

“Easy Cell shape with HK-Means” protocol

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Updates will be available here :

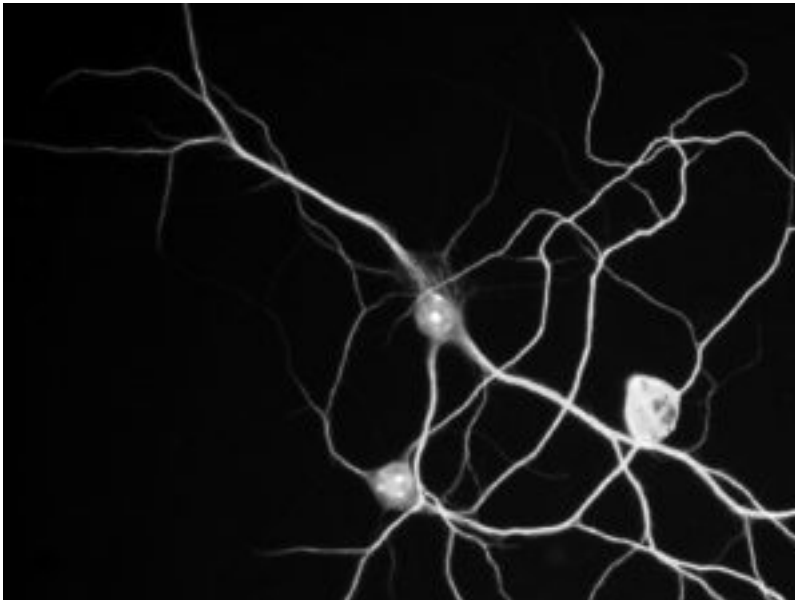
<http://icy.bioimageanalysis.org/protocol/easy-cell-shape-with-hk-means/>

Short Description : This protocol allows you to segment Cell shape thanks to the HK-Means method. It will create an overlay layer of the segmented results over your original picture as a ROI (here in blue over the white signal). This ROI can be saved and used to analyze many parameters of interest (Intensity, area, perimeter, sphericity,... see below) thanks to ROI statistics bloc (Publication ID: ICY-W5T6J4).

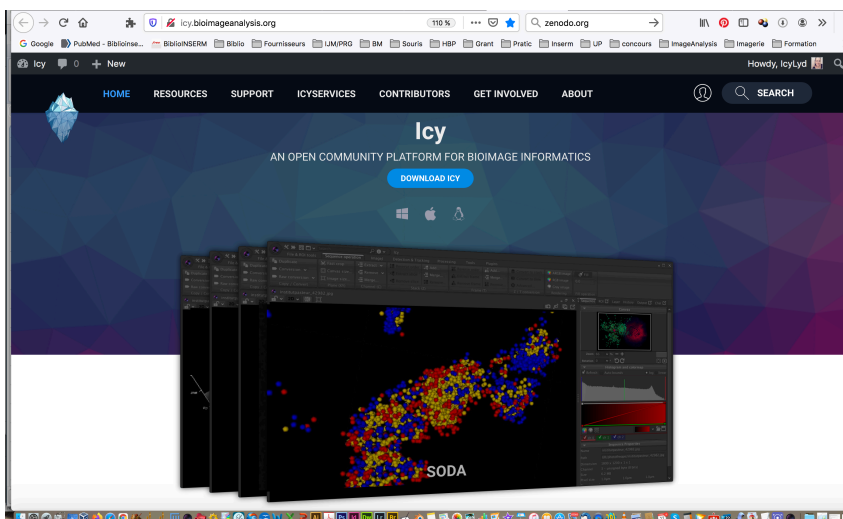
The hk-mean plugin (Publication ID: ICY-V4N7Q1) used in this protocol is documented here : <http://icy.bioimageanalysis.org/plugin/hk-means/> and is related to this paper : A. Dufour, V. Meas-Yedid, A. Grassart, and J.-C. Olivo-Marin, “Automated Quantification of Cell Endocytosis Using Active Contours and Wavelet”, Proc. ICPR 2008, Tampa, FL, USA.

Documentation

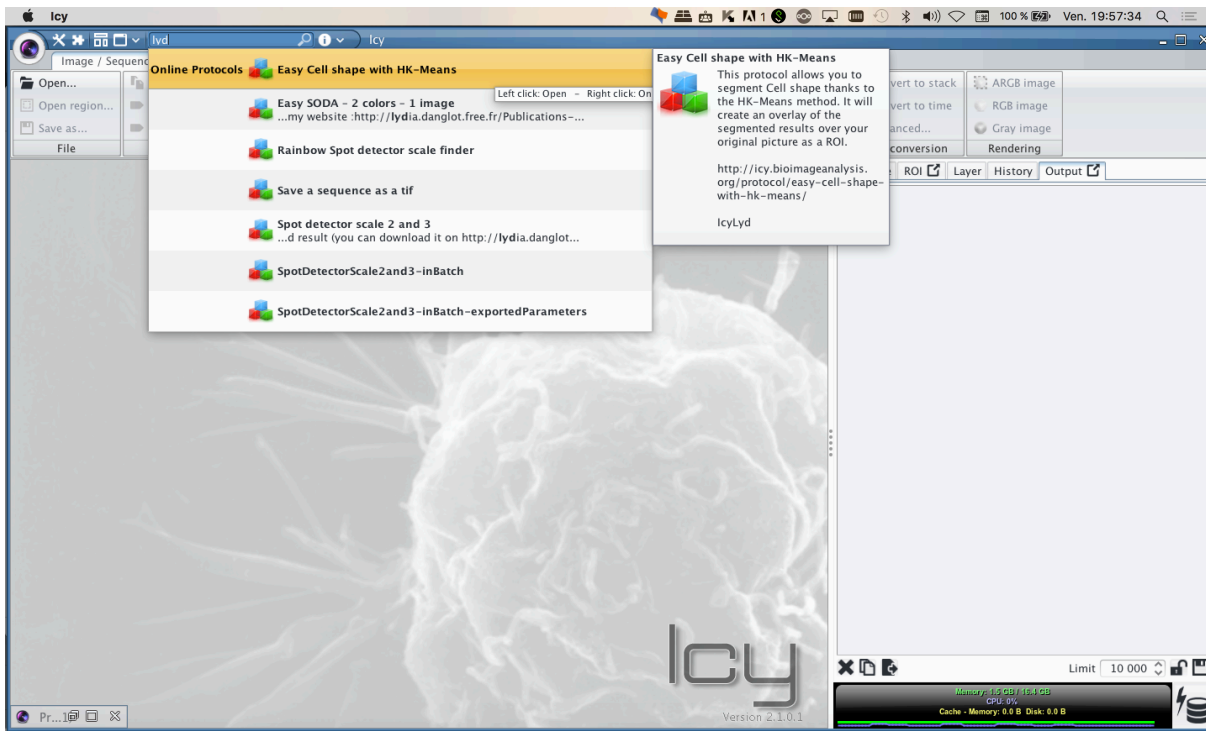
Let's say that you need to segment this cell. Picture is available on <https://doi.org/10.5281/zenodo.4322944>



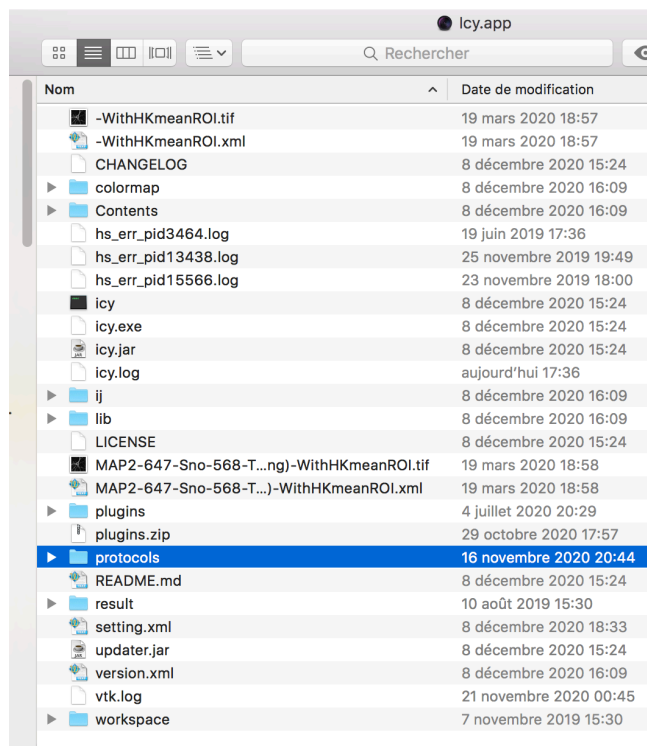
You just have to : Install Icy software by downloading it here : <http://icy.bioimageanalysis.org/>



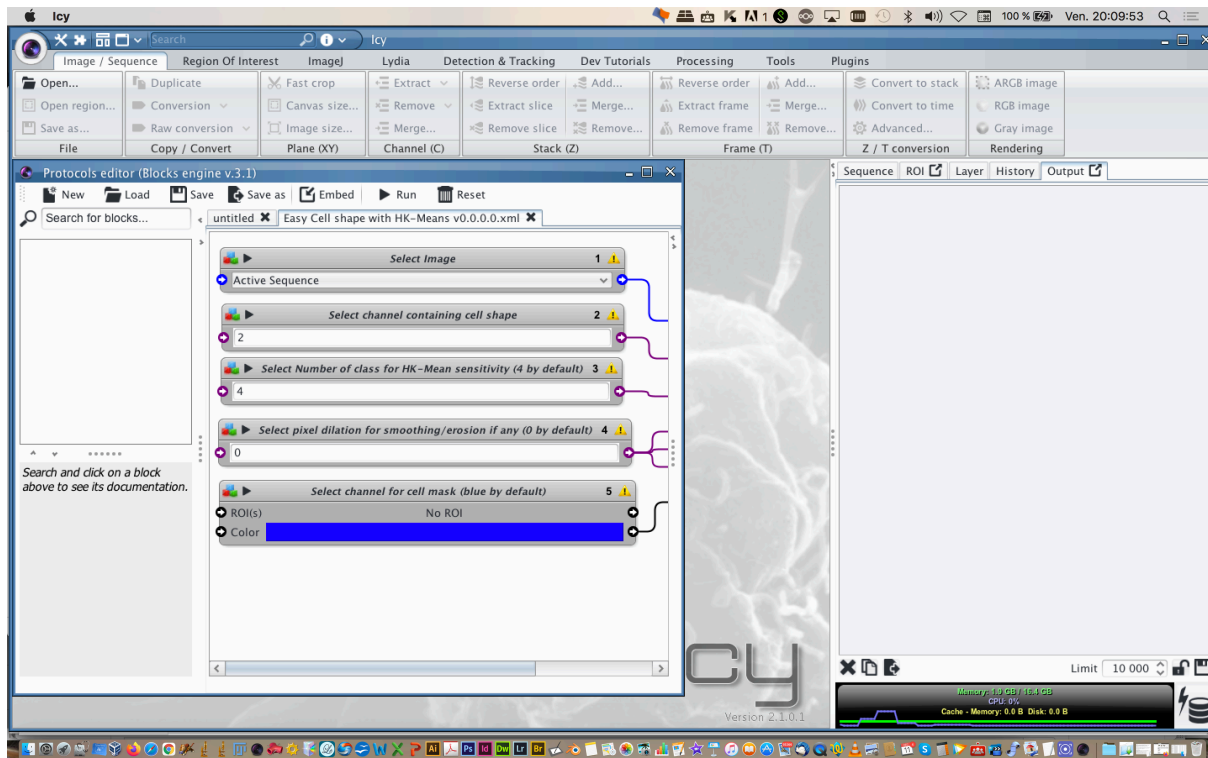
Once installed. Type `lyd` to access my resources list in the search bar on the top and then click on the Easy Cell shape with HK-Means. It will automatically download the protocol file and install it in your icy software.



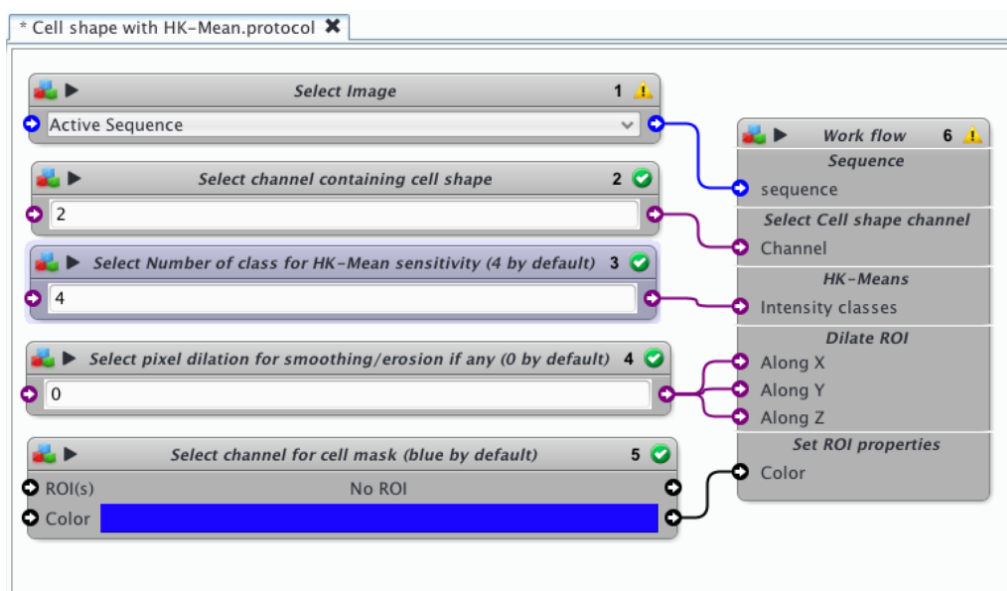
An alternative method is to download the .protocol file and to copy paste it in the protocols folder present within the Icy.app paquet. On mac you can open the Icy.app paquet by right clicking on it and select “open the packet”.



One installed, the Protocols editor plugin that allows graphical programming will open automatically, and give you access to the Easy Cell Shape with HK-Means protocol:

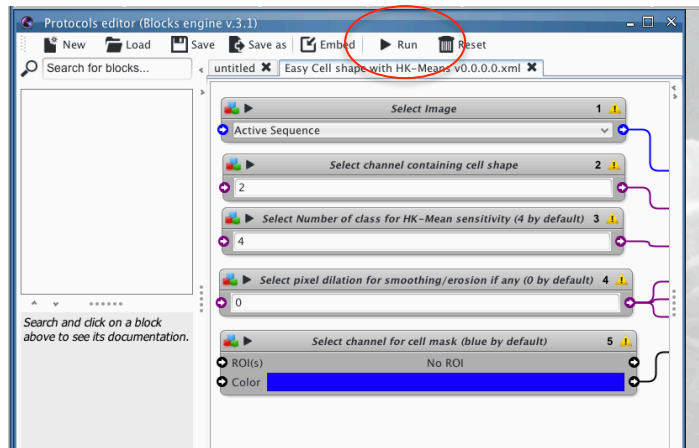


1. Open the desired image, or the image sample available on <https://doi.org/10.5281/zenodo.4322944>
2. Select the desired image in block 1. You can select “active sequence” if the picture is the last selected, or directly select it by name by clicking on the menu.



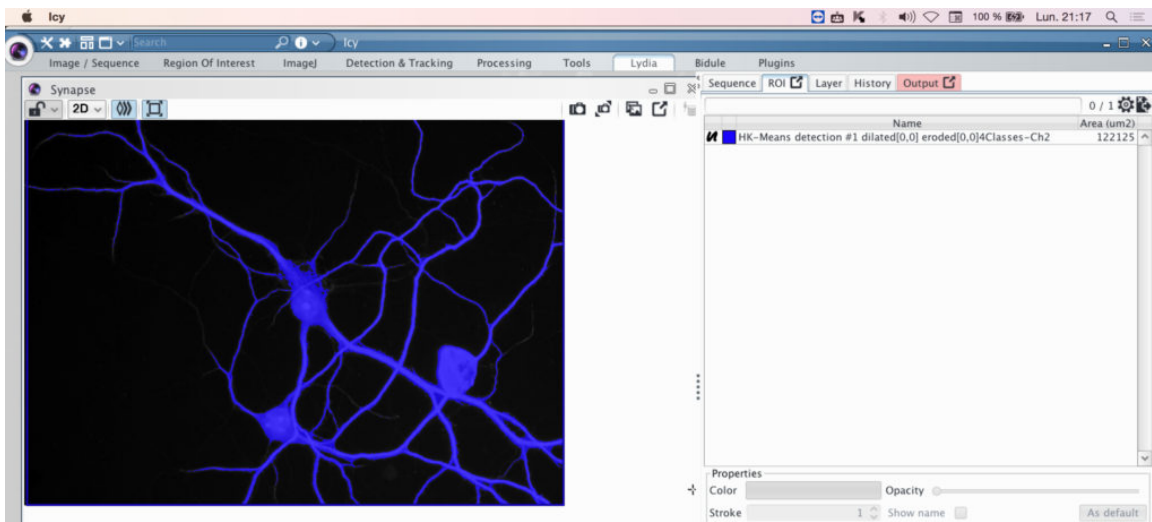
3. Select in block 2 the channel of interest for cell shape (here replace the channel 2 by channel 0 if using sample image). Be careful in icy, channel numbers are following a mathematician numbering : first channel is numbered as #0, second one as #1, third one as #2.
4. Select the number of classes you want to use in HK-Means segmentation. 2 classes would correspond to conventional automatic thresholding. By increasing the number of classes you increase the sensitivity and go deeper in the faint regions. By default this value is set up at 4.
5. In block 4, you can add a facultative step of dilation/erosion that will smooth the mask. By default it is set to 0 pixel.
6. In block 5 choose the color for your ROI. By default it is set to blue, but you can choose your favorite one by clicking on the blue rectangle and pick another color.

- Press the Run button, on the top of the Protocols editor.

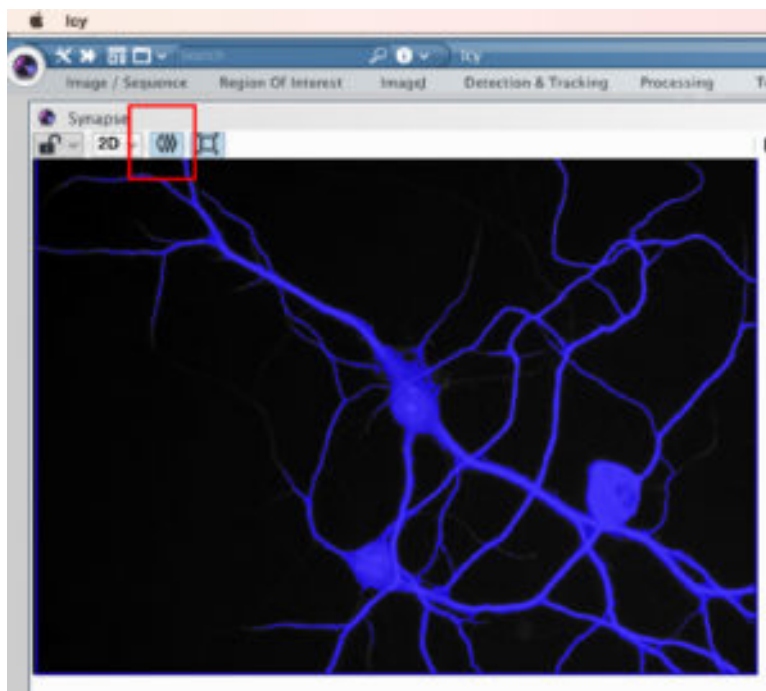


of

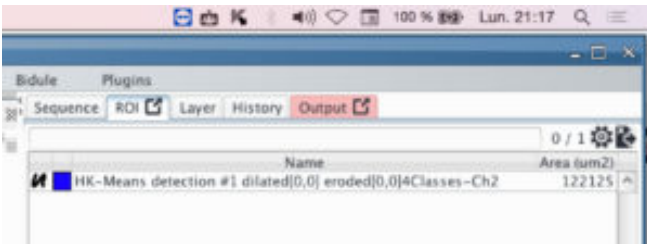
The protocol will run, and show you the segmentation result on the original picture.



Here is the “Cell shape ROI” in blue overlaid on the original picture. You can mask the ROI by clicking on the button indicated in red below. The ROI is an additive layer that does not alter your picture. It is stored in the .xml file close to your picture.



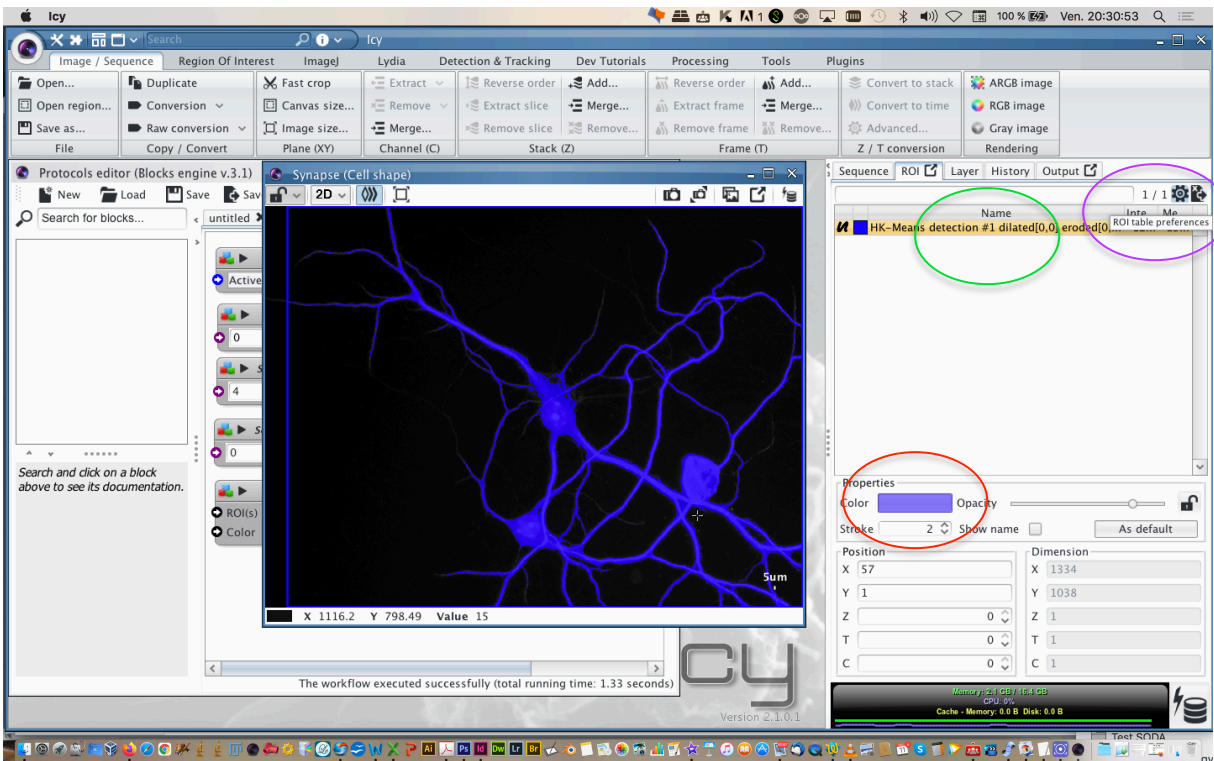
The name of the ROI with the parameters you have selected will appear in the ROI panel.



Here we applied 4 classes on channel 2 with no dilatation/erosion. The ROI is blue.

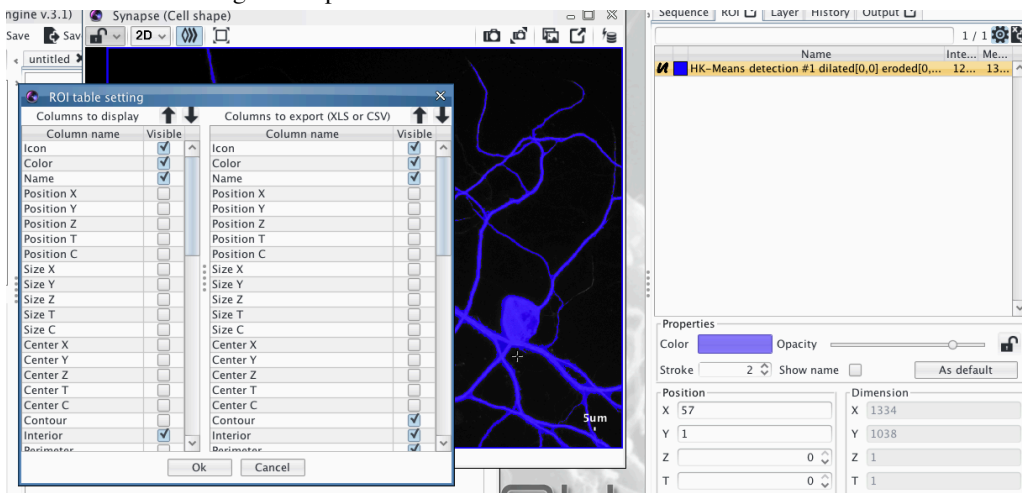
Since the ROI's name depends on the parameters, you can run the protocol several times without crushing your different successive ROIs.

You can edit ROI name by clicking on it in the ROI tab (green circle).

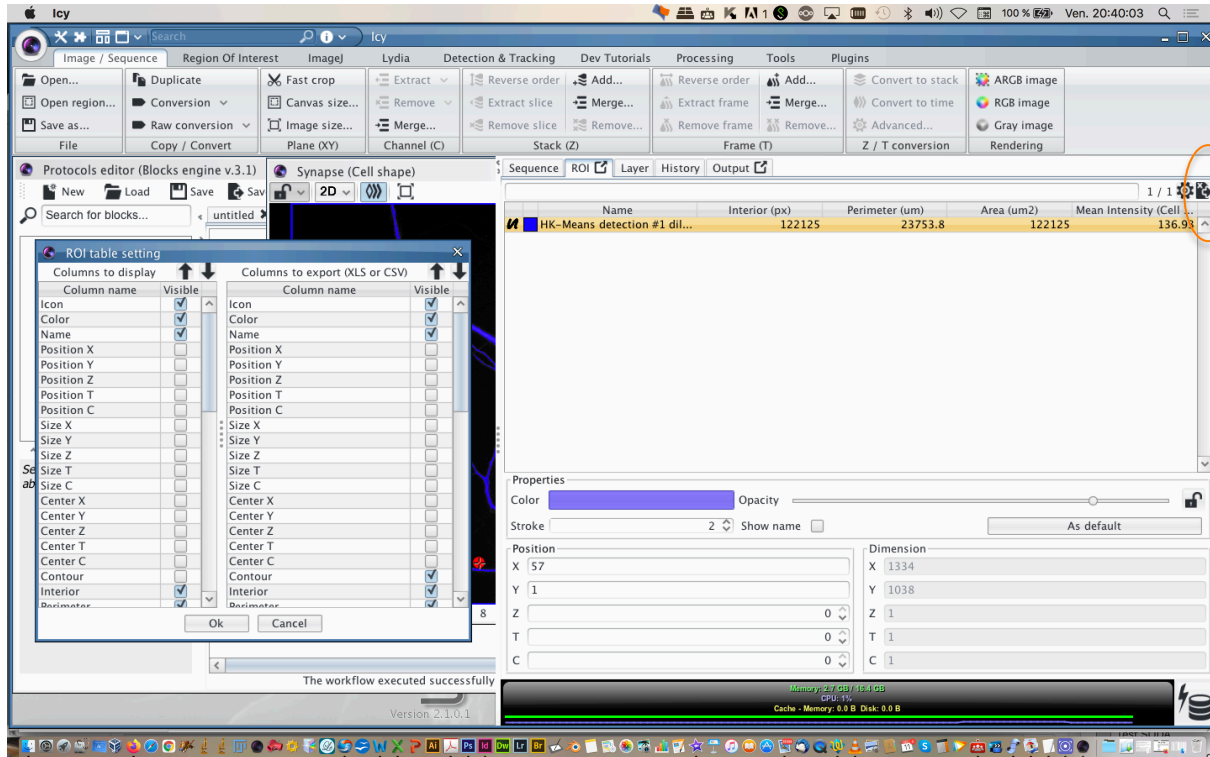


You can edit ROI color by clicking on the color rectangle in ROI panel (red circle).

You can select the ROI parameters to be displayed in the ROI tab by clicking on the preference wheel (purple circle). The ROI table settings will open.



The left column contains parameters that will be displayed in ROI Tab in the software. The right column are the ones that will be present in the excel file if you press the export button (orange circle).

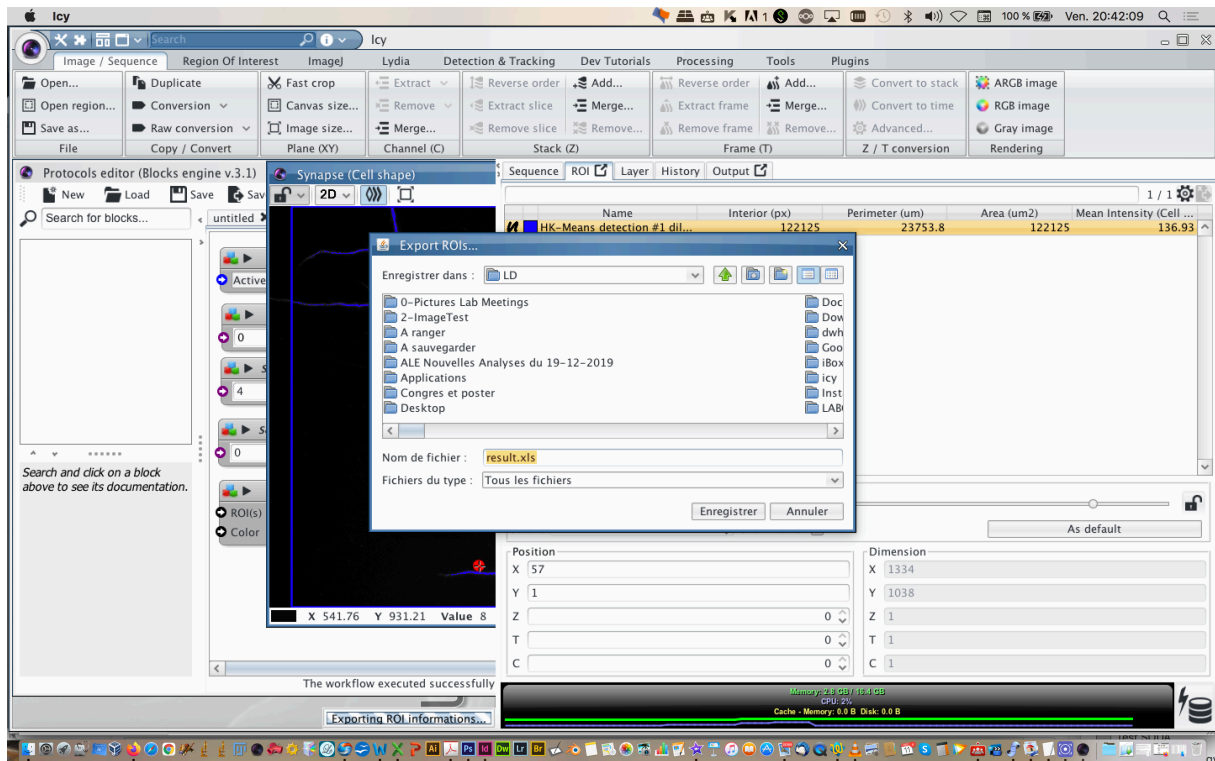


The available parameters are the followings (<http://icy.bioimageanalysis.org/plugin/roi-statistics/>):

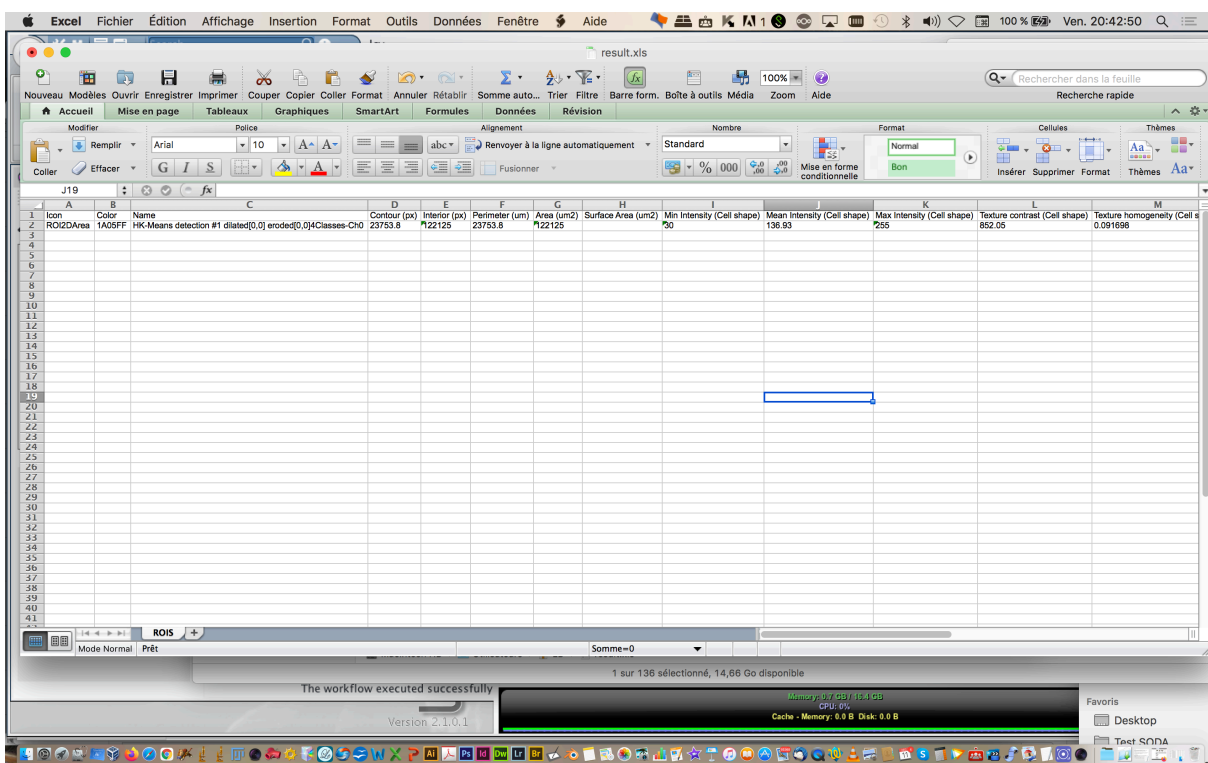
- **Dataset:** the name of the dataset (typically the name of the image or sequence)
- **Icon:** ROI type
- **Color:** ROI color, with hexadecimal code, see the [documentation on ROI blocks](#) for more information on the hexadecimal color coding in Icy
- **Group:**
- **Name:** the name of the region of interest (this can be changed from the “ROI” panel in the Inspector window), by default ROIs are called according to their shape, for instance “Polygon2D”
- **Position X, Y, Z, T, C:** the position of the upper left corner of the bounding box around the ROI along each axis (x, y, z, frame and channel)
- **Size X, Y, Z, T, C:** the size (in pixels) of the bounding box around the ROI along each axis (width, height, depth, number of frames and channels)
- **Center X, Y, Z, T, C:** the coordinates (in pixels) of the center of mass of the ROI along each axis (x, y, z, frame and channel). Note: When selected on the ROI tab it only takes the center of the bounding box instead of computing the mass center.
- **Global center X, Y, Z, T, C:** the coordinates (in microns) of the center of mass of the ROI along each axis (x, y, z) on the sequence, taking into account the pixel size and the sequence position.
- **Contour:** the perimeter (in 2D) or surface area (in 3D), expressed in pixels
- **Interior:** the area (in 2D) or volume (in 3D), expressed in pixels
- **Perimeter:** perimeter given in real units computed from the pixel size information from the image
- **Area:** area given in real units computed from the pixel size information from the image
- **1st, 2nd and 3rd Diameters:** the diameter (along each axis) of the best fitting ellipse (or ellipsoid), expressed in pixels. They are always ordered such that Major > Minor 2D > Minor 3D.

- 1st Diameter (major)
- 2nd Diameter (minor in 2D)
- 3rd Diameter (minor in 3D)
- **Surface area:** 3D only, the surface of the element, generally using the contour pixels.
- **Volume:** 3D only, volume given in real units computed from the pixel size information from the image
- **Intensity statistics:**
 - **Min intensity** = minimum intensity
 - **Mean intensity** = average intensity
 - **Max intensity** = maximum intensity
 - **Sum Intensity** = sum of the intensity distribution for each channel inside the ROI
 - **Standard Deviation** = standard deviation of the intensity distribution for each channel inside the ROI
- **Entropy:** for each channel
- **Contrast:** for each channel
- **Pitch angle:** in degrees
- **Yaw angle:** in degrees
- **Roll angle:** in degrees
- **1st axis:**
- **2nd axis:**
- **3rd axis:**
- **Read only:**
- **Sphericity (as defined by H. Wadell in 1935):** the normalised ratio between the contour and interior, expressed as a percentage (100% for a circle or sphere).
- **Opacity:**
- **Convexity:** the ratio between the volume and the volume of the smallest convex envelope containing the ROI, expressed as a percentage (100% for a convex object)
- **ASM (called Angular Second Moment in the plugin interface):** for each channel
- **Elongation:** a scale factor given by the ratio between the first and second ellipse diameters (see above). The minimum value is 1 (for a non-elongated object).
- **Feret um (called Max. Feret diameter in the plugin interface):** the maximum distance between any 2 points of the surface.
- **Flatness 3D:** 3D only, a scale factor given by the ratio between the second and third diameters (see above). The minimum value is 1 (for an object with perfectly circular section perpendicularly to the major axis)
- **Roundness (following the ISO 1101 standard):** the normalised ratio between the radius of the minimum inscribed and largest circumscribed circles (or spheres), expressed as a percentage (100% for a circle or sphere). This measure is somewhat similar to the sphericity measure, but it much more sensitive to small shape variations (it is used in the manufacturing industry to guarantee the roundness of “round” objects)
- **Homogeneity:** for each channel, based on Haralick texture features, more exactly it's the inverse difference moment. More information about Haralick texture features here:
http://murphylab.web.cmu.edu/publications/boland/boland_node26.html

You can export the data in excel file by pressing this button. Indicate the name and path of the excel file you want to create.



The excel file contain all the Statistical parameters corresponding to the ROI.



Changelog

- Version 1 • Released on: 2020-06-22 21:26:06 [Download](#)