

Getting Started with ImageJ

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The screenshot displays the ImageJ software interface with several windows open, illustrating a workflow for image processing and analysis of a microscopy image.






- Top Left:** The main image window shows a composite of four channels (Max, Channel 1, Channel 2, Channel 3, Channel 4) of a microscopy image.
- Top Right:** The 'Channels' window shows the 'Composite Max' dropdown menu and checkboxes for Channel 1, Channel 2, Channel 3, and Channel 4.
- Middle Right:** The 'Plot of Results' window displays a scatter plot of 'intDen' values for 8 regions of interest (ROIs). The y-axis ranges from 2.0×10^5 to 6.0×10^5 , and the x-axis ranges from 100 to 150.
- Middle:** The 'Results' window shows a table of analysis data for 8 ROIs.
- Bottom Left:** The 'MAX_squares3.nd2 - C=0 (33.3%)' window shows the original image with 8 ROIs highlighted in yellow.
- Bottom Middle:** The 'MAX_squares3.nd2 - C=0-1 (33.3...)' window shows the image with 8 ROIs highlighted in black.
- Bottom Right:** The 'B&C' window shows a histogram and sliders for 'Minimum', 'Maximum', 'Brightness', and 'Contrast' adjustments.

	Area	Mean	Circ.	intDen	RawIntDen	AR	Round	Solidity
1	120.857	2193.322	0.699	265077.656	8216185	1.274	0.785	0.935
2	76.140	3395.058	0.731	258500.904	8012336	1.400	0.714	0.931
3	192.351	3260.593	0.668	627179.035	19439658	1.624	0.616	0.932
4	75.592	2051.162	0.610	155051.225	4805873	1.016	0.985	0.904
5	89.626	3346.513	0.751	299935.325	9296612	1.248	0.801	0.925
6	186.673	3130.649	0.736	584407.511	18113938	1.429	0.700	0.947
7	176.317	3143.618	0.674	554271.803	17179870	1.812	0.552	0.949
8	163.831	3012.859	0.744	493599.165	15299298	1.348	0.742	0.953





What is ImageJ and what can it do?

ImageJ is open source software for processing and analyzing scientific images.

ImageJ is good for...

-  Working with digital photos or other pixel-based images/data
-  Image visualization
-  Image processing
-  Image analysis (quantification)
-  Automation of the above

ImageJ is NOT good for...

-  Working with drawings (vector-based images / line art)
-  Making digital drawings/sketches
-  Annotating (drawing on) images
-  Assembling full figures or posters

Why ImageJ?

- It is free and open source
- It is incredibly versatile and extensible via Plugins
- It has a large and vibrant user/developer community
- There are extensive, free online tutorials and resources
- It is designed for *scientific* image processing and analysis



Which ImageJ?



ImageJ

Basic version

<https://imagej.nih.gov/ij/>

If you're only doing basic stuff or if you want to customize it yourself.



FIJI (FIJI Is Just ImageJ)

“Batteries included” version

<https://fiji.sc>

*Contains lots of useful plugins.
Best all-purpose version.
You want this one.*



IMAGEJ.JS

Powered by  ImJoy

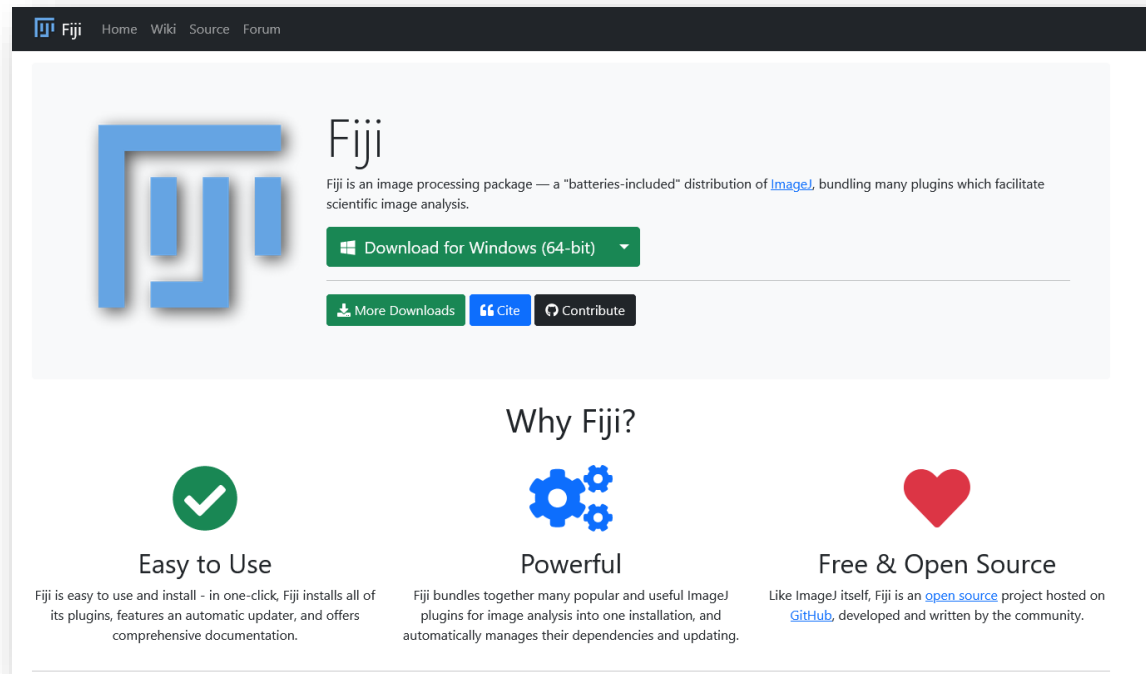
ImJoy ImageJ.JS

Web-browser version

<https://ij.imjoy.io>

*Mostly just basic capabilities.
No installation required.*

Getting and updating FIJI/ImageJ



1. Go to <https://fiji.sc>
2. Download the appropriate version for your computer (Windows, Mac, Linux)
3. Unzip the file to wherever you want to keep it on your computer
4. That's it

Troubleshooting:

<https://imagej.net/downloads>

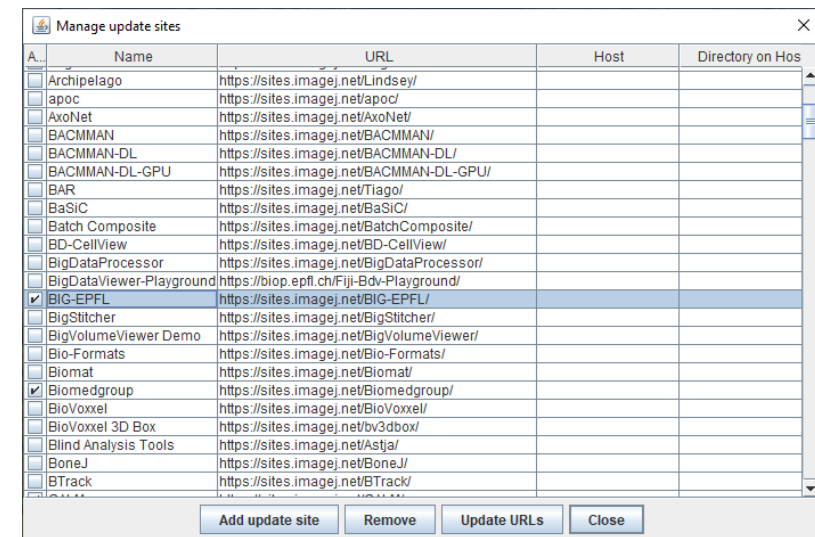
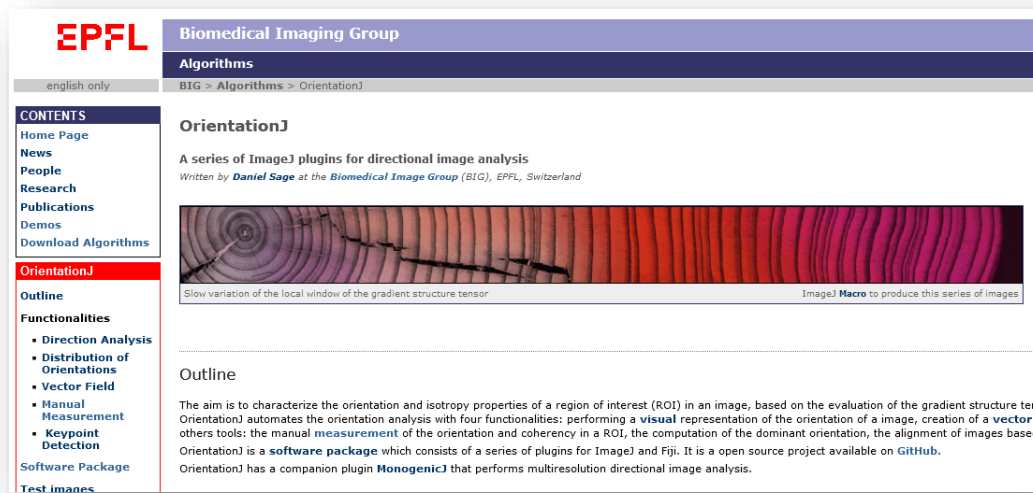
The first time you open FIJI, it will run the Updater to make sure everything is current.

To run the Updater manually, go to Help > Update...

Extending FIJI/ImageJ's capabilities by adding Plugins

FIJI comes pre-loaded with many of the most common/useful plugins, but you can add more...

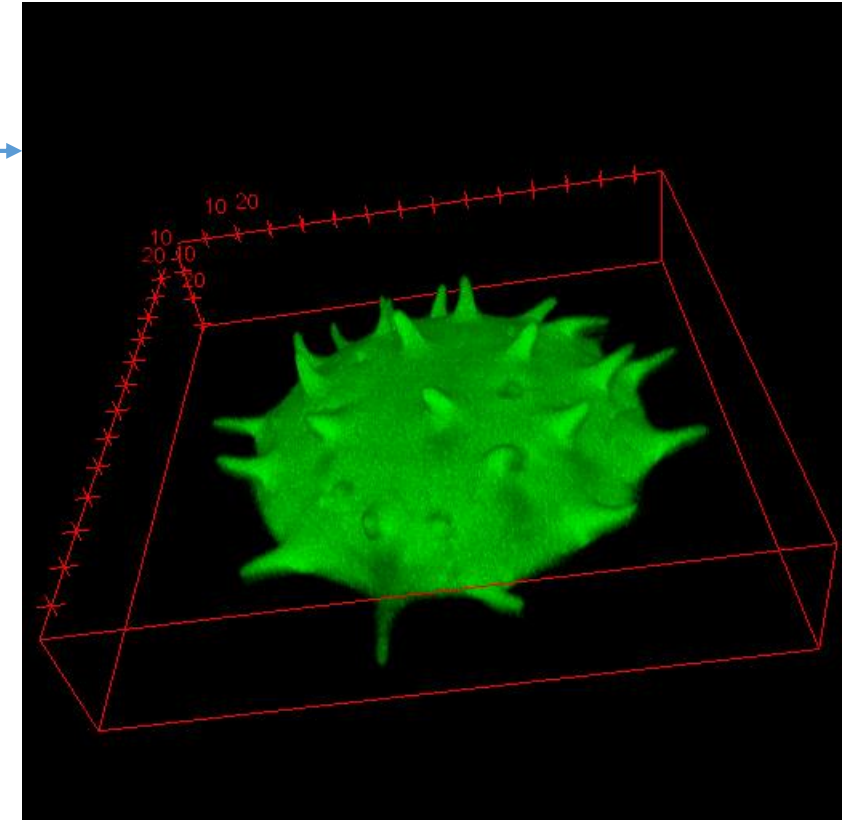
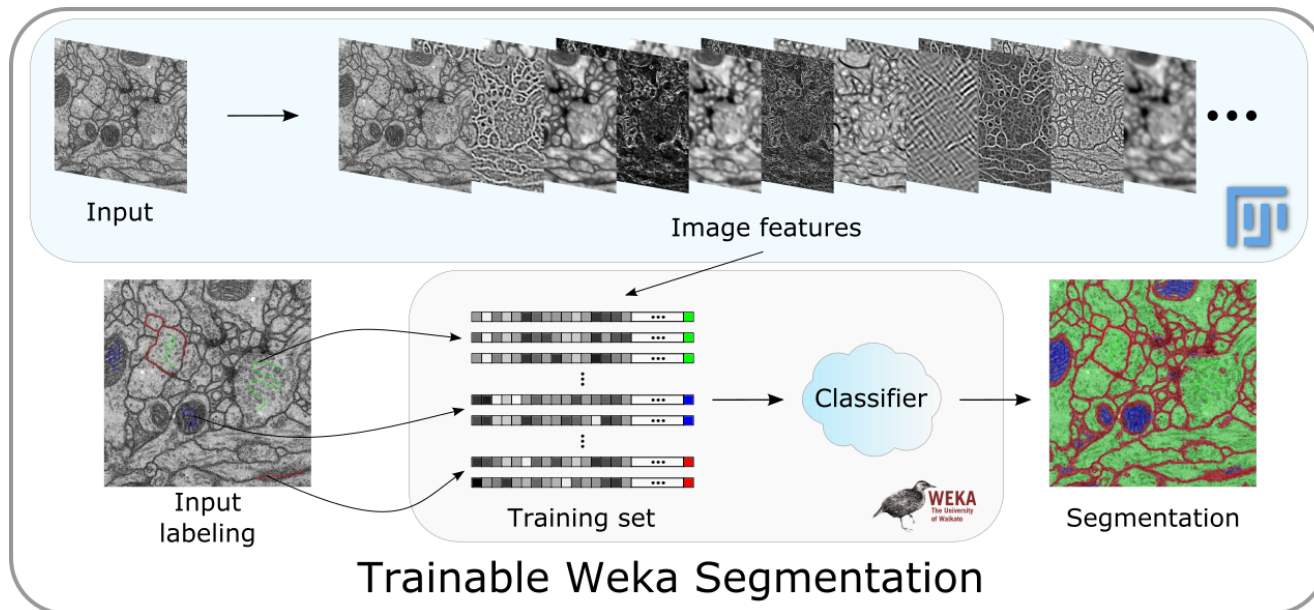
One way to add plugins is to subscribe to the update site that hosts the plugin
(instructions: <https://imagej.net/update-sites/following>)



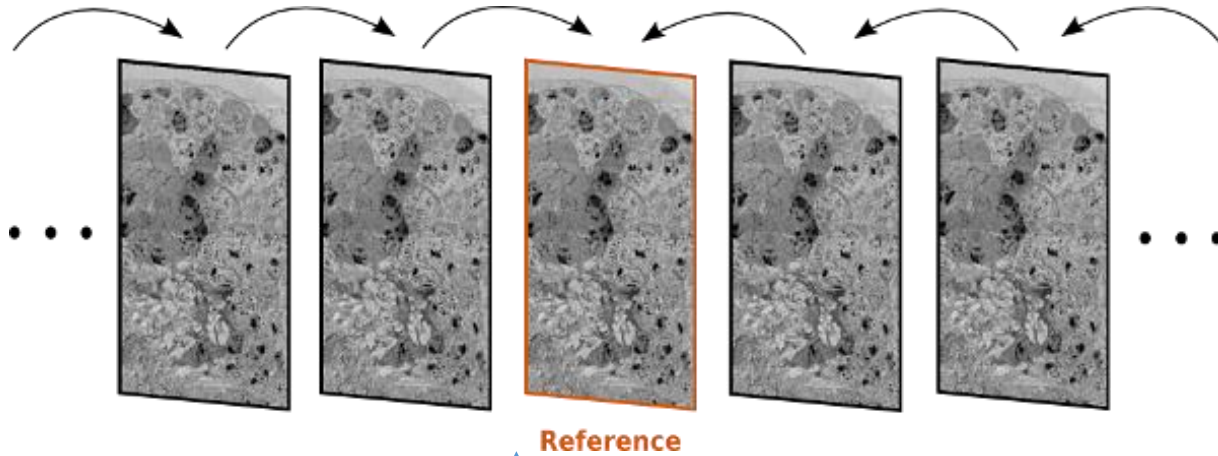
Sometimes you may find a useful plugin that isn't hosted on an update site and will need to be installed manually.
(instructions: <https://imagej.net/plugins/>)

Examples of some additional features of ImageJ

- Wide range of spatial filters (real and Fourier space)
- Image math (adding, subtracting, scaling, etc.)
- Stack and time-series manipulation (rearranging, reducing, etc.)
- Gel analysis
- 3D viewer
- Advanced segmentation algorithms using machine learning
- ...



Examples of some additional features of ImageJ



- ...
- Image registration and alignment
- Orientation/alignment/texture analysis
- Object Tracking
- Color segmentation and deconvolution (for color/histology work, I highly recommend a different program that works in tandem with ImageJ: **QuPath** <https://qupath.github.io/>)
- ...

TrackMate on P31-crop

18/86; 1024x490 pixels; 16-bit; 82MB

P31-crop.tif (600%)

Display options

- Display spots as ROIs
- Spot display radius ratio: 1.5
- Display spot names:
- Color spots by: Track ID
- auto min 0 max 200
- 0 200 382
- Display tracks Show tracks backward in time
- Fade tracks in time:
- Fade range: 30 time-points
- Color tracks by: Track ID
- auto min 0 max 200
- 0 200 382
- Limit drawing Z depth 10 pixel

TrackScheme

Layout Style: full

P31-crop spot features

Plot of Area vs T

Track ID	Quality	X	Y	Z	T	Frame	R	Visibility	Spot color	Mean ch1	Median ch1	Min ch1	Max ch1	Sum ch1	Std ch1	Contr ch1	SNR ch1	El. x0	El. y0	El. long axis	El. sh. axis	El. angle	El. a.r.	Area	Perim.
54	0.7205	384.7	401.0	0.000	17.00	17	4.737	1		2.737e+04	2.667e+04	4980	5.738e+04	1.916e+06	1.368e+04	0.6865	1.629	-0.07379	-0.003487	6.282	3.599	-1.082	1.746	70.50	36.79

daughter B

daughter A

daughter B

Cell division

Track_55

ID5774 ID5746

ID5994

ID6234

ID6406

ID6750 ID6738

ID6692

frame 17 3107

frame 18 3342

frame 19 3604

Next steps – where to learn more

- General introduction to image analysis concepts
 - https://www.youtube.com/watch?v=ETq_9YFUQvU&list=PLXSm9cHbSZBCYxzRpqyFmmtlrqOeZMEq2&index=1
- ImageJ video tutorials on basic functions
 - <https://www.youtube.com/playlist?list=PLXSm9cHbSZBDh7I7muuDecvWVAoxMfmGD>
- More in-depth videos with activities
 - <https://microscopy.unimelb.edu.au/optical-microscopy/capabilities/fiji-workshop-resources>
- Video tutorials on specific moderate-advanced topics
 - <https://www.youtube.com/playlist?list=PL5ESQNfM5Ic7SAMstEu082ivW4BDMvd0U>
- Excellent online “textbook” with examples and activities
 - <https://bioimagebook.github.io/README.html#>
- ImageJ reference
 - <https://imagej.net/imaging/>
- Extensive and well-maintained help forum for scientific image analysis
 - <https://forum.image.sc/>