上課注意事項:

1. 教室內禁止攜帶食物飲料入內, 僅允許“白開水”, 請大家將食物飲料放置於教室外的桌上。
2. 請實體與線上學員掃描以下QR code進行線上簽到。
3. 請線上學員於課程開始前關閉自己的麥克風。
4. 線上學員若有問題, 請先按下“舉手”, 或於聊天室寫下問題, 將於課程結束後在場地時間允許下, 安排QA時間。
5. 現場學員發問時請使用麥克風才可進行收音。

線上簽到

課程材料與資訊連結

ImageJ 顯微影像分析與程式設計-互動式影像分析流程建立

Interactive Bioimage Analysis Workflow with CLIJ

Wei-Chen CHU (朱韋臣)

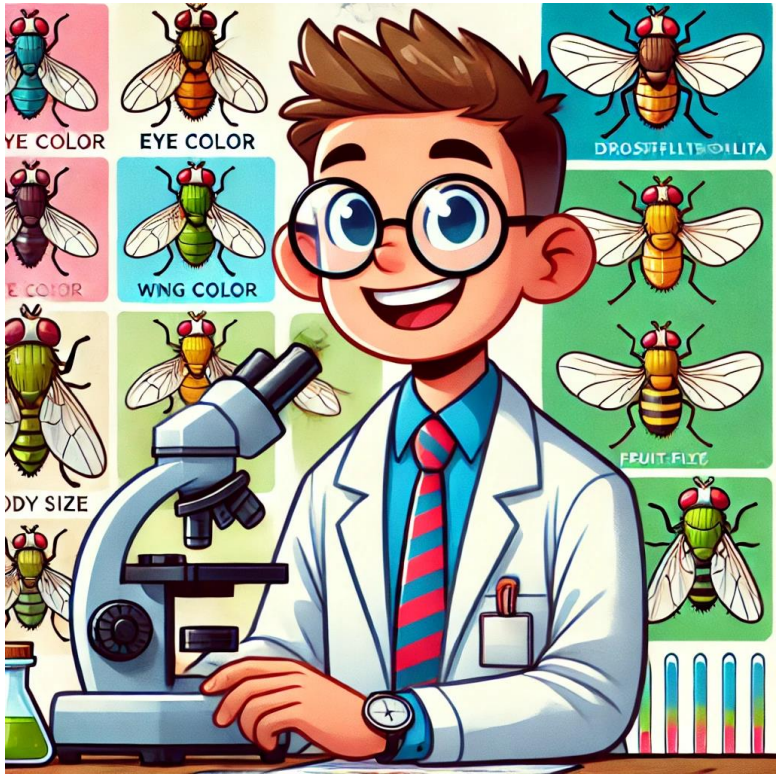
R&D Scientist

ICOB Imaging Core, Academia Sinica, Taiwan

EABIAS (East Asia Bioimage Analysts' Society)
https://eabias.github.io/

My background

Undergraduate ~ Graduate student
@Dept. Life sciences, NCHU

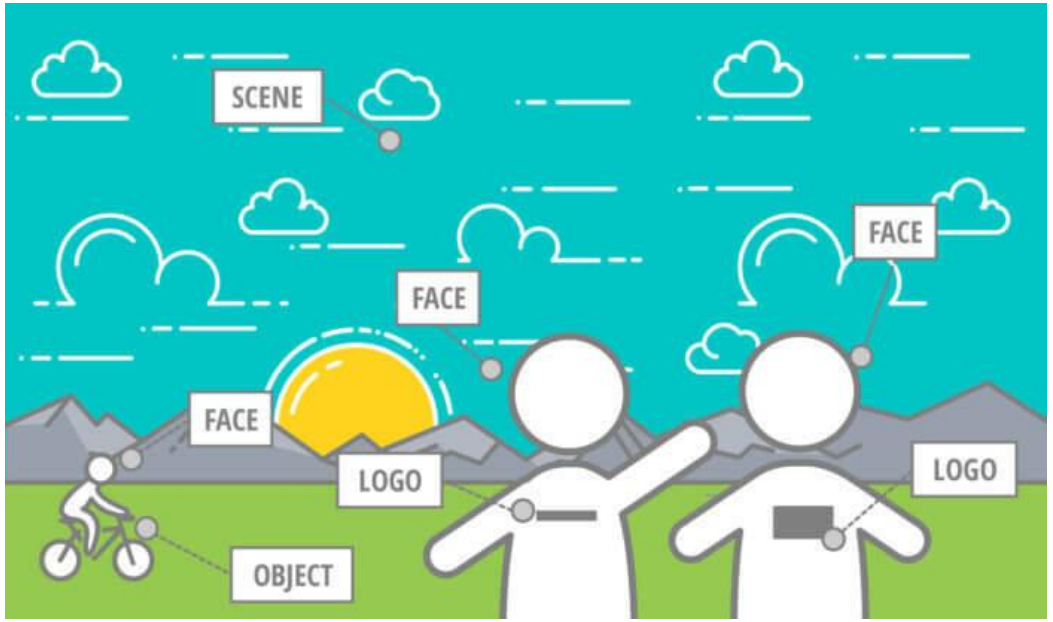


Ph.D. @IMB, Academia Sinica
PostDoc @RIKEN BDR, Japan

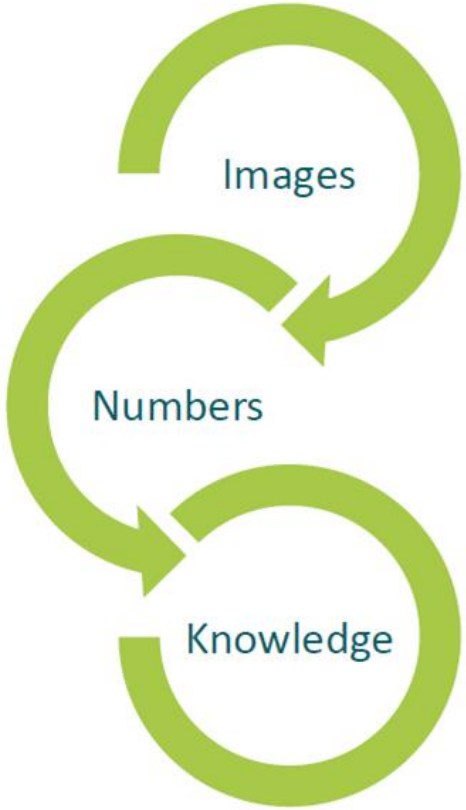
R&D Scientist
@ICOB imaging Core, Academia Sinica

BioImage Analysis?

Image Analysis?
Image analysis (also known as “computer vision” or image recognition) is the ability of computers to **recognize attributes** within an image.



BioImage Analysis?
Understanding and quantifying microscopy, medical or any other calibrated image data.

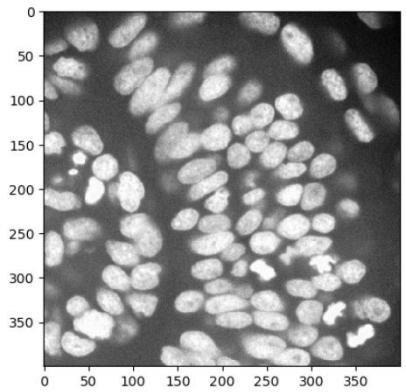


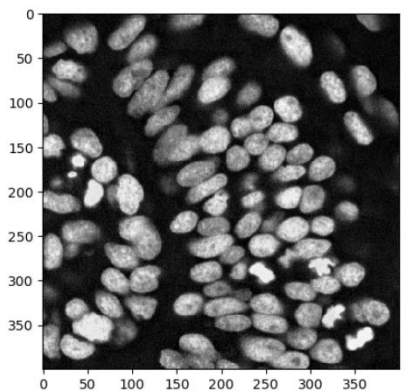
- Objective
- Reliable
- Repeatable
- Reproducible
- Replicable

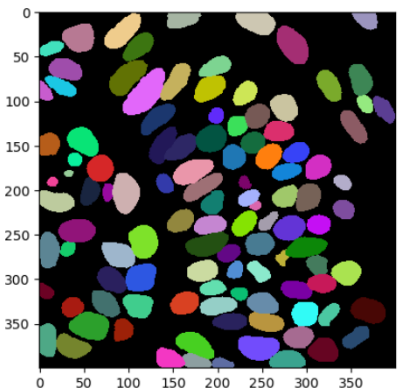
DOI: 10.5281/zenodo.12584729

Bioimage Analysis Workflow

- Goal: **Quantify observations, substantiate conclusions with numbers**

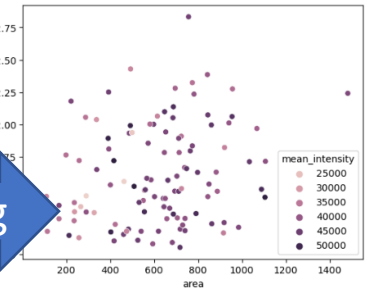






	area	mean_intensity	major_axis_length	minor_axis_length
0	594.0	40572.809764	28.611591	26.537947
1	645.0	43764.872868	33.511511	24.566916
2	1105.0	51970.561991	45.232031	31.456308
3	718.0	47015.487465	31.023274	29.5
4	791.0	49132.515803	36.382253	27.7
...	...	...	...	
105	238.0	30477.126050	20.252197	15.2
106	615.0	32886.154472	41.030760	19.8
107	110.0	33042.445455	14.366347	9.9
108	222.0	43304.180180	25.370549	11.6
109	167.0	43378.808383		

Plotting



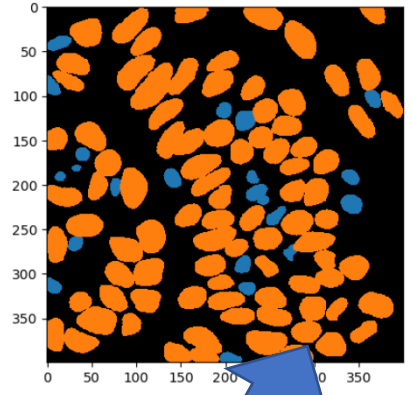


Image filtering → Image segmentation → Feature extraction → Object classification → Visualization

- **Classic image processing**
- **AI-based segmentation**
  - **StarDist**
  - **CellPose… etc.**

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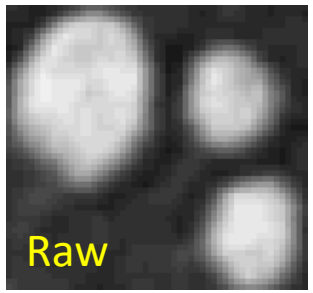
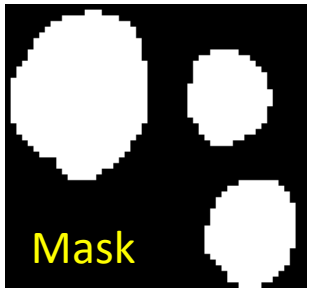
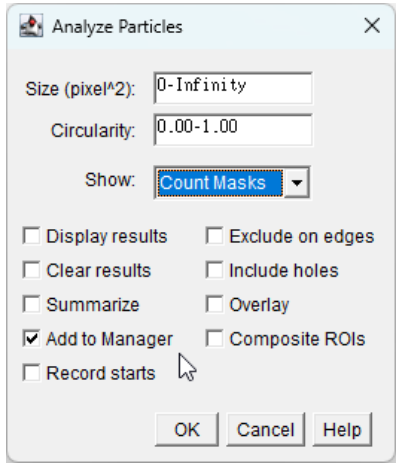
Segmentation with ROI list, Mask, and Label



Thresholding



Analyze Particles
(add to manager)

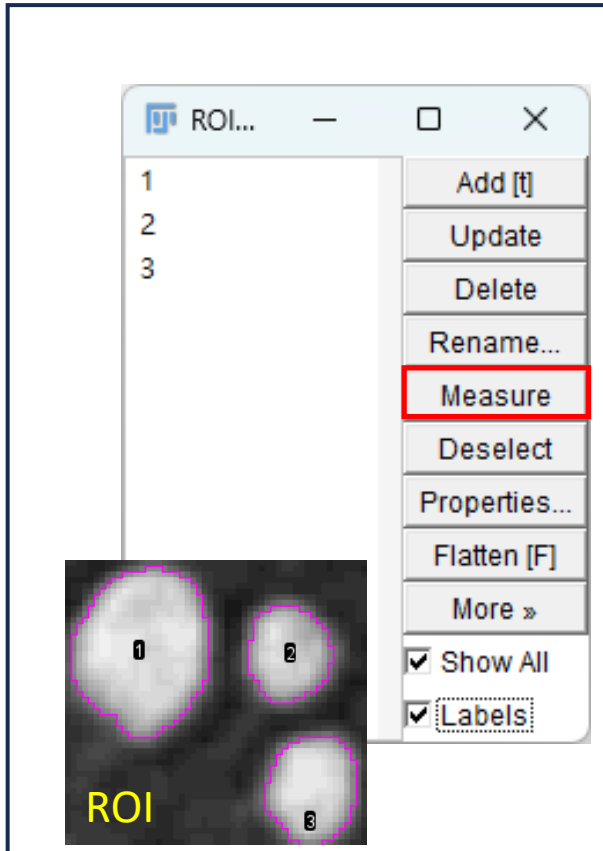


Connected Component Labeling

Analyze Particles
(Count mask)

AI-based segmentation

Labels to ROI



Concept
Component

Recap: Classic workflow in FIJI/ImageJ

RAW Image

Duplicate

Image copy

Median Filter

Filtered image

Thresholding

Binary Mask

Watershed…etc

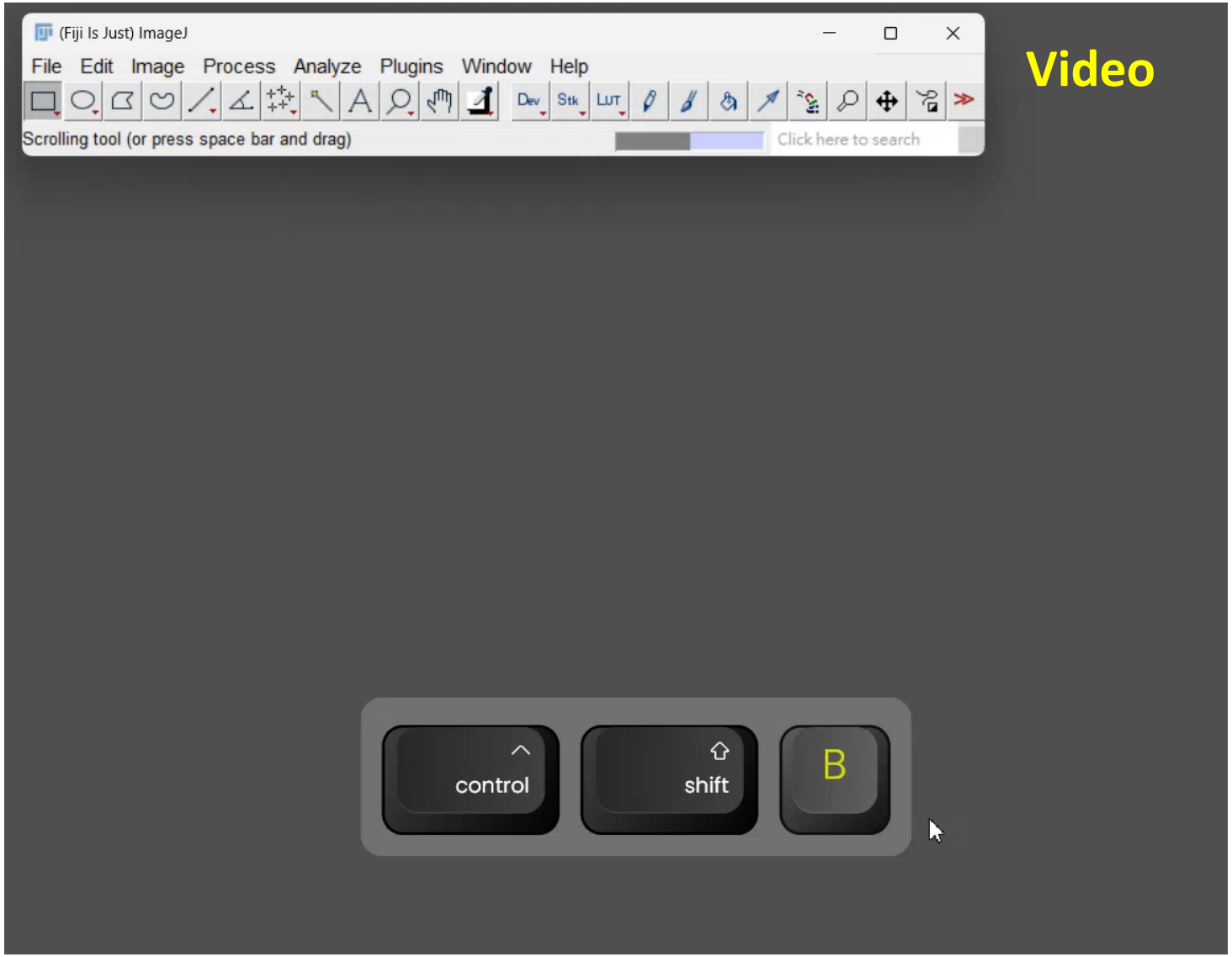
Refined Mask

Analyze Particles

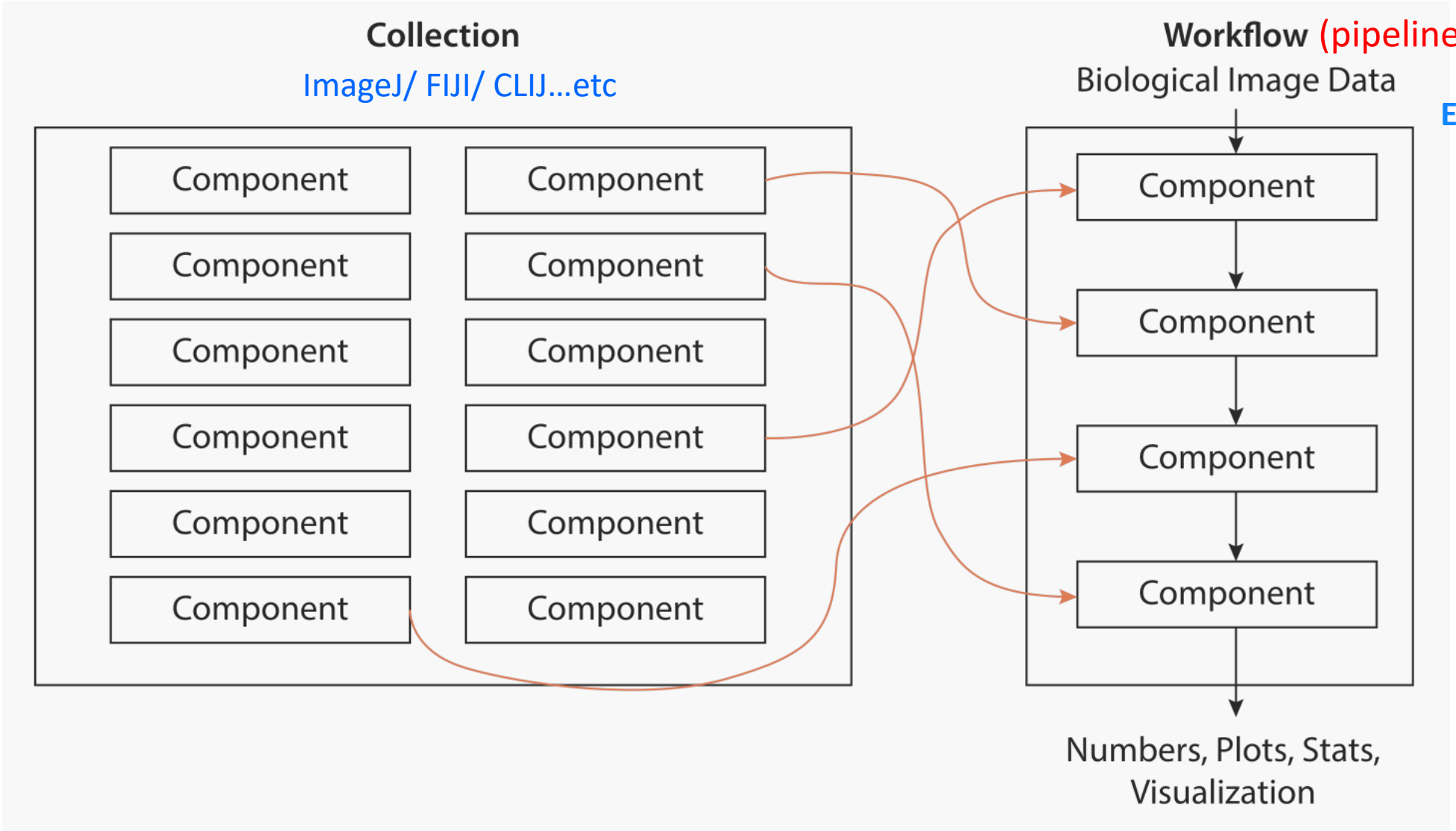
ROIs, Labels

Measure

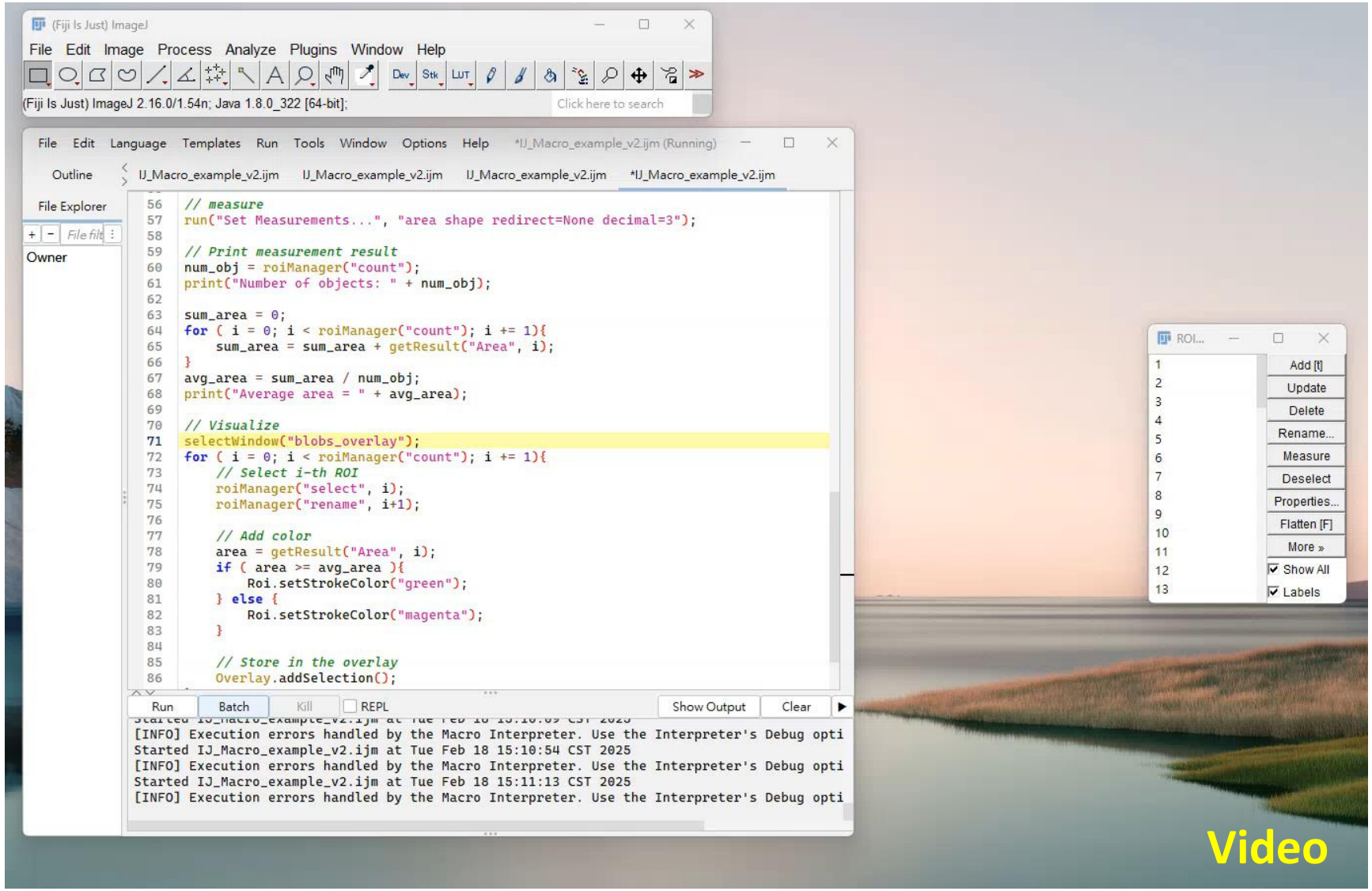
Results



Software -> components, collection and workflow



ImageJ macro allows other people reproduce your workflow and results



ImageJ macro:
Great documentation for your bioimage analysis workflow!

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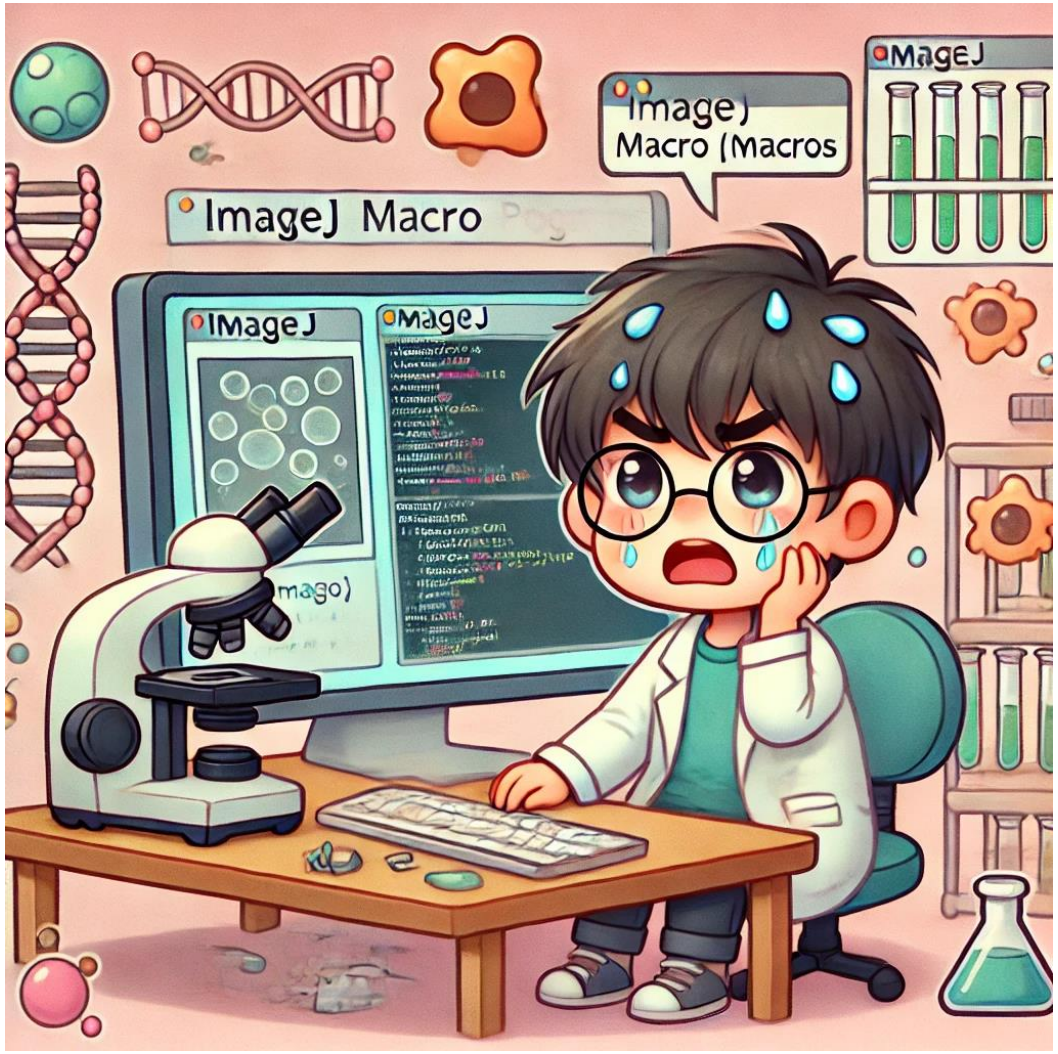
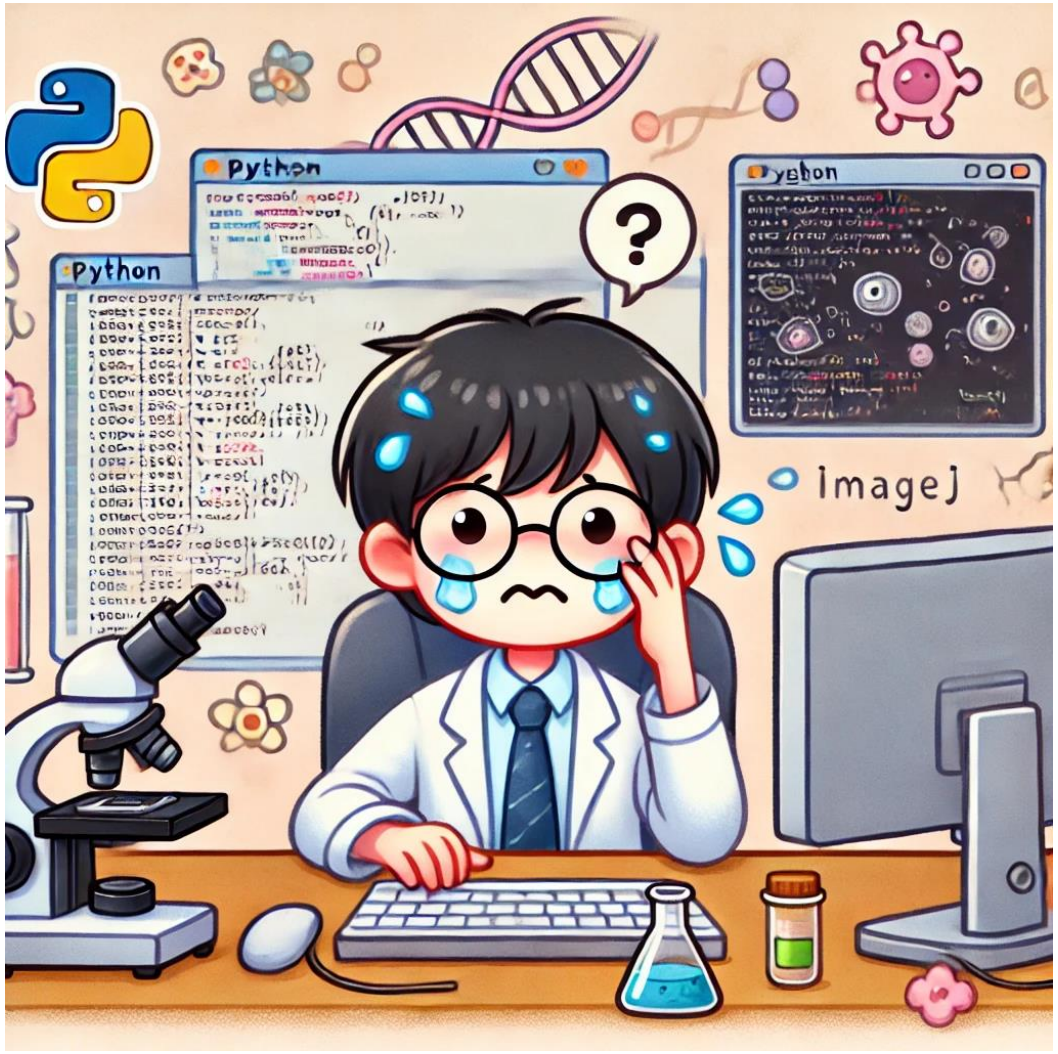
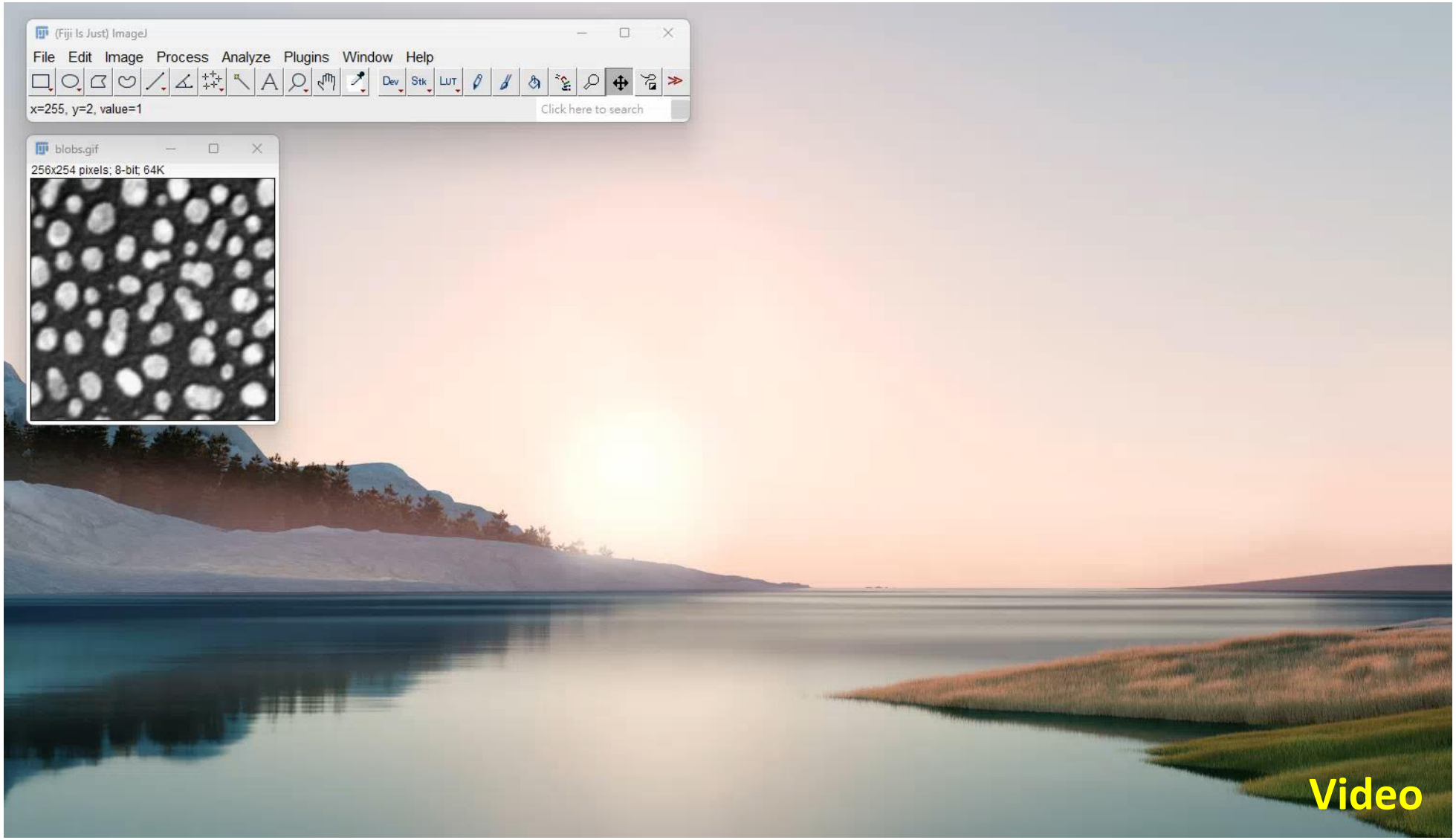
However, many biologists are not good for programming.





How can an image analysis workflow be built more efficiently?

- Interactive workflow
- Code generation
- Data visualization
- Documentation
- GPU acceleration

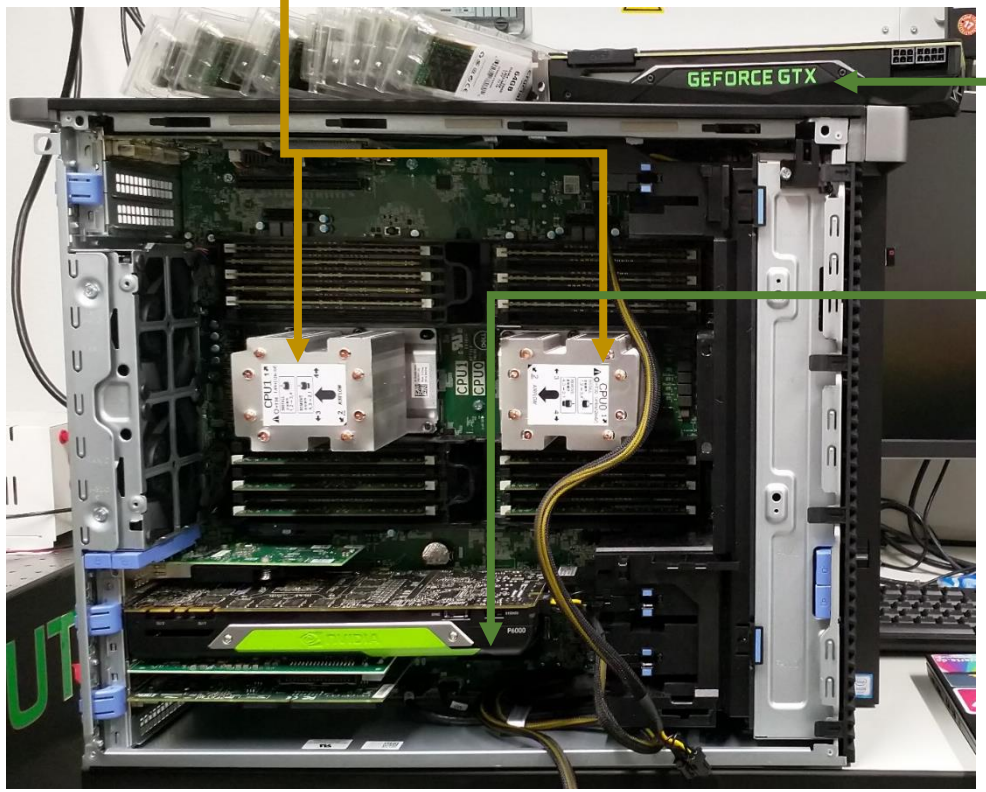


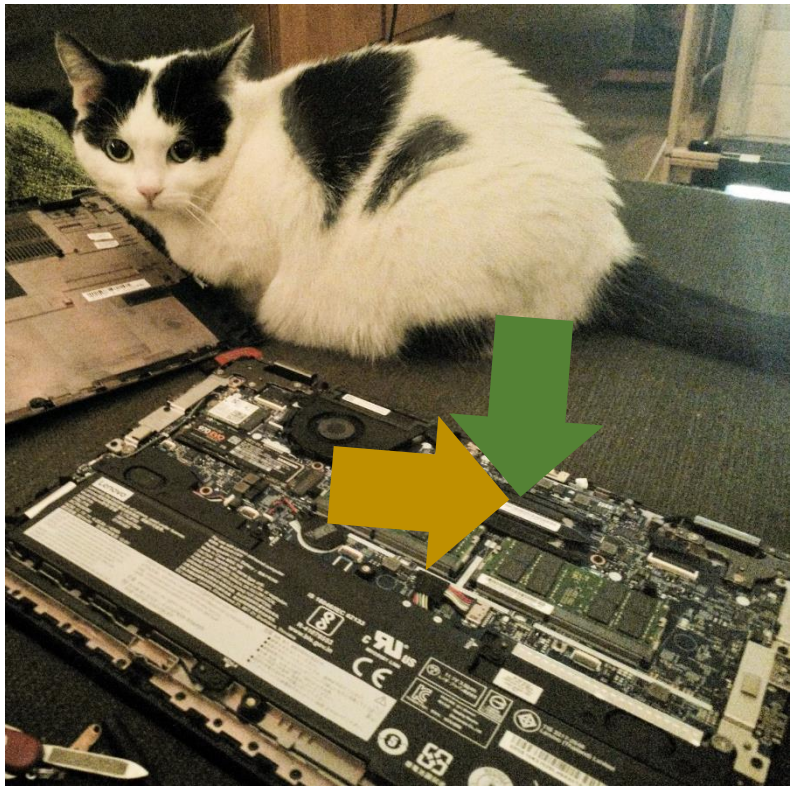
https://clij.github.io/

Graphics Processing Units (GPU)

- **Typical computers contain Graphics Processing Units**

Central Processing Unit (CPU) **Graphics Processing Unit (GPU)**

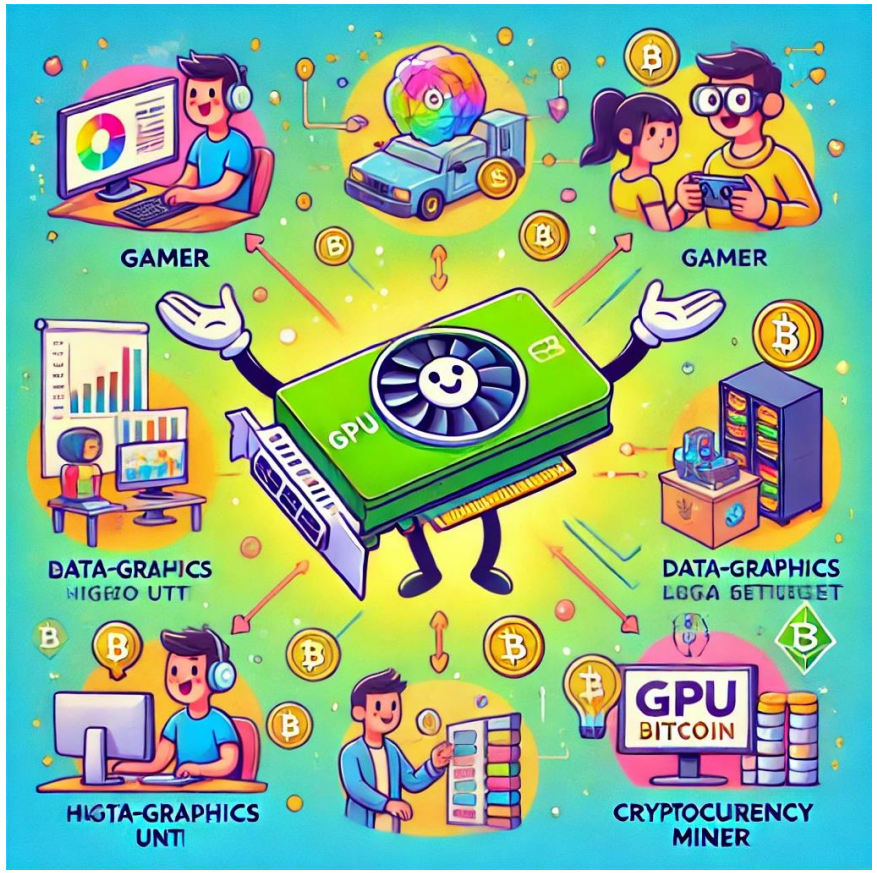




Most laptops contain *integrated* GPUs

Alternative: *external* GPUs

GPU: Graphics processing unit

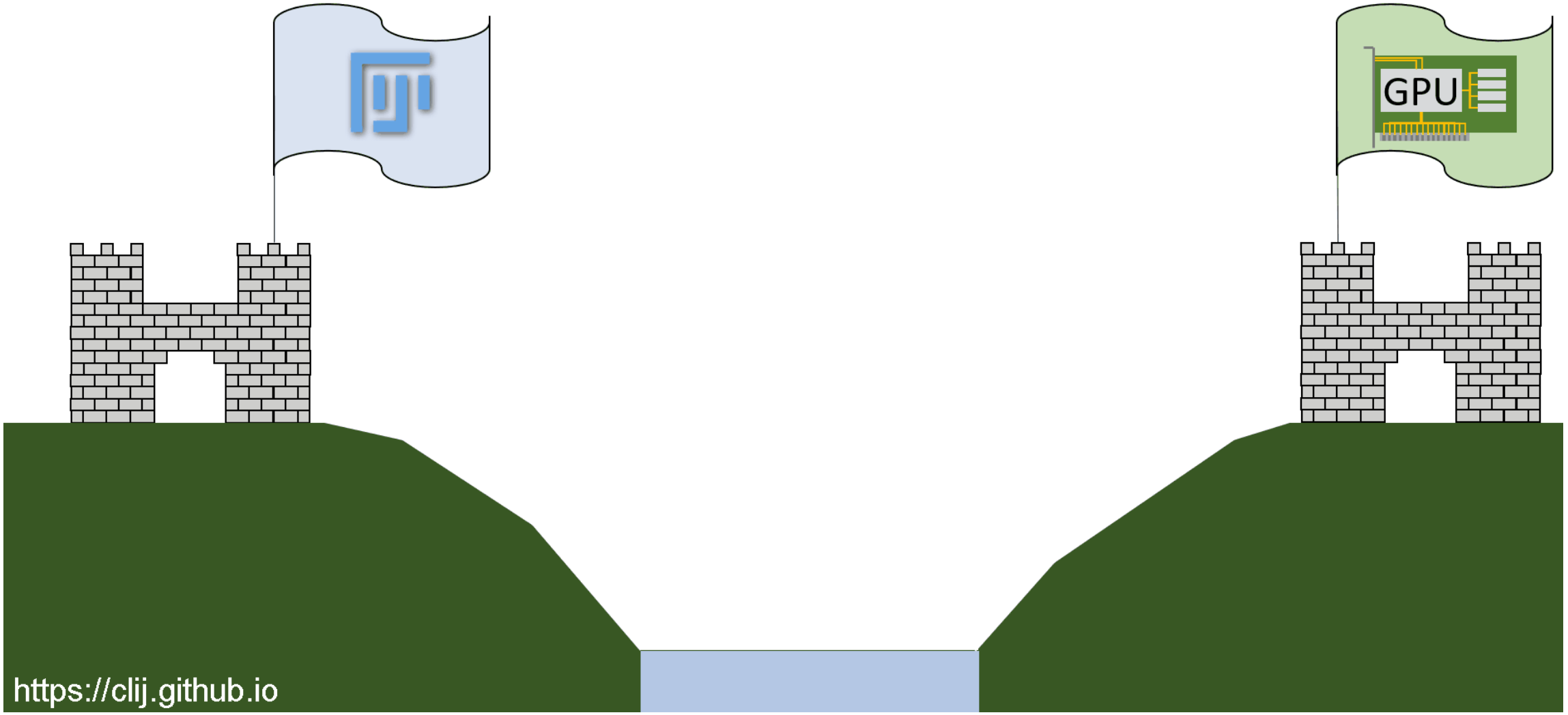


NVIDIA → CUDA (Compute Unified Device Architecture)

AMD → OpenCl (Open Computing Language)

intel → OpenCl (Open Computing Language)

What is CLIJ



Integrated GPU-acceleration into ImageJ / Fiji using ImageJ Macro

Dr. Robert Haase
Center for Scalable Data Analytics and Artificial Intelligence (ScaDS.AI) Dresden/Leipzig, Leipzig University, Germany

CLIJ

OpenCL ImageJ

https://clij.github.io/

clEsperanto

OpenCL 世界語 (International auxiliary language)

https://clesperanto.github.io/

Dr. Stéphane Rigaud
Image Analysus Hub, Institur Pasteur, Paris

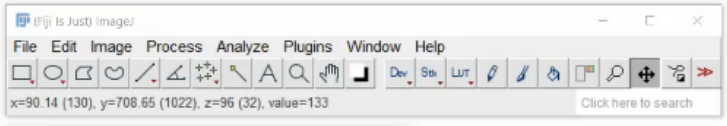
Image processing using FIJI/ ImageJ

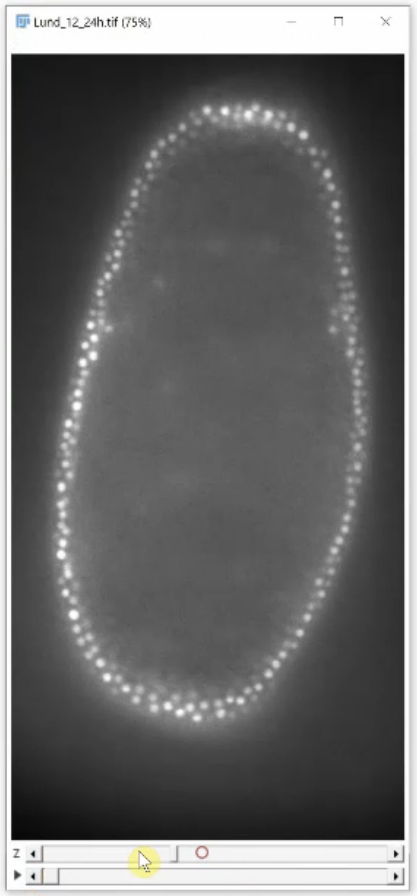
Video

1.4 GB

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

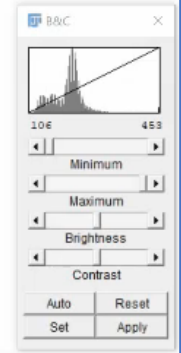
Image processing using CLIJ

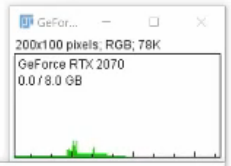




1.4 GB

Video





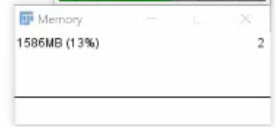
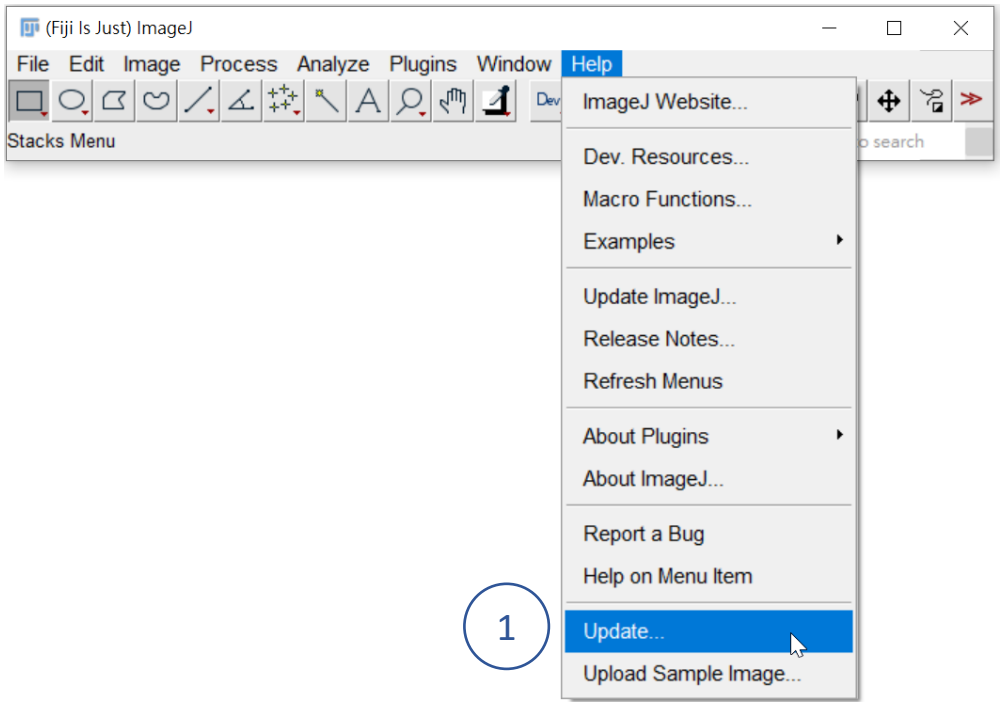
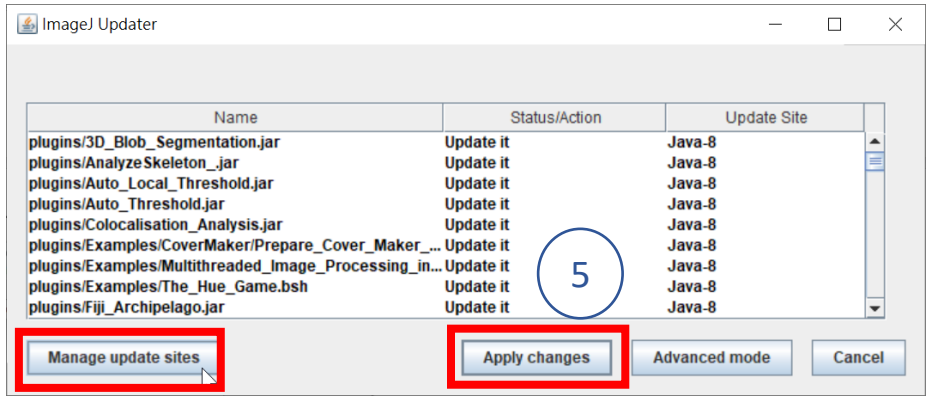


Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

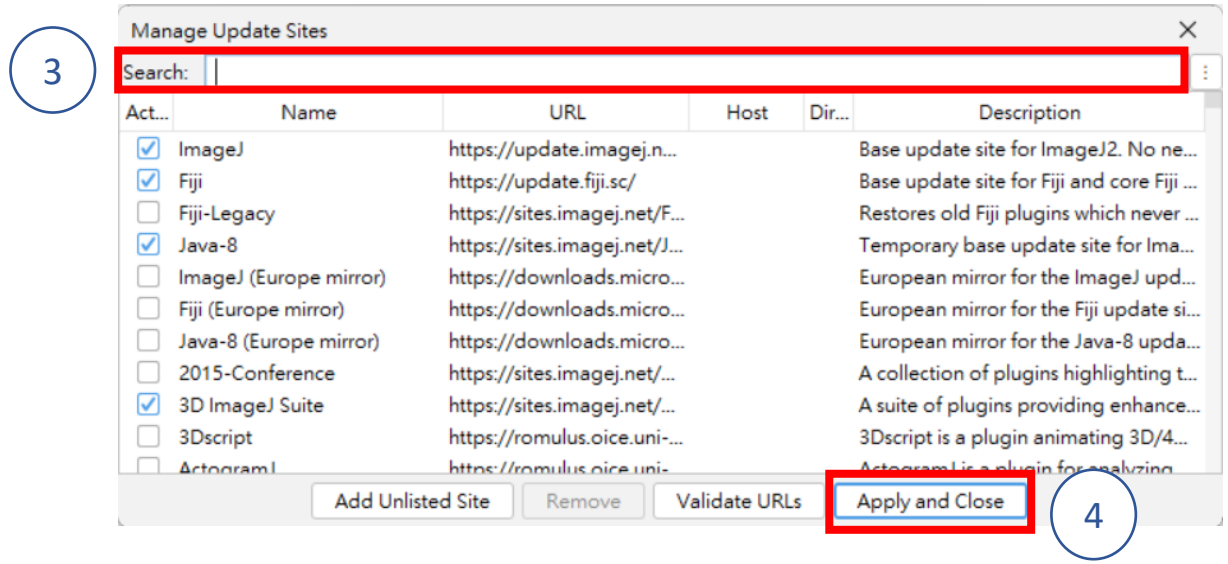
CLIJ: installation





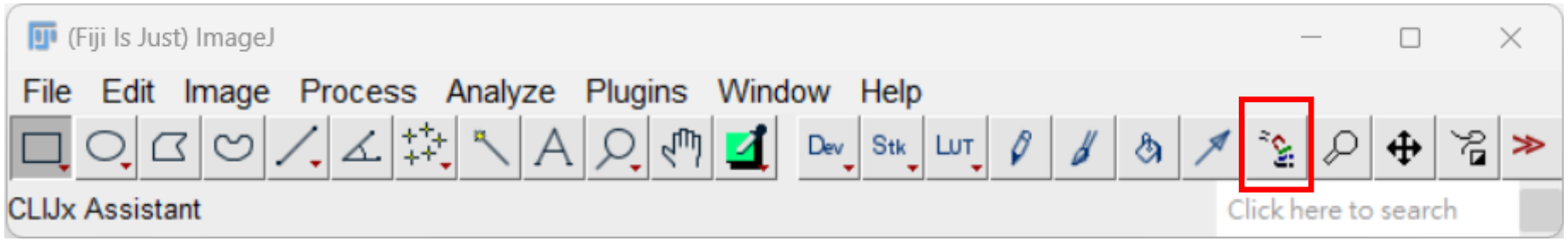
Please select all listed plugins:

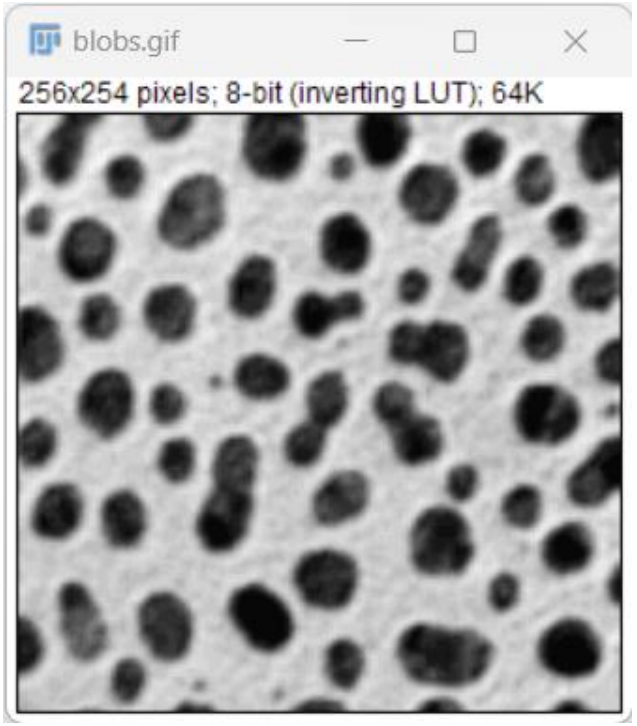
- clij
- clij2
- clijx-assistant
- clijx-assistant-extensions
- 3D ImageJ Suite
- BioVoxxel 3D box
- BoneJ
- IJMND
- IJPB-Plugins



6 Close and restart FIJI

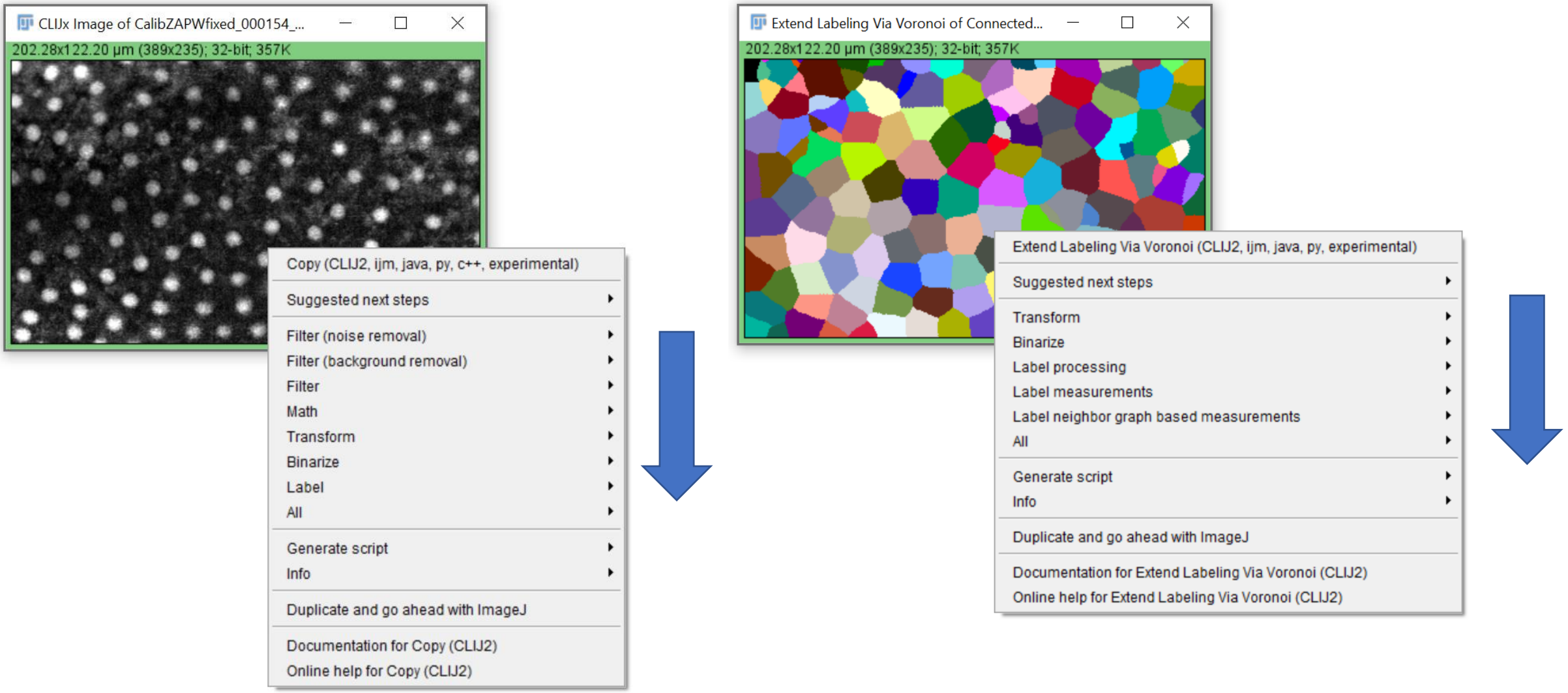
CLIJ-Assistant



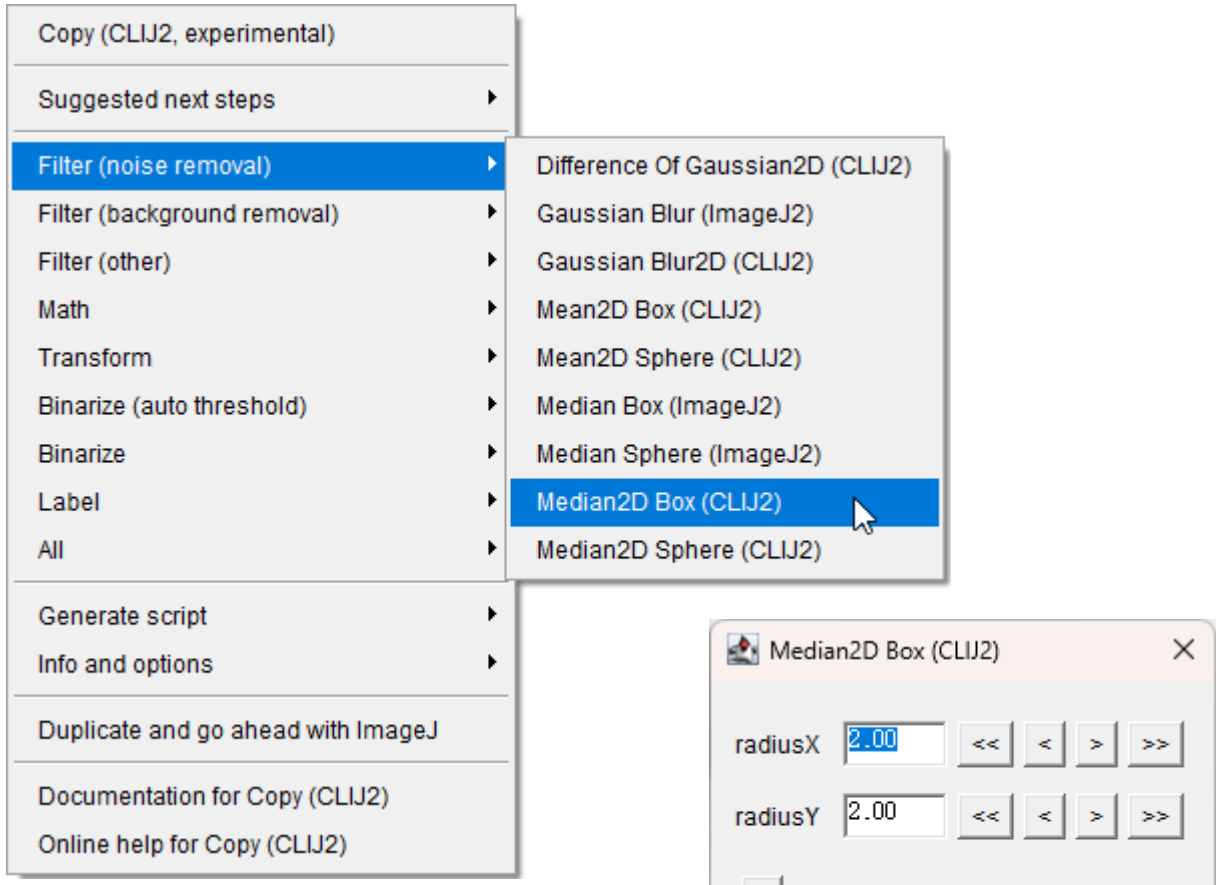


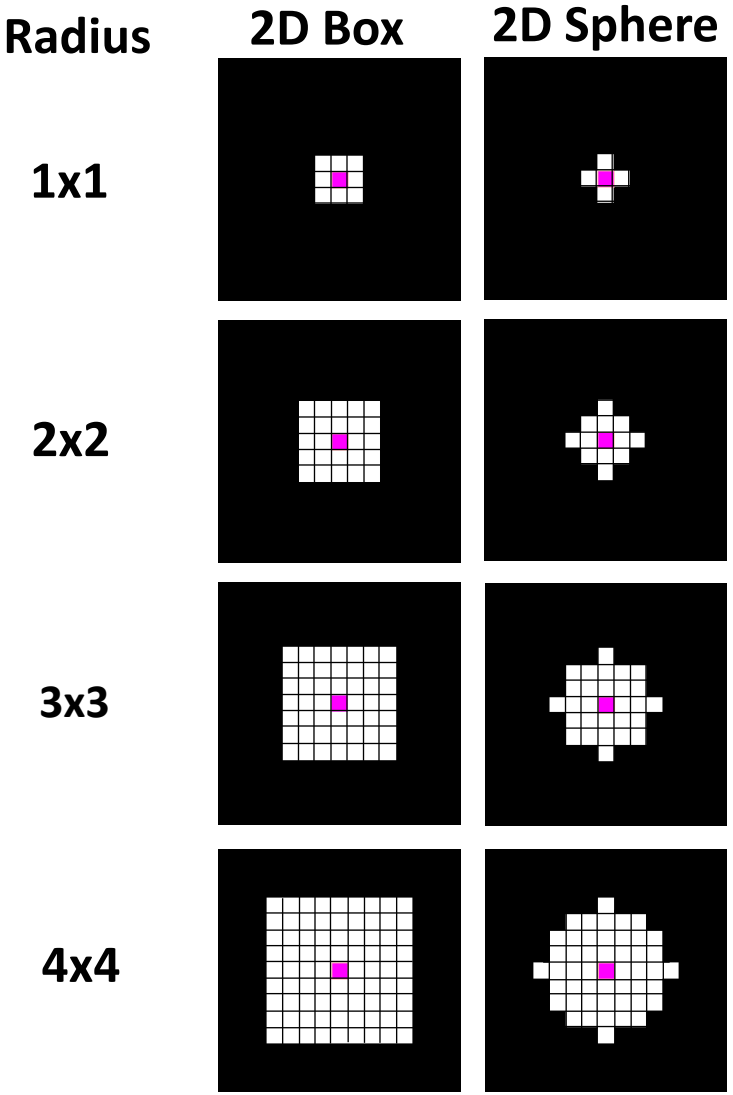
(Default :Auto Brightness and Contrast ON)

The menu order is intentional: From preprocessing to analysis



Filters kernel: Box vs Sphere





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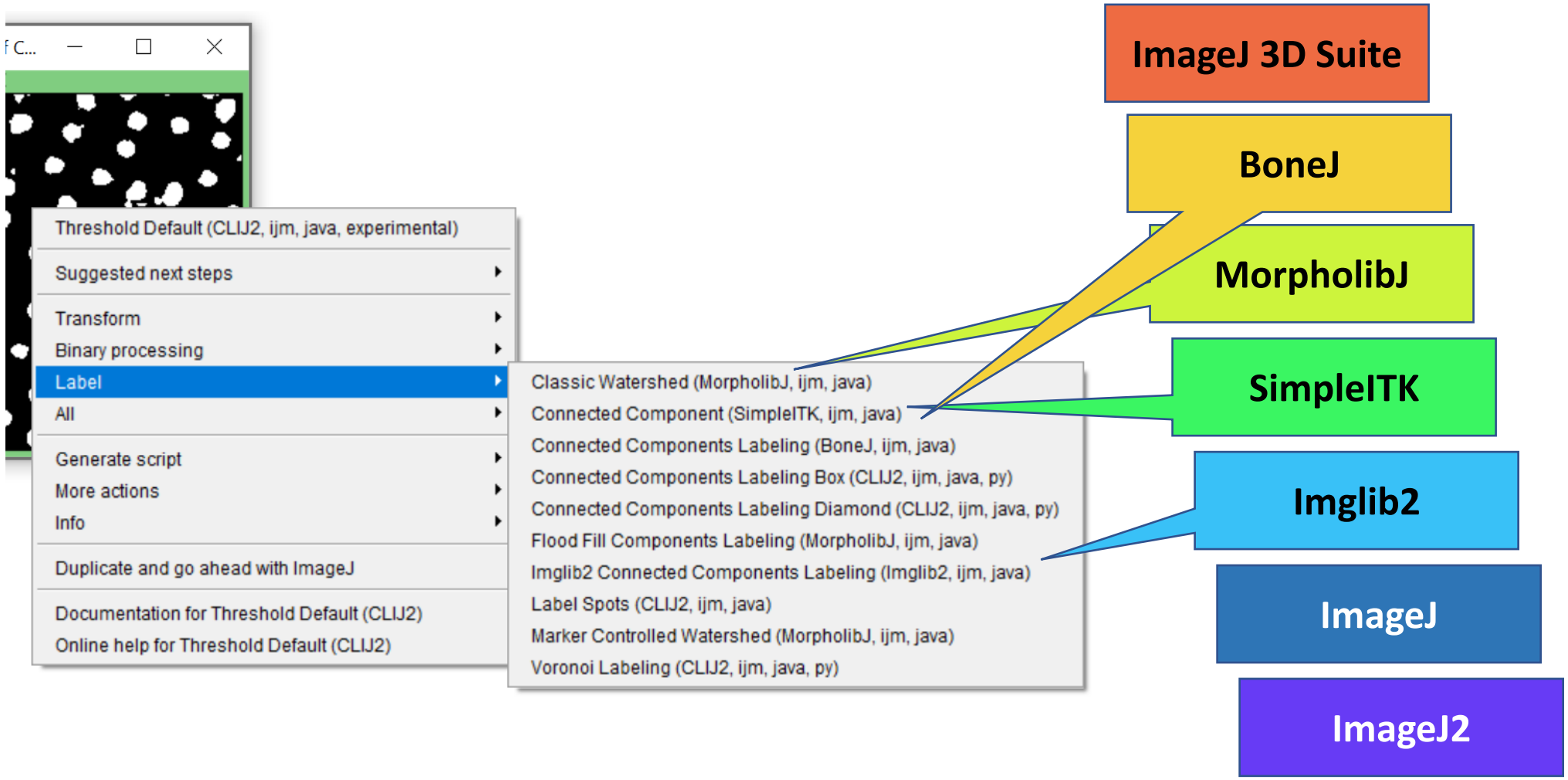
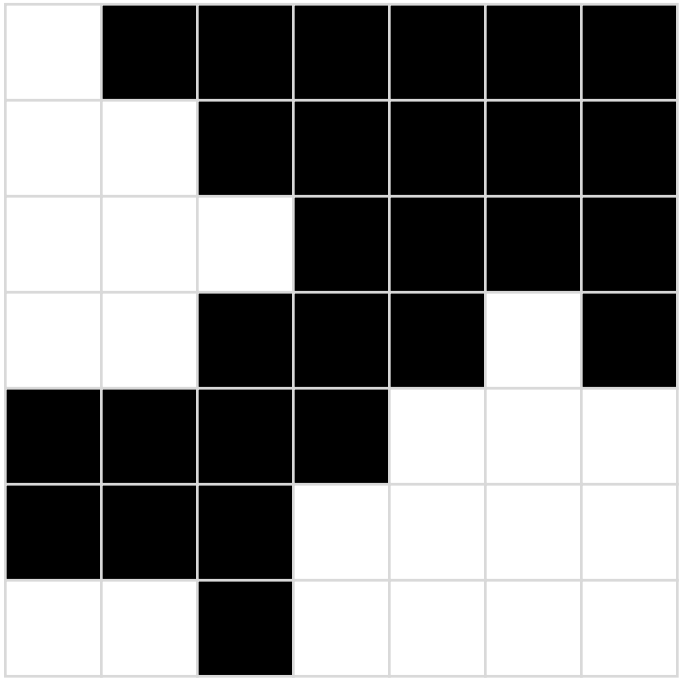
Extensibility

- Install: https://clij.github.io/assistant/installation#extensions



Connected component labeling (CCL / CCA)

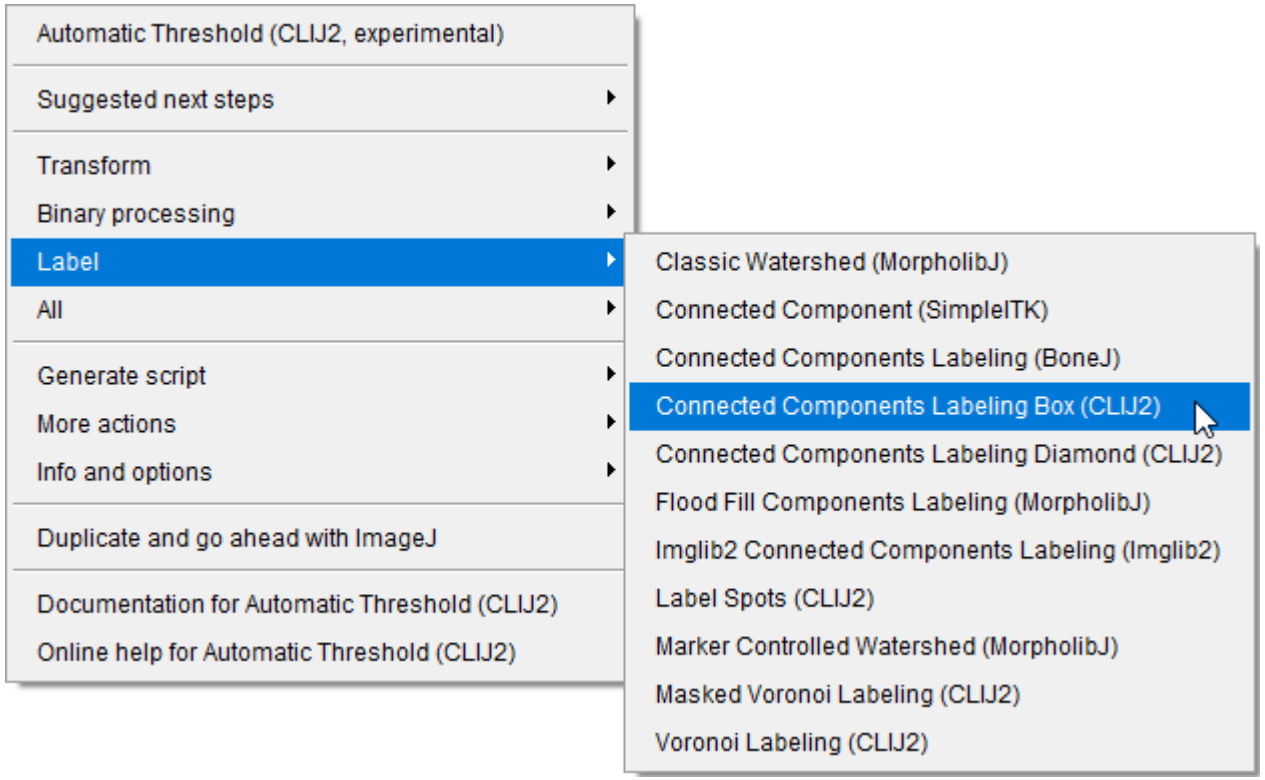
- In order to allow the computer differentiating objects, connected component analysis (CCA) is used to mark pixels belonging to different objects with different numbers
- Background pixels are marked with 0.
- The maximum intensity of a labelled map corresponds to the number of objects.

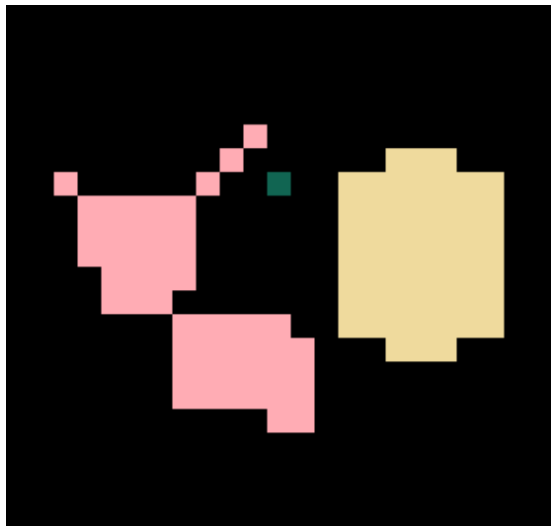


CCL

1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

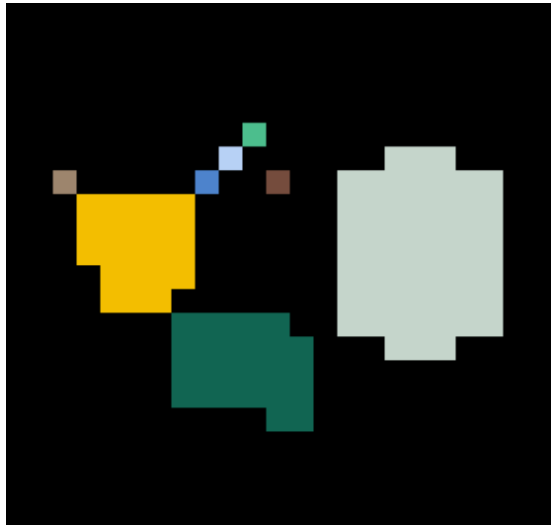
CCL: Box vs Diamond





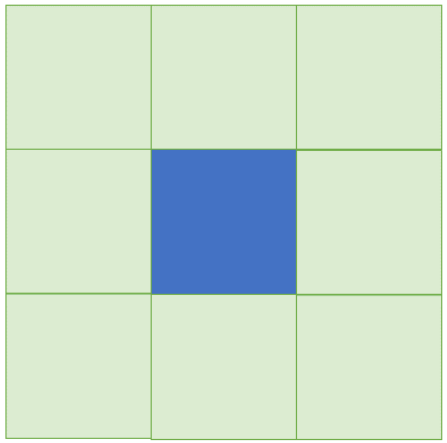
Box (8-connectivity)

ImageJ Analyze Particles: 8-connectivity

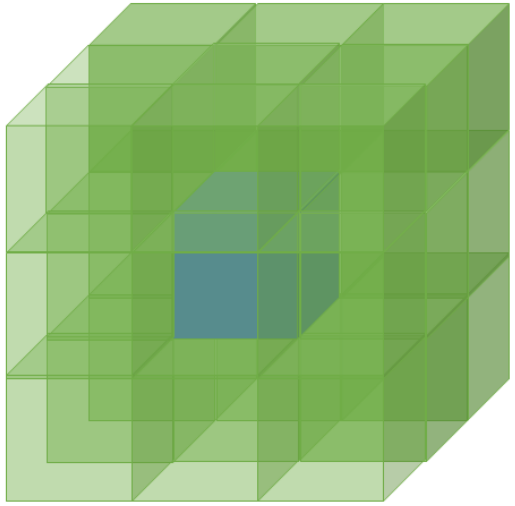


Diamond (4-connectivity)

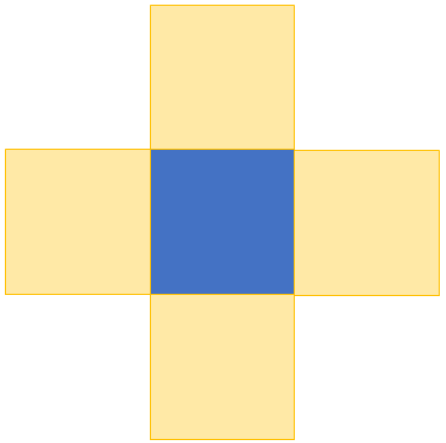
CCL: Box vs Diamond in 2D and 3D



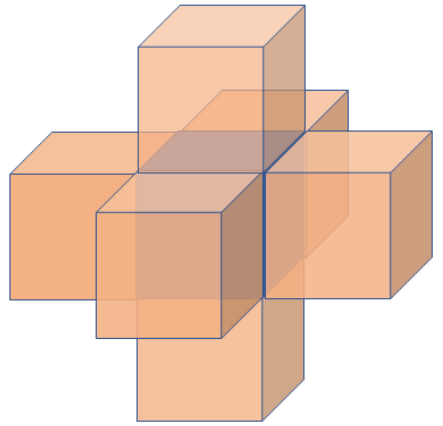
8-connectivity



26-connectivity



4-connectivity



6-connectivity

Box

Diamond

Label processing

Exclude Labels Outside Size Range (CLIJ2, experimental)

Suggested next steps
My favorites

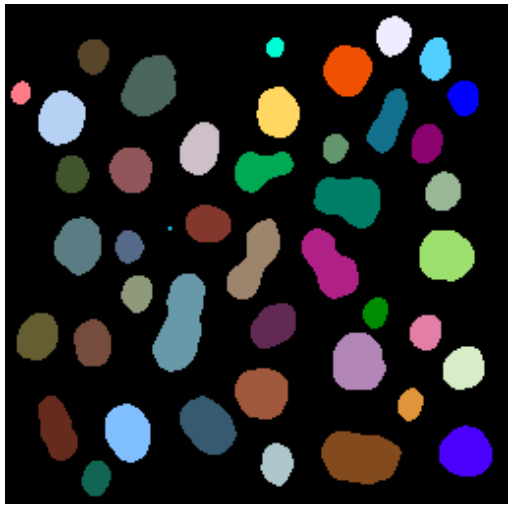
Transform
Binarize
Label processing
Label measurements
Label neighbor filters
Label neighbor graph based measurements
All

Generate script
Info and options

Duplicate and go ahead with ImageJ

Documentation for Exclude Labels Outside Size Range (CLIJ2)
Online help for Exclude Labels Outside Size Range (CLIJ2)

Exclude Label On Edges



Exclude Label Outside Size Range

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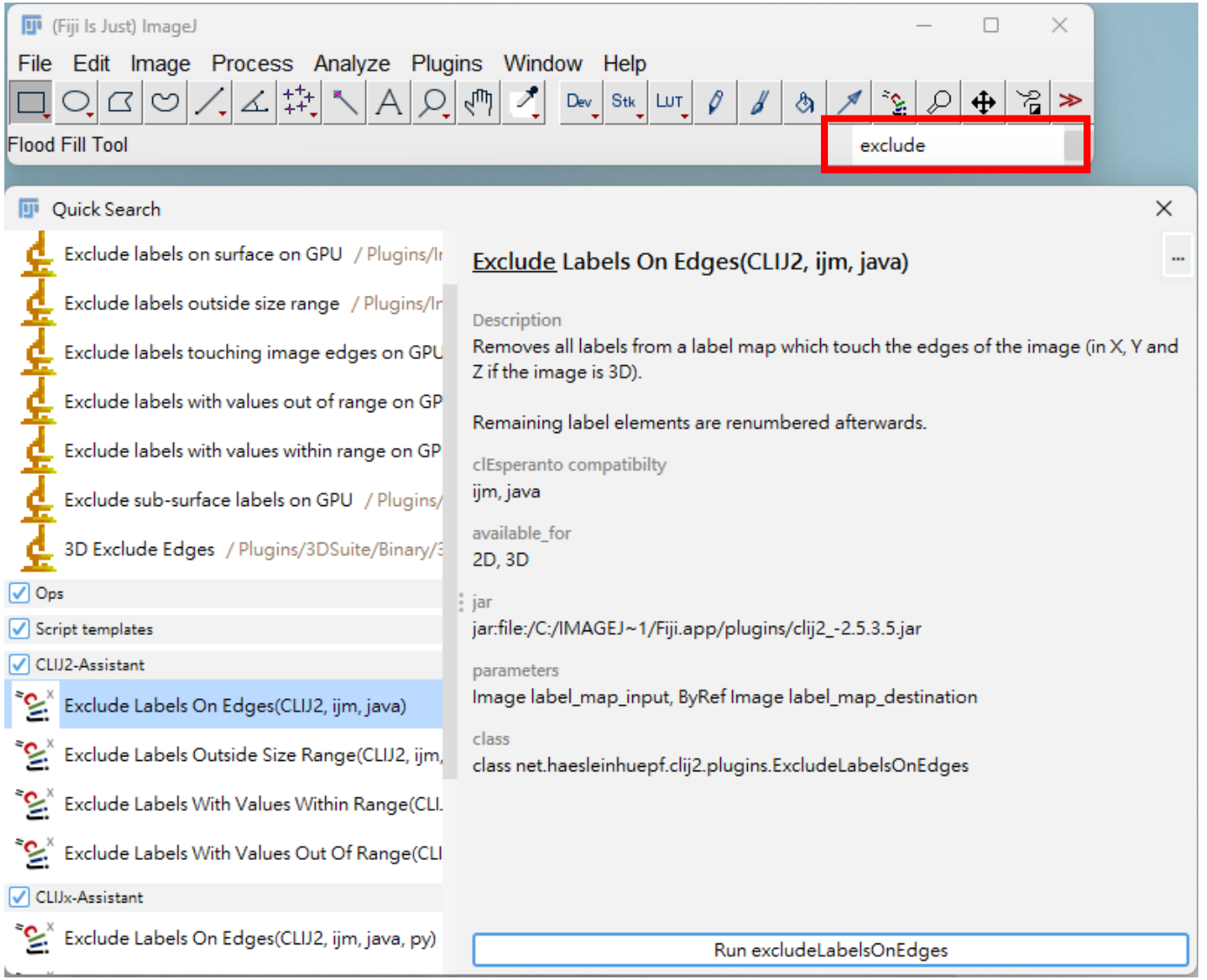
If you start from a mask or label image -> Use search bar

AI-based segmentation tools

CLIJ2-Assistant
CLIJx-Assistant



Label measurements (-> parametric image)

Exclude Labels Outside Size Range (CLIJ2, experimental)

Suggested next steps
My favorites

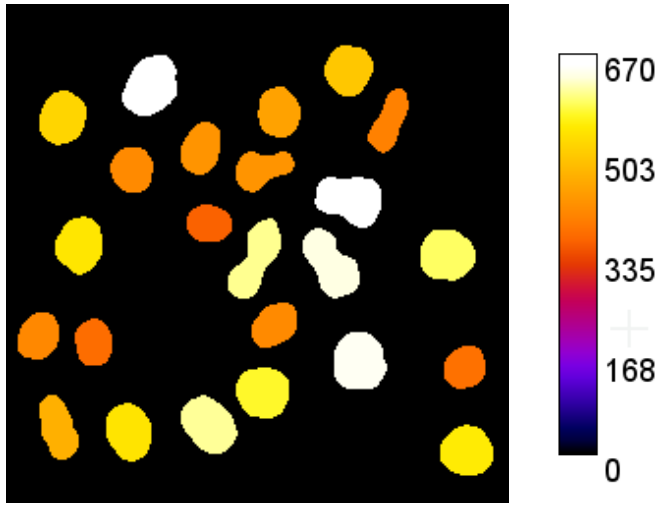
Transform
Binarize
Label processing
Label measurements
Label neighbor filters
Label neighbor graph based measurements
All

Generate script
Info and options

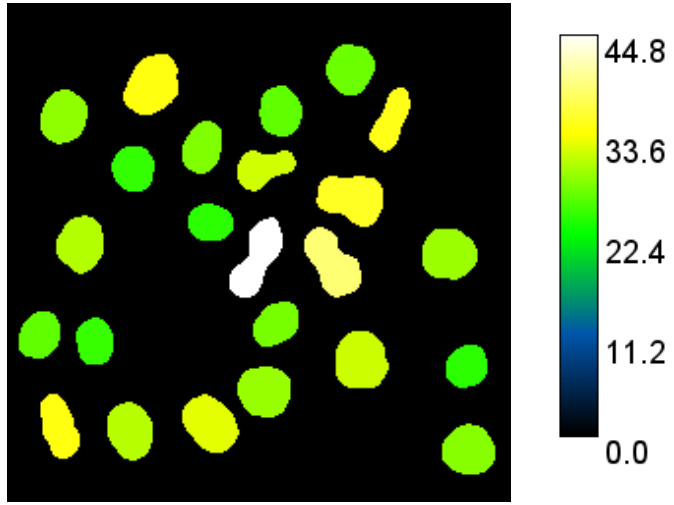
Duplicate and go ahead with ImageJ

Documentation for Exclude Labels Outside Size Range (CLIJ2)
Online help for Exclude Labels Outside Size Range (CLIJ2)

Pixel Count Map

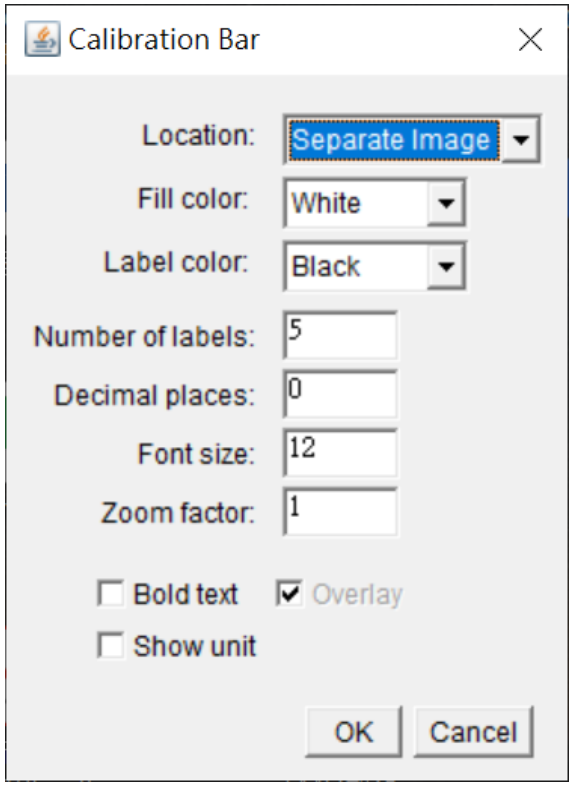


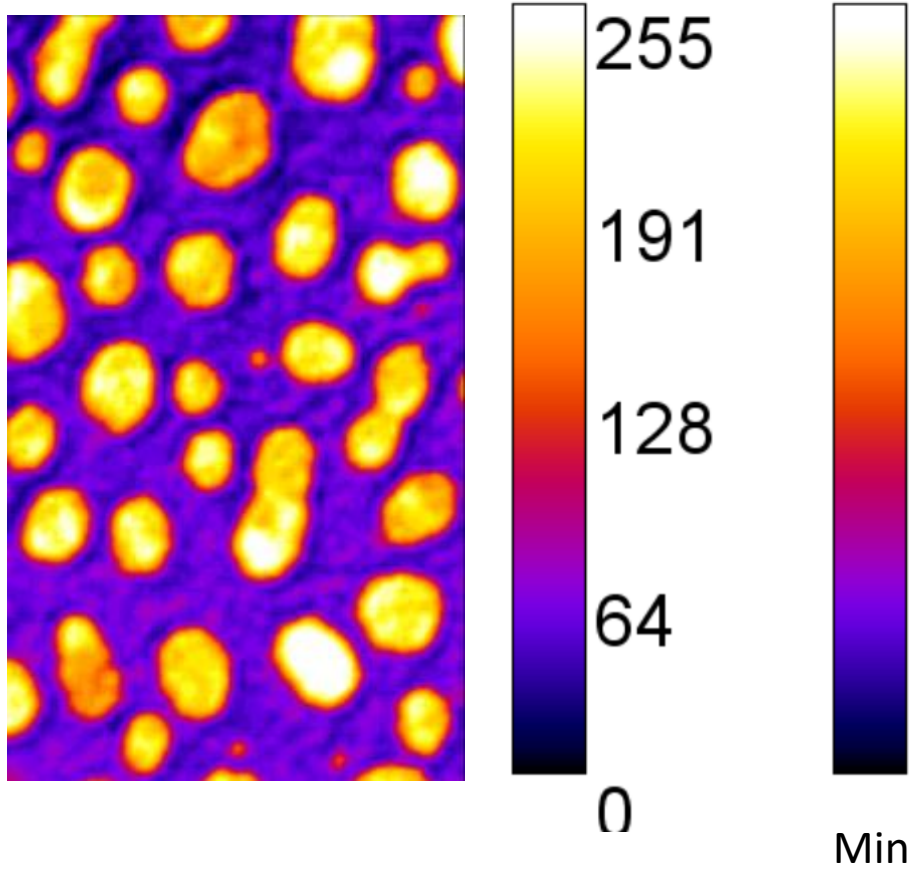
Max. Feret Map



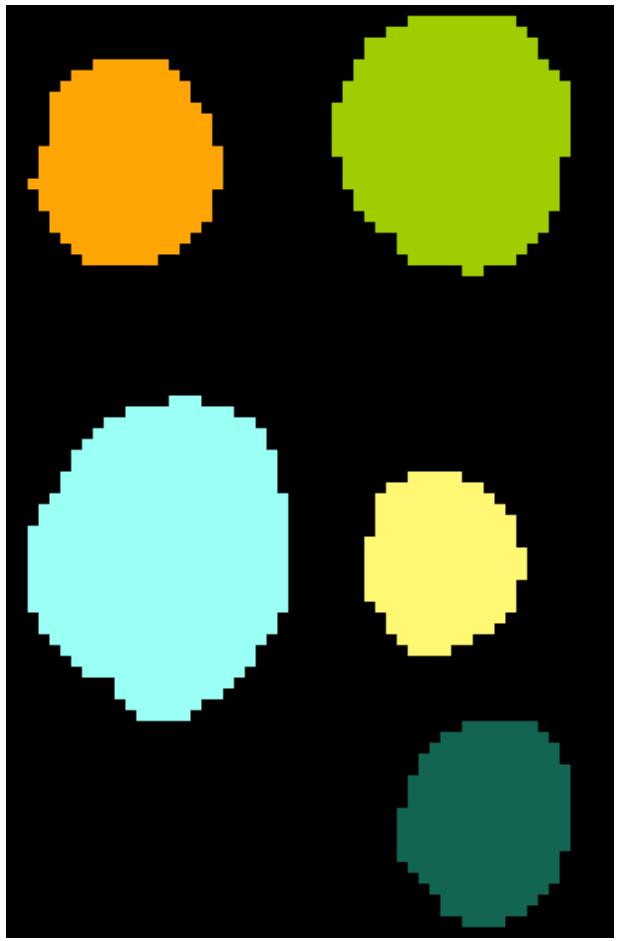
Calibration Bar (Color scale)

- **Analysis -> Tools -> Calibration Bar**
- Only work for single channel image
- Brightness and Contrast setting affect the Calibration Bar!





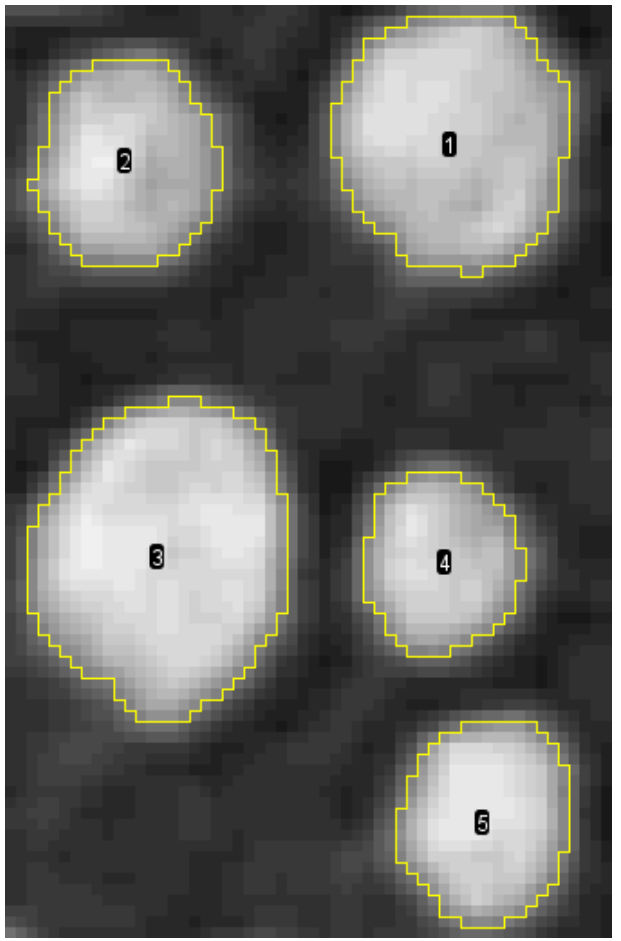
Measurements with labels



Labels

Labels to ROI

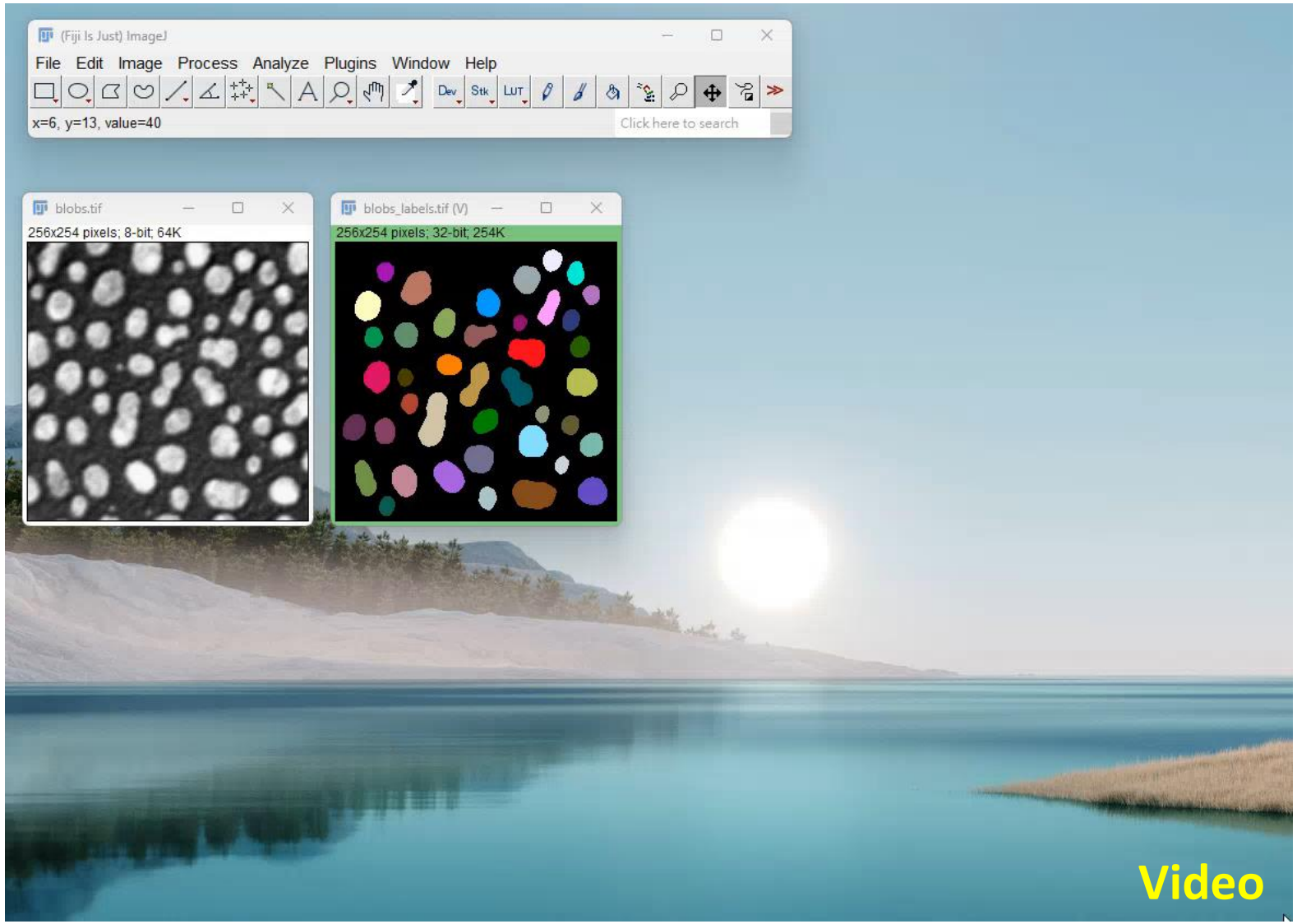
- pullLabelsToROIManager (CLIJ2)
- Label to ROI (BIOP/ijp-LaRoMe)
- **BioVoxxel 3D box**
  - **Labels to 2D Roi Manager**
  - Labels to 3D Roi Manager



ROIs

ImageJ-specific

Label to ROI and measurement



Plugins -> BioVoxxel 3D box
-> Labels to 2D Roi Manager

https://biovoxxel.github.io/bv3dbox/

BioVoxxel 3D Box - GPU-accellerated 2D and 3D Image Processing and Analysis - YouTube

Parametric image and label processing

Image Label Map Input

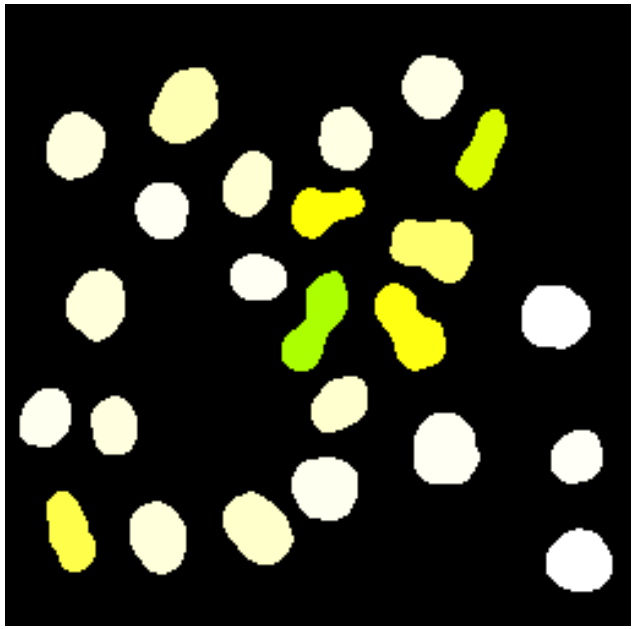
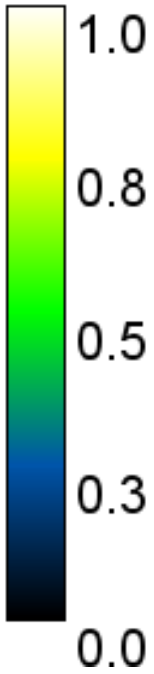


Image Value Vector



Circularity

Exclude the label with circularity Map with value within 0 ~ 0.88

Exclude Labels with value within/out of range

Min/ Mean/ Max Intensity, Circularity, Feret Diameter, Perimeter, Extension ratio….etc.

Label processing demo

Labels image

↓

Exclude labels on edge

↓

Exclude labels outside size range (Filtered-labels)

↓

Max Feret Map

↓

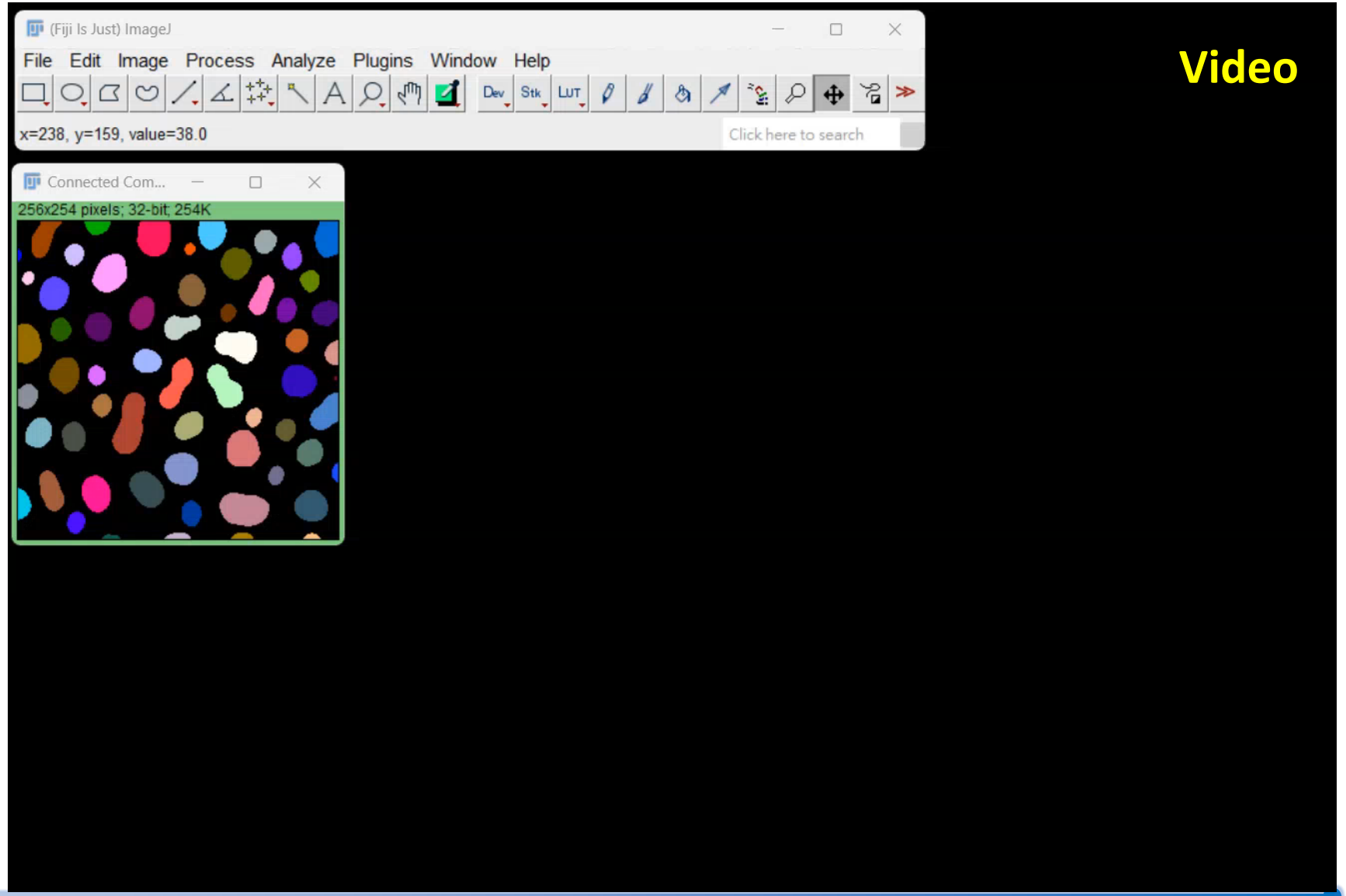
Exclude labels with values outside of range

↓

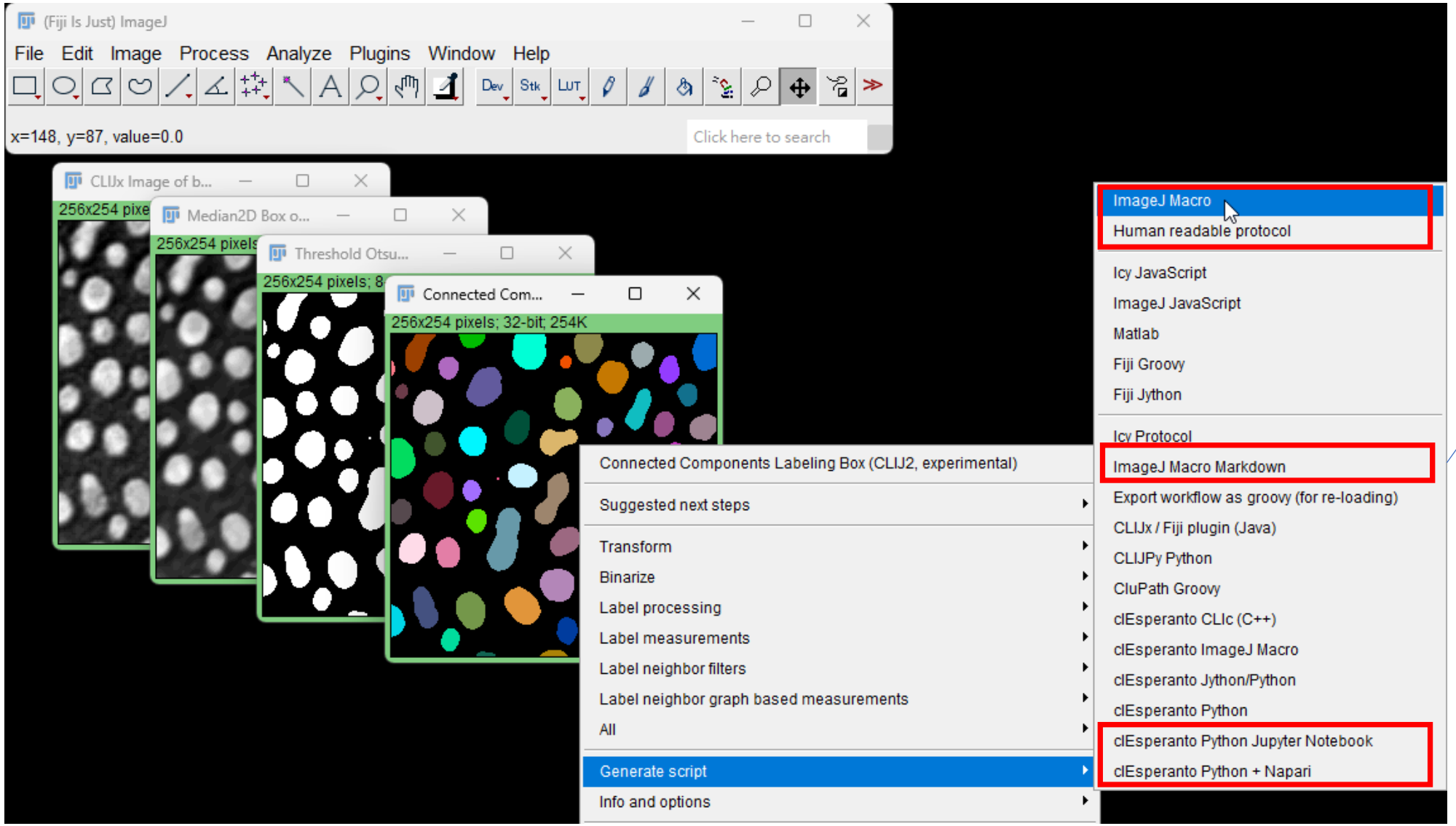
Vector: Max Feret Map
Label: Filtered-labels

↓

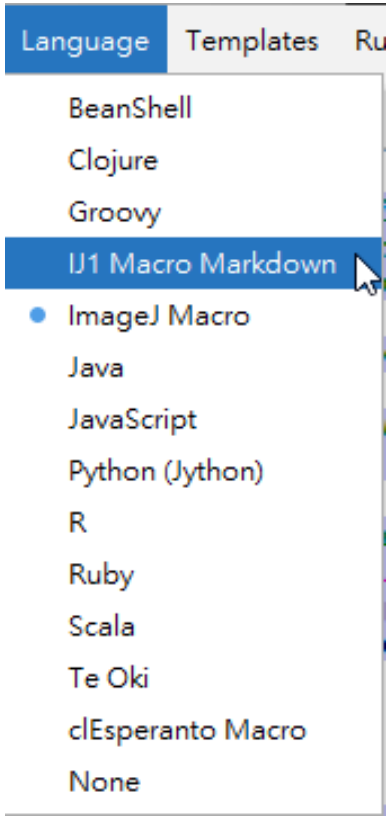
Final filtered-labels



Interactive workflow design and code generation



Fiji Script editor:



CLIJ2: What every ImageJ Macro script must have

Load data

Initialize GPU

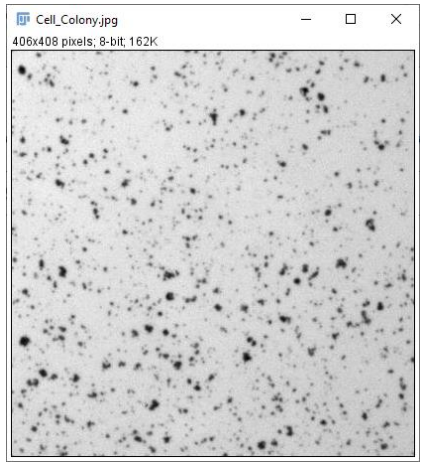
Push

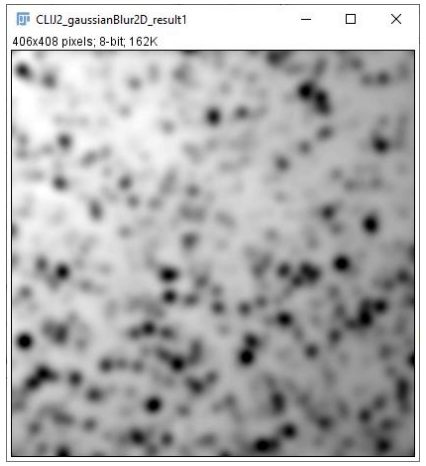
Process images

Pull

Cleanup

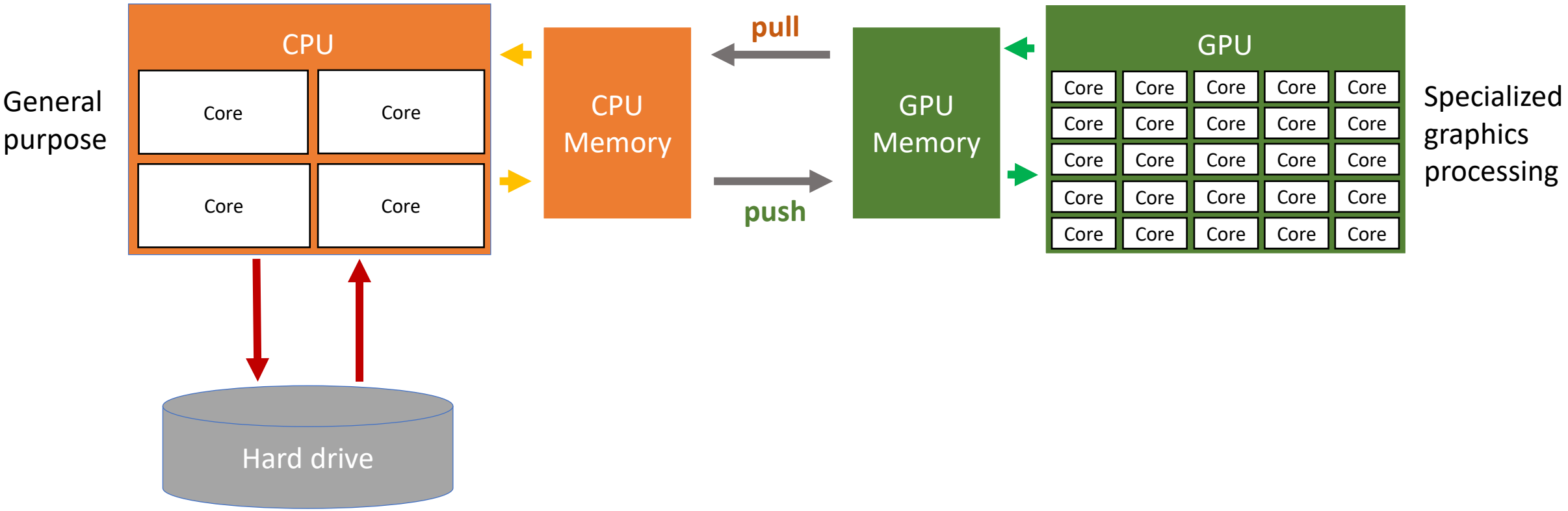
```
// load data
run("Cell Colony (31K)");

// initialize GPU
run("CLIJ2 Macro Extensions", "cl_device=");
Ext.CLIJ2_clear();

// push image to GPU
input_image = getTitle();
Ext.CLIJ2_push(input_image);

// process image
sigma = 5;
Ext.CLIJ2_gaussianBlur2D(input_image, result_image, sigma, sigma);

// optional: release input data
Ext.CLIJ2_release(input_image);

// pull result back from GPU
Ext.CLIJ2_pull(result_image);

// clean up by the end
Ext.CLIJ2_clear();
```



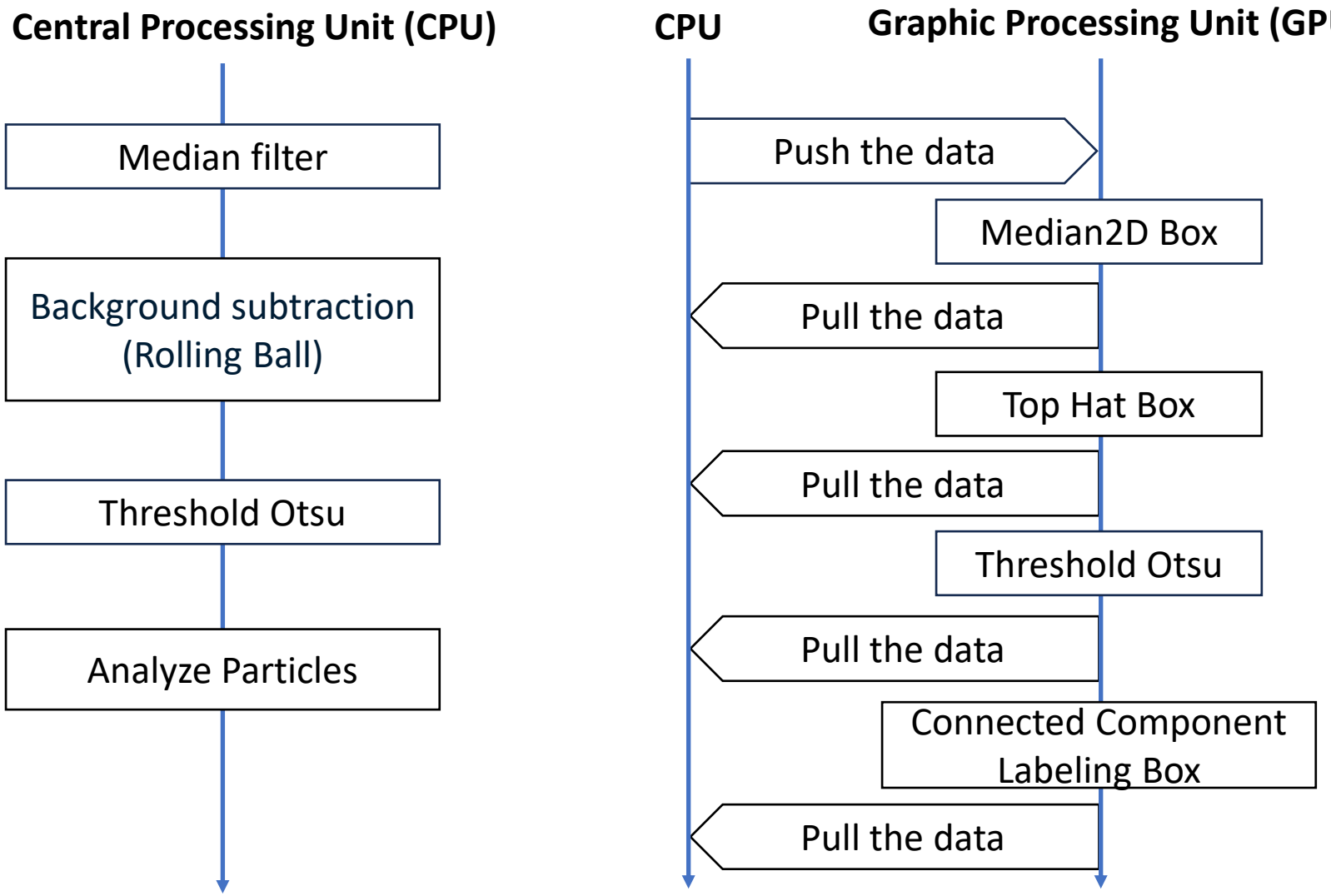


GPUs allow real-time image processing

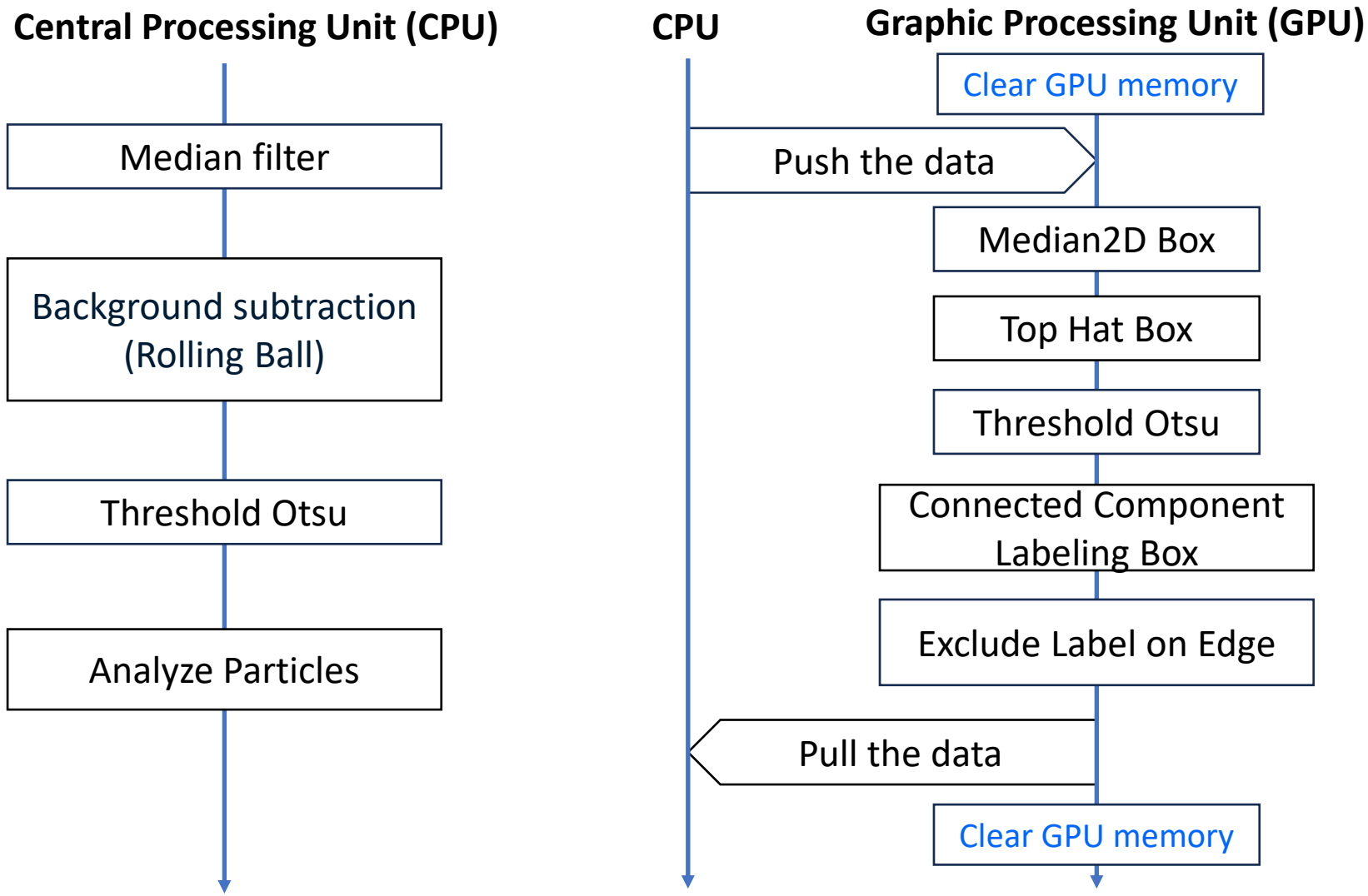
- GPUs are specialised in processing vectors and matrices – super fast



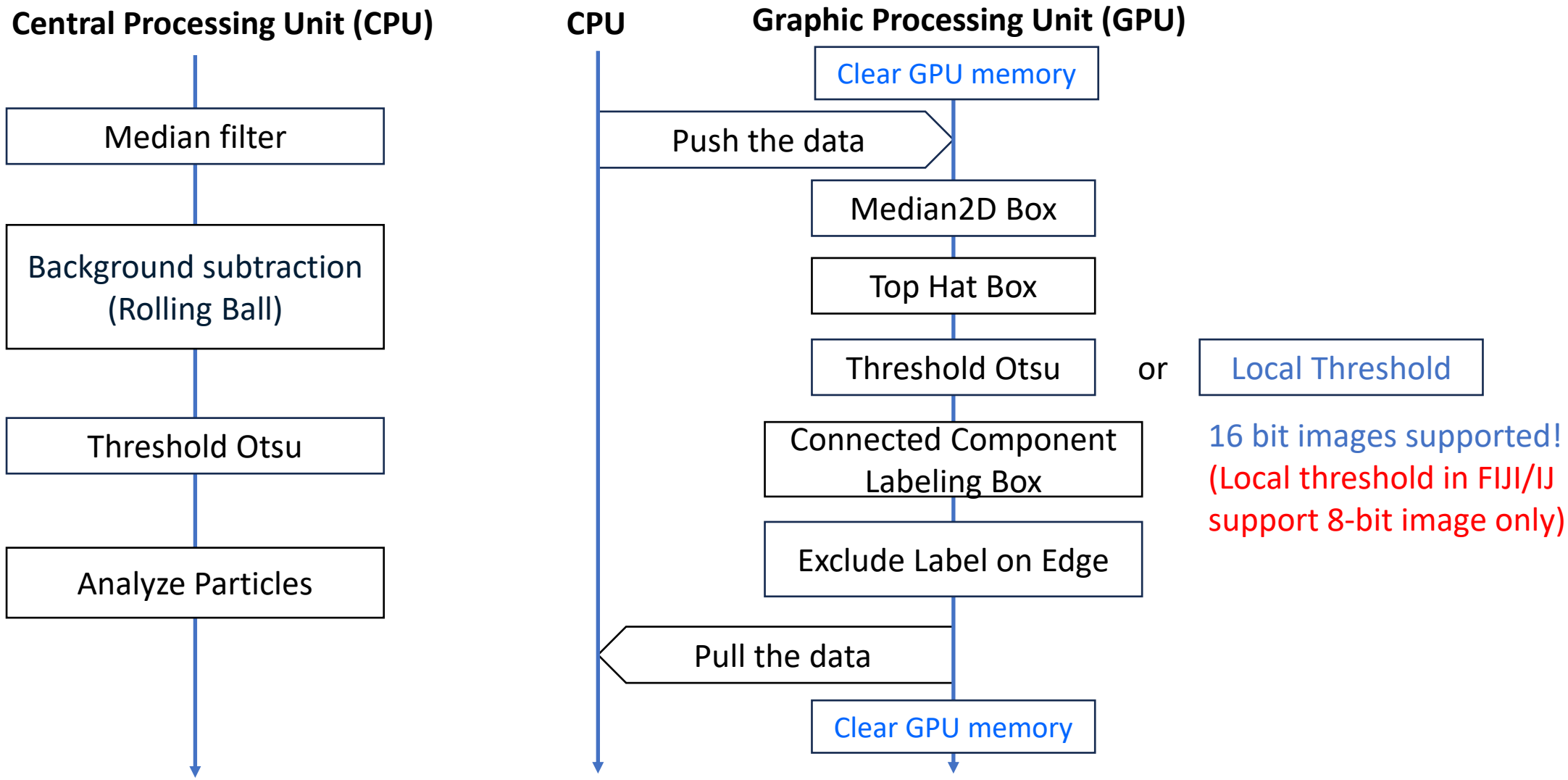
Workflow build (draft by CLIJ-assistant)



Workflow build (improve speed)



Workflow build (improve speed and save the GPU memory)



CLIJ2: Macro refine

```
1  // To make this script run in Fiji, please activate
2  // the clij and clij2 update sites in your Fiji
3  // installation. Read more: https://clij.github.io
4
5  // Generator version: 2.5.1.6
6
7  // Init GPU
8  run("CLIJ2 Macro Extensions", "cl_device=");
9
10 // Load image from disc
11 open("C:/Users/weich/AppData/Local/Temp/temp1740905840354.tif");
12 image_1 = getTitle();
13 Ext.CLIJ2_pushCurrentZStack(image_1);
14
15 // Copy
16 Ext.CLIJ2_copy(image_1, image_2);
17 Ext.CLIJ2_release(image_1);
18
19 Ext.CLIJ2_pull(image_2);
20
21 // Median2D Box
22 radiusX = 2;
23 radiusY = 2;
24 Ext.CLIJ2_median2DBox(image_2, image_3, radiusX, radiusY);
25 Ext.CLIJ2_release(image_2);
26
27 Ext.CLIJ2_pull(image_3);
```

```
1  // To make this script run in Fiji, please activate
2  // the clij and clij2 update sites in your Fiji
3  // installation. Read more: https://clij.github.io
4
5  // Generator version: 2.5.1.6
6
7  // Init GPU
8  run("CLIJ2 Macro Extensions", "cl_device=");
9  Ext.CLIJ2_clear();
10 // Load image from disc
11 //open("C:/Users/weich/AppData/Local/Temp/temp1740905840354.tif");
12 image_1 = getTitle();
13 Ext.CLIJ2_pushCurrentZStack(image_1);
14
15 // Copy
16 Ext.CLIJ2_copy(image_1, image_2);
17 Ext.CLIJ2_release(image_1);
18
19 //Ext.CLIJ2_pull(image_2);
20
21 // Median2D Box
22 radiusX = 2;
23 radiusY = 2;
24 Ext.CLIJ2_median2DBox(image_2, image_3, radiusX, radiusY);
25 Ext.CLIJ2_release(image_2);
26
27 //Ext.CLIJ2_pull(image_3);
```

Remove unnecessary open image

Remove unnecessary pull() calls by the end

⋮

Ext.CLIJ2_clear();
(Clear the GPU memory)

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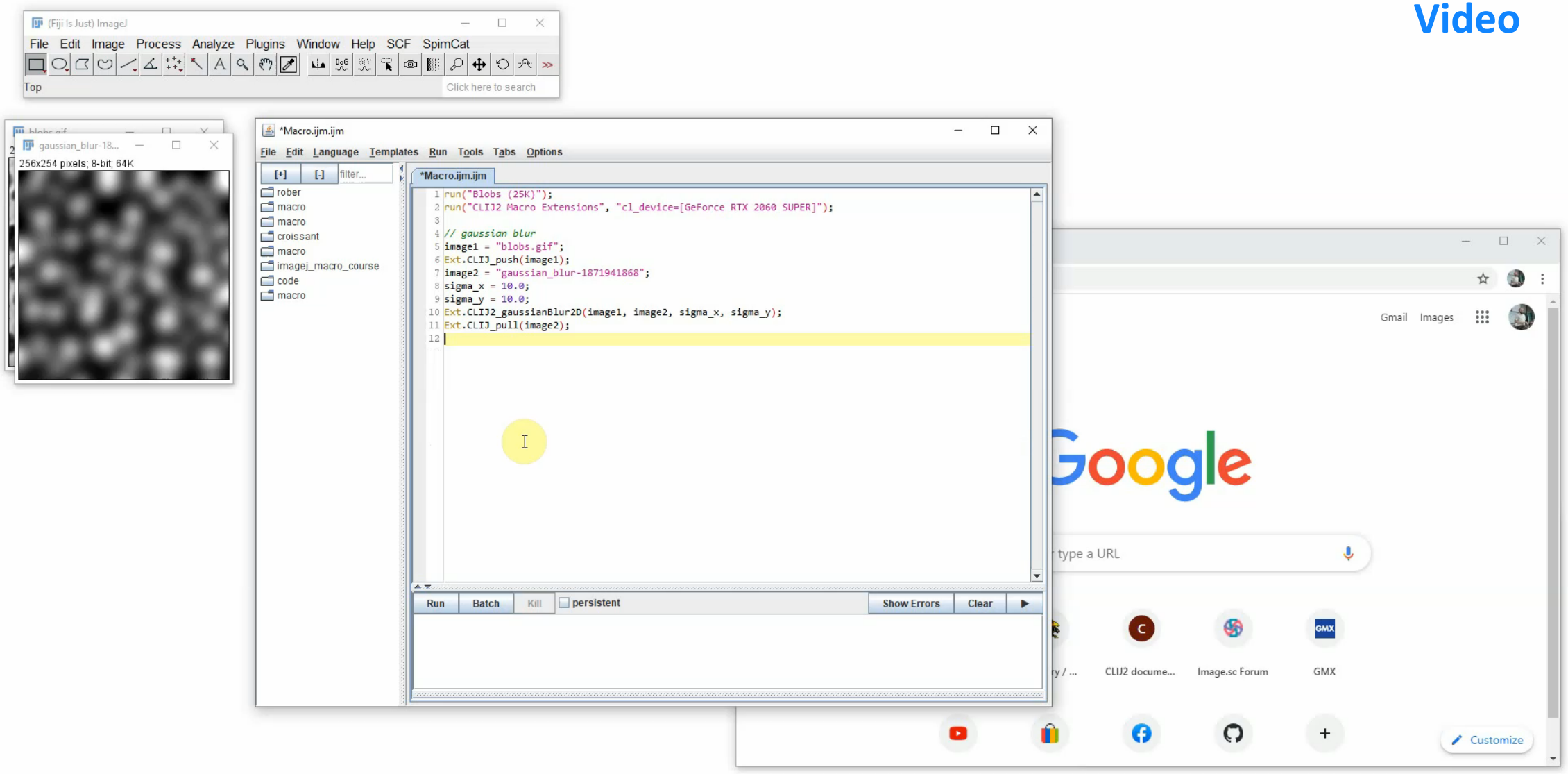
CLIJ2: Macro editing

Video



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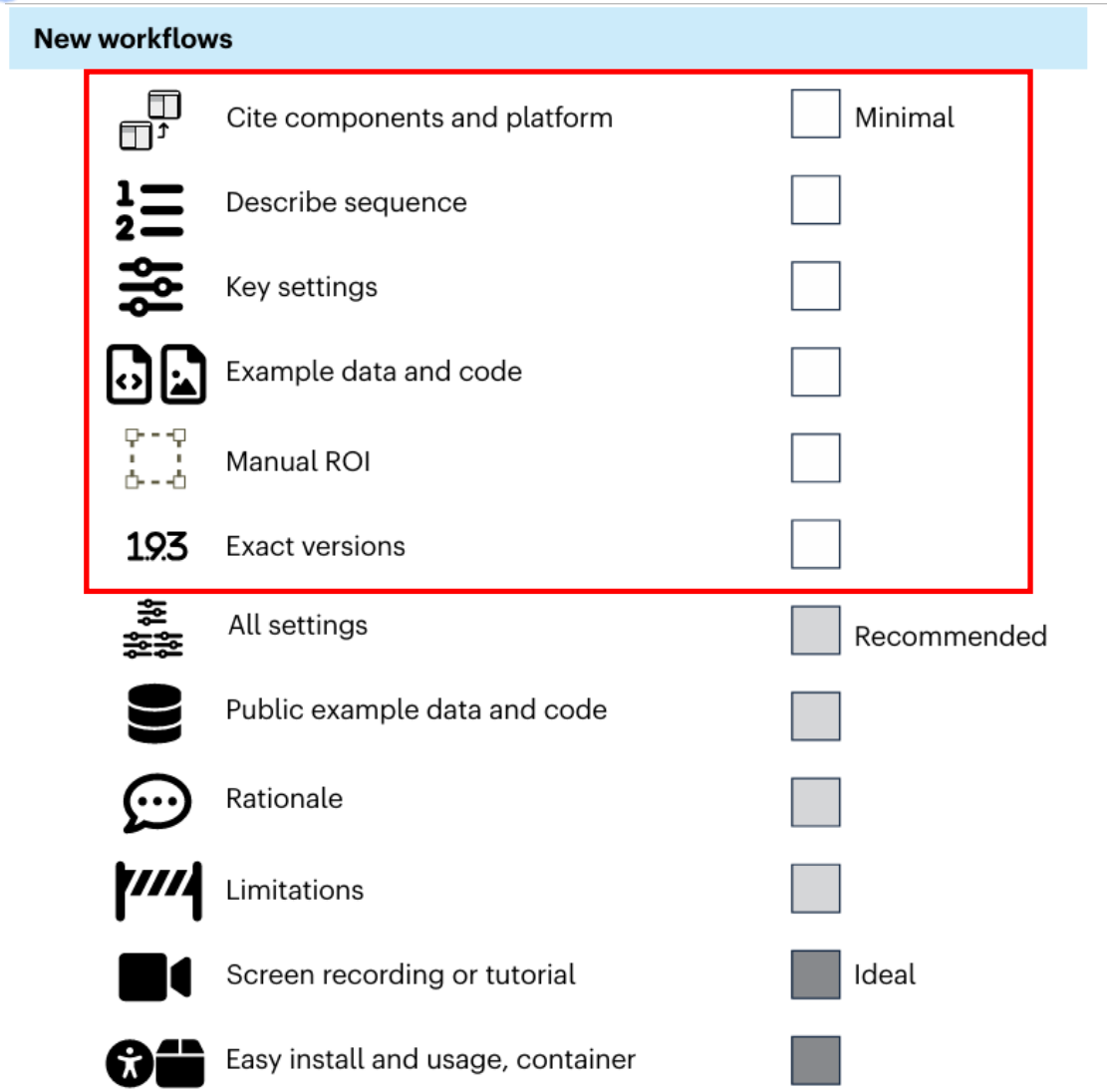
Community-developed checklists for publishing images and image analysis



FIJI

Schindelin, J., Arganda-Carreras, I., Frise, E. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676–682 (2012). https://doi.org/10.1038/nmeth.2019

CLIJ

•Robert Haase, Loic Alain Royer, Peter Steinbach, Deborah Schmidt, Alexandr Dibrov, Uwe Schmidt, Martin Weigert, Nicola Maghelli, Pavel Tomancak, Florian Jug, Eugene W Myers. *CLIJ: GPU-accelerated image processing for everyone*. Nat Methods 17, 5-6 (2020) doi:10.1038/s41592-019-0650-1

•Daniela Vorkel, Robert Haase. *GPU-accelerating ImageJ Macro image processing workflows using CLIJ*. arXiv preprint

•Robert Haase, Akanksha Jain, Stéphane Rigaud, Daniela Vorkel, Pradeep Rajasekhar, Theresa Suckert, Talley J. Lambert, Juan Nunez-Iglesias, Daniel P. Poole, Pavel Tomancak, Eugene W. Myers. *Interactive design of GPU-accelerated Image Data Flow Graphs and cross-platform deployment using multi-lingual code generation*. bioRxiv preprint

BioVoxxel 3D box

Jan Brocher. (2024). biovoxxel/bv3dbox: BioVoxxel 3D Box - v1.22.3 (bv3dbox-1.22.3). Zenodo. https://doi.org/10.5281/zenodo.12571844

Schmied et al (2024) Nature Methods 21, p170–181

Why you should learn CLIJ

- Interactive workflows
- Automatic code generation (multiple languages)
- Easy data visualization
- Comprehensive documentation
- GPU acceleration
- Advanced image processing libraries
- Can be adapted to Python and Napari (with clEsperanto)

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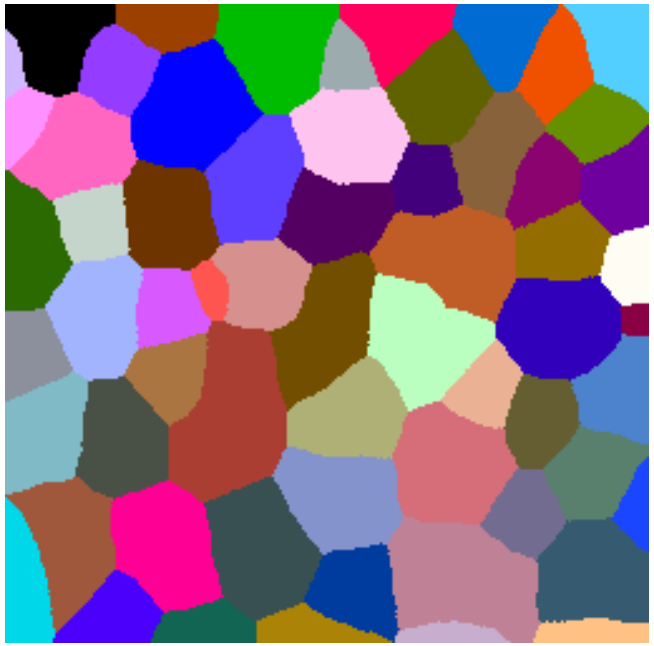
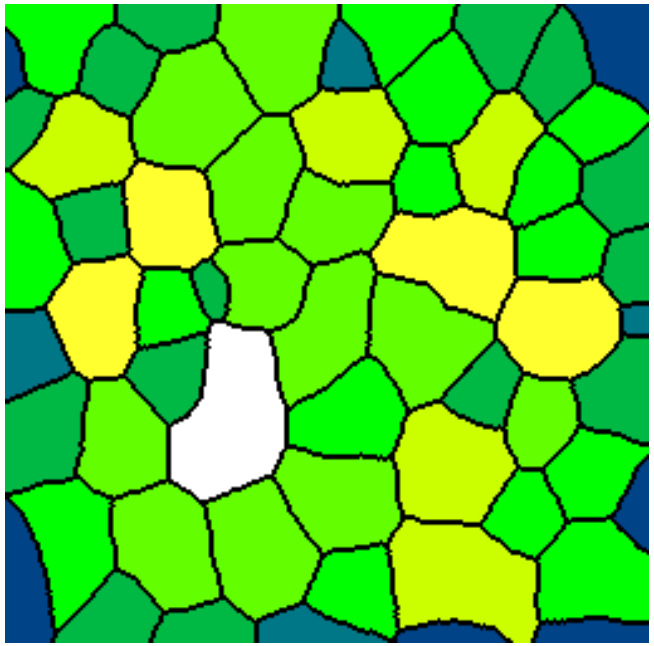
Advanced image processing libraries

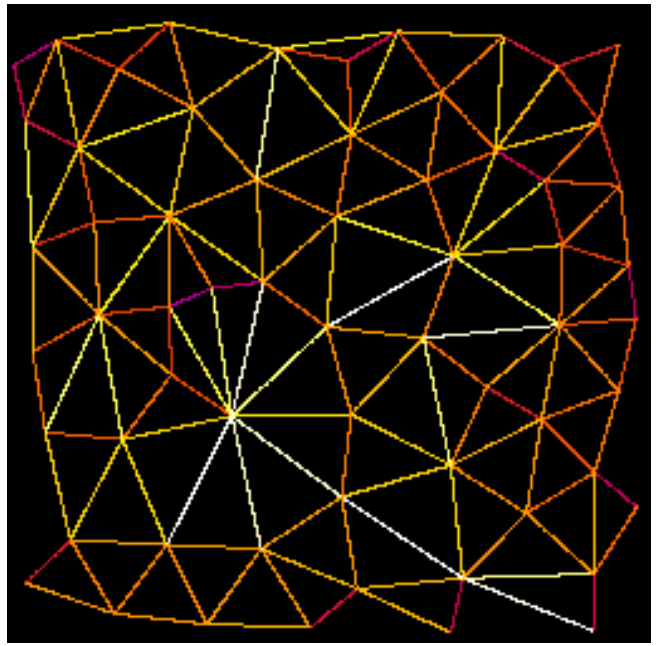
CLIJ2 is particularly effective for studying neighbors



Labels



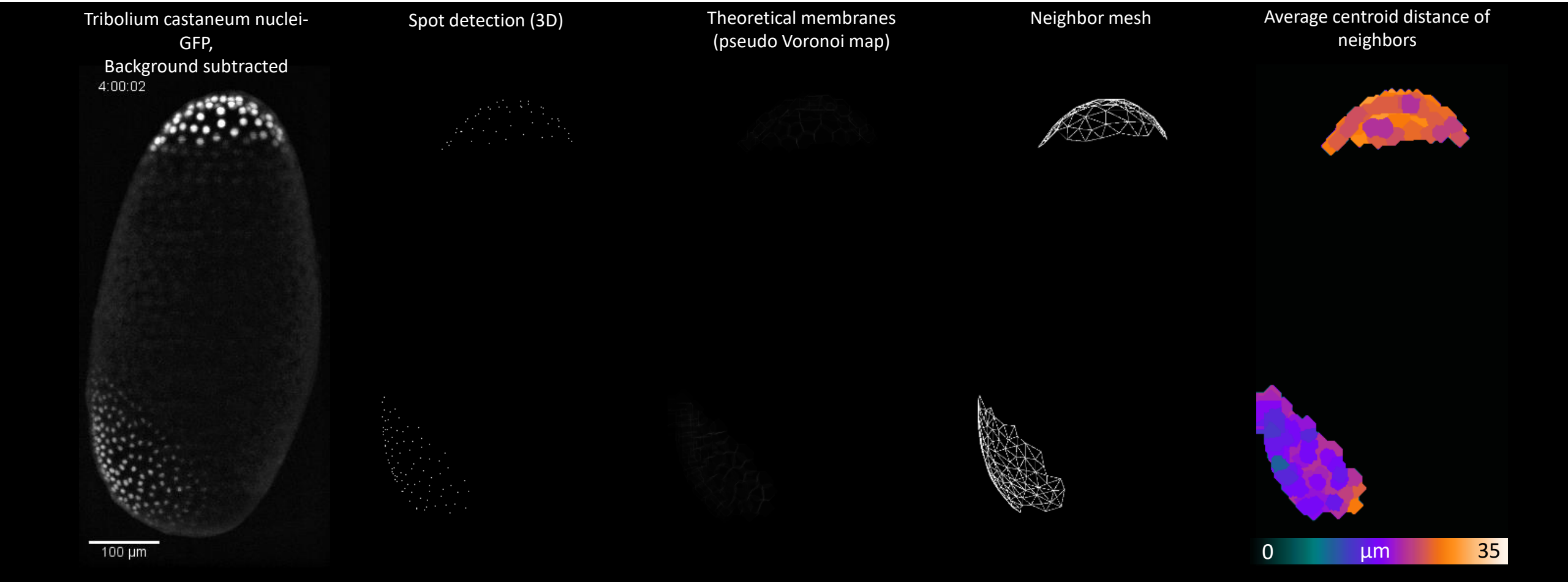
Touching Neighbor count Map



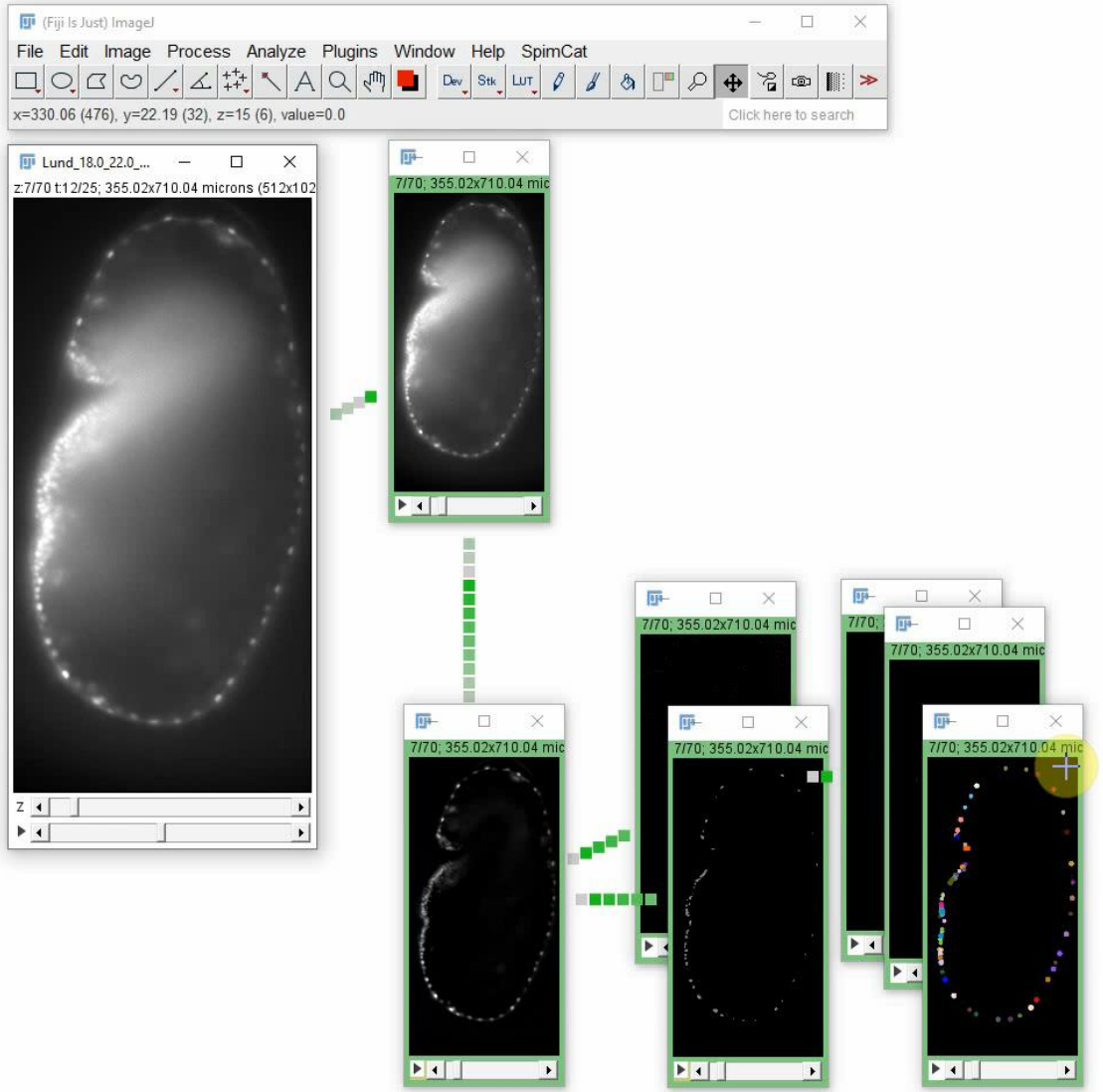
Distance Mesh between Touching Labels

GPU accelerated image processing in life sciences

… to study embryo development



From Fiji/ CLIJ to napari

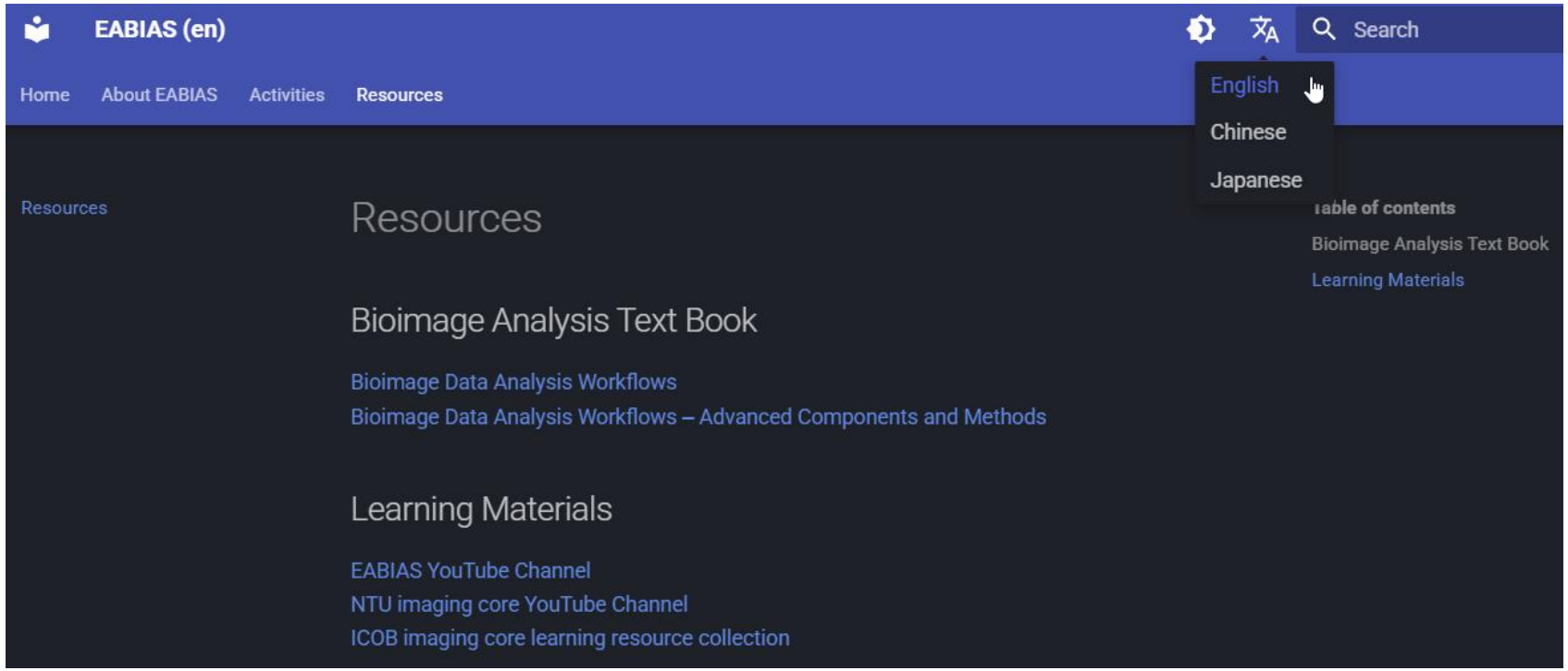


Learn more

- Introduction to Bioimage Analysis by Peter Bankhead
- Lecture BioImage Analysis 2020 by Robert Haase – YouTube, Slides
- Open-Access Training Materials by Robert Haase

- Bioimage Analysis with FIJI/ImageJ and Friends @ICOB (2024, Chinese), Slides
- Open source AI Tools for bioimage analysis workshop @ICOB (2024, Chinese), Slides

- EABIAS: https://eabias.github.io/



Acknowledgements

EABIAS (East Asia Bioimage Analysts' Society)

Dr. Robert Haase

課後意見調查

國立臺灣大學 National Taiwan University

台大醫學院 National Taiwan University College of Medicine

NSTC 國家科學及技術委員會 National Science and Technology Council

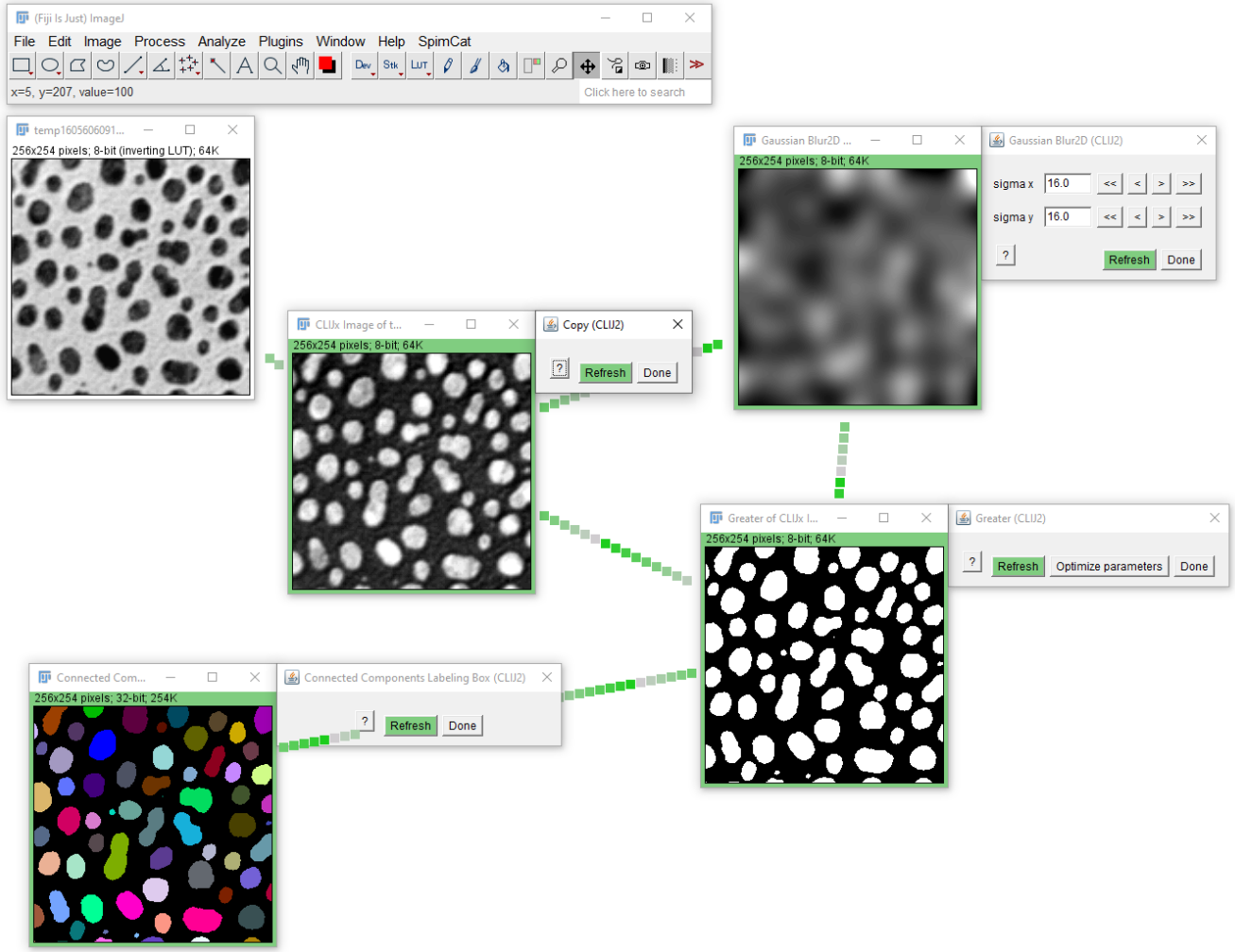
neubias network of european bioimage analysts

GloBIAS Global BioImage Analysts' Society

Excise 1: Segment blobs.gif

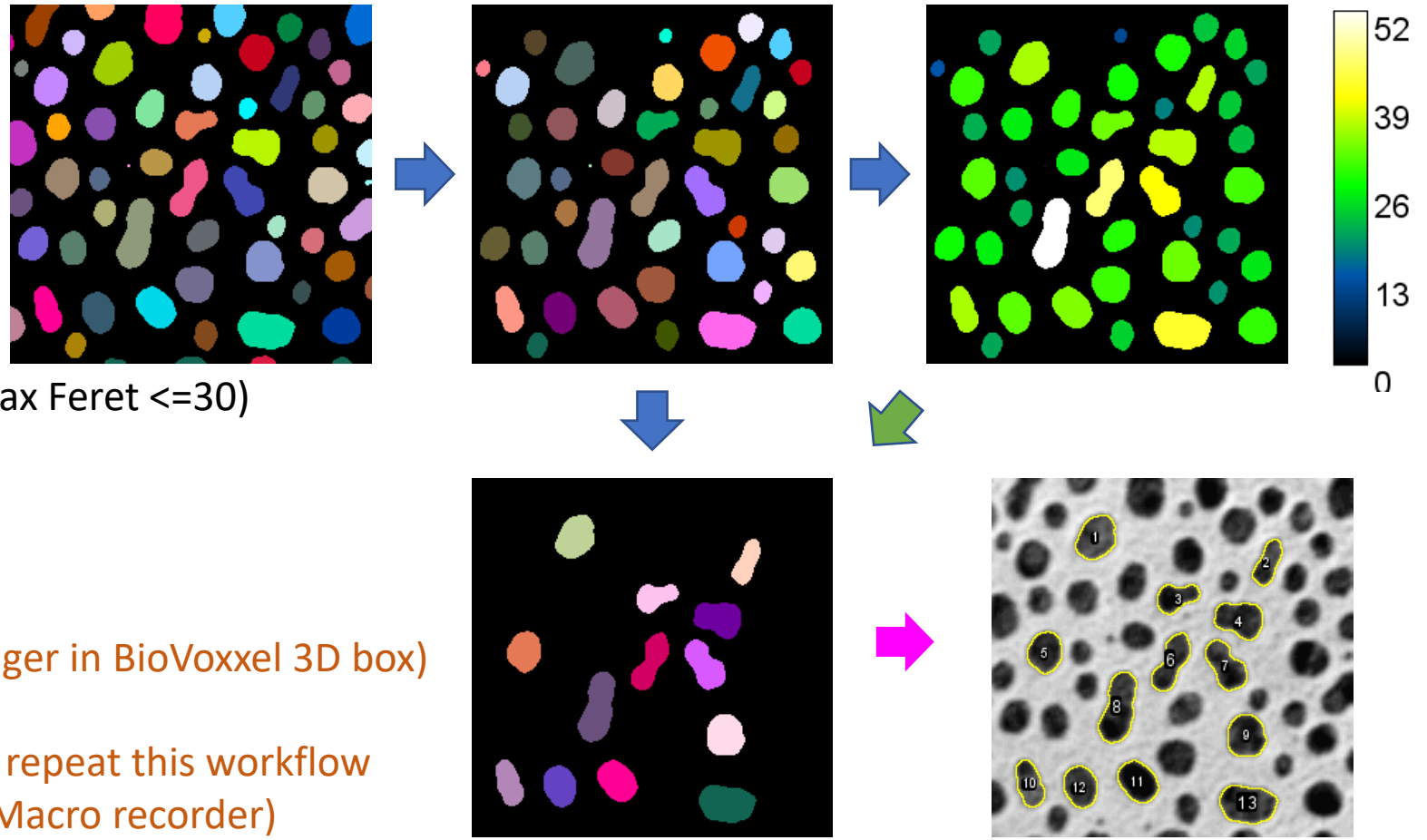
- Design a workflow for segmenting blobs.gif (File > Open Samples…)
- Export the workflow as ImageJ Macro script
- Refine the ImageJ Marco
- Save the final label image

- Optional: Export the workflow as Icy Javascript, as Icy protocol and for QuPath as groovy script. Feel free to generate a Fiji plugin.
- Furthermore, if you only used operations, which are "py" compatbile, export a clEsperanto-based Jupyter notebook and a Python script that uses Napari.



Excise 2: Label processing

- Load the example label image
- Exclude Labels On Edges
  (Hint: using search bar)
- Generate “Maximun Feret Map”
- Exclude the shorter labels (e.g Max Feret <=30)
- Generate an ImageJ Macro
- Convert the final labels to ROIs
  (Hint: using Label to 2d Roi manager in BioVoxxel 3D box)
- Refine the macro to allow you to repeat this workflow
  (Hint: you can also combine the Macro recorder)



Bonus: How to open the raw image files using ImageJ Macro

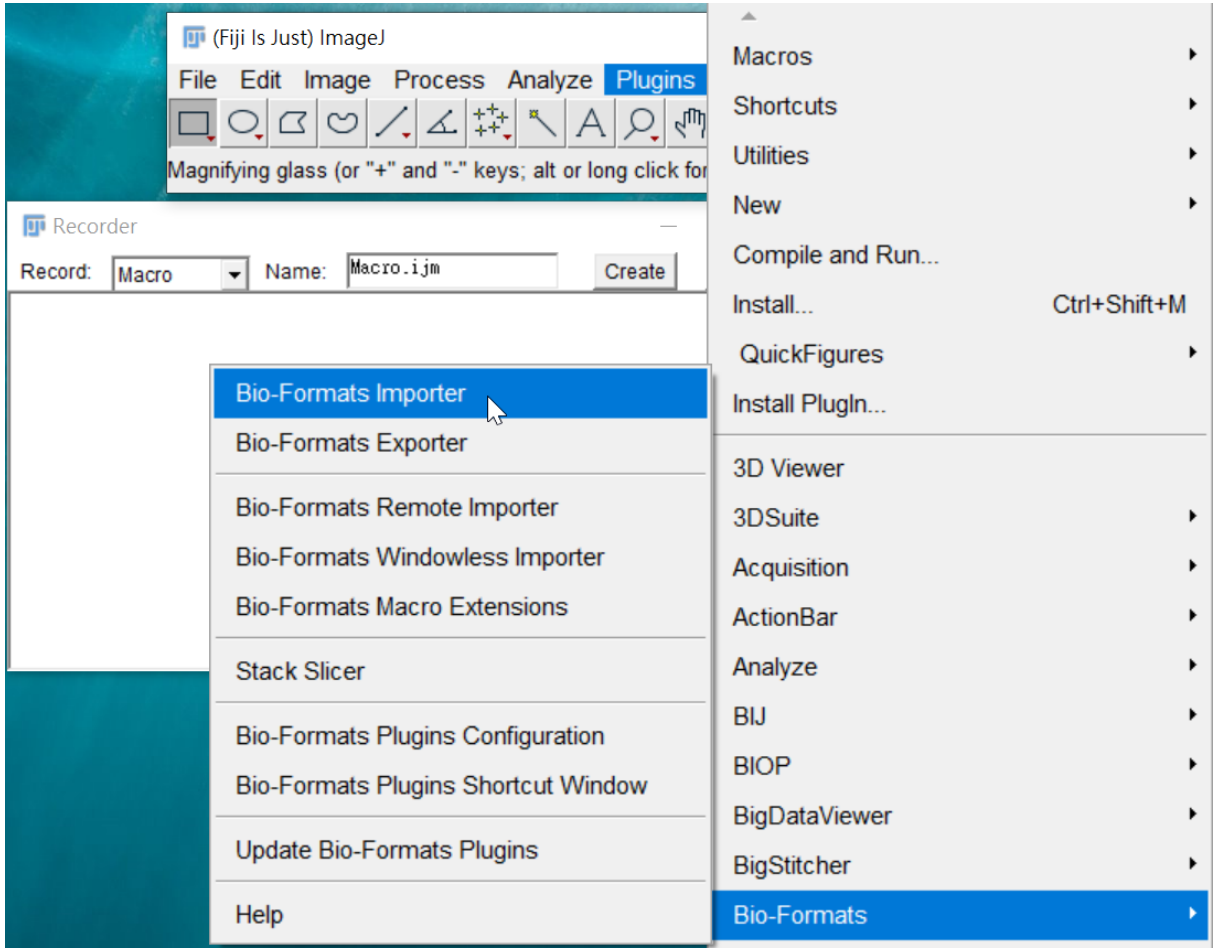
```
open("D:/test.tif");
```

It can be record properly

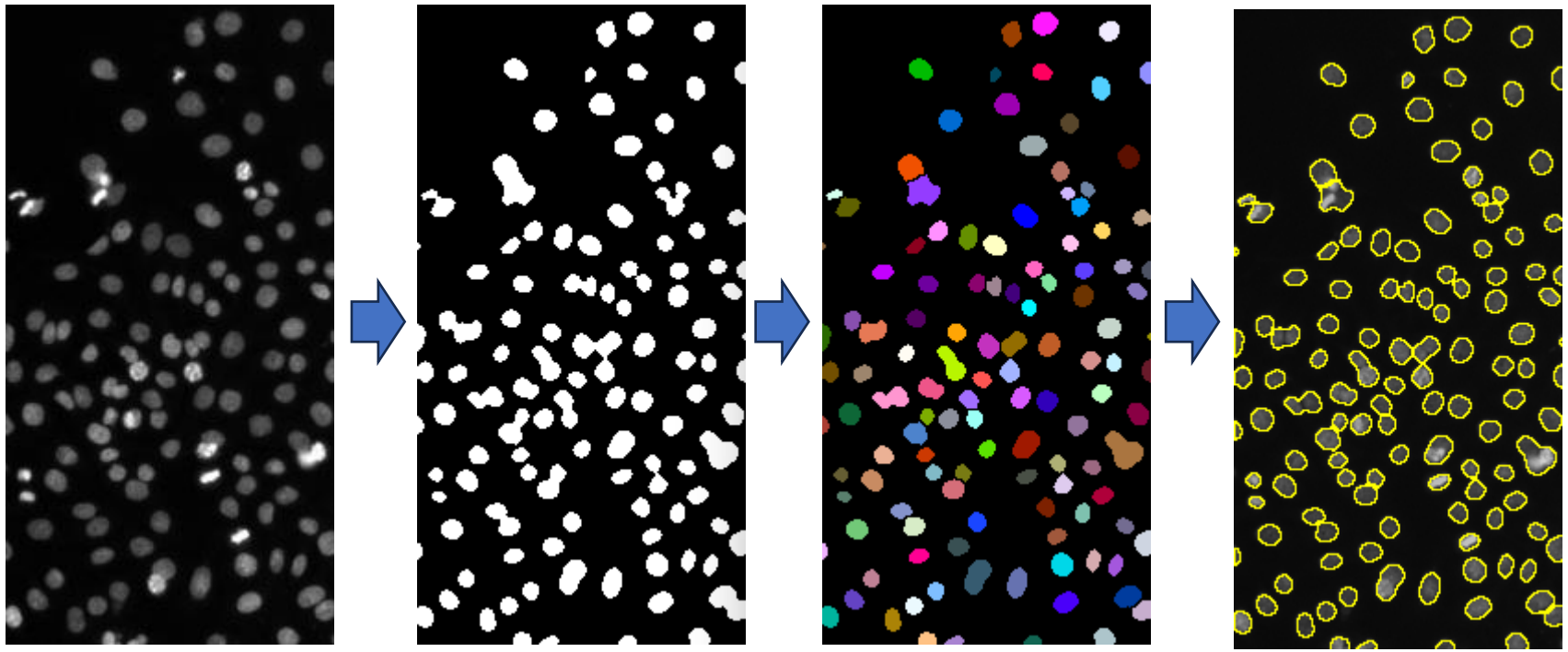
```
open("D:/test.czi");
```

It cannot be recorded properly…

```
run("Bio-Formats Importer", "open=D:/test.czi
color_mode=Composite rois_import=[ROI manager]
view=Hyperstack stack_order=XYCZT");
```



Single channel thresholding issue on intensity measurement



Results

File Edit Font Results

	Label	Area	Mean
1	Mitosis_Crop.tif:1	139	57.676
2	Mitosis_Crop.tif:2	112	61.938
3	Mitosis_Crop.tif:3	97	52.124
4	Mitosis_Crop.tif:4	116	64.293
5	Mitosis_Crop.tif:5	60	51.583
6	Mitosis_Crop.tif:6	78	67.256
7	Mitosis_Crop.tif:7	36	94.111
8	Mitosis_Crop.tif:8	105	64.305
9	Mitosis_Crop.tif:9	136	54.338

- The segmented area is influenced by intensity variations.
  How can we accurately quantify "intensity" in this context?
- To ensure precise measurement of the target signal's intensity, the region of interest (ROI) should be segmented based on a **separate channel**.

Ref1: https://doi.org/10.15252/embj.2020105889
Ref2: Kota's talk in FocalPlane features... reproducibility in imaging

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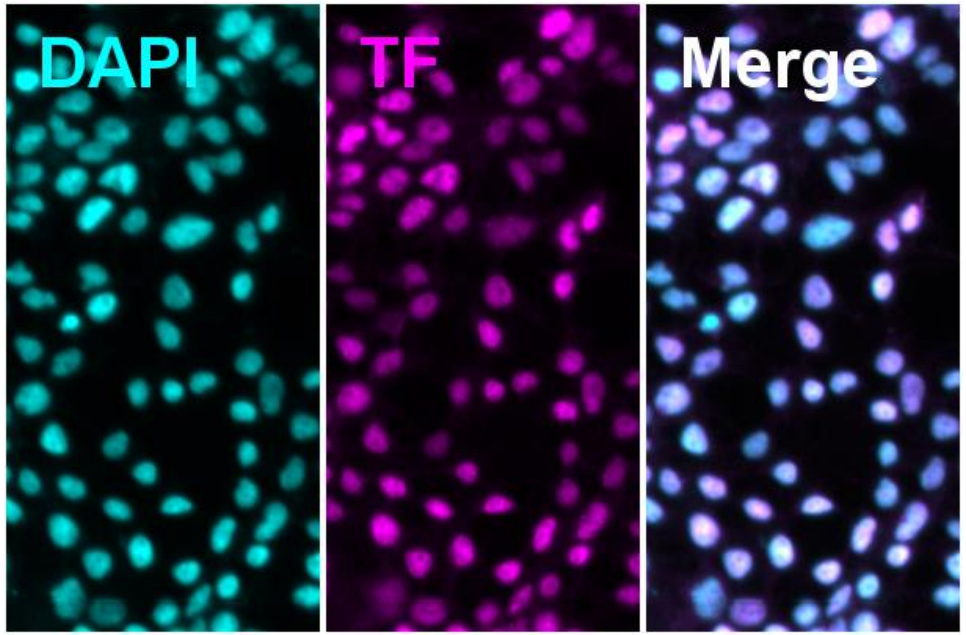
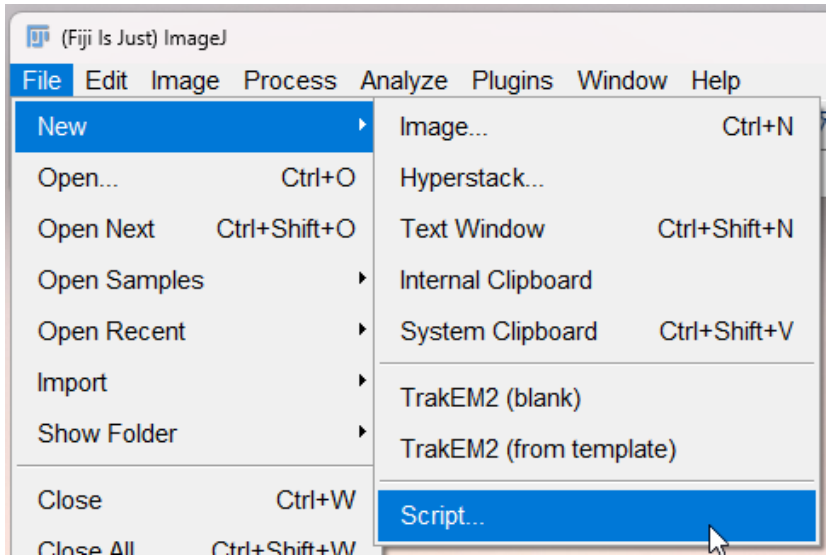
Exercise 3: Full Workflow and Batch Processing





Goal :

- Measure the area and transcription factor (TF) signal intensity based on DAPI segmentation.
- Export an ImageJ macro using CLIJ, then refine it to ensure reusability across different image files.
- Integrate your code into batch processing templates.
- Hint: Split channels first! You can also use the Macro Recorder and refer to the ImageJ Cheat Sheet.

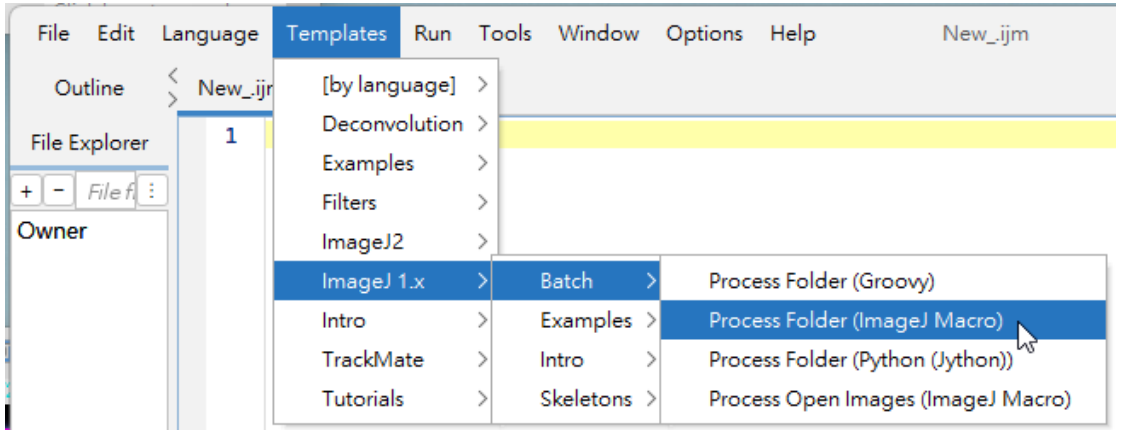


Image credit: Rekha Rey (Dr. Shen-Ju Chou Lab, ICOB, Academia Sinica)

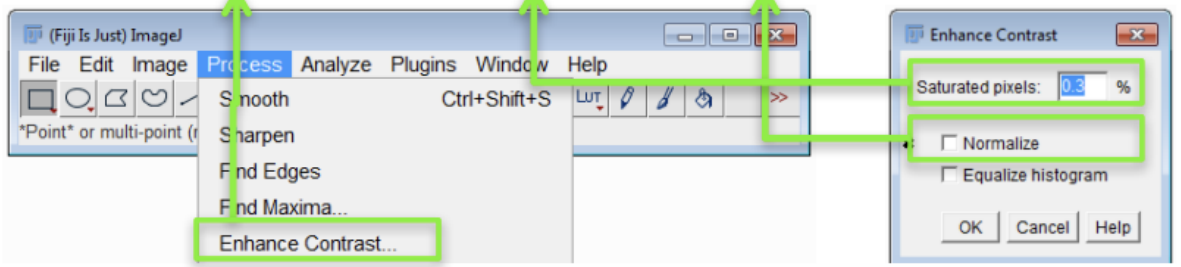
ImageJ macro cheat sheet

Macro language elements

```
// comments for code documentation
numericVariable = 5;
stringVariable = "text value";
builtInCommand();
```

Calling any ImageJ/FIJI menu

```
run("Enhance Contrast...", "saturated=0.3 normalize")
```



Switch between image windows

```
titleOfCurrentImage = getTitle();
selectWindow(titleOfAnyImage);
```

Navigation in image stacks

```
Stack.getDimensions(width, height,
channels, slices, frames);
Stack.setSlice(slice);
Stack.setChannel(channel);
Stack.setFrame(frame);
Stack.setDisplayMode("color");
Stack.setDisplayMode("composite");
Stack.setDisplayMode("grayscale");
```

Handle image files and folders

```
open(folder+imageFilename);
close();
fileList = getFileList(folder);
numFiles = lengthOf(fileList);
for (i=0;i<lengthOf(fileList);i++){
    file = fileList[i];
    open(file);
    // actual image processing...
    close();
}
```

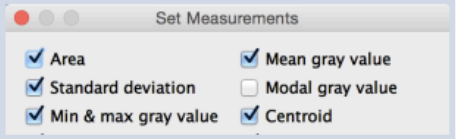
Reading image calibration

```
getPixelSize(unit, pWidth, pHeight);
getVoxelSize(vWidth, vHeight,
vDepth, unit);
```

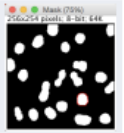
Result tables

```
run("Set Measurements...", "area
mean standard min centroid");
```

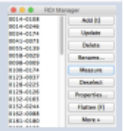
corresp. to this:




```
run("Analyze Particles...",
"add clear display");
```



```
roiManager("Measure");
```



```
rowCount = nResults();
value = getResult("column title",
                  rowNumber);
setResult("column title",
rowNumber, newValue);
saveAs("Results","myResults.xls");
run("Clear results");
```

ROI manager

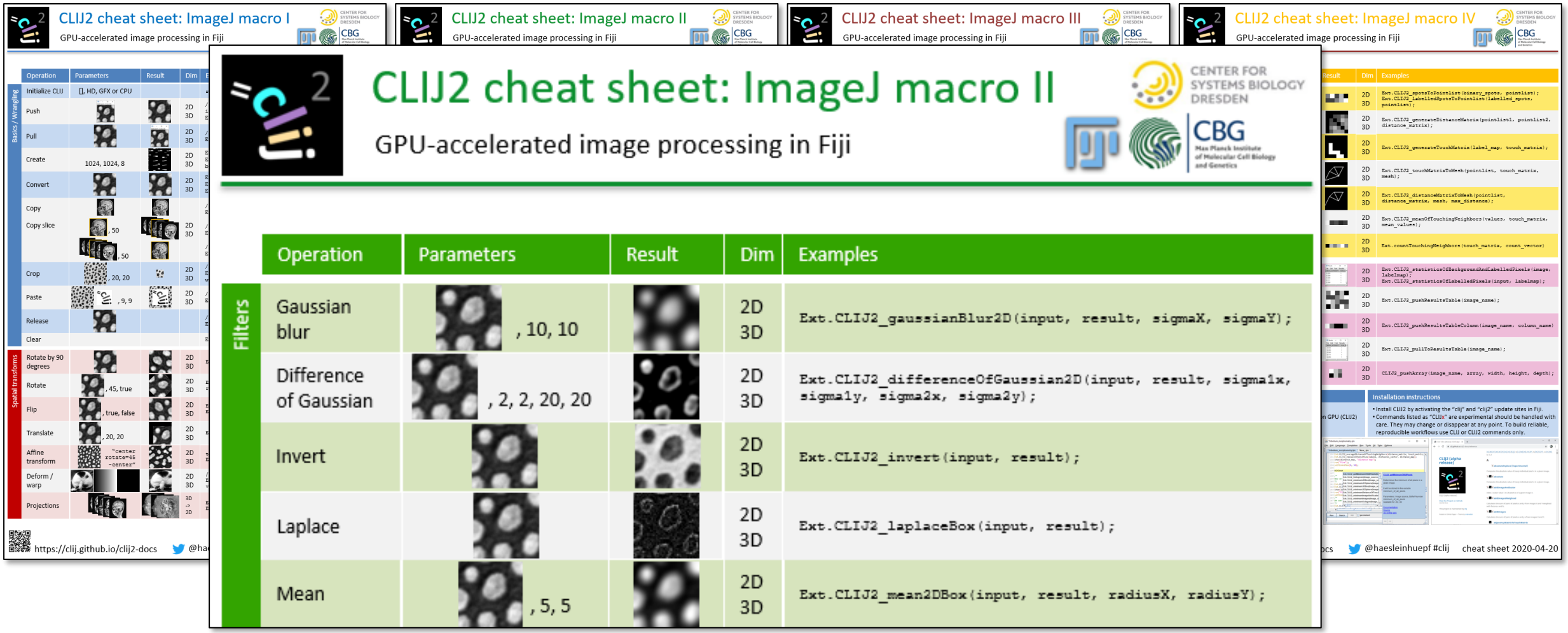
```
roiManager("add");
roiManager("split");
roiManager("delete");
roiManager("reset");
roiManager("measure");
roiManager("count");
roiManager("open", filename);
roiManager("save", filename);
roiManager("save selected", filename);
roiManager("select", index);
roiManager("select", newArray(index1,
index2, ...));
roiManager("deselect");
roiManager("show all");
roiManager("show all with labels");
roiManager("show none");
roiManager("and");
roiManager("combine");
```

Keep in mind:

- Only one active window!
- One activate channel, slices!
- One ROI list
- One result table!

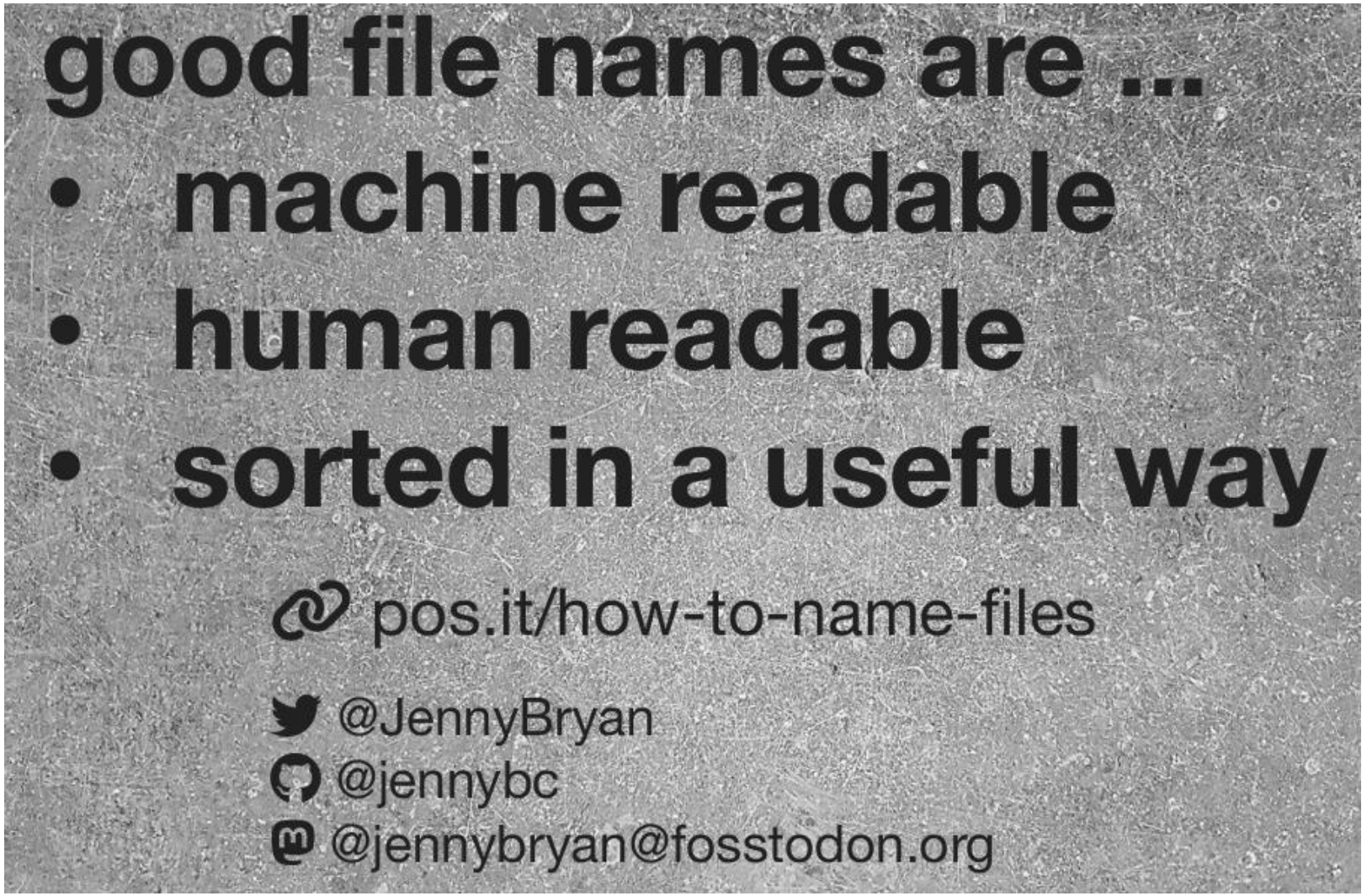
https://www.biodip.de/w/images/0/00/ImageJ_macro_cheatsheet.pdf

CLIJ2 cheat sheet



Adapted from Open-Access Training Materials of Dr. Robert Haase, licensed CC-BY 4.0
https://clij.github.io/clij2-docs/CLIJ2-cheatsheet_V3.pdf

Bonus: How to name files



Bonus: Fix funny filename

- File -> Fix Funny Filenames
- Rename files with problematic file or folder names automatically
  image 001.tif → image_001.tif
- Try it if your ImageJ macro can not find you file or folder!