



MIC-MAQ

Microscopy Images of Cells-Multi Analysis and Quantifications





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## Abstract

- ✓ MIC-MAQ automatically segments cells and/or nuclei on 2D microscopy images
- ✓ The plugin takes Z-stack but works only on 2D projection
- ✓ It provides intensity based and/or morphological measurements in nuclei and/or cells in multi channels experiment
- ✓ The plugin can propose the foci/spots detection

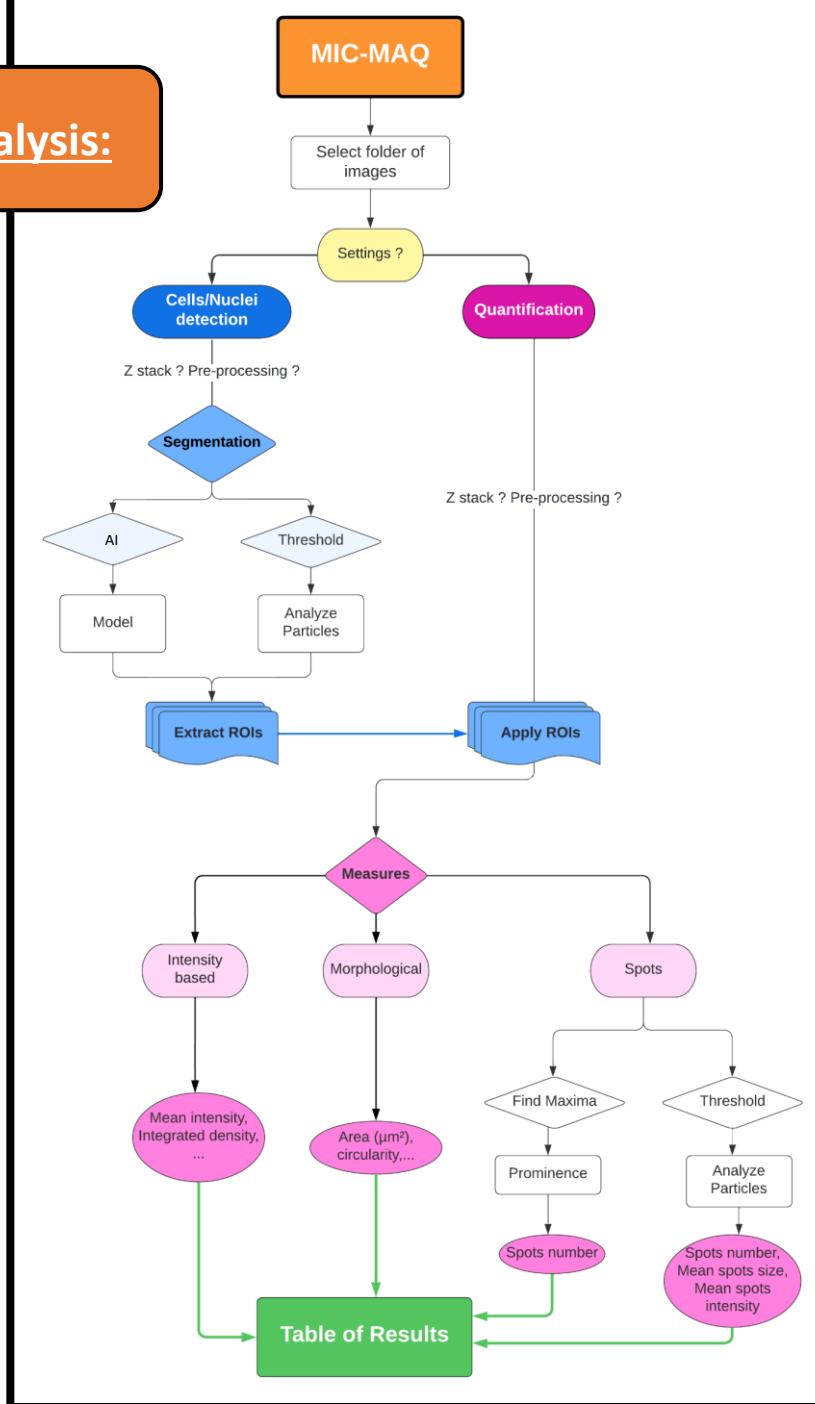
# Workflow

## For MIC-MAQ

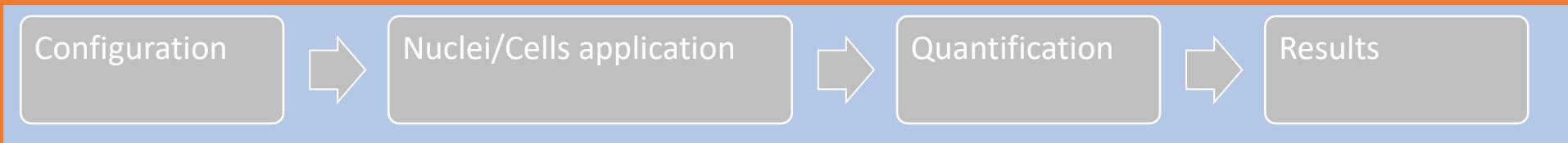


Workflow

## Process for analysis:



# How to use the plugin



## Configuration

- Data
- Calibration

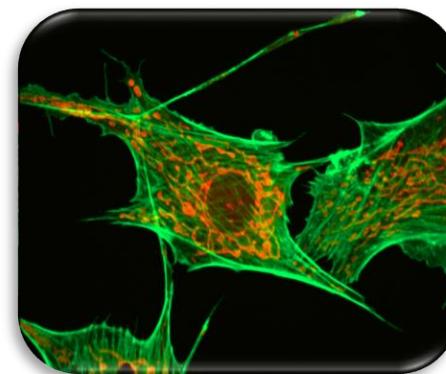
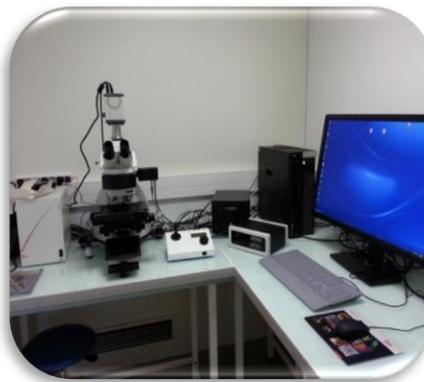
## Nuclei/Cells application

## Quantification

## Results

# Which data can be opened with MIC-MAQ ?

- Classical TIF images
- Any file formats from many life sciences open with the Bio-Format plugin



**.CZI** (Zeiss)  
**.LIF** (Leica)  
**.ND** (Metamorph)  
**.ND2** (Nikon)  
**.VSI** (Olympus)

## Configuration

- Data
- Calibration

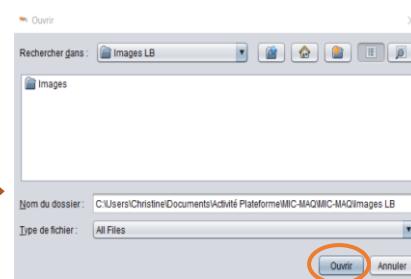
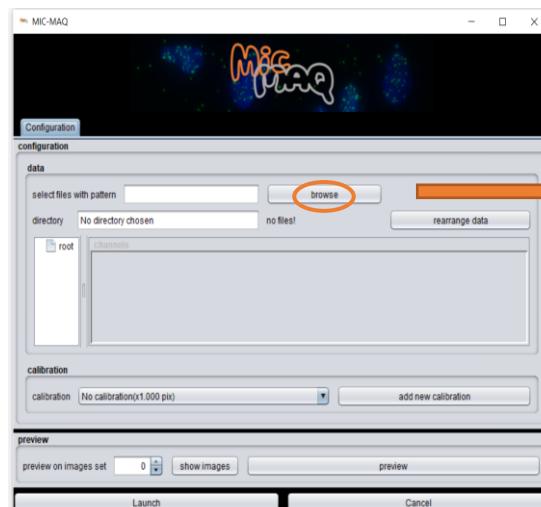
## Nuclei/Cells application

## Quantification

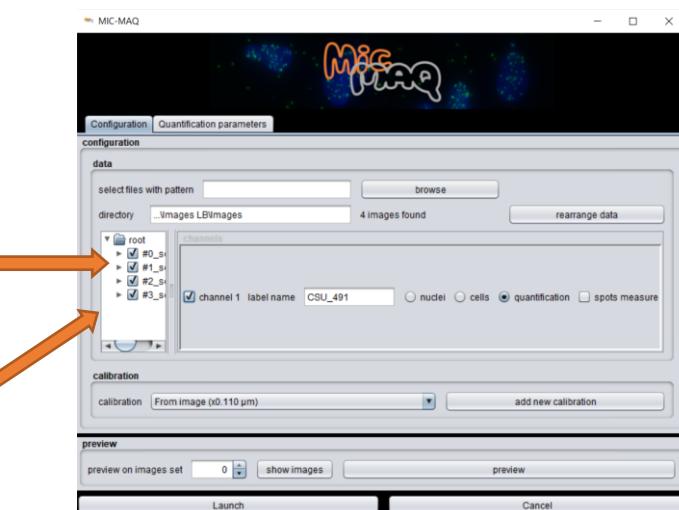
## Results

### A. Open files by selecting directory of images

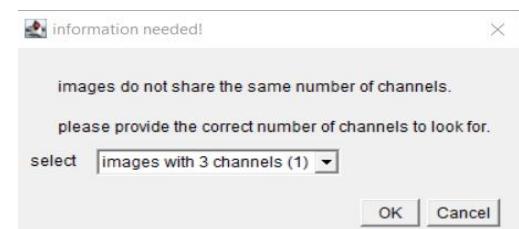
- Select « **browse** » to choose a directory containing images
- Validate the directory by clicking on **Open/Ouvrir**



▼ root  
►  #0\_serie013\_w1CSU-491.TIF#Series\_1: CSU\_491: 660 x 900; 1 plane  
►  #1\_serie013\_w2CSU-Trans.TIF#Series\_1: CSU\_Trans: 660 x 900; 1 plane  
►  #2\_serie020\_w1CSU-491.TIF#Series\_1: CSU\_491: 660 x 900; 1 plane  
►  #3\_serie020\_w2CSU-Trans.TIF#Series\_1: CSU\_Trans: 660 x 900; 1 plane



During the process of opening, if images do not share the same number of channels, you will be asked to select among the list the number of channels to be used for the analysis.



## Configuration

- Data
- Calibration

## Nuclei/Cells application

## Quantification

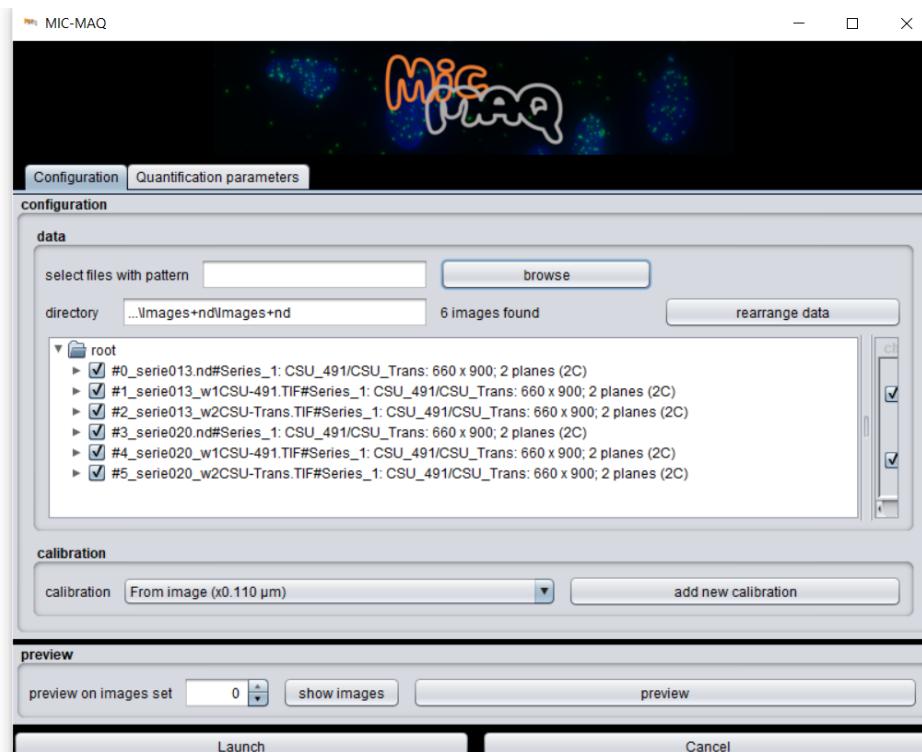
## Results

### B. Open files with pattern

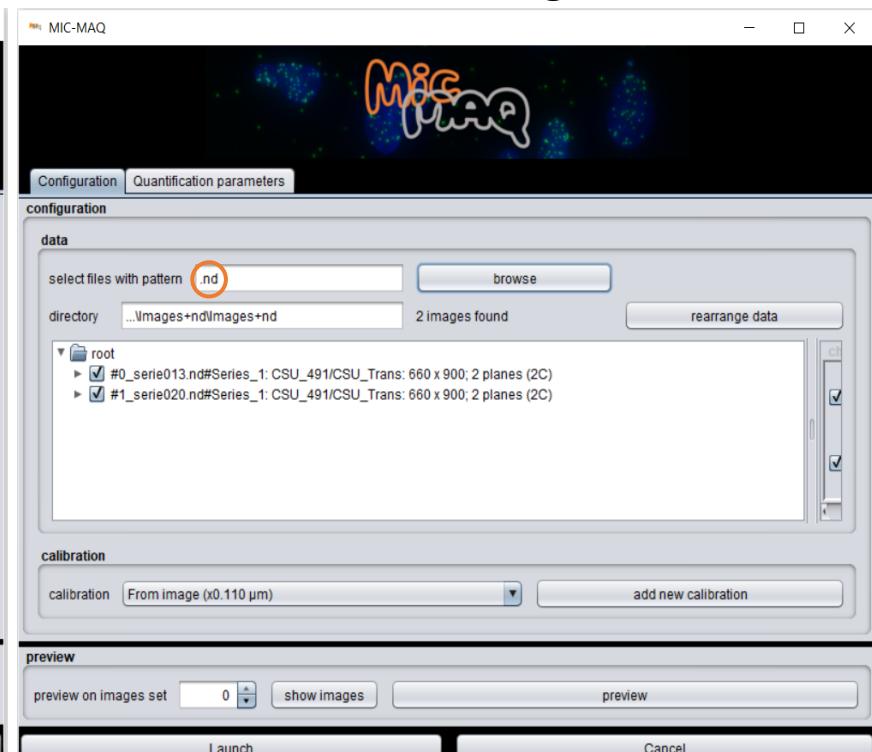
As first step, you can filter the data based on extension file (example: nd, tif) to select only one type of files in the folder of images:

- Enter the extension file in the « select files with pattern box » (example: nd)
- Observe the selected images after filtering in the root box

#### Before filtering



#### After filtering



## Configuration

- Data
- Calibration

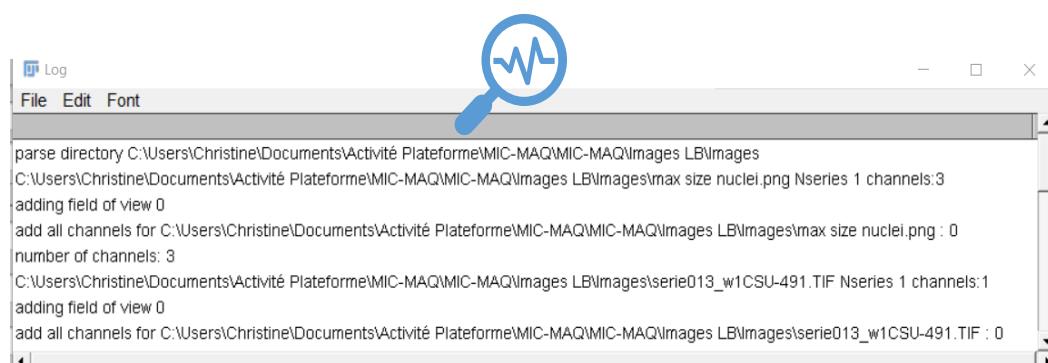
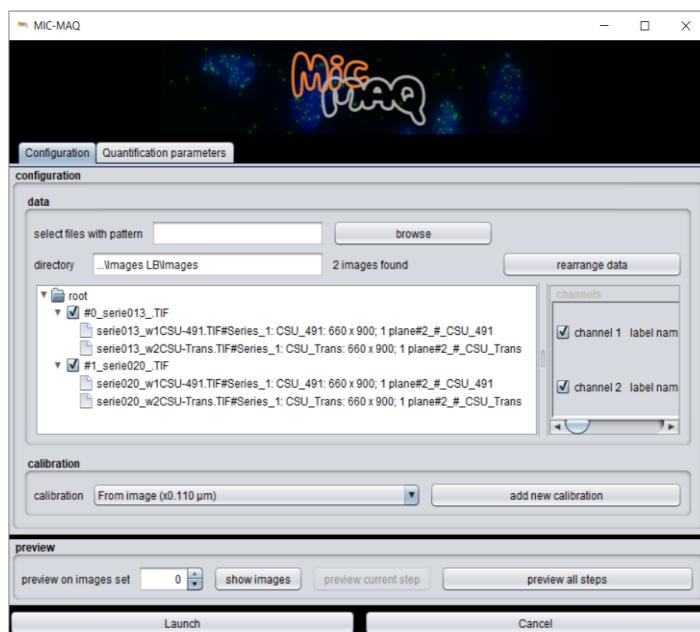
## Nuclei/Cells application

## Quantification

## Results

### Opening process

During the opening process, a window called « log » will appear containing the total number of images. Depend of the number of images, this window can take time to appear.



## Configuration

- Data
- Calibration

## Nuclei/Cells application

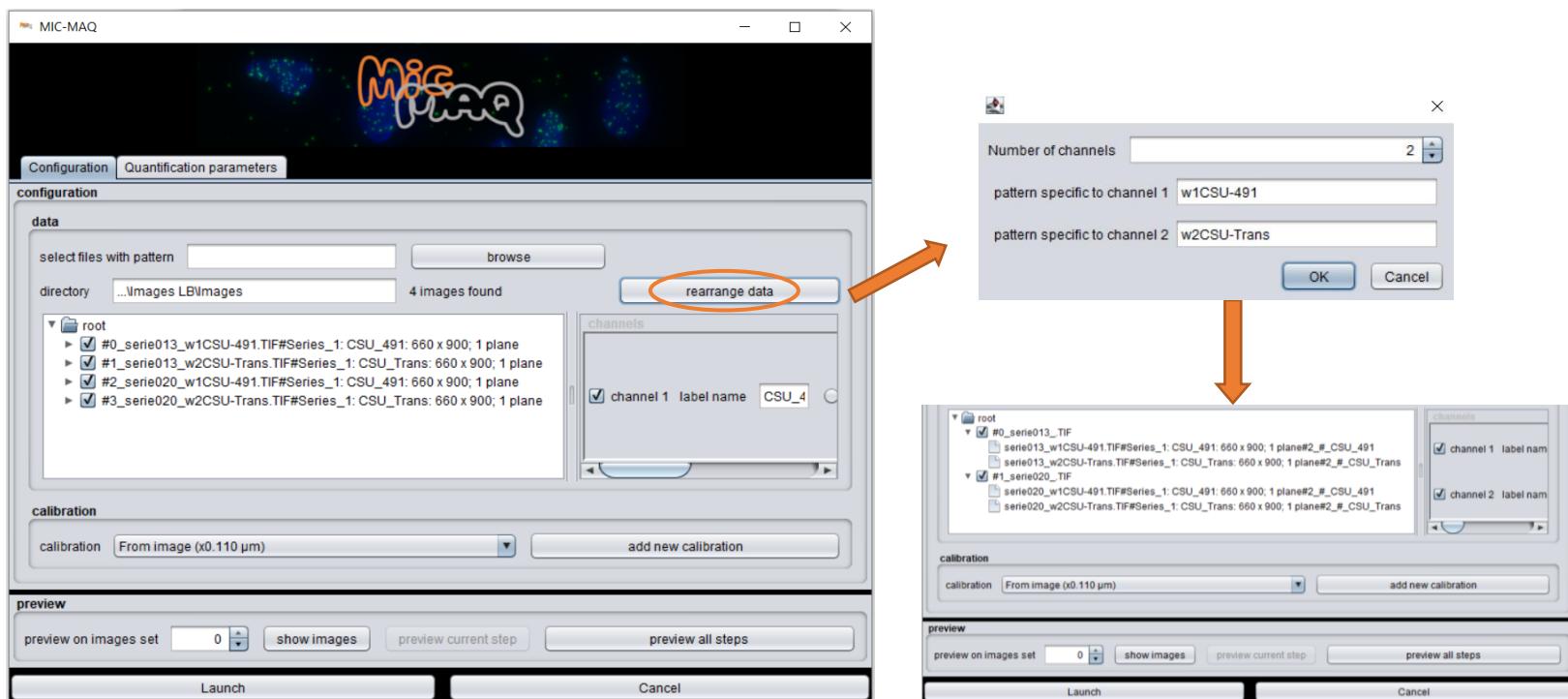
## Quantification

## Results

### Option: Rearrange TIF images by channel association

If you have TIF files with 1 image per channel, you can reorder your data based on the total number of channels and channel name specification:

- Click on « rearrange data »
- Enter the total number of channels in the folder containing images
- Define for each channel the specific filename pattern
- Observe the reorganization of series of images after specifying the filename in the root box



## Configuration

- Data
- Calibration

## Nuclei/Cells application

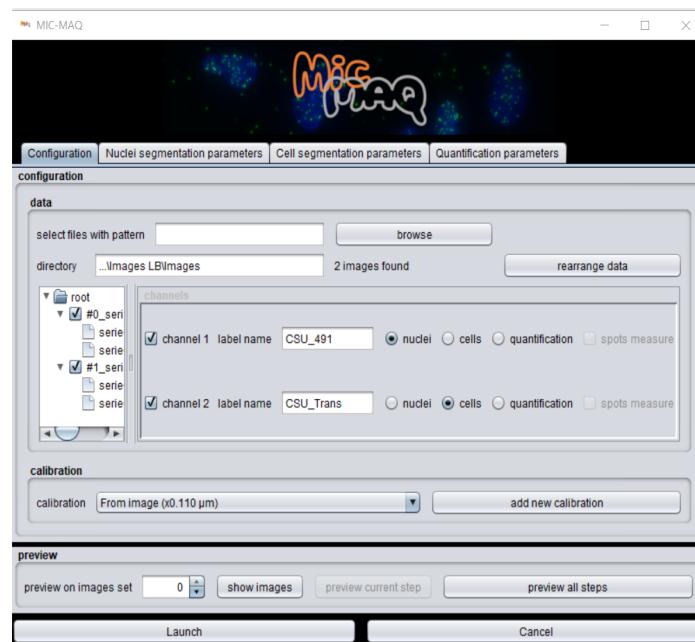
## Quantification

## Results

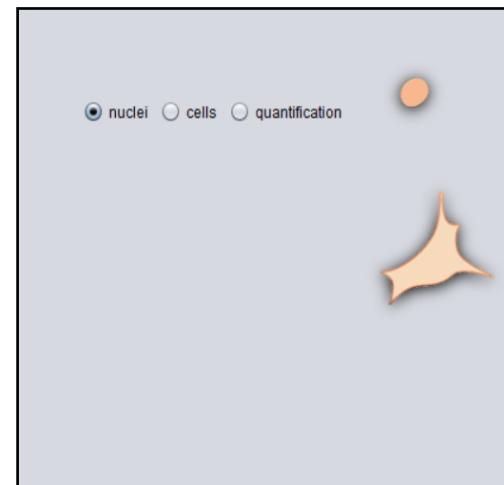
### Configure channels

For each channel:

- Define the label name for the channel
- Choose the application for the channel
  - ✓ « nuclei »: the channel is used to segment nuclei
  - ✓ « cells »: the channel is used to segment cells
  - ✓ « quantification »: the channel is used to measure parameters in nuclei and/or cells regions



*you have only 1 choice for each channel.*



## Configuration

- Data
- Calibration ●●●●

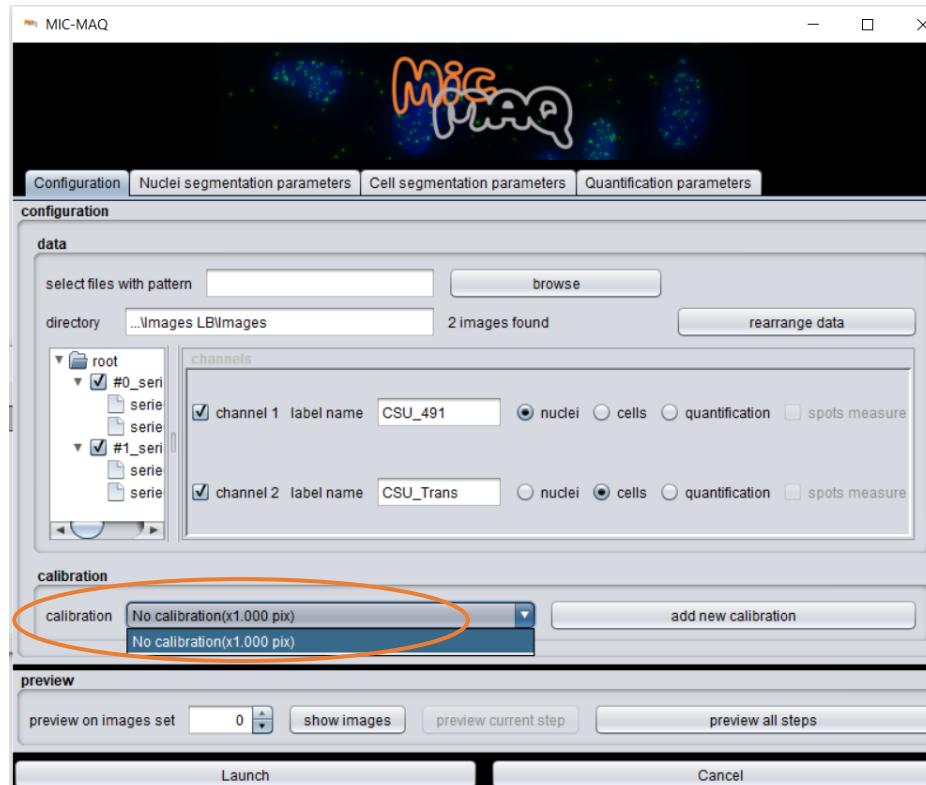
## Nuclei/Cells application

## Quantification

## Results

### Define calibration of images

The default calibration is in pixel (*No calibration (x1.000 pix)*). The measurements (as Area) will be exported in pixels<sup>2</sup>.



If the file format is from a microscope company the calibration directly takes the pixel size recorded in the file metadata (From image (x...  $\mu\text{m}$ )).



## Configuration

- Data
- Calibration

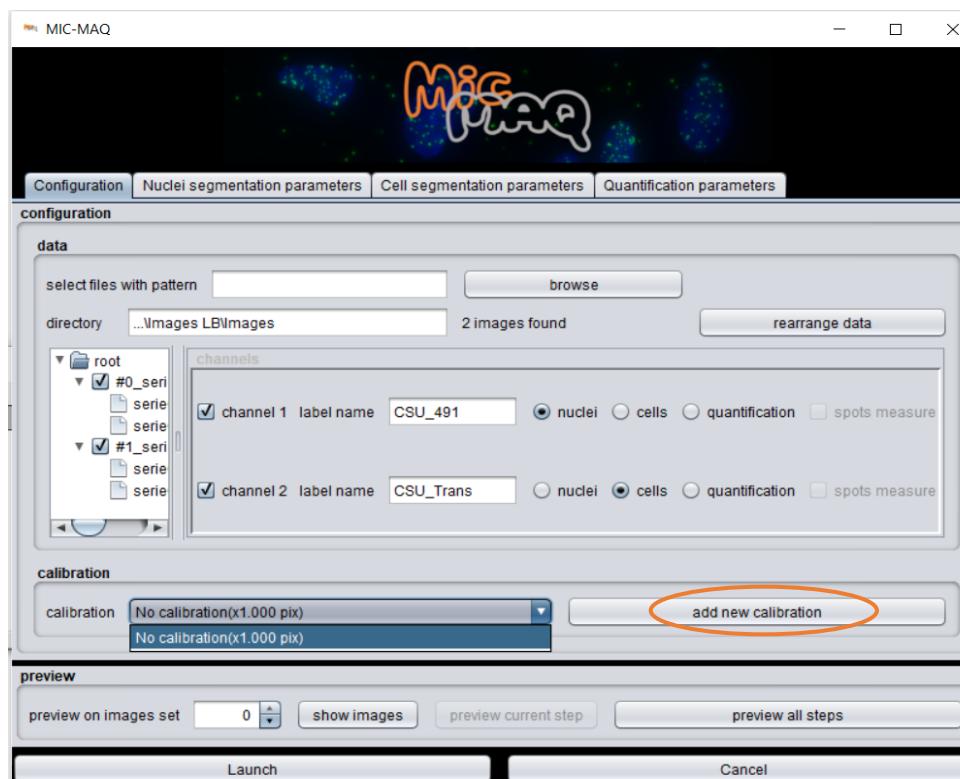
## Nuclei/Cells application

## Quantification

## Results

### Define calibration of images

- i. Click on **Add new measure Calibration**
- ii. A new window « Add a calibration » appears



## Configuration

- Data
- Calibration ● ● ●

## Nuclei/Cells application

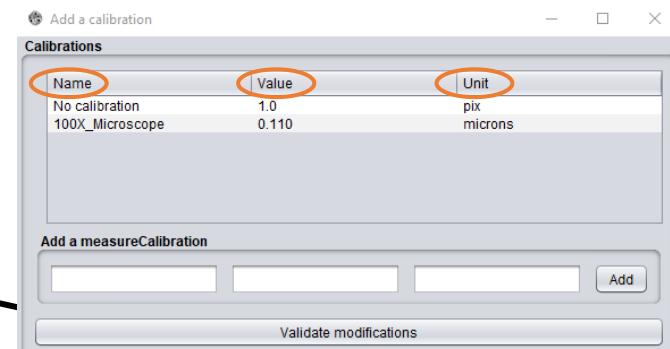
## Quantification

## Results

### Define calibration of images

iii. On this new window, enter a new calibration

- ✓ Name of the calibration
- ✓ The pixel size Value \*
- ✓ The Unit of the length



iv. Click on **Add** to see it on the list

### Pixel size on the microscope ?

Microscope Type	Objective	Pixel size
Ratio 2 Microscope	2.5K	1.82 µm
	5K	0.91 µm
	10K	0.45 µm
	20K	0.23 µm
	40K	0.11 µm
	63K water	0.072 µm
Ratio 1 Microscope	5K	1.82 µm
	10K	0.65 µm
	20K	0.325 µm
	40K	0.163 µm
	63K	0.103 µm
3D SIM Microscope	5K	1.82 µm
	10K	0.65 µm
	20K	0.325 µm
	40K	0.163 µm
	63K	0.103 µm
	100K	0.065 µm

Microscope Type	Objective	Pixel size
3D Dec Microscope	40K	0.161 µm
	63K	0.102 µm
	100K	0.0645 µm
Spinning-Disk Microscope	10K	1.1 µm
	20K	0.55 µm
	40K	0.275 µm
	60K	0.183 µm
	100K oil	0.11 µm
TIRF Microscope	10K	1.6 µm
	20K	0.8 µm
	40K	0.4 µm
	60K oil	0.266 µm
SPS/SPEX CLSM	Dependant of the Zoom and/or the number of pixels in the image	
	100K oil	0.160 µm

\* <https://institutcurie.sharepoint.com/sites/US43-ImageProcessingAnalysis/SitePages/fr/Calibrer%20une%20image.aspx>

## Configuration

✓ Data  
☐ Calibration ● ● ●

## Nuclei/Cells application

## Quantification

## Results

- v. Click on **Validate modifications** and close the window « Add a calibration »
- vi. Select the new calibration appearing on the scroll list

The image shows two windows of the MIC-MAQ software. The main window on the left is titled 'MIC-MAQ' and contains tabs for Configuration, Nuclei segmentation parameters, Cell segmentation parameters, and Quantification parameters. The Configuration tab is active, showing a 'data' section with a file selection dialog and a 'calibration' section where 'No calibration(x1.000 pix)' is selected. An orange arrow points from the 'add new calibration' button in this section to the 'Add a calibration' window on the right. The 'Add a calibration' window has a title 'Calibrations' and displays a table of calibration entries with columns for Name, Value, and Unit. The table includes various entries such as 'No calibration' (Value: 1.0, Unit: pix), '3D SIM 10X' (Value: 0.65, Unit: um), and 'TIRF 100X' (Value: 0.16, Unit: um). At the bottom of the 'Add a calibration' window are buttons for 'Add' and 'Validate modifications'.

Name	Value	Unit
No calibration	1.0	pix
3D SIM 10X	0.65	um
3D SIM 20X	0.325	um
3D SIM 40X	0.163	um
3D SIM 63X	0.103	um
3D SIM 100X	0.065	um
Ratio2 10X	0.65	um
Ratio2 20X	0.325	um
Ratio2 40X	0.163	um
Ratio2 63X	0.103	um
Ratio2 100X	0.065	um
3D Dec 40X	0.161	um
3D Dec 63X	0.102	um
3D Dec 100X	0.0645	um
Ratio1 10X	1.6	um
Ratio1 20X	0.8	um
Ratio1 40X	0.4	um
Ratio1 63X	0.253	um
Ratio1 100X	0.16	um
Spinning 10X	1.1	um
Spinning 20X	0.55	um
Spinning 40X	0.275	um
Spinning 60X	0.183	um
Spinning 100X	0.11	um
Spinning Live-SR 100X	0.065	um
TIRF 60X	0.266	um
TIRF 100X	0.16	um

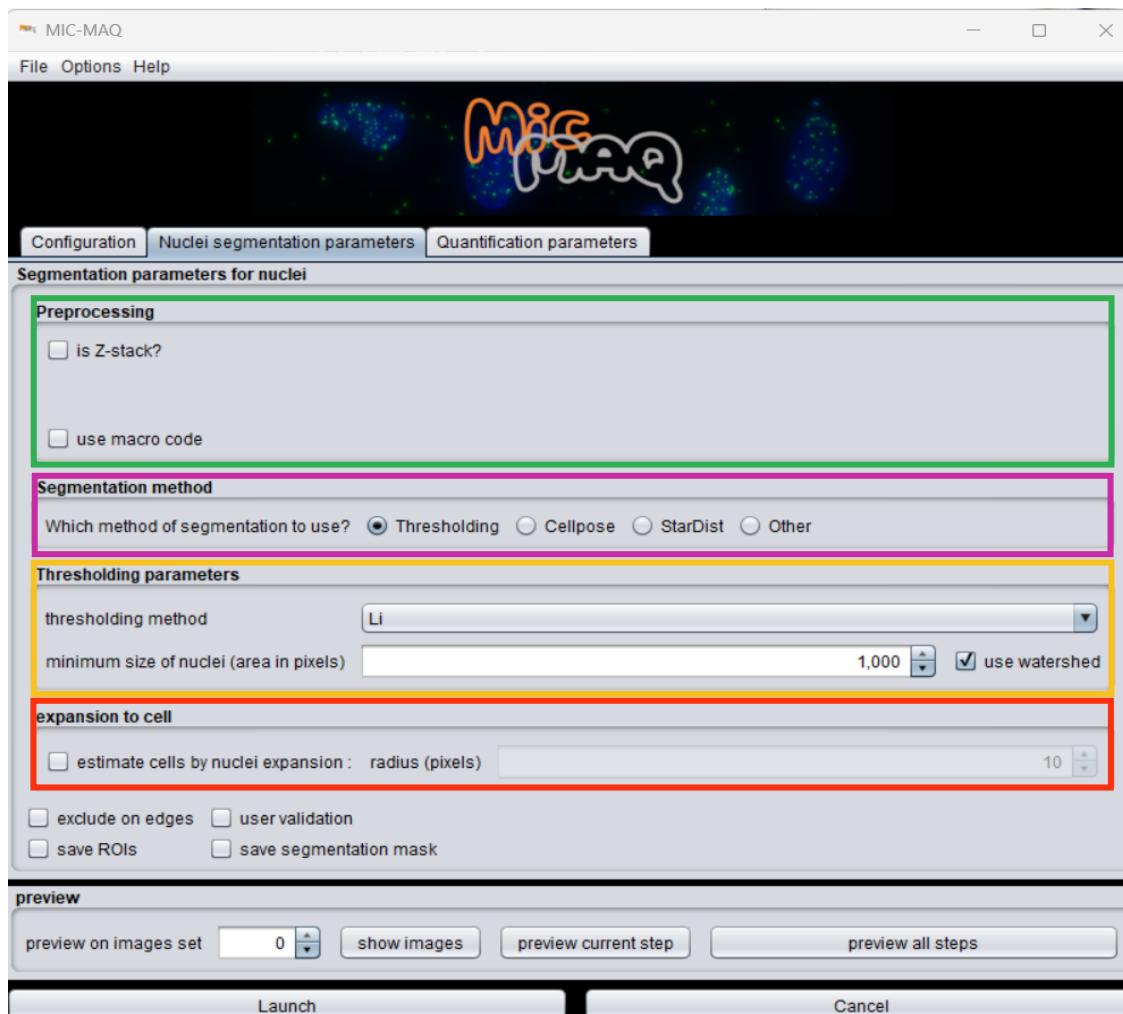
Configuration

Nuclei application

Quantification

Results

## Nuclei application



Preprocessing panel

Segmentation algorithm choice

Segmentation parameters

Expansion to cell (p. 28)

Configuration

Nuclei application

Quantification

Results

## Preprocessing: Z-stack



*MIC-MAQ work only on 2D images, if you have Z stack, the stack should be converted into 2D projection.*

### 2D images

Preprocessing

is Z-stack?

use macro code

### 3D images

Preprocessing

is Z-stack?

use macro code

method of projection

choose slices to use

Maximum projection  
Standard deviation projection  
Sum Slices

- Untick the box « is a Z-stack ? »
- Tick the box « is a Z-stack ? »
- Choose the method of Z projection in the list

*Optional:* You have the possibility to select slices number to create the 2D projection

choose slices to use

1 10

Configuration

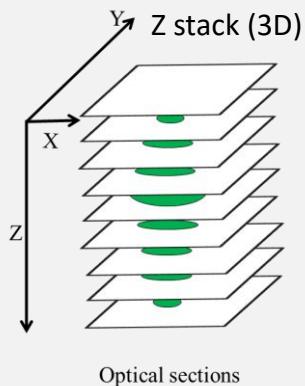
Nuclei application

Quantification

Results

- Preprocessing** ● ● ●
- Segmentation
- Parameters

## Preprocessing: Z-stack

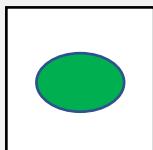


### Method of projection :

- ✓ **Maximum projection** : the 2D projection is calculated based on the maximum voxel value in Z axis
- ✓ **Standard Deviation projection** : the 2D projection is calculated based on the standard deviation value of voxels in Z axis
- ✓ **Sum Slices projection** : the 2D projection is calculated based on the sum voxel values in Z axis

### Method of projection

Z projection (2D)



Configuration

Nuclei application

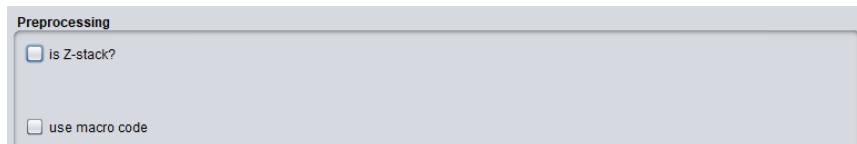
Quantification

Results

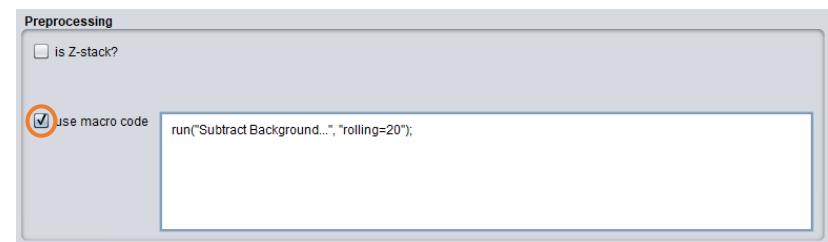
## Preprocessing: use macro code

*MIC-MAQ offers the possibility of applying preprocessing to images using a macro code.*

### Without macro



### With macro



- Untick the box « use macro code »
- Tick the box « use macro code »
- Copy the macro code on the white box

Configuration

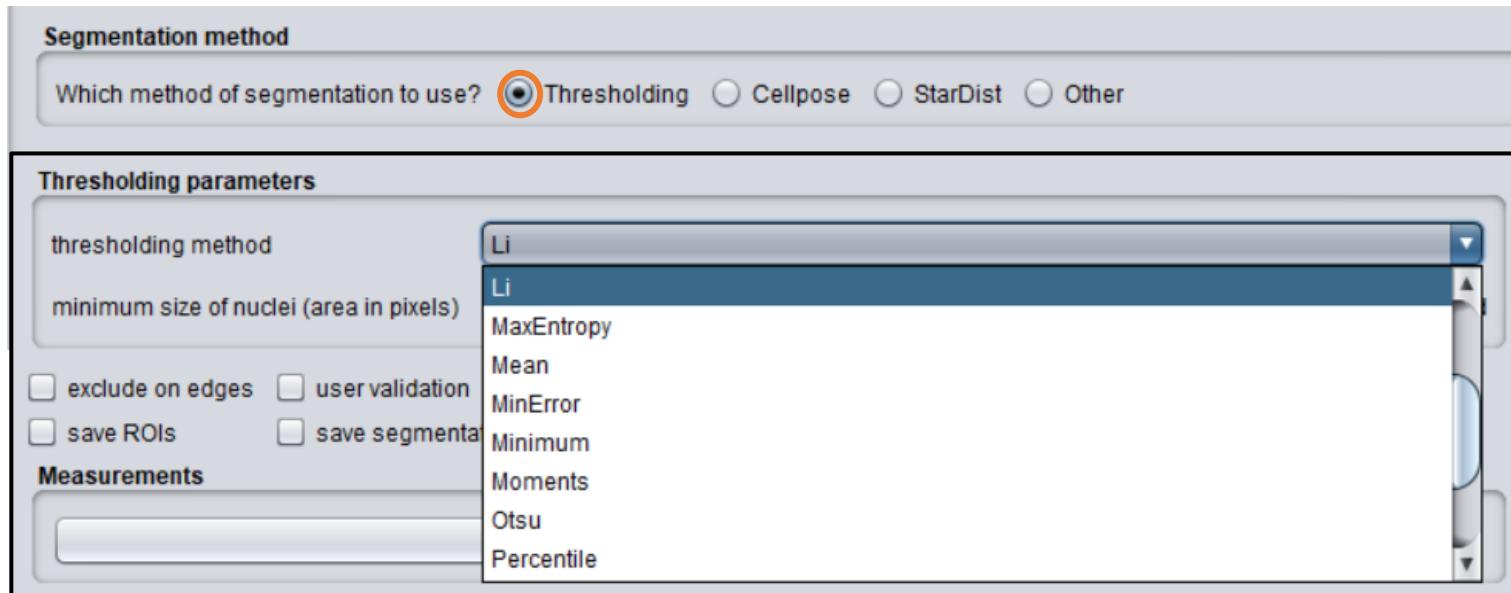
Nuclei application

Quantification

Results

## Segmentation parameters: Thresholding

Nuclei



+ : faster process

- : close proximity between nuclei, intensity variation

Configuration

Nuclei application

Quantification

Results

## Segmentation parameters: Thresholding

Nuclei

Segmentation method

Which method of segmentation to use?  Thresholding  Cellpose  StarDist  Other

Thresholding parameters

thresholding method

minimum size of nuclei (area in pixels)   use watershed

exclude on edges  user validation  
 save ROIs  save segmentation mask

preview

preview on images set

- Li
- Default
- Huang
- Intermodes
- IsoData
- IJ\_IsoData
- Li
- MaxEntropy
- Mean

- Choose the thresholding method among the list 
- Enter the minimum size of nuclei (defined by the area in pixels<sup>2</sup>)

Thresholding parameters

thresholding method

minimum size of nuclei (area in pixels)   use watershed

**Optional:** use watershed to separate fused nuclei

Configuration

Nuclei application

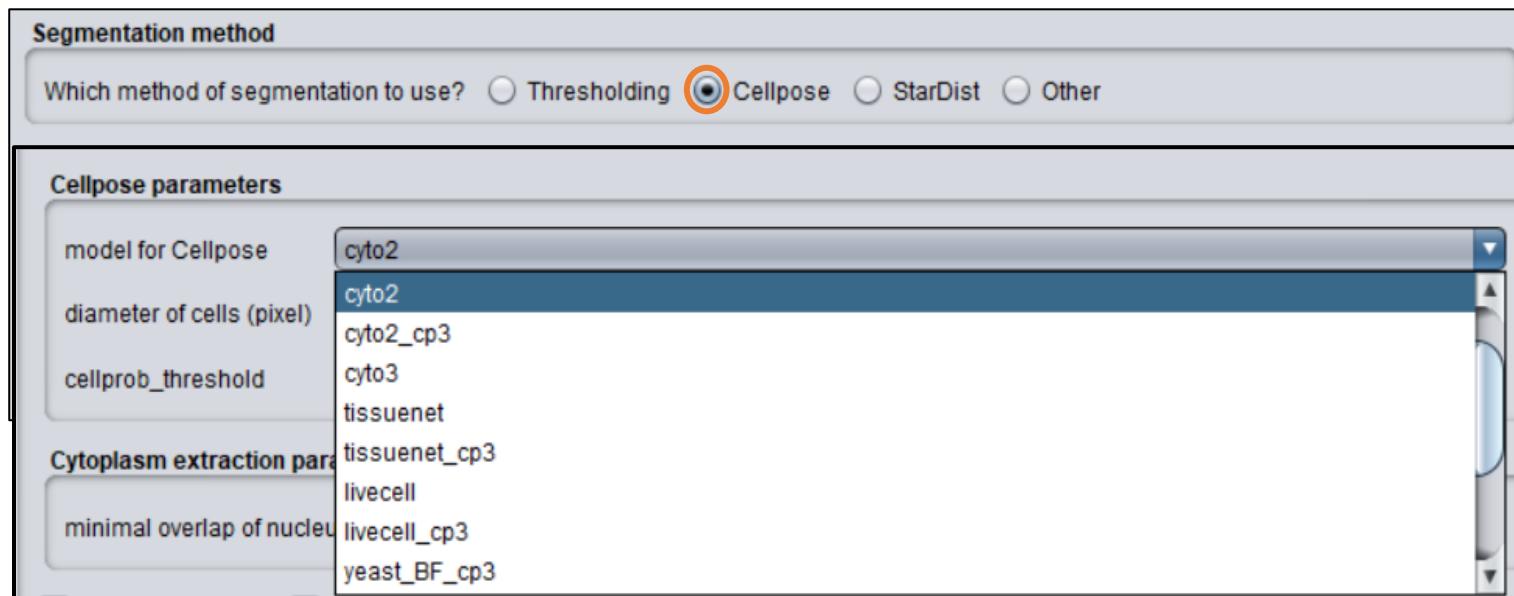
Quantification

Results

## Segmentation parameters: Cellpose

Nuclei

- Choose the segmentation method



+ : close proximity between nuclei

- : slow process

Configuration

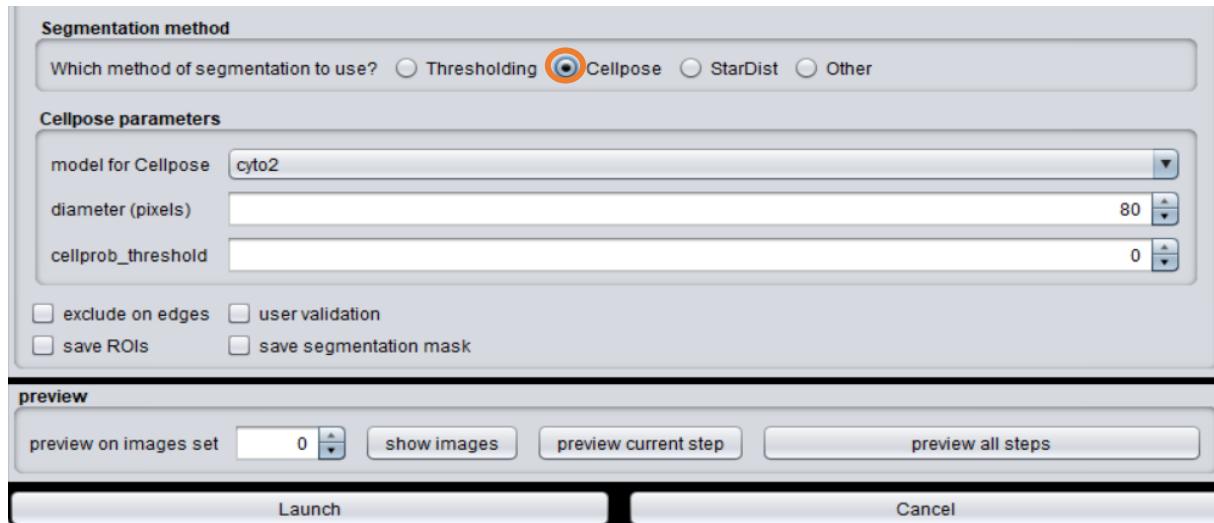
Nuclei application

Quantification

Results

## Segmentation parameters: Cellpose

Nuclei



cyto2  
nuclei  
cyto  
cyto2  
cyto2\_cp3  
cyto3  
tissuenet  
tissuenet\_cp3  
livecell

- Choose the Cellpose model among the list 
- Enter the mean diameter of nuclei that you want to detect (defined in pixels)
- **Optional:** if the detection is too restrictive you can enlarge detection with the cellprob\_threshold value

A close-up view of the 'cellprob\_threshold' input field from the previous screenshot. The value '0' is displayed in the field, with up and down arrows to its right.

Configuration

Nuclei application

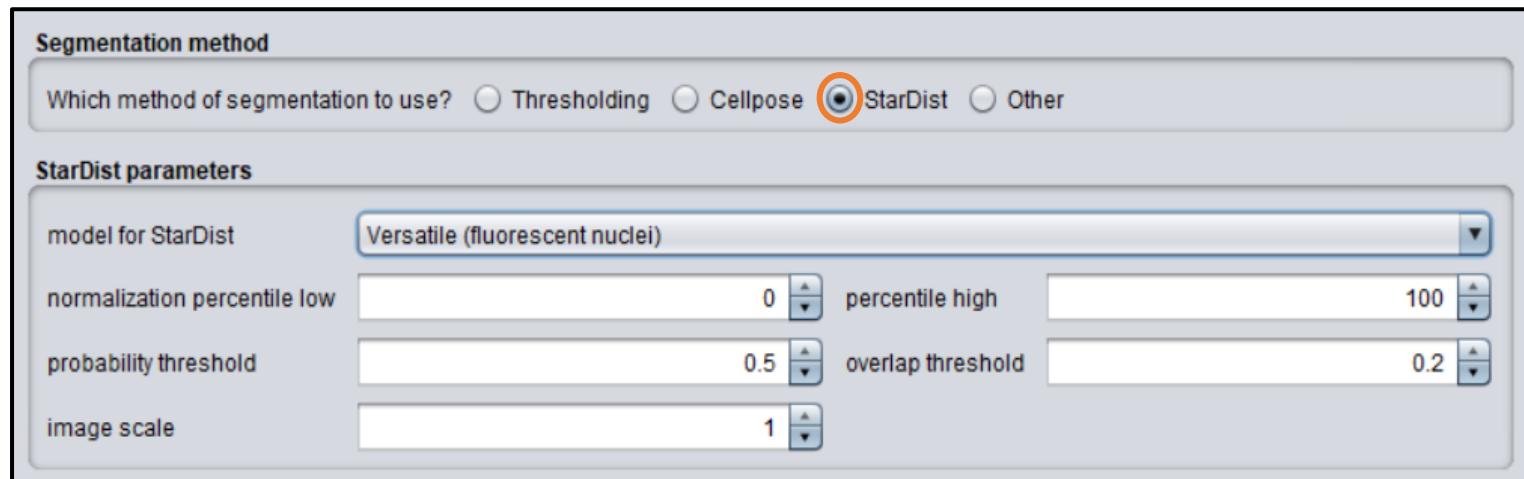
Quantification

Results

## Segmentation parameters: Stardist

Nuclei

- Choose the segmentation method



+ : close proximity between nuclei, fast process

- : The model was developed for detected objects close to 90 pixels

Configuration

Nuclei application

Quantification

Results

## Segmentation parameters: Stardist

Nuclei

Segmentation method

Which method of segmentation to use?  Thresholding  Cellpose  StarDist  Other

StarDist parameters

model for StarDist: Versatile (fluorescent nuclei)

normalization percentile low: 0      percentile high: 100

probability threshold: 0.5      overlap threshold: 0.2

image scale: 1

- Versatile (fluorescent nuclei)
- Versatile (H&E nuclei)
- DSB 2018 (from StarDist 2D paper)
- Model (.zip) from File

- Choose the StarDist model among the list



**Percentile low and high**: is related to the normalization of the images between 0 and 1. The low and high percentiles allow to exclude a percentage of lowest and highest intensities values respectively

**Probability/Score threshold (NMS : non maximum suppression)** allow to select objects based on their probability of detection with the model used

**Overlap**: the model can detect nuclei that are superimposed (higher value allow more overlapping).

- **Optional:** if the detection is not adapted to your objects (detections is smaller than objects), you can change the image scale to optimize the detection

Configuration

Nuclei application

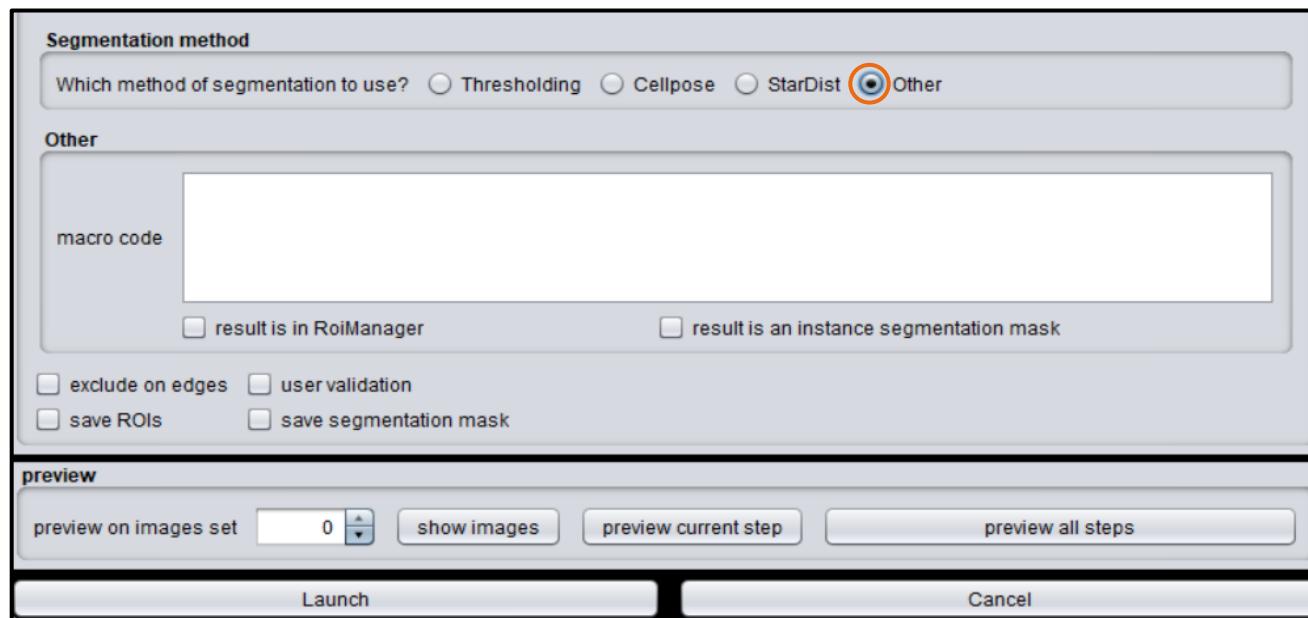
Quantification

Results

## Segmentation parameters: Other

Nuclei

- Choose the segmentation method



+ : Adaptability to another segmentation method

- :

Configuration

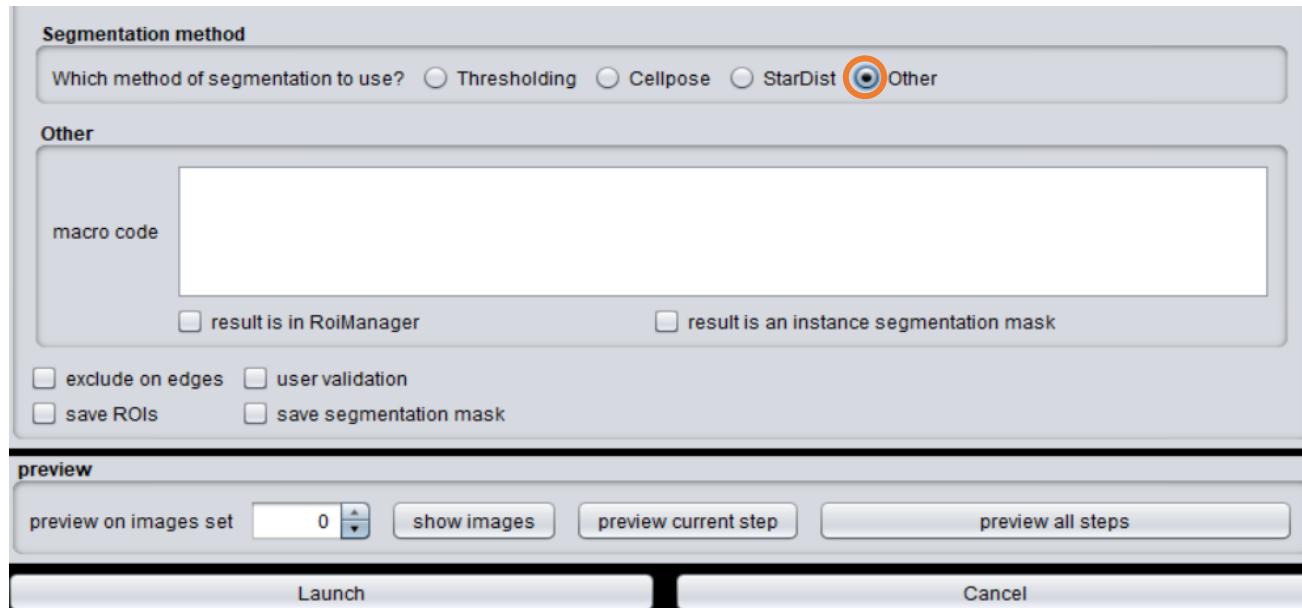
Nuclei application

Quantification

Results

## Segmentation parameters: Other

Nuclei



- Enter the macro lines to run the segmentation process
- For the output, select the export as ROI or as mask of segmentation

result is in RoiManager

result is an instance segmentation mask

Configuration

Nuclei application

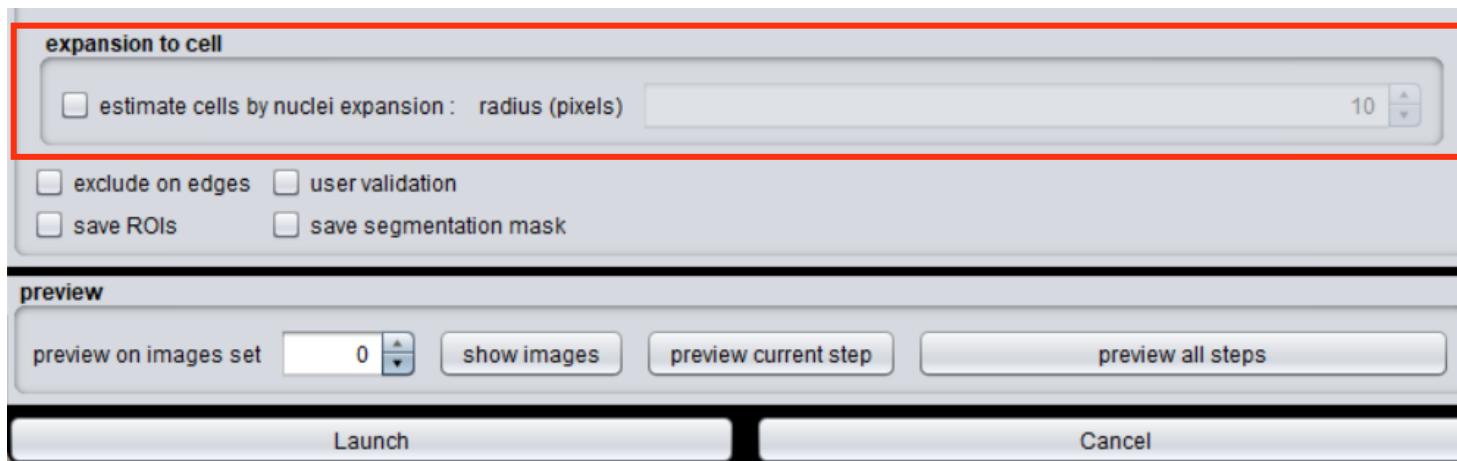
Quantification

Results

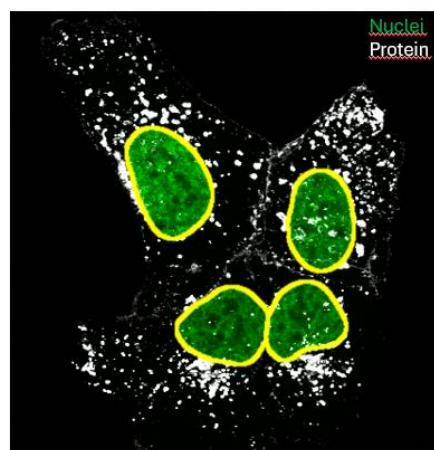
- Preprocessing
- Segmentation
- Parameters : **Cell expansion**

## Segmentation parameters: Cell expansion (in option)

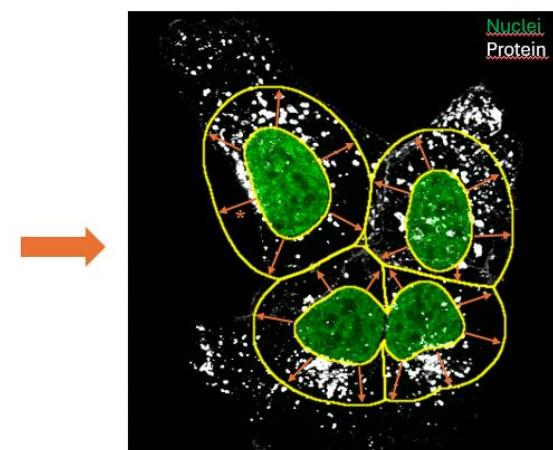
Nuclei



- Choose the estimate cells by nuclei option
- Determine the radius in pixels for the cell expansion



Nuclei detection



Cell expansion (\*: radius of 40 pixels)

Configuration

Nuclei application

Quantification

Results

- Preprocessing
  - Segmentation
  - Parameters**
- 

## Segmentation parameters: Options

<input checked="" type="checkbox"/> exclude on edges	<input checked="" type="checkbox"/> user validation
<input checked="" type="checkbox"/> save ROIs	<input checked="" type="checkbox"/> save segmentation mask

You have the possibility to tick boxes for segmentation:

- **Exclude on edges:** this option will remove automatically regions that touch the edges of the image
- **User validation:** manual step where the user can delete or add regions obtained by segmentation
- **Save ROIs:** this option allows to save automatically the regions of interest obtained by segmentation
- **Save segmentation mask:** this option allows to save automatically the mask of objects obtained by segmentation

Configuration

Nuclei application

Quantification

Results

- Preprocessing
- Segmentation
- Parameters

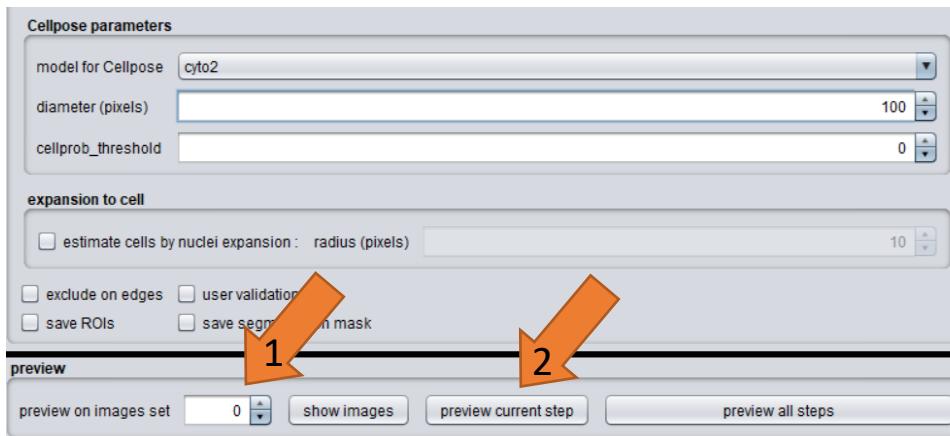


## Test the segmentation



1. Select the set number for the preview to test the thresholding parameters
2. Start the analysis on the previous selected series of images with the button « preview current step »

### Cellpose



To know the association between the preview number and the image name, check the #value in the root box.



root  
►  #0\_serie013\_TIF  
►  #1\_serie020\_TIF

Configuration

Nuclei application

Quantification

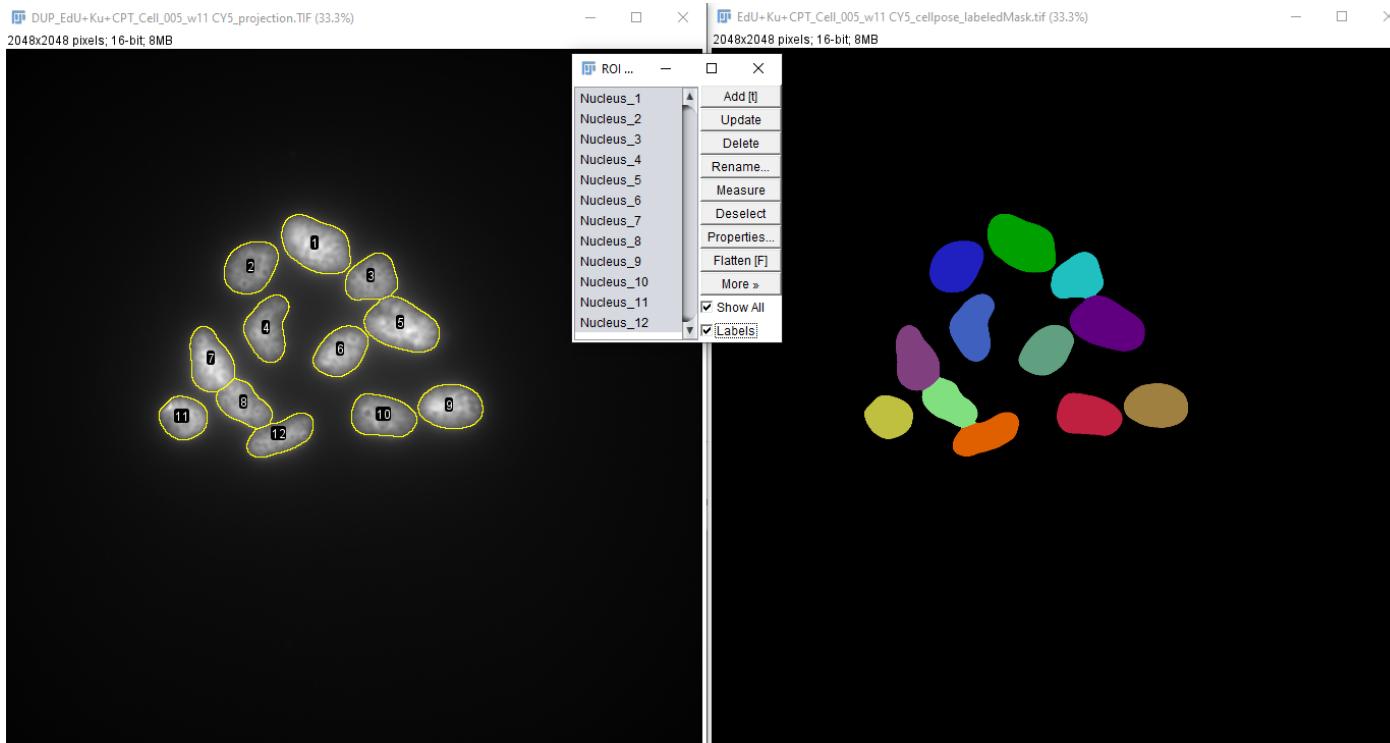
Results

- Preprocessing
- Segmentation
- Parameters



Nuclei

## Examples of segmentation



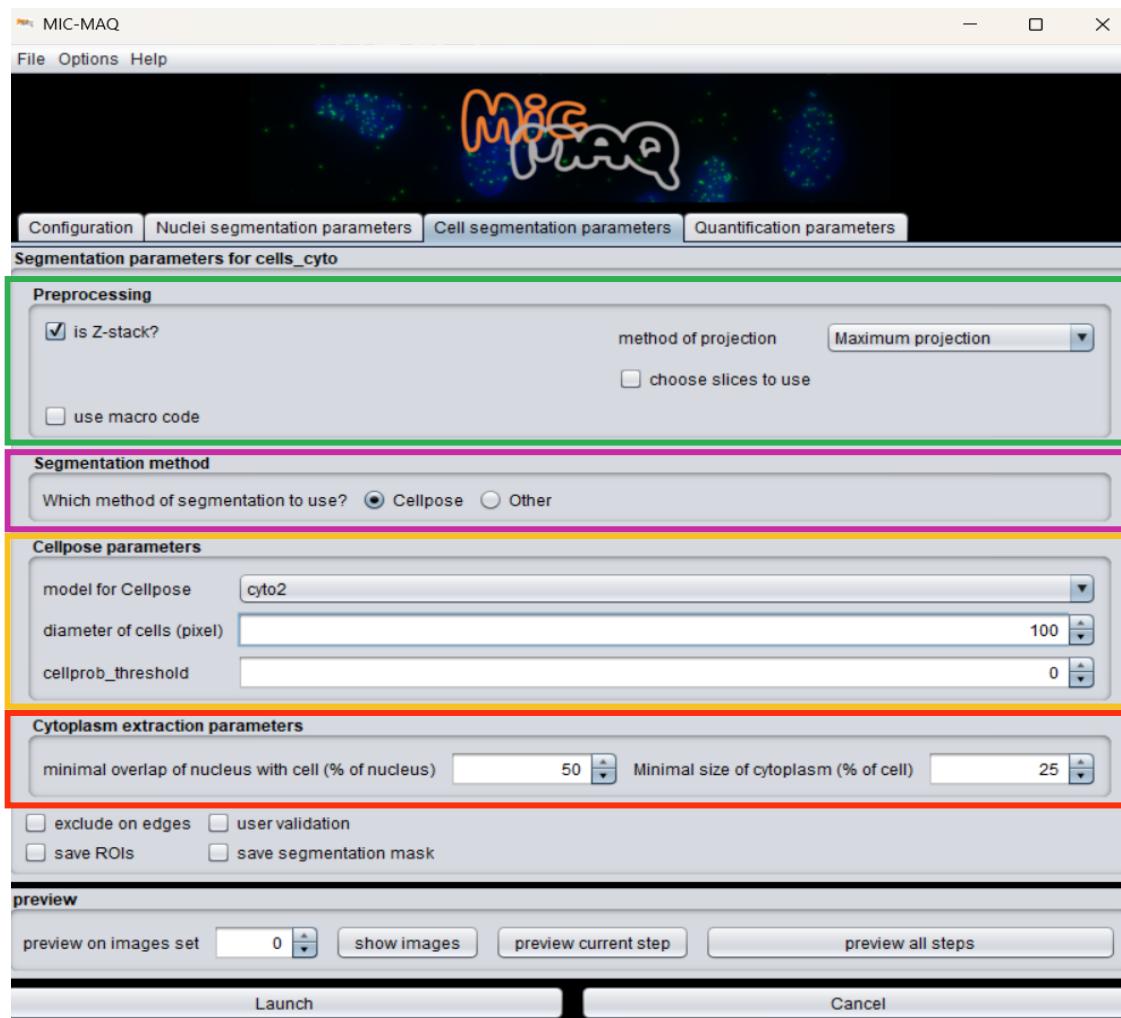
Configuration

Cells application

Quantification

Results

## Cells application



Preprocessing panel

Segmentation algorithm choice

Segmentation parameters

Cytoplasm extraction parameters

Configuration

Cells application

Quantification

Results

## Preprocessing: Z-stack



*MIC-MAQ work only on 2D images, if you have Z stack, the stack should be converted into 2D projection.*

### 2D images

Preprocessing

is Z-stack?

use macro code

### 3D images

Preprocessing

is Z-stack?

use macro code

method of projection

choose slices to use

Maximum projection  
Standard deviation projection  
Sum Slices

- Untick the box « is a Z-stack ? »
- Tick the box « is a Z-stack ? »
- Choose the method of Z projection in the list

*Optional:* You have the possibility to select slices number to create the 2D projection

choose slices to use

1 10

Configuration

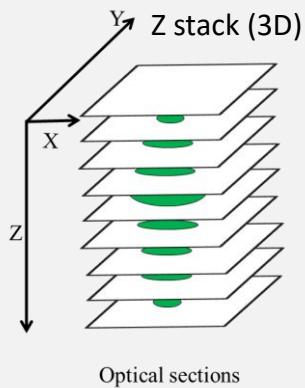
Cells application

Quantification

Results

- Preprocessing** ● ● ●
- Segmentation
- Parameters

## Preprocessing: Z-stack

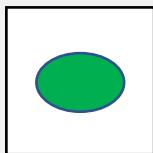


### Method of projection :

- ✓ **Maximum projection** : the 2D projection is calculated based on the maximum voxel value in Z axis
- ✓ **Standard Deviation projection** : the 2D projection is calculated based on the standard deviation value of voxels in Z axis
- ✓ **Sum Slices projection** : the 2D projection is calculated based on the sum voxel values in Z axis

### Method of projection

Z projection (2D)



Configuration

Cells application

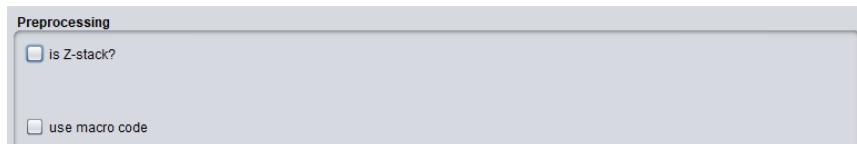
Quantification

Results

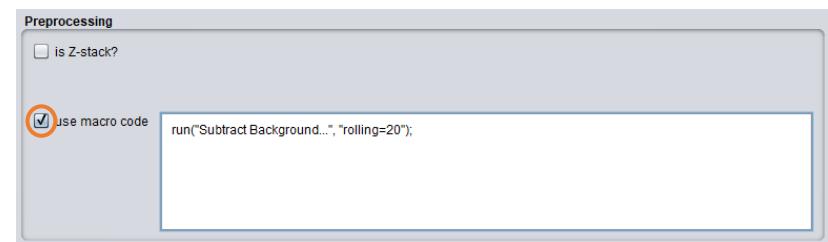
## Preprocessing: use macro code

*MIC-MAQ offers the possibility of applying preprocessing to images using a macro code.*

### Without macro



### With macro



- Untick the box « use macro code »
- Tick the box « use macro code »
- Copy the macro code on the white box

Configuration

Cells application

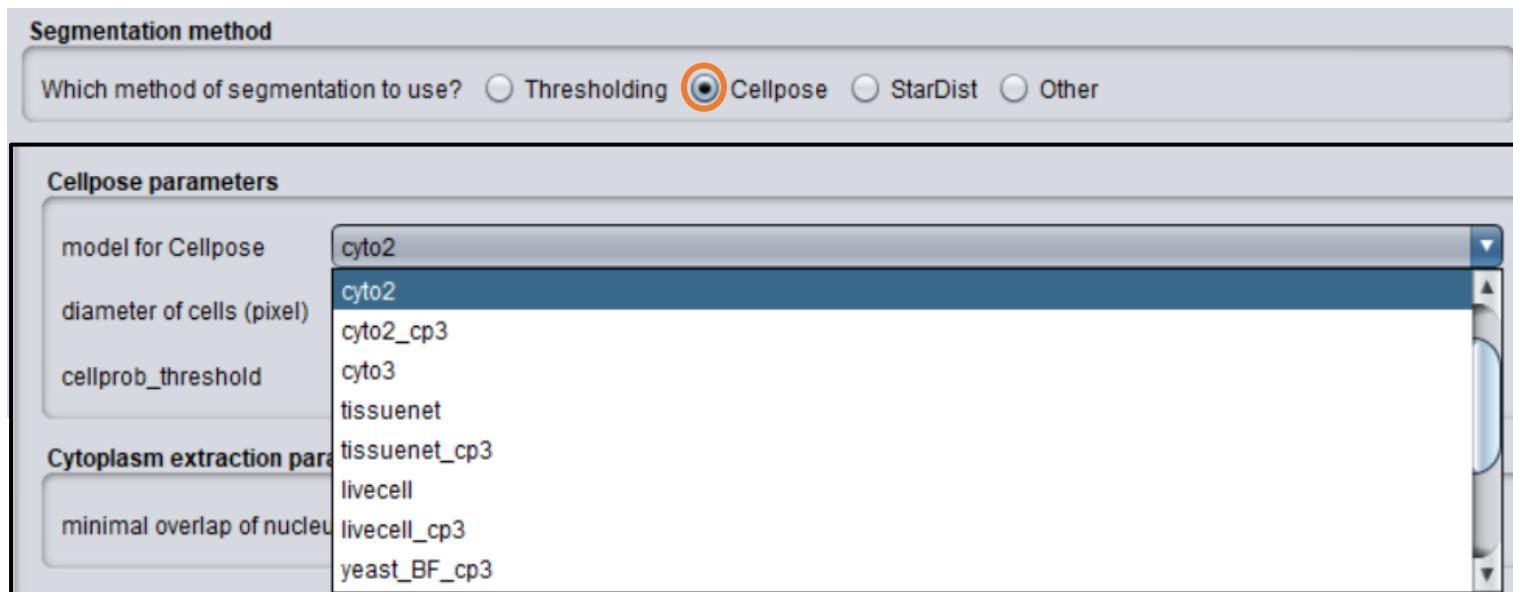
Quantification

Results

## Segmentation parameters: Cellpose

Cells

- Choose the segmentation method



+ : close proximity between nuclei

- : slow process

Configuration

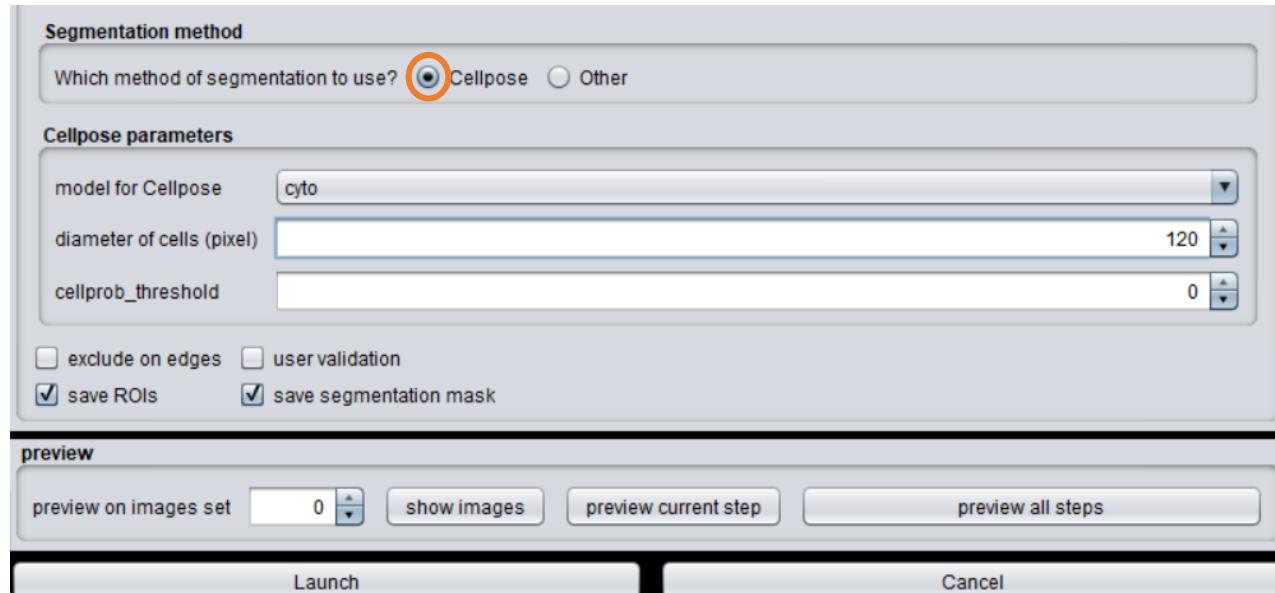
Cells application

Quantification

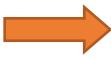
Results

## Segmentation parameters: Cellpose

Cells



cyto2  
nuclei  
cyto  
cyto2  
cyto2\_cp3  
cyto3  
tissuenet  
tissuenet\_cp3  
livecell

- Choose the Cellpose model among the list 
- Enter the mean diameter of nuclei that you want to detect (defined in pixels)
- **Optional:** if the detection is too restrictive you can enlarge detection with the `cellprob_threshold` value

Configuration

Cells application

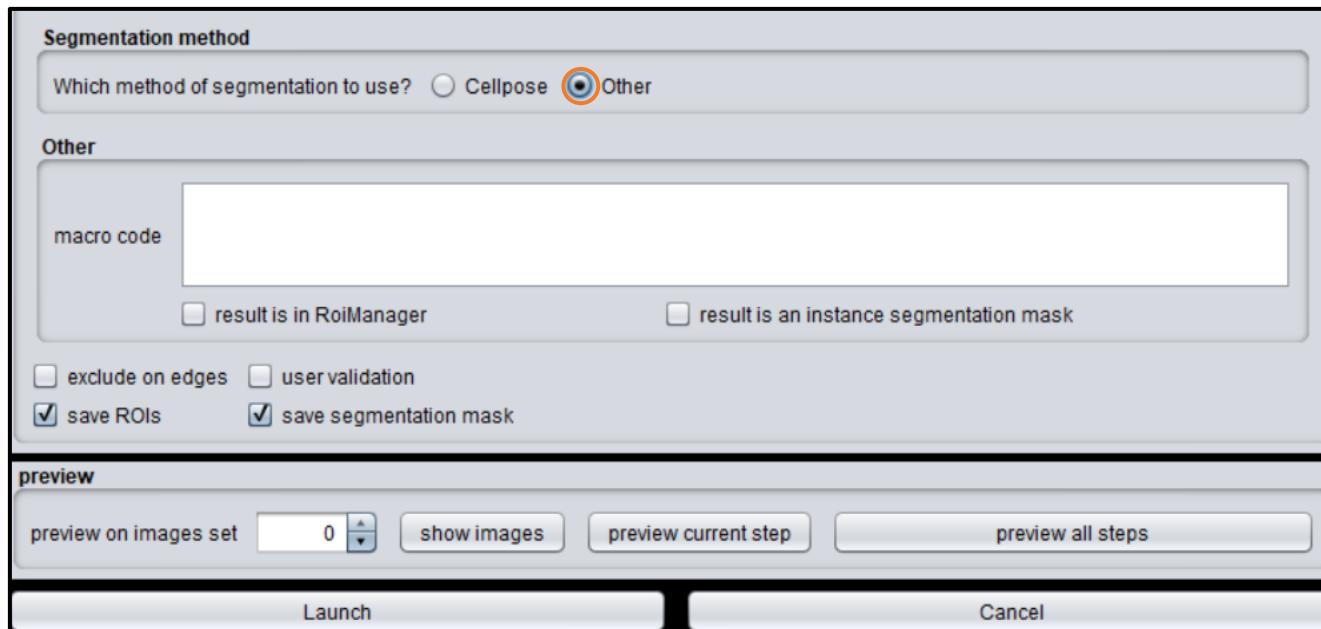
Quantification

Results

## Segmentation parameters: Other

Cells

- Choose the segmentation method



+ : Adaptability to another segmentation method

- :

Configuration

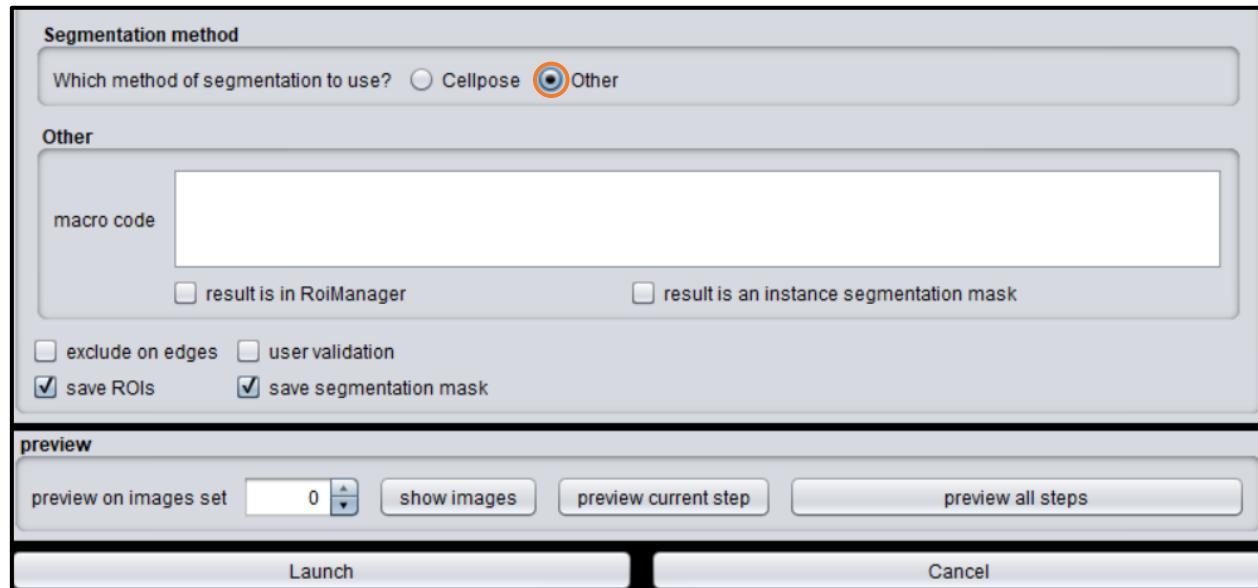
Cells application

Quantification

Results

## Segmentation parameters: Other

Cells



- Enter the macro lines to run the segmentation process
- For the output, select the export as ROI or as mask of segmentation

result is in RoiManager

result is an instance segmentation mask

Configuration

Cells application

Quantification

Results

- Preprocessing
  - Segmentation
  - Parameters**
- 

## Segmentation parameters: Options

<input checked="" type="checkbox"/> exclude on edges	<input checked="" type="checkbox"/> user validation
<input checked="" type="checkbox"/> save ROIs	<input checked="" type="checkbox"/> save segmentation mask

You have the possibility to tick boxes for segmentation:

- **Exclude on edges:** this option will remove automatically regions that touch the edges of the image
- **User validation:** manual step where the user can delete or add regions obtained by segmentation
- **Save ROIs:** this option allows to save automatically the regions of interest obtained by segmentation
- **Save segmentation mask:** this option allows to save automatically the mask of objects obtained by segmentation

Configuration

Cells application

Quantification

Results

- Preprocessing
- Segmentation
- Parameters

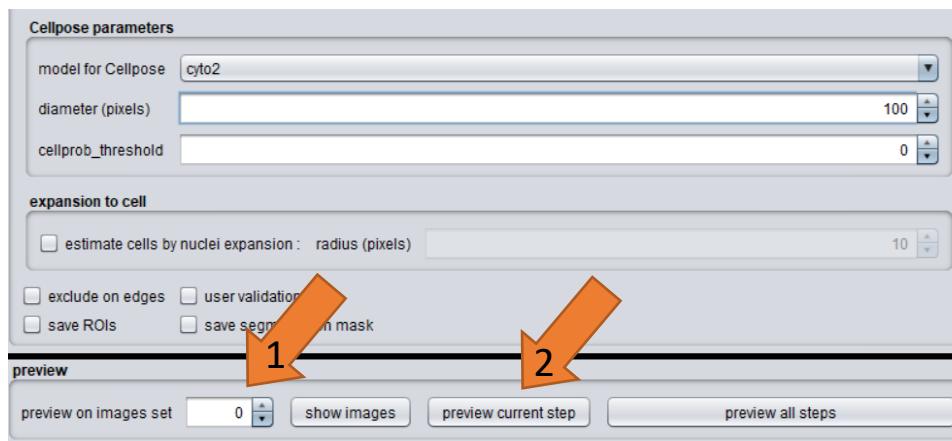


## Test the segmentation



1. Select the set number for the preview to test the thresholding parameters
2. Start the analysis on the previous selected series of images with the button « preview current step »

## Cellpose



To know the association between the preview number and the image name, check the #value in the root box.



root  
►  #0\_serie013\_TIF  
►  #1\_serie020\_TIF

Configuration

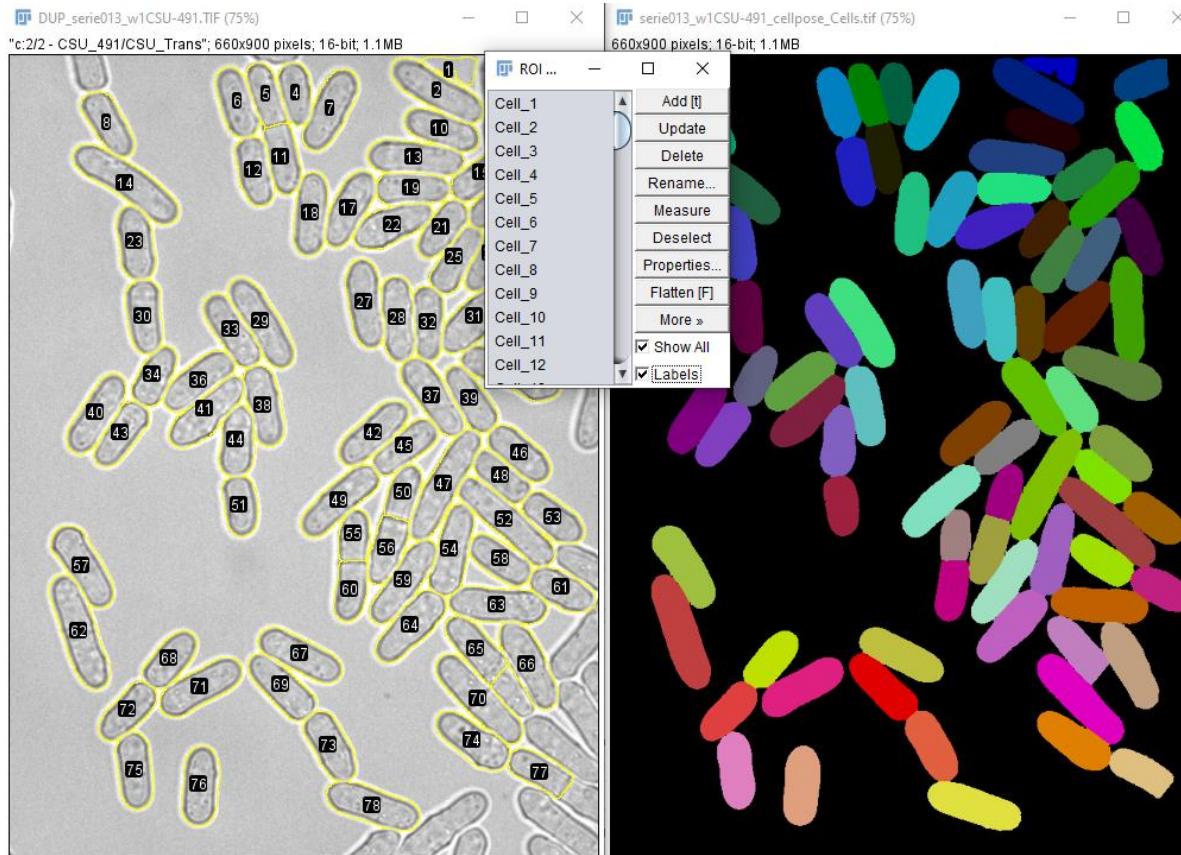
Cells application

Quantification

Results

Cells

## Examples of segmentation



Configuration

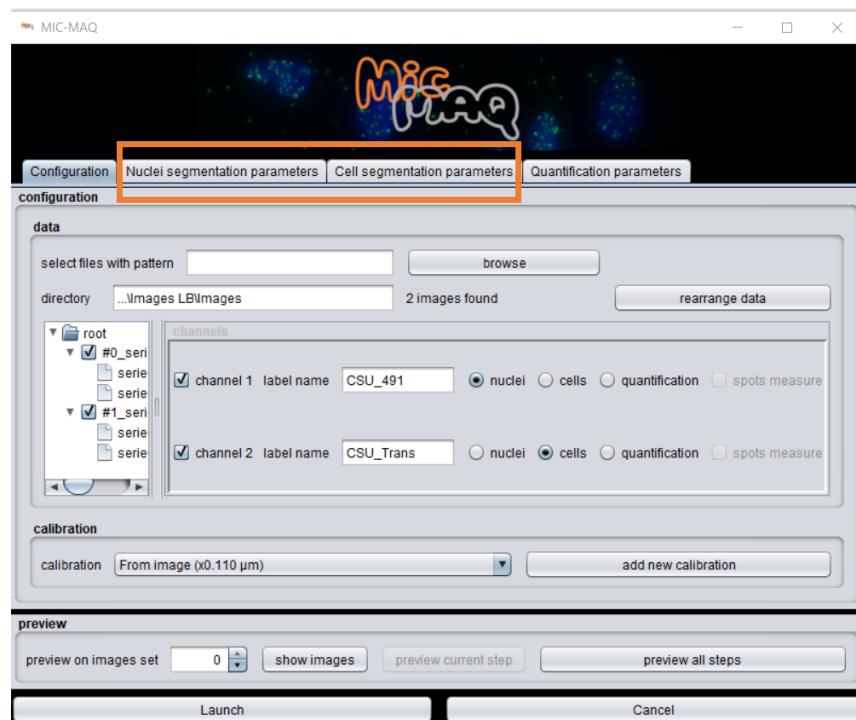
Nuclei/Cells application

Quantification

Results

If you have at least 2 channels, one channel for detecting nuclei and another one for detecting cells, the quantification will be done in nuclei, cells and cytoplasm.

The cytoplasm regions are obtained by removing nuclei regions from cells.



Nuclei + Cells

Measurements are apply on these regions:



Configuration

Nuclei/Cells application

Quantification

Results

- Preprocessing
- Segmentation
- Parameters : **Cytoplasms**

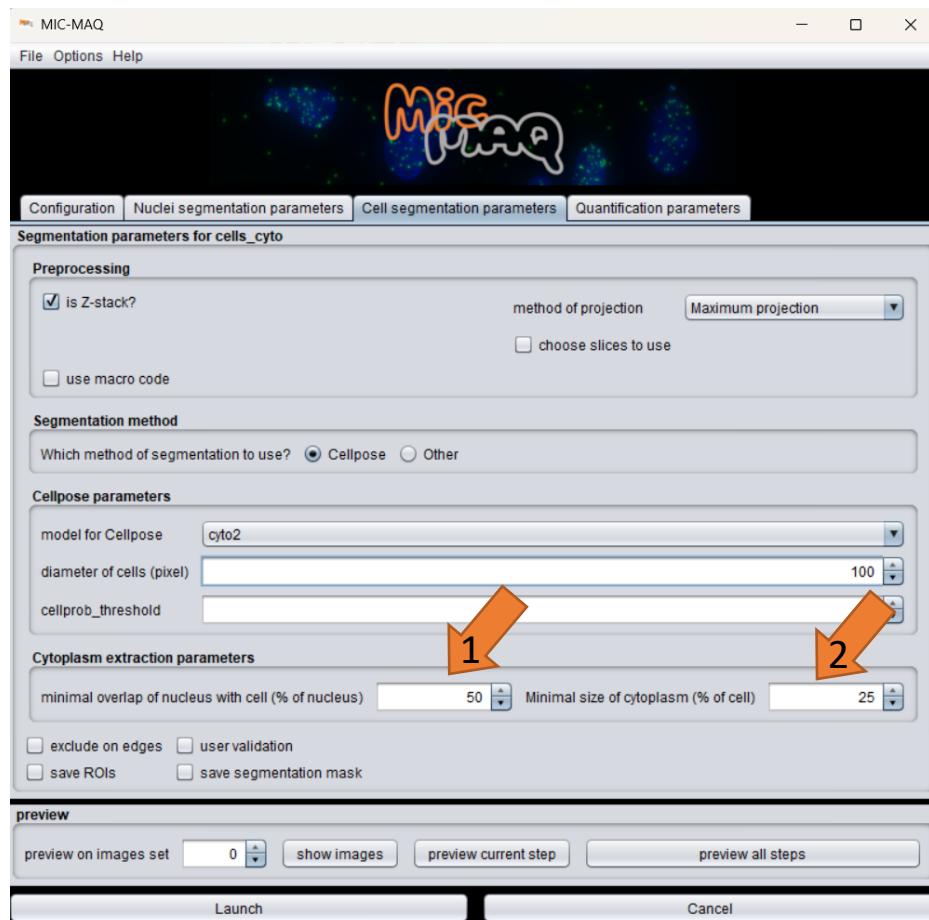
## Cytoplasm extraction parameters

1. Configure nuclei
2. Configure cells
3. Configure the cytoplasm

Nuclei + Cells

In the cell segmentation parameters window:

1. Define the minimal overlap of nucleus with cell to be associated together (% of nucleus) : *default value: 50%*
2. Enter the minimal size of cytoplasm to be quantify (% of cell) : *default value 25%*



Configuration

Nuclei/Cells application

Quantification

Results

- Preprocessing** ● ● ●
- Measurements
- Optional: Spot detection

## Preprocessing: Z-stack



*MIC-MAQ work only on 2D images, if you have Z stack, the stack should be converted into 2D projection.*

**2D images**

**3D images**

Preprocessing

is Z-stack?

use macro code

Preprocessing

is Z-stack?

use macro code

method of projection

choose slices to use

Maximum projection  
Standard deviation projection  
Sum Slices

- Untick the box « is a Z-stack ? »
- Tick the box « is a Z-stack ? »
- Choose the method of Z projection in the list

*Optional:* You have the possibility to select slices number to create the 2D projection

choose slices to use

1 10

Configuration

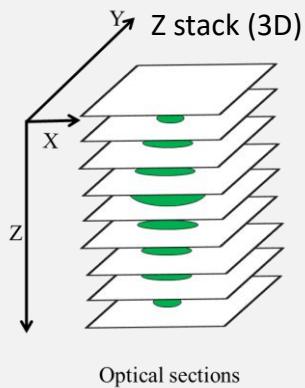
Nuclei/Cells application

Quantification

Results

- Preprocessing** 
- Measurements
- Optional: Spot detection

## Preprocessing: Z-stack

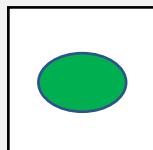


### Method of projection :

- ✓ **Maximum projection** : the 2D projection is calculated based on the maximum voxel value in Z axis
- ✓ **Standard Deviation projection** : the 2D projection is calculated based on the standard deviation value of voxels in Z axis
- ✓ **Sum Slices projection** : the 2D projection is calculated based on the sum voxel values in Z axis

### Method of projection

Z projection (2D)



Configuration

Nuclei/Cells application

Quantification

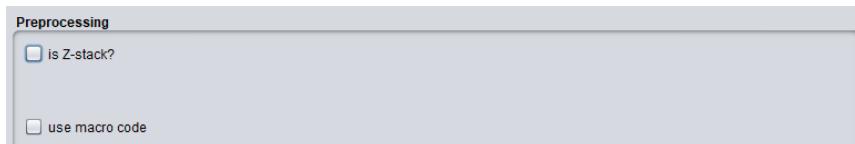
Results

- Preprocessing** ●●●
- Measurements
- Optional: Spot detection

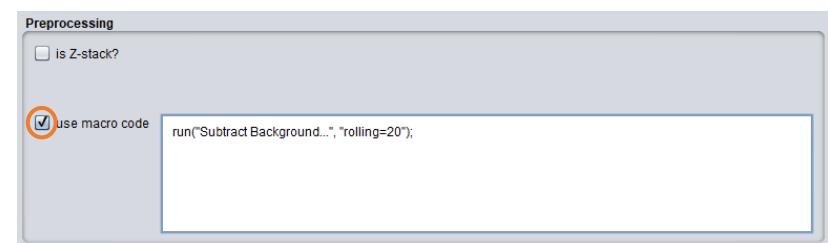
## Preprocessing: use macro code

*MIC-MAQ offers the possibility to apply preprocessing on the images with macro code.*

### Without macro



### With macro



- Untick the box « use macro code »
- Tick the box « use macro code »
- Copy the macro code on the white box

Configuration

Nuclei/Cells application

Quantification

Results

## Quantification parameters

### Measurements

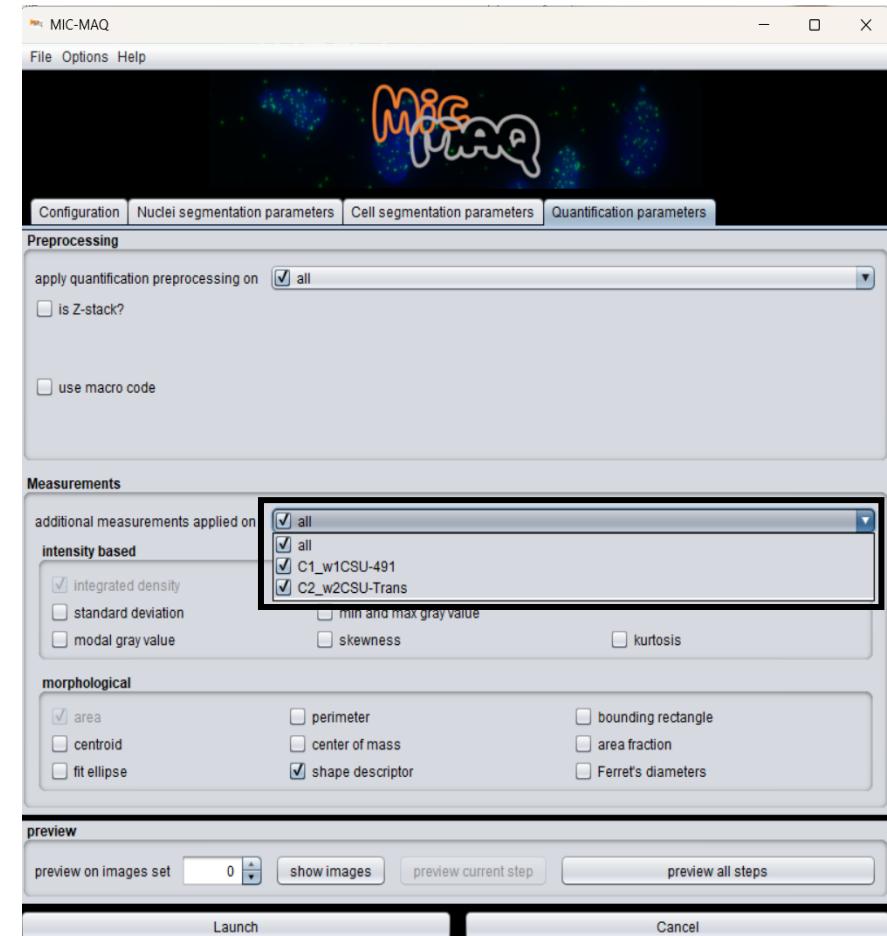
- Among the list, tick the measurement(s) box for the channel(s) used as quantification

Choice of parameters :

Intensity based



Morphological



By default, the mean gray value, integrated density and area are automatically measure on the region of nuclei and/or cells for all channel used as quantification.



Description of measurements: <https://imagej.nih.gov/ij/docs/menus/analyze.html#set>

Configuration

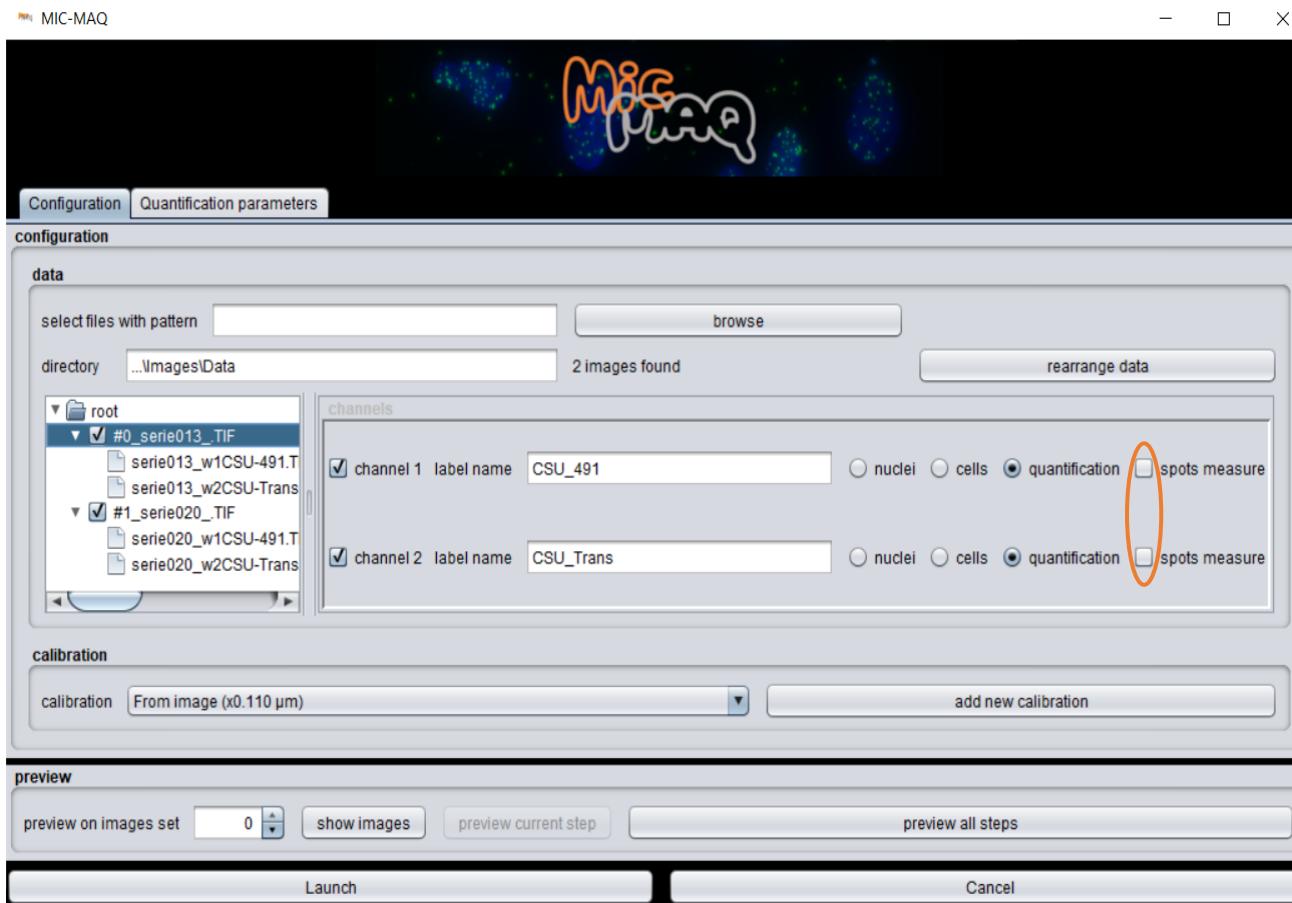
Nuclei/Cells application

Quantification

Results

 Preprocessing Measurements Optional: Spot detection

## Spots detection window



Configuration

Nuclei/Cells application

Quantification

Results

- Preprocessing
- Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

## Spots detection : Preprocessing Z Stack



*MIC-MAQ work only on 2D images, if you have Z stack, the stack should be converted into 2D projection.*

**2D images**

**3D images**

Preprocessing

is Z-stack?

use macro code

Preprocessing

is Z-stack?

use macro code

method of projection

choose slices to use

Maximum projection  
Standard deviation projection  
Sum Slices

- Untick the box « is a Z-stack ? »
- Tick the box « is a Z-stack ? »
- Choose the method of Z projection in the list

*Optional:* You have the possibility to select slices number to create the 2D projection

choose slices to use

1

10

Configuration

Nuclei/Cells application

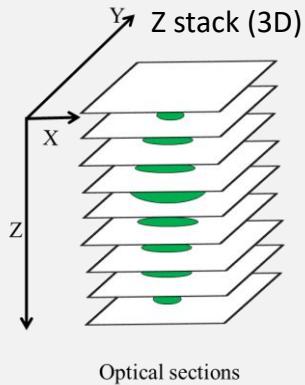
Quantification

Results

- ✓ Preprocessing
- ✓ Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

## Spots detection :

### Preprocessing: Z-stack

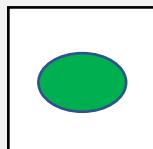


### Method of projection :

- ✓ **Maximum projection** : the 2D projection is calculated based on the maximum voxel value in Z axis
- ✓ **Standard Deviation projection** : the 2D projection is calculated based on the standard deviation value of voxels in Z axis
- ✓ **Sum Slices projection** : the 2D projection is calculated based on the sum voxel values in Z axis

### Method of projection

Z projection (2D)



Configuration

Nuclei/Cells application

Quantification

Results

- ✓ Preprocessing
- ✓ Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

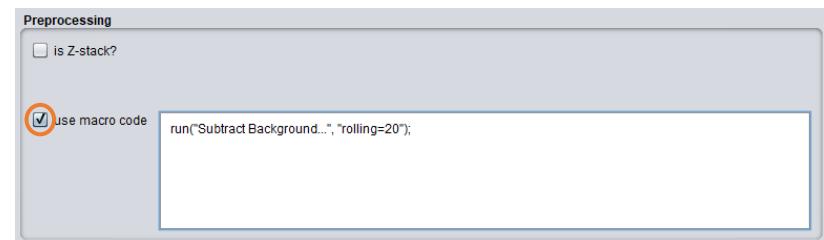
## Spots detection : Preprocessing use macro code

*MIC-MAQ offers the possibility to apply preprocessing on the images with macro code.*

### Without macro



### With macro



- Untick the box « use macro code »
- Tick the box « use macro code »
- Copy the macro code on the white box

Configuration

Nuclei/Cells application

Quantification

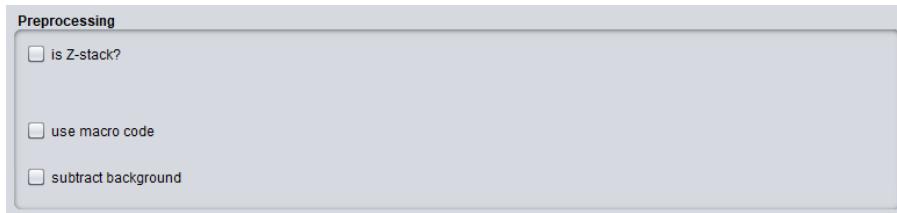
Results

- Preprocessing
- Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

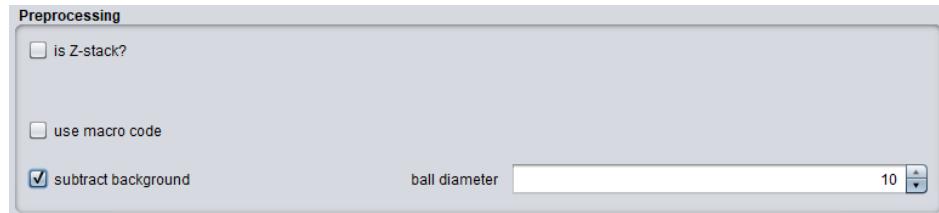
## Spots detection : Preprocessing substract background

*MIC-MAQ offers the possibility to apply preprocessing on the images to remove background and facilitates the spots detection.*

### Without substract background



### With substract background



- Untick the box « substract background »
- Tick the box « substract background »
- Select the ball diameter

Configuration

Nuclei/Cells application

Quantification

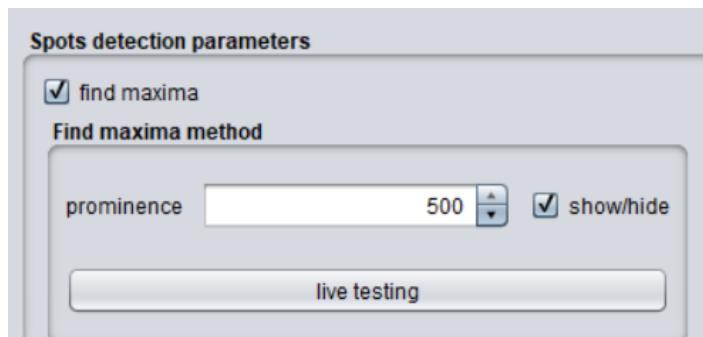
Results

- Preprocessing
  - Measurements
  - Optional: Spot detection**
- 

## Spots detection : Methods

- Choose the spots detection method

### ✓ Find maxima



### ✓ Threshold + Analyze Particles



+ : close proximity between spots

- : spot intensity variation

+ : extract the mean spot size, the mean spot intensity

- : close proximity between spots

Configuration

Nuclei/Cells application

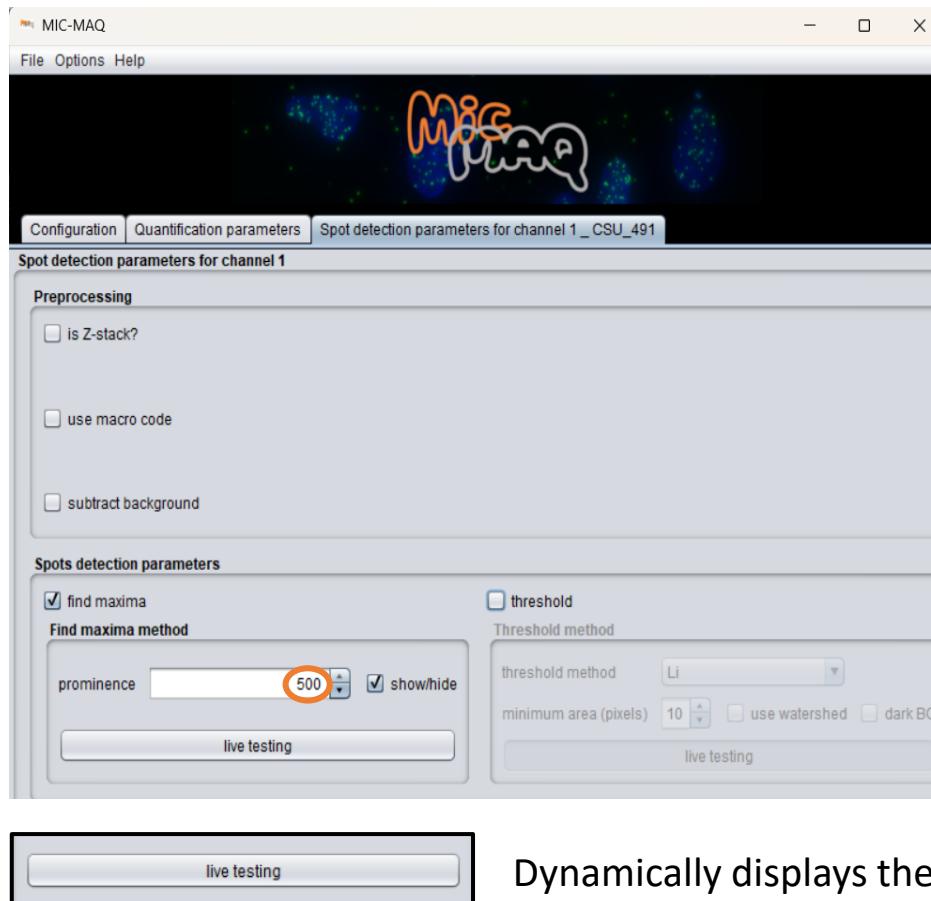
Quantification

Results

- Preprocessing
- Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ●

## Spots detection method: find maxima

- Choose the prominence value to define local maxima corresponding to a spot



Dynamically displays the parameter change

Configuration

Nuclei/Cells application

Quantification

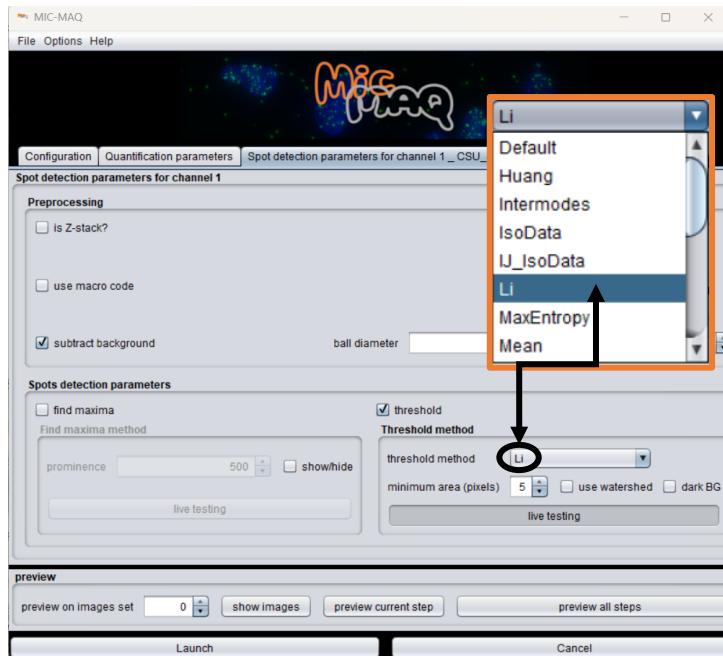
Results

- Preprocessing
- Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

## Spots detection method: Threshold

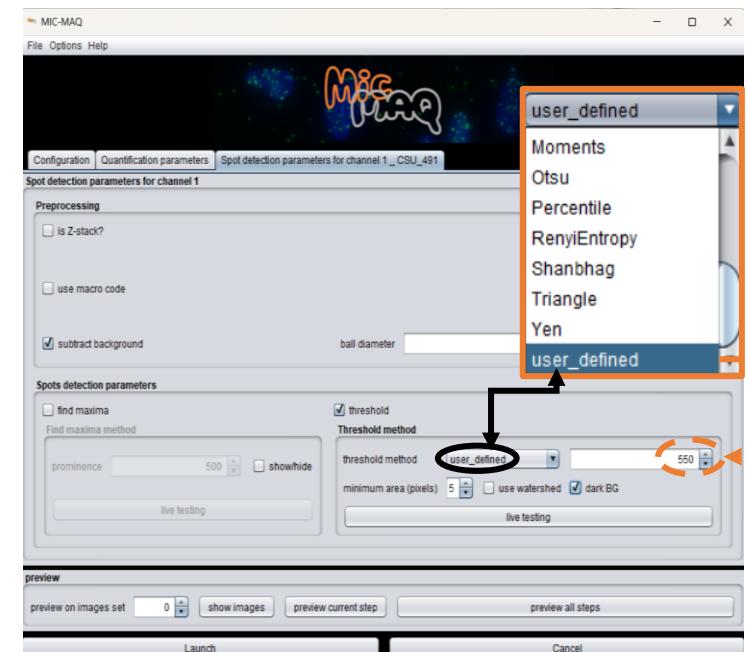
### Threshold: automatic

- Choose an automatic thresholding method



### Threshold: user defined

- Choose "user defined"
- Define the threshold value in the field that appeared



### Options common to both thresholding approach:

 minimum size of spots (pixels<sup>2</sup>) use watershed

**Checked:** separates fused spots.

 dark BG dark BG

**Dark BG checked:** adapted to fluorescence images

**Dark BG unchecked:** adapted to transmission images

Configuration

Nuclei/Cells application

Quantification

Results

- Preprocessing
- Measurements
- Optional: Spot detection**  
● ● ● ● ● ● ● ● ● ●

## Spots detection : Test the spots detection



1. Select the set number for the preview to test the parameters

2. Start the analysis on the previous selected series of images with the button « preview current step »

### Find Maxima

### Threshold

Spots detection parameters

find maxima

**Find maxima method**

prominence     show/hide

live testing

**threshold**

threshold

**Threshold method**

threshold method    Li

minimum area (pixels)     use watershed  dark BG

live testing

preview

preview on images set

Spots detection parameters

find maxima

**Find maxima method**

prominence     show/hide

live testing

**threshold**

threshold

**Threshold method**

threshold method    Li

minimum area (pixels)     use watershed  dark BG

live testing

preview

preview on images set



To know the association between the preview number and the image name, check the #value in the root box.

- ▼ root
  - #0\_serie013\_.TIF
  - #1\_serie020\_.TIF

Configuration

Nuclei/Cells application

Quantification

Results

✓ Preprocessing

✓ Measurements

**Optional: Spot detection**



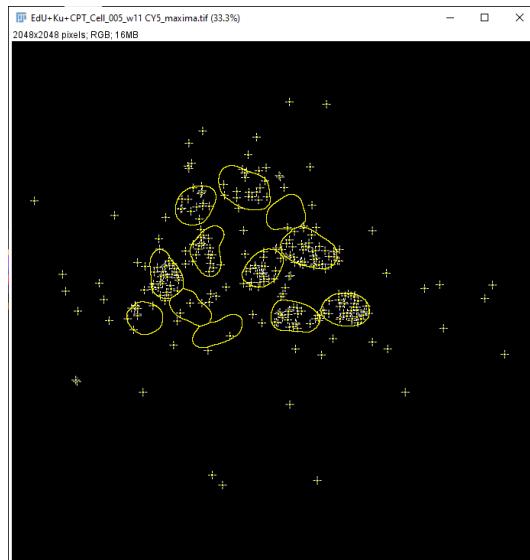
## Examples of spots detection:

### After preprocessing

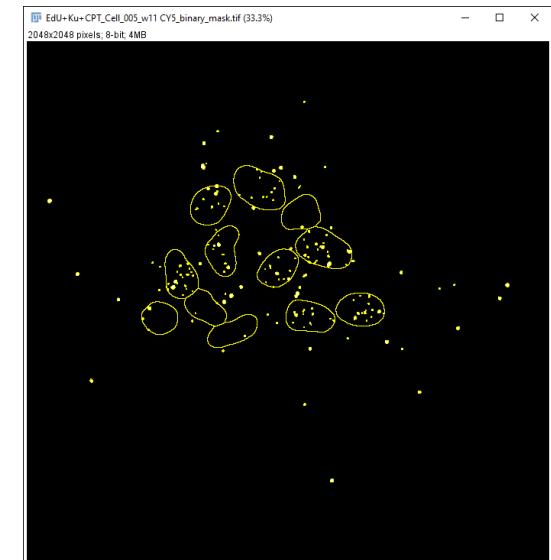


Find Maxima

Analysis



Threshold



Configuration

Nuclei/Cells application

Quantification

Results

## Create summary table

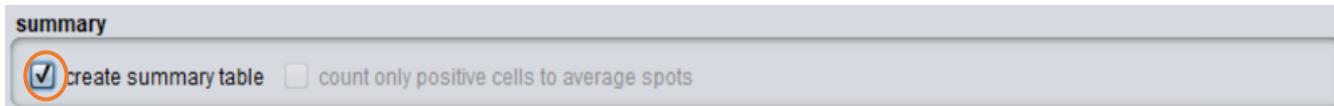
If you tick **Create summary table**:

A new table will be created, that summarize the information in the results and table associated cells nuclei for each image

During the analysis each cell detected will appear as line in the associated table

Depending on the segmentation's parameters, each line will show :

- the number of cells, with averages for all the measurements requested (cell and cytoplasm), and the number of nuclei, with averages for all the measurements requested



Configuration

Nuclei/Cells application

Quantification

Results

## Run the analysis on all images

1. Check that all window have been correctly filled in
2. Test all the steps of analysis on the preview
3. Run the analysis on all images in the directory by clicking on the button « Launch »



During the analysis process, check the window « log » to know the progress of the analysis process.

A screenshot of a 'Log' window. The window title is 'Log' and it has a menu bar with 'File', 'Edit', and 'Font'. The main area contains the following text:

```
out> conda , C:\Users\Christine\anaconda3\envs\cellpose run finished
The binary mask serie020_w2CSU-Trans_cellpose_Cells.tif was saved in C:\Users\Christine\Documents\Activité Plateforme\MIC-MAQ\MIC-MAQ
The cell ROIs of serie020_w2CSU-Trans.TIF were saved in C:\Users\Christine\Documents\Activité Plateforme\MIC-MAQ\MIC-MAQ\Images LB\Im
Quantification channel 1 image:serie020_w1CSU-491.TIF
(cell)number of objects: 100
measurements
Experiment serie020_.TIF is done in :13 seconds
Analysis is done. It took 48 seconds
```

Configuration

Nuclei/Cells application

Quantification

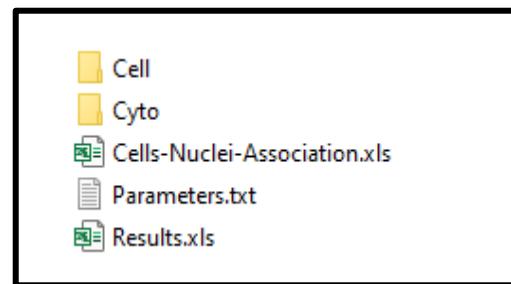
Results

- Summary
- Table
- ROI
- Images

## Explanations about results

After the analysis, a Results folder is automatically created in the folder containing images.

It is composed of :



- **A « Parameters » text file:** resume the parameters used for the analysis
- **One or two « Table » file:** a table of results where all measurements are exported during the analysis
- **several sub-folders :** each contains segmentation of corresponding compartment

Configuration

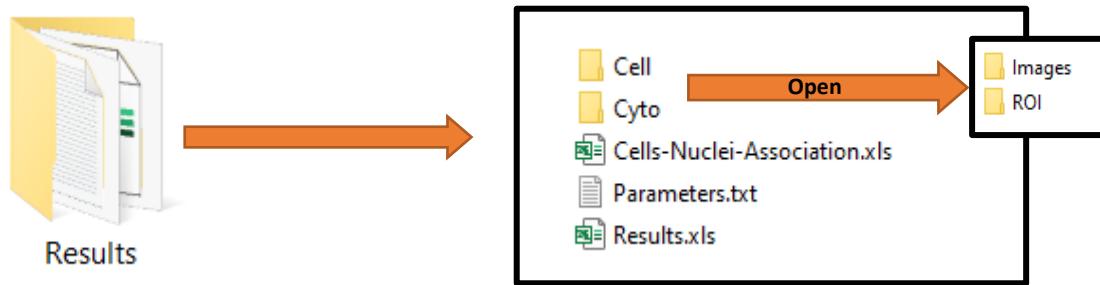
Nuclei/Cells application

Quantification

Results

## Explanations about results

After the analysis, a Results folder is automatically created in the folder containing images.



Each compartment sub-folder:

- **A sub-folder « Images »:** it contains the mask of objects obtained by segmentation
  - **A sub-folder « ROI »:** it contains compartment's regions of interest for each image in a ZIP file
- For spots
- **Each sub-folder « ROI » and « Images » contains subfolders « findmaxima » and « thresholding »** depending on method used.

Configuration

Nuclei/Cells application

Quantification

Results

- Summary ●●●
- Table
- ROI
- Images

## Explanations about results : Parameters file

It resumes the parameters used for the analysis.

```
Parameters.txt - Bloc-notes
Fichier Edition Format Affichage Aide
The configuration is :
CALIBRATION: 100X_Microscope(x0.110 µm)

CHANNEL 1: used
QUANTIFICATION
Quantification Parameters
Preprocessing:
Measurements (intensity based):
    mean
    integrated density
Measurements (morphological):
    area

CHANNEL 2: used
SEGMENTATION cells
Preprocessing:
Use Cellpose:
    Cellpose model: cyto
    Minimum diameter: 100
    Exclude on edges: yes
    Final user validation: no
Quantification Parameters
Preprocessing:
Measurements (intensity based):
    mean
    integrated density
Measurements (morphological):
    area
```



Configuration

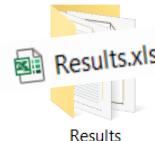
Nuclei or Cells application

Quantification

Results

- Summary
- Table
- ROI
- Images

## Explanations about results : Results table



It is a table where all measurements are compiled during the analysis.

	Name experiment	Cell nr.	Cell_C2_Area (pixel)	Cell_C2_Area (microns)	Cell_C2_Mean	Cell_C2_Circ.	Cell_C2_RawIntDen	Cell_C2_AR	Cell_C2_Round	Cell_C2_Solidity	Cell_C1_Mean	Cell_C1_RawIntDen
1	1 serie013_.TIF	1	1177.00	14.24	1353.66	0.48	1593262.00	2.44	0.41	0.88	174.29	205136.00
2	2 serie013_.TIF	2	3772.00	45.64	1269.72	0.58	4789401.00	3.12	0.32	0.95	190.12	717136.00
3	3 serie013_.TIF	3	2128.00	25.75	1262.12	0.75	2685782.00	1.75	0.57	0.96	183.95	391447.00
4	4 serie013_.TIF	4	2094.00	25.34	1289.56	0.74	2700338.00	2.05	0.49	0.96	196.19	410815.00
5	5 serie013_.TIF	5	2117.00	25.62	1305.75	0.67	2764268.00	2.29	0.44	0.95	197.75	418643.00
6	6 serie013_.TIF	6	2690.00	32.55	1301.26	0.70	3500383.00	2.16	0.46	0.96	197.65	531685.00
7	7 serie013_.TIF	7	3353.00	40.57	1261.89	0.67	4231106.00	2.50	0.40	0.96	202.70	679648.00
8	8 serie013_.TIF	8	2603.00	31.50	1285.35	0.69	3345764.00	2.25	0.44	0.96	196.89	512511.00
9	9 serie013_.TIF	9	2658.00	32.16	1267.91	0.69	3370104.00	2.24	0.45	0.95	196.60	522570.00
10	10 serie013_.TIF	10	2682.00	32.45	1285.89	0.67	3448769.00	2.47	0.40	0.95	199.91	536170.00
11	11 serie013_.TIF	11	2456.00	29.72	1308.34	0.66	3213294.00	2.32	0.43	0.96	206.32	506713.00
12	12 serie013_.TIF	12	2549.00	30.84	1317.12	0.72	3357340.00	2.13	0.47	0.96	202.15	515268.00
13	13 serie013_.TIF	13	3474.00	42.04	1321.22	0.61	4589919.00	2.98	0.34	0.95	206.46	717240.00
14	14 serie013_.TIF	14	4612.00	55.81	1292.44	0.54	5960732.00	3.63	0.28	0.93	203.52	938650.00
15	15 serie013_.TIF	15	2465.00	29.83	1298.13	0.71	3199897.00	2.29	0.44	0.95	201.59	496929.00
16	16 serie013_.TIF	16	3021.00	36.55	1320.08	0.64	3993992.00	2.80	0.36	0.93	205.07	619525.00
17	17 serie013_.TIF	17	2997.00	36.26	1317.19	0.69	3947605.00	2.42	0.41	0.96	207.91	623098.00
18	18 serie013_.TIF	18	3029.00	36.65	1316.81	0.69	3988612.00	2.53	0.39	0.96	203.48	616326.00
19	19 serie013_.TIF	19	2564.00	31.02	1336.25	0.71	3426146.00	2.37	0.42	0.96	208.69	535081.00
20	20 serie013_.TIF	20	2864.00	34.65	1295.11	0.66	3709205.00	2.54	0.39	0.94	214.55	614481.00
21	21 serie013_.TIF	21	2274.00	27.52	1301.39	0.76	2959371.00	2.09	0.48	0.96	207.78	472493.00
22	22 serie013_.TIF	22	3046.00	36.86	1319.26	0.67	4018477.00	2.36	0.42	0.96	227.97	694407.00
23	23 serie013_.TIF	23	2881.00	34.86	1298.81	0.74	3741880.00	2.12	0.47	0.96	210.80	607326.00
24	24 serie013_.TIF	24	2865.00	34.67	1314.52	0.64	3766087.00	2.60	0.38	0.95	217.54	623263.00
25	25 serie013_.TIF	25	2429.00	29.39	1317.20	0.64	3199474.00	2.55	0.39	0.95	209.74	509453.00
26	26 serie013_.TIF	26	3773.00	45.65	1310.74	0.59	4945422.00	3.27	0.31	0.95	214.56	809532.00
27	27 serie013_.TIF	27	3277.00	39.65	1298.17	0.65	4254103.00	2.68	0.37	0.96	224.06	734259.00
28	28 serie013_.TIF	28	3045.00	36.84	1350.10	0.67	4111067.00	2.67	0.37	0.96	216.95	660627.00

This table is the result of an analysis of cells application

Configuration

Nuclei+Cells application

Quantification

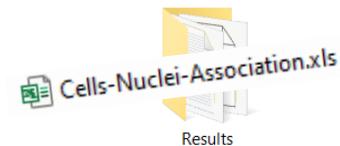
Results

- Summary
- Table
- ROI
- Images

## Explanations about results : Cell-Nuclei-Association table

Nuclei + Cells

Only when Cells and Nuclei are provided, it is a table where correspondence between nucleus and cell is stored as well as nuclei measurements.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Name exper	Nucleus nr.	Cell associat	Nuclei_C1	C Nuclei_C1	CSU_491	RawIntDen										
2	serie013_TII	1	34	463.74	670111.00												
3	serie013_TII	2	41	553.15	608465.00												
4	serie013_TII	3	56	335.56	348646.00												
5	serie013_TII	4	63	362.72	428378.00												
6	serie020_TII	1	28	1395.79	1514435.00												
7	serie020_TII	2	40	1023.46	1117619.00												
8	serie020_TII	3	52	628.51	965397.00												
9	serie020_TII	4	64	673.43	868057.00												
10	serie020_TII	5	-1	1102.77	1650849.00												
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	
21																	
22																	
23																	
24																	

Configuration

Nuclei/Cells application

Quantification

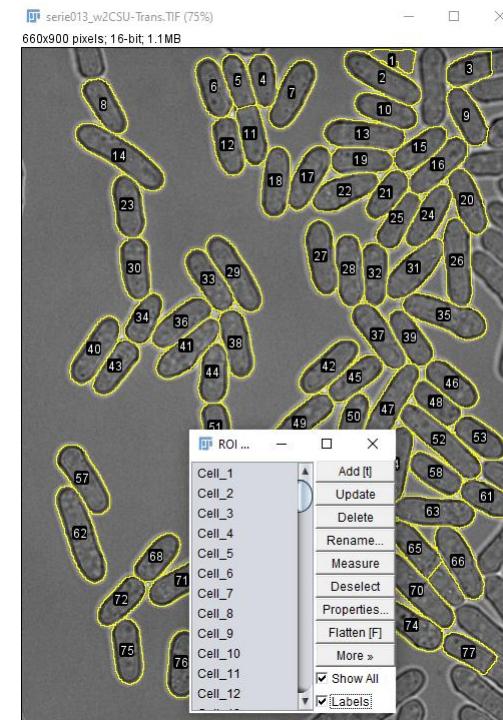
Results

- Summary
- Table
- ROI
- Images

## Explanations about results : A sub-folder « ROI »

It contains nuclei or cells regions for each image in a ZIP file, if the "save ROIs" checkbox was selected in segmentation parameters.

To re-open the nuclei or cells ROI, drag and drop the .Zip file into Fiji. ROI will automatically be loaded to the ROI manager window.



Configuration

Nuclei/Cells application

Quantification

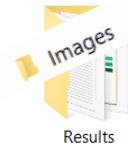
Results

- Summary
- Table
- ROI
- Images**

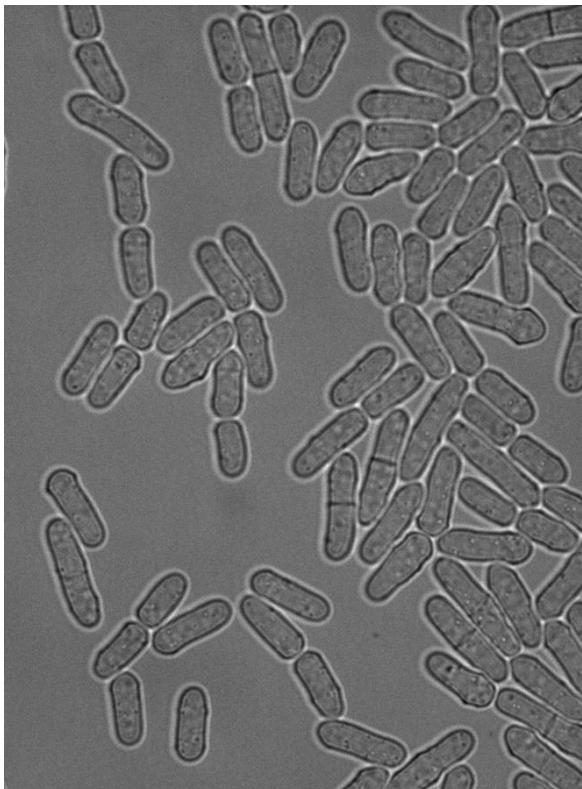
## Explanations about results : A sub-folder « Images »

It contains the mask of objects (nuclei or cells) obtained by segmentation , if the "save segmentation masks" checkbox was selected in segmentation parameters.

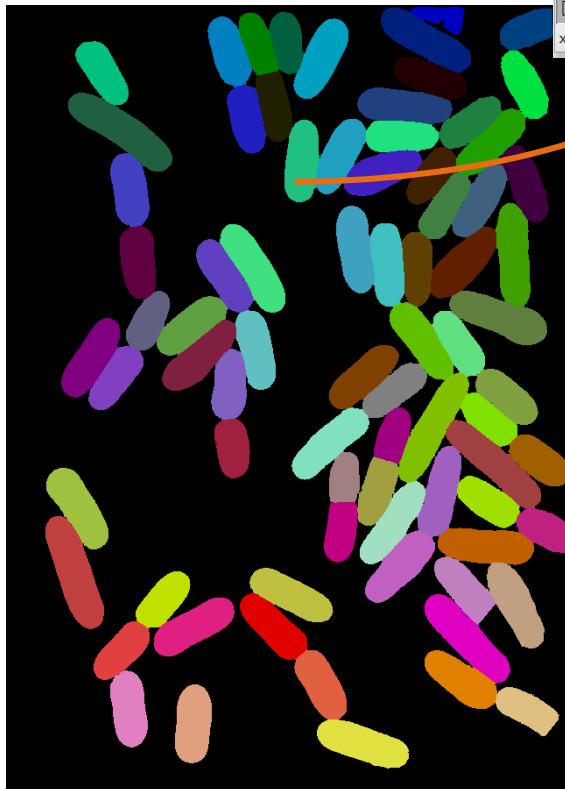
Each detected object is associated to a intensity value on the objects mask image.



Raw data



Objects mask





Informations

## MIC-MAQ version 1.12



<https://github.com/MultimodalImagingCenter/MIC-MAQ>



Images for training

<https://zenodo.org/record/8186508>



If you want to report bugs, ask questions to solve your analyses or request improvements

<https://github.com/MultimodalImagingCenter/MIC-MAQ/issues>