Icy

Icy Training - Level 1 - Introduction

Plan

- What is Icy ?
- Installing Icy
- Graphical User Interface (GUI)
  - Histograms & Colormap / Look up table
  - Basic operations
  - Overlays / Layers
  - 3D view
  - Others image representations
  - Icy Preferences
- Investigate an image...
- Region of Interest (ROI)
- Analysis examples
  - Spot detection
  - Tracking
  - Active contours
- ImageJ inter operability

- The complete archive for the training can be found here:
  http://icy.bioimageanalysis.org/icy_training.zip
- Feel free to use your own images !

What is Icy ?

In a nutshell: Icy is a collaborative photoshop dedicated to image analysis

"Collaborative" as anybody can add features to Icy

What you want,
What you don't want.

Deploiement

User

I want to seek and install a plugin directly from the application

I want my program ready to run

I want everything up to date

I don't want to deal with program installation, anyway I don't understand it.

Developer

I want my plugins to be available to everybody in a few clicks

I want to configure everything online, without using text config file

I want to deploy all my updates by posting it on the website.

What you want,
What you don't want.

Deploiement

Quality

User	Developer
If the program crashes, i want the developer to be aware of it.	If my program crashed, I want to receive a bug report to correct the problem.
I am willing to participate, but by clicking on a button, no more.	I wish to write update and send it right now

What you want,
What you don't want.

Deploiement | Quality | Re-use

User

I want to understand the step involved in the analysis of an image

I can see the analysis I wish can be obtained by tweaking existing scripts or protocols

I want something adapted to my programming skills

Developer

I don't want to write what is already existing in other plugins.

I want to get information on the plugin I build on.

What you want,
What you don't want.

Deploiement | Quality | Re-Use | Deploiement

User

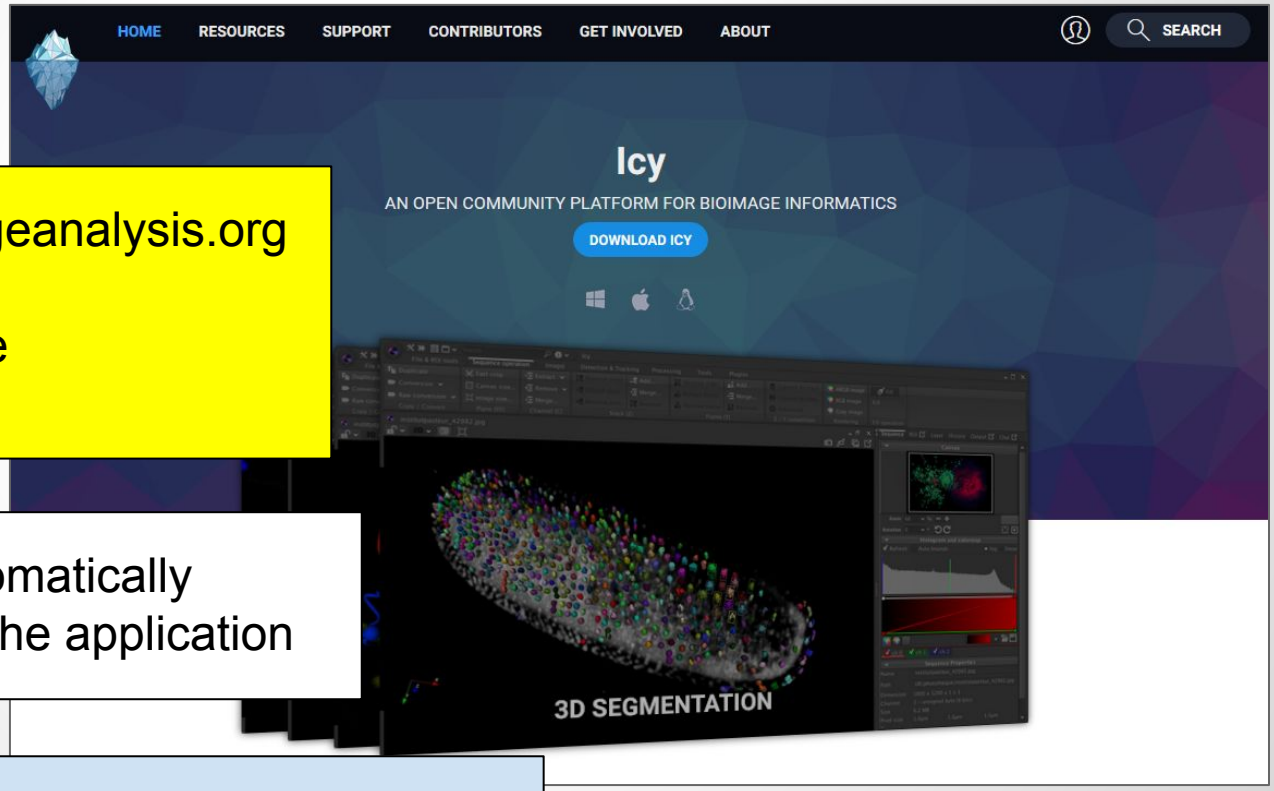
I want to send my scripts or my protocols online. I want to put it in a publication, write a doc and share it with others.

I don't know anything about web hosting

I want the other to download my protocols or my scripts and that everything get automatically installed

Installing Icy

Installation



- Go to icy.bioimageanalysis.org
- Download Icy
- Unzip the archive
- Launch Icy

At each start, Icy automatically updates plugins and the application

Complete training archive is available at : http://icy.bioimageanalysis.org/icy_training.zip

Graphical user interface

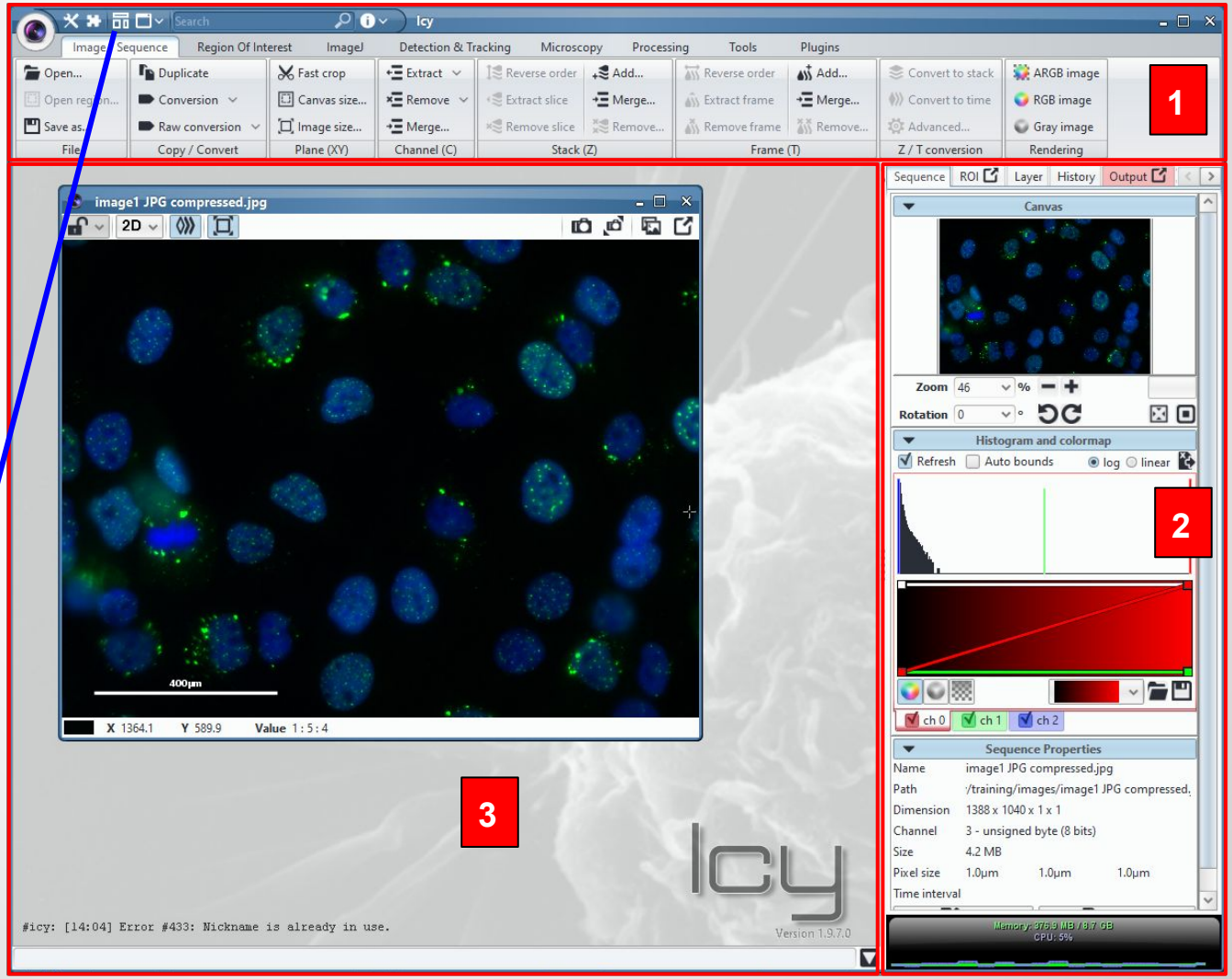
Open the image named 'image 1 JPG compressed'
(you can just use drag and drop)

GUI

The GUI is based on
3 main components :

1 - Ribbon Menu
2 - Inspector
3 - Work space

Tips: Application can also work as floating windows using *detached mode*

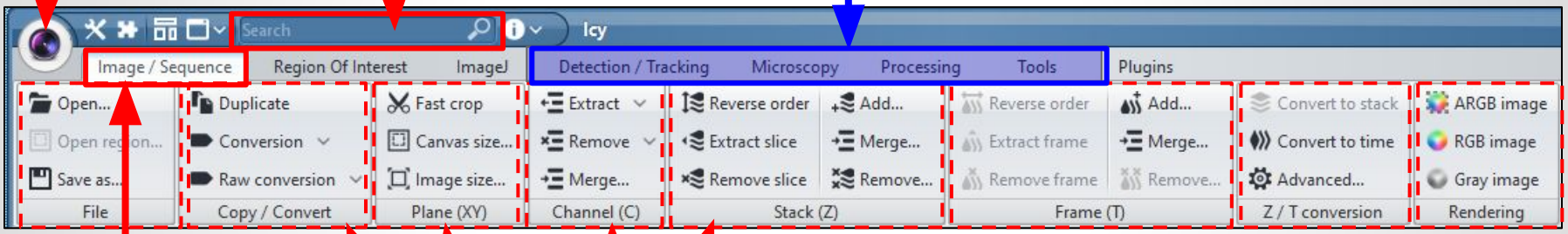


The Ribbon menu

Main menu

Centralized Search (Ctrl+F)

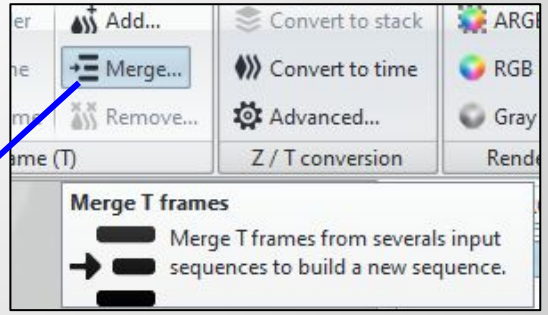
User customizable tasks (*Workspace Editor* plugin)



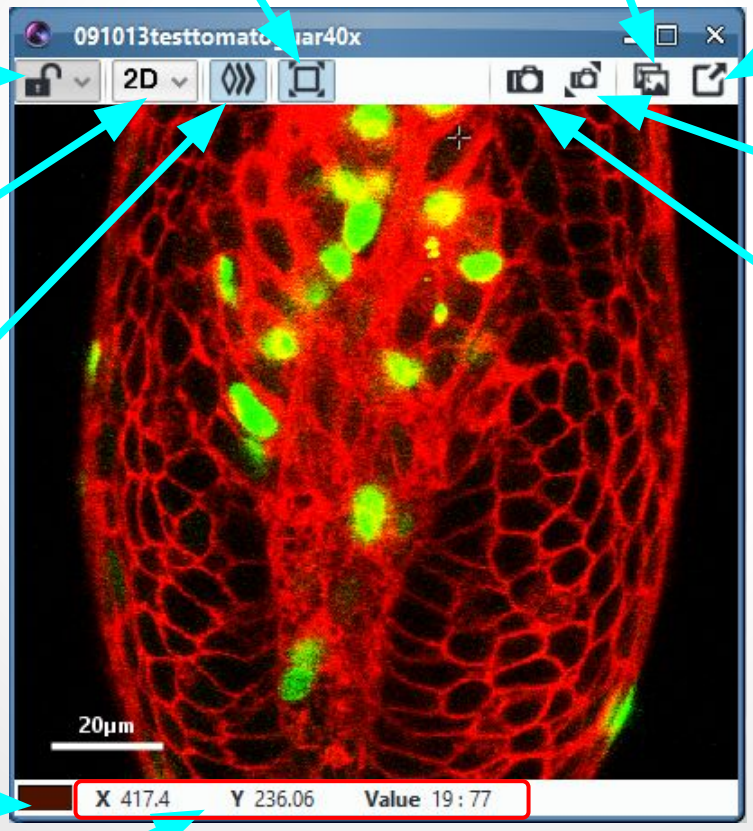
Selected Ribbon task

Actions of current selected task organized by group / band

Tips: Letting mouse cursor on a button will display a tooltip providing more information about the action



The viewer



Keep adjusting image to the window size

Duplicate the viewer over the same data (F2)

Externalize the viewer (floating window) (F3)

Enable view synchronisation of several viewers (0,1,2,3,4)

Create a new "flattened" image (render image with all layers and false color in a RGB image)

Visualization mode selection

Create a cropped "flattened" image using current view

Enable / Disable layers display (L)

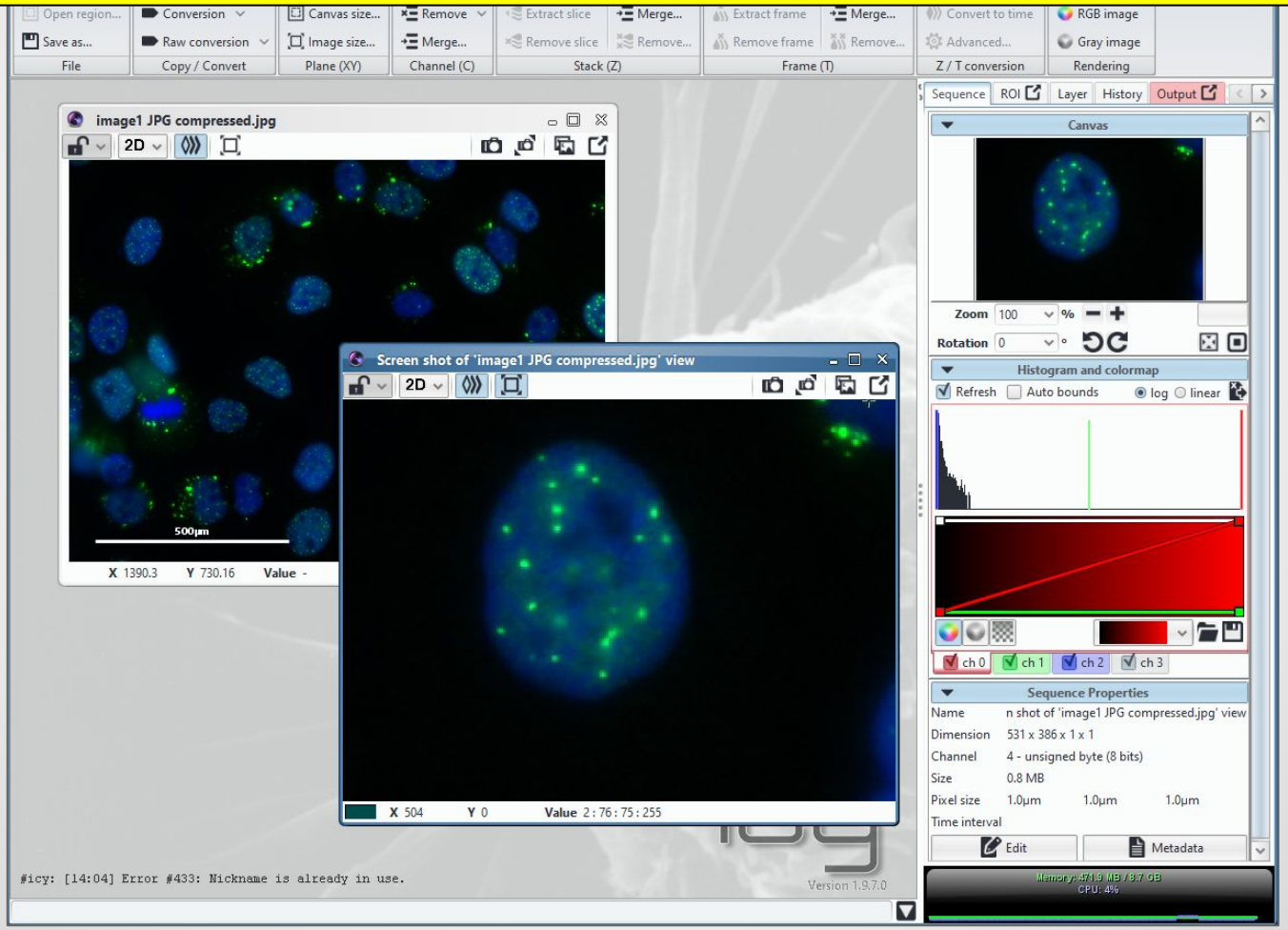
Pixel color under at current mouse position

Current mouse position and values under pointer

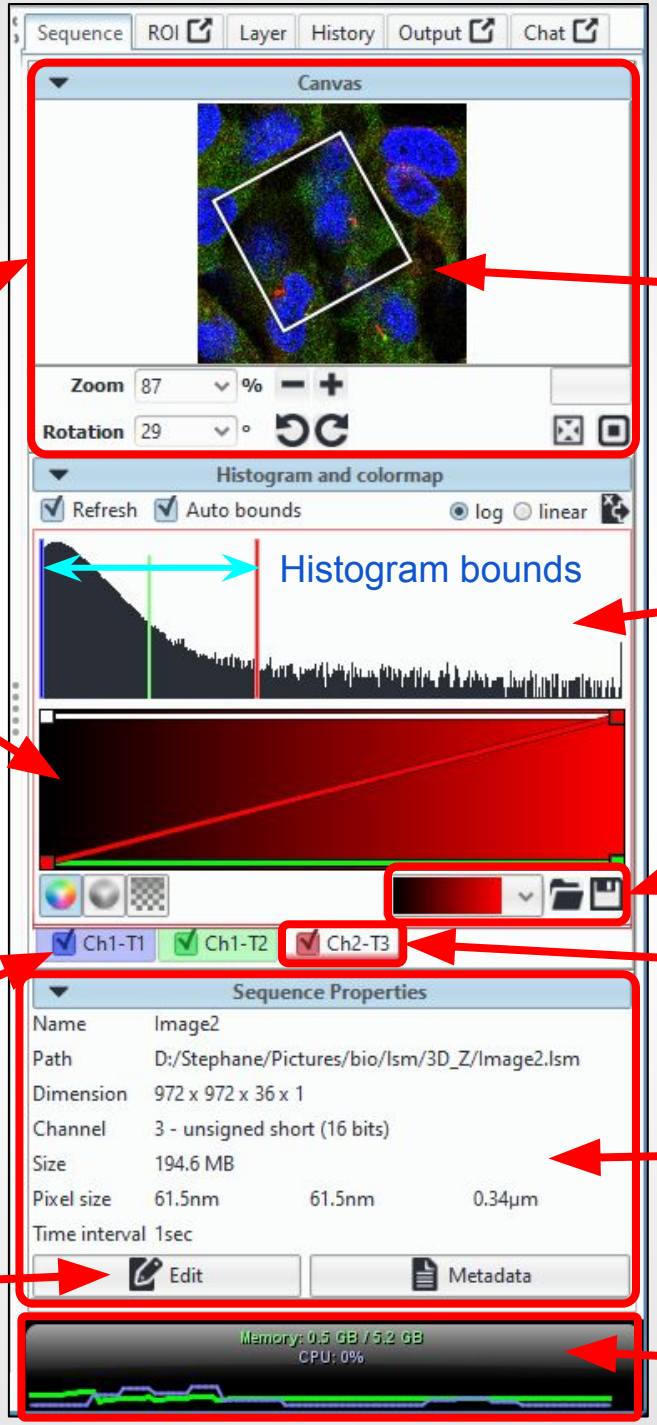
Navigating in the sequence
- Mousewheel = zoom
- Drag with left click = translation
- Drag with right click = rotation

Tips
- use Shift key as aligner
- use Ctrl key as accelerator

- Open the image named 'image 1 JPG compressed'
- Zoom in/out over the viewer and over the navigator
- Pan the view / move the image
- Rotate the view
- Render a flattened image at scale 1:1
- Render a zoomed & cropped flattened image



The Inspector



View of current active Sequence / Viewer

Pan, zoom, rotation

Histogram of the current selected channel

Colormap / LUT of current selected channel

Load or select pre-existing colormap

Tips: Colormap can be manually edited by clicking on a color line and moving the control point (which can be deleted using Shift+click)

Current selected channel

Display / Hide a channel

Properties of the current active Sequence

Edit properties (Sequence name, pixel size, channel name…)

Memory / CPU monitor

Histogram of an image

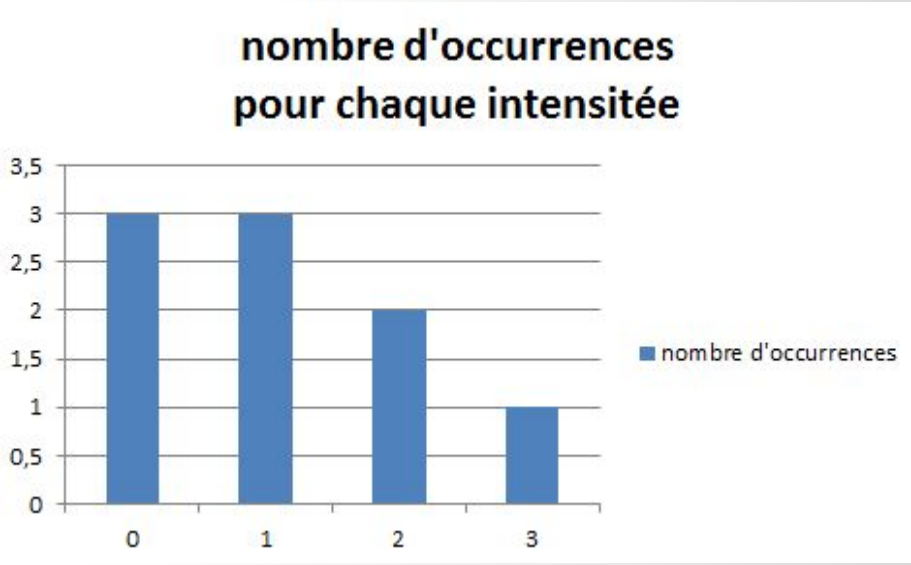
The image is a matrix of values

The bigger it is, the higher is the intensity

For each value of the dynamic of the image, we count the number of corresponding values

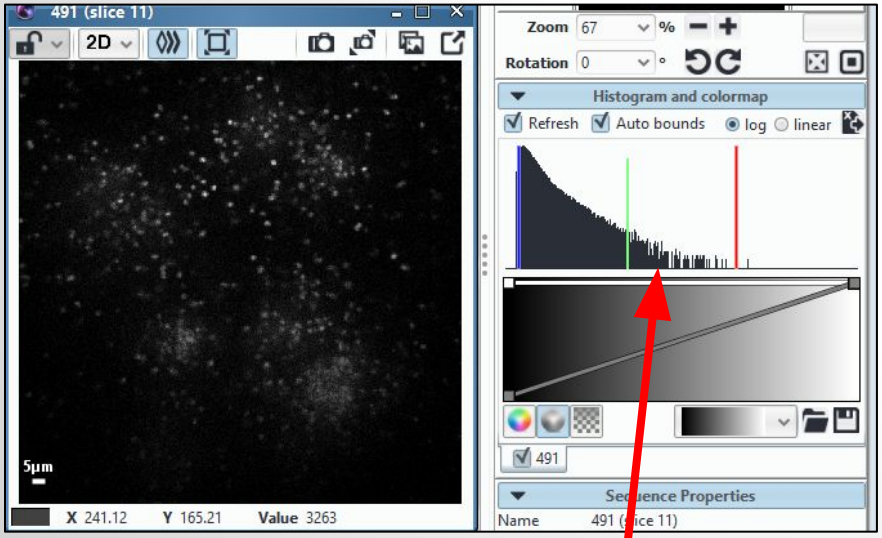
2	3	1
0	1	0
1	0	2

3x3 matrix representing an image of 3x3 pixels.

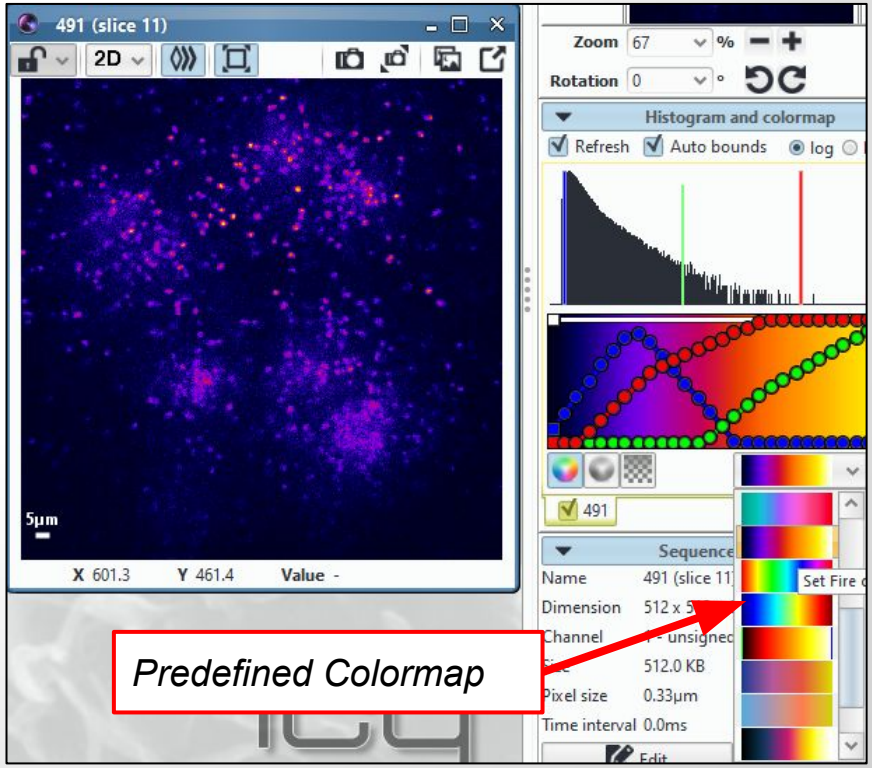


Histogram and colormap / LUT

- Colormaps help at understanding an image.
- Colormap representation **does not affect the real values** of the image.
- The histogram provides **the number of pixel for each intensity** in the image.

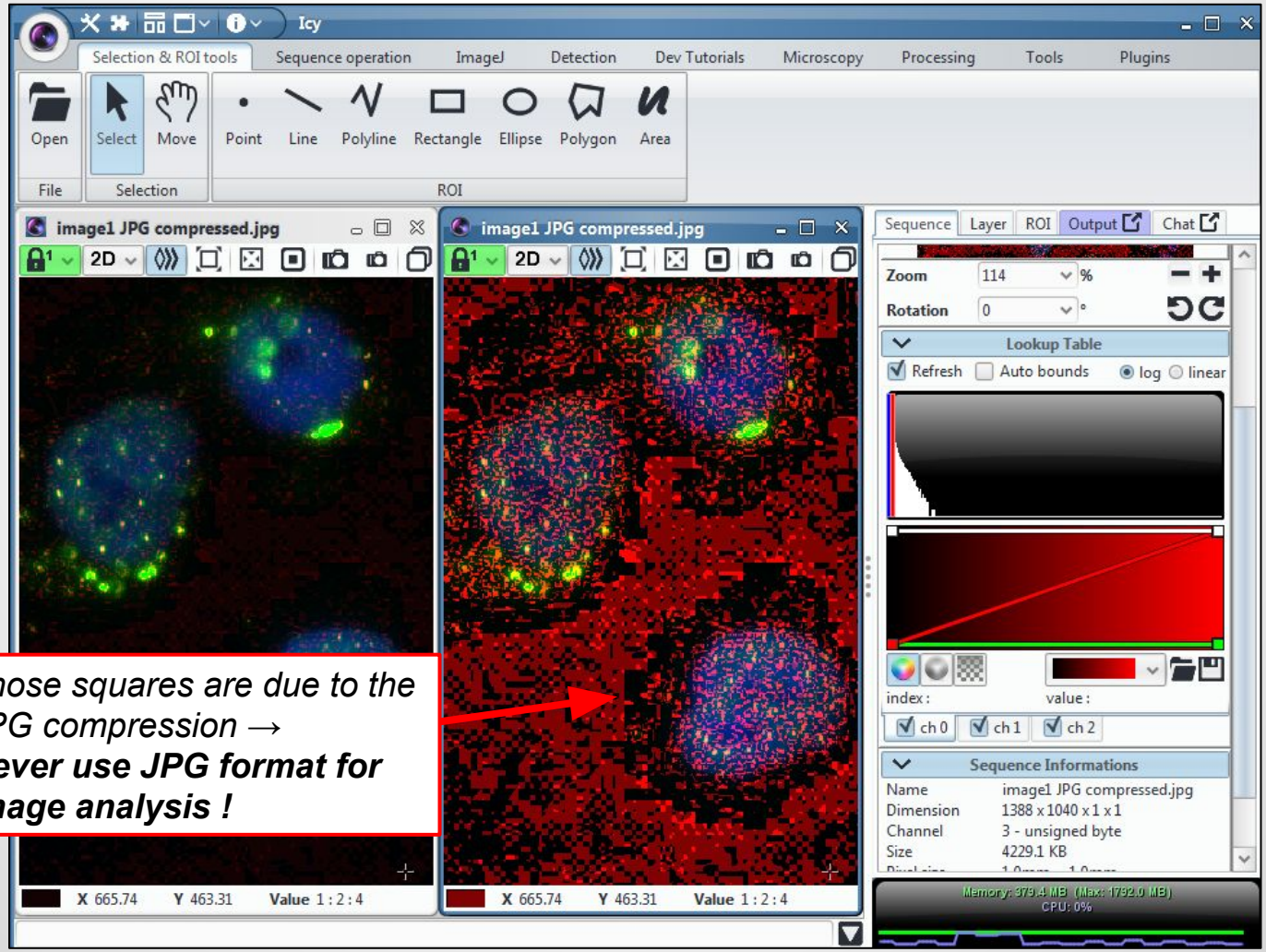


Histogram of selected channel (only 1 channel)



Predefined Colormap

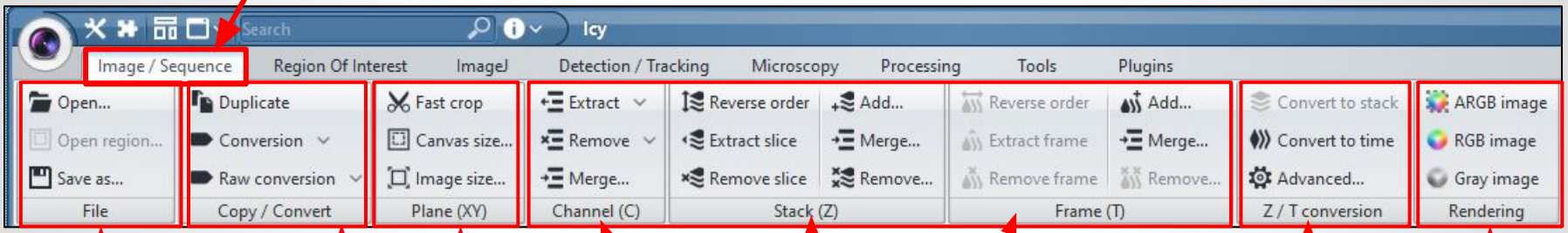
- Deactivate a channel
- Duplicate the view
- Synchronize both view on group 1
- Slide the red bar gradually to the left to increase the contrast of RED channel and watch the squares dues to the JPG compression.



Basics operations

Crop, extract channel, merge...

All basic operations on an image / sequence are located in the first Ribbon task and are organized by group



Open from file / Save to file (also available from the main menu)

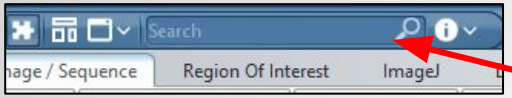
Image Duplicate / data type conversion

XY size operations

Channel / Stack / Frame basic operations: extract, remove, merge...

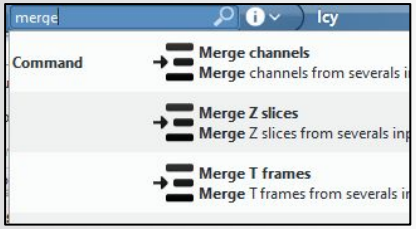
Z (3D stack) / T (TimeLaps) dimension conversion

Gray / RGB / ARGB image conversion (useful to generate JPG, PNG or AVI images)

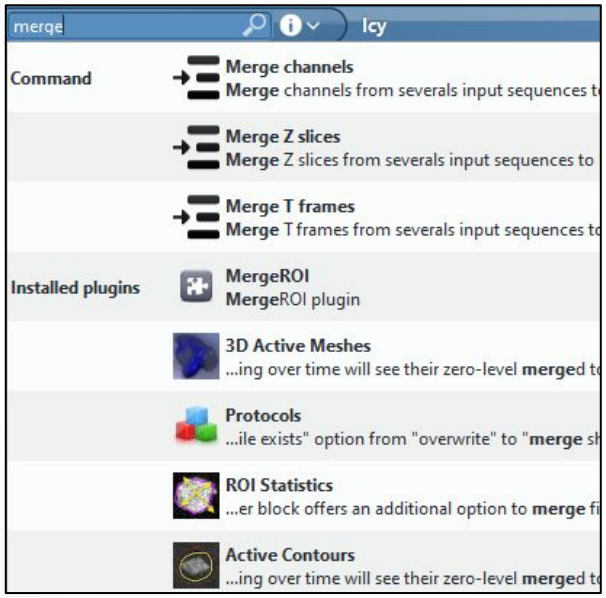


Search for… everything

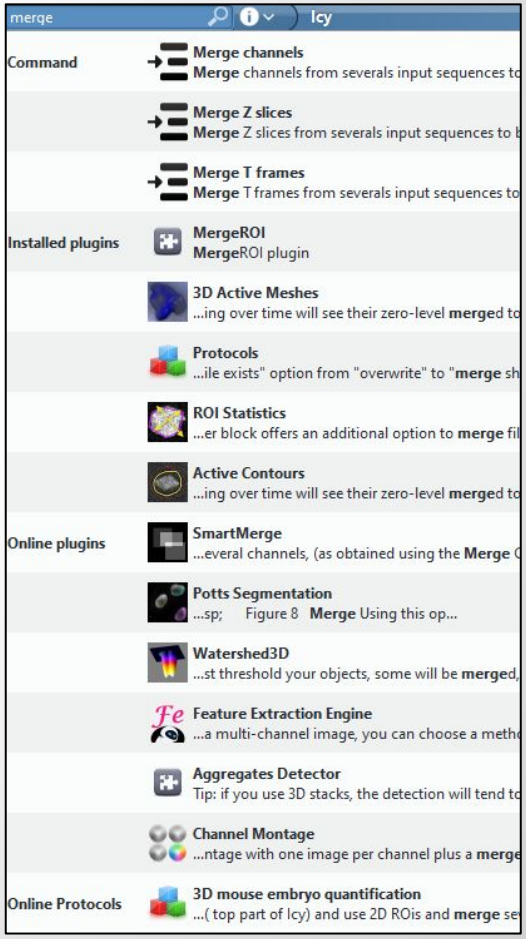
Internal commands...



Installed plugins...



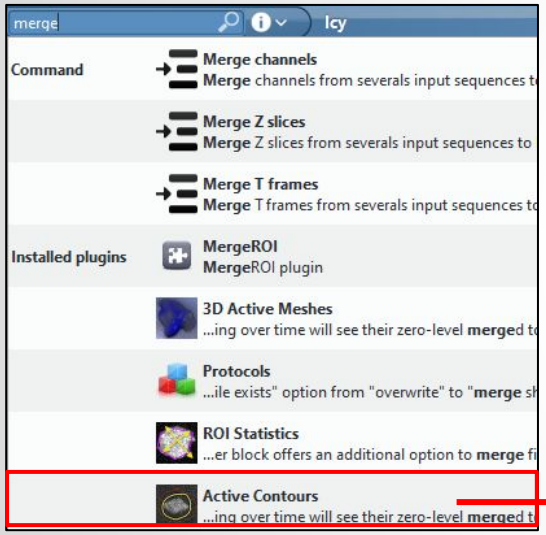
and all online resources !



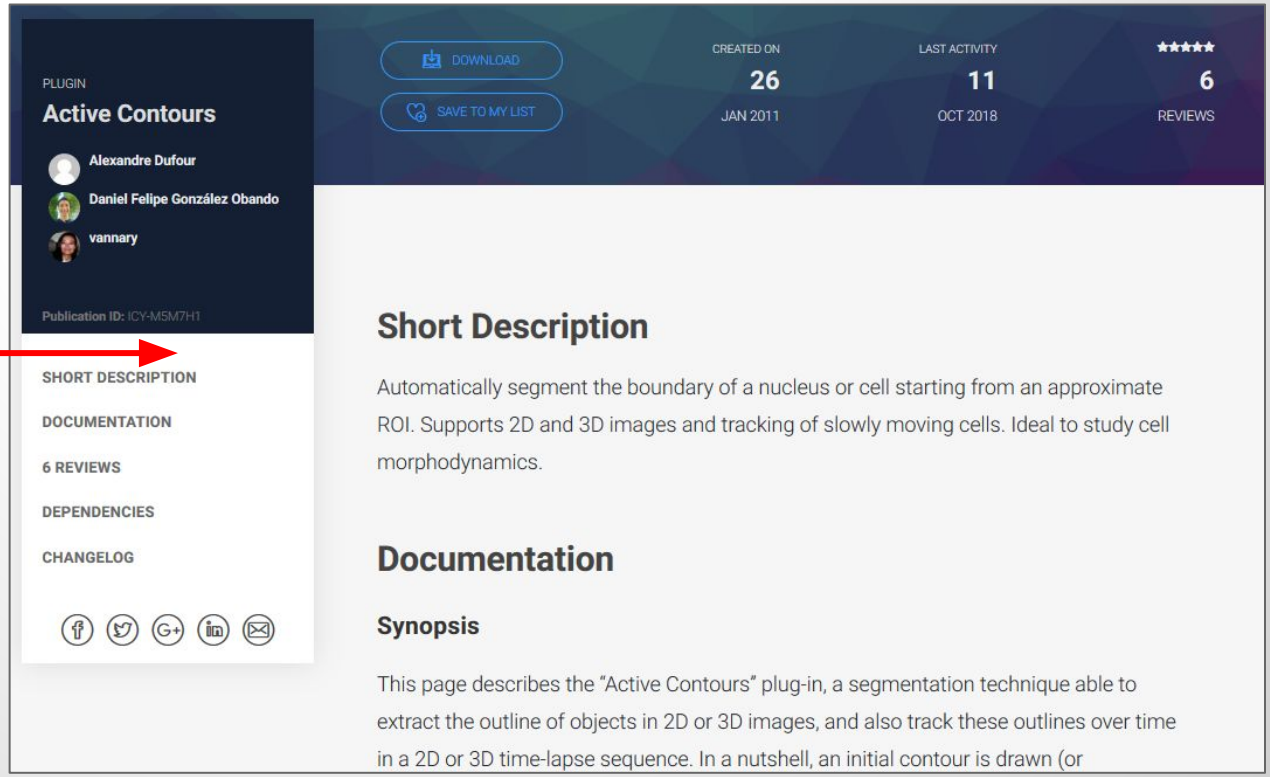
Tips:
- Use CTRL+F for search
- When you don’t find a function or plugin, just use the search bar :)
- Results can be extended by plugin (Protocols, Scripts..)

Plugin Documentation

As a developer creates a plugin, a page is automatically generated.



Tips: Right clicking on a plugin result will redirect you to the online documentation for this plugin

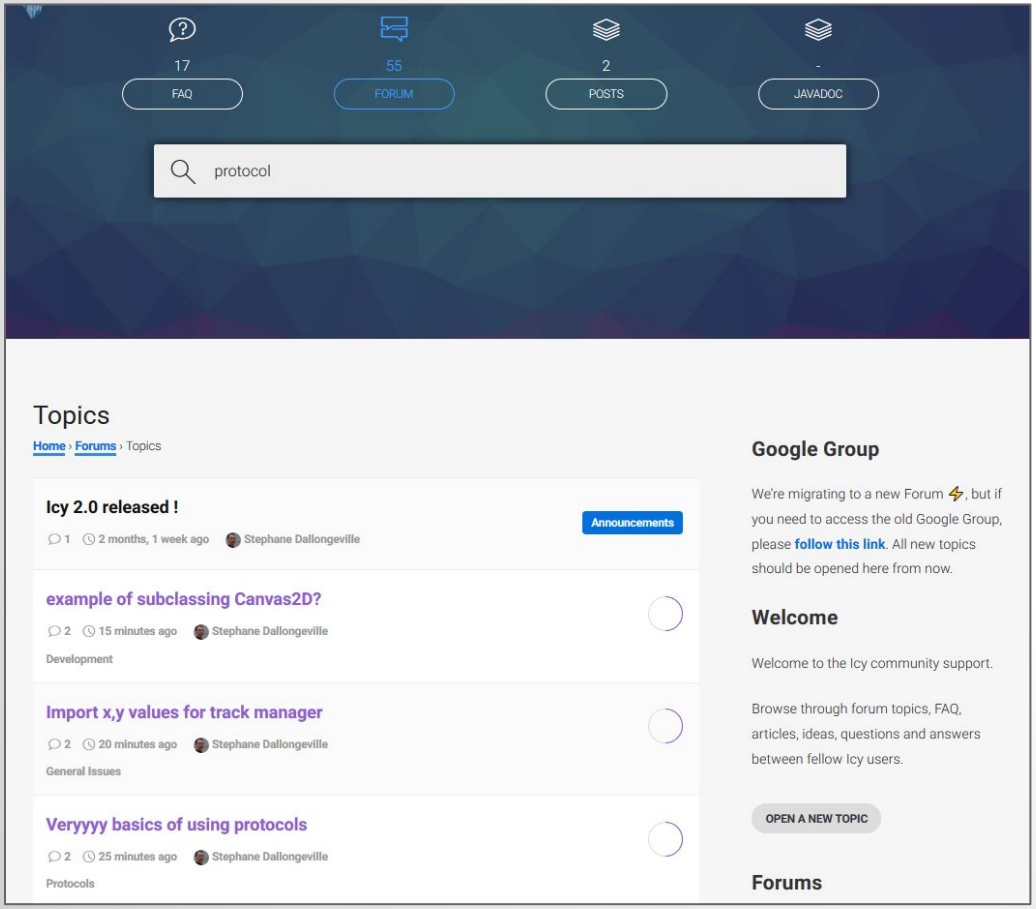


The page contains

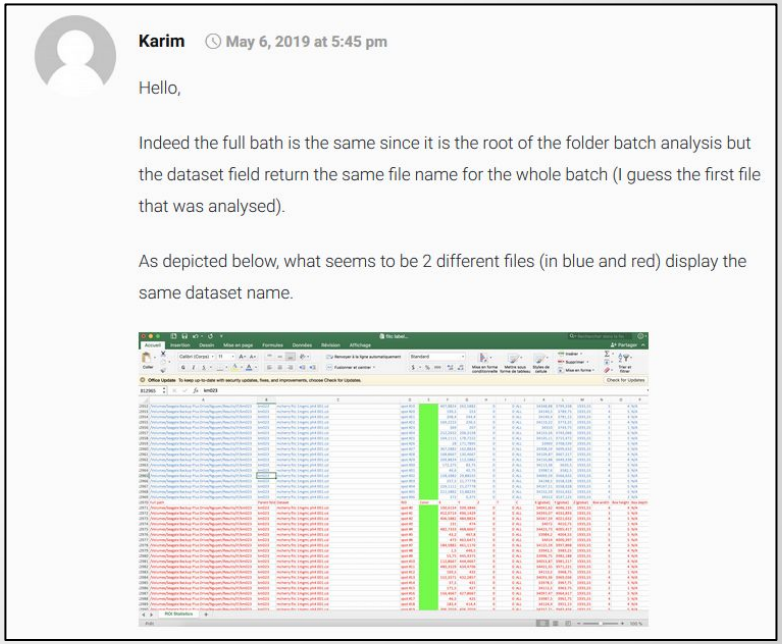
- abstract
- technical infos
- changelog
- documentation
- a rating section

If you still don’t have the solution: Use the forum support

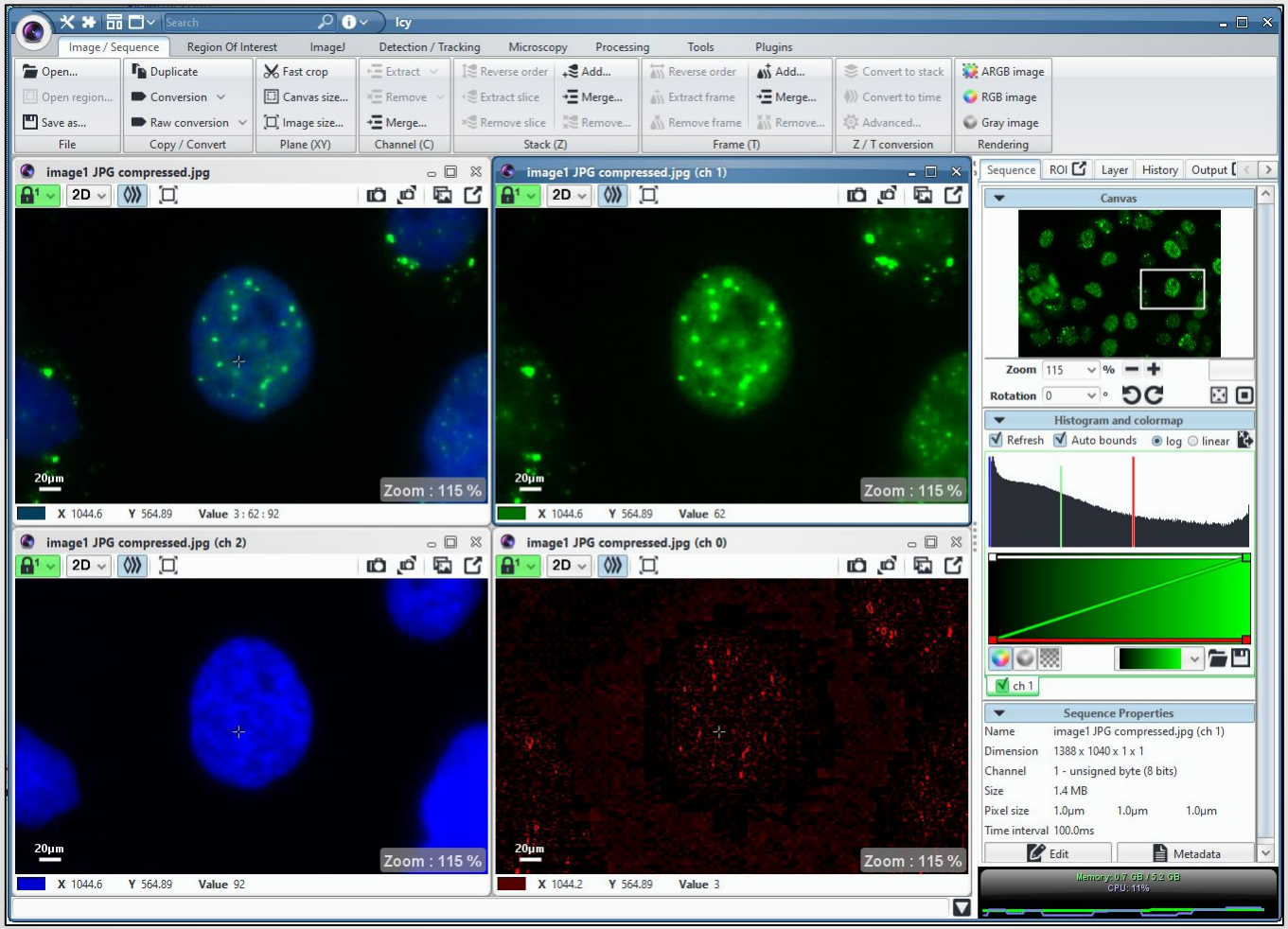
http://icy.bioimageanalysis.org/support



People can illustrate question and answers with code, images and various files.



- Close view with enhanced RED contrast
- Extract all channels of 'image 1 JPG compressed'
- Align all viewers
- Use the histogram to enhance contrast for each channel
- Synchronize all views together in group 1



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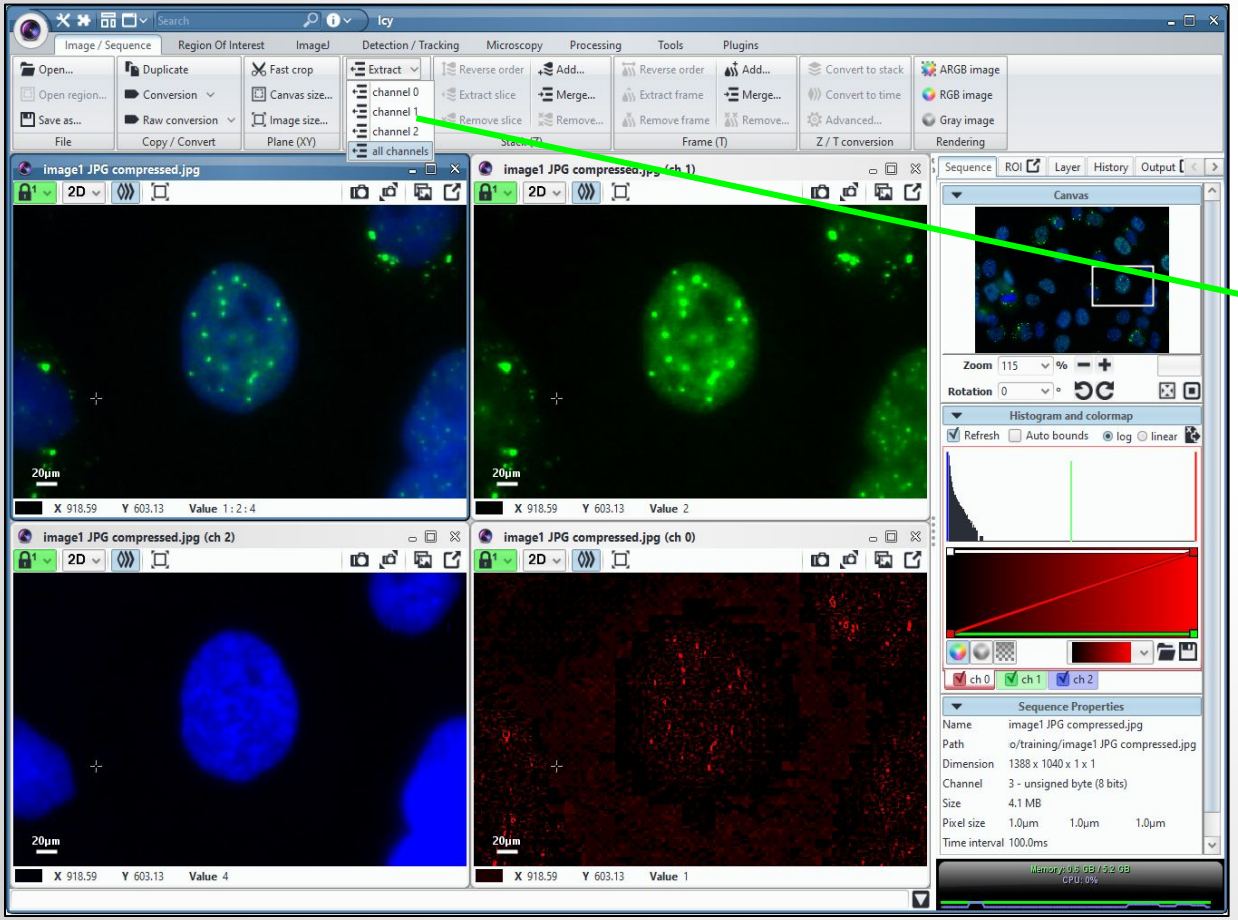
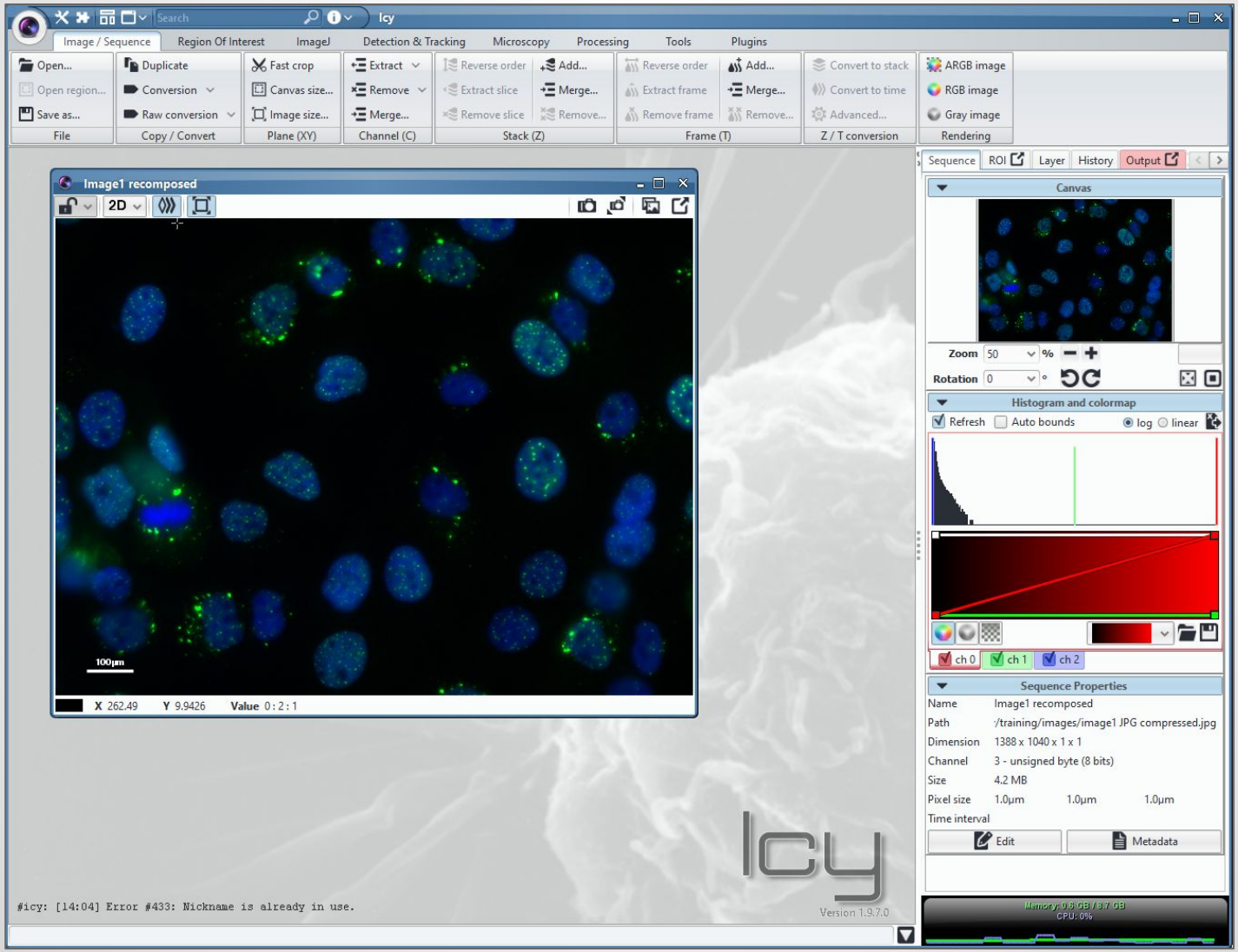


Solution
- Use *extract all channels* command
- Use *Tile grid* align command (Shift+G)
- Use group 1 synch command *(Shift+1)*

- Close the original 'image 1 JPG compressed' image (3 channels)
- Merge channels to rebuild it from single channel images
- Close all single channel images
- Rename the remaining image 'image 1 recomposed'



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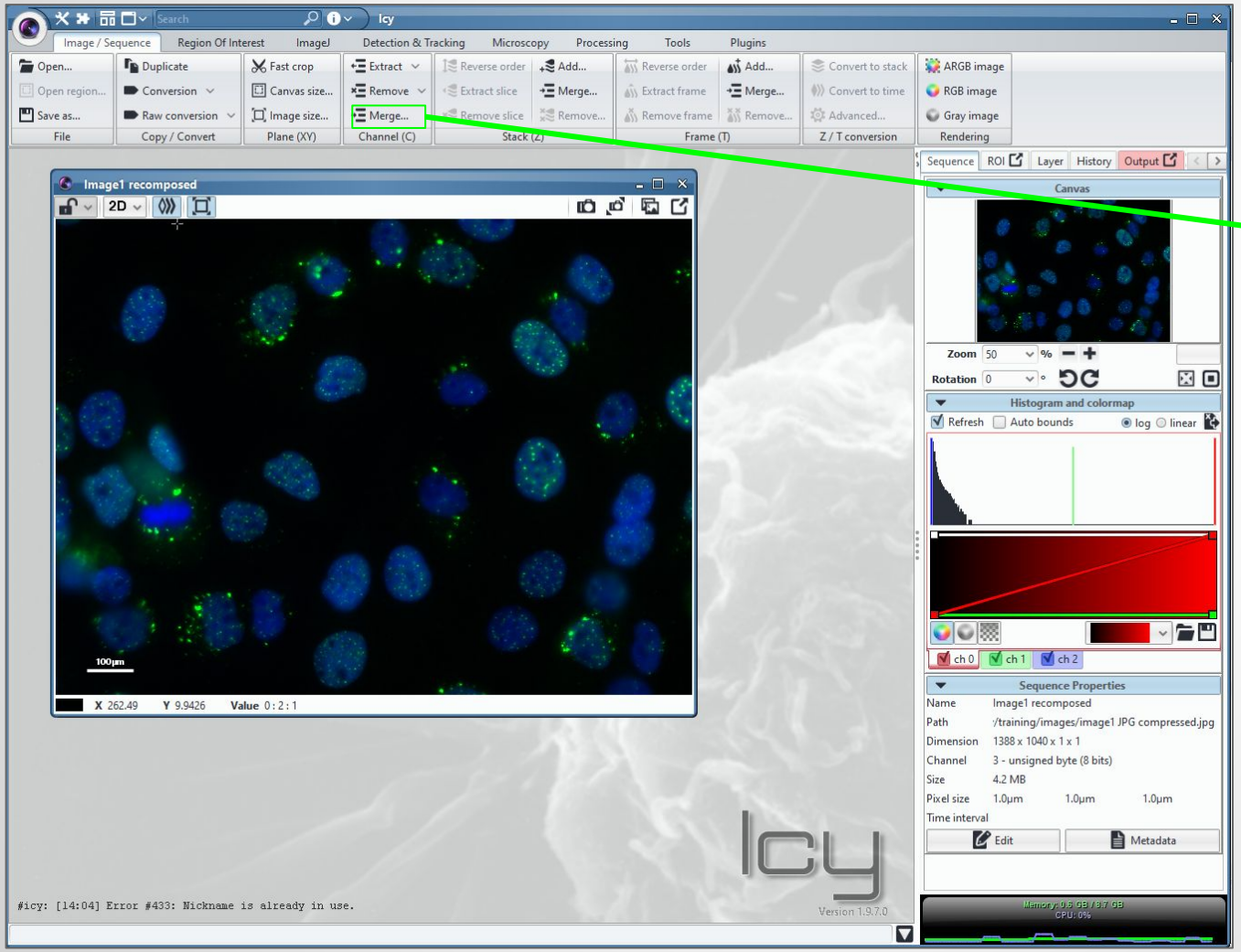
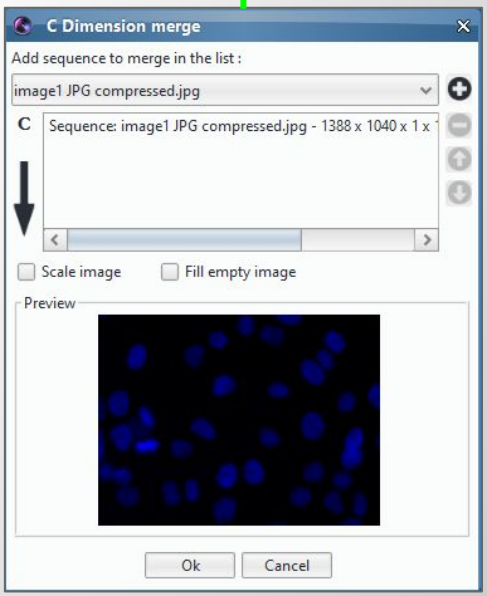


Solution
Use “Merge…” command in “Channel” section



Opening an image

- Can use the classic drag and drop
- Or use the open operation from the main menu to get more options

Try to open a sub part of 'image 1 JPG compressed' :
XY region “500,270 - 220,150” (X,Y - W,H)

Open main menu
by clicking here

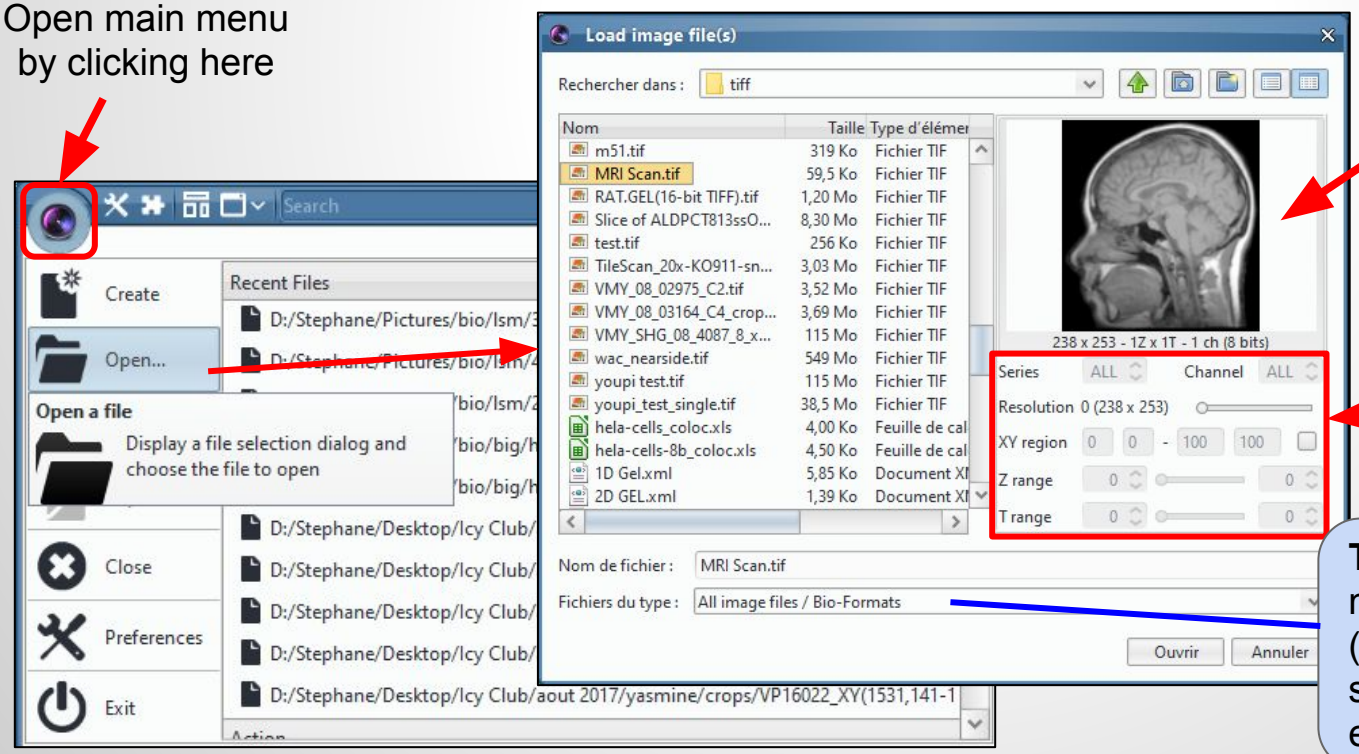
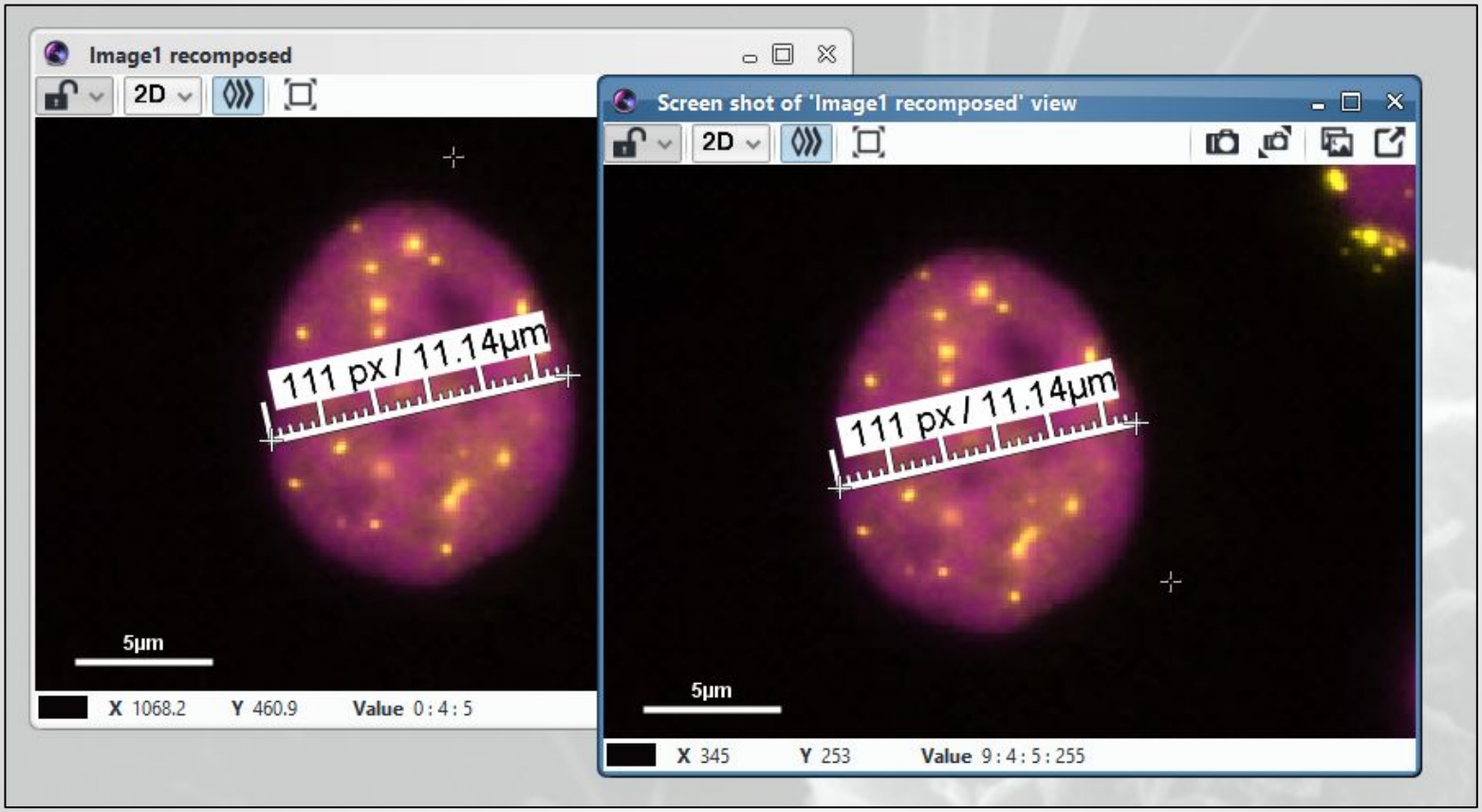


Image preview &
basic infos

Options to open
only sub part of
the image

Tips: Icy supports almost all microscope image format (CZI, LSM, ZVI, LIF, …) and supported format list can be extended using plugins !

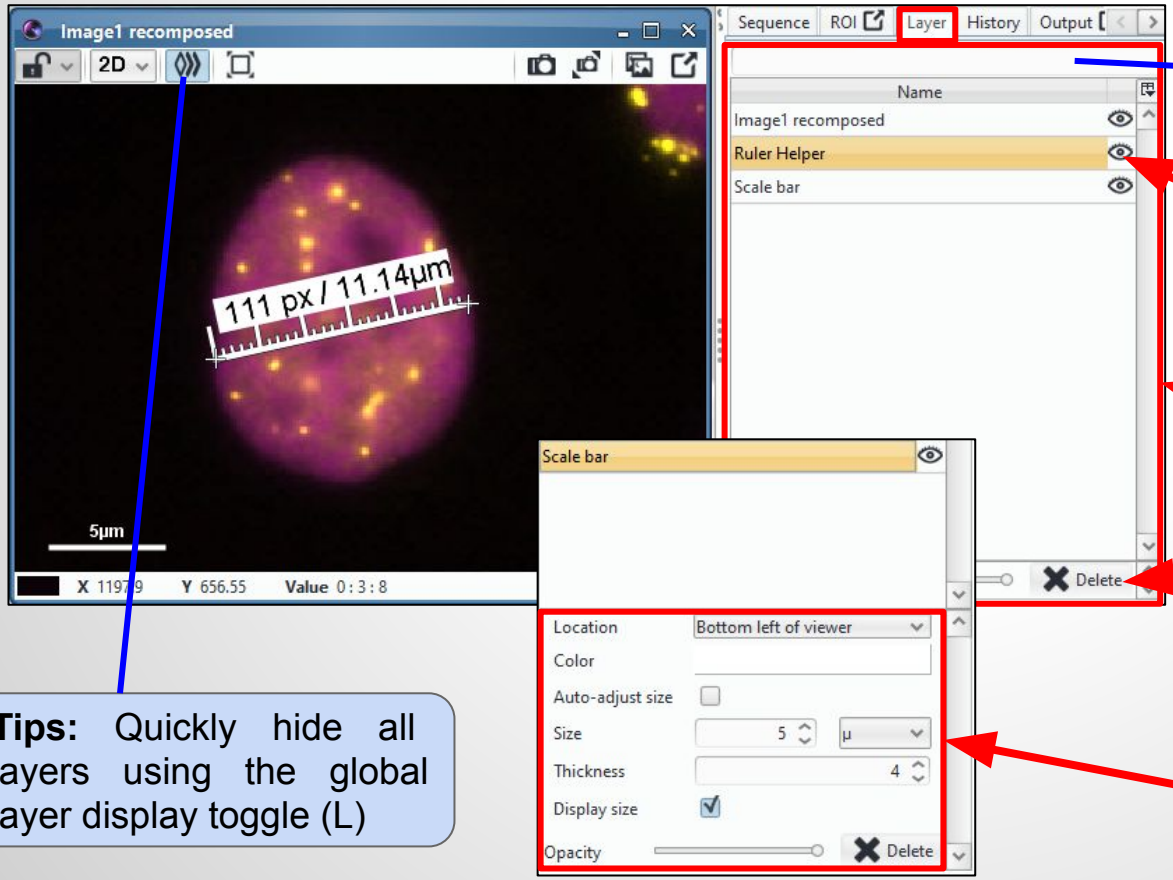
- Change channel 2 color to yellow and channel 3 color to magenta
- Change pixel size X / Y to 0.1 µm
- Zoom on 1 cell and measure its width with the ruler
- Take a screenshot of current view
- Save the result in JPG format



What is the difference between these 2 images ??

Overlays / Layers

It’s possible to enrich the image with various informations over it, still we don’t want to modify the image pixels so we use layers / overlays to display them.



Tips: layers can be filtered by name

Enable / Disable display of a specific layer

Layer panel displaying all layers visible on this image

We can definitely remove some layers if needed

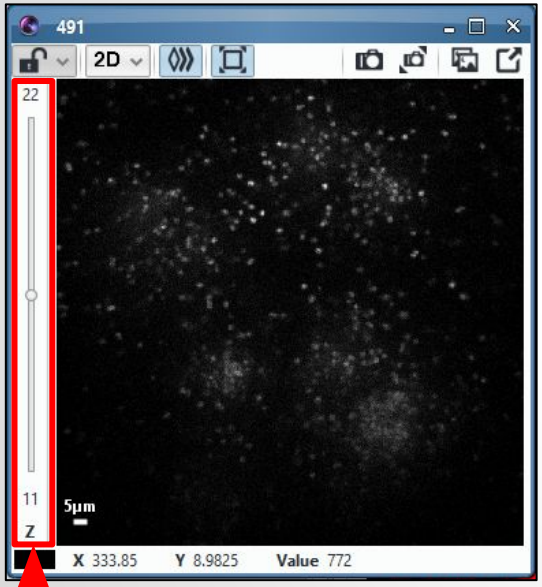
Some layer may display extra settings when you select them

Tips: Quickly hide all layers using the global layer display toggle (L)

3D / 4D / 5D image

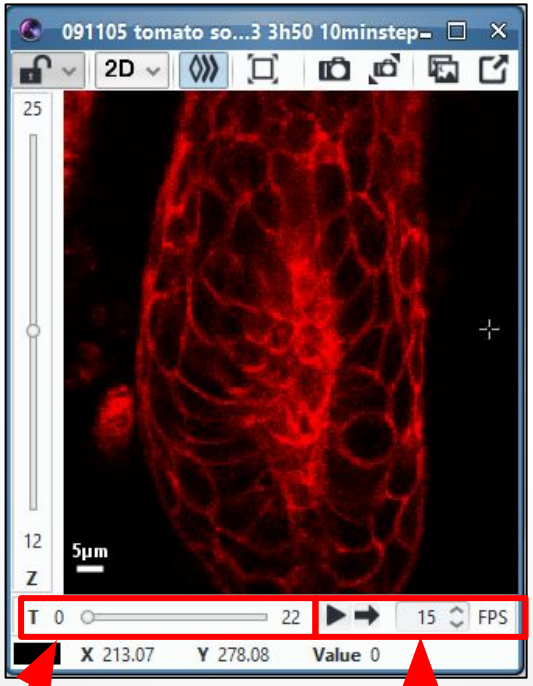
Icy supports up to 5D images (XYZTC) of any data type (byte, short, int, double..)

3D image
Z stack - single channel



Z slider to navigate through Z slices

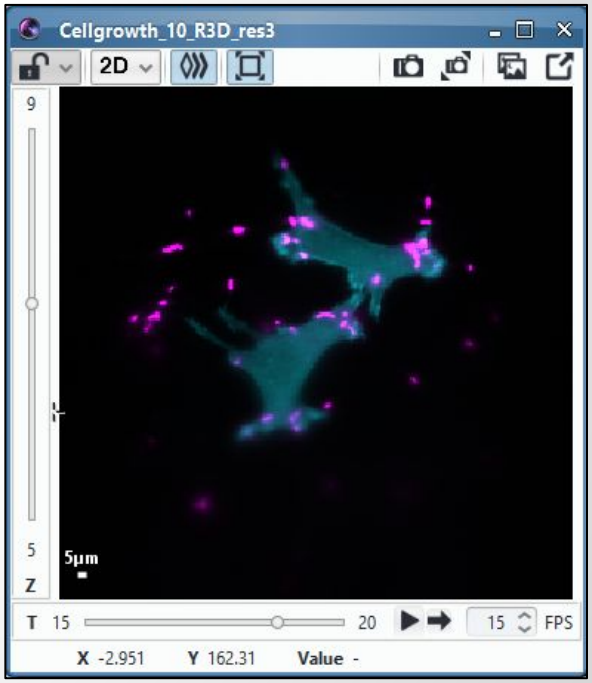
4D image
timelaps of Z stack - single channel



T slider to navigate through frames

Play / pause, loop, frame rate control

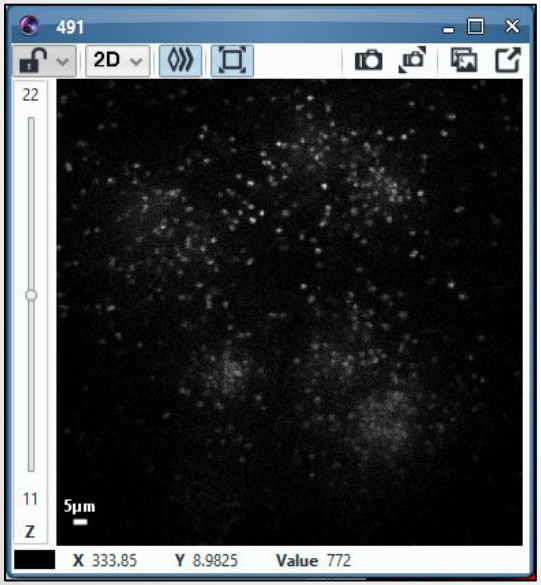
5D image
timelaps of Z stack - multi channel

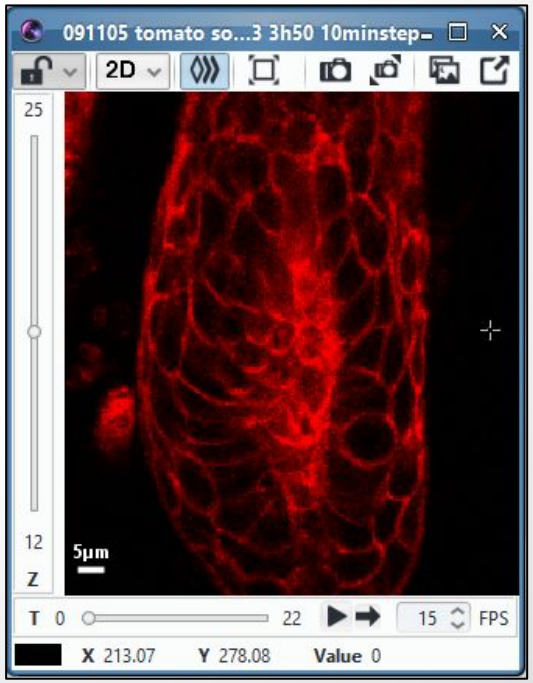


Tips: you can use *arrow* keys to navigate through slices or frames.

3D / 4D / 5D image

- Open ‘3D stack.tif’ and ‘4D hair.tif’ files
- Use the Z / T slider to change slice / frame then use arrow keys to navigate one slice / frame at once
- Play the timelaps in repeat mode at 30 FPS



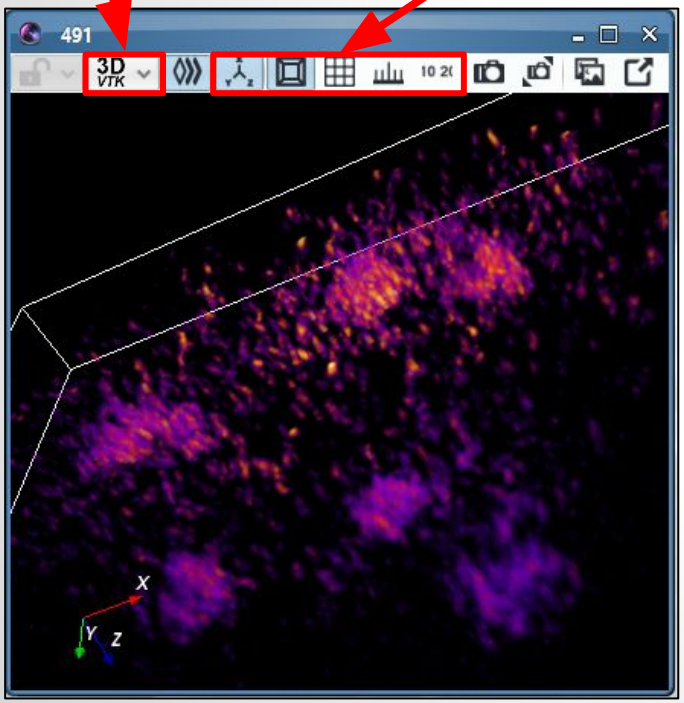


Use 3D view

Icy natively support VTK for 3D raycasting rendering

Select 3D VTK view

Enable / disable axis, bounding box, grid and scale display

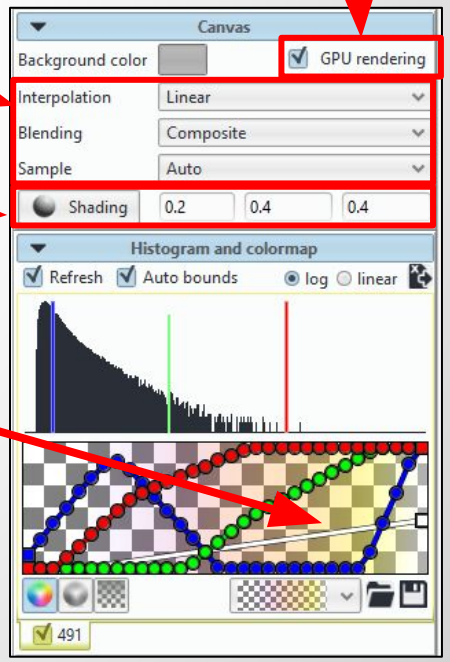


Can use GPU for fast rendering

General rendering settings

Light settings

Note that alpha component in colormap (FIRE here) is useful here

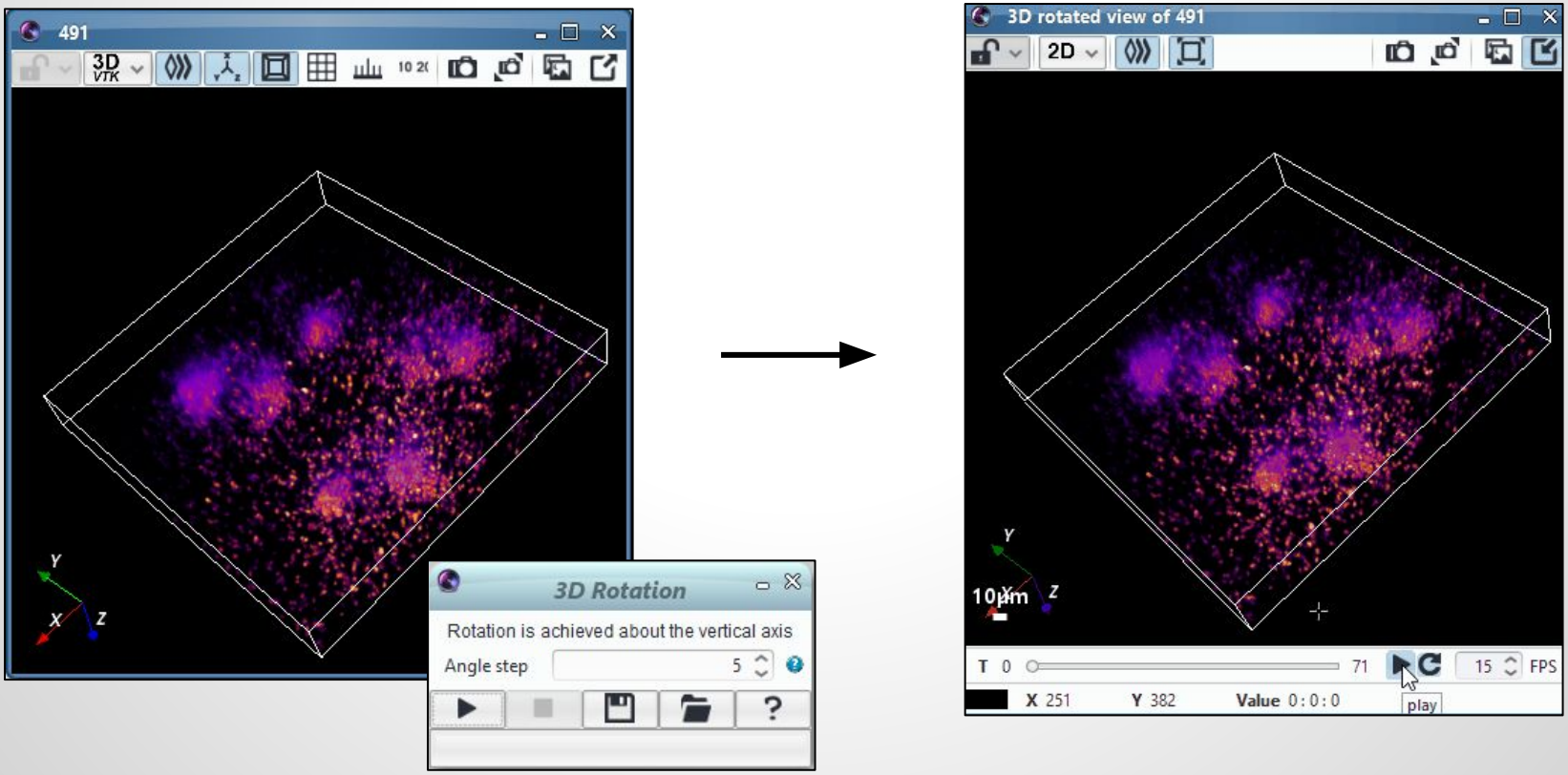


Tips: use *R* key to reset view and Shift+R to reset view and colormap / histogram bounds

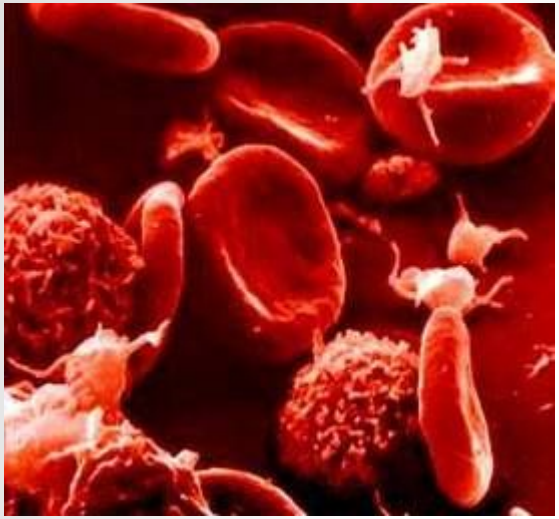
- Switch ‘3D stack.tif’ or ‘4D hair.tif’ to 3D VTK view
- Use the 3D Rotation plugin to make a nice animation :)

Before

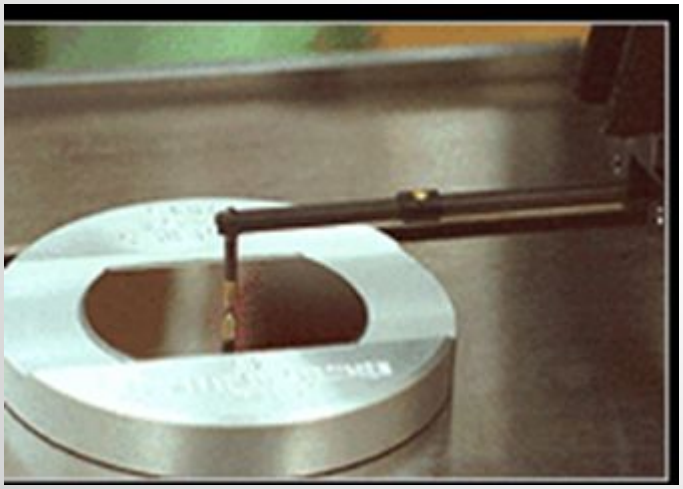
After

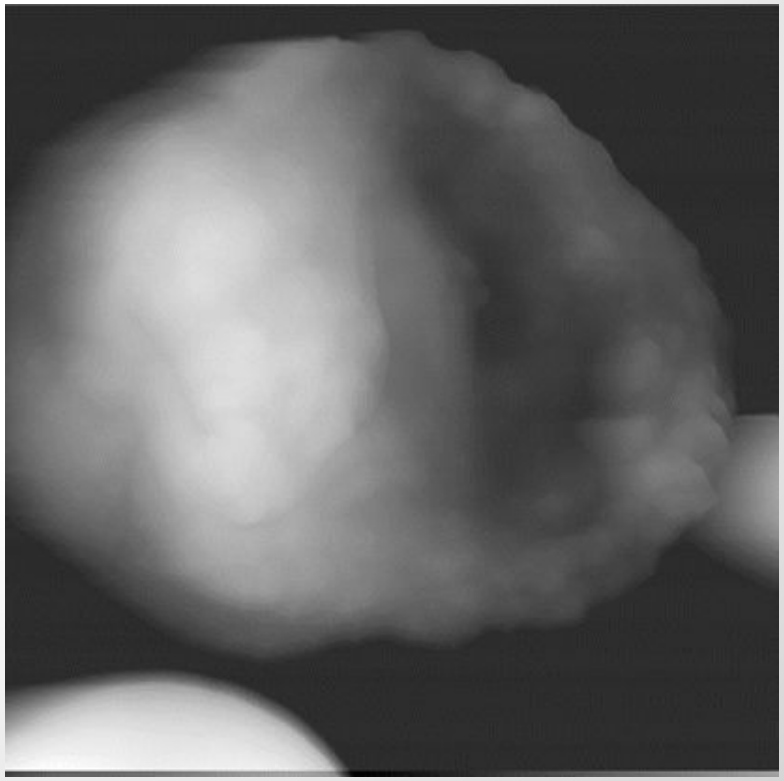


Representing an image on screen



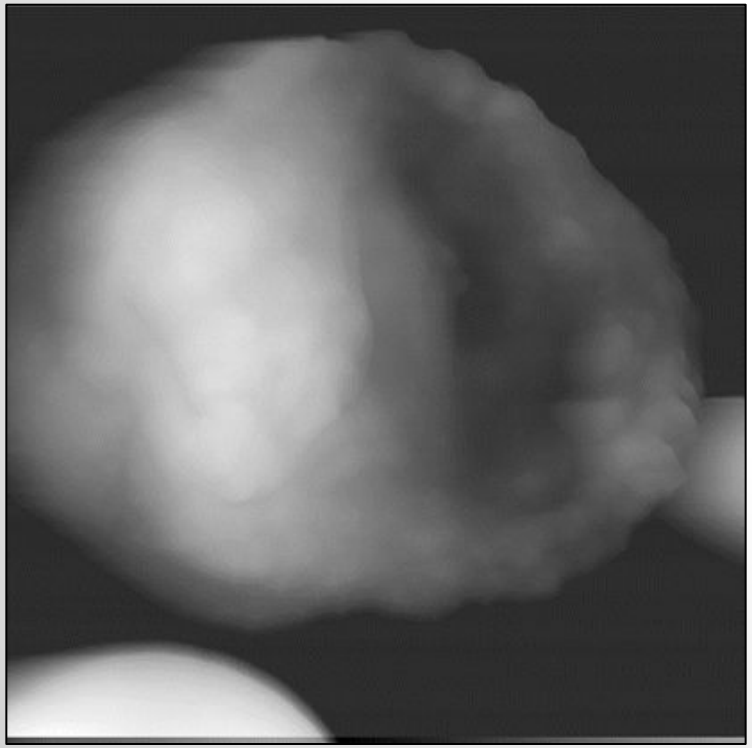
The image below (blood cell) is obtained with an *atomic force microscop*y. Each intensity value is an height: an altitude.





Representing an image on screen

Have you seen the smalls knobs on the left hand side image ? No ? That precisely what we are looking for !
The same image displayed in 3D (on the right) allow to see them.



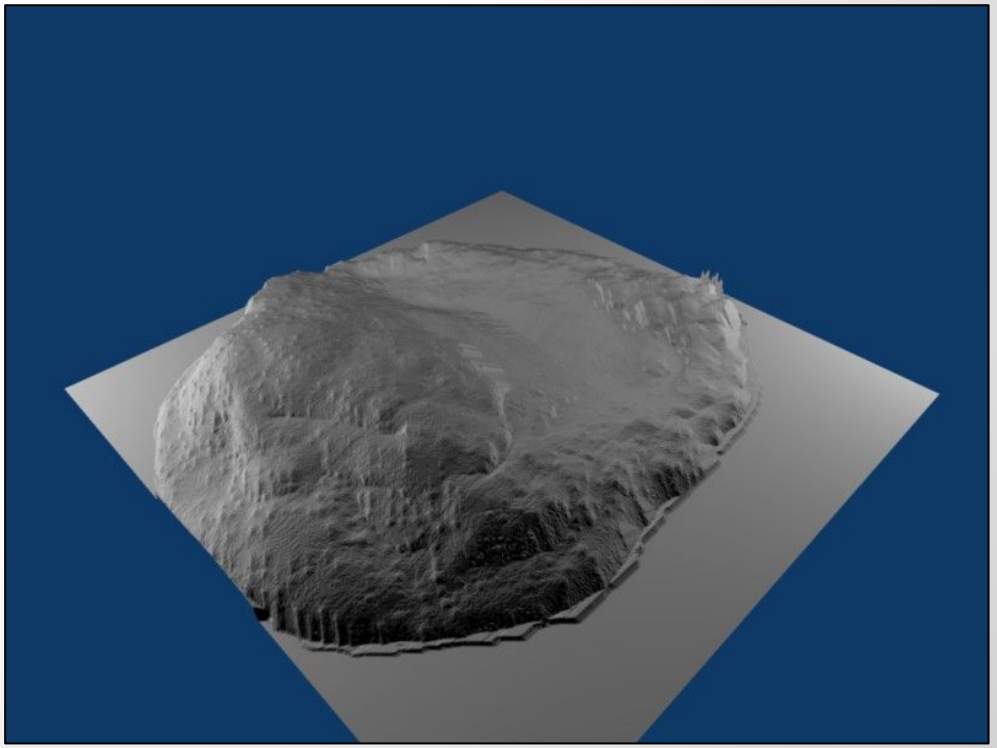
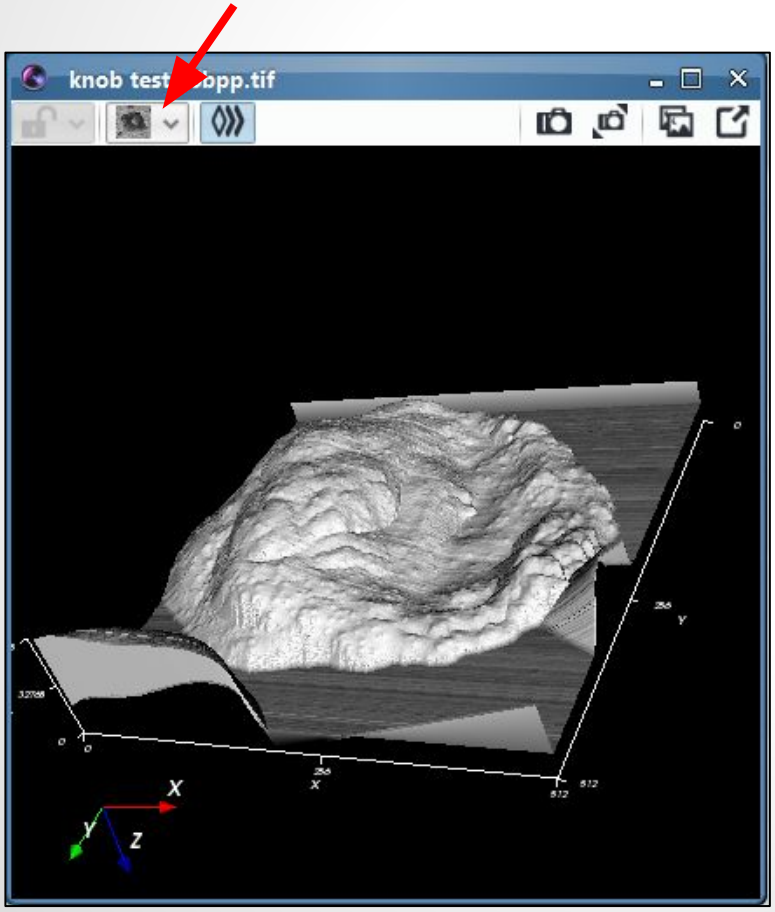


Image rendered with Blender

Representing an image on screen

The image can be seen in 3D in Icy using the *3D Elevation Map* visualization mode.
This is not the same than the 3D VTK volume view !



Tips: Elevation map is a plugin ! It should be included in Icy by default but it may be missing if the plugin is not installed. That means visualisation modes can be extended by plugin. When you install a new visualization plugin, it will be directly integrated here.

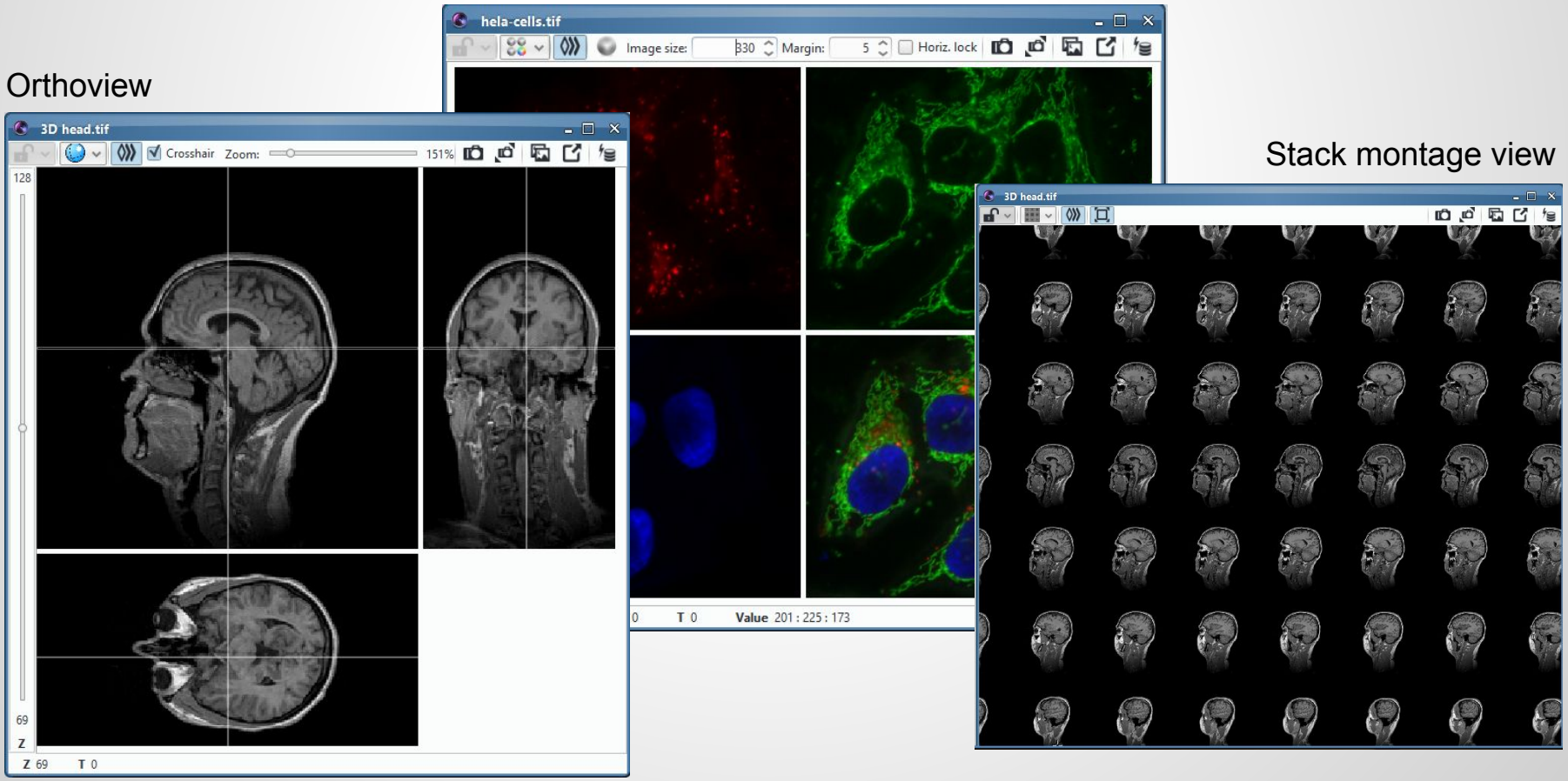
Others image representation..

Use the image representation you need !

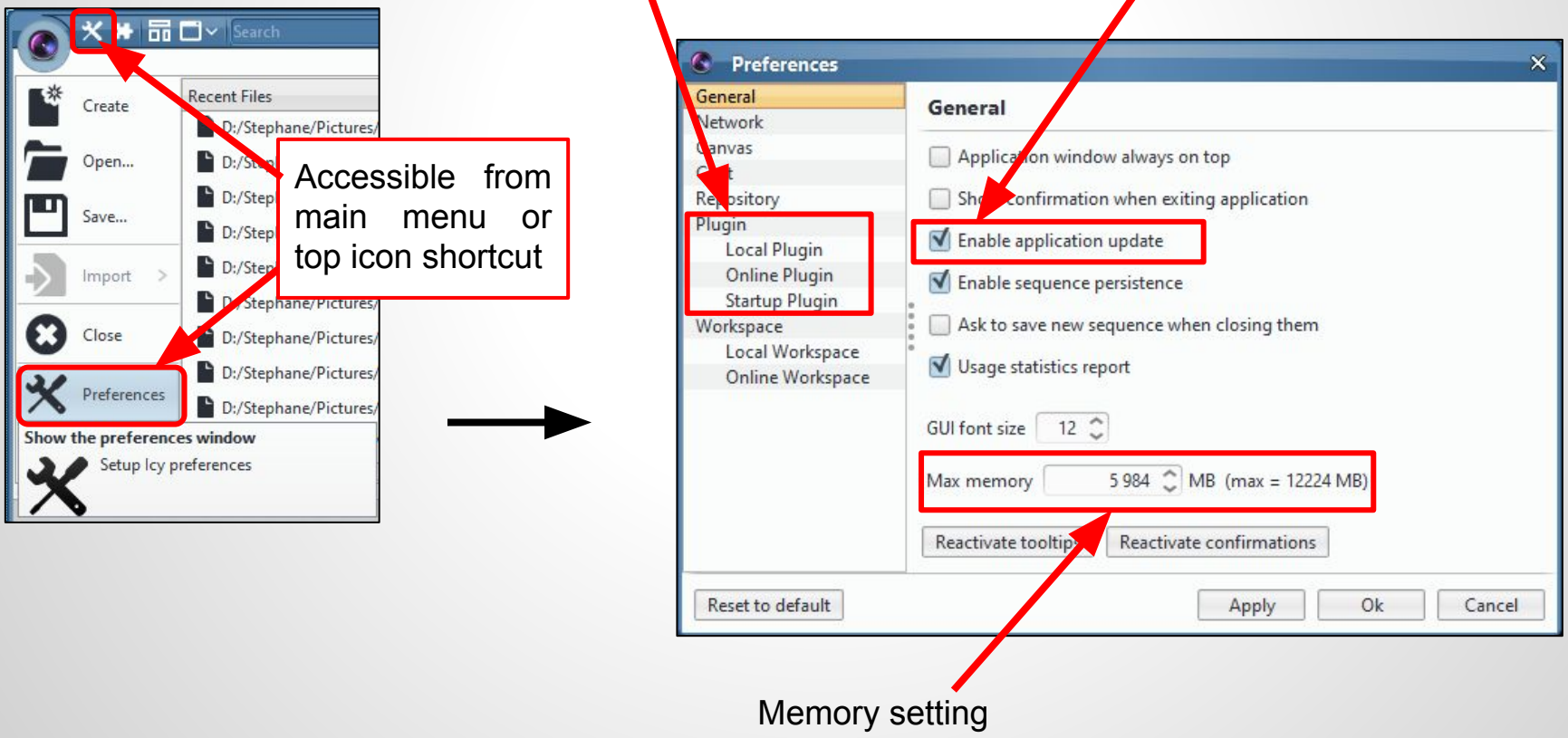
Channel montage view

Orthoview

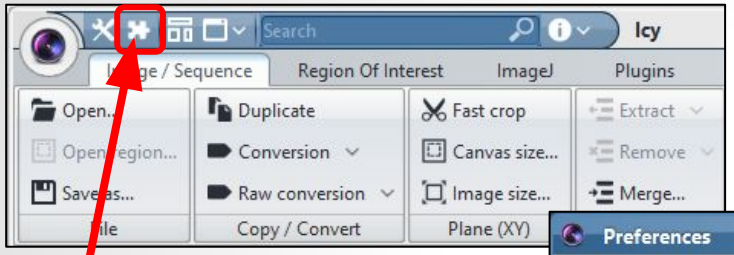
Stack montage view



Icy preferences



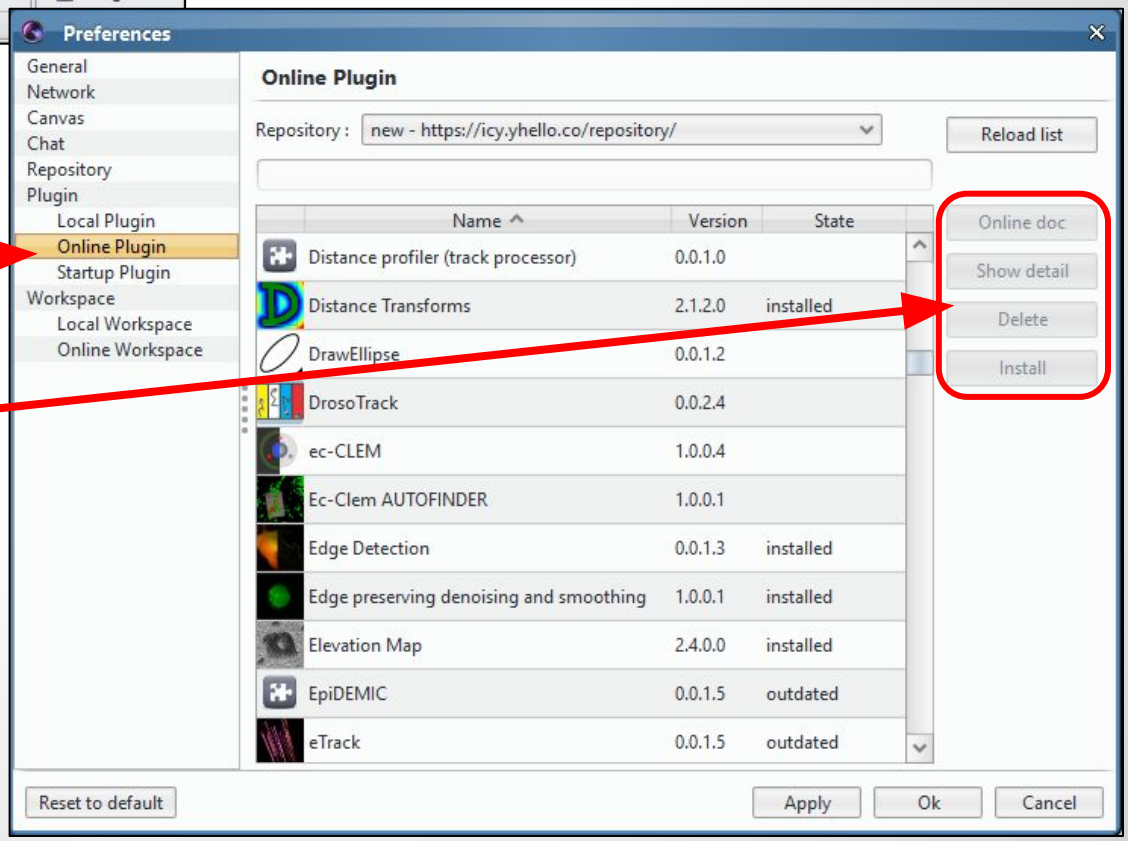
Icy's plugins



All plugins are centralized on a single website

Online plugins can be browsed using the top icon shortcut or *Online Plugin* section in Preferences

Access online documentation, Delete, update or install new plugin

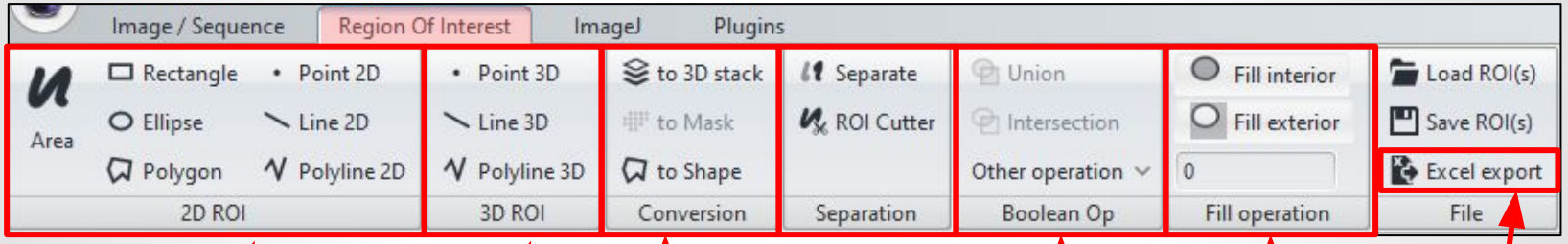


Tips: You can install plugin and access its documentation directly through the search bar !

Region of Interest

ROI (Region of interest)

ROI are a very important aspect in Icy, they will give you quantification results from your image. Almost all plugins generate ROI as results, and some also use ROI as input information.



2D ROI tools, this is the common ROI we use

3D ROI tools, we use these ROIs only in very specific cases

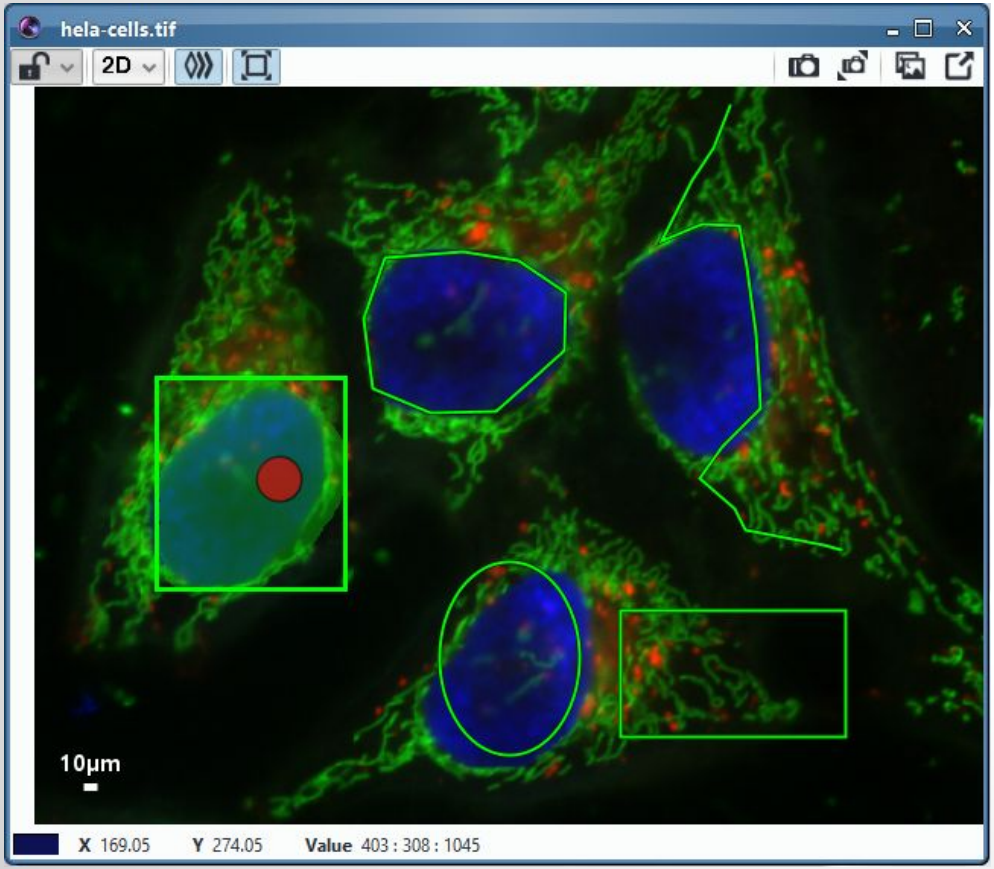
ROI conversion tool, allow to convert 2D to 3D roi. Transform mask to polygon and vice versa

Boolean operations between ROI

Used to paint image inside or outside ROI (rarely used)

Export all ROI information to an excel file (see later)

ROI (Region of interest)



- Open an image (hela-cells.tif)
- Draw each type of 2D ROI
- Try to remove a point then adding a new point to the *Polygon* ROI
- Try changing pencil size and erase some part in *Area* ROI

Tips

- Use the ROI tooltip (when you let mouse cursor over ROI tool) to know how to interact with the ROI.
- Use Ctrl+Z to undo the last operation and Ctrl+Y to redo it.

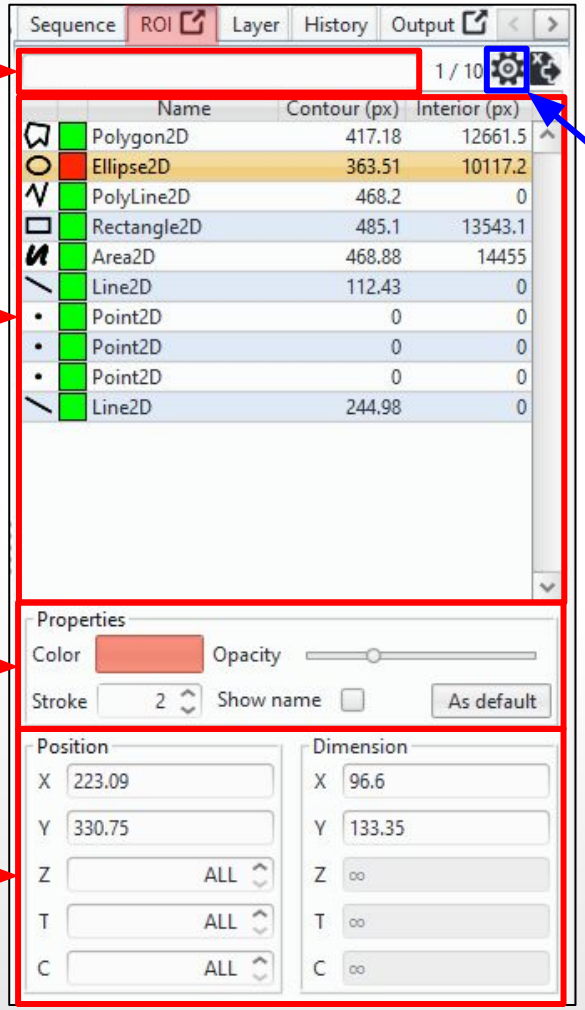
ROI (Region of interest)

Filter ROIs on their name

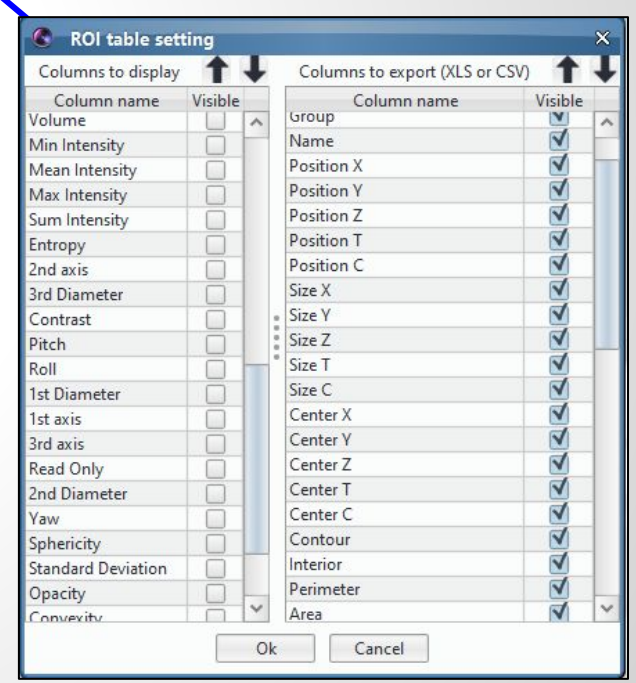
ROIs table displaying various information about ROI

Visualization ROI properties

ROI position and dimension
Note that ROI has 5D position



Configure ROI fields to display in the ROI table and in the ROI Excel export



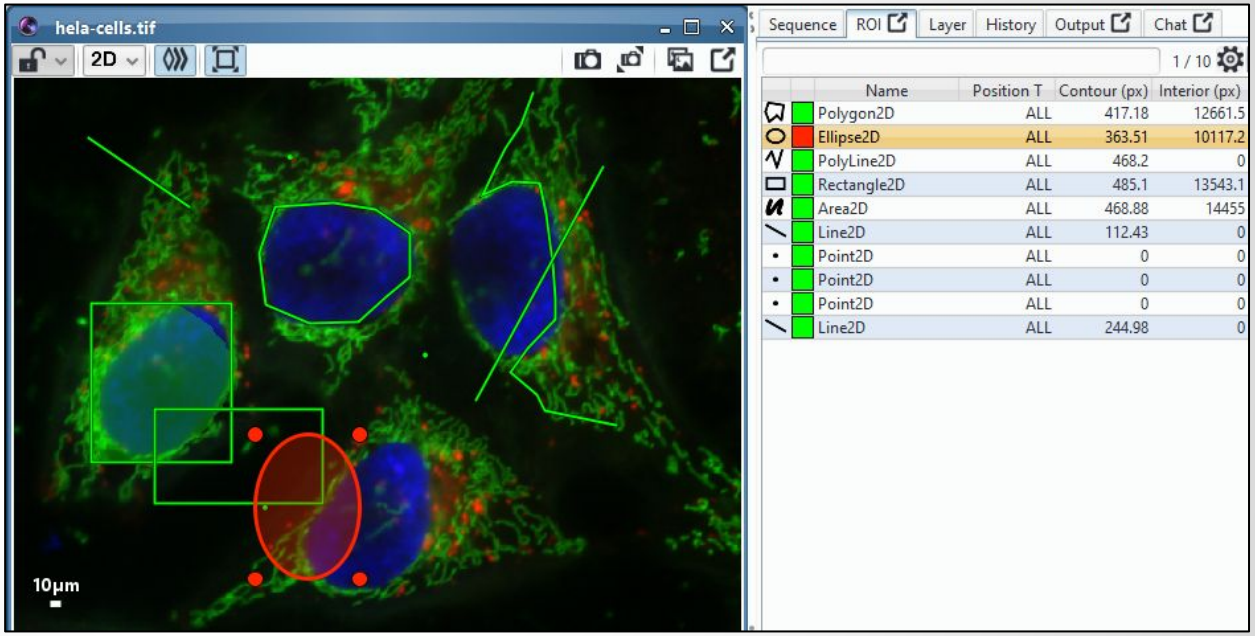
This list is not fixed and can be extended by plugin

ROI (Region of interest)

All ROIs can be selected even if they are overlapping

The ROIs can be colored and renamed

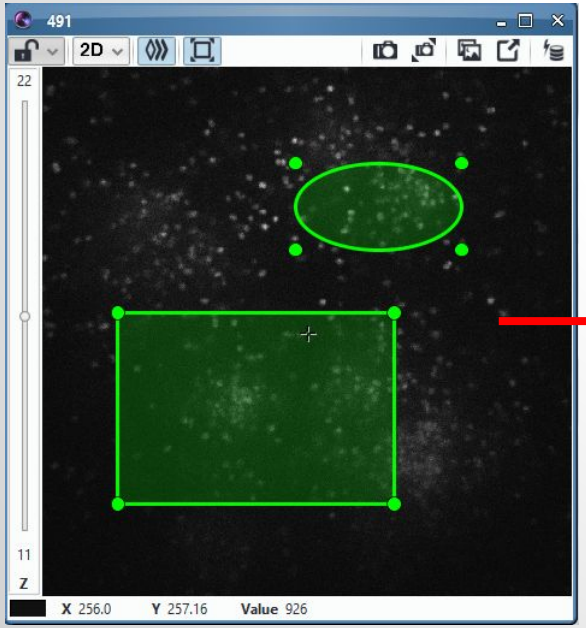
ROIs are persistent, this means that you don't need to worry about saving them: they reappear automatically as you reopen the image.

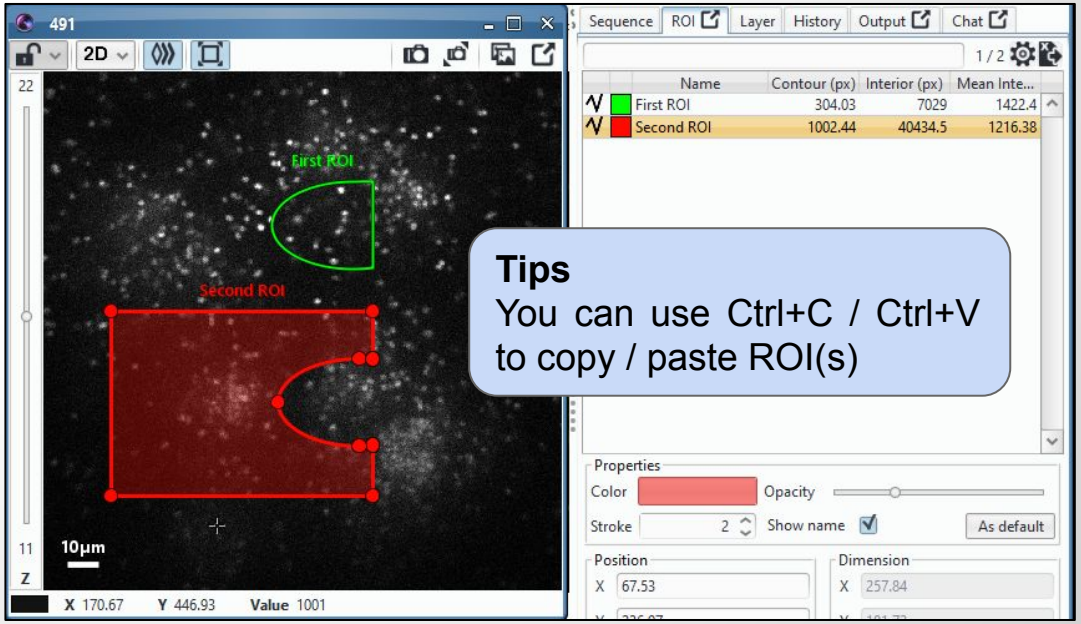


- Move / Edit the existing ROIs
- Rename ROIs, change their colors.
- Close a sequence and reopen it, you will see that ROIs are restored !

ROI (Region of interest)

- Open ‘3D stack.tif’ and try to build these 2 ROIs using only 1 Rectangle and 1 Ellipse together with the Boolean Operation.
- Add the field ‘mean intensity’ in the ROI table
- Set the Z position of the second ROI to 5, why does the mean intensity value change ?





ROI (Region of interest)

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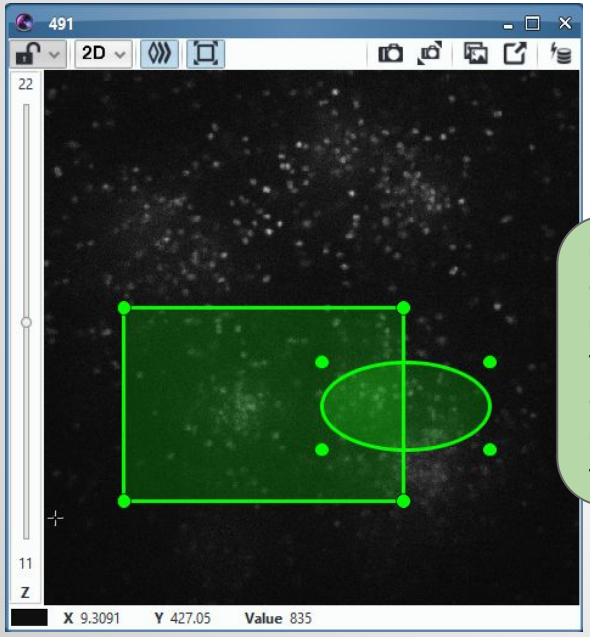
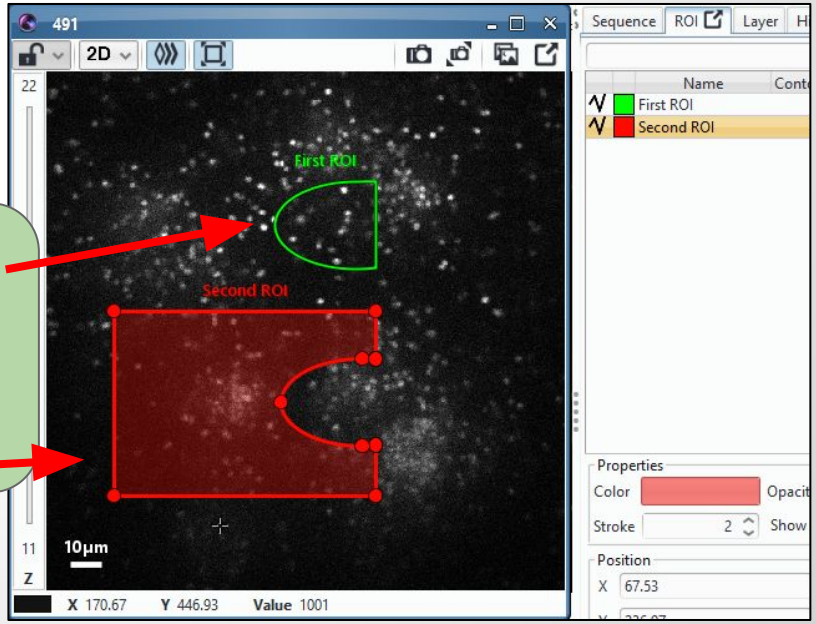


Solution
- Use “Intersection” command in “Boolean Op” for the first ROI
- Use “Subtraction” command in “Boolean Op” for the second ROI



ROI (Region of interest)

ROIs are 3D friendly !
You can see them and you can also interact with them

- Duplicate view
- Set second view mode to 3D
- Try to move ROIs on a view and the other

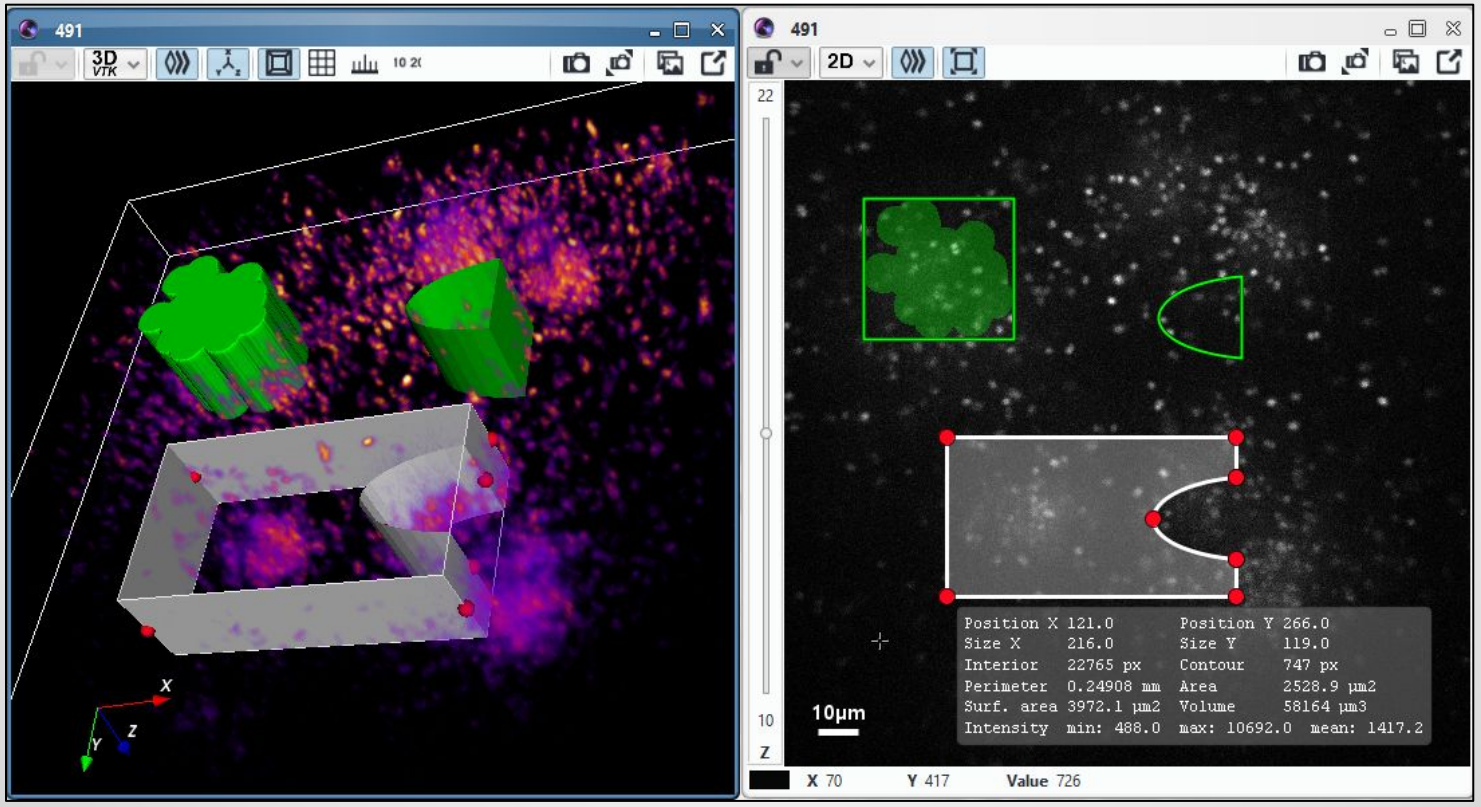
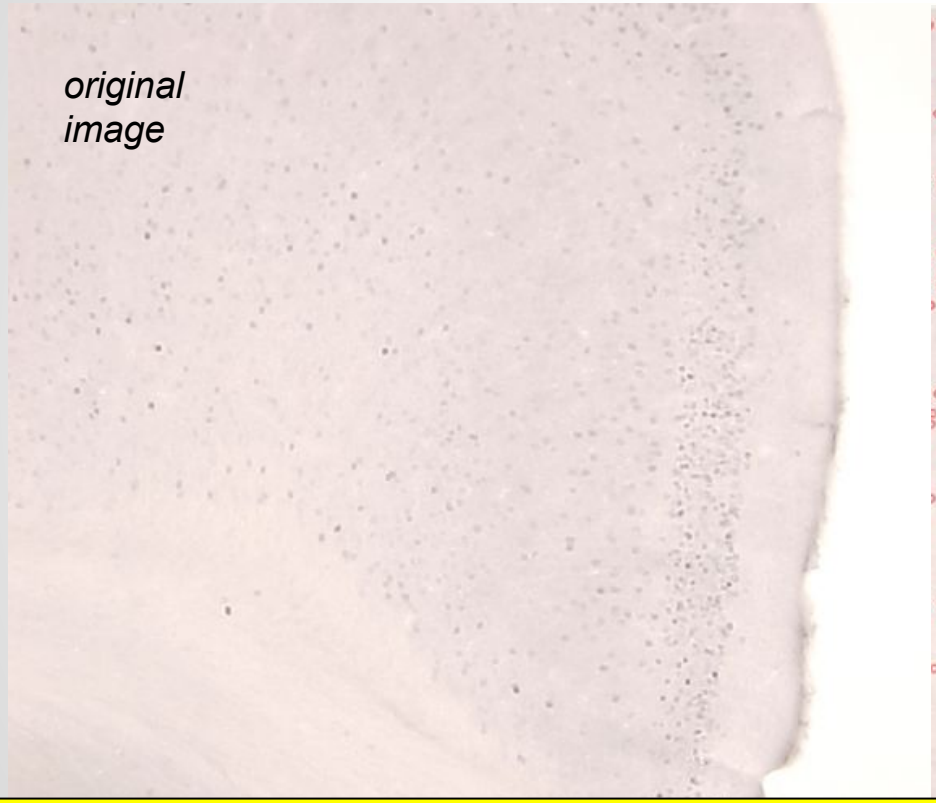
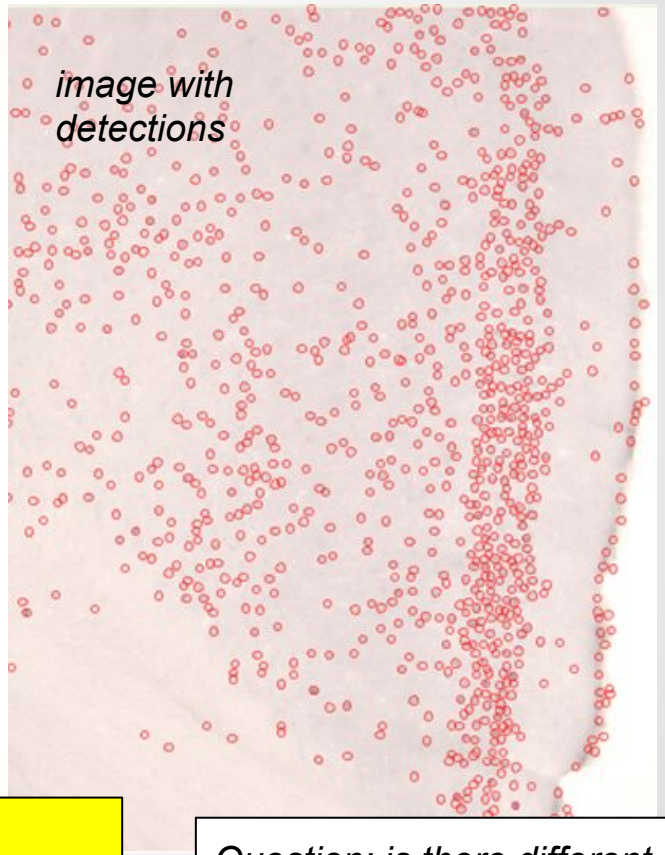


Image analysis examples

Spot detection



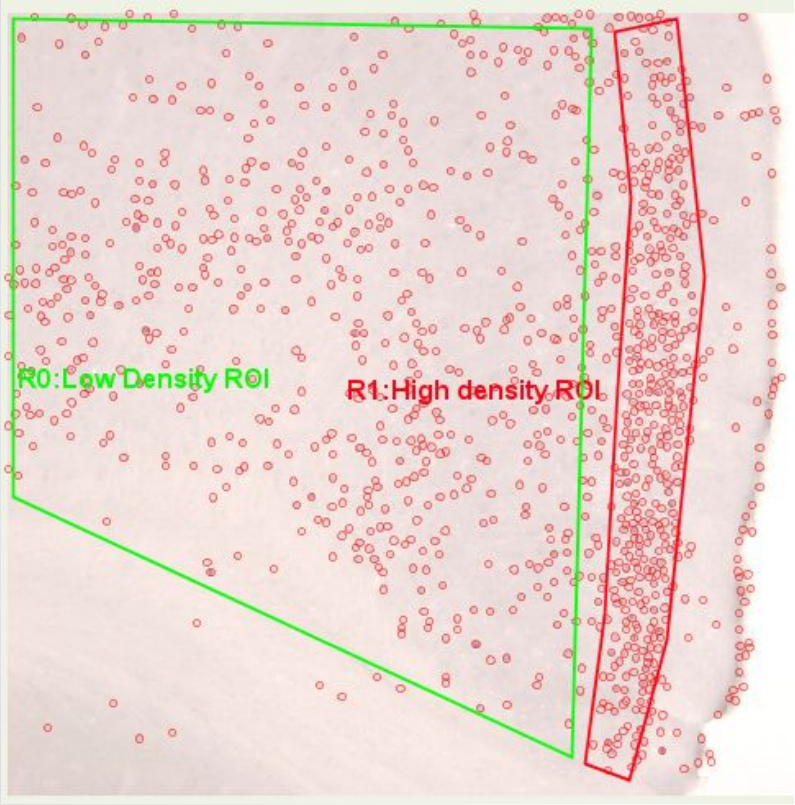


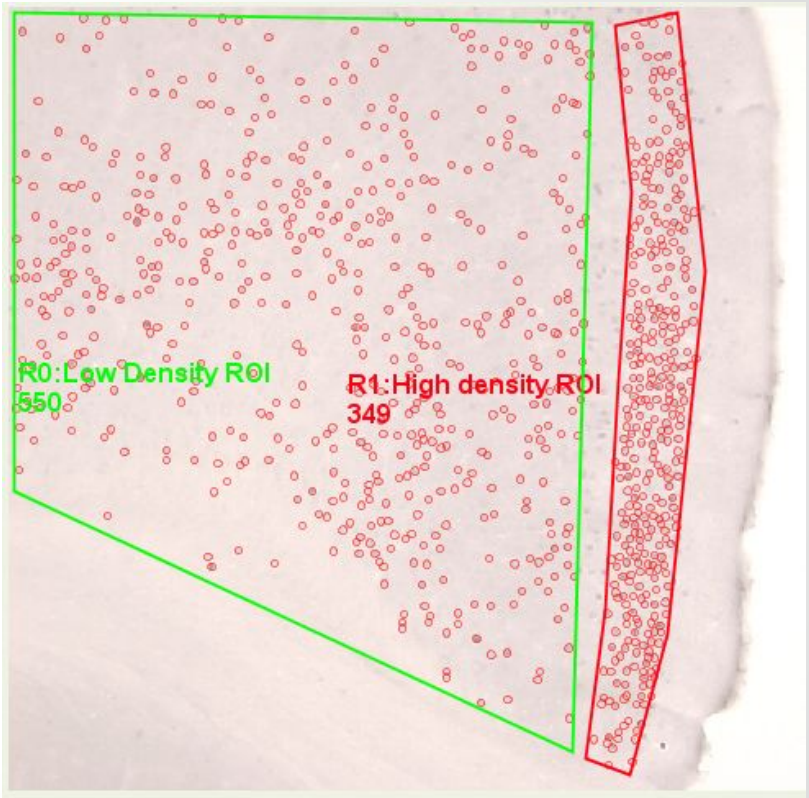
- Load the file *P7.JPG*
- Launch the plugin "*spot detector*"
- Click "*start*"

Question: is there different densities of detection over the image ?

- Yes, and we will quantify it !

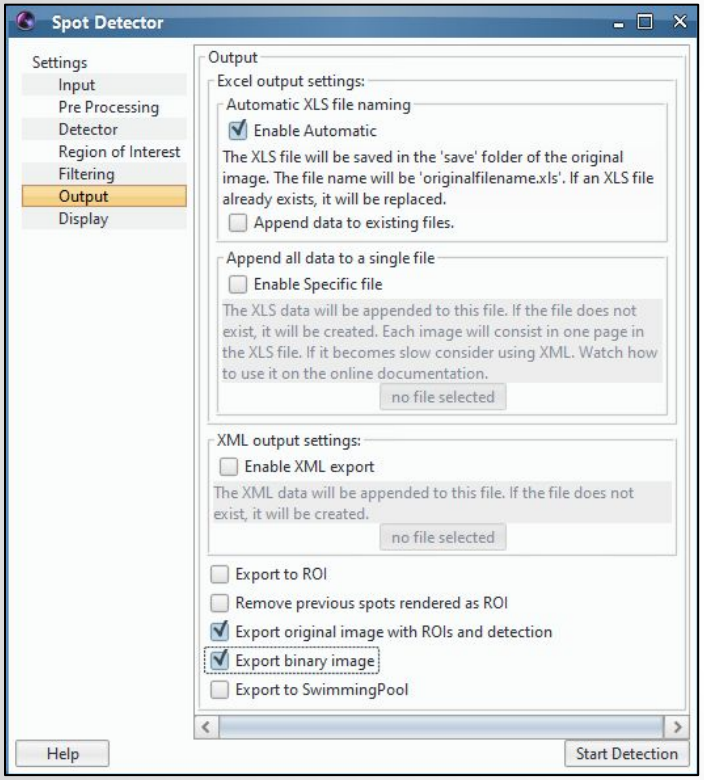
Spot detection using input ROIs



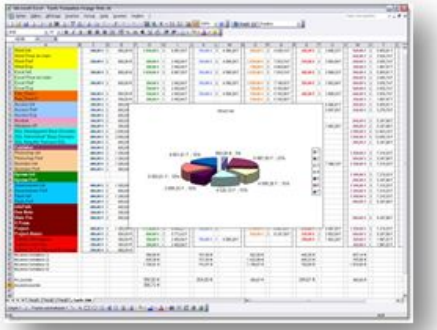


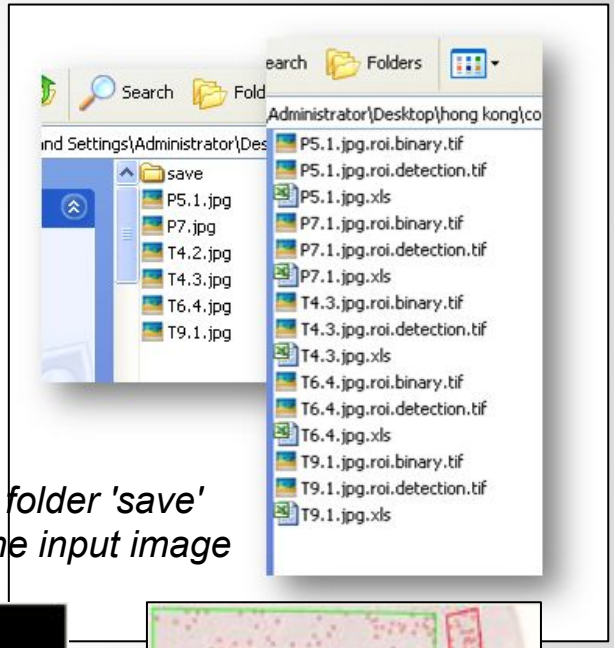
- Draw 2 ROIs corresponding to each area.
- Restart the detection (click *start* again*)*
- Detection are now linked to the ROIs

Example of quantification using input ROIs



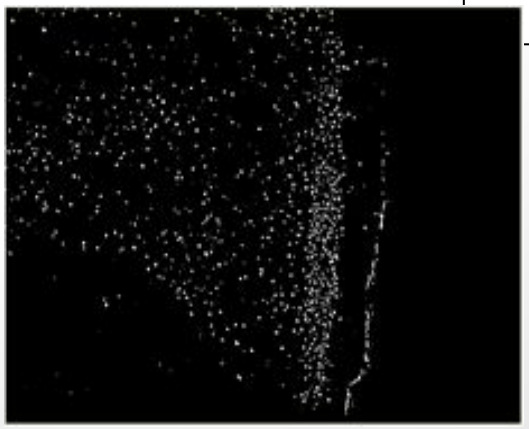
Excel



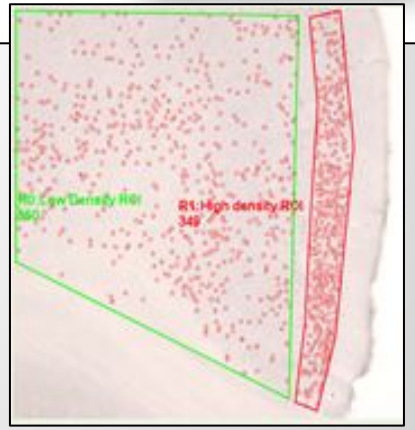


sub folder 'save' of the input image

- Enable XLS export from Spot detector
- Enable export original and binary images
- Restart the detection (again)



Binary image



Original Image with ROIs and detection

Density quantification using input ROIs

Excel

Pre processor					
Pre proces	Band selector				
Band Sele	0				

Detector					
Detector:	UDWT Wavelet Detector				
Parameters:					
Scale 1	Disabled	Threshold:	100		
Scale 2	Enabled	Threshold:	100		

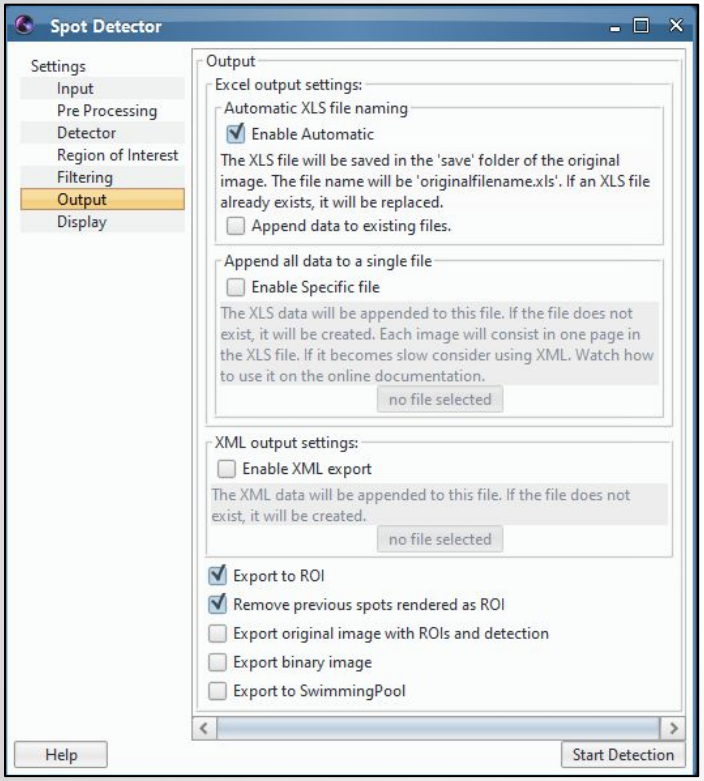
Region of interest					
Region of i	ROI From Sequence module				
ROI numbe	ROI name	ROI surface	ROI nb detection	ROI tag(s)	Density
0	Polygon2D	333626	569		0,001706
1	Polygon2D	56700	367		0,006473

Add a density column in excel result:
Density = nb detection / surface

Question:

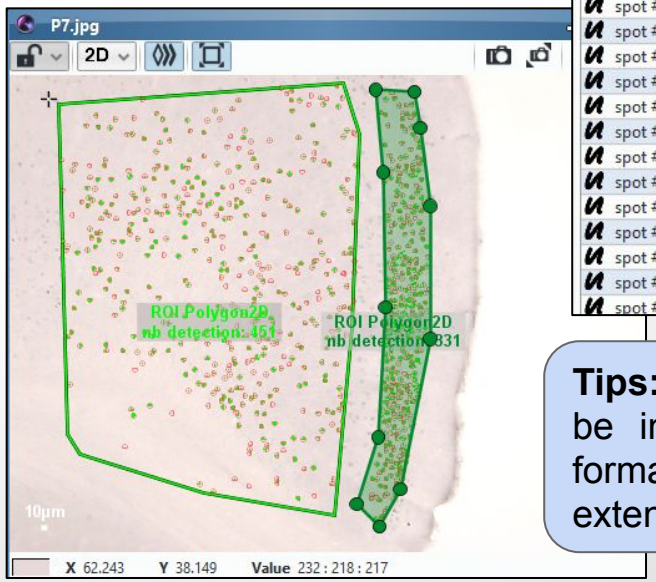
- why we all have different density ?
- why we have more variation on the high density ROI ?

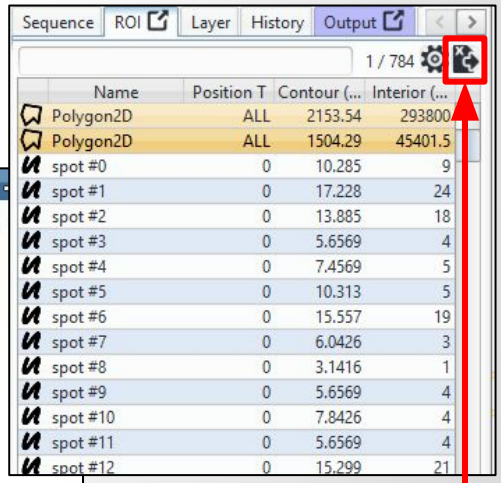
Good practices in Icy : get results as ROI(s)



- Remove XLS and images export
- Enable ROI export
- Restart the detection

Spot detector is a “all in one” tool (for historical reasons) but it’s better to just use it to detect spot as ROIs then complete / customize your analysis workflow using “Protocols”.

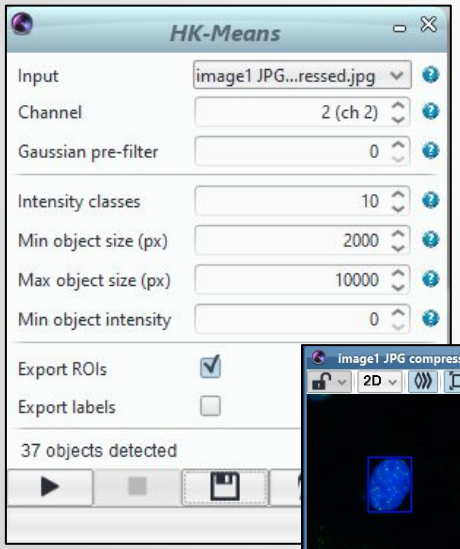


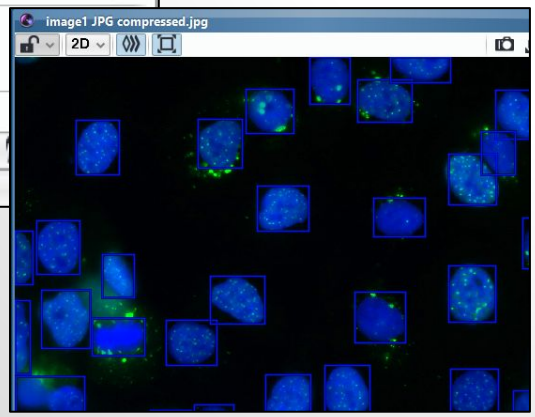


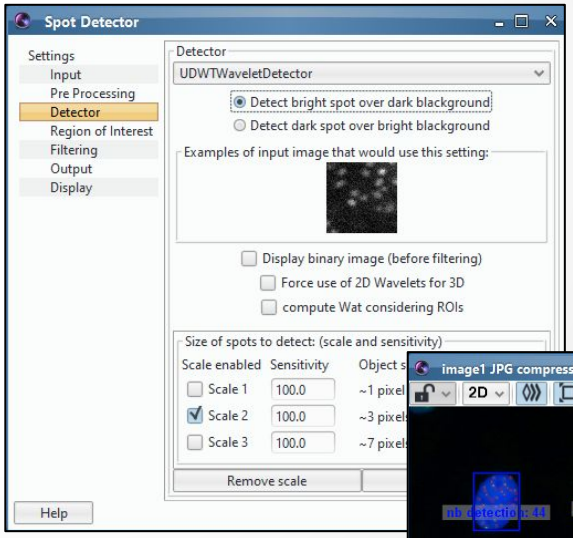
Tips: ROI Excel export can be in XLS or CSV / TXT format depending the file extension we provide.

Spot detector - detect in cells only

- Open ‘image1 JPG compressed.jpg’
- Use HK-Means plugin to detect cell on channel 2 (do it together)
- Then use Spot Detector to detect spots in channel 1 for each cell (don’t forget to set cell ROIs C position to ‘ALL’ before)

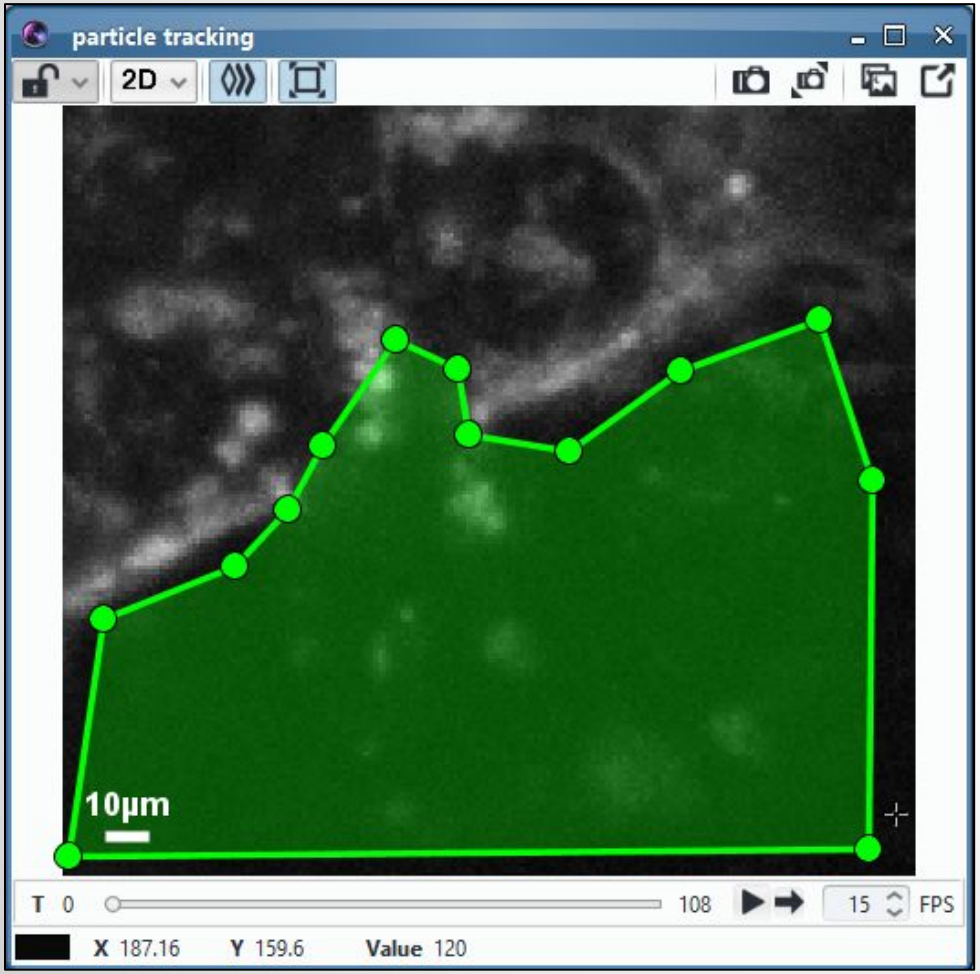






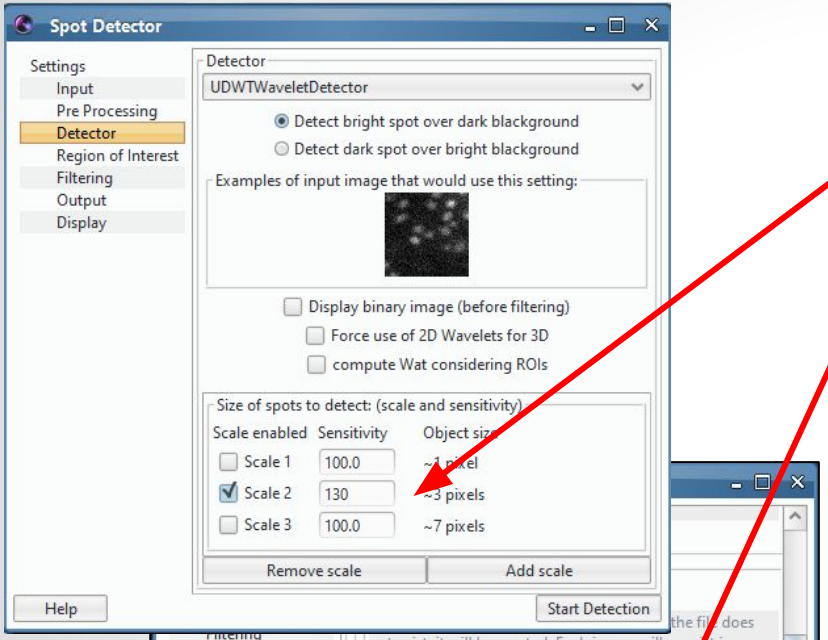
Tracking

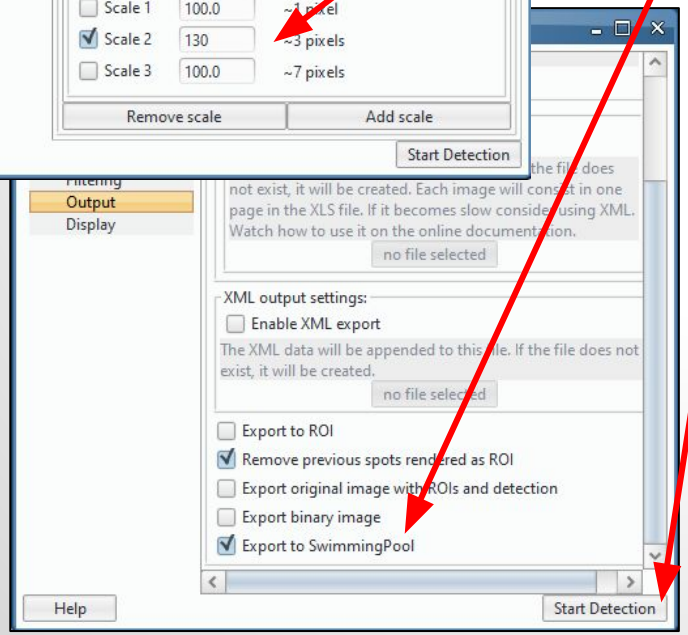
1. Create detections



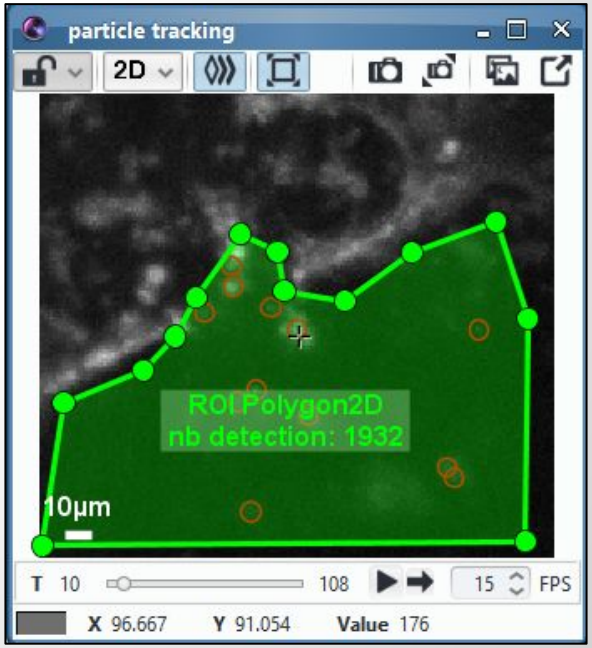
- Open ‘particle tracking’ folder
- Draw an ROI over the area where we want to do the tracking

1. Create detections

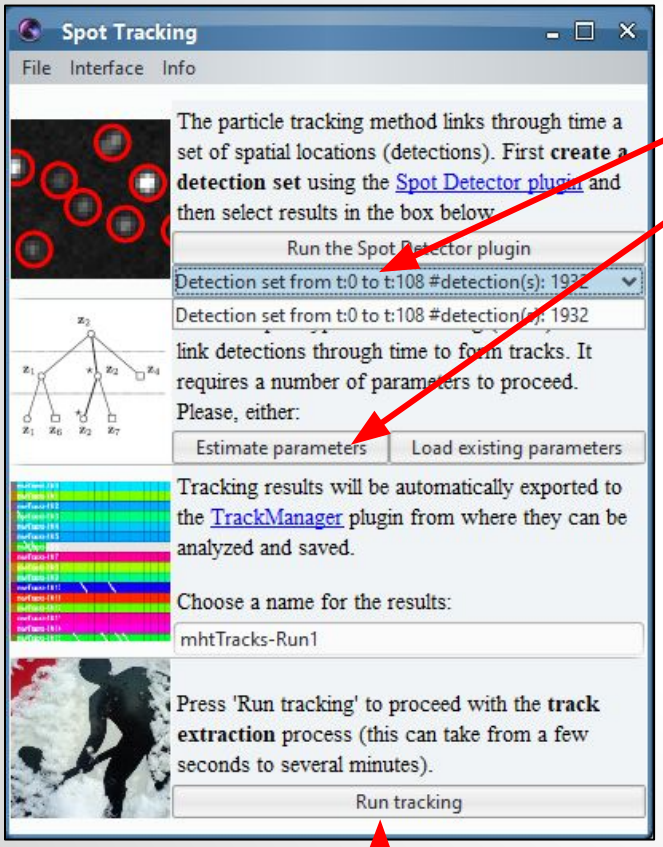




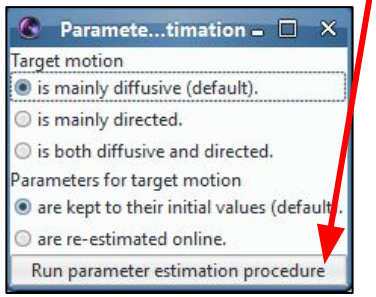
- Use the spot detector
- Set parameters for correct detection
- Enable the SwimmingPool export
- Start detection

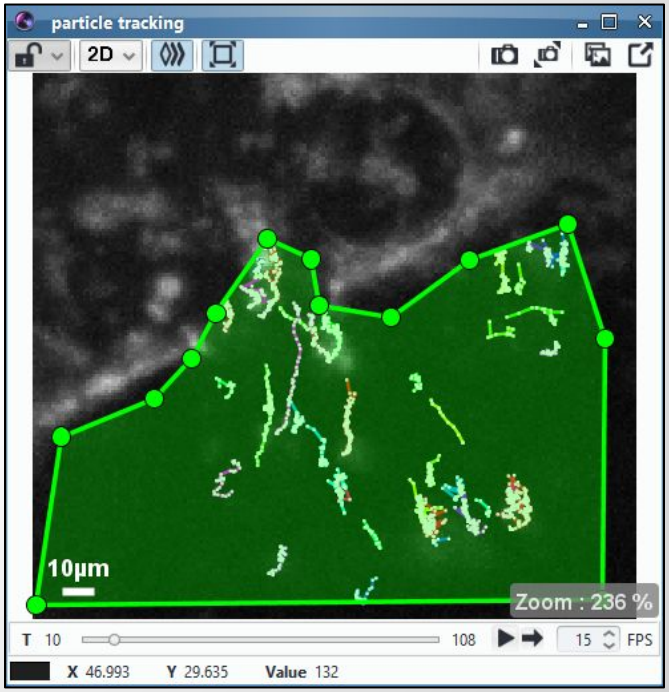


2. Link detections to create tracks



- Starts the Spot Tracking plugin
- Select the detection set we just made
- Start parameters estimation with default setting



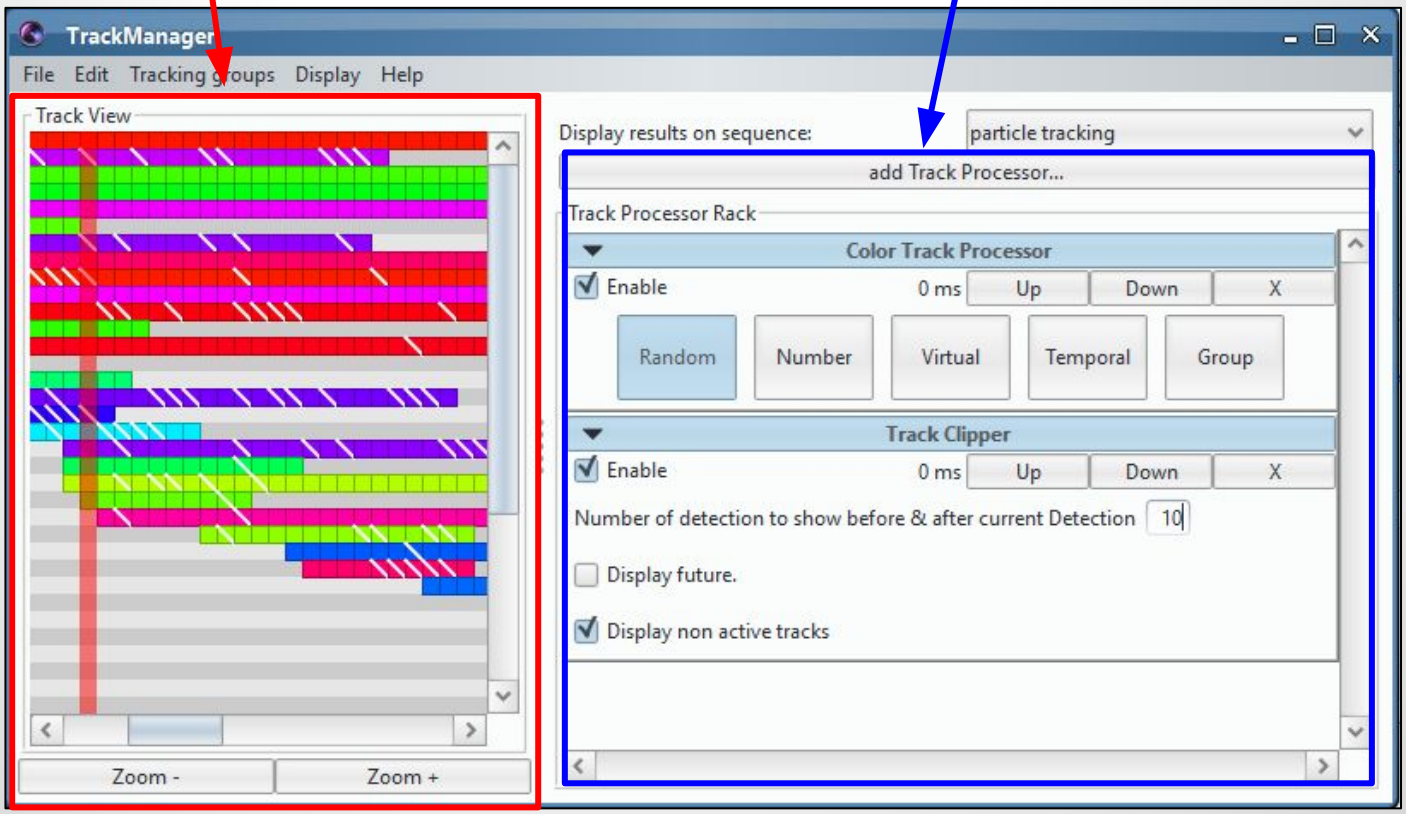


Then click '*Run tracking*' !

The track manager

Track View panel displaying all tracks

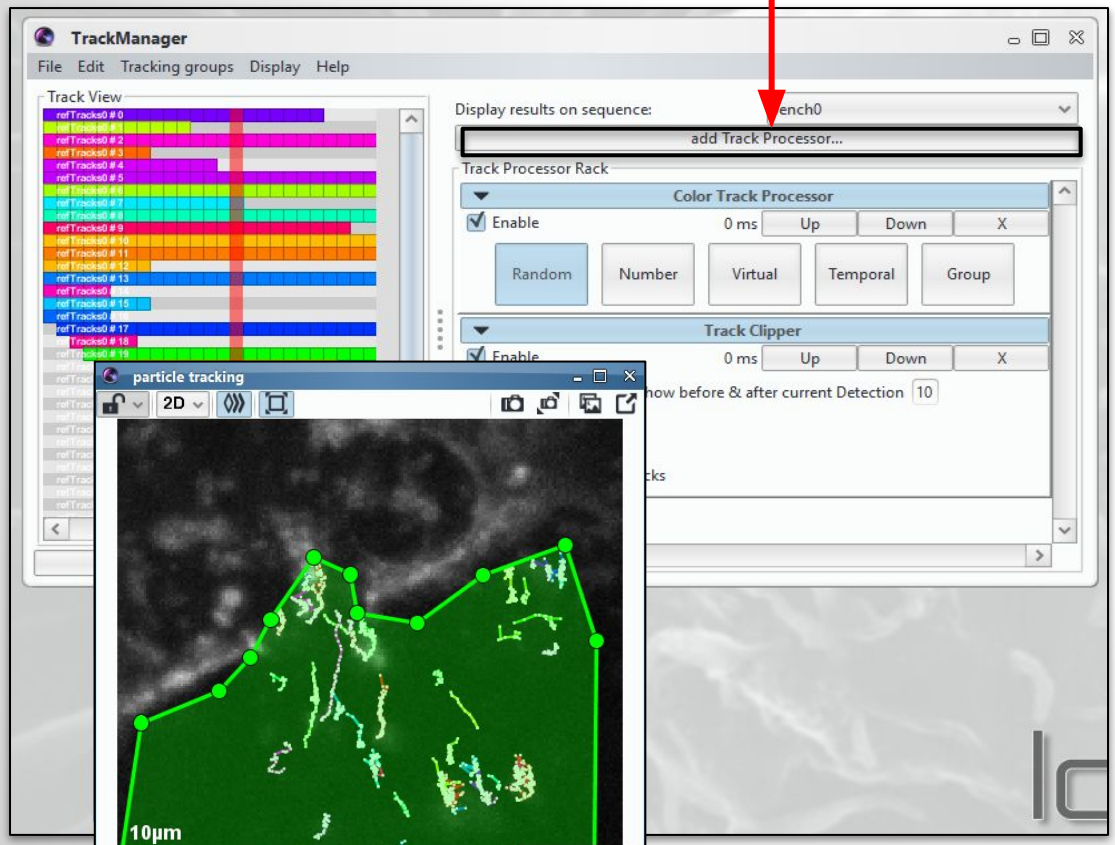
Track Processor Rack: allows customizing processing / visualization / quantification on obtained tracks.

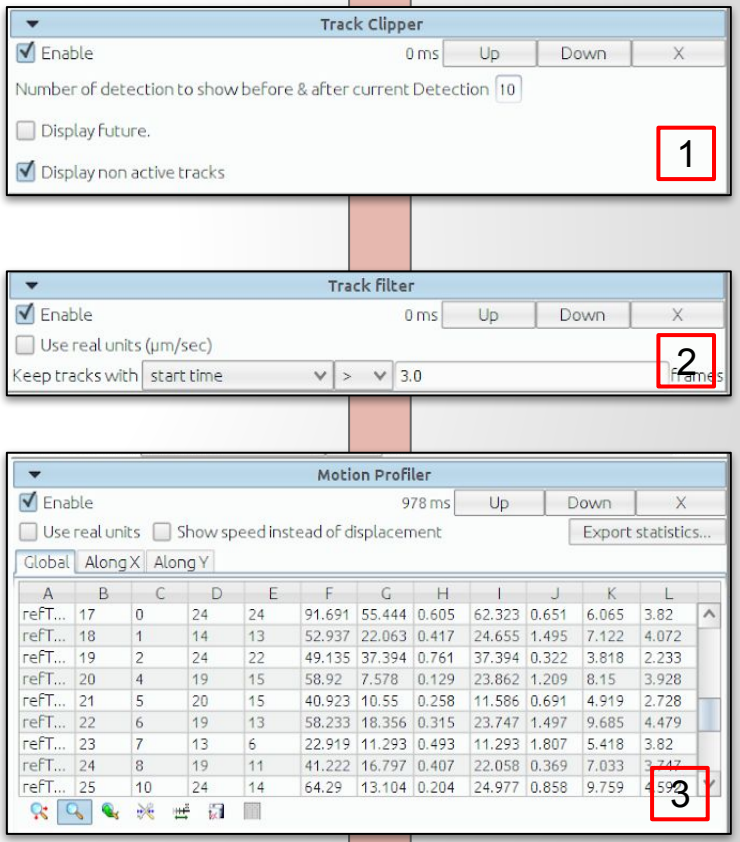


Track Processors

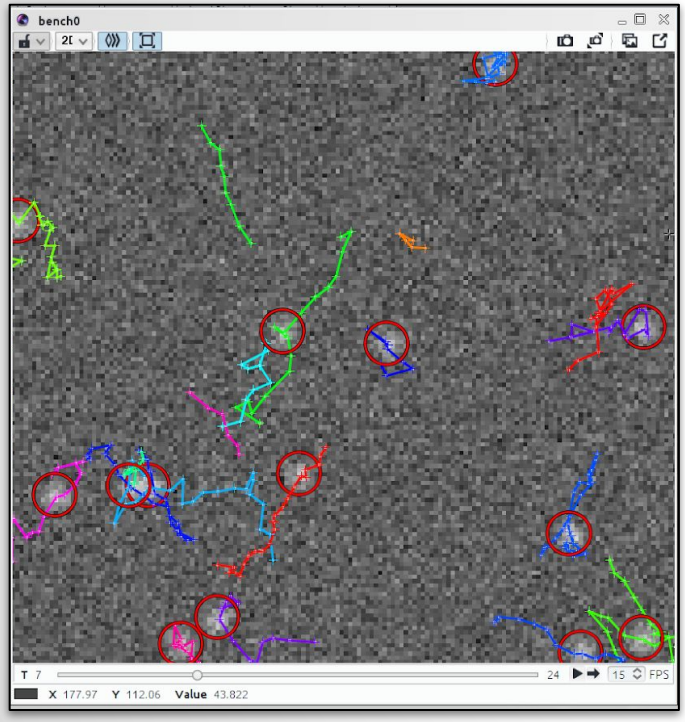
Button to add track processors

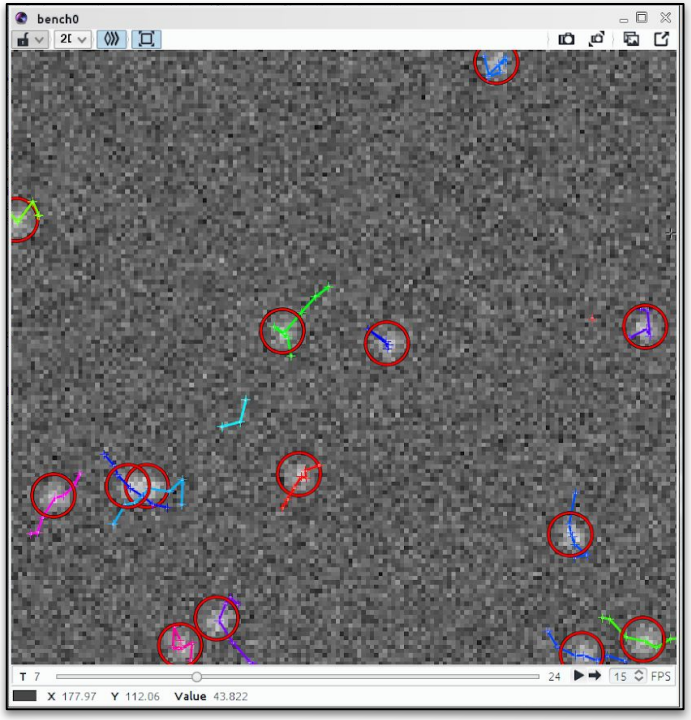
1. add “Track Processor Time Clip” and clip track display to 10 frames
2. add “Filter Track Processor” and filter out tracks with speed < 5 µm/s”
3. add “Motion Profiler Processor” to inspect track parameters

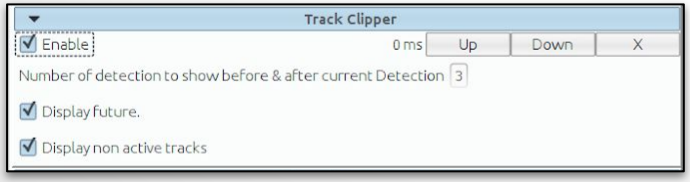


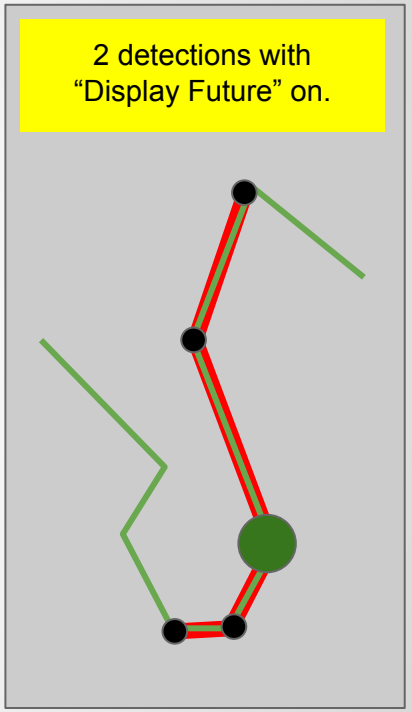


Time clip processor

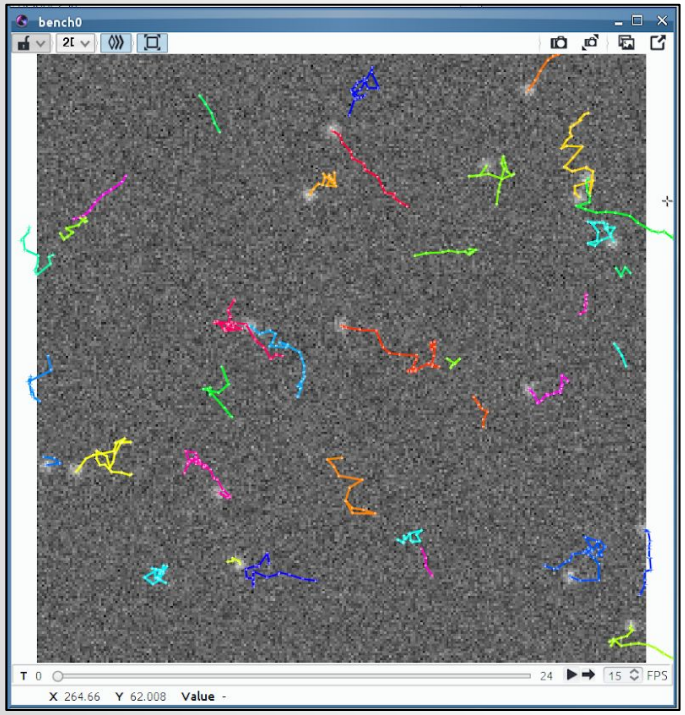


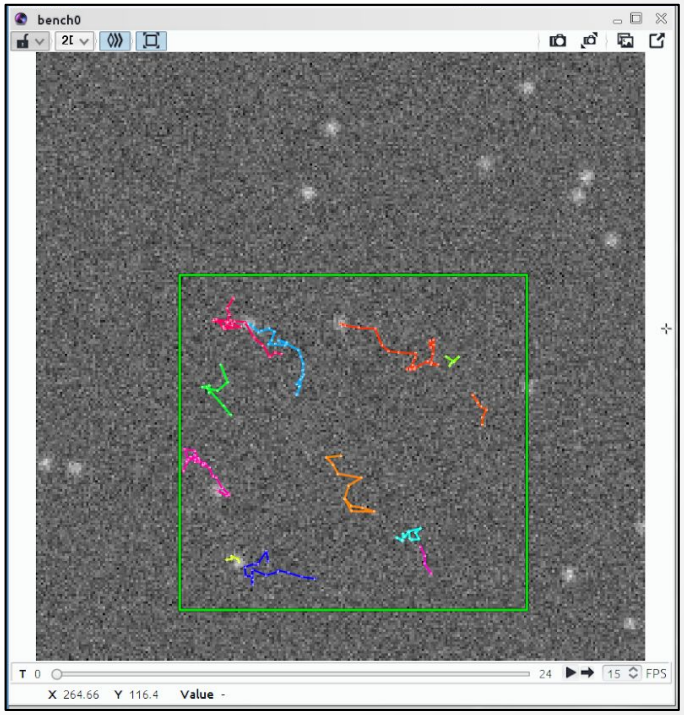




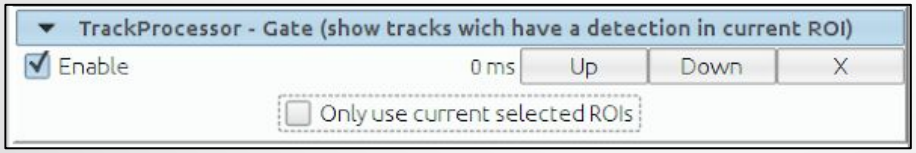


ROI Gate processor

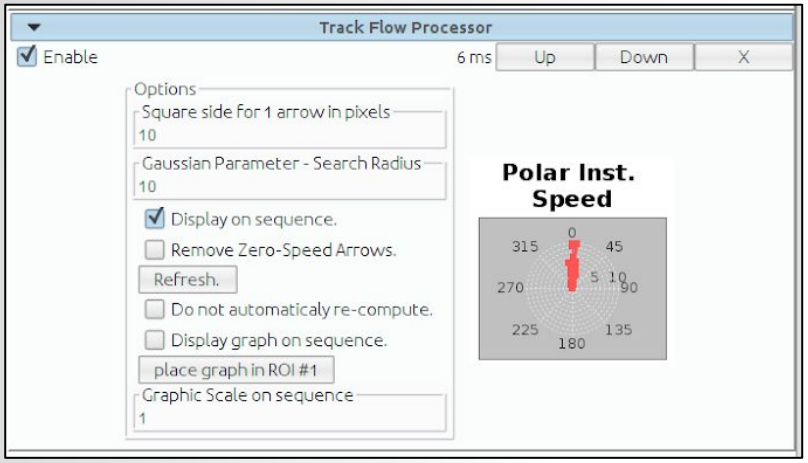


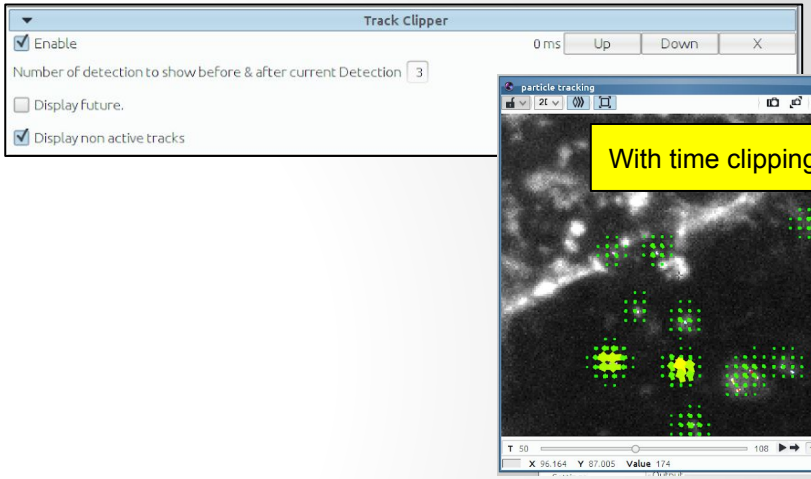


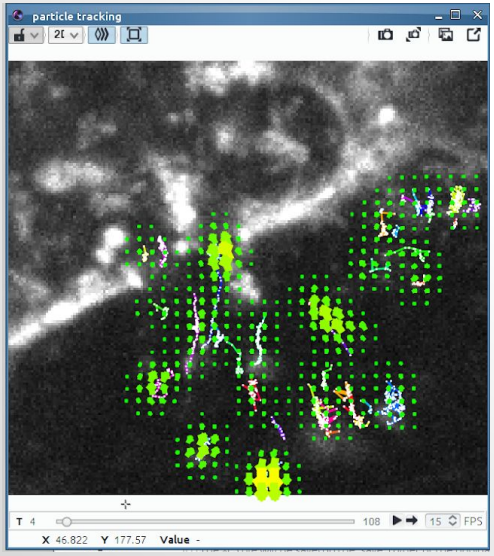
- add Track Processor ROI Gate
- Draw ROI

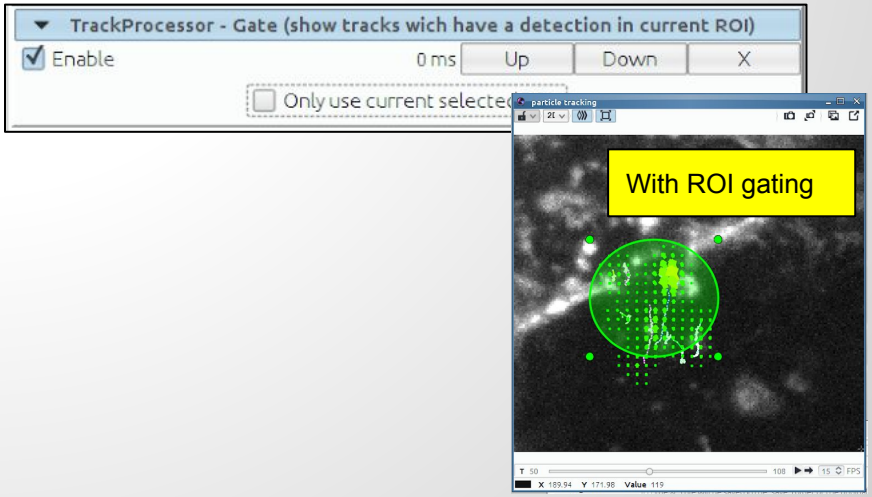


Flow track processor





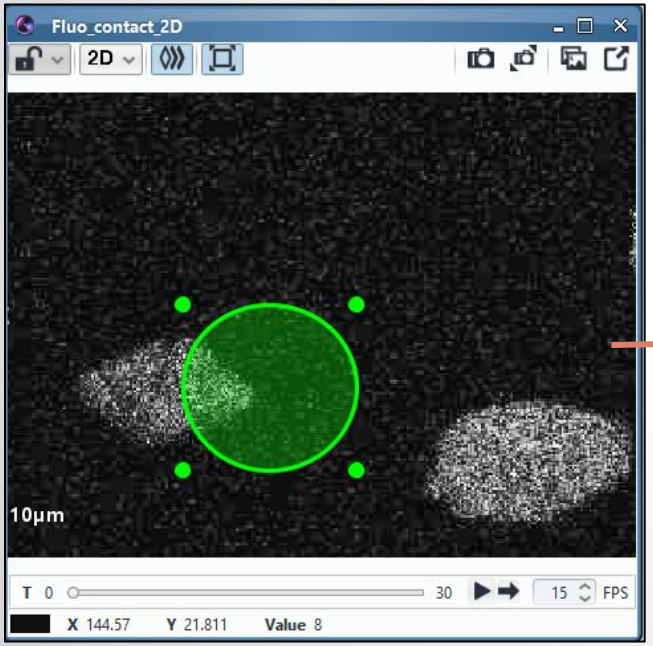


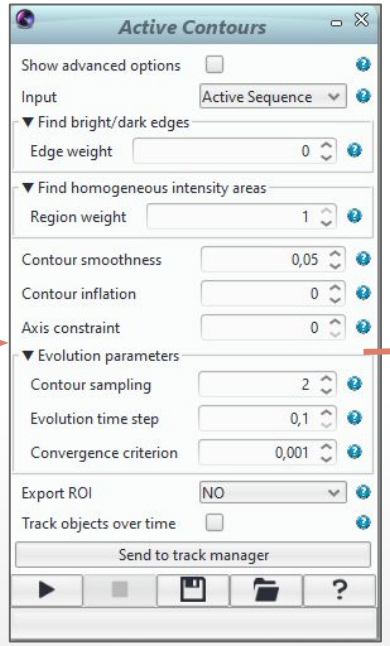


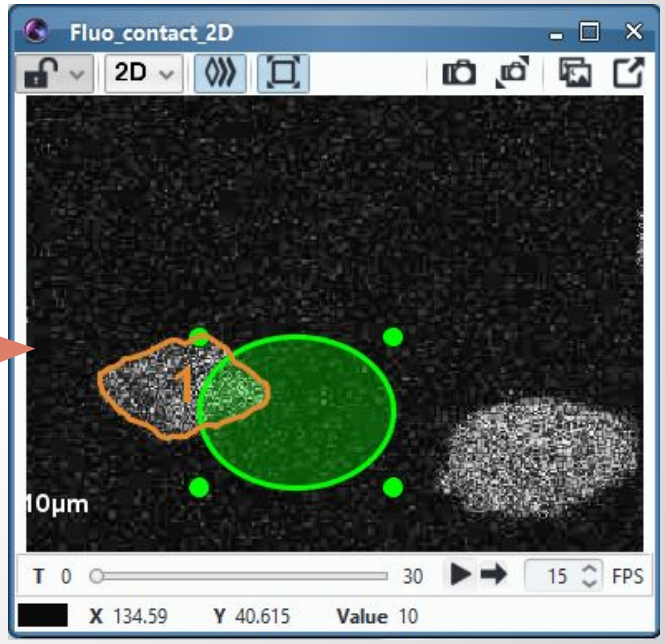
Active Contour

Active contours - noisy image

- Open "*Fluo_contact_2D.lsm*" from "*active contours*" folder
- Launch the "*Active Contours*" plugin
- Draw an ellipse overlapping partially left cell on the left
- Run "*Active Contours*" (default parameter using region)

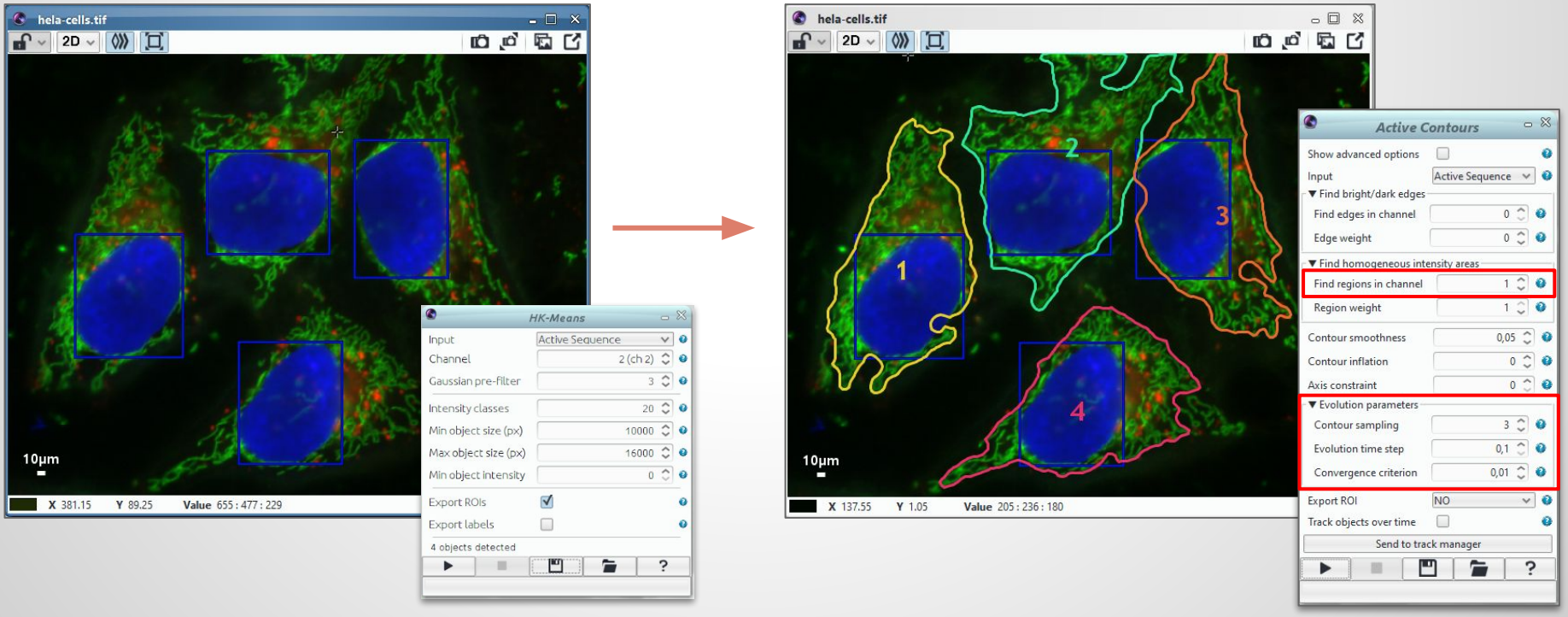






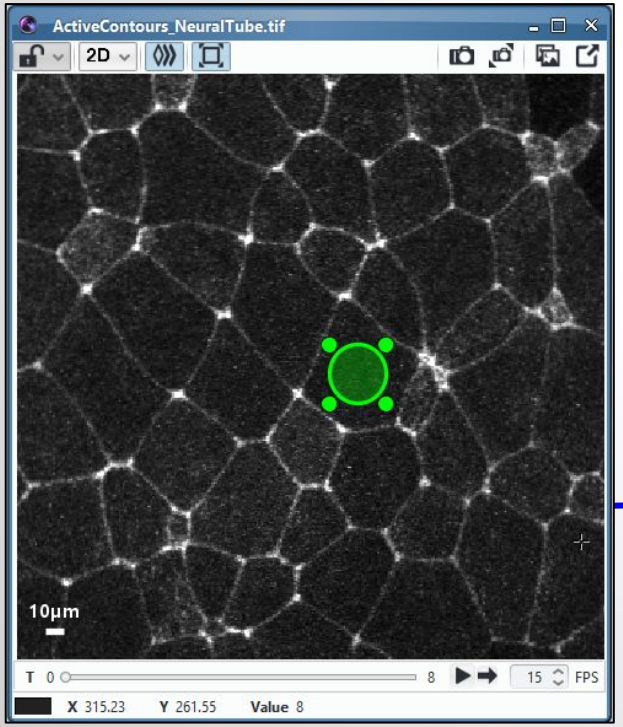
Active contours - object separation

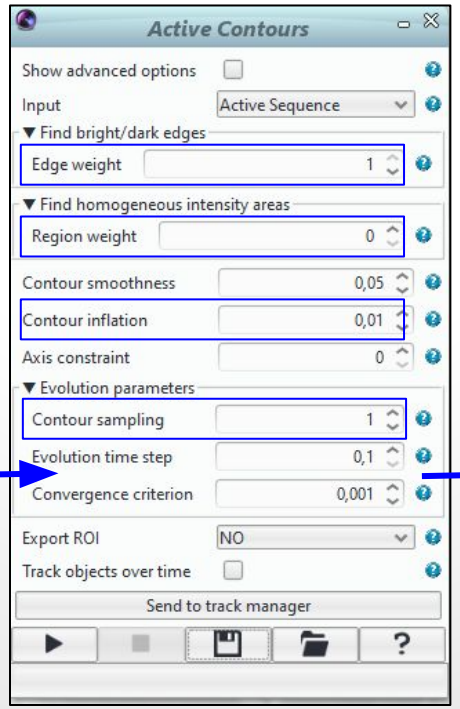
- Open “Hela Cells” image
- Use **HK-Means** to segment nucleus in blue channel
- Set “*channel region”* to 1 as we want to segment from green channel
- Set “*contour sampling”* to 3 to “*convergence criterion”* to 0.01 (faster)
- Run “*Active Contours*”

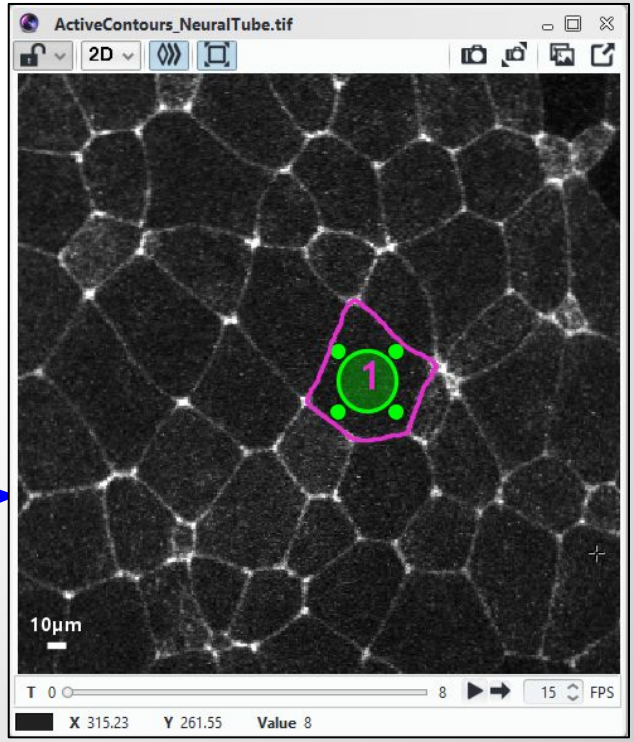


Active contours - find membrane

- Open "*NeuralTube.tif*" from "*active contours*" folder
- Enhance contrast with histogram and draw an ellipse inside a cell
- Set "*Region weight"* to 0 and "*Edge weight"* to 1 (contour attachment)
- Set "*Contour inflation"* to 0,01 and "*Contour sampling"* to 1
- Run "*Active Contours*"

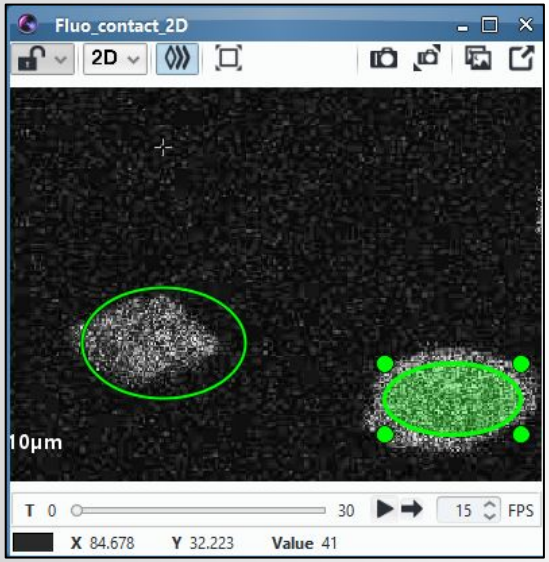


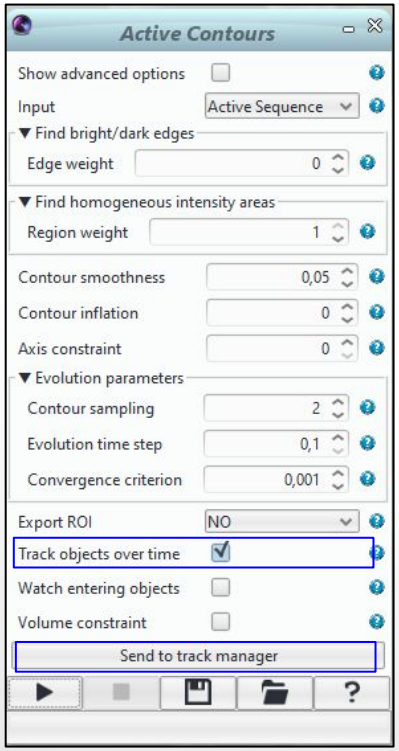


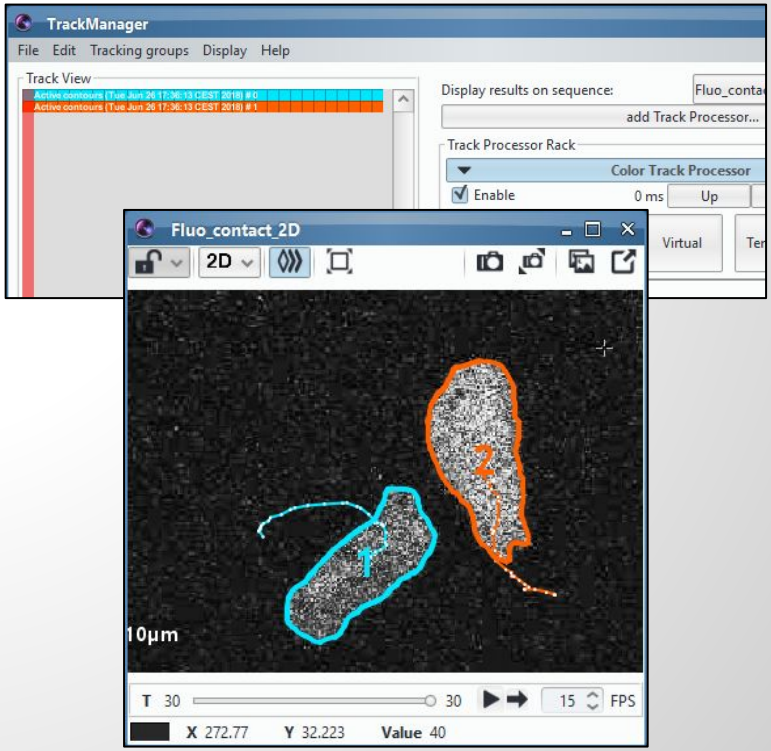


Active contours - tracking

- Open “*Fluo_contact_2D.lsm*” from “*active contours*” folder
- Draw 2 ellipses on the cells (initialization)
- Check “Track objects over time” parameter
- Run “*Active Contours*”
- Click on “Send to track manager” button



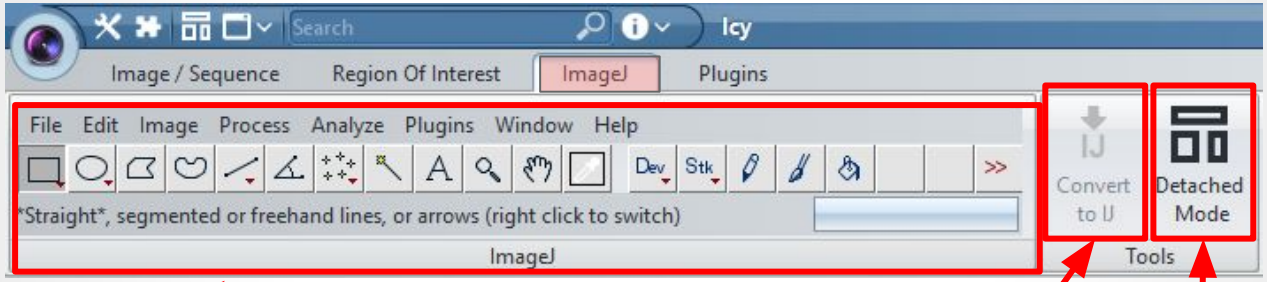




ImageJ in Icy

Use ImageJ inside Icy

You can use ImageJ directly from Icy, this make interaction between Icy and ImageJ very easy



This is ImageJ running in Icy !

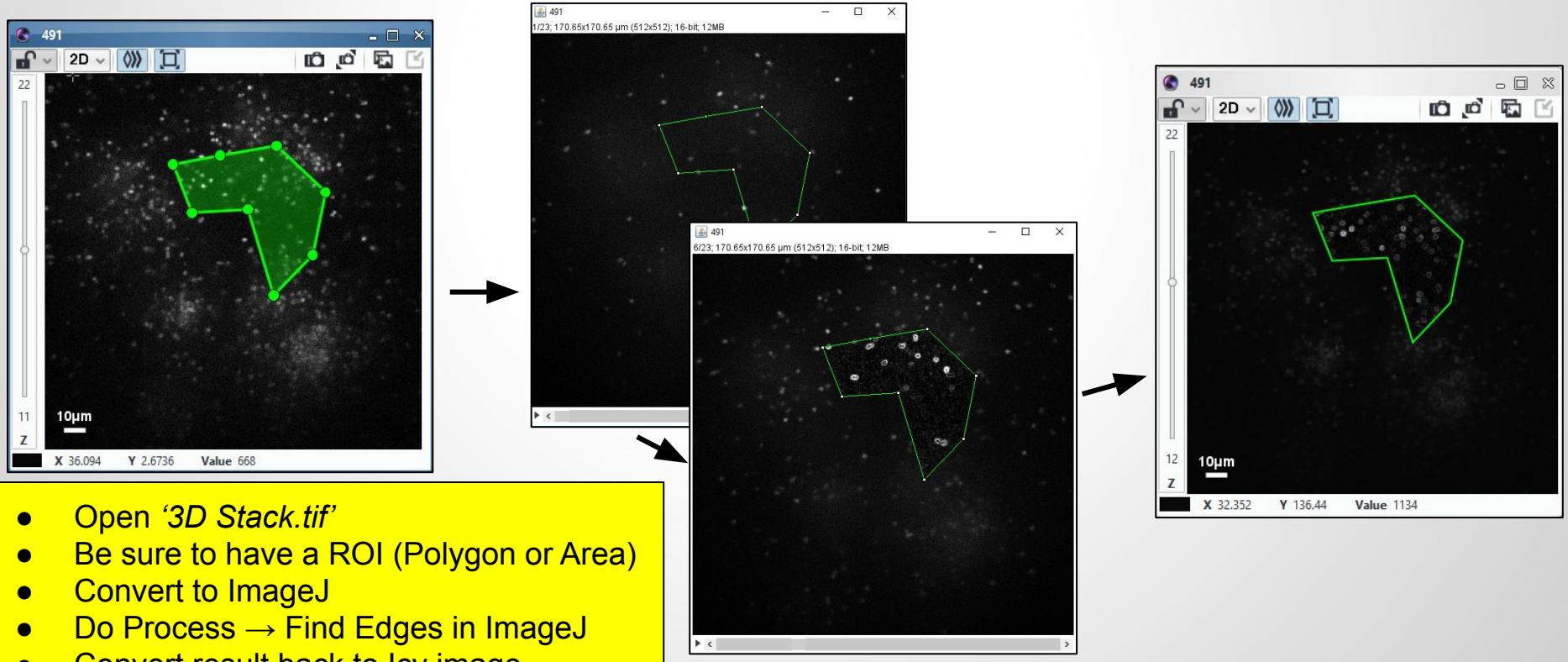
ImageJ to Icy / Icy to ImageJ image conversion

Better to use the “Detached mode” (floating windows) when using ImageJ as ImageJ use floating windows

Use ImageJ inside Icy

Basically you need to :

- convert your Icy image into an ImageJ image
- do your operations with ImageJ
- convert back the ImageJ image in Icy image if needed



- Open *'3D Stack.tif'*
- Be sure to have a ROI (Polygon or Area)
- Convert to ImageJ
- Do Process → Find Edges in ImageJ
- Convert result back to Icy image

Conclusion

Keep in touch !

@Icy_BioImaging

Support forum

http://icy.bioimageanalysis.org/support

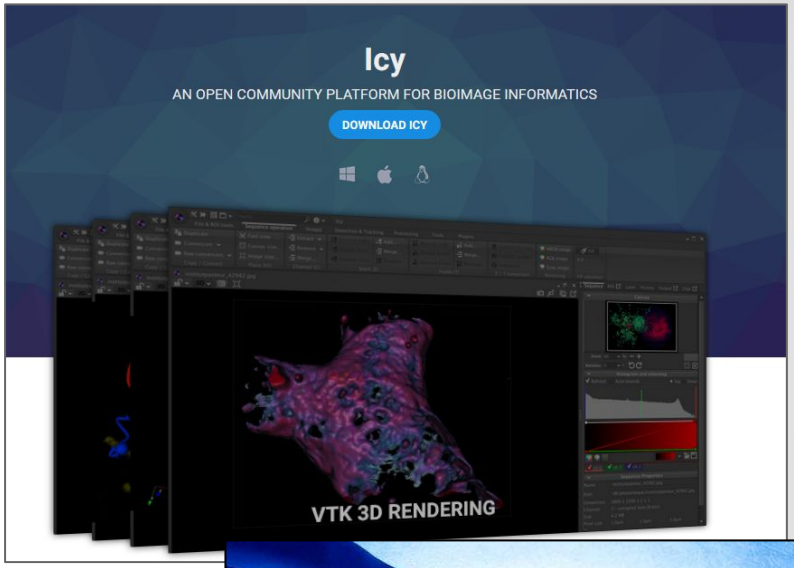


Image Analysis Hub Open Desk

Every other Thursday 9h30-12h30

Pasteur - François Jacob Building

https://research.pasteur.fr/en/news/image-analysis-opendesk/

Don’t forget to cite and acknowledge us :)