

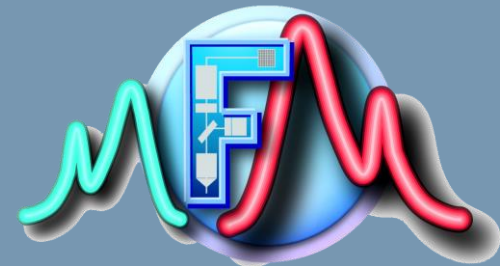
Analyses d'images avec le logiciel Icy

Webinar RTMFM

2021-01-12

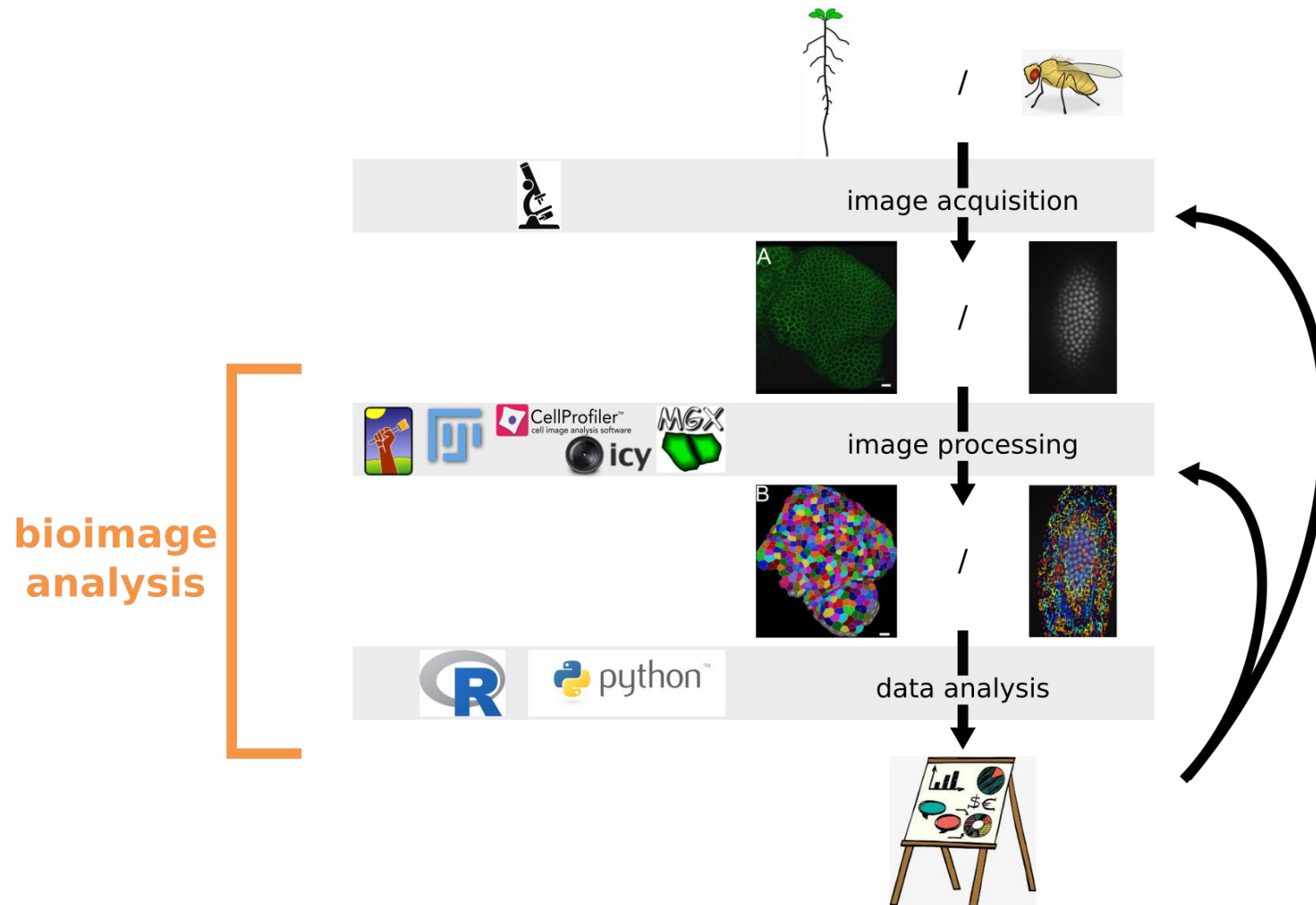
Marion Louveaux

Institut Pasteur



MFM
Microscopie de
Fluorescence
Multidimensionnelle

Icy a bioimage analysis software



Input images

- 2D/3D, timelapse, multichannels
- Mo to Go
- Supported formats:
<https://docs.openmicroscopy.org/bio-formats/5.8.2/supported-formats.html>

Processing possibilities

- Object counting, object detection
- Object segmentation, pixel classification
- Particle and object tracking
- ...

Output formats

- Images
- Spreadsheets (.xls, .csv)



From Pasteur institute



Bioimage analysis unit



FRANCE-BIOIMAGING

 de Chaumont et al. Icy: an open bioimage informatics platform for extended reproducible research. Nat Methods 9, 690–696 (2012). <https://doi.org/10.1038/nmeth.2075>

Original kernel developers



Fabrice de Chaumont



Alexandre Dufour



Stéphane Dallongeville

Project leader



Jean-Christophe Olivo-Marin

Current kernel developers



Stéphane Dallongeville



Daniel Gonzalez Obando



Amandine Tournay



Give access to **state-of-the-art & cutting-edge image processing tools**



Gather biologists, bioimage analysts, developers & computer vision researchers



Promote and facilitate **quantitative approaches & reproducibility**



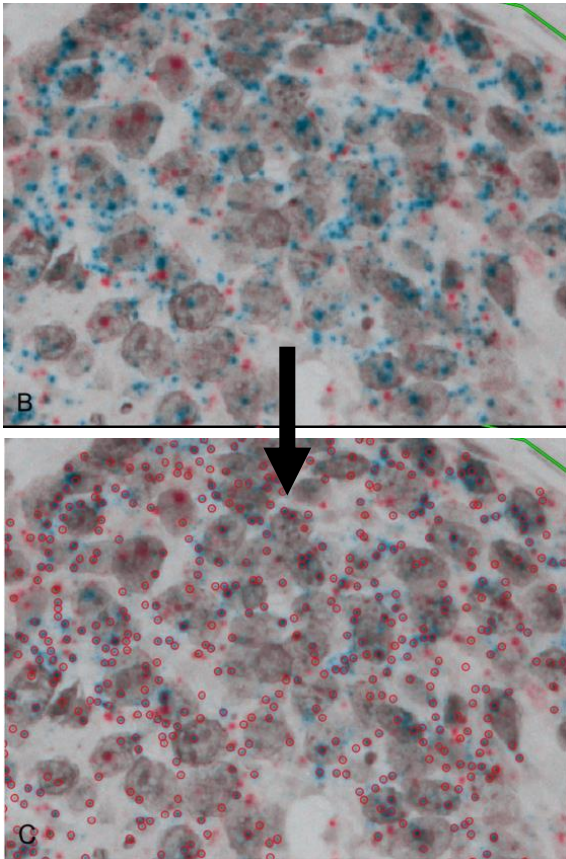
Give access to state-of-the-art & cutting edge image processing tools

- Rich bioimage analysis toolbox
 - Basic tools: filters (Gaussian blur...), morphological operations (erosion, dilation...)...
 - Advanced tools: active contours, tracking...
- Supported and constantly enriched by the Bioimage Analysis unit at Institut Pasteur
- Also constantly enriched by other computer vision labs



Examples of applications

Object Detection
Spot Detector



Seung et al. (2020) *Plos One*

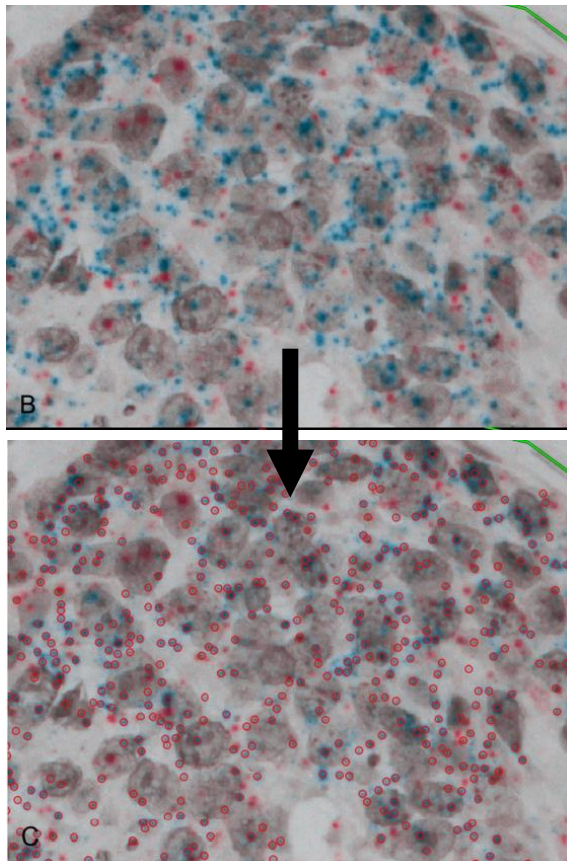
2021-01-12



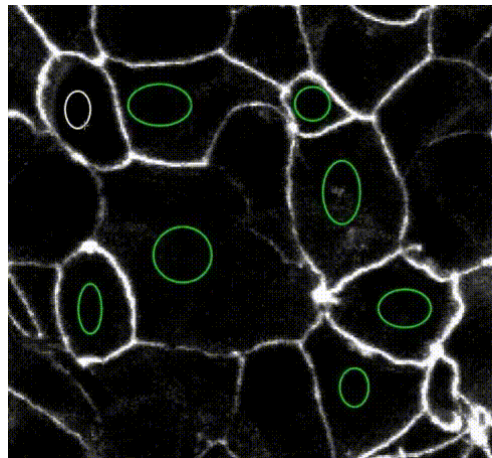
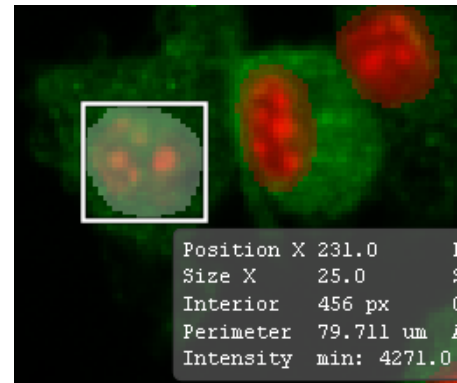
Examples of applications

Object Detection
Spot Detector

Object Segmentation
HK-Means, Active Contours...



Seung et al. (2020) *Plos One*
2021-01-12

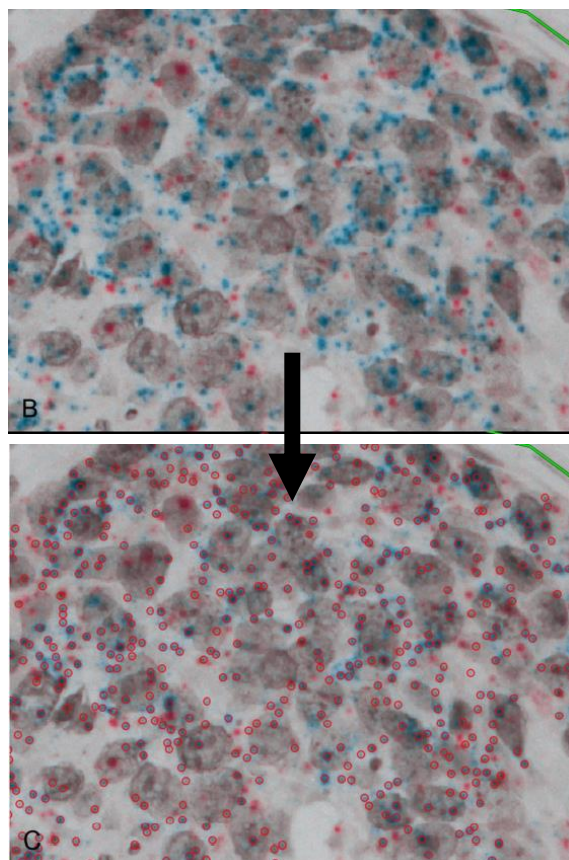


Bottom image: D. Gaboriau



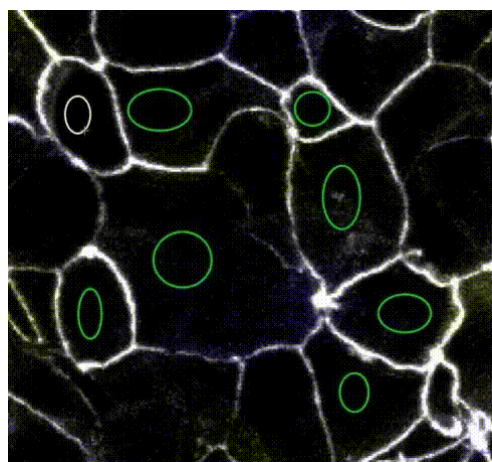
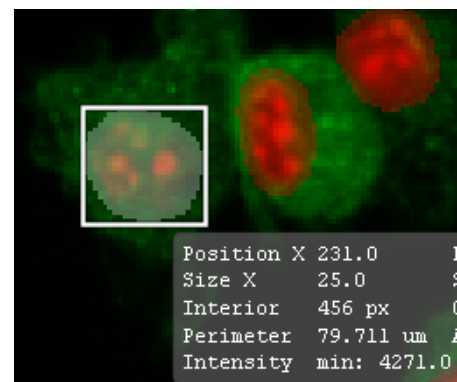
Examples of applications

Object Detection Spot Detector



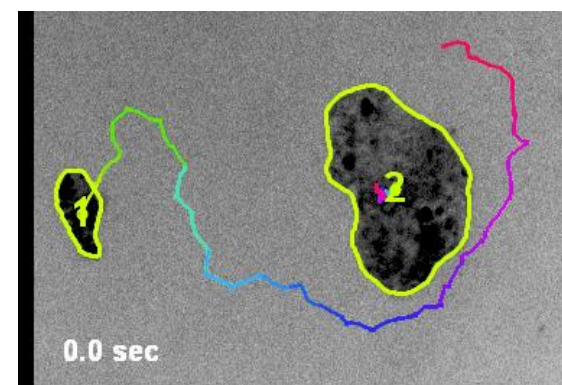
Seung et al. (2020) *Plos One*
2021-01-12

Object Segmentation HK-Means, Active Contours...



Bottom image: D. Gaboriau

Particle Tracking Track manager, Bioflow

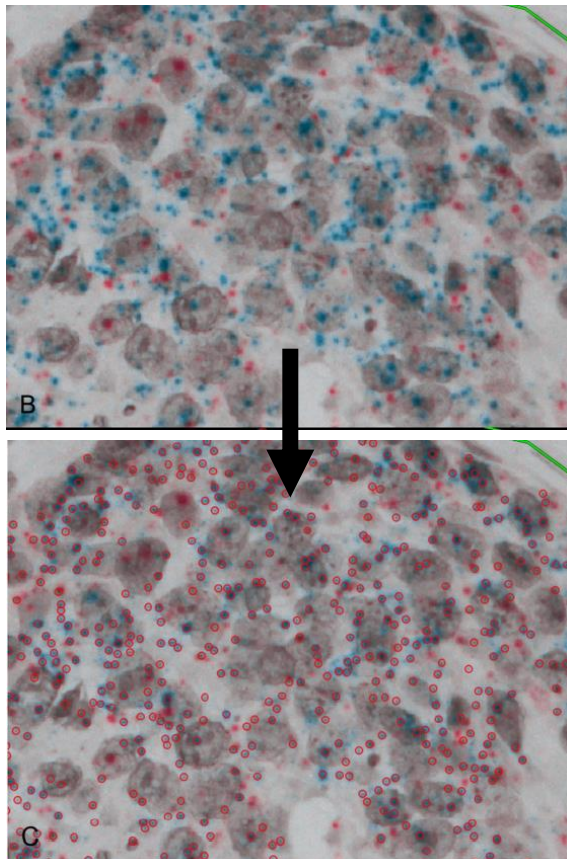


Top: M. Manich; Bottom: Boquet-Pujadas et al. (2017) *Nature Scientific Reports*
Webinar RTMFM



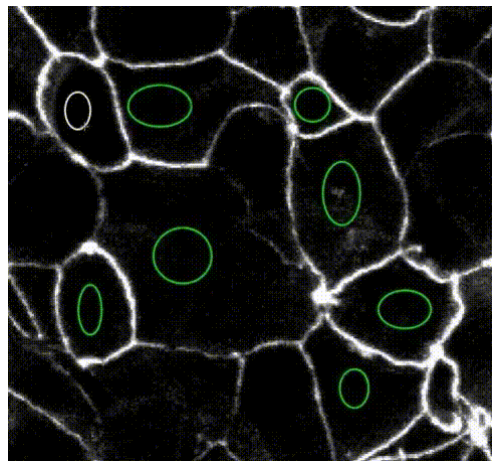
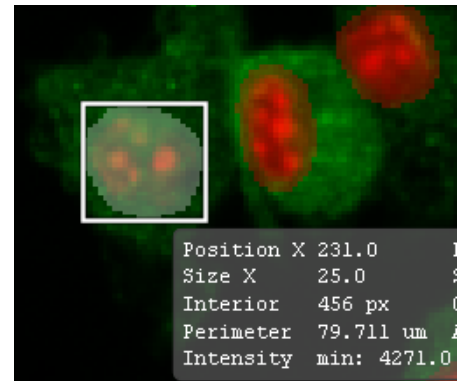
Examples of applications

Object Detection Spot Detector



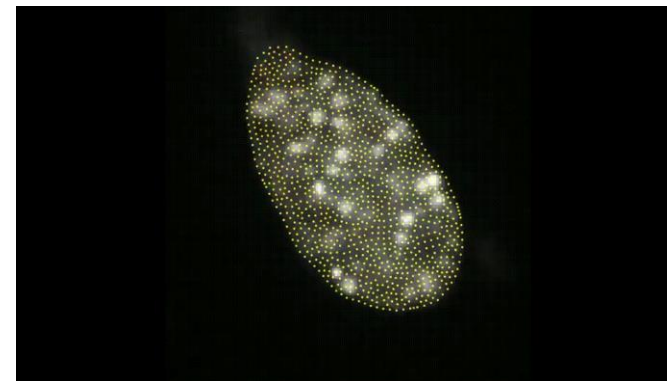
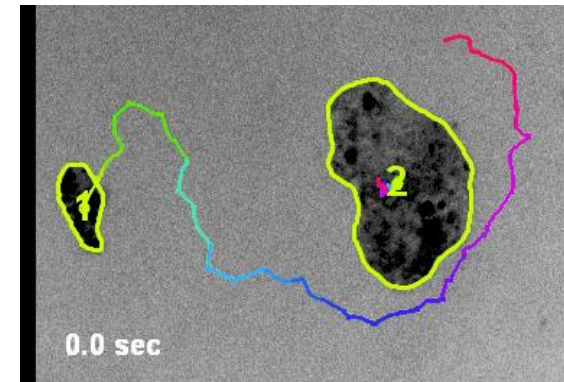
Seung et al. (2020) *Plos One*
2021-01-12

Object Segmentation HK-Means, Active Contours...



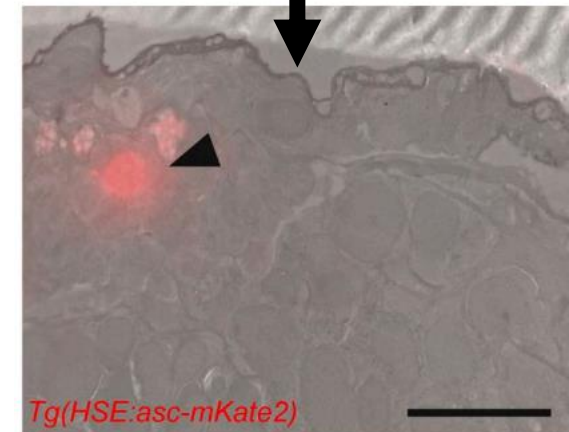
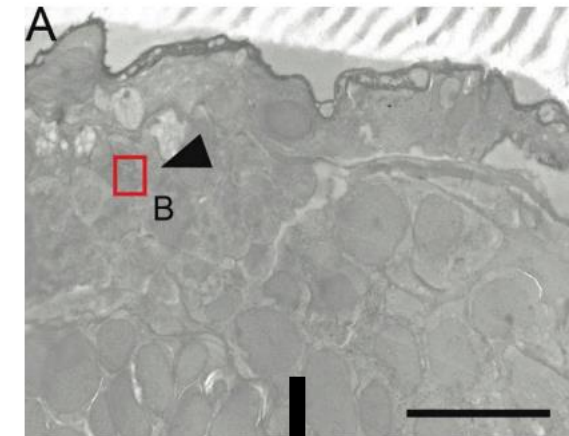
Bottom image: D. Gaboriau

Particle Tracking Track manager, Bioflow



Top: M. Manich; Bottom: Boquet-Pujadas et al. (2017) *Nat Sci Rep*
Webinar RTMFM

Registration ec-CLEM



Kuri et al. (2017), *JCB*

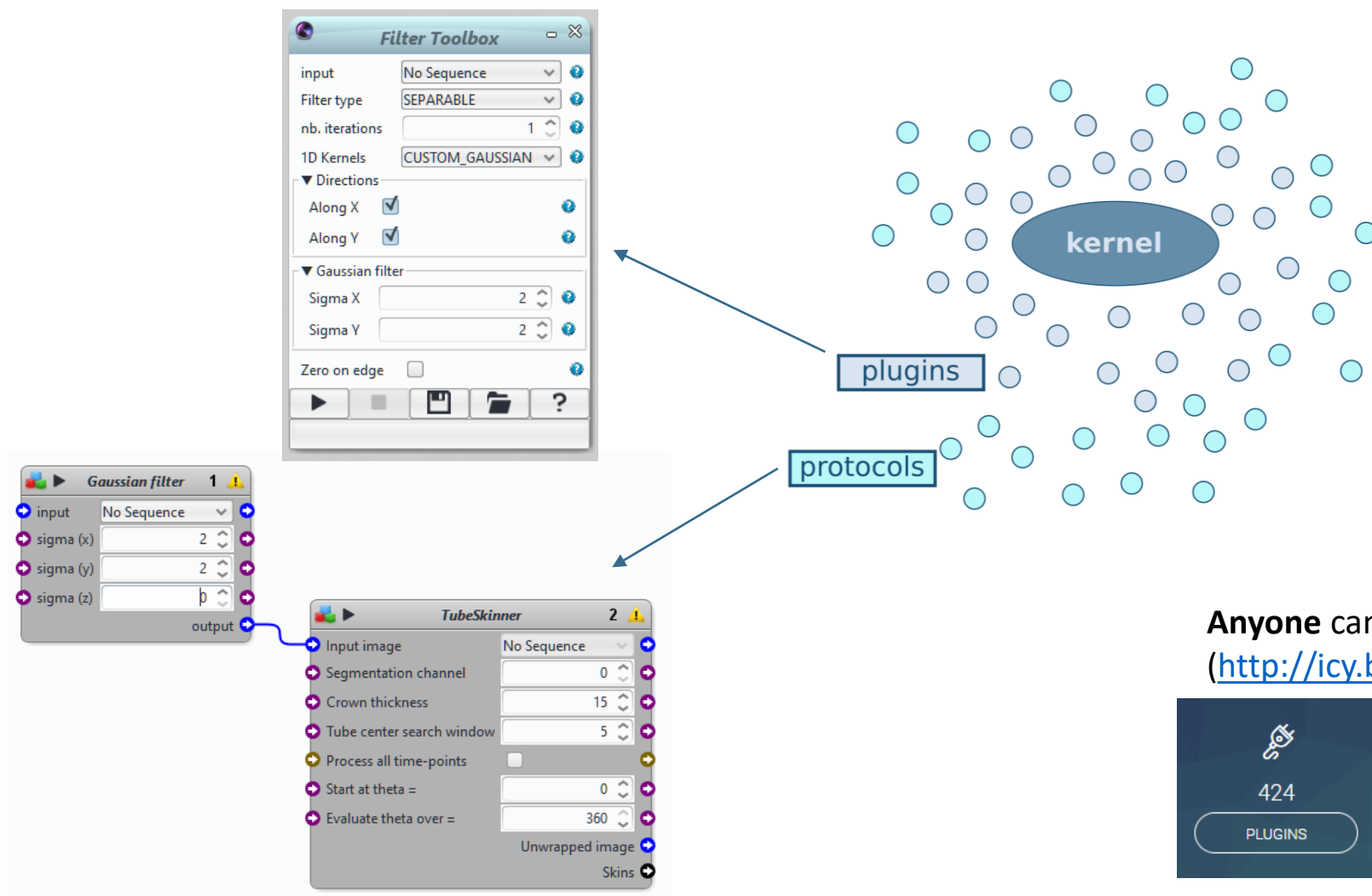


Gather several communities

- **Modular**: new algorithms are packaged for end-users as « plugins » (developed in Java) and **open source**
- **Suited for nonprogrammers as well as developers**
 - A rich graphical user interface
 - Tasks automation and workflows building even without programming knowledge (**protocols**)
 - **Scripting** possibilities
- **Documented and supported** by a community of users and developers through
 - the **image.sc forum**: help and discussion forum
 - the **Icy website**: central repository for plugins and protocols



Modular and open source

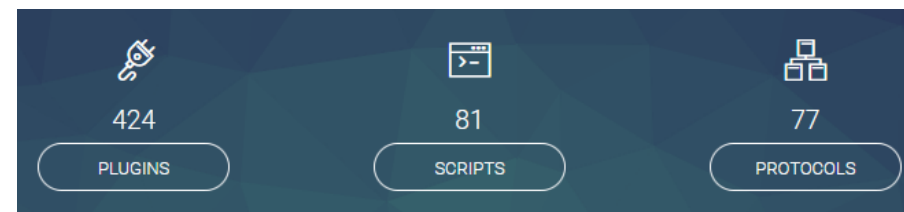


GPL3 license

Source code:

<https://gitlab.pasteur.fr/bia/icy>

Anyone can contribute to plugins and protocols
(<http://icy.bioimageanalysis.org/plugins/>)



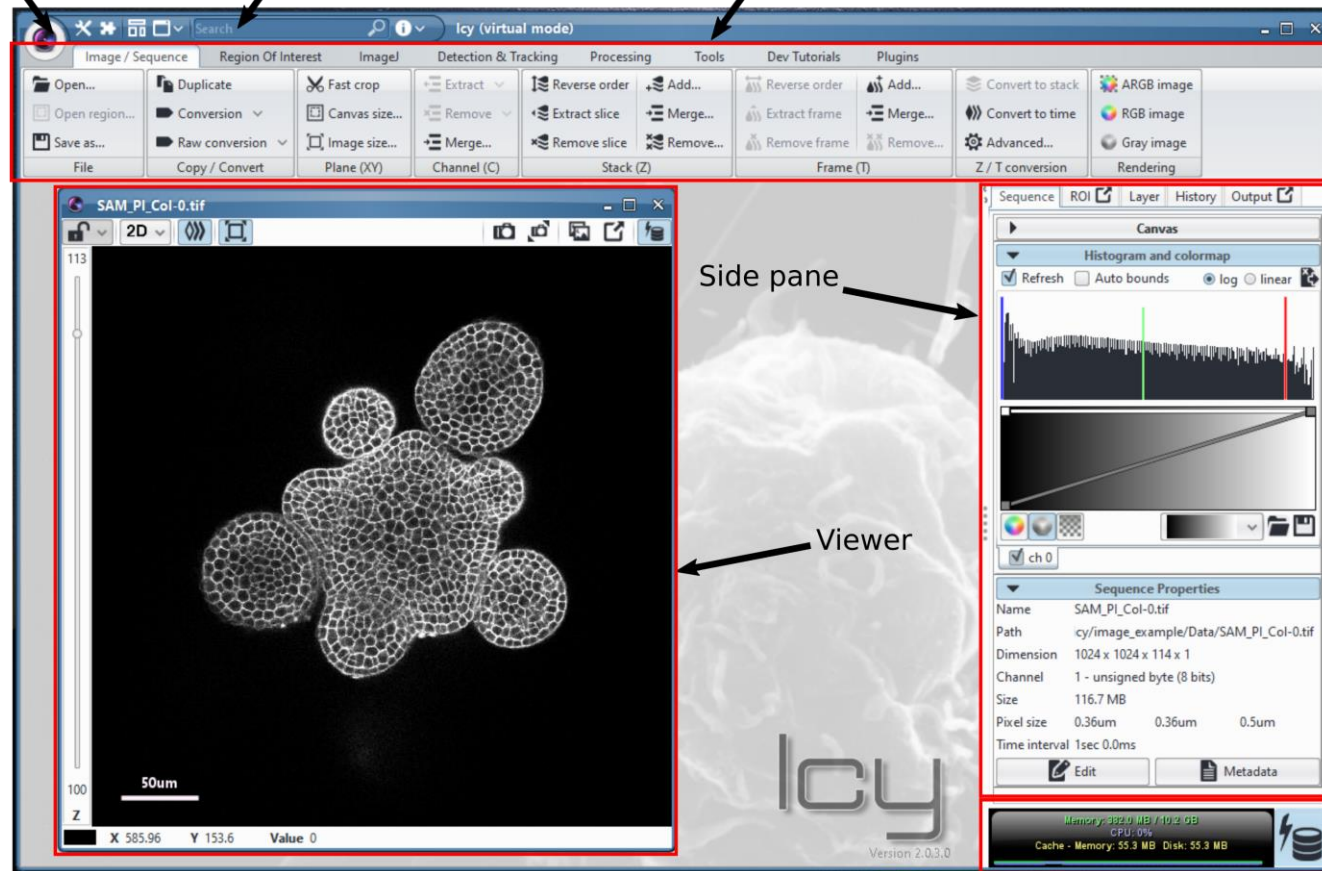
A rich Graphical User Interface (GUI)

Main menu

Search bar

Ribbon menu

 **Detached mode:**
windows are floating



Side pane

Viewer

Memory
usage



Display options & sequence properties

The screenshot displays the Icy software interface with several panels and windows. The main window shows a 3D visualization of a cluster of cells. The 'Canvas' panel on the left shows a zoomed-in view of the cells. The 'Sequence Properties' panel at the bottom left provides details about the loaded file. The 'Histogram and colormap' panel on the right shows the current histogram and colormap settings. The 'Icy (virtual mode)' window in the center shows the main menu and toolbar.

Canvas

Zoom: 52 %
Rotation: 0 °

Sequence Properties

Name	SAM_PI_Col-0.tif		
Path	icy/image_example/Data/SAM_PI_Col-0.tif		
Dimension	1024 x 1024 x 114 x 1		
Channel	1 - unsigned byte (8 bits)		
Size	116.7 MB		
Pixel size	0.36um	0.36um	0.5um
Time interval	1sec 0.0ms		

Histogram and colormap

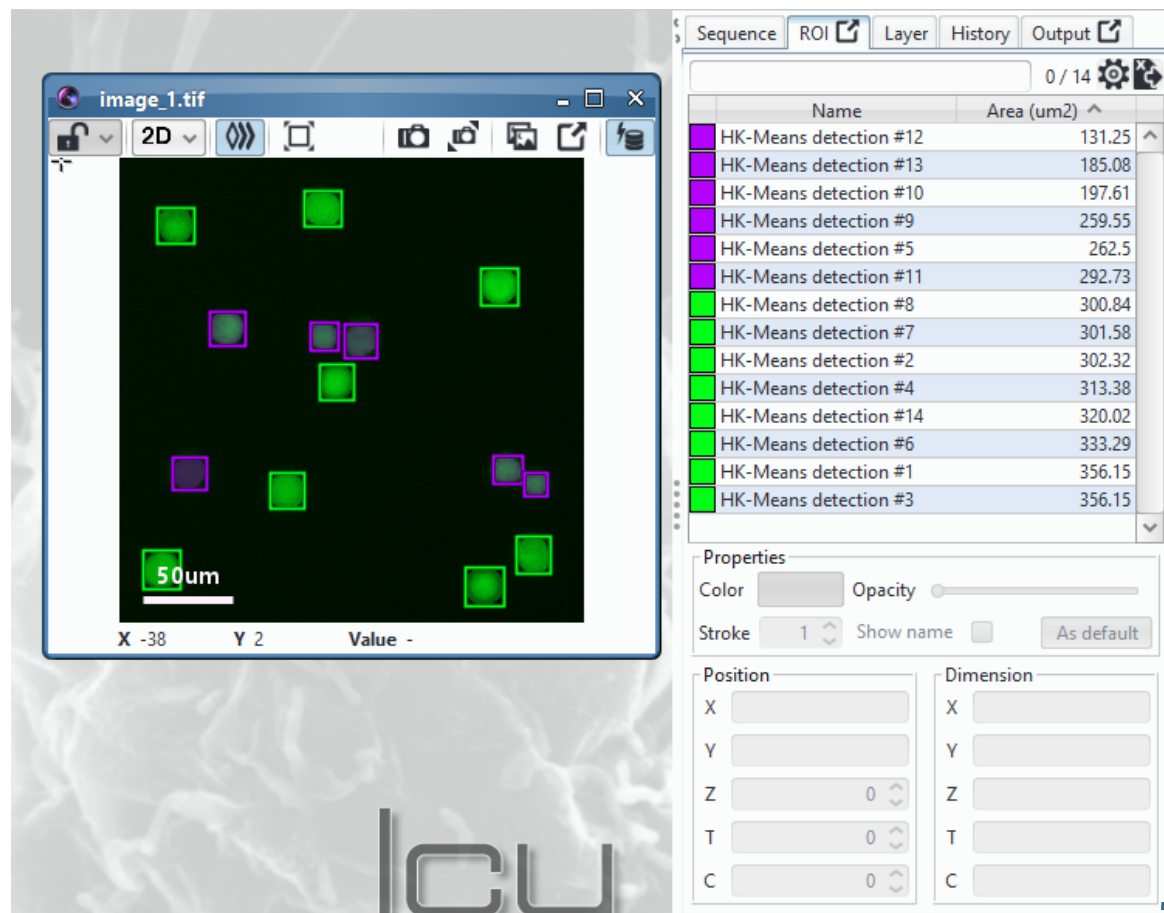
☒ Refresh ☐ Auto bounds ☒ log ☐ linear

Sequence Properties

Name	SAM_PI_Col-0.tif		
Path	icy/image_example/Data/SAM_PI_Col-0.tif		
Dimension	1024 x 1024 x 114 x 1		
Channel	1 - unsigned byte (8 bits)		
Size	116.7 MB		
Pixel size	0.36um	0.36um	0.5um
Time interval	1sec 0.0ms		



Regions Of Interest (ROI)



How to...

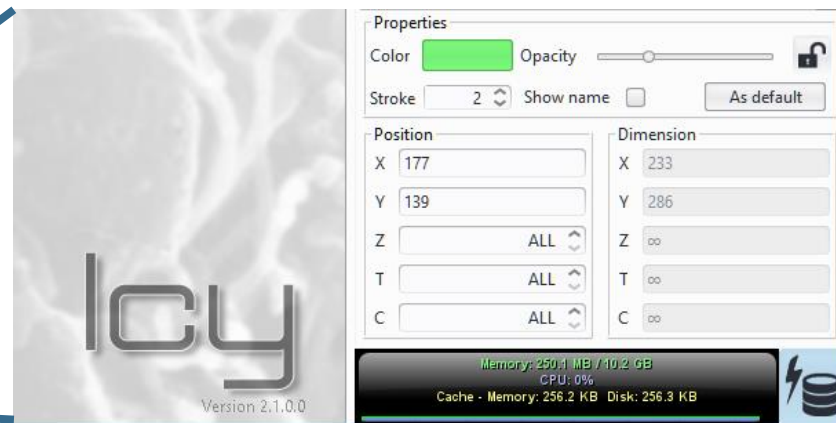
Sort ROIs: click on the name of the column (« Name », « Area »...)

Select a group of ROIs: Shift + click

Delete ROIs: select + press delete

Change the color of one or several ROIs: select + color

Prevent accidental ROIs modifications:



Regions Of Interest – get features

The screenshot displays the 'ROI table setting' dialog box, which is used to configure the features extracted from Regions of Interest (ROIs). The dialog is divided into two main sections: 'Columns to display' and 'Columns to export (XLS or CSV)'. Both sections contain a table with 'Column name' and 'Visible' columns. The 'Columns to display' table has 15 rows, with 'Color' and 'Name' checked. The 'Columns to export' table has 15 rows, with 'Position X' highlighted. The background shows a grayscale image with several green and purple ROIs and a table of ROI data.

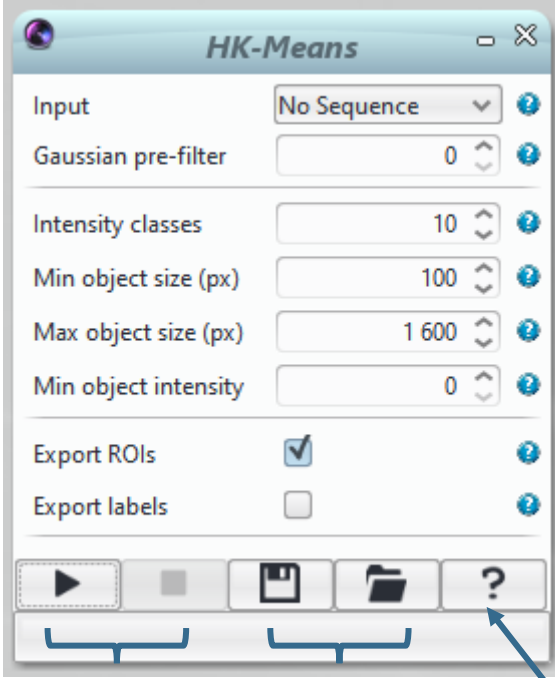
Name	Area (um2)
HK-Means detection #12	131.25
HK-Means detection #13	185.08
HK-Means detection #10	197.61
HK-Means detection #9	259.55
HK-Means detection #5	262.5
HK-Means detection #11	292.73
HK-Means detection #8	300.84
HK-Means detection #7	301.58
HK-Means detection #2	302.32
HK-Means detection #4	313.38
HK-Means detection #14	320.02
HK-Means detection #6	333.29
HK-Means detection #1	356.15
HK-Means detection #3	356.15

Column name	Visible
Icon	☐
Color	☒
Group	☐
Name	☒
Position X	☐
Position Y	☐
Position Z	☐
Position T	☐
Position C	☐
Size X	☐
Size Y	☐
Size Z	☐
Size T	☐
Size C	☐
Center X	☐
Center Y	☐
Center Z	☐
Center T	☐
Center C	☐
Contour	☐
Interior	☐

Column name	Visible
Icon	☐
Color	☐
Group	☒
Name	☒
Position X	☒
Position Y	☒
Position Z	☒
Position T	☒
Position C	☒
Size X	☒
Size Y	☒
Size Z	☒
Size T	☒
Size C	☒
Center X	☒
Center Y	☒
Center Z	☒
Center T	☒
Center C	☒
Contour	☒
Interior	☒



Image processing tools are packaged into plugins



The screenshot shows the 'HK-Means' plugin window. It has a title bar with a close button. The main area contains several input fields and checkboxes. On the left, there are three blue brackets with labels: 'Image to process' pointing to the 'Input' field, 'Input variables' pointing to the 'Gaussian pre-filter', 'Intensity classes', 'Min object size (px)', 'Max object size (px)', and 'Min object intensity' fields, and 'Output variables' pointing to the 'Export ROIs' and 'Export labels' checkboxes. At the bottom, there are five buttons: a play button, a square button, a save button, a folder button, and a question mark button. Below these buttons are three labels: 'Run/Pause' under the play button, 'Save/Load parameters' under the save and folder buttons, and 'Online documentation' under the question mark button. An arrow points from the 'Online documentation' label to the question mark button.

Image to process

Input variables

Output variables

ROIs = Region Of Interest
Labels = new image with a given pixel value per object

Run/Pause

Save/Load parameters

Online documentation

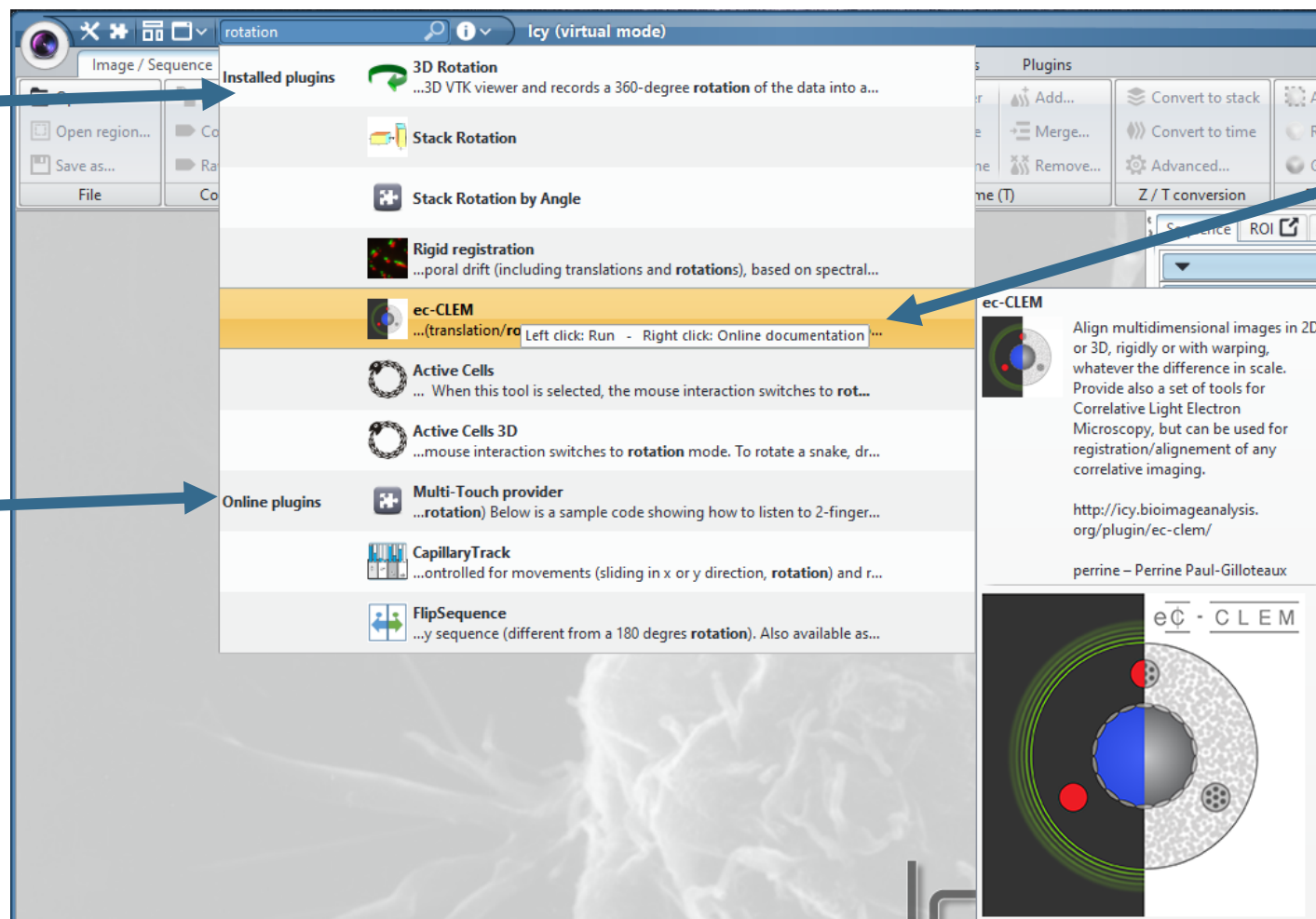


How to find a plugin: the search bar

Installed locally on the computer in the /plugin folder

Plugin stored on the Icy website
<http://icy.bioimageanalysis.org/>

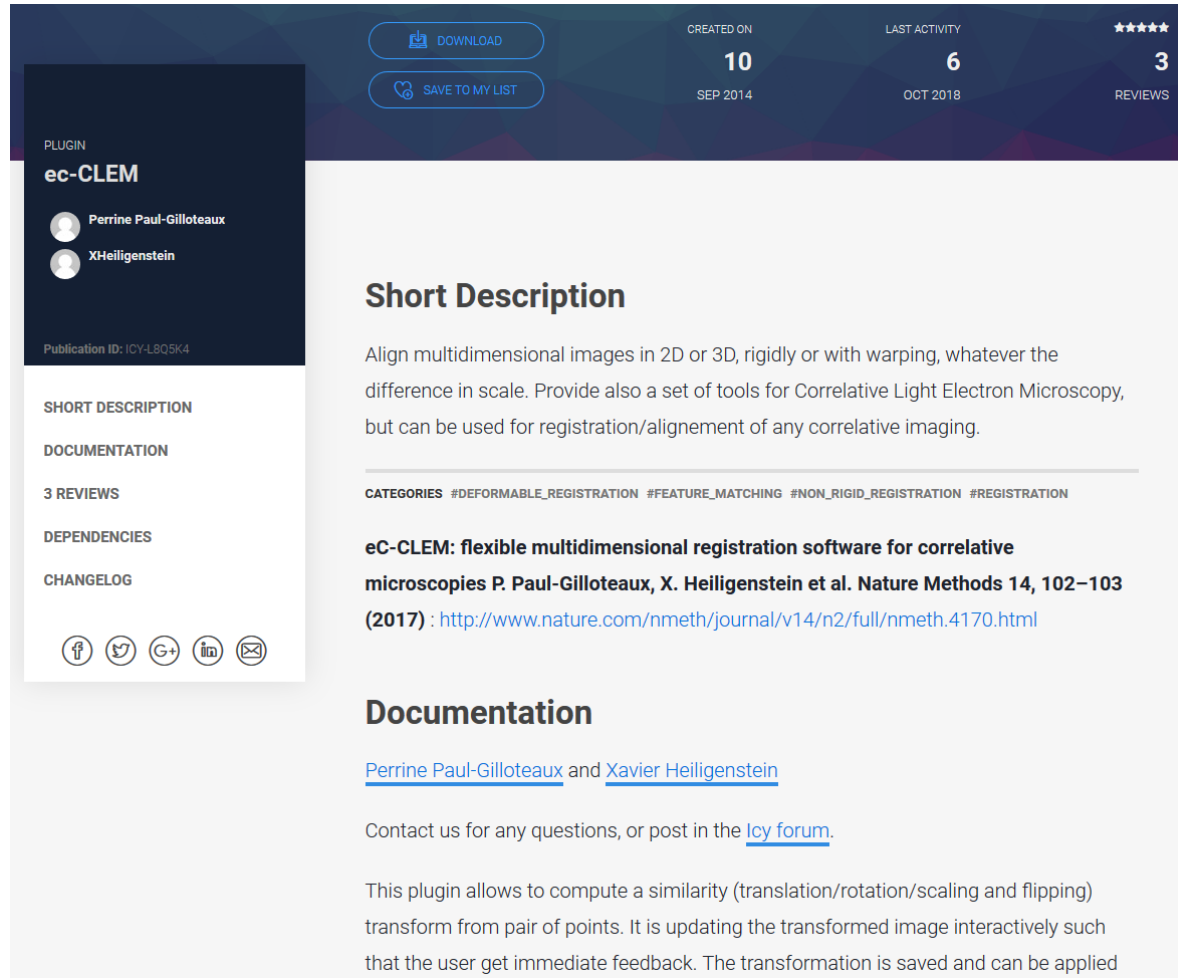
Documentation on the Icy website



Use the search bar to search for plugins, protocols, commands...



How to use a plugin: the documentation



The screenshot shows the Icy website interface for the 'ec-CLEM' plugin. The top navigation bar includes a 'DOWNLOAD' button, a 'SAVE TO MY LIST' button, and statistics: 'CREATED ON SEP 2014' with a count of 10, 'LAST ACTIVITY OCT 2018' with a count of 6, and '★★★★★ 3 REVIEWS'. The left sidebar lists navigation options: 'PLUGIN', 'ec-CLEM', 'Perrine Paul-Gilloteaux', 'XHeiligenstein', 'Publication ID: ICY-LBQSK4', 'SHORT DESCRIPTION', 'DOCUMENTATION', '3 REVIEWS', 'DEPENDENCIES', and 'CHANGELOG'. The main content area features a 'Short Description' section with the text: 'Align multidimensional images in 2D or 3D, rigidly or with warping, whatever the difference in scale. Provide also a set of tools for Correlative Light Electron Microscopy, but can be used for registration/alignement of any correlative imaging.' Below this is a 'Documentation' section with the text: 'eC-CLEM: flexible multidimensional registration software for correlative microscopies P. Paul-Gilloteaux, X. Heiligenstein et al. Nature Methods 14, 102–103 (2017) : <http://www.nature.com/nmeth/journal/v14/n2/full/nmeth.4170.html>'. At the bottom of the documentation section, it says: 'Contact us for any questions, or post in the [Icy forum](#). This plugin allows to compute a similarity (translation/rotation/scaling and flipping) transform from pair of points. It is updating the transformed image interactively such that the user get immediate feedback. The transformation is saved and can be applied'.

Direct access to plugin documentation via

- The search bar of the software
- The plugin window
- The search bar of the Icy website



Where to get help

- Forum image.sc: <https://forum.image.sc/>



 Create a new Topic

Type title, or paste a link here

■ Usage & Issues ▼ icy +

 Type here. Use Markdown, BBCode, or HTML to format. Drag or paste images.

+ Create Topic cancel

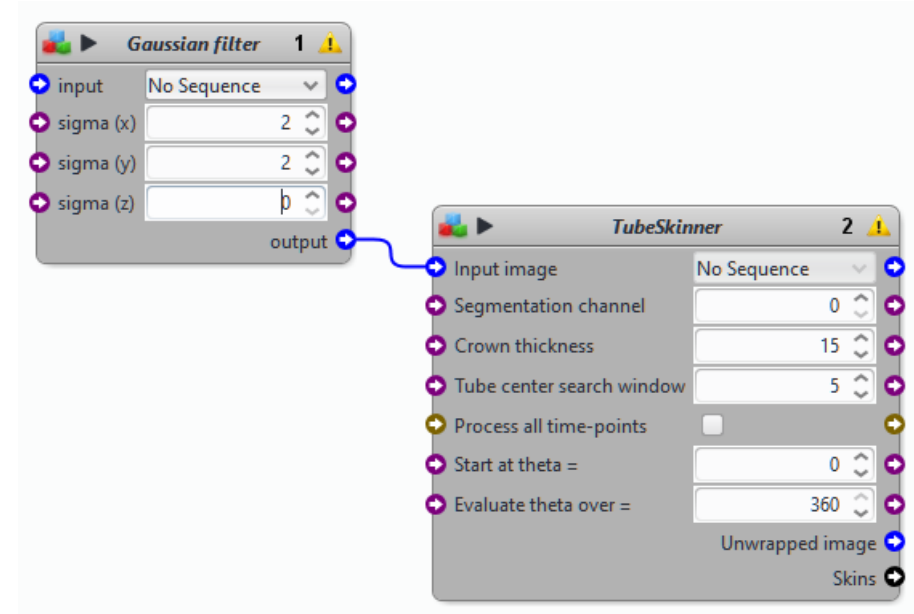
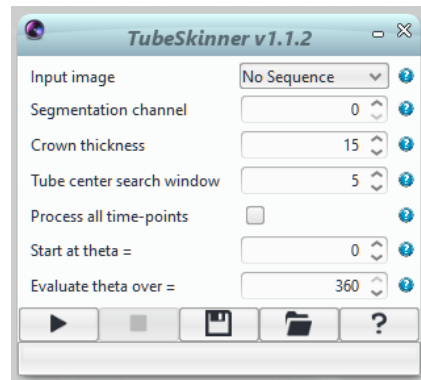
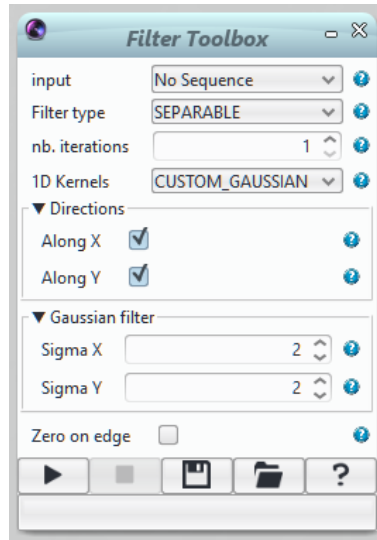


Promote and facilitate the use of quantitative approaches & reproducibility

- Outputs to spreadsheets for further data analyses
- Possibility to save and reload plugins parameters
- Graphical programming to build bioimage analysis workflows with protocols



Protocols overcome plugins limitations



Advantages

- Graphical user interface with nice little buttons
- Possibility to save/reload parameters

Disadvantages

- One image at a time
- One image processing step at a time
- Not easy to reproduce

Advantages

- Workflow: several processing steps
- Batch processing: several images
- Some kind of graphical user interface
- Reproducible and easy to share (light xml file)

More info:

<http://icy.bioimageanalysis.org/plugin/protocols/>



How to cite

- In scientific publications

- **Cite Icy:** de Chaumont et al. Icy: an open bioimage informatics platform for extended reproducible research. Nat Methods 9, 690–696 (2012).
<https://doi.org/10.1038/nmeth.2075>
- **Cite the plugins** you use. Look for the reference in the online documentation
- **Publish your protocols:** <http://icy.bioimageanalysis.org/tutorial/how-to-publish-a-protocol/>

- On Twitter

- Did you publish recently a paper using Icy? Did follow a course on Icy ? Do you have a favorite plugin? Are you proud of your last protocol? Share it!



- @Icy_Bioimaging to notify the Icy team
- Follow @Icy_Bioimaging to get news from the Icy team



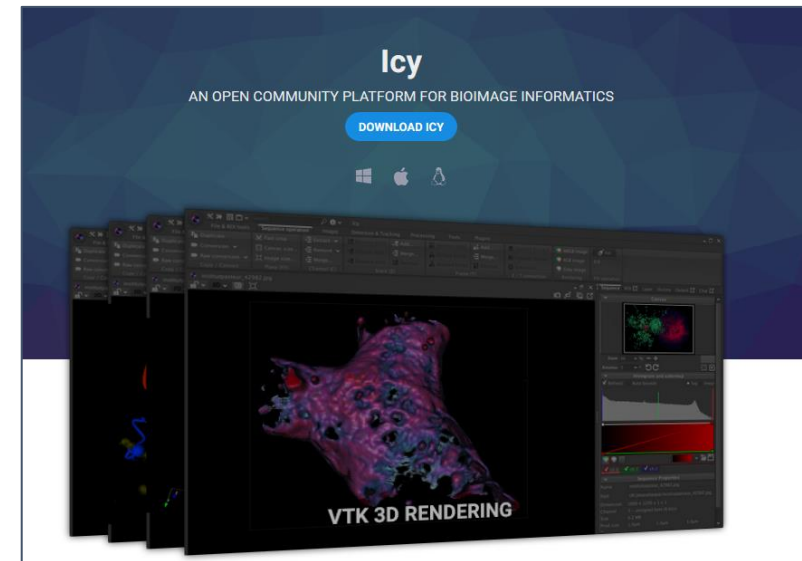
Have fun with Icy and keep in touch with us!

 @Icy_BioImaging

Support forum

<https://forum.image.sc/tag/icy>

Don't forget to cite Icy and its plugins ;)



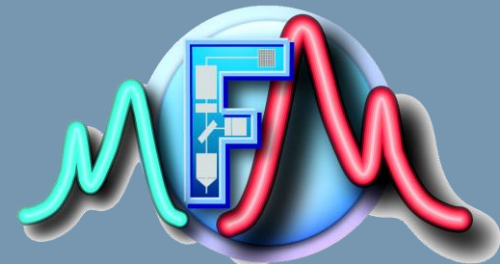
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