



ICY

Icy Training Level 1 - Introduction

Plan

General overview of Icy

Installing Icy

 how to install plugins

Graphical User Interface (GUI)

Analysis example

 Spot detection

 Tracking

- All images of the training are on the USB key
- 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.

Deploiemtent

User

Developer

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.

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.

Deployment

Quality

User

Developer

If the program crashes,
I want the developer to be aware of
it.

I am willing to participate, but by
clicking on a button, no more.

If my program crashed, I want to
receive a bug report to correct the
problem.

I wish to write update and send it
right now

What you want, What you don't want.

Deployment

Quality

Re-use

User

Developer

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

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 automaticaly installed



Installing Icy

Installation

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

At each start, Icy automatically updates its plugins and the application.



The screenshot shows the official website for Icy. At the top, there's a navigation bar with links for Home, Blog, Downloads, Plugin Resources, Workspaces, FAQ, Forum, Developers, Videos, and About Us. A user icon for 'Register' and 'Login' is also present. The main content area features a large, colorful fluorescence microscopy image of a tissue sample with red and green staining. Overlaid on this image is a semi-transparent circular watermark containing the word 'ICY'. Below the image, the text reads: 'ICY: The open source community software for bio-imaging'. A paragraph explains the software's purpose: 'Over the past decades the image analysis community has put substantial efforts into developing algorithms for various applications, yet their visibility often remained clustered to a handful of people, due to lack of proper means of communication and advertising. Meanwhile, numerous experts in the biology community have been turning to image analysis to answer their questions, and yet often fail to find adapted (or affordable) tools to fit their needs.' Another paragraph describes Icy's integrated platform: 'ICY provides an integrated platform that aims at bridging the gap between developers and users, by combining: a) an open-source image analysis software, offering a powerful and'. To the right of the main text is a large, dark button with the text 'Download Icy' in white. At the bottom right, there's a 'News' section with a list of recent updates:

- 25 Nov: New plugin: Montage2D
- 24 Nov: SSM toolbox updated to v1.0.0.1
- 24 Nov: EzPlug updated to v1.4.0.0b
- 23 Nov: Spot Detector updated to v1.3.0.0
- 22 Nov: Track Manager updated to v1.2.0.8
- 22 Nov: Track Manager updated to v1.2.0.7



Graphical user interface

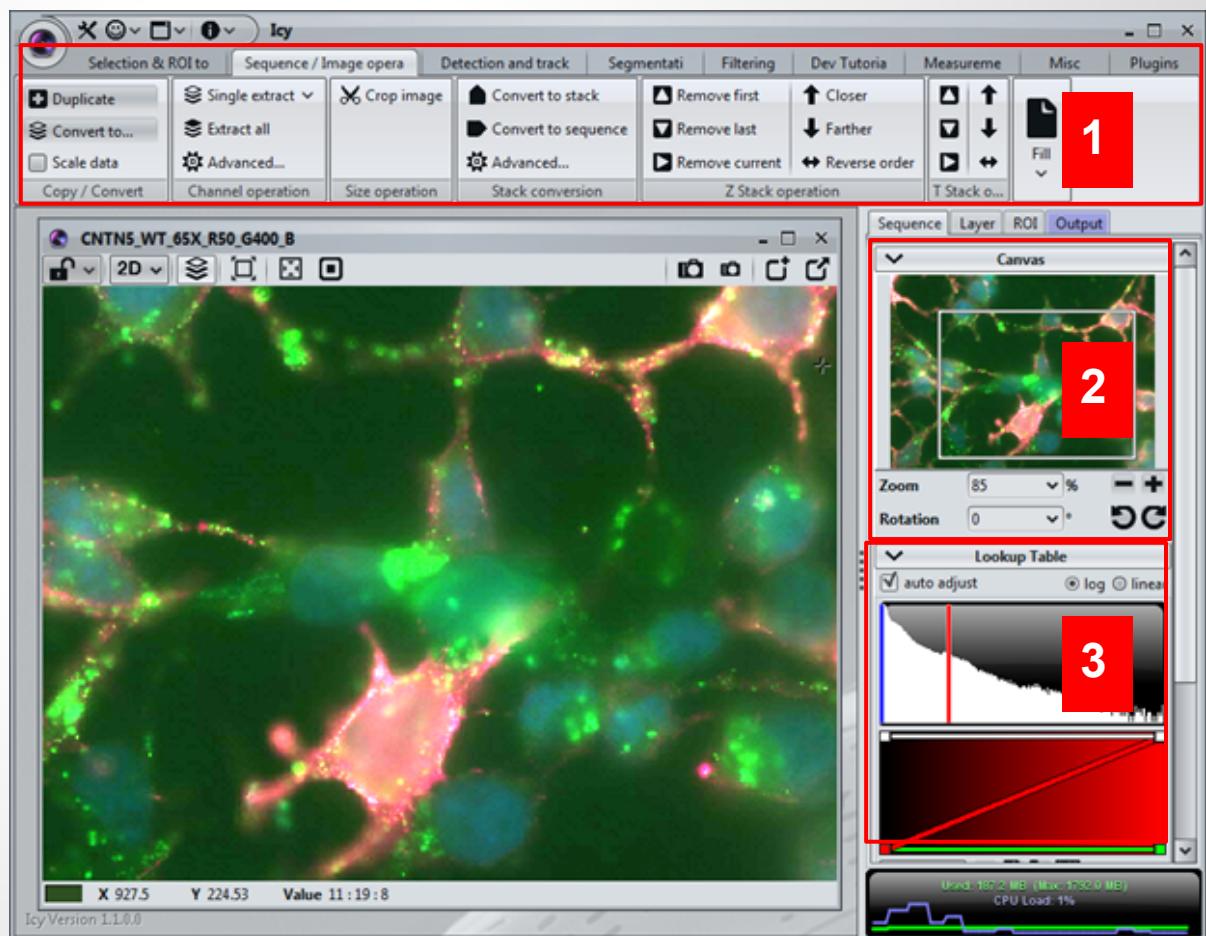
GUI

The GUI is based on well-known components:

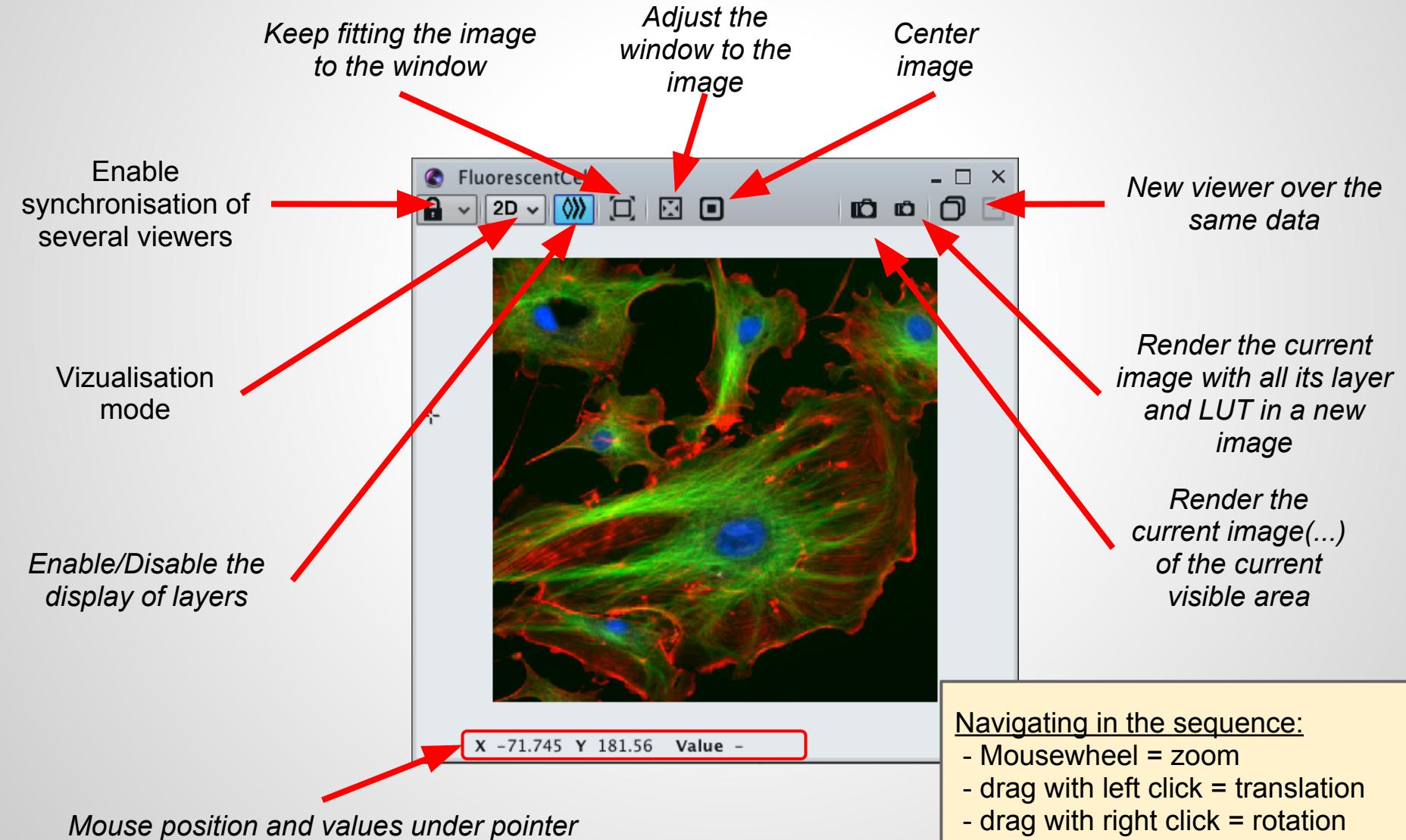
1 - Ribbons

2 - Navigator.

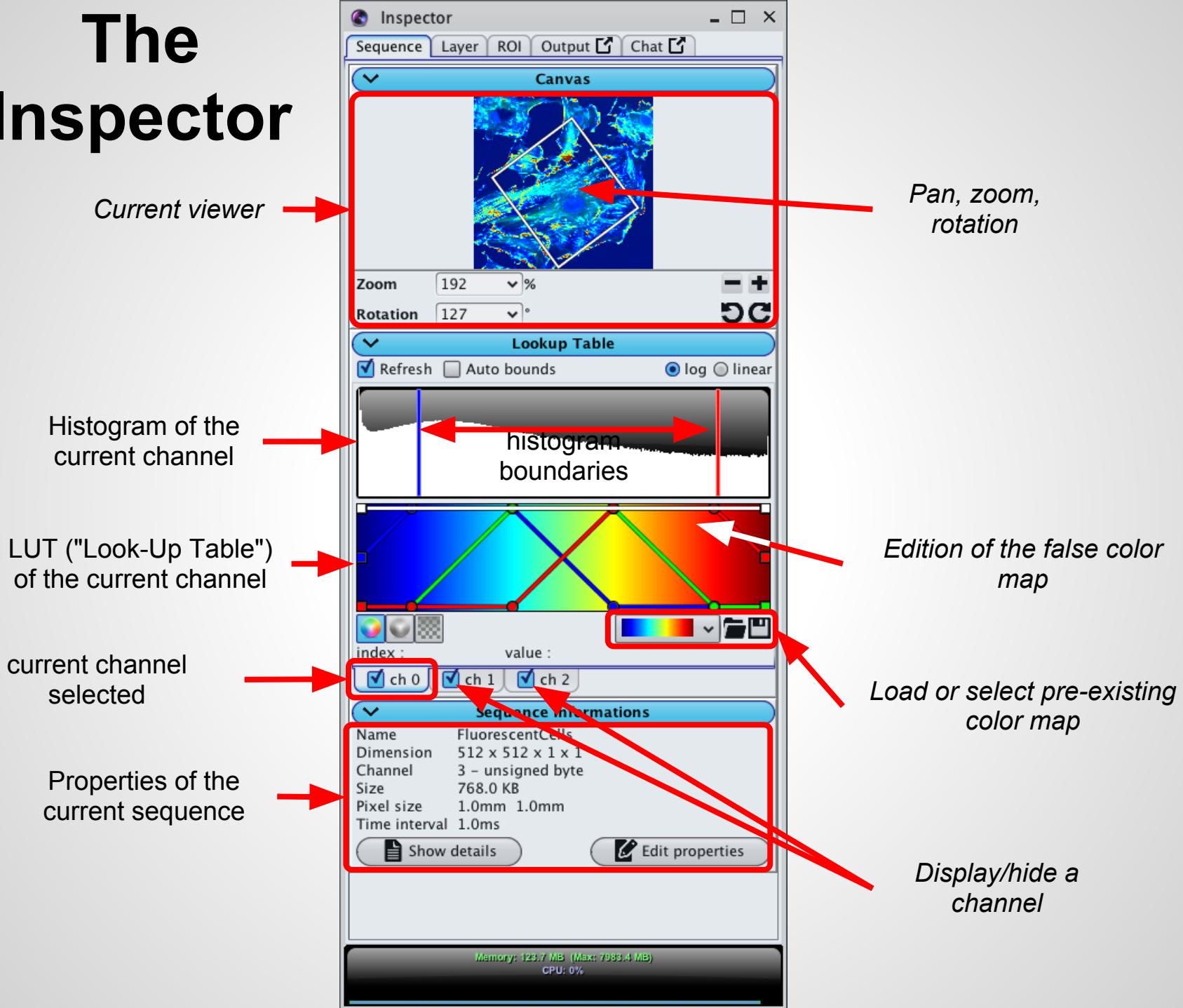
3 - Look-up table. (edition of false-color maps)



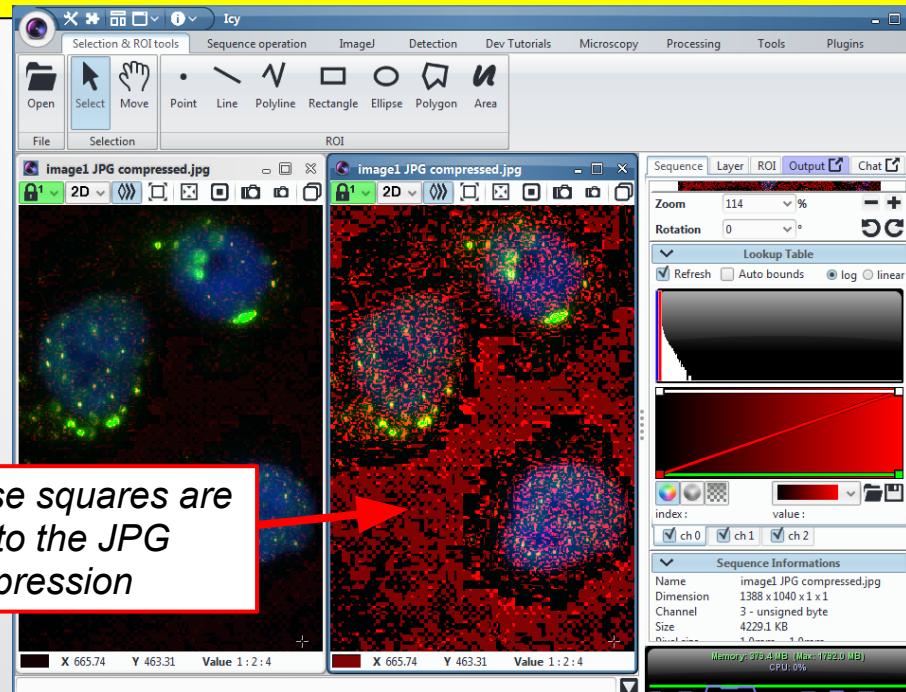
Le viewer



The Inspector



- Open the image named 'image 1 JPG compressed'
- Zoom in/out over the viewer and over the navigator. (mouse wheel)
- Pan the view
- Deactivate a channel
- Rotate the view
- Render the image at scale 1:1
- Render the image with the other camera
- Change the boundaries of the histogram in the different channels
- Slide the red bar gradually to the right to increase the contrast of the image and watch the squares dues to the JPG compression.

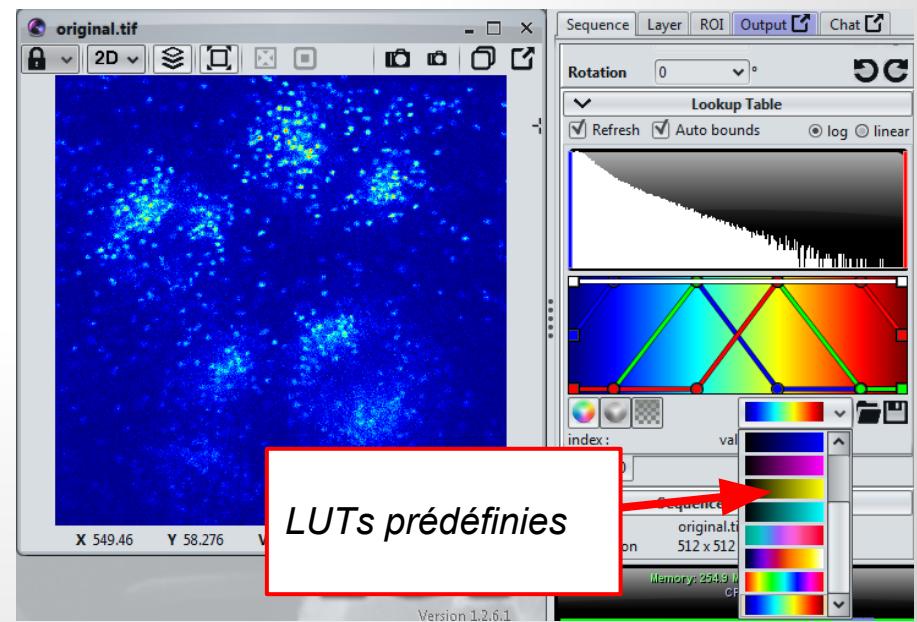
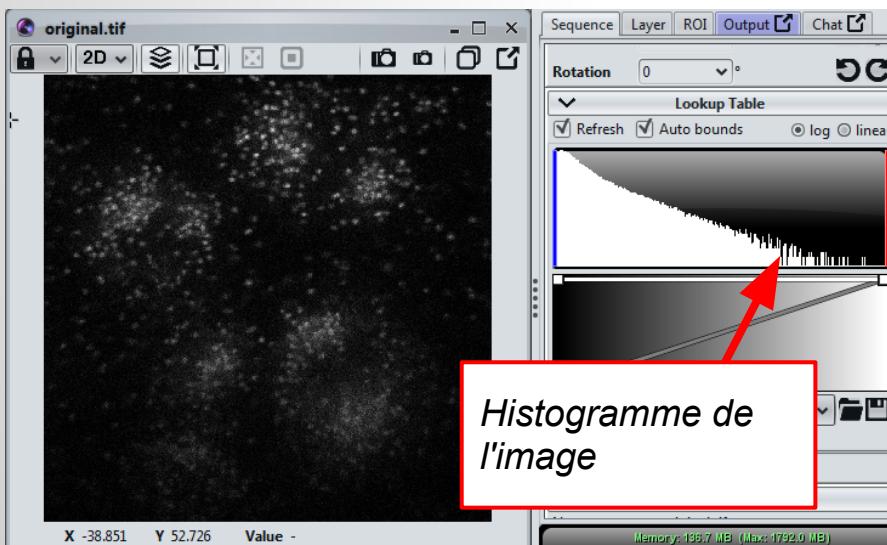




Histograms & Look up table

Exploring the image Histogram and look-up table. (or false-color representation)

- The LUTs help at understanding an image.
- The LUT representation does not affect the real values of the images
- The histogram provides the number of pixel of each intensity in the image.



Creating the histogram of an image

The image is a matrix of values

2 3 1

0 1 0

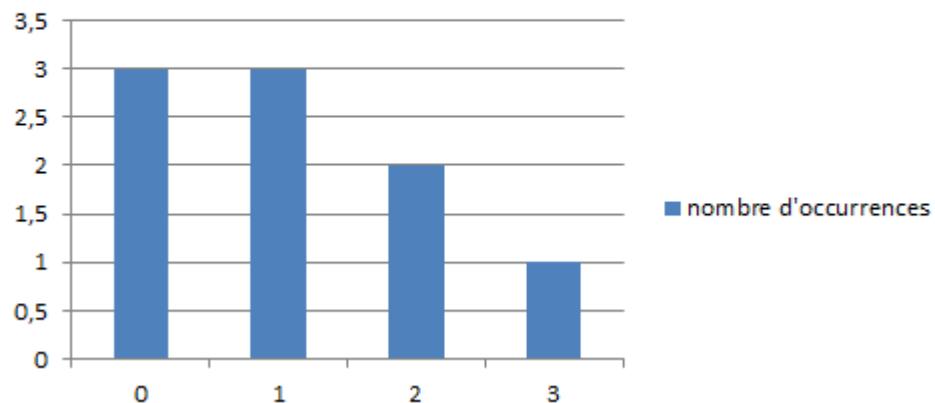
1 0 2

3x3 matrix representing an image of 3x3 pixels.

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

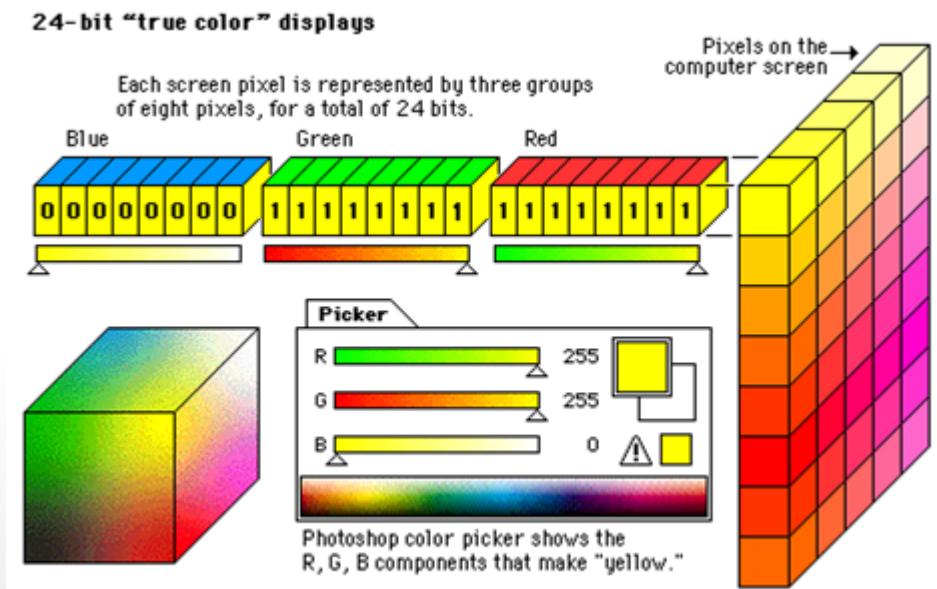
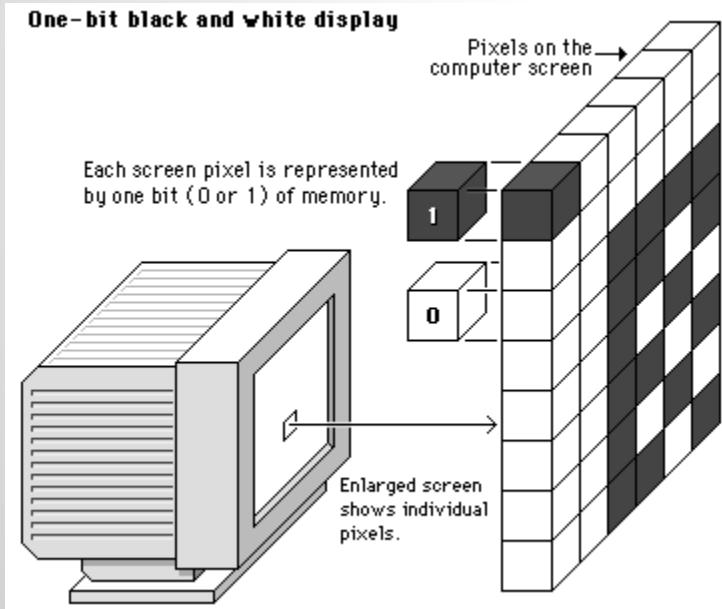
nombre d'occurrences pour chaque intensité



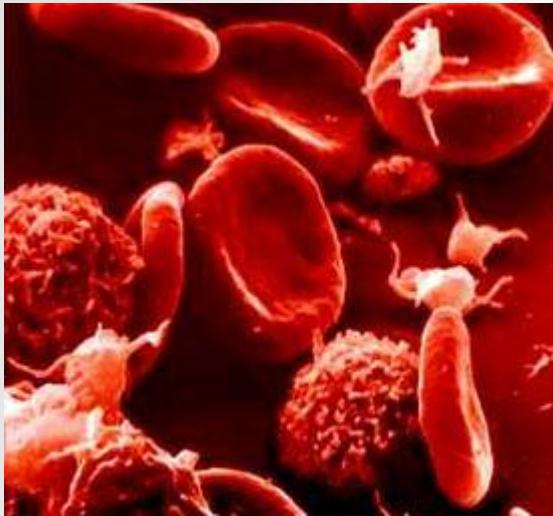
Representing an image on screen

How to display an image with 256 grayshade on a black and white monitor ?

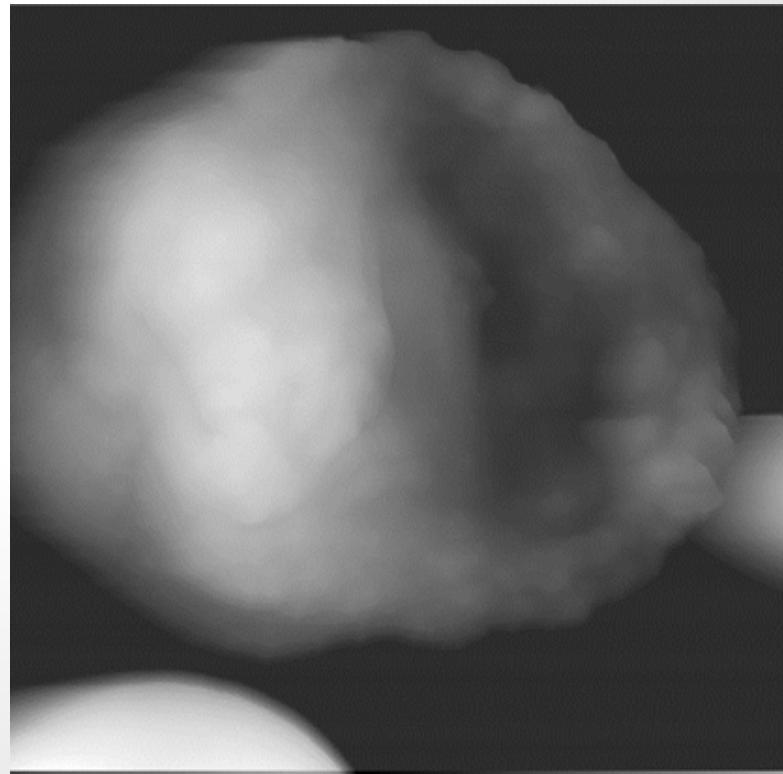
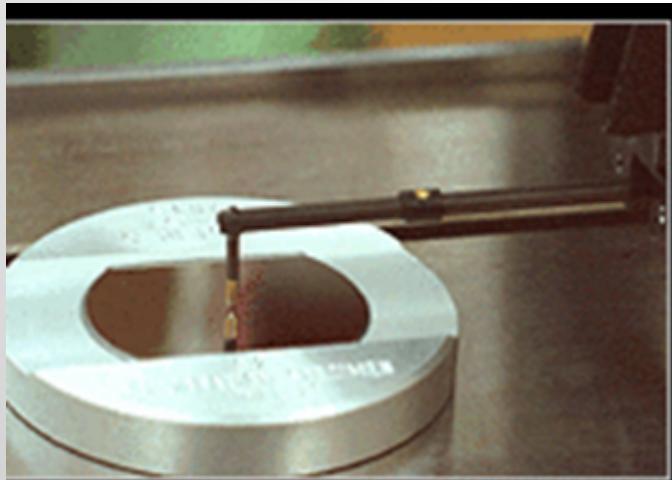
How to display 16bits values on our current screens which only support 8bits per components ?



Representing an image on screen

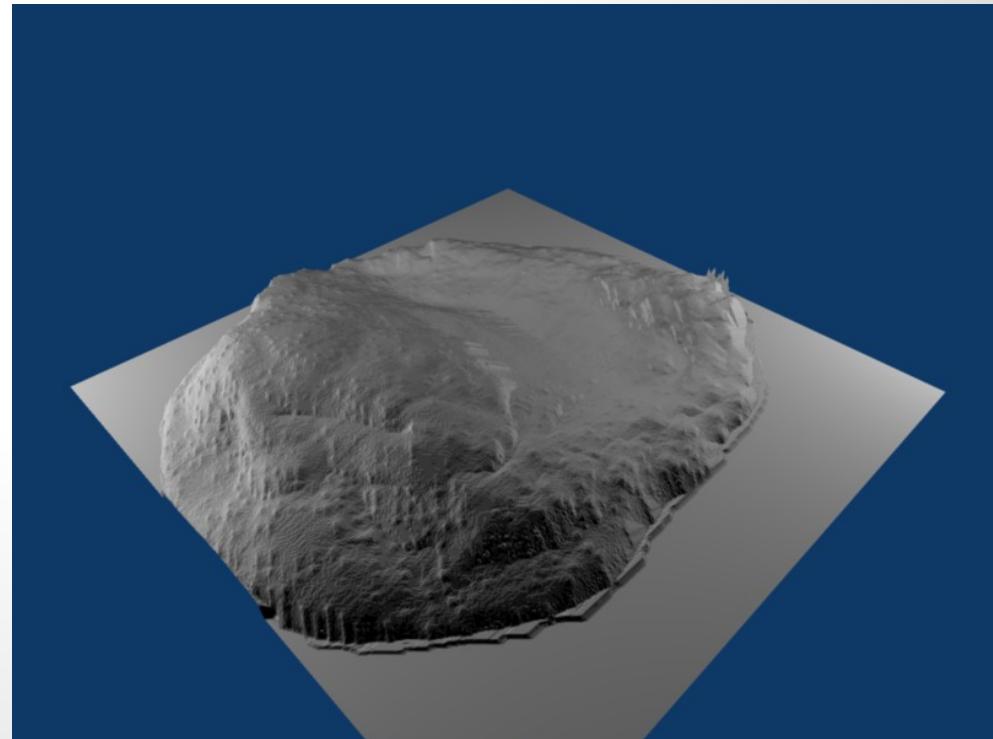
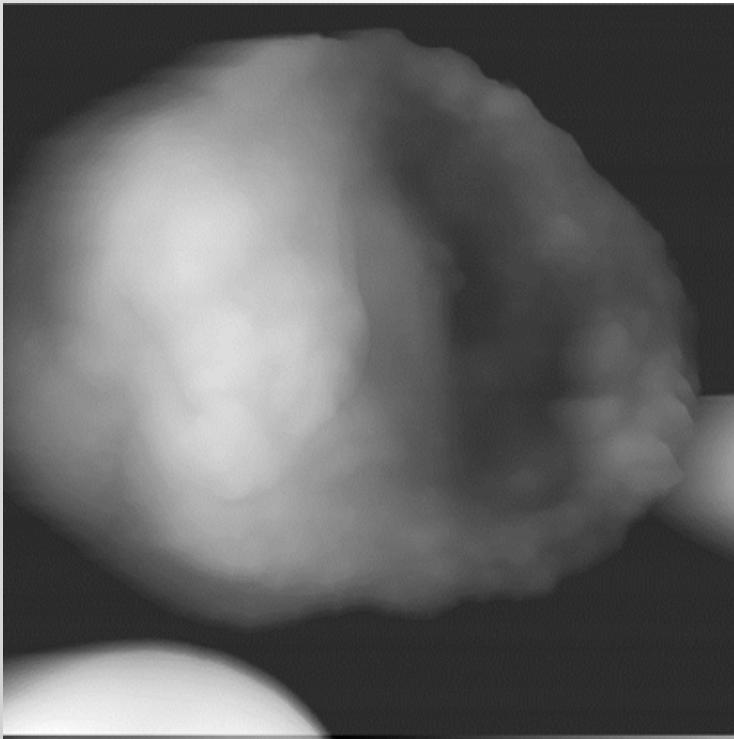


The image below is obtained with an *atomic force microscopy*. Each intensity value is an height, an altitude.



Representing an image on screen

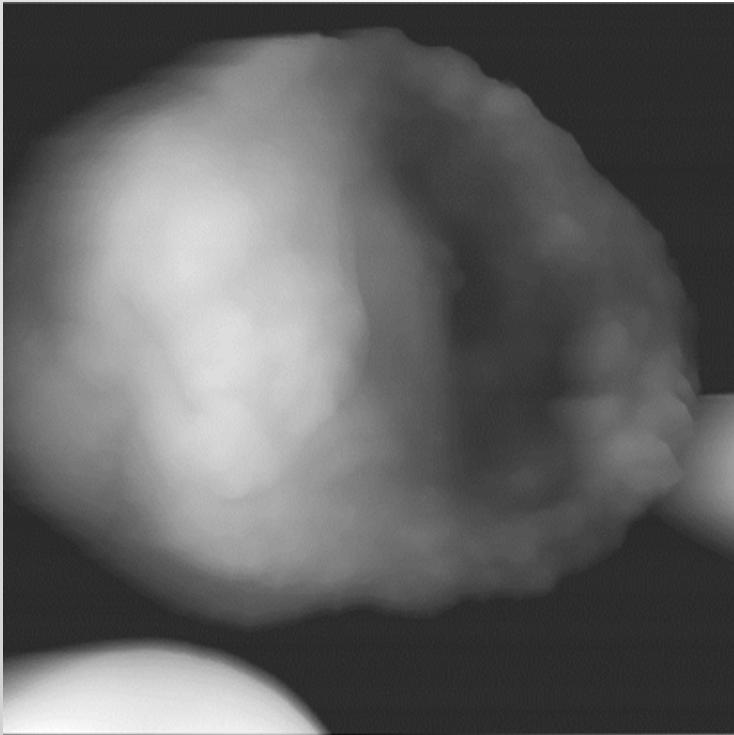
Here is the same image displayed in 3D



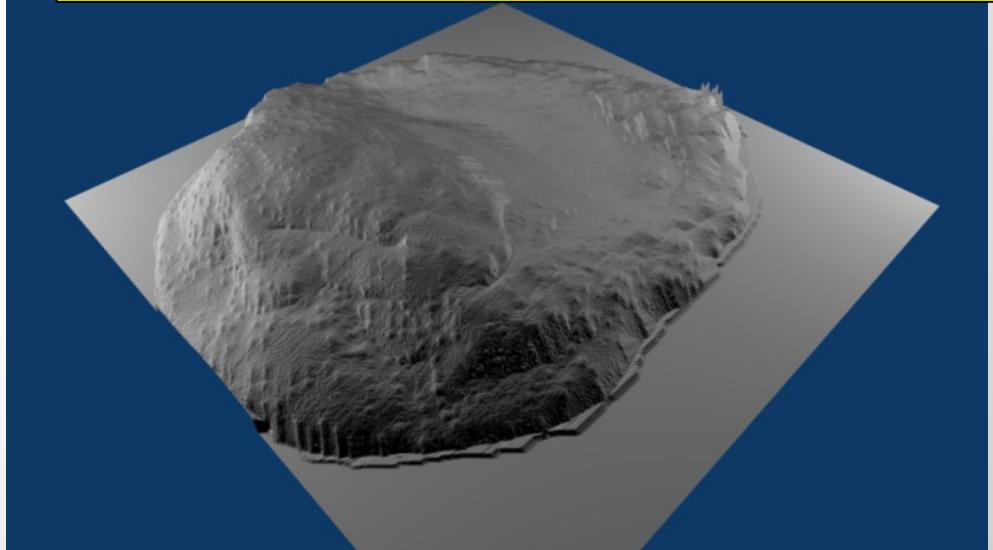
Representing an image on screen

Here is the same image displayed in 3D

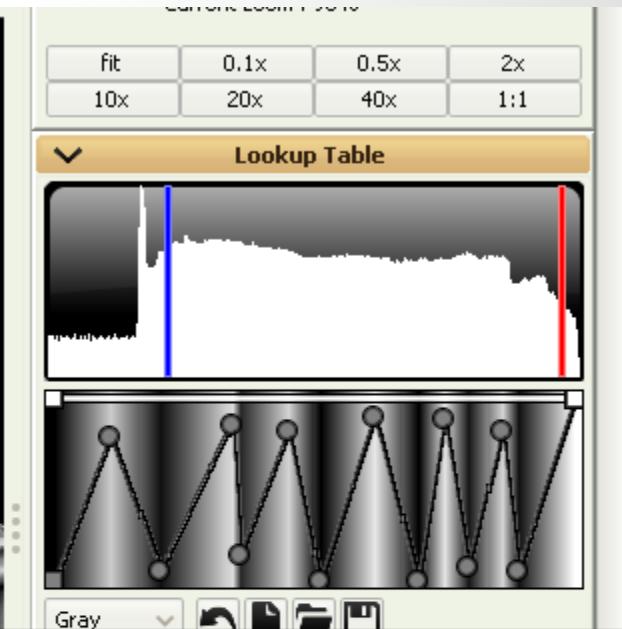
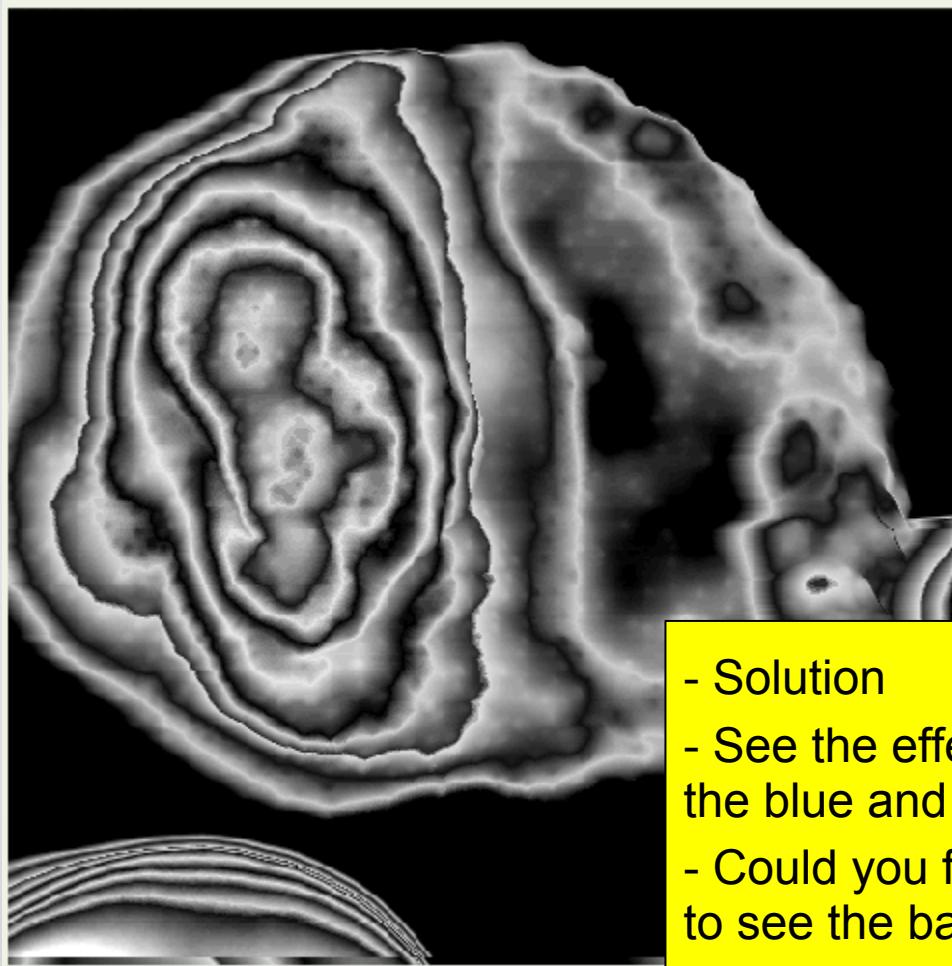
Have you seen the knobs on the left hand side image ? No ? That precisely what we are looking for.



- Load the image *knob test 16bpp.tif*
- Try to find a look up table able to display the knobs in the 2D view

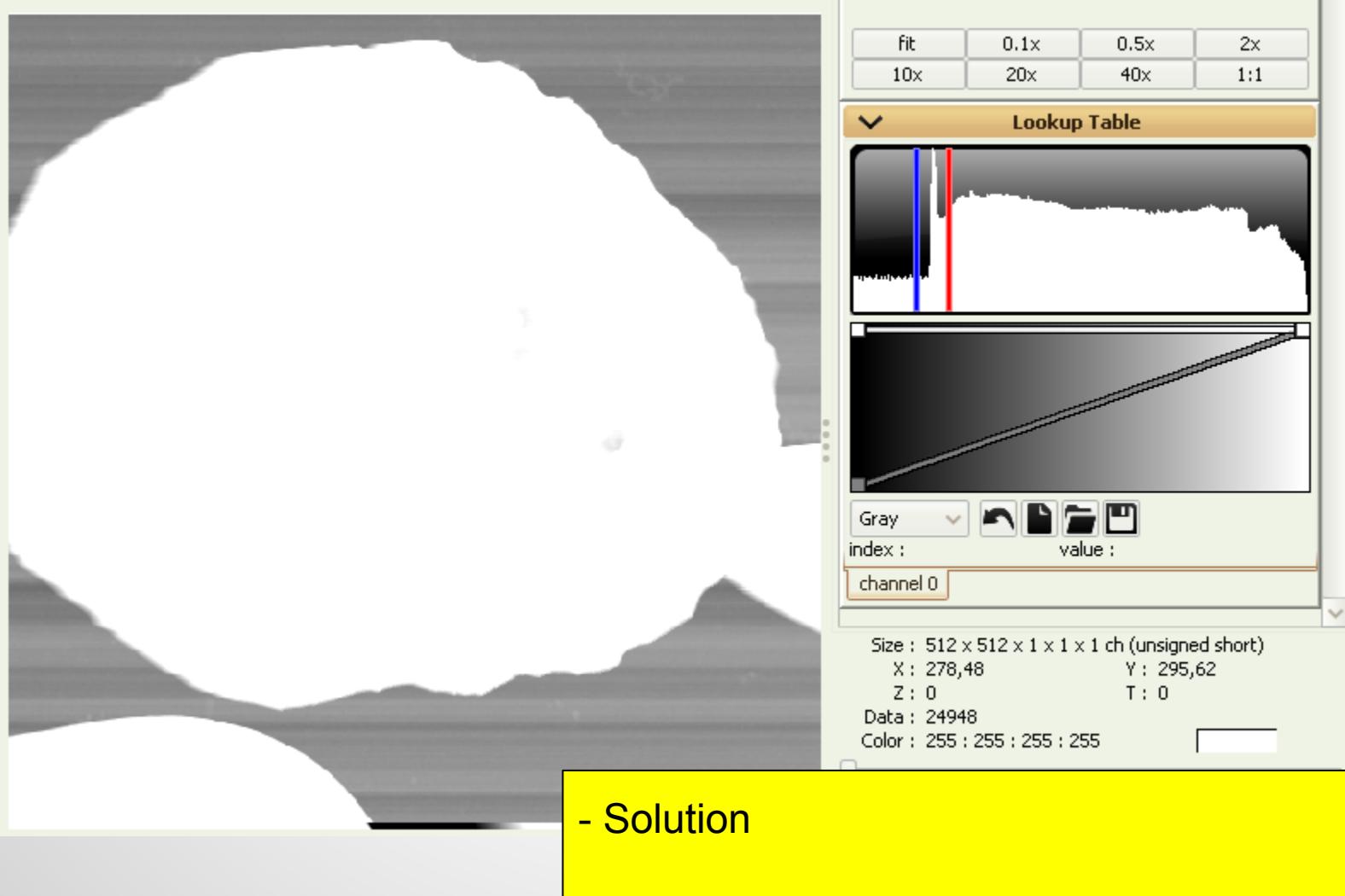


Representing an image on screen



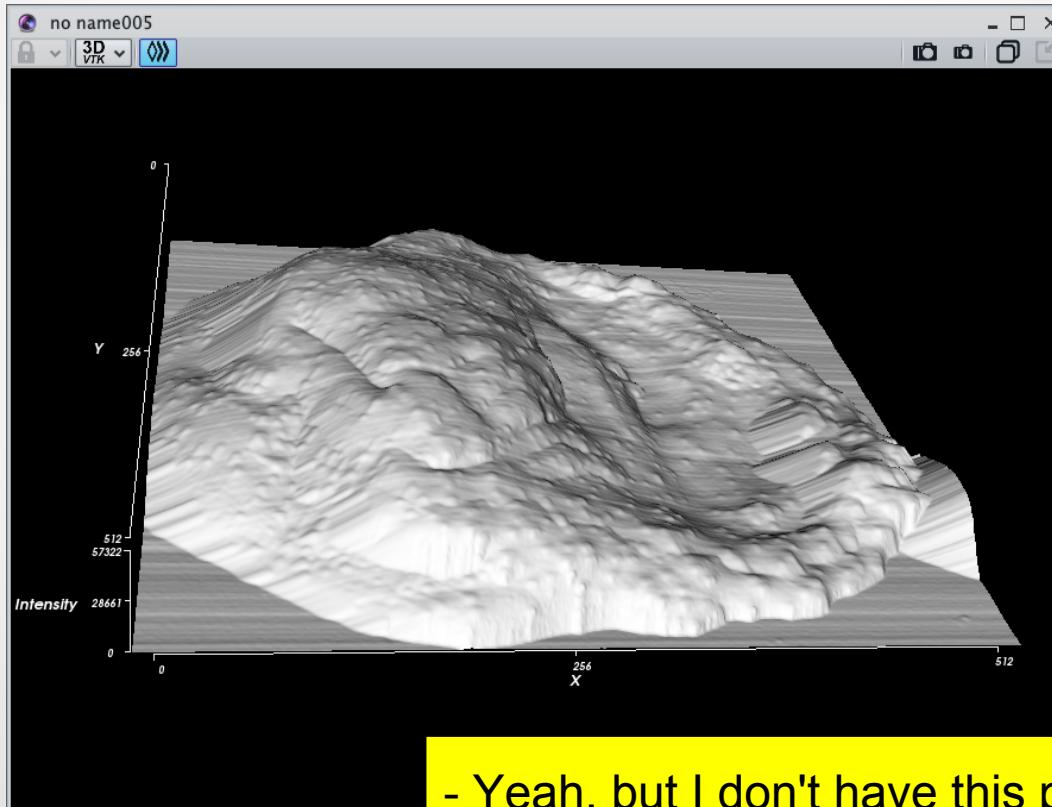
- Solution
- See the effect on the image if you play with the blue and red vertical bars
- Could you find where to put the vertical bars to see the background ?

Representing an image on screen



Representing an image on screen

- The image can be seen in 3D as a deformable surface thanks to the *Elevation Map* plugin



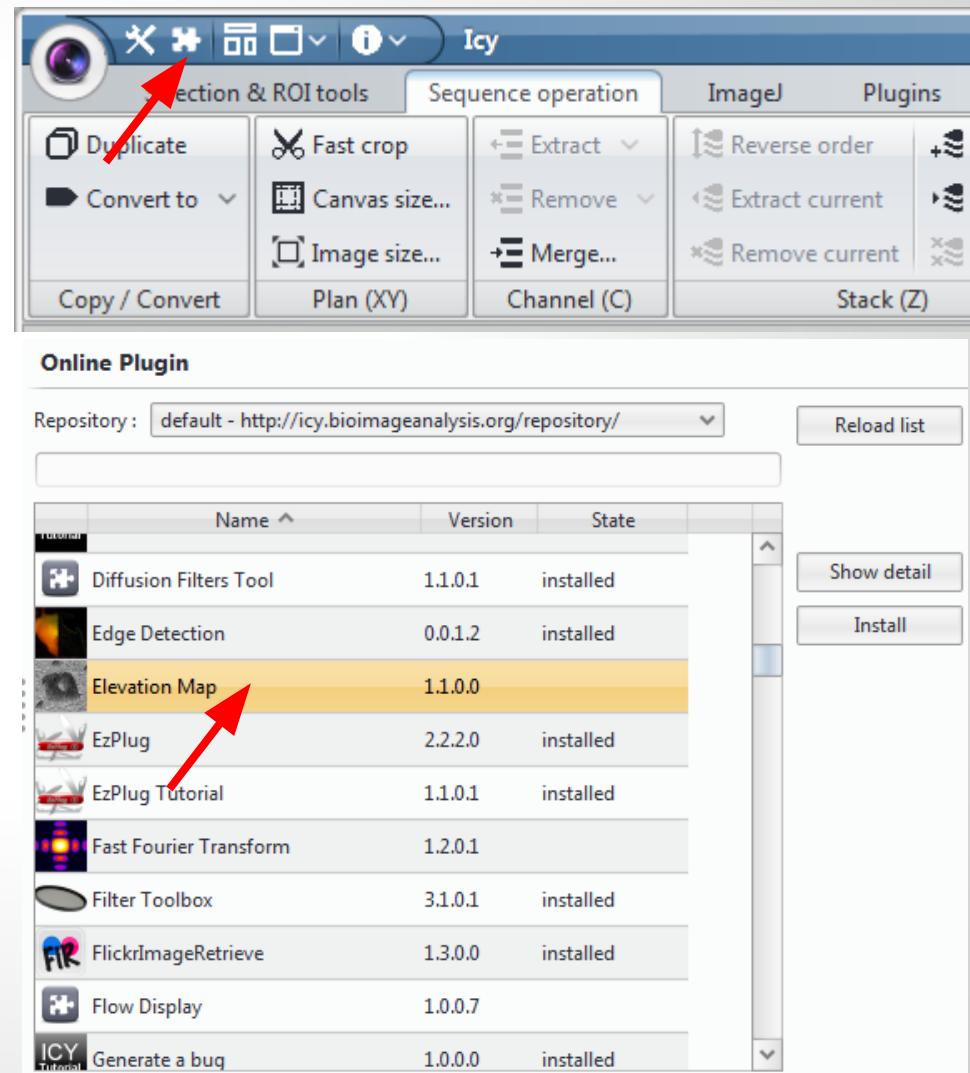
- Yeah, but I don't have this plugin !



How to Install a plugin ?

Icy's plugins

All plugins are centralized on a single website.



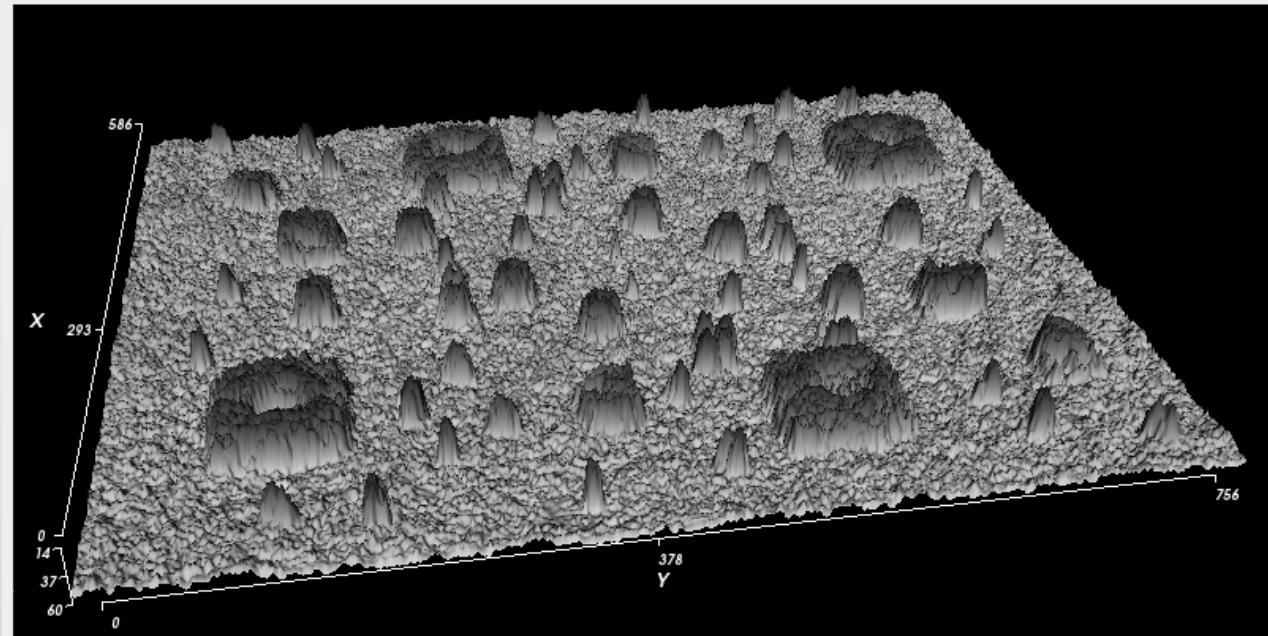
- Installez the plugin *Elevation Map*

Documentation

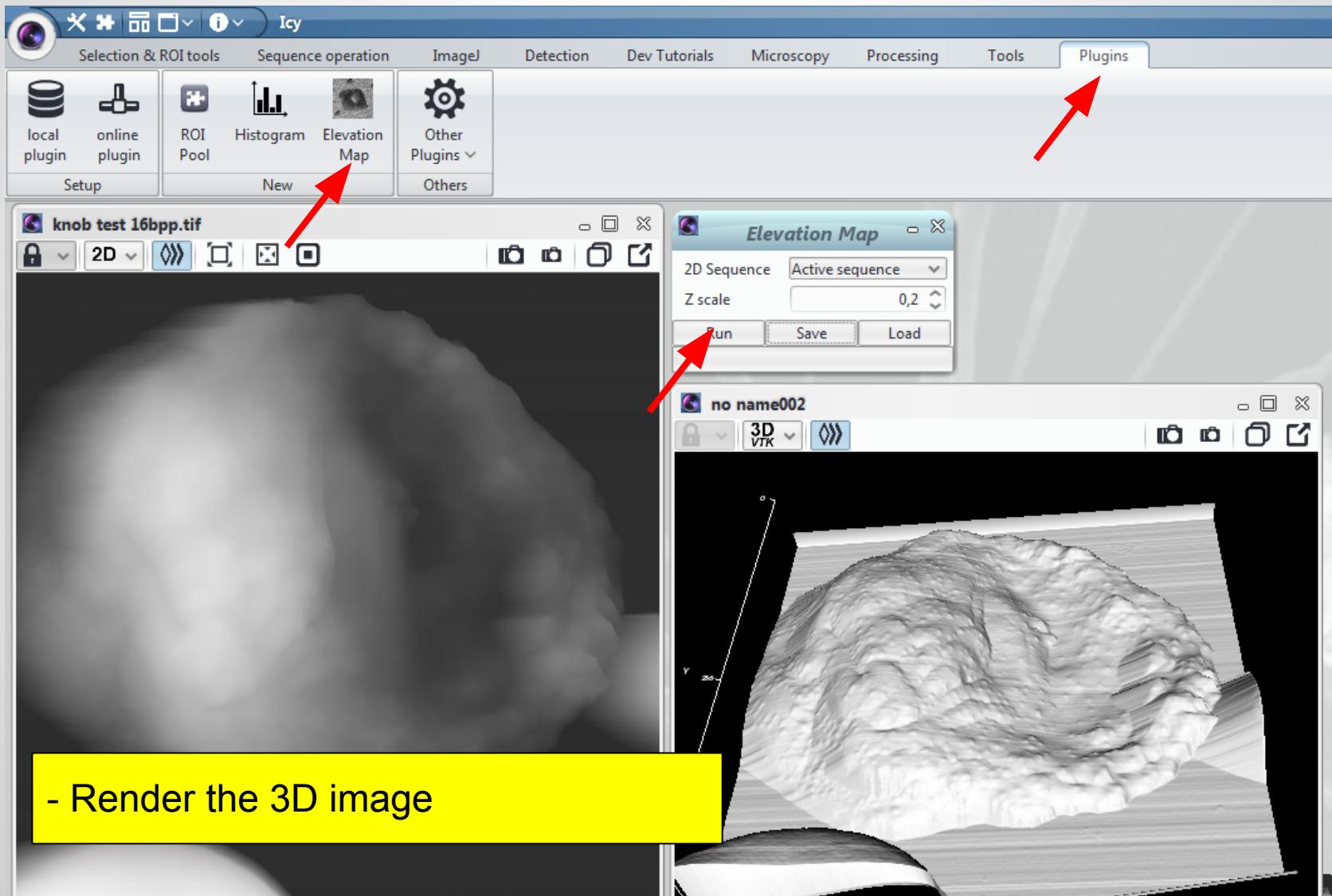
As a developer creates a plugin, a documentation page is automatically generated.

Elle est disponible sur le site *Icy > Plugin > Plugin List*

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



Using the plugin *Elevation Map*



Support: mailing list et forum.

<http://icy.bioimageanalysis.org/support>

High activity, high reactivity

The screenshot shows the Icy support forum interface. At the top, there is a navigation bar with links for Home, Blog, Plugins and Protocols resources, Forum & Support, FAQ, Developers, Videos, About, and Download Icy. Below the navigation bar, there is a search bar and a "POST A QUESTION" button. The main content area displays a list of topics under the heading "Icy imaging". The topics listed are:

- Spot tracking
- Cells Tracking
- Optical Flow protocol
- name and title of images
- Icy 1.2.6.0 will not start
- Plug-in IcyFrame resizing
- Convert a plugin to be useable as a block in protocol.
- Connected Components in blocks

Each topic entry includes the poster's name, the number of posts, views, and the last update time. Some topics have status indicators like "Answered" or "feature-request".

People can illustrate question and answers with:

- images
- scripts
- protocols
- code java
- data files
- configuration files

The screenshot shows a specific forum post. The user "MLB" asks a question about black ROI, asking how to define it and distinguish it from other types. The user "Marie Laure" replies, explaining that the ROI drawn by the users are used by the detector. If no ROI is present, the whole image is considered. If one or more ROIs are present, then the program only considers detections inside ROIs. The program recognizes the black ROIs as removed area (could be scratches, destroyed tissue...). All results in the final Excel file will then be classified by ROI, providing its id, number of detection and its area in pixels² (see output section for details). Black ROIs are subtracted to all other presents ROIs.

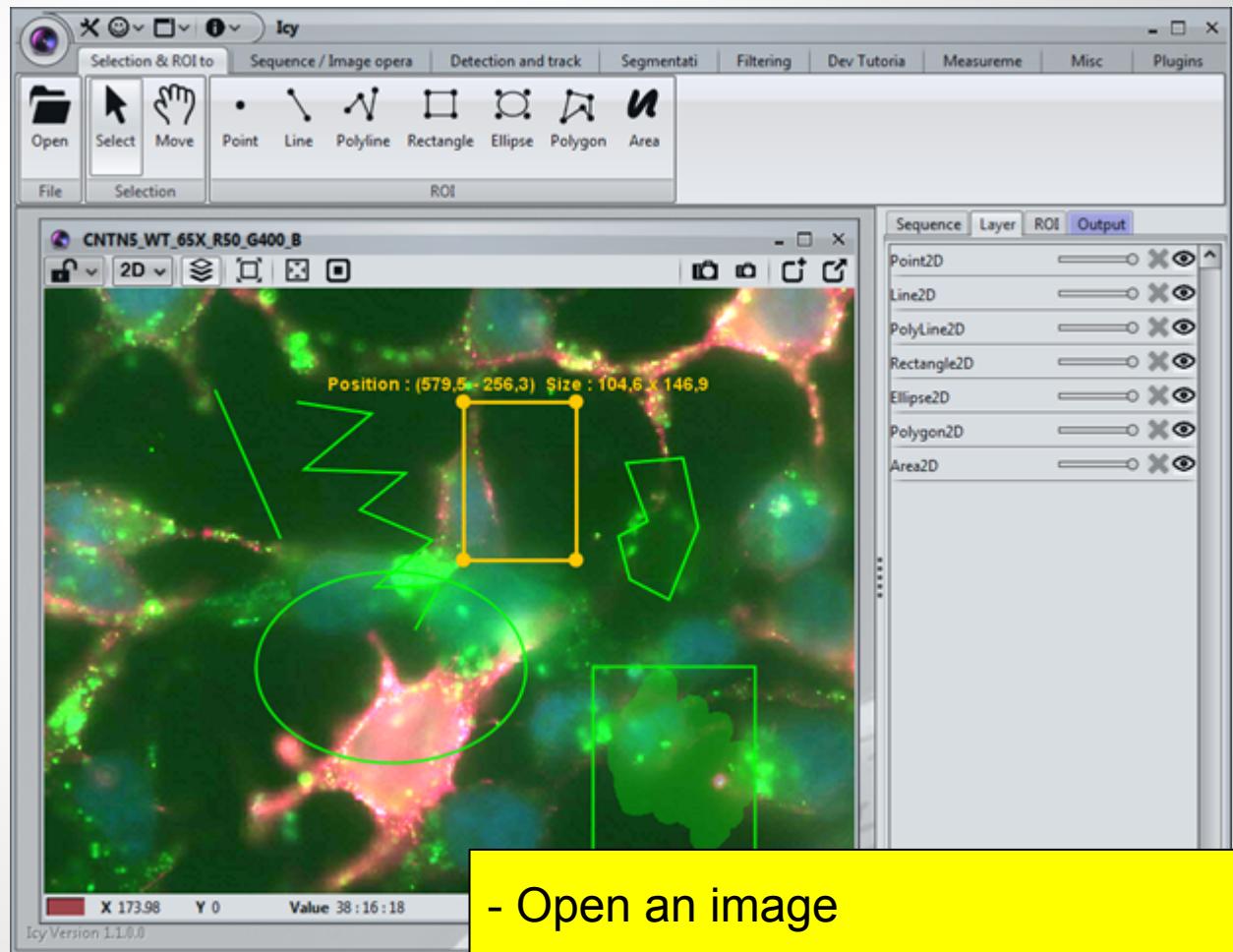
The user "me (Fab change)" provides a "Best answer" with a note "- show quoted text -". They attach a file named "black roi.png" which is a screenshot of a software interface showing a circular ROI on a grayscale image.

ROIs (Region of interest)

Icy comes with 7 default ROIs, their design is inspired d'Osirix.

- pixel
- line
- broken line
- Rectangle
- ellipse
- polygon
- pain bucket

New ROIs can be introduced by plugins



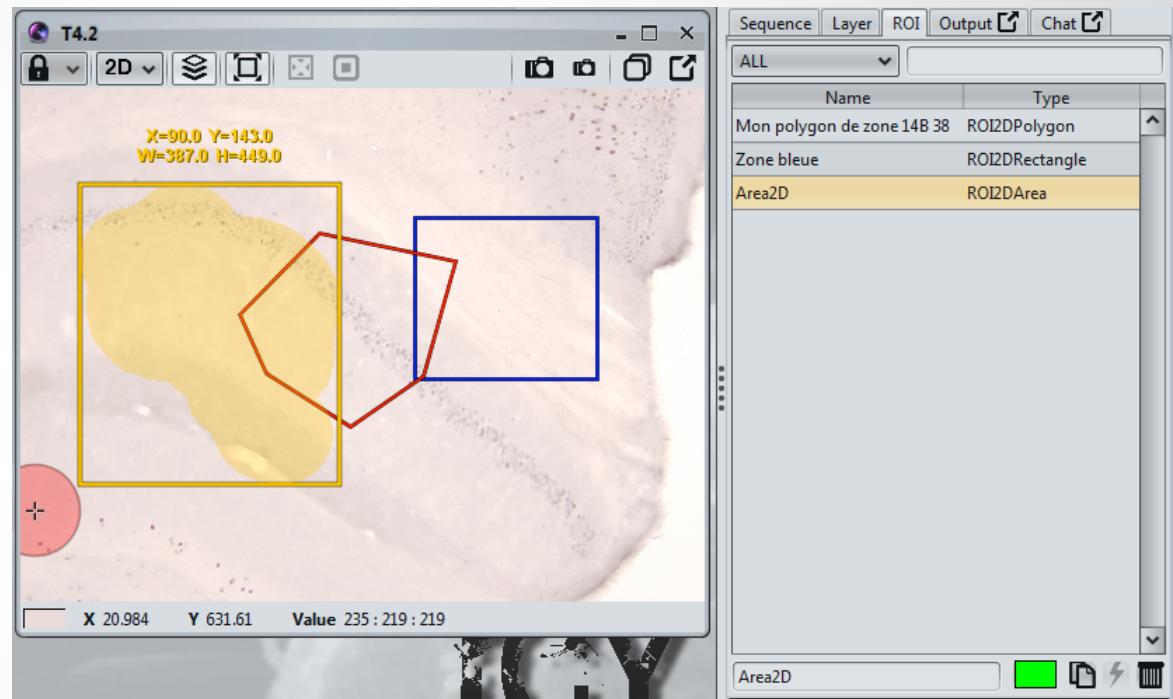
- Open an image
- Draw each type of ROI

ROIs (Region of interest)

All ROI can be catch, even if they are overlaping

The ROIs can be colored and renamed

ROIs are persistants, this means that you don't need to save them: they reappear automatically as you reopen the image.

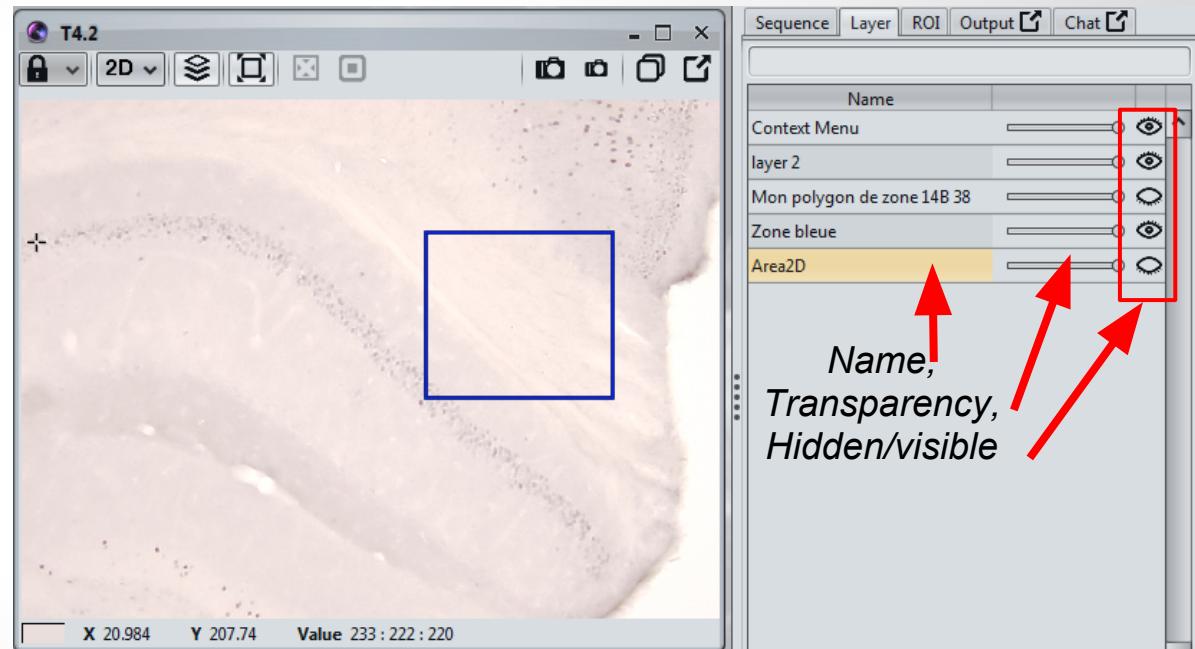


- Edit the existing ROIs
- Rename ROIs, change their colors.
- Close a sequence and reopen it, you will see that ROIs are restored.

ROIs (Region of interest)

The layer is the same as in photoshop: you can display or add layers, or affect its transparency.

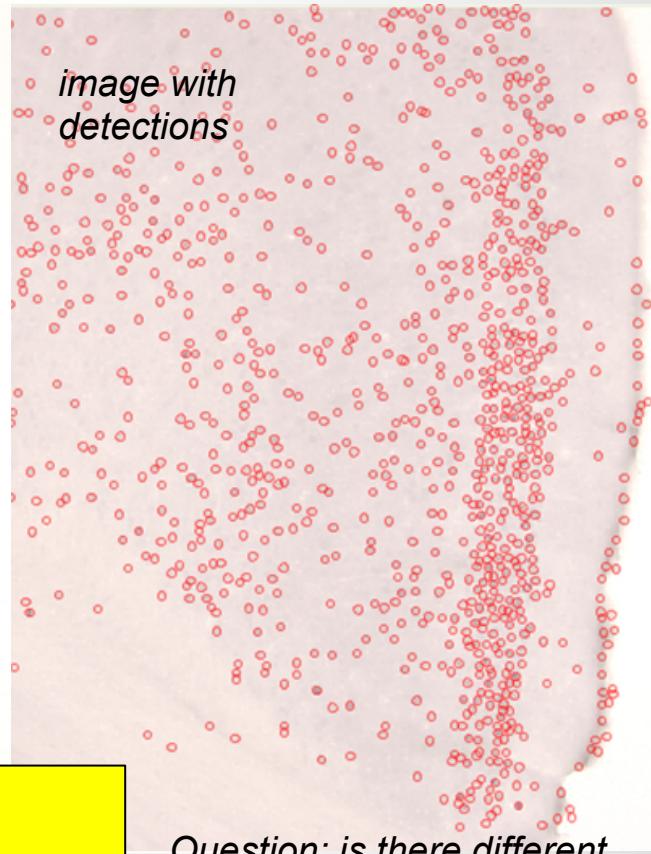
Even if the layer is not visible, algorithms still consider them. (ie: an hidden ROI is still considered for a detection)



- In the layer tab, click on the eyes to display/hide a layer.

Example of quantification using ROIs

*original
image*

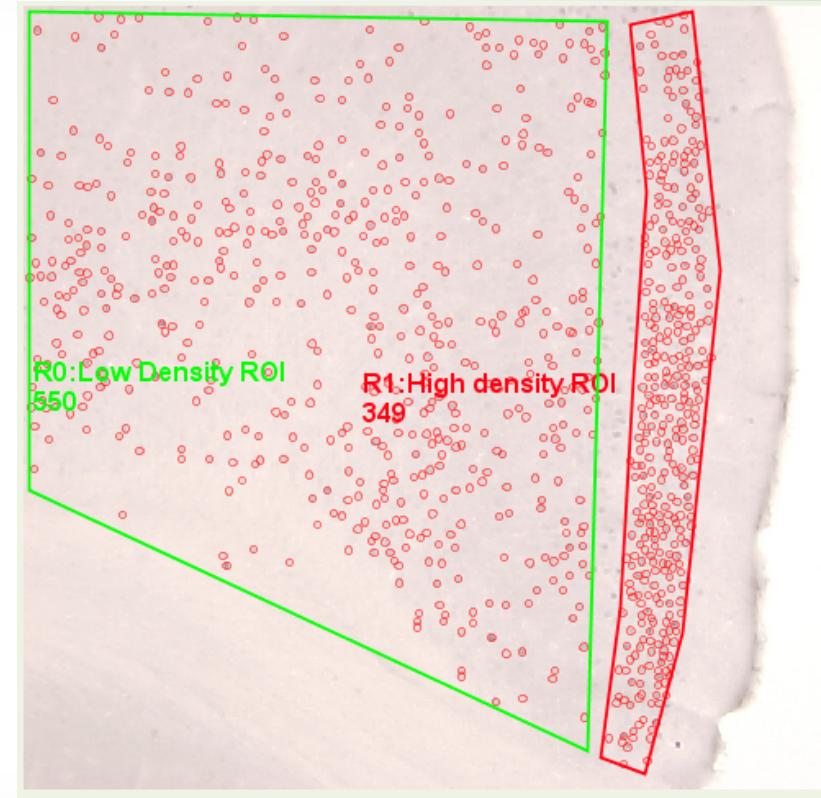
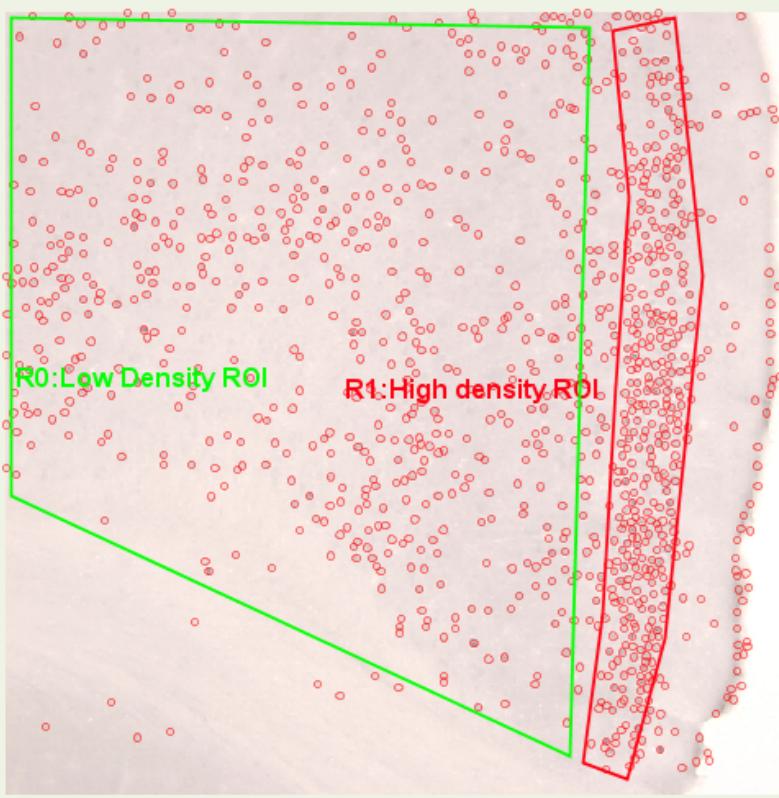


- Load the file *P7.JPG*
- Launch the plugin *spot detector*.
- Click *start*.

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

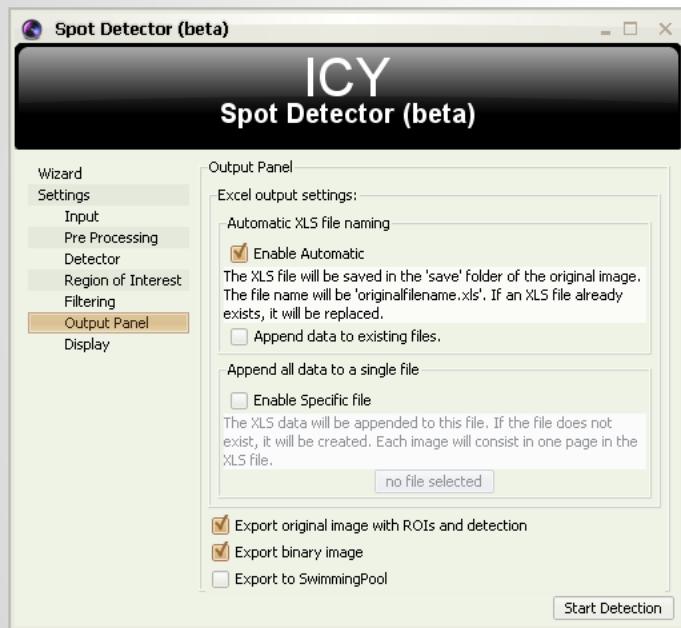
- Yes, and I will quantify it.

Example of quantification using ROIs



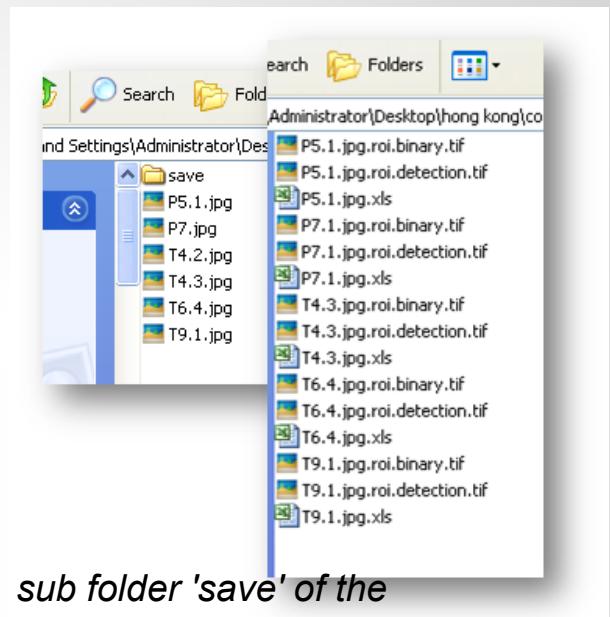
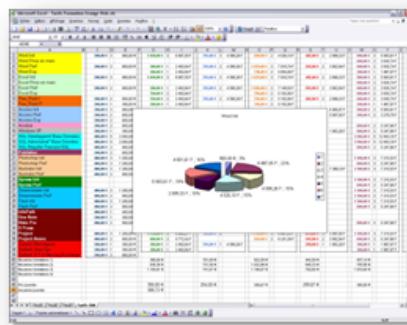
- Draw 2 ROIs corresponding to each area.
- restart the detection (click *start* again)
- Detection are now linked to the ROIs

Example of quantification using ROIs

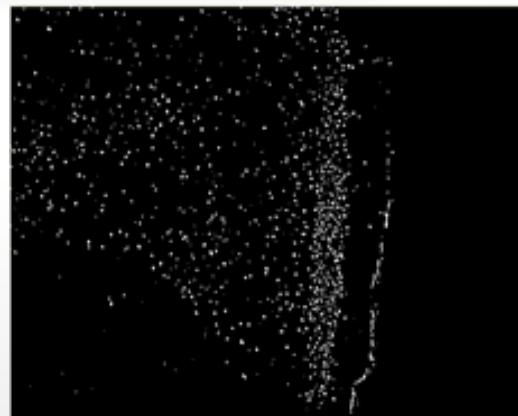


Export option of the spot detector

Excel



sub folder 'save' of the input image



Binary image

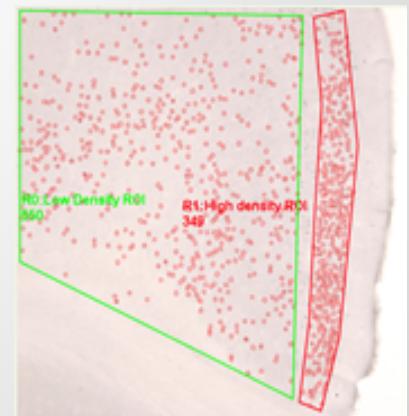
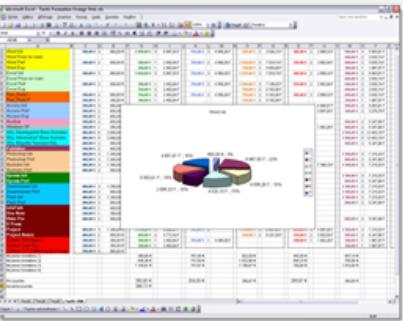


Image with ROIs and detection

Example of quantification using ROIs

Excel



Pre processor				
Band selector				
Band Sele: 0				
Detector				
Detector: UDWL Wavelet Detector				
Parameters:				
Scale 1	Disabled	Threshold:	100	
Scale 2	Enabled	Threshold:	100	
Region of interest				
Region of i ROI From Sequence module				
ROI number	ROI name	ROI surface	ROI nb detection	ROI tag(s)
0	Polygon2D	333626	569	
1	Polygon2D	56700	367	
				Density
				0,001706
				0,006473

Add in excel:
$$\text{Density} = \text{nb detection} / \text{surface}$$

Densities

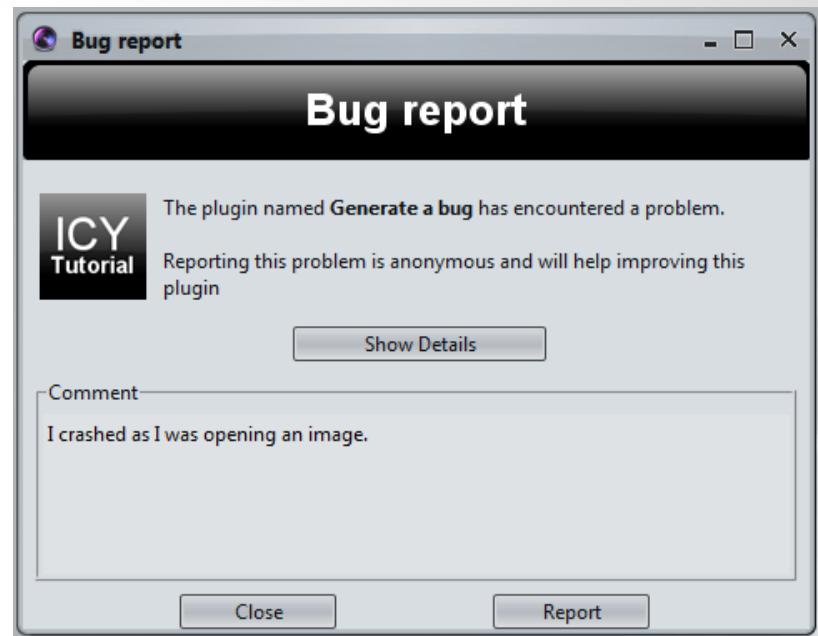
Robustness of plugins

The author of a plugin cannot foresee all the usage of his plugin. Untested contexts can lead to crashes.

If a plugin crashes, a window ask the user if he wants to send an anonymous bug report containing the infos needed to correct the plugin.

The author fix the problem, and publishes the update on the website

The new plugin is immediately deployed over all the clients

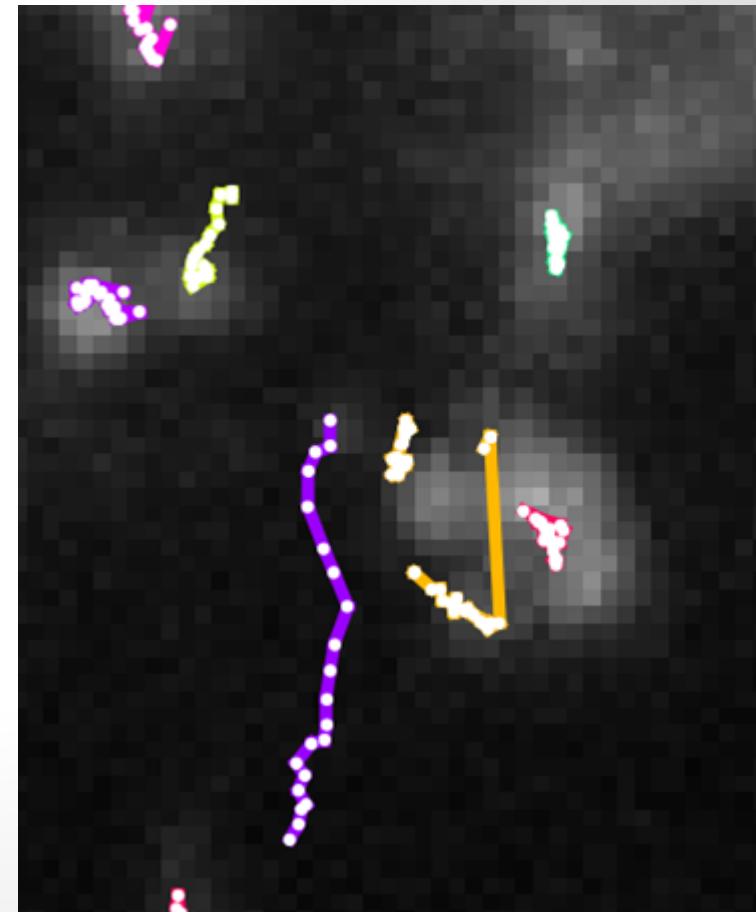
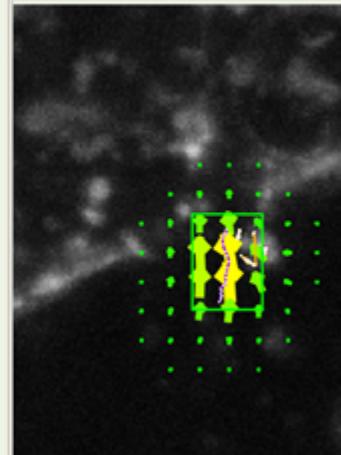
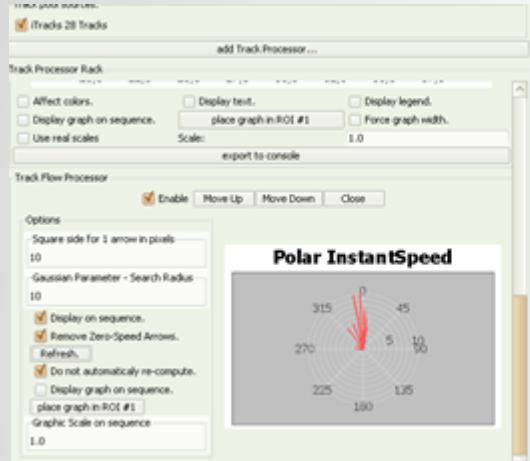


In 1 year, 2114 bug report have been posted. Thanks to this, 768 corrected version of plugins have been posted.

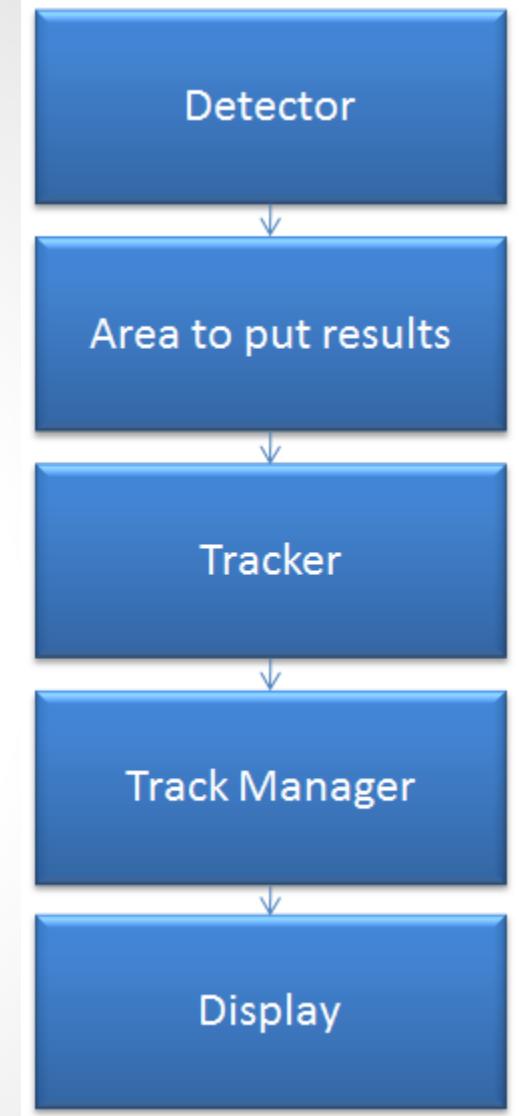
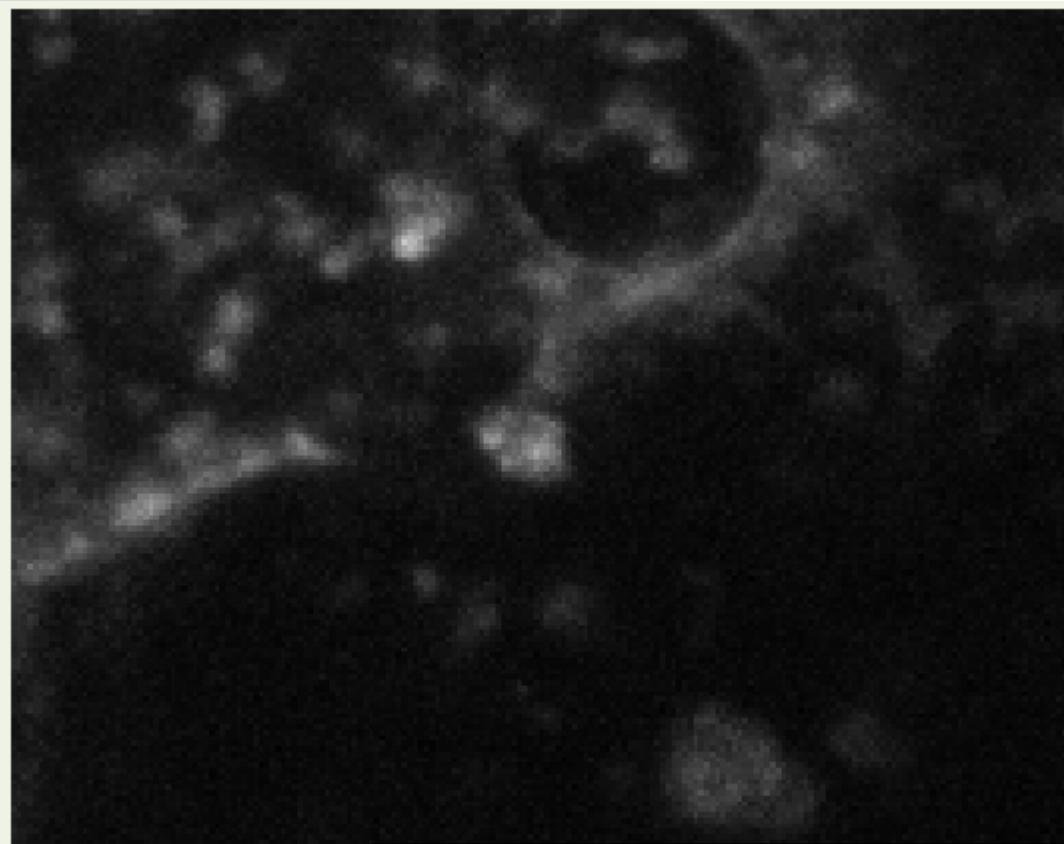


Tracking

Particle tracking

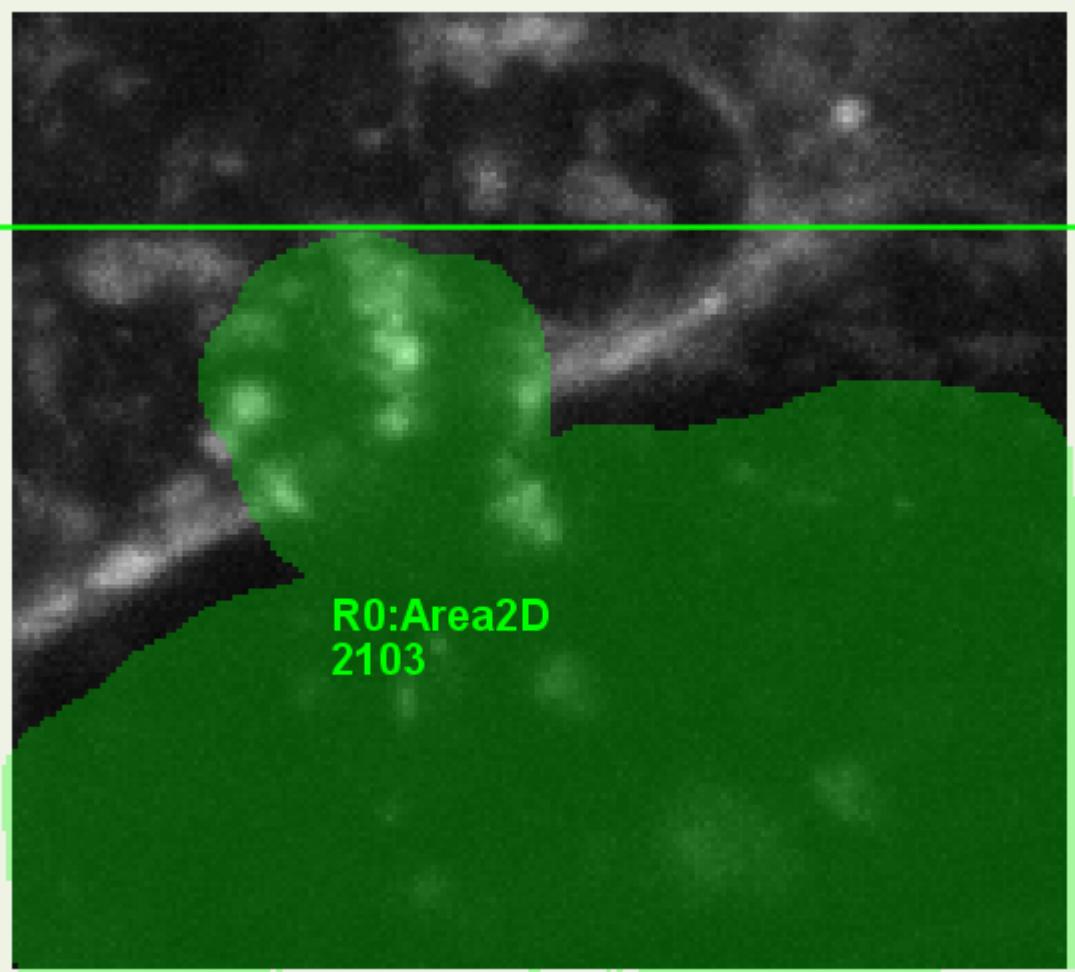


Particle tracking



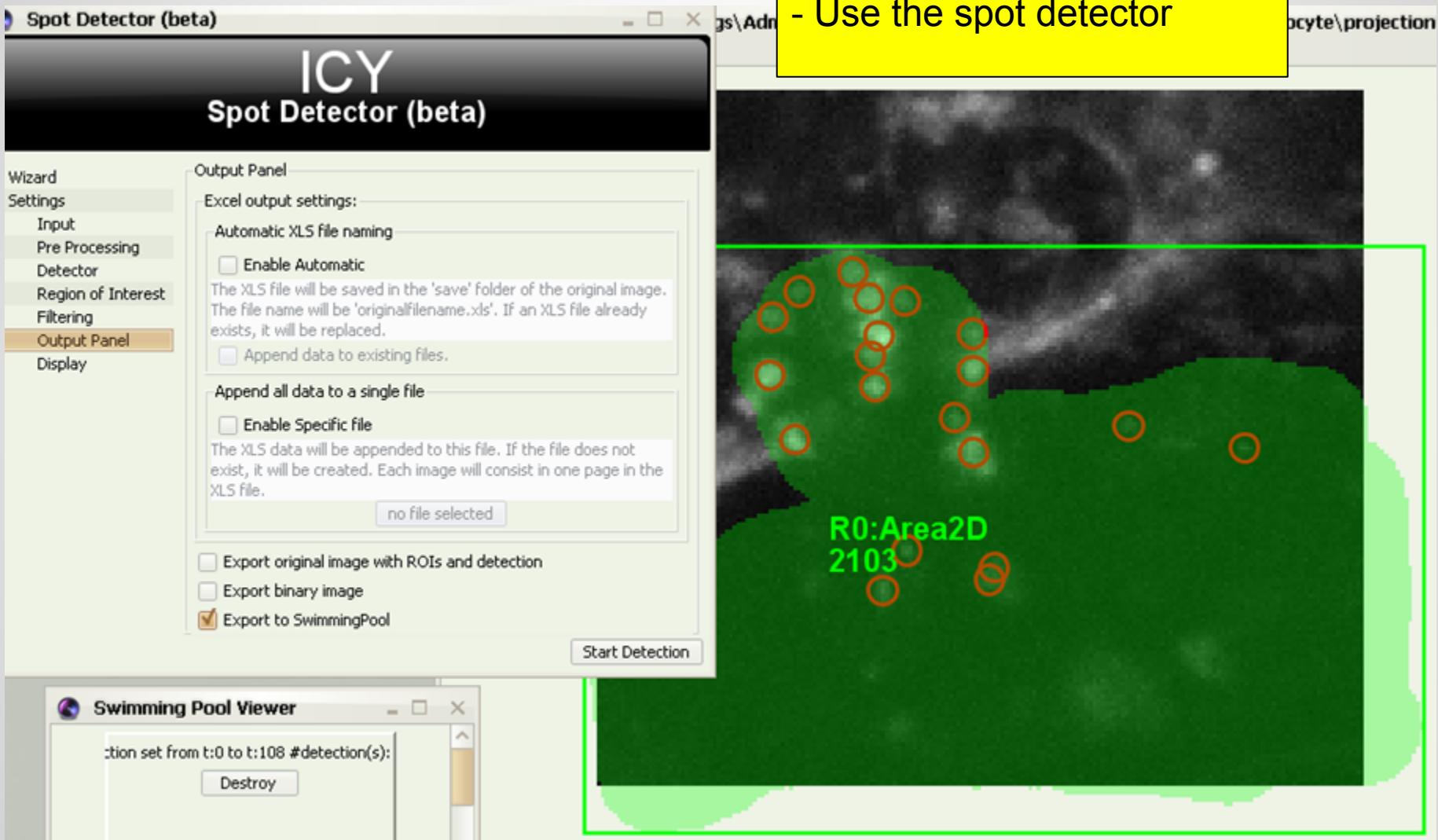
- Ouvrez le répertoire *tracking/fly oocyte*

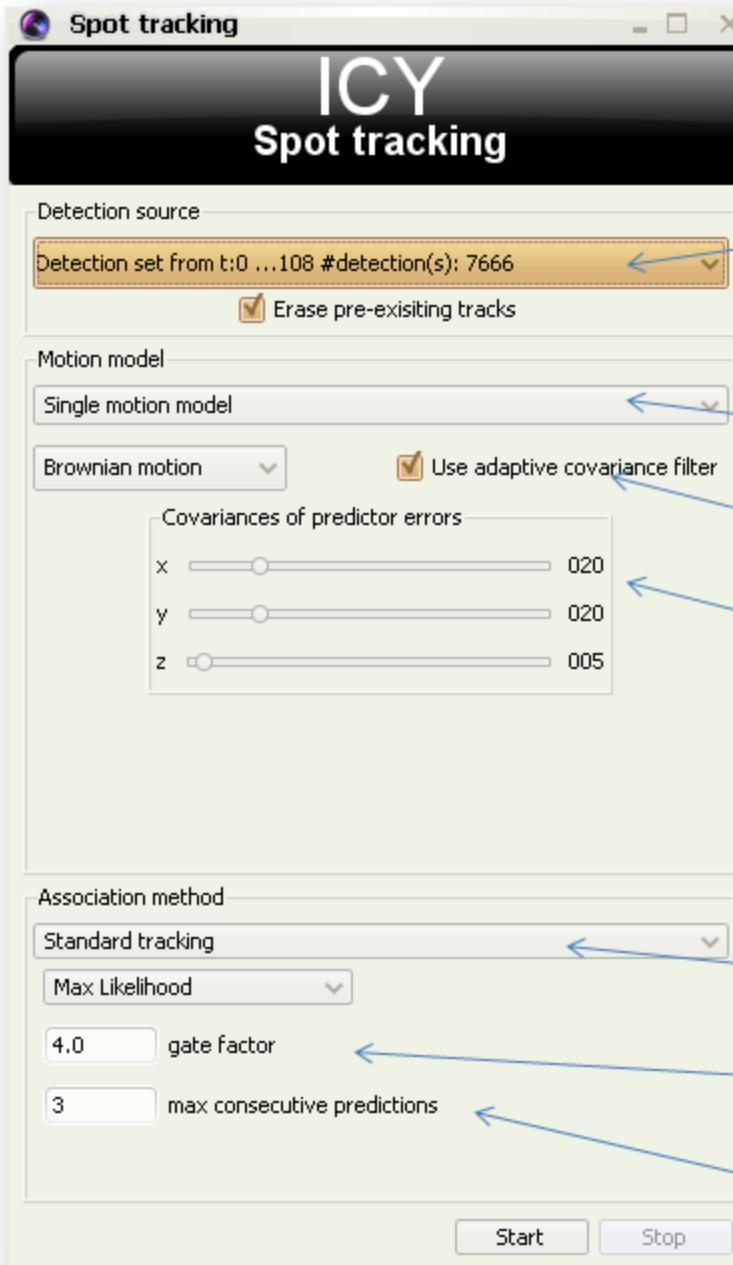
1. Create detections



- Draw an ROI area on the sequence

- Use the spot detector





The set of detections in the swimming pool

Number of motion model

Should the model be adaptive ?

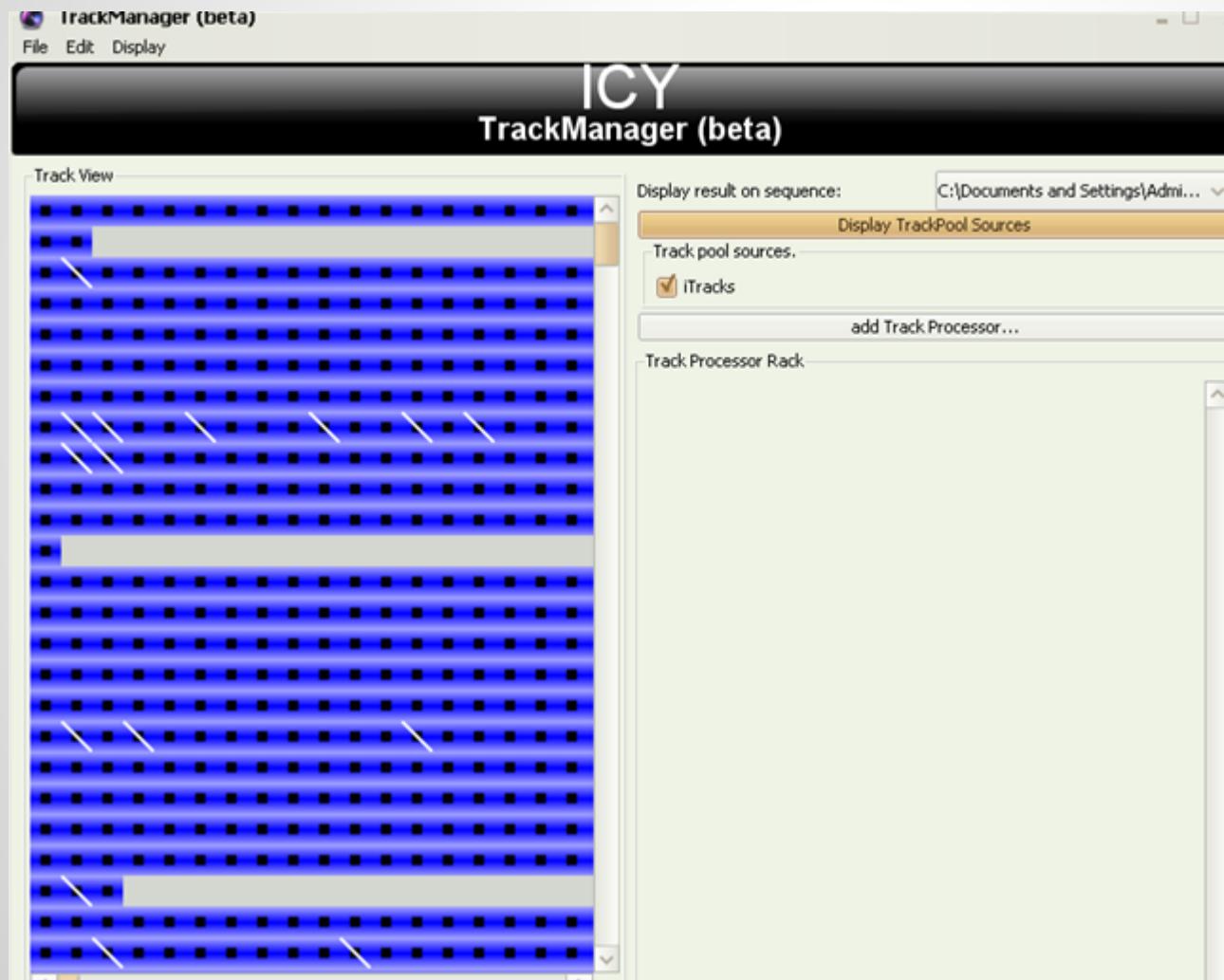
Parameters of the motion models
(known speed of particles)

Association between spots

Confidence in tracking

Number of max consecutive prediction
When detections vanish

The track manager



Spot tracking

ICY Spot tracking

Detection source

Detection set from t:0 ...108 #detection(s): 2103

Erase pre-existing tracks

Motion model

Single motion model

Brownian motion

Use adaptive covariance filter

Covariances of predictor errors

x: 003

y: 003

z: 005

Association method

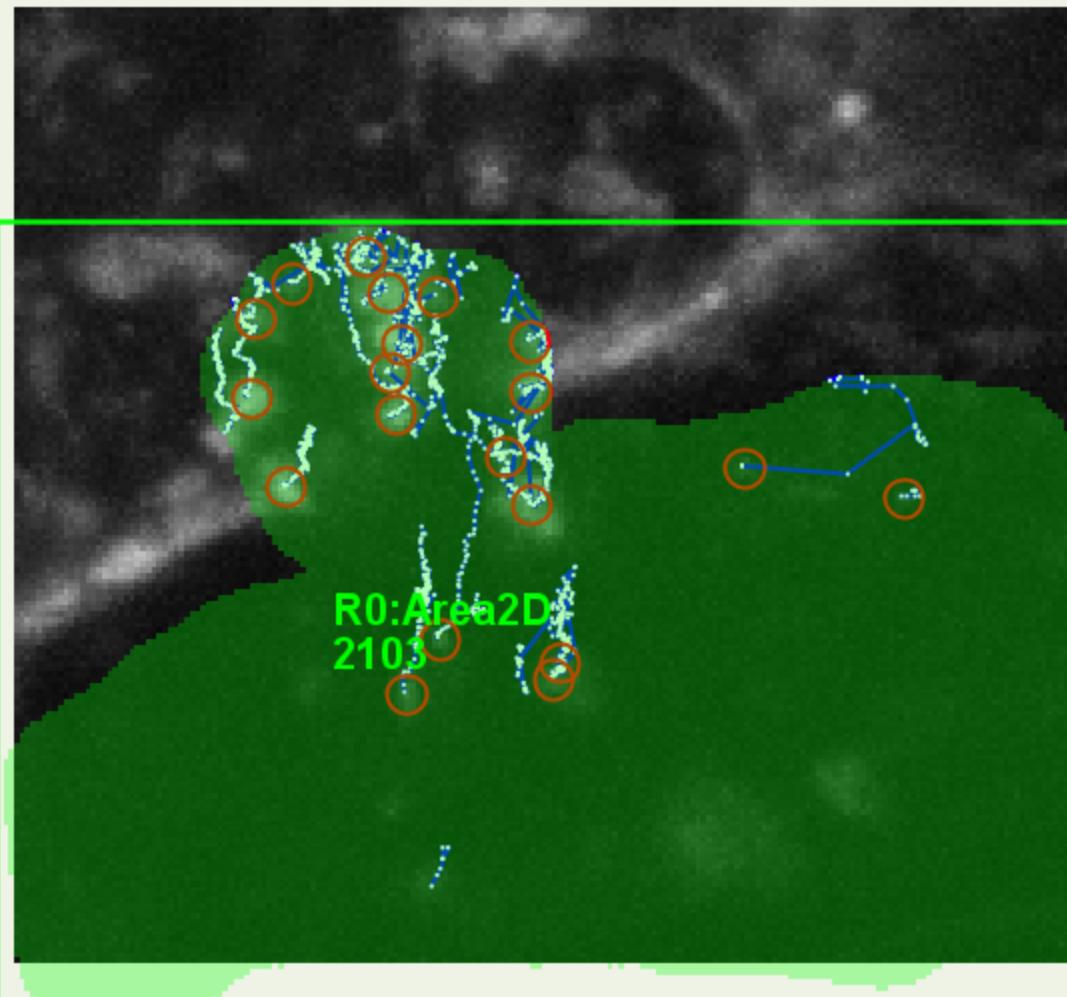
Standard tracking

Max Likelihood

3.5 gate factor

3 max consecutive predictions

ents and Settings\Administrator\Desktop\hong...ourse material\tracking\fly oocyte\proj



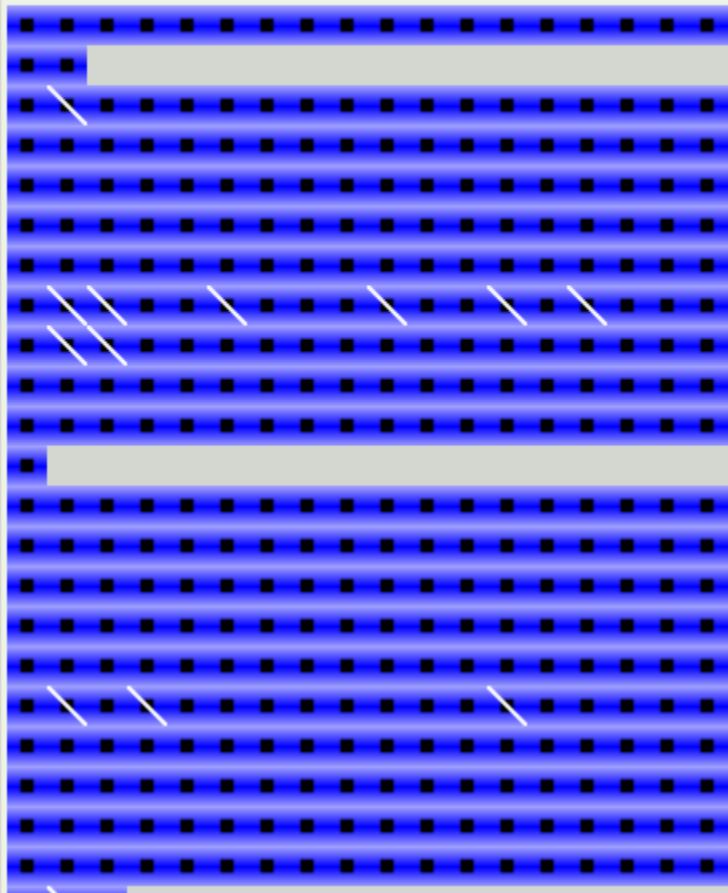
TrackManager (beta)

File Edit Display

ICY

TrackManager (beta)

Track View



Display result on sequence:

C:\Documents and Settings\Admini...

Display TrackPool Sources

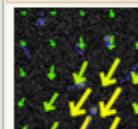
Track pool sources.

iTracks

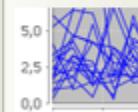
Add Track Processor Plugin...



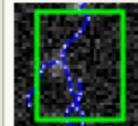
class plugins.fab.trackmanager.processors.TrackProcessorColorTrack



class plugins.fab.trackmanager.processors.TrackProcessorFlow



class plugins.fab.trackmanager.processors.TrackProcessorInstantSpeed

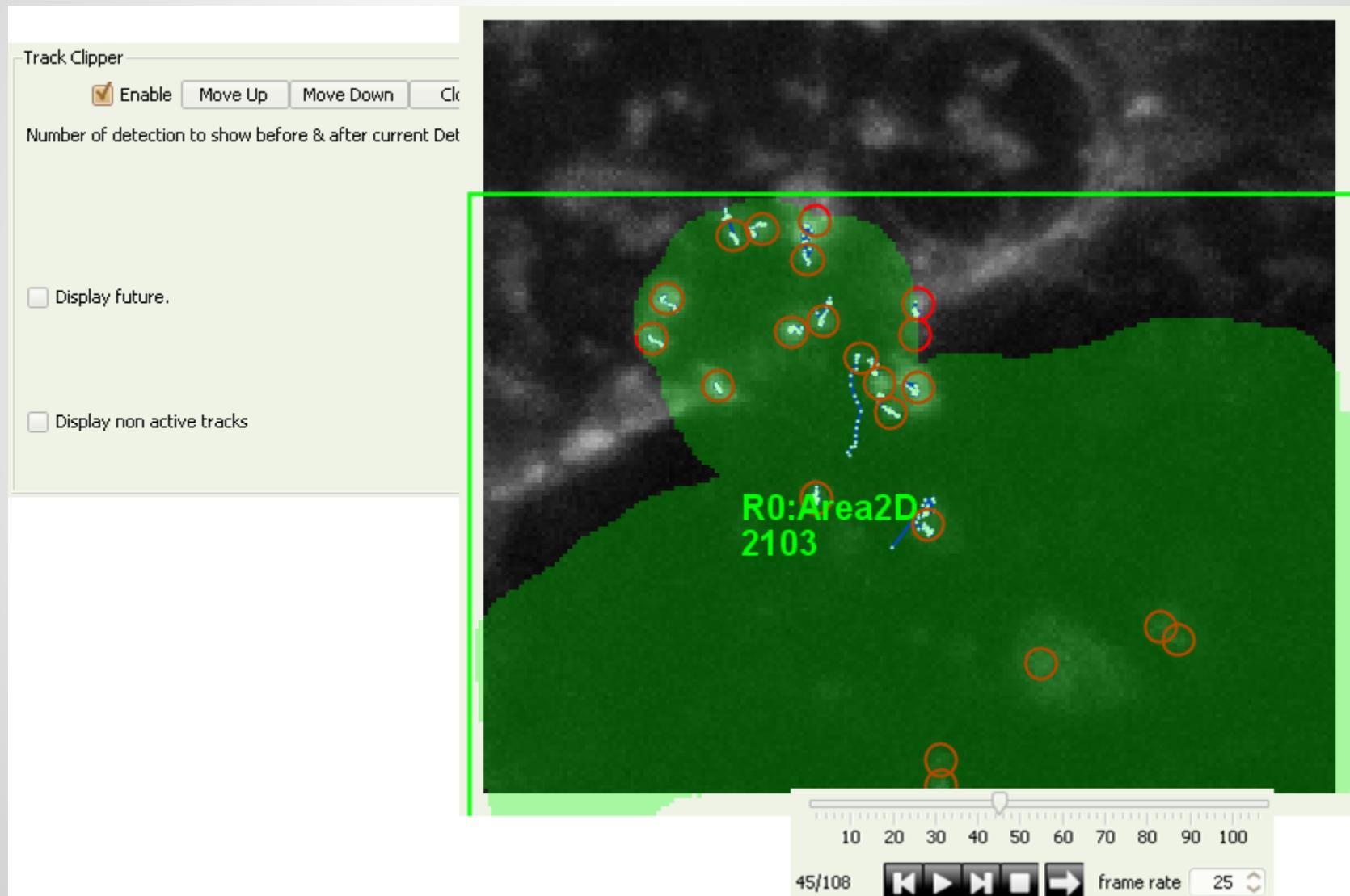


class plugins.fab.trackmanager.processors.TrackProcessorROIGate



class plugins.fab.trackmanager.processors.TrackProcessorTimeClip

Time clip processor



Color Processor

Track View

iTracks

add Track Processor...

Track Processor Rack

Track Clipper

- Enable
- Move Up
- Move Down
- Close

Number of detection to show before & after current Detection 20

Display future.

Display non active tracks

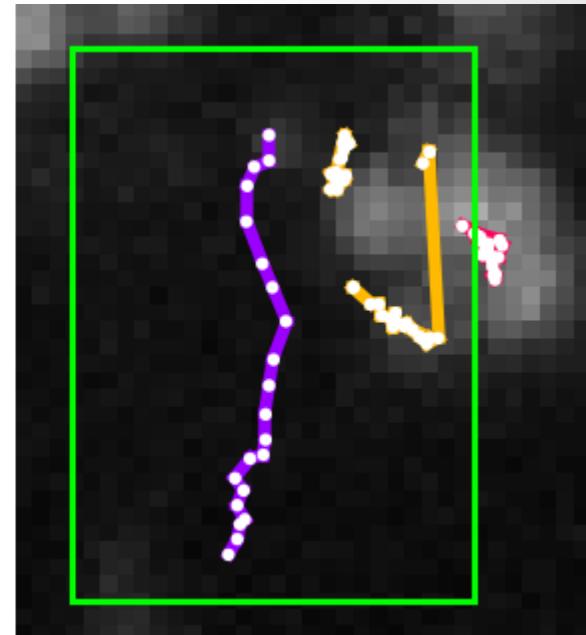
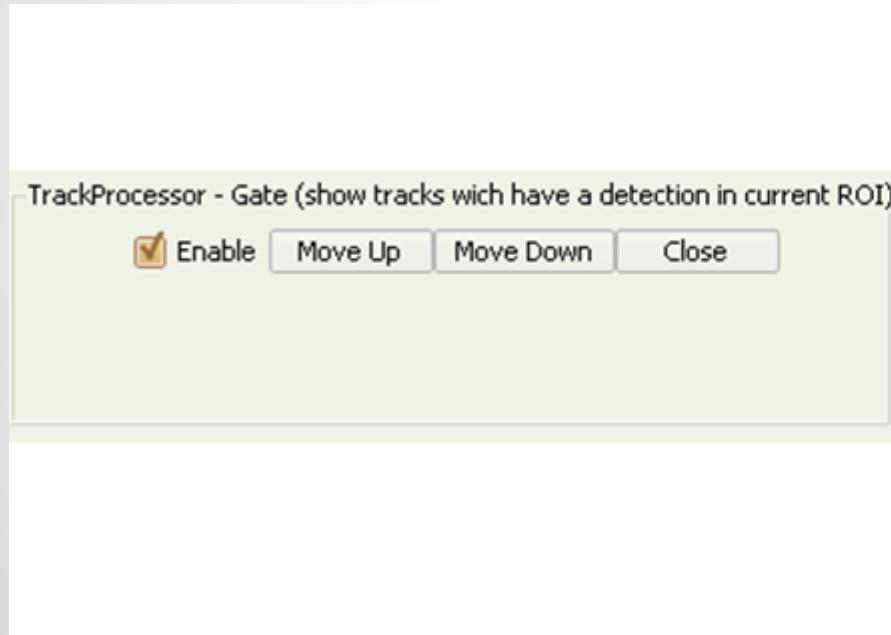
Color Track Processor

- Enable
- Move Up
- Move Down
- Close

Colors depends on start / end of the track.

Default color :

ROI Gate processor



Instant speed track processor

add Track Processor...

Track Processor Rack

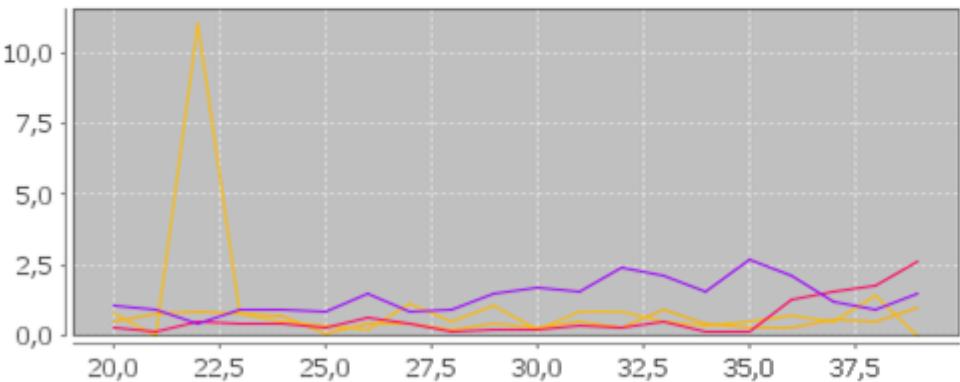
Default color :

TrackProcessor - Gate (show tracks which have a detection in current ROI)

Enable Move Up Move Down Close

Instant Speed Track Processor

Enable Move Up Move Down Close

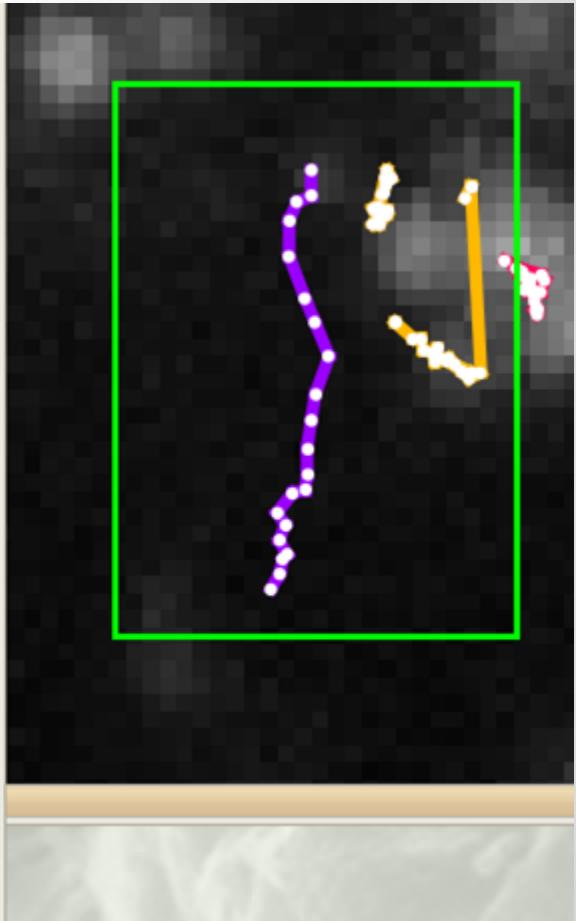


Affect colors. Display text. Display legend.

Display graph on sequence. place graph in ROI #1 Force graph width.

Use real scales Scale: 1.0

export to console



Flow track processor

TRACK POOL SOURCES:

iTracks 28 Tracks

[add Track Processor...](#)

Track Processor Rack

Affect colors. Display text. Display legend.
 Display graph on sequence. place graph in ROI #1 Force graph width.
 Use real scales Scale: 1.0

[export to console](#)

Track Flow Processor

Enable [Move Up](#) [Move Down](#) [Close](#)

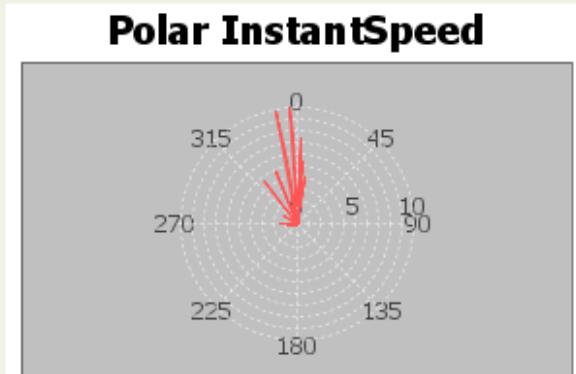
Options

Square side for 1 arrow in pixels: 10

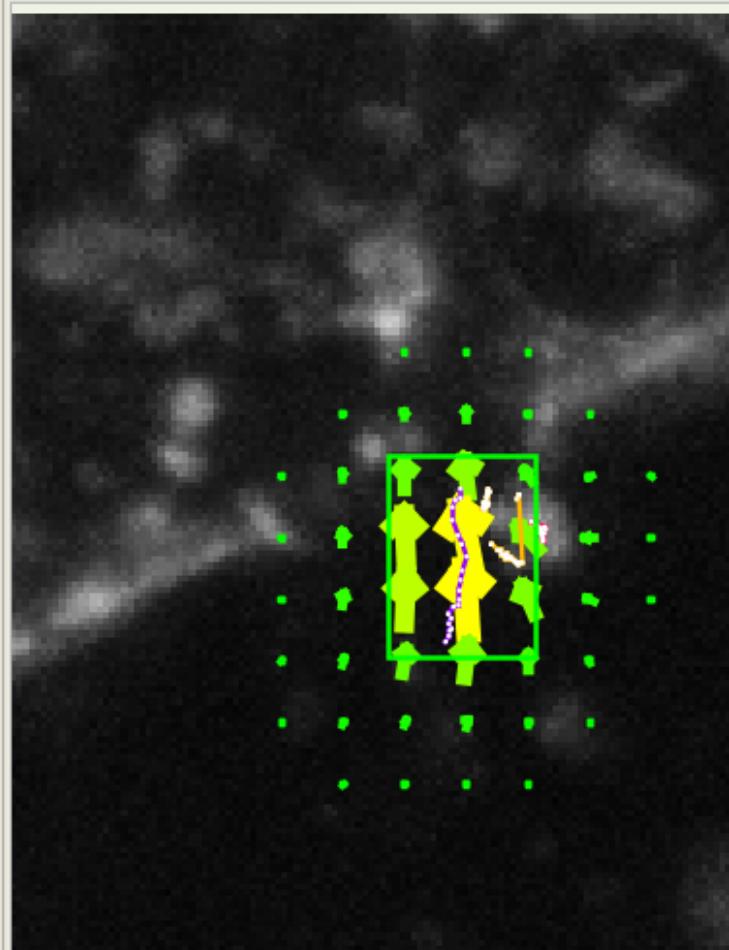
Gaussian Parameter - Search Radius: 10

Display on sequence.
 Remove Zero-Speed Arrows.
[Refresh.](#)
 Do not automatically re-compute.
 Display graph on sequence.
[place graph in ROI #1](#)

Polar InstantSpeed



Graphic Scale on sequence: 1.0



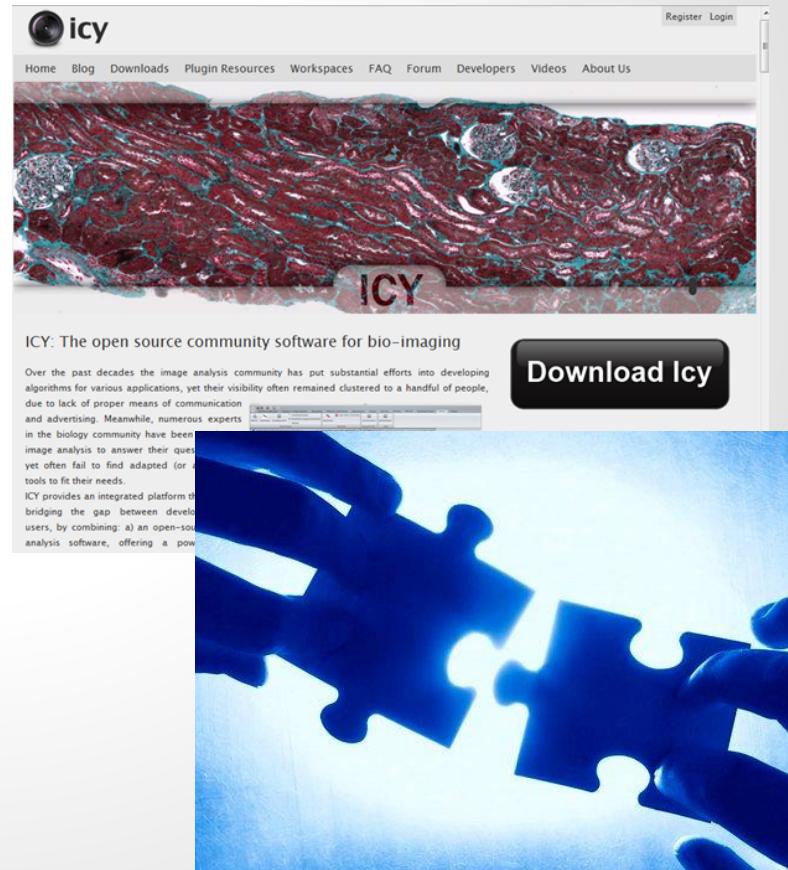


Conclusion

Keep in touch !

Support:

icy.bioimageanalysis.org/support



Going further...

Every wednesday mornings

Icy Club

9h30-13h

**Quantitative image analysis lab,
Pasteur - Calmette Building, first floor,
room 2**

