IJ 1.46r
Revised edition

# ImageJ User Guide

ImageJ/Fiji 1.46

IJ User Guide — Booklet 1

# ImageJ User Guide

## IJ 1.46r

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### Foreword

The *ImageJ User Guide* provides a detailed overview of ImageJ (and inherently Fiji), the standard in scientific image analysis (*see* XXVI Focus on Bioimage Informatics).

It was thought as a comprehensive, fully-searchable, self-contained, annotatable manual (*see* Conventions Used in this Guide). A HTML version is also available as well as printer-friendly booklets (*see* Guide Formats). Its latest version can always be obtained from http://imagej.nih.gov/ij/docs/guide. The source files are available through a Git version control repository at http://fiji.sc/guide.git.

Given ImageJ's heavy development this guide will always remain incomplete. All ImageJ users and developers are encouraged to contribute to the ImageJ documentation resources (*see* Getting Involved).

## Contents

## Summarized Release Notes for ImageJ 1.46r



Please note that this is not an extensive list. Detailed release notes for version 1.46r are available on the ImageJ news web site: http://imagej.nih.gov/ij/notes.html.



## Noteworthy

## Macro Listings





## Guide Formats

This guide is available in the following formats:

**Enhanced PDF** Optimized for electronic viewing and highly enriched in hypertext links (*see* Conventions Used in this Guide). Available at http://imagej.nih.gov/ij/docs/guide/user-guide.pdf.

**HTML document** available online at http://imagej.nih.gov/ij/docs/guide/. For offline usage a downloadable ZIP archive is also available at http://imagej.nih.gov/ij/docs/user-guide.zip.

**Printable booklets** Two-sided booklets that can be printed on a duplex unit printer by setting the automatic duplex mode to "short edge binding". Two formats are available: A4 (http://imagej.nih.gov/ij/docs/guide/user-guide-A4booklet.pdf) and letter size paper (http://imagej.nih.gov/ij/docs/guide/user-guide-USbooklet.pdf).

## Conventions Used in this Guide

Throughout the guide, internal links are displayed in gray (e.g., Part IV ImageJ User Interface). Links to external URLs, such as the ImageJ website, http://imagej.nih.gov/ij/, are displayed in dark blue.

ImageJ commands are typed in sans serif typeface with respective shortcut keys flanked by square brackets (e.g.: Image ▷ Duplicate. . . [D]). As explained in Using Keyboard Shortcuts, this notation implies Shift-modifiers (i.e., [D] means pressing Shift D, [d] only the D key) and assumes that *Require control key for shortcuts* in Edit ▷ Options ▷ Misc. . . is unchecked. Note that references to the Ctrl key include the ⌘ Cmd key of Macintosh keyboards.

Useful tips and reminders are placed in 'Noteworthy notes' numbered with upper case roman numerals (e.g., I Frontmost Window and Window Activation). The full list of these notes is available on page viii.

Filenames, directories and file extensions are typed in monospaced font marked by a related icon, e.g., file `StartupMacros.txt` in folder `/Applications/ImageJ/macros/`.

Macro functions and code snippets are typed in monospaced font, e.g., `resetMinAndMax()`. Scripts and macros are numbered with arabic numerals included in parentheses (e.g., (2) Replace Red with Magenta.ijm (Using Image ▷ Color ▷ Channels. . . ) on page 17) and typeset with the same syntax markup provided by the Fiji Script Editor. The full list of macro listings is available on page ix.

Selected highlights of version 1.46r are listed on page vii and flagged with colored marginal notes. These should be interpreted as:

| | |
|---|---|
| NEW IN IJ 1.46R | A new feature implemented in ImageJ 1.46r. |
| IMPROVED IN IJ 1.46R | A routine that has been improved since previous versions. Typically, a faster or more precise algorithm, a command with better usability, or a task that has been extended to more image types. |
| CHANGED IN IJ 1.46R | A pre-existing command that has been renamed or moved to a different menu location in ImageJ 1.46r. |

# Part I

# Getting Started

This part provides basic information on ImageJ installation, troubleshooting and update strategies. It discusses Fiji and ImageJ2 as well as third-party software related to ImageJ. Being impossible to document all the capabilities of ImageJ without exploring technical aspects of image processing, external resources allowing willing readers to know more about digital signal processing are also provided.

## 1 Introduction

ImageJ is a public domain Java image processing and analysis program inspired by NIH Image for the Macintosh. It runs, either as an online applet or as a downloadable application, on any computer with a Java 1.5 or later virtual machine. Downloadable distributions are available for Windows, Mac OS X and Linux. It can display, edit, analyze, process, save and print 8–bit, 16–bit and 32–bit images. It can read many image formats including TIFF, GIF, JPEG, BMP, DICOM, FITS and 'raw'. It supports 'stacks' (and hyperstacks), a series of images that share a single window. It is multithreaded, so time-consuming operations such as image file reading can be performed in parallel with other operations[1].

It can calculate area and pixel value statistics of user-defined selections. It can measure distances and angles. It can create density histograms and line profile plots. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering.

It does geometric transformations such as scaling, rotation and flips. Image can be zoomed up to 32 : 1 and down to 1 : 32. All analysis and processing functions are available at any magnification factor. The program supports any number of windows (images) simultaneously, limited only by available memory.

Spatial calibration is available to provide real world dimensional measurements in units such as millimeters. Density or gray scale calibration is also available.

ImageJ was designed with an open architecture that provides extensibility via Java plugins. Custom acquisition, analysis and processing plugins can be developed using ImageJ's built in editor and Java compiler. User-written plugins make it possible to solve almost any image processing or analysis problem.

Being public domain open source software, an ImageJ user has the four essential freedoms defined by the Richard Stallman in 1986: 1) The freedom to run the program, for any purpose; 2) The freedom to study how the program works, and change it to make it do what you wish; 3) The freedom to redistribute copies so you can help your neighbor; 4) The freedom to improve the program, and release your improvements to the public, so that the whole community benefits.

ImageJ is being developed on Mac OS X using its built in editor and Java compiler, plus the *BBEdit* editor and the *Ant* build tool. The source code is freely available. The author, Wayne Rasband (wsr@nih.gov), is a Special Volunteer at the National Institute of Mental Health, Bethesda, Maryland, USA.

See also: History of ImageJ at imagejdev.org

[1] A somehow outdated list of ImageJ's features is available at http://imagej.nih.gov/ij/features.html





## 2 Installing and Maintaining ImageJ

ImageJ can be downloaded from http://imagej.nih.gov/ij/download.html. Details on how to install ImageJ on Linux, Mac OS 9, Mac OS X and Windows [1] are available at http://imagej.nih.gov/ij/docs/install/ (Help ▷ Installation... command). Specially useful are the platform-specific *Troubleshooting* and *Known Problems* sections. Fiji installation is described at http://fiji.sc/wiki/index.php/Downloads.

The downloaded package may not contain the latest bug fixes so it is recommended to upgrade ImageJ right after a first installation. Updating IJ consists only of running Help ▷ Update ImageJ..., which will install the latest `ij.jar` in the ImageJ folder (on Linux and Windows) or inside the ImageJ.app (on Mac OS X).

Help ▷ Update ImageJ... can be used to upgrade (or downgrade) the `ij.jar` file to *release updates* or *daily builds*. Release updates are announced frequently on the IJ news website and are labelled alphabetically (e.g., v. 1.43m). Typically, these releases contain several new features and bug fixes, described in detail on the ImageJ News page. *Daily builds,* on the other hand, are labelled with numeric sub-indexes (e.g., v. 1.43n4) and are often released without documentation. Nevertheless, if available, release notes for daily builds can be found at http://imagej.nih.gov/ij/source/release-notes.html. When a release cycle ends (v. 1.42 ended with 1.42q, v. 1.43 with 1.43u, etc.) an *installation package* is created, downloadable from http://imagej.nih.gov/ij/download.html. Typically, this package is bundled with a small list of add-ons (Macros, Scripts and Plugins).

See also: Luts, Macros and Tools Updater, a macro toolset that performs live-updating of macros listed on the ImageJ web site

### 2.1 ImageJDistributions

ImageJ alone is not that powerful: it's real strength is the vast repertoire of Plugins that extend ImageJ's functionality beyond its basic core. The many hundreds, probably thousands, freely available plugins from contributors around the world play a pivotal role in ImageJ's success [22]. Running Help ▷ Update ImageJ..., however, will not update any of the plugins you may have installed[1].

ImageJ add-ons (Plugins, Scripts and Macros) are available from several sources (ImageJ's plugins page [Help ▷ Plugins...], ImageJ Information and Documentation Portal and Fiji's webpage, among others) making manual updates of a daunting task. This reason alone, makes it extremely convenient the use of ImageJDistributions bundled with a pre-organized collection of add-ons.

Below is a list of the most relevant projects that address the seeming difficult task of organizing and maintaining ImageJ beyond its basics. If you are a life scientist and have doubts about which distribution to choose you should opt for Fiji. It is heavily maintained, offers an automatic updater, improved scripting capabilities and ships with powerful plugins. More specialized adaptations of ImageJ are discussed in Software Packages Built on Top of ImageJ.

#### Fiji

Fiji (*Fiji Is Just ImageJ—Batteries included*) is a distribution of ImageJ together with Java, Java 3D and several plugins organized into a coherent menu structure. Citing its developers,

[1] Certain plugins, however, provide self-updating mechanisms (e.g., ObjectJ and the OME Bio-Formats).

"Fiji compares to ImageJ as Ubuntu compares to Linux". The main focus of Fiji is to assist research in life sciences, targeting image registration, stitching, segmentation, feature extraction and 3D visualization, among others. It also supports many scripting languages (BeanScript, Clojure, Jython, Python, Ruby, *see* Scripting in Other Languages). Importantly, Fiji ships with a convenient updater that knows whether your files are up-to-date, obsolete or locally modified. Comprehensive documentation is available for most of its plugins. The Fiji project was presented publicly for the first time at the ImageJ User and Developer Conference in November 2008.

### MBF ImageJ

The MBF ImageJ bundle or *ImageJ for Microscopy* (formerly WCIF-ImageJ) features a collection of plugins and macros, collated and organized by Tony Collins at the MacBiophotonics facility, McMaster University. It is accompanied by a comprehensive manual describing how to use the bundle with light microscopy image data. It is a great resource for microscopists but is not maintained actively, lagging behind the development of core ImageJ.

Note that you can add plugins from MBF ImageJ to Fiji, combining the best of both programs. Actually, you can use multiple ImageJ distributions simultaneously, assemble your own ImageJ bundle by gathering the plugins that best serve your needs (probably, someone else at your institution already started one?) or create symbolic links to share plugins between different installations.

See also: Description of all ImageJ related projects at ImageDev

## 2.2 Related Software

### 2.2.1 Software Packages Built on Top of ImageJ

**Bio7** Bio7 is an integrated development environment for ecological modeling with a main focus on individual based modeling and spatially explicit models. Bio7 features: Statistical analysis (using R); Spatial statistics; Fast communication between R and Java; BeanShell and Groovy support; Sensitivity analysis with an embedded flowchart editor and creation of 3D OpenGL (Jogl) models (*see also* RImageJ in ImageJ Interoperability).

**BoneJ** BoneJ is a collection of tools for trabecular geometry and whole bone shape analysis.

**µManager** Micro-Manager is a software package for control of automated microscopes. It lets you execute common microscope image acquisition strategies such as time-lapses, multi-channel imaging, z-stacks, and combinations thereof. µManager works with microscopes from all four major manufacturers, most scientific-grade cameras and many peripherals used in microscope imaging.

**MRI–CIA** MRI Cell Image Analyzer, developed by the Montpellier RIO Imaging facility (CNRS), is a rapid image analysis application development framework, adding visual scripting interface to ImageJ's capabilities. It can create batch applications as well as interactive applications. The applications include the topics "DNA combing", "quantification of stained proteins in cells", "comparison of intensity ratios between nuclei and cytoplasm" and "counting nuclei stained in different channels".

**ObjectJ** ObjectJ, the successor of object-image, supports graphical vector objects that non-destructively mark images on a transparent layer. Vector objects can be placed manually or by macro commands. Composite objects can encapsulate different color-coded marker





structures in order to bundle features that belong together. ObjectJ provides back-and-forth navigation between results and images. The results table supports statistics, sorting, color coding, qualifying and macro access.

**SalsaJ** SalsaJ is a student-friendly software developed specifically for the EU-HOU project. It is dedicated to image handling and analysis of astronomical images in the classroom. SalsaJ has been translated into several languages.

**TrakEM2** TrakEM2 is a program for morphological data mining, three-dimensional modeling and image stitching, registration, editing and annotation [13]. TrakEM2 is distributed with Fiji and capable of:

**3D modeling** Objects in 3D, defined by sequences of contours, or profiles, from which a skin, or mesh, can be constructed, and visualized in 3D.

**Relational modeling** The extraction of the map that describes links between objects. For example, which neuron contacts which other neurons through how many and which synapses.

See also: BioImageXD, Endrov, Image SXM

### 2.2.2 ImageJ Interoperability

Several packages exist that allow ImageJ to interact with other applications/environments:

**Bitplane Imaris** ImarisXT can load and execute ImageJ plugins. bpImarisAdapter (Windows only and requiring valid licenses for Imaris and ImarisXT) allows the exchange of images between Imaris and ImageJ.

**CellProfiler** CellProfiler [17] features RunImageJ, a module that allows ImageJ plugins to be run in a CellProfiler pipeline.

**Icy** Icy, an open source community software for bio-imaging, executes ImageJ plugins with almost 100% plugin compatibility.

**Knime** Knime (Konstanz Information Miner) contains several image processing nodes (KNIP) that are capable of executing ImageJ plugins and macros.

**Open Microscopy Environment** All Open Microscopy Environment projects such as Bio-Formats, VisBio and OMERO integrate well with ImageJ.

**RImageJ — R bindings for ImageJ** Bindings between ImageJ and R (GNU S) — The free software environment for statistical computing and graphics. The documentation for RImageJ is available at http://cran.r-project.org/web/packages/RImageJ/RImageJ.pdf (*see also* Bio7 in Software Packages Built on Top of ImageJ).

**MIJ — Matlab–ImageJ bi-directional communication** A Java package for bi-directional data exchange between Matlab and ImageJ, allowing to exchange images between the two imaging software. MIJ also allows MATLAB to access all built-in functions of ImageJ as well as third-party ImageJ plugins. The developers provide more information on the MIJ and Matlab File Exchange websites. Fiji features `Miji.m`, which makes even more convenient to use the libraries and functions provided by Fiji's components from within Matlab.

See also: ImageJ related links, list of related imaging software on the ImageJ2 website

### 2.3 ImageJ2

ImageJDev is a federally funded, multi-institution project dedicated to the development of the next-generation version of ImageJ: "ImageJ2". ImageJ2 is a complete rewrite of ImageJ, that includes the current, stable version ImageJ ("ImageJ1") with a compatibility layer so that old-style plugins and macros can run the same as they currently do in ImageJ1. Below is a summary of the ImageJDev project aims:

- To create the next generation version of ImageJ and improve its core architecture based on the needs of the community.
- To ensure ImageJ remains useful and relevant to the broadest possible community, maintaining backwards compatibility with ImageJ1 as close to 100% as possible.
- Expand functionality by interfacing ImageJ with existing open-source programs.
- To lead ImageJ development with a clear vision, avoiding duplication of efforts
- To provide a central online resource for ImageJ: program downloads, a plugin repository, developer resources and more.

Be sure to follow the ImageJ2 project news and the ImageDev blog for updates on this exciting project.

# 3 Getting Help

### 3.1 Help on Image Analysis

Below is a list of online resources (in no particular order) related to image processing and scientific image analysis, complementing the list of external resources on the IJ web site.

#### Ethics in Scientific Image Processing

- Online learning Tool for Research Integrity and Image Processing
  This website, created by the Office of Research Integrity, explains what is appropriate in image processing in science and what is not.
- Digital Imaging: Ethics (at the Cellular Imaging Facily Core, SEHSC)
  This website, compiled by Douglas Cromey at the University of Alabama – Birmingham, discusses thoroughly the topic of digital imaging ethics. It is recommended for all scientists. The website contains links to several external resources, including:
  1. What's in a picture? The temptation of image manipulation (2004) M Rossner and K M Yamada, J Cell Biology 166(1):11–15, doi:10.1083/jcb.200406019
  2. Not picture-perfect (2006), Nature 439, 891–892, doi:10.1038/439891b.

#### Scientific Image Processing

- What you need to know about scientific image processing
  Simple and clear, this Fiji webpage explains basic aspects of scientific image processing.





- imagingbook.com
  Web site of *Digital Image Processing: An Algorithmic Introduction using Java* by Wilhelm Burger and Mark Burge [11]. This technical book provides a modern, self-contained, introduction to digital image processing techniques. Numerous complete Java implementations are provided, all of which work within ImageJ.
- Hypermedia Image Processing Reference (HIPR2)
  Developed at the Department of Artificial Intelligence in the University of Edinburgh, provides on-line reference and tutorial information on a wide range of image processing operations.
- IFN wikipage
  The Imaging Facility Network (IFN) in Biopolis Dresden provides access to advanced microscopy systems and image processing. The website hosts high quality teaching material and useful links to external resources.
- stereology.info
  Stereology Information for the Biological Sciences, designed to introduce both basic and advanced concepts in the field of stereology.

SEE ALSO: ImageJ Related Publications on page 174

### 3.2 Help on ImageJ

Below is a list of the ImageJ help resources that complement this guide (*see* Guide Formats). Specific documentation on advanced uses of ImageJ (macro programming, plugin development, etc.) is discussed in Extending ImageJ.

1. The ImageJ online documentation pages
   Can be accessed via the Help ▷ Documentation... command.
2. The Fiji webpage:
   http://fiji.sc/
3. The ImageJ Information and Documentation Portal (ImageJ wikipage):
   http://imagejdocu.tudor.lu/doku.php
4. Video tutorials on the ImageJ Documentation Portal and the Fiji YouTube channel:
   http://imagejdocu.tudor.lu/doku.php?id=video:start&s[]=video and http://www.youtube.com/user/fijichannel. New ImageJ users will probably profit from Christine Labno's video tutorial.
5. The ImageJ for Microscopy manual
   http://www.macbiophotonics.ca/imagej/
6. Several online documents, most of them listed at:
   http://imagej.nih.gov/ij/links.html and http://imagej.nih.gov/ij/docs/examples/
7. Mailing lists:
   (a) **ImageJ** — http://imagej.nih.gov/ij/list.html
   General user and developer discussion about ImageJ. Can be accessed via the Help ▷ Mailing List... command. This list is also mirrored at Nabble and Gmane. You may find it easier to search and browse the list archives on these mirrors. Specially useful are the RSS feeds and the *frames and threads* view provided by Gmane.

(b) **Fiji users** — http://groups.google.com/group/fiji-users
For user discussion specific to Fiji (rather than core ImageJ).

(c) **Fiji-devel** — http://groups.google.com/group/fiji-devel
For developer discussion specific to Fiji.

(d) **ImageJ-devel** — http://imagejdev.org/mailman/listinfo/imagej-devel
For communication and coordination of the ImageJDev project.

(e) **Dedicated mailing lists** for ImageJ related projects
Described at http://imagejdev.org/mailing-lists .

### Using Mailing-lists

If you are having problems with ImageJ, you should inquiry about them in the appropriated list. The ImageJ mailing list is an unmoderated forum subscribed by a knowledgeable worldwide user community with ≈2000 advanced users and developers. To have your questions promptly answered you should consider the following:

1. Read the documentation files (described earlier in this section) before posting. Because there will always be a natural lag between the implementation of key features and their documentation it may be wise to check briefly the ImageJ news website (Help ▷ ImageJ News. . . ).
2. Look up the mailing list archives (Help ▷ Mailing List. . . ). Most of your questions may have already been answered.
3. If you think you are facing a bug try to upgrade to the latest version of ImageJ (Help ▷ Update ImageJ. . . ). You should also check if you are running the latest version of the Java Virtual Machine for your operating system. Detailed instructions on how to submit a bug report are found at http://imagej.nih.gov/ij/docs/faqs.html#bug.
4. Remember that in most cases you can find answers within your own ImageJ installation without even connecting to the internet since the heuristics for finding commands or writing macros have been significantly improved in later versions (*see* Finding Commands and Extending ImageJ).
5. As with any other mailing list, you should always follow basic netiquette, namely:
   (a) Use descriptive subject lines – *Re: Problem with Image>Set Scale command* is much more effective than a general *Re: Problem.*
   (b) Stay on topic – Do not post off-topic messages, unrelated to the message thread.
   (c) Be careful when sending attachments – Refrain from attaching large files. Use, e.g., a file hosting service instead.
   (d) Edit replies – You should include only the minimum content that is necessary to provide a logical flow from the question to the answer, i.e., quote only as much as absolutely necessary and relevant.



# Part II
# Working with ImageJ

This part introduces some basic aspects of ImageJ so that you can use the software more efficiently. It also introduces some important terms and concepts used throughout this guide. You may skip it if you already use the program efficiently and are familiar with terms such as Virtual Stacks, Hyperstacks, Pseudocolor Images, Color Composite Images or Composite Selections.

## 4 Using Keyboard Shortcuts

You'll learn more and more shortcut keys as you use ImageJ, because (almost) all shortcuts are listed throughout ImageJ menus. Similarly, in this guide each command has its shortcut key listed on its name (flanked by square brackets). Please note that the notation for these key-bindings is case sensitive, i.e., Shift-modifiers are not explicitly mentioned (a capital A means Shift–A) and assumes that *Require control key for shortcuts* in Edit ▷ Options ▷ Misc. . . is unchecked (i.e., except when using the IJ Editor or the Text Tool, you won't have to hold down the Control key to use menu shortcuts). For example, the command Edit ▷ Invert [I] can be evoked by Shift I or Ctrl Shift I if *Require control key for shortcuts* is checked. The full list of ImageJ shortcuts (*see* Keyboard Shortcuts) can be retrieved at any time using the Plugins ▷ Utilities ▷ List Shortcuts. . . command.

There are three modifier keys in ImageJ:

**Control** (**Command Key** on Apple keyboards) Denoted by 'Ctrl' or Ctrl in this document. Although a control key is typically present on Apple keyboards, on a Macintosh computer running ImageJ the Command key ⌘ Cmd replaces the functionality of the Control key of other operating systems. For sake of simplification, 'Ctrl' will always refer to both throughout this guide.

**Shift** Denoted by 'Shift' or Shift in this document.

**Alt** Denoted by 'Alt' or Alt in this document. This is also the 'Option' or 'Meta' key on many keyboards. In ImageJ, it is also used to type special unit symbols such as µ (Alt M) or Å (Alt Shift A).

SEE ALSO: Keyboard Shortcuts, Plugins ▷ Shortcuts ▷

## 5 Finding Commands

Navigating through the extensive list of ImageJ commands, macros and plugins may be quite cumbersome. Through its built-in Command Finder / Launcher[48], ImageJ offers an expedite alternative that allows you to retrieve commands extremely fast: Plugins ▷ Utilities ▷ Find Commands. . . [l].

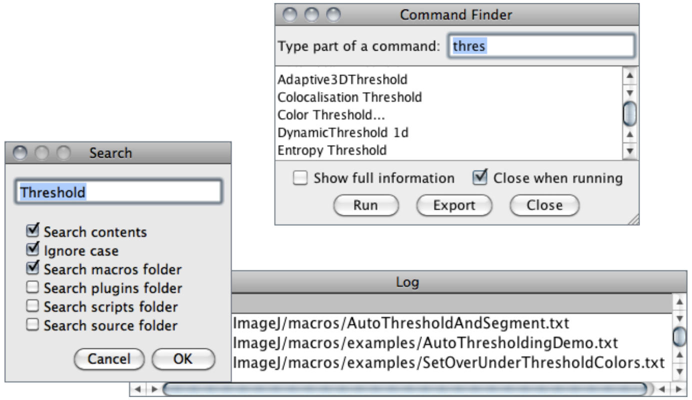
In addition, ImageJ features a find function that locates macros, scripts and plugins source (.java) files on your computer: the Plugins ▷ Utilities ▷ Search. . . command. Because most of IJ source files contain circumstanced comments, you can use this utility to retrieve files

### I Frontmost Window and Window Activation

In ImageJ, all operations are performed on the active (frontmost) image (which has its title bar highlighted). If a window is already open it will activate when its opening command is re-run, e.g., if the B&C window is already opened (Image ▷ Adjust ▷ Brightness/Contrast... [C]), pressing its keyboard shortcut ( Shift C ) will activate it.

Pressing Enter on any image will bring the Main ImageJ window to the foreground. In addition, it is also possible to permanently place the main window above all other windows (*see* Floating Behavior of Main Window).

Plugins ▷ Utilities ▷ Find Commands... [l]




Plugins ▷ Utilities ▷ Search...

related not only to a image processing routine (e.g., *background* or *co-localization*) but also to a practical context such as *radiogram*, *cell* or *histology*. Indeed, ImageJ source files contain detailed annotations useful to both developers and regular users that want to know more about ImageJ routines and algorithms.

Search... and Find Commands... [l] are described in detail in Plugins ▷ Utilities ▷.

See also: Control Panel... [U], Keyboard Shortcuts and `SourceCodeRetriever`, a macro that searches for a menu entry and retrieves the source file of the respective command

## 6 Undo and Redo

Probably the first thing you will notice is that ImageJ does not have a large undo/redo buffer. Undo (Edit ▷ Undo [z]) is currently limited to the most recent image editing / filtering operation. With time you will appreciate that this is necessary to minimize memory overhead. Nevertheless, with IJ 1.45 and later, Undo [z] is, in most cases, undoable and can be applied to multiple images if *Keep multiple undo buffers* is checked in Edit ▷ Options ▷ Memory & Threads...

If you cannot recover from a mistake, you can always use File ▷ Revert [r] to reset the image lo its last saved state. For selections, Edit ▷ Selection ▷ Restore Selection [E] can be used to recover any misdealt selection.





In ImageJ the equivalent to 'Redo' is the Process ▷ Repeat Command [R], that re-runs the previous used command (skipping Edit ▷ Undo [z] and File ▷ Open... [o] commands).

See also: Plugins ▷ Utilities ▷ Reset..., Multi Undo plugin

## 7 Image Types and Formats

Digital Images are two-dimensional grids of pixel intensities values with the width and height of the image being defined by the number of pixels in $x$ (rows) and $y$ (columns) direction. Thus, pixels (picture elements) are the smallest single components of images, holding numeric values – pixel intensities – that range between black and white. The characteristics of this range, i.e., the number of unique intensity (brightness) values that can exist in the image is defined as the bit–depth of the image and specifies the level of precision in which intensities are coded, e.g.: A 2–bit image has $2^2 = 4$ tones: 00 (black), 01 (gray), 10 (gray), and 11 (white). A 4–bit image has $2^4 = 16$ tones ranging from 0000 (0) to 1111 (16), etc. In terms of bits per pixel (bpp), the most frequent types of images (Image ▷ Type ▷) that ImageJ deals with are (ImageJ2 supports many more types of image data):

**8–bit** Images that can display 256 ($2^8$) gray levels (integers only).

**16–bit** Images that can display 65,536 ($2^{16}$) gray levels (integers only).

**32–bit** Images that can display 4,294,967,296 ($2^{32}$) gray levels (real numbers). In 32-bit images, pixels are described by floating point values and can have any intensity value including *NaN* (Not a Number).

**RGB Color** Color Images that can display 256 values in the Red, Green and Blue channel. These are 24–bit ($2^{3\times8}$) images. RGB color images can also be 32–bit color images (24–bit color images with additional eight bits coding alpha blending values, i.e., transparency).

### Native Formats

Natively (i.e. without the need of third-party plugins) ImageJ opens the following formats: **TIFF**, **GIF**, **JPEG**, **PNG**, **DICOM**, **BMP**, **PGM** and **FITS**. Many more formats are supported with the aid of plugins. These are discussed in Non–native Formats.

**TIFF** (Tagged Image File Format) is the 'default' format of ImageJ (cf. File ▷ Save [s]). Images can be 1–bit, 8–bit, 16–bit (unsigned[1]), 32–bit (real) or RGB color. TIFF files with multiple images of the same type and size open as Stacks or Hyperstacks. ImageJ opens lossless compressed TIFF files (*see* II Image Types: Lossy Compression and Metadata) by the LZW, PackBits and ZIP (Deflate/Inflate) [2] compression schemes. In addition, TIFF files can be opened and saved as ZIP archives.
Tiff tags and information needed to import the file (number of images, offset to first images, gap between images) are printed to the Log Window when ImageJ is running in *Debug Mode* (Edit ▷ Options ▷ Misc..., *see* Settings and Preferences).

[1] A numeric variable is signed if it can represent both positive and negative numbers, and unsigned if it can only represent positive numbers.

**DICOM** (Digital Imaging and Communications in Medicine) is a standard popular in the medical imaging community. Support in ImageJ is limited to uncompressed DICOM files. DICOM files containing multiple images open as Stacks.
Use Image ▷ Show Info... [i] to display the DICOM header information. A DICOM sequence can be opened using File ▷ Import ▷ Image Sequence... or by dragging and dropping the folder on the 'ImageJ' window. Imported sequences are sorted by image number instead of filename and the tags are preserved when DICOM images are saved in TIFF format. ImageJ supports custom DICOM dictionaries, such as the one at http://imagej.nih.gov/ij/download/docs/DICOM_Dictionary.txt. More information can be found at the Center for Advanced Brain Imaging.

**FITS** (Flexible Image Transport System) image is the format adopted by the astronomical community for data interchange and archival storage. Use Image ▷ Show Info... [i] to display the FITS header. More information here.

**PGM** (Portable GrayMap), **PBM** (Portable BitMap) and **PPM** (Portable PixMap) are simple image formats that use an ASCII header. More information here.

**AVI** (Audio Video Interleave) is a container format which can contain data encoded in many different ways. ImageJ only supports uncompressed AVIs, various YUV 4:2:2 compressed formats, and PNG or JPEG-encoded individual frames. Note that most MJPG (motion-JPEG) formats are not read correctly. Attempts to open AVIs in other formats will fail.

See also: Non–native Formats, II Image Types: Lossy Compression and Metadata, X Warning on JPEG Compression

### Non–native Formats

When opening a file, ImageJ first checks whether it can natively handle the format. If ImageJ does not recognize the type of file it calls for the appropriate reader plugin using HandleExtraFileTypes, a plugin bundled with ImageJ. If that fails, it tries to open the file using the OME Bio-Formats library (if present), a remarkable plugin that supports more than one hundred of the most common file formats used in microscopy. If nevertheless the file cannot be opened, an error message is displayed.

Because both these plugins are under active development, it is important that you keep them updated. The OME Bio-Formats library can be updated using its self-updating plugin (Plugins ▷ LOCI ▷ Update LOCI Plugin...) or Fiji↑'s built-in updater (Help ▷ Update Fiji...). The following websites provide more information on the OME Bio-Formats:

- http://loci.wisc.edu/bio-formats/imagej
- http://fiji.sc/Bio-Formats
- http://loci.wisc.edu/bio-formats/using-bio-formats

In addition, the ImageJ web site lists more than sixty plugins that recognize more 'exotic' file formats. The ImageJ Documentation Portal also maintains a (somewhat outdated) list of file formats that are supported by ImageJ.

See also: Native Formats, File ▷ Import ▷, II Image Types: Lossy Compression and Metadata, X Warning on JPEG Compression, Acquisition plugins, Input/Output plugins





**II Image Types: Lossy Compression and Metadata**

Two critical aspects to keep in mind when converting images:

**Lossy compression** Transcoding an image into a format that uses lossy compression will alter the original data, introducing artifacts (*see* X Warning on JPEG Compression). This is the case, e.g., for JPEG formats (with the exception of some JPEG2000 images that use lossless compression). As such, these types of data are intended for human interpretation only and are not suitable for quantitative analyses

**Metadata** In ImageJ, metadata associated with the image, such as scale, gray value calibration and user comments is only supported in tiff and zip (compressed tiff) images. In addition, selections and Overlays are also saved in the TIFF header (cf. File ▷ Save [s]). None of the above is saved in other formats (cf. Native Formats).

## 8 Stacks, Virtual Stacks and Hyperstacks

### Stacks

ImageJ can display multiple spatially or temporally related images in a single window. These image sets are called stacks. The images that make up a stack are called slices. In stacks, a pixel (which represents 2D image data in a bitmap image) becomes a voxel (volumetric pixel), i.e., an intensity value on a regular grid in a three dimensional space.

All the slices in a stack must be the same size and bit depth. A scrollbar provides the ability to move through the slices and the slider is preceded by a play/pause icon that can be used to start/stop stack animation. Right-clicking on this icon runs the Animation Options... [Alt /] dialog box.

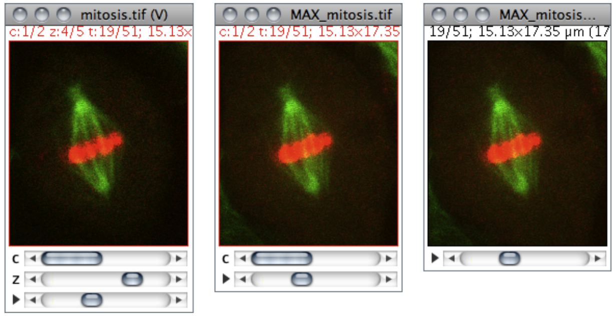
Most ImageJ filters will, as an option, process all the slices in a stack. ImageJ opens multi-image TIFF files as a stack, and saves stacks as multi-image TIFFs. The File ▷ Import ▷ Raw... command opens other multi-image, uncompressed files. A folder of images can be opened as a stack either by dragging and dropping the folder onto the 'ImageJ' window or or by choosing File ▷ Import ▷ Image Sequence... To create a new stack, simply choose File ▷ New ▷ Image... [n] and set the *Slices* field to a value greater than one. The Image ▷ Stacks ▷ submenu contains commands for common stack operations.

See also: Stacks Menu, Stack Manipulations on Fiji website, Image5D

### Virtual Stacks

Virtual stacks are disk resident (as opposed to RAM resident) and are the only way to load image sequences that do not fit in RAM. There are several things to keep in mind when working with virtual stacks:

- Virtual stacks are read-only, so changes made to the pixel data are not saved when you switch to a different slice. You can work around this by using macros (e.g., Process Virtual Stack) or the Process ▷ Batch ▷ Virtual Stack... command
- You can easily run out of memory using commands like Image ▷ Crop [X] because any stack generated from commands that do not generate virtual stacks will be RAM resident.




**Stacks and Hyperstacks in ImageJ:** File ▷ Open Samples ▷ Mitosis (26MB, 5D stack). Hyperstacks dimensionality can be reduced using Image ▷ Hyperstacks ▷ Reduce Dimensionality. . . , Image ▷ Stacks ▷ Z Project. . . or Image ▷ Hyperstacks ▷ Channels Tool. . . [Z] The '(V)' on the window title denotes a virtual image (*see* Virtual Stacks).

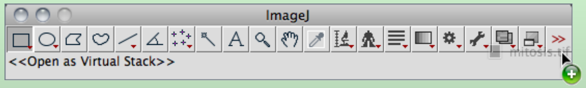
- TIFF virtual stacks can usually be accessed faster than JPEG virtual stacks. A JPEG sequence can be converted to TIFF by opening the JPEG images as a virtual stack and using File ▷ Save As ▷ Image Sequence. . . to save in TIFF format

ImageJ appends a '(V)' to the window title of virtual stacks and hyperstacks (*see* Hyperstacks). Several built-in ImageJ commands in the File ▷ Import ▷ submenu have the ability to open virtual stacks, namely: TIFF Virtual Stack. . . , Image Sequence. . . , Raw. . . , Stack From List. . . , AVI. . . (cf. Virtual Stack Opener). In addition, TIFF stacks can be open as virtual stacks by drag and drop (cf. III Opening Virtual Stacks by Drag & Drop).

SEE ALSO: LOCI Bio-Formats and RegisterVirtualStackSlices plugins, Process Virtual Stack and VirtualStackFromList macros

### III Opening Virtual Stacks by Drag & Drop

TIFF stacks with a `.tif` extension open as virtual stacks when dragged and dropped on the » toolbar icon.




## Hyperstacks

Hyperstacks are multidimensional images, extending image stacks to four (4D) or five (5D) dimensions: $x$ (width), $y$ (height), $z$ (slices), $c$ (channels or wavelengths) and $t$ (time frames). Hyperstacks are displayed in a window with three labelled scrollbars (*see* Stacks and Hyperstacks). Similarly to the scrollbar in Stacks, the frame slider ($t$) has a play/pause icon.

SEE ALSO: Image ▷ Hyperstacks ▷ submenu





# 9 Color Images[1]

ImageJ deals with color mainly in three ways: pseudocolor images, RGB images, RGB/ HSB stacks, and composite images.

## Pseudocolor Images

A pseudocolor (or indexed color) image is a single channel gray image (8, 16 or 32–bit) that has color assigned to it via a lookup table or LUT. A LUT is literally a predefined table of gray values with matching red, green and blue values so that shadows of gray are displayed as colorized pixels. Thus, differences in color in the pseudo-colored image reflect differences in intensity of the object rather than differences in color of the specimen that has been imaged.

8-bit indexed color images (such as GIFs) are a special case of pseudocolor images as their lookup table is stored in the file with the image. These images are limited to 256 colors (24–bit RGB images allow 16.7 million of colors, *see* Image Types and Formats) and concomitantly smaller file sizes. Reduction of true color values to a 256 color palette is performed by color quantization algorithms. ImageJ uses the Heckbert's median-cut color quantization algorithm (*see* Image ▷ Type ▷ menu), which, in most cases, allows indexed color images to look nearly identical to their 24-bit originals.

SEE ALSO: Image ▷ Lookup Tables ▷ and LUT Menu

## True Color Images

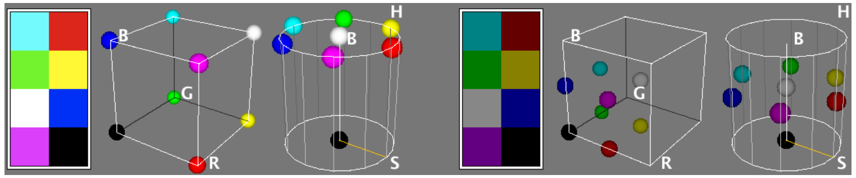
As described in Image Types and Formats, true color images such as RGB images reflect genuine colors, i.e., the green in an RGB image reflects green color in the specimen. Color images are typically produced by color CCD cameras, in which color filter arrays (Bayer masks) are placed over the image sensor.

## Color Spaces and Color Separation

Color spaces describe the gamut of colors that image-handling devices deal with. Because human vision is trichromatic, most color models represent colors by three values. Mathematically, these values (color components) form a three-dimensional space such as the RGB, HSB, CIE Lab or YUV color space.

RGB (Red, Green, Blue) is the most commonly-used color space. However, other alternatives such as HSB (Hue, Saturation, Brightness) provide significant advantages when processing color information. In the HSB color space, *Hue* describes the attribute of pure color, and therefore distinguishes between colors. *Saturation* (sometimes called "purity" or "vibrancy") characterizes the shade of color, i.e., how much white is added to the pure color. *Brightness* (also know as *Value* – HSV system) describes the overall brightness of the color (*see* e.g., the color palette of Color Picker window). In terms of digital imaging processing, using the HSB system over the traditional RGB is often advantageous: e.g., since the Brightness component of an HSB image corresponds to the grayscale version of that image, processing only the brightness channel in

[1]This section is partially extracted from the MBF ImageJ online manual at http://www.macbiophotonics.ca/imagej/colour_image_processi.htm.




**Representation of an eight pixel color image in the RGB and HSB color spaces.** The RGB color space maps the RGB color model to a cube with *Red* (R) values increasing along the x-axis, *Green* (G) along the y-axis and *Blue* (B) along the z-axis. In the HSB cylindrical coordinate system, the angle around the central vertical axis corresponds to *Hue* (H), the distance from the axis corresponds to *Saturation* (S), and the distance along the axis corresponds to *Brightness* (B). In both cases the origin holds the black color. The right panel shows the same image after brightness reduction, easily noted by the vertical displacement along the HSB cylinder. Images produced using Kai Uwe Barthel's 3D Color Inspector plugin.

routines that require grayscale images is a significant computational gain[1]. You can read more about the HSB color model here.

In ImageJ, conversions between image types are performed using the Image ▷ Type ▷ submenu. Segmentation on the HSB, RGB, CIE Lab and YUV color spaces can be performed by the Image ▷ Adjust ▷ Color Threshold... command [20]. Segregation of color components (specially useful for quantification of histochemical staining) is also possible using Gabriel Landini's Colour Deconvolution plugin. In addition, several other plugins related to color processing can be obtained from the ImageJ website.

### Conveying Color Information[2]

People see color with significant variations. Indeed, the popular phrase "One picture is worth ten thousand words" may not apply to certain color images, specially those that do not follow the basic principles of Color Universal Design. Citing Masataka Okabe and Kei Ito:

> Colorblind people can recognize a wide ranges of colors. But certain ranges of colors are hard to distinguish. The frequency of colorblindness is fairly high. One in 12 Caucasian (8%), one in 20 Asian (5%), and one in 25 African (4%) males are so-called 'red–green' colorblind.
>
> There are always colorblind people among the audience and readers. There should be more than TEN colorblind in a room with 250 people (assuming 50% male and 50% female).
>
> [...] There is a good chance that the paper you submit may go to colorblind reviewers. Supposing that your paper will be reviewed by three white males (which is not unlikely considering the current population in science), the probability that at least one of them is colorblind is whopping 22%!

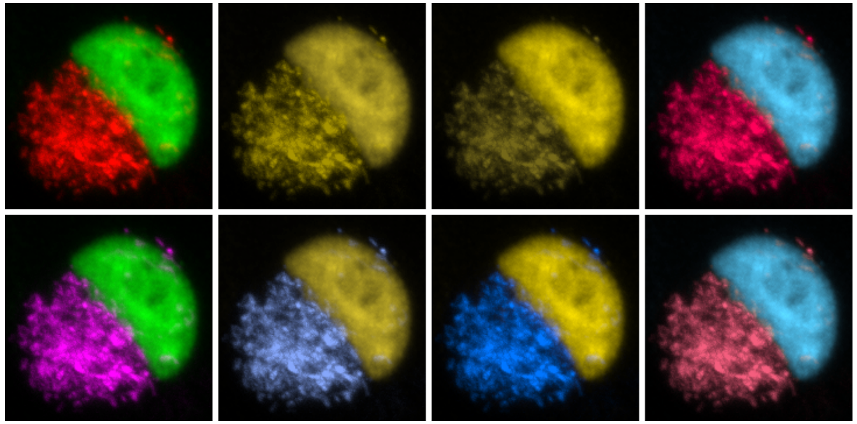
One practical point defined by the Color Universal Design is the use of magenta in red–green overlays (*see also* [66]). Magenta is the equal mixture of red and blue. Colorblind people that

[1] *See* Wootton R, Springall DR, Polak JM. Image Analysis in Histology: Conventional and Confocal Microscopy. *Cambridge University Press*, 1995, ISBN 0521434823

[2] This section is partially extracted from Masataka Okabe and Kei Ito, *Color Universal Design (CUD) — How to make figures and presentations that are friendly to Colorblind people*, http://jfly.iam.u-tokyo.ac.jp/color/, accessed 2009.01.15







**Red–green images and partial color blindness.** Deuteranopia (second panel), protanopia (third panel) are the most common types of partial color blindness (red / green confusion). Tritanopia (blue / orange confusion, fourth panel) is quite rare. Replacing Red with Magenta in RGB Images (bottom row) is a simple way to compensate for color vision deficiencies.

have difficulties recognizing the red component can easily recognize the blue hue. The region of double positive becomes white, which is easily distinguishable for colorblind. In ImageJ this is easily accomplished using the Image ▷ Color ▷ Merge Channels..., or using the ImageJ macro language (*see* IV Replacing Red with Magenta in RGB Images).

### IV Replacing Red with Magenta in RGB Images

When building RGB images, magenta can be obtained using the Image ▷ Color ▷ Merge Channels... Previously created RGB images can be converted to 'MGB' using Image ▷ Color ▷ Channels Tool... [Z]. Alternatively, the Process ▷ Image Calculator... command can be used to add the red channel to the blue channel. Both these approaches can be automated using the ImageJ macro language as exemplified by Macros (2) and (1). Once saved in the `ImageJ/plugins/` folder these Macros are treated as regular ImageJ commands.

In Fiji, as expected, the procedure of modifying RGB images is simpler: one just needs to run Image ▷ Color ▷ Replace Red with Magenta. For even more convenience, Fiji provides an analogous command that replaces the system clipboard's image with a magenta-green one.

It is also possible to simulate color blindness using the Vischeck or Dichromacy plugins[1], or in Fiji, using the Image ▷ Color ▷ Simulate Color Blindness command.

### Color Composite Images

In a composite image colors are handled through channels. The advantages with this type of image over plain RGB images are:

[1] One advantage of Dichromacy over the Vischeck plugin is that it can be recorded and called from scripts and macros, without user interaction.

**(1) Replace Red with Magenta.ijm (Using Process ▷ Image Calculator. . . )**

```
/* This macro replaces Red with Magenta in RGB images using Process>Image ↩
   Calculator...  command. */
  if (bitDepth!=24)
      exit("This macro requires an RGB image");
setBatchMode(true);
  title= getTitle();
  r= title+" (red)"; g= title+" (green)"; b= title+" (blue)";
  run("Split Channels");
  imageCalculator("Add", b, r);
  run("Merge Channels...", "red=&r green=&g blue=&b");
  rename(title + " (MGB)");
setBatchMode(false);
```

1. Each channel is kept separate from the others and can be turned on and off using the 'Channels' tool (Image ▷ Color ▷ Channels Tool. . . [Z]). This feature allows, e.g., to perform measurements on a specific channel while visualizing multiple.
2. Channels can be 8, 16 or 32–bit and can be displayed with any lookup table
3. More than 3 channels can be merged or kept separate

**(2) Replace Red with Magenta.ijm (Using Image ▷ Color ▷ Channels. . . )**

```
/* This macro replaces Red with Magenta in RGB images using the ↩
   Image>Color>Channels... tool. */
  if (bitDepth!=24)          // Ignore non-RGB images
      exit("This macro requires an RGB image");
setBatchMode(true);          // Enter `Batch' mode
  title = getTitle();        // Retrieve the image title
  run("Make Composite");     // Run Image>Color>Make Composite
  run("Magenta");            // Run Image>Lookup Tables>Magenta on channel 1
  run("RGB Color");          // Run Image>Type>RGB Color
  rename(title + " (MGB)"); // Rename the image
setBatchMode(false);         // Restore `GUI' mode
```

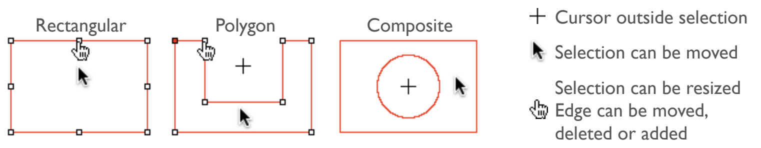
# 10 Selections

Selections (regions of interest, ROIs), are typically created using the Toolbar Tools. Although ImageJ can display simultaneously several ROIs (see Overlays and ROI Manager) only one selection can be active at a time. Selections can be measured (Analyze ▷ Measure. . . [m]), drawn (Edit ▷ Draw [d]), filled (Edit ▷ Fill [f]) or filtered (Process ▷ Filters ▷ submenu), in the case of area selections. In addition it is also possible to hold multiple ROIs as non-destructive Overlays.

Selections can be initially outlined in one of the nine ImageJ default colors (*Red*, *Green*, *Blue*, *Magenta*, *Cyan*, *Yellow*, *Orange*, *Black* and White). Once created, selections can be contoured or painted with any other color using Edit ▷ Selection ▷ Properties. . . [y]. Selection Color can be changed in Edit ▷ Options ▷ Colors. . . , by double clicking on the Point Tool, or using hot keys (*see* (7) Using a Keyboard Shortcut to Change Selection Color). It is highlighted in the center of the Point Tool and Multi-point Tool.








**Three types of area selections In ImageJ.** Notice the cursor changes: to an *arrow* when it is within the selection, to a *cross-hair* when outside the selection, to a *hand* when over a selection vertex or 'handler'. Notice also the filled handler in the polygon selection and the absence of point handlers in Composite Selections. Overlays, i.e., non-active selections displayed in the non-destructive image overlay, are also displayed without handlers.

## 10.1 Manipulating ROIs

Most of commands that can be useful in defining or drawing selections are available in the Edit ▷ Selection ▷ submenu and summarized in ROI manipulations. Listed below are the most frequent manipulations involving selections:

**Adjusting** Area selections can be adjusted with the Brush Selection Tool. In addition, vertexes of selections created with the Polygon Selection Tool and Segmented Line Selection Tool can be adjusted by Alt/Shift-clicking.

**Deleting** Choose any of the selection tools and click outside the selection, or use Edit ▷ Selection ▷ Select None [A]. Use Edit ▷ Selection ▷ Restore Selection [E] to restore a selection back after having deleted it. With Overlays, an activated ROI can be deleted by pressing the Backspace (Delete on Mac) key.

**Managing** A selection can be transferred from one image window to another by activating the destination window and runnig Edit ▷ Selection ▷ Restore Selection [E]. Alternatively, Analyze ▷ Tools ▷ Synchronize Windows to create ROIs across multiple images. Multiple selections can be stored as Overlays or in the ROI Manager list (Analyze ▷ Tools ▷ ROI Manager. . . ).

**Moving** Selections can be moved by clicking and dragging as long as the cursor is within the selection and has changed to an . The status bar displays the coordinates of the upper left corner of the selection (or the bounding rectangle for non-rectangular selections) as it is being moved. To move the contents of a selection, rather than the selection itself, Edit ▷ Copy [c], Edit ▷ Paste [v], and then click within the selection and drag.

**Nudging** Selections can be 'nudged' one pixel at a time in any direction using the arrow keys. Note that the up and down keys zoom the image in and out in the absence of selections (*see* Arrow Keys shortcuts).

**Resizing** The Brush Selection Tool can be used to perform fine adjustments of ROI contours. Most ROIs can be resized one pixel at a time by holding Alt while using the arrow keys. In general (*see* Area Selection Tools and Line Selection Tools for details), selections are resized by dragging one of the selection handlers. While dragging, holding Ctrl resizes the selection around its center, holding Alt imposes a fixed aspect ratio and holding Shift forces a 1:1 aspect ratio.

SEE ALSO: Key Modifiers

## 10.2 Composite Selections

Composite selections are non-contiguous ROIs containing more than one cluster of pixels and/or ROIs containing internal holes. Composite ROIs are typically originated with the Brush Selection Tool but they can be defined with any other selection tool using key modifiers.

The following modifier keys can be use to create composite selections:

Shift Drawing outside current selection while pressing Shift creates new content. To add a non-square rectangle or ellipse, the Shift key must be released after adding the selection

Alt Drawing inside current selection while pressing Alt creates a hole removing content from the ROI

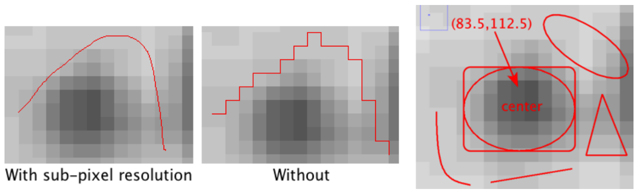
Note that some operations may not be performed properly on complex ROIs. In these cases, it may be useful to convert a composite ROI into a polygon using the Edit ▷ Selection ▷ Enlarge... command as explained in XIII Converting Composite Selections.

See also: Wand Tool, ROI2PolylineROI macro

New in IJ 1.46r

## 10.3 Selections With Sub-pixel Coordinates

Since ImageJ 1.46, selections can be defined with subpixel accuracy, beyond the nominal pixel resolution of the image: Floating point selections. Line Selections (*see* Line Selection Tools) are created with floating-point coordinates if the *Sub-pixel resolution* checkbox is active in Edit ▷ Options ▷ Profile Plot Options... Sub-pixel coordinates of pre-existing selections can be interpolated using the Edit ▷ Selection ▷ Interpolate command. Interpolated points are easily noticeable on small selections created on images zoomed 1200% or greater.




**Interpolated selections.** ROIs drawn with (left) or without (middle) sub-pixel accuracy. For line selections (*see* Line Selection Tools), this option can be enabled in Edit ▷ Options ▷ Profile Plot Options... by activating the *Sub-pixel resolution* checkbox. Pixel coordinates of area selections (*see* Area Selection Tools), can be interpolated using Edit ▷ Selection ▷ Interpolate. The image on the right is the output of `SubPixelSelections.js`, a script that demonstrates how to create selections at sub-pixel resolution without the need of setting any option in ImageJ.

See also: Zoom ▷, Magnifying Glass

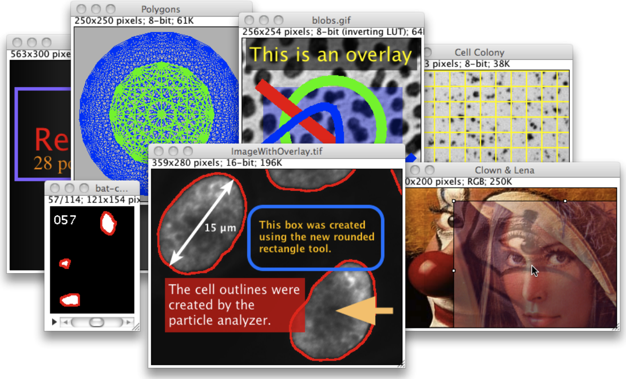
Improved in IJ 1.46r

# 11 Overlays

Overlays are non-active selections displayed 'over' the pixel data, on the image overlay, and are the core of non-destructive image processing in ImageJ. In a way you can think of the image








**Non-destructive operations using the image overlay.** Overlays can be used to annotate images, store ROIs and blend images (ImageROIs) at multiple opacity levels. Refer to the Image ▷ Overlay ▷ documentation for further examples. You can download the frontmost image to practice overlay editing.

overlay as an invisible ROI Manager in which selections are being added, allowing ROIs to be on 'hold'. This concept of multiple distinct selections has been dramatically improved in ImageJ2 so we urge you to download IJ2 if multiple ROIs are important in your workflows.

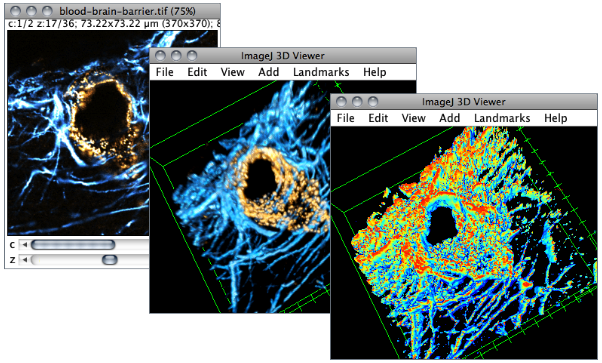
Importantly, overlay selections are vector graphics composed of mathematically-defined paths (as opposed to raster graphics in which objects are defined by pixels) and are not affected by scaling, i.e., do not become pixelated. Most of overlay-related commands are listed in the Image ▷ Overlay ▷, and in the ROI Manager window (Analyze ▷ Tools ▷ ROI Manager...). Appearance of overlay selections can be adjusted using Image ▷ Overlay ▷ Overlay Options.../Labels...

As mentioned in II Image Types: Lossy Compression and Metadata, overlays are saved in the header of tif images, and do not need to be saved externally when using TIFF, the default file format of ImageJ. The major advantages of overlays are summarized below:

**Storage of ROIs** In ImageJ it is only possible to have a single ROI at a time. However, it is possible to add selections to the image overlay using B (Image ▷ Overlay ▷ Add Selection... [b]). Once added to the image overlay, ROIs can be re-activated by Alt-clicking, Control-clicking or long-pressing (1/4 second or longer). Activated ROIs can be deleted by pressing the Backspace key. Selections can also be added and recovered in bulk, using the Image ▷ Overlay ▷ From ROI Manager/To ROI Manager commands. New in IJ 1.46r

**Non-destructive annotations** Overlays are the best way of annotating images in ImageJ (examples). As vector graphics, overlays do not change pixel values, can be scaled without loss of quality even at high zoom levels (*see* XVIII Working with Zoomed Canvases) and can be displayed at different opacity values (*see* XIX Hexadecimal Color Values). RGB snapshots of the image with embedded overlays can be created by holding Shif F, the shortcut for Image ▷ Overlay ▷ Flatten [F]. 'Flattened' images with the overlay rendered as pixel data are also created when saving the image as PNG or JPEG (File ▷ Save As ▷), or when printing the image canvas (File ▷ Print... [p]). The Flatten command is also listed in the ROI Manager.

**Image ROIs** An imageROI (image selection) is a ROI that displays an image as an overlay. As described in Edit ▷ Selection ▷ Image to Selection... and Image ▷ Overlay ▷ Add Image..., this allows multiple images to be blended on a single image canvas.



**3D Viewer (Fiji 1.46o), bringing hardware-accelerated 3D visualization to ImageJ.** As explained in 3D Volumes, most of plugins that truly extend ImageJ functionally to multi-dimentional data are bundled as part of Fiji.

## 12 3D Volumes

Currently, the support for 3D ROIs (selections containing contiguous cluster of voxels) is somewhat limited in ImageJ. This limitation has been addressed by ImageJ2 and several IJ1 plugins. The list below summarizes some of the ImageJ plugins that deal effectively with multi-dimensional objects. Note that a manual installation of these tools as standalone ImageJ plugins is a challenging task given their special dependencies, reason why they are all bundled as part of Fiji.

**3D Filters** Specialized 3D filters such as Process ▷ Filters ▷ Gaussian Blur 3D... can be installed to perform 3D operations. Examples are the 3D processing package by Thomas Boudier [51] and the 3D binary filters by Benjamin Schmid.

**3D Object Counter** 3D Object Counter (3D-OC) counts and qualifies 3D objects in a stack [8], similarly to the 2D analysis performed by Analyze ▷ Analyze Particles... It is complemented by 3D Roi Manager [51], a companion plugin that adds a 3D ROI Manager to ImageJ

**3D Viewer** 3D Viewer brings powerful hardware-accelerated 3D visualization to ImageJ [101], extending the limited functionality of Image ▷ Stacks ▷ 3D Project... In the ImageJ 3D Viewer stacks can be displayed as texture-based volume renderings, surfaces or orthoslices. It is macro-recordable and can be used by other plugins as a high-level programming library for 3D visualization

**Simple Neurite Tracer** Simple Neurite Tracer allows semi-automated segmentation of tubular structures in 3D [71]

**TrakEM2** As mentioned earlier, TrakEM2 features powerful tools for multi-dimensional regions of interest [13]

SEE ALSO: Image ▷ Stacks ▷ 3D Project.../Orthogonal Views [H], Analyze ▷ Surface Plot..., XXI Skeletonize vs Skeletonize 3D, 3D tools in Fiji, Three Pane Crop, 3D image processing tutorials on the ImageJ wikipage





## 13 Settings and Preferences

IMPROVED IN IJ 1.46R

ImageJ preferences are automatically saved in a preferences file, the `IJ_prefs.txt` text file. This file is stored in `~/Library/Preferences/` on Mac OS X, in `~/.imagej/` on Linux and Windows (with ∼ referring to the user's home directory). Several macros and plugins also write parameters to this file. If the `IJ_prefs.txt` is erased using Edit ▷ Options ▷ Reset..., ImageJ will create a new one the next time it is opened resetting all parameters to their default values.

Sometimes, it may be useful to override (or restore) certain settings that may have been changed during a working session. For example, the *Limit to threshold* option (Analyze ▷ Set Measurements...) will affect most measurements performed on thresholded images. Thus, it may be wise to check the status of this parameter before each analysis, specially when working on multiple computers.

**(3) Ensuring Specific Settings at Launch**

```
macro "AutoRun" {
  setOption("DebugMode", true);
  setOption("Bicubic", true);
  setOption("Display Label", true);
  setOption("Limit to Threshold", false);
  setOption("BlackBackground", true);
  run("Colors...", "foreground=white background=black"); //this line ↩
     could be substituted by: setBackgroundColor(0,0,0); ↩
     setForegroundColor(255,255,255);
  run("Profile Plot Options...", "width=350 height=200 draw");
  run("Brightness/Contrast...");
}
```

The `setOption()` macro function can be used to set this and several other ImageJ options. Calling this function from the "AutoRun" macro in the `StartupMacros.txt` file ensures preferences are set each time ImageJ starts. The macro (3) Ensuring Specific Settings at Launch exemplifies this approach ensuring that the following settings are enforced at startup:

1. TIFF tag values are displayed by ImageJ (*Debug Mode* in Edit ▷ Options ▷ Misc...)
2. Bicubic interpolation is preferred over bilinear (e.g., Edit ▷ Selection ▷ Straighten...)
3. The name of the measured image name is recorded in the first column of the Results Table (*Display Label* in Analyze ▷ Set Measurements...)
4. Measurements are not restricted to thresholded pixels (*Limit to Threshold* in Analyze ▷ Set Measurements...)
5. Binary images are processed assuming white objects on a black background (*Black background* in Process ▷ Binary ▷ Options..., *see* XXII Interpreting Binary Images)
6. *Background color* is black and *foreground color* is white (Edit ▷ Options ▷ Colors...)
7. ImageJ plots contain grid lines and are always 350 × 200 pixels in size (Edit ▷ Options ▷ Profile Plot Options...)
8. Open the B&C widget at its last saved screen position (Image ▷ Adjust ▷ Brightness/Contrast... [C])

SEE ALSO: Customizing the ImageJ Interface, FAQs on ImageJ wikipage, VII Organizing Commands in the Menu Bar

# Part III

# Extending ImageJ

ImageJ capabilities can be extended by loadable code modules in the form of macros, scripts or plugins. 300+ macros, 500+ plugins and 20+ scripts are available through the ImageJ web site. Below is a short description of these three type of ImageJ add-ons:

Macros — The easiest way to execute a series of ImageJ commands. The ImageJ macro language – a *Java-like* language – contains a set of control structures, operators and built-in functions and can be used to call built-in commands and other macros. Macro code is stored in text files (.txt and .ijm extensions).

Plugins — Much more powerful, flexible and faster than macros (most of ImageJ's built-in menu commands are actually plugins) but harder to write and debug. Plugins are written in the Java programming language (.java source files) and compiled to .class files.

Scripts — ImageJ uses the Mozilla Rhino interpreter to run JavaScripts. Similarly to plugins, scripts have full access to all ImageJ and Java APIs but do not need to be compiled (scripts and macros run interpretively). On the other hand, scripts lack the simplicity of macro language and feel less integrated in ImageJ.

## 14 Macros

A macro is a simple program that automates a series of ImageJ commands. The easiest way to create a macro is to record a sequence of commands using the command recorder (Plugins ▷ Macros ▷ Record...).

A macro is saved as a text file (.txt or .ijm extension) and once installed executed by selecting the macro name in the Plugins ▷ Macros ▷ submenu, by pressing a key or, in the case of Macro tools, by clicking on an icon in the ImageJ toolbar. In addition, any macro file placed in ImageJ/plugins with an .ijm extension will be installed in the Plugins ▷ menu like any other plugin (before version 1.41 only files with an underscore in the name would be listed).

There are more than 300 example macros, on the ImageJ Web site. To try one, open it in a browser window and drag it directly to the Main ImageJ window or, copy it to the clipboard (Ctrl A, Ctrl C), switch to IJ, and run File ▷ New ▷ System Clipboard [V] (Ctrl Shift V), pasting the macro into a new Editor window. Run it using the editor's Macros ▷ Run Macro command (Ctrl R). Most of the example macros are also available in the macros folder, inside the ImageJ folder.

### Macro Programming

The ImageJ community has created excellent tutorials on macro programming. These resources are indispensable guides to the ImageJ macro language:

1. *The ImageJ Macro Language — Programmer's Reference Guide* by Jérôme Mutterer and Wayne Rasband. This booklet compiles most of the documentation dispersed throughout the web related to ImageJ's macro programming. It provides an up to date printable manual for the ImageJ macro language:
   http://imagej.nih.gov/ij/docs/macro_reference_guide.pdf





2. The Built-in Macro Functions webpage (Help ▷ Macro Functions... and Macros ▷ Function Finder... [F] in the Editor) is the indispensable guide to the built-in functions that can be called from the ImageJ macro language. It is thoroughly documented and constantly updated:
   http://imagej.nih.gov/ij/developer/macro/functions.html
3. Tutorials on the Fiji webpage:
   http://fiji.sc/wiki/index.php/Introduction_into_Macro_Programming
4. How-tos and tutorials on the ImageJ Documentation Portal
   http://imagejdocu.tudor.lu/

Improved in IJ 1.46r

See also: Scripts, Plugins, Editor, Fiji Script Editor

## 15 Scripts

JavaScript scripting was introduced in ImageJ 1.41 in order to bring full access to ImageJ and Java APIs (*see* Advantages and disadvantages of JavaScript). ImageJ uses the Mozilla Rhino interpreter built into Java 1.6 for Linux and Windows to run JavaScript. Mac users, and users of earlier versions of Java, must download JavaScript.jar into the plugins folder. This JAR file is available on the ImageJ website and is included with the Mac version of ImageJ in ImageJ/plugins/jars.

Example JavaScript programs are available at imagej.nih.gov/ij/macros/js/. Thread safe JavaScript code can be generated using the Recorder (Plugins ▷ Macros ▷ Record...). Scripts can be opened in the editor as any other macro. Scripts with the extension .js can be run using Macros ▷ Run Macro otherwise Macros ▷ Evaluate JavaScript (Ctrl J) must be used.

### JavaScript Programming

Resources on ImageJ JavaScript scripting include:

1. The ImageJ web site, with growing documentation:
   http://imagej.nih.gov/ij/developer/javascript.html
2. Tutorials on the Fiji webpage:
   http://fiji.sc/wiki/index.php/Javascript_Scripting
3. Online scripts repository:
   http://imagej.nih.gov/ij/macros/js/

See also: Macros, Plugins, Editor, Fiji Script Editor

## 16 Plugins

Plugins are a much more powerful concept than Macros and Scripts and most of ImageJ's built-in menu commands are in fact implemented as plugins. Quoting Werner Bailer [3]:

**Advantages and disadvantages of JavaScript in ImageJ.** A thorough comparison between different scripting languages is available on the Fiji webpage.

| JavaScript Advantages | JavaScript Disadvantages |
|---|---|
| Full access to ImageJ and Java APIs | Slower, especially starting up |
| Standardized | No equivalent of macro sets |
| Richer language (objects, `?` operator, `break`, `continue`, etc.) | Cannot use most of ImageJ's 360+ built in macro functions |
| Extensive documentation | Requires knowledge of complex ImageJ and Java APIs |
| | No support for "batch mode" |
| | Cannot create Custom Tools and toolbar menus |
| | Not compatible with Function Finder... [F] and CodeBar[1] |
| | No debugger |

[1]CodeBar is a convenient 'ActionBar' that retrieves snippets and common tasks frequently used in macro writing. 'ActionBars' provide one or many easy to use button bar(s) that extend ImageJ's graphical user interface. You can read more about the ActionBar plugin at the ImageJ Documentation Portal.

Plugins are implemented as Java classes, which means that you can use all features of the Java language, access the full ImageJ API and use all standard and third-party Java APIs in a plugin. This opens a wide range of possibilities of what can be done in a plugin.

The most common uses of plugins are filters performing some analysis or processing on an image or image stack and I/O plugins for reading/writing not natively supported formats from/to file or other devices. But as you can see when looking at the plugins listed on the ImageJ plugins page, there are many other things you can do with plugins, such as rendering graphics or creating extensions of the ImageJ graphical user interface.

Plugins in the `ImageJ/plugins/` folder are listed at the bottom of the Plugins ▷ menu (*see* VII Organizing Commands in the Menu Bar). Only `.class` and `.jar` files in the plugins folder with at least one underscore in their name will be installed. Note that, with IJ 1.44d an later, ImageJ no longer automatically installs, at startup, plugins in JAR file directories that start with a lower case letter.

### Developing ImageJ Plugins

More information on how to develop ImageJ plugins can be obtained on the following documents:

1. Developer Resources Page on the ImageJ website (Help ▷ Dev. Resources...): http://imagej.nih.gov/ij/developer/index.html

2. Dedicated tutorials on Fiji's webpage: http://fiji.sc/wiki/index.php/Introduction_into_Developing_Plugins

3. Dedicated tutorials on the ImageJ Documentation Portal: http://imagejdocu.tudor.lu/

4. Dedicated tutorials on the ImageJDev webpage: http://developer.imagej.net/ides

SEE ALSO: Macros, Scripts, Editor, Fiji Script Editor





## 17 Scripting in Other Languages

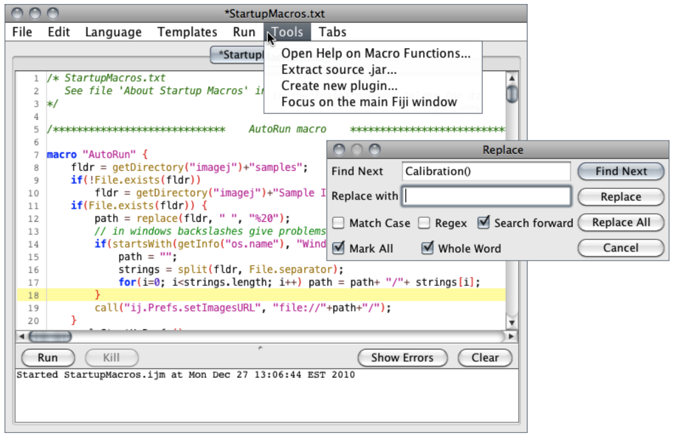
Support for other languages is possible in ImageJ using Fiji and its powerful editor. Fiji adds extra support for BeanShell, Clojure, Python and Ruby. The following documents will introduce you to the advanced scripting capabilities of Fiji:

1. The extensive tutorial on scripting Fiji with Jython by Albert Cardona: http://www.ini.uzh.ch/~acardona/fiji-tutorial/

2. Dedicated tutorials on the Fiji webpage: http://fiji.sc/wiki/index.php/Scripting_comparisons

### Fiji Script Editor

Fiji features a more powerful script editor than ImageJ's built-in Editor. The Fiji editor is an invaluable help when writing scripts in any of Fiji's supported languages, including the ImageJ macro language. The editor features full undo support, syntax highlighting, tabs, bookmarks and several other tools that simplify scripting workflows in ImageJ. For more information visit Fiji's editor website at http://fiji.sc/wiki/index.php/Script_Editor.




**The Fiji Script Editor (ImageJA 1.44m).** The Fiji Editor is an advanced text editor, supporting BeanShell, Jython, JRuby and other scripting languages. It does not support Function Finder... [F] but selecting a built-in macro function and running Tools ▷ Open Help on Macro Functions... retrieves the documentation for the selected function.

SEE ALSO: Scripting in Other Languages, Running ImageJ From the Command Line, IJ_ED, a plugin by Jérôme Mutterer that binds jEdit to ImageJ

## 18 Running ImageJ from the Command Line

ImageJ was devised as a desktop application. It can, however, run without a graphics environment (headless mode) by adding a special library (`headless.jar`) to the `ij.jar` classpath that overrides key ImageJ classes to work better headlessly. As described on the Fiji website, this strategy is implemented in Fiji through the **--headless** command line flag (*see also* Running ImageJ in headless mode and Using Cluster for Image Processing with IJ). Headless operations are simplified in ImageJ2.

ImageJ recognizes the following command line options:

**"file-name"** Opens a file. Examples:
`blobs.tif`
`/Users/wayne/images/blobs.tif`
`e81*.tif`

**-ijpath path** Specifies the path to the directory containing the plugins directory. Example:
`-ijpath /Applications/ImageJ`

**-port** Specifies the port ImageJ uses to determine if another instance is running. Examples:
`-port1` (use default port address + 1)
`-port2` (use default port address + 2)
`-port0` (do not check for another instance (OtherInstance)

**-macro path [arg]** Runs a macro or script, passing it an optional argument, which can be retrieved using `getArgument()`. Examples:
`-macro analyze.ijm`
`-macro analyze /Users/wayne/images/stack1`

**-batch path [arg]** Runs a macro or script in batch mode (no GUI), passing it an optional argument. ImageJ exits when the macro finishes.

**-eval "macro code"** Evaluates macro code. Examples:
`-eval "print('Hello, world');"`
`-eval "return getVersion();"`

**-run command** Runs an ImageJ menu command. Example:
`-run "About ImageJ..."`

**-debug** Runs ImageJ in debug mode.

SEE ALSO: Linux installation, ImageJ Documentation Portal: Command line





Part IV

# ImageJ User Interface

Unlike most image processing programs ImageJ does not have a main work area. ImageJ's main window is actually quite parsimonious containing only a menu bar (at the top of the screen on the Mac) containing all the Menu Commands, a Toolbar, a Status bar and a Progress bar. Images, histograms, profiles, widgets, etc. are displayed in additional windows. Measurement results are displayed in the Results Table. Most windows can be dragged around the screen and resized.

**The ImageJ window (version 1.46j).**


1 Rectangular Selection Tool and Rounded Rectangular Selection Tool
2 Oval Selection Tool, Elliptical Selection Tool and Brush Selection Tool
3 Polygon Selection Tool
4 Freehand Selection Tool
5 Straight Line Selection Tool, Segmented Line Selection Tool, Freehand Line Selection Tool and Arrow Tool
6 Angle Tool
7 Point Tool and Multi-point Tool
8 Wand Tool
9 Text Tool
10 Magnifying Glass
11 Scrolling Tool
12 Color Picker Tool
13 *More Tools* Menu
A–H Customized tools installed from `StartupMacros.txt`, `macros/toolsets/`, `macros/tools/` or `plugins/Tools/`

## Toolbar

The ImageJ toolbar contains tools for making selections, drawings, zooming and scrolling, etc. In addition, the right-side of the toolbar contains seven slots that can host any of the 60+ tools and 15+ toolsets available on the ImageJ website (*see* Custom Tools).

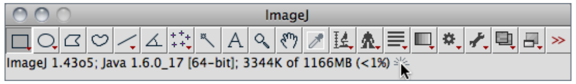
All ImageJ tools share common features:

- The ▾ on the bottom right corner of some icons in the toolbar depicts a contextual menu that can be accessed by right-clicking on the tool icon (e.g., Stacks Menu).
- If an 'Options' dialog is available for a particular tool, it can be accessed by double clicking on the tool icon (e.g., Wand Tool).

## Status bar

When the cursor is over an image, pixel intensities and coordinates are displayed in the status bar. After running a filter, elapsed time and processing rate (in pixels / second) are also displayed. When clicking on the status bar the ImageJ version, the Java version, memory in use, memory available and percent memory used will be displayed. As Selections are created or resized, selection properties (e.g., location, width, etc.) are displayed on the status bar.

In addition, clicking on ImageJ's status bar, forces the Java garbage collector to run, which may help to reclaim unused memory (*see* Edit ▷ Options ▷ Memory & Threads. . . ). You can assess this by running Plugins ▷ Utilities ▷ Monitor Memory. . . : each click on the Status bar should lead to a spike in the ImageJ's memory utilization.




SEE ALSO: Plugins ▷ Utilities ▷ ImageJ Properties. . . , Help ▷ About ImageJ. . .

### V Toggling Calibrated Units

If a spatial scale has been defined in Image ▷ Properties. . . [P] or Analyze ▷ Set Scale. . . , selection properties are displayed in the Status bar in calibrated units. Resizing or moving while holding down Alt forces this information to be displayed in pixels.

### Progress bar

The progress bar, located to the right of the status bar, shows the progress of time-consuming operations. It will not appear if the operation requires less then approximately one second.

# 19 Tools

## 19.1 Area Selection Tools

These tools share the first four toolbar slots. As described in Toolbar, use the right click drop-down menu to switch a different tool. Selection Color can be changed by double clicking on the Point Tool/Multi-point Tool.

### 19.1.1 Rectangular Selection Tool

Location, width, height, and aspect ratio are displayed in the status bar during drawing (*see* V Toggling Calibrated Units).

Modifier keys:

- Shift — Selection is constrained to a square
- Alt — Current aspect ratio is maintained while resizing
  With arrow keys, width and height are changed one pixel at a time
- Ctrl — Selection is resized around the center

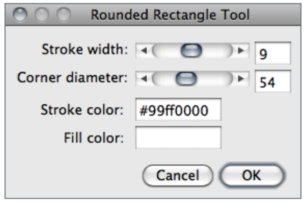
SEE ALSO: Rounded Rectangular Selection Tool, Specify. . . , XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts





### 19.1.2 Rounded Rectangular Selection Tool

Improved in IJ 1.46r




This tool creates rectangular shapes with rounded corners. It shares the same toolbar slot and the same modifier keys with the Rectangular Selection Tool. Double clicking on its icon opens the depicted dialog in which is possible to specify:

***Stroke width*** The width of the contour.

***Corner diameter*** The arc size at the vertices.

***Stroke/Fill Color*** The contour (stroke) color or the filling color of the rounded rectangle. As explained in Edit ▷ Selection ▷ Properties. . . [y], selections can be either filled or contoured, but not both. The nine default selection colors (*black*, *blue*, *cyan*, *green*, *magenta*, *orange*, *red*, *white*, *yellow*) can be typed as text. Any other color must be typed in hex notation (*see* XIX Hexadecimal Color Values).

SEE ALSO: Rectangular Selection Tool, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

### 19.1.3 Oval Selection Tool

Location, width, height, and aspect ratio are displayed in the status bar during drawing (*see* V Toggling Calibrated Units).

Modifier keys:

- Shift — Selection becomes circular
- Alt — Current aspect ratio is maintained while resizing
  With arrow keys, width and height are changed one pixel at a time
- Ctrl — Selection is resized around the center

SEE ALSO: Elliptical Selection Tool, Specify. . . , V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

### 19.1.4 Elliptical Selection Tool

Improved in IJ 1.46r

Ellipse properties are adjusted by dragging the four handlers on its antipodal points [4]. To rotate or resize, drag the handlers on its major axis (transverse diameter). To adjust eccentricity, drag the handlers on its minor axis (conjugate diameter).

SEE ALSO: Oval Selection Tool, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

IMPROVED IN IJ 1.46R

#### 19.1.5 Brush Selection Tool

Adjusts (refines) the shape of area selections using a circular 'brush' [5]. Clicking inside the area selection and dragging along its boundary will expand the boundary outwards. Clicking outside the area selection and dragging along its boundary will shrink the boundary inwards. Once the tool has been applied, ImageJ will treat the adjusted ROIs as Composite Selections. The brush diameter can be adjusted by double clicking on the tool icon.

Modifier keys:

- Shift — Holding Shift forces the Brush Selection Tool to add pixels to the selection
- Alt — Holding Alt forces the Brush Selection Tool to subtract pixels from the selection

SEE ALSO: XIII Converting Composite Selections, Toolbar Shortcuts

#### 19.1.6 Polygon Selection Tool

Creates irregularly shaped selections defined by a series of line segments. Segment length and angle are displayed in the status bar during drawing (*see* V Toggling Calibrated Units). To create a polygon selection, click repeatedly with the mouse to create line segments. When finished, click in the small box at the starting point (or double click), and ImageJ will automatically draw the last segment. The vertex points that define a polygon selection can be moved and modifier keys can be used to delete or add new vertexes to the polygon.

Modifier keys:

- Shift — Shift-clicking on an existing vertex of the polygon adds a new corner point, smoothing the polygon edge
- Alt — Alt-clicking on an existing vertex of the polygon removes it

SEE ALSO: Segmented Line Selection Tool, Enlarge..., V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

#### 19.1.7 Freehand Selection Tool

As with the polygon selection tool, ImageJ automatically draws the last segment. Location and intensity of starting pixel are displayed in the status bar during drawing.

SEE ALSO: Freehand Line Selection Tool, Polygon Selection Tool, Enlarge..., V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

### 19.2 Line Selection Tools

Use these tools to create line selections. The three line selection tools share the same toolbar slot. As described in Toolbar, use the right click drop-down menu to switch between line tools.

Double click on any line tool to specify the line width by opening the Image ▷ Adjust ▷ Line Width... widget, on which is also possible to apply a cubic spline fit to a polyline selection. Check the *Sub-pixel resolution* checkbox in Edit ▷ Options ▷ Profile Plot Options... to create line selections with floating-point coordinates (*see* Selections With Sub-pixel Coordinates).





#### 19.2.1 Straight Line Selection Tool

Length and line angle are displayed in the status bar during drawing (*see* V Toggling Calibrated Units).

Modifier keys:

- Shift — Forces the line to be either horizontal or vertical
- Alt — Keeps the line length fixed while moving either end of the line
  Forces the two points that define the line to have integer coordinates when creating a line on a zoomed image
- Ctrl — While moving either end of the line, the line is rotated/resized about its center

SEE ALSO: Calibration Bar..., iSet Scale..., V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

IMPROVED IN IJ 1.46R

#### 19.2.2 Segmented Line Selection Tool

Works exactly as described for the Polygon Selection Tool: Create a segmented line selection by repeatedly clicking with the mouse. Each click will define a new line segment. Double click when finished, or click in the small box at the starting point. The points that define a segmented line selection can be moved or deleted, and new points can be added. Length and line angle are displayed in the status bar during drawing (*see* Toggling Calibrated Units).

Modifier keys:

- Shift — Shift-clicking on an existing vertex adds a new one, adding a new segment to the segmented line
- Alt — Alt-clicking on an existing vertex of the segmented line removes it

SEE ALSO: Polygon Selection Tool, Freehand Selection Tool, V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

IMPROVED IN IJ 1.46R

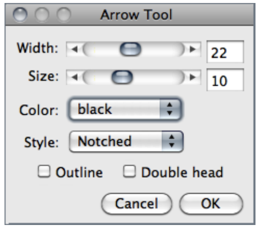
#### 19.2.3 Freehand Line Selection Tool

Select this tool and drag with the mouse to create a freehand line selection.

SEE ALSO: Freehand Selection Tool, Overlay Brush, V Toggling Calibrated Units, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

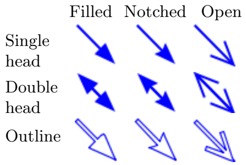
### 19.3 Arrow Tool

This tool shares the same toolbar slot with the Line Selection Tools and can also be installed on a dedicated toolbar slot using the *More Tools* Menu menu (*see* Arrow). Double clicking on the tool icon opens its *Options* prompt [6].




Being an annotation tool, arrows are created using foreground color (*see* Color Picker. . . [K]) and not selection color (*see* Point Tool).

*Width* and *Size* (in pixels) can be adjusted by dragging the respective sliders or by direct input. Apart from the arrow styles listed here, a *Headless* option is also available. As for painting tools (Brush, Flood Filler and Pencil), the *Color* dropdown menu provides a convenient way to reset the foreground color to one of the default options.




As with any other selection, add arrows to the non-destructive overlay by pressing B (Image ▷ Overlay ▷ Add Selection. . . [b]) or D (Edit ▷ Draw [d]) to permanently draw the arrow on the image (*see* XVII Embedding Color Annotations in Grayscale Images when working with non-RGB images).

The same modifier keys described to the Straight Line Selection Tool apply to the arrow tool:

| | |
|---|---|
| Shift | Forces the line to be either horizontal or vertical |
| Alt | Keeps the line length fixed while moving either end of the line<br>Forces the two points that define the line to have integer coordinates when creating a line on a zoomed image |
| Ctrl | While moving either end of the line, the line is rotated/resized about its center |

SEE ALSO: Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Brush, Overlay Brush, Pencil, Text Tool, Toolbar Shortcuts

## 19.4 Angle Tool

This tool allows you to measure an angle defined by three points. Double click on the angle tool icon to enable the measurement of reflex angles. The angle is displayed in the status bar while the selection is being created or adjusted. Press M (Analyze ▷ Measure. . . [m]) to record the angle in the Results Table.

SEE ALSO: Toolbar Shortcuts

IMPROVED IN IJ 1.46R

## 19.5 Point Tool

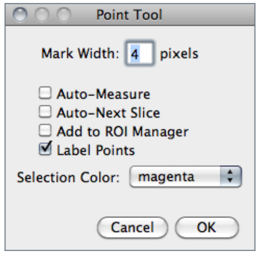
Use this tool to create a point selection, to count objects or to record pixel coordinates.

Modifier keys:

| | |
|---|---|
| Shift | Shift-clicking adds more points, creating a multi-point selection (*see* Multi-point Tool). Point count is displayed on the Status bar |
| Alt | Alt-clicking on a point deletes it. Alt-clicking and dragging with the Rectangular Selection Tool or Oval Selection Tool deletes multiple points |








Double clicking on the point tool icon (or running Edit ▷ Options ▷ Point Tool. . . ) displays its configuration dialog box.

***Mark Width*** If greater than zero, a mark of the specified diameter will be permanently drawn in the current foreground color (cf. Color Picker. . . [K]). Note that marks modify the image (it may be wise to work with a copy) and color marks are only available with RGB images (*see* XVII Embedding Color Annotations in Grayscale Images).

***Auto-Measure*** If checked, clicking on the image records the pixel location and intensity. Note that if *Mark Width* is not zero, every time a point selection is measured a mark will be painted (cf. Measure. . . [m]). If unchecked, Edit ▷ Draw [d] can be used to paint the mark (*Mark Width* diameter) at the location of each point.

***Auto-Next Slice*** If checked, ImageJ will automatically advance to the next stack slice. Note that this feature will only allow one point per slice.

***Add to ROI Manager*** If checked, points will be automatically added to the ROI Manager. . .

***Label Points*** If checked, each point selection will be displayed with an accompanying numeric label. IMPROVED IN IJ 1.46R

***Selection Color*** Specifies Selections color, chosen from one of the nine default colors: *red*, *green*, *blue*, *magenta*, *cyan*, *yellow*, *orange*, *black* and *white*. The chosen color is highlighted in the center of the Point/MultiPoint Tool. It can also be specified using Edit ▷ Options ▷ Colors. . .

SEE ALSO: Multi-point Tool, Using a Keyboard Shortcut to Change Selection Color, Cell Counter plugin, Toolbar Shortcuts

## 19.6 Multi-point Tool

IMPROVED IN IJ 1.46R

The Multi-point Tool selects multiple points behaving as the Point Tool when Shift is pressed, *Label Points* is checked and *Auto-Measure* and *Auto-Next Slice* are deselected. As described for the Point Tool, Alt can also be used to remove points. Similarly, when using Edit ▷ Draw [d] marks are painted with the diameter of *Mark Width*.

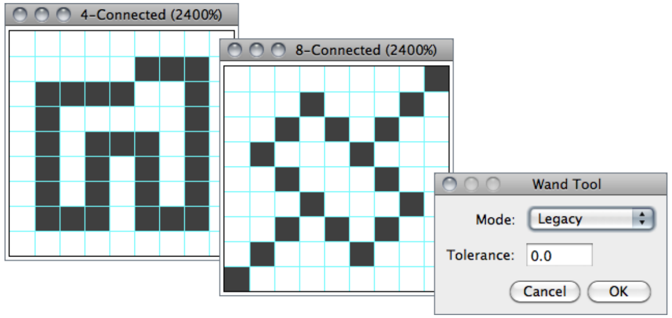
SEE ALSO: Point Tool, Cell Counter plugin, Toolbar Shortcuts

## 19.7 Wand Tool

Creates a selection by tracing objects of uniform color or thresholded objects. To trace an object, either click inside near the right edge, or outside to the left of the object. To automatically outline and measure objects have a look, e.g., at the WandAutoMeasureTool macro.

To visualize what happens, imagine a turtle that starts moving to the right from where you click looking for an edge. Once it finds the edge, it follows it until it returns to the starting point. Note that the wand tool may not reliably trace some objects, especially one pixel wide lines, unless they are thresholded (highlighted in red) using Image ▷ Adjust ▷ Threshold. . . [T].

Double clicking on the wand tool icon (or running Edit ▷ Options ▷ Wand Tool. . . ) opens the configuration dialog box in which three modes (4–connected, 8–connected or 'Legacy') plus a tolerance value can be set [7].




**The** Wand Tool**.** 4/8–connected particles can be traced within an intensity range.

***Tolerance*** The wand takes the pixel value where you click as an initial value. It then selects a contiguous area under the condition that all pixel values in that area must be in the range *initial value − tolerance* to *initial value + tolerance*.

***4–connected*** Only the four neighbors of a pixel are considered neighbors. E.g., the wand does not follow a one-pixel wide diagonal line because the pixels of that line are not four-connected.

***8–connected*** Each pixel is considered to have eight neighbors. So the wand follows a diagonal line if you click onto it. On the other hand, if you have an area of constant value dissected by a one-pixel wide diagonal line, the 8–connected wand will 'jump over the line' and include the other part of that area.

***Legacy*** In this mode no neighbor is checked and no tolerance is used. This is the default mode of the Wand Tool in ImageJ 1.42 and earlier.

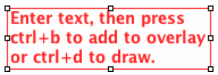
Modifier keys:

Shift Shift-clicking appends the traced area to previously traced selections

Alt Alt-clicking removes the traced area from previously traced selections

SEE ALSO: Analyze ▷ Analyze Particles. . . , Flood Filler, Versatile Wand plugin, Composite Selections, Toolbar Shortcuts

## 19.8 Text Tool




Use this tool to add text to images. It creates text ROIs, rectangular selections containing one or more lines of text. Note the following when using the Text Tool:

– Font style and text alignment is specified in the *Fonts* widget, activated by double clicking on A or by running Edit ▷ Options ▷ Fonts. . . Text is drawn in foreground color (*see* Color Picker. . . [K])

– Use the keyboard to add characters to the text and the backspace key to delete characters. Use Alt to type special unit symbols such as µ (Alt M) or Å (Alt Shift A). Note that menu shortcuts require holding down Ctrl while using the Text Tool (*see* Using Keyboard Shortcuts)





– Use Ctrl Y (Edit ▷ Selection ▷ Properties. . . [y]) to re-adjust font color and size, text justification and to specify a background color for the text selection. XIX Hexadecimal Color Values provides instructions on how to define semi-transparent colored backgrounds (*see also* DrawTextWithBackground macro)

– Use Ctrl B (Image ▷ Overlay ▷ Add Selection. . . [b]) to create non-destructive text annotations (*see* Overlays; OverlayDrawStringDemo, TextOverlay macros). Alternatively, use Ctrl D (Edit ▷ Draw [d]) to permanently draw the text on the image. In the latter case, the background of the text selection is not drawn (*see also* XVII Embedding Color Annotations in Grayscale Images)

SEE ALSO: Arrow Tool, Brush, Overlay Brush, Pencil, TextDemo macro, Toolbar Shortcuts

## 19.9 Magnifying Glass

Magnifies and reduces the view of the active image. Activate the tool and click on the image to zoom in. Right-click (or Alt-click) to zoom out. The current magnification is shown in the image's title bar. Double click on the magnifying glass icon to revert to the image's original magnification. As explained in Image ▷ Zoom ▷ In [+], there are 21 possible magnification levels: 3.1, 4.2, 6.3, 8.3, 12.5, 16.7, 25, 33.3, 50, 75, 100, 150, 200, 300, 400, 600, 800, 1200, 1600, 2400 and 3200%.

Modifier keys:

Shift Clicking and dragging while holding down the Shift key runs Image ▷ Zoom ▷ To Selection

Alt Image zooms out (right-click behavior)

SEE ALSO: XVIII Working with Zoomed Canvases, Zoom ▷ commands, Toolbar Shortcuts

## 19.10 Scrolling Tool

Allows you to scroll through an image that is larger than its window. You can temporarily activate this tool (except when using the Text Tool) by holding down the space bar.

SEE ALSO: XVIII Working with Zoomed Canvases, Toolbar Shortcuts

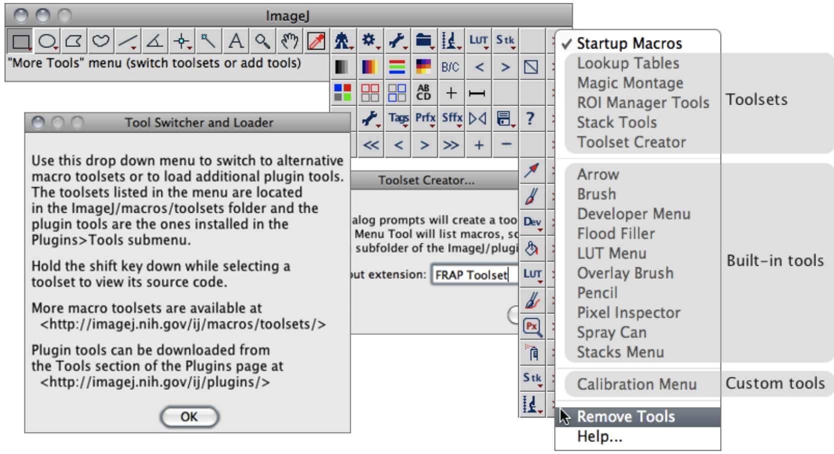
## 19.11 Color Picker

Sets the foreground drawing color by 'picking up' colors from any open image. Colors can also be picked up from the Color Picker (CP) window (Image ▷ Colors ▷ Color Picker. . . [K]) using any tool. In the icon, the 'eye dropper' is drawn in the current foreground color while the frame around it is drawn in the current background color. Edit ▷ Draw [d] and Edit ▷ Fill [f] use the foreground color. Edit ▷ Clear, Clear Outside and Cut [x] use the background color. Double clicking on the tool icon will display the Color Picker window.

Modifier key:

Alt Alt-clicking with the Color Picker Tool on the image canvas 'picks-up' background color

SEE ALSO: Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts, Temporary Activation of a Tool




*More Tools* Menu **(IJ 1.46n).** The menu lists tools from StartupMacros.txt in ImageJ/macros/, Toolsets installed in ImageJ/macros/toolsets/, built-in tools loaded from ij.jar (Arrow, Brush, Developer Menu, Flood Filler, LUT Menu, Overlay Brush, Pencil, Spray Can and Stacks Menu) and Single Tools installed in ImageJ/plugins/Tools/. While toolsets replace all the eight slots in the toolbar, single tools are installed in the first available slot, or in the last slot if no free slots are available.

Improved in IJ 1.46r

Changed in IJ 1.46r

## 19.12 *More Tools* Menu

The eight Toolbar slots between the Color Picker Tool and the *More Tools* Menu can be customized using this drop-down menu (named *Toolset Switcher* in previous IJ versions). Tool configurations are stored in the ImageJ preferences file (*see* Settings and Preferences) and retrieved across restarts.

The *More Tools* list is populated by `StartupMacros.txt` in `ImageJ/macros/`, Toolsets installed in `ImageJ/macros/toolsets/`, built-in tools loaded from `ij.jar` (Arrow, Brush, Developer Menu, Flood Filler, LUT Menu, Overlay Brush, Pencil, Spray Can and Stacks Menu) and Single Tools installed in `ImageJ/plugins/Tools/`.

At startup, the default set of tools is typically loaded from `StartupMacros.txt`. Later on, tools can be appended or replaced. Single Tools are installed in the first available slot, or in the last slot if no free slots are available. Toolsets replace all the eight slots in the toolbar. Choose *Remove Tools* to reset the toolbar.

The icons for drawing tools installed from this menu reflect the foreground color (*see* Color Picker... [K]) and are updated when the foreground color changes.

Modifier key:

| | |
|---|---|
| Shift | Shift-clicking on the menu icon will open the selected macro (`.txt` and `.ijm` files) |

SEE ALSO: Custom Tools, Toolbar Shortcuts





## 19.13 Arrow

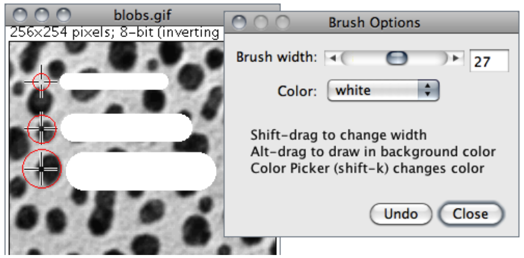
New in IJ 1.46r

Installs a copy of the Arrow Tool on the first available toolbar slot (or the last if no free slots are available), so that it can be accessed without the need of selecting it on the Line Selection Tools dropdown menu. Refer to the original Arrow Tool for details and modifier keys.

SEE ALSO: Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Brush, Overlay Brush, Pencil, Text Tool, Toolbar Shortcuts

## 19.14 Brush

New in IJ 1.46r




A freehand paintbrush tool that draws invasively (as opposed to the Overlay Brush that draws on a non-destructive image overlay (*see* Overlays and Image ▷ Overlay ▷ commands).

Double clicking on the tool icon opens its *Options* dialog box in which is possible to specify the *Brush width* (in pixels) and *Color*.

Being an annotation tool, the paintbrush paints in foreground color as reflected its icon (*see* XVII Embedding Color Annotations in Grayscale Images when working with non-RGB images). The *Color* dropdown menu provides a convenient way to reset the foreground color to one of the default options, bypassing the need of opening the Color Picker window, evoked using Ctrl K . As previously described (*see* Undo and Redo), undo is restricted to last drawing step. The Brush and Pencil tools are in all similar, differing only on brush (stroke) size.

Modifier keys:

| | |
|---|---|
| Shift | Shift-dragging on the canvas will adjust the brush size |
| Alt | Holding Alt makes the brush paint in background color |

SEE ALSO: Overlay Brush, Pencil, Freehand Line Selection Tool, Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

## 19.15 Developer Menu

New in IJ 1.46r

A drop-down menu collecting several online resources and commands that are useful when writing Macros, Plugins or troubleshooting ImageJ operations.

*Debug mode* activates ImageJ's debugging mode (Edit ▷ Options ▷ Misc...).

SEE ALSO: Extending ImageJ, Editor, Help ▷, Plugins ▷ Macros ▷ /Utilities ▷ /New ▷, Common Commands Menu Tool, Stacks Menu, LUT Menu

NEW IN IJ 1.46R

## 19.16 Flood Filler

A *paint bucket* tool that fills with the current foreground color adjacent pixels that have the same value as the clicked pixel. Double click on the tool icon to specify the flood type in terms of pixel connectivity: *4–connected* or *8–connected*.

To spread the fill to contiguous pixels within an intensity range, use the Wand Tool instead: Double click on the Wand Tool icon to set a *Tolerance* value, then press F (Edit ▷ Fill [f]) to fill with foreground color (highlighted in the Flood Filler icon) or Backspace/Del (Edit ▷ Clear) to fill with background color (*see* Color Picker... [K]).

Modifier keys:

Alt Alt-clicking makes the brush paint in background color

SEE ALSO: `floodFill(x,y)` macro function, Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

NEW IN IJ 1.46R

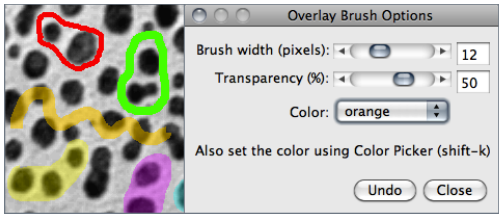
## 19.17 LUT Menu

A drop-down menu listing all the Image ▷ Lookup Tables ▷ commands. It is a convenient way to deal with a large collection of lookup tables that otherwise would only be accessed through the menu bar. Note that although it is not possible to organize LUTs into subfolders, it is possible to rename the most frequently used lookup tables with a numeric prefix (e.g, `01-glasbey.lut`, `02-Termal.lut`, etc.) so that they are listed earlier in the menu.

SEE ALSO: Pseudocolor Images, `Show_All_LUTs` (a macro that creates a graphical palette of all the installed lookup tables), Stacks Menu, Common Commands Menu Tool, Developer Menu

NEW IN IJ 1.46R

## 19.18 Overlay Brush




A freehand paintbrush that draws on a non-destructive image overlay (*see* Overlays), as opposed to the Brush tool that draws invasively over the canvas.

Double clicking on the tool icon opens its *Options* dialog box in which is possible to specify the *Brush width* (in pixels), *Transparency (%)* and *Color*.

As previously described (*see* Brush and Pencil tools), the *Color* dropdown menu changes the foreground color, bypassing the Color Picker window (activated by Ctrl K). Press *Undo* to remove the last painted stroke from the overlay. Overlay manipulations are described in Image ▷ Overlay ▷.

SEE ALSO: Freehand Line Selection Tool, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts





NEW IN IJ 1.46R

## 19.19 Pencil

A freehand painting tool that draws invasively in foreground color. It is in all similar to the Brush tool but it is typically used with thinner strokes. Double clicking on the tool icon opens its *Options* dialog box in which is possible to specify the *Pencil width* (in pixels) and *Color*. Refer to the Brush tool tools for details.

Modifier keys:

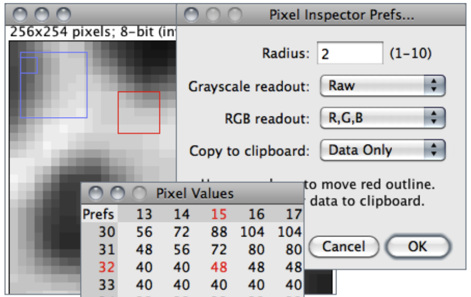
Shift Shift-dragging on the canvas will adjust the brush size

Alt Holding Alt makes the brush paint in background color (*see* Color Picker... [K])

SEE ALSO: Freehand Line Selection Tool, Overlay Brush, Color Picker window, XVII Embedding Color Annotations in Grayscale Images, Toolbar Shortcuts

NEW IN IJ 1.46R

## 19.20 Pixel Inspector




The Pixel inspector displays the values of a square neighborhood around the current cursor position as a table [9]. Values are updated in real time as the mouse is dragged over the image. It is useful to examine how a filter changes the pixel data. E.g., load Pixel Inspector, move the cursor over an image and run Process ▷ Filters ▷ Gaussian Blur...: When toggling the *Preview* checkbox you will be able to monitor in real time how different *Sigma* radius change pixel values.

In the *Pixel Values* table, columns and row headers (x & y positions) are expressed in pixel coordinates. The y-axis direction is determined by the *Invert Y coordinates* value in Analyze ▷ Set Measurements... The center position (current cursor) is printed in red ($x$, $y$, *value*). When the table is in the foreground, the arrow keys can be used to nudge the neighborhood square (outlined in red) and the table can be copied into the clipboard by pressing C. For settings, press the *Prefs* button at the top left of the table:

***Radius*** Specifies the size of the table, $3 \times 3$ for $radius = 1$; $5 \times 5$ for $radius = 2$, etc.

***Grayscale readout*** The numeric output for 8 and 16–bit grayscale images. Can be *Raw* [the default], *Calibrated* [*see* Analyze ▷ Calibrate...] or Hexadecimal (*Hex*).

***RGB readout*** The numeric output for RGB images. Can be *R,G,B* triplets, *Gray Value* or Hexadecimal (*Hex*) [*see* XIX Hexadecimal Color Values]. The mean grayscale value is determined by the weighting factors specified in Edit ▷ Options ▷ Conversions...

***Copy to clipboard*** Specifies which data is copied to the clipboard. Choose *Data only* to copy the table without headers, *x,y and Data* to copy the current position (x,y) values followed by remaining data or *Header and Data* to copy the table with headers. Tables are copied as tab-delimited values.

SEE ALSO: Text Images, Image ▷ Transform ▷ Image to Results/Results to Image, File ▷ Save As ▷ Text Image..., Import ▷ Text Image..., Toolbar Shortcuts

New in IJ 1.46r

### 19.21 Spray Can

The Spray Can (*Airbrush* tool) draws random pixels in the current foreground color (*paint*) (*see* Color Picker... [K] and XVII Embedding Color Annotations in Grayscale Images). It behaves as a traditional airbrush or spray paint: Holding the main mouse button (without moving the cursor) will build up *paint*, as if pressing the nozzle of an aerosol paint can. *Spray width*, *Dot size* and *Flow rate* can be specified by double clicking on the tool icon.

This tool is useful to generate random spot noise. Use it to, e.g., assess the effectiveness of median filtering: Load the Spray Can tool, apply it over an image and toggle the *Preview* option in the Process ▷ Filters ▷ Median... prompt.

SEE ALSO: Process ▷ Noise ▷ Add Noise, Salt and Pepper, Toolbar Shortcuts

New in IJ 1.46r

### 19.22 Stacks Menu

A drop-down menu collecting several commands related to Stacks and Hyperstacks, otherwise accessed through the hierarchy of Image ▷ Stacks ▷, Image ▷ Hyperstacks ▷ and File ▷ Open Samples ▷ submenus. The list makes a particular emphasis on commands that have no keyboard shortcuts assigned.

SEE ALSO: Plugins ▷ Shortcuts ▷, LUT Menu, Stacks Menu, Common Commands Menu Tool, Developer Menu

## 20 Custom Tools

Customized tools are add-ons (macros and plugins) that allow custom interactions with the ImageJ toolbar and/or the image canvas. They are installed on the right side of the Toolbar between the Color Picker Tool and the *More Tools* Menu. At startup, the default set of tools is loaded from `ImageJ/macros/StartupMacros.txt`. Later on, tools can be appended or replaced using the *More Tools* Menu menu. As mentioned, custom tool configurations are saved in the preferences file, and thus remembered across restarts (*see* Settings and Preferences).

It is worth it to mention some differences between the installation of single tools and toolsets:

**Single Tools** Single tools are appended to the first available toolbar slot or installed in the last slot if no free slots are available. Tools can be macros (`.txt` and `.ijm` files) or plugins (`.class` and `.jar` files) and are listed on the *More Tools* Menu menu if placed in the `ImageJ/plugins/Tools/` directory. In addition to the macro tools distributed with ImageJ and saved in `ImageJ/macros/tools/`, a vast repertoire of tools is available on the ImageJ website.

New in IJ 1.46r

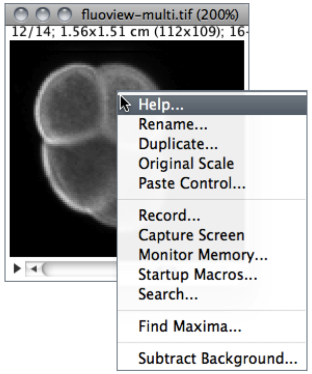
**Toolsets** Toolsets are macro files (`.txt` and `.ijm` files) containing up to eight macro tools, along with any number of ordinary macros. Toolsets are listed on the *More Tools* Menu menu if installed in the `ImageJ/macros/toolsets/` directory. Choosing a toolset (e.g., *Lookup Tables*) replaces all previously installed tools.
As mentioned, `ImageJ/macros/StartupMacros.txt` contains the tools loaded at startup. This file can be customized using Plugins ▷ Macros ▷ Startup Macros... or by holding Shift when choosing *Startup Macros* from the *More Tools* Menu menu.
ImageJ feature several pre-installed toolsets [8] and many others are available on the ImageJ website. Toolsets can also be created by choosing *Toolset Creator*, a convenient way to create groups of Menu Tools listing Plugins ▷ commands.





SEE ALSO: *More Tools* Menu, Tools documentation, Plugins ▷ New ▷ Macro Tool, Plugin Tool

## 21 Contextual Menu




As mentioned earlier macros and macro tools in the `StartupMacros.txt` are automatically installed in the Plugins ▷ Macros ▷ submenu and in the toolbar when ImageJ starts up.

In addition, the `StartupMacros.txt` file also installs the contextual (popup) menu displayed when right-clicking on an image. Other macros and toolsets (e.g., Magic Montage) may also replace the default menu with specialized ones. In this case, re-installing the StartupMacros (using the *More Tools* Menu) will revert the contextual menu to its default.

*The ImageJ Macro Language — Programmer's Reference Guide* explains how this menu can be customized:

> The menu that is displayed when a user right-clicks (or ctrl-clicks) on an image window can be customized through installation of the `"Popup Menu"` macro. Any menu has a name and a list of menu items. The `newMenu(name, items)` macro function allows the creation of a new menu. This menu passes the chosen item as a simple string to the `"Popup Menu"` macro. From this point you can decide what to do, according to what item was chosen.

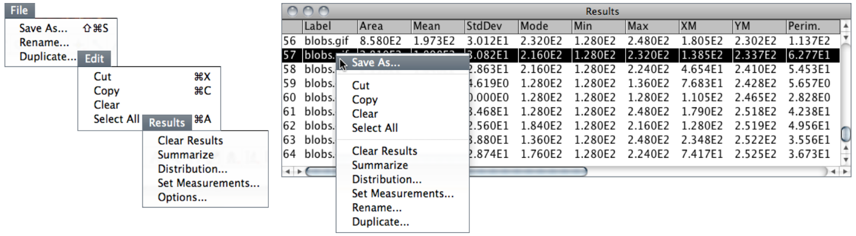
**(4) Customizing the Image Popup Menu**

```
/* The "Popup Menu" macro defines the menu that is displayed when right ↩
   clicking (or ctrl-clicking) on an image. It is part of the startup ↩
   macros (StartupMacros.txt) and several other macro toolsets
*/
var pmCmds= newMenu("Popup Menu", newArray("Help...", "Rename...", ↩
   "Duplicate...", "Original Scale", "Paste Control...", "-", ↩
   "Record...", "Capture Screen ", "Monitor Memory...", "Startup ↩
   Macros...", "Search...", "-", "Find Maxima..."));

macro "Popup Menu" {
 cmd= getArgument();
 if (cmd=="Help...")
    showMessage("About Popup Menu",
           "To customize this menu, edit the line that starts with\n"+
           "\"var pmCmds\" in ImageJ/macros/StartupMacros.txt.");
 else
    run(cmd);
}
```

So, e.g., to add the ability to run the Process ▷ Subtract Background... command from the contextual menu one can simply add that command to the list of items defining the `PopUp Menu`. Note that "-" defines menu separators:

```
var pmCmds= newMenu("Popup Menu", newArray("Help...", "Rename...", ↩
   "Duplicate...", "Original Scale", "Paste Control...", "-", ↩
   "Record...", "Capture Screen ", "Monitor Memory...", "Startup ↩
   Macros...", "Search...", "-", "Find Maxima...", "-", "Subtract ↩
   Background..."));
```




**ImageJ Results table (version 1.44k).** Columns width can be adjusted by clicking on and dragging the vertical lines that separate the column headings. Selected rows can be deleted by pressing the backspace key. The arrow keys can be used to vertically scroll the window.

## 22 Results Table

Most of ImageJ analyses are printed to the Results table. Table commands are organized in four menus: File ▷, Edit ▷, Font ▷ and Results ▷. A contextual menu listing the majority of these commands can be accessed by right-clicking anywhere in the Results window.

**File ▷ Save As...** Exports the measurements as a tab-delimited or comma-delimited text file as defined in Results ▷ Options...

**File ▷ Rename...** Renames the table. Because ImageJ outputs measurements exclusively to the *Results* table, renaming the table will freeze its contents.

**File ▷ Duplicate...** Creates a new table containing a copy of the data. Note that ImageJ will not output measurements to duplicated tables.

**Font ▷** This menu contains commands to adjust font size.

**Results ▷ Clear Results...** Alias for the Analyze ▷ Clear Results command.

**Results ▷ Summarize** Alias for the Analyze ▷ Summarize command.

**Results ▷ Distribution...** Alias for the Analyze ▷ Distribution... command.

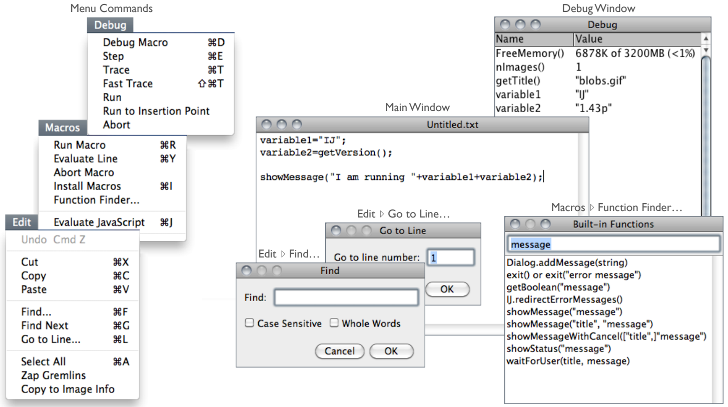
**Results ▷ Set Measurements...** Alias for the Analyze ▷ Set Measurements... command.

**Results ▷ Options...** Opens the Edit ▷ Options ▷ Input/Output... dialog in which is possible to specify if column headers and row numbers should be saved or copied from ImageJ tables (including the Summarize table, cf. Analyze ▷ Analyze Particles...). In addition, it allows to specify the file extension to be used when saving data. Custom extensions (e.g., `.csv`, `.xls` or `.ods`) allow ImageJ tables to be imported seamlessly by spreadsheet applications. ImageJ tables are saved in CSV format if *File extension for tables* is `.csv`.

See also: Plugins ▷ New ▷ Table...








**The ImageJ editor (version 1.43n).** The editor is a simple text editor featuring Function Finder... [F], an essential tool when writing Macros. The Fiji Script Editor is a more advanced editor featuring syntax highlight and support to all of Fiji's scripting languages.

## 23 Editor

Macros ▷, Scripts and Plugins can be opened and executed in the ImageJ editor. The editor commands are organized in five menus: File ▷, Edit ▷, Font ▷, Macros ▷ and Debug ▷.

**File ▷** Basic file operations (Open, Save, Print, etc.) are listed in this menu. The last saving directory is kept in `IJ_prefs.txt`, the IJ preferences file (*see* Settings and Preferences).

**Edit ▷** Similarly to any other text editor this menu contains commands related to text handling as well as commands for locating text. Specially useful are:

- **Go to Line... [l]** Ctrl L, This dialog box enables you to quickly go to a specified line of code.
- **Zap Gremlins** This command finds and deletes the extraneous non-visible, non-printing characters that sometimes appear when cutting and pasting from other sources, such as email messages that may contain extraneous control characters, or any non-ASCII characters.
- **Copy to Image Info** This command will copy the selected text (or the entire contents of the editor if no selection is present) to the image header, being available through the Image ▷ Show Info... [i] command. Note that the copied text will substitute any other information present in the file header and will only be available in images saved as TIFF (*see* II Image Types: Lossy Compression and Metadata).

**Font ▷** This menu contains commands to adjust font size and type.

**Macros ▷** This menu contains commands that allow you to run, install or evaluate macro code:

- **Run Macro [r]** Ctrl R, Runs the macro or the selected line(s) of code.
- **Evaluate Line [y]** Ctrl Y, Runs the line of code that contains the insertion point.
- **Abort Macro** Exits the macro.

**Install Macros [i]** Ctrl I, Adds the macro(s) contained in the editor to Plugins ▷ Macros ▷ submenu (Plugins ▷ Help ▷ Macro Functions... command).

**Macro Functions... [M]** Ctrl Shift M, Opens the Macro Functions reference page, the indispensable guide to the built-in functions that can be called from the ImageJ macro language (alias for Help ▷ Macro Functions...).

**Function Finder... [F]** Ctrl Shift F, [3] Retrieves macro functions in the same way Find Commands... [l] retrieves commands. Functions are read from the `functions.html` file stored in the macros folder (a local copy of http://imagej.nih.gov/ij/developer/macro/functions.html). This file is deleted by Help ▷ Update ImageJ... command every time ImageJ is updated to a release version (i.e., not a *daily build*, *see* Installing and Maintaining ImageJ), forcing Function Finder to download a fresh copy the next time it is launched.

**Evaluate JavaScript [j]** Ctrl J, Runs JavaScript code in the editor window. Note that Run Macro runs JavaScript code if the title of the file ends with '.js'.

**Debug ▷** This menu contains seven commands related to the macro debugging. You can debug a macro using the commands in the Debug menu. You start a debugging session initiating Debug Macro. You can then single step through the macro code by repeatedly running Step.

**Debug Macro [d]** Ctrl D, Starts running the macro in debug mode and opens the 'Debug' window, which initially displays the memory usage, number of open images, and the active image's title. The macro stops running at the first executable line of code, which is highlighted. Use one of the following commands to continue execution.

**Step [e]** Ctrl E, Executes the highlighted statement and advances to the next. The variable names and values in the 'Debug' window are updated.

**Trace [t]** Ctrl T, Runs the macro, displaying variable names and values in the 'Debug' window as they are encountered.

**Fast Trace [T]** Ctrl Shift T, Same as above, but faster.

**Run** Runs the macro to completion at normal speed (similarly to Macros ▷ Run Macro).

**Run to Insertion Point** Ctrl Shift E, Runs the macro to a statement that was previously defined by clicking the mouse on an executable line of code.

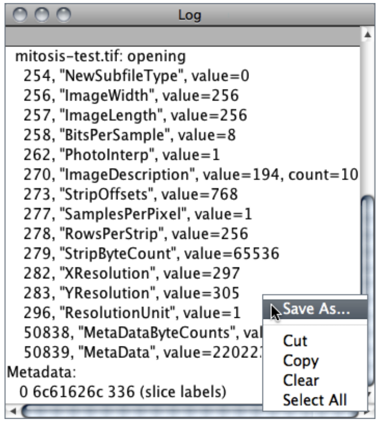
**Abort** Exits debug mode.

SEE ALSO: Extending ImageJ (Macros and Scripts), The ImageJ Macro Language – Programmer's Reference Guide, Fiji's Introduction into Macro Programming, Plugins ▷ Macros ▷ Record..., Fiji Script Editor, IJ_ED





## 24 Log Window



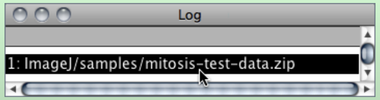

The Log window is used to display useful information about ongoing operations. It is frequent for plugins and macros to send messages to the Log window reporting progress, errors or troubleshooting information.

If you are troubleshooting a problem, you can check *Debug mode* in Edit ▷ Options ▷ Misc... to have ImageJ outputting messages to the Log window (ImageJ will exit debug mode as soon as the Log window is closed).

In addition, Tiff tags and information needed to import files are printed to the log window when ImageJ runs in Debug Mode.

Most of the general shortcut keys described in Editor apply to the Log window.

### VI Opening File Paths in the Log Window




In the Log Window, double click on a file path to have it open by ImageJ.

## 25 Customizing the ImageJ Interface

Most settings determining the look and feel of ImageJ are listed in Edit ▷ Options ▷, namely Edit ▷ Options ▷ Appearance... and Edit ▷ Options ▷ Misc... (*see also* Settings and Preferences). However, other aspects of the ImageJ interface can also be personalized.

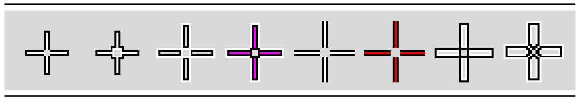
### 25.1 Floating Behavior of Main Window

It is possible to place the Main ImageJ window above all other windows at all time using a simple JavaScript instruction: `IJ.getInstance().setAlwaysOnTop(true);`.

To test it, copy this one line script to the clipboard (or download `Always_on_Top.js` from the online scripts repertoire), switch to ImageJ, type Shift V (File ▷ New ▷ System Clipboard [V]), then type Ctrl J (Macros ▷ Evaluate JavaScript [j]). To create an "Always on Top" command, save this script in the plugins folder as `Always_on_Top.js` and run Help ▷ Refresh Menus to start using the new command. Macro (5) Customizing the Float Behavior of IJ's Main Window exemplifies how to set this option at launch.

SEE ALSO: I, I Frontmost Window and Window Activation

**Examples of modified crosshair pointers, more visible on grayscale images.** The default crosshair cursor can be replaced by any image saved as crosshair-cursor.gif in ImageJ/images/.



New in IJ 1.46r

## 25.2 Pointer

At startup, ImageJ will search for a GIF image named crosshair-cursor.gif in the ImageJ/images/ directory. If present, it will be used to replace the default crosshair cursor. The pointer can also be changed to an arrow by toggling *Use pointer cursor* on Edit ▷ Options ▷ Misc. . .

**(5) Customizing the Float Behavior of IJ's Main Window**

```
// These macros can be added to the ImageJ/macros/StartupMacros.txt file ↩
   in order to set the floating behavior of the ImageJ main window

// option 1) Run ImageJ/plugins/Always_on_Top.js command at launch, by ↩
   adding it to the "AutoRun" macro
macro "AutoRun" {
  run("Always on Top");
}

// option 2) Execute the script at launch, by adding it to "AutoRun"
macro "AutoRun" {
  eval("script", "IJ.getInstance().setAlwaysOnTop(true)");
}

// option 3) Toggle the setAlwaysOnTop option using a shortcut, e.g., F1
var afloat;
macro "Toggle AlwaysOnTop [F1]" {
  booleans = newArray("true", "false");
  eval("script","IJ.getInstance().setAlwaysOnTop("+ booleans[afloat] +")");
  afloat = !afloat;
}
```



# Part V

# Menu Commands

As described in ImageJ User Interface, the menu bar lists all ImageJ commands. It is organized in eight menus:

- File ▷ Basic file operations (opening, saving, creating new images). Most are self-explanatory.
- Edit ▷ Editing and drawing operations as well as global settings.
- Image ▷ Conversion and modification of images including geometric transformations.
- Process ▷ Image processing, including point operations, filters and arithmetic operations.
- Analyze ▷ Statistical measurements, profile and histogram plotting and other operations related to image analysis.
- Plugins ▷ Commands for creating, editing and managing add-ons (*see* Extending ImageJ), listing all the user-installed Macros, Scripts and Plugins installed in the ImageJ/plugins/ directory.
- Window ▷ Selection and management of open windows.
- Help ▷ Updates, documentation resources and version information.

### VII Organizing Commands in the Menu Bar

The Plugins ▷ menu can become easily cluttered after the installation of several plugins. Since Plugins ▷ reflects the hierarchy of directories in ImageJ/plugins/ (up to two subfolders), submenus (i.e., subfolders) can be created to keep the menu organized, preventing it from running off the bottom of the screen. E.g, to move the EPS Writer plugin into a Plugins ▷ Input-Output ▷ PDF ▷ submenu, one would move EPS_Writer.class into ImageJ/plugins/Input-Output/PDF/.

In addition, checking the *Move isolated plugins to Misc. menu* checkbox in Edit ▷ Options ▷ Misc. . . will compact the menu list by moving to Plugins ▷ Miscellaneous ▷ all the plugins with only one command that try to install themselves in submenus.

Note that external plugins can be installed in any of the ImageJ menus. This is the case of plugins packaged in JAR files containing a configuration file (plugins.config) specifying the location of the new commands implemented by the plugin. You can rename, reorganize or move commands implemented by external plugins by editing their plugins.config file as described on the JAR demo documentation page. If you don't know in which menu a plugin has been registered, use *Show full information* in the command Finder (Plugins ▷ Utilities ▷ Find Commands. . . [l]) to find out the location of the installed .jar files.

With Fiji, Scripts and Macros can be registered in any menu by saving into Fiji.app/plugins/Scripts/menu name/submenu name/. E.g., a certain macro (.ijm file) saved in Fiji.app/plugins/Scripts/File/Import/ is registered in the File ▷ Import ▷ submenu.

See also: ImageJ's plugin architecture on the Fiji website

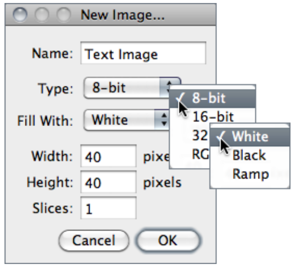
# 26 File ▷

## 26.1 New ▷

Contains commands for creating new images, stacks, hyperstacks or text windows.

SEE ALSO: Plugins ▷ New ▷

### 26.1.1 Image... [n]




Creates a new image window or stack. A dialog box allows you to specify the image title, type, dimensions and initial content.

*Name* is the title that will be used for the Window. *Type* is the image type: 8–bit grayscale, 16–bit grayscale (unsigned), 32–bit (float) grayscale or RGB color. *Fill With* (*White*, *Black* or *Ramp*) specifies how the image is initialized. *Width* and *Height* specify the image dimensions in pixels. Set *Slices* to a value greater than one to create a stack.

SEE ALSO: Image ▷ Hyperstacks ▷ New Hyperstack..., Image Types and Formats

### 26.1.2 Hyperstack...

Alias for the Image ▷ Hyperstacks ▷ New Hyperstack... command.

### 26.1.3 Text Window [N]

Creates a new text window with the title 'Untitled.txt'.

SEE ALSO: Plugins ▷ New ▷ Text Window..., Macro, Table...

### 26.1.4 Internal Clipboard

Opens the contents of the internal ImageJ clipboard.

SEE ALSO: Edit ▷ Copy [c], Cut [x], Paste Control...

### 26.1.5 System Clipboard [V]

Opens the contents of the operating system clipboard.

SEE ALSO: Edit ▷ Copy to System, Cut [x], Paste Control...





## 26.2 Open... [o]

Opens an image and displays it in a separate window. Image files must be in TIFF, GIF, JPEG, DICOM, BMP, PGM or FITS format, or in a format supported by a reader plugin. Also opens:

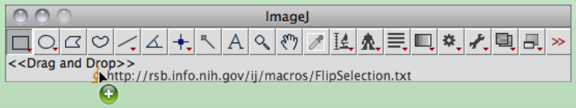
- ImageJ and NIH Image lookup tables (`.lut` extension).
- Tables (in tab-delimited text format) (`.xls` or `.csv` extension, *see* Results Table)
- Selections (`.roi` or `.zip` extension)
- Text files (`.txt`, `.ijm`, `.js` and `.java` extensions)
- ...

SEE ALSO: File ▷ Import ▷, Image Types and Formats, Virtual Stacks, VIII Opening Files: File ▷ Open..., File ▷ Import ▷ and Drag & Drop

### VIII OPENING FILES: File ▷ Open..., File ▷ Import ▷ AND DRAG & DROP

While the File ▷ Open... [o] command opens formats natively supported by ImageJ (images and non-images files), the File ▷ Import ▷ submenu provides access to plugins for additional file types (e.g., reading 'raw' files, images in ASCII format or loading images over the network). Most of ImageJ's Input/Output plugins are installed on this submenu.

Note that almost every format known to ImageJ can be opened by dragging and dropping the file into the Main ImageJ window. E.g., in the illustration below a remote macro file is opened by dragging its URL directly from a Web browser.




## 26.3 Open Next [O]

Closes the current image and opens the next image (if any) in its directory. Holding Alt opens the previous image (if any) in its directory.

## 26.4 Open Samples ▷

Opens example images hosted on the ImageJ Web site. These sample images are useful for creating, testing and debugging macros since routines can be applied to the same image, regardless of where the macro is run. Among all, probably the most used is *blobs.gif*: Open Samples ▷ Blobs (25K) [B].

Sample images can be downloaded from http://imagej.nih.gov/ij/images/ or, in bulk, from either http://imagej.nih.gov/ij/download/sample-images.zip, or in Fiji, by running File ▷ Open

Samples ▷ Cache Sample Images. The 'AutoRun' macro in the StartupMacros.txt file can then be used to change the default path of sample images, allowing a complete off-line usage of the File ▷ Open Samples ▷ submenu:

**(6) Setting File ▷ Open Samples ▷ for Offline Usage**

```
/* This macro calls the Prefs.setImageURL() method to change the default ↩
  path of Sample Images (http://imagej.nih.gov/ij/images/) to a local ↩
  subfolder of ImageJ's directory named "samples". Note that Fiji ↩
  provides this feature by default.
 */

macro "AutoRun" {
  dir= getDirectory("imagej") + "samples";
  if (File.exists(dir)) {
      dir= replace(dir, " ", "%20");
      if (startsWith(getInfo("os.name"), "Windows"))
          dir= "/"+ replace(dir, File.separator, "/");
      call("ij.Prefs.setImagesURL", "file://"+ dir +"/");
  }
```

## 26.5 Open Recent ▷

The submenu shows a list of the 15 recently opened files. Click on a filename to open it.

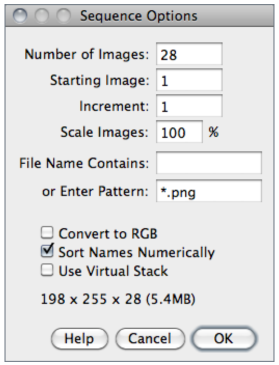
## 26.6 Import ▷

This submenu lists the installed image reader plugins.

SEE ALSO: Non–native Formats, Acquisition plugins, Input/Output plugins, VirtualStackFromList macro, VIII Opening Files: File ▷ Open. . . , File ▷ Import ▷ and Drag & Drop

IMPROVED IN IJ 1.46R

### 26.6.1 Image Sequence. . .




Opens a series of images in a chosen folder as a stack. Images may have different dimensions and can be of any format supported by ImageJ (*see* Image Types and Formats and HandleExtraFileTypes plugin). Non-image files (Scripts, .lut, .roi, RoiSet.zip, etc. ) are ignored.

Information – width×height×depth (size) – of the stack to be created is displayed at the bottom of the dialog.

***Number of Images*** Specifies how many images to open.

***Starting Image*** If set to $n$, import will start with the $n^{th}$ image in the folder.

***Increment*** If set to '2' every other image will be opened, if set to '3' to every third image will be opened, etc.

***File Name Contains*** Enter a string into this field and ImageJ will only open files whose name contains that string.





***Enter Pattern*** Regular expressions (regex) can be typed here for advanced filtering (*see* Basic syntax of regular-expressions).

***Scale Images*** Setting a value less than 100% will reduce memory requirements. E.g., entering 50 reduces the amount of memory needed to open a stack by 25% (two-dimensional images: $0.5 \times 0.5 = 0.25$ of the original data). This value is ignored if *Use Virtual Stack* is checked.

***Convert to RGB*** Allows a mixture of RGB and grayscale images to be opened by converting all the sequence to RGB. Note that if this option is unchecked and the first imported image is 8–bit then all the remaining images in the sequence will be converted to 8–bit. Checking this option, circumvents this issue.

IMPROVED IN IJ 1.46R

***Sort Names Numerically*** When checked, the stack will be opened in NUMERIC file name order (e.g., 'name1.tif', 'name2.tif', 'name10.tif') instead of alphanumeric order (e.g., 'name1.tif', 'name10.tif', 'name2.tif'). DICOM files in the same series (tag# 0020, 0011) are always sorted by the image number (tag# 0020,0013). The *List Stack Tags* macro, part of the ListDicomTags macro set, lists the values of the image number and image series tags.

***Use Virtual Stack*** When checked, images are opened as a read-only virtual (disk-resident) stack using a version of the Virtual Stack Opener plugin. This allows image sequences too big to fit in RAM to be opened, but access time is slower and changes are lost when switching to a different image in the stack (*see* Virtual Stacks). Note the following consequences of enabling this option:

- Image Overlays are not loaded
- If the folder contains tiff stacks, only the first slice of those stacks will be imported (with RAM resident stacks, all slices are imported and concatenated into the sequence [*see* Image ▷ Stacks ▷ Tools ▷ Concatenate. . . ] )

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/file.html#seq1.

SEE ALSO: File ▷ Save As ▷ Image Sequence. . . , OpenSeriesUsingFilter macro, Image ▷ Overlay ▷

**Regular-expressions basic syntax summary.** For more information on regex filtering see http://download.oracle.com/javase/tutorial/essential/regex/.

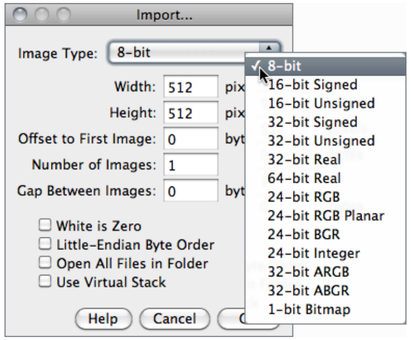
| | Regex Syntax (Character Classes) | Example | Meaning |
|---|---|---|---|
| [] | Delimit the set of characters to match | [aA] | Either lower or upper case A |
| - | Character ranges | [0-9] | Any digit (from 0 through 9) |
| . | Any character | [0-9]. | A digit plus any other character |
| * | Zero or more of the preceding item | .* | Any character sequence |
| ? | Zero or one of the preceding item | [0-9]? | An optional digit |
| + | One or more of the preceding item | [0-9]+ | At least a digit |
| ^ | Negation | [^0-9] | Any character that is not a digit |
| && | AND (Intersection) | [0-9&&[^3]] | A digit that is not 3 |
| \| | OR (Alternation) | [0-9]\|[a-zA-Z] | A digit or lower or upper case letter |

### IX Reducing Memory Requirements When Importing Images

Since ImageJ 1.44d, the File ▷ Import ▷ ▷ Image Sequence... command no longer features the *Convert to 8-bit Grayscale* checkbox. This option was used to reduce memory requirements but used different scaling for each imported image.

As a replacement, use the *Use virtual stack* option and then convert to 8–bit using File ▷ Type ▷ 8–bit. Memory requirements can also be reduced by using the *Scale Images (%)* option. The amount of memory allocated to ImageJ can be adjusted in Edit ▷ Options ▷ Memory & Threads...

### 26.6.2 Raw...




Use this command to import images that are not in a file format directly supported by ImageJ. You will need to know certain information about the layout of the image file, including the size of the image, and the offset to the beginning of the image data.

Interleaved RGB images have pixels stored contiguously (rgbrgbrgb...) in a single image plane. Planar RGB images have the red, green and blue image data stored in separate 8–bit sample planes. ImageJ saves RGB images (both TIFF and raw) in interleaved format.

***Image Type*** There are fourteen choices depicted above. 16–bit signed integer images are converted to unsigned by adding 32,768. 1–bit Bitmap images are converted to 8–bit.

***Image Width*** The number of pixel in each row of image data.

***Image Height*** The number of rows in the image.

***Offset to First Image*** The number of bytes in the file before the first byte of image data.

***Number of Images*** The number of images stored in the file. If this value is greater than the actual number of images the resulting stack will get truncated to the actual size.

***Gap Between Images*** The number of bytes from the end of one image to the beginning of the next. Set this value to width×height×bytes-per-pixel×$n$ to skip $n$ images for each image read.

***White is Zero*** Should be checked if black pixels are represented using numbers that are less than the numbers used for white pixels. If your images look like photographic negatives, changing this field should fix the problem.

***Little-Endian Byte Order*** Probably needs to be checked when importing 16–bit or 32–bit grayscale images from little-endian machines such as Intel based PCs.

***Open All Files in Folder*** If checked, ImageJ will import all the images in the folder as a stack. The images must all be the same size and type.

***Use Virtual Stack*** Images are imported as virtual stacks.





***Help*** Opens http://imagej.nih.gov/ij/docs/menus/file.html#raw.

SEE ALSO: Image Types and Formats

### 26.6.3 LUT...

Opens an ImageJ or NIH Image lookup table, or a raw lookup table. The raw LUT file must be 768 bytes long and contain 256 reds, 256 blues and 256 greens. If no image is open, a 256×32 ramp image is created to display the LUT. Note that lookup tables with file names ending in `.lut` can also be opened using File ▷ Open... [o] or drag and drop.

### 26.6.4 Text Image...

IMPROVED IN IJ 1.46R

Opens a tab-delimited text file as a 32–bit real image (*see* Text Images). The image's width and height are determined by scanning the file and counting the number of words and lines. For text files with integer values no larger than 255, use Image ▷ Type ▷ 8–bit to convert to 8–bit. Before converting, disable *Scale When Converting* in Edit ▷ Options ▷ Conversions... to prevent the image from being scaled to 0–255.

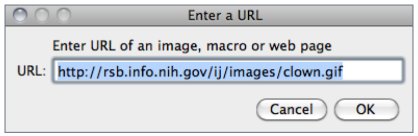
SEE ALSO: Text Images, Pixel Inspector, Image ▷ Transform ▷ Image to Results/Results to Image, Save As ▷ Text Image..., `OpenTextImagesAsStack` macro

### 26.6.5 Text File...

Opens a text file. Note that text files can also be opened using File ▷ Open... [o] or drag and drop.

### 26.6.6 URL...

IMPROVED IN IJ 1.46R




Downloads and displays known formats to ImageJ specified by a URL. Other URLs ending with '/' or '.html' are opened in the user's default browser. The Input URL is saved in the ImageJ preferences file and retrieved across IJ restarts.

It is also possible to open zip archives, using a URL, that contain multiple DICOM images. Some example URLs are:

- http://imagej.nih.gov/ij/images/ct.dcm
- file:///Macintosh HD/images/Nanoprobes.tif
- file:///D:images\neuron.tif
- http://imagej.nih.gov/ij/ (opens the ImageJ website)

### 26.6.7 Results...

Opens an ImageJ table, or any tab or comma-delimited text file (*see* Results Table). Note that `.csv` and `.xls` files can also be opened by drag and drop.

### 26.6.8 Stack From List...

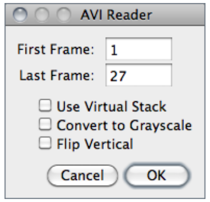
Opens a stack, or virtual stack, from a text file or URL containing a list of image file paths [11]. The images can be in different folders but they must all be the same size and type. The Virtual Stack From List macro demonstrates how to generate a list of images and then use that list to open the images as a virtual stack. The OpenStackUsingURLs macro demonstrates how to how to open an image series from a remote server.

### 26.6.9 TIFF Virtual Stack...

Opens a TIFF file as virtual stack (*see* Virtual Stacks and III Opening Virtual Stacks by Drag & Drop).

Improved in IJ 1.46r

### 26.6.10 AVI...




Uses a built in version of the AVI reader plugin to open an AVI file (JPEG or PNG compressed, or uncompressed) as a stack or virtual stack (one slice per video frame) [12]. Animation speed is retrieved from image frame rate. AVI files can also be opened using File ▷ Open... [o] or drag and drop but macros must use this command to gain access to the dialog box options.

ImageJ supports a restricted number of AVI formats including MJPG (motion-JPEG) and various YUV 4:2:2/4:2:0 compressed formats (cf. plugin source code). The OME Bio-Formats library (*see* Non–native Formats) extends support to MSRLE and MSV1 encoded formats.

The dialog promt allows you to choose if frames should be converted to 8–bit grayscale or flipped vertically. For large files, an option to open the movie as a virtual stack is also available (*see* Virtual Stacks). It is also possible to specify the starting and ending frame. Enter 0 (zero) to specify the last frame, −1 to specify the second last frame, etc.

See also: File ▷ Save As ▷ AVI...

New in IJ 1.46r

### 26.6.11 XY Coordinates...

Imports a two column text file, such as those created by File ▷ Save As ▷ XY Coordinates..., as a polygon selection. The selection is displayed in the current image or, if the current image is too small, in a new blank image. Coordinates of active selection (at evenly spaced one pixel intervals) can be retrieved using the *List coordinates* options in Edit ▷ Selection ▷ Properties... [y].

## 26.7 Close [w]

Closes the active image.

## 26.8 Close All


Closes all open images. An alert is displayed if there are unsaved changes.





## 26.9 Save [s]

Saves the active image in TIFF format, the 'default' format of ImageJ (cf. II Image Types: Lossy Compression and Metadata). To save only a selected area, create a rectangular selection and use the Image ▷ Duplicate... [D] command. Note that Save [s] and File ▷ Save As ▷ Tiff... are redundant commands.

## 26.10 Save As ▷

Use this submenu to save the active image in TIFF, GIF, JPEG, or 'raw' format. Can also be used to save measurement results, lookup tables, selections, and selection XY coordinates.

### 26.10.1 Tiff...

Saves the active image or stack in TIFF format in redundancy with File ▷ Save [s]. TIFF is the only format (other than 'raw') that supports all ImageJ data types (8–bit, 16–bit, 32–bit float and RGB) and the only format that saves spatial and density calibration. In addition Selections and Overlays are also saved in the TIFF header.

By default, 16–bit and 32–bit images are saved using big-endian byte order. Check *Save TIFF and Raw in Intel Byte Order* in the Edit ▷ Options ▷ Input/Output... dialog box to save using little-endian byte order.

See also: Native Formats, II Image Types: Lossy Compression and Metadata, X Warning on JPEG Compression

### 26.10.2 Gif...

Improved in IJ 1.46r

Saves the active image in GIF format. RGB images must first be converted to 8–bit color using using Image ▷ Type ▷ 8–bit Color. The value to be used as the transparent index (0–255) can be set in the Edit ▷ Options ▷ Input/Output... dialog box. Stacks are saved as animated GIFs. Use Image ▷ Stacks ▷ Tools ▷ Animation Options... [Alt /] (or right-click on the on the play/pause icon that precedes the stack slider) to set the frame rate.

### 26.10.3 Jpeg...

Saves the active image in JPEG format. Edit *JPEG Quality* Edit ▷ Options ▷ Input/Output... dialog box to specify the JPEG compression level (0–100). This value is shown on the title of the save dialog prompt. Lower values produce smaller files but poorer quality. Larger values produce larger files but better quality. Color sub-sampling is disabled when the value is set to 100, reducing the likelihood of color artifacts. By default, the DPI in the JPEG header is set to 72. For a higher value, use a unit of *inch* in the Analyze ▷ Set Scale... dialog. E.g., setting *Distance in Pixels* to 300, *Known Distance* to 1 and *Unit of Length* to 'inch' will set the DPI to 300.

Overlays are embedded when saving in Jpeg format (*see* Image ▷ Overlay ▷ Flatten [F]).

See also: II Image Types: Lossy Compression and Metadata, X Warning on JPEG Compression

**X WARNING ON JPEG COMPRESSION**

The JPEG format uses Lossy compression that leads to severe artifacts that are not compatible with quantitative analyses. As such, it should only be used for presentation purposes (if file size is an issue), but even then a lossless format such as PNG is probably more suitable.

The illustration below exemplifies the consequences of saving images in a lossy format. To replicate it, open the Mandrill sample image (by drag and drop, or alternatively using File ▷ Import ▷ URL... and typing the image's path, http://imagej.nih.gov/ij/images/baboon.jpg), duplicate it (Image ▷ Duplicate... [D]), save the duplicate as JPEG (File ▷ Save As ▷ Jpeg...), run File ▷ Revert [r] (so that the saved version is reloaded by ImageJ) and calculate the difference between the two images using Process ▷ Image Calculator...

By adjusting the Brightness/Contrast... [C], you will notice that the imperceptible JPEG artifacts are most pronounced along regions of higher contrast changes. In addition to this edge artifact, the JPEG algorithm may shift colors to improve compression which may lead to artificial colocalization. These artifacts are intrinsic to the format and may persist even if *JPEG Quality* has been increased to 100% in Edit ▷ Options ▷ Input/Output... Once an image has been lossy compressed there is no way of reverting it to the original. Given all this, and since Metadata is poorly supported in lossy formats, it is unreasonable to use JPEG in image processing.

Original | JPEG copy (75% quality) | Difference

#### 26.10.4 Text Image...

Saves the active image as a spreadsheet compatible tab-delimited text file. Calibrated and floating-point images are listed with the precision specified by *Decimal places* in Analyze ▷ Set Measurements... For RGB images, each pixel is converted to grayscale using the formula gray = (red + green + blue)/3 or the formula gray = 0.299 × red + 0.587 × green + 0.114 × blue if *Weighted RGB to Grayscale Conversion* is checked in Edit ▷ Options ▷ Conversions...

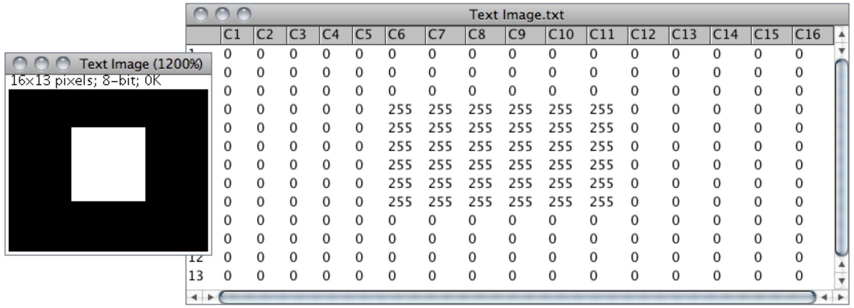
SEE ALSO: Text Images, Pixel Inspector , Image ▷ Transform ▷ Image to Results/Results to Image, Import ▷ Text Image...

#### 26.10.5 Zip...

Saves the active image or stack as a TIFF file inside a compressed ZIP archive.





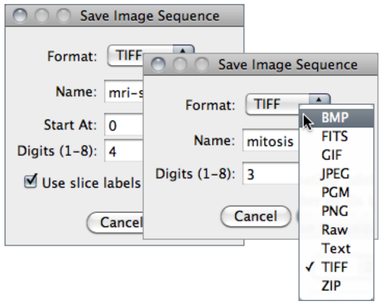



**Text Images: File ▷ Import ▷ Text Image... and File ▷ Save As ▷ Text Image...**

#### 26.10.6 Raw Data...

Saves the active image or stack as raw pixel data without a header. 8–bit images are saved as unsigned bytes, unsigned 16–bit images are saved as unsigned shorts and signed 16–bit images (e.g., File ▷ Open Samples ▷ CT (420K, 16–bit DICOM)) are saved as signed shorts. 32–bit images are saved as floats and RGB images are saved in three bytes per pixel (24–bit interleaved) format. 16–bit and 32–bit (float) images are saved using big-endian byte order unless *Export Raw in Intel Byte Order* is checked in the Edit ▷ Options ▷ Input/Output... dialog box.

#### 26.10.7 Image Sequence...




Saves a Stack or a hyperstack as an image sequence.

***Format*** Specifies the output format that can be set to either *BMP, FITS, GIF, JPEG, PGM, PNG, Raw, Text Image, TIFF,* or *Zip* (cf. Image Types and Formats). In IJ 1.44 and later, Overlays are embedded when saving in JPEG or PNG format.

***Name*** Specifies the leading string that will be common to all numeric filenames.

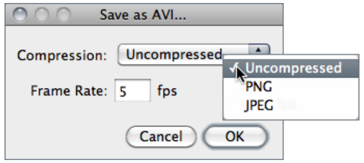
***Start At*** (Stacks only) Specifies the starting number of the sequence.

***Digits (1–8)*** The number digits of the incremental sequence. Filenames are padded with leading zeroes.

***Use slice labels as filenames*** (Stacks only) If checked, each slice will be saved with its own label (the image subtitle displayed above the image, *see* Remove Slice Labels) and no numeric sequence will be used.

With hyperstacks, images are saved using 'Name_t *d*_z *d*_c *d*' in which *d* is the incremental number of specified *Digits; t,* the frame; *z,* the slice and *c,* the channel, so e.g., for the depicted snapshot the first image would be saved as 'mitosis_t001_z001_c001.tif'.

#### 26.10.8 AVI...




Exports a stack or hyperstack as an AVI file [12]. Note that AVI files larger than 2 GB are not correctly written.

***Compression*** *JPEG*, *PNG* or *Uncompressed*. With IJ 1.44 and later, Overlays are embedded when saving in JPEG or PNG format. The default compression is *JPEG*.

**Frame** Specifies the frame frequency. The proposed value is read from Image ▷ Stacks ▷ Tools ▷ Animation Options... [Alt /] and Image ▷ Properties... [P], as long as the unit of *Frame Interval* is 'sec'.

See also: File ▷ Import ▷ AVI...

#### 26.10.9 PNG...

Saves the active image in PNG (Portable Network Graphics) format. All image types, except RGB, are saved as 8–bit PNGs. 16–bit images are saved as 16–bit PNGs. With 8–bit images, the value to be used as the transparent index (0–255, -1 for "*none*") can be set in the Edit ▷ Options ▷ Input/Output... dialog box. Overlays are embedded when saving in PNG format.

#### 26.10.10 FITS...

Saves the active image in FITS (Flexible Image Transport System) format [13].

#### 26.10.11 LUT...

Saves the active image's lookup table to a file. The 768 byte file consists of 256 red values, 256 green values and 256 blue values.

#### 26.10.12 Results...

Exports the contents of the 'Results' window as a tab-delimited or comma-delimited (.csv) text file. Prior to ImageJ 1.44b this command used to be named *'Measurements...'*.

#### 26.10.13 Selection...

Saves the current area selection boundary to a file, that can be later retrieved using File ▷ Open... [o] to restore the selection. Active Selections and Overlays are saved in the TIFF header by default (*see* File ▷ Save As ▷ Tiff...).

Improved in IJ 1.46r

#### 26.10.14 XY Coordinates...

Exports the XY coordinates of the active ROI as a two column, tab-delimited text file. ROI coordinates can also be retrieved using the *List coordinates* option in Edit ▷ Selection ▷ Properties... [y], that tabulates ROI coordinates at evenly spaced one pixel intervals.

See also: Selections With Sub-pixel Coordinates, File ▷ Import ▷ XY Coordinates...



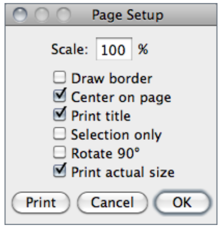


### 26.11 Revert [r]

Reloads the active image, stack or hyperstack from disk, reverting it to its last saved state. It is actually a shortcut for closing the window without saving, and then reopening it. Note that it may not work with Non–native Formats opened through external plugins such as the OME Bio-Formats library.

See also: Undo and Redo

### 26.12 Page Setup...




The Page Setup dialog allows you to control the size of printed output, plus other printing options:

***Scale*** Values less than 100% reduce the size of printed images and values greater than 100% increase the size. 100% corresponds to 72 pixels per inch (ppi), about the unzoomed screen size of the image. The size of the printed image is determined by the *Scale* value and the width and height of the image in pixels. Spatial calibration is ignored.

***Draw border*** If checked, ImageJ will print a one pixel wide black border around the image.

***Center on page*** If checked, the image will be printed in the center of the page instead of in the upper left corner.

***Print title*** If checked, the title of the image will be printed at the top of the page.

***Selection only*** If checked, current selection will be printed instead of the entire image.

***Rotate 90°*** If checked, the image will be rotated 90° to the left before being printed.

***Print actual size*** Considers the DPI information in the image header (typically 72, cf. Jpeg...). For a higher value, use a unit of *inch* in the Analyze ▷ Set Scale... dialog. E.g., setting *Distance in Pixels* to 300, *Known Distance* to 1 and *Unit of Length* to 'inch' will set the DPI to 300.

### 26.13 Print... [p]

Prints the active image. The size of the printed image will normally be slightly less its size on the screen (unzoomed). Use the Page Setup... dialog to increase of decrease the size of printed images. Images larger than the page are scaled to fit. Overlays are embedded when printing images.

### 26.14 Quit

Prompts you to save all unsaved images and then exits. You can also exit ImageJ by clicking on the close button in its window's title bar.

# 27 Edit ▷

## 27.1 Undo [z]

Described in Undo and Redo.

## 27.2 Cut [x]

Copies the contents of the current image selection to the internal clipboard, filling the selection with the current background color.

See also: Edit ▷ Copy to System, Paste Control...

## 27.3 Copy [c]

Copies the contents of the current image selection to the internal clipboard. If there is no selection, copies the entire active image. The amount of image data copied is shown in the status bar.

See also: File ▷ Internal Clipboard, Paste Control...

## 27.4 Copy to System

Copies the contents of the current image selection to the system clipboard.

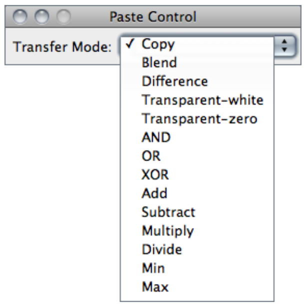
See also: File ▷ New ▷ System Clipboard [V], Copy [c], Paste Control...

## 27.5 Paste [v]

Inserts the contents of the internal clipboard (or from the system clipboard if the internal clipboard is empty) into the active image. The pasted image is automatically selected, allowing it to be dragged with the mouse. Click outside the selection to terminate the paste. Select Edit ▷ Undo [z] to abort the paste operation.

See also: Paste Control...

## 27.6 Paste Control...




After pasting, use the Paste Control pop-up menu to control how the image currently being pasted is transferred to the destination image.

Except for Blend and Transparent, the Paste Control transfer modes are the same as those listed in the description of Process ▷ Image Calculator... The Blend mode is the same as the Image Calculator Average mode. In Transparent mode, white/black pixels are transparent and all other pixels are copied unchanged.

See also: Image ▷ Overlay ▷ Add Image...





## 27.7 Clear

Erases the contents of the selection to the current background color. Backspace and Del keys are shortcuts to this command. With stacks, a dialog is displayed offering the option to clear the selection in all stack images. Clear by pressing Backspace to avoid this dialog.

See also: Clear Outside, Fill [f], Color Picker Tool

## 27.8 Clear Outside

Erases the area outside the current image selection to the background color.

See also: Clear, Fill [f], Color Picker Tool

## 27.9 Fill [f]

Fills the current selection with the current foreground color. With stacks, a dialog is displayed offering the option to fill the selection in all stack images. Fill the selection by pressing F to avoid this dialog.

See also: Clear, Draw [d], Color Picker Tool

## 27.10 Draw [d]

Outlines the current selection using the current foreground color and line width. The foreground and background colors can also be set using the Edit ▷ Options ▷ Colors... command. Use the Edit ▷ Options ▷ Line Width... command, or double click on the line tool, to change the line width.

With stacks, a dialog is displayed offering the option to draw the selection in all stack images. Draw the selection by pressing D to avoid this dialog.

See also: Analyze ▷ Label, Color Picker Tool, XVII Embedding Color Annotations in Grayscale Images, XI Drawing Lines Wider Than One–Pixel

### XI Drawing Lines Wider Than One–Pixel

If the line width is an even number, the selection boundary is at the center of the line. If the line width is odd (1, 3, ...), the center of the line drawn is displaced from the selection edge by $1/2$ pixel to the bottom right. Thus the line center (the line in case of $line\,width = 1$) is inside the selection at the top and left borders, but outside at the bottom and right borders. Rectangular selections (but not polygonal selections or traced selections that happen to be rectangular) are an exception to this rule: For rectangular selections, one–pixel wide outlines are always drawn inside the rectangle. Thicker lines are drawn as for the other selection types.

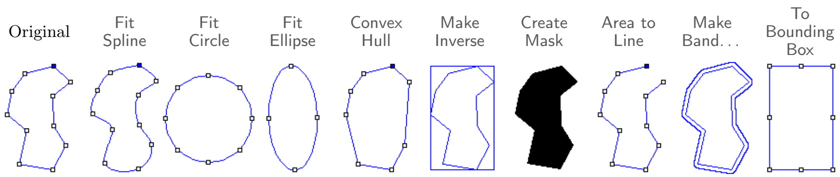
## 27.11 Invert [I]

Creates a reversed image, similar to a photographic negative, of the entire image or selection. For 8–bit and RGB images (*see* Image Types and Formats), Invert always uses $min = 0$ and $max = 255$, regardless of the data values. For 16–bit and 32–bit images, the actual minimum and maximum are used (rather than the full range of the pixel type).

SEE ALSO: Image ▷ Lookup Tables ▷ Invert LUT

IMPROVED IN IJ 1.46R

## 27.12 Selection ▷




**ROI manipulations using Edit ▷ Selection ▷ commands.** General handling of ROIs and Overlays is described in Selections.

### 27.12.1 Select All [a]

Creates a rectangular selection that is the same size as the image.

### 27.12.2 Select None [A]

Deactivates the selection in the active image.

### 27.12.3 Restore Selection [E]

Restores the previous selection to its original position. A selection is saved when you:

- Delete the selection by clicking outside of it
- Draw a new selection
- De-activate the image containing the selection
- Close the image containing the selection
- Use a command that deletes or modifies the selection

SEE ALSO: Analyze ▷ Tools ▷ ROI Manager...

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### 27.12.4 Fit Spline

Fits a cubic spline curve to a polygon or polyline selection (*see* ROI manipulations).

SEE ALSO: Straighten..., Interpolate





**XII TRANSFERRING SELECTIONS BETWEEN IMAGES**

You can transfer a selection from one image to another by activating the image with the selection, activating the destination image, then pressing Shift E (the keyboard shortcut for Edit ▷ Selection ▷ Restore Selection [E]). This shortcut can also be used to restore accidentally deleted ROIs. Alternative ways to transfer ROIs across images involve the ROI Manager and the cursor synchronization features provided by Analyze ▷ Tools ▷ Synchronize Windows.

### 27.12.5 Fit Circle

Fits a circle to a multipoint (with at least 3 points) or area selection [14] (*see* ROI manipulations). Composite selections are not supported. With open shapes (lines and points), the fitting algorithm (*Newton-based Pratt* fit) described in Pratt V, "Direct least-squares fitting of algebraic surfaces", *Computer Graphics*, Vol. 21, pp 145–152 (1987) is used. With closed shapes, the command creates a circle with the same area and centroid of the selection.

IMPROVED IN IJ 1.46R

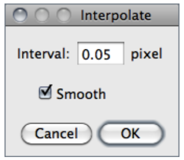
### 27.12.6 Fit Ellipse

Replaces an area selection with the best fit ellipse (*see* ROI manipulations). The ellipse will have the same area, orientation and centroid as the original selection. The same fitting algorithm is used to measure the major and minor axis lengths and angle when *Fit Ellipse* is selected in Analyze ▷ Set Measurements...

SEE ALSO: DrawEllipse macro

NEW IN IJ 1.46R

### 27.12.7 Interpolate




Converts the active selection into a sub-pixel resolution ROI of floating-point coordinates spaced *interval* pixels apart. If *Smooth* is checked, traced and freehand selections (*see* Area Selection Tools) are first smoothed using a 3–point running average. Refer to Selections With Sub-pixel Coordinates for more details.

SEE ALSO: Floating point selections, Fit Spline, Edit ▷ Options ▷ Profile Plot Options...

### 27.12.8 Convex Hull

Replaces a polygon of freehand selection with its convex hull (*see* ROI manipulations), determined by the gift wrap algorithm. The convex hull can be thought of as a rubber band wrapped tightly around the points that define the selection.

SEE ALSO: Fit Ellipse, `ConvexitySolidarity` macro, Convex_Hull_Plus plugin

### 27.12.9 Make Inverse

Creates an inverse selection (*see* ROI manipulations). What is 'inside' the selection will be 'outside', and vice versa.

### 27.12.10 Create Mask

Creates a new 8–bit image called 'Mask' whose pixels have a value of 255 inside the selection and 0 outside (*see* ROI manipulations). By default, this image has an inverting LUT, so black is 255 and white is 0 unless *Black Background* in Process ▷ Binary ▷ Options. . . is checked.

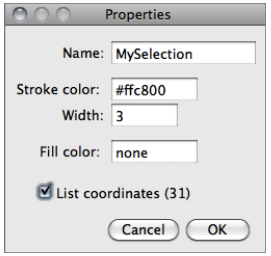
SEE ALSO: Process ▷ Binary ▷ Convert to Mask, XX Creating Binary Masks, XXII Interpreting Binary Images

### 27.12.11 Create Selection

Creates a selection from a thresholded image or a binary mask [15].

IMPROVED IN IJ 1.46R

### 27.12.12 Properties. . . [y]




Opens a dialog box that allows the user to assign a contour color (*Stroke color*) and a contour width (*Width*) to the active selection or a filling color. Note that selections can be either filled or contoured, but not both. The default selection colors (*black*, *blue*, *cyan*, *green*, *magenta*, *orange*, *red*, *white*, *yellow*) can be typed textually. Any other color must be typed using hex notation (*see* XIX Hexadecimal Color Values).

Set *Stroke width* to 0 to have selections drawn using a width of one pixel regardless of the image magnification (*see* XVIII Working with Zoomed Canvases).

With Text Tool selections, it is also possible to specify the font size and text alignment. Choose *List coordinates* to retrieve a dedicated table of XY coordinates from the active selection at evenly spaced one pixel intervals.

Note that while this command can only be applied to the active selection, the ROI Manager's *Properties. . .* command (Analyze ▷ Tools ▷ ROI Manager. . . ) can be applied to multiple ROIs.

SEE ALSO: Selections, Image ▷ Overlay ▷ Add Selection. . . [b], File ▷ Import ▷ XY Coordinates. . . , File ▷ Save As ▷ XY Coordinates. . .

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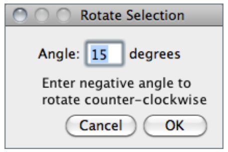
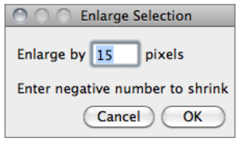
### 27.12.13 Rotate. . .




Rotates the selection (using floating-point coordinates) by the specified number of degrees. A negative number indicates counter-clockwise rotation. This command runs the RotateSelection macro in `ij.jar`.

SEE ALSO: FlipSelection macro

### 27.12.14 Enlarge. . .




Grows an area selection by a specified number of pixels. Enter a negative value to shrink the selection. This command runs the EnlargeSelection macro in `ij.jar`. ShrinkSelection is a variation of this macro that does not shrink polygonal selections from the edges of the image.

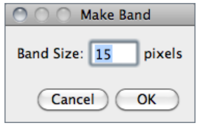




**XIII CONVERTING COMPOSITE SELECTIONS**

Enter zero in the Edit ▷ Selection ▷ Enlarge. . . dialog box to convert Composite Selections into polygon selections. Note, however, that the conversion may fail if the composite ROI is composed of more than one piece and/or contains internal holes.

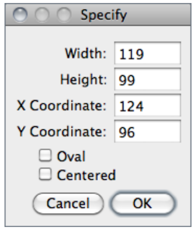
### 27.12.15 Make Band. . .




Takes an area selection and creates a band with a thickness of the specified number of pixels (*see* ROI manipulations). If you imagine the band as a doughnut shape, then the original selection corresponds to the hole (i.e. the band is made by growing out the original selection).

SEE ALSO: MakeSelectionBand, the macro that implemented this command in previous IJ versions.

### 27.12.16 Specify. . .




Opens a dialog that allows the user to define a rectangular or elliptical selection. *Width* and *Height* are the dimensions of the selection. *X Coordinate* and *Y Coordinate* define the position of the selection. Check *Oval* to create an elliptical selection. If *Centered* is checked, the selection is positioned so *X Coordinate* and *Y Coordinate* define the center of the selection, otherwise they define the upper left corner.

This command is also available through the ROI Manager *More≫* drop-down menu (*see* Analyze ▷ Tools ▷ ROI Manager. . . ).

### 27.12.17 Straighten. . .

This command straightens a curved object in an image (*see* Straightening filamentous objects). The curved object must first be outlined using the Segmented Line Selection Tool. Double click on the line tool icon to open the Image ▷ Adjust ▷ Line Width. . . widget, in order to adjust the width of the line selection. By default, the Straighten. . . command fits a cubic spline curve to the points that define the line, so it is not necessary to check the *Spline Fit* checkbox. Note that Straighten. . . also works with straight line selections. In this case, the object defined by the line selection is rotated to be horizontal.

SEE ALSO: Straighten plugin, Image ▷ Transform ▷

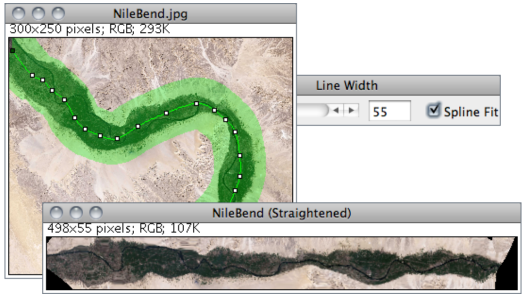
### 27.12.18 To Bounding Box

Converts a non-rectangular selection to the smallest rectangle that completely contains it.

IMPROVED IN IJ 1.46R

### 27.12.19 Line to Area

Converts a line selection to an area (traced) ROI.




**Edit ▷ Selection ▷** Straighten. . . . As described in Segmented Line Selection Tool, the points of a polyline selection can be repositioned (dragged), deleted (using Alt-click) or duplicated (using Shift-click). Press Shift E (Edit ▷ Selection ▷ Restore Selection [E]) to restore accidentally deleted lines.

### 27.12.20 Area to Line

Converts a non-composite area selection to its enclosing outline (*see* ROI manipulations). The obtained line will have the width specified in the Image ▷ Adjust ▷ Line Width. . . widget. Note that by design Area to Line does not create closed paths. E.g., the converted outline of a rectangular selection will be composed of only three segments, with the first and fourth corner points of the rectangle being disconnected.

SEE ALSO: Composite Selections, Line to Area

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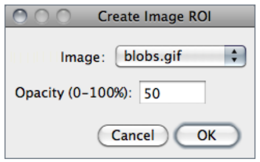
### 27.12.21 Image to Selection. . .




Creates an image selection (ImageROI). Image selections are Overlays that can be moved around the canvas (*see* Image ▷ Overlay ▷ Add Image. . . ). Once created, opacity of the blended image can be re-adjusted at any time using Edit ▷ Selection ▷ Properties. . . [y]. Use Shift E (Edit ▷ Selection ▷ Restore Selection [E]) to recover the blending image after clicking outside its limits. Use Shift F (Flatten [F]) to finally embed the imageROI.

Note that image selections behave only partially as regular selections (e.g., can be added to the ROI Manager list, can be moved beyond image boundaries but cannot be resized or rotated). However they are stored in the TIFF header and can be saved and restored when saving images in TIFF format.

SEE ALSO: Paste Control. . . (Blend transfer mode), Image ▷ Stacks ▷ Tools ▷ Insert. . . , ROI Manager. . .

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### 27.12.22 Add to Manager [t]

Adds the current selection to the ROI Manager (Analyze ▷ Tools ▷ ROI Manager. . . ). If there is no selection the ROI Manager is opened.

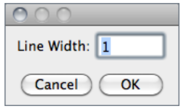




## 27.13 Options ▷

Use commands in this submenu to change various ImageJ user preference settings.

### 27.13.1 Line Width. . .




Displays a dialog box that allows to change the line width (in pixels) of line selections (*see* Line Selection Tools) and concomitantly the lines generated by the Edit ▷ Draw [d] command. This legacy command has been superseded by the Image ▷ Adjust ▷ Line Width. . . widget, but required since the latter is not recordable (*see* Plugins ▷ Macros ▷ Record. . . ).

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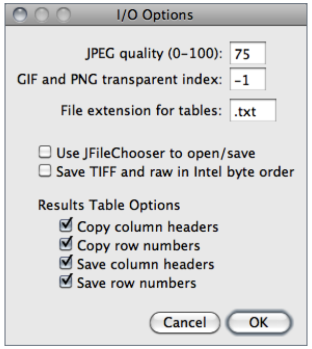
### 27.13.2 Input/Output. . .




***JPEG quality (0–100)*** Specifies the compression level used by File ▷ Save As ▷ Jpeg. . . Requesting a higher degree of compression (a lower value) will result in smaller files, but poorer image quality. Note that lossy JPEG compression creates serious artifacts, *see* II Image Types: Lossy Compression and Metadata.

***GIF and PNG transparent index*** Specifies the transparent color used for images saved in GIF and PNG formats. Use -1 for "none". Note that PNG and GIF transparency only works with 8–bit images.

***File extension for tables*** Sets the default extension to be used when saving Results Tables. Files with `.txt` and `.xls` extensions are saved in tab-delimited format and files with `.csv` extensions are saved in comma-delimited format.

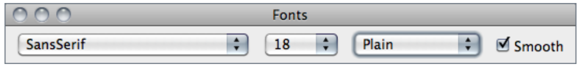
***Use JFileChooser to open/save*** Enables versions of File ▷ Open and File ▷ Save As that use the Java Swing's `JFileChooser` instead of the native OS dialogs. The main advantage of `JFileChooser` is the ability to open multiple files by Shift-clicking to select multiple contiguous files and control-clicking to select more than one individual file. On the other hand, it is slower, uses more memory, and does not behave like the file open and save dialogs used in other applications. It requires Java 2, which is included with the Linux and Windows distributions of ImageJ and is built into Mac OS X.

***Save TIFF and raw in intel byte order*** Specifies the byte order used when saving 16–bit and 32–bit images using File ▷ Save As ▷ Raw Data. . . , or File ▷ Save As ▷ Image Sequence. . . when *Raw* is chosen as the format. Check this option to export images using the order used by Intel ×86 based processors (little-endian). This Wikipedia article has more information.

***Results Table Options*** Specifies if column headers and row numbers should be saved or copied from ImageJ tables such as the *Results* and *Summarize* windows (*see* Results Table).

### 27.13.3 Fonts...

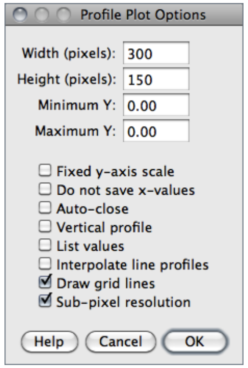
Opens a small widget with three pop-up menus for specifying the typeface, size, style and antialiasing (*Smooth* checkbox) of the font used by the Text Tool and Image ▷ Stacks ▷ Label... It is also possible to adjust the horizontal text alignment using the *style* drop-down menu: *Left* (the default), *Right*, or *Centered*. The widget is more easily opened by double clicking on the Text Tool.




### 27.13.4 Profile Plot Options...

Use this dialog to control how plots generated by ImageJ are displayed (Image ▷ Stacks ▷ Plot Z-axis Profile..., Analyze ▷ Plot Profile [k], Analyze ▷ Calibrate..., Analyze ▷ Tools ▷ Curve Fitting..., *Multi Plot* [Analyze ▷ Tools ▷ ROI Manager...], etc.).

***Plot Width* and *Plot Height*** Specify the length (in pixels) of the X-axis (*Plot Width*) and Y-axis (*Plot Height*).




***Fixed y-axis Scale*** If checked, the Y-axis range is fixed and the specified ***Minimum Y*** and ***Maximum Y*** values are used, otherwise, plots are scaled based on the minimum and maximum gray values.

***Do not Save x–values*** If checked, '*List*', '*Save...*' and '*Copy...*' buttons will appear in profile plot windows.

***Auto–close*** If checked, profile plot windows will be automatically closed when 'List', 'Save' and 'Copy' are clicked on.

***Vertical Profile*** If checked, row average plots of rectangular areas (or line selections wider than 1 pixel) will be generated instead of the default column average plots. Note that evoking Plot Profile [k] with Alt B will generate vertical profiles.

***List values*** *If checked,* the list of values will be automatically opened. If *Auto–close* is also checked, the plot is closed and only the list of values remains open.

***Interpolate line profiles*** If checked, Analyze ▷ Plot Profile [k] will use bilinear interpolation to retrieve intensity values along non-straight line selections.

***Draw grid lines*** If checked, gray grid lines will be drawn in the plot.

New in IJ 1.46r

***Sub-pixel resolution*** If checked, line selections created on zoomed images will use floating-point coordinates, resulting in smoother curves (*see* Selections With Sub-pixel Coordinates and Edit ▷ Selection ▷ Interpolate).

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/edit.html#plot-options.





### 27.13.5 Rounded Rect Tool...

*See* Rounded Rectangular Selection Tool.

### 27.13.6 Arrow Tool...

*See* Arrow Tool.

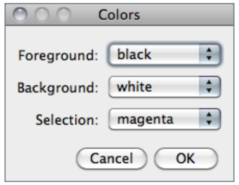
### 27.13.7 Point Tool...

*See* Point Tool.

### 27.13.8 Wand Tool...

*See* Wand Tool.

### 27.13.9 Colors...




Displays a dialog box that allows you to set *Foreground*, *Background* and *Selection color*. As mentioned earlier, the selection color is highlighted in the Point Tool and Wand Tool icons. Drawing colors are displayed in the Color Picker Tool (foreground and background colors) and drawing tools such as the Arrow, Brush, Flood Filler and Pencil (foreground color only).

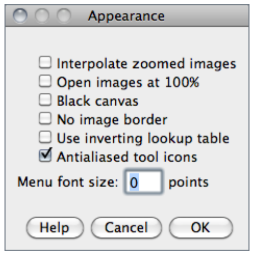
SEE ALSO: Image ▷ Color ▷ Color Picker... [K], Using a Keyboard Shortcut to Change Selection Color

**(7) Using a Keyboard Shortcut to Change Selection Color**

```
/* This macro loops through the all the possible Selection colors using ↩
   "q" as a keyboard shortcut */
 var cIdx;
macro "Change Selection Color [q]" {
  color= newArray("red", "green", "blue","magenta", "cyan", "yellow", ↩
    "orange", "black", "white");
  run("Colors...", "selection="+ color[cIdx++]);
  if (cIdx==color.length) cIdx= 0;
}
```

### 27.13.10 Appearance...

Improved in IJ 1.46r




This dialog contains options that control how images are displayed, an option to display better looking toolbar icons, and an option to set the menu font size.

***Interpolate zoomed images*** Uses interpolation instead of pixel replication when displaying zoomed images.

***Open Images at 100%*** Newly open images are displayed using 100% magnification (1 image pixel = 1 screen pixel).

***Black Canvas*** Causes the image canvas (white by default) to be rendered in black. This is useful when looking at X-ray images in order to avoid high contrasting intensities at the image edges.

***No image border*** Displays images without the default one pixel wide black border.

***Use inverting lookup table*** Causes newly opened 8–bit images to have inverted pixel values, where white= 0 and black= 255. This is done by both inverting the pixel values and inverting the LUT. Use the Image ▷ Lookup Tables ▷ Invert LUT command to invert an image without changing the pixel values.

***Double Buffer Selections*** Reduces flicker when working with complex selections but it also increases memory usage and slows screen updates. It is not needed on Mac OS X, which has built in double buffering.

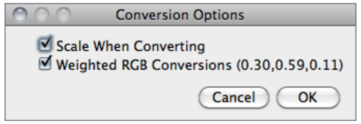
***Antialiased tool* icons** Smooths and darken the tool icons in the Main ImageJ window. This option is enabled by default on all operating systems. On Windows XP, enable Clear Type sub-pixel anti-aliasing to improve the quality of text in menus.

***Menu font size*** Specifies the size of the ImageJ window menu font. Use a size of 0 (zero) to use Java's default menu font size. Changing the font size requires the restarting of ImageJ. This option is ignored on Mac OS X.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/edit.html#appearance.

SEE ALSO: Customizing the ImageJ Interface

### 27.13.11 Conversions...




Use this dialog to set options that control how images are converted from one type to another.

***Scale When Converting*** ImageJ will scale from min–max to 0–255 when converting from 16–bit or 32–bit to 8–bit or to scale from min–max to 0–65535 when converting from 32–bit to 16–bit. Note that *Scale When Converting* is always checked after ImageJ is restarted.

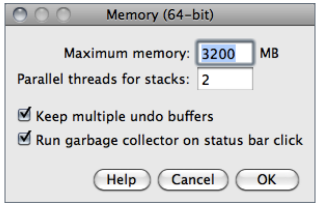
***Weighted RGB Conversions (0.30, 0.59, 0.11)*** When checked, the formula gray $= 0.299 \times$ red $+ 0.587 \times$ green $+ 0.114 \times$ blue is used to convert RGB images to grayscale. If it is not checked, the formula gray $=$ (red $+$ green $+$ blue)/3 is used. The default weighting factors $(0.299, 0.587, 0.114)$, which are based on human perception, are the ones used to convert from RGB to YUV, the color encoding system used for analog television. The weighting factors can be modified using the `setRGBWeights()` macro function.

SEE ALSO: Image Types and Formats, Embedding Color Annotations in Grayscale Images





### 27.13.12 Memory & Threads...




Use this dialog to specify the maximum amount of memory available to ImageJ and the number of threads used by filters when processing stacks. Java applications such as ImageJ will only use the memory allocated to them (typically 640 MB) but this dialog allows the user to allocate more than the default.

Note that specifying more than 75% of real RAM could result in virtual RAM being used, which may cause ImageJ to become slow and unstable. Also note that this dialog cannot be used to set the memory allocation if ImageJ is run from the command line or by double clicking on `ij.jar`.

***Maximum memory*** 64-bit OS and a 64–bit version of Java are required to use more than ≈1700 MB of memory. Windows users must be running a 64–bit version of Windows and must install a 64–bit version of Java. Mac users must be running OS X 10.5 or later and may need to use the Java Preferences utility (in /Applications/Utilities/Java) to select a 64–bit version of Java. They may also need to switch to the ImageJ64 application. Linux users need to be running 64–bit versions of Linux and Java. The title of the *Memory & Threads* dialog box changes to *Memory (64–bit)* when ImageJ is running on a properly configured 64–bit system.

***Parallel threads for stacks*** Determines the number of parallel threads used by commands in the Process ▷ Filters ▷ and the Process ▷ Math ▷ submenus when processing stacks. The default value is the number of available processors.

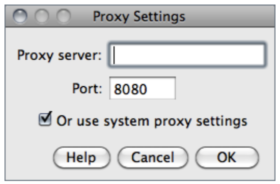
***Keep multiple undo buffers*** If checked, the undo buffer will be preserved when switching images. Edit ▷ Undo [z] remains restricted to the most recent operation, but is available for each opened image, as long as the buffer allows it. If *Keep multiple undo buffers* is unchecked, the undo buffer is reset every time the active (frontmost) image changes.

***Run garbage collector on status bar click*** If checked, forces the Java garbage collector to run every time the user clicks on the Status bar, which may help to reclaim unused memory (*see also* Plugins ▷ Utilities ▷ Monitor Memory...).

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/edit.html#memory.

SEE ALSO: FAQs on the ImageJ wikipage

### 27.13.13 Proxy Settings...

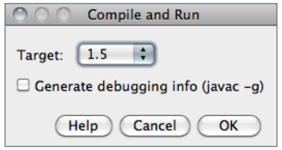



Use this dialog to modify the proxy settings of the Java Virtual Machine. This may be required for ImageJ to connect to the internet in certain machines running behind HTTP proxies. For example, proxy settings may be required to update ImageJ using the Help ▷ Update ImageJ... command or to open the images in the File ▷ Open Samples ▷ submenu.

To use the system proxy settings enable the *Or use system proxy settings* option (this will set the `java.net.useSystemProxies` property to `true`). To configure your proxy settings manually specify the address of the HTTP proxy in *Proxy server* and the port the proxy listens on (normally 8080) in *Port*. Settings will be saved in the ImageJ preferences file (`IJ_Prefs.txt`).

### 27.13.14 Compiler...

Displays a dialog box with options for the Plugins ▷ Compile and Run... command.

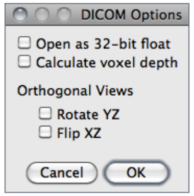



***Target*** Specifies the Java version of the class files created by Plugins ▷ Compile and Run... Plugins compiled with a *Target* of 1.6 will not run on earlier versions of Java. A *Target* of 1.4 should be used to create plugins capable of running on all versions ImageJ. *Target* cannot be set higher than the version of Java ImageJ is currently running on.

***Generate Debugging Info (javac -g)*** If checked, information needed by Java debuggers will be included in the class files.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/edit.html#compiler.

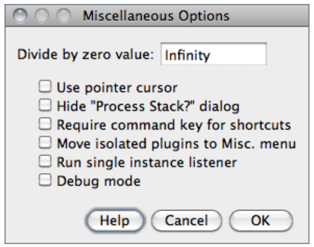
### 27.13.15 DICOM...




This dialog sets options related to the handling of DICOM images. Namely, if ImageJ should open DICOM images as 32–bit float, if voxel depth should be calculated (based on the distance between the first and last slice) and if coronal/transverse sections should be mirrored when using the Image ▷ Stacks ▷ Orthogonal Views [H] command. With IJ 1.45, the DICOM reader applies the Rescale Slope value when *Open as 32-bit float* is enabled and tag 0028,1053 is not 1.0.

See also: Image Types and Formats

### 27.13.16 Misc...




Displays a dialog box for configuring several (advanced) settings that do not fit elsewhere in the ImageJ interface.

***Divide by zero value*** Specifies the value used when Process ▷ Image Calculator... detects a divide by zero while dividing one 32–bit real image by another. The default is infinity. In addition to numeric values, 'infinity' (positive or negative infinity), 'max' (largest positive value) and 'NaN' (Not-a-Number) can be entered as the *Divide by zero value*.

***Use pointer cursor*** If checked, ImageJ will use an arrow cursor instead of the default crosshair that is sometimes difficult to see on grayscale images in areas of medium brightness. This option can also be used to work around a bug on Windows where the text cursor is sometimes used in place of the crosshair. Note that the default crosshair can be customized as explained in Customizing the ImageJ Interface.





***Hide "Process Stack?" dialog*** If checked, ImageJ will suppress the dialog that asks 'Process all xx slices?' (only the current slice will be processed).

***Require control / command key for shortcuts*** If checked, requires the Control key (Command key on Macs) to be pressed when using keyboard shortcuts for menu commands.

***Move isolated plugins to Misc. menu*** This option can reduce the size of the Plugins menu, preventing it from running off the bottom of the screen. When this option is enabled, plugins that attempt to install themselves in a submenu with only one command are instead installed in the Plugins ▷ Miscellaneous submenu. An example of such a plugin is TurboReg, which normally creates a Plugins ▷ TurboReg submenu that contains only one command.

***Run single instance listener*** If checked, ImageJ will use sockets to prevent multiple instances from being launched [16]. On Windows, this avoids the problem where another copy of ImageJ starts each time an image is dragged and dropped on the ImageJ icon. It also prevents multiple instances when running ImageJ from the command line. Note that you may get a security alert the first time ImageJ starts with this option enabled. ImageJ does not require external socket access so it is okay to deny it access in the security alert. This option is set by default with new Windows installations. Improved in IJ 1.46r

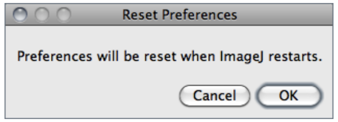
***Debug mode*** If checked, causes ImageJ to display debugging messages in the Log Window. In addition, some commands (e.g., Process ▷ Binary ▷ Skeletonize and Watershed) produce detailed outputs when *Debug mode* is active. Close the Log window to disable display of debugging messages.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/edit.html#misc.

See also: Customizing the ImageJ Interface

### 27.13.17 Reset...

New in IJ 1.46r




Causes the IJ preferences file (`IJ_prefs.txt`) to be deleted when ImageJ quits, resetting all parameters to their default values when ImageJ is restarted.

As mentioned in Settings and Preferences, `IJ_prefs.txt` holds all the settings and preferences of ImageJ and is stored in `~/Library/Preferences/` on Mac OS X, in `~/.imagej/` on Linux and Windows (with ~ referring to the user's home directory). Several macros and plugins also write parameters to this file.

See also: (3) Ensuring Specific Settings at Launch, Plugins ▷ Utilities ▷ Reset...

# 28 Image ▷

## 28.1 Type ▷

Use this submenu to determine the type of the active image or to convert it to another type. An attempt to perform an unsupported conversion causes a dialog box to be displayed that lists the possible conversions.

**Supported conversions in ImageJ (Image ▷ Type ▷ submenu).** Note that ImageJ2 supports many more types of image data.

| ▽From To▷ | 8–bit | 16–bit | 32–bit | 8–bit color | RGB color | RGB stack | HSB stack |
|---|---|---|---|---|---|---|---|
| **8–bit** | ... | I, S | I, S | | I, S | | |
| **16–bit** | I, S | ... | I, S | | I, S | | |
| **32–bit** | I, S | I, S | ... | | I, S | | |
| **8–bit color** | I, S | | | ... | I | | |
| **RGB color** | I, S | | | I, S | ... | I, S | I, S |
| **RGB stack** | | | | | I | ... | |
| **HSB stack** | | | | | I | | ... |

I: Single images only; S: Stacks

***8–bit*** Converts to 8–bit grayscale. ImageJ converts 16–bit and 32–bit images to 8–bit by linearly scaling from min–max to 0–255, where *min* and *max* are the two values displayed in the Image ▷ Adjust ▷ Brightness/Contrast... [C]. Image ▷ Show Info... [i] displays these two values as *Display range*. Note that this scaling is not done if *Scale When Converting* is not checked in Edit ▷ Options ▷ Conversions... RGB images are converted to grayscale using the formula gray $= (\text{red} + \text{green} + \text{blue})/3$ or gray $= 0.299 \times \text{red} + 0.587 \times \text{green} + 0.114 \times \text{blue}$ if *Weighted RGB Conversions* is checked in Edit ▷ Options ▷ Conversions...

***16–bit*** Converts to unsigned 16–bit grayscale.

***32–bit*** Converts to signed 32–bit floating-point grayscale.

***8–bit Color*** Converts to 8–bit indexed color using Heckbert's median-cut color quantization algorithm. A dialog box allows the number of colors (2–256) to be specified. The active image must be RGB color.

***RGB Color*** Converts to 32–bit RGB color.

***RGB Stack*** Converts to a 3–slice (red, green, blue) stack. The active image must be RGB color.

***HSB Stack*** Converts to a 3–slice (hue, saturation and brightness) stack. The active image must be RGB color.

## 28.2 Adjust ▷

This submenu contains commands that adjust brightness/contrast, threshold levels and image size.



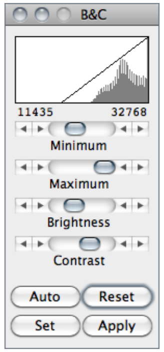


**XIV Applying Auto Brightness/Contrast to Entire Stacks**

The Process ▷ Enhance Contrast... command can be used to adjust the brightness and contrast of each slice in a stack, according to either the optimal for each individual slice (if *Use Stack Histogram* is unchecked) or the overall stack (by ticking *Use Stack Histogram*). The default behavior of the B&C tool (Image ▷ Adjust ▷ Brightness/Contrast... [C]) is to use the overall stack histogram.

### 28.2.1 Brightness/Contrast... [C]

Use this tool to interactively alter the brightness and contrast of the active image. With 8–bit images, brightness and contrast are changed by updating the image's lookup table (LUT), so pixel values are unchanged. With 16–bit and 32–bit images, the display is updated by changing the mapping from pixel values to 8–bit display values, so pixel pixel values are also unchanged. Brightness and contrast of RGB images are changed by modifying the pixel values.




***Histogram*** The line graph at the top of the window, which is superimposed on the image's histogram, shows how pixel values are mapped to 8–bit (0–255) display values. The two numbers under the plot are the minimum and maximum displayed pixel values. These two values define the display range, or 'window'. ImageJ displays images by linearly mapping pixel values in the display range to display values in the range 0–255. Pixels with a value less than the minimum are displayed as black and those with a value greater than the maximum are displayed as white.

***Minimum and Maximum sliders*** Control the lower and upper limits of the display range. Holding down Shift will simultaneously adjust all channels of a composite image (e.g., File ▷ Open Samples ▷ HeLa Cells (1.3 M, 48–bit RGB)).

***Brightness slider*** Increases or decreases image brightness by moving the display range. Holding down Shift will simultaneously adjust all channels of a composite image.

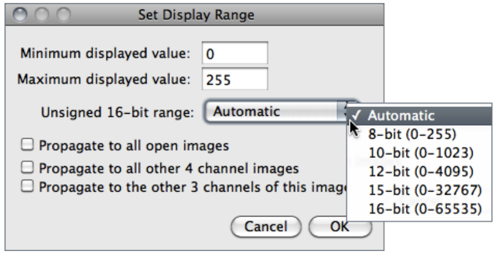
***Contrast slider*** Increases or decreases contrast by varying the width of the display range. The narrower the display range, the higher the contrast. Holding down Shift will simultaneously adjust all channels of a composite image.

***Auto*** ImageJ will automatically optimize brightness and contrast based on an analysis of the image's histogram. Create a selection, and the entire image will be optimized based on an analysis of the selection. The optimization is done by allowing a small percentage of pixels in the image to become saturated (displayed as black or white). Each additional click on *Auto* increases the number of saturated pixels and thus the amount of optimization.
A `run("Enhance Contrast", "saturated=0.35")` macro call is generated if the command recorder (Plugin ▷ Macro ▷ Record...) is running.

***Reset*** Restores the original brightness and contrast settings. The display range is set to the full pixel value range of the image. A `resetMinAndMax()` macro call is generated if the command recorder is running. Holding down Shift restores original settings in all channels of a composite image.

***Set*** Allows to enter the minimum and maximum display range values in a dialog box. A `setMinAndMax()` macro call is generated if the command recorder is running.

A 16–bit image consists of 65536 possible gray levels. Most of times, however, the relevant image information is contained only within a narrow range of the grayscale. This is the case, e.g., in low light microscopy, in which signal is restricted to the lower end of the grayscale. The *Set Display Range* dialog allows you to choose how to scale the range of gray levels of 16–bit images.




***Automatic*** Automatically selects the best range given the intensity values of the image based on the percentage of the total number of pixel values from the lowest to highest pixel value.

***8–bit (0–255)*** Gray level range of 0–255.

***10–bit (0–1023)*** Gray level range of 0–1023.

***12–bit (0–4095)*** Gray level range of 0–4095.

***15–bit (0–32767)*** Gray level range of 0–32767.

**16–bit (0-65535)** Gray level range of 0–65535.

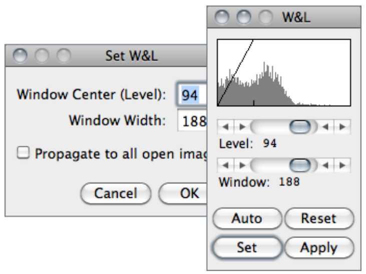
IMPROVED IN IJ 1.46R

Check ***Propagate to all open images*** to apply these values to the rest of the images currently open. With multi-channel images, the option to propagate the specified range to the remaining channels is also available.

***Apply*** Applies the current display range mapping function to the pixel data. If there is a selection, only pixels within the selection are modified. This option currently only works with 8–bit images, 8–bit stacks and RGB stacks. **This is the only B&C option that alters the pixel data of non-RGB images**.

SEE ALSO: Window/Level. . . , Enhance Contrast. . . , Color Balance. . . , XIV Applying Auto Brightness/Contrast to Entire Stacks, XVI Brightness/Contrast of High Bit–Depth Images, XV Display Range of DICOM Images

### 28.2.2 Window/Level. . .




This command and Brightness/Contrast. . . [C] (*B&C*) are redundant, but Window/Level. . . (W&*L*) behaves in a manner closer to that implemented on medical image terminals by interactively adjusting the *Window* – range of minimum and maximum (*Contrast*) – and *Level* – position of that range in the grayscale intensity space (*Brightness*).

If the *B&C* window is opened, it will be closed and the *W&L* window will be opened at the same location.





### XV Display Range of DICOM Images

With DICOM images, ImageJ sets the initial display range based on the *Window Center* (0028, 1050) and *Window Width* (0028, 1051) tags. Click *Reset* on the W&L (Image ▷ Adjust ▷ Window/Level. . . ) or B&C (Image ▷ Adjust ▷ Brightness/Contrast. . . [C]) window and the display range will be set to the minimum and maximum pixel values.

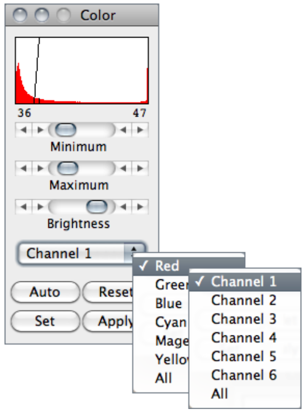
As an example, the File ▷ Open Samples ▷ CT (420K, 16–bit DICOM) image has a Window Center of 50 and Window Width of 500, so the display range is set to -200 to 300 (center-width/2 to center+width/2). Click *Reset* and the display range is set to -719 to 1402. Press H (Analyze ▷ Histogram [h]) and you will see that the minimum pixel value in the image is -719 and the maximum is 1402.

To display the DICOM tags, press I (Image ▷ Show Info. . . [i]). Press R (File ▷ Revert [r]) to revert to the initial display range.

SEE ALSO: Enhance Contrast. . . , Color Balance. . . , XIV Applying Auto Brightness/Contrast to Entire Stacks, XVI Brightness/Contrast of High Bit–Depth Images,XV Display Range of DICOM Images

### 28.2.3 Color Balance. . .

This panel makes adjustments to the brightness and contrast of a single color of a standard RGB image (8–bit per color channel).




For multi-channels Stacks and Hyperstacks (Color Composite Images) it adjusts each of the color channels independently. Use the drop-down menu to specify which color / channel will be adjusted (the histogram is drawn for the selected channel).

*Maximum* and *Minimum* sliders, *Auto*, *Set* and *Apply* work as described for Image ▷ Adjust ▷ Brightness/Contrast. . . [C]. Similarly to the Window/Level. . . tool, if the *B&C* window is opened, it will be closed and the *Color* window will be opened at the same location.

NB: When switching from one color to another, the changes made to one color will be lost unless *Apply* is clicked before. Also, note that for 48–bit color images that load as a stack, Brightness/Contrast. . . [C] works on single stack slices, i.e., colors, and the color settings of the *Color* panel are ignored.

SEE ALSO: Brightness/Contrast. . . [C], Color ▷ submenu

### 28.2.4 Threshold. . . [T]

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Use this tool to automatically or interactively set lower and upper threshold values, segmenting grayscale images into features of interest and background. Use Analyze ▷ Measure. . . [m]) (with *Limitto Threshold* in Analyze ▷ Set Measurements. . . checked) to measure the aggregate of the

### XVI Brightness/Contrast of High Bit–Depth Images

When displayed, the intensity of each pixel that is written in the image file is converted into the *grayness* of that pixel on the screen. How these intensities are interpreted is specified by the image type. From the ImageJ website:

> 16–bit and 32–bit grayscale images are not directly displayable on computer monitors, which typically can show only display 256 shades of gray. Therefore, the data are mapped to 8–bit by windowing. The window defines the range of gray values that are displayed: values below the window are made black, while values above the window are white. The window is defined by minimum and maximum values that can be modified using Image ▷ Adjust ▷ Brightness/Contrast. . . [C].

It may happen that the initial windowing performed by ImageJ on these high bit–depth (or HDR) images is suboptimal. Please note that windowing does not affect image data (cf. the HDRexplorerTool).

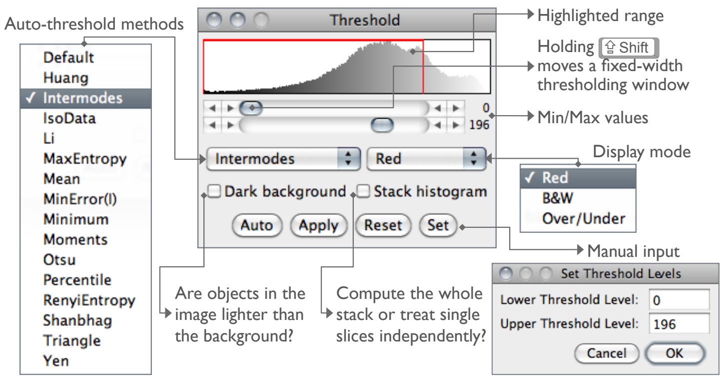



**Image ▷ Adjust ▷ Threshold. . . [T] (ImageJ 1.45m).**

selected features. Use Analyze ▷ Analyze Particles. . . to measure features individually. Use the Wand Tool to outline a single feature.

***Upper slider*** Adjusts the minimum threshold value. Hold Shift while adjusting the minimum to move a fixed-width thresholding window across the range of gray values.

***Lower slider*** Adjusts the maximum threshold value.

***Method*** Allows any of the 16 different automatic thresholding methods to be selected [18]. These methods are described on Fiji's Auto Threshold website. The *Default* method is the modified IsoData algorithm used by ImageJ 1.41 and earlier. Note that these are global thresholding methods that typically cannot deal with unevenly illuminated images (such as in brightfield microscopy). In these cases, local algorithms are more appropriated, by allowing the threshold to smoothly vary across the image.. These are implemented by the Auto Local Threshold plugin, pre-installed in Fiji.

***Display*** Selects one of three display modes:





***Red*** Displays the thresholded values in red.

***B&W*** Features are displayed in black and background in white. This mode respects the *Black background* flag set in Process ▷ Binary ▷ Options. . .

***Over/Under*** Displays pixels below the lower threshold value in blue, thresholded pixels in grayscale, and pixels above the upper threshold value in green. These colors can be changed from a macro by calling the `ImageProcessor.setOverColor()` and `setUnderColor()` methods (example).

***Dark background*** To be checked when features are lighter than the background. The state of the checkbox is remembered across restarts.

***Stack histogram*** If checked, ImageJ will first compute the histogram of the whole stack (or hyperstack) and then compute the threshold based on that histogram. As such, all slices are binarized using the single computed value. If unchecked, the threshold of each slice is computed separately.

***Auto*** Uses the currently selected thresholding method to automatically set the threshold levels based on an analysis of the histogram of the current image or selection.

***Apply*** Sets thresholded pixels to black and all other pixels to white. For 32–bit float images *Apply* will also run Process ▷ Math ▷ NaN Background.

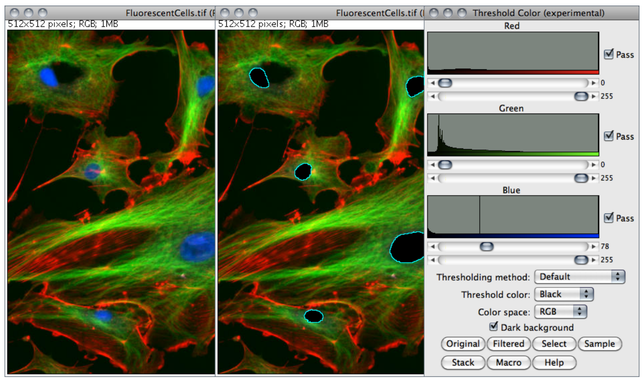
***Reset*** Disables thresholding and updates the histogram.

***Set*** New threshold levels can be entered into this dialog box.

See also: XX Creating Binary Masks, Color Threshold. . . , Wand Tool, Analyze ▷ Analyze Particles. . .

### 28.2.5 Color Threshold. . .

Thresholds 24–bit RGB images based on Hue Saturation and Brightness (HSB), Red Green and Blue (RGB), CIE Lab or YUV components. Ranges of the filters can be set manually or based on the pixel value components of a user-defined ROI. This command, implemented in version 1.43l, is an experimental built-in version of the Threshold Colour plugin [20] and is not yet fully integrated into ImageJ.




**Segmentation of DAPI stained nuclei using Image ▷ Adjust ▷ Color Threshold. . .**

***Pass*** If checked, values within range are thresholded and displayed (band-pass filter), otherwise, values outside the selected range are thresholded (band-reject filter).

***Thresholding Method*** Allows any of the 16 different automatic thresholding methods to be selected (*see* Threshold... [T]).

***Threshold Color*** Selects the threshold color: either *Red*, *Black*, *White* or *Black & White* (*see* Threshold... [T]).

***Color space*** Selects the color space: *HSB*, *RGB*, *CIE Lab* or *YUV* (*see* Color Spaces and Color Separation).

***Dark background*** To be checked when features are lighter than the background (*see* XXII Interpreting Binary Images). The state of the checkbox is remembered across restarts.

***Original*** Restores the original image and updates the buffer when switching to another image.

***Filtered*** Shows the filtered image. Note that the final thresholded image type is RGB, not 8–bit gray (*see* Image Types and Formats).

***Select*** Creates a ROI selection based on the current settings. The selection is made according to the settings defined in the Process ▷ Binary ▷ Options... dialog.

***Sample*** (Experimental) Sets the ranges of the filters based on the pixel value components in a user-defined ROI.

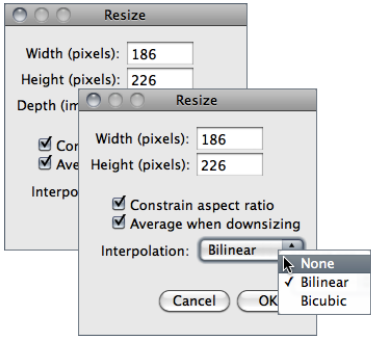
***Stack*** Processes the remaining slices of the stack (if any) using the current settings.

***Macro*** Creates a macro based on the current settings which is sent to the Macro Recorder window (Plugins ▷ Macros ▷ Record...), if open.

***Help*** Opens the built-in help dialog.

SEE ALSO: 3D Color Inspector/Color Histogram, Threshold... [T], Wand Tool, Analyze ▷ Analyze Particles...

### 28.2.6 Size...




Scales the active image or selection to a specified *Width* and *Height* in pixels.

Check *Constrain aspect ratio* and ImageJ will adjust either the *Height* or the *Width* to maintain the original aspect ratio. When applicable, other dimensions can also be resized: *Depth (images)* in stacks, *Depth (slices)* and *Time (frames)* in hyperstacks.

Check the *Average when downsizing* checkbox for better results when scaling down images [19]. Two resampling methods are possible: *Bilinear* and *Bicubic* interpolation. The implementation of the bicubic method (Catmull-Rom interpolation) is derived from Burger and Burge, 2008 [11].

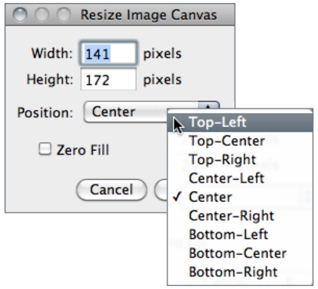
SEE ALSO: Canvas Size..., Image ▷ Scale... [E], Image ▷ Transform ▷ Bin...





### 28.2.7 Canvas Size...

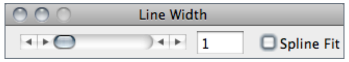
IMPROVED IN IJ 1.46R




Changes the canvas size without scaling the actual image. *Width* and *Height* may be either expanded or contracted. If the canvas size is increased, the border is filled with the current background color (*see* Color Picker... [K]), or, if *Zero Fill* is checked, the border is filled with pixels that have a value of zero. The position of the old image within the new canvas may also be specified.

SEE ALSO: Size..., Image ▷ Scale... [E], Image ▷ Transform ▷ Bin..., XVII Embedding Color Annotations in Grayscale Images

### 28.2.8 Line Width...




This widget is used to adjust the width of line selections (in pixels). It is opened more easily by double clicking on the Line Selection Tools icon. Checking *Spline Fit* fits a cubic spline curve to the points that define the line.

SEE ALSO: Edit ▷ Options ▷ Line Width..., Edit ▷ Selection ▷ Fit Spline

## 28.3 Show Info... [i]

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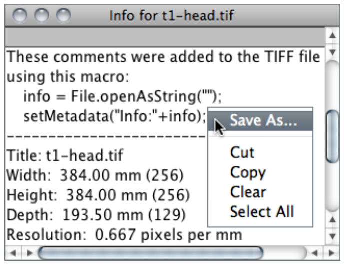
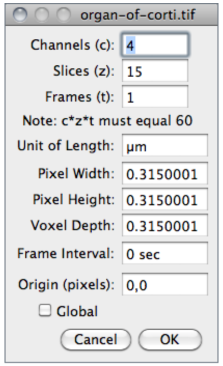



Opens a text window containing information about the active image (including the pixel or voxel size, since IJ 1.44k). For DICOM and FITS images, also displays file header information. Use the popup menu (right-click in the Info window) to save the information to a text file or copy it to the system clipboard.

SEE ALSO: Image ▷ Properties... [P]

## 28.4 Properties. . . [P]




Use this command to display and set various properties of the current image or stack.

The number of *Channels (c)*, *Slices (z)* and *Frames (t)* in the image can be changed as long as the product of $c$, $z$, and $t$ is equal to the number of images in the stack.

*Unit of Length* is a string describing the measuring unit of *Pixel Width*, *Pixel Height* and *Voxel Depth*. These three dimensions are automatically converted if *Unit of Length* is changed from one of ImageJ's known unit ('nm', 'µm' [or 'um' or 'micron'], 'mm', 'cm', 'meter', 'km' or 'inch') to another. µ and Å symbols can be typed using Alt M and Alt Shift A, respectively.

With t–series stacks, the *Frame Interval* in seconds (reciprocal of the frame rate) can be viewed and set. If the unit is 'sec', setting the *Frame Interval* will also set the frame rate used by Animation Options. . . [Alt /].

*Origin* is the reference point 0, 0 (always in pixels) of the image coordinate system (*see also Invert Y coordinates* in Analyze ▷ Set Measurements. . . ).

As explained in XXIII Global Calibrations, check *Global* to make the current settings global, i.e., applied to all images opened during the current session.

SEE ALSO: Analyze ▷ Set Scale. . . , Image ▷ Show Info. . . [i]

## 28.5 Color ▷

This submenu contains commands that deal with color images.

### 28.5.1 Split Channels

Splits an RGB image (or stack) into three 8–bit grayscale images containing the red, green and blue components of the original. The window names have an appended *(red)*, *(green)* and *(blue)*. With composite images and / or hyperstacks (e.g., File ▷ Open Samples ▷ Organ of Corti (2.8M, 4D stack)) this command splits the stack into separate channels.

SEE ALSO: Merge Channels. . .

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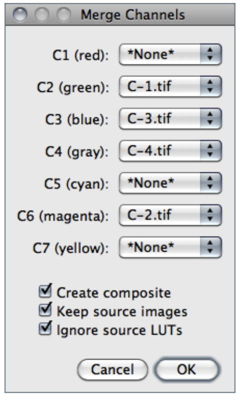
### 28.5.2 Merge Channels. . .

Merges 2–7 images into an RGB image or multi-channel composite image. Select the channel order/color using the *C1–C7* dropdown menus. Select **None** to skip a channel.








***Create composite*** If checked, a multi-channel composite image (*see* Color Composite Images) will be created. If unchecked, an RGB image is created instead. When creating composite images, original LUTs and display ranges are preserved unless *Ignore source LUTs* is checked. Source LUTs are always ignored when creating RGB images.

***Keep source Images*** If checked, source images will not be disposed.

***Ignore source LUTs*** If checked, LUTs of source images are ignored. In this case, merged channels will adopt the lookup table mentioned besides the channel choice, i.e., *red*, *green*, *blue*, *gray*, *cyan*, *magenta*, *yellow*. As mentioned, this option is assumed when merging into RGB.

SEE ALSO: Channels Tool. . . [Z], Lookup Tables ▷ and IV Replacing Red with Magenta in RGB Images

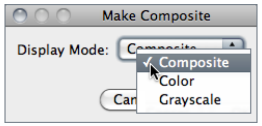
### 28.5.3 Channels Tool. . . [Z]

Alias for Image ▷ Hyperstacks ▷ Channels Tool. . . [Z].

### 28.5.4 Stack to RGB

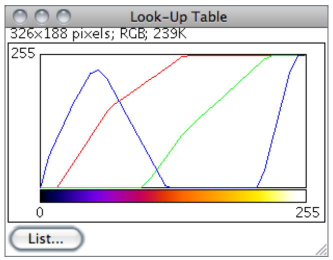
Converts a two or three slice stack into an RGB image, assuming that the slices are in R, G, B order. The stack must be 8–bit or 16–bit grayscale. Also converts composite images (e.g., File ▷ Open Samples ▷ HeLa Cells (1.3M, 48-bit RGB)) into RGB.

### 28.5.5 Make Composite




Converts in place an RGB image, a 2–7 image stack or a 2–7 channel hyperstack into a composite color image. Use the Channels Tool. . . [Z] tool (Shift Z) to enable and disable the channels of a composite image. Use Brightness/Contrast. . . [C] (Shift C) to adjust the brightness and contrast of the current channel.

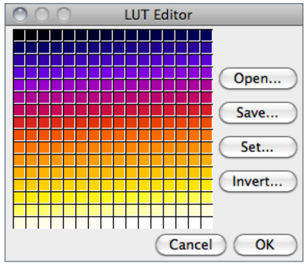
### 28.5.6 Show LUT




Displays a plot of the active image's lookup table (LUT) . The lookup table, or color table, describes the color that is displayed for each of the 256 possible pixel values. For 16 and 32–bit images, the range of displayed pixel values is mapped to 0–255. A bar under the plot displays the color representation of the pixel values. Note that RGB color images do not use a lookup table. Use the *List. . .* radio button to export the LUT as a CSV file.

SEE ALSO: Edit LUT. . .

### 28.5.7 Edit LUT...

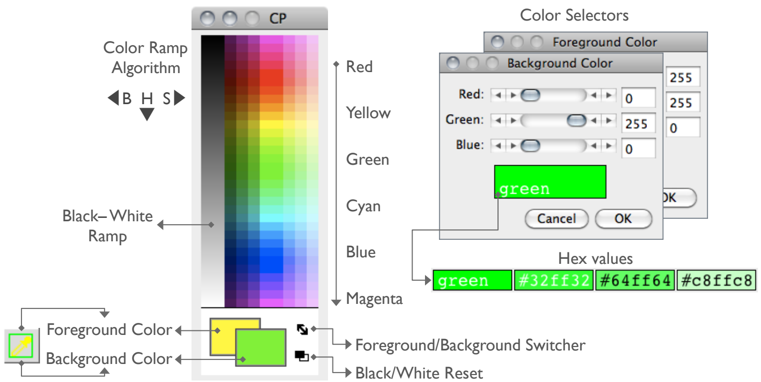



Opens the ImageJ LUT (Lookup Table) Editor. A lookup table in ImageJ has up to 256 entries. The entry index, and the three values (red, green and blue) associated with it, are displayed in the ImageJ status bar as you move the cursor over the LUT Editor window. Click on an entry to edit the red, green and blue values for that entry using a Color Selector window (cf. Color Picker... [K]).

SEE ALSO: Show LUT

### 28.5.8 Color Picker... [K]

The Color Picker [17] enables the user to select foreground and background colors, which affects Edit ▷ Fill [f], Draw [d], Clear and other drawing commands. It displays current foreground and background colors in the selection boxes at the bottom of the window. It has two modes: *Foreground* and *Background*. To change modes, click on the desired selection box. Clicking on the *Foreground / Background Switcher* button sets the current foreground to the background and vice versa. The *Black / White* Reset button sets the foreground to black and the background to white.




**Color Picker and Color Choosers (IJ 1.46c).** The CP window can be activated using Shift K (the keyboard shortcut for Image ▷ Color ▷ Color Picker... [K]) or by double clicking on the Color Picker Tool on the ImageJ Toolbar. Color Choosers are evoked by double clicking on a color of the CP window and can be used to retrieve Hexadecimal Color Values. The 'eye dropper' is drawn in the current foreground color while the frame around it is drawn in background color. Foreground color is also reflected in drawing tools such as the Arrow, Brush, Flood Filler and Pencil tools.

The color palette is based on HSB (Hue, Saturation and Brightness) color model (*see* Color Spaces and Color Separation). Hue increases as you go down the palette while saturation and brightness values are split horizontally. The left half of the palette varies only in brightness while the right half varies only in saturation. At the center of the color ramp are enlarged red, green, blue, cyan, magenta, and yellow colors for quick selection. To the left of the color palette is a grayscale ramp that goes from pure black to pure white.





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Double clicking on a color brings up the ColorChooser, a widget with three sliders used to specify the RGB values of the foreground or background color. The title of the ColorChooser widget (*Foreground Color* or *Background Color*) indicates the current selection mode. To get precise colors, manually change the values in the text boxes. The hex value of the final color is also displayed, offering a convenient way to retrieve custom colors to, e,g, personalize Overlays (*see* XIX Hexadecimal Color Values).

As mentioned earlier, the Color Picker Tool tool can be used to 'pick-up' foreground/background colors from an image canvas. Foreground color can also be changed using the *Color* dropdown menu in the *Options* dialog of drawing tools such as Arrow, Brush, Overlay Brush and Pencil tools.

SEE ALSO: XVII Embedding Color Annotations in Grayscale Images, Draw [d], Fill [f], Clear, Clear Outside, Image Types and Formats, Temporary Activation of a Tool

**XVII EMBEDDING COLOR ANNOTATIONS IN GRAYSCALE IMAGES**

Color marks are only available with color images or grayscale images that have been converted to RGB (Image ▷ Type ▷ submenu). For non-RGB images, background / foreground color will be drawn in equivalent gray levels, e.g.: For a 8–bit image, if the foreground color is red (RGB: 255, 0, 0) intensity of drawn selections will be $(255 + 0 + 0)/3 = 85$.

Colored Overlays (*see* Image ▷ Overlay ▷, Overlay Brush tool), on the other hand, can be created on all image types becoming the easiest way to annotate grayscale images.

## 28.6 Stacks ▷

This submenu contains commands related to Stacks. Operations specifically related to Hyperstacks are listed in the Image ▷ Hyperstacks ▷ submenu.

### 28.6.1 Add Slice

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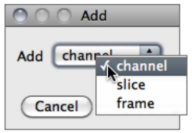
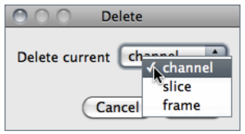



Inserts a blank slice after the currently displayed slice. Holding Alt inserts a blank slice before the current slice. With Hyperstacks, a dialog prompt allows to insert either a channel, z-slice or t-frame.

SEE ALSO: Delete Slice

### 28.6.2 Delete Slice

IMPROVED IN IJ 1.46R




Deletes the currently displayed slice. With Hyperstacks, it can delete the current channel, z-slice or t-frame.

SEE ALSO: Add Slice

### 28.6.3 Next Slice [>]

Displays the slice that follows the currently displayed slice. Holding Alt > will skip ten slices forward.

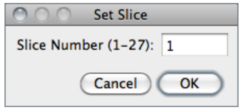
SEE ALSO: Arrow Keys

### 28.6.4 Previous Slice [<]

Displays the slice that precedes the currently displayed slice. Holding Alt < will skip ten slices backward.

SEE ALSO: Arrow Keys

### 28.6.5 Set Slice...

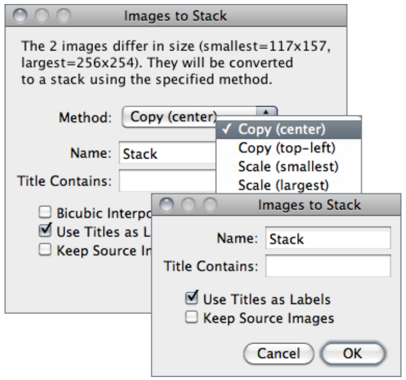



Displays a specified slice. The user must enter a slice number greater than or equal to one and less than or equal to the number of slices in the stack.

### 28.6.6 Images To Stack

Creates a new stack from images currently displayed in separate windows.

***Method*** If images differ in size, a drop-down menu allows to choose a conversion method:




***Copy (center)* and *Copy (top-left)*** Stack will have the width of the widest open image and the height of the highest open image. Smaller images will then be copied (either to the center or to the upper left corner) of the slice. Borders are filled with pixels that have a value of zero.

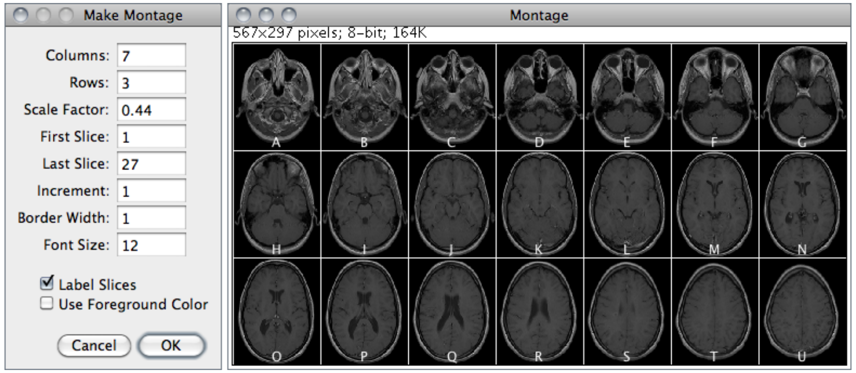
***Scale (smallest)* and *Scale (largest)*** Stack will have the dimensions of the smallest / largest open image. Other images are scaled to the new slice dimensions. Bicubic interpolation is used if *Bicubic interpolation* is checked (cf. Image ▷ Size... and Image ▷ Scale... [E]).

***Name*** Specifies the title of the stack to be created.

***Title Contains*** Enter a string into this field and ImageJ will only convert to stack images whose name contains that string.








***Bicubic Interpolation*** If checked, bicubic interpolation (cf. Adjust ▷ Size...) will be used if any of the *Scale* methods was previously chosen.

***Use Title as Labels*** If checked, image titles (without extension) will be used as stack labels. As described in Stacks ▷ Tools ▷ Remove Slice Labels, these labels (up to 60 characters) correspond to the image subtitle, the line of information above the image.

***Keep Source Images*** If checked, original images are kept.

### 28.6.7 Stack To Images

Converts the slices in the current stack to separate image windows.

SEE ALSO: Stacks ▷ Images To Stack

### 28.6.8 Make Montage...

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Produces a single grid-image containing the individual images that compose Stacks and 4D Hyperstacks. This can be useful for visual comparisons of a series of images stored in a stack and to create 'panel figures' for publication and presentations. A dialog box allows you to specify the magnification level at which the images are copied, and to select the layout of the resulting grid.

***Label Slices*** If checked, montage panels are labelled with slice labels. Slice labels (up to 60 characters) correspond to the image subtitle, the line of information above the image. These labels are part of the stack metadata, typically created by File ▷ Import ▷ Image Sequence... or Stacks ▷ Images To Stack. If no slice metadata exists (the `setMetadata("Label", string)` macro function can be used to customize slice labels) images are labelled with slice numbers. Note that the Stacks ▷ Label... command can be used to draw labels in stack slices. Labels are typeset in sans-serif typeface.

***Use Foreground Color*** If checked, borders and labels are drawn in the foreground color and blank areas of the panel are filled with the background color.

SEE ALSO: Stacks ▷ Tools ▷ Montage to Stack..., Stacks ▷ Tools ▷ Remove Slice Labels, RC Montage plugin, Magic Montage — a macro toolset to reorder and manipulate images in the montage (a video tutorial can be found here)

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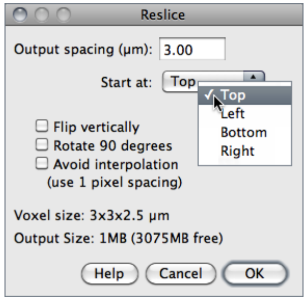
### 28.6.9 Reslice... [/]




Reconstructs one or more orthogonal slices through the image volume represented by the current stack or hyperstack [21].

The estimated size of the output stack and the amount of available memory are displayed at the bottom of the dialog. Increase *Output spacing* to reduce the size of the output stack.

A dialog allows you to specify the spacing of the reconstructed slices.

***Output spacing*** Determines the number of orthogonal slices that will be reconstructed. Increasing *Output spacing* reduces the size of the output stack.

***Start at*** Determines the image edge (*top, left, bottom* or *right*) from which reconstruction starts. *Start at* is replaced by *Slice count* if there is a line selection. With lines selections, a stack is created by shifting (by *Output spacing*) the line down and to the left to generate additional slices for the output stack. In this case, the size of the output stack in determined by *Slice count.*

***Flip vertically*** If checked, each slice in the output stack will be flipped vertically.

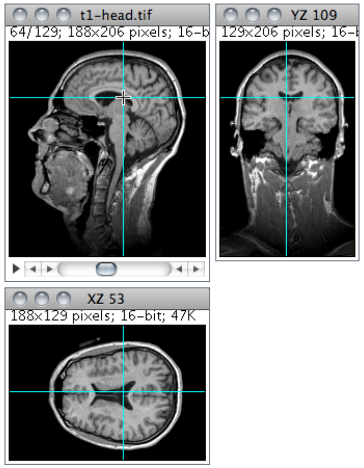
***Rotate 90 degrees*** If checked, each slice in the output stack will be rotated 90°.

***Avoid interpolation*** If checked, no interpolation will be done.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/image.html#reslice.

SEE ALSO: Dynamic Reslice and Radial Reslice plugins

### 28.6.10 Orthogonal Views [H]




Provides an orthogonal view display of the current stack or hyperstack [22]. E.g., if a stack displays sagittal sections, coronal (YZ projection image) and transverse (XZ projection image) will be displayed through the data-set.

The two extra planar views are displayed in 'sticky' panels next to original image and can be toggled using Shift H, the command shortcut.

The intersection point of the three views follows the location of the mouse click and can be controlled by clicking and dragging in either the XY, XZ or YZ view. XY and XZ coordinates are displayed in the title of the projection panels. The mouse wheel changes the screen plane in all three views.

Voxel dimensions can be adjusted in Image ▷ Properties... [P].





SEE ALSO: 3D Project..., and 3D Viewer [101], Volume Viewer, Stack Slicer Display3_TP plugins, Edit ▷ Options ▷ DICOM...

### 28.6.11 Z Project...

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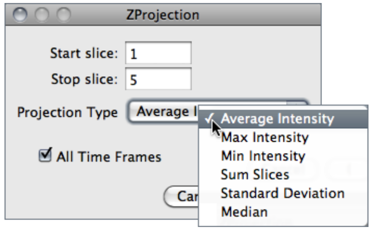
Projects an image stack along the axis perpendicular to image plane (the so-called $z$ axis) [21]. With hyperstacks, the projection is performed on the active time frame, or for all time points if *All Time Frames* is checked. Five different projection types are supported. The preferred projection method is stored in the preferences file.




***Average Intensity*** projection outputs an image wherein each pixel stores average intensity over all images in stack at corresponding pixel location.

***Maximum Intensity*** projection (MIP) creates an output image each of whose pixels contains the maximum value over all images in the stack at the particular pixel location.

***Sum Slices*** projection creates a real image that is the sum of the slices in the stack.

***Standard Deviation*** projection creates a real image containing the standard deviation of the slices.

***Median*** projection outputs an image wherein each pixel stores median intensity over all images in stack at corresponding pixel location.

SEE ALSO: Grouped Z Project..., 3D Project..., Plot Z-axis Profile...

### 28.6.12 3D Project...

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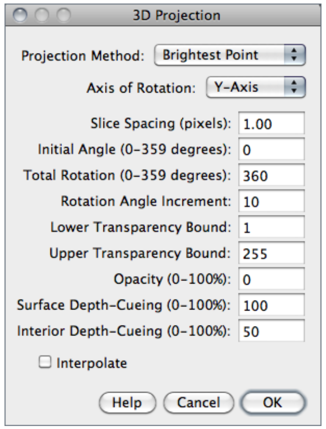



This command creates a sequence of projections of a rotating volume (stack or hyperstack) onto a plane using *nearest-point* (surface), *brightest-point*, or *mean-value* projection or a weighted combination of nearest point projection with either of the other two methods (partial opacity) [?]. The user may choose to rotate the volume about any of the three orthogonal axes (x, y, or z), make portions of the volume transparent (using thresholding), or add a greater degree of visual realism by employing depth cues.

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.

***Projection Method*** 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).

***Slice Spacing*** The interval, in pixels, between the slices that make up the volume. ImageJ 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*.

***Lower / Upper Transparency Bound*** 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 also use Image ▷ Adjust ▷ Threshold... [T] to set the transparency bounds.

***Opacity*** Can be used to reveal hidden spatial relationships, especially on overlapping objects of different colors and dimensions. 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 *Opacity* to a value greater than zero and select either *Mean Value* or *Brightest Point* projection.

***Surface / Interior Depth–Cueing*** 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.

***Interpolate*** Check *Interpolate* to generate a temporary z-scaled stack that is used to generate the projections. Z-scaling eliminates the gaps seen in projections of volumes with slice spacing greater than 1.0 pixels. This option is equivalent to using the Scale plugin from the TransformJ package to scale the stack in the z-dimension by the slice spacing (in pixels). This checkbox is ignored if the slice spacing is less than or equal to 1.0 pixels.

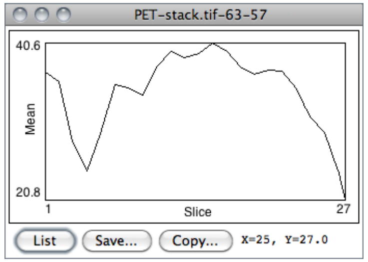




***Help*** Opens http://imagej.nih.gov/ij/docs/menus/image.html#project.

SEE ALSO: Orthogonal Views [H], Z Project..., Grouped Z Project... and 3D Viewer [101], Volume Viewer plugins

### 28.6.13 Plot Z-axis Profile...




Plots the ROI selection mean gray value versus slice number. Requires a point or area selection.

Coordinates of the upper left corner of the selection (or the bounding rectangle for non-rectangular selections) are displayed in the graph title.

SEE ALSO: Profile Plot Options..., Plot Profile [k]

### 28.6.14 Label...

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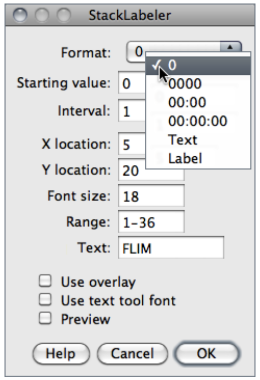



Adds a sequence of numbers (e.g., timestamps) and/or a label to a stack or hyperstack. Numbers and label are drawn in the current foreground color (cf. Image ▷ Colors ▷ Color Picker... [K]).

The initial *X, Y location*, and *Font size* of the label are based on the existing rectangular selection, if any. Slices outside the *Range* are not affected.

***Format*** Specifies the structure of the label. *0*: Unpadded sequence; *0000*: Pads each number with leading zero(s); *00 : 00*: Converts the label into a *minutes : seconds* timestamp; *00 : 00 : 00* Converts the label into a *hours : minutes : seconds* timestamp; *Text*: Stamps only the contents of the *Text* field. *Label*: Displays slice labels.

***Starting value* and *Interval*** Specify the first value and the numeric steps to be applied. Note that with timestamps, metric time values must be used, e.g., an *Interval* of 3600 will create 1 hour increments.

***Text*** The string to be drawn after each number when the *Format* chosen is either *0* or *Text* (label without numeric sequence).

***Use overlay*** If checked, labels will be created as non-destructive image Overlays. Note that previously added overlays will be removed. Also, note that Virtual Stacks and Hyperstacks can only have overlay labels.

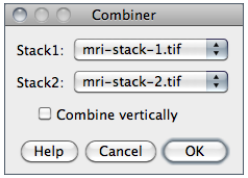
***Use text tool font*** If checked, labels will be created using the typeface and style specified in the Fonts... widget. If unchecked, labels are typeset using ImageJ's default font: sans-serif typeface.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/image.html#label.

SEE ALSO: Make Montage..., Stacks ▷ Tools ▷ Insert...

### 28.6.15 Tools ▷

#### 28.6.15.1 Combine...




Combines two stacks [*Width*×*Height*×*Depth*] ($W_1 \times H_1 \times D_1$ and $W_2 \times H_2 \times D_2$) to create a new $W_1+W_2 \times max(H_1,H_2) \times max(D_1,D_2)$ stack. E.g., a 256×256×40 and a 256×256×30 stack would be combined to create one 512×256×40 stack.

If *Combine vertically* is enabled, creates a new $max(W_1+W_2) \times (H_1+H_2) \times max(D_1,D_2)$ stack.

Unused areas in the combined stack are filled with background color (cf. Color Picker... [K]).

SEE ALSO: Concatenate...

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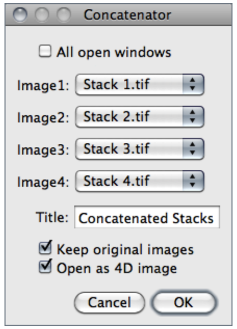
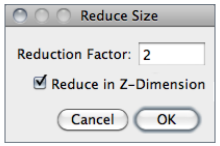
#### 28.6.15.2 Concatenate...




Concatenates multiple images or stacks. Images with mismatching type (*see* Image Types and Formats) and dimensions are omitted [24]. Stacks with the same number of slices can be concatenated as a 4D Hyperstacks, if *Open as 4D image* is checked. In this case, chosen stacks will be appended as time-points.

SEE ALSO: Combine...

#### 28.6.15.3 Reduce...




Reduces the size of stacks and hyperstacks by the specified *Reduction Factor*. E.g., For a 30 slices stack and a *Reduction Factor* of 2, the reduced stack will be be composed of 15 slices with every second slice being removed. Virtual stacks/hyperstacks are supported

With Hyperstacks, the default reduction is performed in the T-Dimension, but a choice is available to *Reduce in Z-Dimension* instead.

SEE ALSO: Hyperstacks ▷ Reduce Dimensionality...

#### 28.6.15.4 Reverse

Alias for the Image ▷ Transform ▷ Flip Z command.





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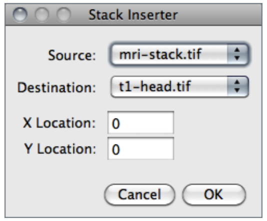
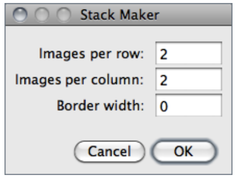
#### 28.6.15.5 Insert...




Inserts a *Source* image into a *Destination* image at the specified *X* and *Y Location* (pixel coordinates). *Source* and *Destination* can be single images or stacks but must be of the same type (*see* Image Types and Formats). The *Destination* image will be permanently modified once *Source* has been inserted. Note that when *Source* is a single image, Edit ▷ Selection ▷ Image to Selection... can be used (together with Image ▷ Overlay ▷ Add Image...) to create image selections (ImageROIs), a more convenient way of blending two open images.

SEE ALSO: Image ▷ Type ▷, Image ▷ Stacks ▷ Label...

#### 28.6.15.6 Montage to Stack...




Converts a montage image to an image stack based on the specified number of rows and columns, taking into account a *Border width*. This is the opposite of what the Image ▷ Stacks ▷ Make Montage... command does.

SEE ALSO: Demontager plugin

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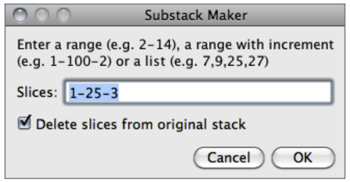
#### 28.6.15.7 Make Substack...




Extracts selected images from the active stack copying them to a new stack in the order of listing or ranging [25].

Extracted slices will be removed from the source stack if *Deleted slices from original stack* is checked. Currently, it does not work with hyperstacks and takes one of three types of input:

1. A range of images. E.g.: `2-14` [*extract slices 2 through 14*]
2. A range of images with increment, which can be used to de-interleave slices. E.g.: `2-14-2` [*extract slices 2 and 14 and every second slice in between*]
3. A list of images. E.g.: `2,4,7,9,14`

SEE ALSO: Image ▷ Duplicate... [D]

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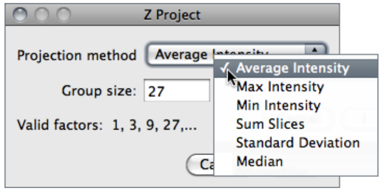
#### 28.6.15.8 Grouped Z Project...

Creates a substack of *Stack size/Group size* slices with each slice being the result of a Z Projection performed over the range of *Group size*.




*Group size* must divide evenly into the stack size. This limitation can be overcome by the Image ▷ Transform ▷ Bin. . . , that can also create grouped Z-projections.

The first ten divisors of *Stack size* are suggested at the bottom of the dialog.

SEE ALSO: Image ▷ Stacks ▷ Z Project. . . , Image ▷ Adjust ▷ Size. . . , Image ▷ Adjust ▷ Scale. . . [E]

#### 28.6.15.9 Remove Slice Labels

Removes slice labels from stacks. The first line of a slice label (up to 60 characters) is displayed in parentheses in the image subtitle, the line of information above the image. The macro functions `setMetadata("Label", string)` and `getMetadata("Label")` can be use to set and retrieve the current slice label.

SEE ALSO: Stacks ▷ Label. . .

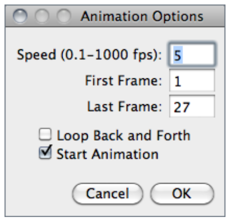
#### 28.6.15.10 Start Animation [\]

Animates the active stack by repeatedly displaying its slices (frames) in sequence. It is run more easily by clicking on the play icon preceding stack sliders (*see* Stacks). To stop the animation, click on the slider pause icon, click on the image or use Stop Animation [\], evoked by the same shortcut. As such, stacks animation can be toggled using \. The frame rate is displayed in the status bar. Open the Animation Options. . . [Alt /] dialog box to specify the animation speed (pressing Alt \ or right-clicking on the on the slider play/pause icon opens the Animation Options. . . dialog). Note that more than one stack can be animated at a time.

#### 28.6.15.11 Stop Animation [\]

Terminates animation of the active stack (*see* Start Animation [\]).

#### 28.6.15.12 Animation Options. . . [Alt /]




Use this dialog to set the animation speed in frames per second, the starting and ending frame, or to enable 'oscillating' animations. Selecting *Start Animation* animates the stack as soon as the dialog is dismissed.

Note that setting the *Frame Interval* in Image ▷ Properties. . . [P] sets the animation speed as long as the unit used is 'sec'.

This dialog can also be accessed by right-clicking on the play/pause icon that precedes the slice slider in Stacks and frame slider in Hyperstacks (*see* Stacks and Hyperstacks).

SEE ALSO: File ▷ Save As ▷ Gif. . . , AVI. . .

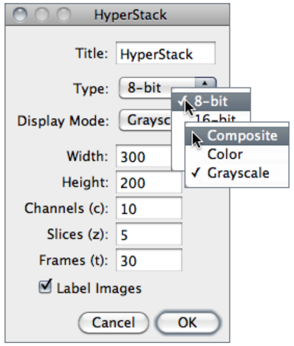




## 28.7 Hyperstacks ▷

This submenu hosts commands specifically related Hyperstacks, images that have four (4D) or five (5D) dimensions. General operations related to Stacks are listed in the Image ▷ Stacks ▷ submenu.

### 28.7.1 New Hyperstack. . .




Creates a new hyperstack. Hyperstacks have *Width*, *Height*, *Channels* (c dimension), *Slices* (z dimension) and time *Frames* (t dimension).

*Image Type* (*see* Image ▷ Type ▷ and Image Types and Formats) and *Display Mode* (*see* Channels Tool. . . [Z]) can be specified.

Checking *Label Images* will draw the channel number, slice number and frame number on each image in the hyperstack.

SEE ALSO: File ▷ New ▷ Hyperstack. . . (an alias of this command) and File ▷ New ▷ Image. . . [n]

### 28.7.2 Stack to Hyperstack. . .

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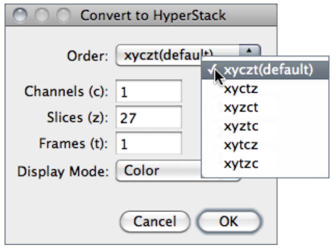


Converts a stack into a hyperstack. RGB stacks are converted into 3 channel hyperstacks.

*Order* is the order of the channels ($c$), slices ($z$) and frames ($t$) within the stack. ImageJ hyperstacks are always in *czt* order. Stacks not in *czt* order will be shuffled to be in czt order. The channel *Display Mode* can be *Composite*, *Color* or *Grayscale* (cf. Channels Tool. . . [Z]).

SEE ALSO: Hyperstack to Stack

### 28.7.3 Hyperstack to Stack

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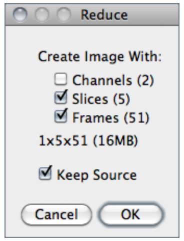
Converts a hyperstack into a stack (in *czt* order).

SEE ALSO: Stack to Hyperstack. . .

### 28.7.4 Reduce Dimensionality. . .

This command [26] reduces the dimensionality of an hyperstack by creating a new hyperstack with, for example, all the channels and time points at a given z position or all the z slices for the current channel and time point.



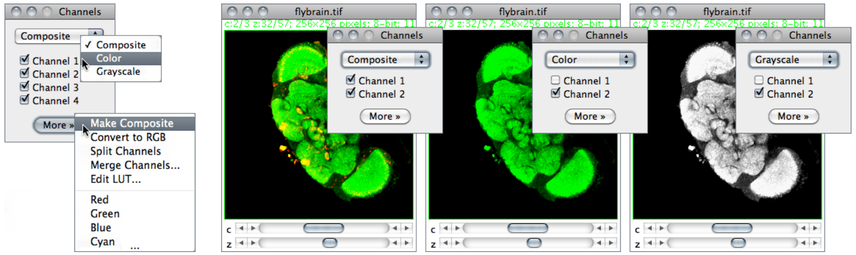

Uncheck *Channels (n)* to delete all but the current channel, *Slices (n)* to delete all but the current z slice and *Frames (n)* to delete all but the current time point. Check *Keep Source* and the original stack will not be deleted.

The expected dimensions and size of the reduced stack are displayed in the dialog.

SEE ALSO: Hyperstack to Stack

#### 28.7.5 Channels Tool... [Z]

Opens the Channels... widget, or brings it to the front if it is already open. Shift Z is the keyboard shortcut for this command. This tool allows to select the *Display mode* of composite images. In addition, several commands hosted in the Image ▷ Color ▷ submenu can easily be accessed through the *More≫* drop-down menu. The same drop-down menu also provides a convenient list of primary colors (additive: red, green and blue, subtractive: cyan, magenta, yellow) that can be used to pseudocolor Color Composite Images.




**Channel manipulations in Color Composite Images using the Image ▷ Color ▷ Channels Tool... [Z] tool.**

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### 28.8 Crop [X]

Crops the image or stack based on the current rectangular selection.

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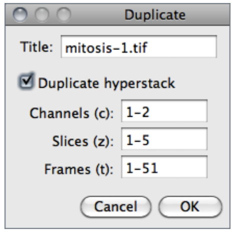
### 28.9 Duplicate... [D]




Creates a new window containing a copy of the active image or rectangular selection. For stacks and hyperstacks it is possible to specify the range of *Channels (c)*, *Slices (z)* and *Frames (t)* to be duplicated.

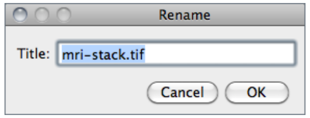
With single images, hold Alt to skip the dialog box.

SEE ALSO: Rename...





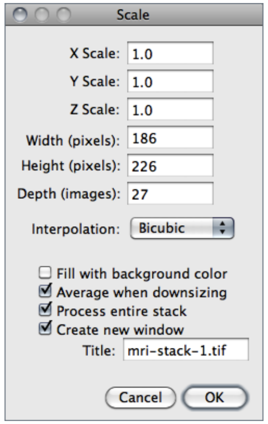
### 28.10 Rename...




Renames the active image.

SEE ALSO: Duplicate... [D]

### 28.11 Scale... [E]




Resizes the image or current area selection by scale factors entered into a dialog box. As with Image ▷ Size..., two resampling methods are possible: *Bilinear* and *Bicubic* interpolation.

For the best looking results, particularly with graphics and text, use integer scale factors (2, 3, 5, etc.) and check *Average when downsizing* with scale factors less than 1.0 [19]. Also, when downsizing, smoothing the source image prior to scaling may produce better looking results.

Scaled image/selection are copied to a new image named *Title* if *Create new window* is checked. If scaling a selection that will not be copied to a new image check *Fill with Background Color* to fill with the background color instead of zero.

Entire stacks (or hyperstacks in the Z Dimension) will be scaled if *Process entire stack* is checked.

SEE ALSO: Image ▷ Adjust ▷ Size..., Image ▷ Transform ▷ Bin..., Image ▷ Stacks ▷ Tools ▷ Grouped Z Project...

### 28.12 Transform ▷

This submenu contains commands that perform geometrical image transformation on the active image or stack.

#### 28.12.1 Flip Horizontally

Replaces the image or selection with a x-mirror image of the original.

#### 28.12.2 Flip Vertically

Turns the image or selection upside down (y-mirror).

#### 28.12.3 Flip Z

Reverses the order of the slices in a stack (z-mirror).

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#### 28.12.4 Rotate 90 Degrees Right

Rotates the entire image or stack clockwise 90°.

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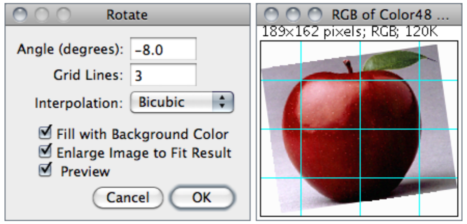
### 28.12.5 Rotate 90 Degrees Left

Rotates the entire image or stack counter-clockwise 90°.

### 28.12.6 Rotate...

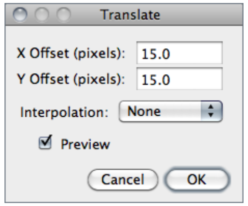



Use this dialog to rotate the active image or selection clockwise the specified number of *degrees*.

Set *Grid Lines* to a value greater than zero to superimpose a grid on the image in *Preview* mode. Two resampling methods are possible: *Bilinear* and *Bicubic* interpolation (cf. Image ▷ Size...).

With 8–bit and RGB images, check *Fill with Background Color* to fill with the background color instead of zero (cf. Color Picker... [K]). Check *Enlarge to Fit Result* and the image will be enlarged as needed to avoid clipping.

### 28.12.7 Translate...



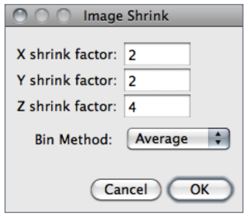

Translates (moves) the image in the x and y directions by a specified number of pixels. With stacks, you can translate either the current image or all the images in the stack. Two resampling methods are possible: *Bilinear* and *Bicubic* interpolation (cf. Image ▷ Size...).

Check *Preview* to see how the translation will affect the image. The background at the edges of the image will be set to 0.

SEE ALSO: Align_Slice and Align_RGB_planes plugins

NEW IN IJ 1.46R

### 28.12.8 Bin...




Reduces the size of an image by binning groups of pixels of user-specified size (*X, Y, Z shrink factor*) [27]. The resulting pixel can be calculated as *Average*, *Median*, *Maximum*, or *Minimum*. Undo support is restricted to 2D images (non stacks).

Z binning produces equivalent results to Image ▷ Stacks ▷ Tools ▷ Grouped Z Project... However, there are two main differences between the two commands: While Bin... replaces the original image, Grouped Z Project... creates a new substack; and while *Z shrink factor* takes any value, *Group size* must divide evenly into the stack size.

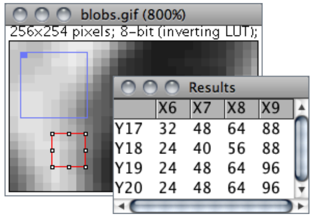
SEE ALSO: Image ▷ Adjust ▷ Size..., Image ▷ Adjust ▷ Scale... [E], Image ▷ Stacks ▷ Tools ▷ Grouped Z Project...





### 28.12.9 Image to Results

NEW IN IJ 1.46R




Prints the active area selection to the Results Table, clearing previous results. The entire image is processed when no area ROI exists. XY coordinates are detailed in column and row headers. Calibrated and floating-point images are listed with the precision specified by *Decimal places* in Analyze ▷ Set Measurements...

For RGB images, each pixel is converted to grayscale using the formula gray = (red + green + blue)/3 or the formula gray = $0.299 \times$ red $+ 0.587 \times$ green $+ 0.114 \times$ blue if *Weighted RGB to Grayscale Conversion* is checked in Edit ▷ Options ▷ Conversions...

SEE ALSO: Text Images, Pixel Inspector, Results to Image, File ▷ Save As ▷ Text Image..., Import ▷ Text Image..., Stack_to_Results.js, a script that displays the contents of a stack using one image per column

### 28.12.10 Results to Image

NEW IN IJ 1.46R

The reverse of Image to Results, converting the tabular data in the Results Table into a 32–bit image named *Results Table*. Column and row headers are ignored.

SEE ALSO: Text Images, Pixel Inspector, File ▷ Save As ▷ Text Image..., Import ▷ Text Image...

## 28.13 Zoom ▷

This submenu contains commands that control how the current image is displayed. The [+] and [−] or [↑] and [↓] keys are the preferred way to use the In [+] and Out [-] commands. When a selection exists, zooming with the Arrow Keys requires holding down either [Shift] or [Ctrl].

### 28.13.1 In [+]

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Zooms in to next higher magnification level and, if possible, enlarges the window. As explained in Magnifying Glass, there are 21 possible levels (shown in the title bar): 3.1, 4.2, 6.3, 8.3, 12.5, 16.7, 25, 33.3, 50, 75, 100, 150, 200, 300, 400, 600, 800, 1200, 1600, 2400 and 3200%.

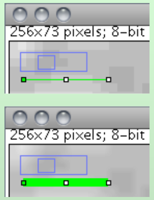
### 28.13.2 Out [-]

Zooms out to next lower magnification level and, if needed, shrinks the window.

### 28.13.3 Original Scale [4]

Displays the image at the magnification used when the image was first opened. As a shortcut, double click on the Magnifying Glass tool.

## XVIII Working with Zoomed Canvases




Images are magnified using [+] and [−], or [↑] and [↓] if no selection exists. Magnification occurs around the cursor, or to the center of the image when the cursor lays outside the image canvas. The *Zoom indicator* in the upper left corner of magnified images shows what portion of the image is currently displayed. At high magnification levels the pixel grid becomes visible by default unless *Interpolate zoomed images* is checked in Edit ▷ Options ▷ Appearance. . . To scroll a magnified image, hold down the space bar (Scrolling Tool shortcut) while dragging the cursor.

By default, Overlays and the active selection are displayed with a 1–pixel wide contour regardless of the image magnification (*0 Width*). To thicken ROI edges at higher zoom levels, set *Stroke width* to a non-zero value in Edit ▷ Selection ▷ Properties. . . [y], Image ▷ Overlay ▷ Overlay Options. . . or ROI Manager's *Properties. . .*

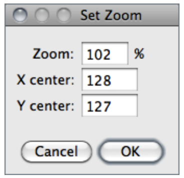
### 28.13.4 View 100% [5]

Displays the image using 100% magnification (1 image pixel = 1 screen pixel). Enable *Open Images at 100%* in the Edit ▷ Options ▷ Appearance. . . dialog to have images automatically opened at 100% magnification.

### 28.13.5 To Selection

Zooms in based on the current selection. Note that in the absence of a selection, this command zooms the image to a *fit to screen* level (*see* Magnifying Glass).

### 28.13.6 Set. . .




Zooms the active image to an exact value (e.g. 37.4%) overcoming the predefined zoom steps described in Image ▷ Zoom ▷ In [+]. The zoomed canvas will be centered at *X, Y center* (pixel coordinates) [28].

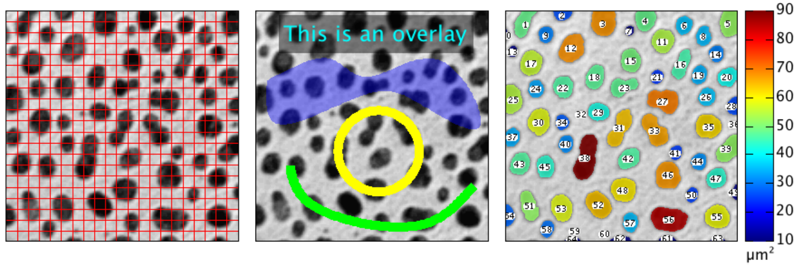
## 28.14 Overlay ▷

As mentioned previously, this submenu contains commands for creating and working with non-destructive image Overlays. An overlay consists of one or more selections: arrows, lines, points, shapes and text (*see* ImageJ Toolbar). Overlays can also be composed of image selections (imageROIs) that behave partially as regular Selections (*see* Add Image. . . ).

Press [B] (Add Selection. . . [b]) to add the current selection to the overlay. Press [Shift] [F] (Flatten [F]) to create an RGB image with the overlay embedded in it. The overlay is preserved when an image is saved in TIFF format (cf. II Image Types: Lossy Compression and Metadata).



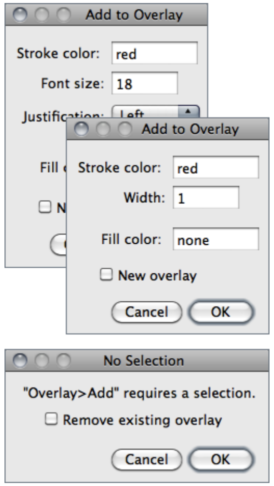





**Non-destructive image** Overlays**.** Outputs from Grid_Overlay, MakeOverlay and ROI Color Coder that exemplify the usage of most Image ▷ Overlay ▷ submenu commands. The Overlay Brush tool can also be used to create freehand annotations.

### 28.14.1 Add Selection. . . [b]

Improved in IJ 1.46r




Selections are immediately added to the current overlay when pressing [B]. Pressing [Alt] [B] will display a dialog box in which *Stroke color* and *width* and *Fill color* can be set.

Except for text selections, and as explained in Edit ▷ Selection ▷ Properties. . . [y], *Stroke* (contour) color and *Width* are ignored if a *Fill color* is specified. Colors are specified using the name of one of the default selection colors (*black, blue, cyan, green, magenta, orange, red, white* and *yellow*) or using hex notation (*see* XIX Hexadecimal Color Values).

Previously added Overlays are removed if *New overlay* is checked. Also, if no selection exists and the command is run, a warning message is displayed in which is possible to remove the existing overlay, by running Remove Overlay.

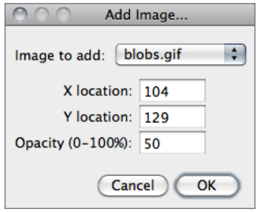
Note that measured selections (Analyze ▷ Measure. . . [m]) can be added automatically to the image overlay by selecting the *Add to overlay* checkbox in Analyze ▷ Set Measurements. . .

See also: Overlay Options. . . , Labels. . . , Selection ▷ Properties. . . [y],, XIX Hexadecimal Color Values, ROI Manager. . .

### 28.14.2 Add Image. . .

Changed in IJ 1.46r

Blends two open images by adding an image to the overlay of frontmost image. The image to be blended can be of any type (*see* Image Types and Formats) but cannot be larger than the host image. A blending alpha value can be specified in the *Opacity (0–100%)* field. The initial X,Y location is based on the existing rectangular selection, if any.




By default the created overlay image cannot be moved around the canvas, i.e,, is not a image selection (ImageROI), but are stored in the TIFF header and can be saved and restored when saving images in TIFF format. On the other hand, image selections can be created using Edit ▷ Selection ▷ Image to Selection. . . or by running Image ▷ Overlay ▷ To ROI Manager after adding the image to the overlay.

SEE ALSO: Paste Control. . . (Blend transfer mode), Image ▷ Stacks ▷ Tools ▷ Insert. . .

## XIX Hexadecimal Color Values

Hexadecimal (hex) notation is frequently used in computing to summarize binary code in a human-friendly manner. Here are some decimal/hexadecimal equivalents:

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | . . . | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | . . . | 255 |
| Hex | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | A | B | C | D | E | F | 10 | 11 | 12 | 13 | 14 | . . . | 1E | 28 | 32 | 3C | 46 | 50 | 5A | 64 | 6E | . . . | FF |

Decimal RGB color values that typically range from 0 to 255 are succinctly represented by two hexadecimal digits ranging from 00 through FF. A hex color value is a 6–digit, three–byte hexadecimal number (hex triplet) in the form #RRGGBB, in which RR specifies the red, GG the green and BB the blue value.

Opacity of hex triplets can be modified by an optional transparency (alpha) channel, giving rise to a 8–digit, four–byte hex number in the form #AARRGGBB with AA specifying the alpha blending value. In ImageJ, the alpha channel codes for opacity and ranges from 00 (fully transparent) to FF (solid color). Alpha values can be omitted for fully opaque colors. The table below provides some hexadecimal color values in 10% transparency increments, and can be used to create semi-transparent Overlays.

| Color | RGB triplet | Opacity value 100% | 90% | 80% | 70% | 60% | 50% | 40% | 30% | 20% | 10% | 0% | Hex triplet |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blue | 0, 0, 255 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | 00 00 FF |
| Cyan | 0, 255, 255 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | 00 FF FF |
| Green | 0, 255, 0 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | 00 FF 00 |
| Magenta | 255, 0, 255 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | FF 00 FF |
| Red | 255, 0, 0 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | FF 00 00 |
| Orange | 255, 150, 0 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | FF 96 00 |
| Yellow | 255, 255, 0 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | FF FF 00 |
| White | 255, 255, 255 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | FF FF FF |
| Gray | 127, 127, 127 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | 7F 7F 7F |
| Black | 0, 0, 0 | #FF | #E5 | #CC | #B2 | #99 | #7F | #66 | #4C | #33 | #19 | #00 | 00 00 00 |

Alpha blending values can be added to the beginning of hex triplets to modulate color transparency: e.g., #7F FF 00 00 defines red at 50% opacity. In ImageJ the hash (#) prefix is optional.

ColorChoosers in the Color Picker window display hex values of RGB colors. The built-in macro function `toHex()` returns hexadecimal representations of decimal numbers and can also be used to convert RGB color values (*see* RGBtoHEX macro). Several other macros (e.g., MakeOverlay) exemplify how to annotate images using hex colors.





### 28.14.3 Hide Overlay

Causes ImageJ to stop displaying the overlay displayed by Show Overlay.

### 28.14.4 Show Overlay

Displays an overlay that was hidden by Hide Overlay.

### 28.14.5 From ROI Manager

Creates an overlay from the selections on the ROI Manager list (*see* Analyze ▷ Tools ▷ ROI Manager. . . ). Note that previously added Overlays will be removed.

### 28.14.6 To ROI Manager

Copies the selections and images in the current overlay to the ROI Manager, where they can be edited (moved, resized or re-colored) (*see* ROI Manager. . . ). Note that previous items in the ROI Manager list will be deleted.

### 28.14.7 Remove Overlay

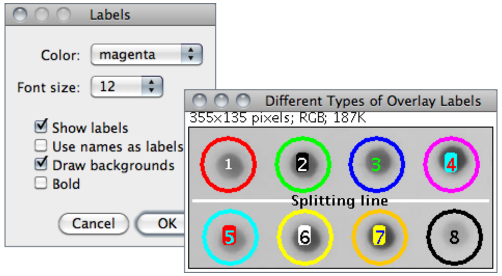
Permanently clears the overlay so that it cannot be restored using Show Overlay.

### 28.14.8 Flatten [F]

Creates a new RGB image that has the overlay rendered as pixel data. The RGB image is the same size as the active image, unlike Plugins ▷ Utilities ▷ Capture Image, which creates a WYSIWYG (What You See Is What You Get) image that is the same size as its window. Stacks must first be converted to RGB (Image ▷ Type ▷ submenu, *see also* Image Types and Formats) when flattening all slices in the stack.

### 28.14.9 Labels. . .

NEW IN IJ 1.46R




This prompt defines if and how Overlays should be labelled.

It sets the behavior of Image ▷ Overlay ▷ Add Selection. . . [b], Analyze ▷ Measure. . . [m] (after activating the *Add to overlay* option in Analyze ▷ Set Measurements. . . ), Analyze ▷ Analyze Particles. . . and the ROI Manager *Show All* display.

***Color*** The color of the label as one of the default selection colors.

***Font size*** Specifies the font size of the label (12–72 pt).

***Show labels*** If overlays should be decorated with a text label. This option is inactive by default.

***Use names as labels*** If checked, ROI names are used instead of the default numeric labels. If unchecked, the size (selection count) of the current overlay is used. Selections can be renamed using the Edit ▷ Selection ▷ Properties... [y] command, or, when using the ROI Manager, either *Rename...* or *Properties...*

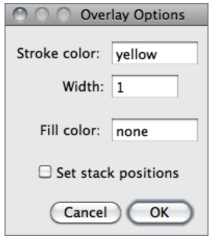
***Draw backgrounds*** If checked, text will be displayed on complementary colored background. This option produces similar labels to those produced by the ROI Manager *Show All* option when *Labels* is activated.

***Bold*** If checked, labels (typeset in sans-serif font) are displayed in boldface.

SEE ALSO: Overlay Options..., Add Selection... [b]

New in IJ 1.46r

### 28.14.10 Overlay Options...




Use this command to define the default overlay *Stroke color*, *Stroke width* and *Fill color*. As mentioned in Edit ▷ Selection ▷ Properties... [y] and Image ▷ Overlay ▷ Add Selection... [b], *Stroke* (contour) color and *Width* are ignored if a *Fill color* is specified. Set *Stroke width* to 0 to have selections drawn using a width of one pixel regardless of the image magnification (*see* XVIII Working with Zoomed Canvases).

As usual, colors are specified using the name of one of the ImageJ default colors (*black*, *blue*, *cyan*, *green*, *magenta*, *orange*, *red*, *white* and *yellow*) or using Hexadecimal Color Values.

With Stacks and Hyperstacks, selecting the *Set stack positions* checkbox will make Overlays visible only when browsing their respective slice or frame. If unchecked, overlays will be displayed throughout the stack (*see also* ROI Manager..., *More*≫Options...).

SEE ALSO: Labels..., *Remove Slice Info* (Analyze ▷ Tools ▷ ROI Manager...), Settings and Preferences

## 28.15 Lookup Tables ▷

This submenu contains a selection of color lookup tables that can be applied to grayscale images to produce Pseudocolor Images. In addition, it lists all the lookup tables installed in the `ImageJ/luts/` directory. More than 100 additional lookup tables are available from the ImageJ website as individual files or, in bulk, as a `ZIP archive`.

As explained earlier, it is not possible to organize LUTs into subfolders. However, the most frequently used lookup tables can be renamed with a numeric prefix (e.g, `01-glasbey.lut`, `02-Termal.lut`, etc.) so that they are listed earlier in the menu. This submenu can also be accessed from the Toolbar by loading the LUT Menu.

When loading a lookup table is loaded and no image is open, a 256×32 ramp image is created to display the color table.

SEE ALSO: Channels... widget, `Show_All_LUTs`, a macro that creates a graphical palette of all the installed lookup tables.





### 28.15.1 Invert LUT

Inverts the current lookup table. For 8–bit images, the value ($v$) of each entry in the table is replaced by $255 - v$. With inverted LUTs, pixels with a value of zero are white and pixels with a value 255 are black. Unlike the Edit ▷ Invert [I] command, pixels values are not altered, only the way the image is displayed on the screen.

SEE ALSO: Image ▷ Color ▷ Show LUT/Edit LUT...

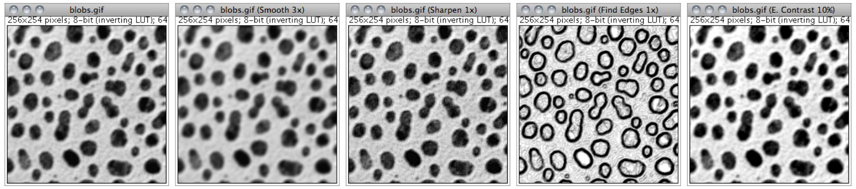
### 28.15.2 Apply LUT

Applies the current lookup table function to each pixel in the image or selection and restores the default identity function. This modifies the gray values so that when the image is viewed using the default grayscale lookup table it will look the same as it did before. This command is equivalent to clicking on *Apply* in Image ▷ Adjust ▷ Brightness/Contrast... [C]. For thresholded images, it is equivalent to clicking on *Apply* in Image ▷ Adjust ▷ Threshold... [T].

SEE ALSO: Image ▷ Color ▷ Show LUT/Edit LUT...

# 29 Process ▷

This menu lists all commands related to image processing, including point operations, filters, and arithmetic operations between multiple images [11]. The File ▷ Open Samples ▷ ▷ Blobs (25K) [B] image will be used in most of the illustrations of this section.




**Process ▷ submenu: Smooth [S], Sharpen, Find Edges and Enhance Contrast...**

## 29.1 Smooth [S]

Blurs the active image or selection. This filter replaces each pixel with the average of its 3×3 neighborhood.

## 29.2 Sharpen

Increases contrast and accentuates detail in the image or selection, but may also accentuate noise. This filter uses the following weighting factors to replace each pixel with a weighted average of the 3×3 neighborhood:

$$\begin{matrix} -1 & -1 & -1 \\ -1 & 12 & -1 \\ -1 & -1 & -1 \end{matrix}$$

## 29.3 Find Edges

Uses a Sobel edge detector to highlight sharp changes in intensity in the active image or selection. Two 3×3 convolution kernels (shown below) are used to generate vertical and horizontal derivatives. The final image is produced by combining the two derivatives using the square root of the sum of the squares.

$$\begin{matrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{matrix} \qquad \begin{matrix} 1 & 0 & -1 \\ 2 & 0 & -2 \\ 1 & 0 & -1 \end{matrix}$$

Improved in IJ 1.46r

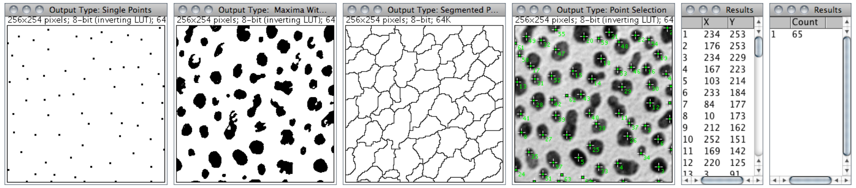
## 29.4 Find Maxima...

Determines the local maxima in an image and creates a binary (mask-like) image of the same size with the maxima, or one segmented particle per maximum, marked [29]. Analysis is performed on the existing rectangular selection or on the entire image if no selection is present.

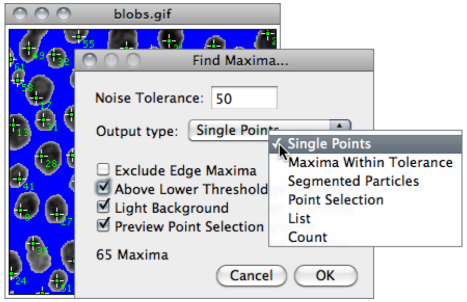
For RGB images, maxima of luminance are selected, with the luminance defined as weighted or unweighted average of the colors depending on how *Weighted RGB to Grayscale Conversion* is set in Edit ▷ Options ▷ Conversions....








**Process ▷ Find Maxima... (ImageJ 1.43s).** Six outputs are possible: *Single Points, Maxima Within Tolerance, Segmented Particles, Point Selection, List* and *Count*.




***Noise Tolerance*** Maxima are ignored if they do not stand out from the surroundings by more than this value (calibrated units for calibrated images). In other words, a threshold is set at the maximum value minus noise tolerance and the contiguous area around the maximum above the threshold is analyzed. For accepting a maximum, this area must not contain any point with a value higher than the maximum. Only one maximum within this area is accepted.

***Output Type*** Can be (*see* Process ▷ Find Maxima... outputs):

- ***Single Points*** Results in one single point per maximum.
- ***Maxima Within Tolerance*** All points within the *Noise Tolerance* for each maximum.
- ***Segmented Particles*** Assumes that each maximum belongs to a particle and segments the image by a watershed algorithm applied to the values of the image (in contrast to Process ▷ Binary ▷ Watershed, which uses the Euclidian distance map – EDM). See Process ▷ Binary ▷ Voronoi for EDM-based segmentation of binary images.
- ***Point Selection*** Displays a multi-point selection with a point at each maximum.
- ***List*** Displays the XY coordinates of each maximum in the Results window.
- ***Count*** Displays the number of maxima in the Results window.

***Exclude Edge Maxima*** Excludes maxima if the area within the noise tolerance surrounding a maximum touches the edge of the image (edge of the selection does not matter).

***Above Lower Threshold*** (Thresholded images only) Finds maxima above the lower threshold only. The upper threshold of the image is ignored. If *Segmented Particles* is selected as *Output Type*, the area below the lower threshold is considered a background. This option cannot be used when finding minima (image with light background and inverted LUT).

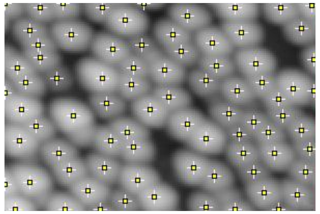
***Light Background*** To be checked if the image background is brighter than the objects to be found, as it is in the Cell Colony image in the illustration below.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/process.html#find-maxima.

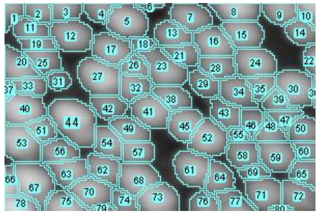
Output is a binary image, with foreground 255 and background 0, using an inverted or normal LUT depending on the *Black Background* option in Process ▷ Binary ▷ Options... (*see* XXII Interpreting Binary Images).

The number of particles (as obtained by Analyze ▷ Analyze Particles...) in the output image does not depend on the selected *Output Type*. Note that *Segmented Particles* will usually result in particles touching the edge if *Exclude Edge Maxima* is selected. *Exclude Edge Maxima* applies to the maximum, not to the particle.

Find Maxima... does not work on stacks, but the FindStackMaxima macro runs it on all the images in a stack and creates a second stack containing the output images. The FindMaximaRoiManager macro demonstrates how to add particles found by Find Maxima... to the ROI Manager...



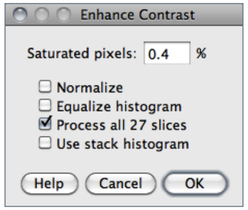
Points at maxima (Multi-point selection)



Segmented Particles (ROIs obtained with Analyze ▷ Analyze Particles...)

Improved in IJ 1.46r

## 29.5 Enhance Contrast...




Enhances image contrast by using either histogram stretching or histogram equalization. Both methods are described in detail in the Hypermedia Image Processing Reference – Contrast Stretching and Histogram Equalization.

This command does not alter pixel values as long as *Normalize*, *Equalize Histogram* or *Normalize All n Slices* (in the case of stacks) are not checked.

***Saturated Pixels*** Determines the number of pixels in the image that are allowed to become saturated. Increasing this value will increase contrast. This value should be greater than zero to prevent a few outlying pixel from causing the histogram stretch to not work as intended.

***Normalize*** If checked, ImageJ will recalculate the pixel values of the image so the range is equal to the maximum range for the data type, or 0–1.0 for float images. The contrast stretch performed on the image is similar to the '*Auto*' option in the Brightness/Contrast... [C] window, except that with stacks, each slice in the stack is adjusted independently, according to the optimal for that slice alone (if *Use Stack Histogram* is unchecked). The maximum range is 0–255 for 8–bit images and 0–65535 for 16–bit images.
With stacks another checkbox, *Normalize All n Slices*, is displayed. If checked, normalization will be applied to all slices in the stack. Note that normalization of RGB images is not supported, and thus this option will not be available on RGB stacks.

***Equalize Histogram*** If checked, ImageJ will enhance the image using histogram equalization [30]. Create a selection and the equalization will be based on the histogram of that selection.





Uses a modified algorithm that takes the square root of the histogram values. Hold Alt to use the standard histogram equalization algorithm. The *Saturated Pixels* and *Normalize* parameters are ignored when *Equalize Histogram* is checked.

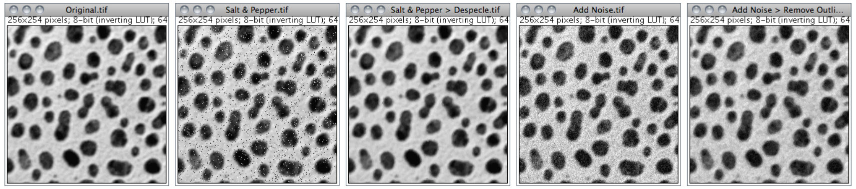
***Use Stack Histogram*** If checked, ImageJ will use the overall stack histogram instead of individual slice histograms, that allow optimal adjustments for each slice alone. This option may be specially relevant when performing enhancements based on a ROI.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/process.html#enhance.

See also: Brightness/Contrast... [C] , XIV Applying Auto Brightness/Contrast to Entire Stacks

## 29.6 Noise ▷

Use the commands in this submenu to add noise to images or remove it.




**Process ▷ Noise ▷: Salt and Pepper, Despeckle, Add Noise and Remove Outliers...**

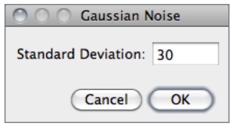
See also: RandomJ (Binomial, Exponential, Gamma, Gaussian, Poisson and Uniform) a Java package for image randomization by Erik Meijering

### 29.6.1 Add Noise

Adds random noise to the image or selection. The noise is Gaussian (normally) distributed with a mean of zero and standard deviation of 25.

See also: Filters ▷ Gaussian Blur...

### 29.6.2 Add Specified Noise...




Adds Gaussian noise with a mean of zero and a chosen standard deviation.

See also: Filters ▷ Gaussian Blur...

### 29.6.3 Salt and Pepper

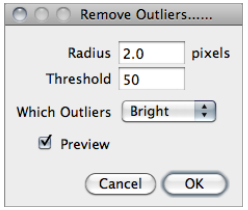
Adds *salt and pepper* noise to the image or selection by randomly replacing 2.5% of the pixels with black pixels and 2.5% with white pixels. This command only works with 8–bit images.

See also: Spray Can, Filters ▷ Gaussian Blur...

#### 29.6.4 Despeckle

This is a median filter. It replaces each pixel with the median value in its 3×3 neighborhood. This is a time consuming operation because, for each pixel in the selection, the nine pixels in the 3×3 neighborhood must be sorted and the center pixel replaced with the median value (the fifth). Median filters are good at removing *salt and pepper* noise.

#### 29.6.5 Remove Outliers...




Replaces a pixel by the median of the pixels in the surrounding if it deviates from the median by more than a certain value (the threshold). Useful for correcting, e.g., hot pixels or dead pixels of a CCD camera.

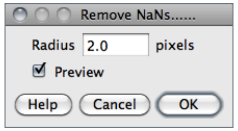
***Radius*** Determines the area (uncalibrated, i.e., in pixels) used for calculating the median. Run Process ▷ Filters ▷ Show Circular Masks... to see how radius translates into an area.

***Threshold*** Determines by how much the pixel must deviate from the median to get replaced, in raw (uncalibrated) units.

***Which Outliers*** Determines whether pixels brighter or darker than the surrounding (the median) should be replaced.

SEE ALSO: Despeckle

#### 29.6.6 Remove NaNs...




This filter replaces NaN (Not-a-Number) pixels in 32–bit (float) images by the median of the neighbors inside the circular kernel area defined by *Radius* [31]. It does not remove patches of NaNs larger than the kernel size, however.

Note that some ImageJ filters, such as Process ▷ Filters ▷ Gaussian Blur..., Mean..., and Variance... destroy the surrounding of NaN pixels by setting it also to NaN. Other filters may produce invalid results in the position of NaN pixels.

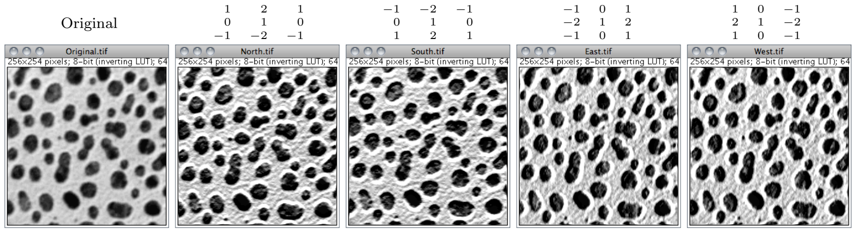
SEE ALSO: NaNs.txt, a macro that demonstrates how to create, count and remove NaNs

### 29.7 Shadows ▷

Commands in this submenu produce a shadow effect, with light appearing to come from a direction corresponding to the command name (East, North, Northeast, Northwest, South, Southeast, Southwest and West). The commands use Convolve 3 × 3, ImageJ's 3 × 3 convolution function. The Shadows Demo command uses all eight kernels to demonstrate the speed of Convolve 3 × 3. The illustration below shows four of the Shadows convolution kernels.








**Shadows ▷: North, South, East, and West kernels.**

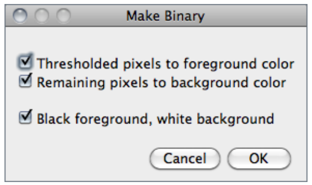
### 29.8 Binary ▷

This submenu contains commands that create or process binary (black and white) images. They assume that objects are black and background is white unless *Black Background* is checked in the Process ▷ Binary ▷ Options... dialog box (*see* XXII Interpreting Binary Images).

**Summary of morphological operators (Process ▷ Binary ▷ submenu).**

| Original | Make Binary | Erode | Dilate | Open | Close– | Outline | Skeletonize |
|---|---|---|---|---|---|---|---|
|  | | | | | | | |
| Adjust ▷ Threshold... [T] | | Minimum... (grayscale) | Maximum... (grayscale) | Erode then Dilate | Dilate then Erode | 1 pixel wide outline | 1 pixel wide skeleton |

#### 29.8.1 Make Binary



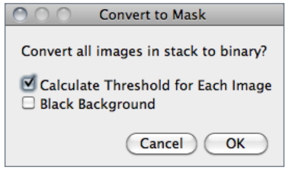

Converts an image to black and white. If a threshold has been set using the Image ▷ Adjust ▷ Threshold... [T] tool, the depicted dialog is displayed. The value of the *Black foreground, white background* checkbox reflects and sets the global *Black Background* value of Process ▷ Binary ▷ Options...

If a threshold has not been set, Make Binary will analyze the histogram of the current selection, or of the entire image if no selection is present, and set an automatic threshold level to create the binary image (*'Auto-thresholding'* is displayed in the Status bar, cf. Threshold... [T]).

With stacks the Convert to Mask dialog box is displayed. Note that for non-thresholded images and stacks Make Binary and Convert to Mask behave similarly.

SEE ALSO: Edit ▷ Selection ▷ Convert to Mask, XX Creating Binary Masks, XXII Interpreting Binary Images

### 29.8.2 Convert to Mask




Converts an image to black and white.

The mask will have an inverting LUT (white is 0 and black is 255) unless *Black Background* is checked in the Process ▷ Binary ▷ Options... dialog box. If a threshold has not been set, automatic threshold levels will be calculated (cf. Make Binary). Note that for non-thresholded images and stacks Make Binary and Convert to Mask behave similarly.

With stacks, the depicted dialog is displayed.

***Calculate Threshold for Each Image*** If checked, threshold levels will be calculated for each individual slice, otherwise the calculated threshold of the currently displayed slice will be used for all slices

***Black Background*** Defines whether the background is black and the foreground is white. Note that the value of this checkbox reflects and sets the global *Black Background* value of Process ▷ Binary ▷ Options...

SEE ALSO: Make Binary, Edit ▷ Selection ▷ Convert to Mask, XX Creating Binary Masks,

#### XX Creating Binary Masks

Four ImageJ commands can be used to create binary masks:

1. Edit ▷ Selection ▷ Create Mask
2. Process ▷ Binary ▷ Make Binary
3. Process ▷ Binary ▷ Convert to Mask
4. Image ▷ Adjust ▷ Threshold... [T] (*Apply*)

By default these commands will produce binary images with inverted LUTs, so that black is 255 and white is 0 (*see* Invert LUT). This behavior can be reversed by checking *Black Background* in Process ▷ Binary ▷ Options... before running the above commands (i.e., an inverting LUT will not be used: black will be 0 and white 255). This choice can be confirmed when running Make Binary and Convert to Mask on thresholded images. It can also be imposed at startup (*see* Settings and Preferences).

SEE ALSO: XXII Interpreting Binary Images

### 29.8.3 Erode

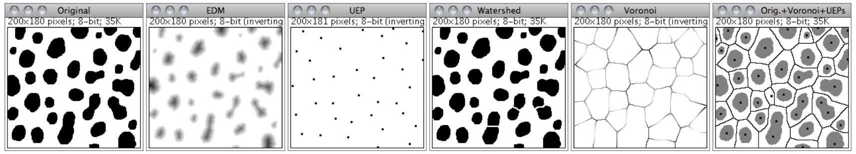
Removes pixels from the edges of objects in a binary image. Use Filters ▷ Minimum... to perform grayscale erosion on non-thresholded images.

SEE ALSO: Binary ▷ Options..., XXII Interpreting Binary Images





Original | Distance Map | Ultimate Points | Watershed | Voronoi | Original + Voronoi + Ultimate Points




**Process ▷ Binary ▷ commands.**

### 29.8.4 Dilate

Adds pixels to the edges of objects in a binary image. Use Filters ▷ Maximum... to perform grayscale dilation on non-thresholded images.

SEE ALSO: Binary ▷ Options..., XXII Interpreting Binary Images

### 29.8.5 Open

Performs an erosion operation, followed by dilation. This smoothes objects and removes isolated pixels.

SEE ALSO: Binary ▷ Options..., XXII Interpreting Binary Images

### 29.8.6 Close–

Performs a dilation operation, followed by erosion. This smoothes objects and fills in small holes. The command has a tailing hyphen to differentiate it from File ▷ Close [w] .

SEE ALSO: Binary ▷ Options..., XXII Interpreting Binary Images

### 29.8.7 Outline

Generates a one pixel wide outline of foreground objects in a binary image. The line is drawn inside the object, i.e., on previous foreground pixels.

### 29.8.8 Fill Holes

This command fills holes (4–connected background elements) in objects by filling the background [34].

### 29.8.9 Skeletonize

IMPROVED IN IJ 1.46R

Repeatably remove pixels from the edges of objects in a binary image until they are reduced to single-pixel-wide shapes (topological skeletons). As explained in XXI Skeletonize vs Skeletonize 3D, there are several skeletonization algorithms. ImageJ implements a thinning algorithm from Zhang and Suen. A fast parallel algorithm for thinning digital patterns. *CACM* 27(3):236–239, 1984, in which a lookup table indexes all the 256 possible 3×3 neighborhood configurations for each foreground pixel. The algorithm calculates the index number for each object pixel, and uses the lookup table to decide if the pixel is eliminable. This process is repeated until no pixel can be eliminated.

When debugging is enabled in Edit ▷ Options ▷ Misc..., Skeletonize creates an animation documenting the iterations of the thinning algorithm.

SEE ALSO: AnalyzeSkeleton plugin, BinaryProcessor source code

### XXI Skeletonize vs Skeletonize 3D

Skeletonize3D is a ImageJ plugin written by Ignacio Arganda-Carreras [35] that offers several advantages over Process ▷ Binary ▷ Skeletonize, the legacy skeletonization algorithm of ImageJ:

- Skeletonize works only with binary 2D images. Skeletonize3D works with 8-bit 2D images and stacks, expecting the image to be binary. If not, Skeletonize3D considers all pixel values above 0 to be white (255).
- While Skeletonize relies on *Black background* value in Binary ▷ Options... (*see* XXII Interpreting Binary Images), the output of Skeletonize3D always has a value of 255 at the skeleton and 0 at background pixels, independently of the *Black background* option.

In Fiji, Skeletonize 3D is already pre-installed as Plugins ▷ Skeleton ▷ Skeletonize (2D/3D). In ImageJ, it can be downloaded and installed from the Skeletonize3D homepage.

Maximum projections (Image ▷ Stacks ▷ Z Project...) of skeletons produced by Skeletonize (middle) and Skeletonize3D (right). The left image is the maximum projection of the original stack, File ▷ Open Samples ▷ Bat Cochlea Volume (19K). Topographic skeletons can be analyzed using the AnalyzeSkeleton plugin.

### 29.8.10 Distance Map

Generates a Euclidian distance map (EDM) from a binary image [69]. Each foreground pixel in the binary image is replaced with a gray value equal to that pixel's distance from the nearest background pixel (for background pixels the EDM is 0). The Ultimate Points, Watershed and Voronoi operations are based on the EDM algorithm.

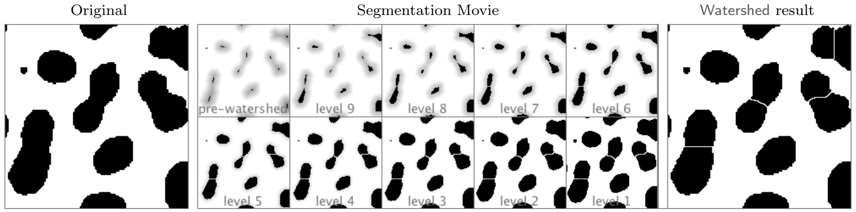
The output type (*Overwrite*, *8–bit*, *16–bit* or *32–bit*) of this command can be set in the Binary ▷ Options... dialog box. Note that when selecting '*Overwrite*' or '*8–bit output*', distances larger than 255 are labelled as 255.

### 29.8.11 Ultimate Points

Generates the ultimate eroded points (UEPs) of the Euclidian distance map (EDM, *see* Distance Map) from a binary image. Ultimate Eroded Points are maxima of the EDM. In the output, the points are assigned the EDM value, which is equal to the radius of the largest circle that fits into the binary particle, with the UEP as the center. The output type (*Overwrite*, *8–bit*, *16–bit* or *32–bit*) of this command can be set in the Binary ▷ Options... dialog box.








Process ▷ Binary ▷ Watershed **running in Debug mode.**

### 29.8.12 Watershed

Watershed segmentation is a way of automatically separating or cutting apart particles that touch. It first calculates the Euclidian distance map (EDM) and finds the ultimate eroded points (UEPs). It then dilates each of the UEPs (the peaks or local maxima of the EDM) as far as possible – either until the edge of the particle is reached, or the edge touches a region of another (growing) UEP. Watershed segmentation works best for smooth convex objects that don't overlap too much.

Enable debugging in Edit ▷ Options ▷ Misc... and the Watershed command will create an animation that shows how the watershed algorithm works (cf. online example).

SEE ALSO: Find Maxima... (Segmented Particles output) for watershed segmentation of grayscale images.

### 29.8.13 Voronoi

Splits the image by lines of points having equal distance to the borders of the two nearest particles. Thus, the Voronoi cell of each particle includes all points that are nearer to this particle than any other particle. When particles are single points, this process is a Voronoi tessellation (also known as Dirichlet tessellation).

The output type (*Overwrite*, *8–bit*, *16–bit* or *32–bit*) of this command can be set in the Process ▷ Binary ▷ Options... dialog box. In the output, the value inside the Voronoi cells is zero; the pixel values of the dividing lines between the cells are equal to the distance between the two nearest particles. This is similar to a medial axis transform of the background, but there are no lines in inner holes of particles.

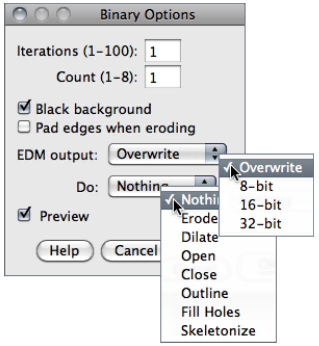
SEE ALSO: Find Maxima... (Segmented Particles output), Delaunay_Voronoi plugin

### 29.8.14 Options...

Specifies several settings used by Binary ▷ commands.

***Iterations*** Specifies the number of times erosion, dilation, opening, and closing are performed. Iterations can be aborted by pressing Esc.

***Count*** Specifies the number of adjacent background pixels necessary before a pixel is removed from the edge of an object during erosion and the number of adjacent foreground pixels necessary before a pixel is added to the edge of an object during dilation.




***Black background*** If checked, binary images will be created without using an inverted LUT (cf. XX Creating Binary Masks) and commands in the Process ▷ Binary ▷ submenu will assume that images contain white objects on a black background (*see* XXII Interpreting Binary Images). Macros can set this option using `setOption("BlackBackground", true);` (*see* XXII Interpreting Binary Images and Settings and Preferences).

***Pad edges when eroding*** If checked, Binary ▷ Erode does not erode from the edges of the image. This setting also affects Binary ▷ Close–, which erodes from the edges unless this checkbox is selected.

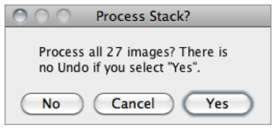
***EDM output*** Determines the output type for the Binary ▷ Distance Map, Ultimate Points and Voronoi commands. Set it to '*Overwrite*' for 8–bit output that overwrites the input image; '*8–bit*', '*16–bit*' or '*32–bit*' for separate output images. 32–bit output has floating point (subpixel) distance resolution.

***Do*** This drop-down menu allows one to test the chosen settings by previewing each binary operation (Erode, Dilate, Open, Close–, Outline, Fill Holes, Skeletonize) on the active image. This option is only available when the active image is binary.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/process.html#options.

## 29.9 Math ▷

The commands in this submenu add (subtract, multiply, etc.) a constant to each pixel in the active image or selection. A '*Preview*' option is available for most operations.




With stacks, the dialog depicted on the left is displayed. Choose '*Yes*' to process entire stack or '*No*' to process only the active slice. The dialog is not displayed if *Hide "Process Stack?" dialog* is checked in Edit ▷ Options ▷ Misc. . .

SEE ALSO: Memory & Threads. . .

### 29.9.1 Add. . .

Adds a constant to the image or selection. With 8–bit images, results greater than 255 are set to 255. With 16–bit signed images, results greater than 65,535 are set to 65,535.

### 29.9.2 Subtract. . .

Subtracts a constant from the image or selection. With 8–bit and 16–bit images, results less than 0 are set to 0.





**XXII INTERPRETING BINARY IMAGES**

Binary images are thresholded to only two values, typically 0 and 1, but often – as with ImageJ – 0 and 255, that represent black and white on an 8–bit scale (*see* Image Types and Formats).

The interpretation of binary images is not universal. While some software packages will always perform binary operations on 255 values (or 1, or any non-zero value), ImageJ takes into account the foreground and background colors of the binary image.

0: Black (Background), 255: White

0: White (Background), 255: Black

In ImageJ, the *Black background* option in Process ▷ Binary ▷ Options. . . defines not only how new binary images will be created (*see* XX Creating Binary Masks) but also how previously created images are interpreted. This means object(s) will be inferred on a image-per-image basis. As such, inverting the LUT (*see* Invert LUT) of a binary image without updating the *Black background* option may lead to unexpected results, such as the aberrant thinning operation (Process ▷ Binary ▷ Skeletonize) depicted here. This issue can be avoided by imposing adequate preferences at startup, as described in Settings and Preferences.

You can use the Process ▷ Binary ▷ Options. . . dialog to assess the impact of the *Black background* option: Create a binary image, choose an operation from the *Do* drop-down menu, activate the preview feature and toggle the *Black background* checkbox.

SEE ALSO: Settings and Preferences, XX Creating Binary Masks

### 29.9.3 Multiply. . .

Multiplies the image or selection by the specified real constant. With 8–bit images, results greater than 255 are set to 255. With 16–bit signed images, results greater than 65, 535 are set to 65, 535.

### 29.9.4 Divide. . .

Divides the image or selection by the specified real constant. Except for 32–bit (float) images, attempts to divide by zero are ignored. With 32–bit images, dividing by zero results in *Infinity*, -*Infinity* or *NaN* (0/0) pixels when the source pixels are positive, negative or zero. The divide-by-zero value can be redefined using Edit ▷ Options ▷ Misc. . . .

### 29.9.5 AND. . .

Does a bitwise AND of the image and the specified binary constant.

### 29.9.6 OR. . .

Does a bitwise OR of the image and the specified binary constant.

### 29.9.7 XOR. . .

Does a bitwise XOR of the image and the specified binary constant.

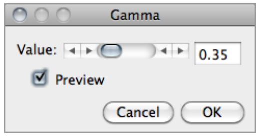
### 29.9.8 Min...

Pixels in the image with a value less than the specified constant are replaced by the constant.

### 29.9.9 Max...

Pixels in the image with a value greater than the specified constant are replaced by the constant.

### 29.9.10 Gamma...




Applies the function $f(p) = (p/255)^{\gamma} \times 255$ to each pixel ($p$) in the image or selection, where $0.1 \leq \gamma \leq 5.0$. For RGB images, this function is applied to all three color channels. For 16–bit images, the image min and max are used for scaling instead of 255.

SEE ALSO: GammaCorrectionTool macro

### 29.9.11 Set...

Fills the image or selection with the specified value.

### 29.9.12 Log

For 8–bit images, applies the function $f(p) = \ln(p) \times 255/\ln(255)$ to each pixel ($p$) in the image or selection. For RGB images, this function is applied to all three color channels. For 16–bit images, the image min and max are used for scaling instead of 255. For float images, no scaling is done. To calculate $\log_{10}$ of the image, multiply the result of this operation by 0.4343 ($1/\ln(10)$).

### 29.9.13 Exp

Performs an exponential transform on the active image or selection.

### 29.9.14 Square

Performs a square transform on the active image or selection.

### 29.9.15 Square Root

Performs a square root transform on the active image or selection.

### 29.9.16 Reciprocal

Generates the reciprocal (multiplicative inverse) of the active image or selection, transforming each pixel ($p$) into $1/p$. Requires 32–bit float images (*see* Image Types and Formats).





### 29.9.17 NaN Background

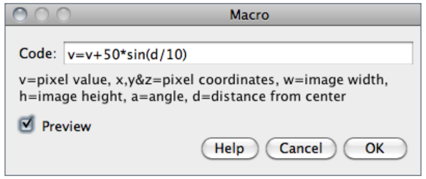
Sets non-thresholded pixels in 32–bit float images to the *NaN* (Not a Number) value. For float images, the *Apply* option in Image ▷ Adjust ▷ Threshold... [T] runs this command.

Pixels with a value of Float.NaN (0f/0f), Float.POSITIVE_INFINITY (1f/0f) or Float. NEGATIVE_INFINITY (-1f/0f) are ignored when making measurements on 32–bit float images.

### 29.9.18 Abs

Generates the absolute value of the active image or selection. Works only with 32–bit float or signed 16–bit image images.

### 29.9.19 Macro...




This command performs image arithmetic using an expression specified by the user [32]. It can be used to create fully-synthetic images or to perform precise pixel manipulations on existing images. The Math-MacroDemo macro demonstrates the usage of this command.

SEE ALSO: Expression plugin

## 29.10 FFT ▷

The commands in this submenu support frequency domain display, editing and processing. They are based on an implementation of the 2D Fast Hartley Transform (FHT) contributed by Arlo Reeves, the author of the ImageFFT, spinoff of NIH Image[1]. 3D FHT can be performed using Bob Dougherty's 3D Fast Hartley Transform plugin.

The frequency domain image is stored as 32–bit float FHT attached to the 8–bit image that displays the power spectrum. Commands in this submenu, such as Inverse FFT, operate on the 32–bit FHT, not on the 8–bit power spectrum. All other ImageJ commands only 'see' the power spectrum.

Two FFT dedicated tutorials are available on the ImageJ website: FFT Measurements and FFT Filtering.

### 29.10.1 FFT

Computes the Fourier transform and displays the power spectrum. Polar coordinates of measured point selections are recorded by Analyze ▷ Measure... [m].

If the mouse is over an active frequency domain (FFT) window, its location is displayed in polar coordinates. The angle is expressed in degrees, while the radius is expressed in pixels per cycle (p/c). The radius is expressed in [units] per cycle (e.g. mm/c) if the spatial scale of the image was defined using Image ▷ Properties... [P] or Analyze ▷ Set Scale...

[1] Although outdated, the ImageFFT documentation summarizes important frequency domain methodologies.

#### 29.10.2 Inverse FFT

Computes the inverse Fourier transform. You can filter or mask spots on the transformed (frequency domain) image and do an inverse transform to produce an image which only contains the frequencies selected or which suppresses the frequencies selected. Use ImageJ's selection tools and fill / clear commands to draw black or white areas that mask portions of the transformed image. Black areas (pixel value = 0) cause the corresponding frequencies to be filtered (removed) and white areas (pixel value = 255) cause the corresponding frequencies to be passed. It is not, however, possible to both filter and pass during the same inverse transform.

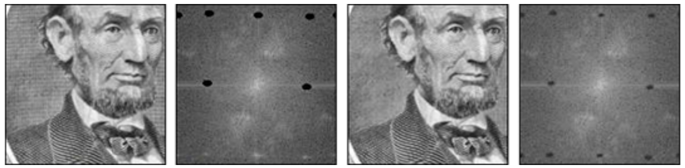
Note that areas to be filtered in the frequency domain image must be zero filled and areas to be passed must be filled with 255. You can verify that this is the case by moving the cursor over a filled area and observing that the values displayed in the status bar are either 0 or 255. Thus, you should always confirm that masked areas are not some other gray value, by using the black & white reset option in the Color Picker window widgets when defining foreground (Edit ▷ Fill [f]) and background (Edit ▷ Clear) colors.

| Original | Power spectrum with mask that filters low frequencies | Result of inverse transform | Power spectrum with mask that passes low frequencies | Result of inverse transform |
|---|---|---|---|---|

**FFT: Example of low frequencies filtering.**

With off-center selections, the same spatial frequency appears twice in the power spectrum, at points opposite from the center. It is sufficient to fill / clear only one of these.

| Original | Edited power spectrum | Inverse transform | Power spectrum after filtering |
|---|---|---|---|



**FFT: Example of low frequencies filtering.**

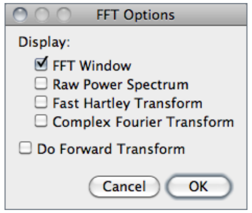
#### 29.10.3 Redisplay Power Spectrum

Recomputes the power spectrum from the frequency domain image (32–bit FHT). This command allows you to start over after mis-editing the 8–bit power spectrum image.





#### 29.10.4 FFT Options...




Displays the FFT Options dialog box. The first group of checkboxes specifies which image(s) are created by the FFT command:

***Display FFT Window*** The standard output. It consists of an 8–bit image of the power spectrum and the actual data, which remain invisible for the user. The power spectrum image is displayed with logarithmic scaling, enhancing the visibility of components that are weakly visible. The actual data are used for the Inverse FFT command.

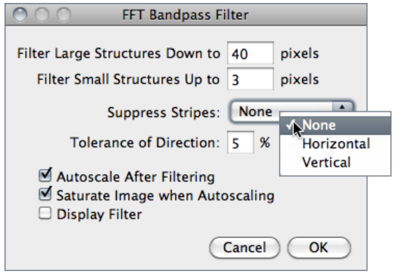
***Display Raw Power Spectrum*** The power spectrum without logarithmic scaling.

***Display Fast Hartley Transform*** The internal format used by the command, which is based on a Hartley transform rather than Fourier transform.

***Display Complex Fourier Transform*** A stack with two slices for the real and imaginary parts of the FFT.

***Do Forward Transform*** If checked, the current image is transformed immediately when closing the FFT Options dialog.

#### 29.10.5 Bandpass Filter...




Removes high spatial frequencies (blurring the image) and low spatial frequencies (similar to subtracting a blurred image). It can also suppress horizontal or vertical stripes that were created by scanning an image line by line [33].

The Bandpass Filter uses a special algorithm to reduce edge artifacts (before the Fourier transform, the image is extended in size by attaching mirrored copies of image parts outside the original image, thus no jumps occur at the edges).

***Filter Large Structures Down to*** Smooth variations of the image with typical sizes of bright or dark patches larger than this value are suppressed (background).

***Filter Large Structures Up to*** Determines the amount of smoothing. Objects in the image smaller than this size are strongly attenuated. Note that these values are both half the spatial frequencies of the actual cutoff. The cutoff is very soft, so the bandpass will noticeably attenuate even spatial frequencies in the center of the bandpass unless the difference of the two values is large (say, more than a factor of 5 or so).

***Suppress Stripes*** Select whether to eliminate *Horizontal* or *Vertical* stripes. Removal of horizontal stripes is similar to subtracting an image that is only blurred in the horizontal direction from the original.

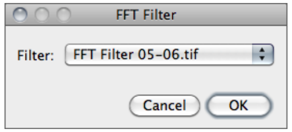
***Tolerance of Direction*** This is for *Suppress Stripes*; higher values remove shorter stripes and/or stripes that are running under an angle with respect to the horizontal (vertical) direction.

***Autoscale After Filtering*** If checked, puts the lowest intensity to 0 and the highest intensity to 255, preserving all intensities.

***Saturate Image when Autoscaling*** If checked, allows some intensities to go into saturation, and produces a better visual contrast. *Saturate Image when Autoscaling* only has an effect when *Autoscale After Filtering* is enabled.

***Display Filter*** If checked, shows the filter generated. Note that this disables Undo of the filter operation on the original image.

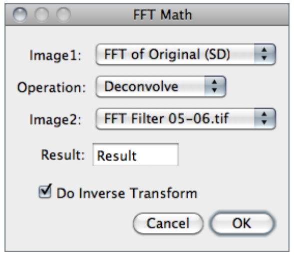
### 29.10.6 Custom Filter. . .




This command does Fourier space filtering of the active image using a user-supplied spatial domain (non-FFT) image as the filter.

This image will be converted to 8–bit. For pixels that have a value of 0, the corresponding spatial frequencies will be blocked. Pixel with values of 255 should be used for passing the respective spatial frequencies without attenuation. Note that the filter should be symmetric with respect to inversion of the center: Points that are opposite of the center point (defined as x = *width*/2, y = *height*/2) should have the same value. Otherwise, artifacts can occur. For some examples, see the FFTCustomFilterDemo and FFTRemoveStreaks macros.

### 29.10.7 FD Math. . .




This command correlates, convolves or deconvolves two images.

It does this by converting *Image1* and *Image2* to the frequency domain, performing conjugate multiplication or division, then converting the result back to the space domain. These three operations in the frequency domain are equivalent to correlation, convolution and deconvolution in the space domain.

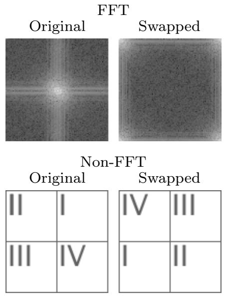
Refer to the DeconvolutionDemo and MotionBlurRemoval macros for examples.

### 29.10.8 Swap Quadrants

This command transforms between the 'user friendly' display of Fourier transforms with the lowest frequencies at the center and the 'native' form with the lowest frequencies at the four corners.








Swap Quadrants swaps quadrants I with III and II with IV (counterclockwise starting from 'Northeast') so that points near the center are moved towards the edge and vice versa. Another way to see this command is to imagine that the image is periodically repeated and the origin is shifted by *width*/2 in x and by *height*/2 in y direction.

For Fourier transforms, Swap Quadrants affects only the image displayed, not the actual FHT data. Therefore, editing an image with swapped quadrants for filtering or masking may lead to undesired results.

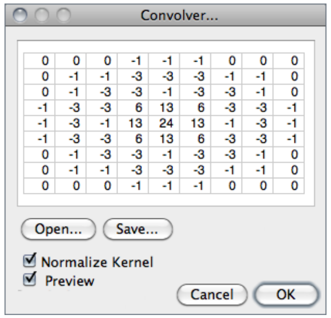
## 29.11 Filters ▷

This submenu contains miscellaneous filters [36] (including those installed by the Plugins ▷ Utilities ▷ Install Plugin. . . command).

More information on image filters can be obtained by looking up related keywords (*convolution, Gaussian, median, mean, erode, dilate, unsharp*, etc.) on the Hypermedia Image Processing Reference index.

SEE ALSO: Memory & Threads. . .

### 29.11.1 Convolve. . .




Does spatial convolution using a kernel entered into a text area.

A kernel is a matrix whose center corresponds to the source pixel and the other elements correspond to neighboring pixels. The destination pixel is calculated by multiplying each source pixel by its corresponding kernel coefficient and adding the results. If needed, the input image is effectively extended by duplicating edge pixels outward. There is no arbitrary limit to the size of the kernel but it must be square and have an odd width.

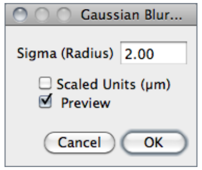
Rows in the text area must all have the same number of coefficients, the rows must be terminated with a carriage return, and the coefficients must be separated by one or more spaces. Kernels can be pasted into the text area using Ctrl V

Checking *Normalize Kernel* causes each coefficient to be divided by the sum of the coefficients, preserving image brightness.

The kernel shown is a 9 × 9 "Mexican hat", which does both smoothing and edge detection in one operation. Note that kernels can be saved as a text file by clicking on the *'Save'* button, displayed as an image using File ▷ Import ▷ Text Image. . . , scaled to a reasonable size using Image ▷ Adjust ▷ Size. . . and plotted using Analyze ▷ Surface Plot. . .

SEE ALSO: ConvolutionDemo macro

#### 29.11.2 Gaussian Blur...




This filter uses convolution with a Gaussian function for smoothing [37].

*Sigma* is the radius of decay to $e^{-0.5}$ ($\approx 61\%$), i.e., the standard deviation ($\sigma$) of the Gaussian (this is the same as in Adobe®Photoshop®, but different from ImageJ versions till 1.38q, in which *radius* was $2.5\times\sigma$ (cf. GaussianBlur.java).

Like all ImageJ convolution operations, it assumes that out-of-image pixels have a value equal to the nearest edge pixel. This gives higher weight to edge pixels than pixels inside the image, and higher weight to corner pixels than non-corner pixels at the edge. Thus, when smoothing with very high blur radius, the output will be dominated by the edge pixels and especially the corner pixels (in the extreme case, with a blur radius of e.g. $10^{20}$, the image will be replaced by the average of the four corner pixels).

For increased speed, except for small blur radii, the lines (rows or columns of the image) are downscaled before convolution and upscaled to their original length thereafter.

SEE ALSO: Noise ▷ Add Noise, Accurate Gaussian Blur plugin, AnimatedGaussianBlur macro

New in IJ 1.46r

#### 29.11.3 Gaussian Blur 3D...

This command calculates a three dimensional (3D) gaussian lowpass filter using a 3-D Gaussian. It works with Stacks and Hyperstacks but not single-slice Color Composite Images. Refer to Gaussian Blur... for more information on sigma values.

SEE ALSO: Gaussian Blur 3D source code

#### 29.11.4 Median...

Reduces noise in the active image by replacing each pixel with the median of the neighboring pixel values.

#### 29.11.5 Mean...

Smooths the current image by replacing each pixel with the neighborhood mean.

#### 29.11.6 Minimum...

This filter does grayscale erosion by replacing each pixel in the image with the smallest pixel value in that pixel's neighborhood.

SEE ALSO: Binary ▷ Erode

#### 29.11.7 Maximum...

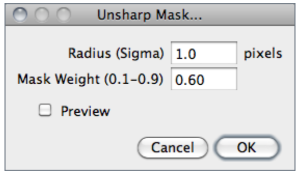
This filter does grayscale dilation by replacing each pixel in the image with the largest pixel value in that pixel's neighborhood.

SEE ALSO: Binary ▷ Dilate





#### 29.11.8 Unsharp Mask...




Sharpens and enhances edges by subtracting a blurred version of the image (the unsharp mask) from the original.

Unsharp masking subtracts a blurred copy of the image and rescales the image to obtain the same contrast of large (low-frequency) structures as in the input image. This is equivalent to adding a high-pass filtered image and thus sharpens the image.

***Radius*** The standard deviation ($\sigma$ blur radius, cf. Gaussian Blur...) of the Gaussian blur that is subtracted. Increasing the Gaussian blur radius will increase contrast.

***Mask Weight*** Determines the strength of filtering, whereby $MaskWeight = 1$ would be an infinite weight of the high-pass filtered image that is added. Increasing the *Mask Weight* value will provide additional edge enhancement.

#### 29.11.9 Variance...

Highlights edges in the image by replacing each pixel with the neighborhood variance.

#### 29.11.10 Show Circular Masks...

Generates a stack containing examples of the circular masks used by the Median..., Mean..., Minimum..., Maximum..., and Variance... filters for various neighborhood sizes.

### 29.12 Batch ▷

This submenu allows the execution of commands in a series of images without manual intervention.

Batch ▷ commands are non-recursive, i.e., they are applied to all the images of the chosen *Input* folder but not its subfolders. Nevertheless a directory hierarchy can be transversed using ImageJ macro language (cf. BatchProcessFolders macro).

Three critical aspects to keep in mind when performing batch operations that modify processed images:

- Files can be easily overwritten since the batch processor will silently override existing files with the same name.
- The destination *Output* folder should have adequate disk space to receive the created images.
- In the case of non-native formats, batch operations will be influenced by the behavior of the reader plugin or library (cf. Non–native Formats).

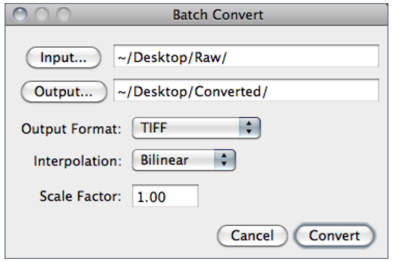
### 29.12.1 Measure...

This command measures all the images in a user-specified folder, by running the Analyze ▷ Measure... [m] command in all images of the chosen directory.

Note that measurements are performed on non thresholded images. In the case of TIFF images saved with active selections measurements are performed on the ROI and not the whole image.

SEE ALSO: Analyze ▷ Set Measurements..., Batch ▷ Macro...

### 29.12.2 Convert...




Batch converts and/or resizes multiple images from a specified folder.

***Input...*** Selects the source folder containing the images to be processed.

***Output...*** Selects the destination folder where the processed images will be stored.

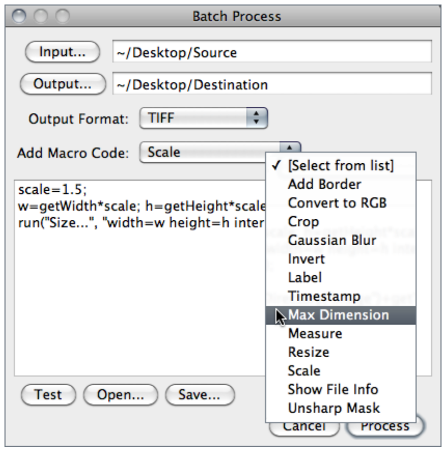
***Output Format*** Specifies the output format that can be set to *TIFF, 8–bit TIFF, JPEG, GIF, PNG, PGM, BMP, FITS, Text Image, ZIP* or *Raw* (cf. Image Types and Formats and File ▷ Save As ▷ submenu).

***Interpolation*** The resampling method to be used in case *Scale Factor* is not 1.00 (*see* Image ▷ Size... and Image ▷ Scale... [E]). For better results, *Average when downsizing* is automatically selected when scaling down images.

***Scale Factor*** Specifies if images should be resized (*see* Image ▷ Scale... [E]).

IMPROVED IN IJ 1.46R

### 29.12.3 Macro...




Runs a macro over a specified folder. The last used macro is stored in the /ImageJ/macros/batchmacro.ijm file and remembered across restarts..

***Input...*** Selects the source folder containing the images to be processed.

***Output...*** Selects the destination folder where the processed images will be stored. Note that original files will not be saved if this field is left empty.

***Output Format*** Specifies the output format that can be set to *TIFF, 8–bit TIFF, JPEG, GIF, PNG, PGM, BMP, FITS, Text Image, ZIP* or *Raw* (cf. Image Types and Formats and File ▷ Save As ▷ submenu).





***Add Macro Code*** This drop-down menu contains macro snippets that can be combined to create the processing macro. Other statements can be pasted from the macro recorder or ImageJ's editor while the dialog box is opened [38]. Previously written macros can be imported using *Open...* When editing the macro beware of any statements that may interfere with the normal operation of the batch processor (such as `Close()` or `Open()` calls).

***Test*** Tests the macro on the first image of the *Input...* folder (the processed image will be displayed).

***Open...*** Imports previously written macros.

***Save...*** Saves the assembled macro.

SEE ALSO: Plugins ▷ Macros ▷ Record..., Batch ▷ Virtual Stack...

### 29.12.4 Virtual Stack...

IMPROVED IN IJ 1.46R

This command, that shares the same interface of Batch ▷ Macro... (cf. `BatchProcesser.java`), allows virtual stack manipulations. E.g., Cropping a virtual stack can be performed by executing the following steps:

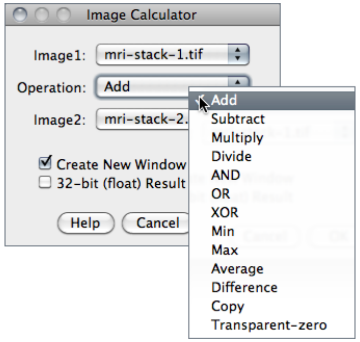
1. Open a virtual stack
2. Run Process ▷ Batch ▷ Virtual Stack...
3. Select an *Output folder* and *Output format*
4. Select '*Crop*' from the *Add Macro Code* drop-down menu
5. Edit the macro code as needed and press the *Test* button to verify the macro
6. Click *Process* to create the cropped virtual stack

Note that cropped images are not loaded into memory but are saved to disk as they are cropped (*see* Virtual Stacks).

## 29.13 Image Calculator...

Performs arithmetic and logical operations between two images selected from popup menus described in the Image operations table. *Image1* or both *Image1* and *Image2* can be stacks. If both are stacks, they must have the same number of slices. *Image1* and *Image2* do not have to be the same data type or the same size.

With 32–bit (float) images, pixels resulting from division by zero are set to *Infinity*, or to *NaN* (Not a Number) if a zero pixel is divided by zero. The divide-by-zero value can be redefined in Edit ▷ Options ▷ Misc...



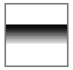
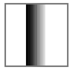

***Operation*** Selects one of the thirteen available operators (*see* Image operations).

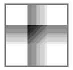
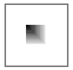
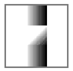
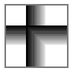
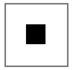
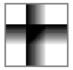
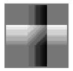
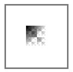
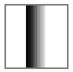
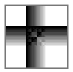
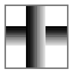
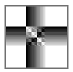
***Create New Window*** If checked, a new image is created to hold the result. If unchecked, the result of the operation is applied directly to *Image1*.

***32–bit (float) Result*** If checked, source images will be converted to 32–bit floating point before performing the operation.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/process.html#calculator.

Image Calculator... **operations.** On these examples source and destination images (8–bit grayscale) are displayed with inverted LUTs (White= 0; Black= 255) (cf. Lookup Tables ▷ submenu). Note that calculations between images can also be performed using copy and paste and the Edit ▷ Paste Control... command.

Source image (*img*1):  Destination image (*img*2): 

| Operator | Result | Operator | Result |
|---|---|---|---|
| **Add**: $img1 = img1 + img2$ |  | **Min**: $img1 = \min(img1, img2)$ |  |
| **Subtract**: $img1 = img1 - img2$ |  | **Max**: $img1 = \max(img1, img2)$ |  |
| **Multiply**: $img1 = img1 \times img2$ |  | **Average**: $img1 = (img1 + img2)/2$ |  |
| **Divide**: $img1 = img1 \div img2$ |  | **Difference**: $img1 = \lvert img1 - img2 \rvert$ |  |
| **AND**: $img1 = img1 \wedge img2$ |  | **Copy**: $img1 = img2$ |  |
| **OR**: $img1 = img1 \vee img2$ |  | **Transparent–zero** |  |
| **XOR**: $img1 = img1 \oplus img2$ |  | | |

SEE ALSO: Calculator Plus plugin

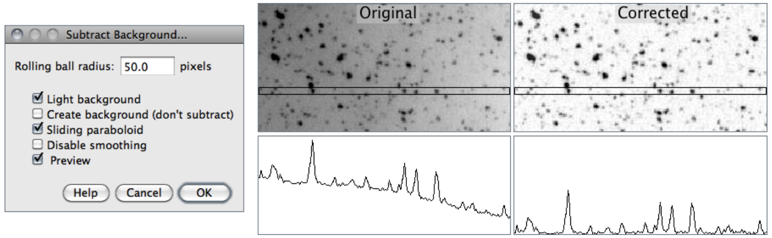
## 29.14 Subtract Background...

Removes smooth continuous backgrounds from gels and other images [39]. Based on the concept of the 'rolling ball' algorithm described in Sternberg Stanley, Biomedical image processing, *IEEE*





*Computer*, Jan 1983). Imagine that the 2D grayscale image has a third dimension (height) by the image value at every point in the image, creating a surface. A ball of given radius is rolled over the bottom side of this surface; the hull of the volume reachable by the ball is the background to be subtracted.




**Process ▷ Subtract Background...** This command uses a 'sliding paraboloid' or a legacy 'rolling ball' algorithm that can be used to correct uneven illuminated background as shown in the profiles (Analyze ▷ Plot Profile [k]) below each image. *Rolling ball radius* should be set to at least the size of the largest object that is not part of the background.

***Rolling Ball Radius*** The radius of curvature of the paraboloid. As a rule of thumb, for 8–bit or RGB images it should be at least as large as the radius of the largest object in the image that is not part of the background. Larger values will also work unless the background of the image is too uneven. For 16–bit and 32–bit images with pixel value ranges different from 0–255, the radius should be inversely proportional to the pixel value range (e.g., for 16–bit images (pixel values 0–65535), typical values of the radius are around 0.2 to 5).

***Light Background*** Allows the processing of images with bright background and dark objects.

***Separate Colors*** (RGB images only) If unchecked, the operation will only affect the brightness, leaving the hue and saturation untouched.

***Create Background (Don't Subtract)*** If checked, the output is not the image with the background subtracted but rather the background itself. This option is useful for examining the background created (in conjunction with the *Preview* option). *Create Background* can be also used for custom background subtraction algorithms where the image is duplicated and filtered (e.g. removing 'holes' in the background) before creating the background and finally subtracting it with Process ▷ Image Calculator...

***Sliding Paraboloid*** If checked, the 'rolling ball' is replaced by a paraboloid that has the same curvature at the apex as a ball of that radius. This option allows any value of the radius> 0.0001 (the 'rolling ball' algorithm requires a radius of at least 1). The 'sliding paraboloid' typically produces more reliable corrections since the 'rolling ball', a legacy algorithm (only kept for backward compatibility), is prone to edge artifacts.
To reduce the computing time the 'rolling ball' algorithm downscales the image in a inconsistent way. The 'sliding paraboloid' algorithm does not use downscaling and thus produces no downscaling artifacts. Nevertheless, the 'sliding paraboloid' is also an approximation, since it does not use a *de facto* paraboloid (an exact implementation would require a great computing effort) but it rather slides parabolae in different directions over the image.

***Disable Smoothing*** For calculating the background ('rolling the ball'), images are maximum-filtered (3×3 pixels) to remove outliers such as dust and then smoothed to reduce noise (average over (3×3 pixels). With *Disable Smoothing* checked, the unmodified image data are used for creating the background. Check this option to make sure that the image data after subtraction will never be below the background.

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/process.html#background.

SEE ALSO: How to correct background illumination in brightfield microscopy by G. Landini, Auto Local Threshold, command's source code, Rolling Ball Background Subtraction (the plugin that implemented this command in versions up to 1.39e)

### 29.15 Repeat Command [R]

Reruns the previous command. The Edit ▷ Undo and File ▷ Open commands are skipped.

SEE ALSO: Undo and Redo





## 30 Analyze ▷

This menu contains commands related to statistical measurements on image data, profile and histogram plotting and plugins related to image analysis.

### 30.1 Measure... [m]

IMPROVED IN IJ 1.46R

Based on the selection type, calculates and displays on the Results Table either area statistics, line lengths and angles, or point coordinates. Performed measurements can be specified in the Set Measurements... dialog box.

Area statistics are calculated for the complete image if there is no selection or for a selected subregion defined by one of the Area Selection Tools. For linear selections (Straight, Segmented and Freehand lines, *see* Line Selection Tools) length and angle (straight lines only) are also calculated. For Point selections (*see* Point Tool and Multi-point Tool), the X and Y coordinates are recorded. Note that Measure... [m] will paint (invasively) a mark over the measured point in foreground color unless *Mark Width* in the Point Tool options dialog box is set to zero.

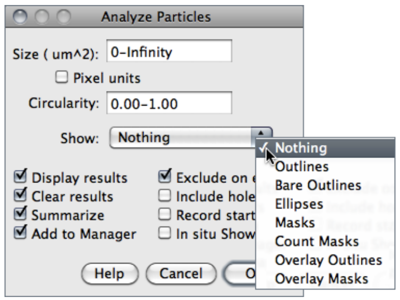
With RGB images, results are calculated using brightness values. RGB pixels are converted to brightness values using the formula value = (red+green+blue)/3 or value = 0.299 × red + 0.587 × green + 0.114 × blue if *Weighted RGB Conversions* is checked in Edit ▷ Options ▷ Conversions....

Intensity statistics (*Mean, Modal, Median, Min. & Max. Gray Value, Standard Deviation* and *Integrated Density*) can be performed on area, line and multi-point selections. With lines, these parameters are calculated from the values of the pixels along the line (*see* Plot Profile [k]).

With area selections, the following parameters can measured: *Area*, *Center of Mass*, *Centroid*, *Perimeter*, *Bounding Rectangle*, *Shape Descriptors*, *Fitted Ellipse, Feret's Diameter*, *Skewness*, *Kurtosis* and *Area Fraction*.

SEE ALSO: Results Table, Analyze Particles..., Summarize, Distribution..., Set Measurements..., Batch ▷ Measure...

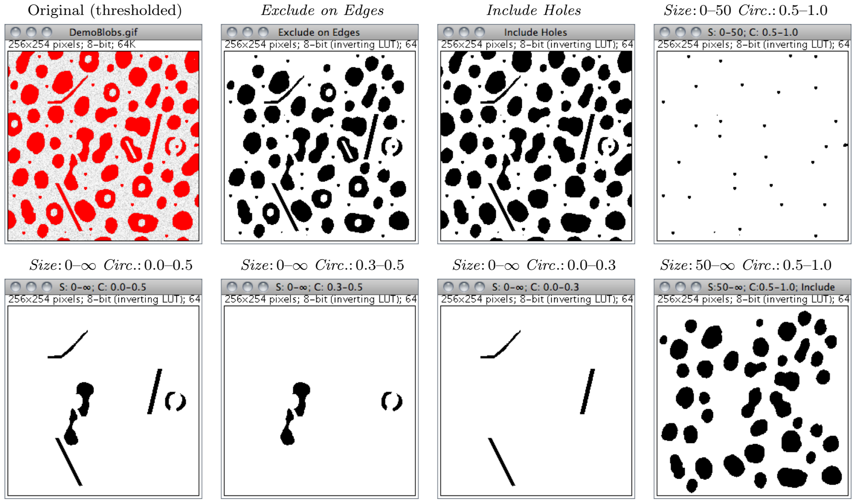
### 30.2 Analyze Particles...




This command counts and measures objects in binary or thresholded images (Image ▷ Adjust ▷ Threshold... [T] or Color Threshold...). Analysis is performed on the existing area selection or on the entire image if no selection is present.

It works by scanning the image or selection until it finds the edge of an object. It then outlines the object using the Wand Tool, measures it using the Measure... [m] command, fills it to make it invisible, then resumes scanning until it reaches the end of the image or selection. Press Esc to abort this process.

***Size*** Particles with size (area) outside the range specified in this field are ignored. Values may range between 0 and 'Infinity'. For spatial scaled images (cf. Set Scale...) values are expressed in physical size square units or in pixels if *Pixel Units* is checked. Enter a single value and particles smaller than that value will be ignored.




**Particle Analyzer (Analyze ▷ Analyze Particles. . . ).** Features of thresholded images can be extracted by specifying suitable *Size* and *Circularity* ranges and/or by choosing if particles should be traced by their outer edge or by flood filling (*Include Holes* checkbox).

***Circularity*** Particles with size circularity values outside the range specified in this field are also ignored. Circularity ($4\pi \times \frac{[Area]}{[Perimeter]^2}$, *see* Set Measurements. . . ) ranges from 0 (infinitely elongated polygon) to 1 (perfect circle).

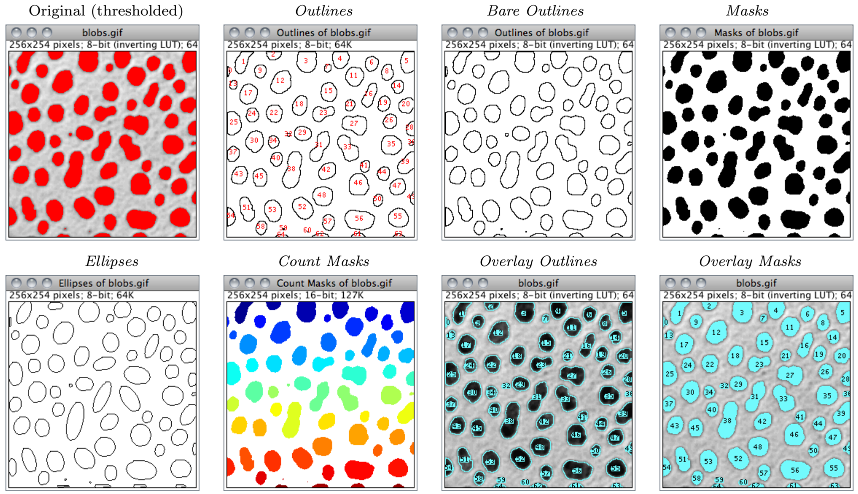
***Show*** This drop-down menu specifies which image (or overlay) should ImageJ display after the analysis (*see* Display options of ParticleAnalyzer). Size, color and background of text labels can be adjusted in the Image ▷ Overlay ▷ Labels. . . prompt. Non-overlay outputs can be adjusted in a macro by using `call("ij.plugin.filter.ParticleAnalyzer.setFontSize", size);` and `call("ij.plugin.filter.ParticleAnalyzer.setLineWidth", width);` or `ParticleAnalyzer.setFontSize(size); ParticleAnalyzer.setLineWidth(width);` in a script or plugin.

- ***Nothing*** Neither images nor Overlays will be displayed. Note that the particle analyzer will display a blank image when the count of detected particles is zero and *Show* is not *Nothing*.
- ***Outlines*** 8–bit image containing numbered outlines of the measured particles (gray levels: *Outlines*: 0; *Labels*: 1; *Background*: 255). If *In situ Show* is checked, the original image will be replaced by this image.
- ***Bare Outlines*** 8–bit image containing simple outlines of the measured particles without labels (graylevels: *Outlines*: 0; *Background*: 255). If *In situ Show* is checked, the original image will be replaced by this image.
- ***Masks*** 8–bit binary image containing filled outlines of the measured particles (gray levels: *Masks*: 0; *Background*: 255). If *In situ Show* is checked, the original image will be replaced by this image.
- ***Ellipses*** 8–bit binary image containing the best fit ellipse (cf. Edit ▷ Selection ▷ Fit Ellipse) of each measured particle (gray levels: *Ellipses*: 0; *Background*: 255). If *In situ Show* is checked, the original image will be replaced by this image.





- ***Count Masks*** 16–bit image containing filled outlines of the measured particles painted with a grayscale value corresponding to the particle number. If *In situ Show* is checked, the original image will be replaced by this image.
- ***Overlay Outlines*** Displays outlines of the measured particles in the image overlay, removing previously added Overlays.
- ***Overlay Masks*** Displays filled outlines of the measured particles in the image overlay, removing previously added Overlays.




**Display options of ParticleAnalyzer (Analyze ▷ Analyze Particles. . . ), IJ 1.44l.** When displaying Overlays, size, color and background of text labels can be adjusted using Image ▷ Overlay ▷ Labels. . .

***Display Results*** If checked, the measurements for each particle will be displayed in the Results Table.

***Clear Results*** If checked, any previous measurements listed in the Results Table will be cleared.

***Summarize*** If checked, the particle count, total particle area, average particle size, area fraction and the mean of all parameters listed in the Set Measurements. . . dialog box will be displayed in a separate *Summary* table. Note that while single images 'Summaries' are output to the same *Summary* table, stack Summaries are printed in dedicated tables (named *Summary of* [*stack title*]). Also, note that descriptive statistics on Results measurements can be obtained at any time using the Summarize command.

***Add to Manager*** If checked, the measured particles will be added to the ROI Manager. . .

***Exclude on Edges*** If checked, particles touching the edge of the image (or selection) will be ignored.

***Include Holes*** If checked, interior holes will be included. Disable this option to exclude interior holes and to measure particles enclosed by other particles. When this option is enabled, ImageJ finds the extent of each particle by tracing the outer edge. When it is disabled, ImageJ finds the extent by flood filling.

***Record Starts*** This option allows plugins and macros to recreate particle outlines using the doWand(x,y) macro function. The CircularParticles macro demonstrates how to use this feature.

***In situ Show*** If checked, the original image will be replaced by the binary mask specified in the ***Show*** drop-down menu. Note that this option does not apply to *Overlay Outlines* and *Overlay Masks* that are always displayed as non-destructive image Overlays on the measured image.

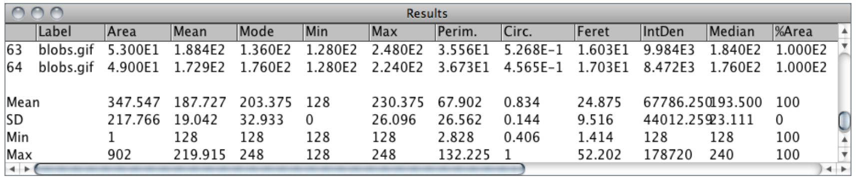
***Help*** Opens http://imagej.nih.gov/ij/docs/menus/analyze.html#ap.

SEE ALSO: Particle Remover plugin, 4/8–Connected Particle Analysis

IMPROVED IN IJ 1.46R

## 30.3 Summarize

For each column in the Results Table, calculates and displays the mean, standard deviation, minimum and maximum of the values in that column. This command is also available by right-clicking on the Results Table.



Results

| | Label | Area | Mean | Mode | Min | Max | Perim. | Circ. | Feret | IntDen | Median | %Area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 63 | blobs.gif | 5.300E1 | 1.884E2 | 1.360E2 | 1.280E2 | 2.480E2 | 3.556E1 | 5.268E−1 | 1.603E1 | 9.984E3 | 1.840E2 | 1.000E2 |
| 64 | blobs.gif | 4.900E1 | 1.729E2 | 1.760E2 | 1.280E2 | 2.240E2 | 3.673E1 | 4.565E−1 | 1.703E1 | 8.472E3 | 1.760E2 | 1.000E2 |
| Mean | | 347.547 | 187.727 | 203.375 | 128 | 230.375 | 67.902 | 0.834 | 24.875 | 67786.250 | 193.500 | 100 |
| SD | | 217.766 | 19.042 | 32.933 | 0 | 26.096 | 26.562 | 0.144 | 9.516 | 44012.259 | 23.111 | 0 |
| Min | | 1 | 128 | 128 | 128 | 128 | 2.828 | 0.406 | 1.414 | 128 | 128 | 100 |
| Max | | 902 | 219.915 | 248 | 128 | 248 | 132.225 | 1 | 52.202 | 178720 | 240 | 100 |

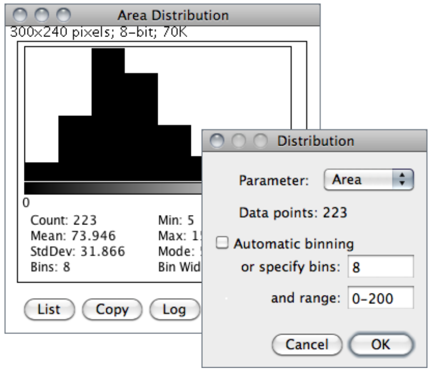
SEE ALSO: Analyze Particles. . . , Distribution. . .

## 30.4 Distribution. . .

Produces a relative frequency histogram from the data of a chosen column of the Results table [40].

Use the *List* or *Copy* buttons to save the histogram data. Mouse over the histogram bars to read the counts for each bin on the window's lower right corner. Analyze ▷ Histogram [h] describes in more detail ImageJ's histogram window.

This command is also available by right-clicking on the Results Table.




***Parameter*** Specifies the parameter in the Results Table to be analyzed.

***Data points*** The number of rows that will be analyzed (informative).

***Automatic binning*** If checked, ImageJ will use the method described by David Scott to assess the optimal histogram bin width (*see* Scott DW, Optimal and data-based histograms. *Biometrika*, 66(3):605–610, Jan 1979). If unchecked, the number of bins can be set with *Specify bins* and the starting and ending limits of the histogram with *range*.





SEE ALSO: Distribution Plotter, a macro that plots relative and cumulative frequencies on a double Y-axis graph

## 30.5 Label

This command labels the active selection with the current measurement counter value, i.e., the number of rows present in the Results Table. Selection outline and label (at the selection centroid) are drawn invasively using current foreground/background colors. As for Edit ▷ Draw [d], use the Edit ▷ Options ▷ Line Width. . . command, or double click on the line tool, to change the width of selection's outline.

Selections can be labelled if they were previously analyzed (Analyze Particles. . . or Measure. . . [m] commands) and the parameter *Centroid* (cf. Set Measurements. . . ) extracted in the Results table.

SEE ALSO: Color Picker Tool, XVII Embedding Color Annotations in Grayscale Images

## 30.6 Clear Results

Erases the results table and resets the measurement counter. This command is also available by right-clicking on the Results Table.

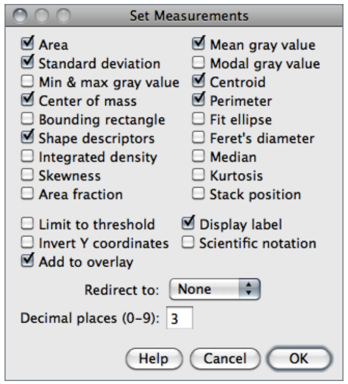
## 30.7 Set Measurements. . .

IMPROVED IN IJ 1.46R

Use this dialog box to specify which measurements are recorded by Analyze ▷ Measure. . . [m], ROI Manager's *Measure* command and Analyze ▷ Analyze Particles. . . Measurements are performed on the current selection, the entire active image if no selection is present. For thresholded images (Image ▷ Adjust ▷ Threshold. . . [T]), measurements can be restricted to highlighted pixels if *Limit to Threshold* is checked.

This command is also available by right-clicking on the Results Table.

The dialog contains two groups of checkboxes: The first group controls the type of measurements that are printed to the Results table. The second group controls measurement settings. The eighteen checkboxes of the first group are:




***Area*** Area of selection in square pixels or in calibrated square units (e.g., $mm^2$, $\mu m^2$, etc.) if Analyze ▷ Set Scale. . . was used to spatially calibrate the image.

***Mean gray value*** 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 Analyze ▷ Calibrate. . . was used to calibrate the image. For RGB images, the mean is calculated by converting each pixel to grayscale using the formula gray = (red + green + blue)/3 or gray = 0.299×red+0.587×green+0.114×blue if *Weighted RGB Conversions* is checked in Edit ▷ Options ▷ Conversions. . .

***Standard deviation*** Standard deviation of the gray values used to generate the mean gray value. Uses the Results Table heading **StdDev**.

***Modal gray value*** Most frequently occurring gray value within the selection. Corresponds to the highest peak in the histogram. Uses the heading **Mode**.

***Min & max gray level*** Minimum and maximum gray values within the selection.

***Centroid*** The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the image or selection. Uses the **X** and **Y** headings.

***Center of mass*** This is the brightness-weighted average of the x and y coordinates all pixels in the image or selection. Uses the **XM** and **YM** headings. These coordinates are the first order spatial moments.

***Perimeter*** The length of the outside boundary of the selection. Uses the heading **Perim.**. With IJ 1.44f and later, the perimeter of a composite selection is calculated by decomposing it into individual selections. Note that the composite perimeter and the sum of the individual perimeters may be different due to use of different calculation methods.

***Bounding rectangle*** The smallest rectangle enclosing the selection. Uses the headings **BX**, **BY**, **Width** and **Height**, where **BX** and **BY** are the coordinates of the upper left corner of the rectangle.

***Fit ellipse*** Fits an ellipse to the selection. Uses the headings **Major**, **Minor** and **Angle**. **Major** and **Minor** are the primary and secondary axis of the best fitting ellipse. **Angle** is the angle between the primary axis and a line parallel to the X-axis of the image. The coordinates of the center of the ellipse are displayed as **X** and **Y** if Centroid is checked. Note that ImageJ cannot calculate the major and minor axis lengths if *Pixel Aspect Ratio* in the Analyze ▷ Set Scale... dialog is not 1.0. There are several ways to view the fitted ellipse:

1. The Edit ▷ Selection ▷ Fit Ellipse command replaces an area selection with the best fit ellipse.
2. The DrawEllipse macro draws (destructively) the best fit ellipse and the major and minor axis.
3. Select *Ellipses* from the *Show:* drop-down menu in the particle analyzer (Analyze ▷ Analyze Particles... ) and it will draw the ellipse for each particle in a separate window.

***Shape descriptors*** Calculates and displays the following shape descriptors:

***Circularity*** $4\pi \times \frac{[Area]}{[Perimeter]^2}$ with a value of 1.0 indicating a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape. Values may not be valid for very small particles. Uses the heading **Circ**.

***Aspect ratio*** The aspect ratio of the particle's fitted ellipse, i.e., $\frac{[Major\ Axis]}{[Minor\ Axis]}$. If ***Fit Ellipse*** is selected the **Major** and **Minor** axis are displayed. Uses the heading **AR**.

***Roundness*** $4 \times \frac{[Area]}{\pi \times [Major\ axis]^2}$ or the inverse of *Aspect Ratio*. Uses the heading **Round**.

***Solidity*** $\frac{[Area]}{[Convex\ area]}$; Note that the Edit ▷ Selection ▷ Convex Hull command makes an area selection convex.

***Feret's diameter*** The longest distance between any two points along the selection boundary, also known as maximum caliper. Uses the heading **Feret**. The angle (0–180 degrees) of the Feret's diameter is displayed as **FeretAngle**, as well as the minimum caliper diameter (**MinFeret**). The starting coordinates of the Feret diameter (**FeretX** and **FeretY**) are also displayed (*see also* Feret's Diameter macro and Chamfer distances and Geodesic diameters plugin).





***Integrated density*** The sum of the values of the pixels in the image or selection. This is equivalent to the product of **Area** and **Mean Gray Value**. With IJ 1.44c and later, **Raw integrated density** (sum of pixel values) is displayed under the heading **RawIntDen** when **Integrated density** is enabled. The Dot Blot Analysis tutorial demonstrates how to use this option to analyze a dot blot assay.

***Median*** The median value of the pixels in the image or selection.

***Skewness*** The third order moment about the mean. The documentation for the Moment Calculator plugin explains how to interpret spatial moments. Uses the heading **Skew**.

***Kurtosis*** The fourth order moment about the mean. Uses the heading **Kurt**.

***Area fraction*** For thresholded images is the percentage of pixels in the image or selection that have been highlighted in red using Image ▷ Adjust ▷ Threshold... [T]. For non-thresholded images is the percentage of non-zero pixels. Uses the heading **%Area**. Improved in IJ 1.46r

***Stack position*** The position (slice, channel and frame) in the stack or hyperstack of the selection. Uses the headings **Slice**, **Ch** and **Frame**.

N.B.: For line selections the heading **Length** is created. For straight line selections, **Angle** is recorded even if ***Fit Ellipse*** is unchecked. Also, note that measurements that do not apply to certain selection types may be listed as *NaN*, *Infinity* or *−Infinity*.

The second part of the dialog controls measurement settings:

***Limit to threshold*** If checked, only thresholded pixels are included in measurement calculations. Use Image ▷ Adjust ▷ Threshold... [T] to set the threshold limits. This setting affects only thresholded images (*see* Settings and Preferences).

***Display label*** If checked, the image name and slice number (for stacks) are recorded in the first column of the Results Table, e.g., mri-stack.tif:9. For renamed selections (Edit ▷ Selection ▷ Properties... [y]) or selections measured via ROI Manager's measure command (*see* ROI Manager...), the selection label is appended, e.g., blobs.gif:0339-0163 or blobs.gif:mySelection.

***Invert Y coordinates*** If checked, the XY origin is assumed to be the lower left corner of the image window instead of the upper left corner (*see also* Image ▷ Properties... [P]).

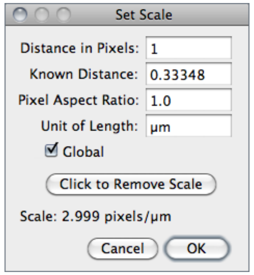
***Scientific notation*** If checked, measurements are displayed in scientific notation, e.g., 1.48E2. Improved in IJ 1.46r

***Add to Overlay*** If checked, measured ROIs are automatically added to the image overlay (*see* Overlays). Appearance of overlay selections can be adjusted using Image ▷ Overlay ▷ Overlay Options... /Labels...

***Redirect to*** The image selected from this popup menu will be used as the target for statistical calculations done by Analyze ▷ Measure... [m] and Analyze ▷ Analyze Particles... commands. This feature allows you to outline a structure on one image and measure the intensity of the corresponding region in another image.

***Decimal places*** This is the number of digits to the right of the decimal point in real numbers displayed in the Results Table and in Histogram windows (Analyze ▷ Histogram [h]). Improved in IJ 1.46r

## 30.8 Set Scale...




Use this dialog to define the spatial scale of the active image so measurement results can be presented in calibrated units, such as mm or µm.

Before using this command, use the straight line selection tool to make a line selection that corresponds to a known distance. Then, bring up the Set Scale... dialog, enter the *Known Distance* and unit of measurement, then click 'OK'. The *Distance in Pixels* field will be automatically filled in based on the length of the line selection.

As described in Image ▷ Properties... [P], µ and Å symbols can be typed using Alt M and Alt Shift A, respectively. µm can also be defined as 'um', or 'micron'.

Setting *Pixel Aspect Ratio* to a value other than 1.0 enables support for different horizontal and vertical spatial scales, e.g., 100 pixels/cm horizontally and 95 pixels/cm vertically. To set the *Pixel Aspect Ratio*:

1. Measure the width and height (in pixels) of a digitized object with a known 1:1 aspect ratio.
2. Enter the measured width (in pixels) in *Distance in Pixels*. Enter the known width in *Known Distance*.
3. Calculate the aspect ratio by dividing the width by the height and enter it in *Pixel Aspect Ratio*

When *Global* is checked, the scale defined in this dialog is used for all opened images during the current session instead of just the active image, *see* XXIII Global Calibrations.

*Click to Remove Scale* resets *Distance in Pixels* field and *Known Distance* to zero and the *Unit of Length* to 'pixel'.

SEE ALSO: Tutorials showing how to use this command: Examples of Image Analysis Using ImageJ by Larry Reinking, Measuring DNA Contour Lengths and Spatial Calibration (Fiji). The Copy_Pixel_Size plugin

IMPROVED IN IJ 1.46R

## 30.9 Calibrate...

Use this dialog box to calibrate an image to a set of density standards, for example radioactive isotope standards or a calibrated optical density step tablet. Note that, in general, calibrations cannot be applied to 32–bit images (the pixel intensity unit of 32–bit images can still be changed, nevertheless).

The calibration procedure is done in three steps:

1. Use Analyze ▷ Clear Results to reset the measurement counter, use one of the Area Selection Tools and Analyze ▷ Measure... [m] to record the mean gray value of each of the standards.
2. When finished making the measurements, select Analyze ▷ Calibrate... to display the *Calibrate...* dialog box. To calibrate the image, enter the known standard values in the right column. The left column will be already populated with the measured mean gray values. Select a curve fitting method from the popup menu, enter the unit of measurement, and click 'OK'. If *Show plot* is checked, ImageJ will then display the calibration function on a separate window. Note that both columns must contain the same number of values.
3. If the calibration function is not satisfactory, bring up the *Calibrate...* dialog box again and select a different curve fitting method.

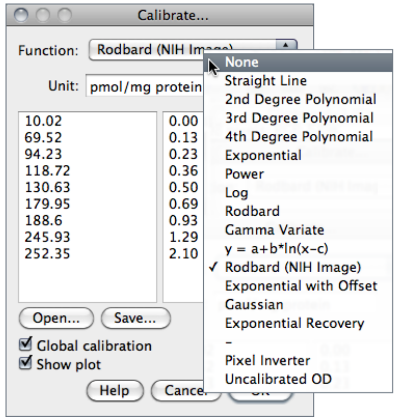


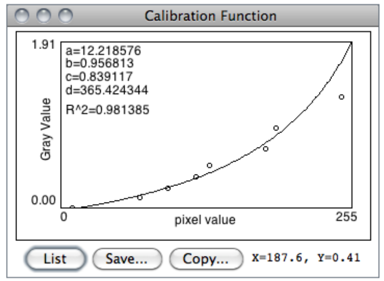


In addition to the functions that can be chosen from the drop-down menu (described in Curve-Fitter's built-in functions) two other functions are available that do not require any measurement of OD standards:

***Uncalibrated OD*** As mentioned in Gels ▷ Gel Analyzer Options..., causes ImageJ to convert gray values from 8–bit images to uncalibrated optical density values using the function Unc. OD $= \log_{10}(255/\text{Pixel value})$. This conversion can only be performed on 8-bit images.

***Pixel Inverter*** Linear function defined by Inverted pixel = *Bit-depth* − 1 − Pixel value, with *Bit-depth* being 255 for 8–bit images or 65535 for 16–bit images.




SEE ALSO: Optical Density Calibration tutorial, Global Calibrations, Calibration Bar..., Curve Fitting..., Image Types and Formats

## 30.10 Histogram [h][1]

IMPROVED IN IJ 1.46R

Calculates and displays a histogram of the distribution of gray values in the active image or selection.

The X-axis represents the possible gray values and the Y-axis shows the number of pixels found for each gray value. The horizontal LUT bar below the X-axis is scaled to reflect the display

[1] This shortcut is shown on Windows and Linux but not on Mac OS X as it conflicts with the system wide 'Hide' shortcut. However, the H shortcut (without holding down Cmd) does work on OS X.

### XXIII Global Calibrations

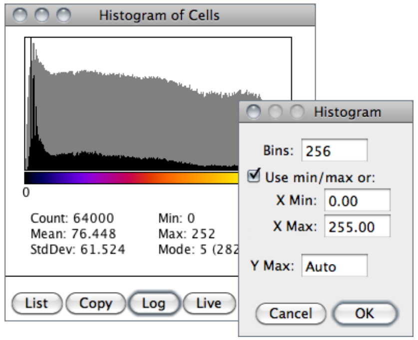
Calibration settings related to spatial (pixel width, height and voxel depth), temporal (frame interval) and luminance (brightness) information can be set globally, i.e., can be applied to all images opened during the current session instead of just the active image. The *Global* flag can be set in three dialog prompts:

1. Image ▷ Properties... [P] (pixel width, height, voxel depth, frame interval)
2. Analyze ▷ Set Scale... (pixel width and height)
3. Analyze ▷ Calibrate... (pixel intensity)


Once *Global* calibration is set, a '(G)' is displayed in all image titles until ImageJ is closed. A warning message is displayed when a calibrated image with conflicting calibration is opened and the *Global* option is enabled.

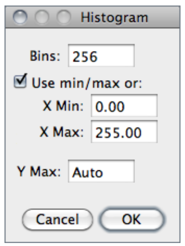
Choose *Disable Global Calibration* to stop using global settings or *Disable these Messages* to keep respecting global settings, ignoring the calibration of the newly open image.

range of the image [41]. The total pixel *Count* is also calculated and displayed, as well as the *Mean*, standard deviation (*StdDev*), minimum (*Min*), maximum (*Max*) and modal (*Mode*) gray value.




Click on *Live* to monitor the histogram while browsing stacks or while moving a ROI. *Value* / *Count* pairs (i.e., grayscale value corresponding to the X-axis cursor position / the number of pixels that have that intensity) are displayed on the bottom right while mousing over the histogram window.

With RGB images, the default histogram is calculated by converting each pixel to grayscale using the formula gray = (red + green + blue)/3 or gray = 0.299 × red + 0.587 × green + 0.114 × blue if *Weighted RGB Conversions* is checked in Edit ▷ Options ▷ Conversions.... However, single-channel RGB histograms can be obtained by repetitively clicking on the *RGB* button.




Use the *List* or *Copy* buttons to save the histogram data. Click on *Log* to display a log-scaled version of the histogram (overlaid in gray).

With 16–bit images, the range of gray values between the *Min* and *Max* values is divided into 256 bins. With 32–bit images, the number of bins is specified in the depicted dialog box. With any image type, an options dialog can be called with Alt H or by holding Alt while clicking on Analyze ▷ Histogram [h].

***Bins*** Specifies the number of bins.





***Use min/max*** If checked, the X-axis range is determined by the minimum and maximum values in the image or selection. If unchecked, *X Min* and *X Max* values can be specified to fix the X-axis range.

***Y Max*** Fixes the Y-axis range. Type 'Auto' to have the range determined by the largest bin count.

The `getHistogram()` and `Plot.getValues()` macro functions can be used to get the 'Value' and 'Count' data displayed when you click the *List* button (cf. (8) Obtaining Histogram Lists).

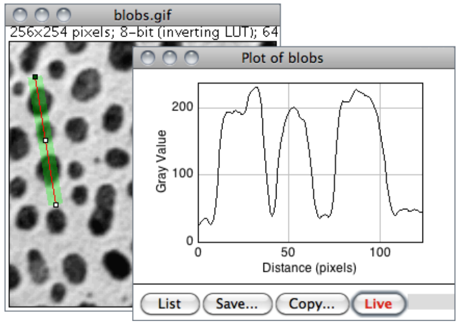
**(8) Obtaining Histogram Lists**

```
// 1. Single images:
run("Blobs (25K)");
getHistogram(values, counts, 256);
for (i=0; i<values.length; i++)
    print(values[i], counts[i]);

// 2. Entire stacks:
run("T1 Head (2.4M, 16-bits)");
run("Histogram", "stack");
Plot.getValues(values, counts);
for (i=0; i<values.length; i++)
    print(values[i], counts[i]);
```

## 30.11 Plot Profile [k]

Improved in IJ 1.46r




Displays a two-dimensional graph of the intensities of pixels along a line or rectangular selection. The X-axis represents distance along the line and the Y-axis is the pixel intensity. *NaN* values in 32–bit images (*see* Image Types and Formats) are ignored.

For rectangular selections or line selections wider than one pixel, displays a 'column average plot', where the X-axis represents the horizontal distance through the selection and the Y-axis the vertically averaged pixel intensity.

To average horizontally, hold Alt K or check *Vertical Profile* in Edit ▷ Options ▷ Profile Plot Options.... For real-time examinations, activate *Live* mode to continuously update the profile as the selection is moved or resized.

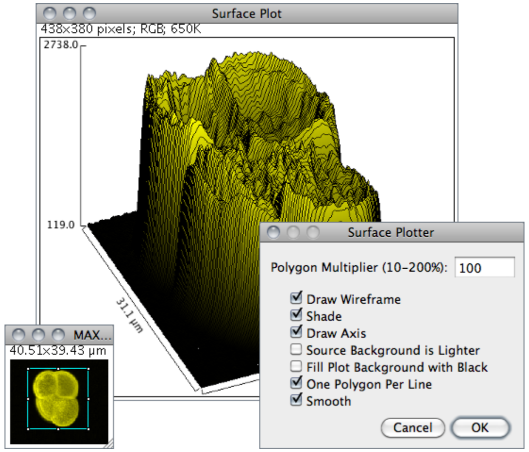
To obtain profiles of several selections in a single plot use the ROI Manager's *Multi Plot* command (Analyze ▷ Tools ▷ ROI Manager...). Other types of area selections such as oval or freehand ROIs can be profiled by first running Edit ▷ Selection ▷ Area to Line, which will convert these ROIs to line selections.

Use the *List*, *Save...* or *Copy...* buttons to view and save the profile data. Use Edit ▷ Options ▷ Profile Plot Options... to adjust how plots are generated.

See also: Rectangular Selection Tool, Line Selection Tools, Plot Z-axis Profile..., Surface Plot..., Dynamic Profiler, Oval Profile Plot, Radial Profile Plot, Radial Profile Extended plugins, StackProfilePlot macro

## 30.12 Surface Plot...

Displays a three-dimensional graph of the intensities of pixels in a grayscale or pseudo color image (non-RGB images). The plot is based on the existing rectangular selection or on the entire image if no selection is present. A stack of plots can be produced when the source image is a stack or hyperstack. In this case, closing the plot stack window will abort the plotting process.




***Polygon Multiplier*** Adjusts the number of profiles used to generate the plot.

***Draw Wireframe*** If checked, the outline of each profile will be drawn in black.

***Shade*** If checked, a shaded plot will be generated using the LUT of source image.

***Draw Axis*** If checked, the three axis will be drawn and labelled.

***Source Background is Lighter*** If checked, lighter areas in the source image will represent lower elevations (valleys) while darker areas in the source image will represent higher elevations (peaks).

***Fill Plot Background with Black*** If checked, the plot is drawn with a black background, otherwise white will be used.

***One Polygon Per Line*** If checked, all polygons will be drawn.

***Smooth*** If checked, sharp fluctuations will be smoothed. Note that some plots can be further improved by adjusting the contrast of the source image or smoothing it.

SEE ALSO: Plot Profile [k], Interactive 3D Surface Plot plugin (it works with all image types and viewing angle, perspective, scale, lighting and smoothing can be interactively adjusted), 3D Color Inspector/Color Histogram





### XXIV Using Scanners in Densitometry

Electrophoretic gels such as Western blots need frequently to be quantified in order to translate biochemical results into statistical values (*see* Gels ▷). Independently of the measurement method, you should be familiar with the Beer–Lambert law, so that you are aware that the optical density (absorbance) of the staining to be measured must be proportional to the concentration of the probed material. An obvious corollary of the Beer–Lambert law is that saturated stains (or overexposed films) cannot be quantified.

Generally speaking, office scanners are not suitable for densitometric analysis. That being said, and in the absence of better alternatives, they can be used to digitize gels and X-ray films if the following guidelines are respected:

**Samples must be scanned in transmission mode** Most flatbed scanners have the light source and detector located on the same side of the instrument (reflection mode). Under these conditions, light interacts with semi-transparent objects in a complex manner with reflected light re-passing through the object on its way to the detector. In addition, thick samples (such as Coomassie-stained gels) are imaged from the surface under reflection light.
Scanners can only serve as densitometers if the light source and detector are on opposite sides of the instrument (transmission mode). This is critical for quantitation of absorbances and requires an adapter for transparencies or negatives to be mounted or built-in on the scanner.

**The scanner must offer a linear intensity response** The response characteristics of most scanner detectors is unknown, therefore the film/gel should always be scanned next to a photographic gray scale image of known optical intensity (optical tablet or step wedge, *see* optical density calibration tutorial). Autogain must be disabled during digitization to ensure generally linear intensity responses.

In addition, images should also be scanned with sufficiently high spatial and dynamic (bit-depth) resolutions (the optical—not interpolated—resolution of a scanner can also be measured: e.g., high contrast patterns 84.6 µm (1/300 inch) apart should be distinguishable on images scanned at 300 dpi). Note that, in general, *a posteriori* background corrections on scanned images should also be avoided.

All these issues have been discussed in detail in Gassmann et al. "Quantifying Western blots: pitfalls of densitometry" [38] (N.B.: The paper benchmarks a legacy version of Process ▷ Subtract Background...).

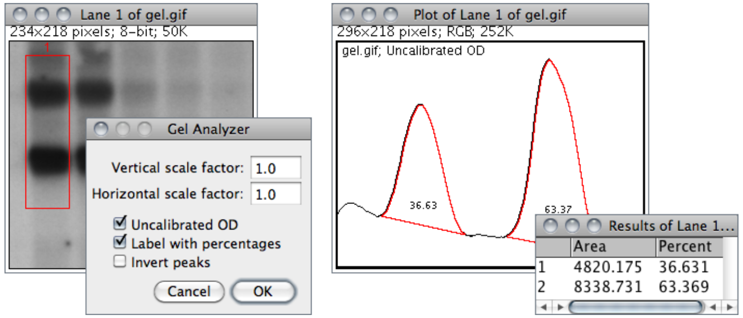
## 30.13 Gels ▷

IMPROVED IN IJ 1.46R

Use the commands in this submenu to analyze one-dimensional electrophoretic gels. Electrophoretic densitometry is discussed in more detail in XXIV Using Scanners in Densitometry. These commands use a simple graphical method that involves generating lane profile plots, drawing lines to enclose peaks of interest, and then measuring peak areas (i.e., definite integrals) using the Wand Tool.... Note that this technique cannot be used to compare bands on different gels unless gels are calibrated to known standards.

The commands listed in this submenu are:

**Select First Lane** [1] Requires a rectangular selection. Note that lanes are assumed to be vertical unless the width of the initial selection is at least twice its height.




**Select Next Lane** [2] To be used after the first rectangular ROI is moved over the adjacent lanes. Note that all selections must have the same dimensions.

**Plot Lanes** [3] Generates the lane profile plots. ImageJ assumes that only one plot is created per analysis. As a consequence, re-running this command more than once within the same analysis will cause an error message: "*You must first use the 'Select First Lane' command*". To recreate plotted profiles use the Re-plot Lanes command.

**Re-plot Lanes** Recreates the lane profile plots. If Plot Lanes has not yet been run an error message is displayed: "*The data needed to re-plot the lanes is not available*".

**Reset** Resets the analysis.

**Label Peaks** Uses the area measurements obtained with the Wand Tool to label lane peaks (*see* Gel Analyzer Options. . . ).

**Gel Analyzer Options. . .** Use this dialog to control the behavior of the gel analyzer.

***Vertical / Horizontal scale factor*** Specifies the scale factor at which the lane profile plots are displayed.

***Uncalibrated OD*** If checked, ImageJ will convert gray values to uncalibrated optical density values. As explained in Analyze ▷ Calibrate. . . , ImageJ converts pixel intensities into optical density using the function: Unc. OD = $\log_{10}(255/\textit{pixel value})$. As mentioned earlier, the conversion can only be performed on 8–bit images. Thus, when dealing with higher bit-depth images (see Image Types and Formats), the gel analyzer works on a 8–bit copy of the gel (kept hidden from the user) when using this option.

***Label With Percentages*** If checked, the Label Peaks command will print to the Results Table table the *peak percentage* and use it to label the plot. The percentage value is obtained by dividing the area of each peak by the sum of all measured peaks from all lanes.

***Invert Peaks*** If checked, peaks will be inverted, i.e, bands darker than background will have positive peaks, bands lighter than background will have negative peaks. This setting does not change the analysis (*see* Image ▷ Lookup Tables ▷ Invert LUT).

For practice, refer to the video tutorial on the ImageJ wikipage and use the File ▷ Open Samples ▷ Gel sample image (1–D gel) to perform the following steps. Note that a copy of the gel image with the lane outlines can be created at any point using the Image ▷ Overlay ▷ Flatten [F] command.

1. Use the rectangular selection tool to outline the first lane. This should be the left most lane if the lanes are vertical or the top lane if the lanes are horizontal.





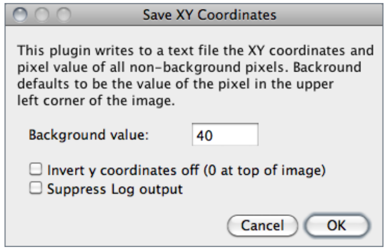
2. Select Gels ▷ Select First Lane ([1]) and the lane will be outlined and 'Lane 1 selected' displayed in the status bar.
3. Move the rectangular selection right to the next lane (or down if the lanes are horizontal) and select Gels ▷ Select Next Lane ([2]). The selected lane is outlined and labelled, and 'Lane $n$ selected' is displayed in the status bar.
4. Repeat the previous step for each remaining lane.
5. Select Gels ▷ Plot Lanes ([3]) to generate the lane profile plots.
6. Use the Straight Line Selection Tool to draw base lines and/or drop lines so that each peak of interest defines a closed area (ImageJ will automatically switch to the Straight Line tool). Note that you can hold [Shift] to constrain lines to be either horizontal or vertical. To access to all the lanes, it may be necessary to scroll the image vertically using the Scrolling Tool (Hold down the space bar to temporarily switch to this tool).
7. For each peak, measure the size by clicking inside the peak with the Wand Tool. If necessary, scroll the image vertically by holding down the space bar and dragging.
8. Select Gels ▷ Label Peaks to label each measured peak with its size as a percent of the total size of the measured peaks.

SEE ALSO: Calibrate. . . , SinglePanelGelAnalyzer macro, Video tutorial on ImageJ wikipage, Luke Miller's tutorial, Dot Blot Analysis, Dot Blot Analyzer toolset

## 30.14 Tools ▷

This submenu provides access to various image analysis plugins.

### 30.14.1 Save XY Coordinates. . .

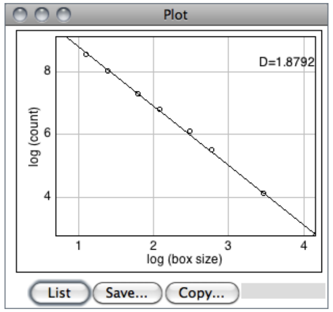



Writes to a text file the XY coordinates and pixel value of all non-background pixels in the active image. Background is assumed to be the value of the pixel at the upper left corner of the image. For grayscale images, writes three values per line (x, y, and value), separated by spaces. For RGB images, writes five values per line (x, y, red, green and blue). The origin of the coordinate system is at the lower left corner of the image.

The number and percentage of non-background pixels is printed to the Log Window if *Suppress Log output* is not checked.

SEE ALSO: Edit ▷ Selection ▷ Properties. . . [y]

### 30.14.2 Fractal Box Count...




Estimates the fractal dimension ($D$) of a binary image. $D$ can be used as a measure of pattern complexity (cell shape, vascularization, textures, etc.) and is specially relevant in cases in which Euclidean measures such as diameter or length are not good descriptors of complexity.

The command counts the number of boxes of an increasing size needed to cover a one pixel binary object boundary and implements the method described in T. G. Smith, Jr., G. D. Lange and W. B. Marks, Fractal Methods and Results in Cellular Morphology, *J Neurosci Methods*, 69:1123–126, 1996.

A plot is generated with the log of size on the X-axis and the log of count on the Y-axis and the data is fitted with a straight line. The slope (S) of the line is the negative of the fractal dimension, i.e., D= $-slope$. 'Size' (S) and 'count' (C) are printed to the Results Table. Refer to the source code for additional information.

SEE ALSO: Fractal Dimension and Lacunarity plugin

### 30.14.3 Analyze Line Graph

This command uses the Particle Analyzer to extract sets of coordinate data from digitized line graphs. The following procedure describes how to use it:

1. Open the image containing the graph. For practice, use the File ▷ Open Samples ▷ Line Graph (21K) sample image. Make sure your graph is a grayscale image (Image ▷ Type ▷ 8–bit). Analyze Line Graph will assume that the graph is displayed on a white background so images with darker backgrounds must be adjusted beforehand (*see* e.g., Edit ▷ Invert [I] and Process ▷ Binary ▷ Make Binary).

2. Set background color to white using the Color Picker Tool or the Color Picker window. Use any of the Area Selection Tools as an eraser (press Backspace to erase) in order to isolate the single graph curve to be measured. Alternatively, use one of the drawing tools (Pencil or Brush) to paint directly in background color.

3. Open the Threshold... [T] tool (Shift T) and adjust the threshold levels so that the curve is highlighted in red.

4. Select the curve with the Wand Tool and run Edit ▷ Clear Outside to erase everything on the canvas but the curve.

5. With the line still highlighted by the threshold widget, run Analyze ▷ Tools ▷ Analyze Line Graph to get the XY coordinates of the traced line (you can hold down Alt while selecting the command to reveal the actual image that is processed ).

6. On the newly obtained plot, select *List*, *Copy* or *Save* (these commands are described in Plot Profile [k]) to export the curve coordinates into a spreadsheet application.

The exported values are tabulated in pixel coordinates, unless the digitzed graph has been spatially calibrated using Analyze ▷ Set Scale... or Image ▷ Properties... [P].

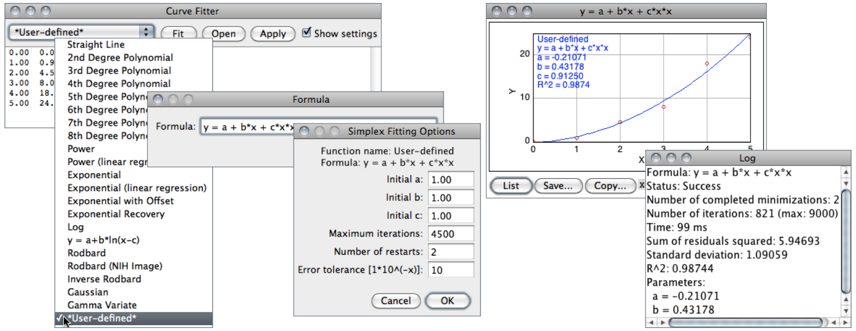




SEE ALSO: Figure Calibration, a simple plugin to retrieve data from other types of graphs such as scatter plots and histograms

### 30.14.4 Curve Fitting...

IMPROVED IN IJ 1.46R




**ImageJ's CurveFitter (Analyze ▷ Tools ▷ Curve Fitting...) – ImageJ 1.46g.**

ImageJ's CurveFitter provides a simple tool for fitting various functions to X- and Y-data, using an improved multithreaded simplex algorithm [42]. This strategy (an iterative method) is a kind of "trial and error" procedure in which the parameters of the fitting model are adjusted in a systematic way until the equation fits the data as close as required. It proceeds by: 1) Making first guesses of all the non-linear parameters; 2) Computing the model, comparing it to the data set and calculating a fitting error; 3) If the fitting error is large, the CurveFitter will systematically change parameters and return to step 2). The loop stops when the fitting accuracy is met, which in difficult cases may never happen. In the latter case, the procedure terminates after an imposed number of iterations or restarts.

The typical usage of this command is listed below:

1. Tabular data is entered or copied in the input window or alternatively, a two column text file is opened by clicking on the *Open* button. Values may be separated by spaces, tabs, commas or semicolons.

2. The function to be fit is selected from the drop-down menu. Several built-in functions are available (*see* CurveFitter's built-in functions). User defined functions with up to six parameters are also possible by choosing **User-defined**. Note that elimination of parameters by linear regression does not take place for user-defined functions. As a consequence, the performance of custom functions does not fully match that of built-in functions.

3. Once the *Fit* button is pressed, ImageJ displays a graph of the data with the fitted curve as depicted in CurveFitter. If *Show Settings* is checked, detailed information about the fit (including measures of goodness of fit) is printed to the Log Window and the user is prompted to re-adjust the simplex fitting options, namely:

   **Maximum number of iterations** The number of maximum iterations in which the CurveFitter will try to improve upon the parameter values to get the best fit. Usually the algorithm reaches optimal convergence before reaching the default value.

**Number of restarts** To ensure that the result is trustworthy (i.e., that it did that it did not get "stuck" or find a local minimum), CurveFitter tries at least twice to find the minimum, with different starting points. If the two results are not the same, *Number of restarts* determines how often it may start two additional runs, until the best two results agree within the error tolerance. There is no limit for the number of restarts, apart from the maximum number of iterations.

4. Click *Apply* to create a 32–bit copy (*see* Image Types and Formats) of the current image transformed with the chosen function.

**CurveFitter's built-in functions.** A more detailed summary of the CurveFitter's abilities is available online. The complete documentation can be accessed through the ImageJ API. Most functions use linear regression to determine the first two coefficients directly, which generally increases the quality of the result [42]. As explained in Curve Fitting..., simplex fitting options can be adjusted by selecting the *Show settings* checkbox. For polynomials, the proposed criteria are known to yield optimal convergence.

| Function | Formula | Comments |
|---|---|---|
| Straight line | $y = a + bx$ | cf. Pixel Inverter (Analyze ▷ Calibrate...) |
| 2nd–8th degree polynomial | $y = a + bx + cx^2 + \ldots + ix^8$ | The range of $x$ values should not be too far from 0, especially for higher-order polynomials<br>4th: should fulfill $\lvert x_{\mathrm{Mean}} \rvert < 2 \times (x_{\mathrm{Max}} - x_{\mathrm{Min}})$<br>5th: should fulfill $\lvert x_{\mathrm{Mean}} \rvert < (x_{\mathrm{Max}} - x_{\mathrm{Min}})/2$<br>7th & 8th: should fulfill $\lvert x_{\mathrm{Mean}} \rvert \ll x_{\mathrm{Max}} - x_{\mathrm{Min}}$ |
| Power† | $y = a \times x^b$ | †Optionally, fit can be performed without linear regression during minimization. Most curve-fitting programs such as Microsoft® Excel® use regression. Fitting without regression assumes equal weight for all data points and is insensitive to zero or negative data |
| Exponential† | $y = a \times e^{bx}$ | |
| Exponential with offset | $y = a \times e^{-(bx)} + c$ | |
| Exponential recovery | $y = a \times (1 - e^{(-bx)}) + c$ | cf. FRAP_Profiler plugin |
| Log | $y = a \times \ln(bx)$<br>$y = a + b \times \ln(x - c)$ | |
| Rodbard | $y = \frac{d+(a-d)}{1+(\frac{x}{c})^b}$ | [42], *see* DeLean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol. 1978 Aug;235(2):E97–102 [PMID: 686171] |
| Rodbard (NIH Image) | $x = \frac{d+(a-d)}{1+(\frac{x}{c})^b}$ | |
| Inverse Rodbard | $y = c \times (\frac{x-a}{d-x})^{1/b}$ | |
| Gaussian | $y = a + (b - a) \times e^{-\frac{(x-c)^2}{2d^2}}$ | [42] |
| Gamma variate | $y = b \times (x - a)^c \times e^{-(\frac{x-a}{d})}$ | |

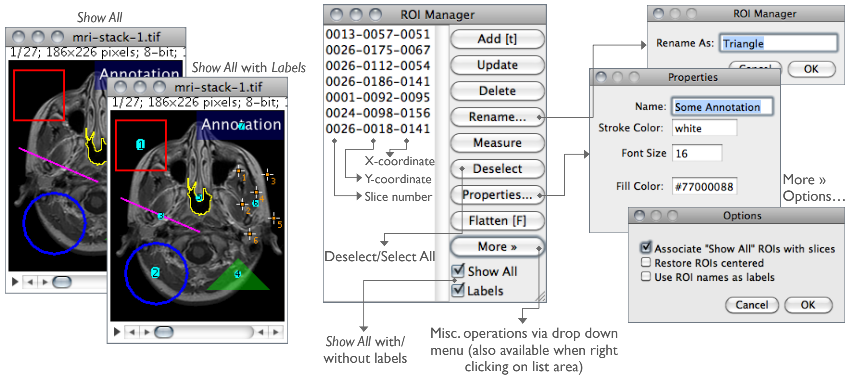
SEE ALSO: CurveFittingDemo, RodbardSigmoidFit and PlotSigmoidDerivatives macros, Profile Plot Options...





### 30.14.5 ROI Manager...

IMPROVED IN IJ 1.46R




**The ROI Manager (Analyze ▷ Tools ▷ ROI Manager...) – ImageJ 1.46p.**

The ROI (Region of Interest) Manager is a tool for working with multiple selections. Selections can be from different locations on an image, from different slices of a stack or from different images. All selection types, including points, lines and text, are supported.

***Add*** Click *Add* to add the current selection to the list, or press T, the keyboard shortcut for the Edit ▷ Selection ▷ Add to Manager [t] command. The ROI manager creates a three part label. The first part (stacks only) is the slice number, the second is the Y-coordinate of the selection and the third is the X-coordinate. Click on a label to restore the associated selection to the current image. With stacks, the selection is restored to the slice it came from. Hold down Shift while clicking *Add* to 'Add and Draw'. Hold down Alt while clicking *Add* to 'Add and Rename'.
Install the ROIManagerMacros macro set and you will be able to add a selection by pressing 1, 'add and rename' by pressing 2, 'add and draw' by pressing 3 and 'add and advance to the next slice' by pressing 4.

***Update*** Replaces the selected ROI on the list with the current selection, updating the z/t-position of the ROI in Stacks and Hyperstacks.

***Delete*** Deletes the selected ROIs from the list. Deletes all ROIs if none is selected.

IMPROVED IN IJ 1.46R

***Rename...*** Renames the selected ROI. The chosen string will be used as label (*Labels* checkbox) if *Use ROI names as labels* is checked in the *More≫Options...* dialog. The selected ROI can also be renamed using the *Properties...* button. Note that while it is not possible to rename multiple ROIs simultaneous, you can use ROI Manager Tools to rename multiple ROIs.

***Measure*** Measures the selected ROIs, or if none is selected, all ROIs on the list. Use Analyze ▷ Set Measurements... to specify the measuring parameters.

***Deselect*** Deselects any selected ROIs on the list. As mentioned in XXV Selecting ROIs in the ROI Manager, when items are deselected subsequent ROI Manager commands are applied to all ROIs.

IMPROVED IN IJ 1.46R

***Properties...*** Similarly to Edit ▷ Selection ▷ Properties... [y], opens a dialog box in which is possible to assign a contour color (*Stroke color*) of a certain *Width* or a *Filling color*. Set

*Stroke width* to 0 to have selections drawn using a width of one pixel regardless of the image magnification (*see* XVIII Working with Zoomed Canvases).

As previously mentioned, selections can be either filled or contoured, but not both. The nine default selection colors (*black*, *blue*, *cyan*, *green*, *magenta*, *orange*, *red*, *white*, *yellow*) can be typed textually. Any other color must be specified using hex notation (*see* XIX Hexadecimal Color Values).

With Text Tool selections, it is also possible to specify the *Font size* and *Justification*.

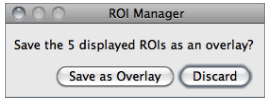
If multiple ROIs have been selected from the ROI Manager's list (*see* XXV Selecting ROIs in the ROI Manager), properties will be applied to the specified *Range* of selections.

***Flatten [F]*** Alias for Image ▷ Overlay ▷ Flatten [F].

Improved in IJ 1.46r

***Show All*** Toggles the display of all ROI Manager Overlays. If *Labels* is active, ROIs will also be labelled. Once *Show All* is checked, ROIs can be re-activated by Alt-clicking, Control-clicking or long-pressing (1/4 second or longer). Re-activated selections that are moved or edited are automatically updated.

New in IJ 1.46r



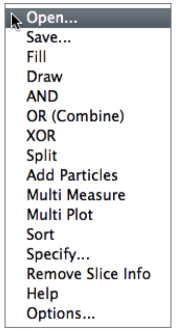

When *Show All* is active and the ROI Manager window is closed, a dialog box is displayed providing the option to save the displayed ROIs by moving them to the image overlay by running Image ▷ Overlay ▷ From ROI Manager. When saving the image as tiff, Overlays are stored in the TIFF header and can later be retrieved using Image ▷ Overlay ▷ To ROI Manager.

Changed in IJ 1.46r

***Labels*** Toggles overlay labels displayed by *Show All*. Labels are customized using *More≫Labels...*, a shortcut to Image ▷ Overlay ▷ Labels...

Improved in IJ 1.46r

***More≫*** Displays a drop-down menu with several additional commands (this menu is also available when right-clicking on the ROI Manager's list area):




***Open...*** Opens a `.roi` file and adds it to the list or opens a ZIP archive (`.zip` file) and adds all the selections contained in it to the list.

***Save...*** Saves the selected ROI as a `.roi` file. If no ROIs are selected, saves all the ROI Manager selections in a ZIP archive.

***Fill*** Alias for Edit ▷ Fill [f].

***Draw*** Alias for Edit ▷ Draw [d].

***AND*** Uses the conjunction operator on the selected ROIs to create a composite selection. All ROIs are considered if none is selected.

***OR (Combine)*** Uses the union operator on the selected ROIs to create a composite selection. Combines all ROIs if none is selected.

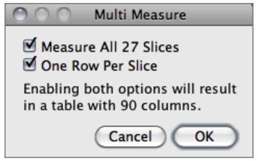
***XOR*** Uses the exclusive or operator on the selected ROIs to create a composite selection [43]. All ROIs are considered if none is selected.

***Split*** Splits the current selection (it must be a composite selection) into its component parts and adds them to the ROI Manager.

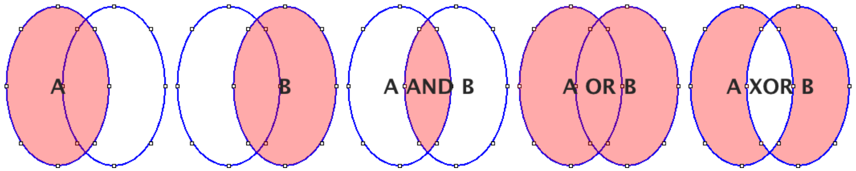
***Add Particles*** Adds objects segmented by the particle analyzer to the ROI Manager. Requires that *Record Starts* be checked in the Analyze ▷ Analyze Particles... dialog box. Particle analyzer objects can also be added to the ROI Manager by checking *Add to Manager* in the Analyze Particles dialog box.








***Multi Measure*** Measures all the ROIs on all slices in the stack, creating a Results Table with either one row per slice (if *One Row Per Slice* is checked in the dialog) or one row per measurement [44].




**Logical operations using the ROI Manager (Analyze ▷ Tools ▷ ROI Manager...).**

Improved in IJ 1.46r

***Multi Plot*** Runs Analyze ▷ Plot Profile [k] on the selected ROIs on a single graph [45]. All selections are plotted if none is selected. When plotting less than seven selections colored lines are drawn: blue (ROI 1), green (ROI 2), magenta (ROI 3), red (ROI 4), cyan (ROI 5) and yellow (ROI 6). Profiles with more selections are drawn in tones of gray.

While Analyze ▷ Plot Profile [k] requires a line or rectangular selection, *Multi Plot* accepts all type of selections by first running Edit ▷ Selection ▷ Area to Line, which converts area and freehand ROIs to line selections.

***Sort*** Sorts the list in alphanumeric order.

***Specify...*** Alias for the Edit ▷ Selection ▷ Specify... prompt that allows the creation of area ROIs at specific locations.

***Remove Slice Info*** Removes the information in the ROI names that associates them with particular stack slices (*see* ROI Manager illustration).

***Help*** Opens http://imagej.nih.gov/ij/docs/menus/analyze.html#manager.

***Labels...*** Alias for Image ▷ Overlay ▷ Labels..., which allow the customization of selection labels when *Show All* is active.

New in IJ 1.46r

***List*** Prints a table detailing the properties of the ROIs stored in the Manager, including: *Index* (cf. `roiManager("index")` macro function), *Name*, *XY* coordinates of ROI center (pixels), and stroke *Color*.

***Options...*** Displays a dialog box (depicted in ROI Manager) that allows you to set several ROI Manager settings:

***Associate "Show All" ROIs with slices*** If checked, *Show All* will only reveal ROIs when browsing their respective slice. If unchecked, ROIs are shown in all stack slices.

***Restore ROIs centered*** If checked, ROIs opened by *More≫Open...* are centered on the image canvas. This option avoids loaded ROIs to be displayed out of boundaries when the image has been resized.

***Use ROI names as labels*** If checked, ROI names are used as selection labels when in the *Labels* checkbox is active. If unchecked, the ROI position in the Manager's list is used. Selections can be renamed using either *Rename* or *Properties...*

### XXV Selecting ROIs in the ROI Manager

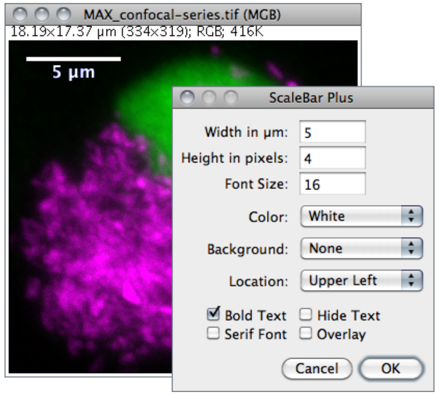
For most ROI Manager operations *Deselect* works as a *Select All* button, e.g., to measure all ROIs in manager one would press *Deselect* then *Measure*. In addition, it is possible to select contiguous ROIs in the ROI Manager list with a single Shift–click. Non-contiguous ROIs can be selected by Control–click (Command–click on Mac OS X).

When *Show All* is active, Overlays that are not stored in the ROI Manager will not be re-activated by Alt-clicking, Control-clicking or long-pressing (1/4 second or longer). This is a reminder that those overlay selections (added to the image overlay via Image ▷ Overlay ▷ Add Selection... [b]) are not under the control of the ROI Manager. You will be able to activate them as soon as *Show All* is unchecked.

See also: ROI Manager Tools, RoiManagerMacros, ROI Color Coder, Edit ▷ Selection ▷, Image ▷ Overlay ▷, Analyze ▷ Tools ▷ Synchronized windows, XIX Hexadecimal Color Values, XII Transferring Selections Between Images

Improved in IJ 1.46r

### 30.14.6 Scale Bar...




Draws a labelled spatial calibration bar.

***Width*** Length of the bar in calibrated units.

***Height*** Height of the bar in pixels.

***Font Size*** Adjusts the font size of the scale bar label.

***Color*** Adjusts the text color (*see* XVII Embedding Color Annotations in Grayscale Images).

***Background*** Adjusts the filling color of the label text box.

***Location*** Adjusts the position of the calibration bar. If there is a selection, the bar is initially drawn at the selection.

***Bold Text / Serif Font*** Specify if label should be typeset in boldface/serif typeface.

***Hide Text*** If checked the bar is drawn without label.

***Overlay*** If checked the bar is created as a non-destructive image overlay (*see* Overlays and Image ▷ Overlay ▷ submenu). If unchecked, the scale bar is drawn invasively.

See also: Analyze ▷ Set Scale..., Image ▷ Properties... [P], XXIII Global Calibrations

### 30.14.7 Calibration Bar...

Creates an RGB copy of the current image and displays a labelled calibration bar on it (*see* Analyze ▷ Calibrate...).

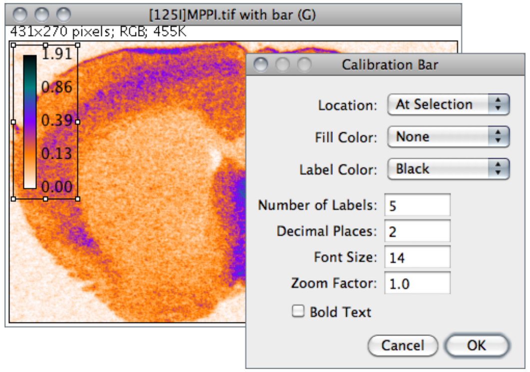




***Location*** Defines the position of the bar. If an area selection is active, the bar is initially drawn at that selection.

***Fill Color*** Defines the bar's background color.

***Label Color*** Adjusts the text color.




***Number of Labels*** Adjusts the total number of values displayed.

***Decimal Places*** Adjust the number of decimal places present in the labels.

***Font Size*** Adjusts labels font size (labels are drawn bold if *Bold Text* is checked).

***Zoom Factor*** Magnifies the entire calibration bar canvas. Enter a value less than 1 to reduce the bar size.

The Calibration Bar Macros can be used to add a calibration bar to a stack or to all the images and stacks in a folder.

See also: Calibrate..., XXIII Global Calibrations

### 30.14.8 Synchronize Windows

New in IJ 1.46r

Synchronizes mouse motion and input between multiple windows so that a ROI drawn in one image is replicated in all other Synchronized windows [46]. A synchronization cursor indicates the location of the mouse across the synchronized window set.

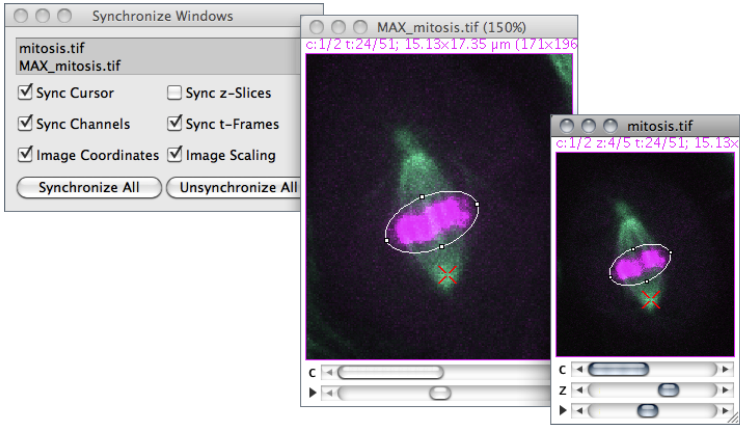
***Synchronized window set*** Images to be synchronized are specified in this list, containing all open images. To select a consecutive group of images, click the first item, press and hold down Shift, and then click the last item. Alternatively, click on the first item and drag it across. To select non-consecutive images, press and hold down Ctrl, then click each item to be selected. Use the *Un/Synchronize All* buttons to de/select all listed images.

***Sync cursor*** If checked, the cursor is synchronized across selected images, with the mouse pointer changing to a red X-shape cursor. When unchecked, mouse movements become restricted to the active image.

***Sync channels*** If checked, the channel slider ($c$) is synchronized across the synchronized window set.

***Image coordinates*** If checked, spatial calibration units will be used instead of pixels coordinates. For proper registration, this option should be unchecked when syncing images with different pixel sizes (*see* Image ▷ Properties... [P]).

***Sync z-slices*** If checked, the depth slider ($z$) is synchronized across the synchronized window set.




**Analyze ▷ Tools ▷ Synchronize Windows, IJ 1.46j**. This command can be used to measure ROIs across images, transfer ROIs from a reference image (e.g., a Maximum Intensity Projection, MIP) to multiple images or to use an un-zoomed copy of the active image as a navigator palette.

***Sync t-frames*** If checked, the frame slider (t) is synchronized across the synchronized window set.

***Image scaling*** If checked, positions to different windows are translated via offscreen coordinates, providing correct registration at different zoom levels.

SEE ALSO: XII Transferring Selections Between Images, ROI Manager





# 31 Plugins ▷

## 31.1 Macros ▷

This submenu contains commands for installing, running and recording macros, as well as any macro commands added by Plugins ▷ Macros ▷ Install... Macros contained in a file named `StartupMacros.txt`, in the macros folder, are automatically added to this submenu when ImageJ starts up. By design, only one set of macros can be installed at a time. As such, the last set of macros installed by Plugins ▷ Macros ▷ Install... (or by the *More Tools* Menu) will always replace previously installed macros.

### 31.1.1 Install...

Adds one or more macros contained in a file to the bottom of this submenu. To install a set of macros, and at the same time view their source code, open the macro file with File ▷ Open and use Editor's Macros ▷ Install Macros command. Macros in the file `ImageJ/macros/StartupMacros.txt` are automatically installed when ImageJ starts up. Similarly, with ImageJ 1.44f and later, newly opened macro sets with two or more macros are also automatically installed in this menu.

SEE ALSO: *More Tools* Menu

### 31.1.2 Run...

Loads and runs a macro without opening it in Editor. To run a macro, and at the same time view its source code, open it with File ▷ Open and use the editor's Macros ▷ Run Macro command.

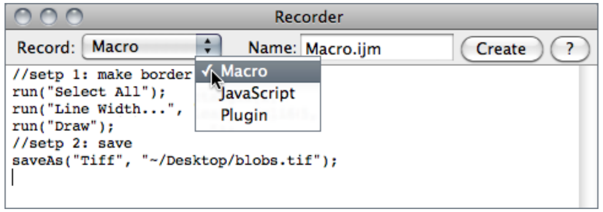
### 31.1.3 Startup Macros...

Opens `ImageJ/macros/StartupMacros.txt`. The same file can be opened by holding Shift while selecting *Startup Macros* from the » drop-down menu (*see More Tools* Menu).

### 31.1.4 Record...

Opens the ImageJ command recorder. To create a macro, open the recorder, use one or more ImageJ commands, then click *Create*. When the recorder is open, each menu command you use generates a macro `run()` function call. The `run()` function has one or two string arguments. The first is the command name. The optional second argument contains dialog box parameters. Examples:

- Create a rectangular, oval or line selection and the recorder will generate a `makeRectangle()`, `makeOval()` or `makeLine()` function call.
- Click on *Auto* or *Set* in the Image ▷ Adjust ▷ Threshold... [T] window to generate a `setThresold()` call, and on *Reset* to generate a `resetThresold()` call.
- Select an image from the Window menu to generate a `selectWindow()` call.
- Click in the Image ▷ Color ▷ Color Picker... [K] window to generate `setForegroundColor()` and `setBackgroundColor()` calls.




Note that you can interact with the recorder window by deleting or commenting lines of code or pasting text from Editor. This may be specially useful when writing your own macros or to generate simple 'Session Logs'. In this case, you would start the Recorder and let ImageJ keep track of the performed actions by generating macro code.

SEE ALSO: Editor, Extending ImageJ, The ImageJ Macro Language — Programmer's Reference Guide, Fiji's Introduction into Macro Programming

## 31.2 Shortcuts ▷

This submenu contains commands for creating keyboard shortcuts and for installing and removing plugins.

SEE ALSO: Toolbar Shortcuts

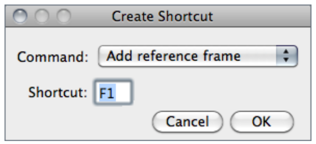
### 31.2.1 List Shortcuts...


This command generates a table with the ImageJ keyboard shortcuts in one column and the commands they call in another. Commands prefixed by '*' refer to shortcuts created with Create Shortcuts... while commands prefixed by '^' refer to installed macros (listed in Plugins ▷ Macros ▷) and override ImageJ default hot-keys.

Note that unless *Require control key for shortcuts* in Edit ▷ Options ▷ Misc... is checked, you do not have to hold down Ctrl to use a keyboard shortcut . E.g., to open an image (File ▷ Open... [o]) simply press O.

SEE ALSO: Keyboard Shortcuts, Create Shortcuts...

### 31.2.2 Create Shortcuts...



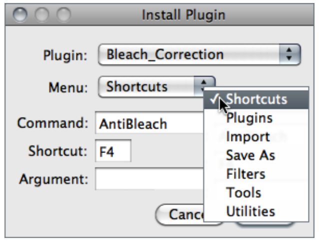

Assigns a keyboard shortcut to an ImageJ menu command and lists the shortcut in the Shortcuts submenu.

Select the command from the popup menu and enter the shortcut in the text field. A shortcut can be a lower or uppercase letter or 'F1' through 'F12'. Use Plugins ▷ Utilities ▷ List Shortcuts... to get a list of shortcuts that are already in use.





### 31.2.3 Install Plugin...




Installs a plugin in a user-specified submenu. Plugins with a showAbout() method are also automatically added to the Help ▷ About Plugins ▷ submenu.

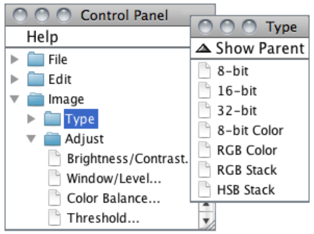
Use the first popup menu to select the plugin and the second to select the submenu it is to installed in. The command must be different from any existing ImageJ command. Shortcut (optional) must be a single letter or 'F1' through 'F12'. Argument (optional) is the string that will passed to the plugin's run method.

### 31.2.4 Remove...

Removes commands added to the Shortcuts submenu by Create Shortcuts... Also removes commands added by Install Plugin... and removes plugins installed in the Plugins menu. The menus are not updated until ImageJ is restarted.

## 31.3 Utilities ▷

### 31.3.1 Control Panel... [U]




This command [47] opens a window containing ImageJ commands in a hierarchical tree structure. Click on a leaf node to launch the corresponding ImageJ command (or plugin). Double click on a tree branch node (folder) to expand or collapse it. Click and drag on a tree branch node (folder) to display its descendants in a separate (child) window. In a child window, click on 'Show Parent' to re-open the parent window.

SEE ALSO: Find Commands... [l]

### 31.3.2 Find Commands... [l]


The quickest way to find a command without having to navigate through all the menus [48].

Evoke the prompt by pressing L (as in 'command Launcher', or 'Locator'). If you type part of a command name, the list will only show commands that match that substring. If only a single command matches then that command can be run by pressing Enter.

If multiple commands match, click with the mouse to select a command to run. Alternatively pressing the up or down keys will move keyboard focus to the list and the selected command can be run by pressing Enter. Pressing Backspace switches focus back to the prompt. Double clicking on a command will run that command. Pressing Esc closes the window.

***Show full information*** If checked, the Command Finder will display the location of the listed menu entries.

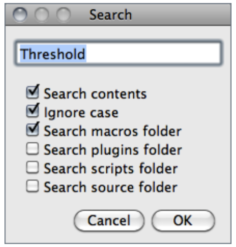
***Fuzzy matching*** (Fiji only) Activates approximate string matching. Useful if you are not sure about the command spelling.

***Close when running*** If checked, the Command Finder will dismiss after choosing *Run* or pressing Enter.

***Export*** Prints the filtered list of commands to an ImageJ table.

SEE ALSO: Control Panel... [U], Search..., Finding Commands

### 31.3.3 Search...




Searches for macros (`.txt`, `.ijm`), scripts (`.js`, `.py`, `.rb`, `.clj`, `.bsh`), plugins source (`.java`) and `.html` files containing a particular string.

Search is performed recursively (subdirectories are included) and results displayed in the Log Window. In the Log window, double click on a file path to have it open.

***Search contents*** Specifies if the search should be restricted to filenames or extended to file contents. If checked, the line number where the string was found is displayed.

***Ignore case*** Specifies if the search should be case-insensitive.

***Search macros folder*** Extends the search scope to `ImageJ/macros/`.

***Search plugins folder*** Extends the search scope to `ImageJ/plugins/`.

***Search scripts folder*** Extends the search scope to `ImageJ/scripts/` (if present).

***Search source folder*** Extends the search scope to `ImageJ/source/`(if present). Requires the ImageJ source code to be downloaded from http://imagej.nih.gov/ij/download/src/ and extracted into the ImageJ folder.

Note that you can perform searches in other directories by choosing none of the folders above mentioned. In this case, you will be asked to choose a target directory on a second dialog prompt.

SEE ALSO: Search.txt, the macro in `ij.jar` implementing this command, Find Commands... [l], Finding Commands

### 31.3.4 Monitor Events...

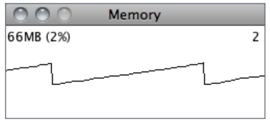
By implementing the `IJEventListener`, `CommandListener` and `ImageLister` interfaces, this command is able to monitor foreground and background color changes, tool switches, Log window closings, command executions and image window openings, closings and updates.

SEE ALSO: Debug mode (Edit ▷ Options ▷ Misc...)





### 31.3.5 Monitor Memory...




Displays a continuously updated graph of ImageJ's memory utilization, which can be useful for detecting memory leaks.

Memory usage and running threads are displayed above the graph. As for the IJ Status bar, clicking on the window will reclaim unused memory by running the Java garbage collector.

Ideally you should be able to open several images, process them, close them, and the amount of memory used will be the same as when you started.

SEE ALSO: Edit ▷ Options ▷ Memory & Threads...

### 31.3.6 Capture Screen [g]

Copies the screen to an RGB image and displays that image in a new window. Holding Ctrl Shift G will capture the screen while a modal dialog box is active if the dialog is based on ImageJ's GenericDialog class.

SEE ALSO: Capture Image, Flatten [F]

### 31.3.7 Capture Image

Copies a WYSIWYG version of active image to an RGB image and displays that image a new window.

SEE ALSO: Flatten [F], Capture Screen [g]

### 31.3.8 ImageJ Properties...

This command displays various ImageJ properties (Java version, OS name and version, path separator, location of directories, screen size, etc.) in a text window. Holding Alt lists all Java properties

SEE ALSO: Status bar, Help ▷ About ImageJ...

### 31.3.9 Threads...

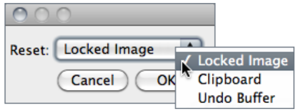
This command lists, in a text window, the currently running threads and their priorities.

### 31.3.10 Benchmark

Runs 62 image processing operations on the current image and displays the elapsed time in the ImageJ status bar. Additional benchmarks, test results, and source code are available in the Benchmarks package of plugins.

SEE ALSO: FAQs on the ImageJ Wikipage

### 31.3.11 Reset...




Use this command to unlock a locked Image, or to reclaim memory used by the clipboard and undo buffers (cf. Undo and Redo).

SEE ALSO: Edit ▷ Options ▷ Reset...

## 31.4 New ▷

This submenu contains commands opening editor windows that can be used to edit and run macros, scripts and plugins. It also has a command that opens a text window of a specified size and a command that opens a table that macros can write to. The editor windows opened by Plugin, Plugin Filter and Plugin Frame contain prototype Java code for the three types of plugins supported by ImageJ.

SEE ALSO: Editor, Extending ImageJ

### 31.4.1 Macro

Opens a blank editor window with the title 'Macro.txt'.

SEE ALSO: Macros, Editor, Text Window..., File ▷ New ▷ Text Window [N]

### 31.4.2 Macro Tool

NEW IN IJ 1.46R

Opens `Macro_Tool.txt`, an example macro tool that creates circular selections.

SEE ALSO: Macro Tools Documentation

### 31.4.3 JavaScript

Opens a blank editor window with the title 'Script.js'.

SEE ALSO: Scripts, Editor

### 31.4.4 Plugin

Opens an editor window containing a prototype plugin that implements the `PlugIn` interface. Plugins of this type open, capture or generate images. The prototype displays *Hello world!*. Press Ctrl R (File ▷ Run Macro) to compile and run it. Note that the name you choose for the plugin should include at least one underscore. Another example is the Step Maker plugin.

### 31.4.5 Plugin Filter

Opens an editor window containing a prototype plugin that implements the `PlugInFilter` interface. Plugins of this type process the active image. The prototype inverts the active image twice. Another example is the Image Inverter.





### 31.4.6 Plugin Frame

Opens an editor window containing a prototype plugin that extends the `PlugInFrame` class. Plugins of this type displays a window containing controls such as buttons and sliders. The prototype opens a window containing a text area. Another example is the IP Demo plugin.

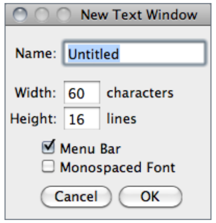
### 31.4.7 Plugin Tool

NEW IN IJ 1.46R

Opens a prototype plugin tool, demonstrating `ij.plugin.tool.PlugInTool` [49]. A plugin tool is a Java plugin that installs in the ImageJ toolbar to interact with the image canvas (*see* Custom Tools). Plugin tools with names ending in `Tool` are listed on the *More Tools* Menu if placed in the `ImageJ/plugins/Tools/` directory.

SEE ALSO: Plugin Tools

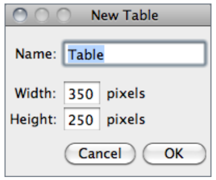
### 31.4.8 Text Window...




Opens a text window of a specified size that macros can write to. PrintToTextWindow, Clock and ProgressBar are examples of macros that write to a text window.

SEE ALSO: Plugins ▷ New ▷ Macro, File ▷ New ▷ Text Window [N]

### 31.4.9 Table...




Opens a blank table, similar to the Results table, that macros can write to. SineCosineTable2 is an example of such a macro.

SEE ALSO: Plugins ▷ New ▷ Text Window...

## 31.5 Compile and Run...

Compiles and runs a plugin. Runs a plugin if the name of the selected file ends in `.class`. Requires that ImageJ be running on a Java Virtual Machine that includes the javac compiler, which is contained in the `Tools.jar` archive. `Tools.jar` is included with the Windows and Linux versions of ImageJ bundled with Java, and it is pre-installed on Mac OS X. Troubleshooting information can be found under 'Compiling Plugins' in the Linux and Windows release notes.

The Edit ▷ Options ▷ Compiler... command can be used to configure the javac compiler. Since ImageJ 1.44c and later, Compile and Run... adds the Bio-Formats plugin (`loci_tools.jar`) to the Java compiler's classpath.

# 32 Window ▷

This menu contains four commands plus a list of all open windows. The currently active image will have a checkmark next to its name. To activate a window, pull down this menu and select the window by name.

## 32.1 Show All [ ] ]

Makes all the windows associated with ImageJ visible.

## 32.2 Put Behind [tab]

Displays the next open image. Repeatedly press the tab key to cycle through all open images. Note that pressing Enter on any image will bring the Main ImageJ window to the foreground.

SEE ALSO: I Frontmost Window and Window Activation

## 32.3 Cascade

Moves all open images to the left side of the screen, slightly offset from each other, and displayed in the order they are listed at the bottom of this menu.

## 32.4 Tile

Shrinks all open image windows and repositions them to fit on the screen without overlapping.





# 33 Help ▷

Many of the commands in this menu use ImageJ's BrowserLauncher to open a Web page using the user's default browser. On Linux, BrowserLauncher looks for 'netscape', 'firefox', 'konqueror', 'mozilla', 'opera', 'epiphany' or 'lynx' and uses the first one it finds.

## 33.1 ImageJ Website...

Opens the ImageJ home page.

## 33.2 ImageJ News...

Opens the News section of the ImageJ website.

## 33.3 Documentation...

Opens the Documentation section of the ImageJ website.

## 33.4 Installation...

Opens the Installation section of the ImageJ website specifically dedicated to the OS in which ImageJ is running, i.e either Linux, Mac OS 9, Mac OS X or Windows. Worth reading are the sections *Known Problems* and *Troubleshooting*.

## 33.5 Mailing List...

Opens the ImageJ Interest Group page on the NIH LISTSERV facility. Here you can search the mailing list archives, post a message to the list, join or leave the list, or update options.

## 33.6 Dev. Resources...

Opens the Developer Resources section of the ImageJ website.

## 33.7 Plugins...

Opens the Plugins page on the ImageJ website, which lists more than 500 ImageJ plugins.

## 33.8 Macros...

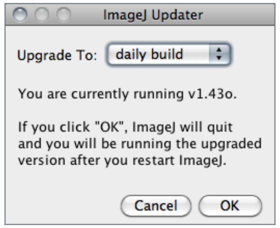
Opens the macros directory on the ImageJ website, which contains more than 400 ImageJ macros.

## 33.9 Macro Functions...

Opens the Macro Functions reference page, an indispensable guide to the built in functions that can be called from the ImageJ macro language.

SEE ALSO: Macros, Function Finder... [F]

### 33.10 Update ImageJ…




Upgrades ImageJ to the latest ij.jar at http://imagej.nih.gov/ij/upgrade/, or downgrades to one of the earlier versions at http://imagej.nih.gov/ij/download/jars/. Select *daily build* from the drop-down menu and ImageJ will be upgraded to the latest daily build at http://imagej.nih.gov/ij/ij.jar.

SEE ALSO: Installing and Maintaining ImageJ

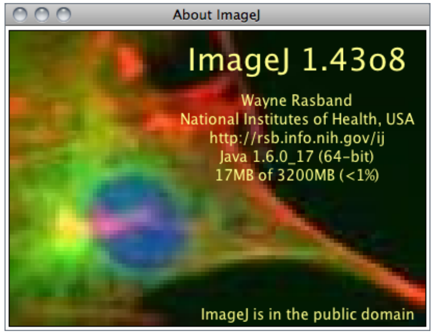
### 33.11 Refresh Menus

Use this command to update ImageJ's menus after adding (or removing) plugins or macros to the plugins folder. Prior to ImageJ 1.44b this command was named *'Update Menus'*.

### 33.12 About Plugins ▷

This submenu displays information about some of the plugins in the ImageJ plugins folder. To be included in this submenu, a plugin must be packaged as a JAR file. There is an example at rsb.info.nih.gov/ij/plugins/jar-demo.html.

### 33.13 About ImageJ…




Opens an image containing information about the ImageJ version, the author, the website, Java version and memory available. Note that clicking in the Status bar is a quicker way to show this information.

SEE ALSO: Plugins ▷ Utilities ▷ ImageJ Properties…



# Part VI

# Keyboard Shortcuts

The following table summarizes the keyboard shortcuts built into ImageJ. You can create additional shortcuts, or override built-in ones, by creating simple macros and adding them to the StartupMacros.txt. You can also assign a function key to a menu command using Plugins ▷ Shortcuts ▷ Create Shortcuts….

Several of these shortcuts accept key modifiers as described in Key Modifiers. Also note that, except when using the Text Tool, you do not need to hold down the control key to use a keyboard shortcut, unless *Require control key for shortcuts* in Edit ▷ Options ▷ Misc… is checked.

SEE ALSO: Using Keyboard Shortcuts, Finding Commands, KeyboardShortcuts.txt macro (demonstrating how assign shortcuts to custom macros), Toolbar Shortcuts

**List of ImageJ 1.46r built-in shortcuts.** This table can be obtained within ImageJ using the Plugins ▷ Shortcuts ▷ List Shortcuts… command.

| Command / Operation | Shortcut | Description |
|---|---|---|
| **File ▷** | | |
| New ▷ Image… [n] | N | Create new image or stack |
| New ▷ Text Window [N] | Shift N | Create new text window |
| New ▷ System Clipboard [V] | Shift V | Create image from system clipboard |
| Open… [o] | O | Open file (any format recognized by ImageJ) |
| Open Next [O] | Shift O | Open next image in folder |
| Open Samples ▷ Blobs (25K) | Shift B | Opens the *Blobs.gif* example image |
| Close [w] | W | Close the active window |
| Save [s] | S | Save active image in Tiff format |
| Revert [r] | R | Revert to saved version of image |
| Print… [p] | P | Print active image |
| **Edit ▷** | | |
| Undo [z] | Z | Undo last operation |
| Cut [x] | X | Copy selection to internal clipboard and clear |
| Copy [c] | C | Copy selection to internal clipboard |
| Paste [v] | V | Paste contents of internal clipboard |
| Clear | Backspace | Erase selection to background color |
| Fill [f] | F | Fill selection in foreground color |
| Draw [d] | D | Draw selection |
| Invert [I] | Shift I | Invert image or selection |
| Selection ▷ Select All [a] | A | Select entire image |
| Selection ▷ Select None [A] | Shift A | Remove selection |
| Selection ▷ Restore Selection [E] | Shift E | Restore previous selection |
| Selection ▷ Properties… [y] | Y | Defines selection properties |
| Selection ▷ Add to Manager [t] | T | Add selection to ROI Manager |
| **Image ▷** | | |
| Adjust ▷ Brightness/Contrast… [C] | Shift C | Adjust brightness and contrast |
| Adjust ▷ Threshold… [T] | Shift T | Adjust threshold levels |
| Show Info… [i] | I | Display information about active image |
| Properties… [P] | Shift P | Display image properties |

**List of ImageJ 1.46r built-in shortcuts.** This table can be obtained within ImageJ using the Plugins ▷ Shortcuts ▷ List Shortcuts… command.

| Command / Operation | Shortcut | Description |
|---|---|---|
| Color ▷ Color Picker… [K] | Shift K | Open Color Picker |
| Stacks ▷ Next Slice [>] | > or → | Go to next stack slice |
| Stacks ▷ Previous Slice [<] | < or ← | Go to previous stack slice |
| Stacks ▷ Reslice… [/] | / | Reslice stack |
| Stacks ▷ Orthogonal Views [H] | Shift H | Toggle orthogonal view display |
| Stacks ▷ Tools ▷ Start Animation [\] | \ | Start/stop stack animation |
| Hyperstacks ▷ Channels Tool… [Z] | Shift Z | Open the 'Channels' tool |
| Hyperstacks | > or → | Next hyperstack channel |
| Hyperstacks | < or ← | Previous hyperstack channel |
| Hyperstacks | Ctrl > | Next hyperstack slice |
| Hyperstacks | Ctrl < | Previous hyperstack slice |
| Hyperstacks | Alt > | Next hyperstack frame |
| Hyperstacks | Alt < | Previous hyperstack frame |
| Crop [X] | Shift X | Crop active image or selection |
| Duplicate… [D] | Shift D | Duplicate active image or selection |
| Scale… [E] | E | Scale image or selection |
| Zoom ▷ In [+] | + or ↑ | Make image larger |
| Zoom ▷ Out [-] | − or ↓ | Make image smaller |
| Zoom ▷ Original Scale [4] | 4 | Revert to original zoom level |
| Zoom ▷ View 100% [5] | 5 | Zoom to 1:1 |
| Overlay ▷ Add Selection… [b] | B | Adds active selection to image overlay |
| **Process ▷** | | |
| Smooth [S] | Shift S | 3×3 unweighted smoothing |
| Repeat Command [R] | Shift R | Repeat previous command |
| **Analyze ▷** | | |
| Measure… [m] | M | Display statistics of active image / selection |
| Histogram [h] | H | Display histogram of active image / selection |
| Plot Profile [k] | K | Display density profile plot of active selection |
| Gels ▷ Select First Lane | 1 | Select first gel lane |
| Gels ▷ Select Next Lane | 2 | Select next gel lane |
| **Plugins ▷** | | |
| Utilities ▷ Control Panel… [U] | Shift U | Open Control Panel |
| Utilities ▷ Capture Screen [g] | Shift G | Grab screenshot (with Ctrl if a dialog box is active) |
| Utilities ▷ Find Commands… [l] | L | List, find and launch commands |
| **Window ▷** | | |
| Show All [ ] ] | ] | Make all windows visible |
| Put Behind [tab] | Tab | Switch to next image window |
| Main ImageJ window | Enter | Bring ImageJ window to front |

# 34 Key Modifiers

## 34.1 Alt Key Modifications

**File ▷ Open Next [O]** Open previous

**File ▷ Revert [r]** Skip dialog prompt





**Edit ▷ Copy [c]** Copy to system clipboard

**Image ▷ Color ▷ Split Channels** Keep original image

**Image ▷ Stacks ▷ Add Slice** Insert before current slice

**Image ▷ Stacks ▷ Next Slice [>]** Skip ten slices

**Image ▷ Stacks ▷ Previous Slice [<]** Skip ten slices

**Image ▷ Stacks ▷ Start Animation [\]** Show options dialog

**Image ▷ Duplicate… [D]** Skip dialog prompt

**Image ▷ Overlay ▷ Add Selection… [b]** Show options dialog

**Process ▷ Enhance Contrast…** Do classic histogram equalization

**Analyze ▷ Histogram [h]** Show dialog prompt

**Analyze ▷ Plot Profile [k]** For rectangular selections, generate row average plot. For wide straight lines, display rotated contents

**Analyze ▷ Gels ▷ Select First Lane** Assumes lanes are horizontal

**Analyze ▷ Tools ▷ Analyze Line Graph** Show intermediate image

**Analyze ▷ Tools ▷ ROI Manager (*Add*)** Name and add selection

**Plugins ▷ Utilities ▷ ImageJ Properties…** List all Java properties

Area Selection Tools Subtract current selection from the previous one

Rectangular Selection Tool and Oval Selection Tool Current aspect ratio is maintained while resizing

Straight Line Selection Tool Keeps the line length fixed while moving either end of the line. Forces the two points that define the line to have integer coordinate values when creating a line on a zoomed image

Segmented Line Selection Tool and Polygon Selection Tool Alt-clicking on a node deletes it

Point Tool Alt-clicking on a point deletes it

Color Picker Tool Alt-clicking on an image 'picks-up' background color

All Tools Show location and size in pixels rather than calibrated units

## 34.2 Shift Key Modifications

**Image ▷ Adjust ▷ Threshold… [T]** Adjusting *Min* also adjusts *Max*

**Image ▷ Adjust ▷ Brightness/Contrast… [C]** Apply adjustments to all channels of a composite image

**Installed Macros and Scripts** Open instead of run

Rectangular Selection Tool and Oval Selection Tool Forces 1:1 aspect ratio

Area Selection Tools Add selection to previous one

Polygon Selection Tool Shift-clicking on a node duplicates it

Straight Line Selection Tool Forces line to be horizontal or vertical

Segmented Line Selection Tool Shift-clicking on a node duplicates it

Point Tool Shift-clicking adds points (Multi-point Tool behavior)

Magnifying Glass Shift-clicking and dragging runs Image ▷ Zoom ▷ To Selection

SEE ALSO: Manipulating ROIs, Tools

### 34.3 Ctrl (or Cmd) Key Modifications

Rectangular Selection Tool and Oval Selection Tool Selection is resized around its center

Straight Line Selection Tool Line is rotated/resized around its center

SEE ALSO: Manipulating ROIs, Tools

### 34.4 Space Bar

**Any Tool** Switch to the Scrolling Tool

### 34.5 Arrow Keys

**Moving** Selections The four arrow keys move selection outlines one pixel at a time

**Resizing** Selections Rectangular and oval selections are resized by holding Alt while using the arrow keys

Stacks **Navigation** The ← and → keys substitute for < and > for moving through a stack. If there is a selection, you must also hold Shift

Hyperstacks **Navigation** The ← and → keys change the channel. Hold Ctrl to move through the slices and Alt to move through the frames

**Zooming** The ↑ and ↓ keys zoom the image in and out. If there is a selection, you must also hold either Shift or Ctrl

SEE ALSO: Manipulating ROIs, Zoom▷

## 35 Toolbar Shortcuts

Keyboard shortcuts cannot be used directly to activate tools in the ImageJ Toolbar (with the exception of the Magnifying Glass and the Scrolling Tool). However, shortcuts can be assigned to macros that use the `setTool()` macro function.

The set of macros listed below (taken from `ToolShortcuts`) exemplify how to assign the function keys F1 through F12 to some of the most commonly used Tools. Once copied to the `ImageJ/macros/StartupMacros.txt` file, they will be automatically installed at startup.

**(9) Assigning Keyboard Shortcuts to ImageJ Tools**

```
/* These macros allow tools to be selected by pressing function keys. Add ↩
   them to ImageJ/macros/StartupMacros.txt and they will be automatically ↩
   installed when ImageJ starts. */

 macro "Rectangle [f1]" {setTool("rectangle")}
 macro "Elliptical [f2]" {setTool("elliptical")}
 macro "Brush [f3]" {setTool("brush")}
 macro "Polygon [f4]" {setTool("polygon")}
 macro "Freehand [f5]" {setTool("freehand")}
 macro "Straight Line [f6]" {setTool("line")}
 macro "Segmented Line [f7]" {setTool("polyline")}
 macro "Arrow [f8]" {setTool("arrow")}
 macro "Angle [f9]" {setTool("angle")}
 macro "Multi-point [f10]" {setTool("multipoint")}
 macro "Wand [f11]" {setTool("wand")}
 macro "Magnifying Glass [f12]" {setTool("zoom")}
```





This approach, however, requires the user to memorize a large number of shortcuts. In addition, it may be difficult to assign so many hot-keys without conflicting with previously defined ones (*see* Plugins▷Shortcuts▷). An alternative way to control the toolbar using the keyboard is to create macros that progressively activate tools from a predefined sequence. The next example demonstrates such strategy. It is composed of two macros activated by F1 and F2 that iterate through the toolbar from left to right (forward cycle) and right to left (reverse cycle).

**(10) Cycling Through the Toolbar Using Keyboard Shortcuts**

```
/* These two macros loop through the tools listed in an array using "F1" ↩
   and "F2" as keyboard shortcuts (forward and reverse cycling). */

 var index;
 var tools = newArray("rectangle", "roundrect", "oval", "ellipse", ↩
    "brush", "polygon", "freehand", "line", "freeline", "polyline", ↩
    "arrow", "wand", "dropper", "angle", "point", "multipoint", "text");

 macro "Cycle Tools Fwd [F1]" {
  setTool(tools[index++]);
  if (index==tools.length) index = 0;
 }

 macro "Cycle Tools Rwd [F2]" {
  if (index<0) index = tools.length-1;
  setTool(tools[index--]);
 }
```

A tool can be defined either by its name or by its position in the toolbar using `setTool(id)`, which allows assigning keyboard shortcuts to Custom Tools and items loaded by the *More Tools* Menu (e.g., `setTool(21);` activates whatever tool has been installed on the last slot of the toolbar). It is also possible to temporarily activate a tool. The macro below (taken from the `Rename and Save ROI Sets` toolset), activates the Color Picker Tool when F3 is pressed, but restores the previously active tool as soon as the mouse is released.

**(11) Temporary Activation of a Tool**

```
 macro "Pick Color Once [F3]" {
   tool = IJ.getToolName;
   setTool("dropper");
   while (true) {
       getCursorLoc(x, y, z, flags);
       if (flags&16!=0)
           { setTool(tool); exit; }
   }
 }
```

# Credits

[C1] The ImageJ installer for Windows is created using the Inno Setup installer generator. `ImageJ.exe` the Windows that launches ImageJ (`ij.jar`) was contributed by George Silva.

[C2] Support for ZIP-compressed TIFFs was contributed by Jason Newton in IJ 1.45g.

[C3] The macro editor's Function Finder (Macros ▷ Find Functions...) was written by Jérôme Mutterer.

[C4] The Elliptical Selection Tool was contributed by Norbert Vischer.

[C5] The Brush Selection Tool is based on the ROI Brush Tool plugin from Tom Larkworthy and Johannes Schindelin.

[C6] Jean-Yves Tinevez and Johannes Schindelin (authors of the Fiji Arrow Tool) contributed code to the Arrow Tool.

[C7] Michael Schmid, added 4–connected and 8–connected tracing with tolerance to the Wand Tool.

[C8] Macro Toolsets distributed with ImageJ have been contributed by Gilles Carpentier, Jérôme Mutterer and Tiago Ferreira.

[C9] The Pixel Inspector is a plugin tool conversion of Michael Schmid's Pixel Inspector plugin.

[C10] In IJ 1.43l and earlier, the File ▷ Import ▷ Results... command was based on Jérôme Mutterer's Import_Results_Table macro.

[C11] Marcel van Herk added URLs support to the File ▷ Import ▷ Stack From List... command in IJ 1.45f.

[C12] Michael Schmid contributed improvements to the AVI reader and AVI writer plugins.

[C13] Karen Collins contributed improvements to the FITS_Writer (File ▷ Save As ▷ FITS... command).

[C14] The Edit ▷ Selection ▷ Fit Circle command, based on a MATLAB script by Nikolai Chernov, was contributed by Michael Doube and Ved Sharma.

[C15] The Edit ▷ Selection ▷ Create Selection command is based on the Threshold_To_Selection plugin written by Johannes Schindelin.

[C16] IJ 1.46f adopted Johannes Schindelin's RMI-based OtherInstance class from Fiji, which works on multi-user machines and is more secure.

[C17] The Color Picker (Image ▷ Color ▷ Color Picker... [K]) was written by Gali Baler, a 2003–2004 intern from Bethesda-Chevy Chase High School.

[C18] The 16 different thresholding methods available in the Image ▷ Adjust ▷ Threshold... [T] tool were implemented by Gabriel Landini.

[C19] Michael Schmid contributed improvements to the downsizing kernel used by Image ▷ Adjust ▷ Size... and Image ▷ Scale... [E] as well as undo support for Image ▷ Scale... [E].

[C20] The Image ▷ Adjust ▷ Color Threshold... command implements Gabriel Landini's Threshold Colour plugin.

[C21] The Reslice and the ZProject plugin (Image ▷ Stacks ▷ Reslice... [/] and Z Project... commands) were contributed by Patrick Kelly and Harvey Karten of the University of California, San Diego.

[C22] The Image ▷ Stacks ▷ Orthogonal Views [H] command is based on Dimiter Prodanov's StackSlicer plugin and Albert Cardona's Updater class. Michael Doube added support for XZ and YZ view control as well as mouse wheel control.

[C23] The Image ▷ Stacks ▷ 3D Project... was written by Michael Castle and Janice Keller of the University of Michigan Mental Health Research Institute (MHRI). Bill Mohler added suport for hyperstacks and 16/32–bit images in IJ 1.44m.

[C24] The Image ▷ Stacks ▷ Tools ▷ Concatenate... command implemented in IJ 1.46e is based on the Concatenate plugin by Jonathan Jackson.

[C25] The Image ▷ Stacks ▷ Tools ▷ Make Substack... command is based on the Substack Maker plugin by Anthony Padua, Daniel Barboriak and Ved Sharma.



[C26] The Image ▷ Hyperstacks ▷ Reduce Dimensionality... command is based Jérôme Mutterer's Reduce HyperStack macro.

[C27] The Image ▷ Transform ▷ Bin... command is based on Nico Stuurman's Binner plugin.

[C28] The Image ▷ Zoom ▷ Set... command is based on Albert Cardona's Zoom Exact plugin.

[C29] The Process ▷ Find Maxima... command is based on a plugin contributed by Michael Schmid.

[C30] The equalization code implemented in Process ▷ Enhance Contrast... was contributed by Richard Kirk.

[C31] The Process ▷ Noise ▷ Remove NaNs... was contributed by Michael Schmid.

[C32] The Process ▷ Math ▷ Macro... command is modeled after Ulf Dittmer's Expression plugin.

[C33] The Process ▷ FFT ▷ Bandpass Filter... is a built in version of Joachim Walter's FFT Filter plugin.

[C34] The Process ▷ Binary ▷ Fill Holes algorithm was contributed by Gabriel Landini.

[C35] The Skeletonize3D plugin was written by Ignacio Arganda-Carreras, based on an ITK implementation by Hanno Homann. It implements a 3D thinning algorithm from Lee et al. Building skeleton models via 3-D medial surface axis thinning algorithms. *CVGIP*, 56(6):462–478, 1994.

[C36] Multi-threading support for all Process ▷ Filters ▷ commands was contributed by Stephan Saalfeld and Michael Schmid in ImageJ 1.45c.

[C37] The faster and more accurate version of Process ▷ Filters ▷ Gaussian Blur... implemented in ImageJ 1.38r was contributed by Michael Schmid.

[C38] The `NonBlockingGenericDialog.class` used by the Process ▷ Batch ▷ Macro... command was added by Johannes Schindelin.

[C39] The rolling ball code of Process ▷ Subtract Background... is based on the NIH Image Pascal version by Michael Castle and Janice Keller. The sliding paraboloid algorithm was written by Michael Schmid.

[C40] The Analyze ▷ Distribution... command was written by Gabriel Landini.

[C41] The scaled color bar implemented in Analyze ▷ Histogram [h] was contributed by Bob Dougherty.

[C42] The much improved CurveFitter (Analyze ▷ Tools ▷ Curve Fitting...) implemented in IJ 1.46f was contributed by Michael Schmid. The Rodboard and Gaussian functions were originally contributed by David Rodbard (NIH) and Stefan Wörz (DKFZ), respectively.

[C43] The ROI Manager(*XOR*) command (Analyze ▷ Tools ▷ ROI Manager...) was added by Johannes Schindelin.

[C44] The ROI Manager(*Multi Measure*) command (Analyze ▷ Tools ▷ ROI Manager...) is based on Bob Dougherty's Multi_Measure plugin.

[C45] The ROI Manager(*Multi Plot*) command (Analyze ▷ Tools ▷ ROI Manager...) was contributed by Philippe Gendre.

[C46] The Analyze ▷ Tools ▷ Synchronize Windows command (an improved version of the SyncWindows_ plugin by Patrick Kelly) was contributed by Joachim Walter.

[C47] The Control Panel (Plugins ▷ Utilities ▷ Control Panel... [U]) was written by Cezar M. Tigare.

[C48] The Command Finder (Plugins ▷ Utilities ▷ Find Commands... [l]) was written by Mark Longair.

[C49] The `PlugInTool` class was inspired by Johannes Schindelin's AbstractTool class in Fiji.

[C50] Other additions, improvements and reproducible bug reports have been contributed by Adrian Daerr, Airen Peraza, Ajay Gopal, Albert Cardona, Alberto Duina, Alden Dima, Andreas Maier, Andrew French, Andrii Savchenko, Arttu Miettinen, Aryeh Weiss, Balazs Nyiri, Barry DeZonia, Bill Mohler, Bob Hamilton, Bob Loushin, Bruno Vellutini, Burri Olivier, Carlos Becker, Carne Draug, Charles Anderson, Cheryl McCreary, Christian Moll, Christophe Leterrier, Christopher Harrison, Damon Poburko, Daniel Hornung, Daniel Kalthoff, Daniel Senff, David Gauntt, David McDonald, Denny Hugg, Dimiter Prodanov, Divakar Ramachandran, Dorai Iyer, Duncan Mak, Eik Schumann, Emmanuel Levy, Erik Meijering, Fabian Svara, Francis Burton, Frank Sprenger, Franklin Shaffer, Frederic Hessman, Gabriel Landini, Gilles Carpentier, Gregory Reneff, Hiroki Hakoshima, Ian Lim, Ingo Bartholomaeus, Jakob Preus, Jamie Robinson, Jan Eglinger, Jan Funke, Jarek Sacha, Jay Unruh, Jean-Pierre Clamme, Jerome Mutterer, Jesper Pedersen, Jim Passmore, Joachim Wesner, Johannes Hermen, Johannes Schindelin, Johannes Weissmann, John Oreopoulos, John Pearl, Jonathan Silver, Jose Wojnacki, Juan Grande, Julian Cooper, Kai Uwe Barthel, Karen Collins, Kees Straatman, Kevin Moll, Kris Sheets, Mark Krebs, Mark Longair, Martin Dressler, Mat Al-Tamimi, Matthew Smith, Michael Cammer, Michael Doube, Michael Ellis, Michael Schmid, Michel Julier, Nagananda Gurudev, Nico Stuurman, Norbert Vischer, Olaf Freyer, Oliver Bannach, Olivier Bardot, Paul Jurczak, Peter Haub, Rainer Engel, Raymond Coory, Reinhard Mayr, Richard Cole, Richie Mort, Robert Dougherty, Shannon Stewman, Simon Roussel, Stefan Starke, Stephan Saalfeld, Steven Green, Thomas Boudier, Thorsten Wagner, Tiago Ferreira, Tomas Karlsson, Tseng Qingzong, Ulf Dittmer, Uwe Walschus, Valerio Mussi, Ved Sharma, Vytas Bindokas, Wilhelm Burger, Winfried Wurm, Xiao Chen, Zeljka Maglica.

### XXVI Focus on Bioimage Informatics

In July 2012 Nature Methods issued a focus dedicated to the role of bioimage informatics in microscopy, the tools that are available for scientific image processing, and the challenges and opportunities in the field. The special issue features a large collection of articles discussing ImageJ, Fiji and Related Software, including:

- Cardona A, Tomancak P: "Current challenges in open-source bioimage informatics" [14]
- Carpenter AE et al.: "A call for bioimaging software usability" [18]
- Eliceiri KW. "Biological imaging software tools" [30]
- Schindelin J et al. "Fiji: an open-source platform for biological-image analysis" [100]
- Schneider CA et al.: "NIH Image to ImageJ: 25 years of image analysis" [102]

A full announcement can be found on the Fiji news channel.



# ImageJ Related Publications

The following references are a small sample (particularly biased towards the life sciences) of the bibliography directly related to ImageJ, the standard in scientific image analysis. These publications include: 1) technical articles and books describing routines implemented in ImageJ, 2) research articles that have made extensive use of ImageJ as a scientific tool or 3) reviews that discuss ImageJ pertinently. A similar list is maintained on the Fiji website.

To cite ImageJ one of the citations is possible (see FAQs):

1. Rasband WS. ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, imagej.nih.gov/ij/, 1997–2012.
2. Abràmoff MD, Magalhães PJ and Ram SJ. "Image Processing with ImageJ", *Biophotonics International*, 11(7):36–42, 2004 (PDF) [1].
3. Schneider CA, Rasband WS and Eliceiri KW. "NIH Image to ImageJ: 25 years of image analysis", *Nature Methods*, pp. 671, 2012 [102]

To cite Fiji:

- Schindelin J et al. "Fiji: an open-source platform for biological-image analysis", *Nature Methods*, pp. 676–82, 2012 [100]

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## List of Abbreviations and Acronyms

Alt ........... Alt, Option or Meta key;
API ........... Application Programming Interface;
ASCII ........ American Standard Code for Information Interchange;
bit ............ Binary digit;
BMP ......... Bitmap Image File (Device Independent Bitmap, DIB);
bpp ........... Bits per pixel;
CCD .......... Charge-Coupled Device;
CP ........... Color Picker;
CSV .......... Comma-Separated Values;
CT ........... Computed Tomography;
Ctrl ........... Control key. In this guide also the Command key in Apple keyboards;
DICOM ....... Digital Imaging and Communications in Medicine;
DPI ........... Dots Per Inch;
EDM ......... Euclidian Distance Map;
FAQ .......... Frequently Asked Questions;
FFT .......... Fast Fourier Transform;
FHT .......... Fast Hartley Transform;
FIJI .......... Fiji Is Just ImageJ;
FITS ......... Flexible Image Transport System;
fps ............ Frames Per Second;
GIF ........... Graphics Interchange Format;
GUI .......... Graphical User Interface;
HDR .......... High Dynamic Range;
HEX .......... Hexadecimal;
HIPR ......... Hypermedia Image Processing Reference;
HSB .......... Hue Saturation Brightness;
HTML ........ HyperText Markup Language;
IDE ........... Integrated Development Environment;
IJ ............. ImageJ;
ITK .......... Insight Segmentation and Registration;
JAR .......... Java ARchive;
JPEG ......... Joint Photographic Experts Group;
Knime ........ Konstanz Information Miner;
KNIP ......... Knime Image Processing;
LUT .......... Lookup table;
LZW .......... Lempel-Ziv-Welch;
MIP .......... Maximum Intensity Projection;
MJPG ........ Motion-JPEG;
NaN .......... Not a Number;
OME ......... Open Microsopy Environment;
OS ............ Operating System;
p/c ........... Pixels per cycle;
PBM ......... Portable BitMap;
PGM ......... Portable GrayMap;
pixel .......... Picture element;
PNG .......... Portable Network Graphics;
ppi ............ Pixels per inch;
PPM ......... Portable PixMap;
pt ............. Point;
RAM ......... Random-Access Memory;
regex ......... Regular expression;
RGB .......... Red Green Blue;
ROI .......... Region Of Interest;
Shift .......... Shift key;
TIFF ......... Tagged Image File Format;
UEPs ......... Ultimate Eroded Points;
URL .......... Uniform Resource Locator;
voxel .......... Volumetric pixel;
WYSIWYG ... What You See Is What You Get;



## Index

# Colophon

The initial contents of this guide have been retrieved in 2009 from the ImageJ website using html2text. Since then, it has been complemented and updated using informations posted on the ImageJ mailing list, ImageJ Documentation Portal, Fiji, ImageJ, and ImageJDev websites and Tony Collins *ImageJ for Microscopy* manual. Nevertheless, because there has never been accompanying documentation for some of the 350+ described commands, several sections have been written from scratch based on the relevant ImageJ source code and authors own experience. Legacy nomenclature that became obsolete with version 1.46r has been intentionally omitted.

The guide was typeset with TEXLive 2012 on Mac OS 10.6.8. All illustrations were created with ImageJ/Fiji, loaded with G. Landini's IJ Robot and J. Schindelin's Tutorial Maker plugins. Screenshots were produced by the `screencapture` shell utility controlled by the following IJ macro:

```
exec("screencapture -ciWo > /dev/null 2>&1 &"); run("System Clipboard");
setLineWidth(1); setForegroundColor(111, 121, 132);
drawRect(0, 0, getWidth, getHeight);
```

The HTML version was produced with eLyXer 1.2 and formatted using CSS code from Alex Fernández and Michael Hüneburg; JavaScript code from Ciarán O'Kelly, Stuart Langridge and Tiago Ferreira. It uses SyntaxHighlighter and icons from the Tango Desktop Project.

## Getting Involved

Your help is needed to improve ImageJ. Even if you are not a programmer, your participation is important:

– Are you a skilled ImageJ user?
  You might want to help out with the documentation effort: Write a FAQ, How-To, Tutorial or Video Tutorial on the ImageJ Documentation Portal; Help us updating the ImageJ User Guide; Share the add-ons you may have created with the community; Subscribe the mailing list and help answering the questions raised by other users.

– Are you know knowledgeable in image processing?
  Please join the mailing list and participate in the discussions. You could also write a Tutorial on the ImageJ Documentation Portal.

– Do you have a strong scientific background?
  Frequently, the most demanding tasks in scientific image processing relate to experimental design. Even if you do not have special expertise in image processing, by participating on the mailing list discussions, your experience will be valuable to others.

– Do you want ImageJ to improve?
  You can report bugs or request new features using the mailing list.

– Do you have experience in graphic/web design?
  If you are able to to improve the look and feel of the guide we welcome your skills.

## The ImageJ Icon

The Hartnack microscope (*ca.* 1870's) depicted on the front page inspired the ImageJ icon for Mac OS X. It is based on a photograph by Tom Grill at arsmachina.com.

Edmund Hartnack (1826–1891) was a renown microscope manufacturer that pioneered the use of correction collars in water-immersion lenses and the adoption of the substage condenser[1]. The precision and robustness of Hartnack optics played a pivotal role in the groundbreaking research by the Nobel laureates Robert Koch[2], Camillo Golgi[3] and Santiago Ramón y Cajal[4].

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# Document Revision History

| Date | Notes |
|---|---|
| September/October 2012 | Reviewed by Michael Schmid |
| July 2012 | Updated for v 1.46r<br>Reviewed by Barry DeZonia |
| June 2012 | Updated for v 1.46p<br>Second major revision with new sections on overlays, 3D volumes, sub-pixel selections, curve fitting and interface customizations<br>Improvements in layout and browsability |
| September 2011 | Updated for v 1.45<br>Deeply revised edition with several new sections in Parts I–IV<br>Available as printable booklets<br>Redesigned HTML version |
| January 2011 | Updated for v 1.44<br>New sections on advanced ImageJ usage, Fiji scripting, command line usage and interoperability with other software packages |
| May 2010 | First HTML version |
| April 2010 | First edition: v 1.43 |

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