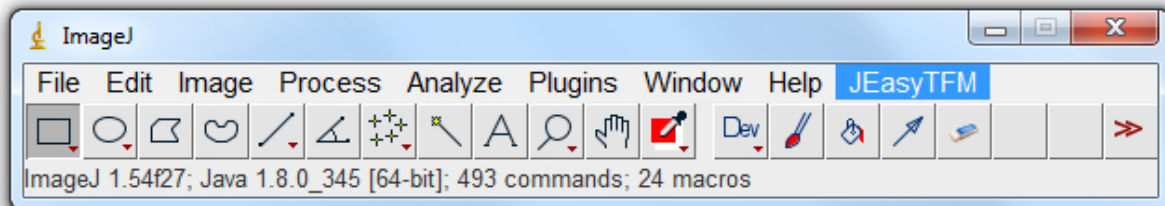# JEasyTFM - User manual

I - <u>Installation</u>

To use the fully functional ImageJ bundle, just download the platform-dependent version of ImageJ bundled with Java file and containing all the tools needed for the package embedded:

- [ImageJ bundled with Java 8 and JEasyTFM under 64-bit Windows](#)        ( 80.6 Mb)
- [ImageJ bundled with Java 8 and JEasyTFM under 64-bit Linux](#)        ( 79.6 Mb)
- [ImageJ bundled with Java 8 and JEasyTFM under 64-bit Mac](#)        (120   Mb)
- [ImageJ bundled with Zulu OpenJDK 13.0.6. and JEasyTFM under 64-bit Mac](#)( 88   Mb)

By unzipping and launching ImageJ, there will a "JEasyTFM" command at the right of the ImageJ menu for using it:



Or

Manual installation: Proceed with the manual installation by downloading the [ImageJ](#) software and unziping it.

Check wether the given ImageJ version is **1.54f11 or higher** and if not replace the **ij.jar** file with its [daily-build](#) version.

Download [JEasy_TFM.jar](#) to the plugins/jars folder, restart ImageJ, and there will a JEasyTFM new command at the right of the ImageJ menu.

But in order to be fully fonctional, the JEasyTFM package uses several third party open source plugins which origin will be indicated next to the needed tool.

Nevertheless, most of the linked plugins have been modified (with at least the the addition of some "direct static methods") and very often improved with the addition of new functionalities and features.

All the modified source codes are available inside the jar files for further editing and improvements.

Thus the following additional tools need to be downloaded from the links below (and not the original sites) to the plugins/jars folder:

- the [MosaicSuite-1.0.25_Full_extended](#)      package,
- the [Extended Depth of Field](#)      plugin,
- the [Template Matching and Slice Alignment](#)      plugin,
- the [Find Focused Slices](#)      plugin,
- the [PIV (Particle Image Velocimetry)](#)      plugin,
- the [Traction Force Microscopy](#)      plugin,
- the [Apache Commons Mathematics](#)      library file,

some of the previous plugins require the javacv library files: [javacv.jar](#), [javacpp.jar](#), [opencv.jar](#).
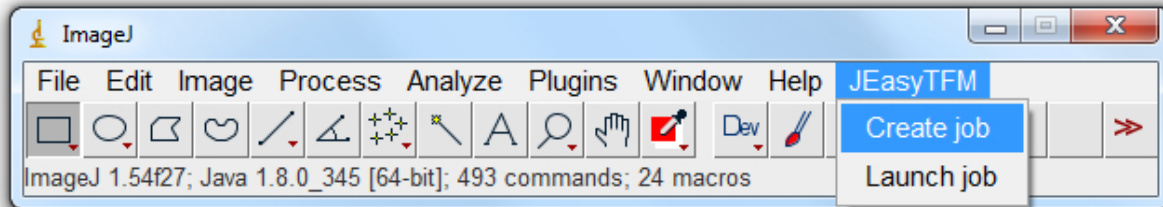
Alternatively the download of all the previously described files can simply be replaced by downloading the [JEasyTFM_files](#) file and unzip it in the plugins/jars folder.

Finally one of the platform-dependent library files need to be downloaded into the plugins/jars folder:

- [32-bit Windows](#)
- [64-bit Windows](#)
- [64-bit Mac](#)
- [32-bit Linux](#)
- [64-bit Linux](#)

II - <u>Configuration of the analysis</u>

The JEasyTFM tool is divided into two submenus:



- JEasyTFM>Create_job - for setting up the analysis configuration job, the data of which will then be saved into a batch file (JEasy.txt) within the ImageJ plugins folder.
- JEasyTFM>Launch_job - will be reading the generated batch file (JEasy.txt) and launch the analysis job it defines.

Splitting the JEasyTFM tool into two steps, with on one side a job creation and on the other side, a job launching operation, provides two main advantages. The first one is to offer traceability of all the analysis settings used to generate the different analysis results. In the case of heavily booked dedicated analysis computers, the second one is to rapidly define and protect the analysis settings, which can then quickly be launched later on once the machine is available.

When launching the JEasyTFM->Create_job tab, the following window will be displayed:



We will now explain each point in details.

<u>Data folder and file names definitions</u>



**Debug**: When the **Debug** checkbox is selected, the analysis is performed by launching the different used plugins going through their GUI, often resulting in images being displayed at each stage for the user to visualize the results of the different processes going on. On the contrary, when the **Debug** checkbox is unselected, all the analyses are performed launching the additional plugins by using some back-doors (i.e. static methods) that have been created to short-cut the plugins GUI resulting in all the images produced in memory and not displayed for a gain of machine time.

**Data_folder**: On clicking on the **Data_folder Browse** Button, a "choose directory" dialog is displayed for the user to choose the directory in which the data to be analyzed are saved. Upon selection validation of the chosen folder, its path will be updated within the field **Data_folder** together with the prefixes file name of the acquired images to be analyzed in the **File** field and the one for the images of the beads at equilibrium (i.e. after the addition of trypsin also called reference images in this document) in the **Reference_images** field.

**Sequence_images**: On clicking on the **File Browse** Button, a "file open" dialog is displayed for the user to choose an image file to be analyzed. Upon selection validation of an image file, the file name prefix of the acquired images to be analyzed will be updated within the **Sequence_images** field.

**Reference_images**: On clicking on the **Reference_images Browse** Button, a "file open" dialog is displayed for the user to choose a reference images file to be analyzed. Upon selection validation of the image file, the file name prefix of the acquired images to be analyzed will be updated within the **Reference_images** field. The reference images selected in **Reference_images** have to be previously saved within a folder whose name is the one defined within **Data_folder** field with the addition of "_-_Reference".

Note that it is also possible to populate the **Data_folder**, **Sequence_images** and/or **Reference_images** fields with a simple Drag&Drop of an image file in the fields.


<u>Sizes definitions</u>



**Number_of_acquired_cells**: Slider defining the number of cells that have been recorded during the acquisitions.

Note that the value specified by the **Number_of_acquired_cells** slider represents the maximum value for the sliders:

- **Cells_starting_folder**

- **Cells_ending_folder**
- **Beads_reference_starting_folder**
- **Beads_reference_ending_folder**
- **Beads_sequence_starting_folder**
- **Beads_sequence_ending_folder**
- **Traction_starting_folder**
- **Traction_ending_folder**
- **Cells_analysis_starting_folder**
- **Cells_analysis_ending_folder**
- **Traction_high_starting_folder**
- **Traction_high_ending_folder**
- **FA_segmentation_starting_folder**
- **FA_segmentation_ending_folder**
- **Traction_force_applied_on_FA_starting_folder**
- **Traction_force_applied_on_FA_ending_folder**

Note that all the sliders values within JEasyTFM>Create_job can either be modified directly by a mouse drag, by using the arrow or Page-Up/Page-Down keys or by typing in the value within the numeric field, but also with a mouse wheel on top of the slider or within the numeric field.

**Slices**: Slider defining the number of z-slices that have been acquired during the acquisitions.

Note that the setting of this field is ignored in the case the source images are saved in the form of hyperstacks in which case the image z-slices properties are taken into account.

**Frames**: Slider defining the number of time-frames that have been acquired during the acquisitions.

Note that the setting of this field is ignored in the case the source images are saved in the form of hyperstacks in which case the image time-frames properties are taken into account.

**Frames_begin**: Slider whose maximum value will be defined by the **Frames** slider and defining the first time-frames number of the data that will be analyzed. This option can be used in the case an analysis process has been canceled before completion to not have to restart the analysis from the first frame.

<u>Experiment definition</u>



**Beads1**: The selection of the **Beads1** and/or **Beads2** checkboxes implies that an experiment of high-resolution TFM has been performed, i.e. with the use of beads with two different colors. Under the high-resolution TFM configuration, the selection of the **Beads1** checkbox will enable the analysis of the data with the beads of color 1.

**Beads2**: Under the high-resolution TFM configuration, the selection of the **Beads2** checkbox will enable the analysis of the data with the beads of color 2.

Note that in the case of the analysis of "regular" TFM experiments, i.e. with the use of a single color, it is easier to proceed with the analysis without selecting any of the **Beads1** or **Beads2** checkboxes.

**Ch1**: The selection of the **Ch1** and/or **Ch2** and/or **Ch3** checkboxes implies that more than one channel has been acquired for imaging the cells. Under these acquisitions conditions, the selection of the **Ch1** checkbox will enable the analysis of the data of the first channel (for example a Bright Field channel).

**Ch2**: The selection of the **Ch2** checkbox will enable the analysis of the data of the second channel (for example an Epifluorescence channel).

**Ch3**: The selection of the **Ch3** checkbox will enable the analysis of the data of the third channel (for example a channel corresponding to a Hoechst staining).

Note that in the case of the analysis of an experiment where only one channel has been acquired for imaging the cells, it is easier to proceed with the analysis without selecting any of the **Ch1, Ch2** or **Ch3** checkboxes. Note also that **Ch1, Ch2** and **Ch3** may correspond to any cell channel being recorded.

**Number of Ch**: Popup menu defining the number of channels that have been acquired. When selecting 2 channels within the **Number of Ch** popup menu, the **Ch3** checkbox will be hidden. And when selecting only 1 channel within the **Number of Ch** popup menu, the **Ch2** and **Ch3** checkboxes will be hidden.

**Kurto1**: The **Kurto1** checkbox will apply a best Kurtosis selection algorithm to the images of the **Ch1** in the case it is selected and the "Extended Depth Field"[1] plugin in the case it is unselected.

**Kurto2**: The **Kurto2** checkbox will apply a best Kurtosis selection algorithm to the images of the **Ch2** in the case it is selected and the "Extended Depth Field"[1] plugin in the case it is unselected.

**Kurto3**: The **Kurto3** checkbox will apply a best Kurtosis selection algorithm to the images of the **Ch3** in the case it is selected and the "Extended Depth Field"[1] plugin in the case it is unselected.

Note that the Kurtosis algorithm should rather be applied on images of a Bright Field channel as the "Extended Depth Field" on images of a fluorescent channel.

**Youngs_modulus_Pa**: Numerical field defining the Young's modulus in Pascal units of the support used for cell plating.

**Precision_for_the_Regularization_factor_calculation**: Popup menu defining the precision for the calculation of the regularization or Lagrange parameter λ value that is used to convert the measured beads displacements to the forces applied from the cells onto their support. The regularization or Lagrange parameter λ value determination is performed by using a binary search algorithm.

Cells images extraction



The **Cells_images** analysis will create a folder for each cell number defined within **Cells_starting_folder** and **Cells_ending_folder** within which all following analysis data will be saved.
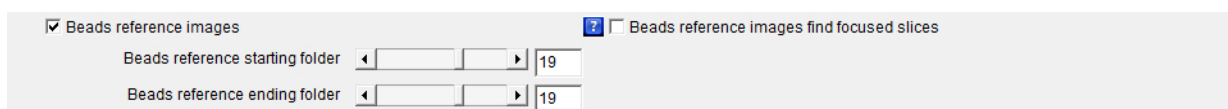
**Cells_images**: The checkbox defines whether cell images should be extracted from all the **Slices** for all the defined **Frames** and for the cells numbers defined between the **Cells_starting_folder** and **Cells_ending_folder**. If the checkboxes **Ch1** and/or **Ch2** and/or **Ch3** is/are activated, this extraction will then be applied to all the selected channels.

**Cells_starting_folder**: Defines the first cell number or folder for which cell images should be extracted from all the **Slices** and for all the defined **Frames**. If the checkboxes **Ch1** and/or **Ch2** and/or **Ch3** is/are activated, this extraction will then be applied to all the selected channels.

**Cells_ending_folder**: Defines the last cell number or folder for which cell images should be extracted from all the **Slices** and for all the defined **Frames**. If the checkboxes **Ch1** and/or **Ch2** and/or **Ch3** is/are activated, this extraction will then be applied to all the selected channels.

**Image_chosen_from_slices**: The automatic selection of the best focused image may be performed by putting the value 0 in this slider. In this case, a kurtosis algorithm will be applied on the **Slices** images in order to extract the one giving the highest value and this for all the defined **Frames**. On the other hand, manual inspection of the z-slices can be performed and the slice number of the selected best focused image can be defined in the Image_chosen_from_slices slider by choosing which cell image number between 1 and the number indicated in **Slices** should be extracted from all the **Slices** for all the defined **Frames**.

Beads reference images best focus extraction



**Beads_reference_images**: The checkbox defines whether images corresponding to the best focused image of the beads extracted within the z-series acquired with the support at equilibrium (i.e. after the cells have been detached from the surface) should be extracted from all the **Slices**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_reference_starting_folder**: Defines the first cell number or folder for which images corresponding to the best focus of the beads within the images acquired with the support at equilibrium (i.e. after the cells have been detached from the surface) should be extracted from all the **Slices**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_reference_ending_folder**: Defines the last cell number or folder for which images corresponding to the best focus of the beads within the images acquired with the support at equilibrium (i.e. after the cells have been detached from the surface) should be extracted from all the **Slices**.
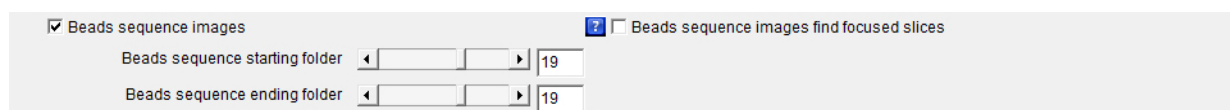
If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_reference_images_find_focused_slices**: The checkbox defines whether the "Find focused slices"[2] plugin should be applied prior to applying the "Extended Depth Field"[1] plugins on the images of the beads with the support at equilibrium from all the **Slices** in order to extract the best focused image. This option can be activated in order to reduce the number of images used as input for the "Extended Depth Field" plugin in the case its output is too noisy for the rest of the analysis to be completed.

Beads sequence images best focus extraction



**Beads_sequence_images**: The checkbox defines whether images corresponding to the best focus of the beads extracted within the images through the acquired time-lapse should be extracted from all the **Slices** for all the defined **Frames**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_sequence_starting_folder**: Defines the first cell number or folder for which images corresponding to the best focus of the beads extracted within the images through the acquired time-lapse should be extracted from all the **Slices** for all the defined **Frames**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_sequence_ending_folder**: Defines the last cell number or folder for which images corresponding to the best focus of the beads extracted within the images through the acquired time-lapse should be extracted from all the **Slices** for all the defined **Frames**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will then be applied to all the selected beads colors.

**Beads_sequence_images_find_focused_slices**: The checkbox defines whether the "Find focused slices"[2] plugin should be applied prior to applying the "Extended Depth Field"[1] plugin on the images of the beads through the acquired time-lapse, defining the **Frames** from all the **Slices** for all the

defined **Frames** to extract the best focused image. This option can be activated in order to reduce the number of images used as input for the "Extended Depth Field" plugin in the case its output is too noisy for the rest of the analysis to be completed.


## Traction force analysis



**Traction_force**: The checkbox defines whether the whole or part of the force calculation processes should be launched. This is the main checkbox for the analysis, which means that if it is not selected, none of the following analysis processes:

- **Alignment_make**
- **Alignment_crop**
- **PIV_calculation**
- **Lambda_calculation**
- **Force_calculation**
- **Force_superposition**

will be launched whether or not they are selected.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the launched analysis will then be applied to all the selected beads colors.

**Traction_starting_folder**: Defines the first cell number or folder for which the whole or part of the force calculation processes should be calculated.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this calculation will then be applied to all the selected beads colors.

**Traction_ending_folder**: Defines the last cell number or folder for which the whole or part of the force calculation processes should be calculated.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this calculation will then be applied to all the selected beads colors.

**Traction_force_alignment**: The checkbox defines whether the whole or part of the images alignment processes should be calculated. This means that if the checkbox **Traction_force_alignment** is not selected none of the following images alignment processes:

- **Alignment_make**
- **Alignment_crop**

will be launched whether or not they are selected.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the launched analysis will then be applied to all the selected beads colors.

**Alignment_make**: The checkbox defines whether an alignment algorithm should be launched on the images. This algorithm consists of applying the "Template Matching and Slice Alignment"[3] plugin on all the images that have been previously generated with the **Beads_sequence_images** feature (that correspond to the best focused images of the beads extracted from all the **Slices** for all the defined **Frames**) taking as reference the image that has been previously generated with the **Beads_reference_images** feature (that correspond to the best focused images of the beads extracted with the support at equilibrium, i.e. after the cells have been detached from the surface) and this for all the defined **Frames**.

Once the alignment is performed on the images of the beads, the obtained displacement is then applied on all the selected channel images **Ch1** and/or **Ch2** and/or **Ch3**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

Note that the **Alignment_make** feature will only be launched if the **Alignment_make, Traction_force_alignment** and **Traction_force** checkboxes are all selected.

**Alignment_crop**: The checkbox defines whether the algorithm that will get rid of the image borders generated by the **Alignment_make** algorithm should be launched on the images. The algorithm automatically detects the maximum displacement during image alignment through all the defined **Frames** and applies cropping on the defined region.

Once the cropping is performed on the images of the beads, the obtained border selection and cropping is then applied on all the selected channel images **Ch1** and/or **Ch2** and/or **Ch3**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this cropping will then be applied to all the selected beads colors.

Note that the **Alignment_crop** feature will only be launched if the **Alignment_crop, Traction_force_alignment** and **Traction_force** checkboxes are all selected.

**Traction_force_calculation**: The checkbox defines whether the whole or part of the images forces calculation processes should be launched. This means that if the checkbox **Traction_force_calculation** is not selected none of the following force calculation processes:

- **PIV_calculation**
- **Lambda_calculation**
- **Force_calculation**
- **Force_superposition**

will be launched whether or not they are selected.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the launched analysis will then be applied to all the selected beads colors.

**PIV_calculation**: The checkbox defines whether a PIV (Particle Image Velocimetry) algorithm should be launched on the images of the aligned and cropped beads in order to generate beads displacement maps.

This algorithm consists of applying the "PIV (Particle Image Velocimetry)"[4] plugin on all the images of the beads that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms taking as reference the image of the beads with the support at equilibrium and this for all the defined **Frames**.

The displacement vectors spacing of the generated displacement map images is of 16 pixels, which in the case of a camera pixel size of 6.5 µm and a used objective magnification of 60× corresponds to 1.733 µm.

If you are using a camera system for which the pixel size isn't equal to 6.5 µm, or the objective magnification equal to 60×, then these values have to be modified manually within the job file "JEasyTFM.txt" (saved in the "ImageJ/plugins" folder) and more precisely the 2 first lines of the file designed by camera_pixel_size and objective_magnification.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

Note that the **PIV_calculation** feature will only be launched if the **PIV_calculation**, **Traction_force_calculation** and **Traction_force** checkboxes are all selected.

**Lambda_calculation**: The checkbox defines whether the regularization or Lagrange parameter λ values should be calculated. This algorithm consists of using a binary search algorithm to calculate the regularization or Lagrange parameter λ values for the different frames. The regularization or Lagrange parameter λ values will be used within the **Force_calculation** analysis step to convert the beads displacement maps previously generated with the **PIV_calculation** feature into force maps by using an a Fourier Transform Traction Cytometry (FTTC) algorithm.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

Note that the **Lambda_calculation** feature will only be launched if the **Lambda_calculation, Traction_force_calculation** and **Traction_force** checkboxes are all selected.

**Force_calculation**: The checkbox defines whether a Fourier Transform Traction Cytometry (FTTC) algorithm should be launched on the beads displacement maps previously generated with the **PIV_calculation** feature in order to create force maps. This algorithm consists of applying the "Traction Force Microscopy"[5] plugins on all the PIV data that have been previously generated with the **PIV_calculation** algorithm and this for all the defined **Frames**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.
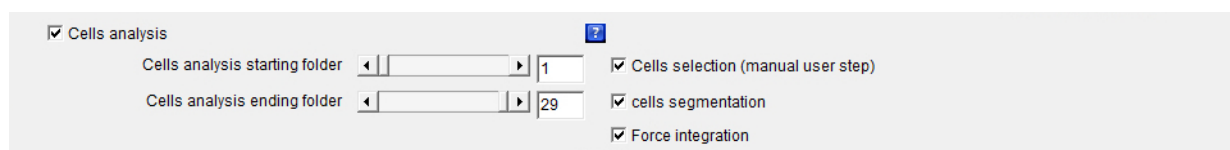
Note that the **Force_calculation** feature will only be launched if the **Force_calculation, Traction_force_calculation** and **Traction_force** checkboxes are all selected.

**Force_superposition**: The checkbox defines whether the force maps previously generated with the **Force_calculation** feature should be superimposed with the images of the cells that have been previously generated with the **Cells_images** feature. If the checkboxes **Ch1** and/or **Ch2** and/or **Ch3** is/are activated, this superimposition will then be applied to all the selected channels.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this extraction will also be applied to all the selected beads colors.

Note that the **Force_superposition** feature will only be launched if the **Force_superposition, Traction_force_calculation** and **Traction_force** checkboxes are all selected.

Cells analysis



**Cells_analysis: T**he checkbox defines whether the whole or part of the cells segmentation and force integration processes should be launched. This is the main checkbox for the analysis, which means that if it is not selected none of the following analysis processes:

- **Cells_segmentation**
- **Cells_selection (manual user step)**
- **Force_integration**

will be launched whether or not they are selected.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the launched analysis will then be applied to all the selected beads colors.

**Cells_analysis_starting_folder**: Defines the first cell number or folder for which the whole or part of the cells segmentation and force integration processes should be launched.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**Cells_analysis_ending_folder**: Defines the last cell number or folder for which the whole or part of the cells segmentation and force integration processes should be launched.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**Cells_segmentation**: The checkbox defines whether a cells segmentation algorithm should be launched on the images of the cells that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms.

The cells segmentation algorithm is split in two, given that it implies some user action. This part of the algorithm consist of applying different image filters on all the images of the cells that have been

previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms and this for all the defined **Frames**.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the filter application algorithm will then be applied to all the selected beads colors.

Note that the **Cells_segmentation** feature will only be launched if the **Cells_analysis** checkbox is selected as well.

**Cells_selection (manual user step)**: The checkbox defines whether a cells segmentation algorithm with some manual user steps should be launched on the images of the cells that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms and pre-filtered with the **Cells_segmentation** algorithm. This part of the algorithm consist of manually defining some cells border as well as threshold settings which will then be applied for all the **Frames** images on all the images of the cells that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms and filtered with the **Cells_segmentation** algorithm.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the filter application algorithm will then be applied to all the selected beads colors.

Note that the **Cells_selection (manual user step)** feature will only be launched if the **Cells_analysis** checkbox is selected as well.

**Force_integration**: The checkbox defines whether a whole cells force integration algorithm should be launched on the traction force images using the cells segmentation ROI (Region Of Interest) data that have been obtained either automatically through a **cells_selection (manual user step)** algorithm or manually and the result together with the ROI drawing superimposed with the images of the cells that have been previously generated with the **Cells_images** feature. If the checkboxes **Ch1** and/or **Ch2** and/or **Ch3** is/are activated, this superimposition will then be applied to all the selected channels.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the filter application algorithm will then be applied to all the selected beads colors.
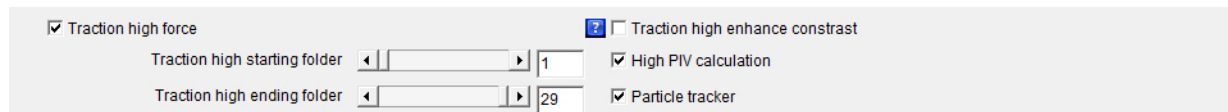
Note that the **Force_integration** feature will only be launched if the **Cells_analysis** checkbox is selected as well.

Beads positions tracking



**Traction_high_force:** The checkbox defines whether the whole or part of the beads positions tracking processes should be launched. This is the main checkbox for the analysis, which means that if it is not selected none of the following analysis processes:

- **Traction_high_enhance_contrast**
- **High_PIV_calculation**
- **Particle_tracker**

will be launched whether or not they are selected.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, the launched analysis will then be applied to all the selected beads colors.

**Traction_high_starting_folder**: Defines the first cell number or folder for which the whole or part of the beads positions tracking processes should be calculated.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**Traction_high_ending_folder**: Defines the last cell number or folder for which the whole or part of the beads positions tracking processes should be calculated.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**Traction_high_enhance_contrast**: The checkbox defines whether a **Subtract_Background...** followed by an **Enhance_Contrast...** filter should be applied on the images in order to enhance their contrast before applying the **High_PIV_calculation** regression.

Note that the **Traction_high_enhance_contrast** feature will only be launched if the **Traction_high_force** checkbox is selected as well.

**High_PIV_calculation**: The checkbox defines whether a PIV (Particle Image Velocimetry) algorithm should be launched on the images of the aligned and cropped beads in order to generate beads displacement maps.

This algorithm consists of applying the "PIV (Particle Image Velocimetry)"[4] plugin on all the images of the beads that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms taking as reference the image of the beads with the support at equilibrium and this for all the defined **Frames**.

The difference between the **High_PIV_calculation** and the **PIV_calculation** is that the displacement vectors spacing of the generated displacement map images are now pushed up to 8 pixels, and the

calculation starts with the results of the 16 pixels displacement vectors spacing maps which have previously been obtained from the **PIV_calculation** algorithm.

The reason why we are pushing the displacement vectors spacing of the generated displacement maps from 16 to 8 pixels is that this precision is needed in order to get consistent results with the tracking algorithm used in the next step.

For information, a displacement vectors spacing of the generated force maps images of 8 pixels corresponds to 0.866 µm in the case of a camera pixel size of 6.5 µm and a used objective magnification of 60×.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

Note that the **High_PIV_calculation** feature will only be launched if the **Traction_high_force** checkbox is selected as well.

**Particle_tracker**: The checkbox defines whether a particle tracking algorithm should be launched on the beads displacement maps previously generated with the **High_PIV_calculation** feature in order to track the beads displacements between images that have been previously generated with the **Beads_sequence_images**.

The beads tracking algorithm used on the images data is an improvement of the ParticleTracker_2D[6] plugin which have initially been developed by the Mosaic group (http://mosaic.mpi-cbg.de/ParticleTracker/index.html). The new additions consist essentially of giving the possibility to input the beads displacement vecto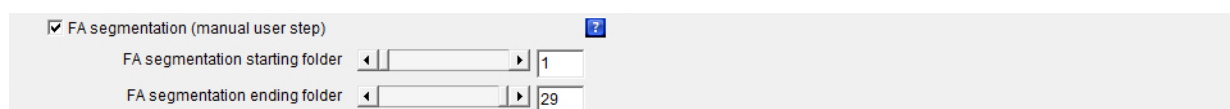r maps that have been previously generated with the **High_PIV_calculation** algorithm as a starting position for the particle linking algorithm as well as giving the possibility to reload and manually correct the trajectories obtained by the algorithm.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this generation will then be applied to all the selected beads colors.

Note that the **Particle_tracker** feature will only be launched if the **Traction_high_force** checkbox is selected as well.

FA segmentation



**FA_segmentation (manual user step)**: The checkbox defines whether a **FA_segmentation (manual user step)** algorithm should be launched on fluorescent images of the cells that have been previously aligned and cropped with the **Alignment_make** and **Alignment_crop** algorithms.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**FA_segmentation_starting_folder**: Defines the first cell number or folder for which the **FA_segmentation (manual user step)** algorithm should be applied.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

**FA_segmentation_ending_folder**: Defines the last cell number or folder for which the **FA_segmentation (manual user step)** algorithm should be applied.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to all the selected beads colors.

Traction force applied on FA



**Traction_force_applied_on_FA**: The checkbox defines whether a **Traction_force_applied_on_FA**[7,8] algorithm should be launched which will calculate the forces applied on the focal adhesion positions that had previously been determined by the **FA_segmentation** algorithm using the beads displacements data obtained by the **Particle_tracker** algorithm.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to the beads displacements and FA positions data merge of all the selected beads colors.

**Traction_force_applied_on_FA_starting_folder**: Defines the first cell number or folder for which the **Traction_force_applied_on_FA** algorithm should be applied.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to the beads displacements and FA positions data merge of all the selected beads colors.

**Traction_force_applied_on_FA_ending_folder**: Defines the last cell number or folder for which the **Traction_force_applied_on_FA** algorithm should be applied.

If the checkboxes **Beads1** and/or **Beads2** is/are activated, this algorithm will then be applied to the beads displacements and FA positions data merge of all the selected beads colors.

1. Forster, B., Van De Ville, D., Berent, J., Sage, D. & Unser, M. Complex Wavelets for Extended Depth-of-Field: A New Method for the Fusion of Multichannel Microscopy Images. *Microsc. Res. Tech.* **65**, 33–42 (2004).

2. Tseng, Q. Find Focused Slices ImageJ Plugin.

   https://sites.google.com/site/qingzongtseng/find-focus.

3. Tseng, Q. Template Matching and Slice Alignment ImageJ Plugin.

   https://sites.google.com/site/qingzongtseng/template-matching-ij-plugin.

4. Tseng, Q. PIV (Particle Image Velocimetry) ImageJ Plugin.

   https://sites.google.com/site/qingzongtseng/piv.

5. Tseng, Q. Traction Force Microscopy ImageJ Plugin.

   https://sites.google.com/site/qingzongtseng/tfm.

6. Sbalzarini, I. F. & Koumoutsakos, P. Feature point tracking and trajectory analysis for
   video imaging in cell biology. *J. Struct. Biol.* **151**, 182–195 (2005).

7. Schwarz, U. S. *et al.* Calculation of forces at focal adhesions from elastic substrate data:
   the effect of localized force and the need for regularization. *Biophys. J.* **83**, 1380–1394
   (2002).

8. Sabass, B., Gardel, M. L., Waterman, C. M. & Schwarz, U. S. High resolution traction
   force microscopy based on experimental and computational advances. *Biophys. J.* **94**, 207–
   220 (2008).