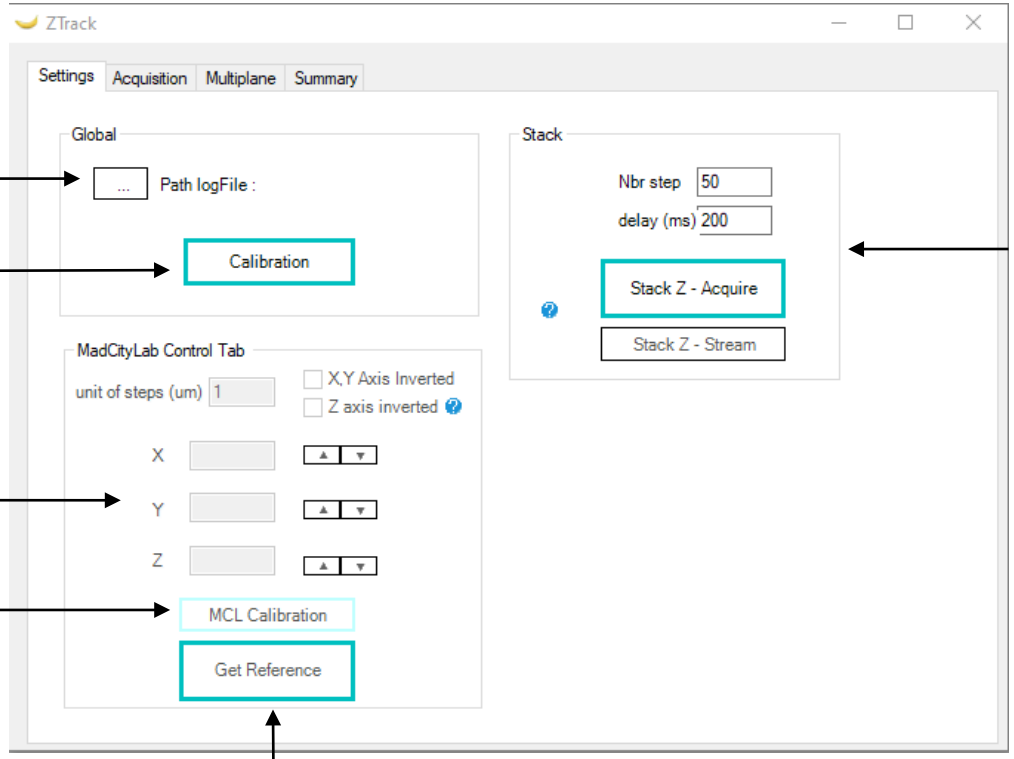
Z-Track V4.0

Manual

: Mandatory to move forward in the process



Head Path of the Arborescence

Calibration

Control Panel of MadCityLab stage

Put the stage at the middle of its capacity of displacement (200um)

Set the Origin coordinates

Create a Z-stack using acquire (image by image or stream)

Define if you want to do a multiplane or single plane

Say if WaveTracer is installed

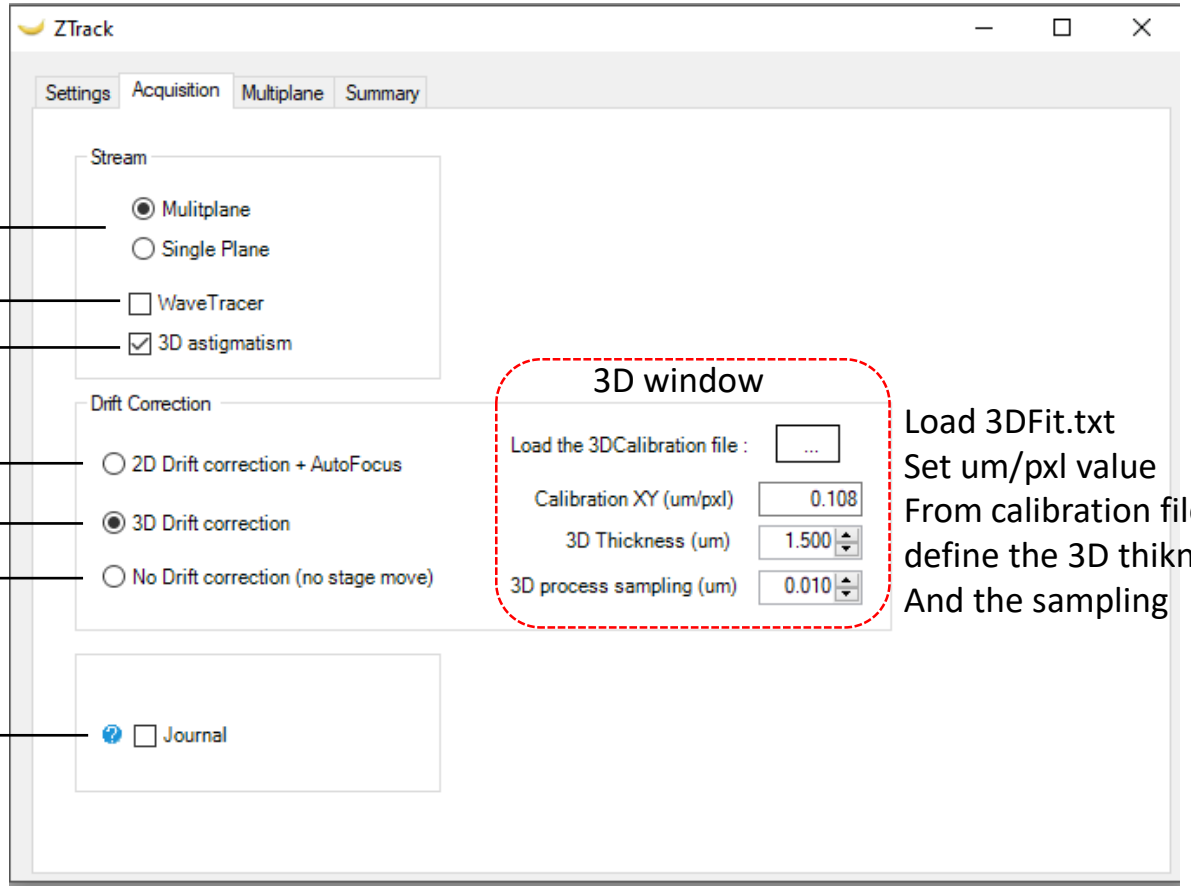
3D or 2D

2D drift correction + PFS

3D drift correction

NO drift correction

Load a journal which will run before each acquisition



Load 3DFit.txt
Set um/pxl value
From calibration file,
define the 3D thikness
And the sampling

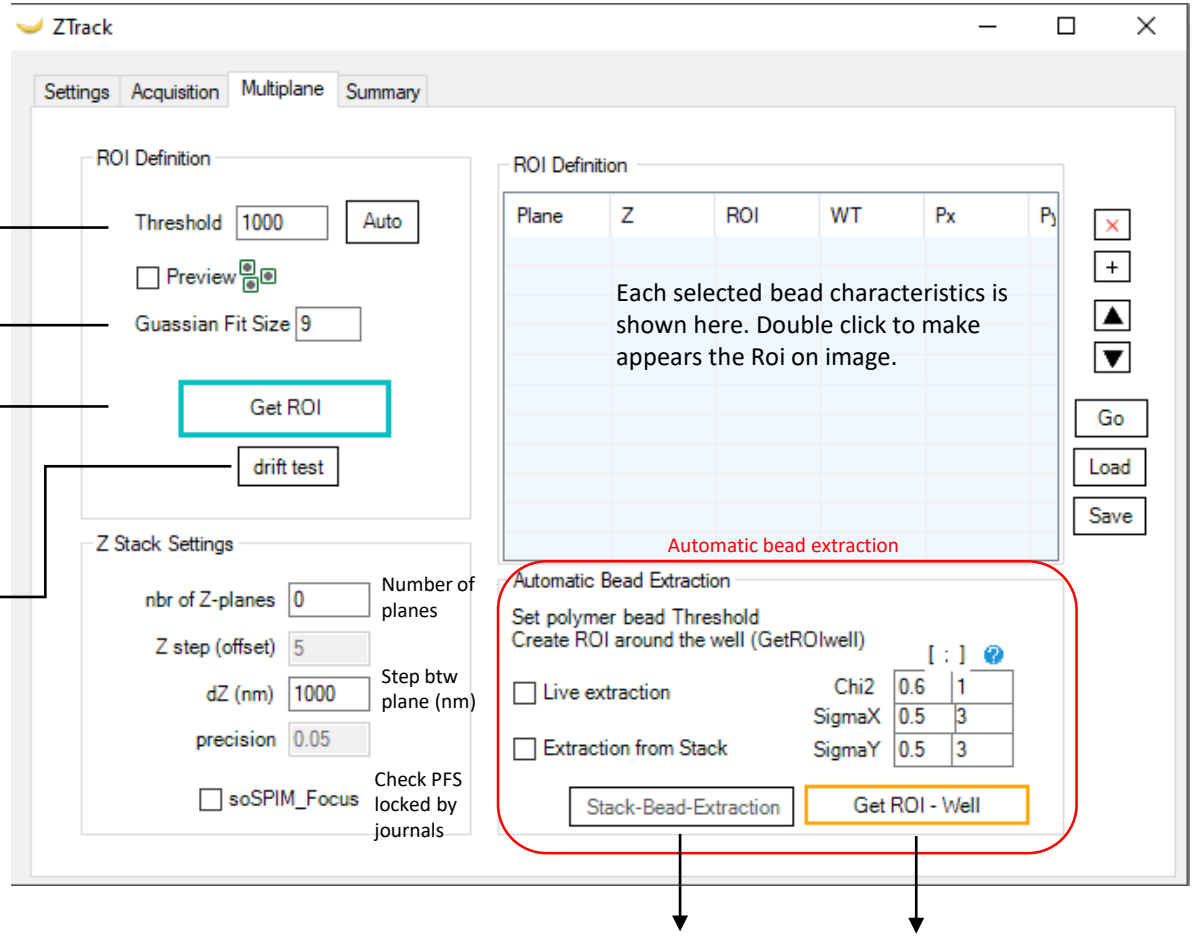
Reference bead = bead used as reference for the automatic drift correction.

Threshold Wavelet

Gaussian fit size

Get the active ROI around the reference bead to track

Replace automatically the stage centered to the reference bead.



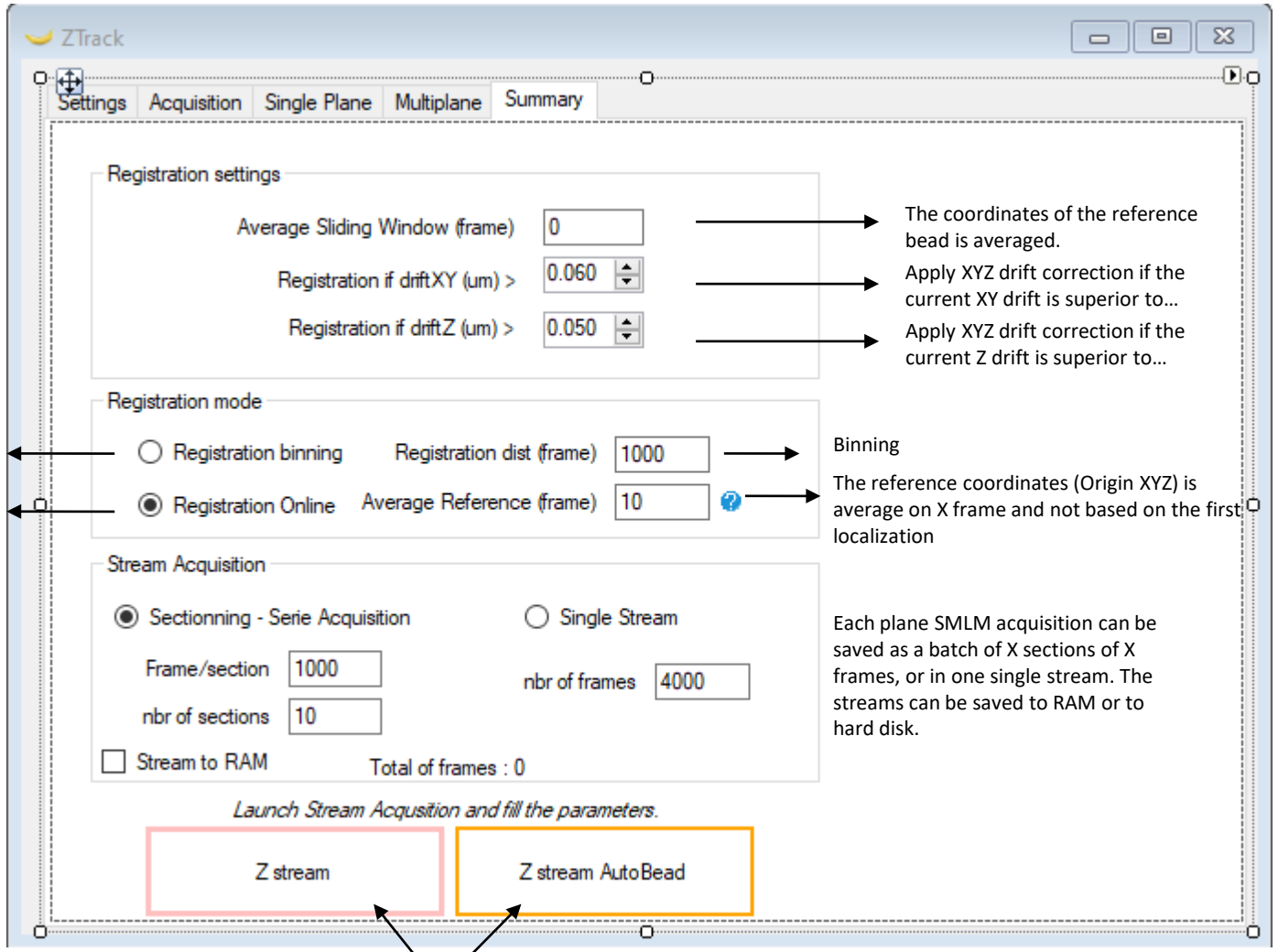
Extract beads from a stack.
Do a Z stack of the cell/well you want acquire. Then from each frame of this stack one bead per plane will be pre-selected as reference for drift correction for each plane.

Select a ROI (active) around the well. Then launch the acquisition for the « live automatic bead extraction » or launch the « bead extraction from stack ». For each plane, one bead will be automatically selected around the well.

Bead extraction :

- **Manual bead extraction**: Select one ROI of the reference bead for each plane you want to acquire manually by using « Get ROI button ». Make sure the bead is catched by the wavelet threshold by using « preview » checkbox.
- **Automatic bead extraction**: First select a ROI around the well and click « GetROI-well ». Then you can automatically extract the reference bead of each planes of your acquisition either **in live** or **from a Z-stack** previoulsy acquired manually. Then for each plane (live or Z-stack), each beads of the FOV (except in the ROI well) will be selected and filtered using the **chi2/sigma filters**. From each plane, the filtered bead with the best score will be set as reference.

- N.B in live mode, for each plane, an image is taken from which a reference bead is selected before the SMLM acquisition. If any beads pass the filters and is selected, then the acquisition is cancelled and move forward the next plane. Using the stack mode, from the panel, the user can easily check if a plane/reference bead is missing and fix it by adapting filters.


ZTrack
Settings
Acquisition
Single Plane
Multiplane
Summary
Registration settings
Average Sliding Window (frame)
0
Registration if driftXY (um) >
0.060
Registration if driftZ (um) >
0.050
Registration mode
Registration binning
Registration dist (frame)
1000
Registration Online
Average Reference (frame)
10
Stream Acquisition
Sectionning - Serie Acquisition
Single Stream
Frame/section
1000
nbr of sections
10
nbr of frames
4000
Stream to RAM
Total of frames : 0
Launch Stream Acqusition and fill the parameters.
Z stream
Z stream AutoBead
The coordinates of the reference bead is averaged.
Apply XYZ drift correction if the current XY drift is superior to...
Apply XYZ drift correction if the current Z drift is superior to...
Binning = Drift correction applied every X frame
Drift correction applied every frame
Binning
The reference coordinates (Origin XYZ) is average on X frame and not based on the first localization
Each plane SMLM acquisition can be saved as a batch of X sections of X frames, or in one single stream. The streams can be saved to RAM or to hard disk.
Launch acquisition