Install and Update Fiji



Download Fiji

https://fiji.sc/





https://imagej.net

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

https://imagej.net/Fiji



- Fiji is distributed as a portable application,
- which means that you do not have to run an installer.
 - Just download (zip file), unpack and start it.



<u>https://fiji.sc/</u>

https://imagej.net/Fiji



Update Fiji (and install/uninstall plugins)

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										Documentation
										Mailing List
										Dev. Resources
										Macros
										Macro Functions
										Examples
										Update ImageJ
										Refresh Menus
										About Plugins
										About ImageJ
										Report a Bug
										Help on Menu Item
										Switch to Modern Mode
										Update
										Upload Sample Image



https://imagej.net

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



Help > Update...

To update Fiji and/or the plugins.

Apply Changes: Install/Update the listed plugins

Manage Update Sites: opens a list of plugins, you can select which one(s) to install in Fiji



You can have more than one Fiji app!





<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Graphic User Interface (GUI)





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

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



<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Graphic User Interface (GUI)

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https://imagej.net

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



Search Bar (L key shortcut)

*Plugins > Shortcuts > List Shortcuts - list of the default shortcuts

****Plugins > Utilities > Find Commands - search for Fiji Commands**





<u>https://fiji.sc/</u>

https://imagej.net/Fiji



Open a file in Fiji

Drag and Drop the file you want to open onto the Status Bar.





https://imagej.net

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

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<u>https://fiji.sc/</u>

https://imagej.net/Fiji

"File" menu "Plugins" menu

Bio-Format Plugin import a file - options

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	(Fiji Is	Just) ImageJ
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		Bio-Formats Import Options
Stack viewing	Metadata only Standard ImageJ	Metadata viewing
View stack with:	✓ Hyperstack	Display metadata
Stack order:	View5D	Display OME-XML metadata
		Display ROIs
		ROIs Import Mode: ROI manager 🗘
Dataset organiz	ation	Memory management
🗹 Group files w	ith similar names	Use virtual stack
Open files in	dividually	Specify range for each series
Swap dimens	ions	Crop on import
Open all serie	es	
Concatenate	series when compatible	Split into separate windows
Stitch tiles		Split channels
Color options		Split focal planes
Color mode:	Default 🗘	Split timepoints
Autoscale		



https://imagej.net

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

https://imagej.net/Bio-Formats



Information

View stack with - The type of image viewer to use when displaying the dataset.

Possible choices are:

- Metadata only Display no pixels, only metadata.
- Standard ImageJ This option is deprecated (i.e. intended for use by old macros only). Please use *Hyperstack* instead.
- Hyperstack Display the pixels in ImageJ's built-in 5D viewer.
- Data Browser Display the pixels in the multidimensional Data Browser viewer. The Data Browser has some additional features on top of the normal ImageJ hyperstack.
- Image5D Display the pixels in Joachim Walter's Image5D viewer. Requires the Image5D plugin.
- View5D Display the pixels in Rainer Heintzmann's View5D viewer. Requires the View5D plugin.

Cancel

Import data from many life sciences file formats (e.g. @NIC .nd2 format)



https://fiji.sc/

ОК

https://imagej.net/Fiji



https://imagej.net/Bio-Formats

"File" menu "Plugins" menu

Bio-Format Plugin import a file - options





https://imagej.net

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

<u>https://fiji.sc/</u>

https://imagej.net/Fiji



https://imagej.net/Bio-Formats

"File" menu "Plugins" menu

Bio-Format Plugin import a file

Drag and Drop

File > Import > Bio-Formats

or

Plugins > Bio-Formats > Bio-Formats Importer



https://imagej.net

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

If you have a tiff file, "Drag and Drop" does not open Bio-Format Importer.







https://fiji.sc/

https://imagej.net/Fiji



what is an image?



A digital image is a matrix of numbers!



Pixel = Picture Element



6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
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9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Images in publications and presentations should be used to **communicate** a finding... not **be** the finding

this is your **data**

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9



this is your **result**

this just helps to communicate the result





Individual Pixel Values in Fiji





https://imagej.net

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

https://fiji.sc/

https://imagej.net/Fiji







A digital image is a matrix of numbers!



Where do these



6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

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Bit depth

Detectors in Fluorescence Microscopy

The detectors used in <u>fluorescence microscopy</u> are **monochromatic**.

separate your fluorophores.







- Cameras or PMTs are not able to distinguish between different wavelengths (they just collect photons), you need fluorescence filters to
- The detector converts photons in digital numbers (linear relation).
 - Each pixel in the digital image has **one digital** value that depends on the intensity of the signal emitted by the sample.
 - Digital Values = Pixel Intensity Value
 - The range of possible digital values is defined by the bit depth.

Bit Depth

The **bit depth** defines the range of possible **digital values** that each pixel can have, usually 8, 12 or 16 bit.

The **bit depth** is expressed in **grey values**.

bit depth of the **image** = **bit depth** of the **detector** (Unless you change that during acquisition)

x bit = a range of 2^X grey values





8 bit image = each pixel can have 2⁸ grey values = 256 grey values = range 0-255 12 bit image = each pixel can have 2¹² grey values = 4096 grey values = range 0-4095 16 bit image = each pixel can have 2^{16} grey values = 65536 grey values = range 0-65535

Digital Value = Pixel Intensity Value = Grey Value

"Image" menu

x bit image

Image Metadata





https://imagej.net

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

Image > Show Info...

(cmd) + i

Show the Metadata stored with the file

e.g. bit depth, camera bit depth



https://fiji.sc/

https://imagej.net/Fiji







Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are <u>displayed</u>



and a start -	lmage (12 bit)	Displayed color
	0	
	1	~
	•••	
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THE Y -	•••	
the second	4095	



<u>LUTs do not change the pixel values</u>



Image > Adjust > Brightness/Contrast

Interactively change the <u>displayed</u> brightness and contrast of the <u>active</u> image.





https://imagej.net

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

(cmd) + shift + c

*You are NOT changing the pixels values, you are just changing how the image is displayed (unless you click on the "Apply" button).





https://fiji.sc/

https://imagej.net/Fiji

Analyze > Histogram



https://imagej.net

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



Pixel Count



jaehyuk-lee: https://jaehyuk-lee.com/animatedimage-histogram/



Pixel Values

<u>https://fiji.sc/</u>

https://imagej.net/Fiji



Analyze > Histogram



Fiji auto-adjust the range (default option)



https://imagej.net

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

Histogram

h

0 65535 N: 640000 Min: 1 Mean: 1328.815 Max: 14039 StdDev: 1273.577 Mode: 1015 (212550) Bins: 256 Bin Width: 255.996 Value: Count:		2001
0 65535 N: 640000 Min: 1 Mean: 1328.815 Max: 14039 StdDev: 1273.577 Mode: 1015 (212550) Bins: 256 Bin Width: 255.996 Value: Count:		
N: 640000 Min: 1 Mean: 1328.815 Max: 14039 StdDev: 1273.577 Mode: 1015 (212550) Bins: 256 Bin Width: 255.996 Value: Count:		
	0	65535

Bit depth

https://fiji.sc/

https://imagej.net/Fiji



Which image has more fluorescence?



Mean:	4803
Display range:	188- 16828

Mean:	4803
Display range:	188- 16828







4803

188-**16828**

Which image has more fluorescence?



Mean:	4803
Display range:	188- 19540

Mean:	4803
Display range:	188-1 9540











188-**19540**

Do NOT trust your eyes, Do NOT trust your eyes, rely on numbers!

"Set" button - visually compare images





https://imagej.net

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

Use the same acquisition parameters

(per channel) if you want to compare images!!! (e.g. same exposure time, Illumination intensity,...)

Example	Exposure time Condition 1	Exposure time Condition2
Channel 1	100 ms	100 ms
Channel 2	200 ms	200 ms

For a meaningful comparison, you have to extract numbers from your images (analysis). Use the images to support your results.

If you save the images as jpeg/png/tiff, they will maintain the display range you set.

<u>https://fiji.sc/</u>

https://imagej.net/Fiji



Segmentation with pixel based classifier—exercises



- 1.3 image inspection
- 1.4 adjust brightness/contrast of all open images

Images and Colors

LUT = how the grey values are <u>displayed</u>

<u>LUTs do not change the pixel values</u>

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	136	106	152	179		
	109	209	236	179		
	103	179	189	132		
	123	186	192	169		



https://imagej.net

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

Lookup Tables (LUTs)



<u>https://fiji.sc/</u>

https://imagej.net/Fiji



Images and Colors Lookup Tables (LUTs)



*Image > Color > Display LUTs - to display default LUTs available



https://imagej.net

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



https://fiji.sc/

https://imagej.net/Fiji





Images and Colors in Fiji Choose the right LUT

Which is brighter?



The human eye evaluates intensity best in grayscale

If you are imaging for example a blue fluorophore, you are <u>NOT FORCED</u> to display it in blue!





Images and Colors in Fiji Choose the right LUT

Color blind people don't distinguish some colors

POINTS OF VIEW

Color blindness

NATURE METHODS | VOL.8 NO.6 | JUNE 2011 | 441

"If a submitted manuscript happens to go to three male reviewers of Northern European descent, the chance that at least one will be color blind is 22 percent"



Images and Colors in Fiji Choose the right LUT

Color blind people don't distinguish some colors



Image > Color > Dichromacy or Image > Color > Simulate Color Blindness



Protanope (no red)



Tritanope (no blue)





MitoTracker Red







RGB Images (still matrix of numbers)

LUTs cannot be applied to RGB Images

RGB image



Image > Type > RGB Color or



https://imagej.net https://imagej.nih.gov/ij/

255 0 234	0 255 0	0 186 28	255 255 1
0	1	255	0
0 255	199 255	0	255
255	255	255	0
0	255	255	0
255	1	255	0
254	117	141	118
105	117	137	0
	255 0 234 0 255 255 0 255 255 255 255 254 105 0	255 0 0 255 234 0 0 1 0 199 255 255 255 255 0 255 255 1 255 1 254 117 105 117 0 117	2550002551862340280125501990255255025525525502552552551255255125525411714110511713701170

RGB Color image (e.g. jpeg, png) = **Red + Green + Blue**

RGB Color image = 8 bit Red, 8 bit Green, 8 bit Blue = R (0-255), G (0-255), B (0-255)

Save As...png, jpeg



<u>https://fiji.sc/</u>

https://imagej.net/Fiji



"Image" menu

Images and Colors in Fiji

The Color menu

🖤 Fiji File Edit	Image Process	Analyze	Plugins Window Help	
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hela_cells.tif (1/3; 34.60x25.03 μm (6	Color Stacks Hyperstacks		Split ChannelsMerge ChannelsArrange ChannelsChannels Tool	ጉ፞፞፞፞፞፞፞፞፞፞፞፞
	Duplicate Rename Scale Transform Zoom Overlay	ひみ入 分光D 彩E ▶ ▶	Stack to RGB Make Composite Show LUT Display LUTs Edit LUT Color Picker	ን ዤዞ
C	Lookup Tables Annotate Drawing Video Editing Axes		Colour Deconvolution Dichromacy Simulate Color Blindness Retinex Replace Red with Magenta Replace Red with Magenta (system clipboard Average Color RGB to CIELAB RGB to Luminance	1)



https://imagej.net

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



Image > Color > Split Channels

Image > Color > Merge Channels

Split or Merge the channels of the active image





<u>https://fiji.sc/</u>

https://imagej.net/Fiji

"Image" menu

Images and Colors in Fiji

The Color menu

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		hala ad	Prope	rties					
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1/5, 54.00	XZJ.UJ	μπ (δ	Stack	S		Merge C	nanneis		
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https://imagej.net

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

	Image > Color > Channels tools
search	
	(cmd) + shift + z
<mark></mark>	
	Interactive visualization/handling of each image channel.
ት 	Choose how to display your multicolour image: "Color" mode (single channel) vs "Composite" mode (overlay)
ta	



<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Segmentation with pixel based classifier—exercises



2.2 composite images - splitting and merging



Pixel Size



Laurence Haren @HarenLaurence

never forget the scale bar! @StearnsLab when biology meets astronomy: cell vs nebula, centrosome vs dying star! @EtienneKlein



Scale Bar and Pixel Size

...



If you want to make some physical measurements of your sample (length, size, ...), you need to know the pixel size of your image.





https://imagej.net

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

Pixel Size and Scale Bar

https://fiji.sc/

https://imagej.net/Fiji







https://imagej.net

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

Pixel Size and Scale Bar

If you want to make some physical measurements of your sample (length, size, ...), you need to know the pixel size of your image.



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https://imagej.net/Fiji

Scale Bar and Pixel Size

What if the pixel size is not stored in the metadata?

Example

Magnification = 100x Objective

Camera = Hamamatsu Orca Flash 4

Product number	C13440-20CU
Imaging device	SCMOS
Cell (pixel) Size (µm²)	6.5×6.5
Pixel Array (horizontal by vertical)	2048×2048
Effective Area (horizontal by vertical in mm)	13.312×13.312

pixel width and height: 6.5 μm / 100X = 0.065 μm



https://imagej.net

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

If you know the magnification and the camera you used for the acquisition, you can estimate the image pixel size.

image pixel size = camera pixel size/magnification



If a z-series was acquired, "Voxel depth" is the z step size choose for the acquisition (cannot estimate).





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"Analyze" menu

Scale Bar and Pixel Size

Add pixel information to your images from a reference (same magnification)





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

Analyze > Set Scale...

Set the pixel size information starting from an object with a known dimension.

*Perform multiple measures and average them to be more precise





https://fiji.sc/

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"Analyze" menu

Scale Bar and Pixel Size

Add scale bar to your images





https://imagej.net

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

Analyze > Tools > Scale Bar...

- "Overlay" means that the scale bar is not embedded in your image. You can remove it: "Image >Overlay > Remove Overlay".
- If you save the image as tiff, the scale bar will be saved as an overlay.
- If you save as jpeg/png, the image will have the scale bar embedded.



https://fiji.sc/

https://imagej.net/Fiji



Segmentation with pixel based classifier—exercises



- 1.6 file handling and non-invasive editing
- 1.7 file handling and invasive editing