



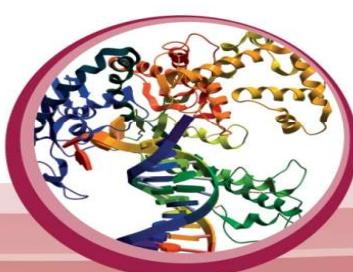
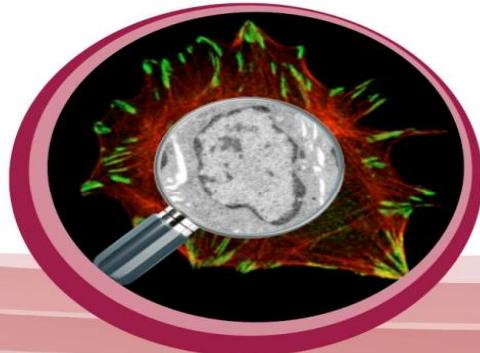
Using ImageJ/Fiji for microscope image processing and analysis

MMC 2025

<https://github.com/KeesStraatman/MMC2025>

Dr Kees Straatman

Advanced Imaging Facility
Core Biotechnology Services
College of Life Sciences
University of Leicester
krs5@le.ac.uk





Programme

- 1.30 pm
 - Introduction
 - Basics
 - Plugins
- Short break
 - Macros
- 4.30 pm end

<https://github.com/KeesStraatman/MMC2025>

Image analysis

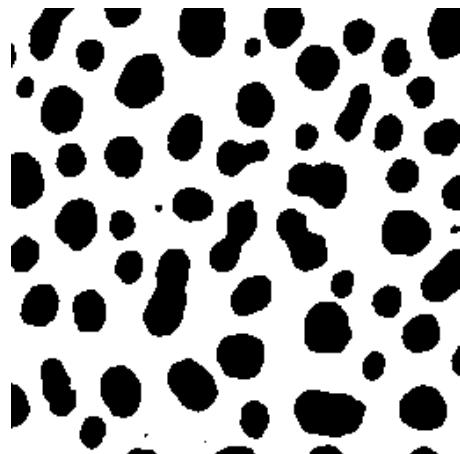
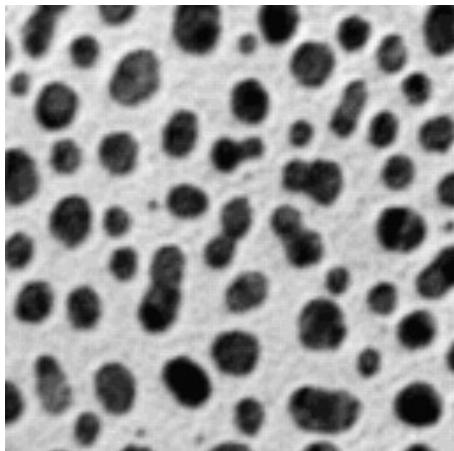
Image analysis is the extraction of meaningful information from digital images by means of digital image processing techniques.

Setup an image analysis workflow



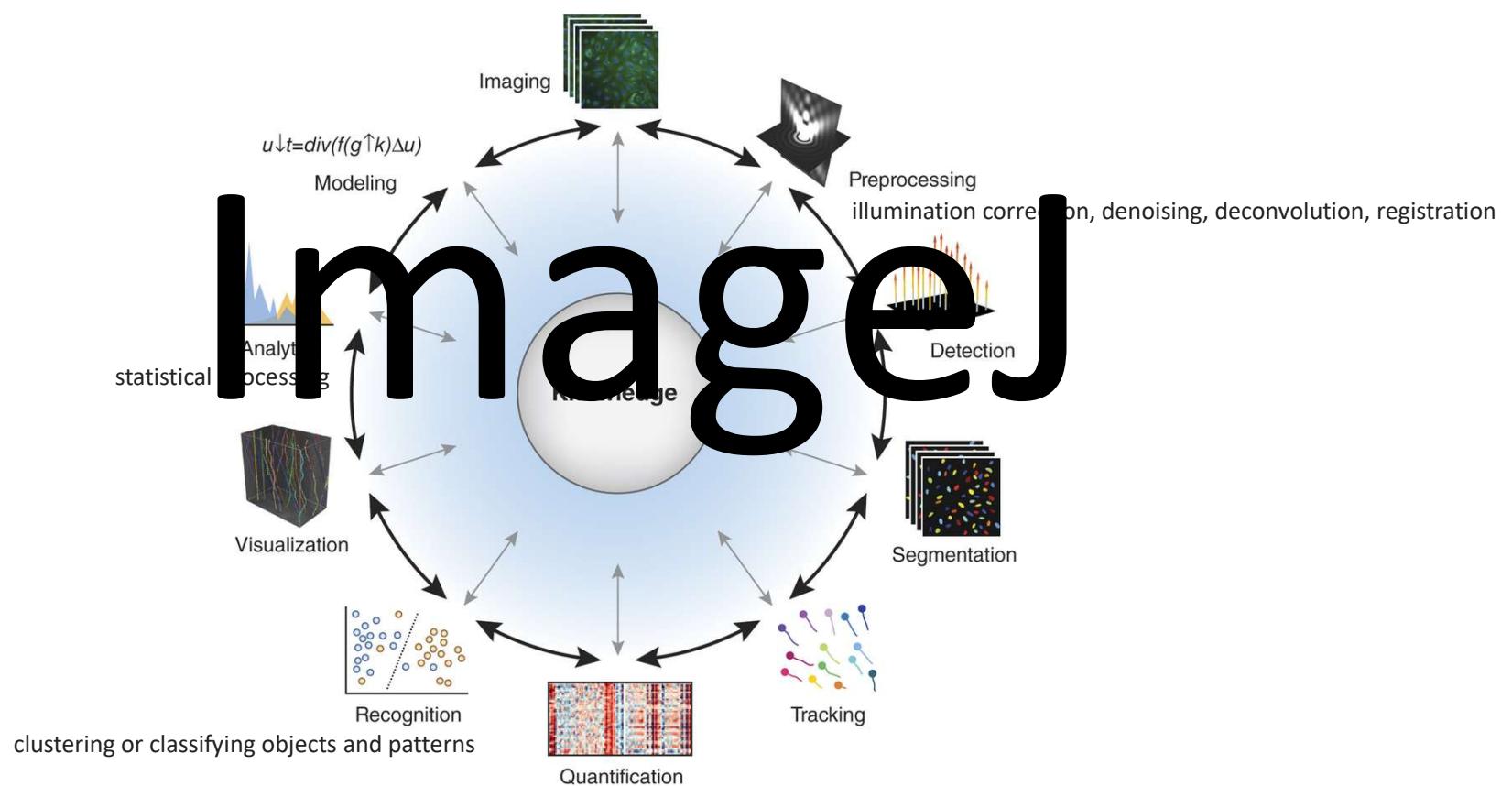
Image analysis

Image analysis is the extraction of meaningful information from digital images by means of digital image processing techniques.

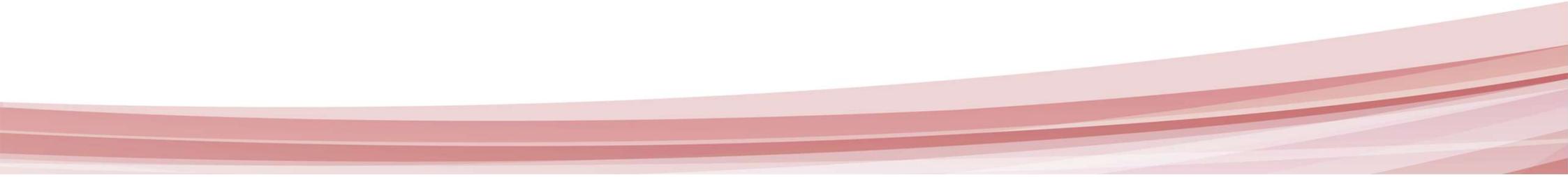


Label	Area	Min	Max	Perim.	Circ.	AR	Round	Solidity	
1	blobs-1.gif	285	255	255	62.184	0.926	1.153	0.867	0.938
2	blobs-1.gif	81	255	255	32.385	0.971	1.204	0.830	0.926
3	blobs-1.gif	278	255	255	63.941	0.854	1.389	0.720	0.919
4	blobs-1.gif	231	255	255	55.698	0.936	1.141	0.877	0.941
5	blobs-1.gif	501	255	255	82.669	0.921	1.075	0.930	0.948
6	blobs-1.gif	660	255	255	98.326	0.686	1.337	0.748	0.952
7	blobs-1.gif	99	255	255	35.799	0.971	1.269	0.788	0.943
8	blobs-1.gif	228	255	255	55.698	0.924	1.142	0.876	0.940
9	blobs-1.gif	448	255	255	78.426	0.915	1.207	0.822	0.947
10	blobs-1.gif	401	255	255	87.497	0.658	2.499	0.400	0.890
11	blobs-1.gif	520	255	255	84.669	0.912	1.182	0.846	0.952
12	blobs-1.gif	425	255	255	77.841	0.881	1.451	0.689	0.953
13	blobs-1.gif	271	255	255	62.184	0.881	1.348	0.742	0.922
14	blobs-1.gif	159	255	255	46.284	0.933	1.225	0.816	0.933
15	blobs-1.gif	412	255	255	75.598	0.906	1.101	0.904	0.945
16	blobs-1.gif	426	255	255	87.497	0.699	1.810	0.553	0.869
17	blobs-1.gif	260	255	255	59.941	0.909	1.153	0.867	0.932
18	blobs-1.gif	289	255	255	62.770	0.922	1.131	0.884	0.935
19	blobs-1.gif	676	255	255	104.326	0.780	1.483	0.675	0.909
20	blobs-1.gif	361	255	255	70.184	0.921	1.222	0.816	0.945
21	blobs-1.gif	545	255	255	86.912	0.907	1.225	0.817	0.948
22	blobs-1.gif	610	255	255	112.569	0.605	2.748	0.364	0.848
23	blobs-1.gif	14	255	255	12.485	1.000	1.020	0.981	0.933
24	blobs-1.gif	641	255	255	106.569	0.709	1.936	0.517	0.881
25	blobs-1.gif	195	255	255	51.113	0.938	1.147	0.872	0.929

Image analysis

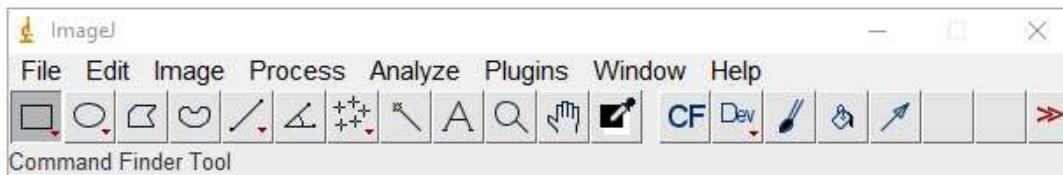


Introduction



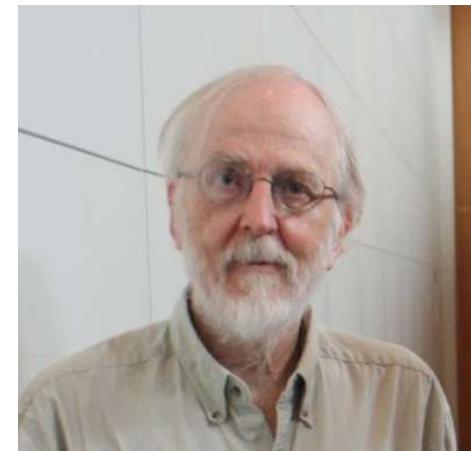
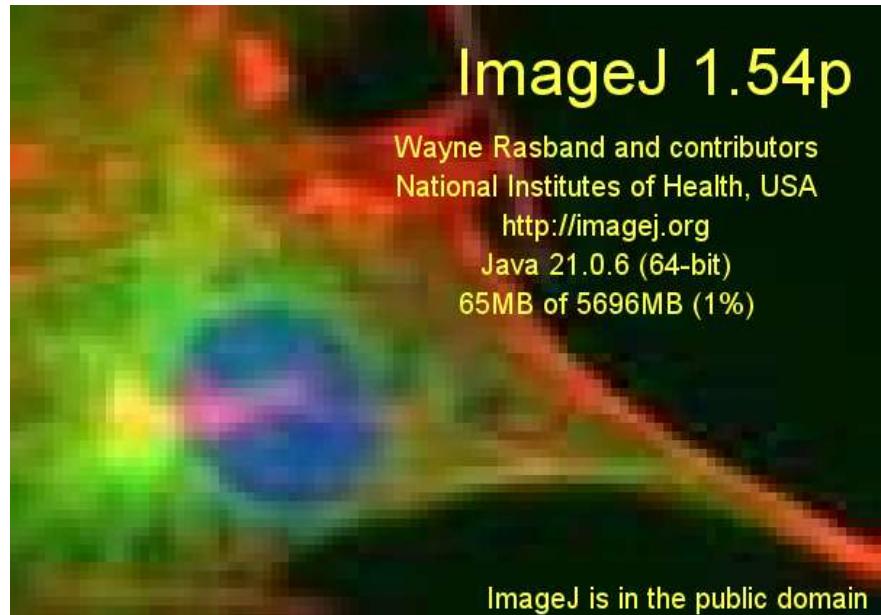
ImageJ

- It provides a Graphical User Interface (GUI) with extensive image analysis commands.
Floating windows.



- Platform independent: Mac OSX/ Linux/Windows: 64 bit version
- Open source (free!!).
- Active community → large number of solutions available or help to find solutions.

ImageJ



Wayne Rasband; Special volunteer
at National Institutes of Health

ImageJ

Download in ZIP format. Unpack in a folder you have access to, like 'My Documents' or a D-drive.

home | docs | download | plugins | resources | list | links

Download

Platform Independent
To install ImageJ on a computer with Java pre-installed, or to upgrade to the latest full distribution (including macros, plugins and LUTs), download the [ZIP archive](#) (6MB) and extract the ImageJ directory. Use the [Help>Update ImageJ](#) command to upgrade to newer versions.

Mac OS X
Download [ImageJ bundled with Java 8](#) (may need to work around Path Randomization). Instructions. With M1 (ARM) Macs, download [ImageJ bundled with Zulu OpenJDK 13.0.6](#).

Linux
Download [ImageJ bundled with Java 8](#). Instructions.

Windows
Download [ImageJ bundled with 64-bit Java 8](#). Instructions.

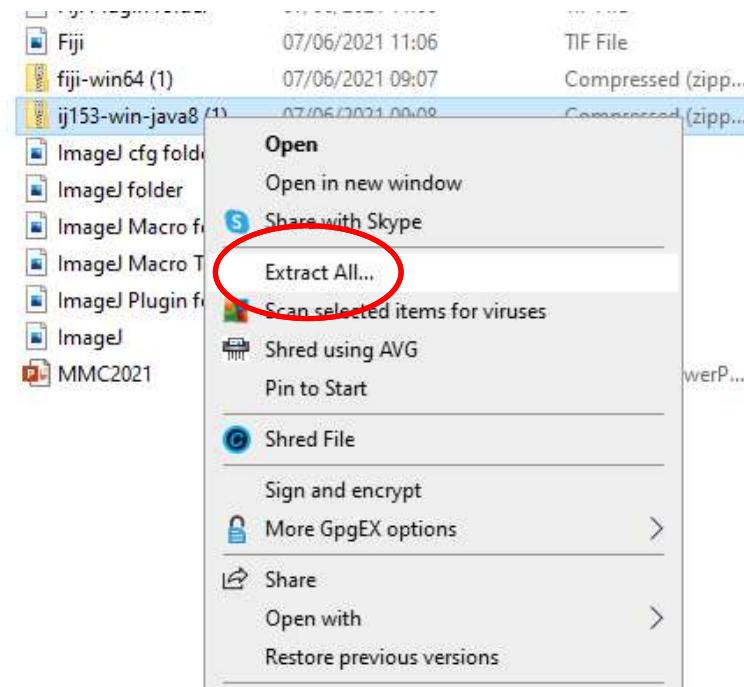
Documentation
Tiago Ferreira's comprehensive [ImageJ User Guide](#) is available as an 8MB PDF document and as a [ZIP archive](#). The online JavaDoc API documentation is also available as a [ZIP archive](#).

Source Code
The ImageJ Java source consists of 160,000 lines of code in 380 files (LineCounter macro). It is available [online](#) and as [zip archives](#).

Example Images
31 downloadable sample images and stacks are available in ImageJ's [File>Open Samples](#) submenu. These images, and more, are also available as a [8.2MB zip archive](#).

You can also browse the ImageJ download directory at imagej.net/ij/download/. Newer ImageJ distributions are available at <http://wsr.imagej.net/distros/>. Refer to the [Release Notes](#) for a list of new features and bug fixes.

top | home | docs | download | plugins | resources | list | links



ImageJ

Download in ZIP format. Unpack in a folder you have access to, like 'My Documents' or a D-drive.

You need access to the ImageJ installation folder:

- To install add-ons
- To update ImageJ
- To change some of the *Options* settings

Windows: if you get the error:

'Unable to update the file "ImageJ.cfg C:\ Program Files\ImageJ\ImageJ.cfg (Access denied)'

ImageJ has been installed in a folder you don't have write permission for.

ImageJ

The functionality of ImageJ can be extended by using:

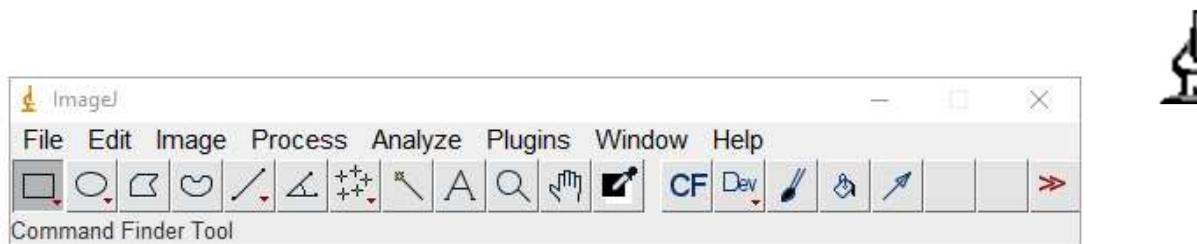
- Plugins: programmes that add functionality to ImageJ;
 - Written in Java.
- Macros: automations, add tools or document your analysis in ImageJ.
 - ImageJ's own scripting language.
- Other scripting languages: JavaScript, BeanShell and Python.

ImageJ

NIH Image (for MAC) from before 1993 developed by Wayne Rasband. Windows version was called Scion Image.

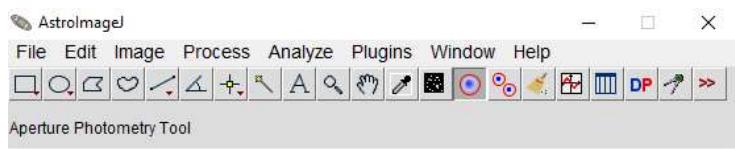
ImageJ since 1997

Schneider, CA, Rasband, WS, Eliceiri, KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9, 671-675.

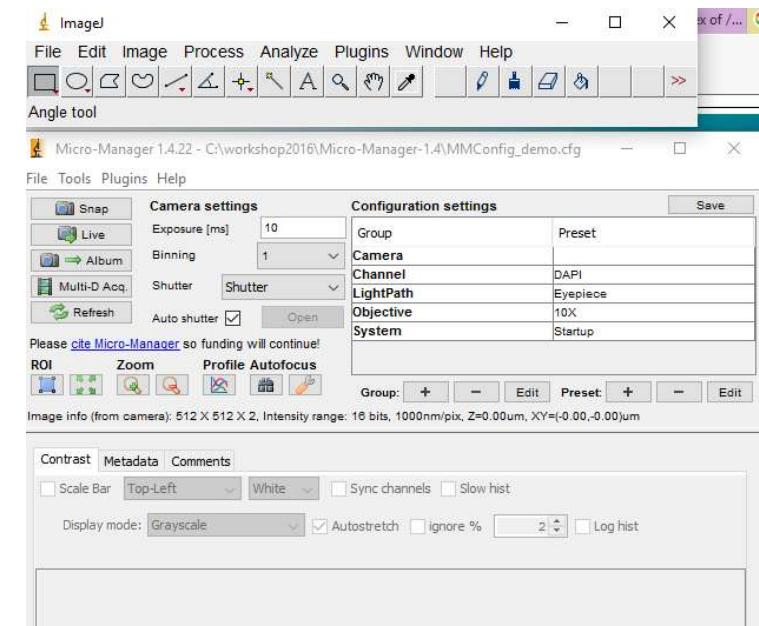


ImageJ

AstrolimageJ : Astronomy



μManager: Image acquisition

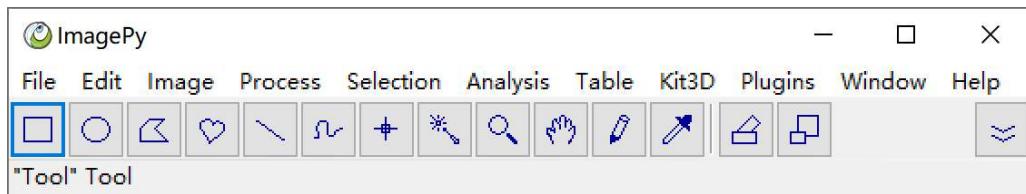


SalsaJ: Astronomy in the class room



ImageJ

ImagePy – 2017: <https://github.com/Image-Py/imagepy>

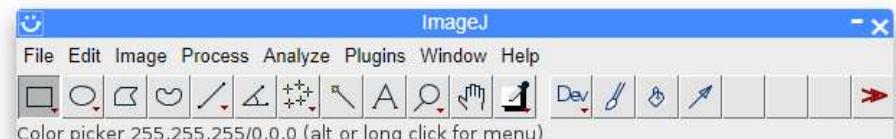


<https://ij.imjoy.io/>



2018

<https://py.imagej.net/en/latest/>



2020: ImageJ running in a web browser

ImageJ

ImageJ is not focussed on a specific field.

No control over plugins

- No quality control
- No control over updates
- Many orphaned plugins

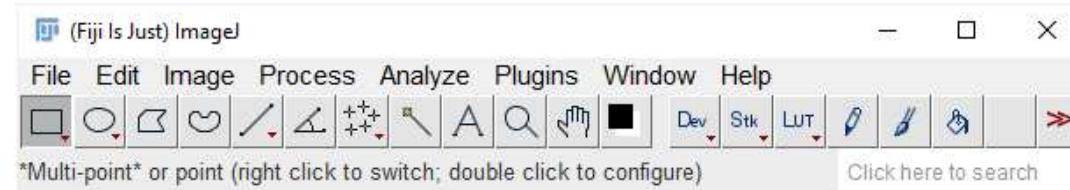
No automatic updates.

It was felt “difficult” for the community to contribute to core code as only Wayne Rasband can implement changes.

Fiji

Fiji since 2007 (Fiji Is Just ImageJ); ImageJ distribution includes > 300+ plugins focussed on biological image analysis.

Schindelin et al. (2012) Fiji: an open-source platform for biological-image analysis. Nature Methods 9, 676–682.



ImageJ2 initiated in 2009

Rueden et al. (2017) ImageJ2: ImageJ for the next generation of scientific image data, BMC Bioinformatics 18:529-555



Fiji

Download (<https://imagej.net/software/fiji/>):

- **Fiji-Latest** is the new version of Fiji that bundles Java 21 and is intended for modern plugin development and usage.
 - JRE = Java Runtime Environment: just to execute Java programs
 - JDK = Java Development Kit: includes the JRE, if you need to compile Java programs
 - Historically, Fiji included JDK
- **Fiji-Stable** is the older version that still uses Java 8, and it includes the Java-8 update site by default.

Fiji

Download include:

ImageJ2 core

ImageJ1 for backward compatibility

Java 21 (JDK or JRE)

- JRE = Java Runtime Environment: just to execute Java programs

- JDK = Java Development Kit: includes the JRE, if you need to compile Java programs

Java3D

Many plugins (+ only few macros and scripts)

Windows: like ImageJ, Fiji is “unpacked” not installed.

Caution: “Program Files” not recommended!



If you are installing ImageJ2 on Windows, we strongly recommend that you store your ImageJ2.app directory somewhere in your user space (e.g., `C:\Users\[your name]\ImageJ2.app`) rather than in `C:\Program Files` or other system-wide directory. If you move ImageJ2.app to such a directory, modern versions of Windows will deny ImageJ2 write permission to its own directory structure, preventing it from being able to update. See also [imagej/imagej#72](#).

Fiji

Contributors:

- Albert Cardona
- Barry DeZonia
- Benjamin Schmid
- Cornelius Sicker
- Curtis Rueden
- Dan White
- Erwin Frise
- Gabriel Landini
- Greg Jefferis
- Ignacio Arganda-Carreras
- Jean-Yves Tinevez
- Johannes Schindelin
- Kota Miura
- Larry Lindsey
- Mark Longair
- Mark Hiner
- Pavel Tomancak
- Rob Bryson-Richardson
- Stephan Preibisch
- Stephan Saalfeld
- Verena Kaynig
- Etc, etc

<https://imagej.net/people/>

Fiji development during Hackathons.

Often in collaboration with third parties like:

- Knime
- Omero
- ImgLib2
- Cellprofiler
- Scifio

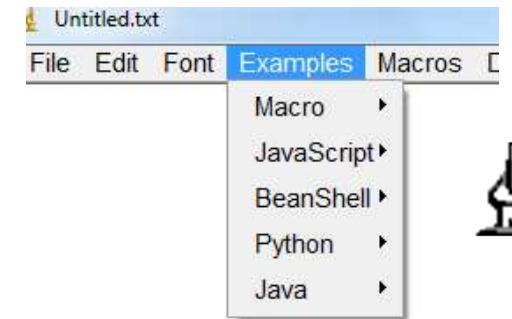
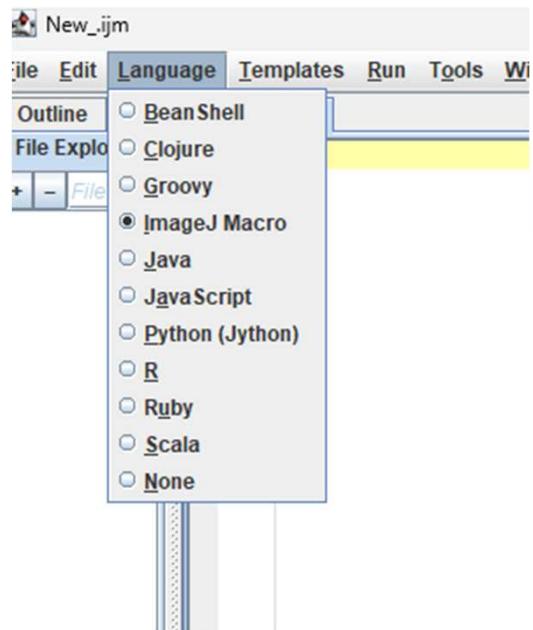
And/or on specific topics like:

- Metadata
- Performance and generality
- Multiview reconstruction

Overview of hackathons

<https://imagej.net/events/hackathons>

Fiji



ImageJ has plugins:
Ruby
Clojure (FunImageJ)
Groovy
Scala

References

[home](#) | [news](#) | [docs](#) | [download](#) | [plugins](#) | [resources](#) | [list](#) | [links](#)

Documentation

- Introduction
- Basic Concepts
- Installation
- *ImageJ User Guide* (download PDF)
 - User Interface and Tools
 - Menu Commands
 - Extending ImageJ
 - Keyboard Shortcuts
- Tutorials and Examples
- ImageJ Forum
- ImageJ Documentation Wiki
- Image Processing with ImageJ
- ImageJ on Wikipedia
- Frequently Asked Questions
- Macro Language (download PDF)
- Complete Release Notes (744 pages)

<https://imagej.net/ij/docs/index.html>



Analyzing fluorescence microscopy images with ImageJ

Peter Bankhead
Queen's University Belfast

May 2014

This work is made available in the hope it will help a few more people develop an interest in image analysis. If you have any comments, corrections or suggestions, please contact me at p.bankhead@qub.ac.uk, so that it might one day get better.



Only online version from 2016 at
<https://petebankhead.gitbooks.io/imagej-intro/content/>

References

ImageJ Documentation Wiki

Log In

Search Recent Changes Media Manager Sitemap

Welcome to the ImageJ Information and Documentation Portal

You are also invited to contribute content and share your knowledge with our community. Feel free to register and contribute additional content to the portal. The system should be easy to use; please read the "Create new content" document first. This will explain in more detail which mechanisms are in place to control the content of the portal.

You only need to register an account if you are planning to publish documentation on this site. To browse the information contained here, no registration is necessary.

ImageJ related events

Quick Links

- Recent changes
- Do you need additional feature or you discovered a bug? Please add it to the suggestion page.
- Want to contribute? Read the "Create new content" document
- Do you have a question about ImageJ? Please join the ImageJ mailing list.

Categories

 Frequently Asked Questions ...about ImageJ	 HOWTOs documents describing common scenarios
 Graphical User Interface ...commands. A reference to the menu commands	 Tutorials ...on various aspects of imaging
 Keyboard shortcuts ...a list of all the built-in shortcuts	 Known problems ...you may encounter and their possible solutions
 Plugins ...and extensions to ImageJ functionality	 Links ...to external image processing resources
 Macros ...to automate ImageJ functionality	 Diverse ...a list of features that do not fit anywhere else
 Wishlist ...functions that are still needed	 Video Tutorials ...to learn ImageJ functionality

Table of Contents

- Welcome to the ImageJ Information and Documentation Portal
 - ImageJ related events
 - Categories

YouTube 08

start

Search

All Related Recently uploaded

Intro to ImageJ/Fiji Harvard Center for Biological 33K views • 1 year ago

Mix - The Jackson Lab YouTube

30 min Introduction to Fiji/ImageJ for... Dominic Waithe 19K views • 1 year ago

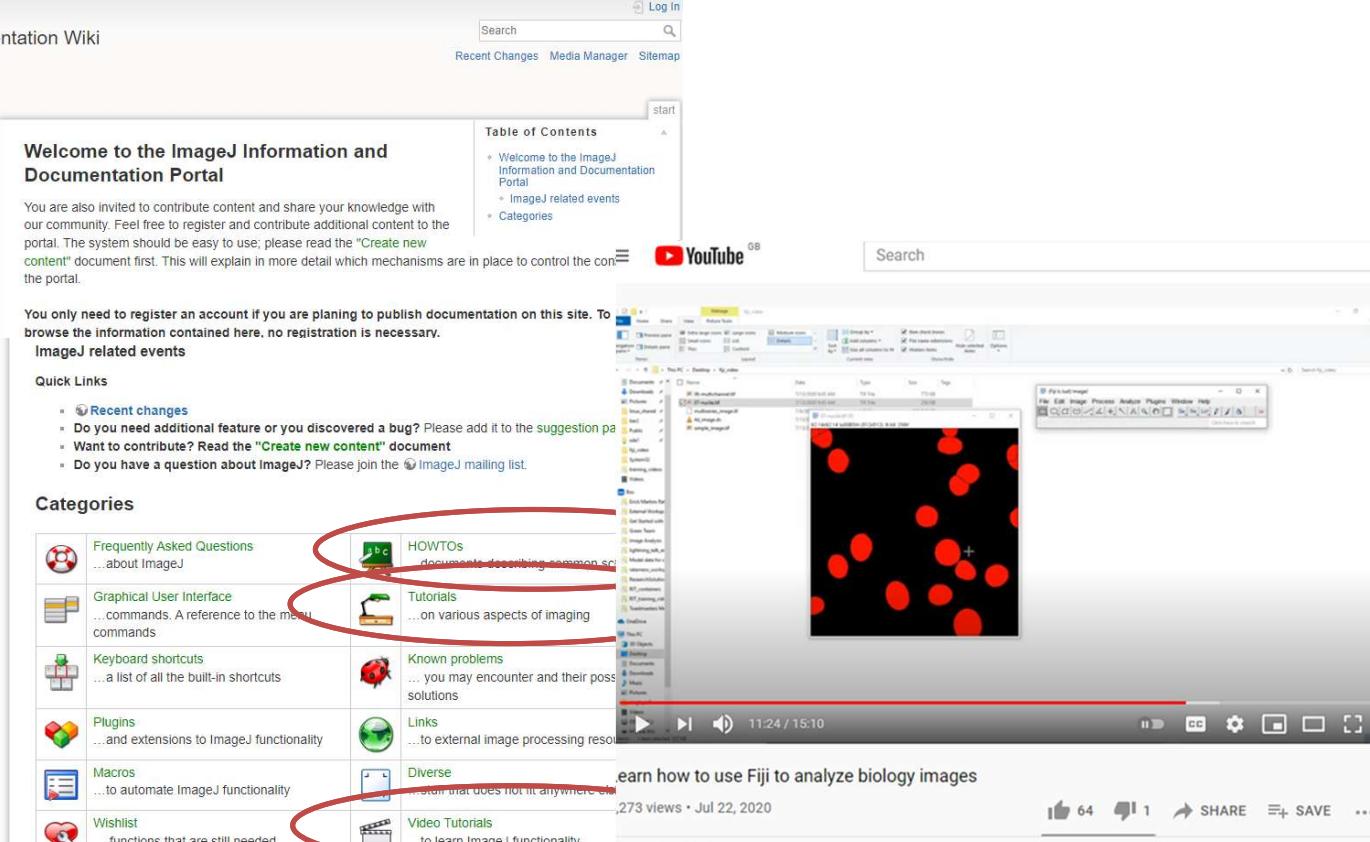
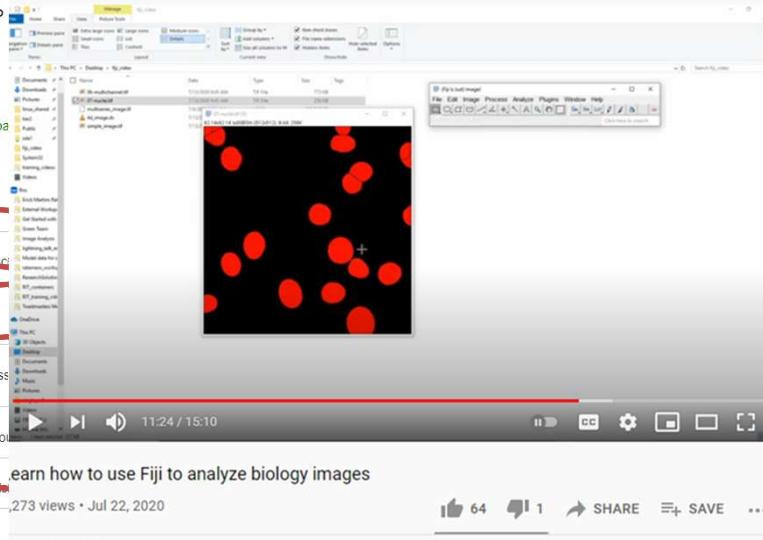
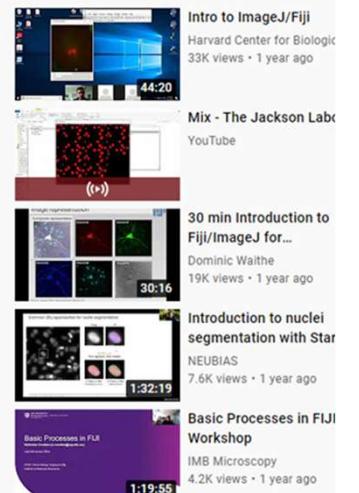
Introduction to nuclei segmentation with Star NEUBIAS 7.6K views • 1 year ago

Basic Processes in FIJI Workshop IMB Microscopy 4.2K views • 1 year ago

earns how to use Fiji to analyze biology images 2,733 views • Jul 22, 2020

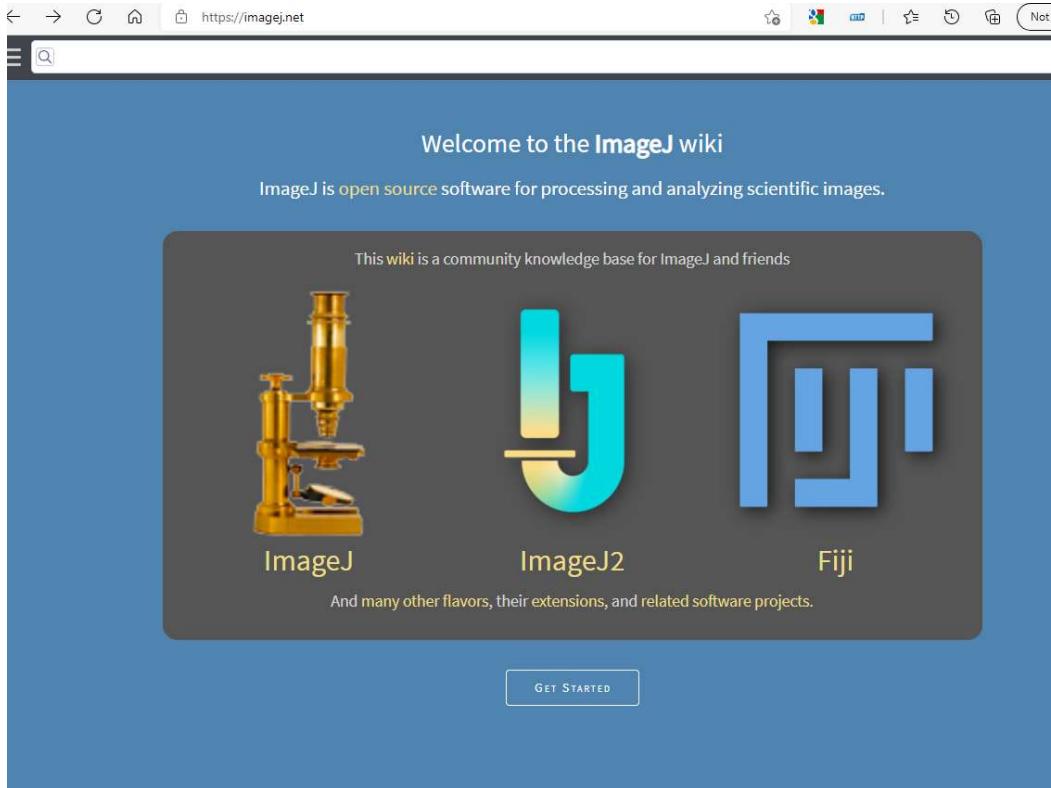
11:24 / 15:10

64 1 SHARE SAVE

<https://imagejdocu.list.lu/start>

References



The screenshot shows the homepage of the ImageJ wiki at <https://imagej.net>. The page has a blue header with the university logo and the title "UNIVERSITY OF LEICESTER". Below the header, there's a navigation bar with links like "Home", "About", "Tutorials", "Documentation", "Source", "Issues", "Contributors", and "Log in". The main content area features a dark banner with the text "Welcome to the **ImageJ** wiki" and "ImageJ is open source software for processing and analyzing scientific images.". It also includes icons for a microscope, the ImageJ logo, and the Fiji logo, along with the text "This wiki is a community knowledge base for ImageJ and friends". A "GET STARTED" button is visible at the bottom of the banner.

Start with the [ImageJ Tutorial Notebooks!](#)

And be sure to read over the [Development](#) pages.

Tutorials on this site

- [Analyze FRAP movies with a Jython script](#)
- [How to apply a common operation to a complete directory](#)
- [How to call a plugin with a range of parameters](#)
- [Correcting drift in FRAP experiments](#)
- [Downsample](#)
- [Edit LUT As Text](#)
- [Gabor Filter script](#)
- [Generate and exploit Kymographs](#)
- [The Hue Game](#)
- [ImageJ2 Python Scripts](#)
- [Linux command line tutorial](#)
- [Mamed](#)
- [Multithreaded Image Processing in Clojure](#)
- [Multithreaded Image Processing in JavaScript](#)
- [Open an image](#)
- [A Tutorial for using OpenCL in ImageJ](#)
- [Plasma Cloud](#)
- [RGB to CMYK](#)
- [Segmentation evaluation after border thinning - Script](#)
- [Segmentation evaluation metrics - Script](#)
- [Image Processing School Pilsen 2009 - Segmentation](#)
- [Stitch and Align a sequence of grid images Tutorial](#)
- [Super Sloppy Surface Reconstruction](#)

To add your tutorial to this list, place the page in the `/tutorials` folder.

See also

<https://imagej.net/tutorials/index>

- [Presentations about ImageJ](#)
- [Fiji YouTube channel](#)
- [ImageJ videos and Fiji videos on YouTube](#)
- [Lecture BioImage Analysis 2020 videos on YouTube by Robert Haase](#)

References



IMAGEJ Home Page

IMAGEJ@LIST.NIH.GOV

Latest Messages

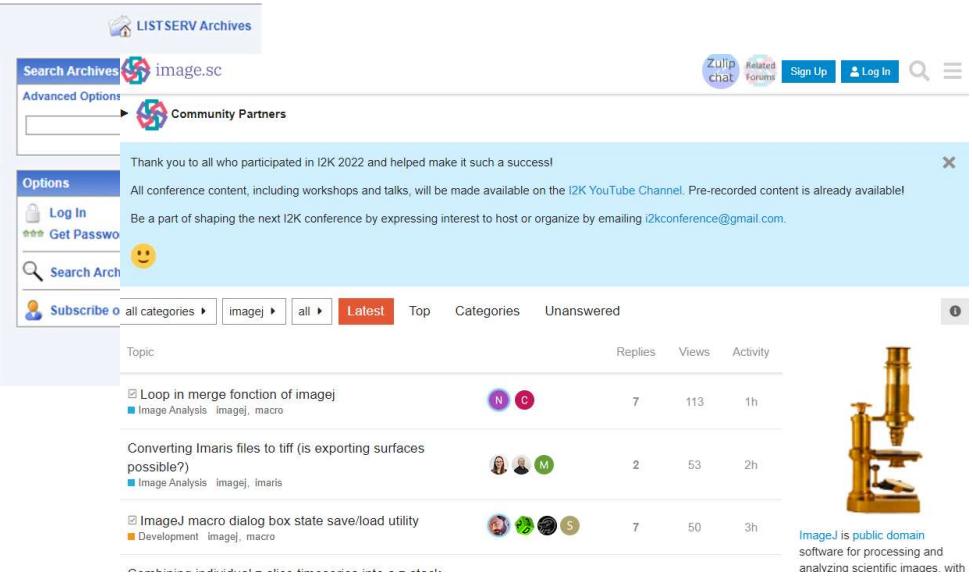
Re: Mystery failure of NonBlockingGenericDialog	Fred Damen < [log in to unmask] >	Tue, 19 Nov 2024 14:21:21 -0600
Re: FolderOpener	Hidenao Iwai < [log in to unmask] >	Tue, 19 Nov 2024 08:24:00 -0500
Re: FolderOpener	Curtis Rueden < [log in to unmask] >	Mon, 18 Nov 2024 11:54:14 -0600

IMAGEJ

Archive-Months: none This is a mailing list for users and developers of ImageJ, a public domain, Java-based and extendable image processing program for Linux, Macintosh and Windows. The ImageJ home page is located at <http://imagej.nih.gov/ij/>.

- November 2024
- October 2024
- September 2024
- August 2024
- July 2024
- June 2024
- May 2024

<https://list.nih.gov/cgi-bin/wa.exe?A0=IMAGEJ>



LISTSERV Archives

Search Archives image.sc

Community Partners

Thank you to all who participated in I2K 2022 and helped make it such a success!

All conference content, including workshops and talks, will be made available on the I2K YouTube Channel. Pre-recorded content is already available!

Be a part of shaping the next I2K conference by expressing interest to host or organize by emailing i2kconference@gmail.com.

Options

Subscribe to all categories ▾ imagej ▾ all ▾ **Latest** Top Categories Unanswered

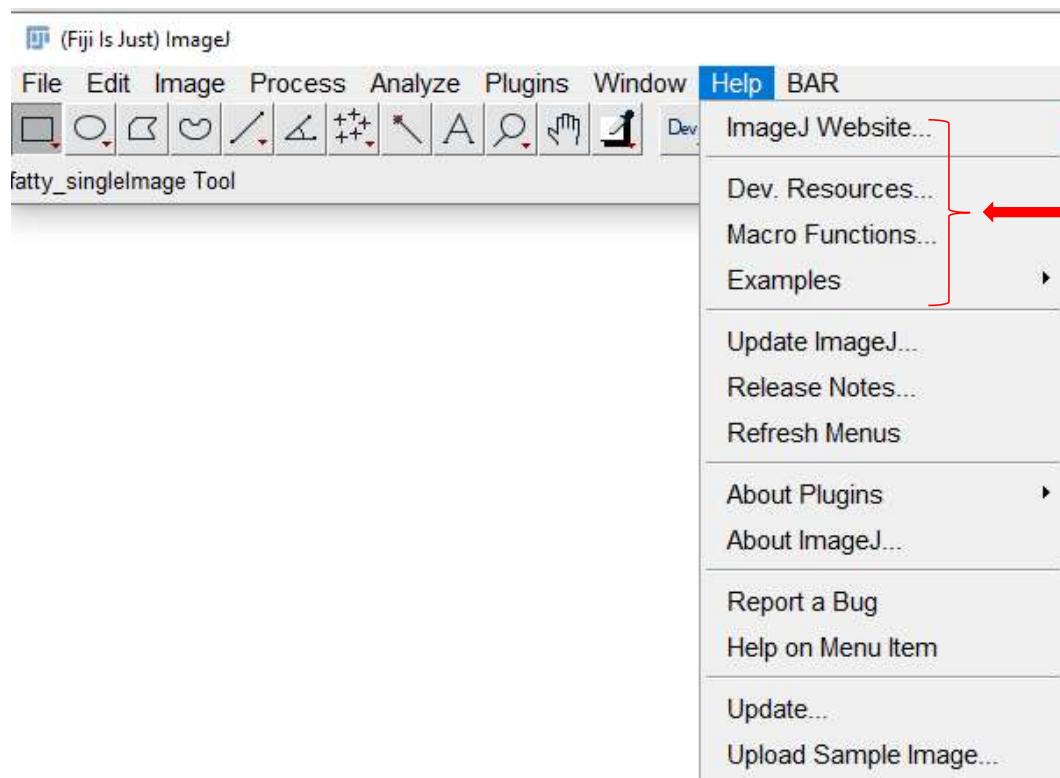
Topic

Loop in merge fonction of imagej	N C	7	113	1h
Converting Imaris files to tiff (is exporting surfaces possible?)	M	2	53	2h
ImageJ macro dialog box state save/load utility	S	7	50	3h

ImageJ is public domain
software for processing and analyzing scientific images. with

Fiji mailing list has merged with other software forums into the Scientific Community Image Forum: <https://forum.image.sc/>.

Documentation



ImageJ website

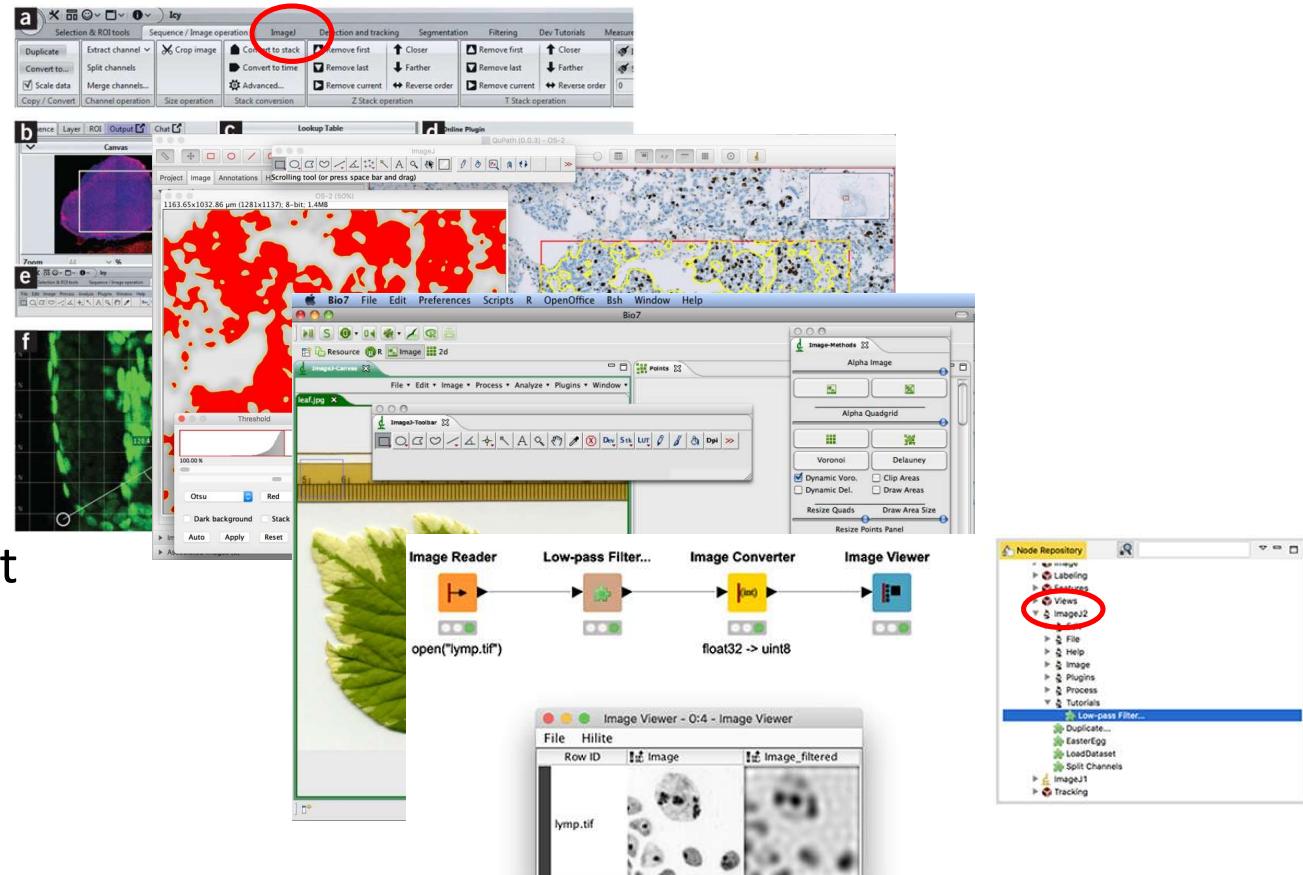
Other projects

Icy; Image analysis software to visualize, annotate and quantify bioimaging data

QuPath; ImageJ1 integrated in digital pathology

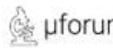
Bio7: ImageJ integrated in an ecological modelling environment including Statistical analysis (R).

Knime: Data analysis pipelines



Other projects

<https://forum.image.sc>

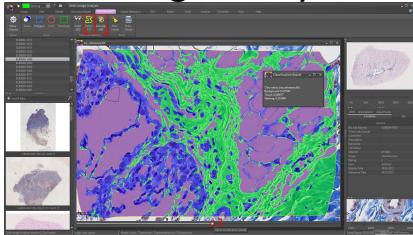
All Topics	AGAVE	AICSImageIO	Aydin	BAND	BIAFLOWS
BiaPy	BiofilmQ	Bio-Formats	BiolmagelO	BoneJ	BrainGlobe
CAREamics	Cell-ACDC	Cellpose	CellProfiler	CLIJ	CytoMAP
Cytomine	DAIM	DeepLabCut	Fiji	FLIMLib	GerBl
GloBIAS	Icy	IDR	ilastik	ImageC	ImageJ
ImageJ2	ImgLib2	ImJoy	iRODS	JIPipe	Mars
MCMICRO	MIA	MIB	μManager	μSAM	Microscopy Nodes
MoBIE	ModularImageAnalysis	MorphoGraphX	MorphoNet	napari	NFDI4BIOIMAGE
OME	OMERO	OpenSPIM	Orbit	Piximi	PolusAI
PYME	Python-Microscope	QUAREP-LiMi	QuPath	SBEMimage	scenery
SCIFIO	scikit-image	sciview	SmartMicroscopy	SpotMAX	StarDist
#teamTomo	TissUUmaps	vedo	webKnossos	ZeroCostDL4Mic	... Your Icon Here
Zzz	Dormant Partners				
RELATED COMMUNITIES  μforum  TK KNIME SPONSORED BY 					



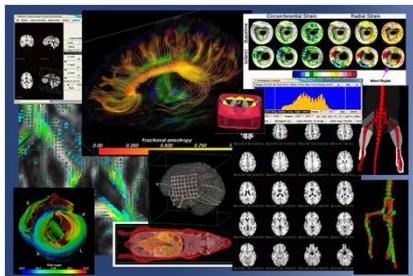
Microscopy Image
Browser (MIP)



Orbit Image Analysis

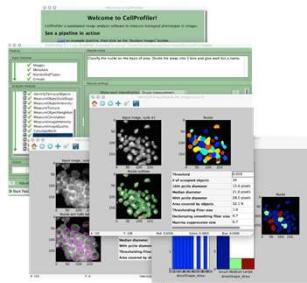


BioImaging Suite

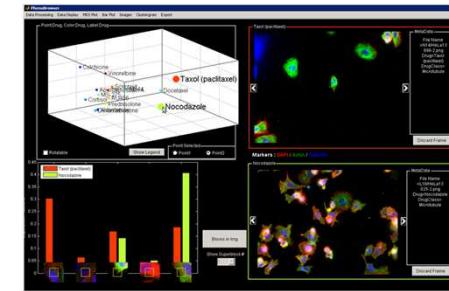


Other projects

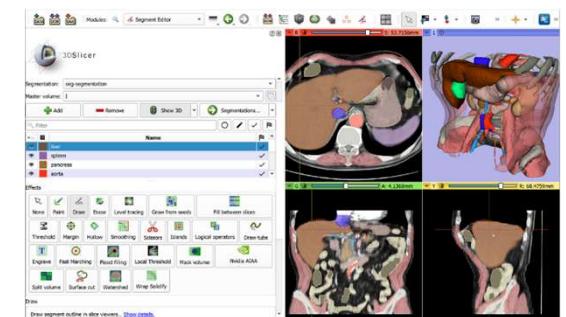
CellProfiler



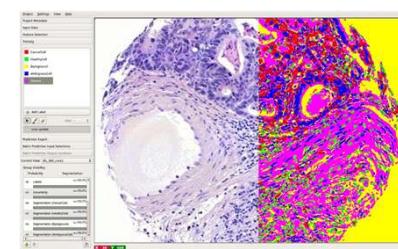
PhenoRipper



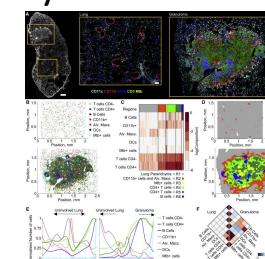
3D Slicer



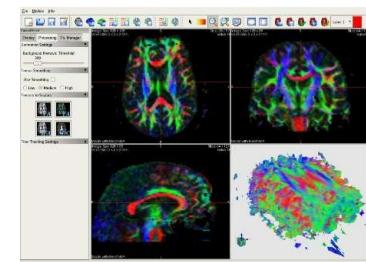
Ilastik



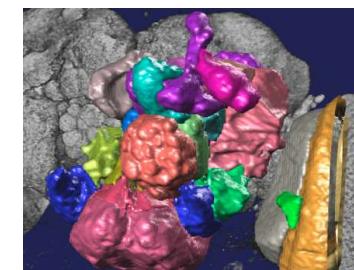
CytoMAP



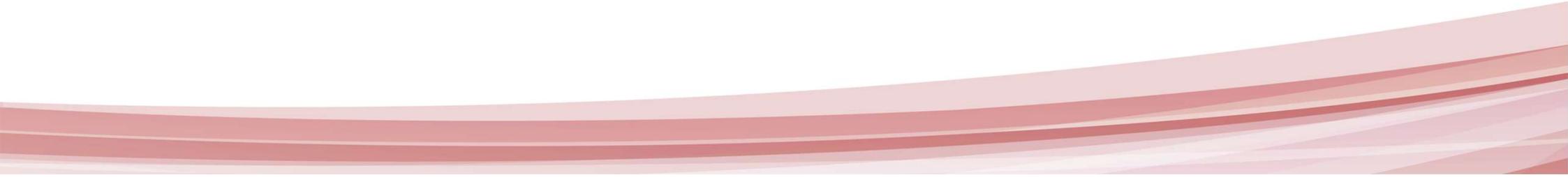
MedINRIA



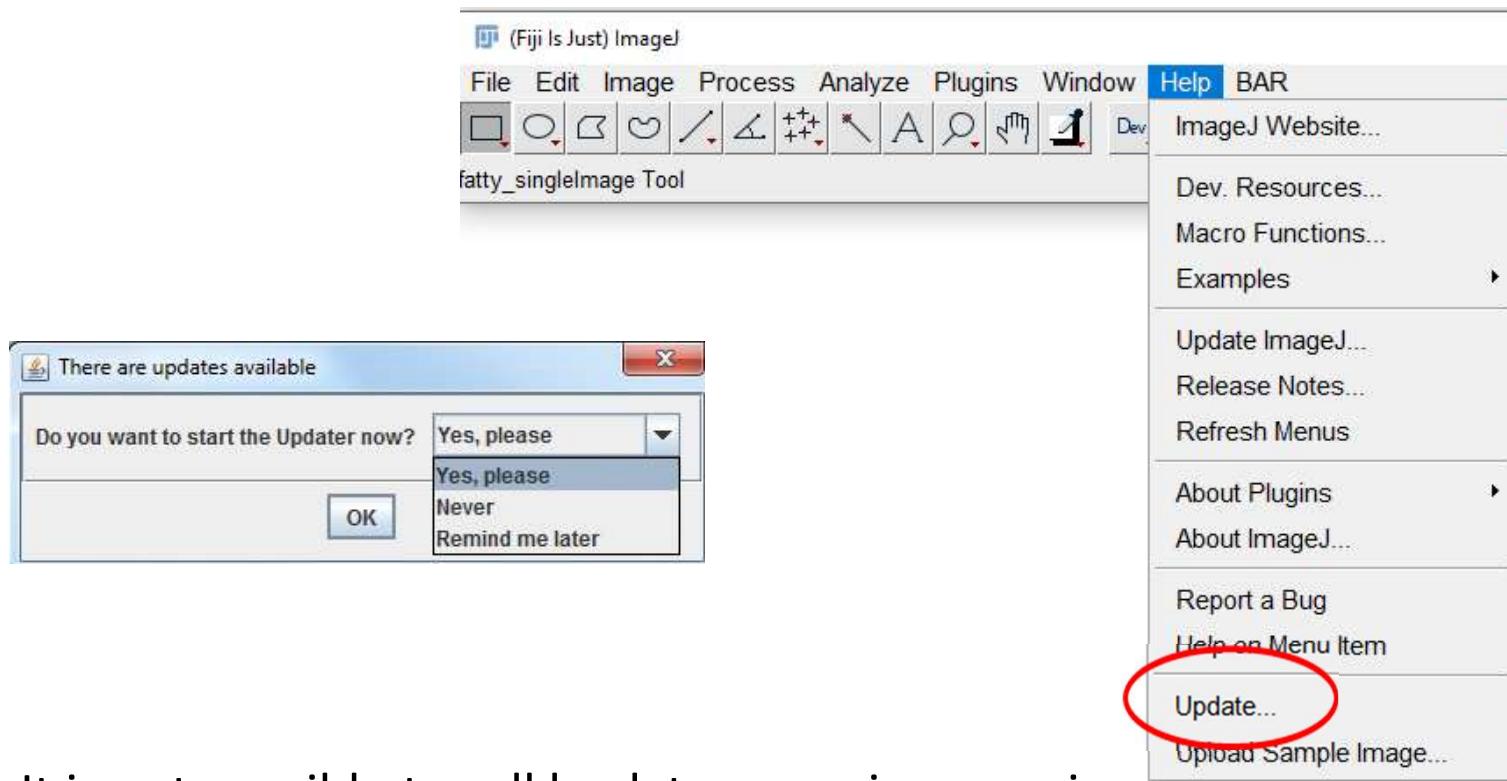
Vaa3D



Some basics

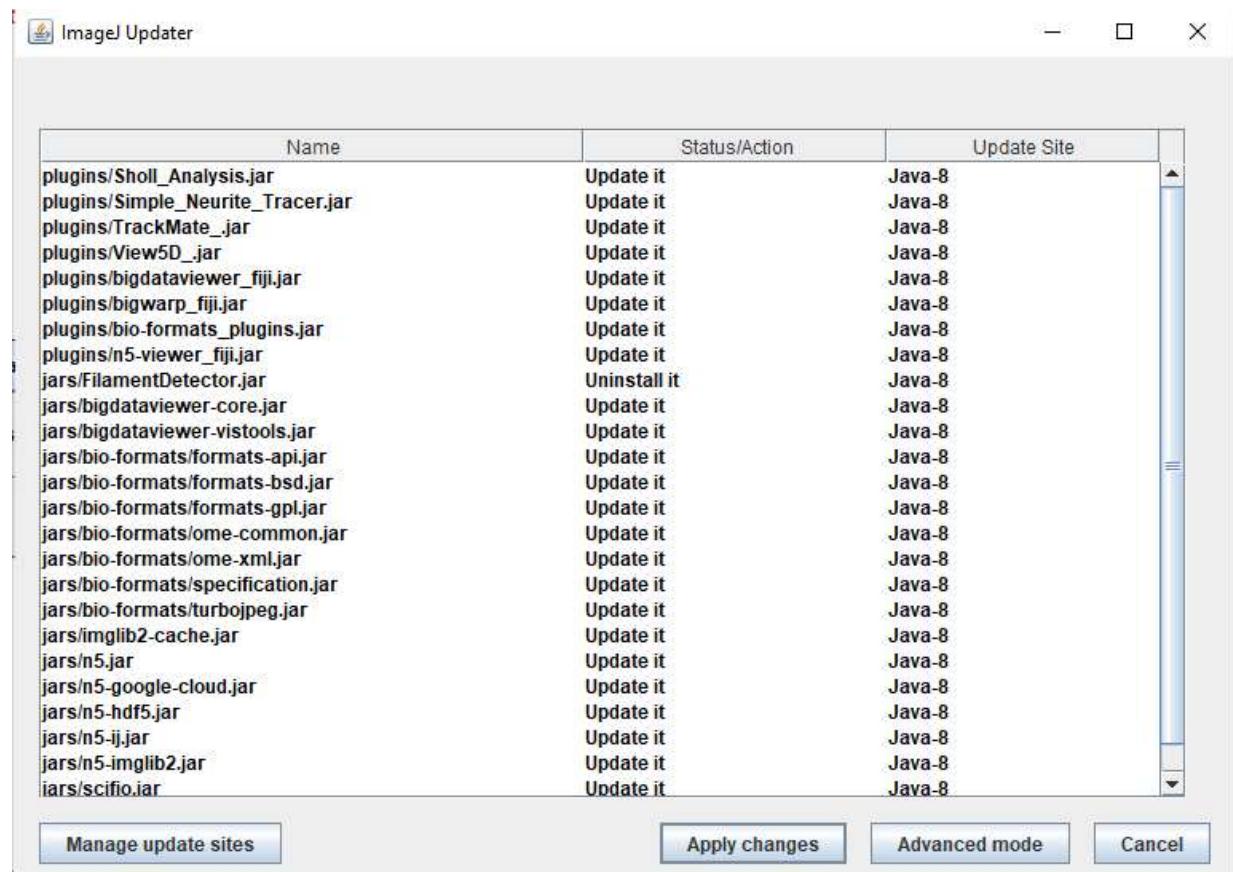


Update Fiji



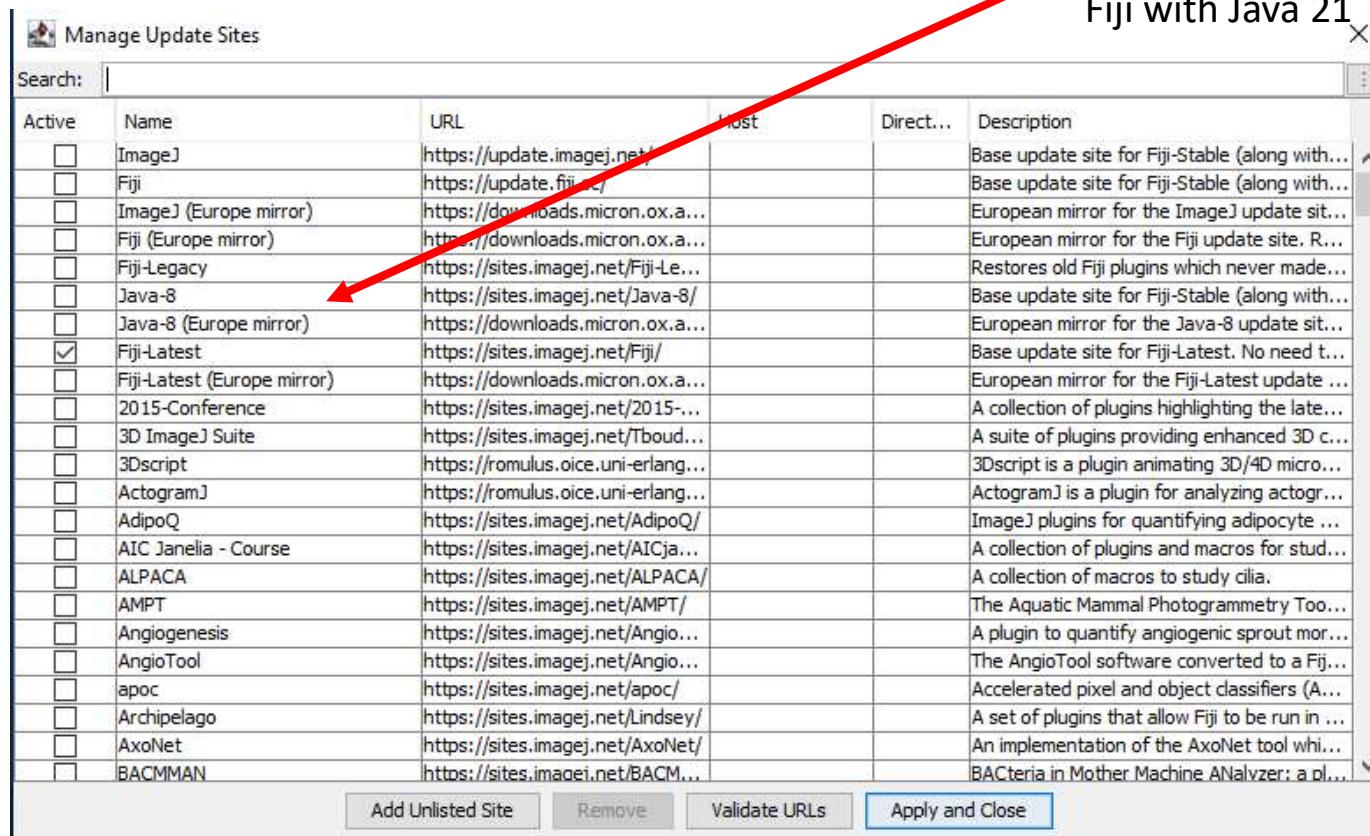
It is not possible to roll back to a previous version.

Update Fiji



Update Fiji

Includes the latest Java-8 versions of all core Fiji and ImageJ components of Fiji-Stable. Not a Java-8 update and not for Fiji with Java 21

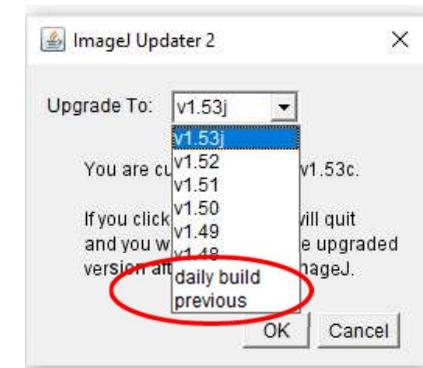
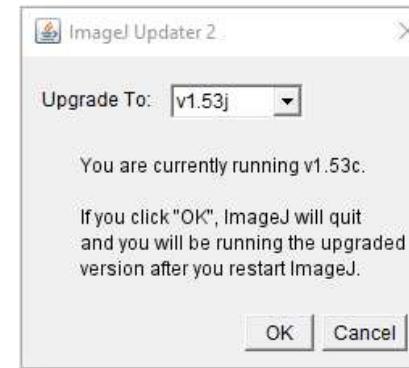
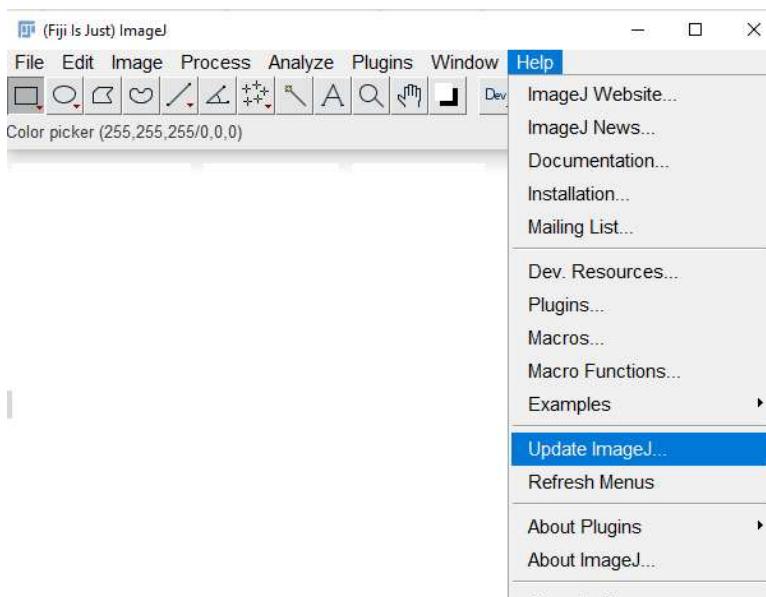
 Manage Update Sites

Active	Name	URL	Host	Direct...	Description
<input type="checkbox"/>	ImageJ	https://update.imagej.net/			Base update site for Fiji-Stable (along with...
<input type="checkbox"/>	Fiji	https://update.fiji/			Base update site for Fiji-Stable (along with...
<input type="checkbox"/>	ImageJ (Europe mirror)	https://downloads.micron.ox.a...			European mirror for the ImageJ update sit...
<input type="checkbox"/>	Fiji (Europe mirror)	https://downloads.micron.ox.a...			European mirror for the Fiji update site, R...
<input type="checkbox"/>	Fiji-Legacy	https://sites.imagej.net/Fiji-Le...			Restores old Fiji plugins which never made...
<input type="checkbox"/>	Java-8	https://sites.imagej.net/Java-8/			Base update site for Fiji-Stable (along with...
<input type="checkbox"/>	Java-8 (Europe mirror)	https://downloads.micron.ox.a...			European mirror for the Java-8 update sit...
<input checked="" type="checkbox"/>	Fiji-Latest	https://sites.imagej.net/Fiji/			Base update site for Fiji-Latest. No need t...
<input type="checkbox"/>	Fiji-Latest (Europe mirror)	https://downloads.micron.ox.a...			European mirror for the Fiji-Latest update ...
<input type="checkbox"/>	2015-Conference	https://sites.imagej.net/2015-...			A collection of plugins highlighting the late...
<input type="checkbox"/>	3D ImageJ Suite	https://sites.imagej.net/Tboud...			A suite of plugins providing enhanced 3D c...
<input type="checkbox"/>	3Dscript	https://romulus.oice.uni-erlang...			3Dscript is a plugin animating 3D/4D micro...
<input type="checkbox"/>	ActogramJ	https://romulus.oice.uni-erlang...			ActogramJ is a plugin for analyzing actogr...
<input type="checkbox"/>	AdipoQ	https://sites.imagej.net/AdipoQ/			ImageJ plugins for quantifying adipocyte ...
<input type="checkbox"/>	AIC Janelia - Course	https://sites.imagej.net/AICja...			A collection of plugins and macros for stud...
<input type="checkbox"/>	ALPACA	https://sites.imagej.net/ALPACA/			A collection of macros to study cilia.
<input type="checkbox"/>	AMPT	https://sites.imagej.net/AMPT/			The Aquatic Mammal Photogrammetry Too...
<input type="checkbox"/>	Angiogenesis	https://sites.imagej.net/Angio...			A plugin to quantify angiogenic sprout mor...
<input type="checkbox"/>	AngioTool	https://sites.imagej.net/Angio...			The AngioTool software converted to a Fiji...
<input type="checkbox"/>	apoc	https://sites.imagej.net/apoc/			Accelerated pixel and object classifiers (A...
<input type="checkbox"/>	Archipelago	https://sites.imagej.net/Lindsey/			A set of plugins that allow Fiji to be run in ...
<input type="checkbox"/>	AxoNet	https://sites.imagej.net/AxoNet/			An implementation of the AxoNet tool whi...
<input type="checkbox"/>	BACMMAN	https://sites.imagej.net/BACM...			BACteria in Mother Machine ANalyzer: a pl...

Add Unlisted Site [Remove](#) [Validate URLs](#) [Apply and Close](#)

Update ImageJ

ImageJ is continuously updated. This is done by Wayne Rasband.
If you update Fiji, only major releases of ImageJ are included.



This allows you to roll ImageJ back to a previous version.

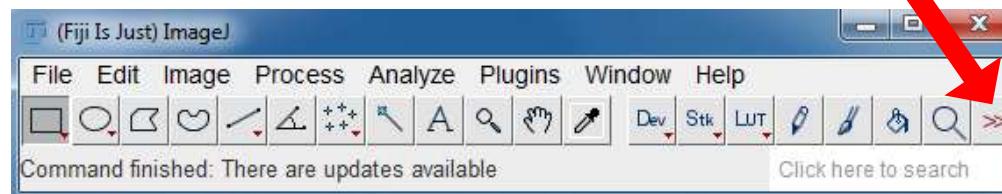
Be aware: ImageJ closes after update!

User interface

Menu Bar →

Tool Bar →

Status Bar →



Menu items are written in Java. Use of menu items will be written in *Italic* in this workshop.

Tools: mostly macros that are added to the ImageJ toolbar.

Tools are saved in subfolder\macros\tools.

Toolsets: a toolset can contain up to 12 macro tools, along with any number of ordinary macros.

Toolsets are saved in subfolder\macros\toolsets.

Toolsets

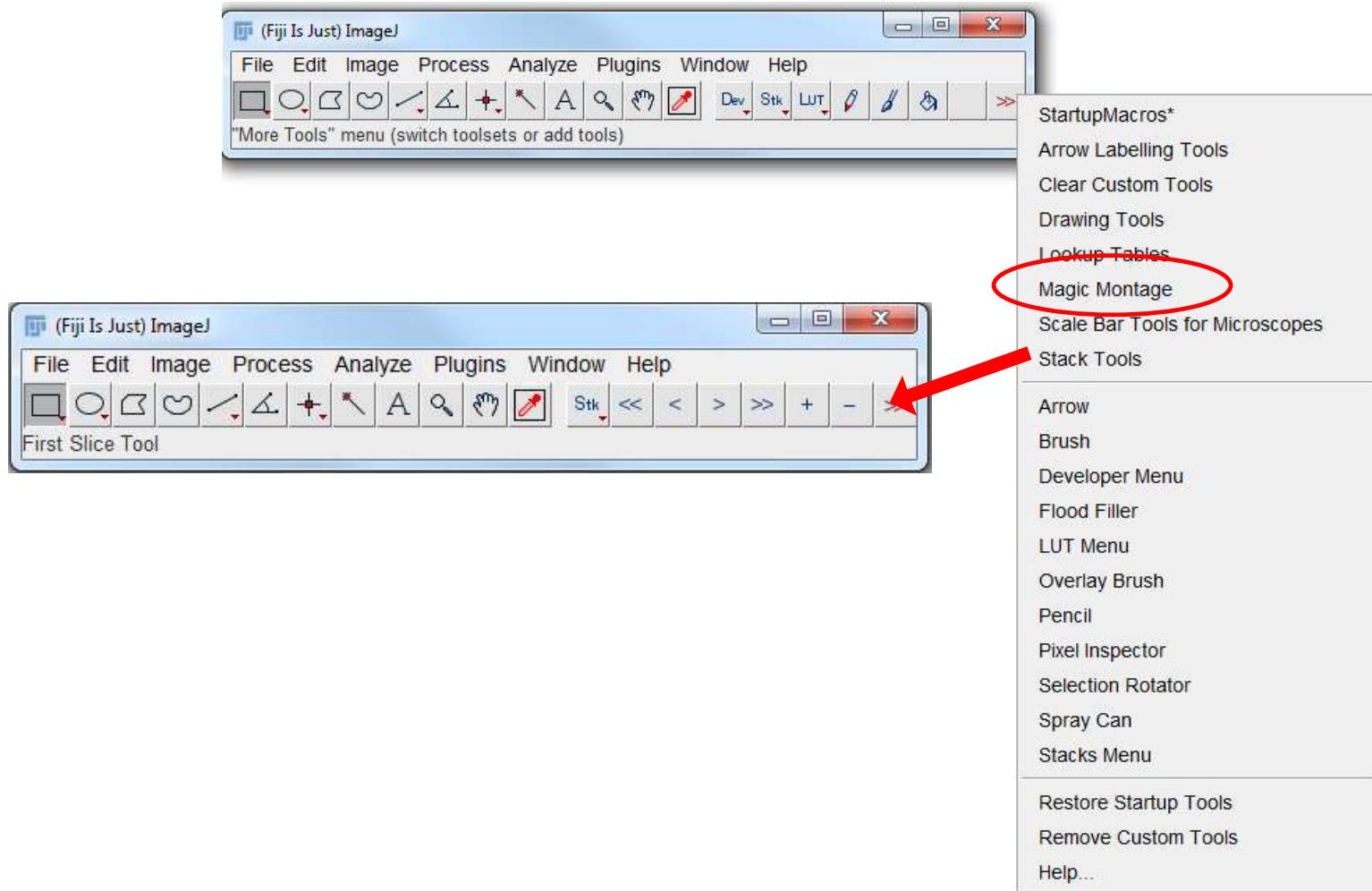
home | news | docs | download | plugins | resources | list | links

Developer Resources

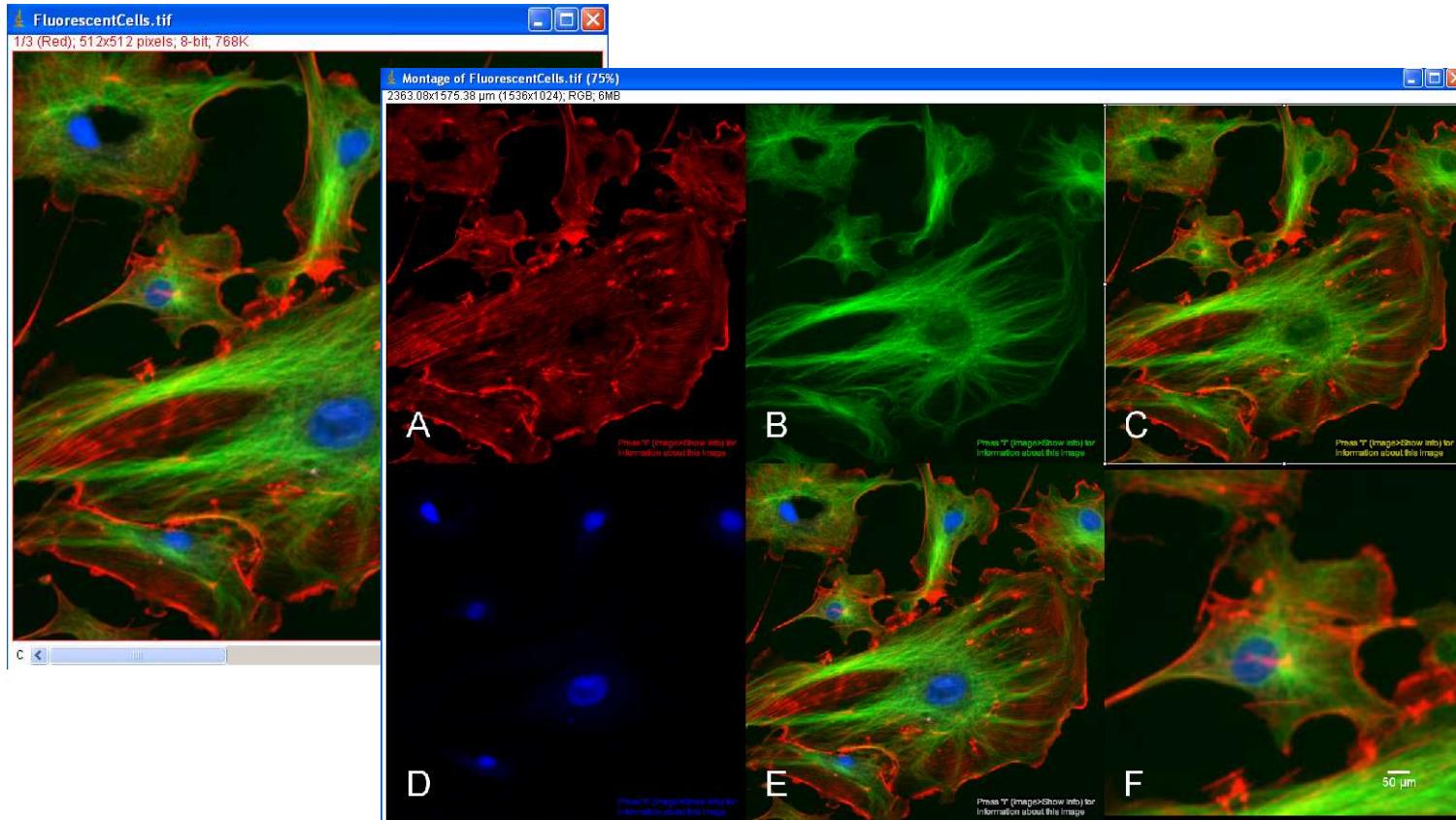
- Macro Language (download PDF)
-
- Built-in Macro Functions
- Introduction to Macro Programming
- Macro Programming in ImageJ (Kota Miura) New
- Macros toolset
- Macros Directory
- Examples
- Macro Tools
- Toolsets
- Scripting
 - JavaScript Examples
 - BeanShell (examples) New
 - Python (examples) New

Name	Last modified
Angiogenesis Analyze..>	2013-12-03 13:35
BioVoxel Toolbox.txt	2013-04-28 21:35
Built-in Tools.txt	2012-07-14 14:44
Developer Tools.txt	2010-07-29 14:18
Dot Blot Analyzer.txt	2013-12-03 23:02
Drawing Tools.txt	2012-07-14 14:43
Dynamic_Stats_and_Hi..>	2010-07-29 14:18
Example Icons.txt	2013-12-03 12:16
Image, Stack and Tim..>	2013-12-03 23:06
Intersects_Method.txt	2014-07-15 13:01
LSM Transmission Deb..>	2010-07-29 14:18
Lookup Tables.txt	2013-12-03 13:45
Luts Macros and Tool..>	2013-12-15 18:22
MBF Tools.txt	2010-07-29 14:18
MagicMontage.pdf	2018-11-29 15:41
Magic Montage.txt	2018-11-29 15:41
Magic Montage2.txt	2010-07-29 14:18
Multi-Channels Image..>	2018-10-16 21:06
Original Drawing Too..>	2012-07-13 22:16
Plugins.txt	2008-10-21 12:25
Protein Array Analyz..>	2018-10-16 21:06
ROI Manager Tools.txt	2013-12-15 18:48
Rename and Save ROI ..>	2010-07-29 14:18

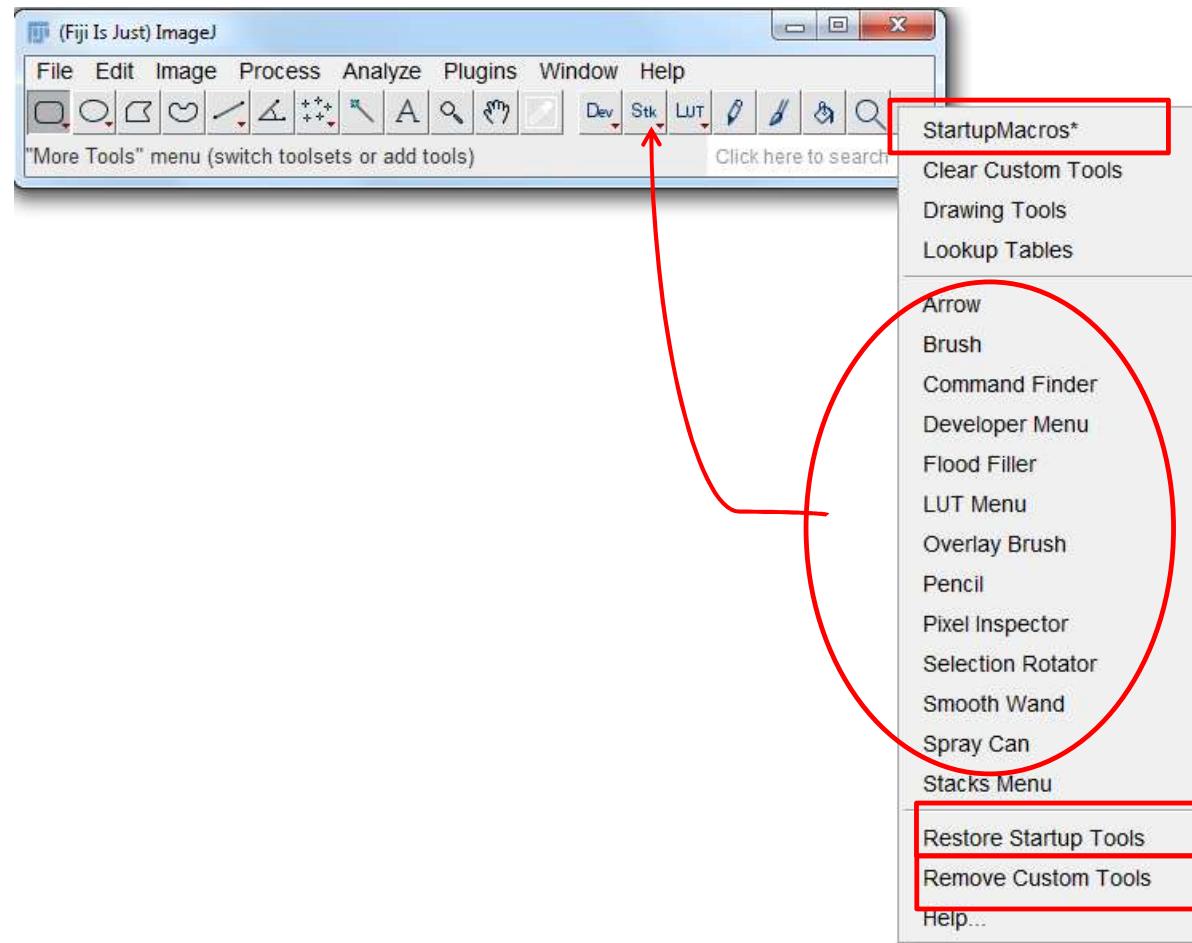
Toolsets



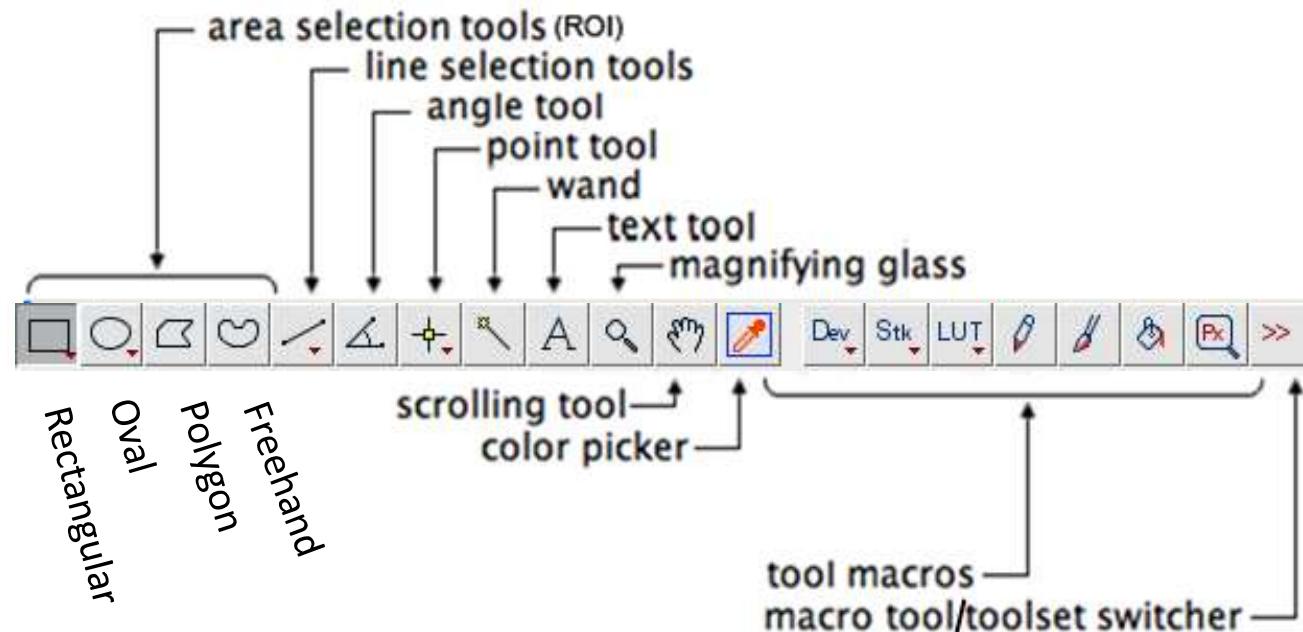
Toolsets



Toolsets



Toolbar

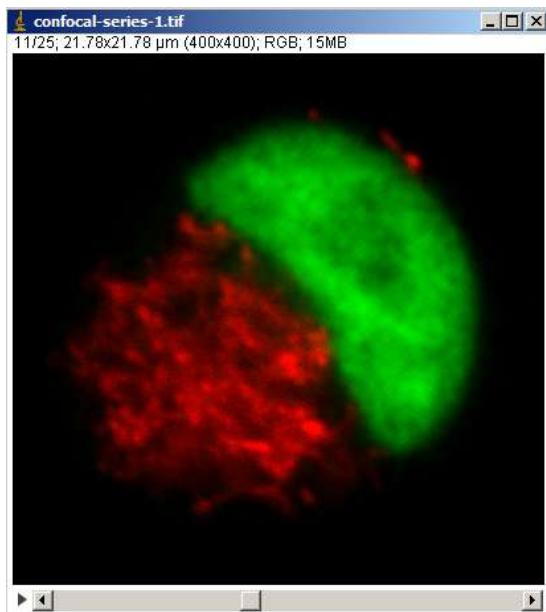


Right mouse click on  : access to different tools

Double mouse click on some tools: access to options menu

Toolbar

File > Open Samples > Confocal-Series

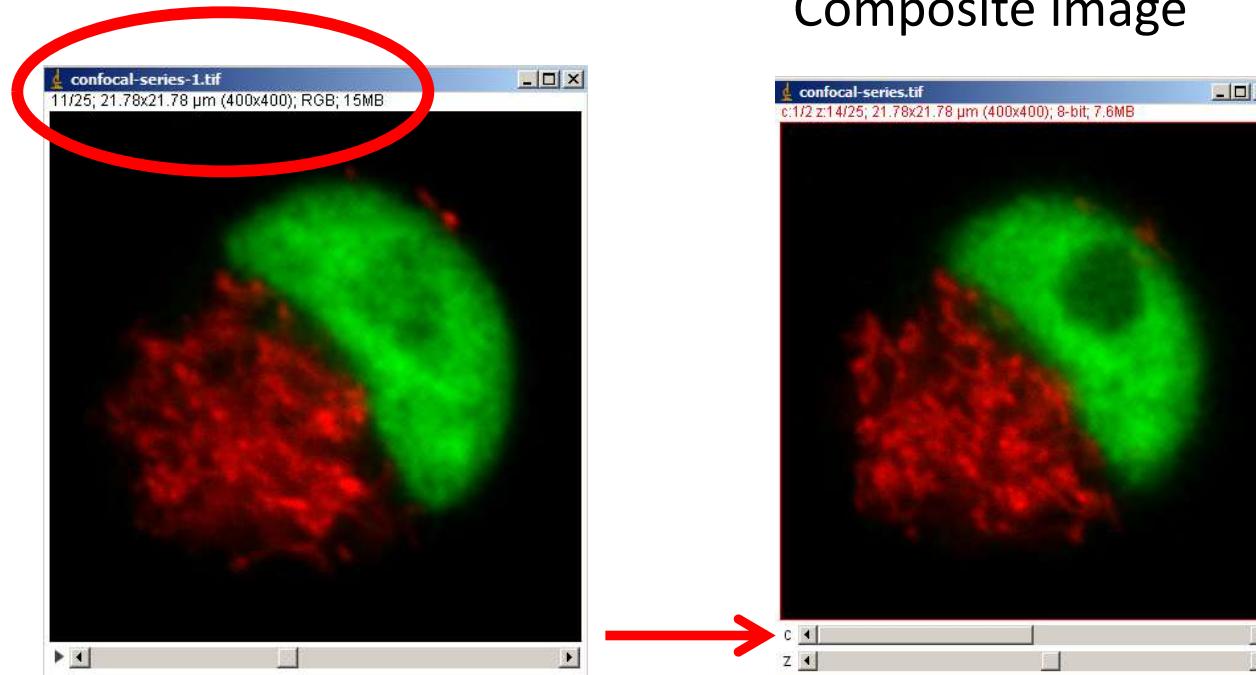


Try different ROI selections

Image types

- 8 bit: 256 grey levels
- 16 bit: 65536 grey levels
- 32 bit: floating point scale; can have any value
- RGB 24 bit: 8 bit Red + 8 bit Green + 8 bit Blue
- RGB 32 bit: as RGB 24 bit + additional eight bits coding for alpha channel transparency information
- Color composites
 - Each channel can be 8, 16 or 32 bit
 - Color channels kept separate
 - More than 3 channels can be merged

Image types



Composite image

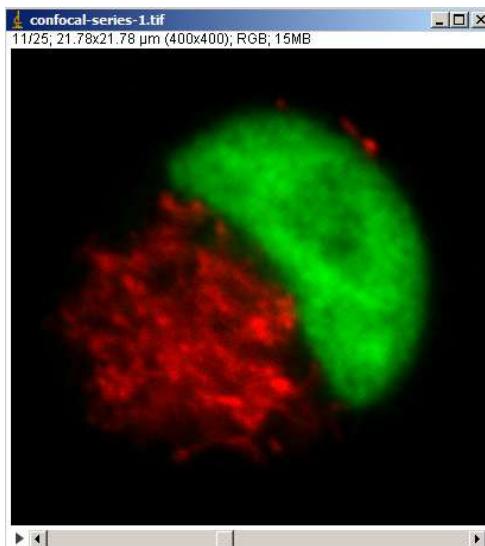
Advantage:

- Each channel is separate
- More than 3 channels possible
- Can work on 1 channel while all are visible

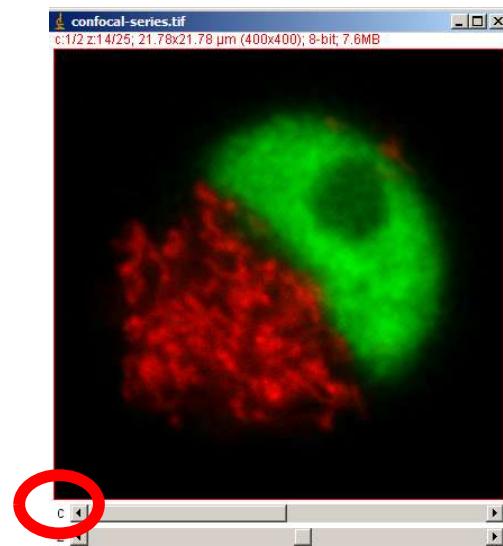
Image types

- Single image (x,y)
- Stack: one channel (x,y,z; x,y,t)
- Hyperstack: multidimensional extended stack (x, y, z, c and/or t)

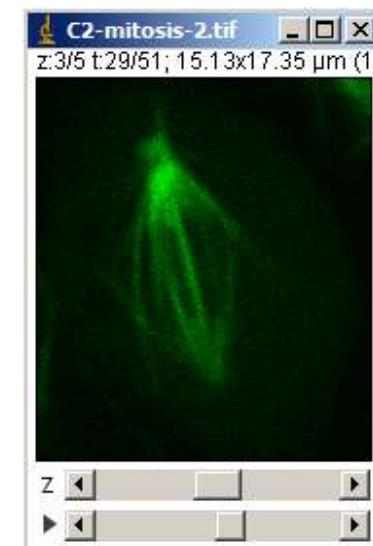
Stack



Hyperstack



= Composite image

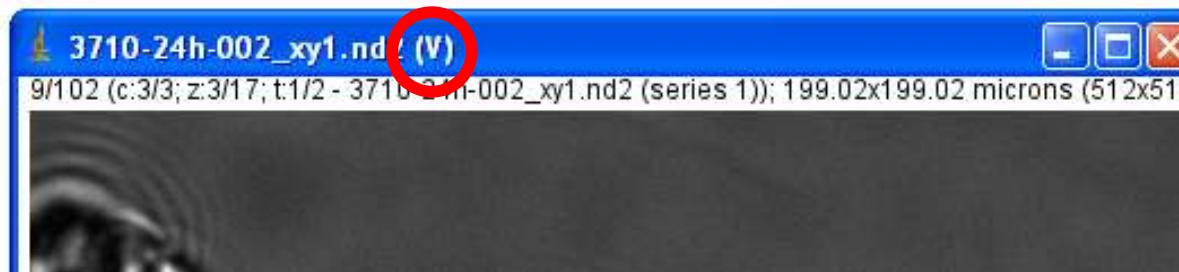


≠ Composite image

Image types

- Single image (x,y)
- Stack: one channel (x,y,z; x,y,t)
- Hyperstack: multidimensional extended stack (x, y, z, c and/or t)

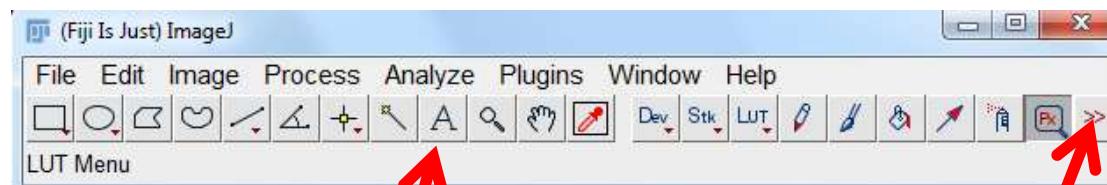
- Virtual Stack: read-only disk-resident (virtual) stack (be aware, changes won't be saved; overlays won't be loaded!). This is useful for large image series.



File Formats

Default image support for BMP, DICOM, GIF, JPEG, PGM, PNG, TIFF and raw data.

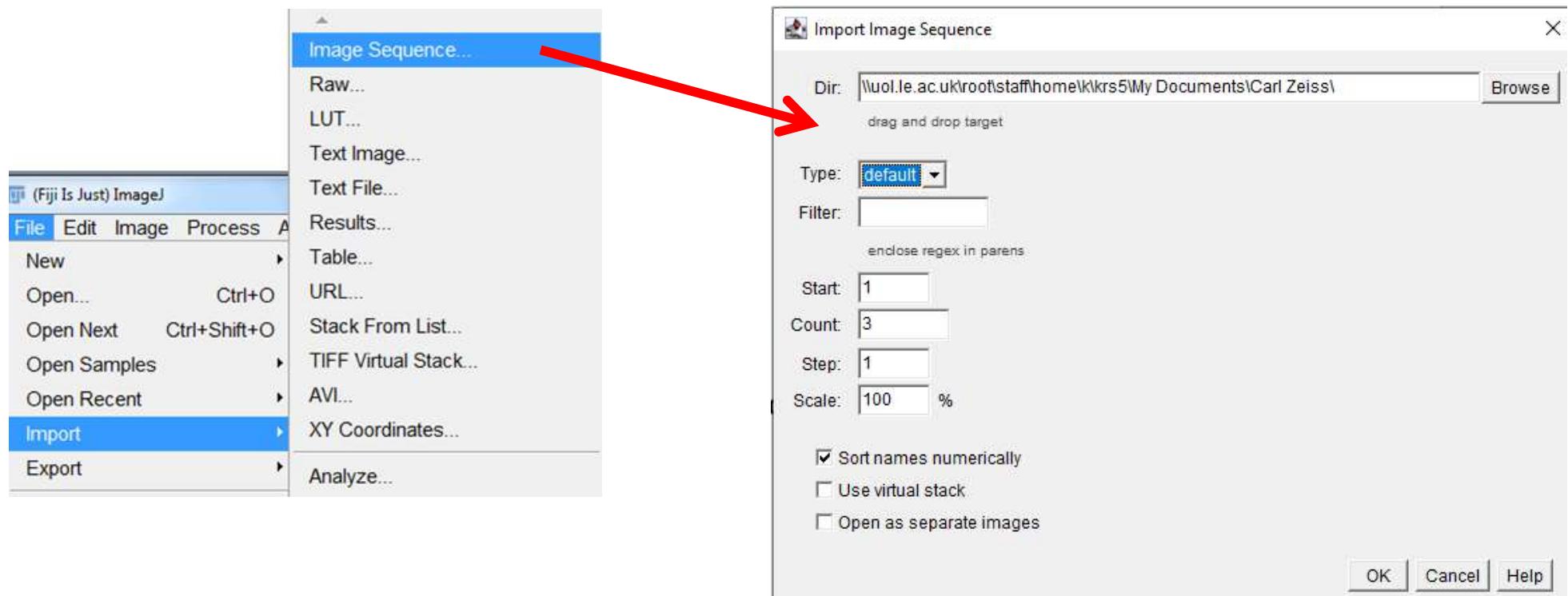
Open images using *File > Open*, drag-and-drop or *File > Import* (for image series):



Drag-and-drop;
Opens file.

Drag-and-drop;
opens TIF stacks
as virtual stack.

File Formats



File Formats

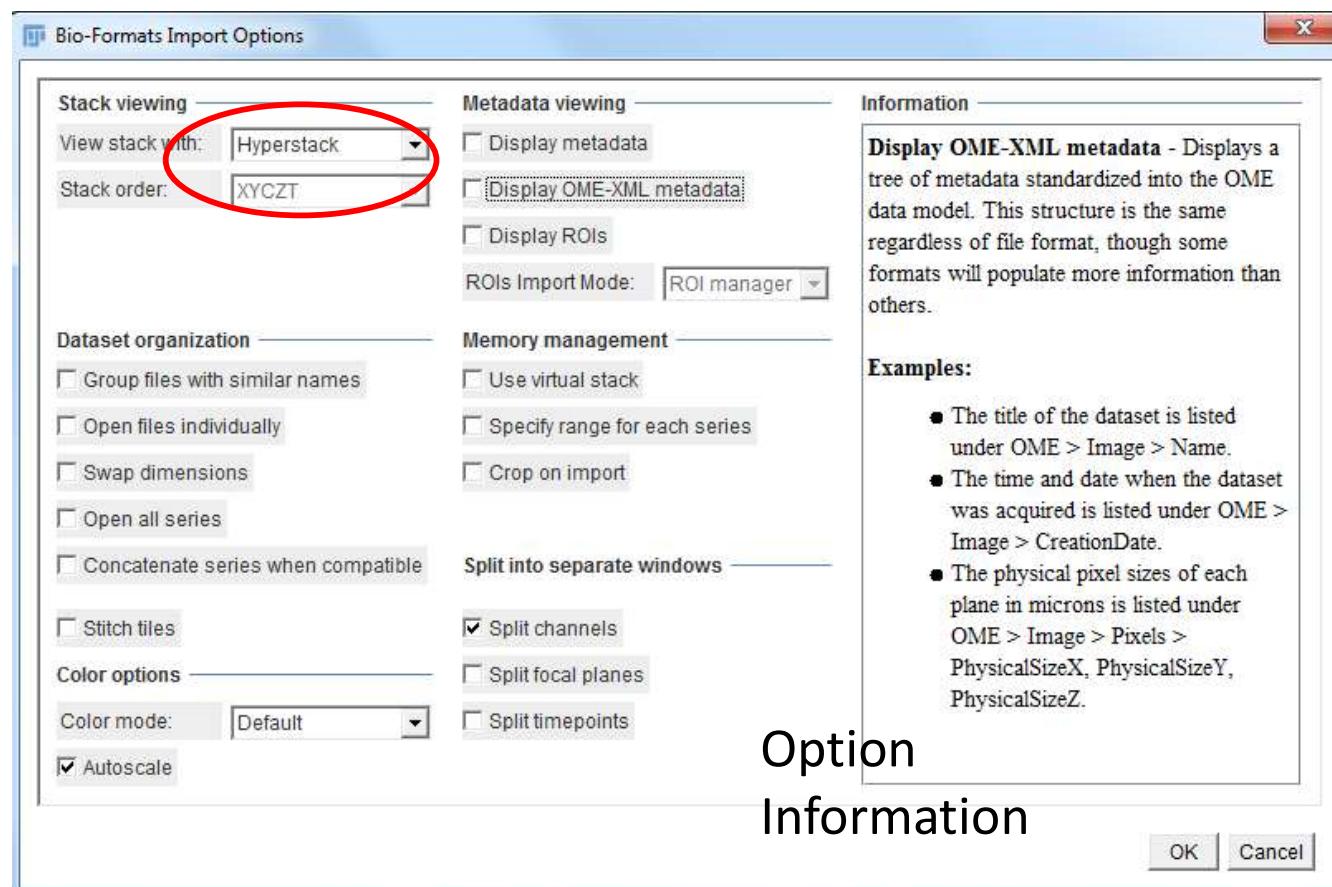
Default image support for BMP, DICOM, GIF, JPEG, PGM, PNG, TIFF and raw data.

Using **Bio-Formats library**. This plugin will allow you to open most microscope file formats directly via *File > Open* or *File> Import > Bio-Formats*.

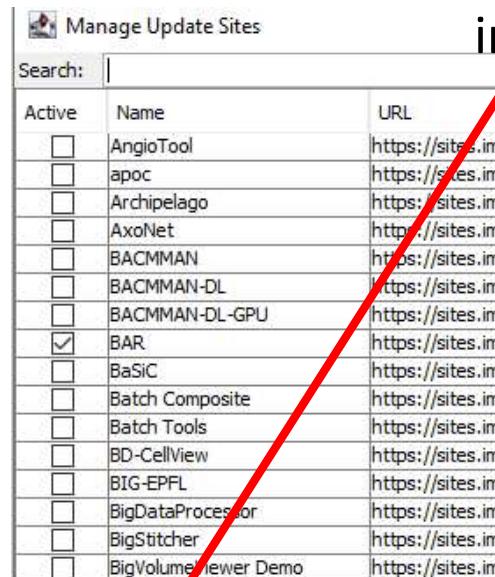
- reads >160 formats
- writes 13 formats

However, it is possible that you have to open an image via
Plugins > Bio-Formats > Bio-Formats Importer

File Formats



Bio-Formats library



Latest development update; Latest stable update is automatically included

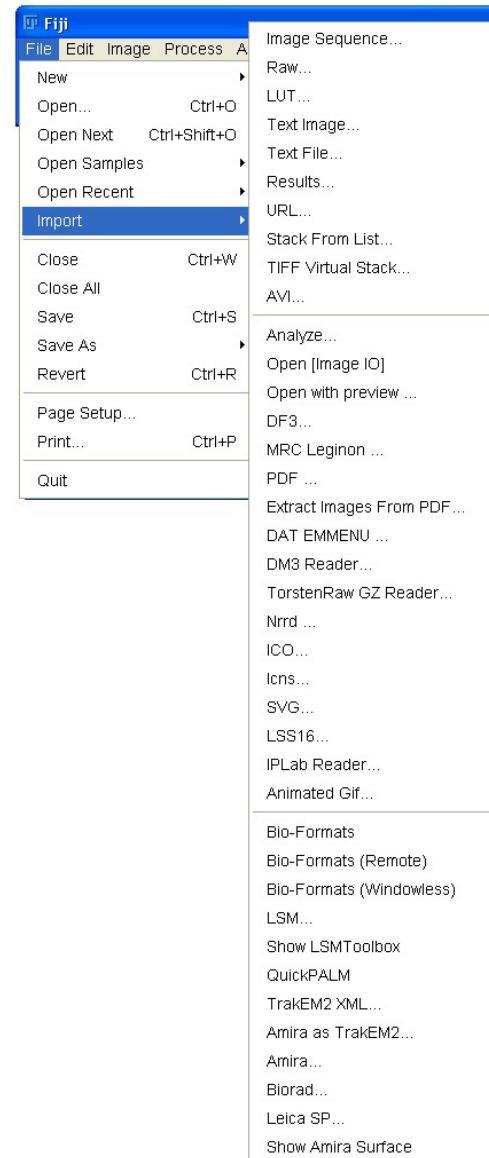
Manage Update Sites					
Active	Name	URL	Host	Direct...	Description
<input type="checkbox"/>	AngioTool	https://sites.imagej.net/AngioTool/			The An...
<input type="checkbox"/>	apoc	https://sites.imagej.net/apoc/			Acceler...
<input type="checkbox"/>	Archipelago	https://sites.imagej.net/Lindsey/			A set of...
<input type="checkbox"/>	AxoNet	https://sites.imagej.net/AxoNet/			
<input type="checkbox"/>	BACMMAN	https://sites.imagej.net/BACMMAN/			
<input type="checkbox"/>	BACMMAN-DL	https://sites.imagej.net/BACMMAN-DL			
<input type="checkbox"/>	BACMMAN-DL-GPU	https://sites.imagej.net/BACMMAN-DL-GPU			
<input checked="" type="checkbox"/>	BAR	https://sites.imagej.net/Tiago/			
<input type="checkbox"/>	BaSiC	https://sites.imagej.net/BaSiC/			
<input type="checkbox"/>	Batch Composite	https://sites.imagej.net/BatchComposite			
<input type="checkbox"/>	Batch Tools	https://sites.imagej.net/BatchTools/			
<input type="checkbox"/>	BD-CellView	https://sites.imagej.net/BD-CellView			
<input type="checkbox"/>	BIG-EPFL	https://sites.imagej.net/BIG-EPFL/			
<input type="checkbox"/>	BigDataProcessor	https://sites.imagej.net/BigDataProcessor			
<input type="checkbox"/>	BigStitcher	https://sites.imagej.net/BigStitcher/			
<input type="checkbox"/>	BigVolumeViewer Demo	https://sites.imagej.net/BigVolumeViewerDemo			
<input type="checkbox"/>	Bio-Formats	https://sites.imagej.net/Bio-Formats			
<input type="checkbox"/>	Biomat	https://sites.imagej.net/Biomat/			
<input type="checkbox"/>	Biomedgroup	https://sites.imagej.net/Biomedgroup			
<input checked="" type="checkbox"/>	BioVoxel	https://sites.imagej.net/BioVoxel/			
<input checked="" type="checkbox"/>	BioVoxel 3D Box	https://sites.imagej.net/bv3dbox/			
<input type="checkbox"/>	BioVoxel Figure Tools	https://sites.imagej.net/bvft/			
<input type="checkbox"/>	Blind Analysis Tools	https://sites.imagej.net/Astja/			
<input checked="" type="checkbox"/>	BoneJ	https://sites.imagej.net/BoneJ/			

Format	Extensions	Pixels	Metadata	Openness	Presence	Utility	Export	SCIFIO
3i SlideBook	.sld	▲	▼	▼	▼	▼	✗	✗
Andor Bio-Imaging Division (ABD) TIFF	.tif	▲	▲	▲	▼	▼	✗	✗
AIM	.aim	■	▲	▼	▼	▼	✗	✗
Aicona 3D	.a13d	▲	▲	▲	▼	▼	✗	✗
Amersham Biosciences Gel	.gel	▲	▲	▲	▼	▼	✗	✗
Amira Mesh	.am, .amiramesh, .grey, .hx, .labels	▲	■	▼	▼	▼	✗	✗
Analyze 7.5	.img, .hdr	▲	■	▲	▼	▼	✗	✗
Animated PNG	.png	▲	▲	▲	▼	▼	✓	✓
Aperio SVS TIFF	.svs	▲	▲	▲	▼	▼	✗	✗
Applied Precision CellWorX	.htd, .pnl	▲	■	■	▼	▼	✗	✗
AVI (Audio Video Interleave)	.avi	■	▲	▼	▼	✓	✓	✓
Axon Raw Format	.arf	▲	▼	▲	▼	▼	✗	✗
BD Pathway	.exp, .tif	▲	▲	▲	▼	▼	✗	✗
Becker & Hickl SPCImage	.sdt	▲	▲	▲	▼	▼	✗	✗
Bio-Rad Gel	.1sc	■	▼	▼	▼	▼	✗	✗
Bio-Rad PIC	.pic, .raw, .xml	▲	▲	▲	▲	▲	✗	✗
Bitplane Imaris	.ims	▲	▲	▲	▼	▼	✗	✗
Bruker MRI	.ims	■	▲	▼	▼	▼	✗	✗
Burleigh	.img	■	▼	▼	▼	▼	✗	✗
Canon DNG	.cr2, .crw	■	▲	▼	▼	▼	✗	✗
Cellomics	.c01	▲	▼	▼	▼	▼	✗	✗

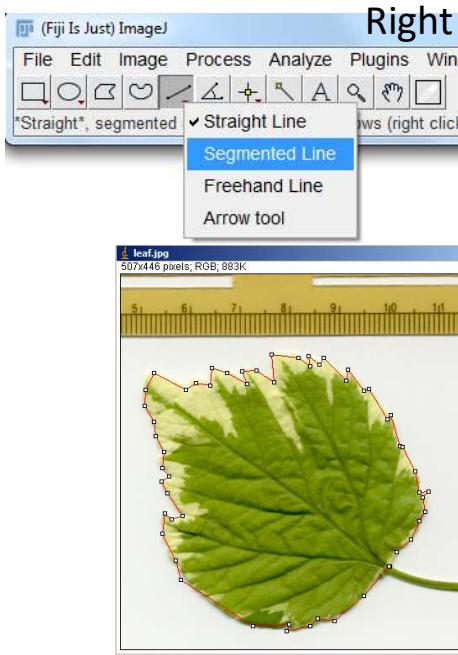
File Formats

Fiji installs with more file formats supported.

If you miss a format or an image does not open correctly using Bio-Formats plugin there might be a plugin available.



File > Open Samples > Leaf

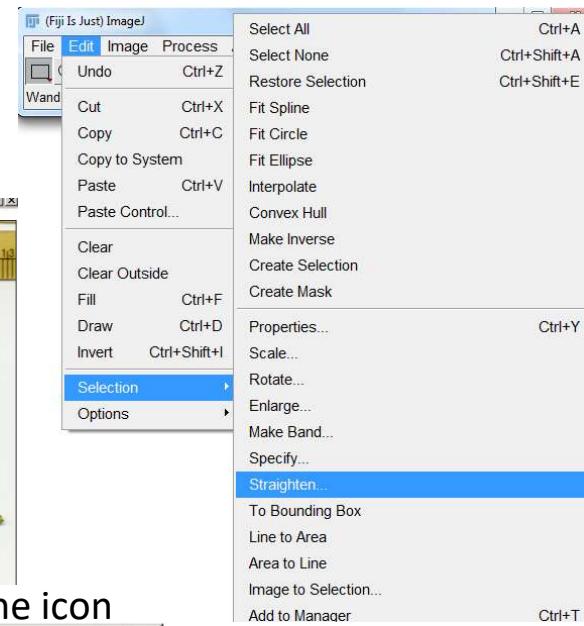


Right mouse click

Selection



Double click on line icon



Change colour of selection line: *Edit > Options > Colors*

Restore selection line: *Edit > Selection > Restore Selection*



Brightness and Contrast

File > Open samples > Fluorescence Cells

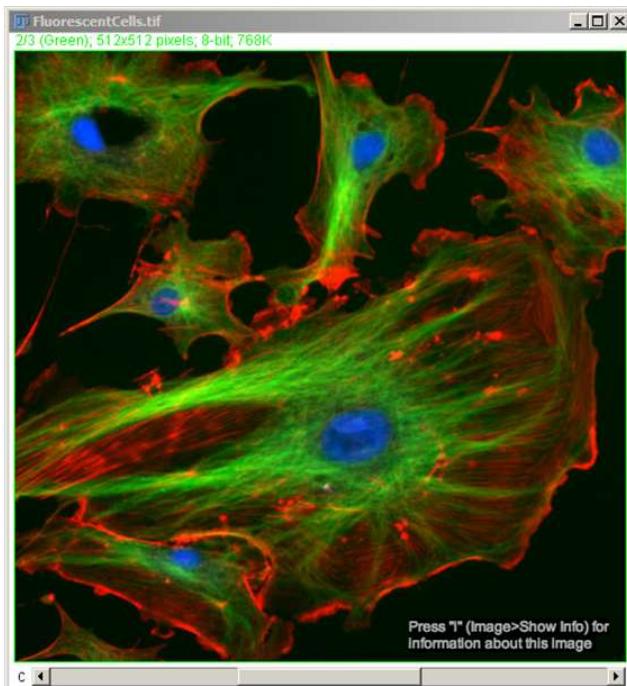
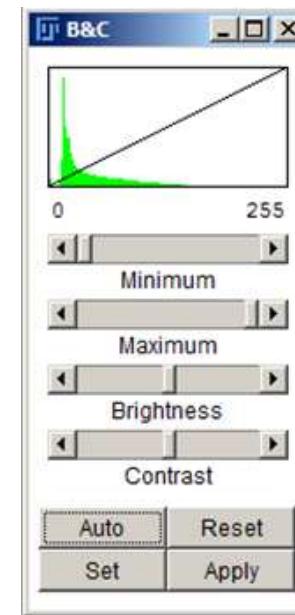
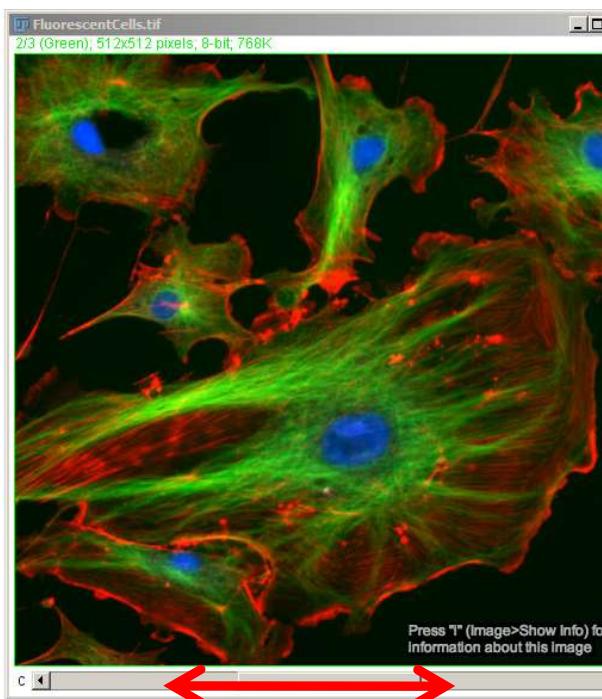


Image > Adjust > Brightness/Contrast



Composite: use slider to make different channels active

Brightness and Contrast



Composite

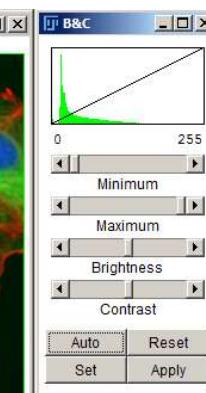
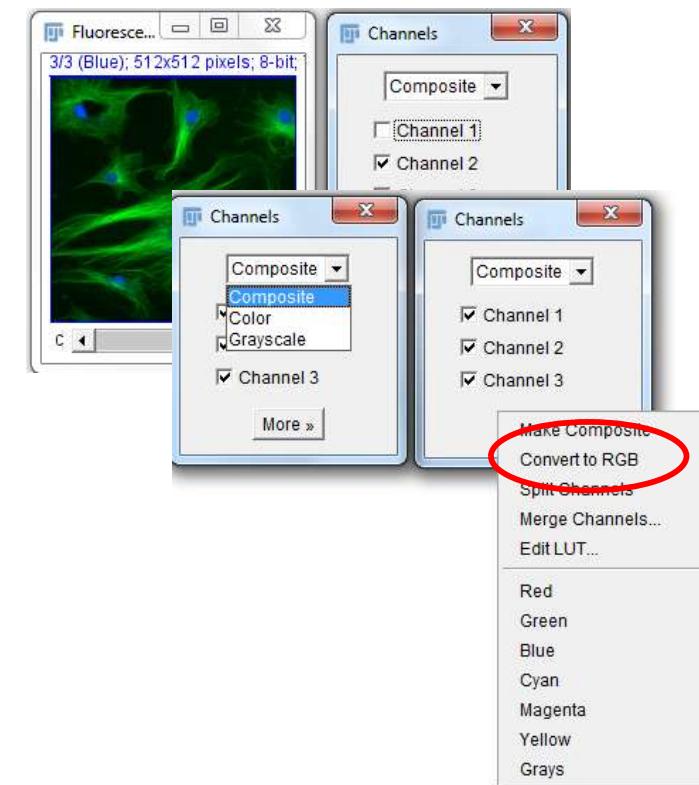
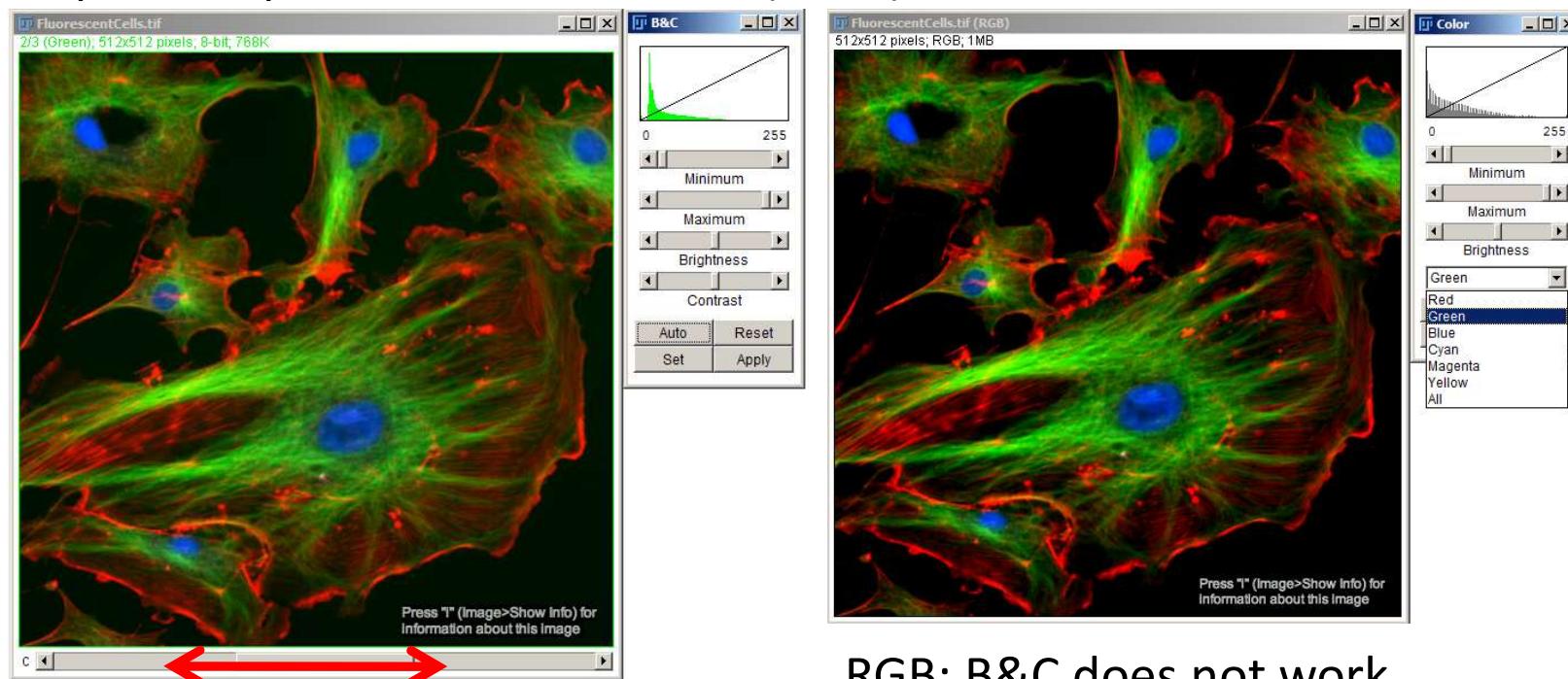


Image > Color > Channels Tools



Brightness and Contrast

Open sample Fluorescence Cells (400K)



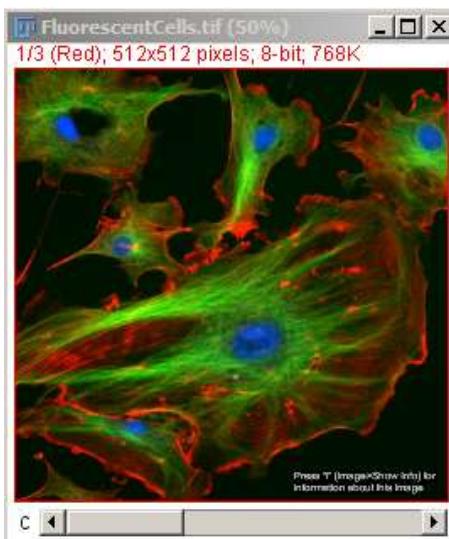
Composite

RGB: B&C does not work.

Use: *Image > Adjust > Color Balance.*

Close the GRB image

Split channels



Split
Channels

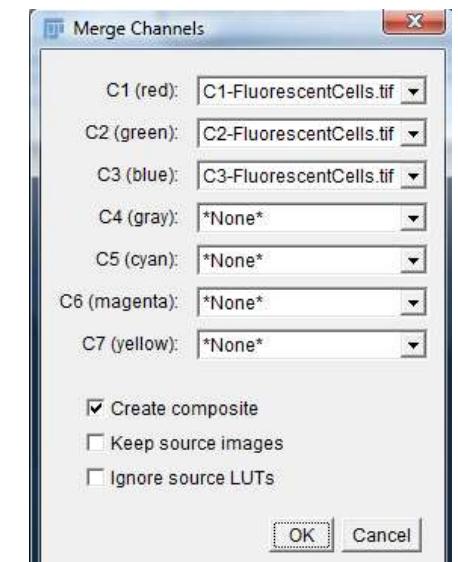
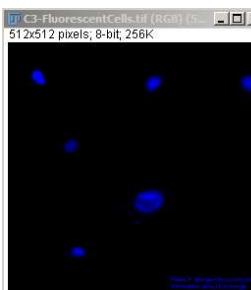
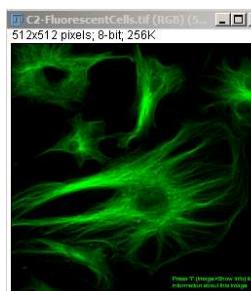
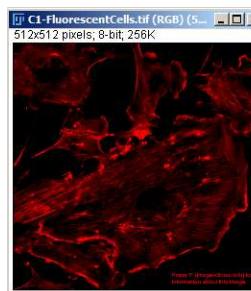
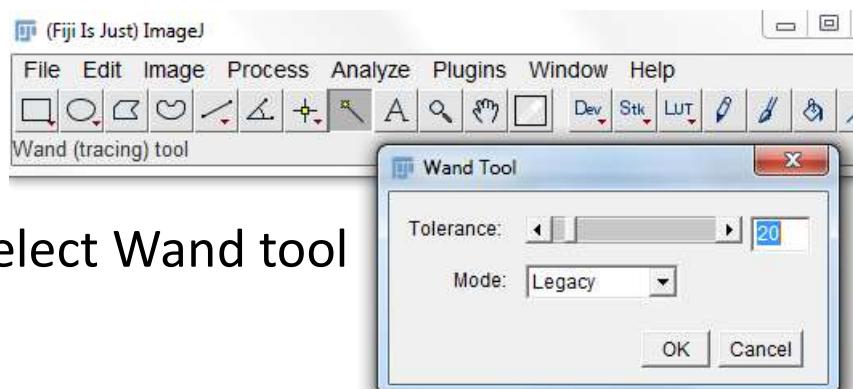
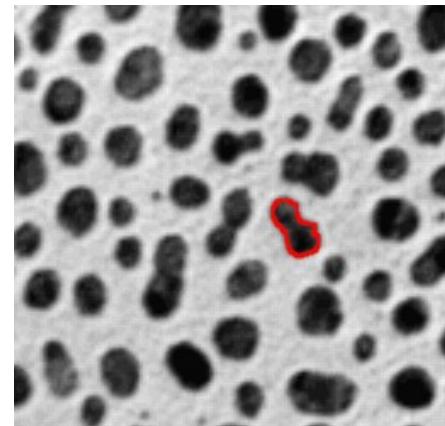
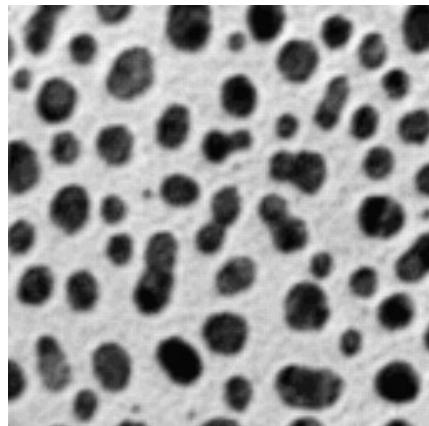


Image > Color > Split Channels

Image > Color > Merge Channels

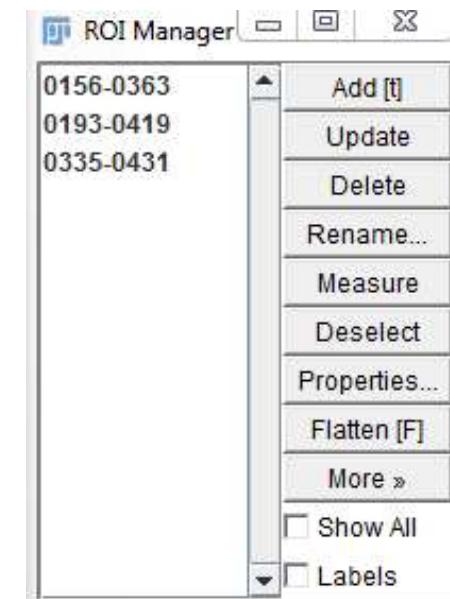
Selections

File > Open samples > Blobs

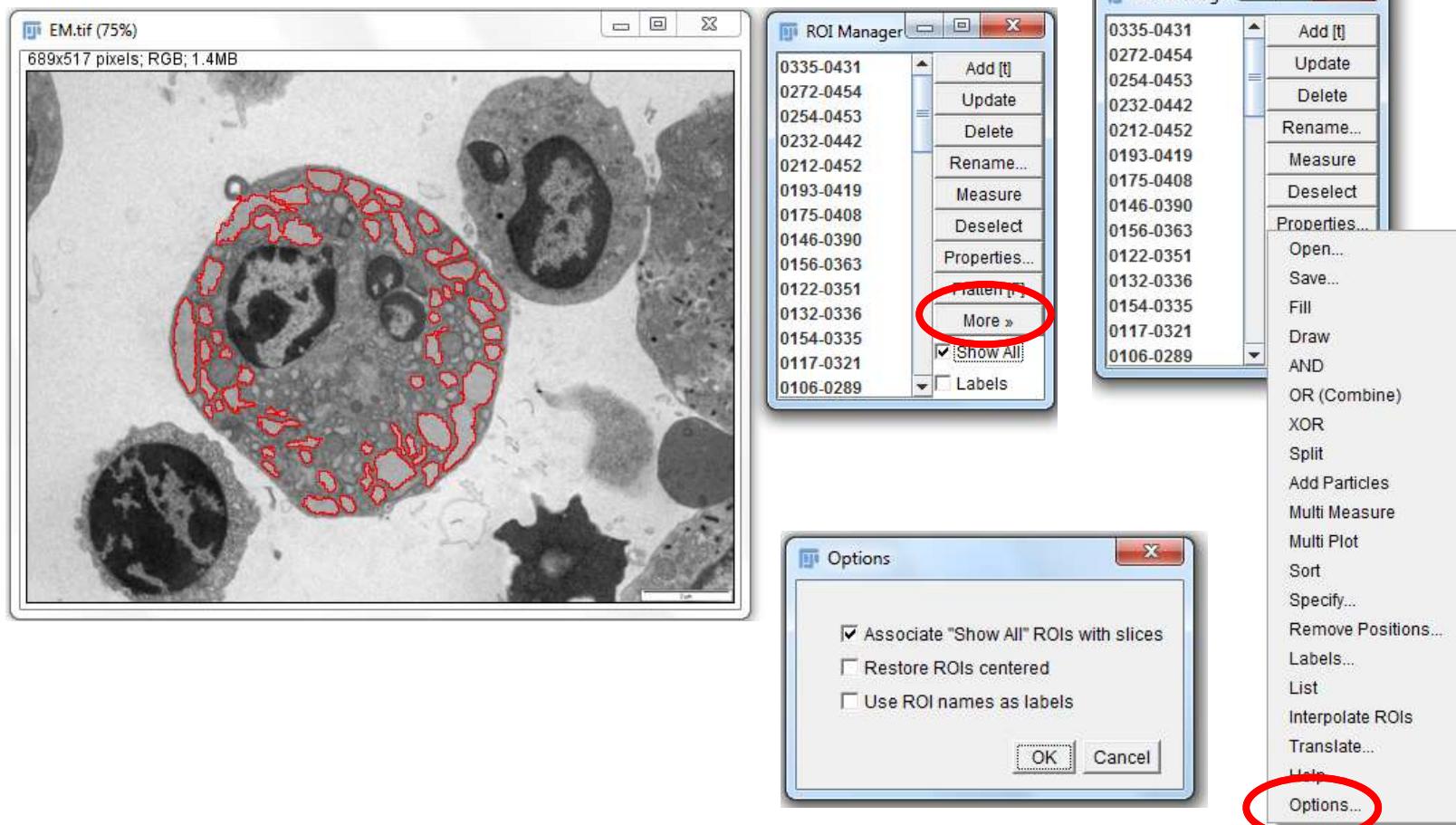


Select Wand tool

Analyse > Tools > ROI manager
(Short cut: t)



Selections



Threshold

We create a binary image (black and white) to extract information

*File > Open Samples
> Blobs (25k)*

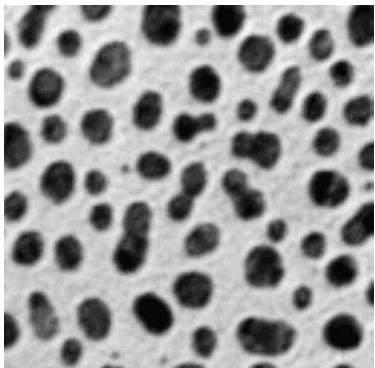
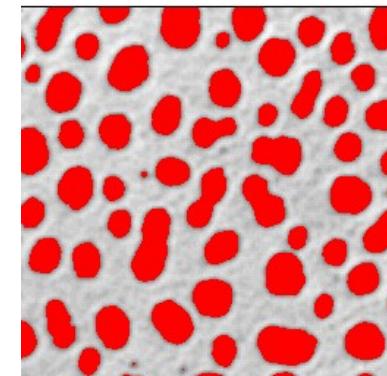
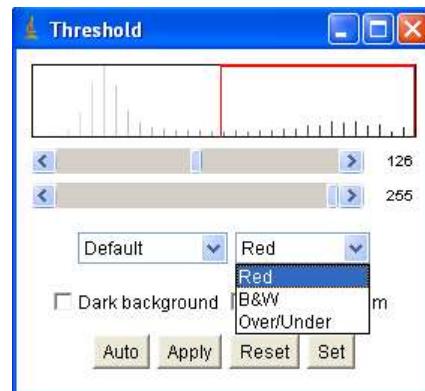
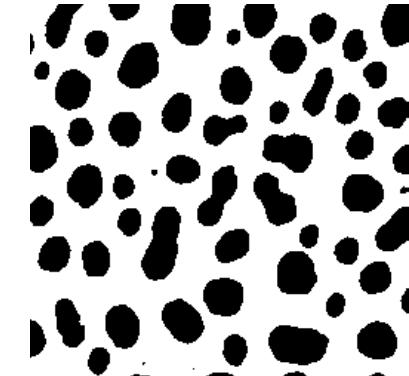


Image > Adjust > Threshold...

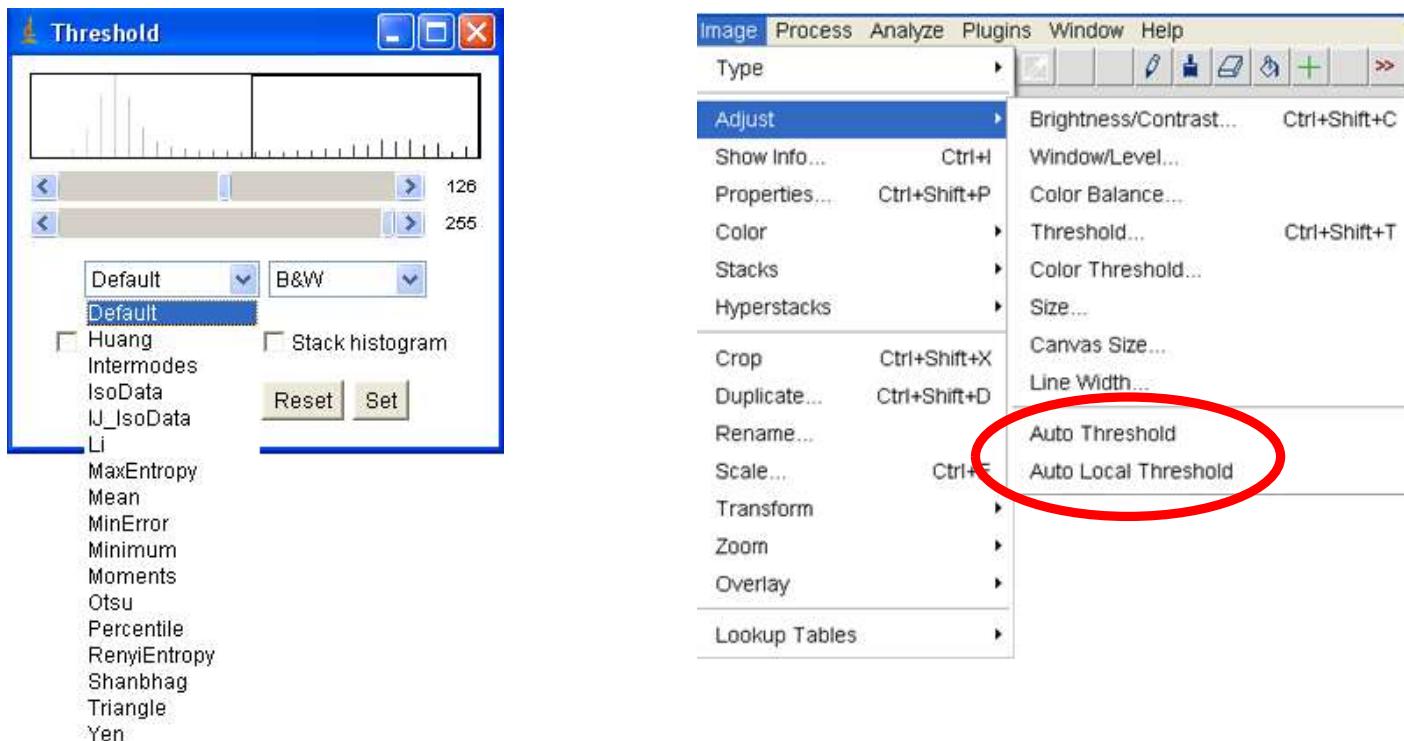


Select “Red”; You can Select “Apply”
see what you select

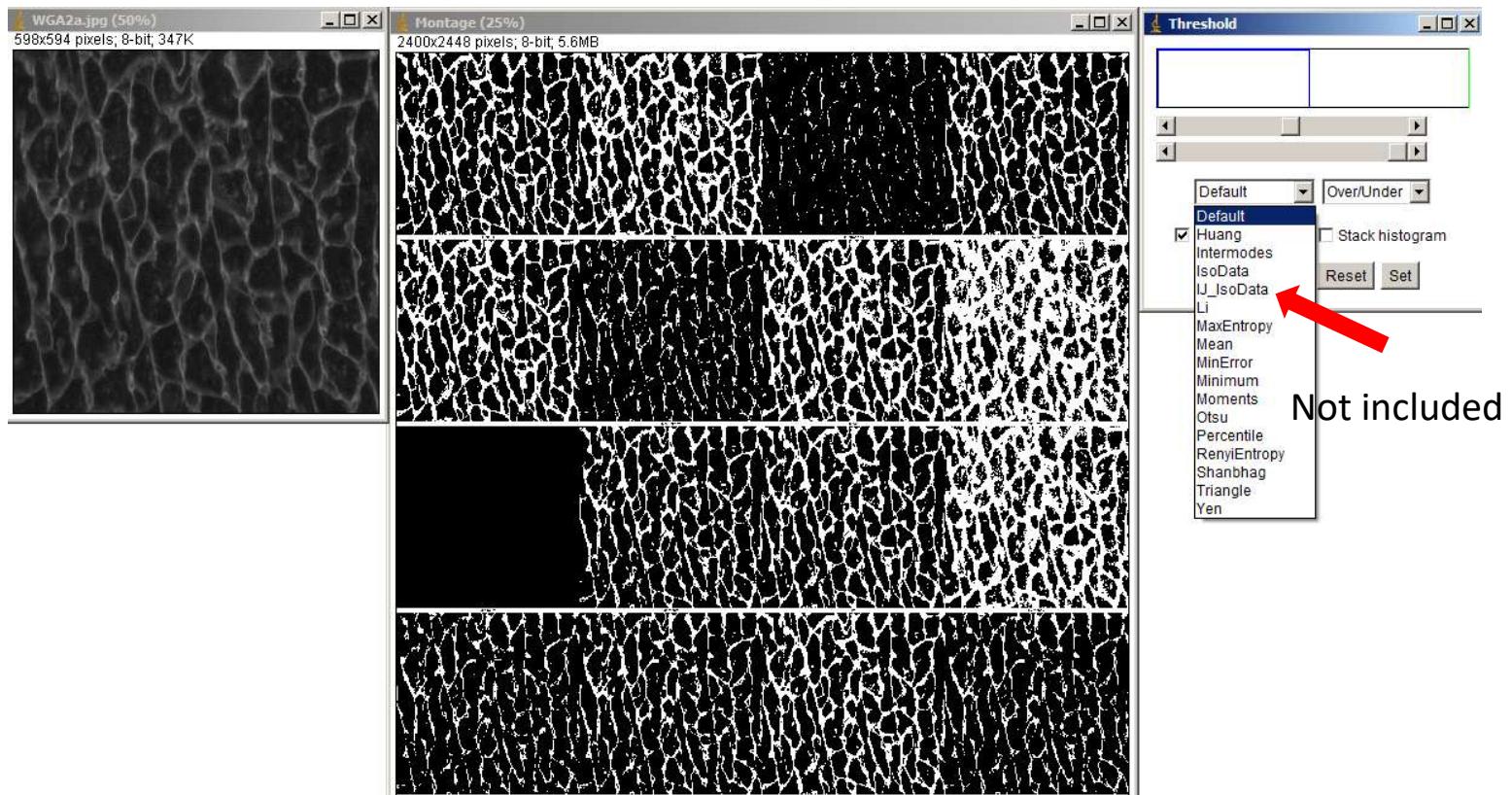


Historically, selected pixels have a value of 255 inside the selection and 0 outside, so black is 255 and white is 0 unless Black Background in *Process > Binary > Options* is checked.

Threshold



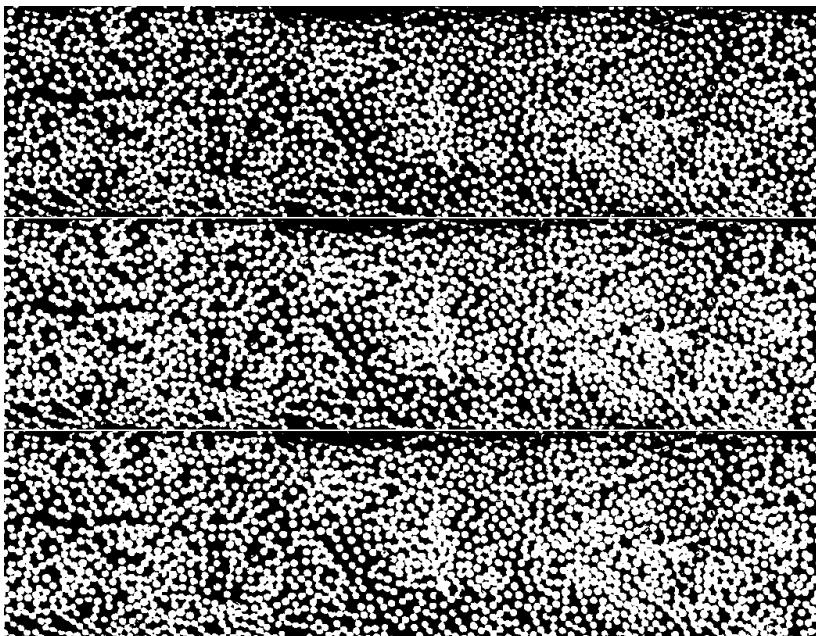
Threshold



Auto Threshold

Threshold

Discussion on the image.sc mailing list <https://forum.image.sc/t/bad-thresholding/113514>



Results

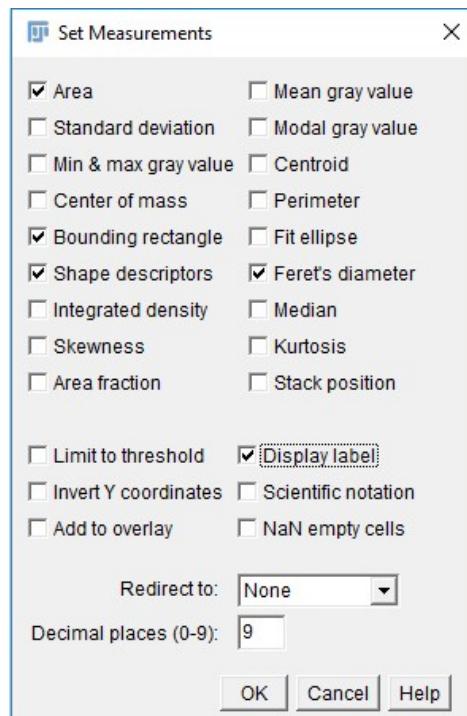
	Area	Mean	Circ.	IntDen	%Area	RawIntDen	AR	Round	Solidity
1	282420	105.031	0.512	29662875	41.189	29662875	3.874	0.258	1.000
2	282420	127.883	0.512	36116670	50.150	36116670	3.874	0.258	1.000
3	282420	125.730	0.512	35508750	49.306	35508750	3.874	0.258	1.000

"There isn't necessarily a better or worse thresholding method, just different ones."

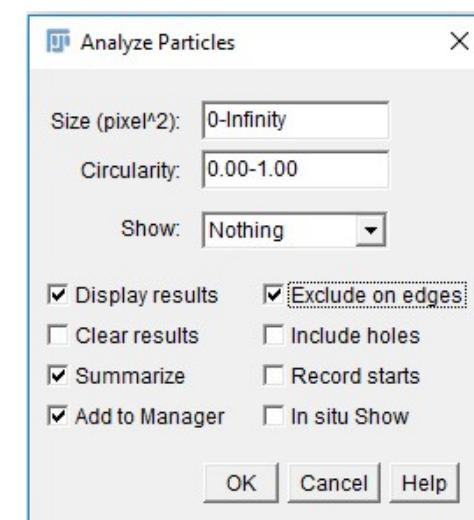
Jorge Ramirez; Institut de Neurosciences de la Timone, Marseille, FR

Measurements

Analyze > Set Measurements...

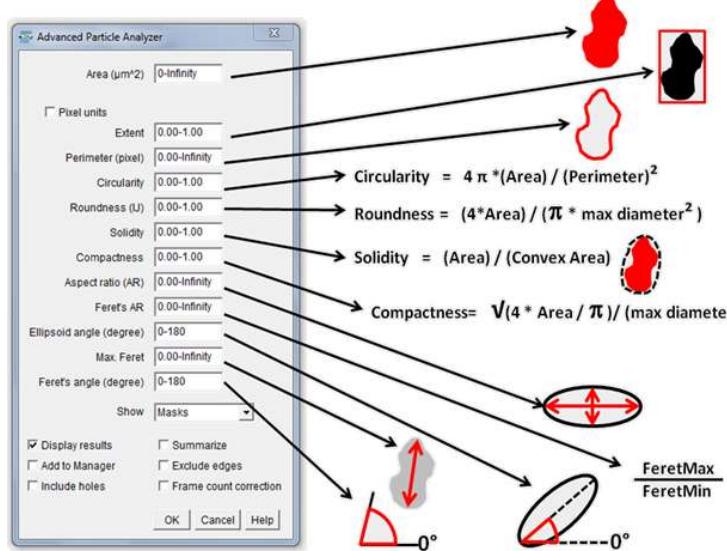


Analyze > Analyze Particles

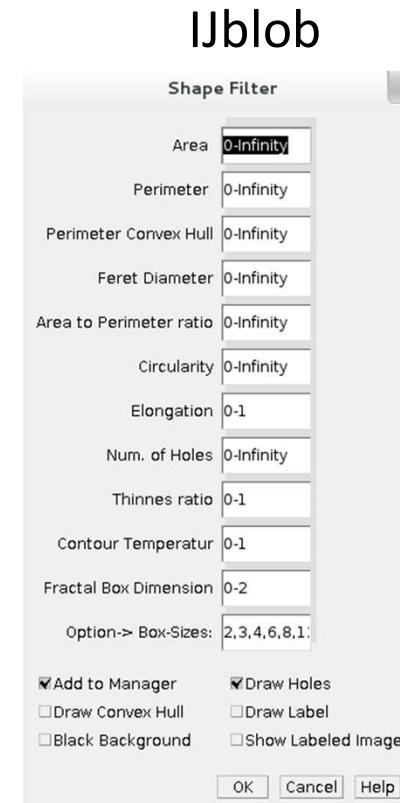


Measurements

BioVoxel Toolset:
includes Advanced Particle Analyzer

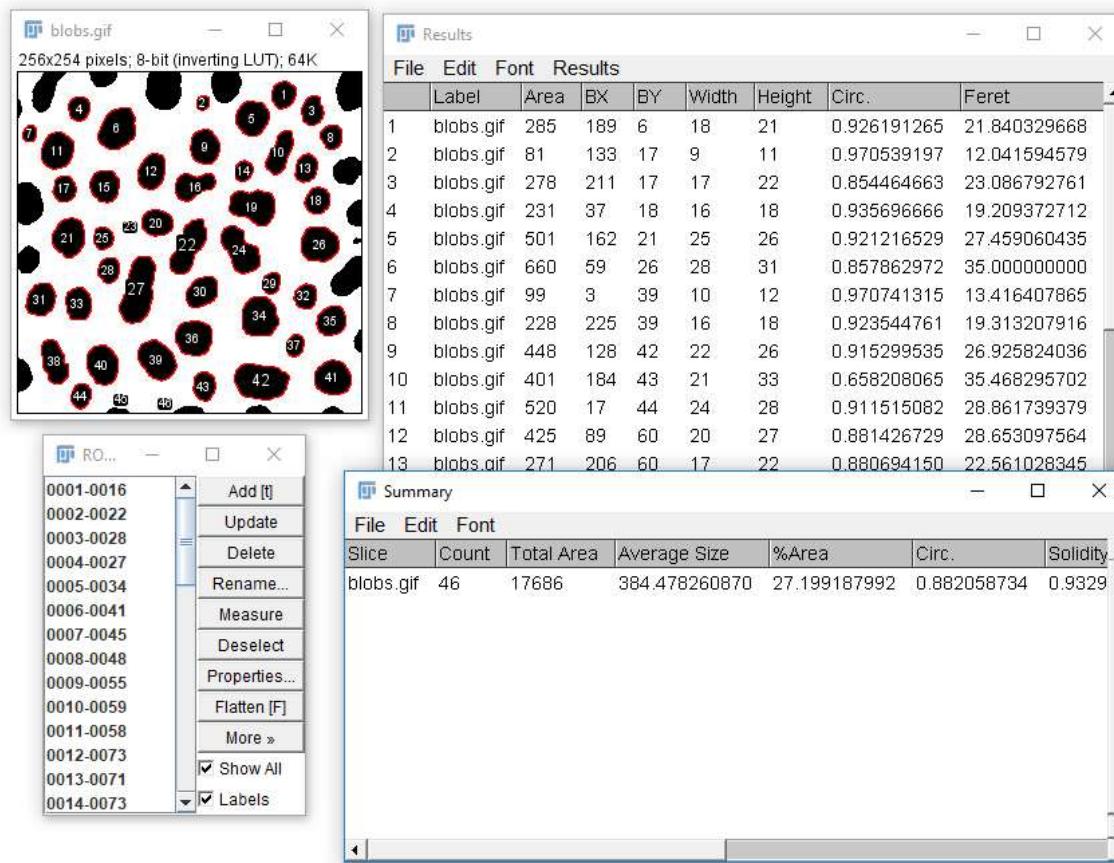


<http://www.biovoxxel.de/macros.html>

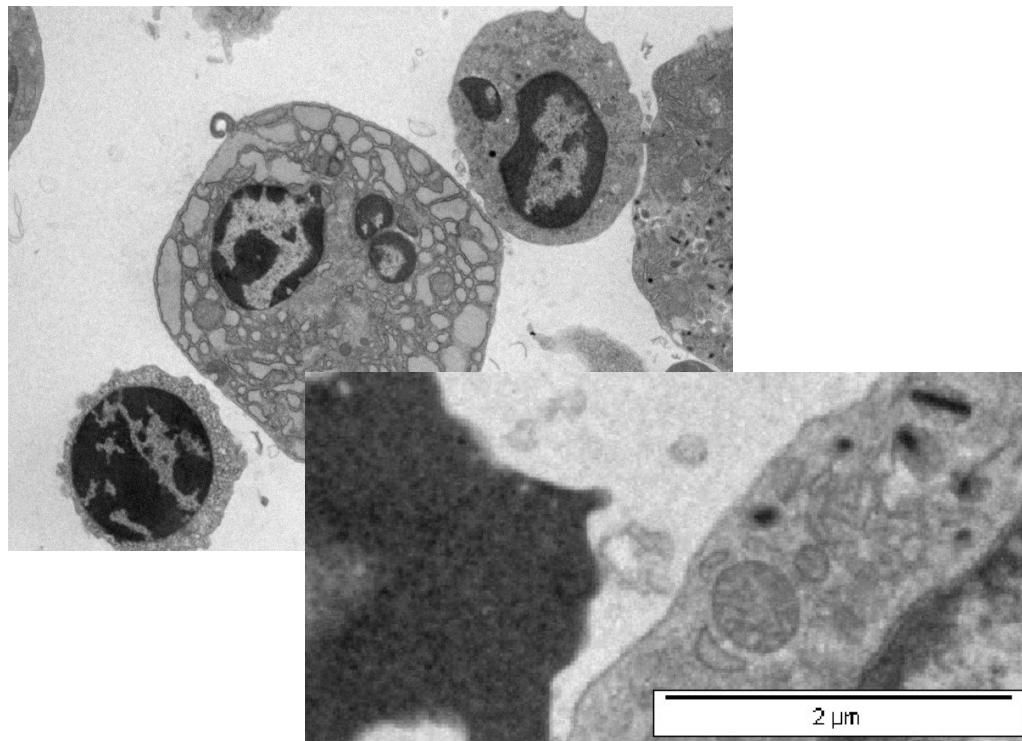


<https://code.google.com/p/ijblob/>

Measurements

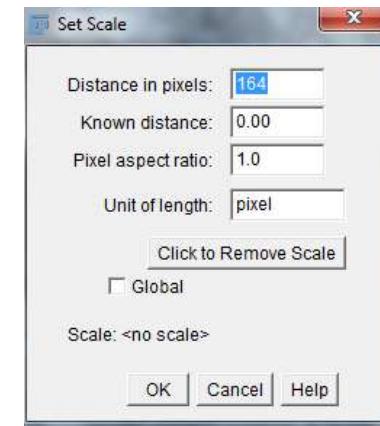


Scale (pixel size)



Make straight line on the scale bar

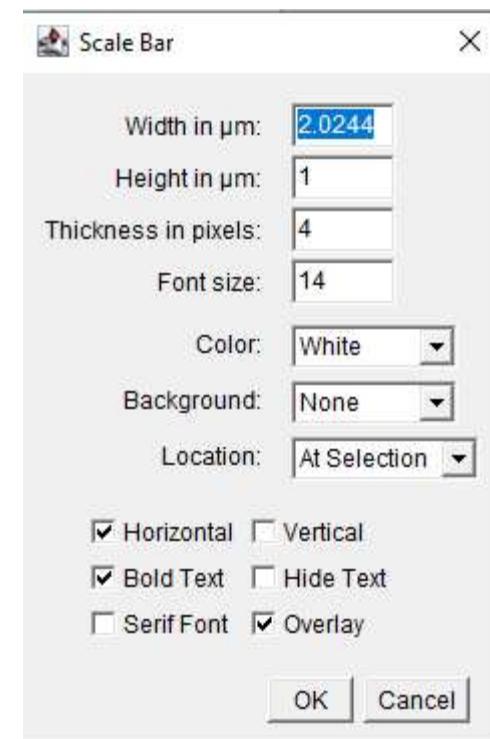
Analyze > Set Scale



- Set known distance (2 in this example)
 - Set unit (μm)
- If 'Global' is selected all successive images will be opened with this scale.

Set scale bar

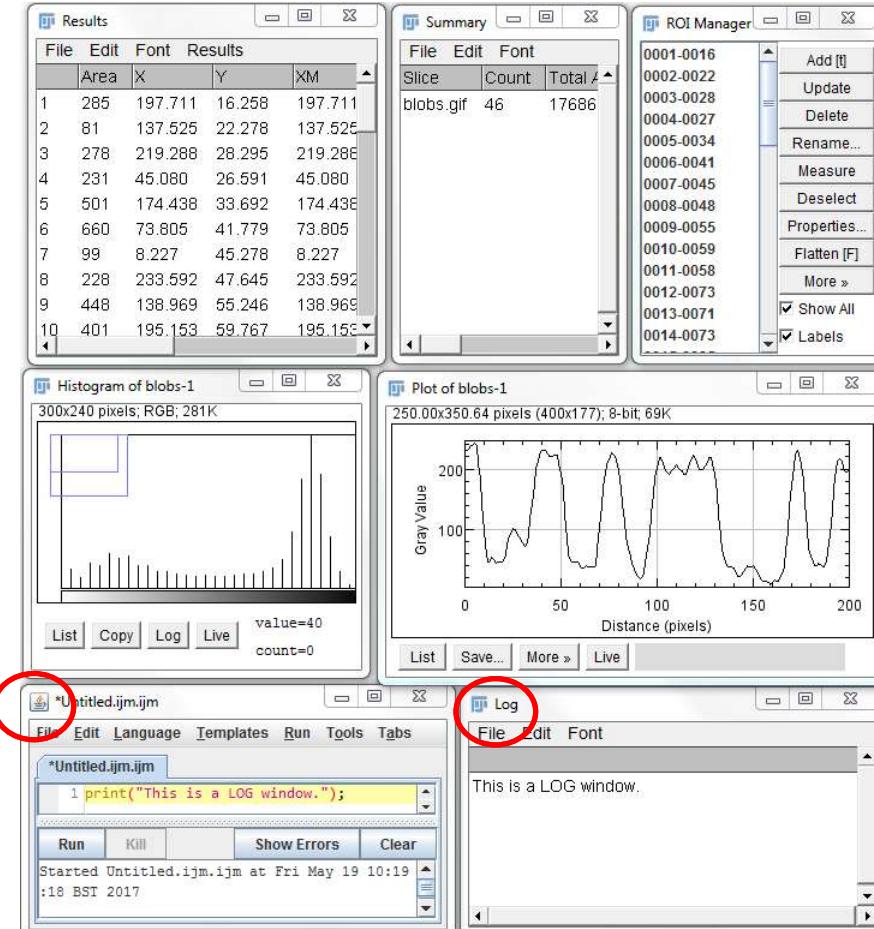
Analyze > Tools > Scale Bar...



Non-image windows

Windows besides image windows:

- Results
- Summary
- ROI manager
- Histogram
- Plots
- Editor
- Log
- Plugins
-



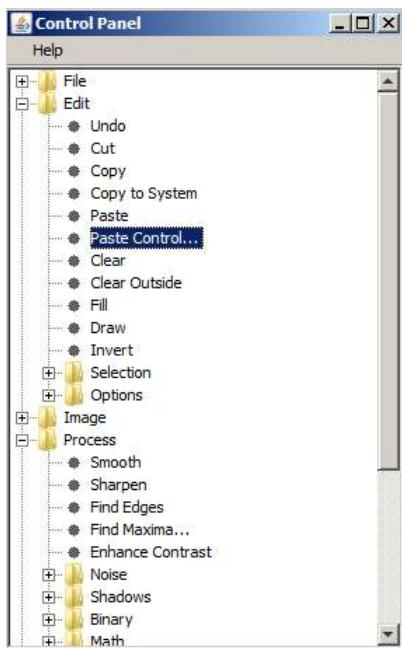
These windows have their own menus.

Find where you are looking for

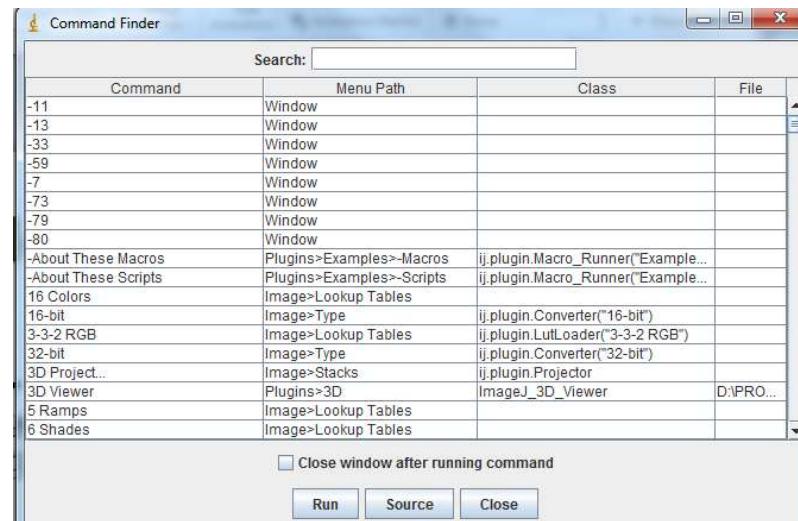
Fiji search bar. Change appearance: *Edit > Options > Search Bar...*

Plugins > Utilities >

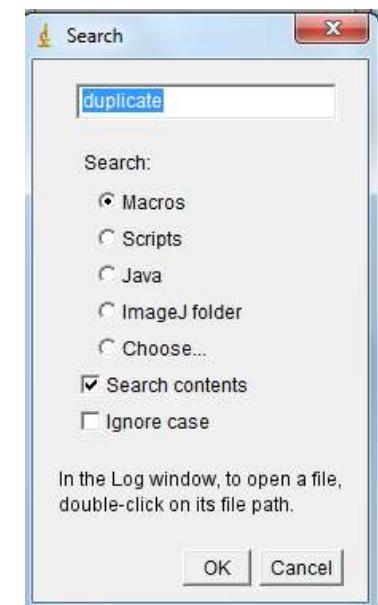
Control Panel



Find Command



Search



Plugins



Plugins

Plugins are special-purpose software extensions written in Java which you can add to ImageJ for added functionality.

If you understand Java you can write ImageJ plugins.

Plugins are compiled (written code is converted to machine code)  run faster than macro's or scripts.

Plugins

There are many websites you can download plugins from. Some examples:

- [ImageJ website](#)
- <https://imagej.net/list-of-extensions>
- ImageJ Tudor Wiki website - <https://imagejdocu.list.lu/>
- Many institutional and personal websites (few examples)
 - <http://bigwww.epfl.ch/algorithms.html>
 - <https://blog.bham.ac.uk/intellimic/g-landini-software/> (Updated)
 - <http://www.sussex.ac.uk/gdsc/intranet/microscopy/UserSupport/AnalysisProtocol/imagej/>
 - <http://research.stowers.org/imagejplugins/>
- Company websites
 - <https://www.optinav.info/>
 - <https://ibidi.com/chemotaxis-analysis/171-chemotaxis-and-migration-tool.html>
- Fiji update sites

Plugins

There are many websites you can download plugins from. Not only for cell biology:

- X-ray, CT scanner and Material sciences: <https://lazzyizzi.github.io/>
- Soil tomography: <https://github.com/ivangtorre/multifrac>; SoilJ
- Rock Art Digital Enhancement: <https://www.dstretch.com/>
- Topographical image analysis: <https://www.gcsca.net/IJPlugins>

Plugins

https://imagej.net/list-of-extensions

Page histo

List of Extensions

Categories filter

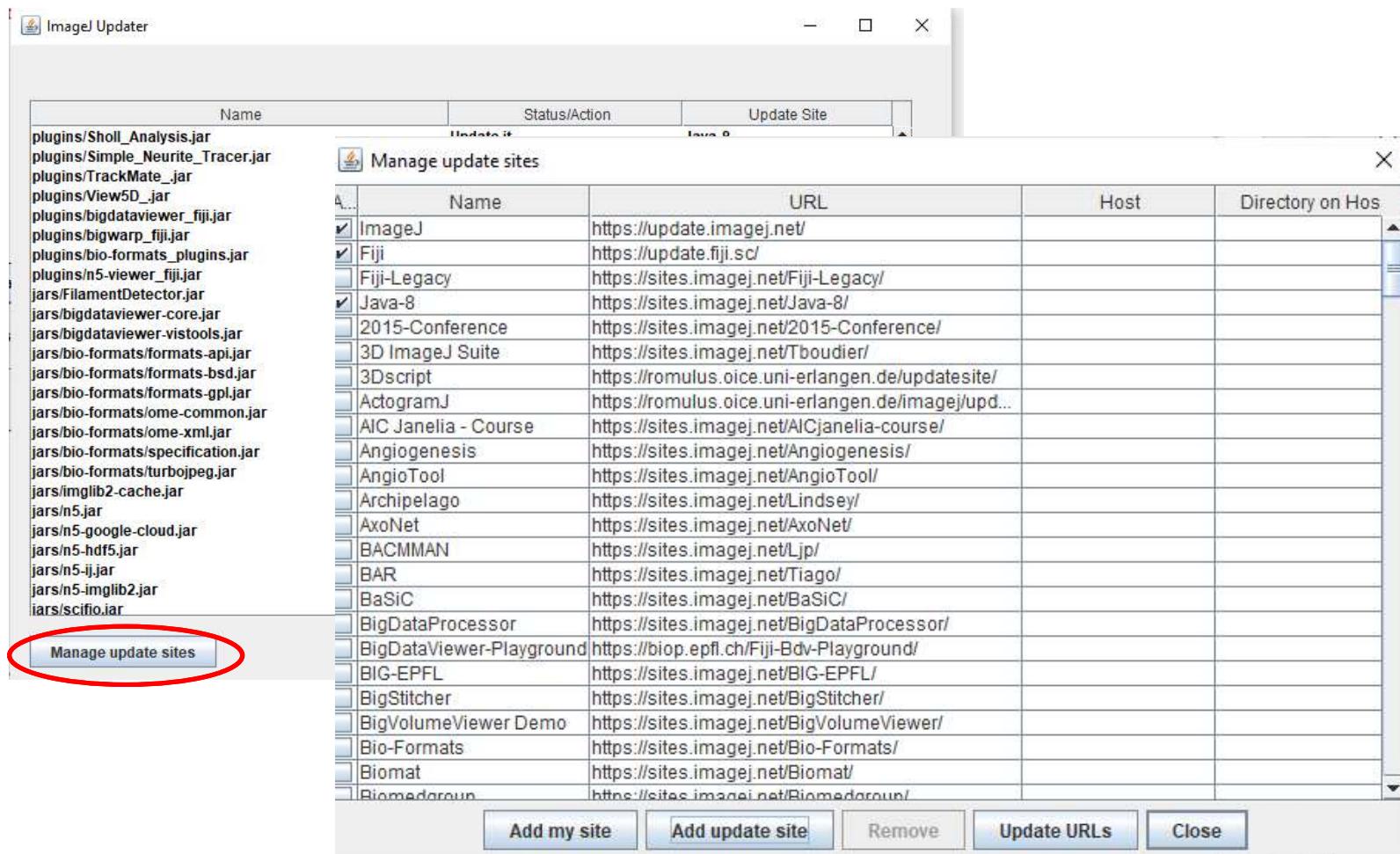
SELECT ALL SELECT NONE All Any

<input checked="" type="checkbox"/> 3D	<input checked="" type="checkbox"/> Example Data	<input checked="" type="checkbox"/> Lisp	<input checked="" type="checkbox"/> Optic Flow	<input checked="" type="checkbox"/> Stacks
<input checked="" type="checkbox"/> Analysis	<input checked="" type="checkbox"/> Feature Extraction	<input checked="" type="checkbox"/> MATLAB	<input checked="" type="checkbox"/> Particle Analysis	<input checked="" type="checkbox"/> Stitching
<input checked="" type="checkbox"/> Automation	<input checked="" type="checkbox"/> Fiji	<input checked="" type="checkbox"/> Machine Learning	<input checked="" type="checkbox"/> Pattern Recognition	<input checked="" type="checkbox"/> Super-resolution
<input checked="" type="checkbox"/> Binary	<input checked="" type="checkbox"/> Filtering	<input checked="" type="checkbox"/> Mathematical Morphology	<input checked="" type="checkbox"/> Perfusion	<input checked="" type="checkbox"/> Tracking
<input checked="" type="checkbox"/> Colocalization	<input checked="" type="checkbox"/> Help	<input checked="" type="checkbox"/> Microscopy	<input checked="" type="checkbox"/> Photography	<input checked="" type="checkbox"/> TrakEM2
<input checked="" type="checkbox"/> Color processing	<input checked="" type="checkbox"/> Image Annotation	<input checked="" type="checkbox"/> Microtubules	<input checked="" type="checkbox"/> Plotting	<input checked="" type="checkbox"/> Transform
<input checked="" type="checkbox"/> Cookbook	<input checked="" type="checkbox"/> ImageJ2	<input checked="" type="checkbox"/> Montage	<input checked="" type="checkbox"/> Projection	<input checked="" type="checkbox"/> Tutorials
<input checked="" type="checkbox"/> Data	<input checked="" type="checkbox"/> ImageScience	<input checked="" type="checkbox"/> Neuroanatomy	<input checked="" type="checkbox"/> Registration	<input checked="" type="checkbox"/> Uncategorized
<input checked="" type="checkbox"/> Deconvolution	<input checked="" type="checkbox"/> ImgLib	<input checked="" type="checkbox"/> Noise	<input checked="" type="checkbox"/> SciJava	<input checked="" type="checkbox"/> Unmaintained
<input checked="" type="checkbox"/> Development	<input checked="" type="checkbox"/> Import-Export	<input checked="" type="checkbox"/> OME	<input checked="" type="checkbox"/> Scripting	<input checked="" type="checkbox"/> User interface
<input checked="" type="checkbox"/> Digital Volume Flattening	<input checked="" type="checkbox"/> Integral Image	<input checked="" type="checkbox"/> Object Detection	<input checked="" type="checkbox"/> Segmentation	<input checked="" type="checkbox"/> Visualization
<input checked="" type="checkbox"/> Digital Volume Unrolling	<input checked="" type="checkbox"/> Interactive	<input checked="" type="checkbox"/> Ops	<input checked="" type="checkbox"/> Skeleton	

Extensions

 Save as animated gif Import-Export	 DeformationTracker Uncategorized	 KymoResliceWide Stacks	 RGB to Montage Color processing
 Bio Formats Import-Export, SciJava, OME	 Deforming Mesh 3D Uncategorized	 Labkit Uncategorized	 Ridge Detection Uncategorized
 DICOM Import-Export	 Delanun Voronoi Uncategorized	 Landmark Correspondences Transform, Registration	 RiFRET Analysis
 FIBSEM importer Import-Export	 Delayed Snapshot Scripting	 Lasso and Blow Tool Segmentation	 Robust clump splitting Uncategorized
 Formats Import-Export	 Denoiseg Uncategorized	 Level Sets Uncategorized	 Roi 1-click tools Image Annotation
 LSM Toolbox Import-Export	 Descriptor-based registration (2d) Uncategorized	 LimeSeg Uncategorized	 ROI Conversions Uncategorized
 LuraWave Import-Export	 Descriptor based registration Uncategorized	 LinearDistance Uncategorized	 ROI Picker Uncategorized
 OlympusImageJPlugin Import-Export	 Detrendr	 Linear Kuwahara Tutorials	 Rolling Ball Background Subtract Filtering

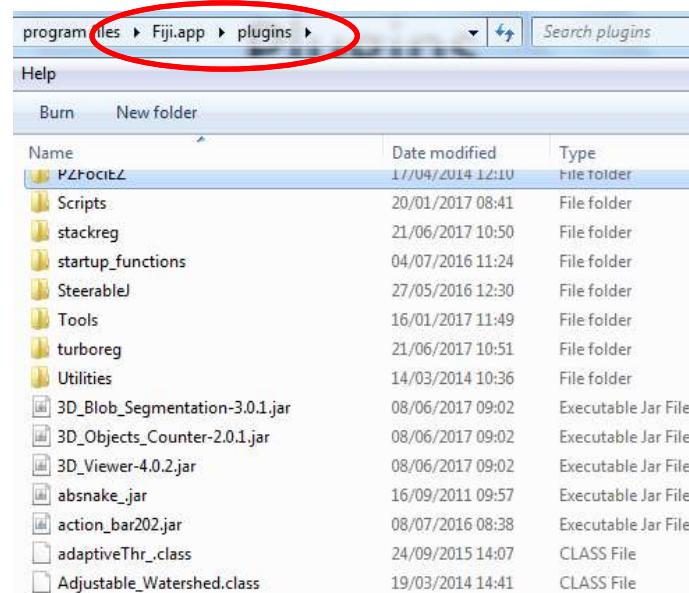
Install plugin via update



Plugins

Download from the website and install in the subfolder ‘plugins’. Restart Fiji.

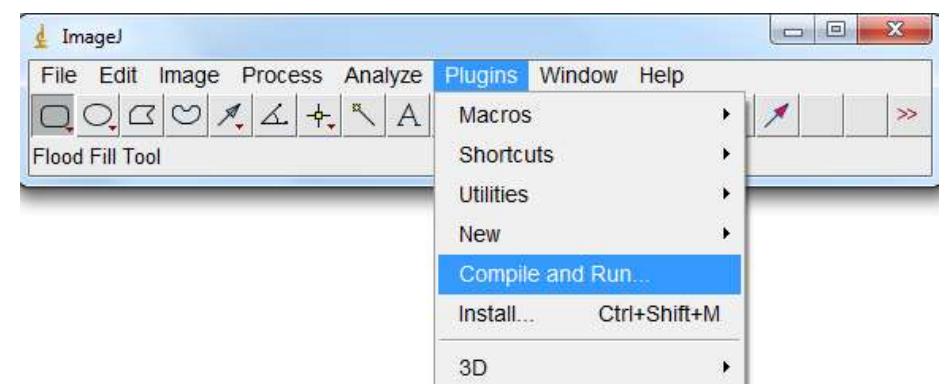
- When *.class and *.jar files.
(*.jar files are *.zip files; collection of files.)



Plugins

Download from the website and install in the subfolder ‘plugins’. Restart Fiji.

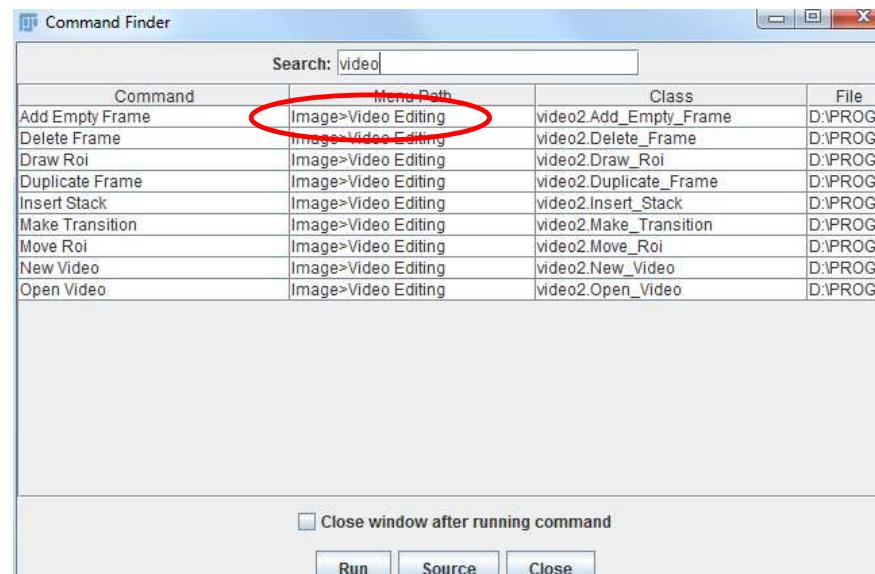
- When *.class and *.jar files.
(*.jar files are *.zip files; collection of files.)
- If plugin file is *.java you have to use submenu: *Plugins > Compile and Run*; **this works only in ImageJ!**
- Take care capitals in name are maintained.



Plugins

Plugins not always in Plugins menu, so how to find them?
For example: Video editing (Video_Editing.jar)

Use search bar or *Plugins > Utilities > Find Commands*



Note

- All ImageJ plugins can be used in Fiji.
- You can have more than one ImageJ/Fiji installation on a computer.
- If you use Fiji, don't forget all those other nice plugins not included in Fiji.

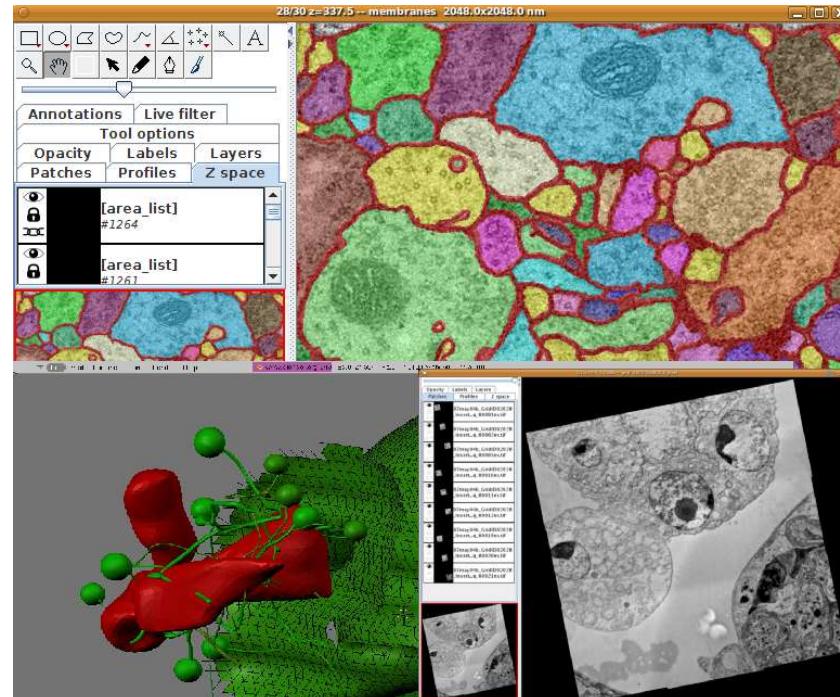
Don't forget to put a reference to the plugin in your publication!!

Plugins

Some plugins are almost applications by themselves, build on top of ImageJ

TrakEM2

TrakEM2 is an ImageJ plugin for morphological data mining, three-dimensional modelling, image stitching, registration, editing and annotation



<https://imagej.net/plugins/trakem2/>

Segmentation

https://imagej.net/list-of-extensions

Benchmark	Digital Volume Flattening	ImageScience	Neuron	Registration	Tutorials
<input type="checkbox"/> Binary	<input type="checkbox"/> Digital Volume Unrolling	<input type="checkbox"/> ImgLib	<input type="checkbox"/> Noise	<input type="checkbox"/> SciJava	<input type="checkbox"/> Uncategorized
<input type="checkbox"/> CellProfiler	<input type="checkbox"/> Entropy	<input type="checkbox"/> Import-Export	<input type="checkbox"/> OME	<input type="checkbox"/> Scripting	<input type="checkbox"/> Unmaintained
<input type="checkbox"/> Classification	<input type="checkbox"/> Example Data	<input type="checkbox"/> Integral Image	<input type="checkbox"/> Object Detection	<input checked="" type="checkbox"/> Segmentation	<input type="checkbox"/> User Interface
<input type="checkbox"/> Colocalization	<input type="checkbox"/> Feature Extraction	<input type="checkbox"/> Interactive	<input type="checkbox"/> Ops	<input type="checkbox"/> Skeleton	<input type="checkbox"/> Visualization
<input type="checkbox"/> Color Processing	<input type="checkbox"/> Fiji	<input type="checkbox"/> Lisp	<input type="checkbox"/> Optic Flow	<input type="checkbox"/> Stacks	<input type="checkbox"/> plugin
<input type="checkbox"/> Complexity	<input type="checkbox"/> Filtering	<input type="checkbox"/> MATLAB	<input type="checkbox"/> Particle Analysis	<input type="checkbox"/> Stitching	
<input type="checkbox"/> Cookbook	<input type="checkbox"/> Fractal dimension	<input type="checkbox"/> Machine Learning	<input type="checkbox"/> Pattern Recognition	<input type="checkbox"/> Super-resolution	
<input type="checkbox"/> Deconvolution	<input type="checkbox"/> Gut	<input type="checkbox"/> Mathematical Morphology	<input type="checkbox"/> Perfusion	<input type="checkbox"/> Time signal analysis	

3D Blob Segmentation 3D, Segmentation
3D ImageJ Suite 3D, Analysis, Filtering, Segmentation, Mathematical Morphology
3D Segmentation 3D, Segmentation
Auto Local Threshold Segmentation
Auto Threshold Segmentation
Automatic optimal filament segments Segmentation
Balloon segmentation Segmentation
BAR Analysis, Filtering, Image Annotation, Scripting, Segmentation
BioVoxel Toolbox BioVoxel Toolbox Particle Analysis, Segmentation
BioVoxel 3D Box Particle Analysis, Segmentation
Classic Watershed Segmentation, Mathematical Morphology
CMP-BIA tools Segmentation
Dendritic Spine Counter Annotation, Segmentation, Feature Extraction
Denoiseg Deep learning, Denoising, Segmentation
Distance Transform Watershed Segmentation, Mathematical Morphology

Dithering Segmentation
FAST Analysis, Segmentation
FiloQuant Analysis, Segmentation, Object Detection
Graph Cut Segmentation, Tutorials
Gray Morphology Segmentation, Mathematical Morphology
Gut Analysis Toolbox Analysis, Segmentation, Neuron, Gut
Interactive Marker-controlled Watersh Segmentation, Mathematical Morphology
Interactive Watershed Segmentation
IsoData Classifier Segmentation
Lasso and Blow Tool Segmentation
LimeSeg Segmentation
LungJ Segmentation, Binary, Filtering, Image Annotation
Marker-controlled Watershed Segmentation, Mathematical Morphology
Mask(s) from ROI(s) Image Annotation, Segmentation
Max Inscribed Circles Segmentation, Feature Extraction

Maximum Entropy Threshold Segmentation
Mixture Modeling Thresholding Segmentation
MorphoLibJ Analysis, Filtering, Segmentation, Mathematical Morphology
Morphological Segmentation Segmentation, Mathematical Morphology
MOSAICsuite Particle Analysis, Filtering, Colocalization, Deconvoluti Segmentation
Multi Otsu Threshold Segmentation
MultiThresholder Segmentation
Public data sets Example Data, Segmentation, Tracking
RATS: Robust Automatic Threshold Se Segmentation, Tutorials
Region Competition Segmentation, Deconvolution
Segment blob in 3D Viewer Segmentation
Segmentation Editor Segmentation
Snakuscule Segmentation
SNT Segmentation, Neuroanatomy

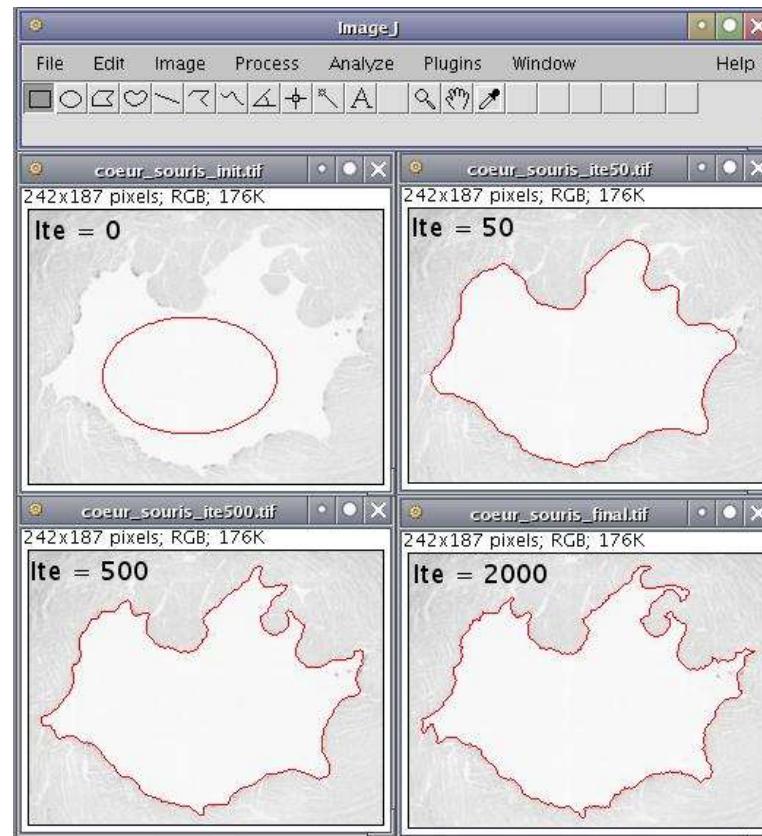
Squash Segmentation, Colocalization, Deconvolut Segmentation
Statistical Region Merging Segmentation, Segmentation
TAPAS Automation, Analysis, Filtering, Segmentation
TrackMate actions Segmentation, Tracking
Export tracking results in the cell track Segmentation, Tracking, Benchmark Segmentation
TrackMate-Helper Segmentation, Tracking, Benchmark
Multi Otsu Threshold Segmentation
TrackMate FAQ Tracking, Segmentation
TrackMate Segmentation, Tracking
Public data sets Example Data, Segmentation, Tracking
Manual editing of tracks using TrackM Segmentation, Tracking
Manual tracking with TrackMate Segmentation, Tracking
Scripting TrackMate Tracking, Segmentation
TrackMate-Cellpose Segmentation, Tracking
Setting keys for TrackMate detectors a Tracking, Segmentation, Scripting
TrackMate display settings Tracking, Segmentation
TrackMate-Ilastik Segmentation, Tracking, Machine Learning

TrackMate-Label-Image-Detector Segmentation, Tracking
TrackMate Mask detector Segmentation, Tracking
TrackMate-MorphoLibJ Segmentation, Tracking
TrackMate-StarDist Segmentation, Tracking, Deep Learning
TrackMate-Thresholding-Detector Segmentation, Tracking
TrackMate v7 detectors Tracking, Segmentation
TrackMate-Weka Segmentation, Tracking, Machine Learning
Using TrackMate from MATLAB Segmentation, Tracking
Trainable Segmentation Plugin (depre Segmentation
TrakEM2 Registration, Segmentation, TrakEM2, Image Annotat Neuroanatomy
Trainable Weka Segmentation Segmentation, Machine Learning
VTEA > Volumetric Tissue Exploration > Segmentation, Visualization
Xlib Analysis, Filtering, MATLAB, Particle Analysis, Segment Skeleton
Zelige tutorial Projection, Segmentation

https://imagej.net/list-of-extensions

Segmentation

Active contour (Snake)

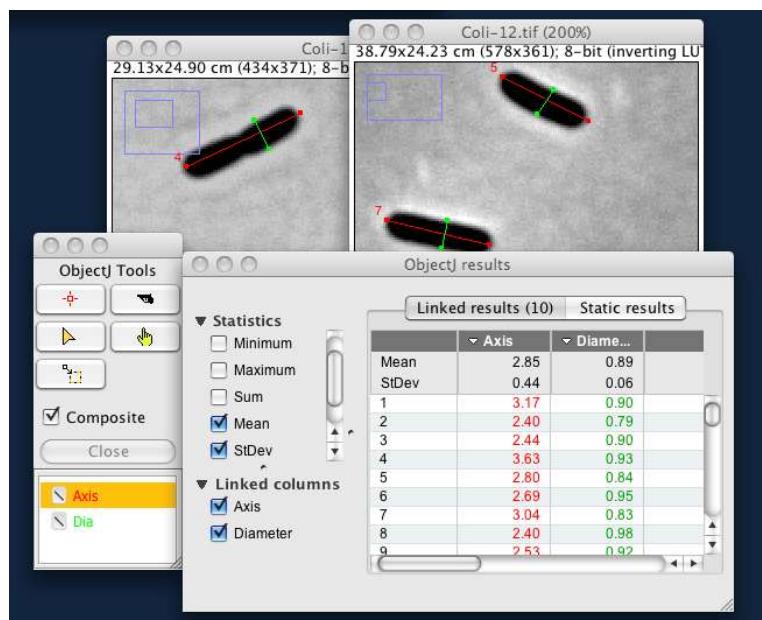


https://imagejdocu.list.lu/plugin/segmentation/active_contour/start

Segmentation

ObjectJ: Non-destructive marking and analysing of images.

Allows Automatic marking and measuring of objects. Many possibilities.



Alphabetic:

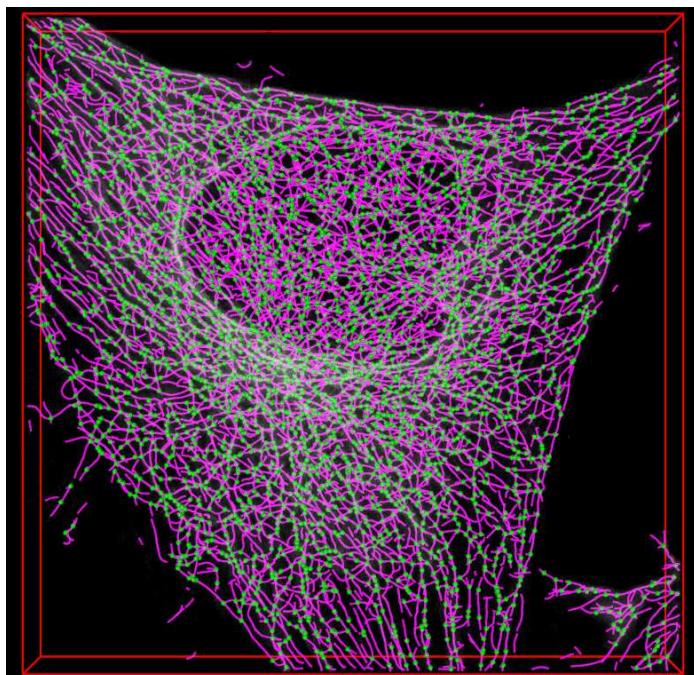
*These examples do not require ObjectJ

- [Align fluorescence channels*](#)
- [BX_macros*](#)
- [CCSim Cell cycle simulation*](#)
- [Cindy's cells](#)
- [ChainTracer](#)
- [Cell Counter](#)
- [Coli-Inspector](#)
- [CrossProfiles](#)
- [Elliptical oocytes](#)
- [Eucalyptus leafs](#)
- [Fish eggs + Hough transform](#)
- [Import_ND2_files*](#)
- [MeiosisTool](#)
- [Otoliths_\(interactive\)](#)
- [PeakFinder Tool*](#)
- [Perspective Stack View*](#)
- [Roi Counter](#)
- [Side-by-side movie*](#)
- [SIPcharts*](#)
- [SporeTracker](#)
- [SporeTrackerX](#)
- [SporeTrackerB](#)
- [Stabilize Movie in Chunks*](#)
- [StampFishEggs](#)
- [ThioBac_Tester*](#)
- [Tree rings](#)
- [utils](#)
- [Weibel Grid Cell counting](#)
- [Zebrafish Immunoreaction](#)

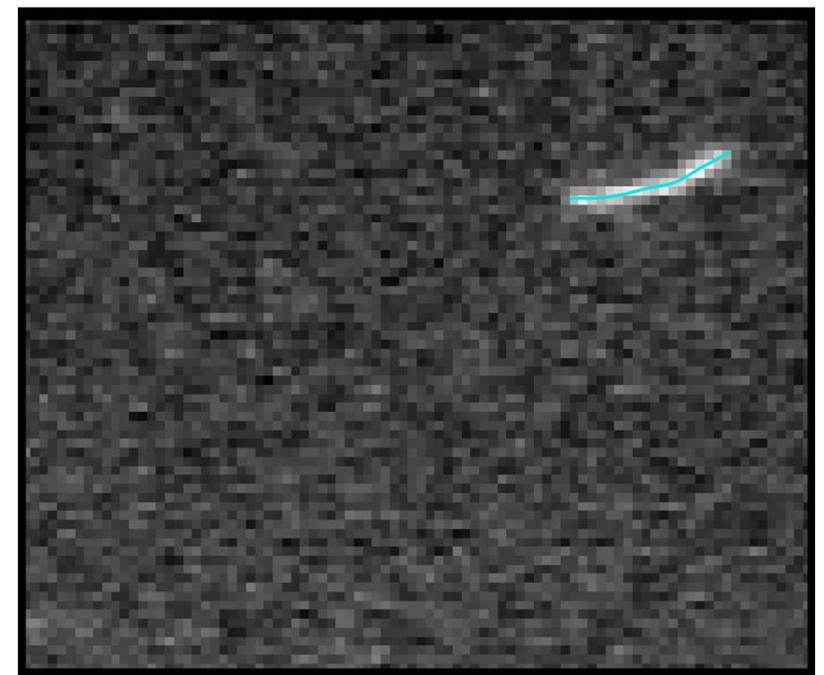
<https://sils.fnwi.uva.nl/bcb/objectj/index.html>

Segmentation

SOAX Detection of filaments in 2D and 3D images



TSOAX – time laps analysis

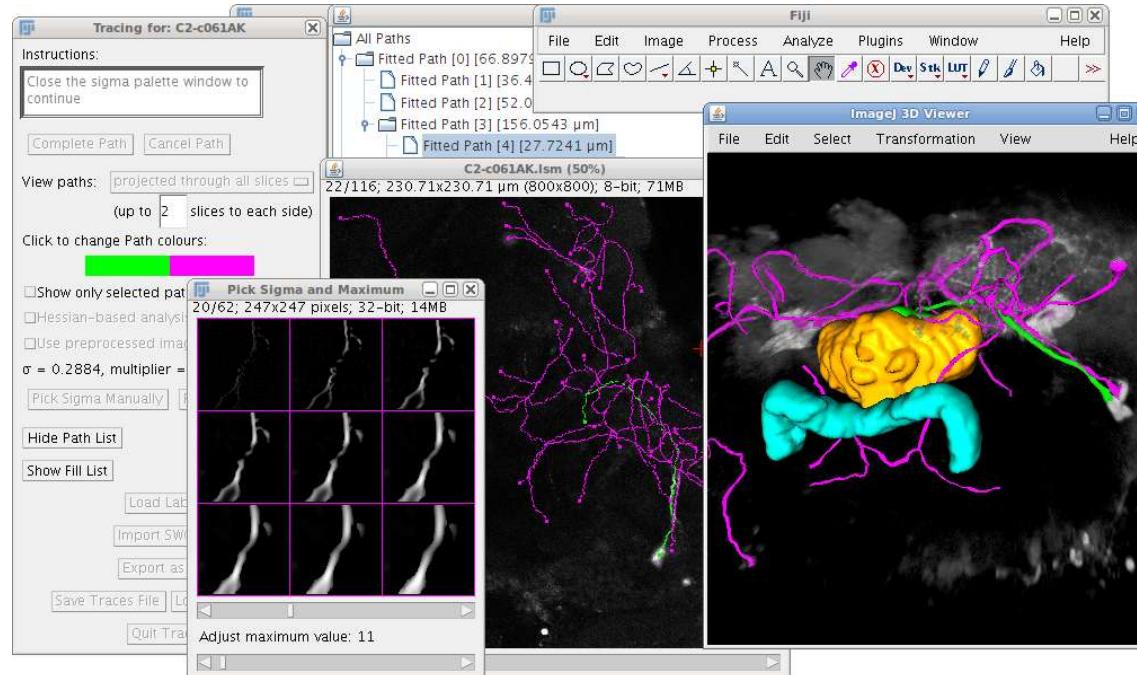


Measures filament lengths, junctions and centrelines

<https://www.lehigh.edu/~div206/soax/>

Segmentation

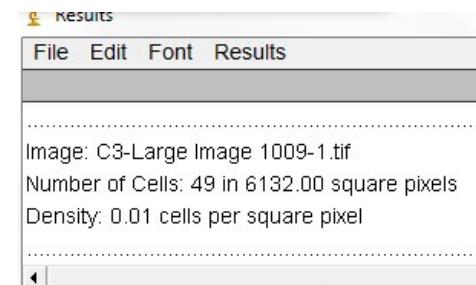
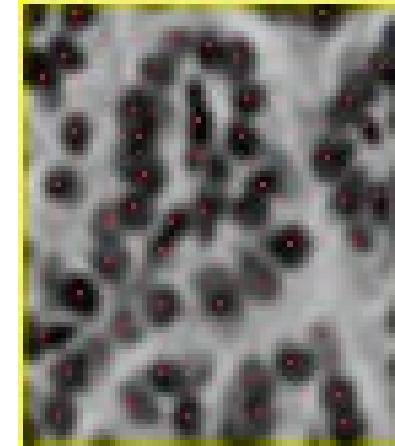
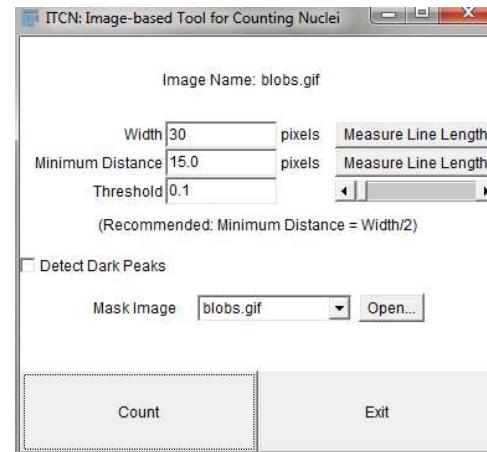
SNT (Simple Neurite Tracer): semi-automatic tracing, analysis, modelling and visualization of neurons or other tube-like structures (e.g. blood vessels) through 3D image stacks.



<https://imagej.net/plugins/snt/>

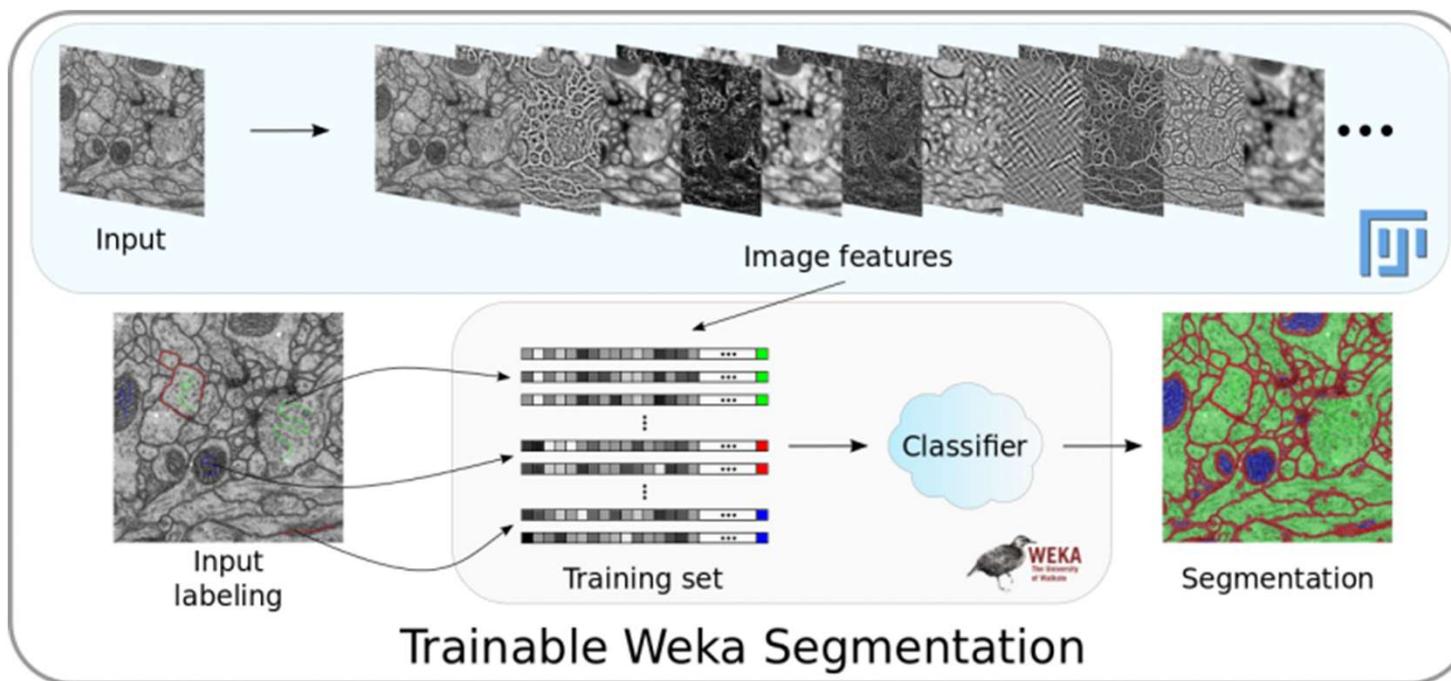
Segmentation

Automatic Nuclei Counter



<http://bioimage.ucsb.edu/docs/automatic-nuclei-counter-plugin-imagej>

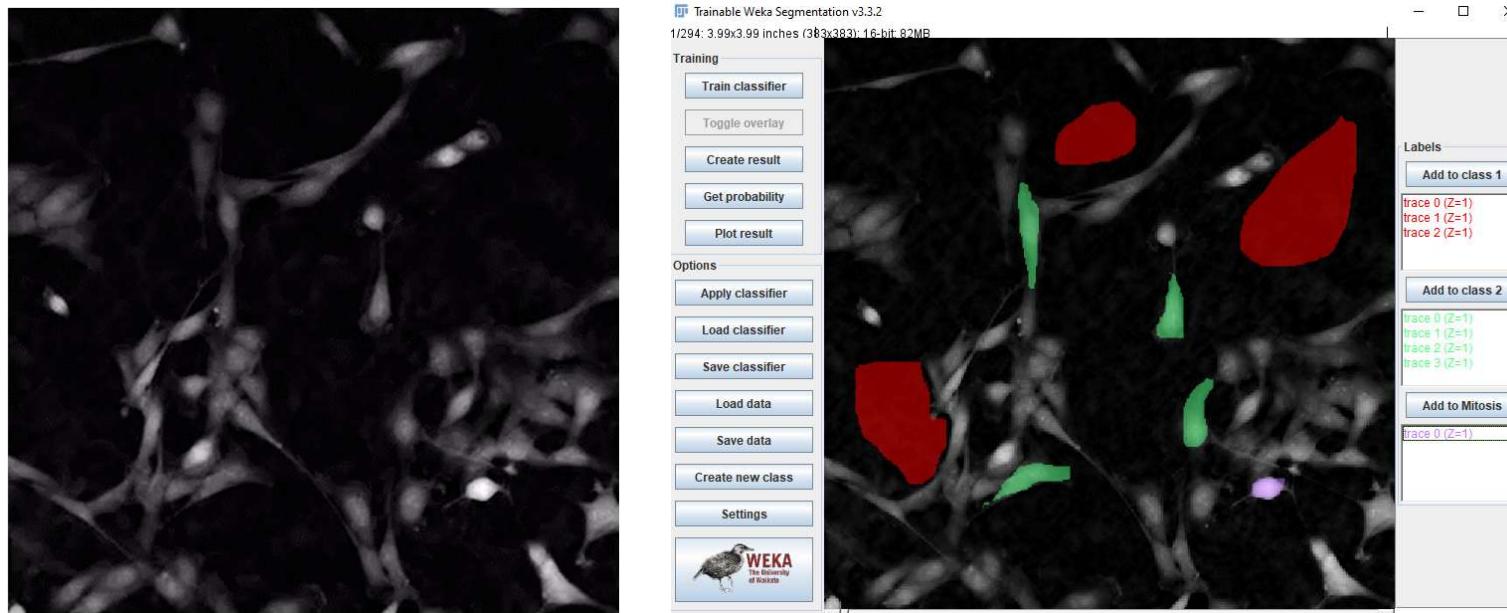
Segmentation



Uses a collection of machine learning algorithms.

Segmentation

Advanced Weka Segmentation:

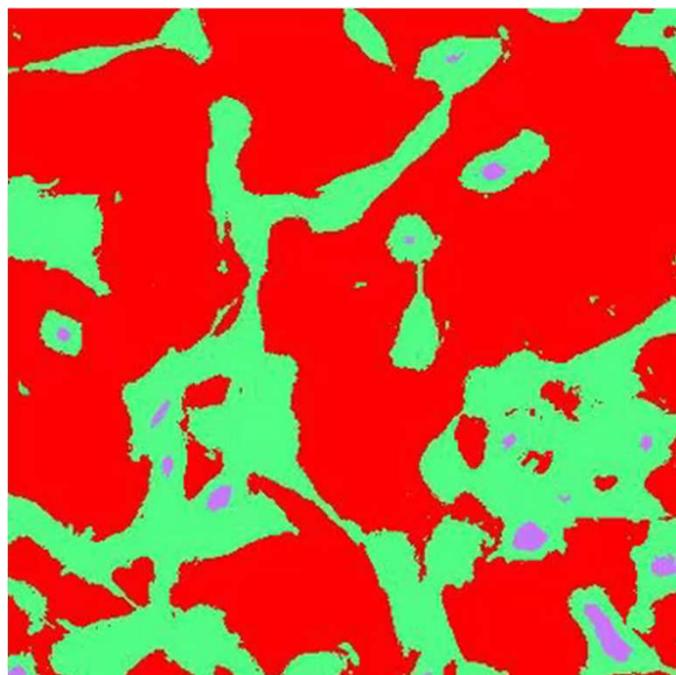


Train classifier

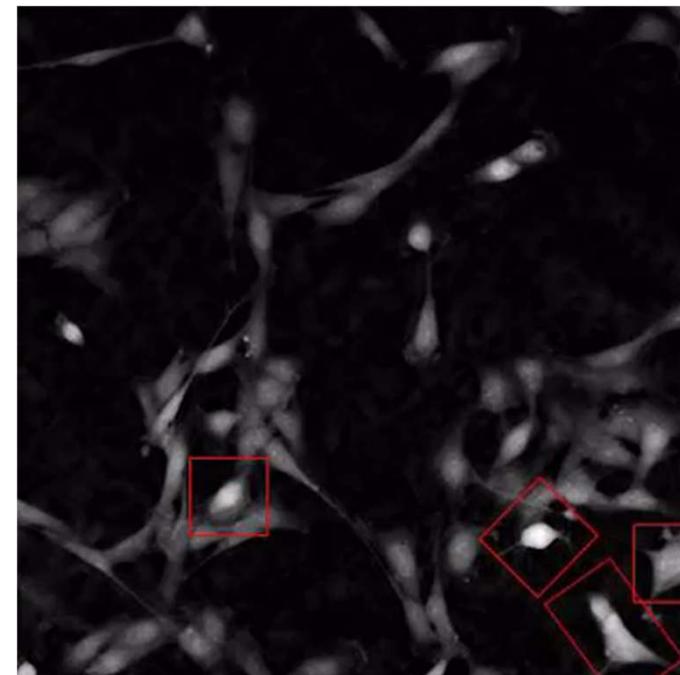
<https://imagej.net/plugins/tws/>

Segmentation

Advanced Weka Segmentation:



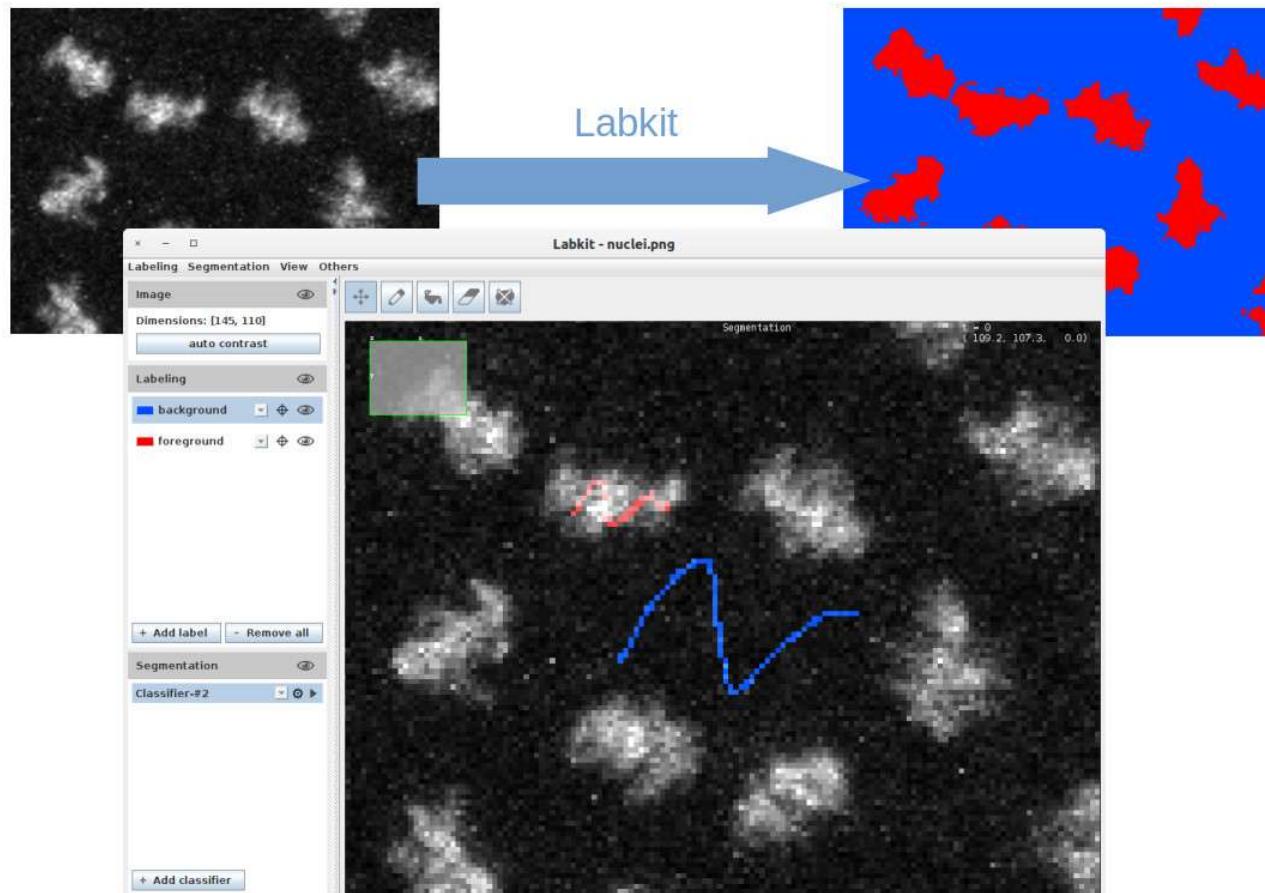
Create results



Extract information

<https://imagej.net/plugins/tws/>

Segmentation



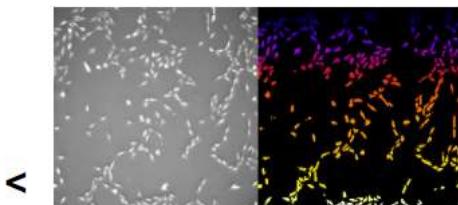
Segmentation

[DeepImageJ](#) is a user-friendly plugin that enables the use of a variety of pre-trained deep learning models in ImageJ and Fiji. The plugin bridges the gap between deep learning and standard life-science applications. DeepImageJ does not require any deep learning expertise.

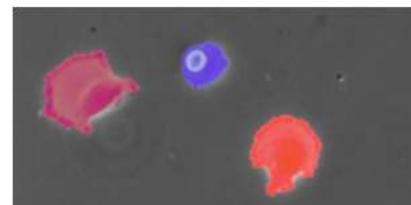
[!\[\]\(a55c25fbb580d84cdfb2c2dffea3b535_img.jpg\) Install deepImageJ](#)[Try it yourself](#)[Biolimage Model Zoo !\[\]\(1e498f4e3b32478700bd65b6a2c7eed3_img.jpg\)](#)[Other models](#)

version 2.1

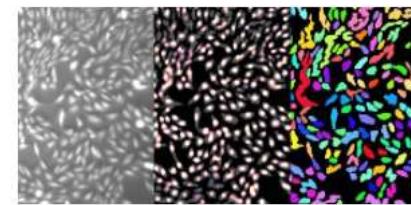
DeepImageJ trained models



U-Net Pancreatic Segmentation
[Binary Segmentation](#) [Phase Contrast](#)
Data: [Cell Tracking Challenge](#)
Training: [deepImageJ & I. Arganda-Carreras](#)



U-Net Glioblastoma Segmentation
[Binary Segmentation](#) [Phase Contrast](#)
Data: [Cell Tracking Challenge](#)
Training: [João Luis Soares Lopes \(EPFL\)](#)



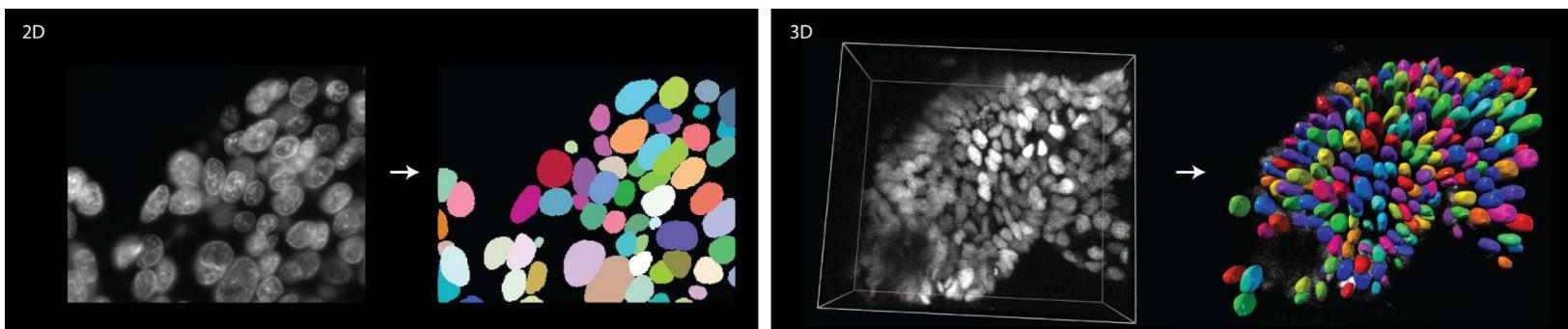
MU-Lux (CTC) PhC-C2DL-PSC cells
[Instance Segmentation](#) [Phase Contrast](#)
F. Lux & P. Matula, arXiv, 2020

A first selection of state-of-the-art models from various groups has been made available in the [Biolimage Model Zoo](#). Beyond its direct use, we expect deepImageJ to contribute to the spread and validation of deep learning models in life-science applications.

[New functionalities of deepImageJ 2.1](#)[News & Social media](#)

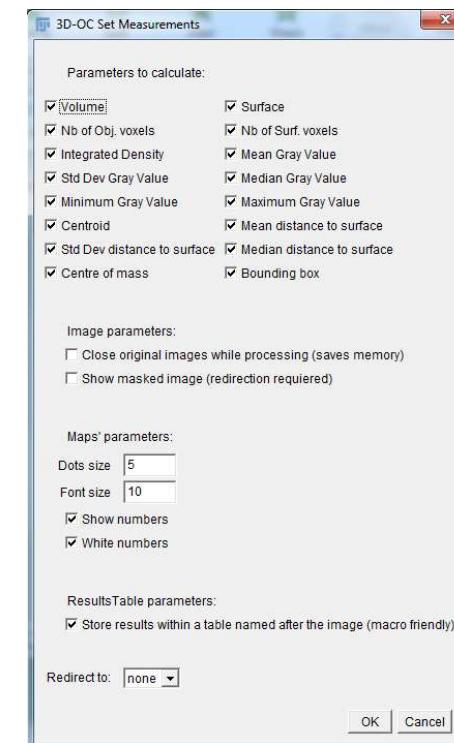
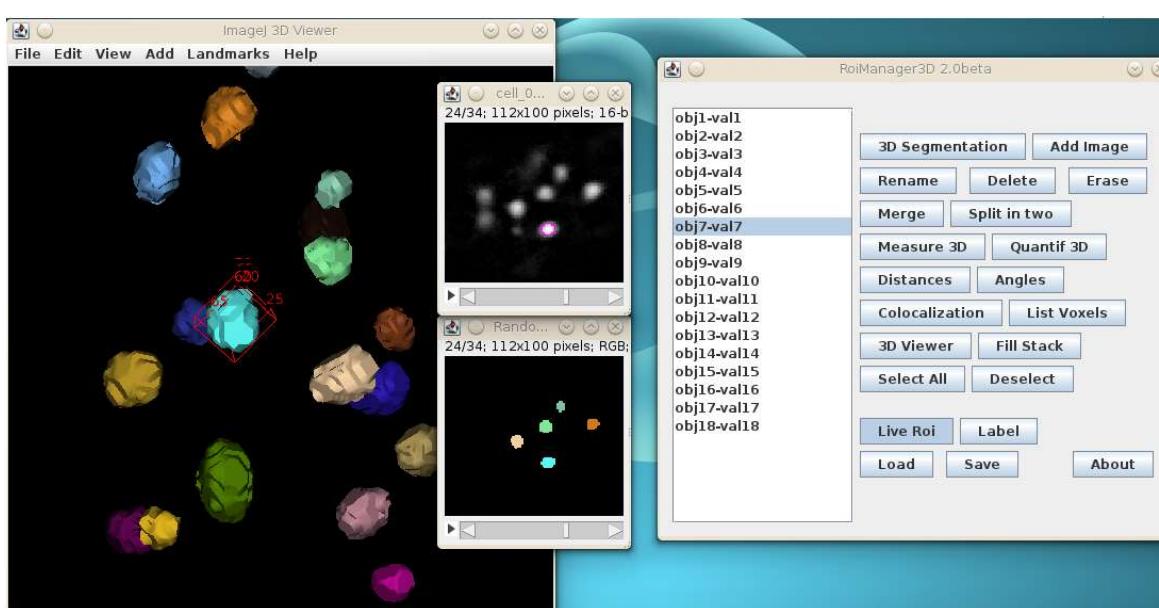
Segmentation

StarDist: a trained model to detect star-convex objects in 2D and 3D images.



<https://github.com/stardist/stardist/>

3D ROI manager

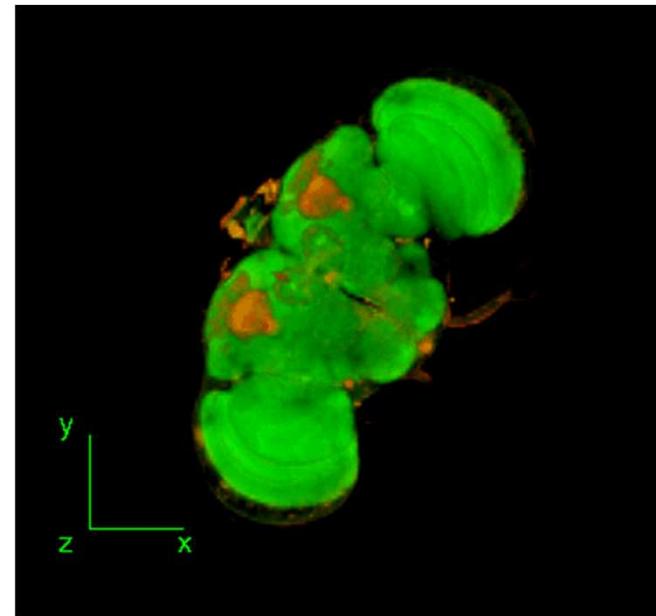


https://imagejdocu.list.lu/plugin/stacks/3d_ij_suite/start

3D viewer

3D Viewer: provides hardware-accelerated 3D visualization of image stacks as volumes, surfaces and orthoslices.

You can export into 3D formats that you can use in other programs like Blender.



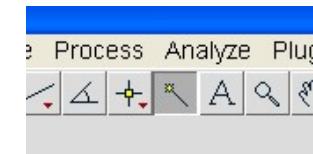
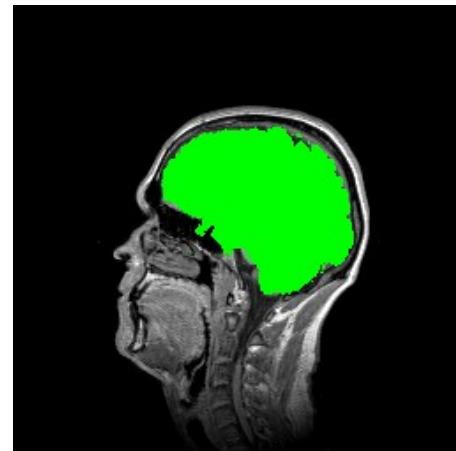
3D data sets

Sample T1-head.tif



Make RGB
(*Image > Type*)

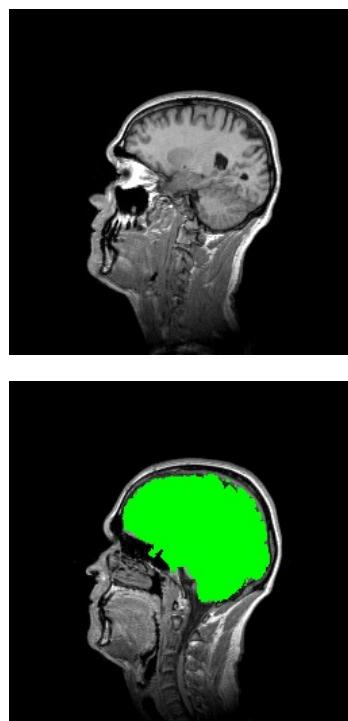
Select using wand tool and
add selection to ROI manager
(*Analyze > Tools > ROI Manager*)



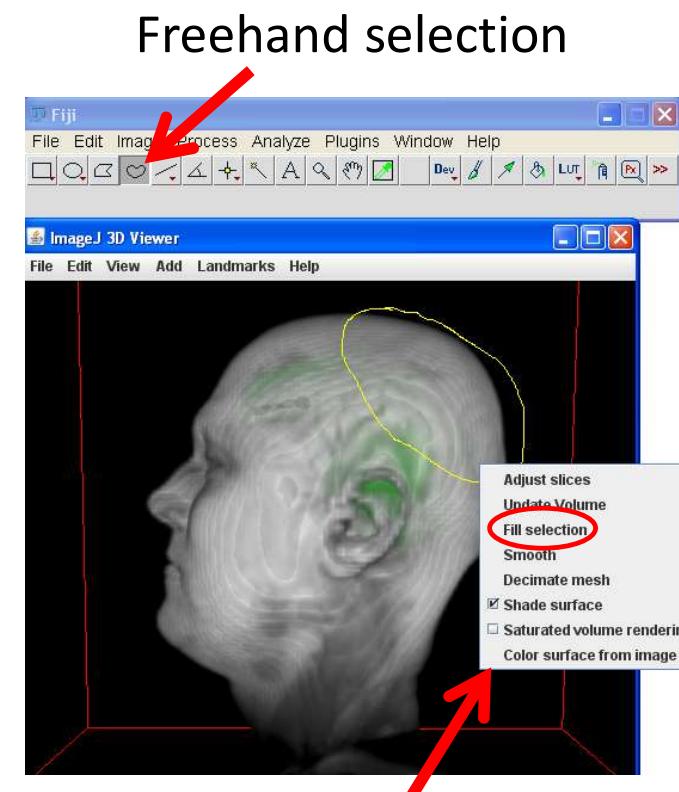
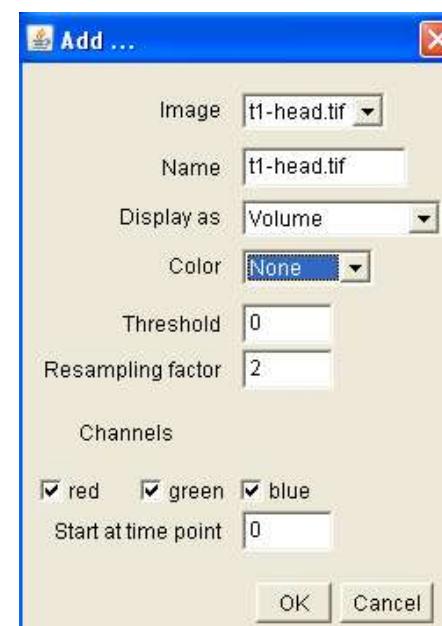
In ROI manager:
More >> Options > select Associate
“Show All” ROIs with Slices.

Wand tool: Tolerance 40; Shift = add selection;
Alt = delete selection;
Ctrl + Shift + E = restore selection

3D viewer



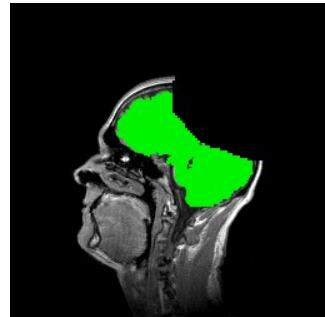
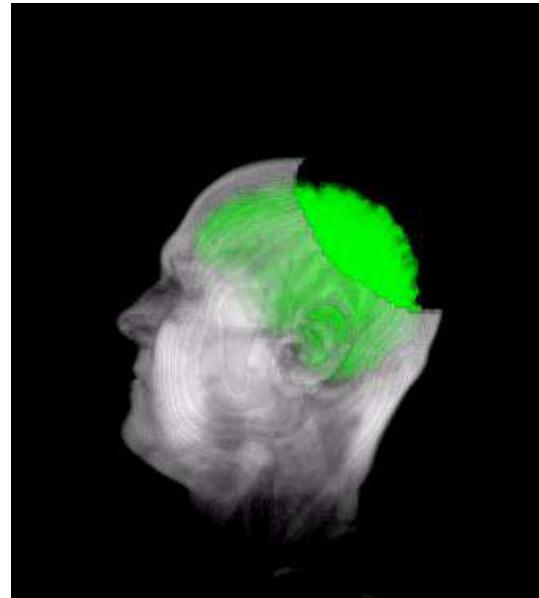
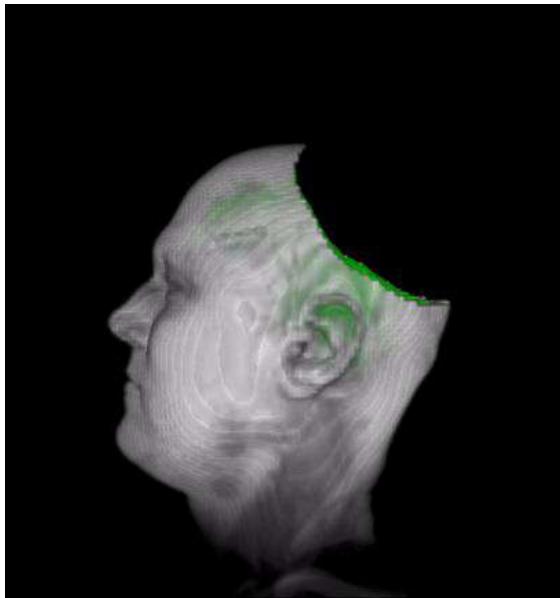
Plugins > 3D Viewer



Freehand selection

Right mouse click in selection

3D viewer



Use ROI manager
to restore ROIS

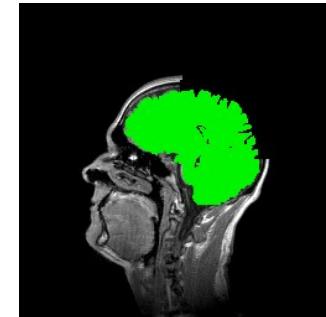


Image stabilization

Image Stabilizer: stabilizes jittery image stacks and supports both grayscale and colour images.

Alternatives (for example):

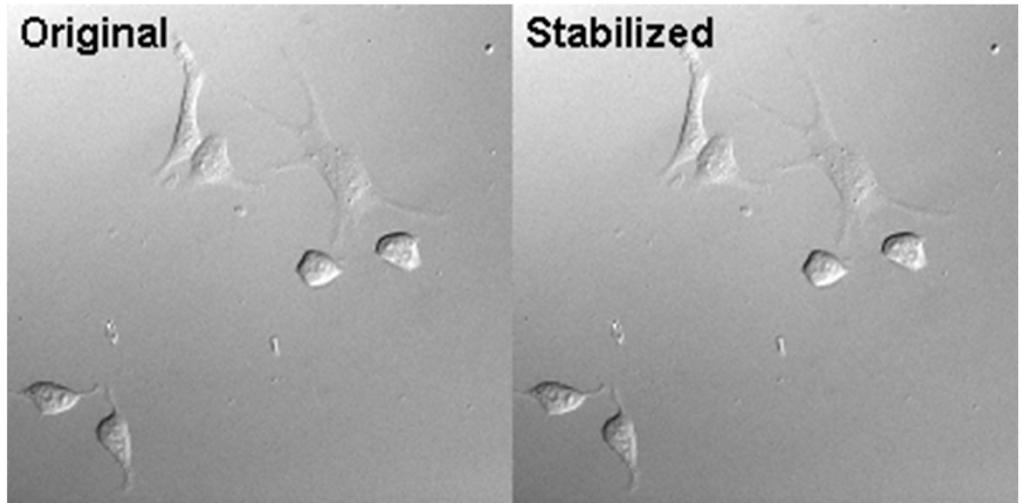
StackReg + TurboReg:

Register Virtual Stack Slices

See also:

Linear Stack Alignment with SIFT

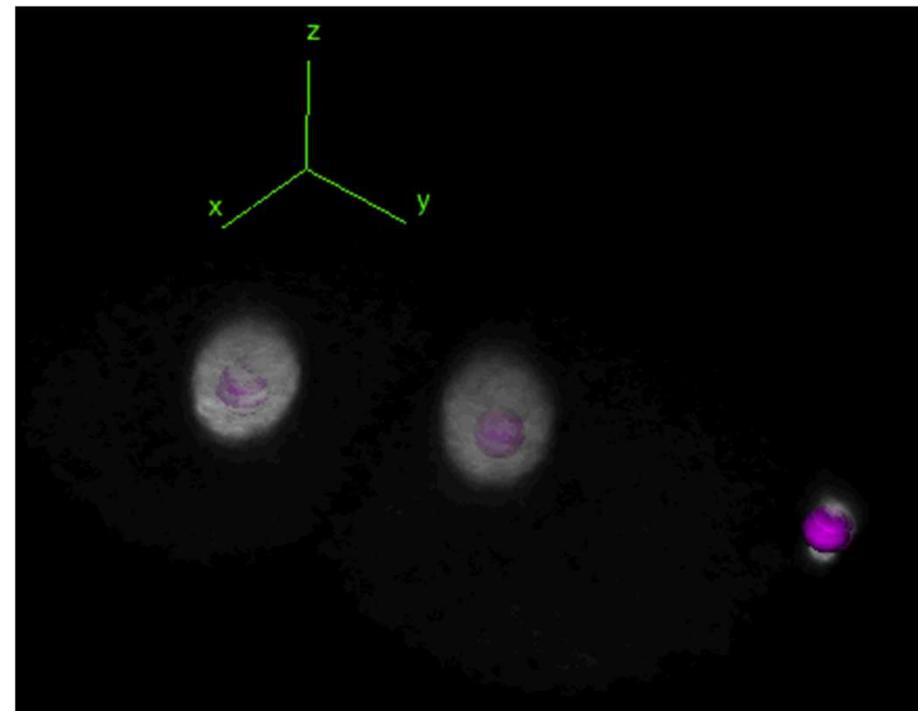
Elastic Alignment and Montage



http://fiji.sc/wiki/index.php/Image_Stabilizer

Particle tracking

TrackMate: automatic particle tracking in 2D and 3D.

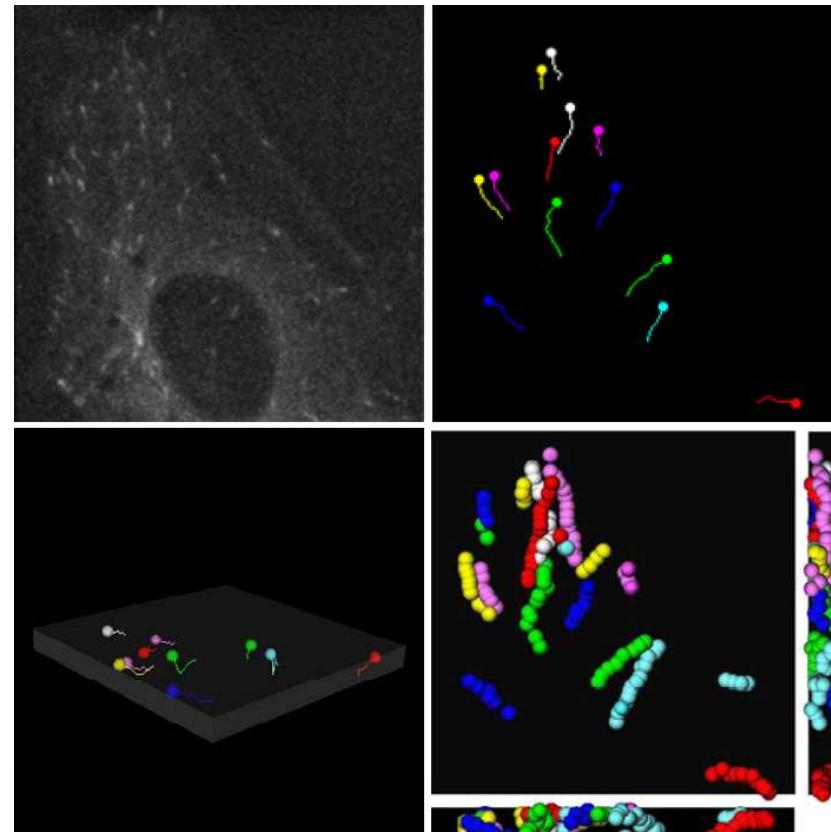


The first 2 hours of a *C.elegans* embryo development, followed in 3D over time using TrackMate (strain: [AZ212](#)). Source: TrackMate website: <https://imagej.net/plugins/trackmate/>

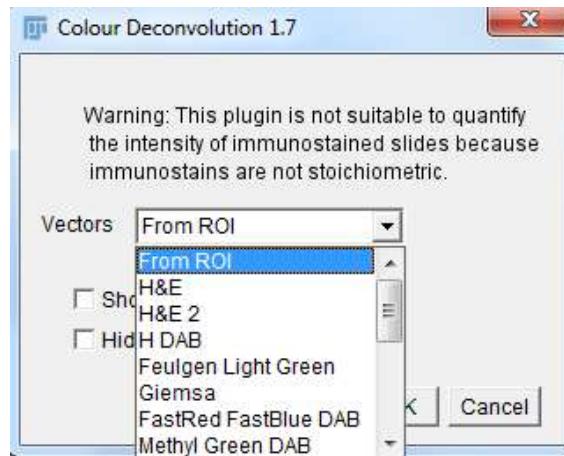
Particle tracking

Manual Tracking: quantify movement of objects between frames of a temporal stack, in 2D and 3D.

Many other tracking plugins.



Immunohistochemistry

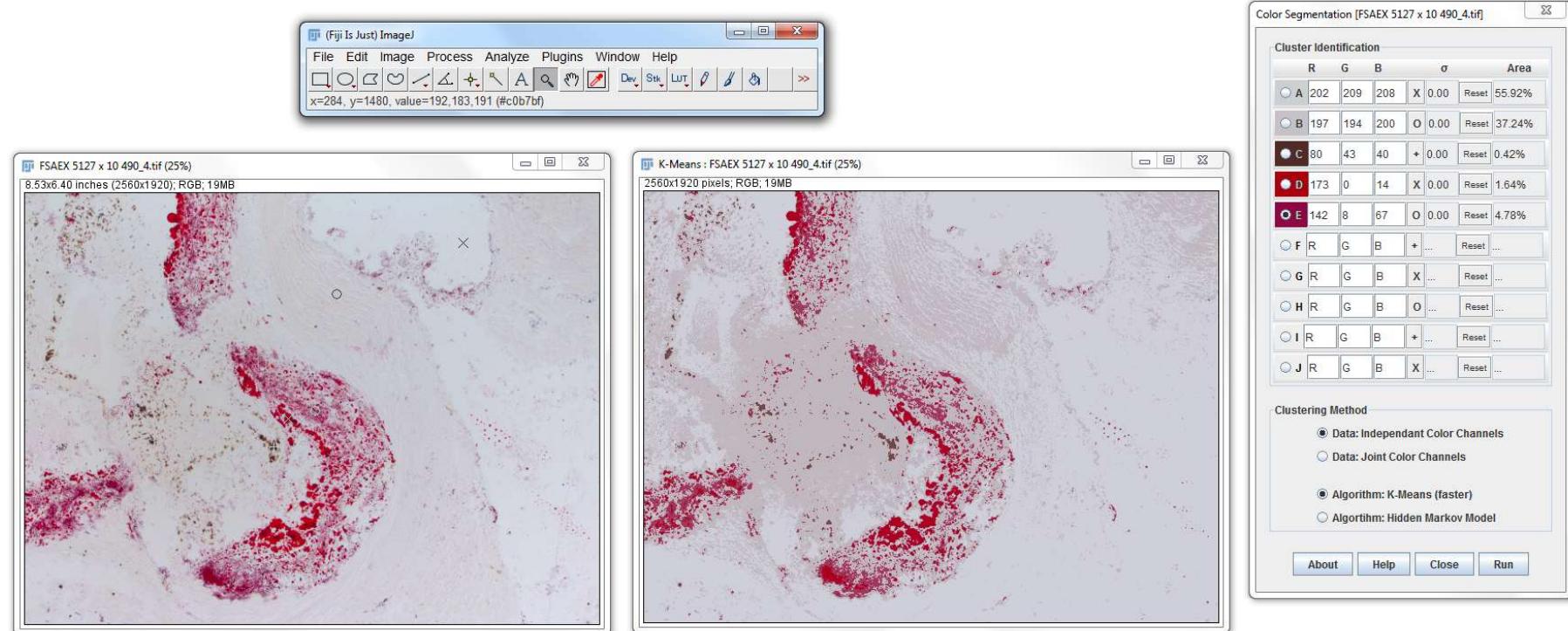


Gabriel Landini

Haematoxylin and Eosin (H&E) x2
Haematoxylin and DAB (H DAB)
Feulgen Light Green
Giemsa
Fast Red, Fast Blue and DAB
Methyl green and DAB
Haematoxylin, Eosin and DAB (H&E DAB)
Haematoxylin and AEC (H AEC)
Azan-Mallory
Masson Trichrome
Alcian blue & Haematoxylin
Haematoxylin and Periodic Acid - Schiff (PAS)
Brilliant blue
RGB subtractive
CMY subtractive
User values entered by hand
Values from rectangular ROIs

Immunohistochemistry

Color Segmentation



<http://bigwww.epfl.ch/sage/soft/colorsegmentation/>

Bar (Broadly Applicable Routines)

scripts using the BAR API

List of BARs

- Analysis
 - LoG-DoG Spot Counter, Multi ROI Profiler, Multichannel Plot Profile, Multichannel ZT-axis Profile, Smoothed Plot Profile
- Annotation
 - Combine Orthogonal Views, Cumulative Z Project, ROI Color Coder
- Data Analysis
 - Create Boxplot, Create Polar Plot, Distribution Plotter, Find Peaks, Fit Polynomial, Interactive Plotting
- Segmentation
 - Shen-Castan Edge Detector, Apply Threshold To ROI, Clear Thresholded Pixels, Remove Isolated Pixels, Threshold From Background, Wipe Background
- Snippets, BAR lib and Tutorials
 - Described in Scripting BARs
- Tools and Toolsets
 - Calibration Menu, List Folder Menu, Segment Profile, Shortcuts Menu, ROI Manager Tools, Toolset Creator
- Utilities
 - Commander

(Fiji Is Just) ImageJ

File Edit Image Process Analyze Plugins Window Help BAR Cookbook

BAR

Strahler Mask.tif
144.77x118.33 pixels (378x355); RGB, 524K

Data Analysis

Area Distribution
blobs.gif
256.00x254.00 µm (356x361); 16-bit; 255K

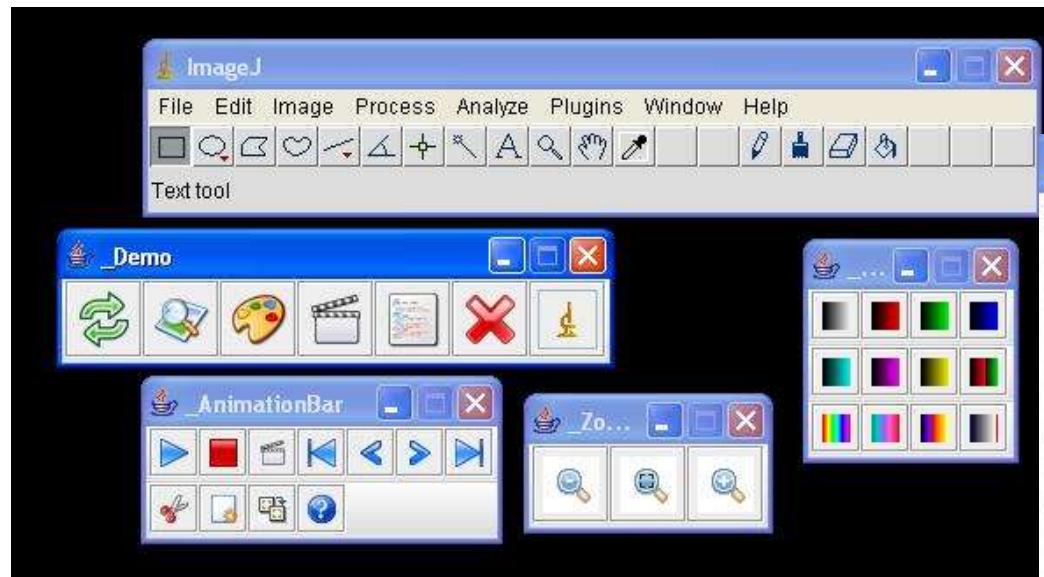
Box Plot

Mean Area
■ Type I ■ Type II

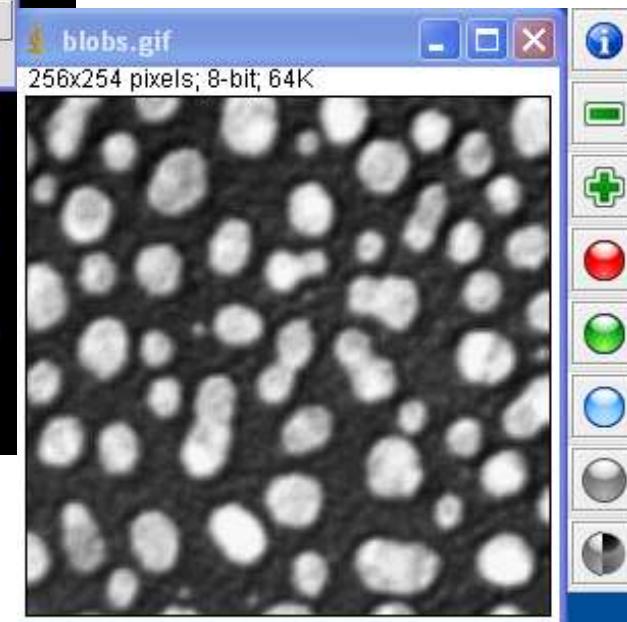
Manage update sites

A..	Name	URL	Host	Directory on Host
<input checked="" type="checkbox"/>	ImageJ	http://update.imagej.net/		
<input checked="" type="checkbox"/>	Fiji	http://fiji.sc/update/		
<input type="checkbox"/>	3D ImageJ Suite	http://sites.imagej.net/Tboudier/		
<input type="checkbox"/>	Angiogenesis	http://sites.imagej.net/Angiogenesis/		
<input type="checkbox"/>	Archipelago	http://sites.imagej.net/Lindsey/		
<input type="checkbox"/>	BAR	http://sites.imagej.net/Tiago/		
<input type="checkbox"/>	BioDataViewer	http://sites.imagej.net/Pietzsch/		
<input checked="" type="checkbox"/>	Bio-Formats	http://sites.imagej.net/Bio-Formats/		
<input type="checkbox"/>	Biomedgroup	http://sites.imagej.net/Biomedgroup/		
<input type="checkbox"/>	BioVoxel	http://sites.imagej.net/BioVoxel/		
<input type="checkbox"/>	Cookbook	http://sites.imagej.net/Cookbook/		
<input type="checkbox"/>	CMCI-EMBL	http://sites.imagej.net/Miura/		
<input type="checkbox"/>	CMP-BIA tools	http://sites.imagej.net/CMP-BIA/		
<input type="checkbox"/>	FFMPEG	http://fiji.sc/~schindelin/ffmpeg-plugins/		
<input type="checkbox"/>	GDSC	http://sites.imagej.net/GDSC/		

Action bar



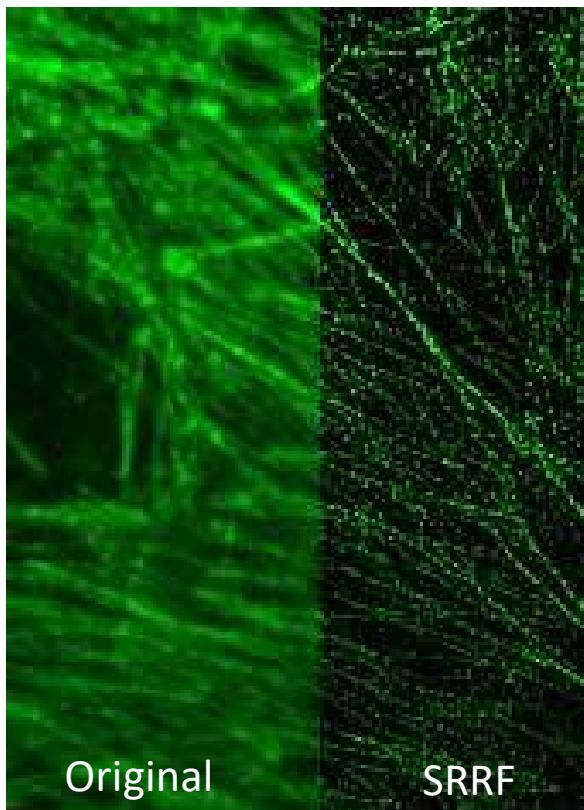
Create own menu bars



Alt-click action bar removes menu

Super-Resolution plugins

Super-Resolution Radial Fluctuations (NanoJ-SRRF plugin),



Fifty images per channel were taken on the VisiTech confocal laser microscope with an exposure time of 100 ms using the 100x/1.47 NA objective.

SRRF calculations were performed using the free available ImageJ plugin nanoJ-SRRF.

No special microscope or sample preparation required!

Gustafsson et al. (2016) Nat Comm DOI: 10.1038

Macros



ImageJ macro

A macro is a program that automates a series of ImageJ commands.

ImageJ has a special and relative easy macro language.

It allows user interaction if needed.

Macros are saved as *.txt or *.ijm and are normally installed in the ImageJ subfolder “macros”.

What can a macro do for you?

Create automations for image analysis pipelines.

Automated or semi-automated analysis of large data sets.

Automation or semi-automation of repetitive tasks.

Consistency in analysis.

Distribute common procedures.

Document analysis steps.

Speed up your work.

Add tools to the toolbar.

You can write simple to very complex macros.

[home](#) | [news](#) | [docs](#) | [download](#) | [plugins](#) | [resources](#) | [list](#) | [links](#)

Developer Resources

- o [Macro Language \(download PDF\)](#)
- o [Built-in Macro Functions](#)
- o [Introduction to Macro Programming](#) New
- o Macros

 - o [Macros Directory](#)
 - o [Examples](#)
 - o [Macro Tools](#)
 - o [Toolsets](#)

- o Scripting
 - o [Examples](#)
- o API Documentation (v1.45s)
- o Browsable Source

 - o [Online Source](#)
 - o [Source Code Archive](#)

- o Git Version Control Repository
- o [Writing ImageJ Plugins - A Tutorial](#)
- o [Programming Tutorials \(Albert Cardona\)](#)
- o [Imaging Book \(Burger and Burge\)](#)
- o [UML Class Diagram \(320KB Jpeg\)](#)
- o [Configuration File \(IJ_Props.txt\)](#)

Functions

[home](#) | [news](#) | [docs](#) | [download](#) | [plugins](#) | [resources](#) | [list](#) | [links](#)

Built-in Macro Functions

[A][B][C][D][E][F][G][H][I][J][K][L][M][N][O][P] [Print List](#)
[Q][R][S][T][U][V][W][X][Y][Z]

A [Top]

abs(n)

Returns the absolute value of *n*.

acos(n)

Returns the inverse cosine (in radians) of *n*.

Array Functions

These functions operate on arrays. Refer to the [ArrayFunctions](#) macro for examples.

Array.concat(array1,array2) - Returns a new array created by joining two or more arrays or values ([examples](#)).

Array.copy(array) - Returns a copy of *array*.

Array.deleteValue(array, value) - Returns a version of *array* where all numeric or string elements in the array that contain *value* have been deleted ([examples](#)). Requires 1.52o.

Array.deleteIndex(array, index) - Returns a version of *array* where the element with the specified index has been deleted. Requires 1.52o.

Array.fill(array, value) - Assigns the specified numeric value to each element of *array*.

Array.filter(array, filter) Returns an array containing the elements of '*array*' that contain 'filter'. Enclose the filter in parans to do regular expression matching. Requires 1.53f.

Array.findMaxima(array, tolerance) - Returns an array holding the peak positions (sorted with descending strength). 'Tolerance' is the minimum amplitude difference needed to separate two peaks. With v1.51n and later, there is an optional 'edgeMode' argument: 0=include edges, 1=exclude edges(default), 2=circular array. [Examples](#).

Array.findMinima(array, tolerance) - Returns an array holding the minima positions.

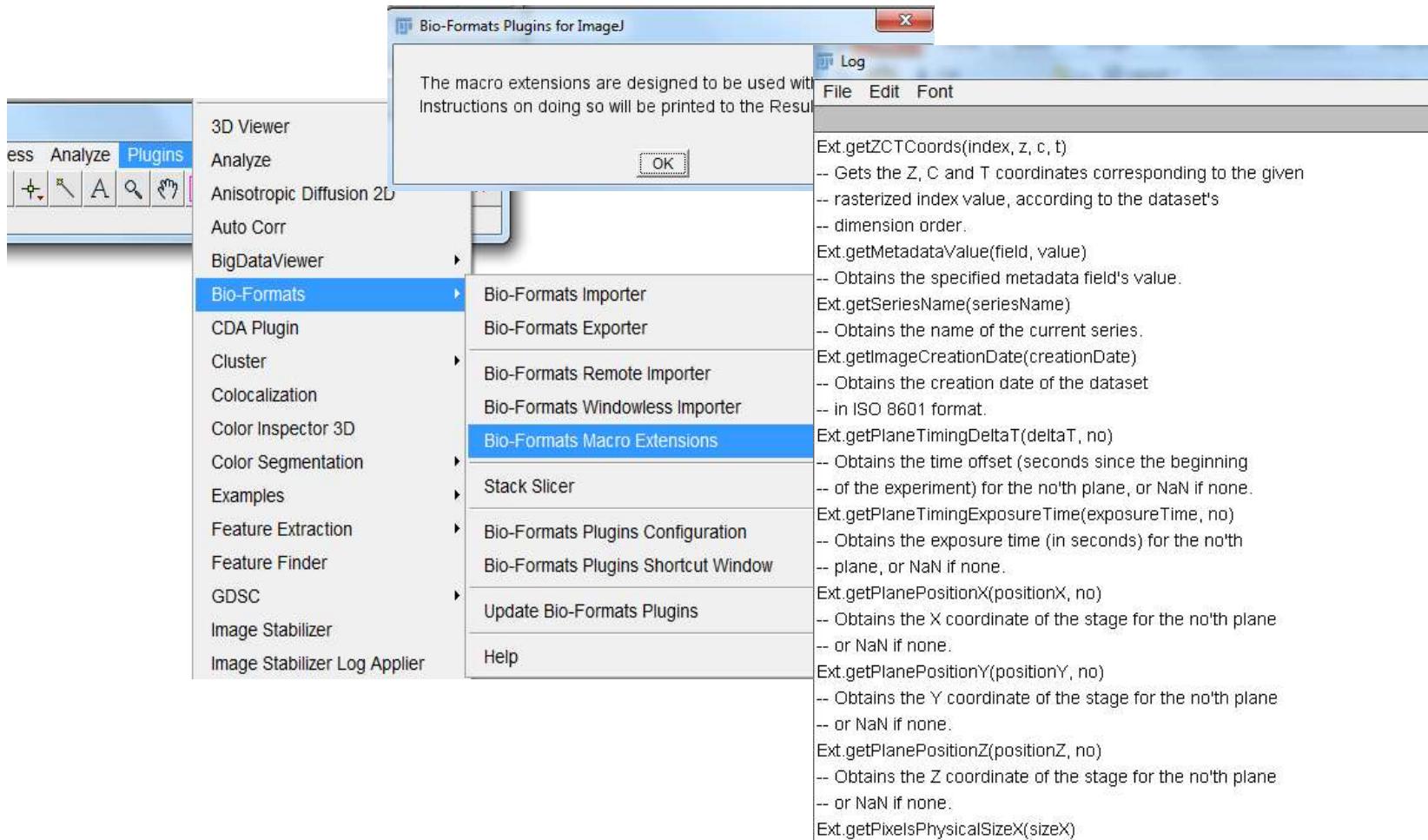
Macro extensions

Macro extensions are extra functions provided by a plugin that can be used in a macro

- Bio-Formats plugin
- PDF Macro extension
- Serial Macro Extensions
- LSM Toolbox
- Image5D Macro Language Extensions
- 3D ROI manager
- CLIJ2- a GPU-accelerated image processing library
-

You can add functions to a macro as well. However they are not general available, only within the macro.

Macro extensions



Resources

ImageJ website

[home](#) | [news](#) | [docs](#) | [download](#) | [plugins](#) | [resources](#) | [list](#) | [links](#)

Developer Resources

- o Macro Language ([download PDF](#))
- o Built-in Macro Functions
- o [Introduction to Macro Programming](#) New
- o Macros

 - o Macros Directory
 - o Examples
 - o Macro Tools
 - o Toolsets

- o Scripting
 - o Examples
- o API Documentation (v1.45s)
- o Browsable Source

 - o Online Source
 - o Source Code Archive

- o Git Version Control Repository
- o Writing ImageJ Plugins - A Tutorial
- o Programming Tutorials (Albert Cardona)
- o Imaging Book (Burger and Burge)
- o UML Class Diagram (320KB Jpeg)
- o Configuration File (IJ_Props.txt)

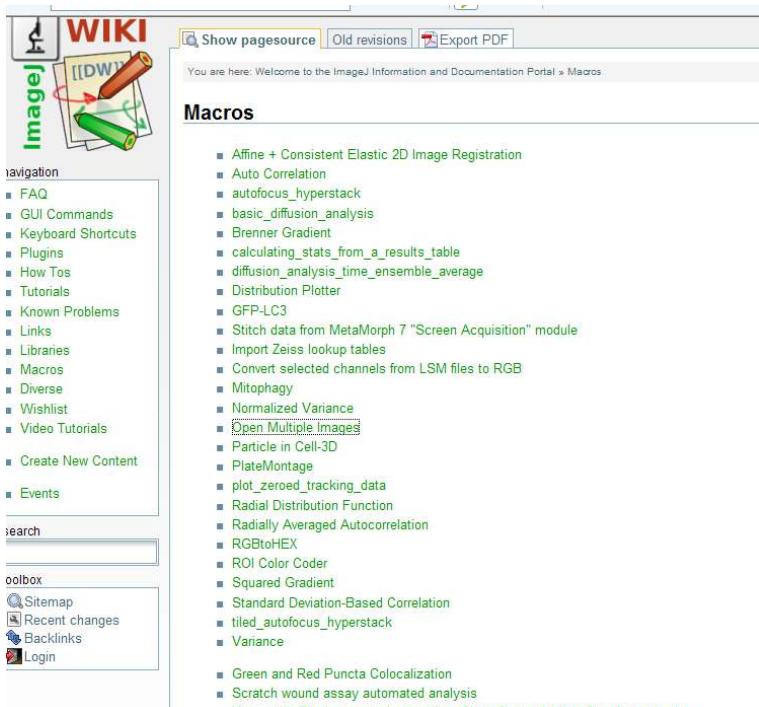
Index of /ij/macros

Name	Last modified	Size	Description
Parent Directory		-	
bsh/	14-Nov-2012 13:20	-	
class/	29-Jul-2010 14:18	-	
data/	29-Jul-2010 14:18	-	
examples/	26-Nov-2012 22:58	-	
images/	29-Jul-2010 14:18	-	
js/	05-Jun-2012 14:20	-	
misc/	12-Nov-2012 22:10	-	
tools/	03-Mar-2012 11:07	-	
toolsets/	18-Oct-2012 17:01	-	
MeasureStack.txt	29-Jul-2010 14:18	193	
ConvertSigned16BitToUnsigned.txt	29-Jul-2010 14:18	194	
Factorials.txt	29-Jul-2010 14:18	246	
ConvertImageToStack.txt	29-Jul-2010 14:18	266	
OpenStackUsingURLs.txt	21-Apr-2011 23:27	274	
build.xml	29-Jul-2010 14:18	275	
CopyResultsToClipboard.txt	29-Jul-2010 14:18	277	
Measure_Stack.txt	24-Jun-2012 18:31	279	
FullRange16BitInvert.txt	29-Jul-2010 14:18	285	
DeleteEdgeRois.txt	28-Feb-2011 15:00	286	
ImageRotator.txt	29-Jul-2010 14:18	289	
SplineFitForStraighten.txt	31-Oct-2011 12:13	289	
SumAreas.txt	29-Jul-2010 14:18	308	
About Startup Macros	29-Jul-2010 14:18	315	
SineCosineTable.txt	08-Sep-2011 22:04	316	

Also via Fiji *Help* menu

Resources

There are 1000s of macros available. Check their code to see how things are done.



The screenshot shows the 'Macros' page on the ImageJ Wiki. The page title is 'Macros'. The content lists numerous macro scripts, each preceded by a small green square icon. The listed macros include:

- Affine + Consistent Elastic 2D Image Registration
- Auto Correlation
- autofocus_hyperstack
- basic_diffusion_analysis
- Brenner Gradient
- calculating_stats_from_a_results_table
- diffusion_analysis_time_ensemble_average
- Distribution Plotter
- GFP-LC3
- Stitch data from MetaMorph 7 "Screen Acquisition" module
- Import Zeiss lookup tables
- Convert selected channels from LSM files to RGB
- Mitophagy
- Normalized Variance
- Open Multiple Images
- Particle in Cell-3D
- PlateMontage
- plot_zeroed_tracking_data
- Radial Distribution Function
- Radially Averaged Autocorrelation
- RGBtoHEX
- ROI Color Coder
- Squared Gradient
- Standard Deviation-Based Correlation
- tiled_autofocus_hyperstack
- Variance
- Green and Red Puncta Colocalization
- Scratch wound assay automated analysis
- Huner with Stacks: manual extraction of best focused slices from hyperstacks

...and many, many
personal websites

Ask the mailing list.

Macro editor

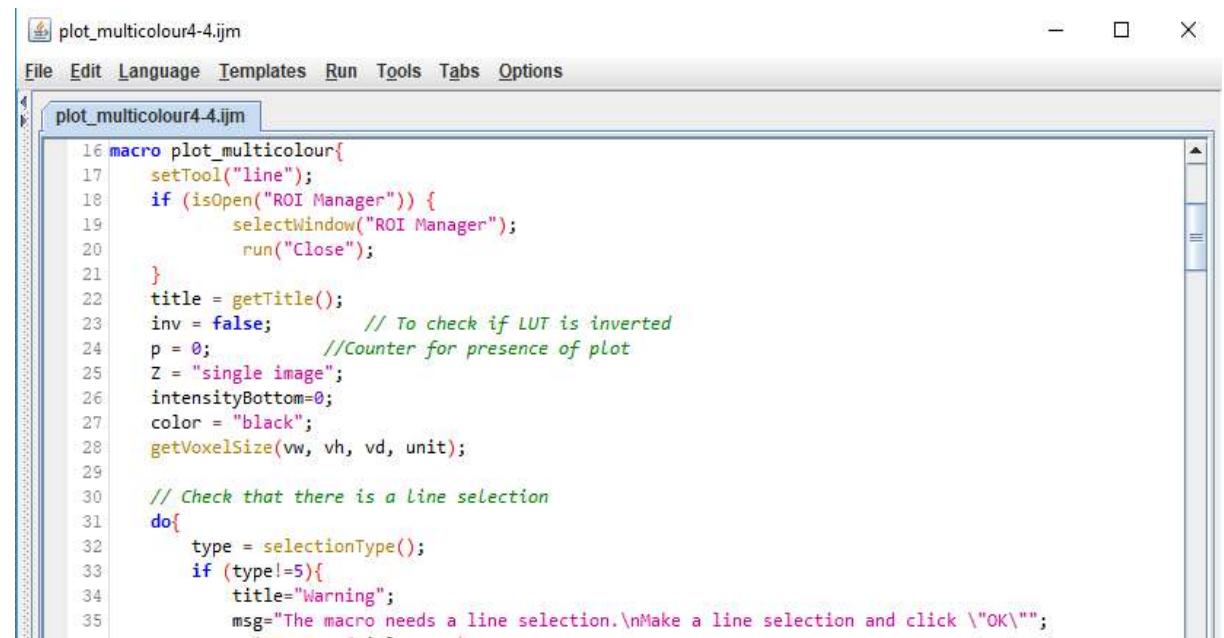
Simple text editor like Notepad

Fiji includes an editor:

File > New > Text Window OR

File > New > Script OR

Plugins > New > Macro



The screenshot shows a window titled "plot_multicolour4.4.ijm" which is a macro script for Fiji. The script content is as follows:

```
16 macro plot_multicolour{
17     setTool("line");
18     if (isOpen("ROI Manager")) {
19         selectWindow("ROI Manager");
20         run("Close");
21     }
22     title = getTitle();
23     inv = false; // To check if LUT is inverted
24     p = 0; //Counter for presence of plot
25     Z = "single image";
26     intensityBottom=0;
27     color = "black";
28     getVoxelSize(vw, vh, vd, unit);
29
30 // Check that there is a Line selection
31 do{
32     type = selectionType();
33     if (type!=5){
34         title="Warning";
35         msg="The macro needs a line selection.\nMake a line selection and click \"OK\"";
36     }
37 }
```

JIPipe



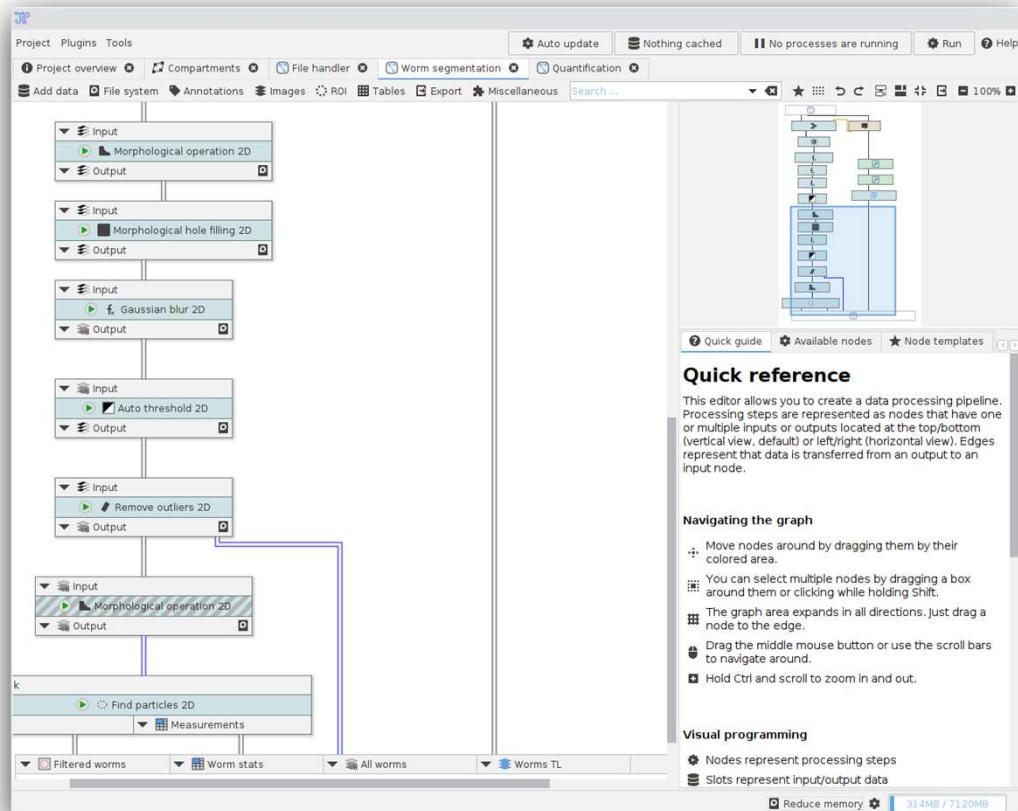
The screenshot shows the JIPipe website homepage. At the top left is the JIPipe logo (a stylized 'J' and 'P' in blue and purple). To its right is the title "JIPipe" in large black font. Below the title is the tagline "Macro programming for everyone!". A brief description follows: "JIPipe is a visual programming language for ImageJ that allows you to create macros without any programming by just creating a flowchart!" Two buttons are present: a green "Download" button and a white "Cite" button. Below these are three news items, each with a small icon and a timestamp:

-  2024/10/17 [JIPipe@I2K](#)
Information for the participants of the JIPipe workshop at the I2K 2024
-  2024/10/09 [Omnipose package 0.2.1.1000 available](#)
Δ Fixed issues with newer GPU
-  2024/10/09 [Cellpose package 2.1.0.1000 available](#)
Δ Fixed issues with newer GPU

<https://jipipe.hki-jena.de/>

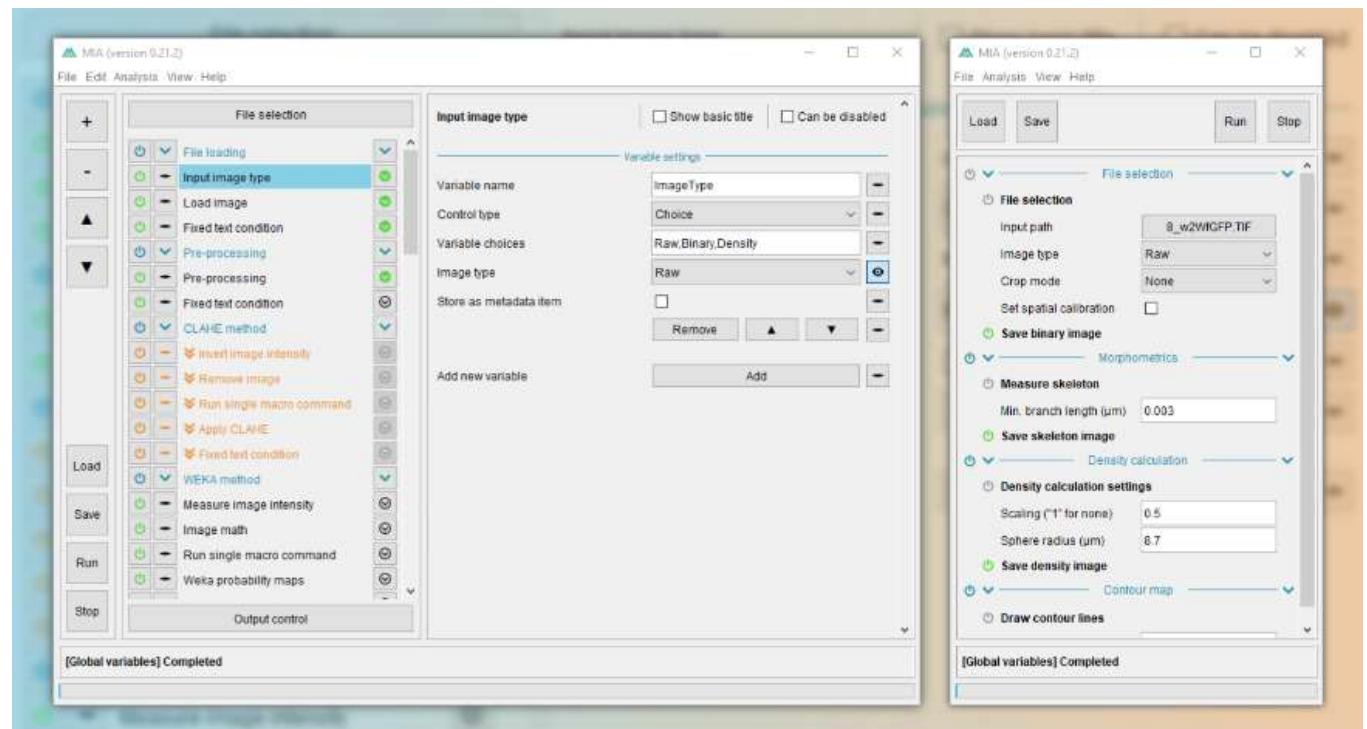
JIPipe

Adds visual macro
programming, creating
pipelines to ImageJ/Fiji



ModularImageAnalysis (MIA)

A modular framework for assembling image and object analysis workflows with batch capability.



<https://imagej.net/plugins/modularimageanalysis/>

AI generative models like ChatGPT or ImageJ Mentor can also create ImageJ macro code.



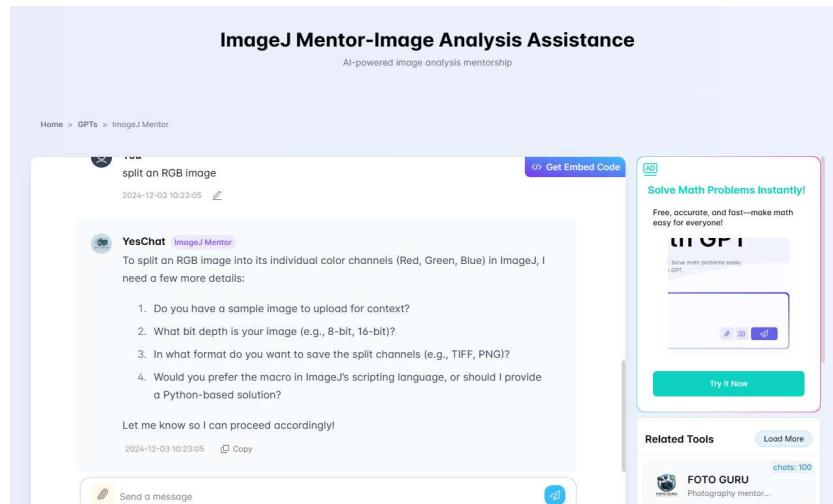
Certainly! Here's an example of an ImageJ macro that splits a four-channel image into separate channels:

```
perl
run("Split Channels");

// Rename channels
rename("Channel 1", "Red");
rename("Channel 2", "Green");
rename("Channel 3", "Blue");
rename("Channel 4", "Channel4");

// Optional: Save individual channels
saveAs("Tiff", "path/to/save/Red.tif");
saveAs("Tiff", "path/");
saveAs("Tiff", "path/");
saveAs("Tiff", "path/");


Microsoft  
Copilot
```



The screenshot shows a web-based AI interface for ImageJ. At the top, it says "ImageJ Mentor-Image Analysis Assistance" and "AI-powered image analysis mentorship". Below this, there's a message from "YesChat" (ImageJ Mentor) asking to split an RGB image. The user has responded with a list of questions:

1. Do you have a sample image to upload for context?
2. What bit depth is your image (e.g., 8-bit, 16-bit)?
3. In what format do you want to save the split channels (e.g., TIFF, PNG)?
4. Would you prefer the macro in ImageJ's scripting language, or should I provide a Python-based solution?

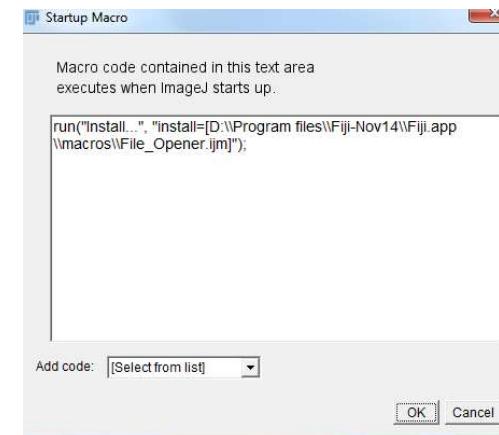
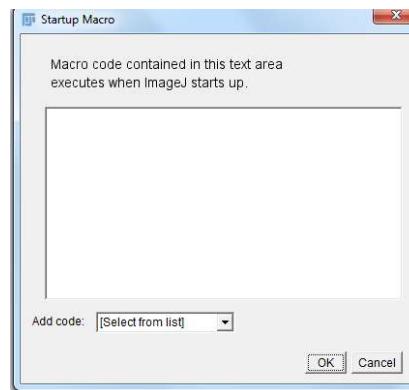
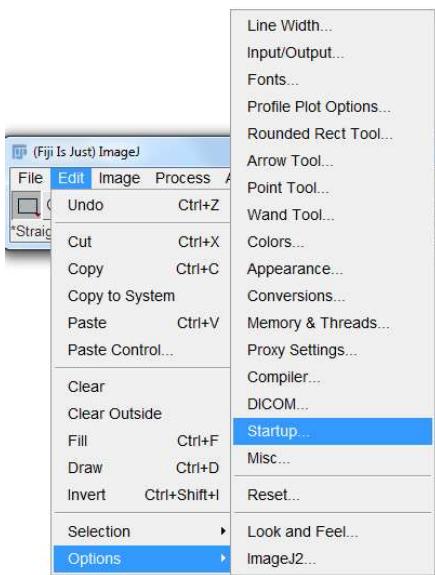
At the bottom, there's a "Send a message" input field and a "Copy" button. To the right, there are ads for "Solve Math Problems Instantly!" and "FOTO GURU".

<https://www.yeschat.ai/gpts-2OToEghs3P-ImageJ-Mentor>

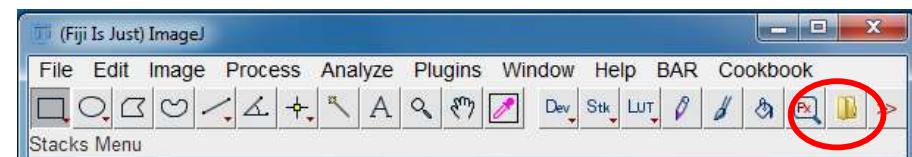
But, you still have to understand the code created!

Start-up Macros

Easiest:

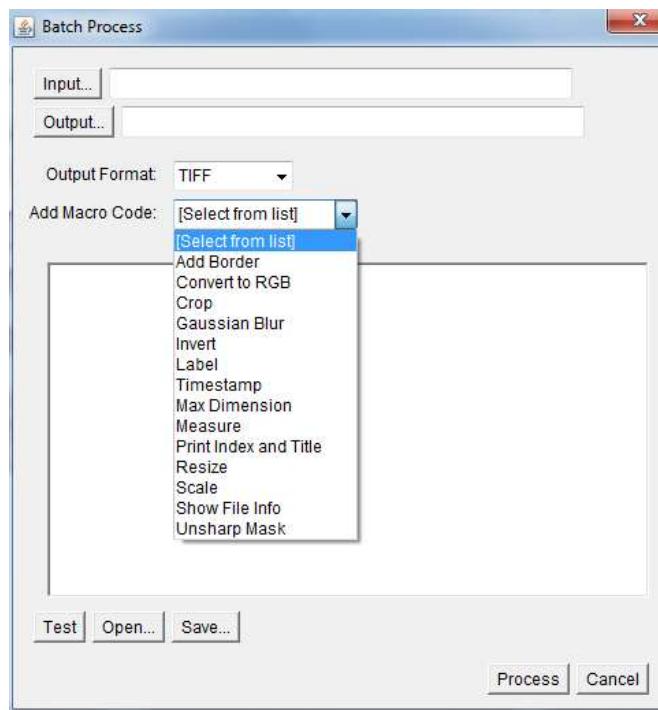


You can Use the
Macro recorder to
collect the code.



Batch

- *Process > Batch > Macro:* allows to run commands on a dataset without user intervention.



Select an action from the dropdown menu

or

simple macro code can be added from the Macro recorder.

Macro recorder

Records a sequence of commands.

Also very useful to see how to pass parameters on to a plugin or macro.

IT RECORDS MANY OF YOUR ACTIONS/CLICKS!

However, the Recorder window is a text area. It is possible to change or delete commands that are not wanted in the macro.

Create a results folder

Create a results folder called MMC2025 to save the results of the macro

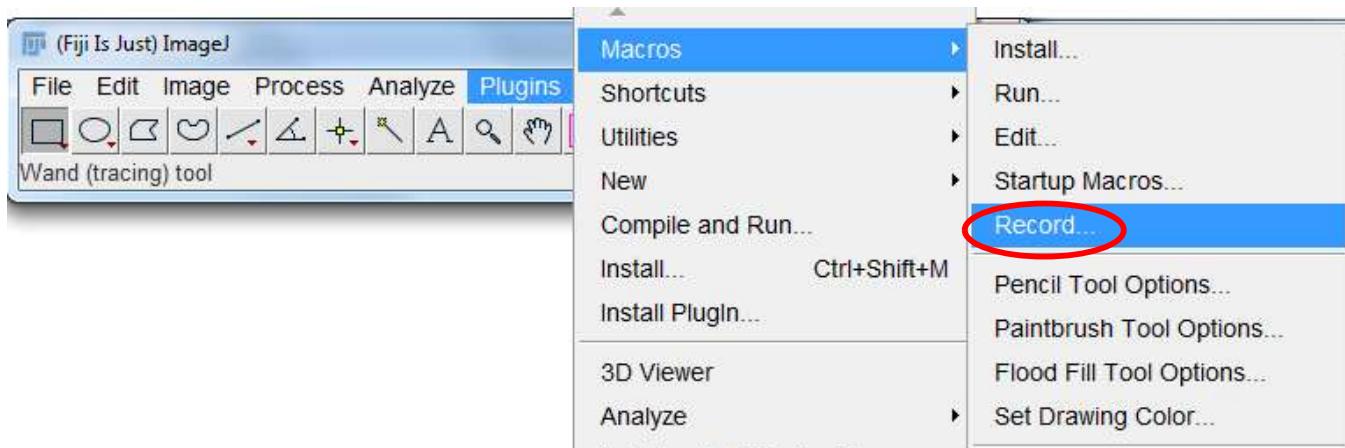
Create some macro code

Close all windows

Open the recorder (*Plugins > Macros > Recorder*)

Remember ALMOST EVERYTHING you do within ImageJ will be recorded! So follow exactly!!

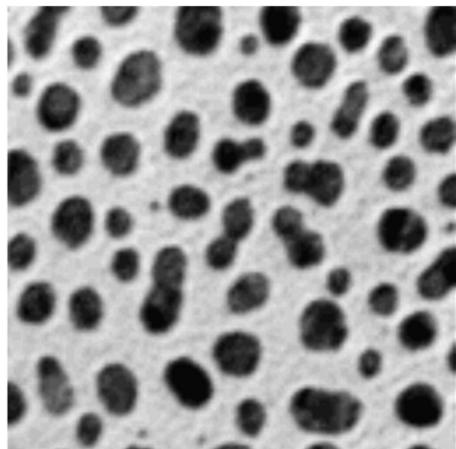
Macro recorder



Create some macro code

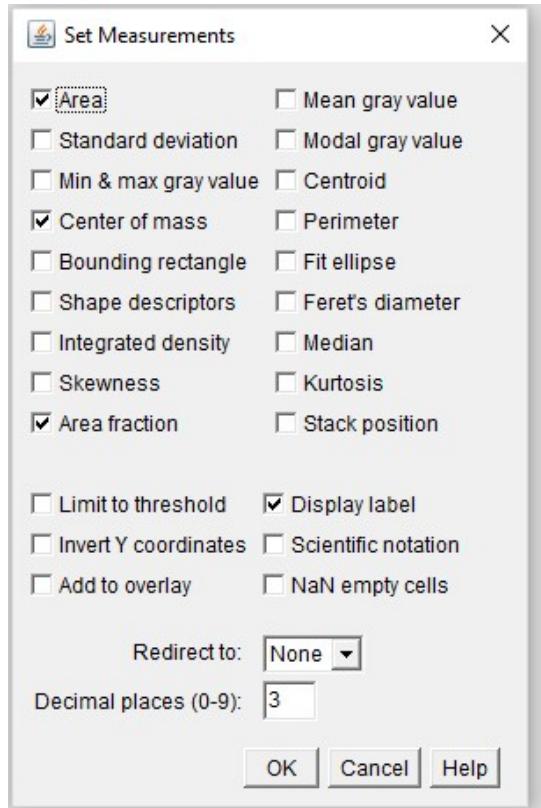
File > Open Samples > Blobs(25k)

Image > Adjust > Threshold - Apply



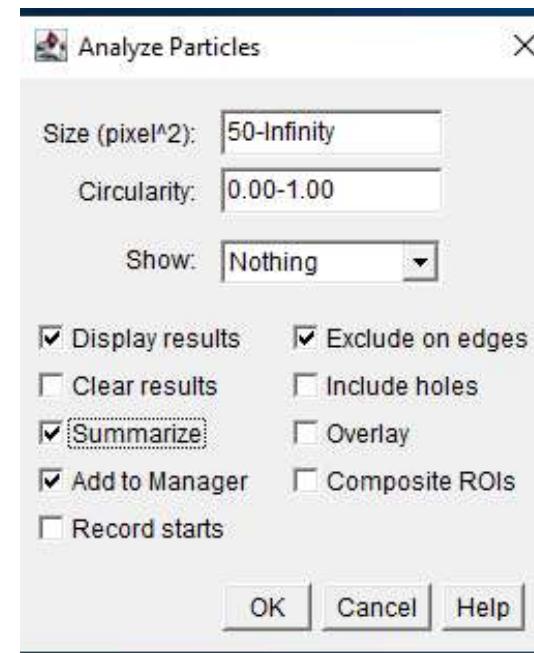
Create some macro code

Analyze > Set Measurements



Select “OK”

Analyze > Analyze Particles

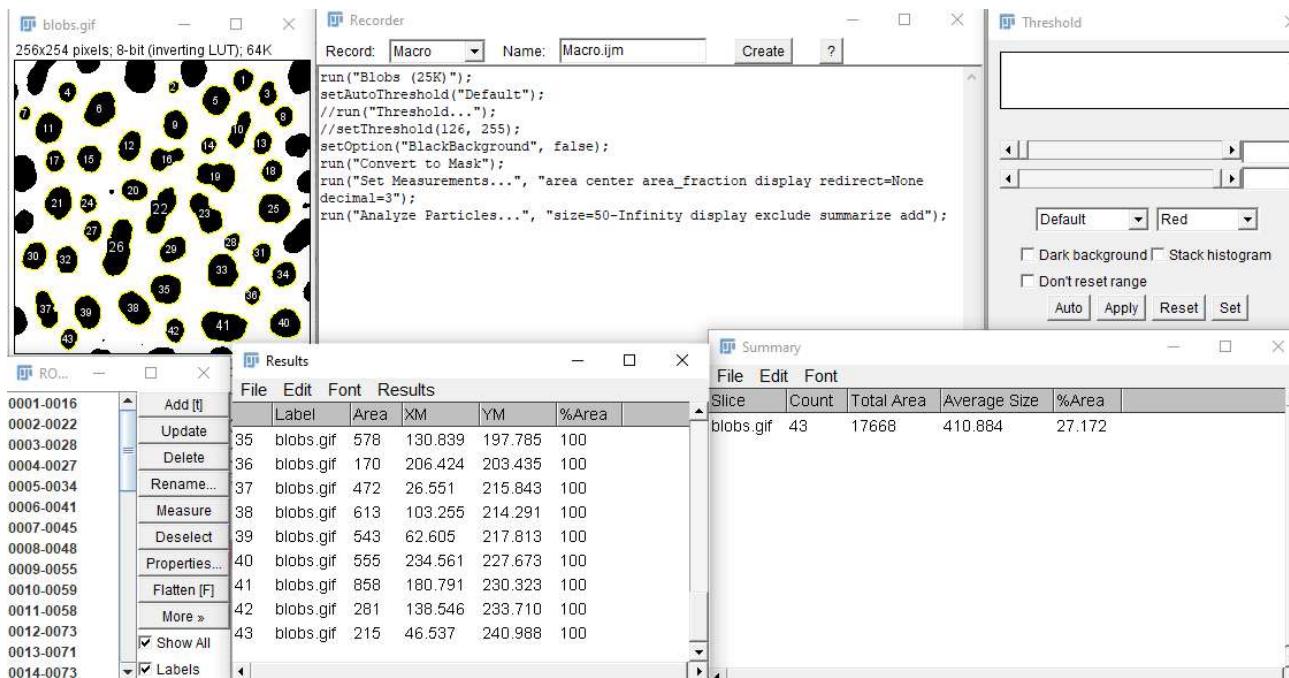


Select “OK”

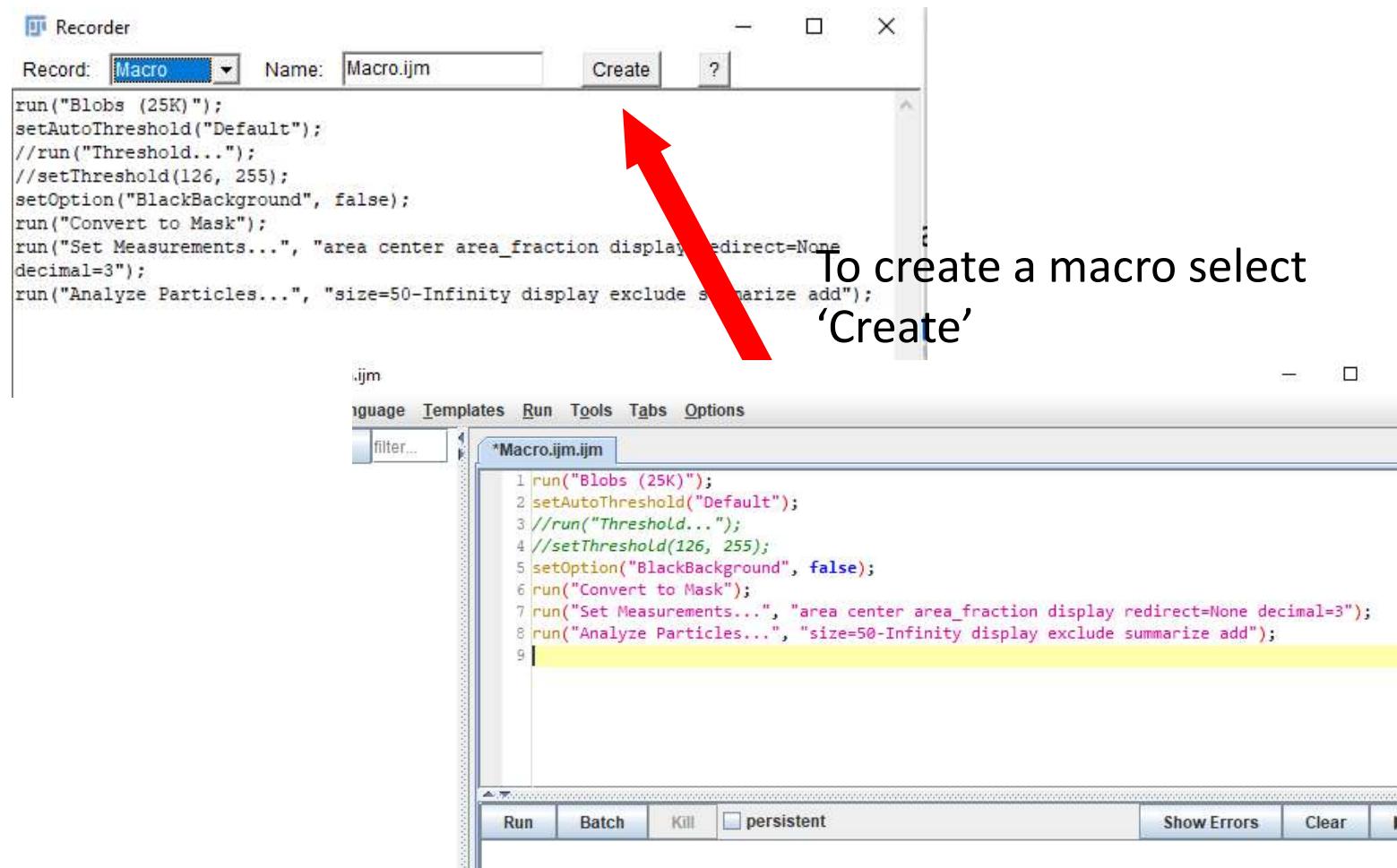
Don’t do anything else!

Create some macro code

Don't do anything else!

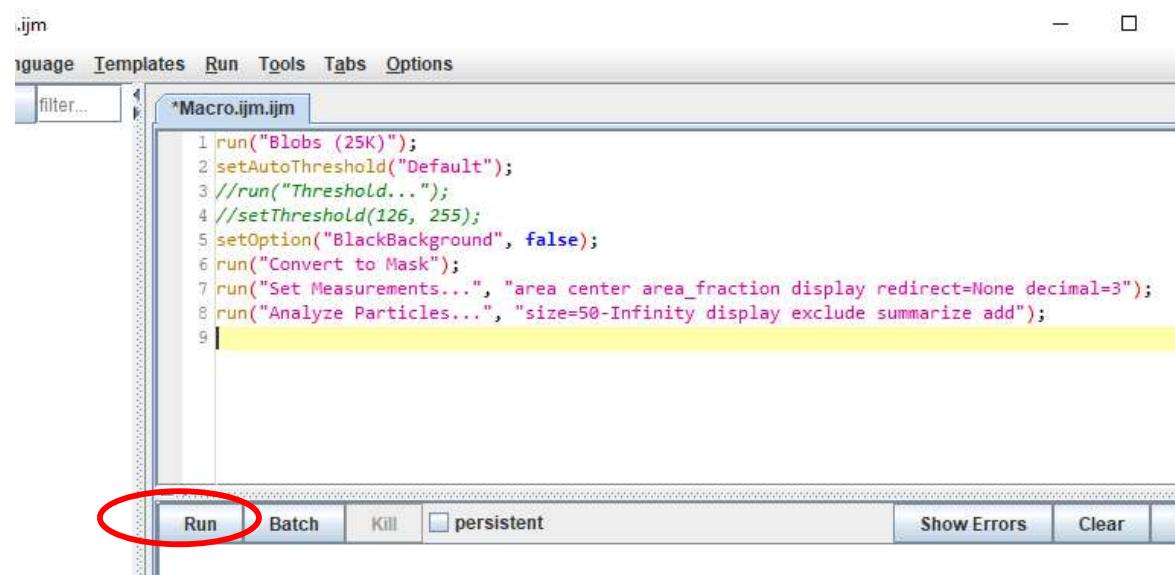


Macro recorder



Macro recorder

Close all windows except the macro editor



Close all windows except the macro editor

Create some test images

File > Open Samples > Blobs(25k)

Image > Duplicate... - OK

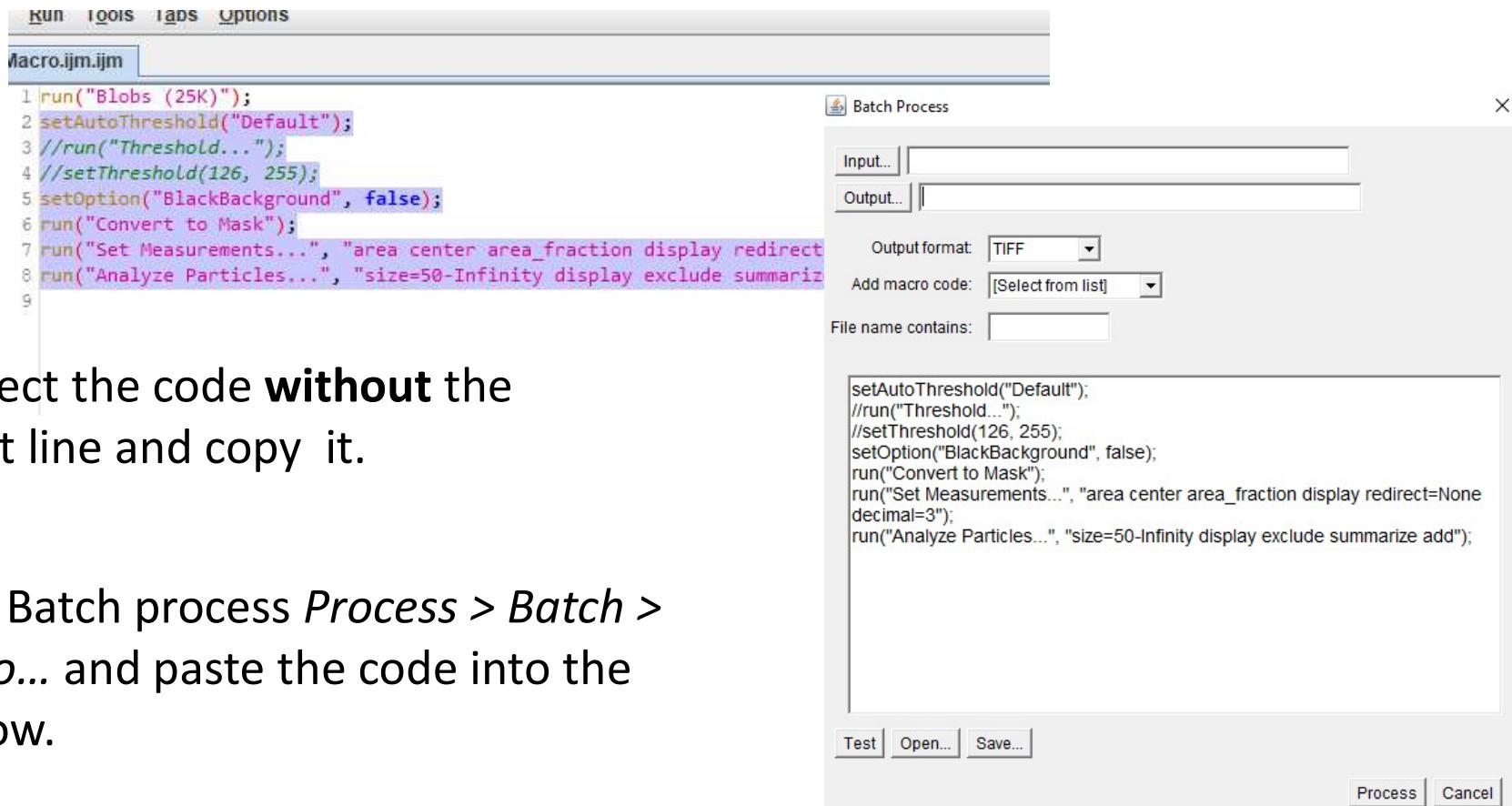
Image > Duplicate... - OK

Image > Duplicate... - OK

Save these files to a folder called Blobs

Close all windows except the macro editor

Create some macro code



The image shows the ImageJ macro editor window on the left and the 'Batch Process' dialog box on the right.

Macro.ijm.ijm (Macro Editor Window):

```
Run Tools Tabs Options
Macro.ijm.ijm
1 run("Blobs (25K)");
2 setAutoThreshold("Default");
3 //run("Threshold...");
4 //setThreshold(126, 255);
5 setOption("BlackBackground", false);
6 run("Convert to Mask");
7 run("Set Measurements...", "area center area_fraction display redirect=None decimal=3");
8 run("Analyze Particles...", "size=50-Infinity display exclude summarize add");
9
```

Batch Process Dialog Box:

- Input... (empty)
- Output... (empty)
- Output format: TIFF
- Add macro code: [Select from list] (dropdown menu open)
- File name contains: (empty)
- Macro code preview:

```
setAutoThreshold("Default");
//run("Threshold...");
//setThreshold(126, 255);
setOption("BlackBackground", false);
run("Convert to Mask");
run("Set Measurements...", "area center area_fraction display redirect=None decimal=3");
run("Analyze Particles...", "size=50-Infinity display exclude summarize add");
```
- Buttons at the bottom: Test, Open..., Save..., Process, Cancel

Select the code **without** the first line and copy it.

Open Batch process *Process > Batch > Macro...* and paste the code into the window.

Select as “Input” your Blobs folder.

Select as “Output” the MMC2025 folder

Hit ‘Process’.

Check the output folder.

You notice that the Results, Summary table and ROI manager are not automatically saved.

