

ZMB/BVC Lunch seminars 2024: BioImage processing and Analysis using Fiji/ImageJ

Joana Delgado Martins / 2024

3 μm

Including adapted material Robert Haase <https://haesleinhuepf.github.io/>
and Pete Bankhead <https://bioimagebook.github.io/chapters/0-preamble/preface/preface.html>
NEUBIAS training Resources <https://neubias.github.io/training-resources/all-modules/index.html>



Lunch Seminar Series

Bioimage Analysis

This lunch seminar series is designed for anyone interested in light and/or electron microscopy and image analysis, offering insights from basic to intermediate levels. It focuses on the usage of open source as well as commercial software tools.

Please register using the QR Code or link below

Online every two weeks on Friday 11:30 AM - 12:30 PM

September 20, 2024 **Bioimage processing and analysis using Fiji/ImageJ**

Joana Delgado Martins, Flurin Sturzenegger (ZMB)

October 4, 2024 **Streamlining Bioimage Analysis with Fiji/ImageJ Macros**

Flurin Sturzenegger, Joana Delgado Martins (ZMB)

October 18, 2024 **Machine learning-based segmentation with ilastik**

Lorenzo Cerrone (BVC)

November 1, 2024 **Deep learning-based segmentation with Cellpose**

Joel Lüthi (BVC)

November 15, 2024 **3D/4D image visualization and analysis workflows with Imaris**

ZMB

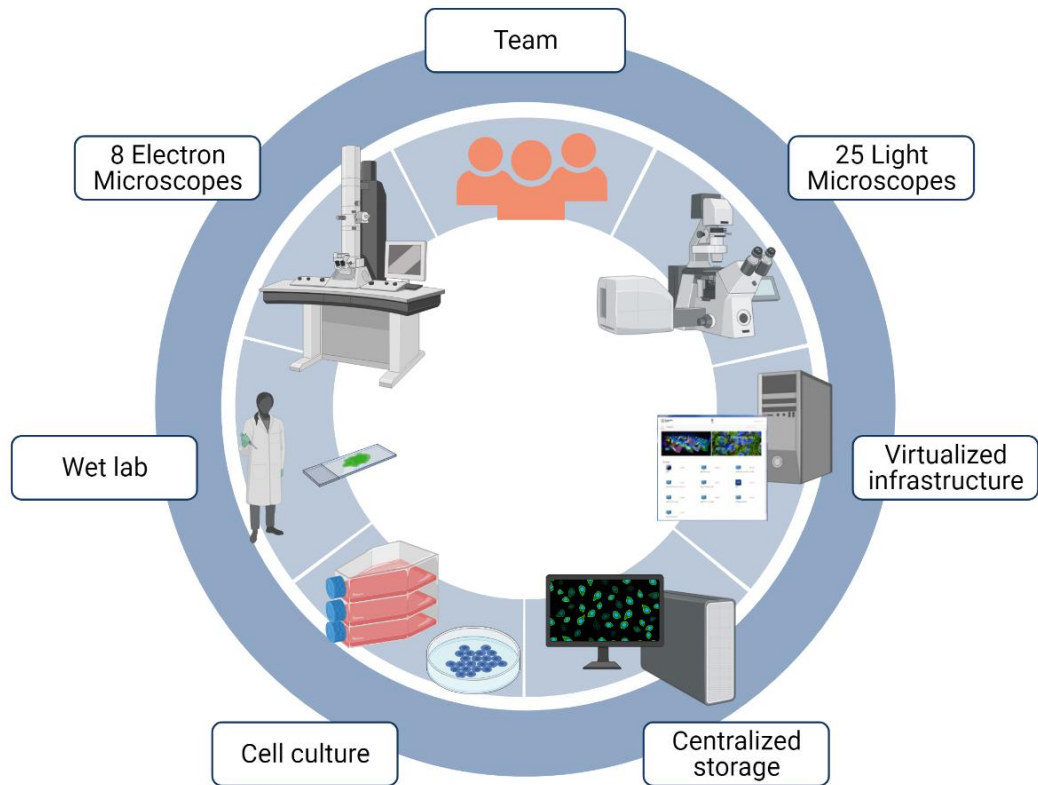
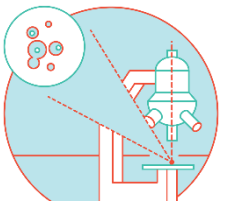
November 29, 2024 **Large-scale bioimage analysis workflows with Fractal**

Joel Lüthi, Lorenzo Cerrone (BVC)

December 13, 2024 **Interacting with the bioimage analysis community on Image.sc**

Virginie Uhlmann (BVC)

> ZMB Quick Facts



Around 2.2h of image processing per 1h of microscopy

700
active users /
year

2000
TB of storage
administrated

10
TB new data
generated / day

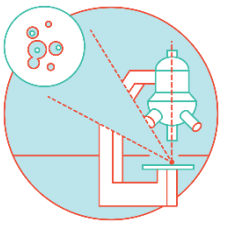
50
Available
instruments

34000
hours / year
Light
Microscopes

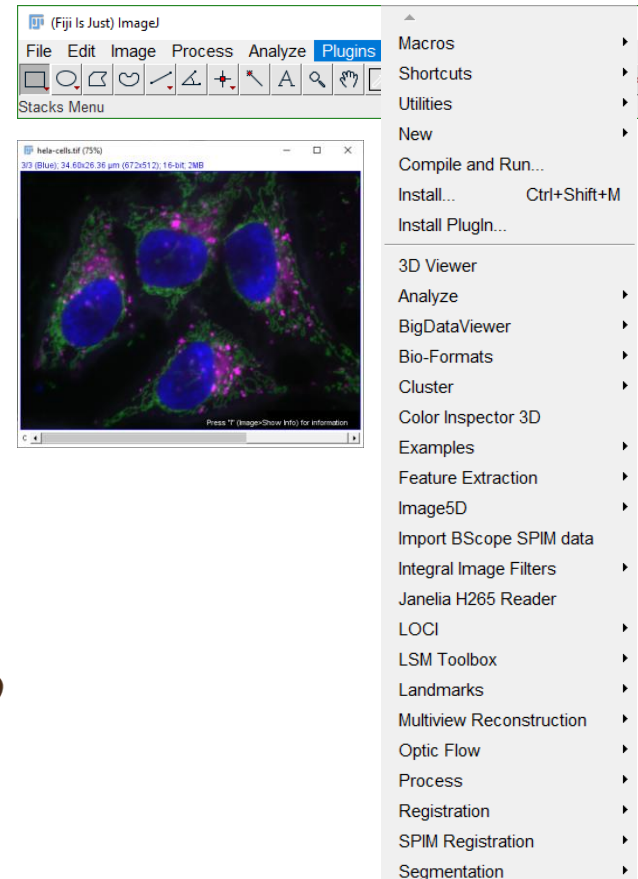
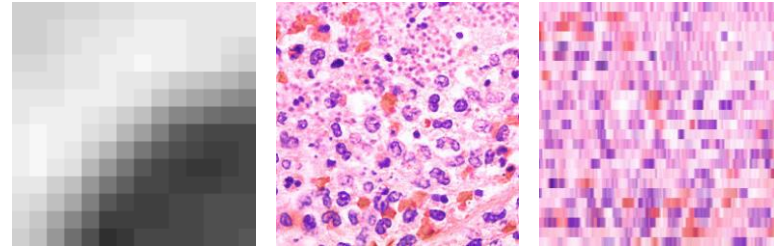
8000
hours / year
Electron
Microscopes

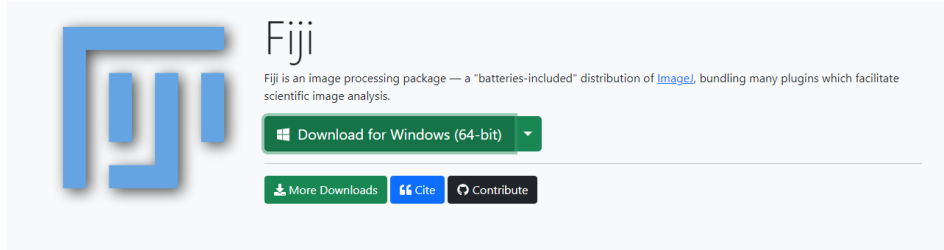
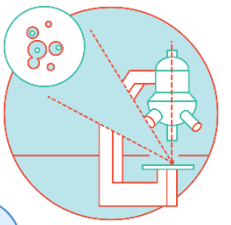
75000
hours / year
Image and Data
processing

> Overview



- Brief intro image analysis
- ImageJ/Fiji
 - Digital Image
 - Display and visualization
 - Histogram
 - Segmentation, Thresholding
 - Analysis particles workflow and basic measurements





<http://fiji.sc/>

Why Fiji?



Easy to Use

Fiji is easy to use and install - in one-click. Fiji installs all of its plugins, features an automatic updater, and offers comprehensive documentation.



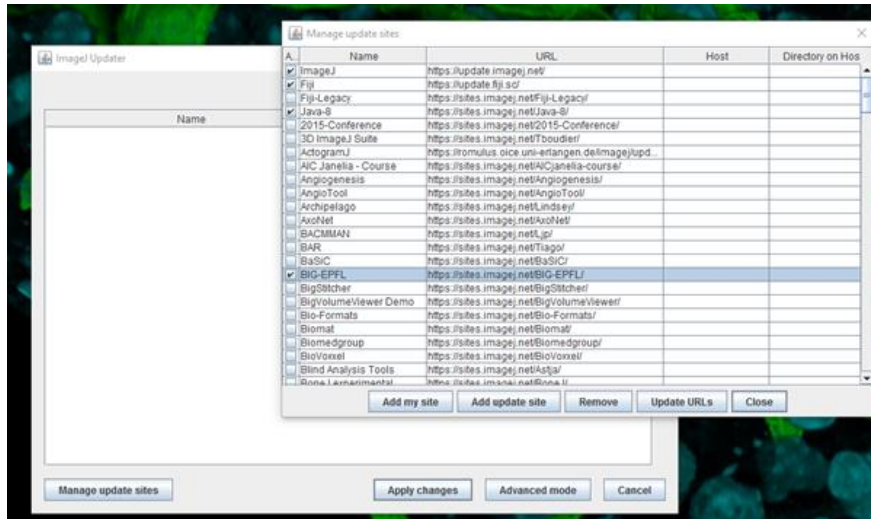
Powerful

Fiji bundles together many popular and useful ImageJ plugins for image analysis into one installation, and automatically manages their dependencies and updating.

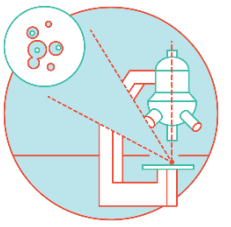


Free & Open Source

Like ImageJ itself, Fiji is an [open source](#) project hosted on [GitHub](#), developed and written by the community.



1. Download the appropriate Fiji version from <http://fiji.sc/>
2. Unzip your copy to a location where you have writing rights. Otherwise, you will not be able to automatically install the latest updates.
3. Check if you have the latest updates by running `Help>Update`
4. Additionally add the "BIG-EPFL" update site.
5. Click 'Update URLs' and 'Apply changes'.



Tutorials & Resources

For getting started in image processing, we have written several tutorials. We wrote them for the users of our facility, we hope they will be helpful to you.

In addition, be sure to check out our other resources.

ImageJ/Fiji

ImageJ/Fiji is one of the most popular free, open source tool for image processing and data extraction at our facility. It is simple, powerful, extendable by plugins and supports scripting languages for an easy automation of image processing tasks.

[Intro Fiji Image processing 2024 \(PDF, 3 MB\)](#)

[Intro Fiji image processing demo data 2024 \(ZIP, 17 MB\)](#)

ImageJ/Fiji

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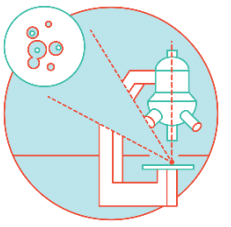
[Intro Fiji Image processing 2024 \(PDF, 3 MB\)](#)

[Intro Fiji image processing demo data 2024 \(ZIP, 17 MB\)](#)

[Intro Fiji macro programming \(PDF, 667 KB\)](#)

[Intro Fiji macro programming demo data \(ZIP, 12 MB\)](#)

<https://www.zmb.uzh.ch/en/Available-Systems/Image-processing/TutorialsAndResources0.html>



Universität
Zürich^{UZH}

Guides Support Team



Center for Microscopy and Image Analysis

This is the official guidebook of the Center for Microscopy and Image Analysis at the University of Zurich. Here you can find guides for light and electron microscopy as well as for image processing. If you want to see private guides, comment on guides or ask questions, please log-in using your ZMB core account credentials.



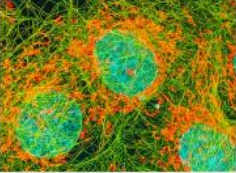
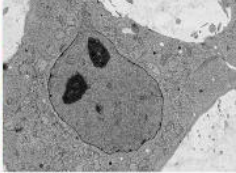
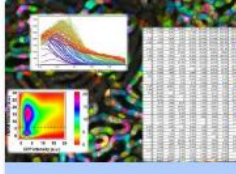

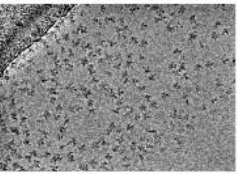
Author: Dozuki System (and 4 other contributors)

Create a Guide

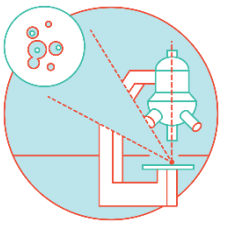
Home / Available Systems /

7 Categories

+ New Page

 <p>Basics, Access and IT</p>	 <p>Sample Preparation</p>	 <p>Light Microscopy</p>	 <p>Electron Microscopy</p>	 <p>Image Analysis</p>
 <p>Internal Guides</p>	 <p>CryoEM</p>			

https://zmb.dozuki.com/c/Image_Analysis



Introduction to Bioimage Analysis

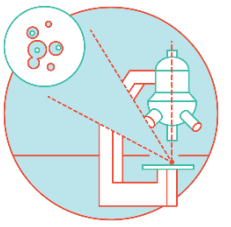
1. **Images in biology are enormously varied.** Almost nothing ‘just works’. I might find a paper describing a marvellous method to detect, classify and track cells – but there is no guarantee the method will work to detect, classify and track *my* cells. Maybe I have a different type of cell. Imaged on a different kind of microscope. At a different spatial and temporal resolution. To answer a different question. In short, I have a very different computational challenge from the one described in the paper – even if the shared theme of ‘tracking cells’ initially made it sound similar.
2. **Bioimage analysis involves a lot of disciplines.** Analysing images in a scientifically justifiable way typically requires (at least a bit of) knowledge across a lot of domains. Of course it’s necessary to know about the scientific question, e.g. the biology. But to really understand the data, you also need to know about the experimental setup, the imaging hardware, fundamental limits like noise and diffraction, and also how digital images are represented, stored and (sometimes) compressed. Then there are a plethora of image processing techniques that might help answer your scientific questions. You need to know not only what these are, but also how to assemble them together into a sequence of steps that work reliably and with minimal bias – either using existing software or by writing new computer code. And finally statistics to bring it all together. It’s a lot.

But amidst all this variety lurk some of the positive things about image analysis: it’s **creative**, it’s **challenging** (most of the time in a good way), and – because it’s rare for any individual to be an expert in all the related domains – it’s usually **collaborative** (or at least it should be).

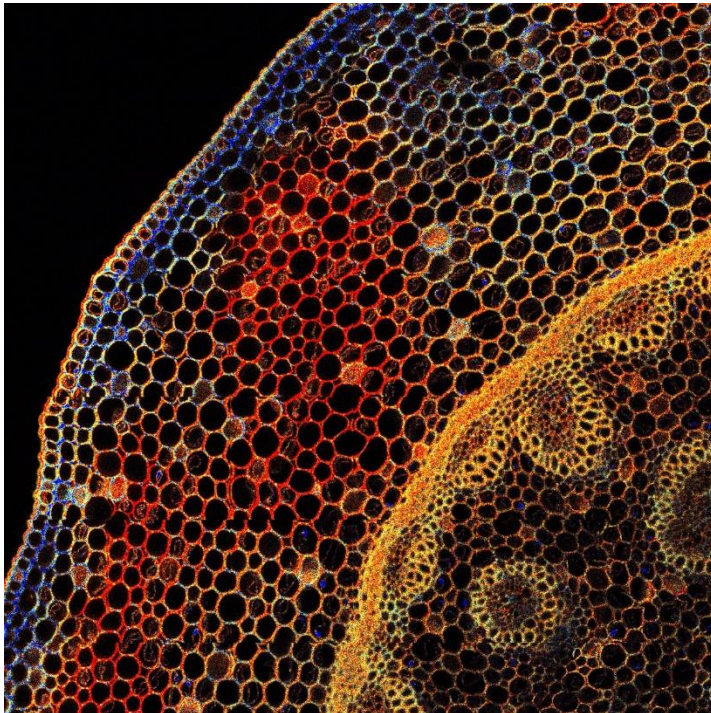
The fact that bioimage analysis is so cross-disciplinary means that pretty much everyone can have valuable insights to contribute.

This underpins my motivation in writing this book: I want to explain the concepts I use every day as an image analyst to people who spend their days differently. No matter who you are, you know a huge amount of stuff I don’t know. My hope is that if we put the stuff we know together, we’ll do better research, faster.

<https://bioimagebook.github.io/index.html>



- Deriving quantitative information from images of biological samples taken with microscopes



How many cells are there in the outer ring?

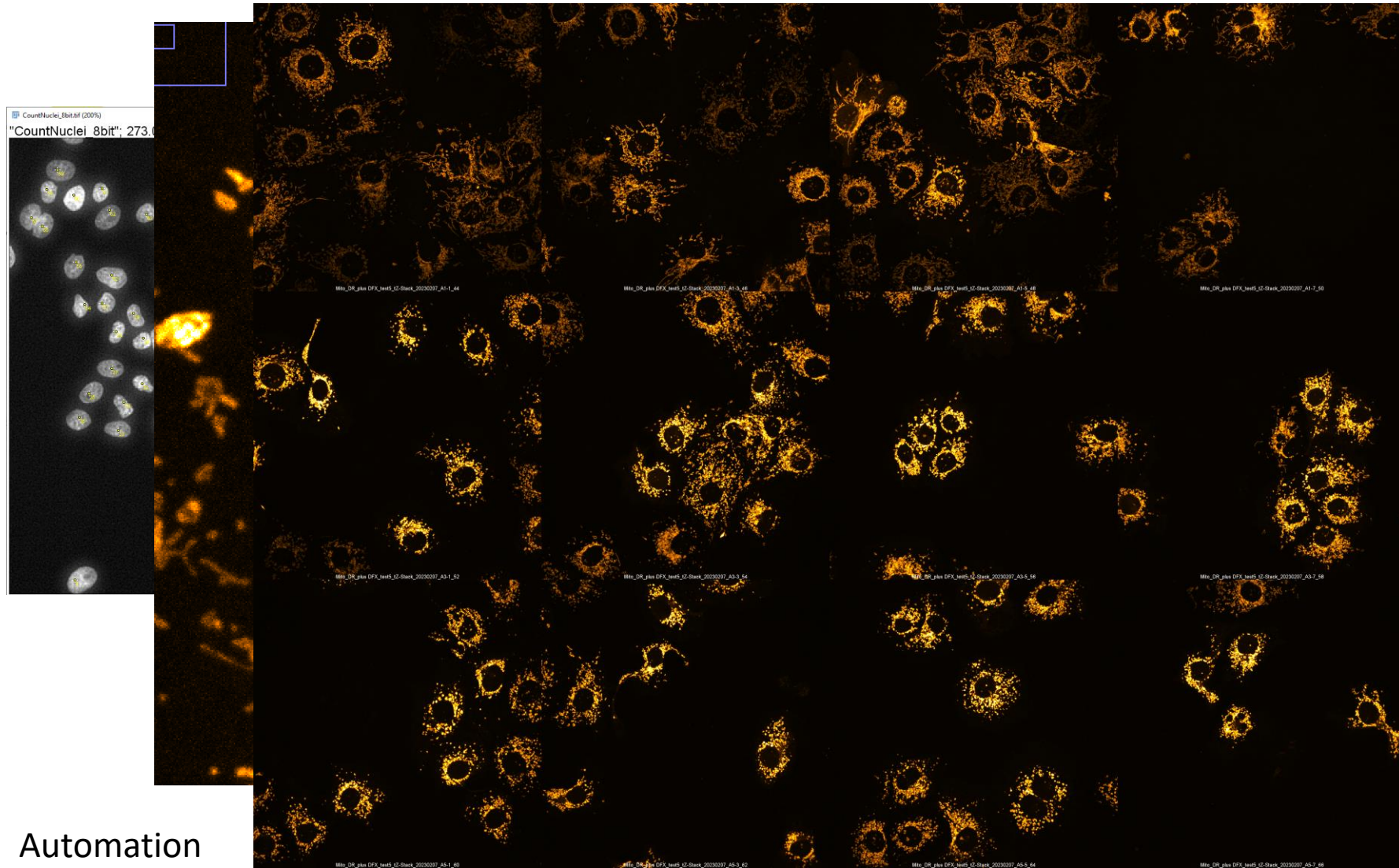
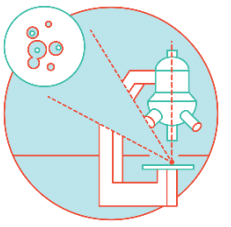
Biological question

How high is the intensity in the inner ring?

How is the signal in the blue channel distributed?

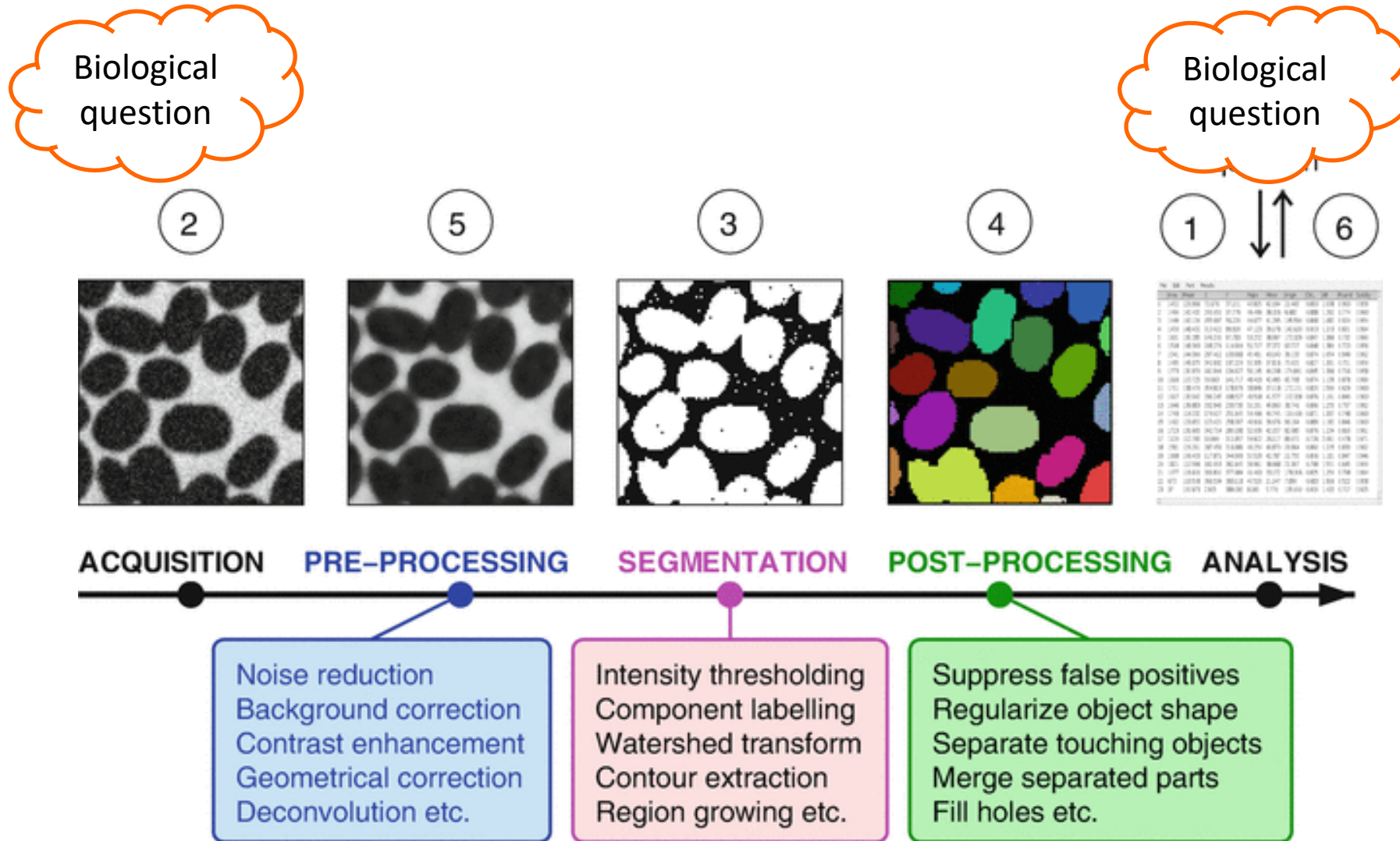
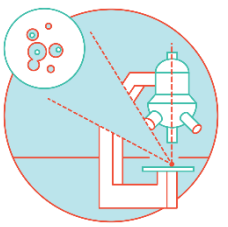
Image data source: Lorenzo Scipioni, U. California / Irvine
@LorenzoScipion3

> Image Processing and Analysis



Automation

> Image Processing workflows



> Image Processing workflows

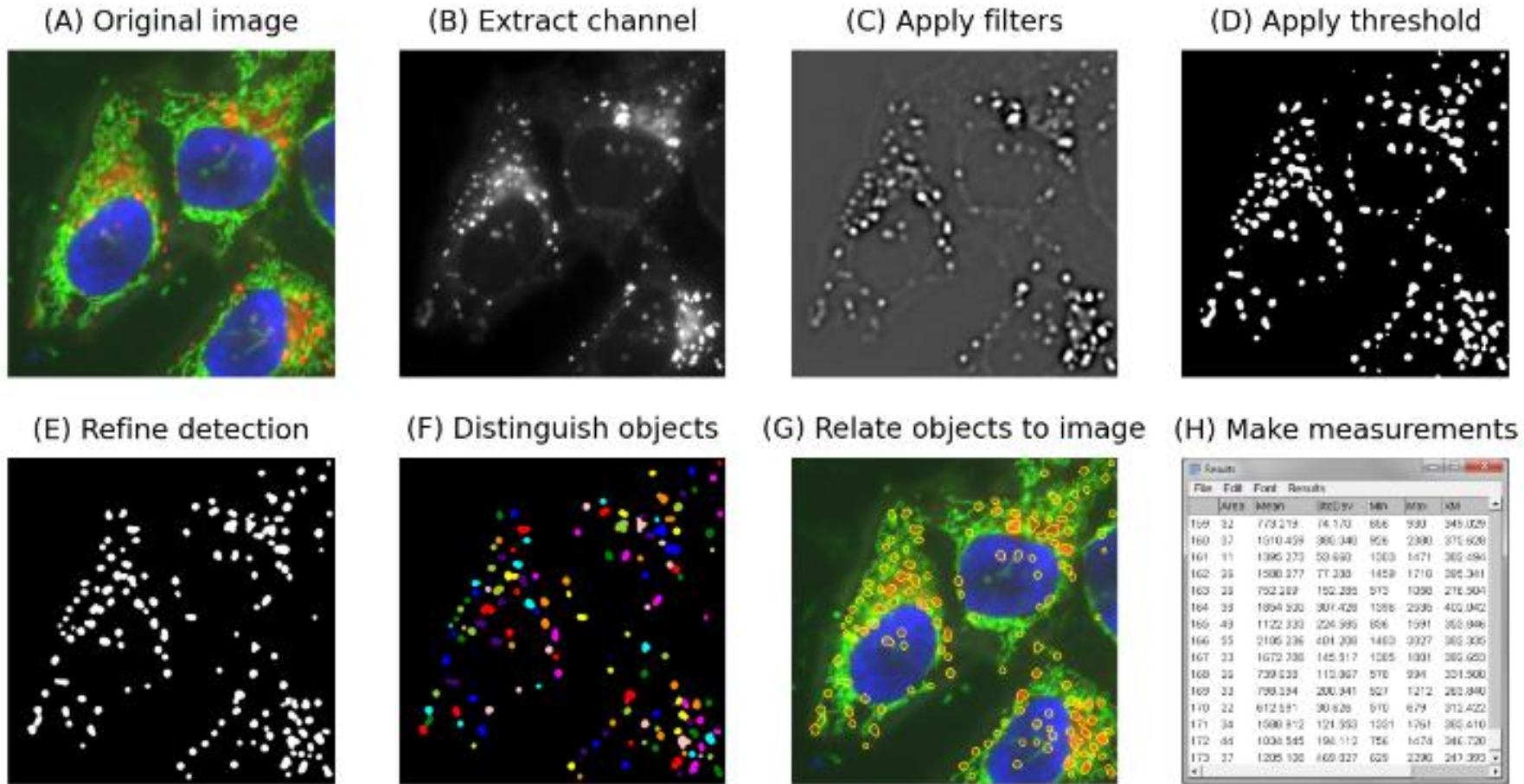
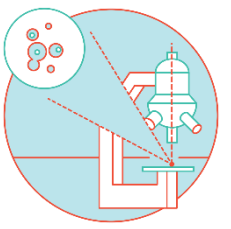
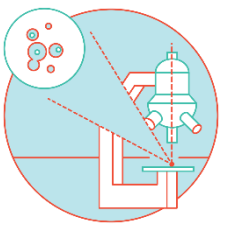


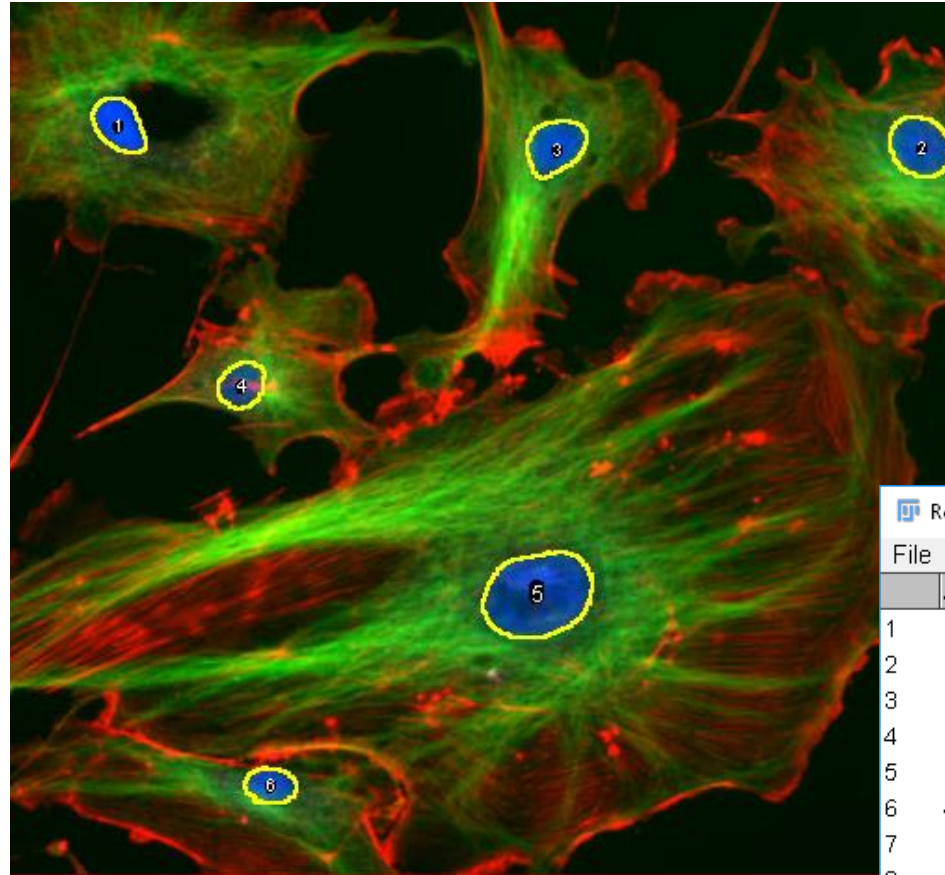
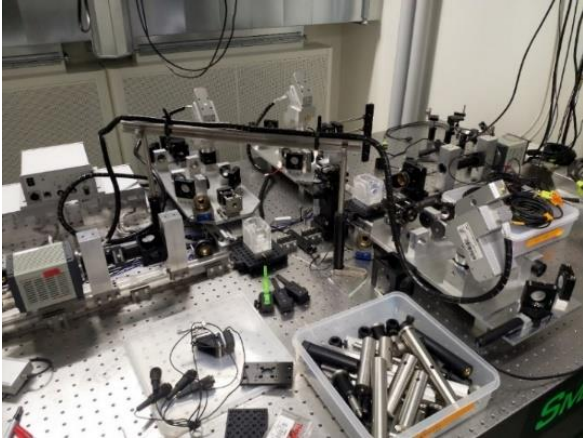
Fig. 56 A simple image analysis workflow for detecting and measuring spots in an image.

<https://bioimagebook.github.io/chapters/0-preamble/preface/preface.html>

> Introduction to Bioimage Analysis



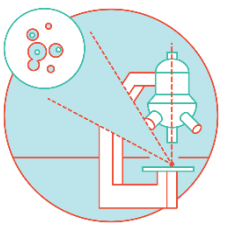
Tools we use are



File	Area	Perim.	Circ.	AR	Round	Solidity
1	2644	182.21	1.00	1.07	0.93	1.00
2	2680	183.78	1.00	1.09	0.92	1.00
3	3176	199.49	1.00	1.02	0.98	1.00
4	2733	185.35	1.00	1.07	0.93	1.00
5	3804	218.34	1.00	1.05	0.96	1.00
6	4188	229.34	1.00	1.09	0.92	1.00
7	2832	188.50	1.00	1.06	0.94	1.00
8	2970	193.21	1.00	1.12	0.89	1.00

URI: <http://imagej.nih.gov/ij/images/FluorescentCells.zip>

> Fiji: Image analysis



1997 NIH Image ImageJ

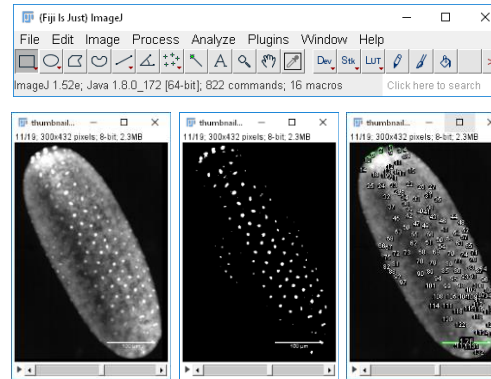
- Fiji is just ImageJ - with batteries included since 2005

Application

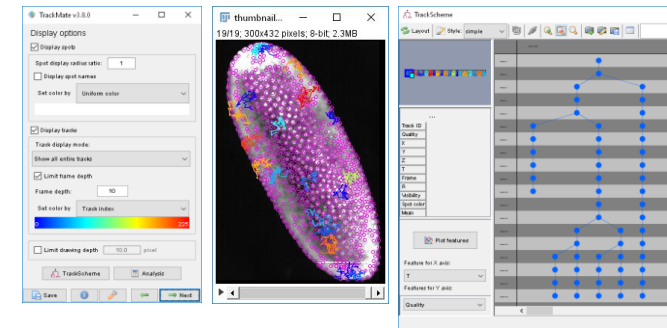
Reusable set of libraries

Shared framework for image analysis

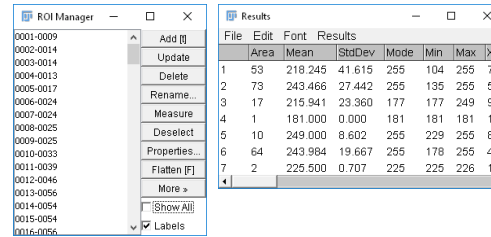
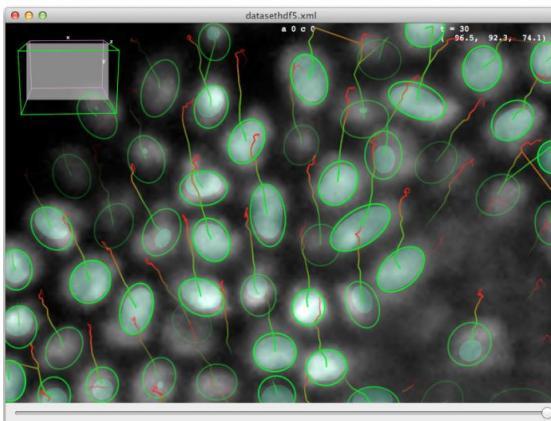
Core ImageJ



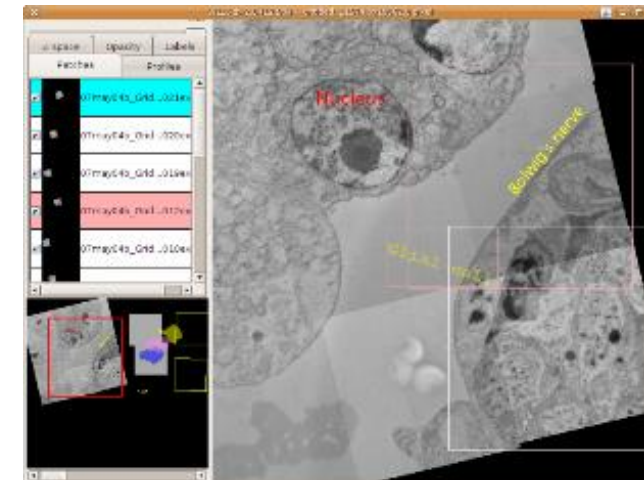
Trackmate



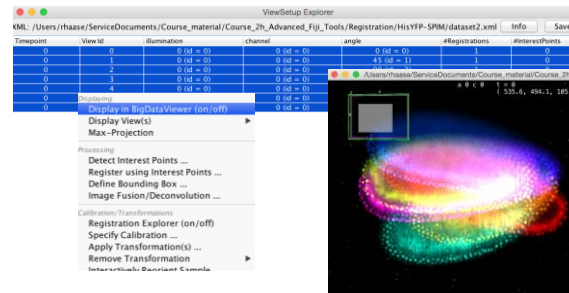
BigDataViewer



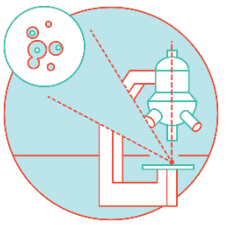
TrakEM



Multiview fusion



> ImageJ, Fiji & friends: community



- Visit <http://forum.image.sc> !

Batch process and Image calculator
Image Analysis fiji, imagej

Monara
Hi all,
I'm trying to use batch Mode for creating one image for each image of a list of images in a folder. how way they can be saved with a different name.

1. Img X and Y are in two different folders;
2. The title of X and Y images is the same, but
3. My image X is always a binary image and

I could do:

```
setBatchMode(true);
open("path_img X");
saveAs("rename img to XX");
XX = getTitle();
open("path_img Y");
Y = getTitle();
imageCalculator("Add create", XX, Y);
```

could anyone help me to improve this macro in calculator in the pairs of them and save the results?

Thank you in advance,
Monara

Batch process and Image calculator
Image Analysis fiji, imagej

CellKai The Last Pixelbender
Hi Monara,
If you have several pairs of images in the loop* in your macro.
You can add "Script Parameters" at the beginning of the macro.
Note that the macro command setBatchMode(true) is not displayed. Working on all your images of the folder.
Here is an example in the imageJ-macro forum.

```
// @File(Label="source director",Label="source director",Label="destination director")
// get a list of all files in the source directory
listx = getFileList(dirX);
listy = getFileList(dirY);

// stop the macro if the number of files is not the same
if (listx.length != listy.length)
    exit("number of images is not the same");

// set batch mode to not see the progress bar
setBatchMode(true);

// start a for loop to process the images
```

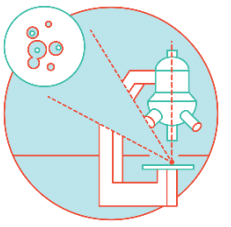
Batch process and Image calculator
Image Analysis fiji, imagej

Monara
Thank you a lot for your answer. I'm gonna check the links 😊

Monara
Thank you so much for the Macro. It works very well after adding "DirXY = getDirectory (XY);" and adjusting the files names 😊 I appreciate a lot the time you spend to make it. That was the last piece of my puzzle.

4 / 5
Sep 4

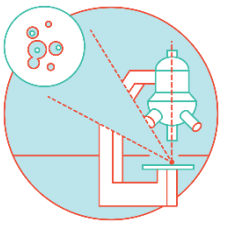
Topic Category Replies Views Activity



FiJi (ImageJ) Demo



> Fiji: user experience

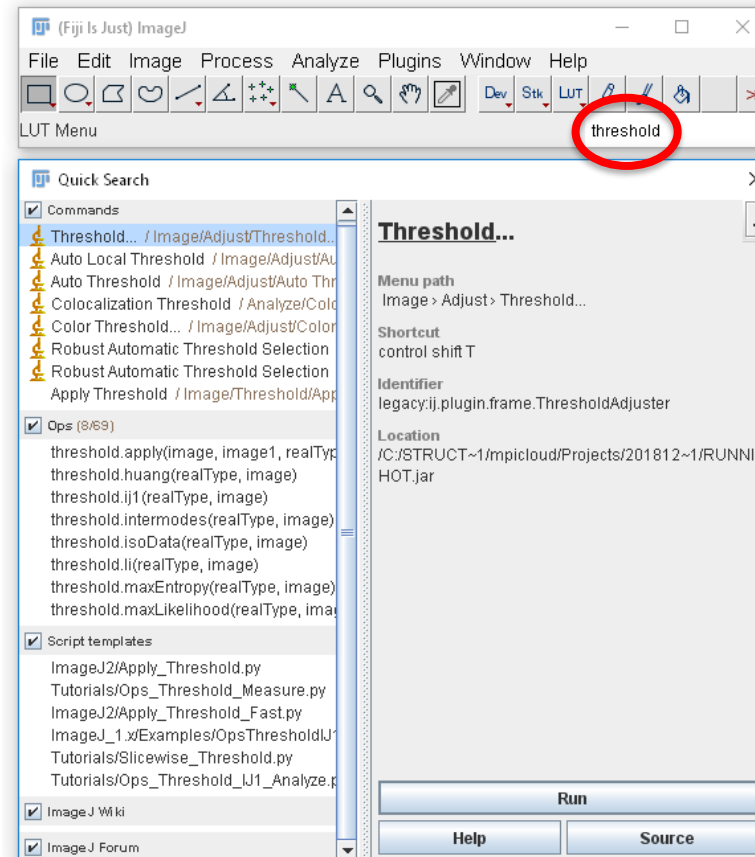


- If you cannot find the command,
 - Use the search field!
 - Press the “L” key and start typing in the search bar.
- It shows you where the plugin is located
- You can run it from here (enter)

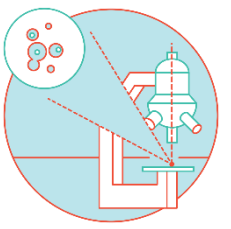


Navigating through confusing menus

*Additional plug-ins can be accessed through the update site
Help < Update*



> Open an image – Bio formats



Formats / Supported Formats [Edit on GitHub](#)

Supported Formats

Ratings legend and definitions

Format	Extensions	Pixels	Metadata	Openness	Presence	Utility	Export	BSD	Multiple Images	Pyramid
OME-TIFF	.ome.tif, .ome.tf2, .ome.tf8, .ome.btf	🟢	🟢	🟢	🔻	🟢	✅	✅	✅	✅
Zeiss Axio CSM	.lms	🟡	🔻	🔻	🔻	🔻	❌	❌	❌	❌
Zeiss AxioVision TIFF	.xml, .tif	🟡	🟡	🟡	🔻	🔻	❌	❌	✅	❌
Zeiss AxioVision ZVI (Zeiss Vision Image)	.zvi	🟢	🟡	🟡	🟡	🟡	❌	❌	❌	❌
Zeiss CZI	.dzi	🟢	🟢	🟡	🔻	🟡	❌	❌	✅	✅
Zeiss LSM (Laser Scanning Microscope) 510/710	.lsm, .mdb	🟢	🟡	🟡	🟡	🟡	❌	❌	✅	❌
Bio-Rad PIC	.xml	🟢	🟢	🟡	🟡	🟡	❌	❌	❌	❌
Bio-Rad SCN	.scn	🟡	🔻	🔻	🔻	🔻	❌	❌	❌	❌
Bitplane Imaris	.ims	🟡	🟡	🟡	🔻	🔻	❌	❌	✅	✅

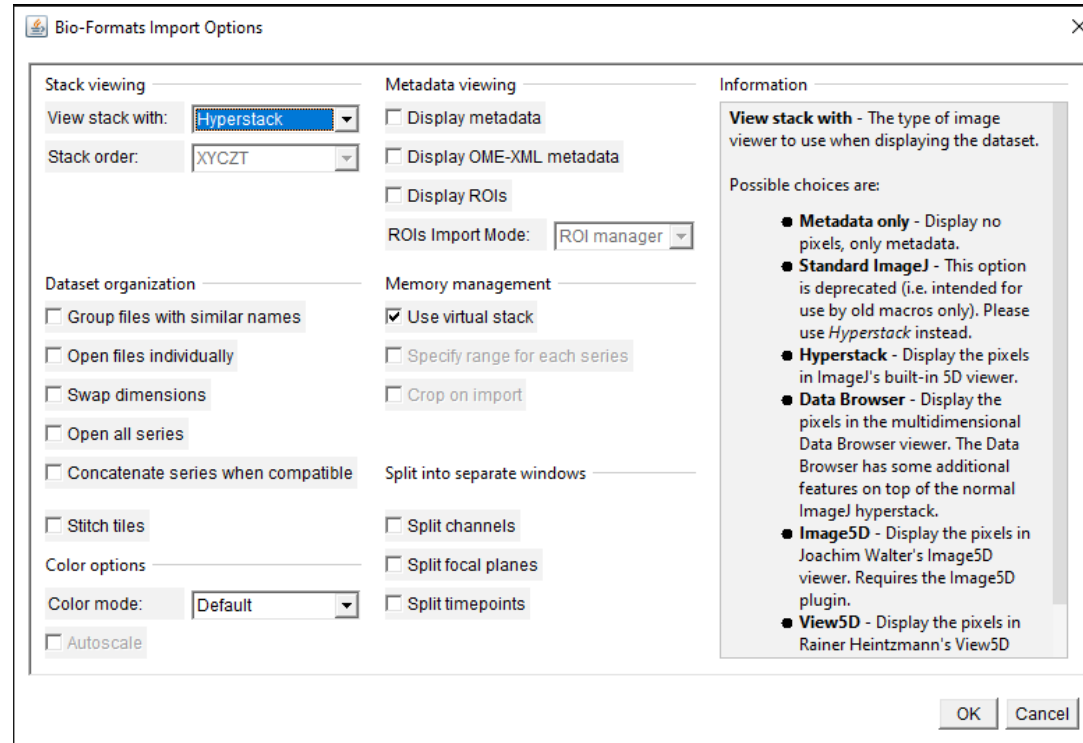
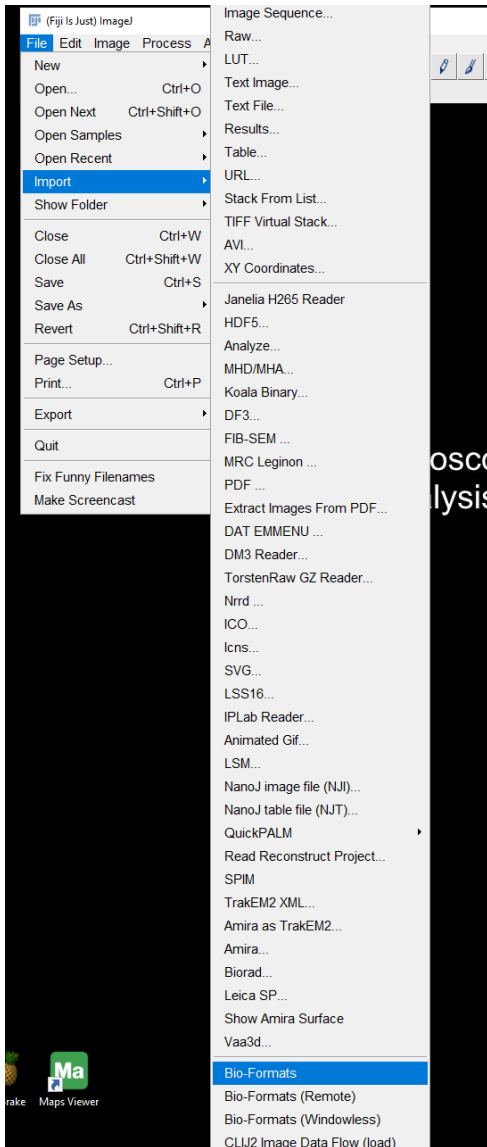
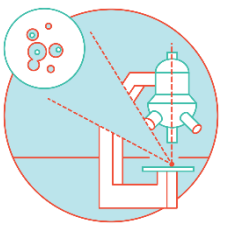
Bio-Formats currently supports 161 formats

Ratings legend and definitions

🟢	Outstanding
🟡	Very good
🟠	Good
🔻	Fair
🔴	Poor

<https://bio-formats.readthedocs.io/en/v7.3.0/supported-formats.html>

> Open an image – Bio formats



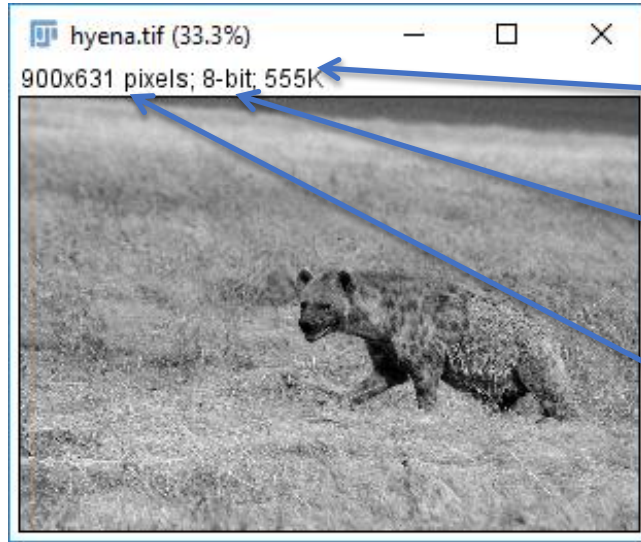
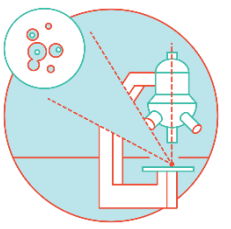
File > Import > Image sequence

Really large data

Memory management

Virtual stack

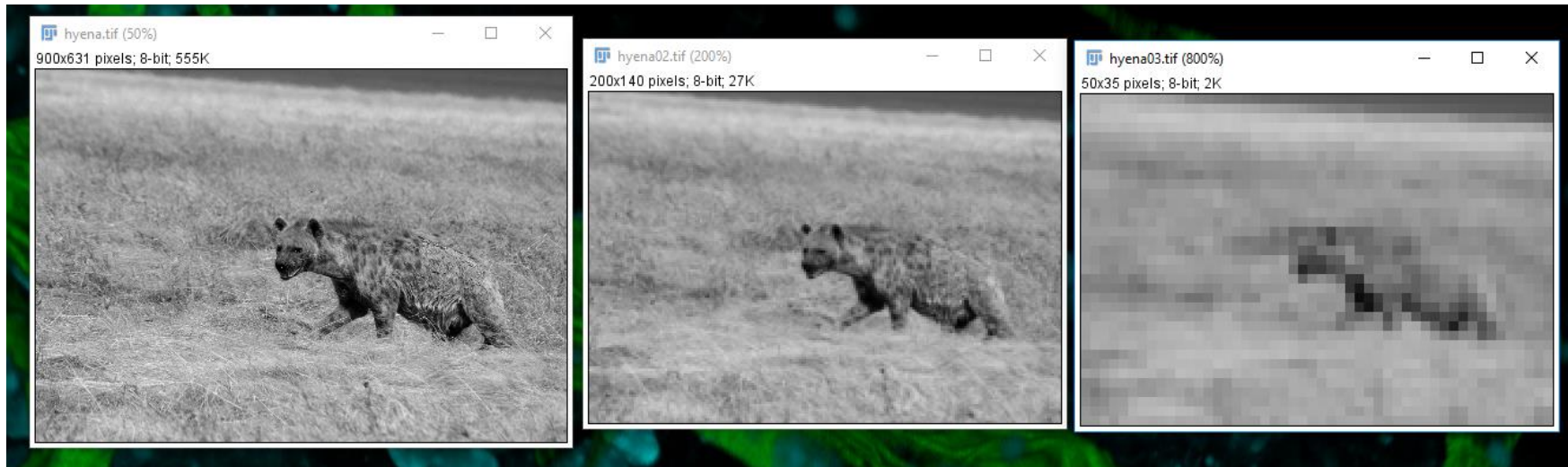
> FiJi: Opening images with FIJI



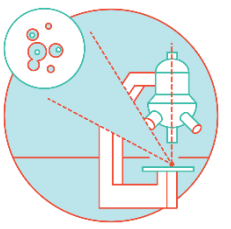
Memory in hard drive

Image bit-depth

Image size in pixels

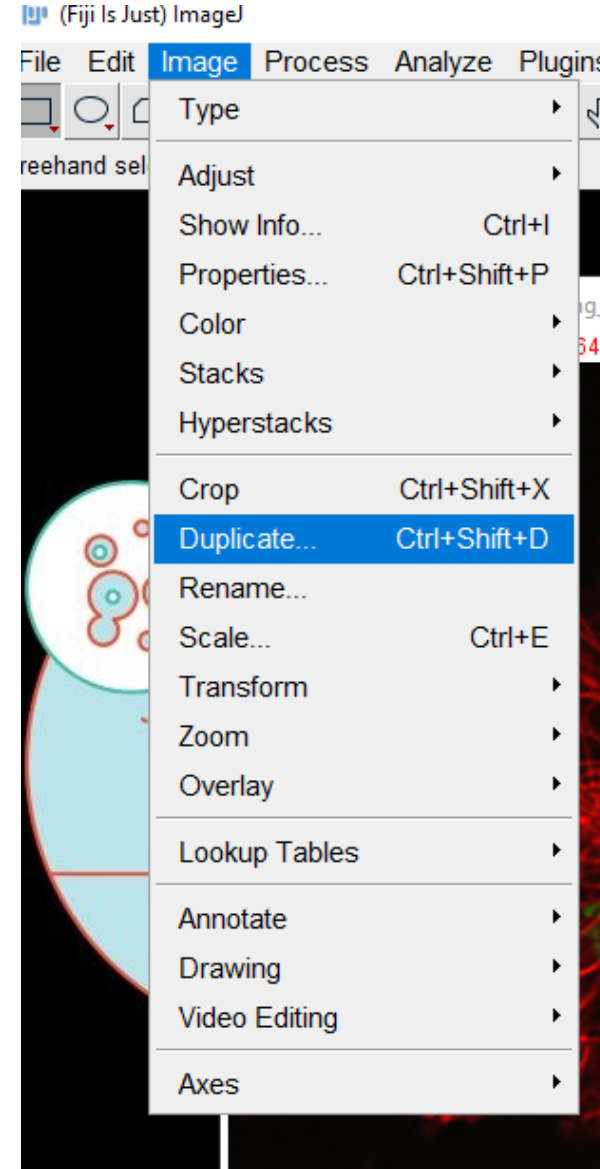


> Duplicate



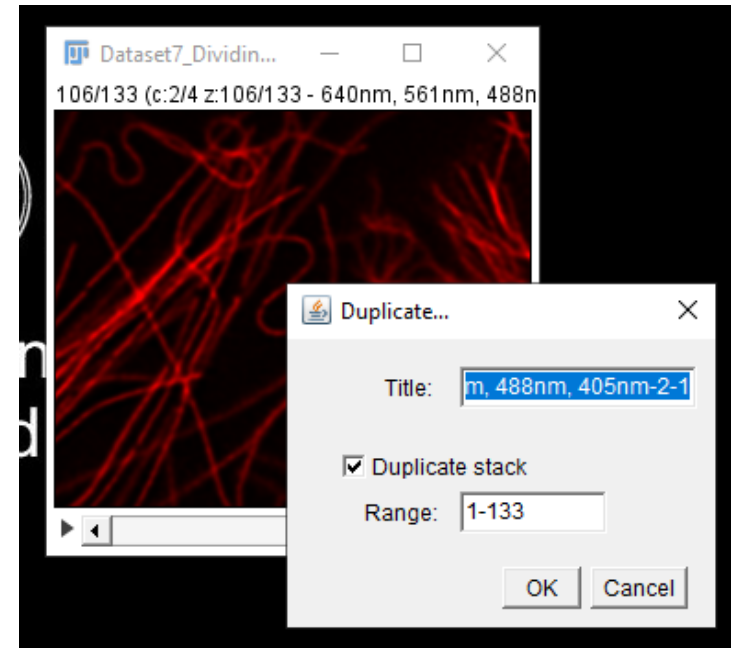
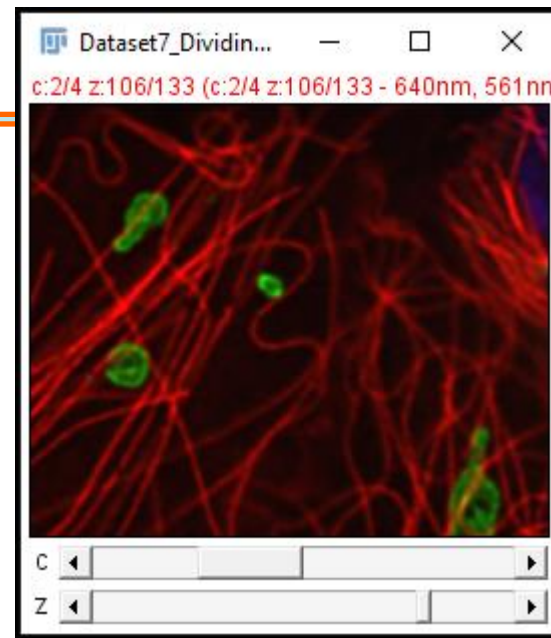
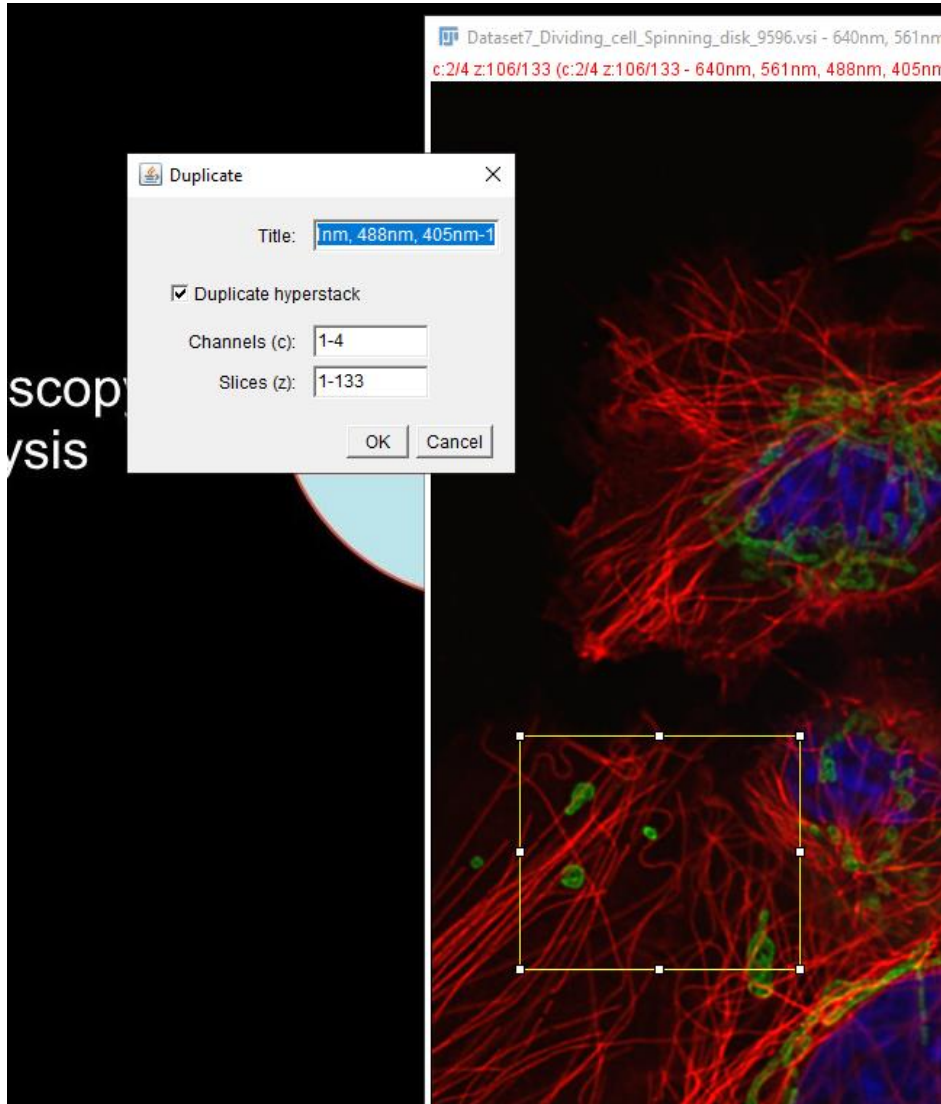
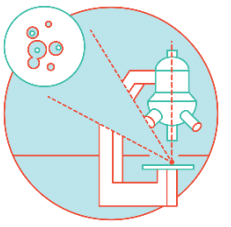
ImageJ provides very limited **Undo** support for 2D slices

If you suspect you might regret a processing step, good practice to always duplicate the image beforehand with

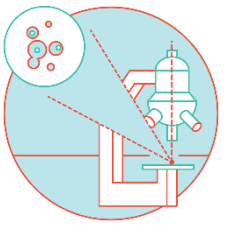


Shift + D

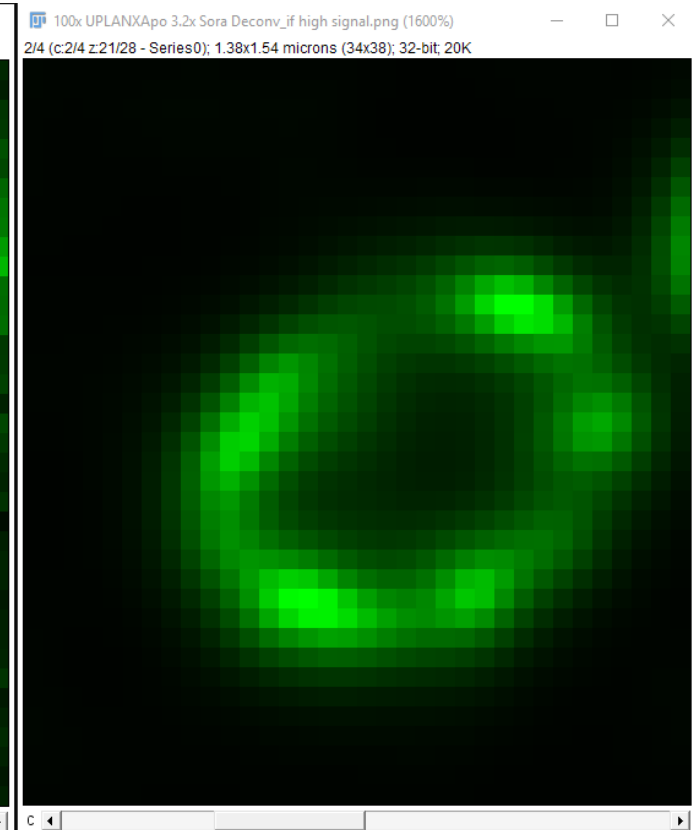
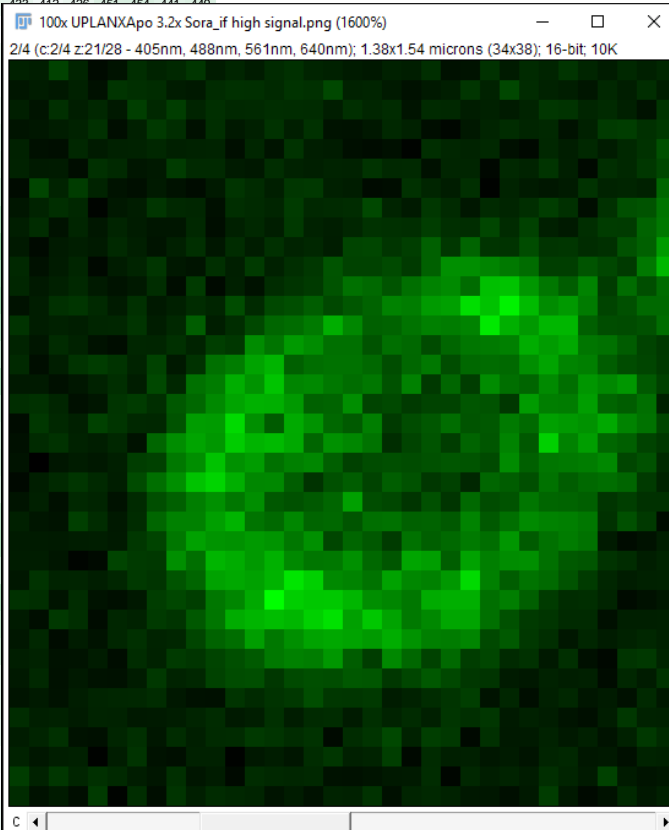
> Duplicate



> Digital images: Matrices of digital numbers



426	423	457	429	405	413	438	428	433	446	421	430	421	435	441	429	426	420	417	426	423	439	411	413	442	420	435	429	445	430	421	433	425	432
415	421	417	426	411	445	440	437	421	418	436	441	440	434	444	441	464	427	429	419	411	418	423	446	427	463	436	427	423	415	432	423	438	422
424	414	440	423	432	418	455	433	454	429	440	433	434	438	437	436	423	440	431	436	414	435	433	422	426	420	428	447	444	430	435	425	412	445
414	416	422	427	441	408	425	450	438	411	439	418	425	443	424	442	413	412	417	433	426	408	401	417	429	437	430	422	412	426	454	454	444	440
426	410	408	432	420	426	427	432	425	427	428	409	431	414	444	423	421	409	406	410	398	439	431	428	433	410	403	422	412	426	454	454	444	440
427	419	420	407	441	434	415	418	452	434	434	420	417	438	427	424	428	427	444	433	434	411	430	430	402	433	428	422	412	426	454	454	444	440
405	461	428	453	428	413	409	419	412	406	419	445	436	424	447	449	425	422	405	407	442	430	422	440	397	428	420	422	412	426	454	454	444	440
415	428	439	432	399	417	422	446	433	431	410	439	435	430	452	433	435	437	464	429	419	448	435	416	435	430	439	422	412	426	454	454	444	440
423	437	412	430	416	429	421	415	415	433	408	425	433	423	450	427	423	431	443	441	439	454	440	438	462	436	457	422	412	426	454	454	444	440
418	404	419	423	419	434	424	414	425	440	416	437	422	441	428	432	422	460	462	446	455	488	448	492	445	444	468	422	412	426	454	454	444	440
433	411	420	430	403	421	429	446	417	414	440	424	451	426	439	448	476	458	458	466	451	488	534	533	523	512	499	422	412	426	454	454	444	440
431	418	425	433	419	413	418	438	407	451	404	425	412	437	445	451	475	504	497	476	502	521	529	499	581	584	541	422	412	426	454	454	444	440
412	422	441	446	434	420	441	418	425	437	430	468	435	464	470	485	464	479	491	521	516	544	550	611	583	630	577	422	412	426	454	454	444	440
444	422	426	423	430	431	445	422	433	485	423	438	464	494	499	513	564	526	488	491	506	519	515	522	622	566	543	422	412	426	454	454	444	440
428	415	436	427	412	407	431	430	441	428	455	448	531	507	544	531	503	503	475	502	496	467	494	509	513	519	563	422	412	426	454	454	444	440
434	408	423	444	453	444	428	458	418	443	503	516	553	570	488	501	505	505	496	469	495	511	469	496	499	497	533	422	412	426	454	454	444	440
418	419	410	416	427	439	422	464	432	504	515	564	538	585	524	528	511	500	510	474	520	450	494	454	531	502	493	422	412	426	454	454	444	440
438	442	430	410	415	445	452	439	480	496	528	554	567	565	543	489	492	533	460	465	451	459	459	478	475	449	545	422	412	426	454	454	444	440
406	448	433	425	457	446	433	432	475	547	545	607	534	483	560	538	496	482	517	451	468	479	500	476	480	513	498	422	412	426	454	454	444	440
410	435	443	427	423	432	412	494	532	554	541	593	570	576	559	493	536	526	506	438	460	476	454	492	478	522	480	422	412	426	454	454	444	440
413	394	415	421	417	424	454	457	511	533	583	536	546	504	519	526	487	457	468	471	468	473	478	476	466	530	488	422	412	426	454	454	444	440
403	423	434	446	434	423	467	502	534	584	601	565	526	527	485	444	472	485	459	486	474	448	457	432	494	485	471	422	412	426	454	454	444	440
414	425	410	433	429	412	435	470	488	517	535	510	533	480	473	481	494	550	469	463	446	472	446	497	468	475	509	422	412	426	454	454	444	440
425	415	413	456	437	418	437	494	520	520	549	570	507	497	526	493	496	477	502	505	489	491	481	522	481	502	529	422	412	426	454	454	444	440
420	420	419	422	423	419	448	460	510	551	539	536	544	539	510	535	462	494	480	452	505	487	497	513	501	540	483	422	412	426	454	454	444	440
421	422	415	399	404	460	451	449	534	502	571	562	543	567	528	509	511	535	496	478	555	548	486	552	518	531	534	422	412	426	454	454	444	440
416	444	428	429	423	431	450	445	476	493	552	558	551	569	618	597	528	522	506	552	526	520	542	607	519	485	506	422	412	426	454	454	444	440
442	439	414	437	413	409	417	454	463	455	510	525	559	644	595	588	589	572	533	525	510	561	565	565	502	517	487	422	412	426	454	454	444	440
420	408	449	439	406	431	427	451	445	480	509	562	571	579	572	597	614	550	588	553	512	527	556	554	503	473	451	422	412	426	454	454	444	440
416	432	406	421	413	431	441	428	431	461	491	501	548	511	534	553	558	553	544	563	538	518	515	524	465	481	449	422	412	426	454	454	444	440
423	427	412	413	421	407	437	427	451	446	460	471	482	511	520	516	541	485	454	527	495	458	497	454	462	435	442	422	412	426	454	454	444	440
418	433	411	418	412	411	421	449	428	432	458	468	470	448	466	471	469	480	478	448	462	480	464	448	445	420	417	422	412	426	454	454	444	440
414	419	412	422	429	431	432	437	417	411	410	445	435	444	449	469	448	447	450	421	427	437	445	455	413	431	408	422	412	426	454	454	444	440
437	424	426	420	428	427	418	419	422	429	436	419	423	441	422	435	437	420	440	428	436	425	446	440	447	419	426	422	412	426	454	454	444	440
421	429	441	412	410	430	440	416	437	424	435	436	450	445	437	443	404	437	445	464	427	455	449	403	441	418	419	422	412	426	454	454	444	440
427	416	423	425	397	422	444	412	409	426	426	397	417	422	415	437	447	425	428	455	403	436	424	425	416	420	414	429	432	416	418	410	428	426
453	427	447	430	441	434	422	415	440	415	421	404	437	433	431	433	412	411	414	418	419	437	400	432	423	428	421	439	429	419	457	420	414	416
436	412	420	408	451	398	438	408	445	424	436	432	429	414	421	412	428	426	443	410	429	433	427	417	416	409	418	453	418	404	412	433	417	425



> Distribution of Intensity Values: Image Histogram

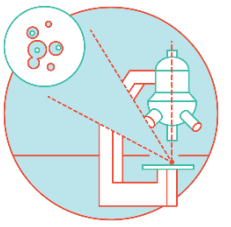
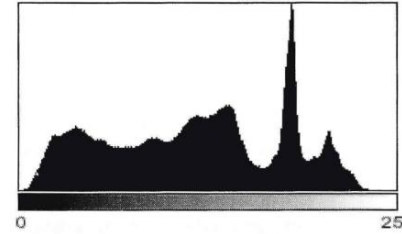
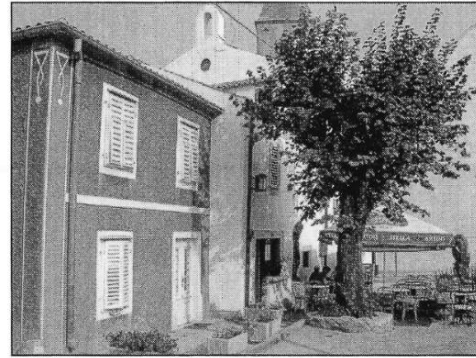
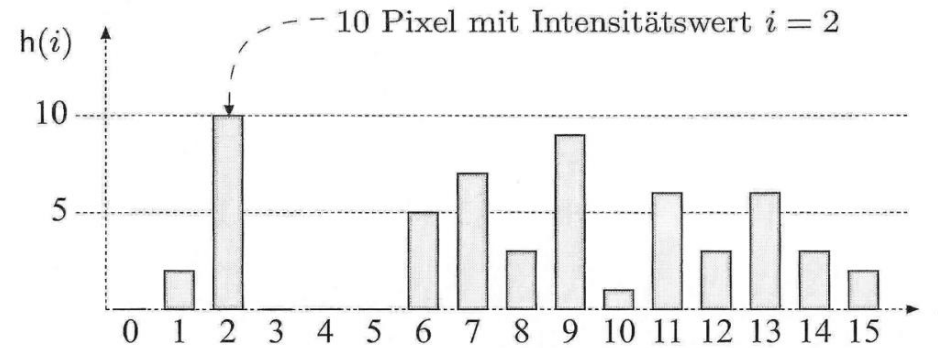


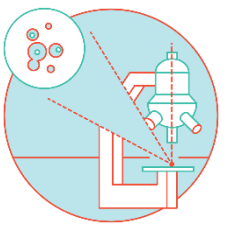
Image Histogram: Important tool to assess image properties and manipulations such as contrast, dynamic range, saturations, compression,...



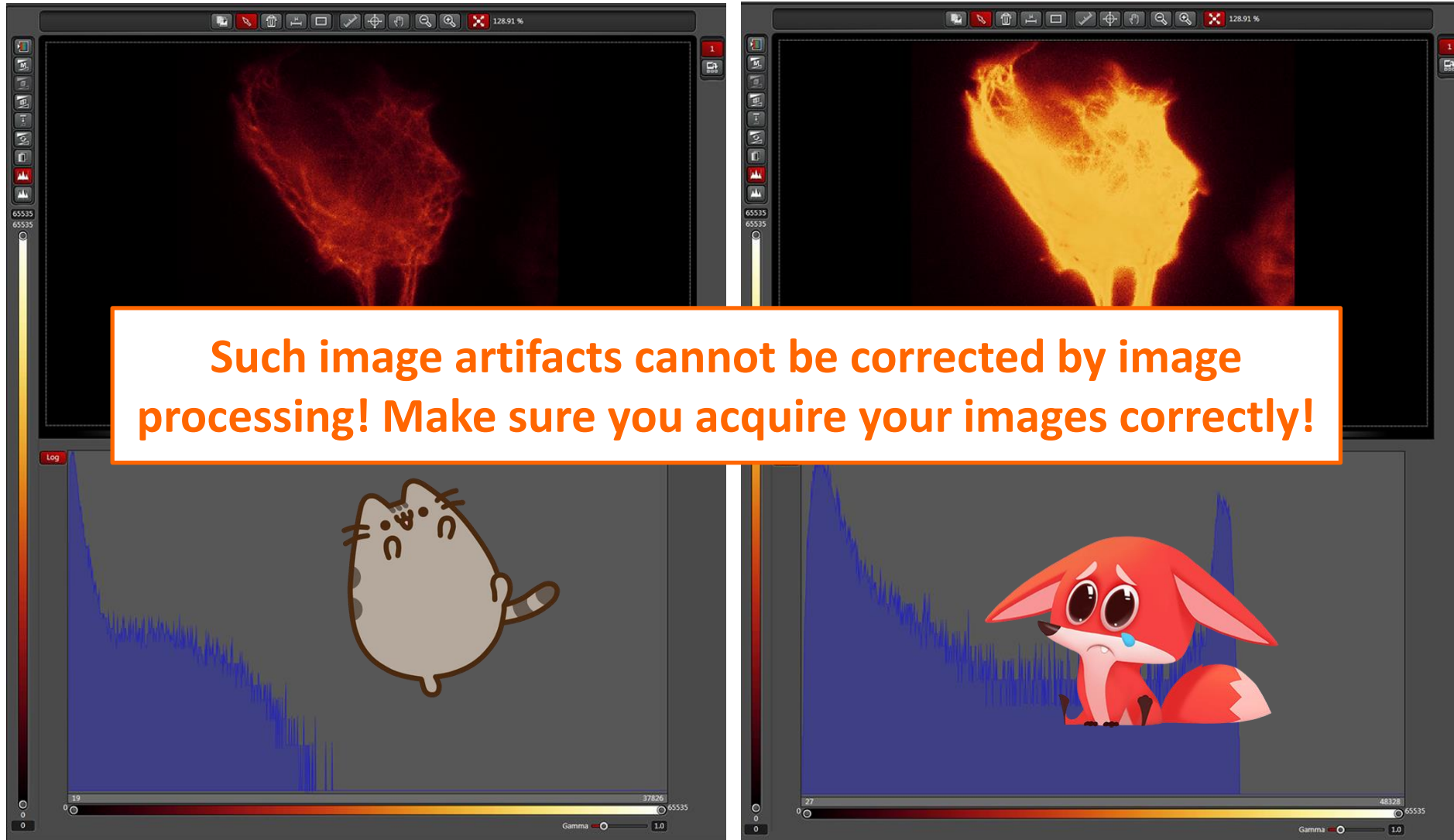
Count: 1920000 Min: 0
Mean: 118.848 Max: 251
StdDev: 59.179 Mode: 184 (30513)

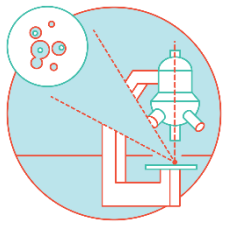


$h(i)$	0	2	10	0	0	0	5	7	3	9	1	6	3	6	3	2
i	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

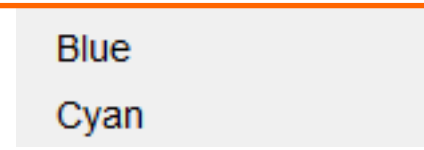
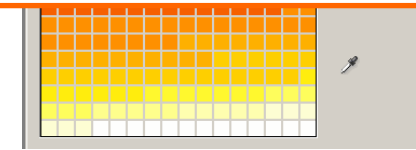
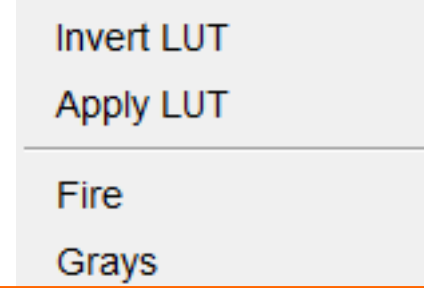
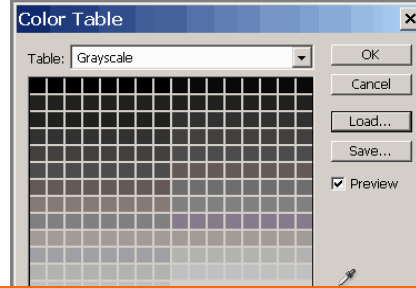
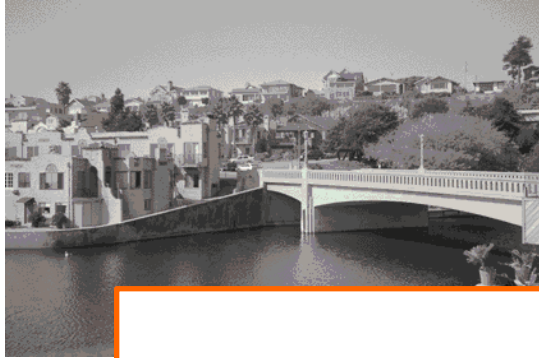


Live histogram as a tool to assess imaging quality: Which one is better?

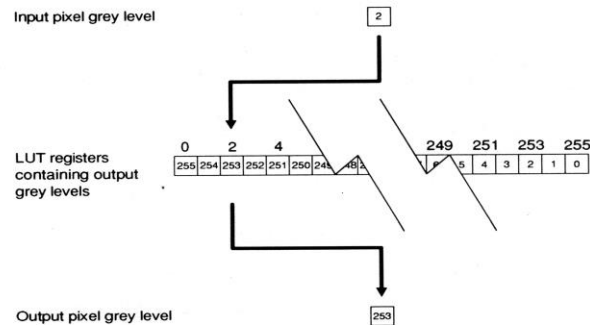




Adjusting look up tables (LUT)

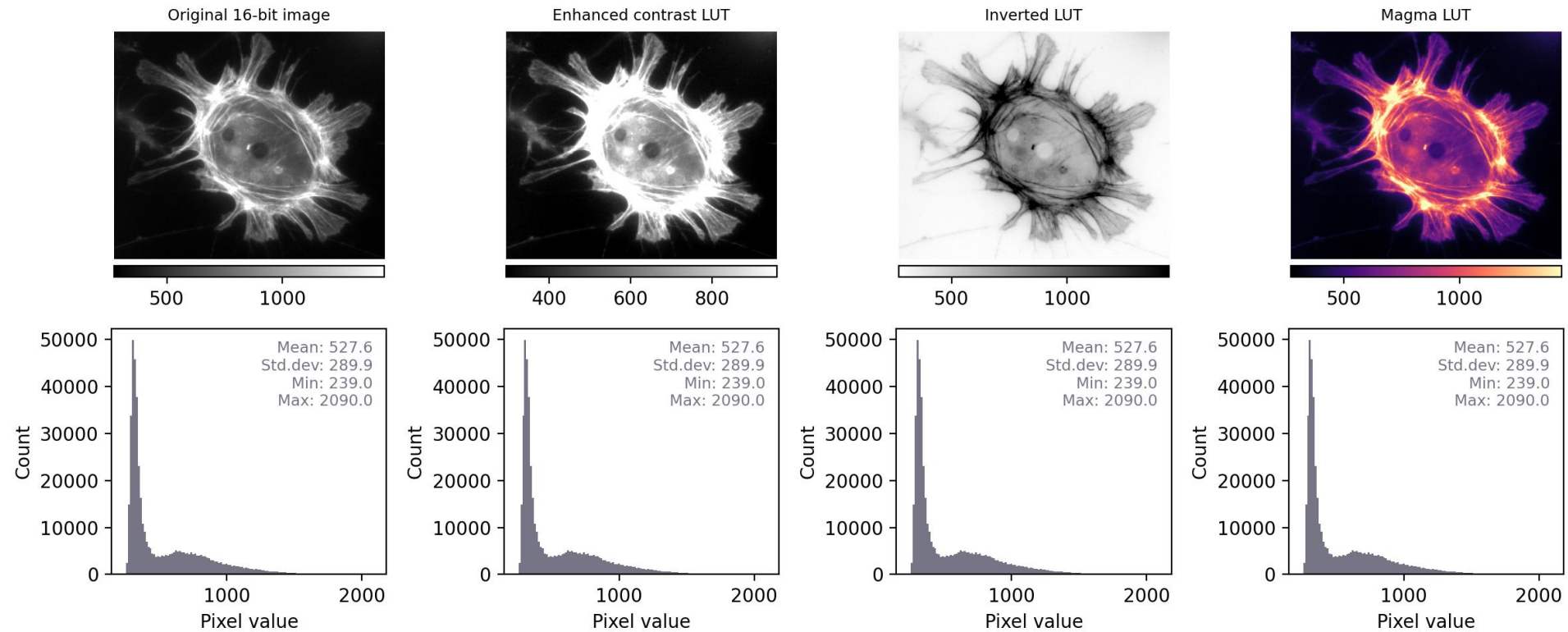
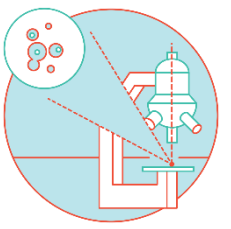


Applying a different lookup table doesn't change the image. All pixel values stay the same, they are just displayed differently.



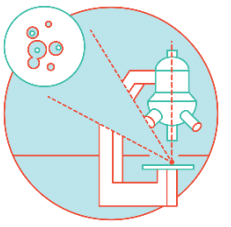
A look up table (LUT) assigns each image intensity value a grey or color value. In imageJ various LUT's can be selected.

> Visualization: Assigning Colors to Image Intensities



<https://bioimagebook.github.io/chapters/1-concepts/2-measurements/measurements.html>

> Lookup tables

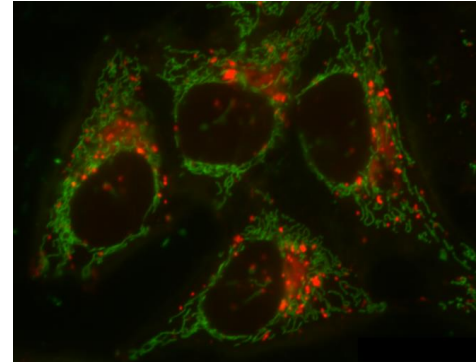


- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!

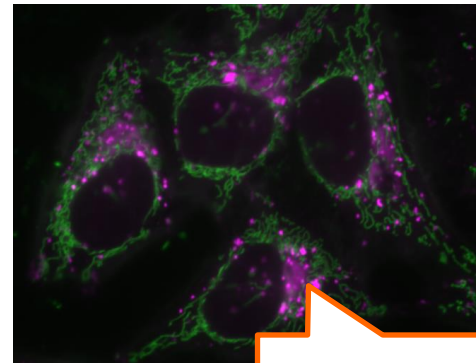
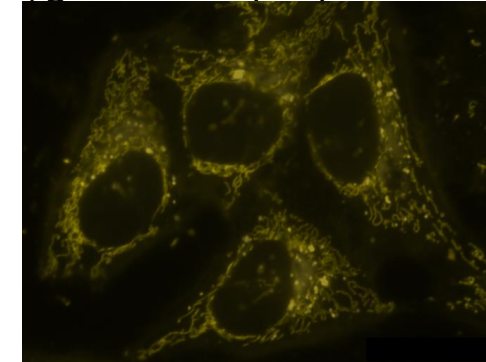
Color-blind friendly Palette

Original	Simulation			Hue	
	Protan	Deutan	Tritan		
1	Black	Black	Black	Black	0°
2	Orange	Orange	Orange	Orange	41°
3	Sky Blue	Sky Blue	Sky Blue	Sky Blue	202°
4	bluish Green	bluish Green	bluish Green	bluish Green	164°
5	Yellow	Yellow	Yellow	Yellow	56°
6	Blue	Blue	Blue	Blue	202°
7	Vermilion	Vermilion	Vermilion	Vermilion	27°
8	reddish Purple	reddish Purple	reddish Purple	reddish Purple	326°

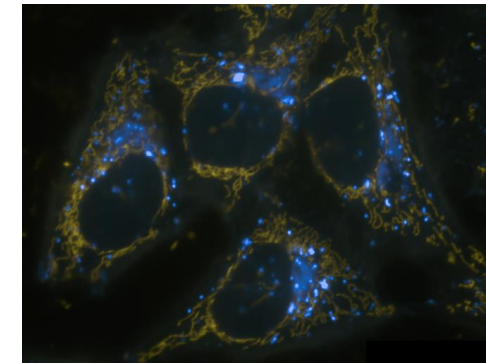
Default view

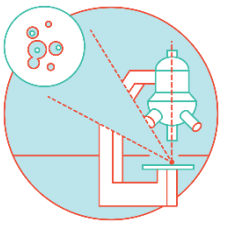


Red/green blind people see it like



Replace red with magenta!

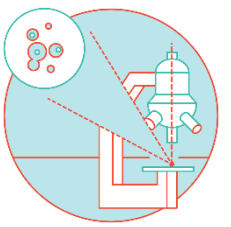




FiJi (ImageJ) Demo



>Macro language



```
Bio401_testing.ijm
File Edit Language Templates Run Tools Tabs
Bio401_testing.ijm
1 run("Green");
2 run("Cyan");
3 run("glow v2");
4 run("Yellow");
5 //run("Channels Tool...");
6 Stack.setDisplayMode("composite");
7 //run("Brightness/Contrast...");
8 run("Enhance Contrast", "saturated=0.35");
9 setMinAndMax(0, 40);
10 setMinAndMax(3, 182);
11 setMinAndMax(3, 38);
12
13
Run Batch Kill Show Errors Clear
```

Commands will automatically appear in brown,

“text in magenta between inverted commas”,

values in blue and

comments in green.

All commands end with ;

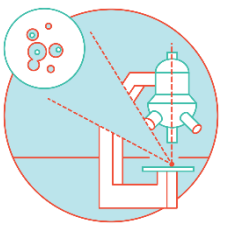
You can check other available scripting languages under the menu Language.

Functions can require additional arguments.

If the command being used by `run` requires extra information of its own, this is included as an extra string.

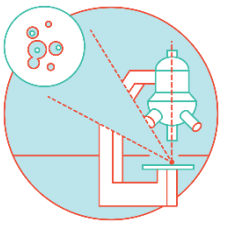
```
run("Duplicate...", "title=New_image.tif");
```

> Very short macro from this session



```
*Macro LS 2024.ijm  *Macro.ijm  *Macro.ijm
1  //this macro was created to demo the functionalities of fiji macro recorder
2  //2024 09 20 ZMB / BVC Lunch seminar series / Bioimage analysis
3
4  //Activate the image you'd like to adjust
5  Stack.setChannel(1);
6  //Display with defined B&C values
7  setMinAndMax(0, 1000);
8  run("Cyan");
9
10 //Display adjusted automatically for channel 2
11 Stack.setChannel(2);
12 run("Enhance Contrast", "saturated=0.35");
13 run("Red");
14
15 //Display adjusted automatically for channel 3
16 Stack.setChannel(3);
17 run("Enhance Contrast", "saturated=0.35");
18 run("Magenta");
19
20
```

> Very short macro from this session



```
< *Macro LS 2024.ijm *Macro.ijm *Macro.ijm (Running)
>
1 //2024 09 Bioimage analysis seminar series
2 //This macro was created during a Fiji/ImageJ Lunch seminar demo
3 //It briefly presents the Analyze particles workflow
4
5 selectImage("CountNuclei_8bit.tif");
6 run("Duplicate...", " ");
7
8
9 //Applies a Gaussian filter, this smooths an image and reduces noise.
10 run("Gaussian Blur...", "sigma=1");
11
12 //Uses Otsu thresholding method (usually performs best on image with a bimodal histogram),
13 //and creates a binary image
14 setAutoThreshold("Otsu dark");
15 setOption("BlackBackground", true);
16 run("Convert to Mask");
17 //Applies watershed transform to separate nuclei
18 run("Watershed");
19
20 //Choose which measurements are displayed
21 run("Set Measurements...", "area standard min shape integrated redirect=None decimal=3");
22
23 run("Analyze Particles...", " show=Nothing display clear summarize add");
```

More on thresholding difficult data:

<https://bioimagebook.github.io/chapters/2-processing/3-thresholding/thresholding.html?highlight=otsu#thresholding-difficult-data>

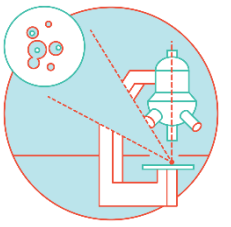
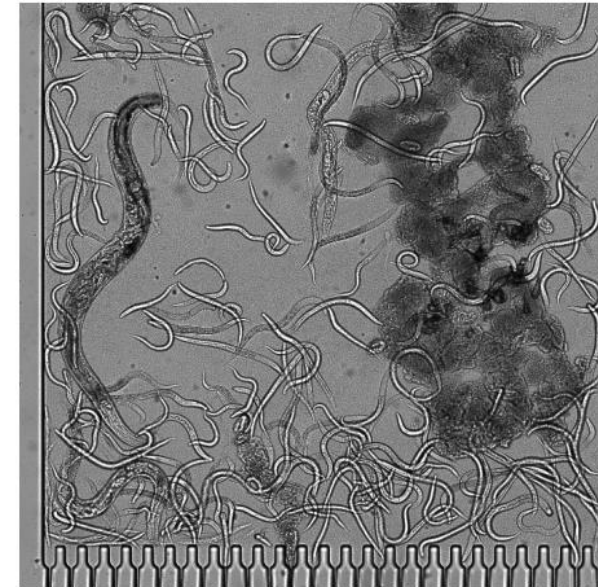
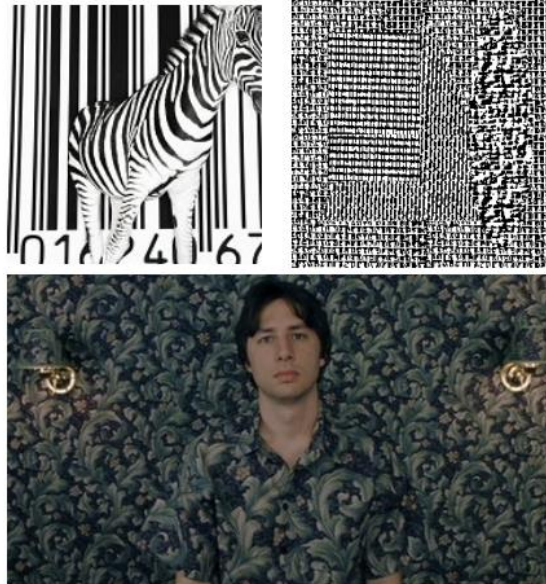
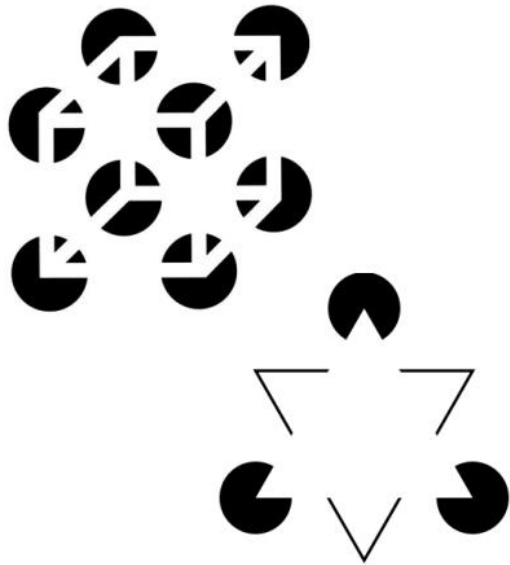
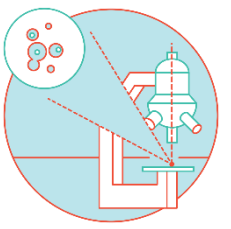
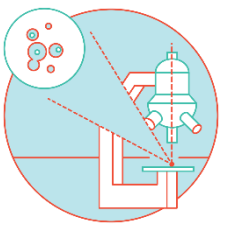


Image Segmentation & Image Analysis

> Why Image Segmentation?



Sometimes boundaries are not completely obvious for the computer point of view...

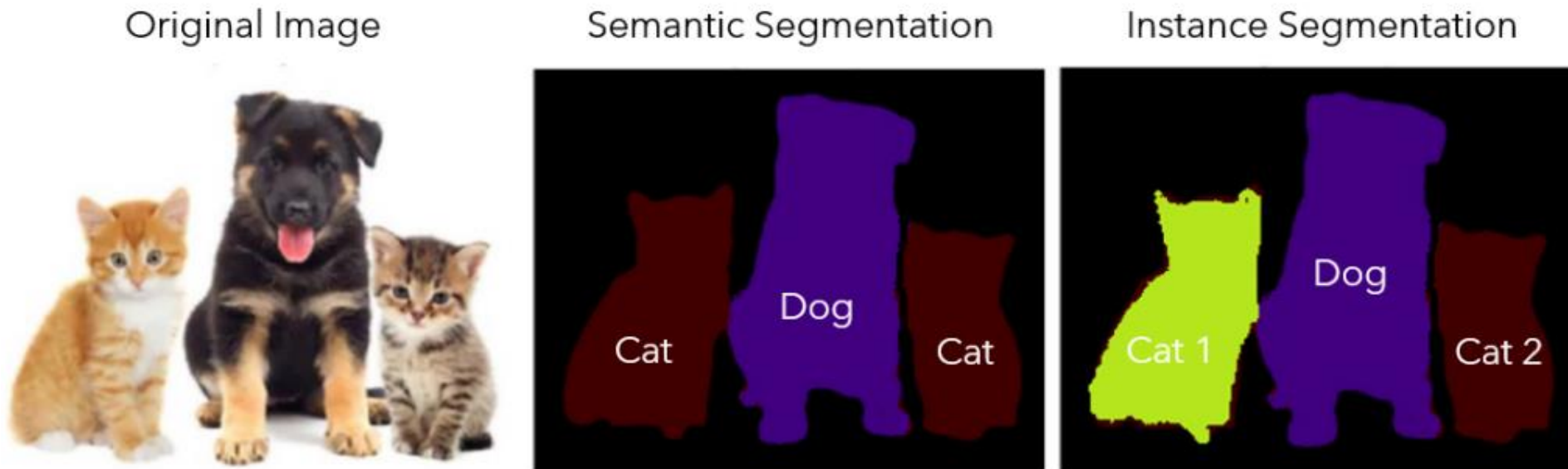


Segmentation

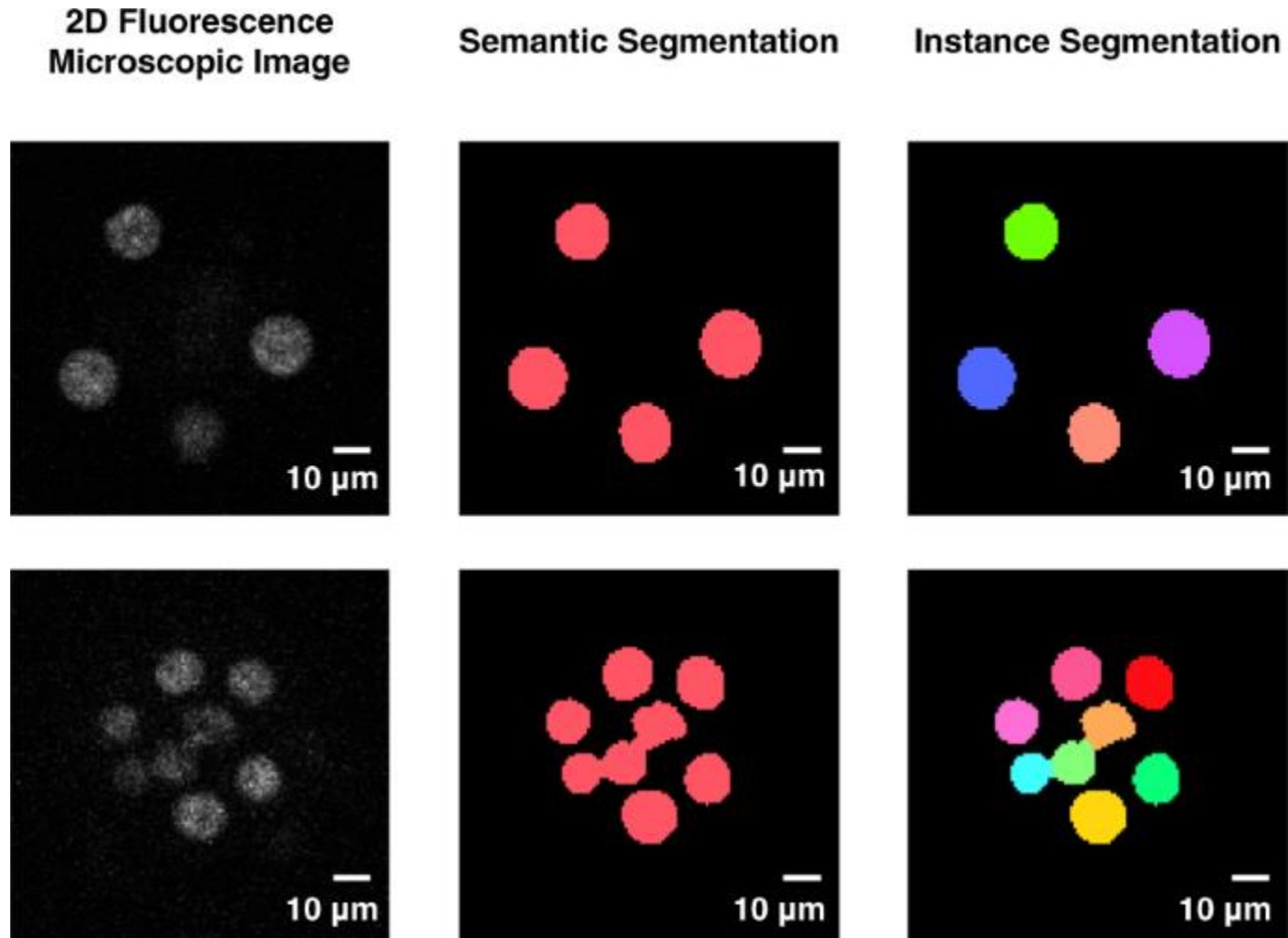
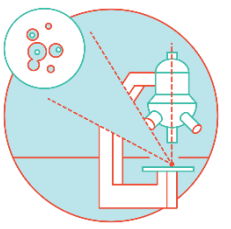
Partitioning of a digital image into **multiple segments** (Assign each pixel a label)

Semantic segmentation – Differentiate pixels into classes. All instances have the same class

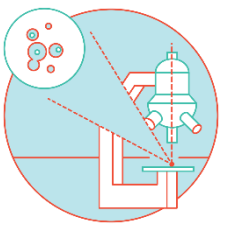
Instance segmentation – Differentiate individual occurrences within each class



> Semantic vs Instance Segmentation



<https://www.nature.com/articles/s41540-020-00152-8>



- Tell Fiji what to measure with *Analyze > Set Measurements*

The screenshot illustrates the workflow in Fiji for particle analysis. It shows the 'blobs.gif' image with red particles, the 'Threshold' window, the 'Set Measurements' dialog box, and the 'Analyze Particles' dialog box. The 'Set Measurements' dialog is configured with the following options:

- Area
- Standard deviation
- Min & max gray value
- Center of mass
- Bounding rectangle
- Shape descriptors
- Integrated density
- Skewness
- Area fraction
- Limit to threshold
- Invert Y coordinates
- Add to overlay
- Redirect to: None
- Decimal places (0-9): 3

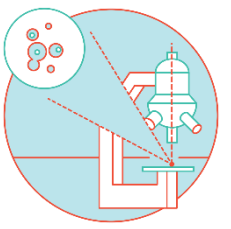
The 'Analyze Particles' dialog is configured with:

- Size (pixel²): 0-Infinity
- Circularity: 0.00-1.00
- Show: Nothing

The 'Results' table displays the following data:

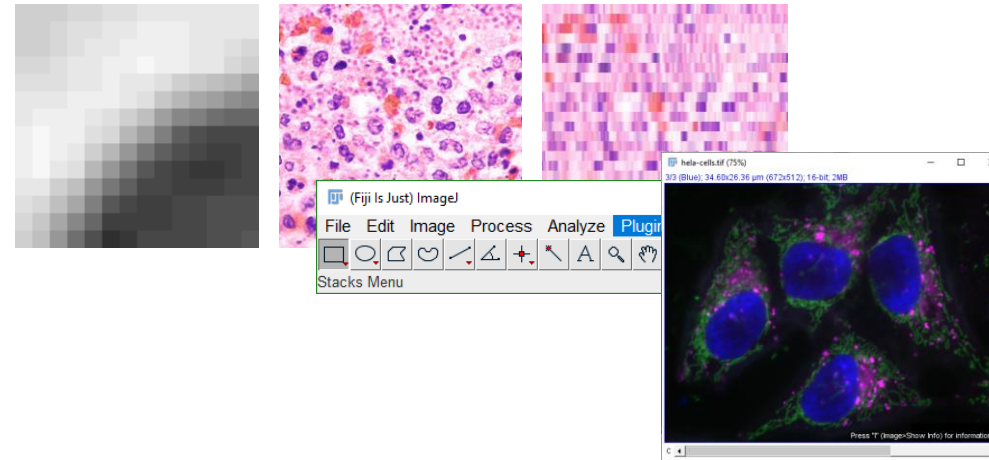
File	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM
54	204	199.608	32.805	232	128	240	4.436	226.314	4.203
55	555	217.139	36.977	248	128	248	234.561	227.673	234.522
56	858	197.277	30.121	232	128	248	180.791	230.323	180.452
57	281	189.779	30.815	216	128	232	138.546	233.710	138.466
58	215	184.037	28.631	216	128	224	46.537	240.988	46.542
59	3	130.667	4.619	128	128	136	76.833	242.833	76.827
60	1	128.000	0.000	128	128	128	110.500	246.500	110.500
61	81	183.407	34.682	128	128	248	179.241	251.636	178.979
62	90	181.511	25.599	184	128	216	128.100	251.822	128.042
63	53	188.377	38.799	136	128	248	234.896	252.066	234.803
64	49	172.898	28.743	176	128	224	74.296	252.418	74.168

> Lecture recap and next session



- Introduction to Bioimage analysis
- Introduction to ImageJ/Fiji

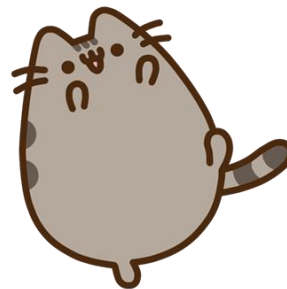
Demo

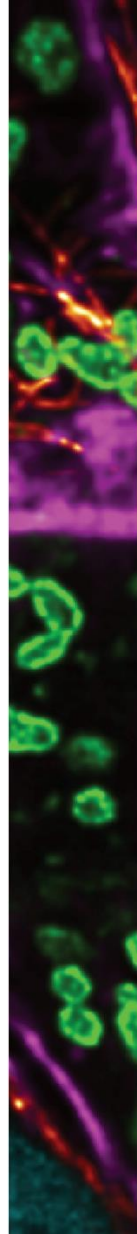
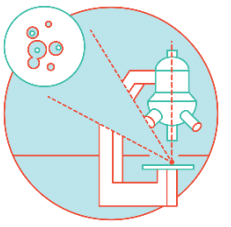


Next session...

- Automation and macros!

HANDS-ON



A vertical strip of microscopy images on the left side of the text area, showing various biological structures in green, purple, and red against a black background.

Lunch Seminar Series

Bioimage Analysis

This lunch seminar series is designed for anyone interested in light and/or electron microscopy and image analysis, offering insights from basic to intermediate levels. It focuses on the usage of open source as well as commercial software tools.

Please register using the QR Code or link below

Online every two weeks on Friday 11:30 AM - 12:30 PM

- September 20, 2024 Bioimage processing and analysis using Fiji/ImageJ**
Joana Delgado Martins, Flurin Sturzenegger (ZMB)
- October 4, 2024 Streamlining Bioimage Analysis with Fiji/ImageJ Macros**
Flurin Sturzenegger, Joana Delgado Martins (ZMB)
- October 18, 2024 Machine learning-based segmentation with ilastik**
Lorenzo Cerrone (BVC)
- November 1, 2024 Deep learning-based segmentation with Cellpose**
Joel Lüthi (BVC)
- November 15, 2024 3D/4D image visualization and analysis workflows with Imaris**
ZMB
- November 29, 2024 Large-scale bioimage analysis workflows with Fractal**
Joel Lüthi, Lorenzo Cerrone (BVC)
- December 13, 2024 Interacting with the bioimage analysis community on Image.sc**
Virginie Uhlmann (BVC)