in a stack without displaying it. ChooseSlice is faster than SelectSlice but changes are not displayed. Use SelectSlice before exiting macro to make sure the stack is correctly displayed.
Exit	Terminate execution of the macro
GetColumn(x,y,length)	Copies a column of pixels from active image to the line buffer
GetLine(x1,y1,x2,y2,LineWidth)	Returns coordinates and width of current straight line selection. Sets x1=-1 if there is no line selection.
GetMouse(x,y)	Returns location of cursor in local pixel coordinates
GetPicSize(width,height)	Returns *width* and *height* of active image
GetRoi(left,top,width,height)	Returns ROI location and size. Sets *width*=0 if no ROI. Returns location and size of bounding rectangle for non-rectangular ROIs and for line selections.
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	Disables the current "marching ants" selection
LineTo(x,y)	Draws a line from current location to (x,y)
MakeLineRoi(x1,y1,x2,y2)	Origin(0,0) is at upper left. Coordinates can be real numbers.
MakeNewStack('name')	Creates a new 1-slice stack. Use SetNewSize to specify size.
MakeRoi(left,top,width,height)	Origin(0,0) is at upper left
MakeOvalRoi(left,top,width,height)	
MoveRoi(dx,dy)	Moves ROI right *dx* pixels and down *dy* pixels

MoveTo(x,y)	Origin is always at upper left corner
MoveWindow(x,y)	Moves current window to global screen coordinates (x,y)
PlotXYZ	Plots XYZ coordinate data stored in a text file
PutColumn(x,y,length)	Copies *length* pixels from line buffer to a column in 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 line buffer to a row in the image
ResetGrayMap	Equivalent to clicking on reset icon in Map window
SaveState	Saves foreground and background color, new window width and height, status of Invert Y flag, text attributes, and various ScaleAndRotate and SetScaling parameters
RequiresVersion(n)	Aborts macro if *Image* version number is less than specified
RestoreState	Restore settings saved by SaveState
SelectPic(n)	Activates the Nth image window. Also accepts PidNumbers (see PidNumber function).
SelectSlice(n)	Display the Nth slice in a stack
SelectWindow('name')	Activate the window with the title *'name'*
SetCounter(n)	Sets the measurement counter to *n*
SetCursor('shape')	Changes cursor shape. Set *'shape'* to 'watch', 'cross', 'arrow' or 'finger'.
SetForegroundColor(c)	$0 <= c <= 255$, were 0=white and 255=black
SetBackgroundColor(c)	$0 <= c <= 255$
SetLineWidth(width)	
SetUser1Label('Label')	Replaces label used for User 1 column in Results window
SetUser2Label('Label')	Replaces label used for User 2 column in Results window
SetPicName('Name')	Renames the currently active image window
SetOption	Equivalent to holding down option key
SetSliceSpacing(n)	Set slice spacing(in pixels) for use by Reslice and Project commands
ShowMessage('message')	Displays *'message'* in the Info window. Accepts multiple arguments like the Write routine. Use a back-slash('\') to start a new line.
SortPalette	Sorts the current LUT by hue
UpdateResults	Redisplays last measurement in Info and Results windows
UpdateLUT	Redisplays the LUT window
UserCode('name',p1,p2,p3)	Calls a user written Pascal routine, where 'name' is the name of the routine and *p1*, *p2* and *p3* are real input parameters. See "Inside NIH Image" for more information.
Wait(seconds)	Fractions of a second are allowed, e.g., wait(1.5)
WaitForTrigger	Waits for external trigger. Requires a QuickCapture or Scion LG-3 frame grabber card. Use "repeat until button" to wait for a mouse down event.
Write(e1,e2,...)	Draws text, variables, or constants at the current location. Like 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 to the right of the decimal point.
Writeln(e1,e2,...)	Similar to Write, but does the equivalent of a line feed and carriage 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, d)	Displays a dialog box and returns with the value entered. *Prompt* is the prompting string. *Default* is the default value. *d* (optional) is the number of digits to the right of the decimal point (default is 2). Use zero to display an integer.
GetPixel(x,y)	Returns the value of the pixel at (*x*,*y*)
GetSliceSpacing	Returns slice spacing (in pixels) of current stack
KeyDown('key')	Checks the state of the specified modifier key. *'key'* is one of: 'option', 'shift', or 'control'. Returns TRUE if the specified key is down.
nCoordinates	Returns the number of XY coordinates for the current selection
nPics	Returns number of image windows
nSlices	Returns number of slices in current stack
PicNumber	Returns number (used by SelectPic) of active image
PidNumber	Returns a negative permanent ID number for the current image
PidExists(pid)	Returns true if image with this PidNumber is still open
rCount	Returns current measurement counter value
SliceNumber	Returns number of current slice in a stack
TickCount	Returns the number of ticks (sixtieths of a second) since system last started
UndoBufferSize	Returns the current size of the Undo and Clipboard buffers
WindowTitle	Returns a string containing the title of the active window

Math Functions

abs(n)	Returns absolute value of *n*
arctan(n)	Returns arctangent of *n* (radians)
BitAnd(n1,n2)	Returns *n1* AND *n2*
BitOr(n1,n2)	Returns *n1* OR *n2*
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* is odd
random	Generates a random number between 0 and 1
round(n)	Converts a real value to an integer with rounding
sin(n)	Returns sine of *n* (radians)
sqr(n)	Returns square of *n*
sqrt(n)	Returns sqrt of *n*
trunc(n)	Converts a real value to an integer with truncation

Paste Control Options (Use immediately after a paste command)

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

Serial I/O and Strings

str:=GetString('Prompt','def.')	Displays a dialog box and returns with the string entered.

	The first argument is the prompting message and the second argument (optional) is the default input string.
str:=concat(str1, str2,...)	Concatenates a series of strings
str:=chr(n)	Converts a positive integer in the range 0..255 to a one character string (e.g. chr(65) = 'A').
Delete(str,index,count)	Removes *count* characters from *str*, beginning at *index*
i:=length(str)	Returns the length of *str*
n:=ord(str)	Returns the ordinal number of the first character in a string (e.g. ord('A')=65). Returns -1 if the string is empty.
i:=pos(substr,str)	Searches for *substr* within *str* and returns an integer that is the index of the first character of *substr* within *str*. Returns zero if *substr* is not found.
n:=StringToNum(str)	Converts a string to a real number. Returns zero if the string contains no digits.
str:=WindowTitle	Returns the title of the currently active window
OpenSerial('Options')	Sets up the modem port for serial I/O. *'Options'* contains '300 baud', '1200 baud', '2400 baud', '9600 baud' or '19200 baud'; 'no parity', 'even parity' or 'odd parity'; 'seven data' or 'eight data'; 'One stop' or 'two stop'. The default is 9600 baud, no parity, eight data, one stop.
str:=GetSerial	Returns the next available character in the serial (modem) input buffer as a one character string or returns an empty string if the buffer is empty.
PutSerial(str)	Writes 'str' to the modem port. Like the Write routine, it accepts multiple arguments(e.g. PutSerial('v=',v:1:2)).

Note that routines that require a file name or window title (MakeNewWindow, MakeNewStack, Open, SaveAs, Import, Export, Duplicate and SetPicName) accept multiple arguments similar to the Write routine, except that numeric fields 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. Several other routines (PutMessage, ShowMessage, PutSerial) also accept multiple arguments.

B. Frame Grabber Cards

Image directly supports three frame grabber cards for acquiring images from video sources, such as TV cameras and VCR's: the Data Translation QuickCapture, the Scion LG-3, and the Scion AG-5. All three cards allow brightness, contrast and pseudocolor to be adjusted during "live" capture. All allow continuous histogram display and "live" paste, which can be useful for image alignment.

The fastest screen refresh rate (about eight frames per second) is achieved on 68040 and Power PC Macs using built-in video. A 16 inch, or larger, monitor is required to see the entire 640x480 frame without panning. *Image* supports the 768 x 512 resolution available with 50Hz (PAL/CCIR) versions of the cards. It also supports external triggering.

Both Scion cards support software control of analog gain and offset and on-chip integration. The LG-3 features a frame buffer that can be expanded to 64MB using standard Mac SIMMS. The AG-5 supports video rate averaging and integration.

1) Data Translation QuickCapture

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

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

2) Scion LG-3 and AG-5

LG-3: $895
AG-5: $2495

Optional cables are required for color capture and on-chip integration

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

C. Cameras and Light Boxes

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.

One CCD camera that is well suited for quantitative densitometry is the Sony XC-77. Other CCD cameras to consider include the Sierra Scientific MS-4030, the COHU Model 4815-5000, and the Dage CCD-72. Sierra Scientific, COHU and Dage also sell lenses and filters. When ordering a camera, particularly the Sony XC-77, make sure it comes with all necessary components, such as power supply, tripod attachment (if needed), and documentation.

Imaging Research, creator of the highly regarded MCID biomedical image analysis system for the IBM-PC, makes a stabilized fluorescent light box 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 Sony and Dage cameras.

For digitizing gels, Fotodyne has an unstabilized light box called the VariQuest 100 that is considerable less expensive than the one from Imaging Research. Fotodyne also distributes a program called "Collage" for imaging of electrophoresis gels, blots and autoradiograms.

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 Macintosh that supports CCDs with a resolution of up to 2K X 2K. The Photometrics system, however, is not directly supported by *Image*. Dage-MTI has a real-time video processor (the DSP-200) that does real-time averaging and integration to enhance noisy, low intensity images. They also have an image intensifier for CCD cameras.

COHU Inc.
San Diego, California
619-277-6700
fax: 619-277-0221

Perceptics Corp.
Knoxville, TN
615-966-9200
fax: 615-966 9330

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

Photometrics Ltd.
Tucson, AZ
602-889-9933
fax: 602-573-1944

Fotodyne Inc.
Hartland, Wisconsin
1-800-362-3642
fax: 414-369-7013

Sierra Scientific
Sunnyvale, CA
408-773-5600

Imaging Research Inc.
Brock University
St. Catharines, Ontario
Canada L2S 3A1
905-688-2040
fax: 905-685-5861

Sony Corporation of America
Component Products Company
10833 Valley View St.
Cypress, CA 90630
714-229-4197
fax: 714-229-4285

D. Alternative Macintosh Analysis Systems

Alice (was DIP Station)
(Medical image analysis, 16–bits, surface rendering, DICOM)
Hayden Image Processing Group
Boulder, Colorado (help@perceptive.com)
303-449-3433, fax: 303-449-3772

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

Image Analyst
Automatix
Billerica, MA
508-667-7900

IPLab Spectrum
(>8-bits precision, 24-bit color, gel analysis, microscopy)
Signal Analytics, Vienna, Virginia
703-281-3277, fax: 703-281-2509
d6196@applelink.apple.com

NCSA Image, PalEdit, Layout, and Gel Reader (all public domain)
(Scientific visualization, palette editing, gels)
NCSA, Champaign, IL
217-738-3160
ftp: zaphod.ncsa.uiuc.edu

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

Synapse Image
(Fluorescence imaging and electrophysiology)
 Synergistic Research Systems
Silver Spring, Maryland
301-942-6601, jimnash@his.com

BDS Image
(Fluorescence microsopy, calcium imaging)
Biological Detection System, Inc.
Rockville, Maryland
301-990-0100, fax: 301-990-8391
72204.70@compuserve.com

Enhance, Color-It!
(Image editing, Photoshop plug-ins)
Micro Frontier
Des Moines, Iowa
515-270-8109

ImportACCESS
(Plug-in for importing medical images in DICOM, ACR-NEMA and other formats)
Designed Access
Chicago, IL 60614
312-880-2034, fax: 312-472-8834
email: daccess@interaccess.com

MedVision
(Medical imaging, multi-modality studies, 16-bits, extendible, DICOM, ACR-NEMA)
Evergreen Technologies, Castine, Maine
207-326-8300, fax:207-326-8333
jsiegel@lunis.nucmed.luc.edu

OSIRIS
(Medical imaging, 16-bits, DICOM, ACR-NEMA)
University Hospital of Geneva
fax:: 41 22 37 26 198
e-mail: yves@cih.hcuge.ch
ftp: expasy.hcuge.ch

PixelTools, TCL-Image
(Video rate processing, 1340x1035 frame grabber)
Perceptics Corporation
Knoxville, TN
615-966-9200
fax: 615-966 9330

Transform, View, Format, and Dicer
(Scientific visualization, presentation graphics, volume visualization)
Spyglass, Inc.
Savoy, IL
217-355-6000

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

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

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

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 IBM PC and VAX systems.

TIFF files created by image can be read using the Import command by selecting *Custom*, *8-bits*, entering *Width* and *Height*, and setting *Offset* to 768. *Slices* should be set to one for a single image and to the number of slices for a stack. Note that you have to hold down the option key when selecting the Import command to override *Image*'s automatic TIFF file detection.

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 zippy.nimh.nih.gov, in the directory /pub/nih-image/documents.

F. Program Limitations

There is no arbitrary limit (other than memory requirements) on the maximum number of lines in an image. The width of images, however, should be limited to 4096 pixels, since that is the size of *Image*'s internal line buffers. *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 Quit, and then restart *Image* before the buffer size change will take effect.

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

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

The maximum size of a macro file is 30,000 bytes. A macro file can contain up to 100 macros. The push-down stack used by the macro language can contain up to 150 variables.

The maximum number of images that can be opened is 250. The maximum number of slices in a stack is 1000.

G. Shortcuts

Double-Click on tool to:

Unzoom →

← Select All

Erase window →

← See measurement options

Set brush size →

← Do density calibration

← Set scale

← Enable/disable density slicing

Set Profile plot options →

Enable/disable density slicing →

← Set spray can size
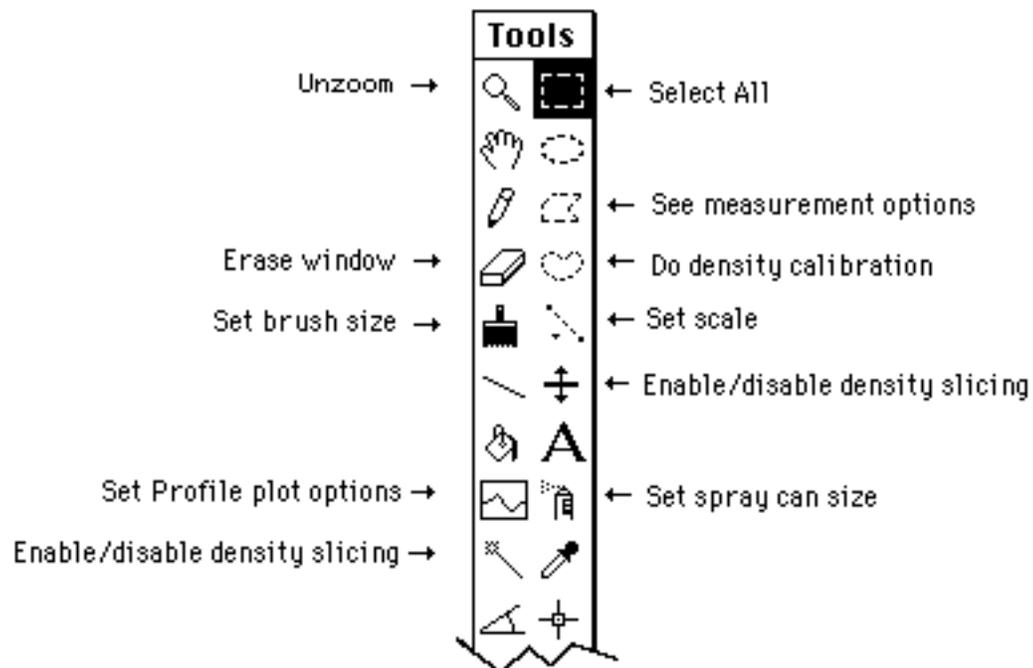
To constrain tool functions hold down the shift key:

pencil →
eraser →
brush →
line selections →
lines →
profile plots →

To force these tools to move only horizontally or vertically
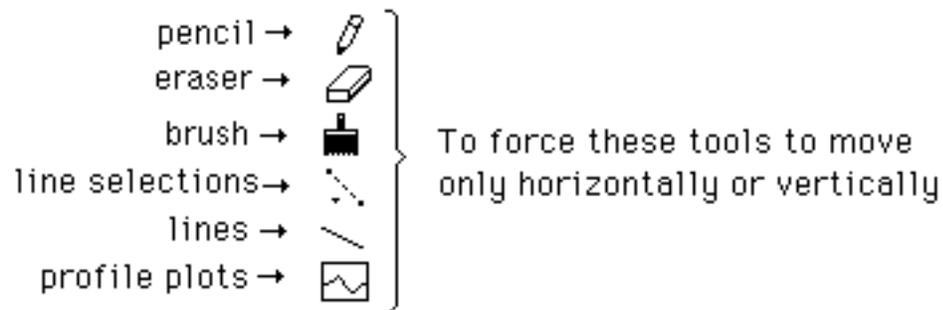
To create squares

To create circles

To move selections precisely horizontally or vertically
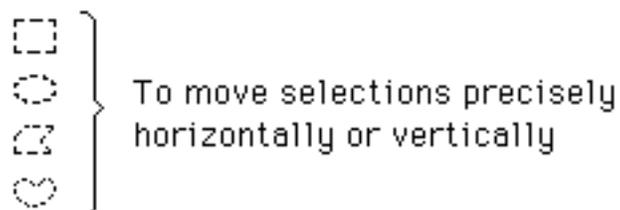
H. References

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I.   Updated Versions and Bug Reports

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

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

2) Via anonymous FTP from zippy.nimh.nih.gov. Enter "anonymous" as the user name and your e-mail address as the password. The /pub/nih-image directory contains the latest version of *Image*, documentation in Word format, and complete Pascal source code. The nih-image directory contains a plain text README file (0README.txt) describing the files, subdirectories and file formats.

3) Library 9 (Graphics Tools) of the MACAPP forum on CompuServe. Source code is in Library 6 of the MACDEV forum.

4) Subscribe to the NIH *Image* mailing list by sending a message containing the line "subscribe nih-image <your name>" to listproc@soils.umn.edu. Next obtain a list of the available NIH *Image* archive files by sending an "index nih-image" command to listproc@soils.umn.edu. These files can then be retrieved by means of a "get nih-image filename" command. The files are Binhexed and broken into chunks less than 32K in size. The NIH mailing is maintained by the Soil Science Department at the University of Minnesota.

6) NTIS (National Technical Information Service), 5285 Port Royal Road, Springfield, VA 22161, phone: 703-487-4650, fax: 703-321-8547, order number PB95-500195GEI ($100 check, VISA, or Mastercard). Note that the zippy.nimh.nih.gov FTP site is likely to have a newer version of *Image* than NTIS.

Bug reports and suggestions are welcome, as are corrections or additions to this manual. Before reporting a bug, please make sure you are using the latest version of *Image*. Also try disabling extensions by holding down the shift key while restarting the computer and try to reproduce the problem on more than one Mac. Send bug reports to Wayne Rasband (wayne@helix.nih.gov), the author of *Image*.

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