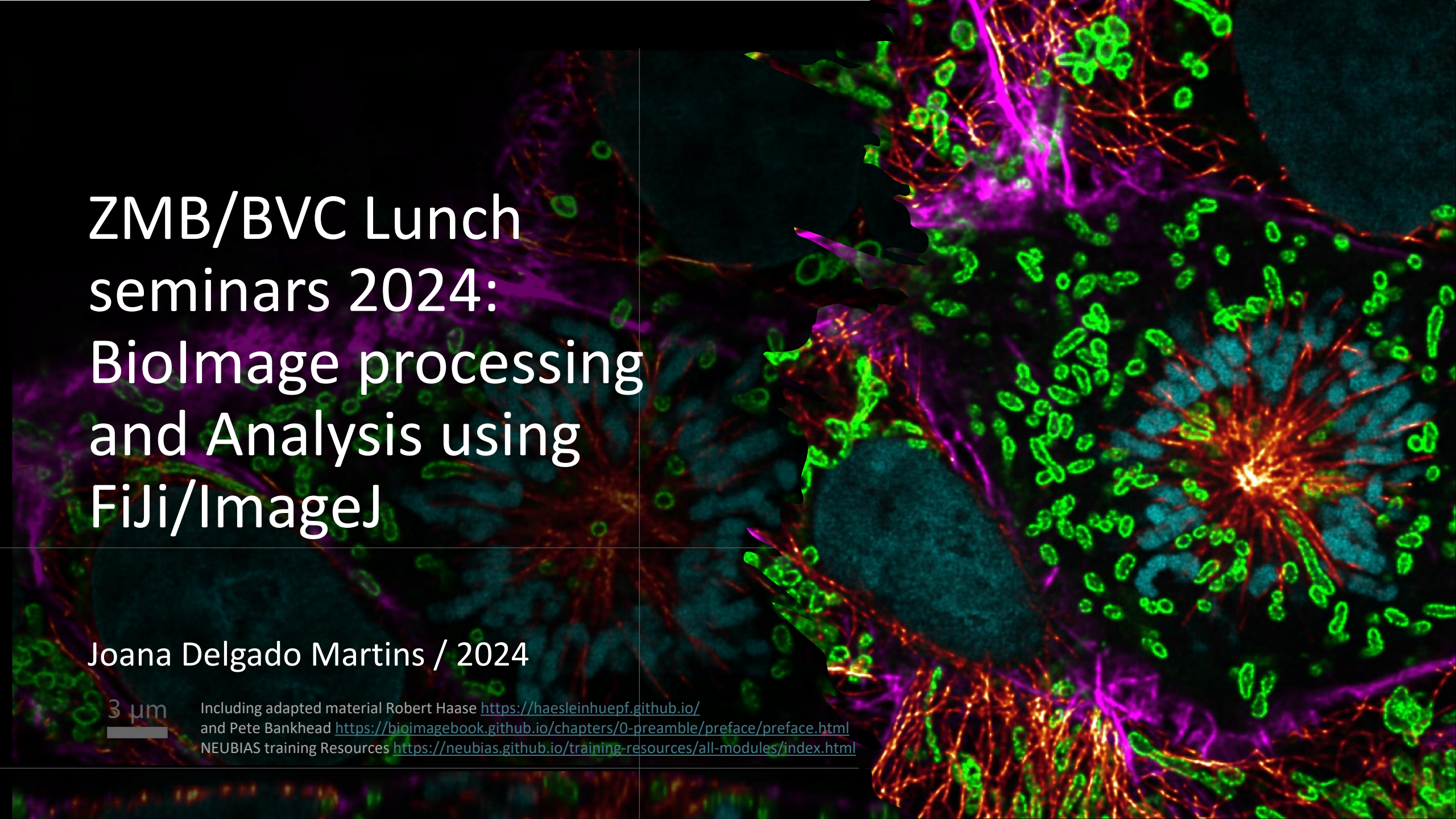
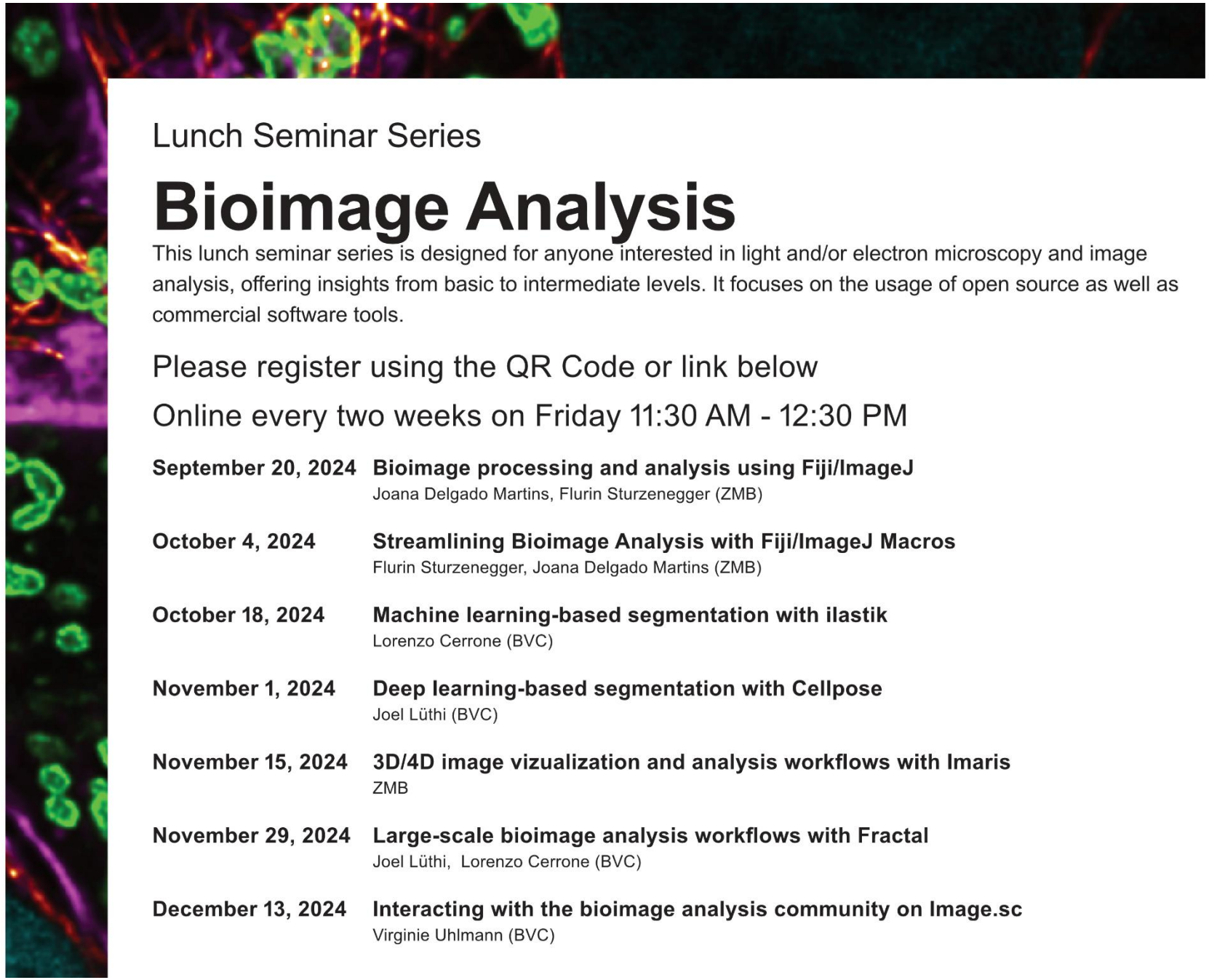
ZMB/BVC Lunch seminars 2024: BioImage processing and Analysis using FiJi/ImageJ

Joana Delgado Martins / 2024



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

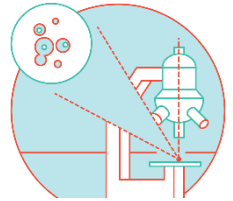
This lunch seminar series is designed for anyone interested in light and/or electron microscopy and image analysis, offering insights from basic to intermediate levels. It focuses on the usage of open source as well as commercial software tools.

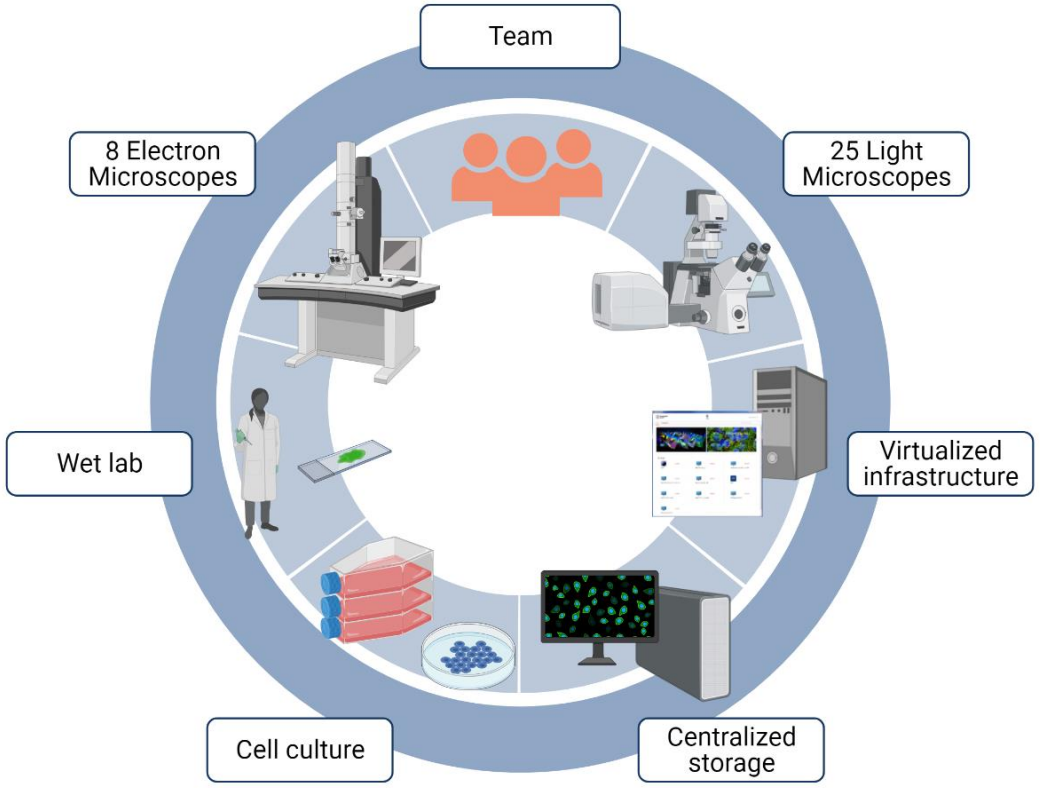
Please register using the QR Code or link below

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

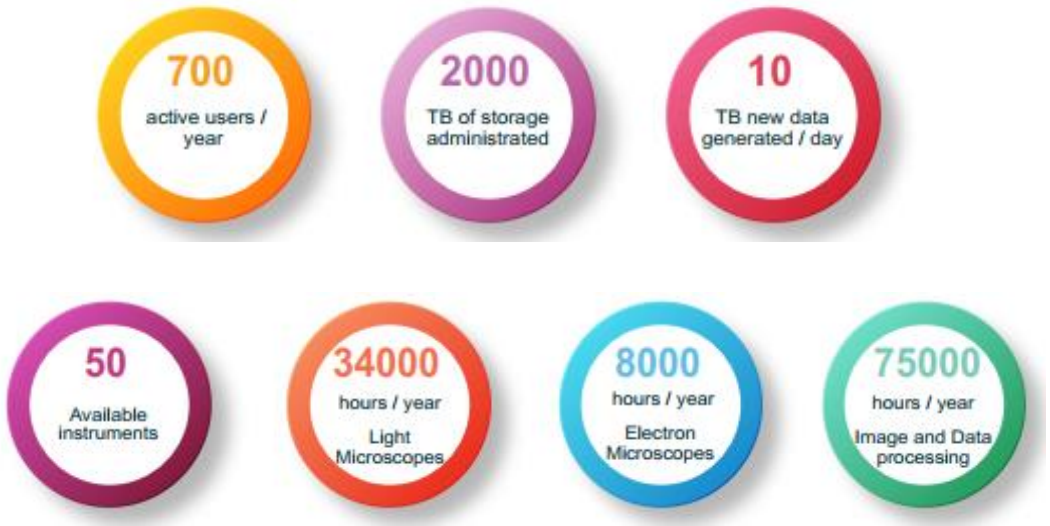
September 20, 2024	**Bioimage processing and analysis using Fiji/ImageJ** Joana Delgado Martins, Flurin Sturzenegger (ZMB)
October 4, 2024	**Streamlining Bioimage Analysis with Fiji/ImageJ Macros** Flurin Sturzenegger, Joana Delgado Martins (ZMB)
October 18, 2024	**Machine learning-based segmentation with ilastik** Lorenzo Cerrone (BVC)
November 1, 2024	**Deep learning-based segmentation with Cellpose** Joel Lüthi (BVC)
November 15, 2024	**3D/4D image vizualization 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





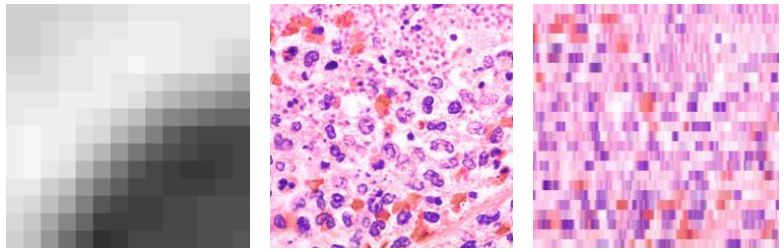
Around 2.2h of image processing per 1h of microscopy

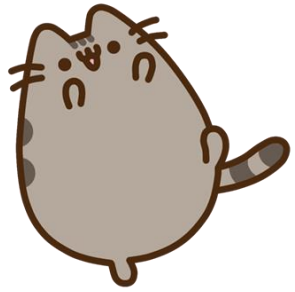


@uzh_microscopy

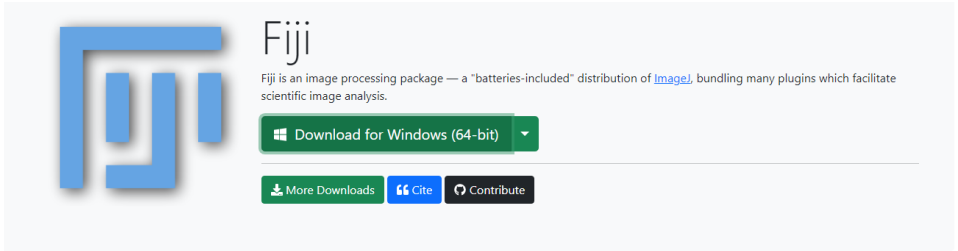
> Overview

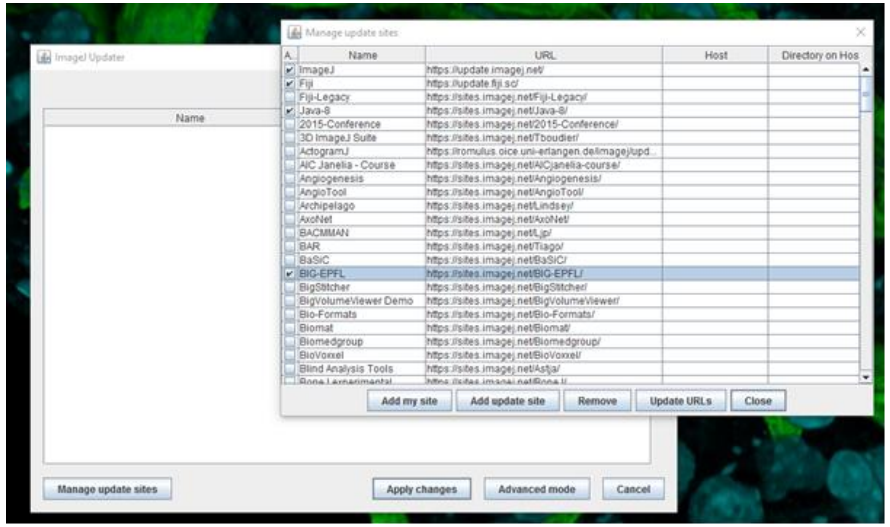
- Brief intro image analysis
- ImageJ/Fiji
  - Digital Image
  - Display and visualization
  - Histogram
  - Segmentation, Thresholding
  - Analysis particles workflow and basic measurements





Download FiJi

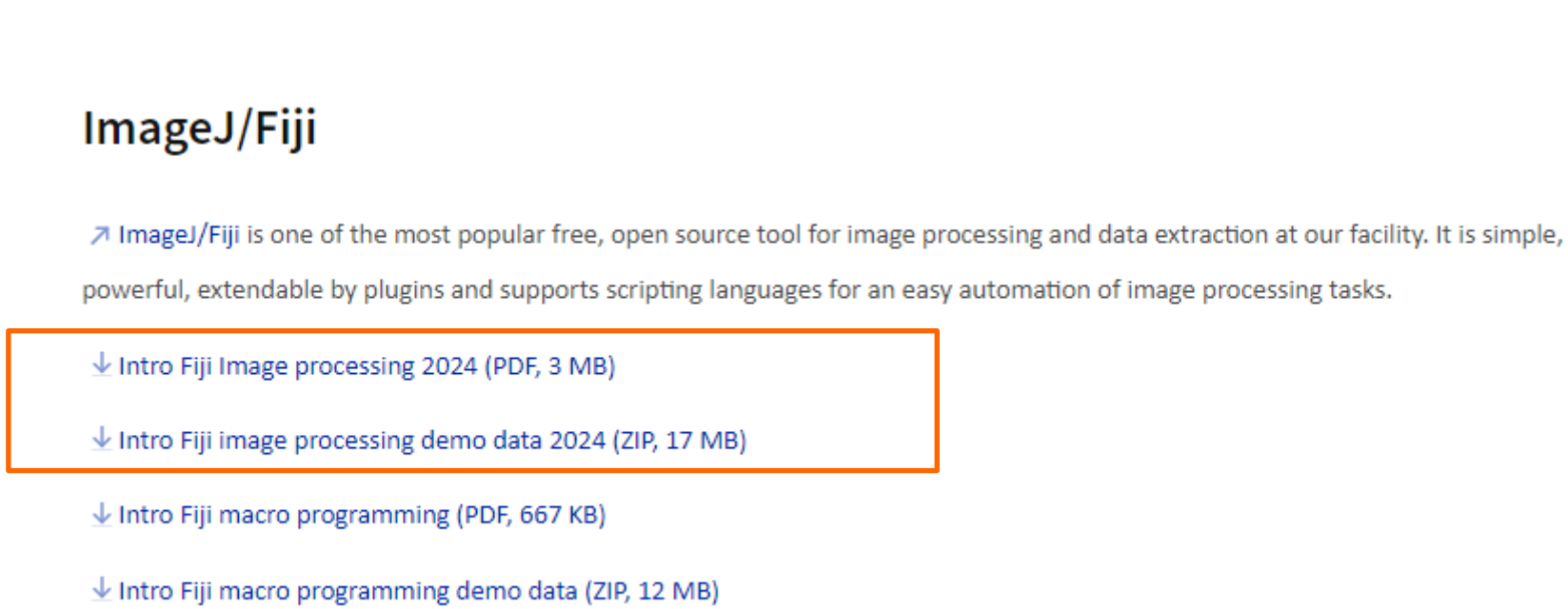




http://fiji.sc/

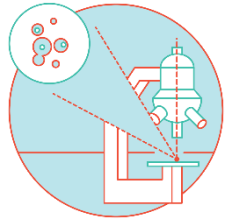
1. Download the appropriate Fiji version from http://fiji.sc/
2. Unzip your copy to a location where you have writing rights. Otherwise, you will not be able to automatically install the latest updates.
3. Check if you have the latest updates by running *Help>Update*
4. Additionally add the “BIG-EPFL”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

> Resources



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

> Resources



Universität Zürich[UZH]

Guides Support Tea

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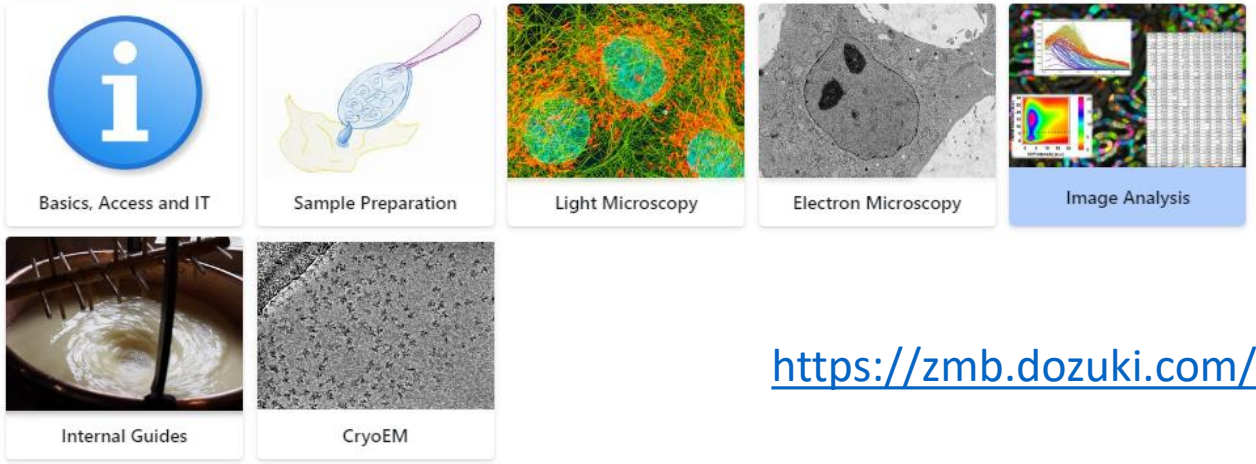
Guides > Center for Microscopy and Image Analysis Edit Options

Dozuki System Automated Release **08/17/2022** by Dozuki System Details

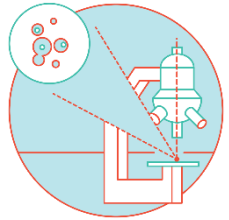
7 Categories + New Page

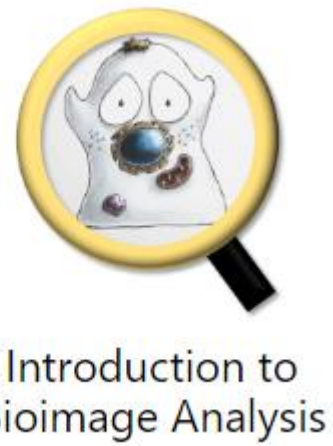


https://zmb.dozuki.com/c/Image_Analysis

@uzh_microscopy

> Image Processing and Analysis





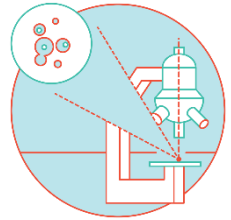
1. **Images in biology are *enormously* varied.** Almost nothing 'just works'. I might find a paper describing a marvellous method to detect, classify and track cells – but there is no guarantee the method will work to detect, classify and track *my* cells. Maybe I have a different type of cell. Imaged 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).

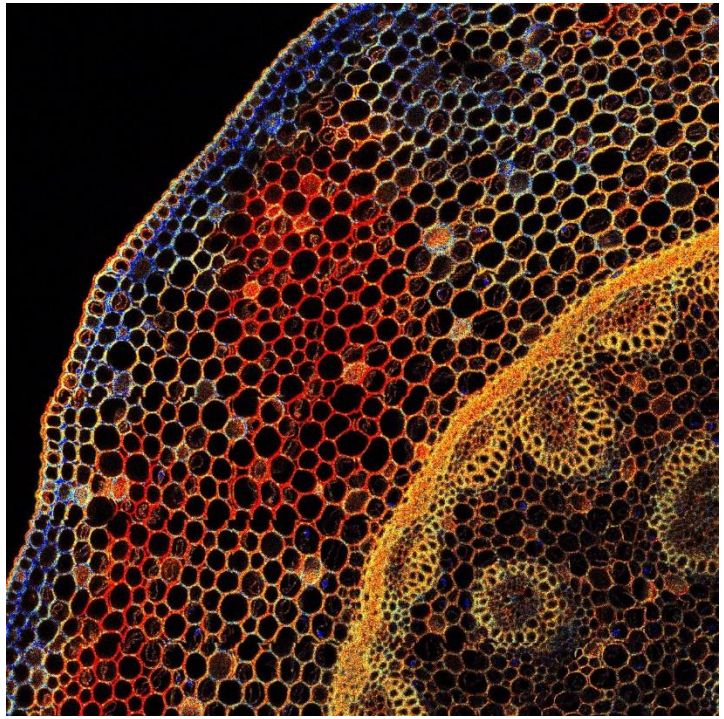
The fact that bioimage analysis is so cross-disciplinary means that pretty much everyone can have valuable insights to contribute.

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?

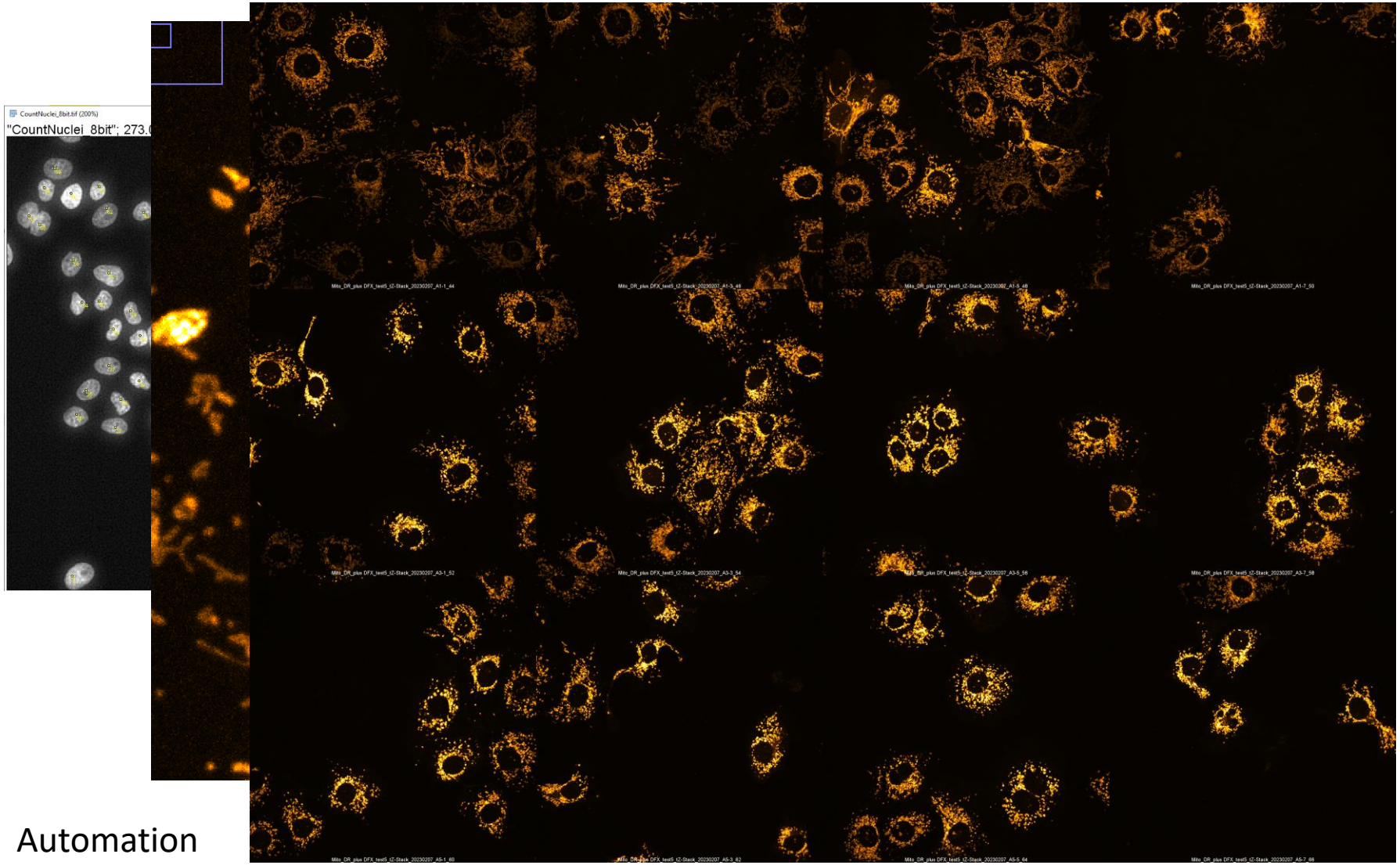
Biological question

How high is the intensity in the inner ring?

How is the signal in the blue channel distributed?

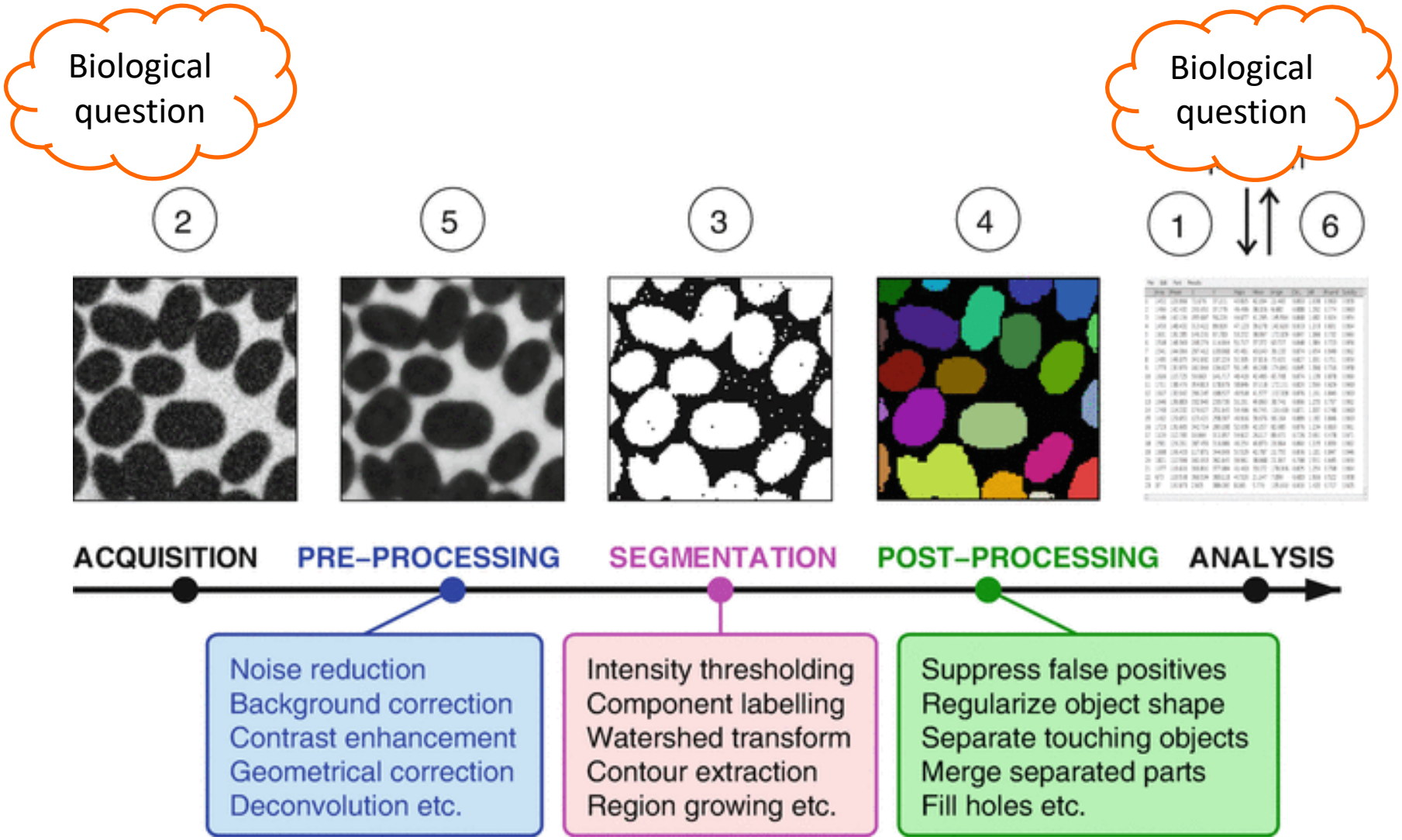
Image data source: Lorenzo Scipioni, U. California / Irvine
@LorenzoScipion3

> Image Processing and Analysis



Automation

> Image Processing workflows



[Arganda-Carreras, Andrey, 2017, https://doi.org/10.1007/978-1-4939-6810-7_13]

> Image Processing workflows

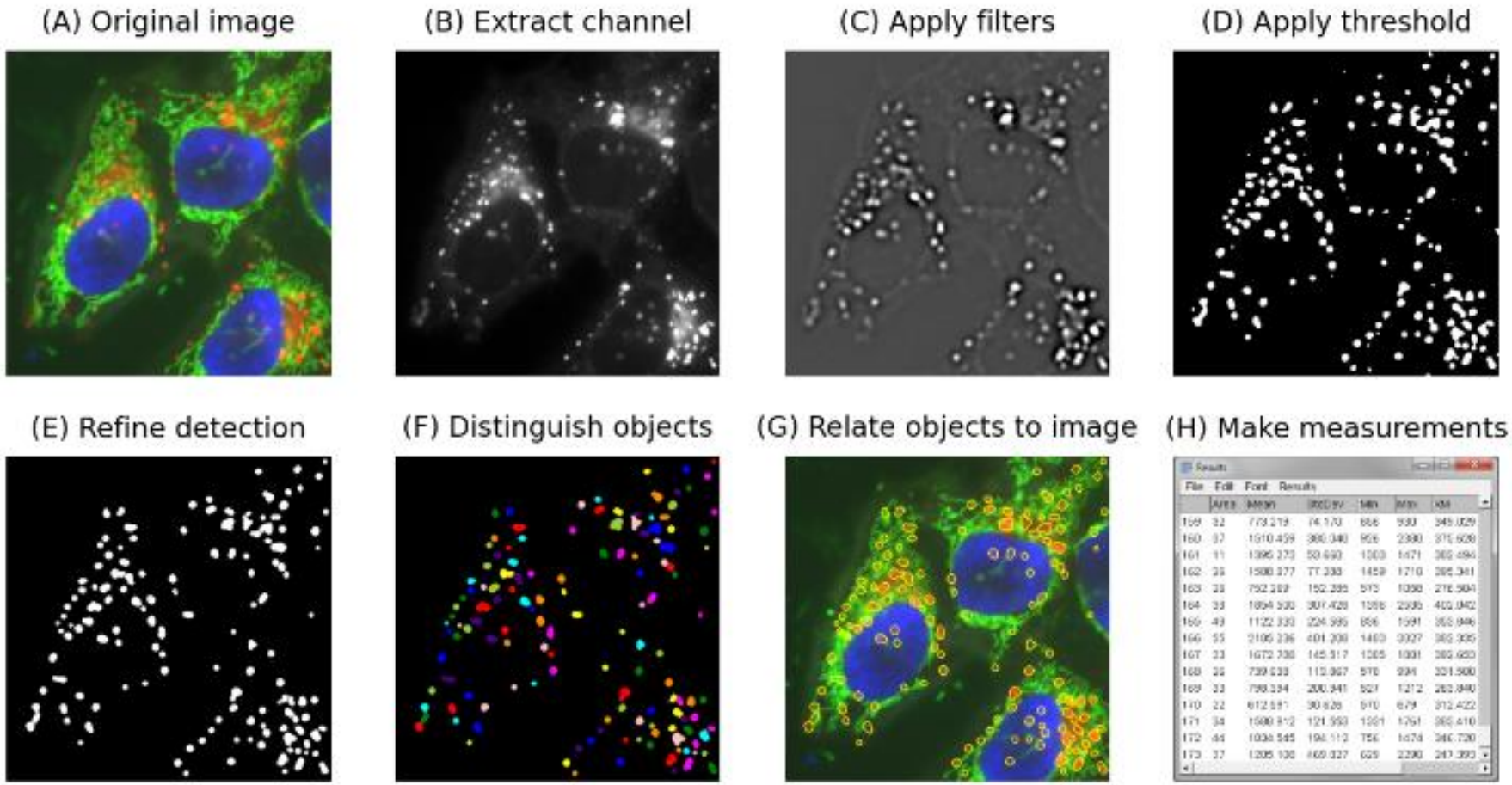
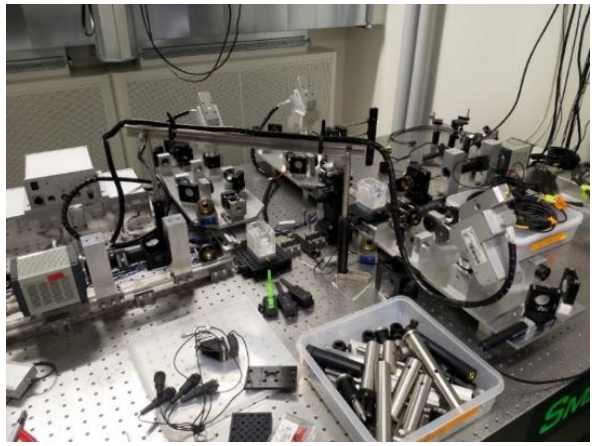
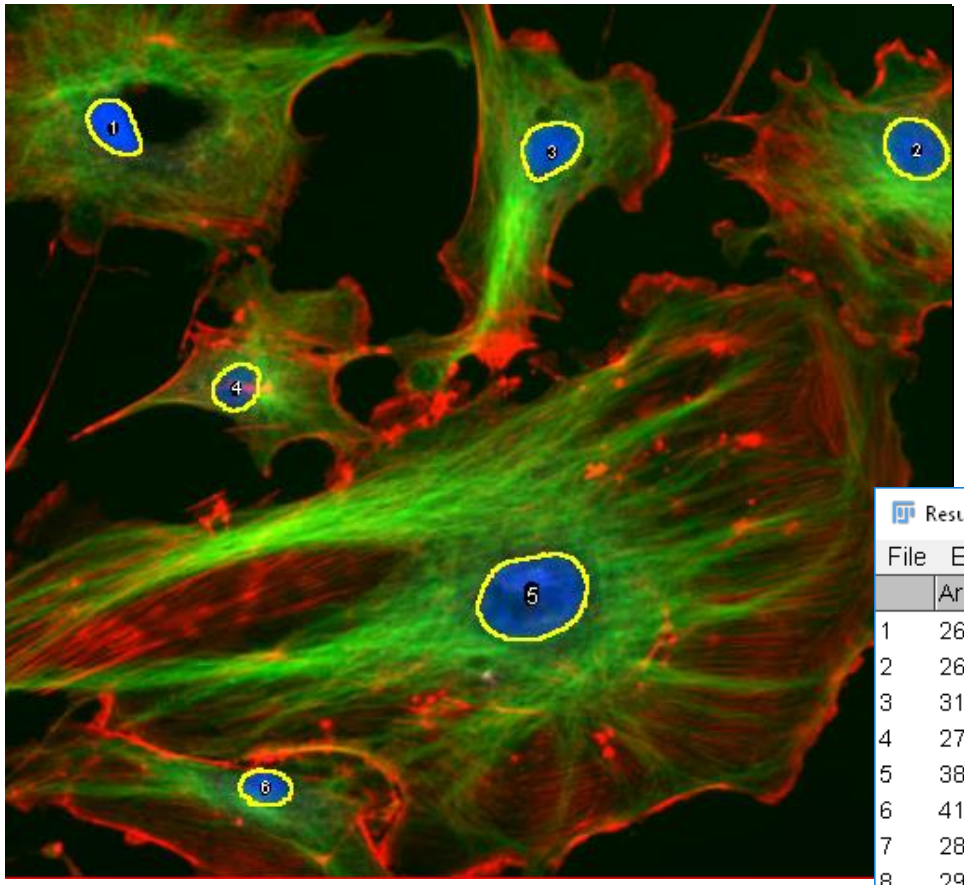


Fig. 56 A simple image analysis workflow for detecting and measuring spots in an image.

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

Tools we use are





Python

Results

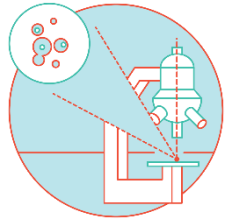
File Edit Font Results

	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

https://commons.wikimedia.org/wiki/User:Acagastya (Mirosope)

URL: http://imagej.nih.gov/ij/images/FluorescentCells.zip

> Fiji: Image analysis



1997 NIH Image ImageJ

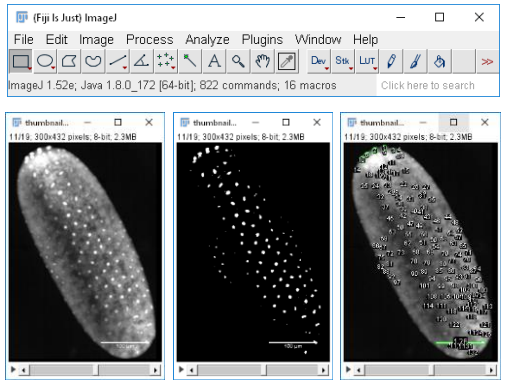
- Fiji is just ImageJ - with batteries included since 2005

Application

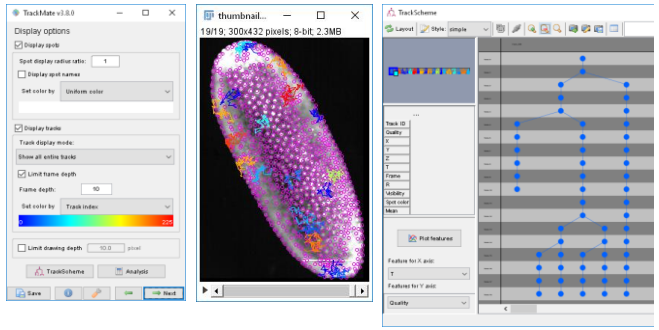
Reusable set of libraries

Shared framework for image analysis

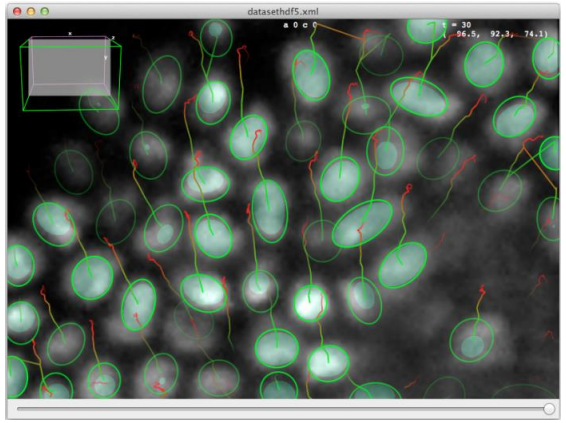
Core ImageJ



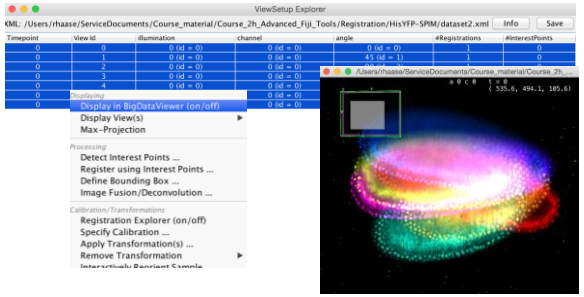
Trackmate



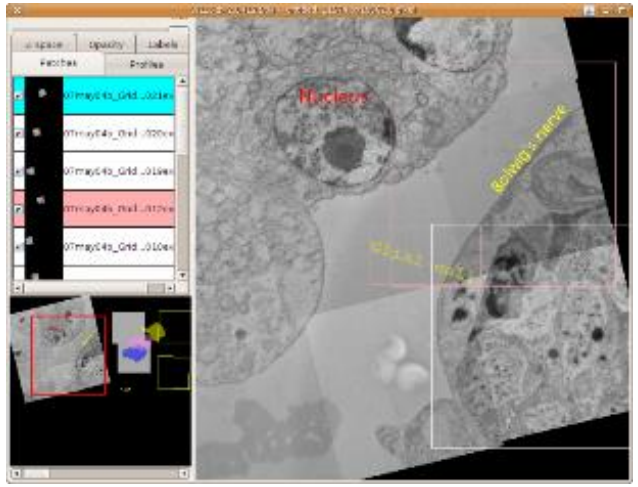
BigDataViewer



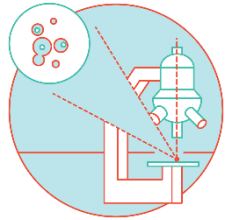
Multiview fusion



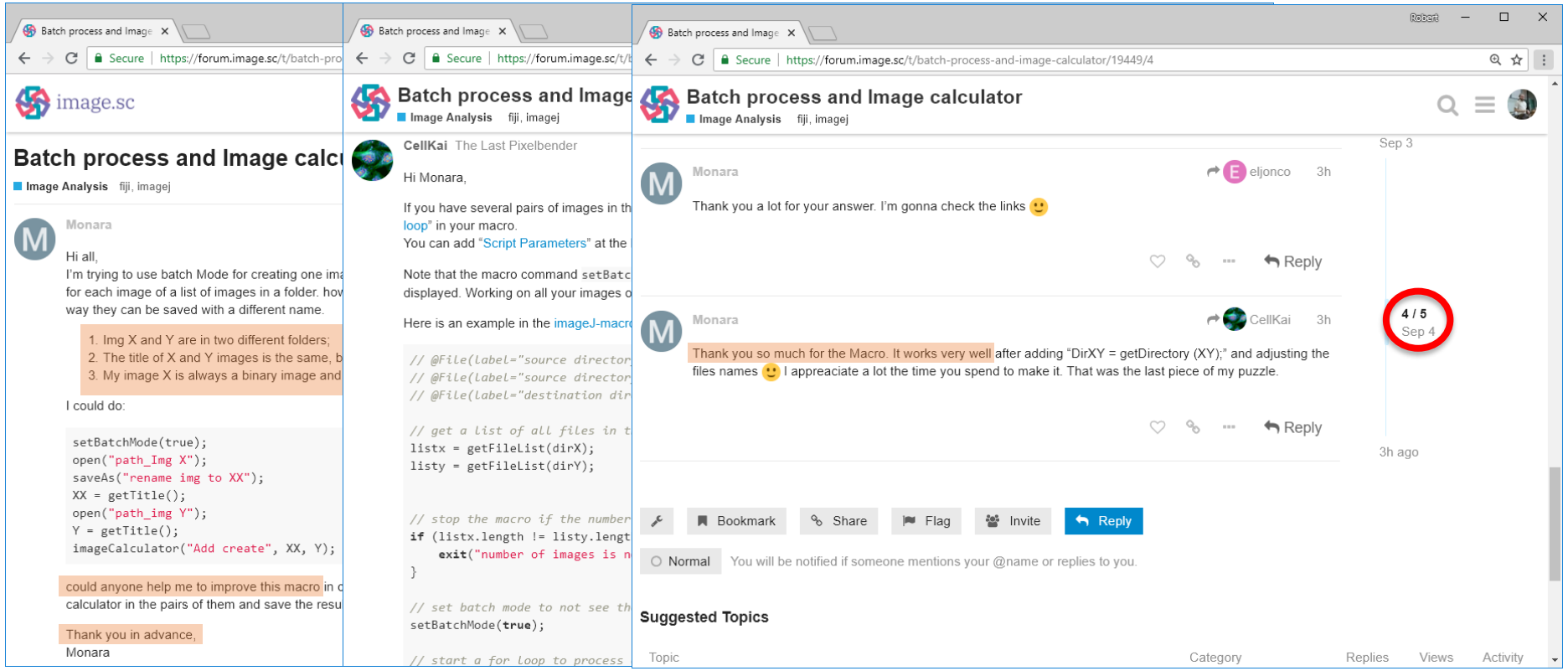
TrakEM



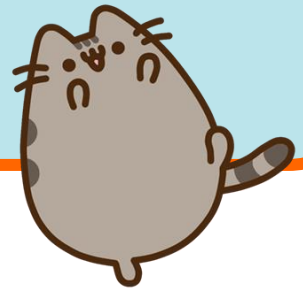
> ImageJ, Fiji & friends: community



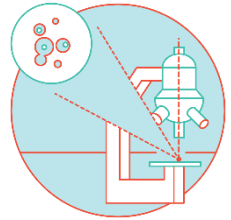
- Visit http://forum.image.sc !



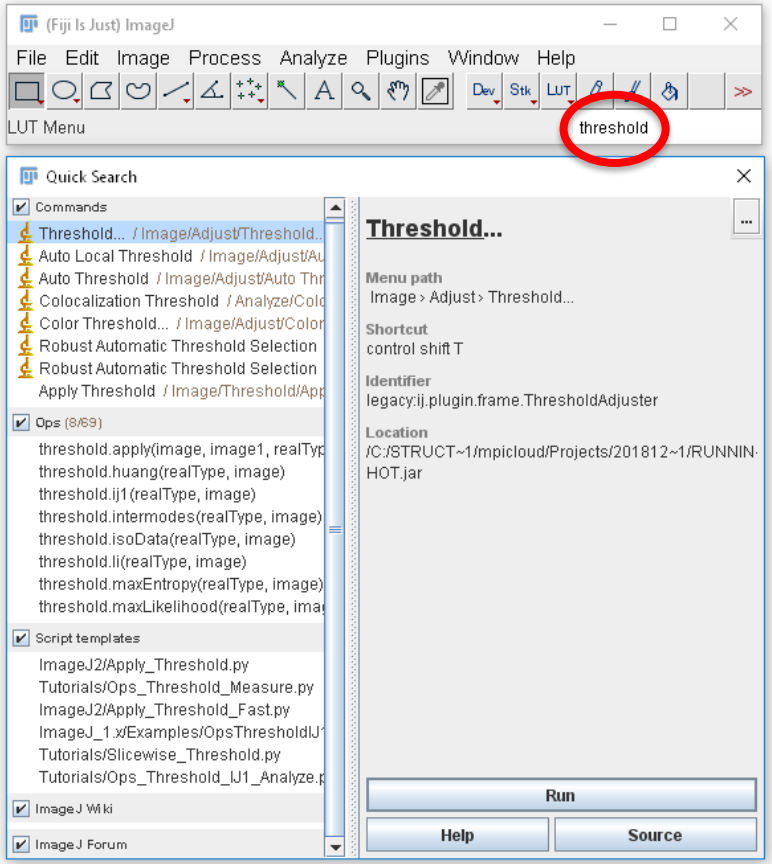
FiJi (ImageJ) Demo



> 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

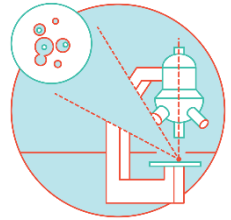
Additional plug-ins can be accessed through the update site
Help < Update

L

Enter

https://imagej.net/ij/docs/shortcuts.html

> 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	.czi	▲	▲	▲	▼	▭	✗	✗	✓	✓
Zeiss LSM (Laser Scanning Microscope) 510/710	.lsm, .mdb	▲	▲	▭	▲	▭	✗	✗	✓	✗
Bio-Rad PIC	.xml	▲	▲	▲	▲	▲	✗	✗	✗	✗
Bio-Rad SCN	.scn	▲	▼	▼	▼	▼	✗	✗	✗	✗
Bitplane Imaris	.ims	▲	▲	▲	▼	▼	✗	✗	✓	✓

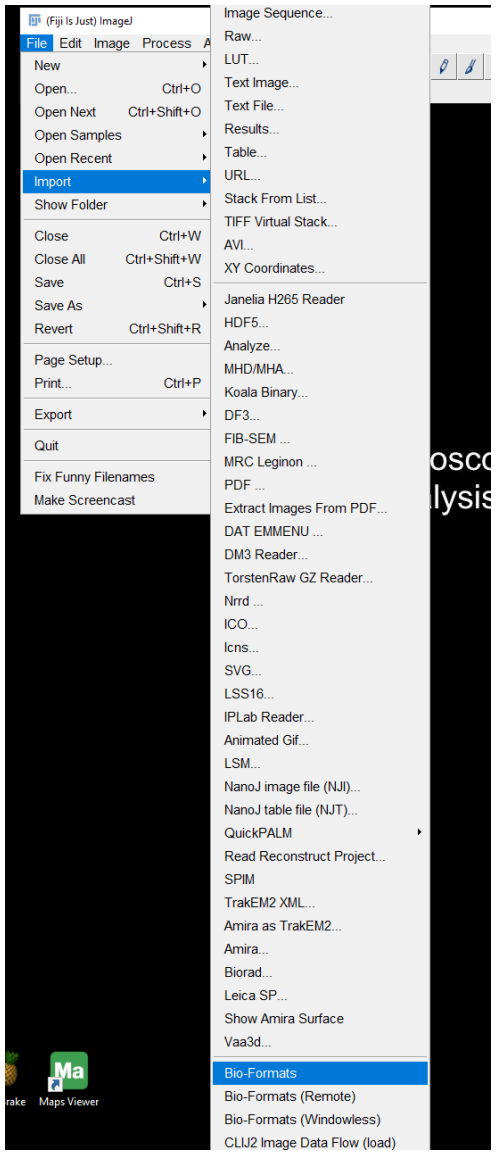
Bio-Formats currently supports **161** formats

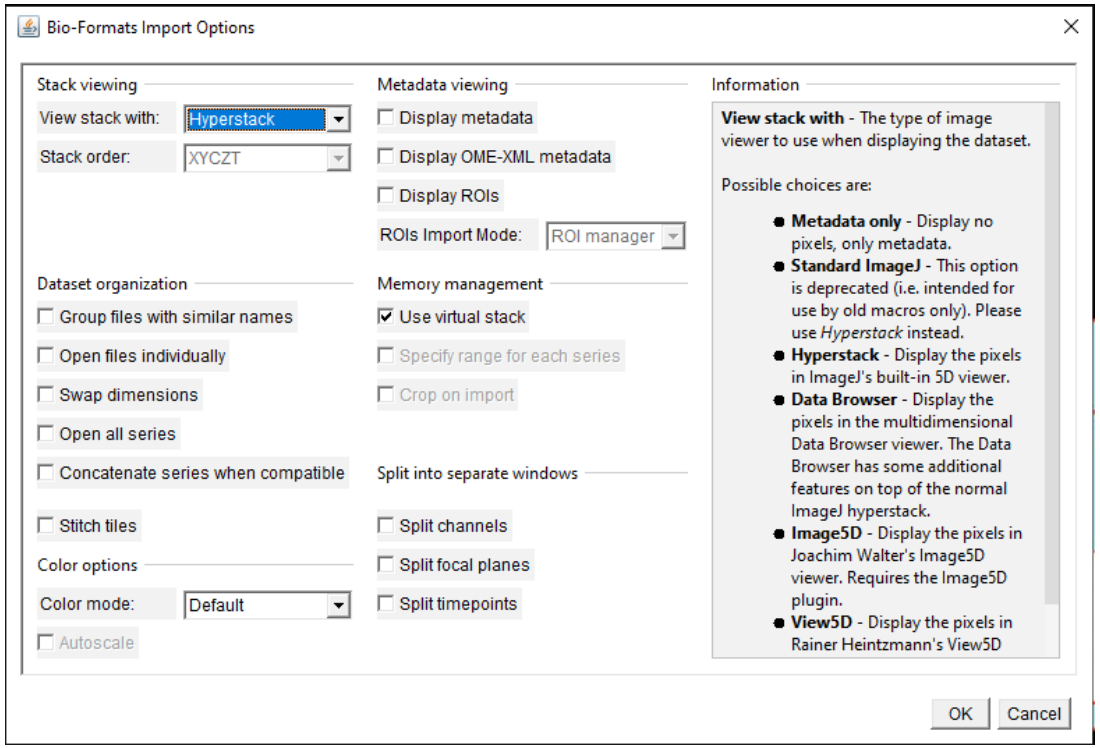
Ratings legend and definitions

▲	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

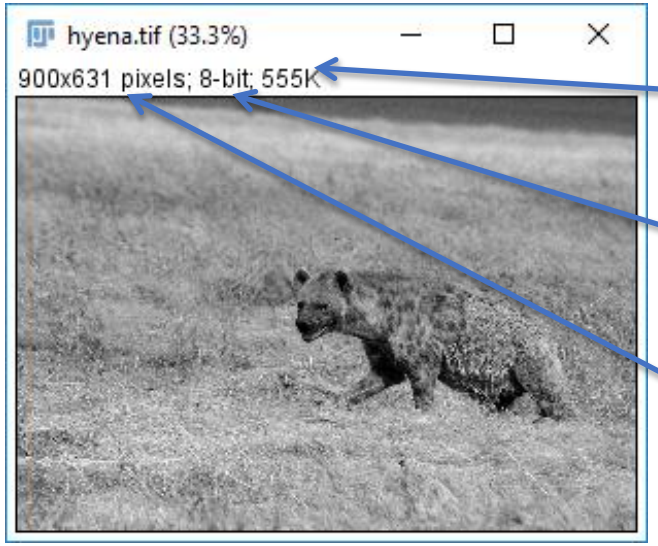
Memory managment

Virtual stack

https://bio-formats.readthedocs.io/en/v7.3.0/formats/bitplane-imaris.html

[2024 06]

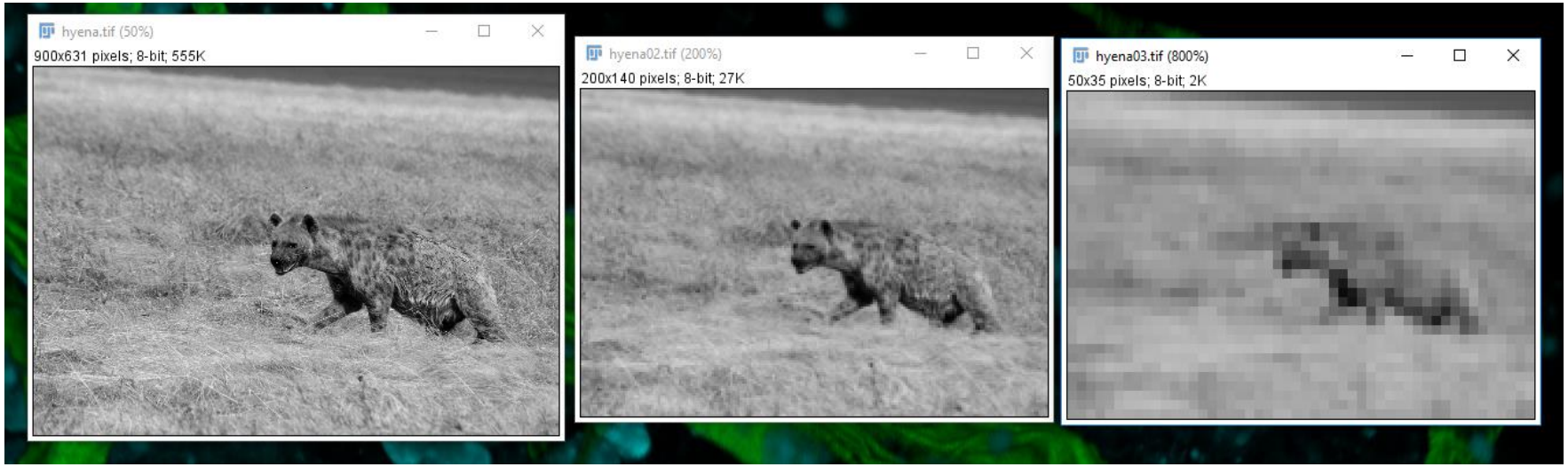
> FiJi: Opening images with FIJI



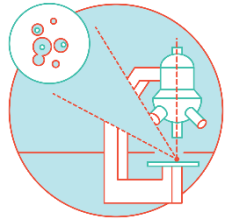
Memory in hard drive

Image bit-depth

Image size in pixels



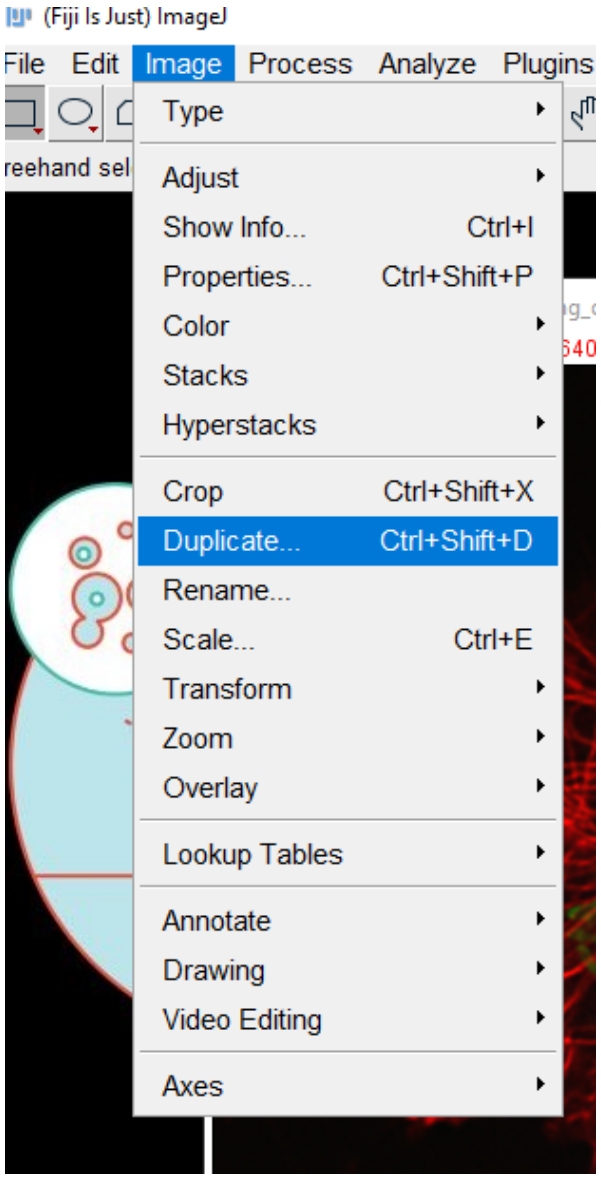
> Duplicate



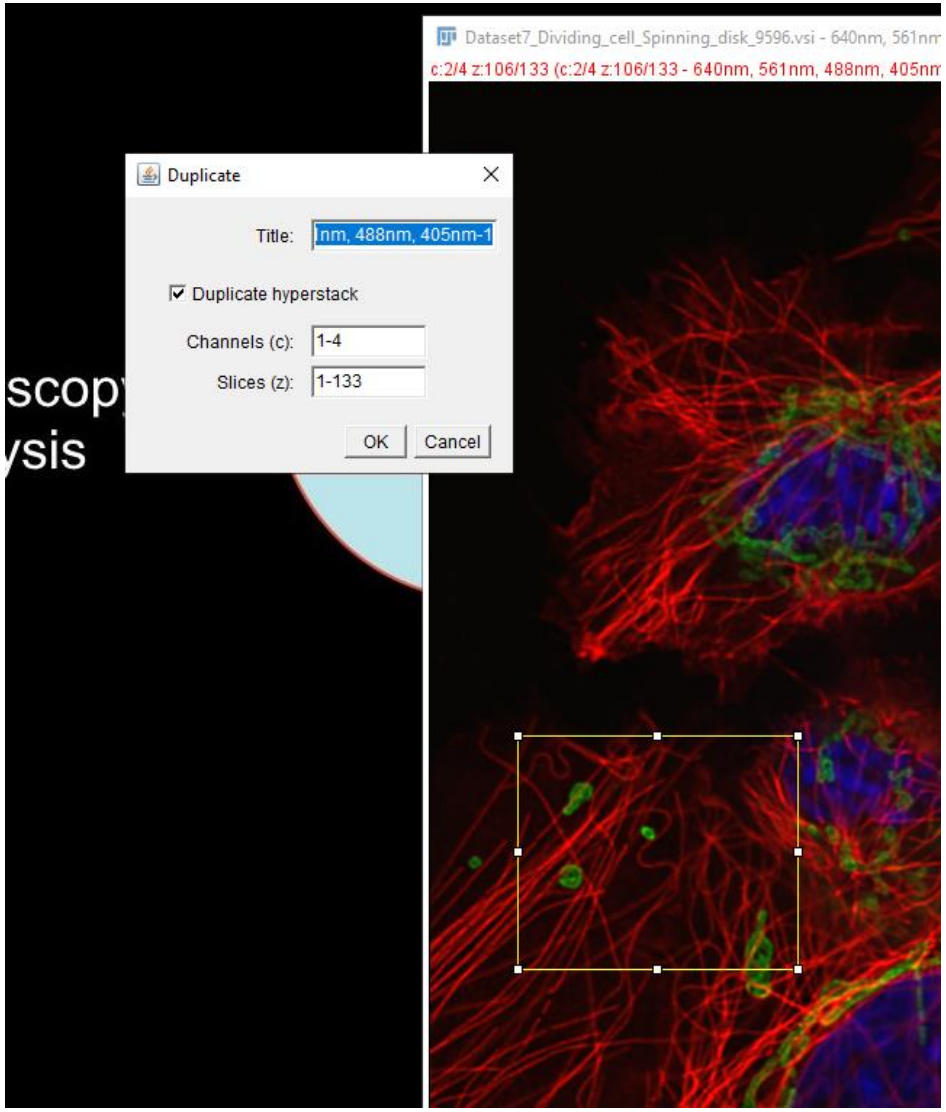
ImageJ provides very limited **Undo** support for 2D slices

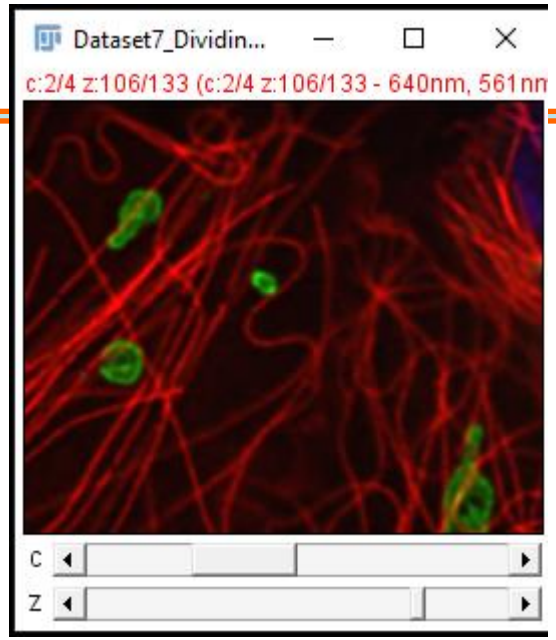
If you suspect you might regret a processing step, good practice to always duplicate the image beforehand with

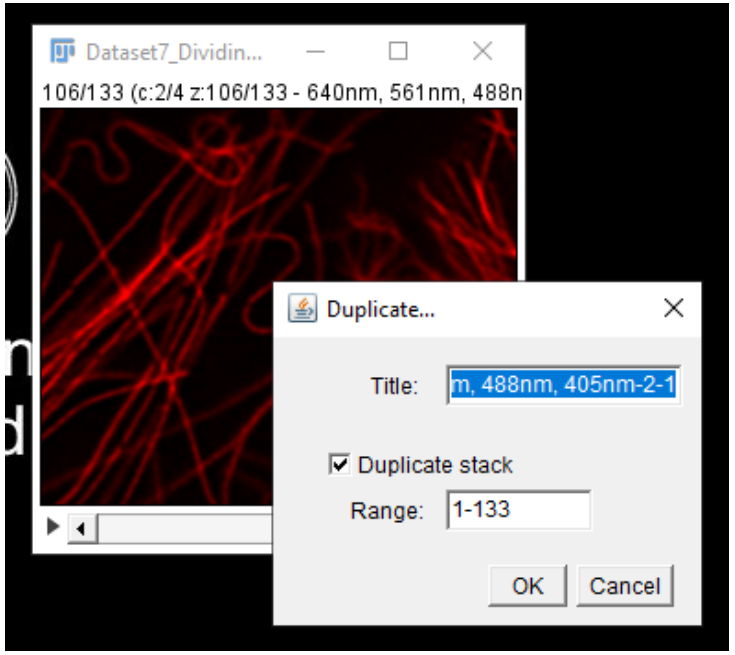
Shift + D



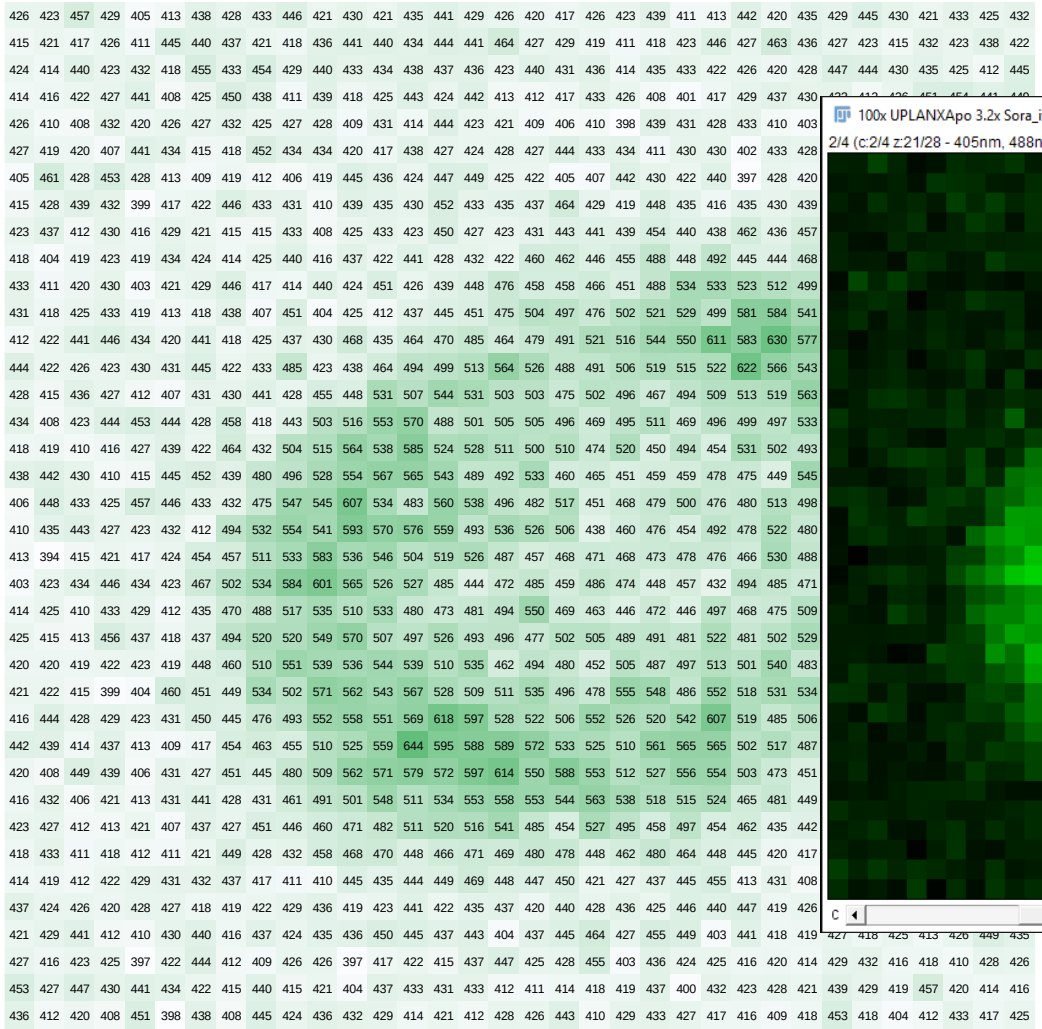
> Duplicate

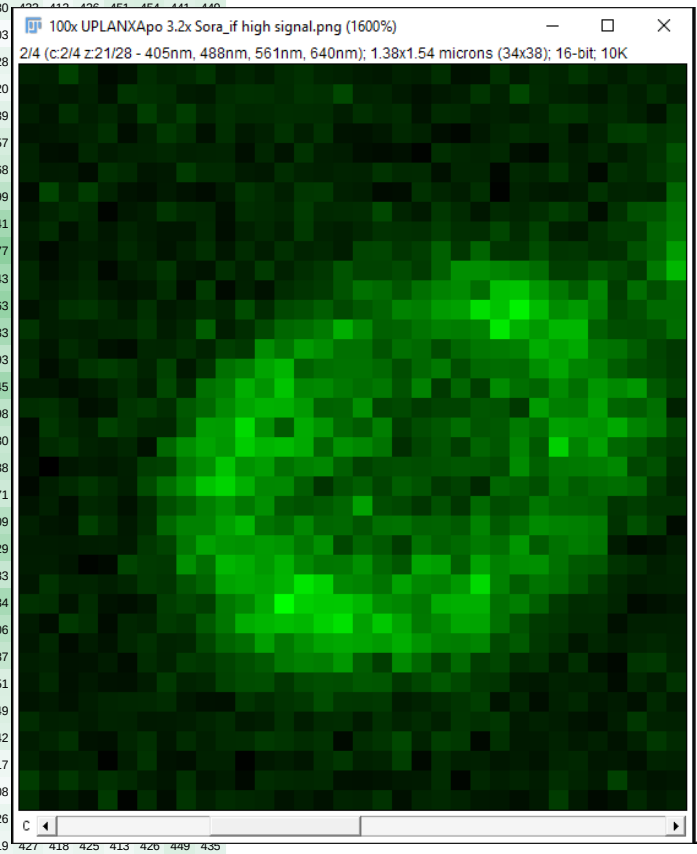

Dataset7_Dividing_cell_Spinning_disk_9596.vsi - 640nm, 561nm
c:2/4 z:106/133 (c:2/4 z:106/133 - 640nm, 561nm, 488nm, 405nm
Duplicate
Title:
nm, 488nm, 405nm-1
Duplicate hyperstack
Channels (c):
1-4
Slices (z):
1-133
OK
Cancel

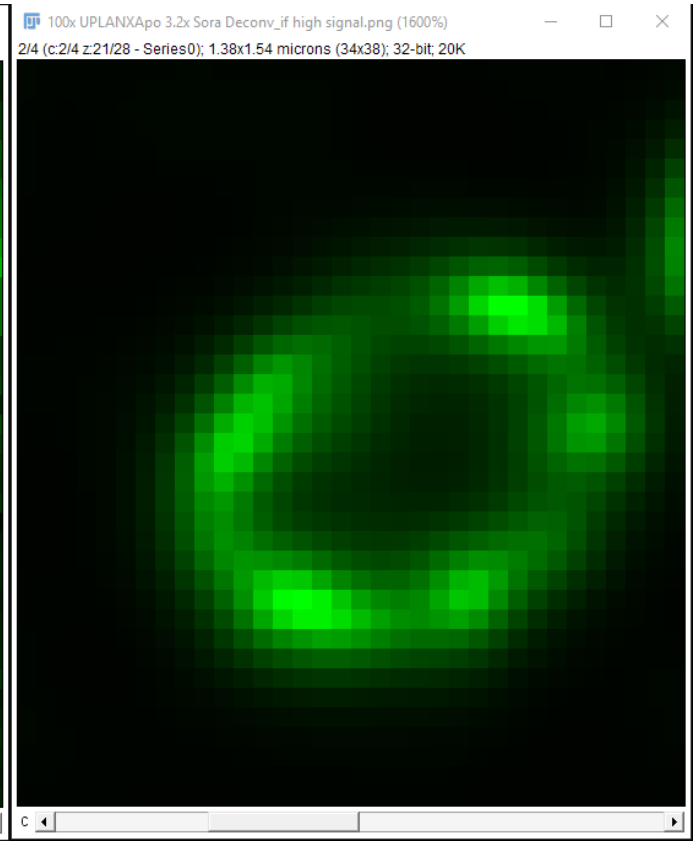

Dataset7_Dividin...
c:2/4 z:106/133 (c:2/4 z:106/133 - 640nm, 561nm
C
Z


Dataset7_Dividin...
106/133 (c:2/4 z:106/133 - 640nm, 561nm, 488n
Duplicate...
Title:
m, 488nm, 405nm-2-1
Duplicate stack
Range:
1-133
OK
Cancel

> Digital images: Matrices of digital numbers

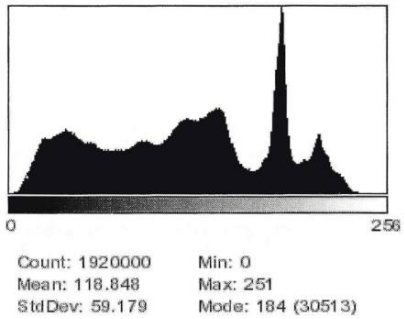


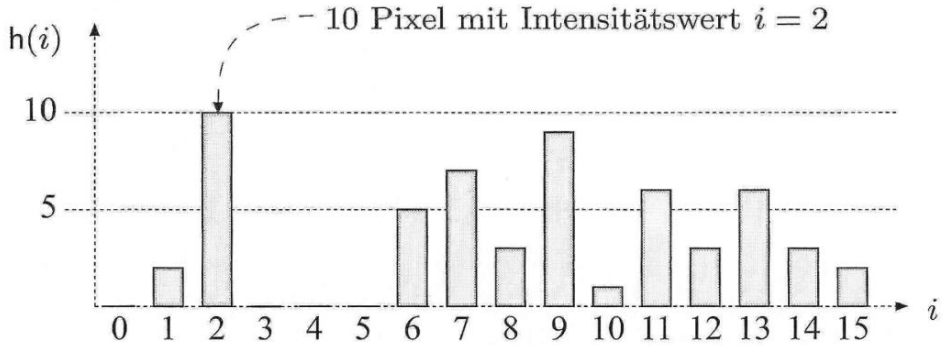




> Distribution of Intensity Values: Image Histogram

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

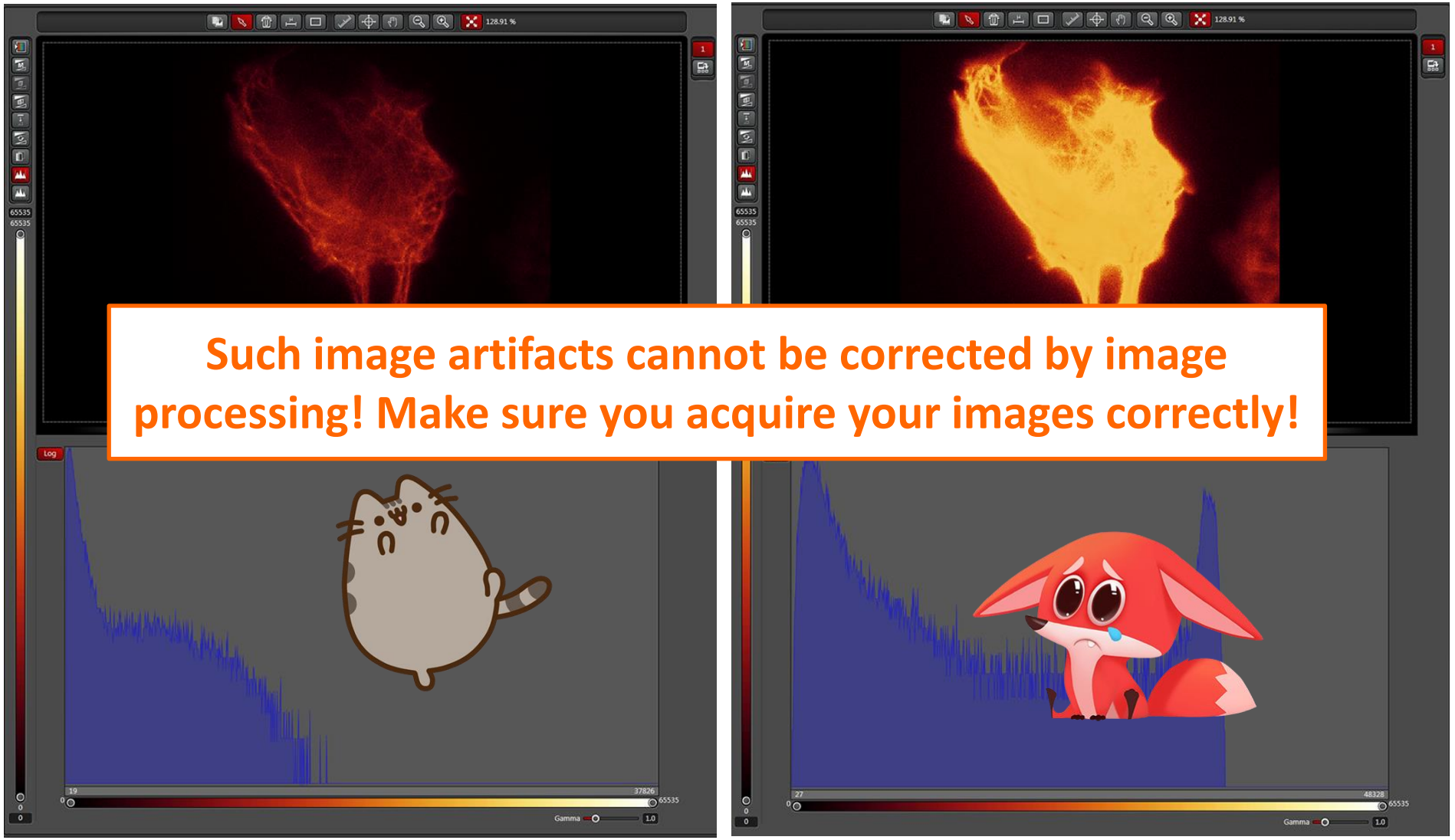




$h(i)$	0	2	10	0	0	0	5	7	3	9	1	6	3	6	3	2
i	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

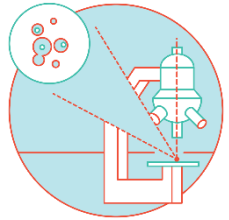
> Image Histogram

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

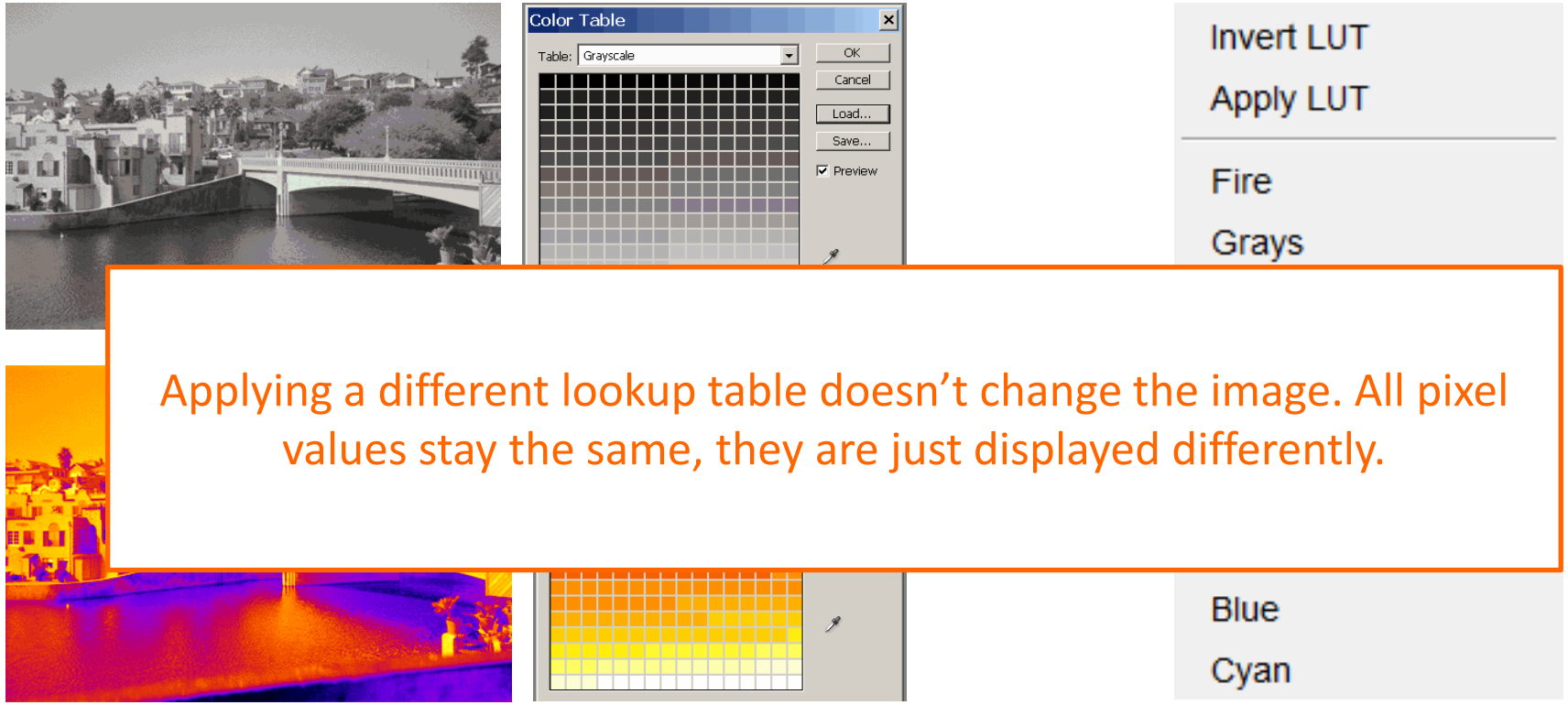


> Visualization: Assigning Colors to Image Intensities

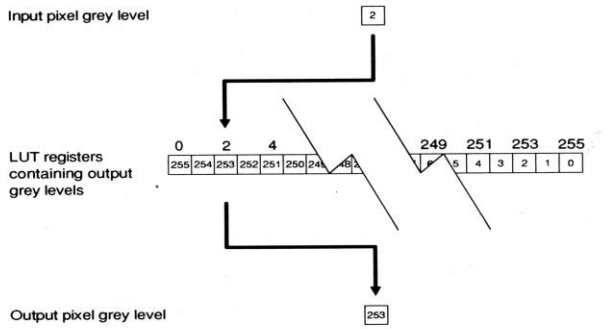
Hands-ON Chapter 11



Adjusting look up tables (LUT)

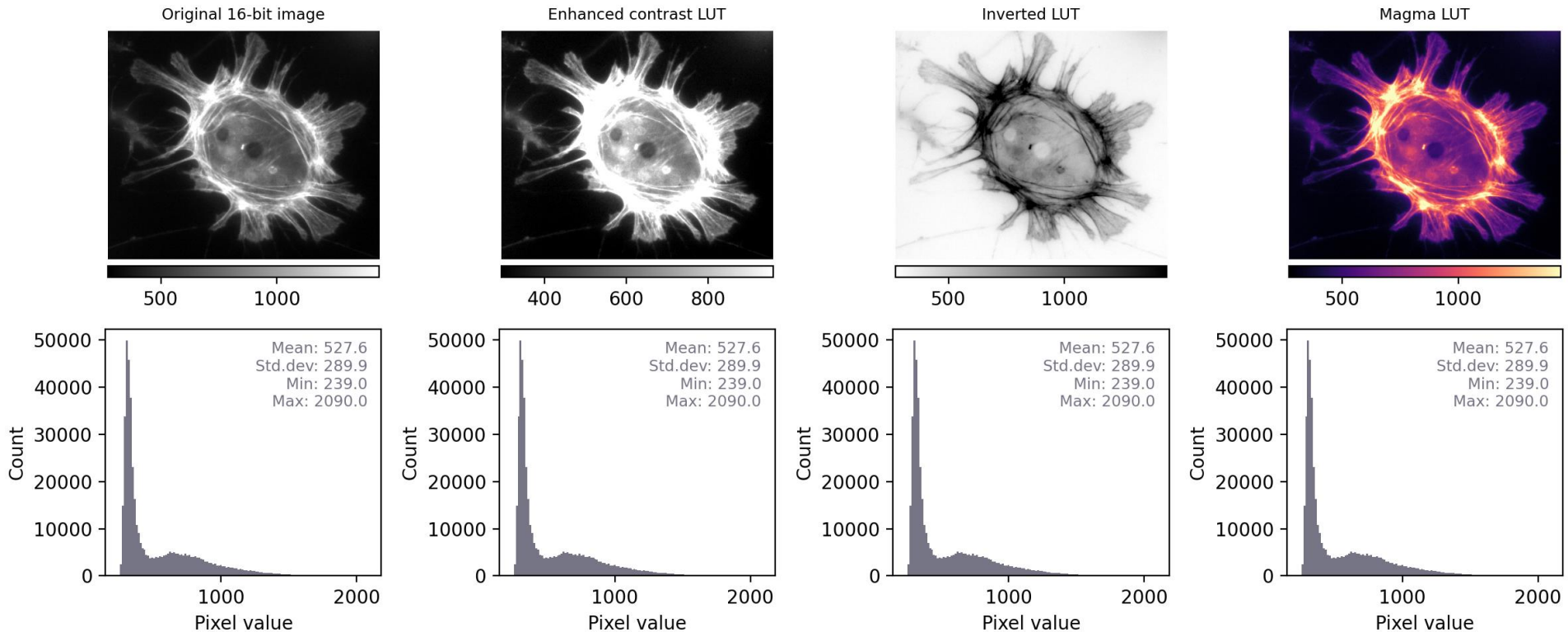


Applying a different lookup table doesn't change the image. All pixel values stay the same, they are just displayed differently.



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

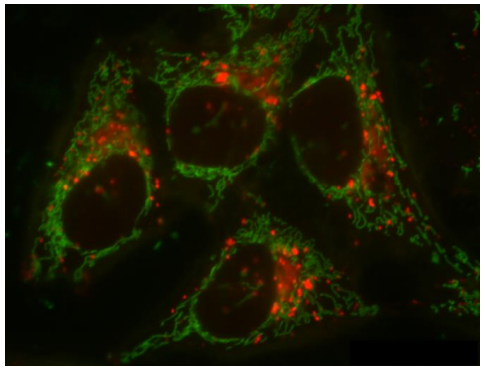
> Lookup tables

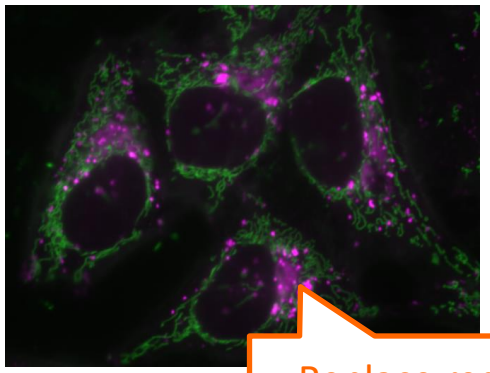
- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!

Color-blind friendly Palette

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

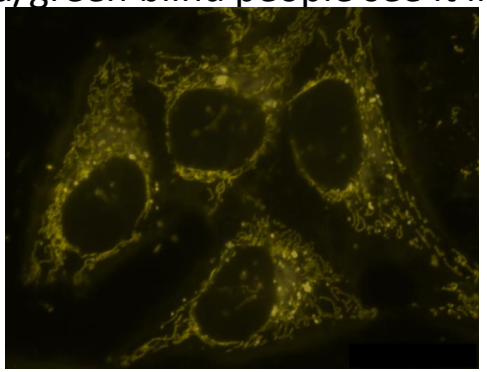
Default view

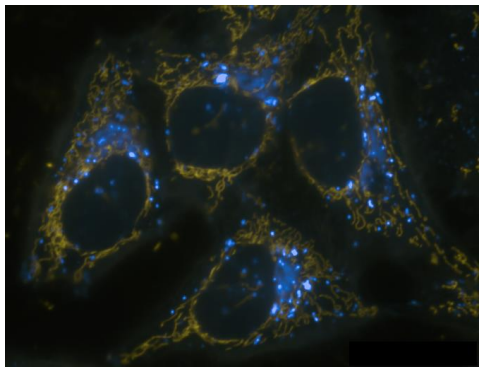




Replace red with magenta!

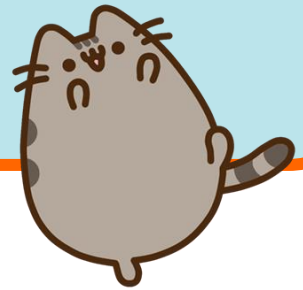
Red/green blind people see it like



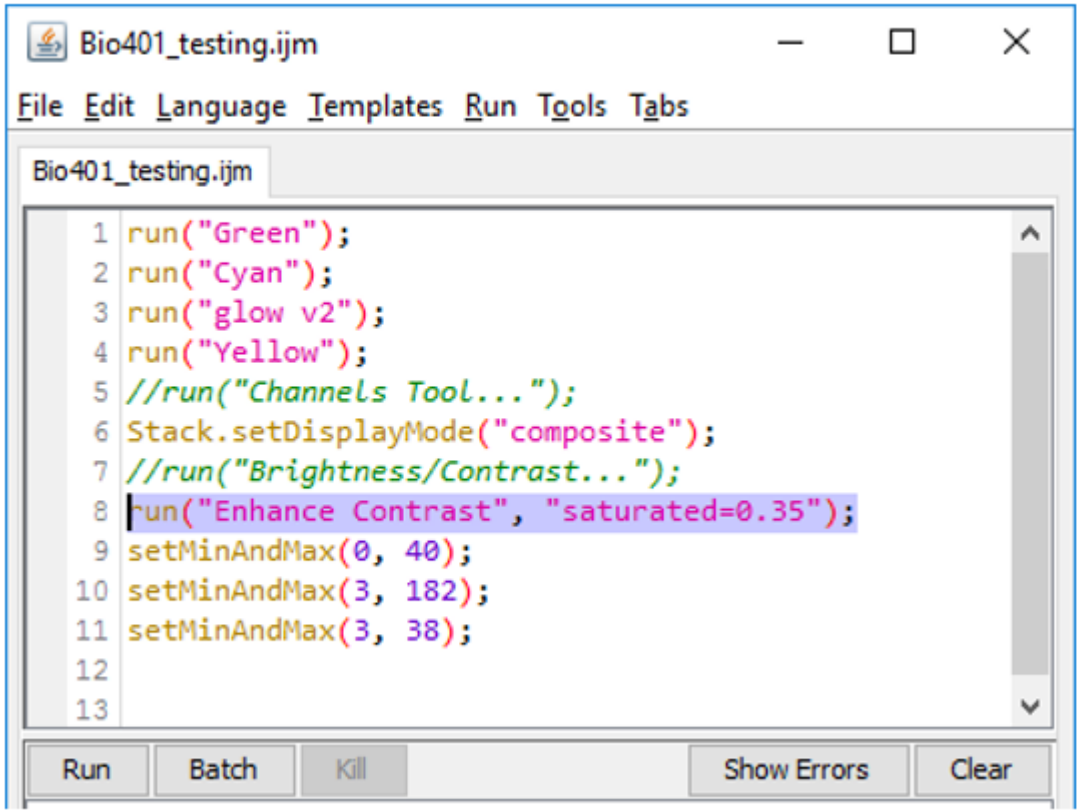


Adapted from https://f1000research.com/slides/10-934

FiJi (ImageJ) Demo



>Macro language



Commands will automatically appear in brown,

"text in magenta between inverted commas",

values in blue and

comments in green.

All commands end with ;

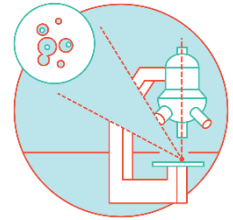
You can check other available scripting languages under the menu Language.

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");

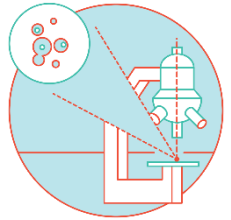
> Very short macro from this session



*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 Analyse 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 tresholding 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

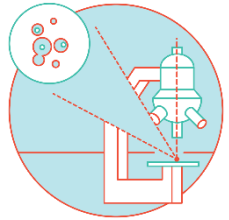
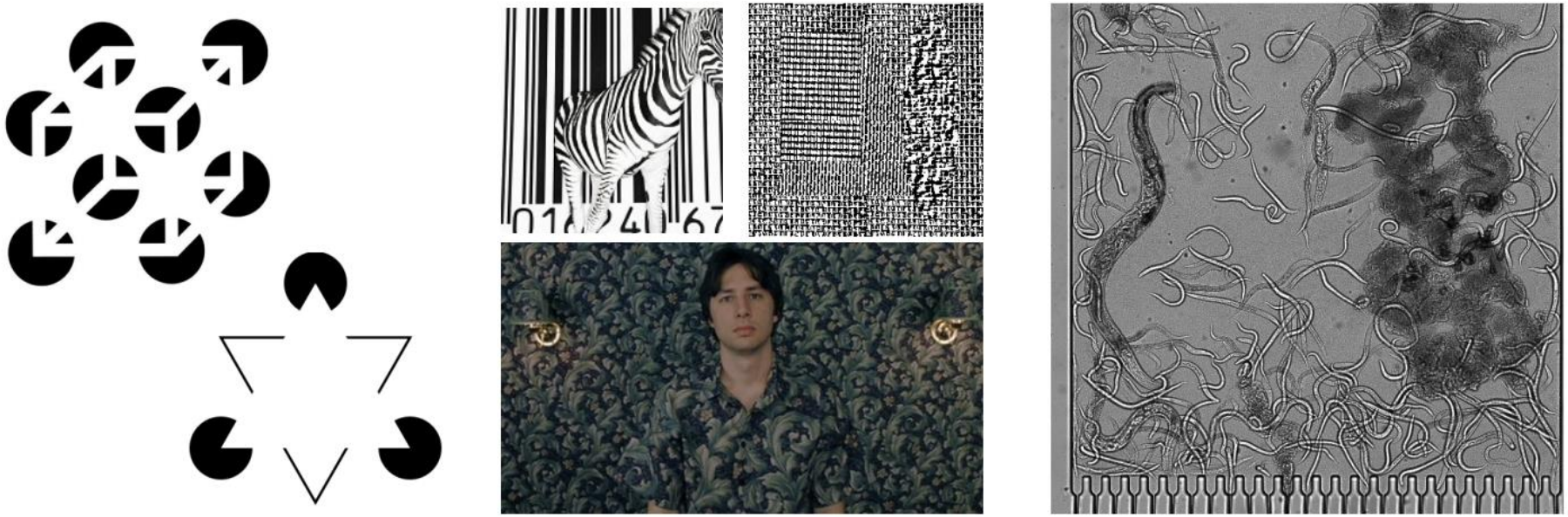


Image Segmentation & Image Analysis

> Why Image Segmentation?



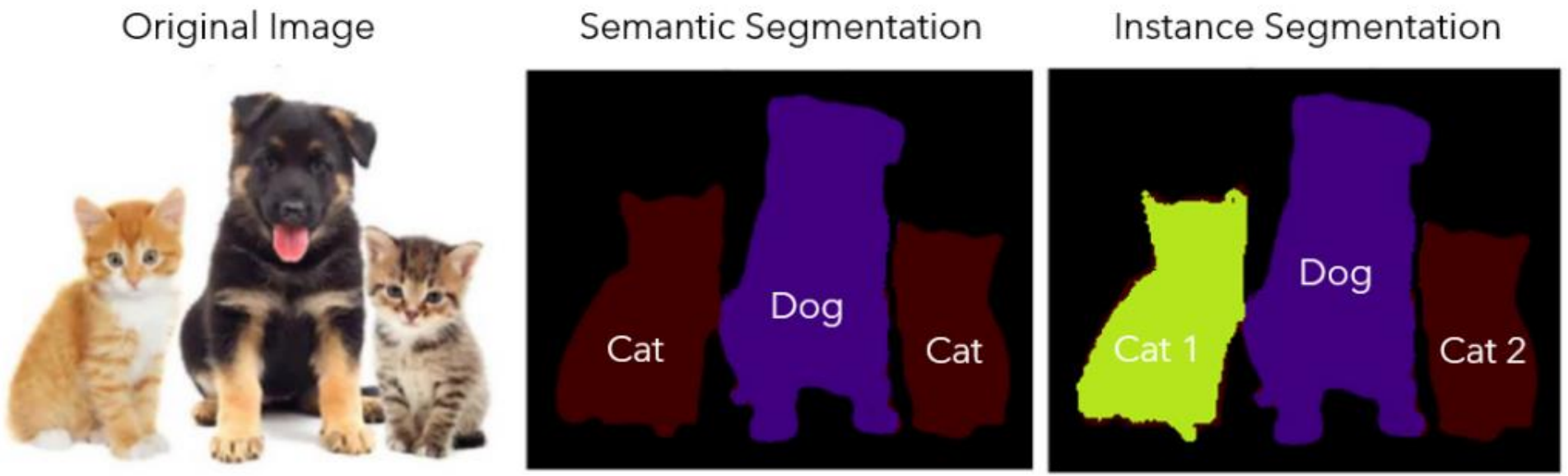
Sometimes boundaries are not complety obvious for the computer point of view…

> Image Segmentation and labelling

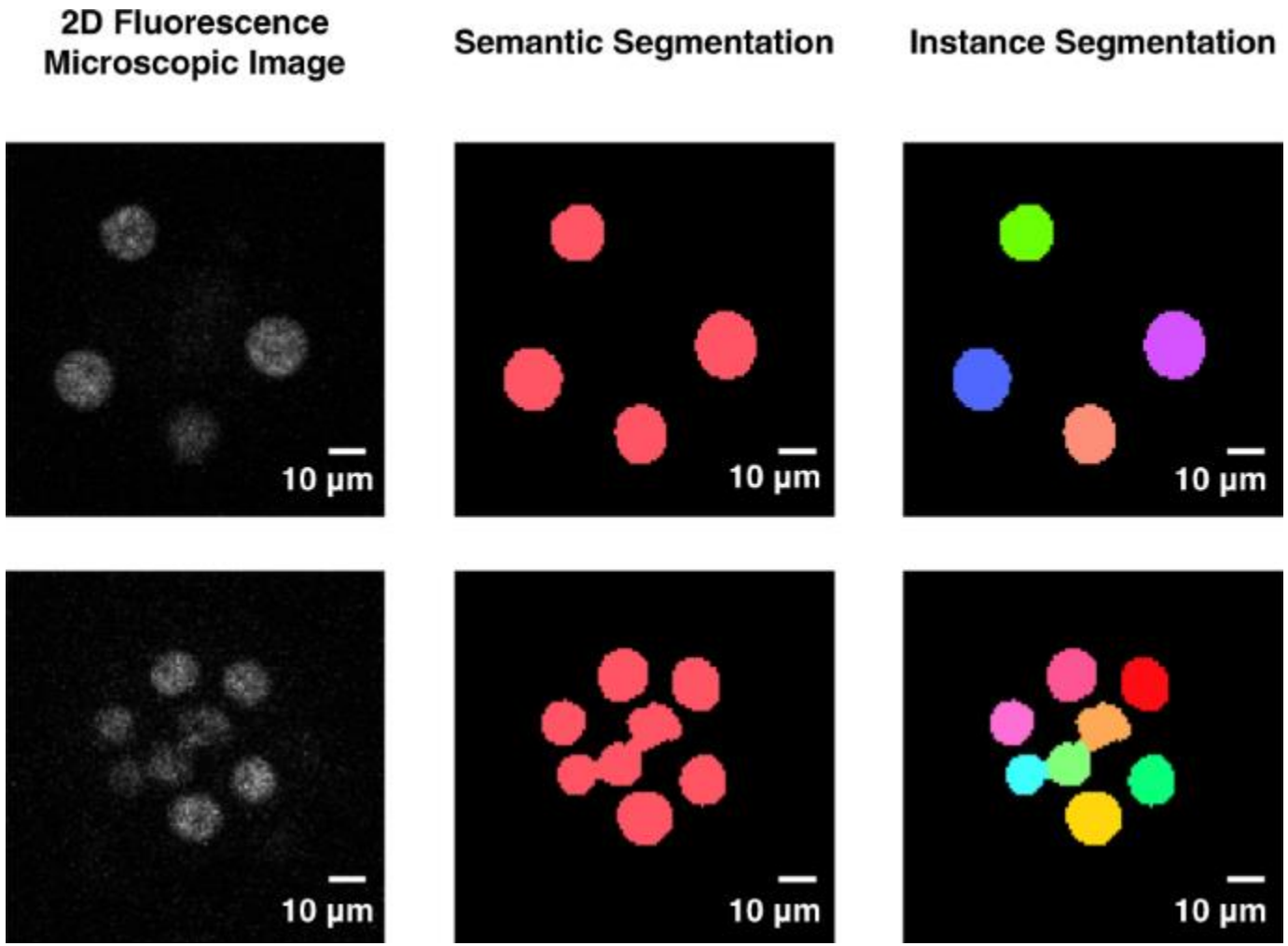
Segmentation
Partitioning of a digital image into **multiple segments** (Assign each pixel a label)

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

Instance segmentation – Differentiate individual occurrences within each class

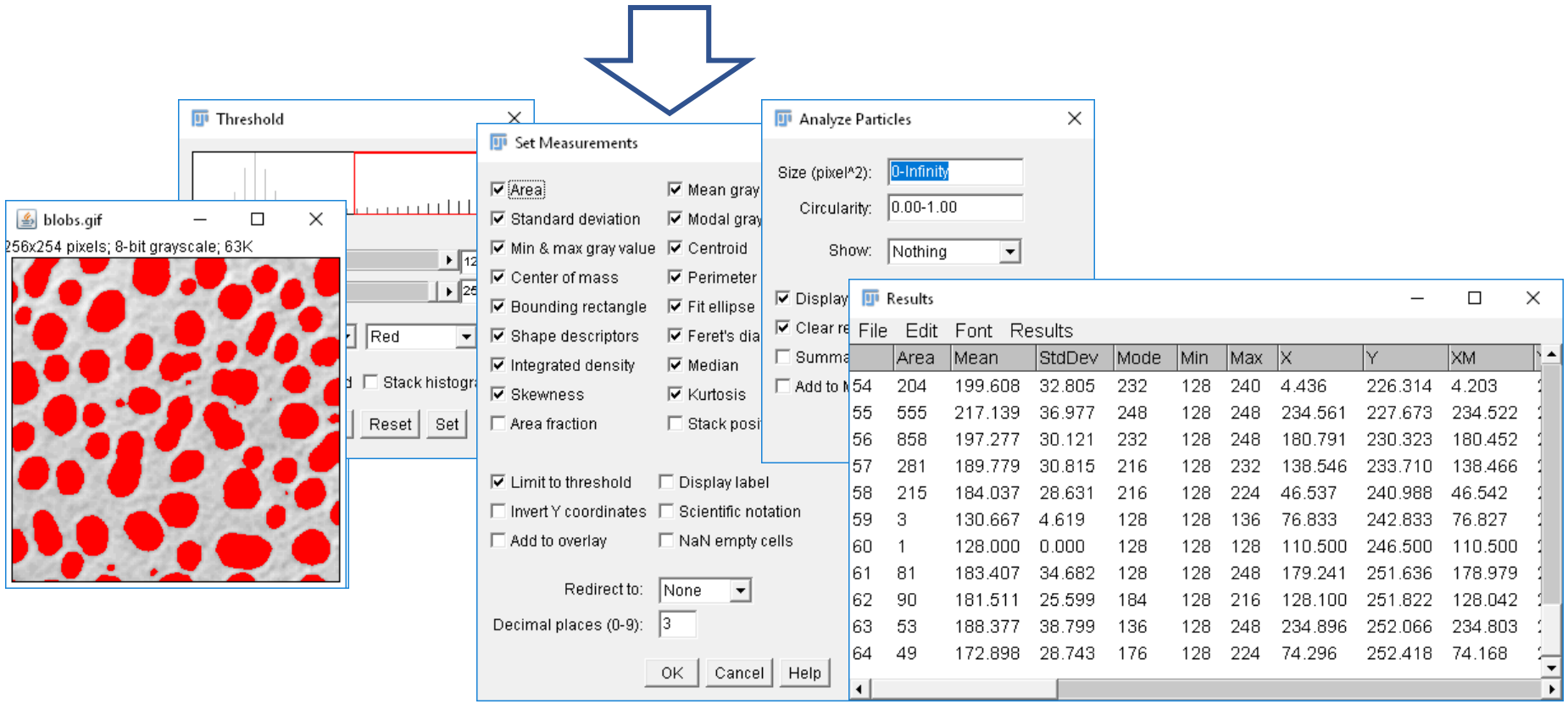


> Semantic vs Instance Segmentation



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

Measurements in 2D: The Particle Analyzer

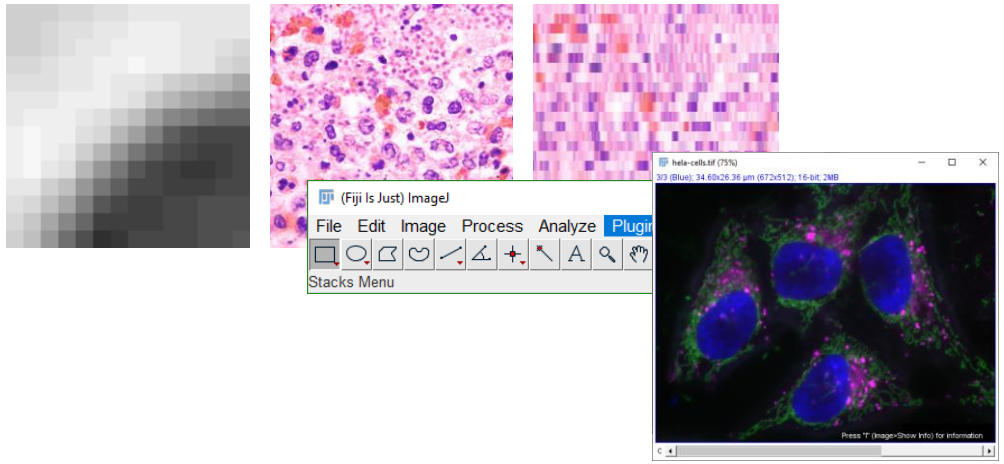


- Tell Fiji what to measure with *Analyze > Set Measurements*

> Lecture recap and next session

- Introduction to Bioimage analysis
- Introduction to ImageJ/Fiji

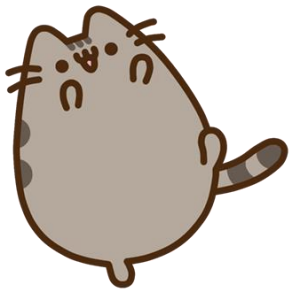
  Demo



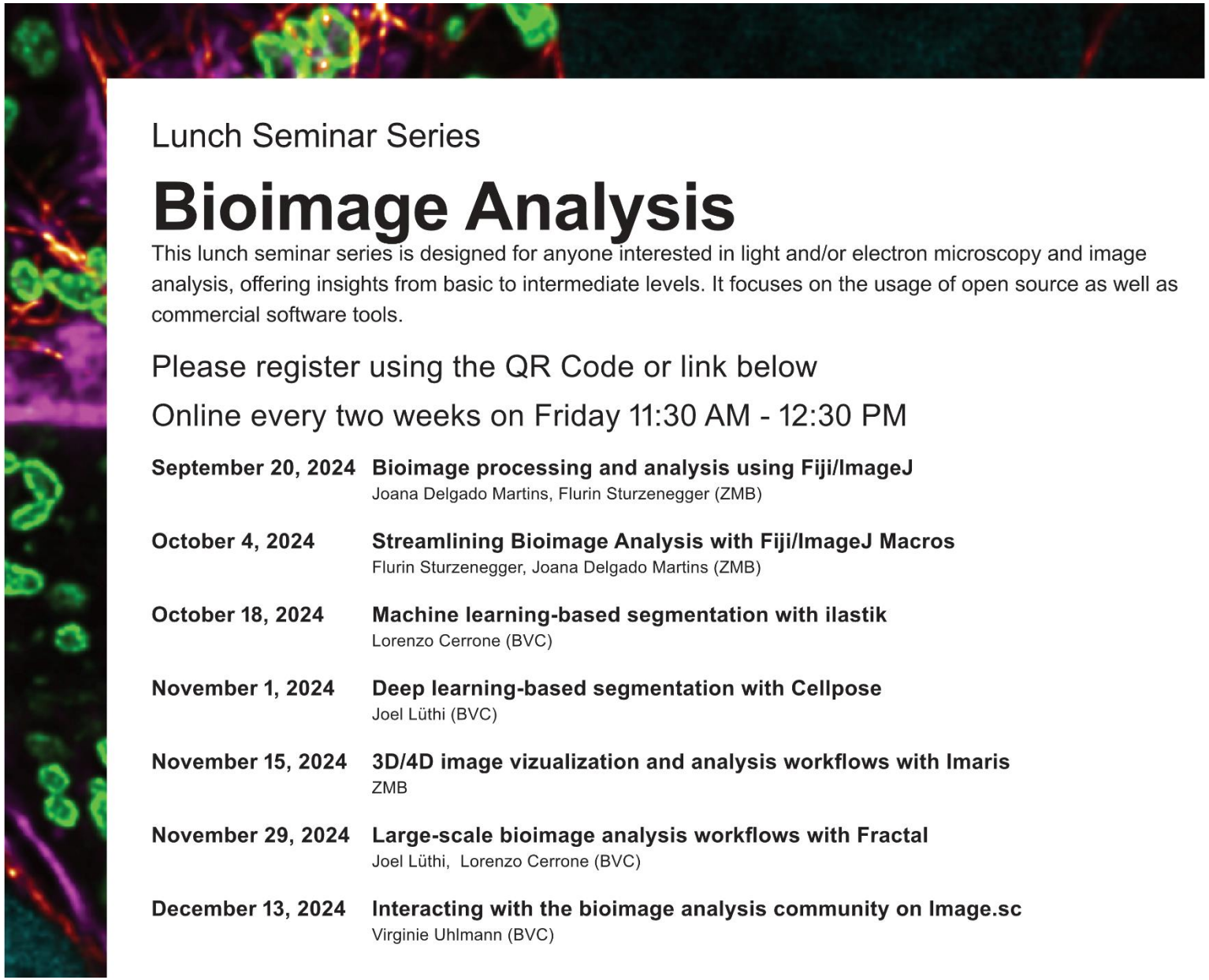
Next session…

- Automation and macros!

HANDS-ON



Thank you! Q&A



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 vizualization 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)