

Z-Track V4.0

Manual



: Mandatory to move forward in the process

Head Path of the Arborescence

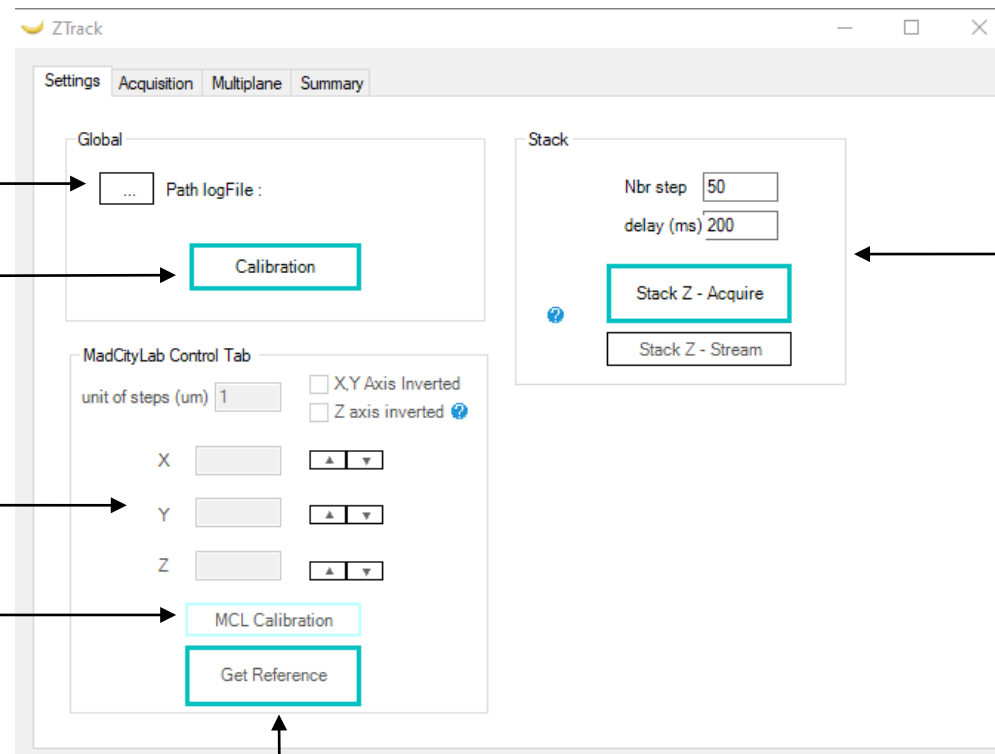


Calibration

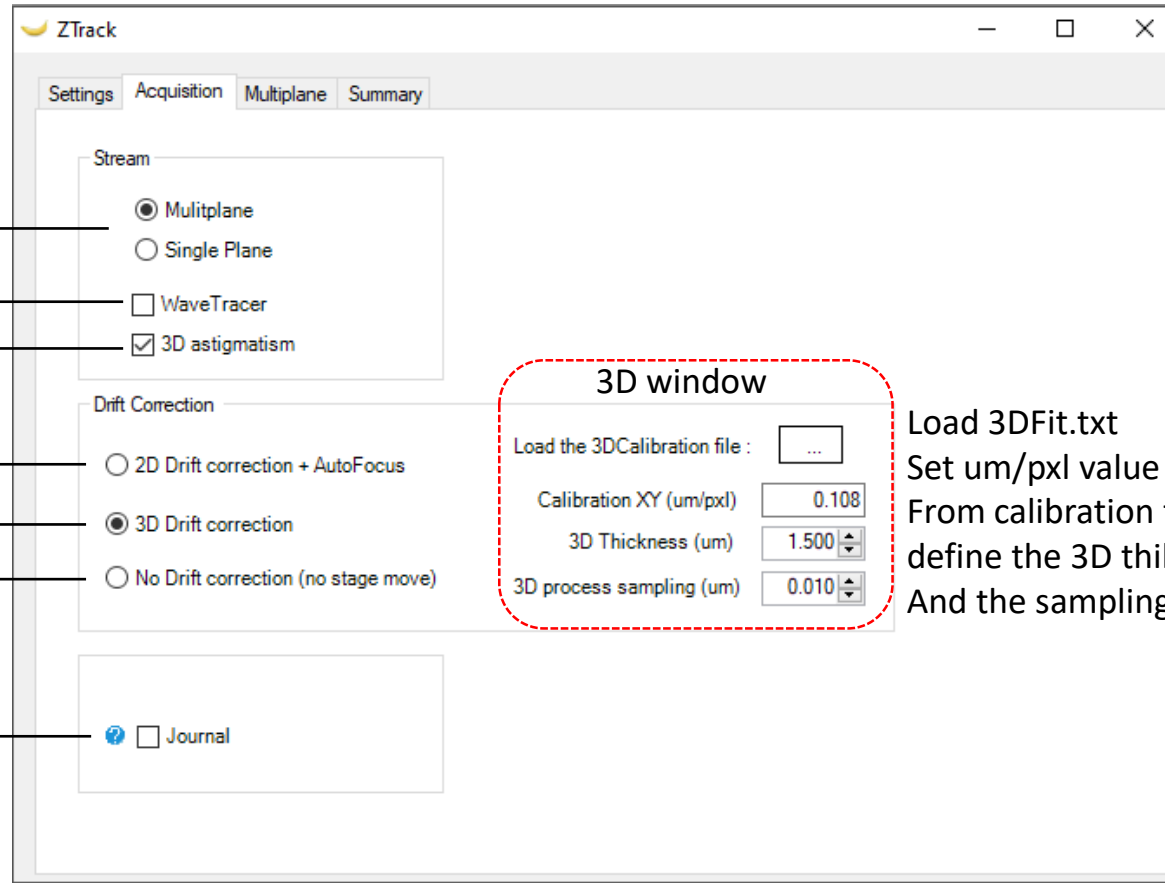
Control Panel of
MadCityLab stage

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

3D window

Load the 3DCalibration file :

...

Calibration XY (um/pxl)

0.108

3D Thickness (um)

1.500

3D process sampling (um)

0.010

Load 3DFit.txt
Set um/pxl value
From calibration file,
define the 3D thickness
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. ←

ROI Definition

Threshold 1000 Auto

☐ Preview

Gaussian Fit Size 9

Get ROI

drift test

Z Stack Settings

nbr of Z-planes 0 Number of planes

Z step (offset) 5

dZ (nm) 1000 Step btw plane (nm)

precision 0.05

☐ soSPIM_Focus Check PFS locked by journals

ROI Definition

Plane	Z	ROI	WT	Px	Py
Each selected bead characteristics is shown here. Double click to make appears the Roi on image.					
Automatic bead extraction					

Automatic Bead Extraction

Set polymer bead Threshold

Create ROI around the well (GetROIwell)

☐ Live extraction

☐ Extraction from Stack

	Chi2	SigmaX	SigmaY
	0.6	0.5	0.5
	1	3	3

Stack-Bead-Extraction

Get ROI - Well

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 caught 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** previously 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.

Binning = Drift correction applied every X frame

Drift correction applied every frame

The screenshot shows the ZTrack software interface with the 'Settings' tab selected. The interface is divided into three main sections: 'Registration settings', 'Registration mode', and 'Stream Acquisition'.
Registration settings: Includes 'Average Sliding Window (frame)' set to 0, 'Registration if driftXY (um) >' set to 0.060, and 'Registration if driftZ (um) >' set to 0.050.
Registration mode: Features two radio buttons: 'Registration binning' (unselected) and 'Registration Online' (selected). Next to 'Registration binning' is a 'Registration dist (frame)' input set to 1000. Next to 'Registration Online' is an 'Average Reference (frame)' input set to 10.
Stream Acquisition: Includes two radio buttons: 'Sectionning - Serie Acquisition' (selected) and 'Single Stream' (unselected). Below these are inputs for 'Frame/section' (1000), 'nbr of sections' (10), and 'nbr of frames' (4000). There is also a checkbox for 'Stream to RAM' and a 'Total of frames : 0' label.
At the bottom, there are two buttons: 'Z stream' (highlighted with a pink box) and 'Z stream AutoBead' (highlighted with an orange box).
Annotations with arrows point to various elements: 'Binning = Drift correction applied every X frame' points to the 'Registration binning' radio button; 'Drift correction applied every frame' points to the 'Registration Online' radio button; three arrows on the right point to the 'Average Sliding Window', 'Registration if driftXY', and 'Registration if driftZ' settings, explaining their functions; an arrow points from the 'Registration dist (frame)' input to the text 'Binning'; another arrow points from the 'Average Reference (frame)' input to the text 'The reference coordinates (Origin XYZ) is average on X frame and not based on the first localization'; and a final arrow points from the 'Z stream' button to the text '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