Fluo_Bac_Tracker tutorial

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Contents

1 What is FluoBacTracker ?

1.1 Uses of the software

FluoBacTracker comes as ImageJ plugins design to segment and track growing cells from microscopic images and to detect protein aggregates in fluorescence images.
It can :

- Select regions of interest in each image (detect the colony)
- Denoise and renormalize images
- Identify each cell in each image (segmentation)
- Follow cells through the whole movie (tracking)
- Detect divisions and construct cell lineage in the population

Algorithms have been developed to do these analysis, see in particular Anne-Sophie Coquel [Coq12] and Maël Primet [Pri12] PHDs.

1.2 Overview

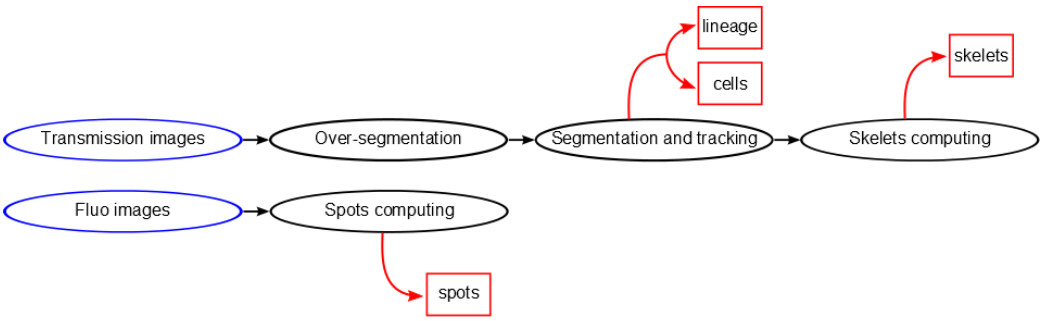

Figure 1: Main workflow of the software (blue : intput; black : processes; red : output)

FluoBacTracker is taking as an input transmission images (as tiff files or stack) and detects and tracks cells through several steps. First images are prepared : they are denoised, grey level is renormalized and a background is defined (see section 3). Then, cells are oversegmented (see section 4). Final segmentation is then done at the same time as lineage tracking (see section 6). The final result is then displayed as a lineage (see section 7).

2 Installation

2.1 To begin

FluoBacTracker comes as plugins of ImageJ software, so you will need ImageJ to use it. To get it go to http://rsbweb.nih.gov/ij/download.html.

Our plugin works with java 6, so be sure to have this version on your computer and that ImageJ is using it. (In ImageJ, go to Edit/Option/Compiler, and select target 1.7).

Also, this software is memory consuming so it is recommended to put java heap space at least at 4 Go. (In ImageJ, go to Edit/Option/Memory and Threads, put maximum memory at least to 4000 and restart ImageJ).

2.2 With .jar file

You can download the FluoBacTracker.jar file from our website `http://fluobactracker.inrialpes.fr/index.php?p=download`.
Once you have the file, you just have to put it in the ImageJ/Plugins directory. It should then appear in the Plugins menu next time you open ImageJ. (see Figure 2).

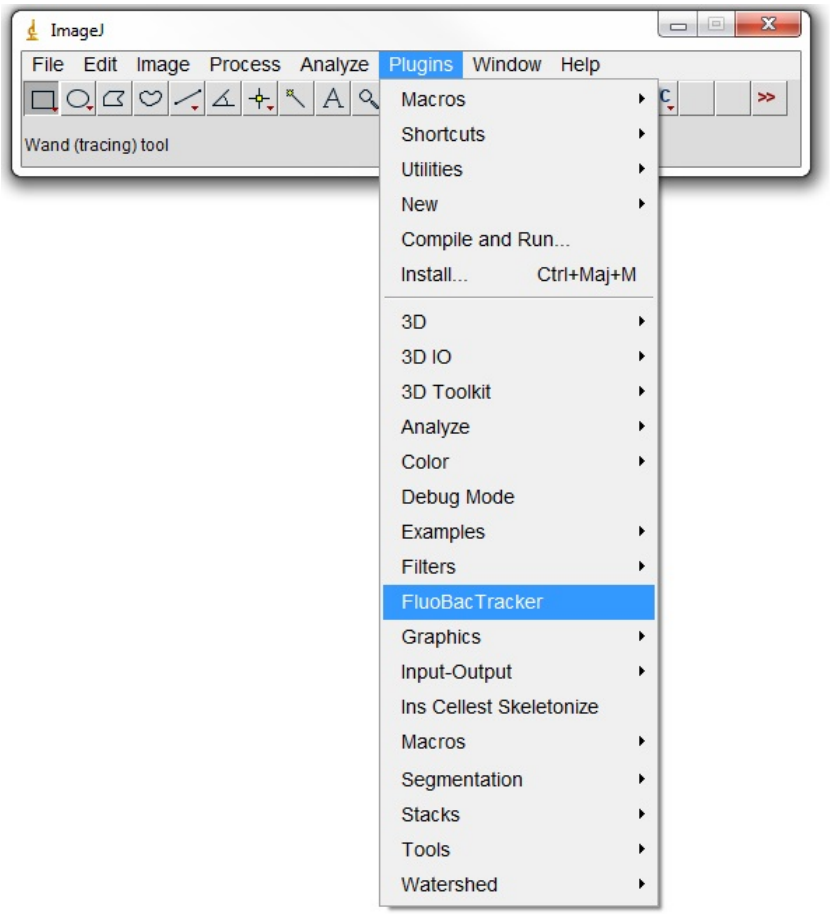

Figure 2: FuoBacTracker appears in Plugins menu

3 Preprocessings

3.1 Overview

In the first window of the software (see Figure 3) images can be imported and preprocessed. Preprocessing consist in three main steps : Denoising [LM11], Renormalizing and Over-segmentation [Pri12].

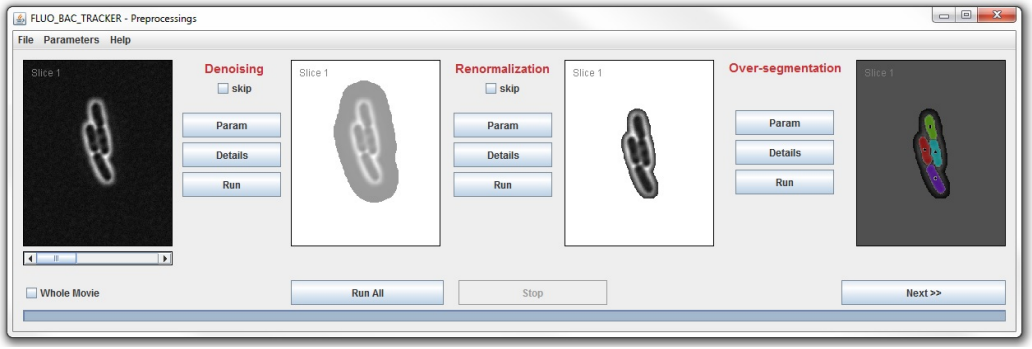

Figure 3: First FluoBacTrackerWindow, Preprocessings

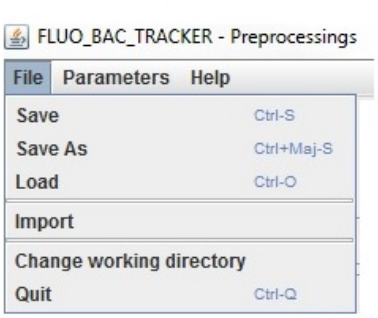

Figure 4: File menu

3.2 MenuBar

3.2.1 File

Save/Save As save current results in a chosen directory.

Load load previously saved results.

Import import new images or stack to work with.

Change working directory change directory were temporary files are writen. This directory is at first the default temp directory of yout computer.

Quit Quit program.

3.2.2 Parameters

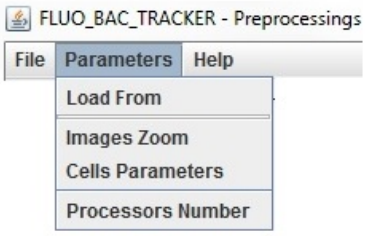

Figure 5: Parameters menu

Load From Load all parameters from previously saved results.

Images Zoom Change images zoom (default value is 100x = 0.064 μm per pixel)

Cells Parameters Change cells maximum width and minimum area. These parameters are used to segment cells.

Processors Number Change number of threads the software can launch at the same time. It is recommended to use your maximum number of processors but youy can also choose to diminish this number to be able to do something else on your computer while processing images.

3.2.3 Help

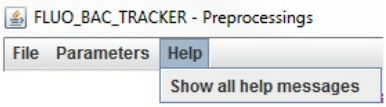

Figure 6: Help menu

Show all help messages Show all help messages again, even when user has clicked the "don't show again" option.

3.3 Bottom Bar

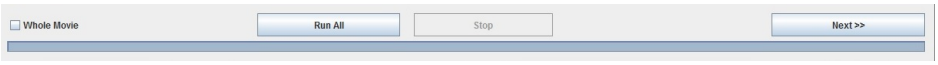

Figure 7: Bottom bar

Whole movie When this checkbox is selected all processing will be done on the whole movie (when clicking Run or Run All buttons). If it is not selected only the current slice will be process.

RunAll Process all the preprocessings either on one slice, either on the whole movie (see Whole movie checkbox). If some steps were already computed on the whole movie (or the current slice), computation will continue from this step.

Stop Stop computation at any time in the process.

Next Go to next window (see section **??**)

3.4 Step buttons

For each preprocessing step, several buttons are available :

Figure 8: Step buttons

Param Opens a dialog wondow to enable user to change main parameters of the step. these are :

- Denoising
  - *Denoising level* :
  - *Patch size* : Size of the square used as patch for the localized mean denoising [LM11].
- Renormalizing
  - *Pseudo seeds threshold* : Threshold use to determine darker part of the image that will be considered as in cell and from wich renormalization will be computed.
  - *Convolution square size* : Size of the convolution square use to smooth masks when renormalizing image.
  - *Convolution iterations number* : Number of iteration of the convolution.
- Over-segmentation
  - *Seeds threshold* : Threshold used to determine seeds. Each pixel darker than the threshold is in a seed.
  - *Blob minimum area* : Blob smaller than this area should be mixied with another blob or deleted.

Details Opens another window showing the substeps of the given step (see section 3.5).

Run Process the step either on one slice, either on the whole movie (see section 3.3 Whole movie checkbox).

Skip CheckBox allowing to skip optional step (denoising and renormalizing). Even when checked, user must click the run button to be able to continue preprocessing further.

3.5 Details Windows of each steps

3.5.1 Denoising

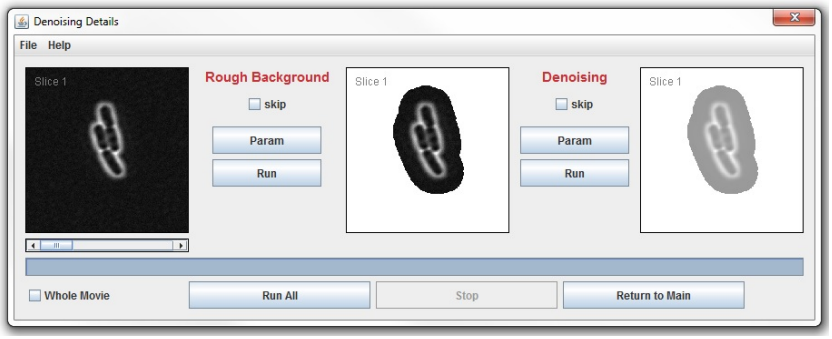

Figure 9: Denoising details dialog

Rough Background In this step a rough background mask of the cell colony is detected. Darker places in the images are detected thanks to a grey level threshold and then this selection is opened and eroded several times untill getting a rough background mask of the cells colony.

Parameters :

- *Rough background threshold* : grey lever threshold to roughly detect where are the cells.
- *Rough background radius* : Radius used for erosion and opening.

Denoising In this step, images are denoised using GTV-means algorithm [LM11].

Parameters :

- *Denoising level* :
- *Reduction coefficient* :
- *Minimal number of patches* :
- *Patch side length in pixels* :

- *Maximum patches distance in pixels* :
- *Precision of denoising* :
- *Aggregation mode* :

3.5.2 Renormalizing

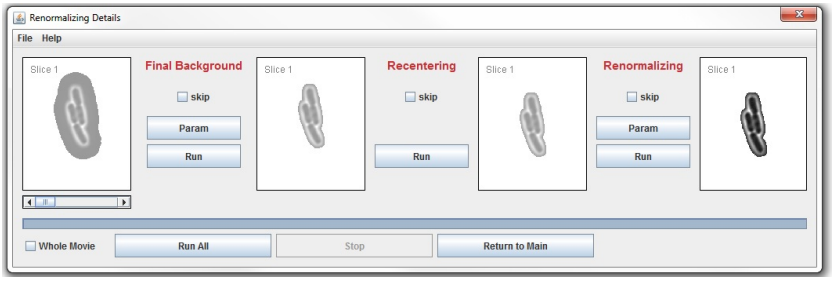

Figure 10: Renormalizing details dialog

Final Background In this step a final background mask of the cell colony is detected. Beginning from the boundaries of the first rough background, pixels are put in background if they are lighter than a grey level threshold. This gives a more accurate background mask. This mask can then be opened to be more accurate.

Parameters :

- *Background threshold* : grey level threshold.
- *Opening iteration number :* Number of times mask is opened.

Recentering In this step, images are recentered so that colony center is in the middle point of the image.

Renormalization In this step images are renormalized so that grey level inside cells is homogeneous. Pseudo seeds (given only by selecting pixels under a grey level threshold) are computed.

Parameters :

- *Pseudo seeds threshold* :
- *Radius of sampling* :
- *Convolution square size* :
- *Convolution iteration number* :

4 Over-segmentation

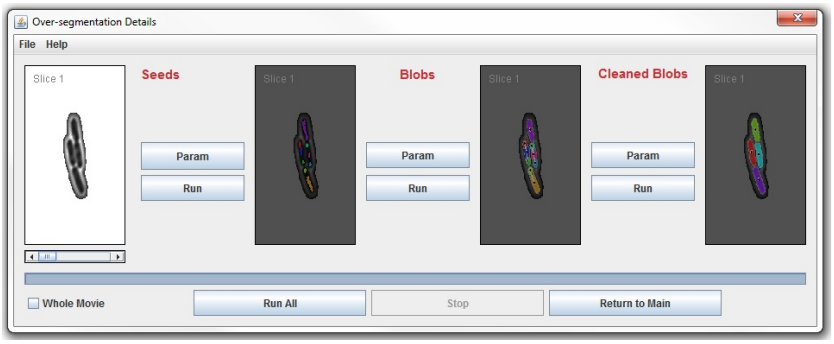

Figure 11: Over-segmentation details dialog

Seeds In this step seeds are detected by selecting pixels under a grey level threshold.

Parameters :

- *Seeds threshold* : grey level threshold. Under this threshold, pixels are in a seed.
- *Seeds computation method* :

Blobs In this step seeds are dilated into blobs with a non homogeneous dilatation [Pri12].

Parameters :

- *First speed parameter* : parameter used to compute viscosity (or dilatation speed) in function of the pixel grey level.

- *Second speed parameter* : parameter used to compute viscosity (or dilatation speed) in function of the pixel grey level.
- *Maximum iteration number* : Maximum dilatation iteration number. This stops dilatation after a while.
- *Minimum significant border between blobs* : This is used to compute which blobs are connected.

Final blobs In this step blobs are cleaned. Small blobs are either merged or deleted.

Parameters :

- *Minimum blob area* : Determines which blobs are too small and must be merged or deleted.
- *Second dilatation iteration number* : After cleaning blobs are dilated a second time.

5 Checking blobs

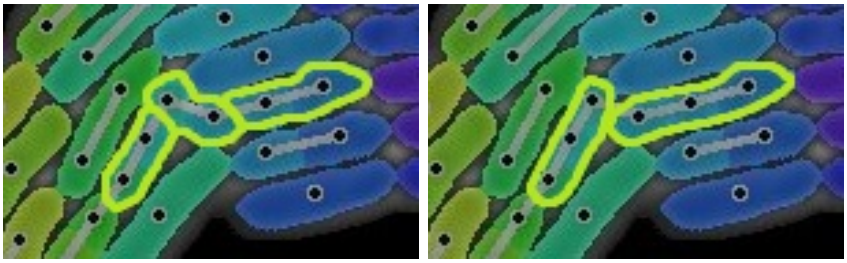

Figure 12: Exemples of possibles cells (highlighted in yellow)

6 BlobSolving

6.1 What is Blobsolving

To fully undertstands how this alorithm works, see Maël Primet PHD [Pri12] (section 2.3 Cell segmentation and tracking).

From blobs (over-segmented cells), segmentation and tracking is done by computing all possible cells (see figure 12) as an addition of blobs and the chose the best ones by choosing cell transitions or divisions one by one. Transitions or divisions with the lowest risk are chosen first.

6.2 Transition risk

The risk $\rho^{A\to B}$ of cell A becoming cell B in next frame is the maximum probability of transition taken over all potential sucessors X of A (B excepted) divided by the probability of transition with B.

$$\rho^{A\to B} = \max_{X\neq B} \frac{Proba_{A\to X}}{Proba_{A\to B}}$$

And the probability of transition $Proba_{A\to B}$ of a cell A to a cell B depends on the probability of the cell to divide P_{div}, and the probability densities of position P_x (cell moving from A position to B position), orientation P_θ (cell rotating from A orientation to B orientation) and area P_A (cell growing from A area to B area).

$$Proba_{A\to B} = (1 - P_{div}) * P_x(\frac{\chi_A - \chi_B}{|\chi_A|}) * P_\theta(|\theta_A - \theta_B|) * P_A(log(\frac{A_A}{A_B}))$$

As reprensented in Figure 13 :

- P_x follows an exponential law
- P_θ follows a Laplace law
- P_A follows a Gaussian law

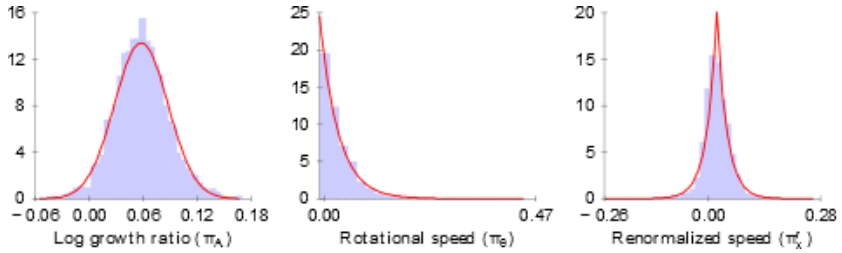

Figure 13: Cell evolution model from Maël Primet PHD

6.3 Parameters

In our software, the blobsolving parameters (see Figure 14) are used to compute risks.

6.3.1 Others parameters

Distance between two linked cells : This is the maximum distance between a mother cell and its daughter cell. Software will not consider as potential daughter cells the cells in follwing frame which center points are further from the mother cell center point than this distance.

- *Increasing this parameter will be time consuming but if it is too small some transitions will not be detected.*

Maximum distance between two sister cells : This is the maximum distance between a mother cell and its sister cell. Software will not consider as potential sister cells the cells which center points are further from the cell center point than this distance.

- *Increasing this parameter will be time consuming but if it is too small some divisions will not be detected.*

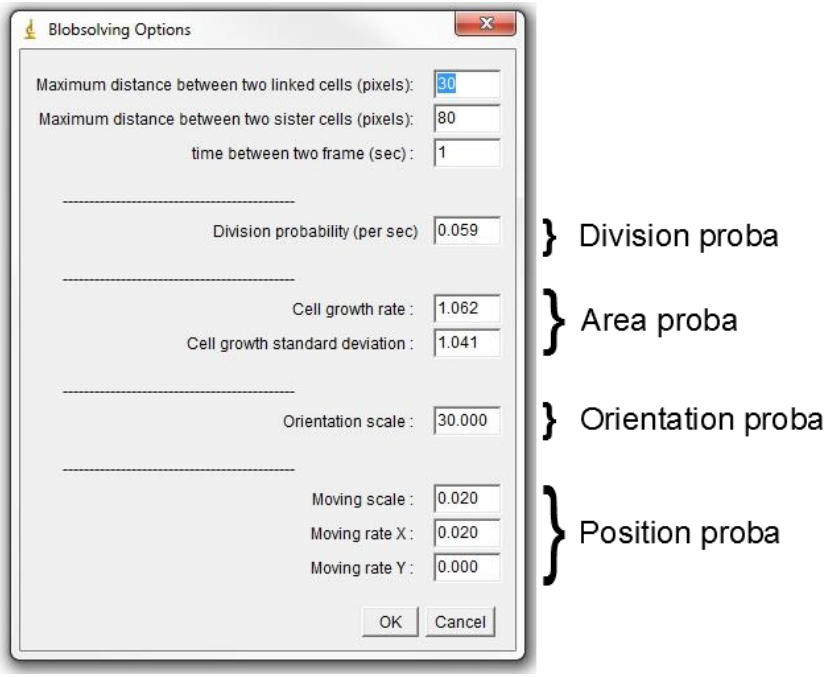

Figure 14: BlobSolving parameters

Time between two frames : Time in second between two frames.

- *This depends on your movie. It is generally 1 second.*

6.3.2 Division parameter

Division probability : This is the probability per second for a cell to divide P_{div}.

- *Increasing this parameter will increase probability of choosing divisions.*

6.3.3 Area parameters

Cell growth rate : The mean cell growth rate per second of the cells in the colony $\mu(\frac{A_A}{A_B})$. Then the logarithm of this value will be used to parameterize P_A function.

- *Transitions and divisions where the cell growth is too far from this parameter will have lower probability to be chosen.*

Cell growth standard deviation : The standard deviation of cell growth rate $\sigma(\frac{A_A}{A_B})$. Then the logarithm of this value will be used to parameterize P_A function.

- *Gives an idea of the possible variation of growth rate between cells.*

$$P_A(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{x-\mu}{\sigma})^2}$$

6.3.4 Orientation parameter

Orientation scale : Scale parameter λ of the exponential law P_θ.

- *The bigger this parameter will be, the less cells will be expected to change orientation.*

$$P_\theta(x) = \lambda e^{-\lambda x} \quad (x \geq 0)$$

6.3.5 Position parameters

Moving scale : Scale parameter λ of the laplace law P_x.

- *The bigger this parameter will be, the less cells will be expected to move differently from the mean move values (see below μ_r and μ_t).*

Moving rate X : Radial mean cell speed μ_r.

Moving rate Y : Tangential mean cell speed μ_t.

$$P_x^{radial}(x) = \frac{1}{2\lambda} * e^{\frac{-|x-\mu_r|}{\lambda}}$$

$$P_x^{tangential}(x) = \frac{1}{2\lambda} * e^{\frac{-|x-\mu_t|}{\lambda}}$$

$$P_x(x) = P_x^{radial}(x) * P_x^{tangential}(x)$$

7 Result Window

Click on the Results button at the bottom of the window. This will open a table with the main results of the lineage analysis, with following columns:

Lineage ID : A unique number that identifies what cell is at the origin of the current lineage in the first image of the movie. If the movie begins with 2 cells, for instance, all the cells deriving from the first cell will have Lineage ID = 0 and all the cells deriving from the second one will have Lineage ID=1

Cell ID : A unique identifier of each cell in each image.

Divisions : Lineage of the current cell. At each division, one daughter is randomly chosen to be the H (for Head) cell and the other is the T (for Tail) one. This H or T is appended to the mother cell lineage to yield the current cell lineage. For instance, cell 'HT' in lineage # 2 is the T cell of the H cell of cell 2 in the initial image. Upon division, cell HT will generate cells HTH and HTT in lineage # 2.

Length Length of the cell (in pixels)

Width Width of the cell (in pixels)

Frame Image number of the image in which the current cell is located

X x-coordinate of the center of mass of the current cell in the current image (in pixels)

Y y-coordinate of the center of mass of the current cell in the current image (in pixels)

Orientation Cell orientation (in radians).

Mother ID Cell ID of the mother cell upon division (=-1 if no mother) or of the current cell in the previous image.

Daughter 1 ID Cell ID of the first daughter upon division or of the current cell in the next image (-1 if no current cell nor division in the next image).

Daughter 2 ID Cell ID of the second daughter upon cell (-1 if no division in the next image).

The Result Table can be saved as an Excel spreadsheet with File/Save of the ImageJ menu.

References

[Coq12] Anne-Sophie Coquel. *Dynamique de l'agrégation protéique chez la bactérie Escherichia Coli.* PhD thesis, INSA-Lyon, 2012.

[LM11] C. Louchet and L. Moisan. Total variation as a local filter. *SIAM Journal on Imaging Sciences*, 4(2):651–694, 2011.

[Pri12] Maël Primet. *Méthodes probabilistes pour le suivi des points et l'analyse d'images biologiques.* PhD thesis, Université Paris Descartes, 2012.

Appendices

A Parameters

A.1 Software parameters

Processors Number number of threads the software can launch at the same time. It is recommended to use your maximum number of processors but you can also choose to diminish this number to be able to do something else on your computer while processing images.

A.2 Movie parameters

Images Zoom images zoom (default value is 100x = 0.064 μm per pixel)

Cells Parameters cells maximum width and minimum area. These parameters are used to segment cells.

A.3 Preprocessings

A.3.1 Denoising

Rough Background

- *Rough background threshold* : grey lever threshold to roughly detect where are the cells.
- *Rough background radius* : Radius used for erosion and opening.

Denoising

- *Denoising level* :
- *Reduction coefficient* :
- *Minimal number of patches* :

- *Patch side length in pixels* :
- *Maximum patches distance in pixels* :
- *Precision of denoising* :
- *Aggregation mode* :

A.3.2 Renormalizing

Final Background

- *Background threshold* : grey level threshold.
- *Opening iteration number :* Number of times mask is opened.

Renormalization

- *Pseudo seeds threshold* :
- *Radius of sampling* :
- *Convolution square size* :
- *Convolution iteration number* :

A.4 Oversegmentation

Seeds

- *Seeds threshold* : grey level threshold. Under this threshold, pixels are in a seed.
- *Seeds computation method* :

Blobs

- *First speed parameter* : parameter used to compute viscosity (or dilatation speed) in function of the pixel grey level.
- *Second speed parameter* : parameter used to compute viscosity (or dilatation speed) in function of the pixel grey level.
- *Maximum iteration number* : Maximum dilatation iteration number. This stops dilatation after a while.
- *Minimum significant border between blobs* : This is used to compute which blobs are connected.

Final blobs

- *Minimum blob area* : Determines which blobs are too small and must be merged or deleted.
- *Second dilatation iteration number* : After cleaning blobs are dilated a second time.