ManZSelect Manual

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I. Goal

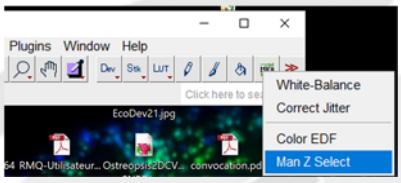
The goal is to extract data from large hyperstack to crop the data following manually the ROI of interested. with a user-friendly manipulation, we can extract either an XYt movie (manual Z selection and manually tracking in XY) or an XYZt hyperstack (manually tracking in XY)

II. How to Use

II-A. Install

1. from MicaJ

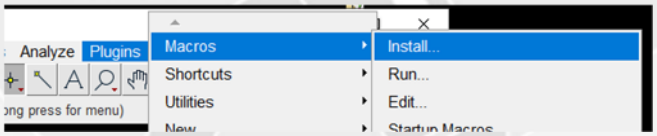
Click on "MICA" > "Man Z Select"



Then the program is installed with all the shortcuts

2. from Standard mode

Click on "Plugins" > "Macros" > Install…" and select the "ManZSelect.ijm" in your computer.



Then the program is installed with all the shortcuts

II-B. Preparation HyperStack

Clicking on [0] "Load & Init". If there is no hyperstack (XYZT) opened, it proposes to open a file (.nd for instance)

If the first time, it open the Parameter Window (can be recalled *[9] "Parameters"*).

II-C. Parameters User-Defined

Clicking on *[9] "Parameters"* you have access to some options:

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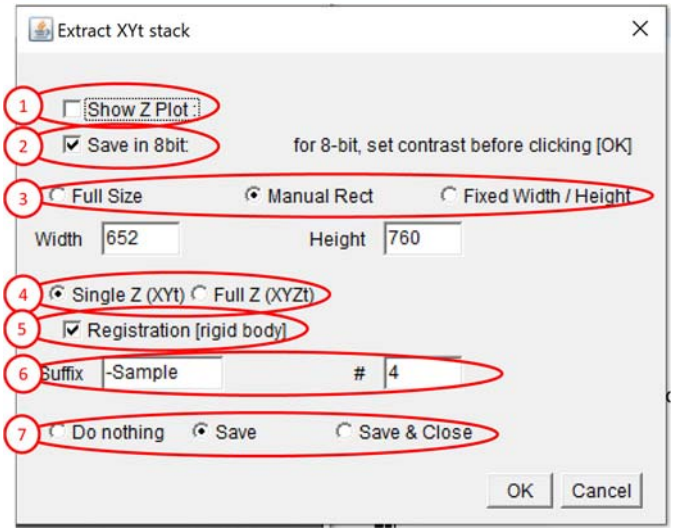
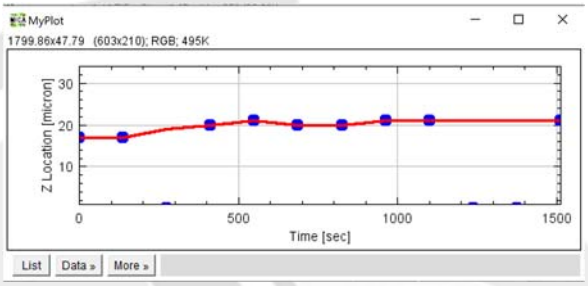


1) **« Show Plot »**
   At each step update a graph with the Z(t), the axial position per time point. Dots are the defined location (see…), the red line is the interpolating curve when missing some Z(t)



2) **"Save 8-bit"**
   Convert the final stack (XYt or XYZt) in 8-bit based on the Gray level limits set before the *[6] "Extract"*
3) **"Region-Of-Interest"**. Set the type of ROI you want:
   a. **"Full Size"** will select all the field of view (then the X(t) and Y(t) have no effect)
   b. **"Manual Rect"** will ask before *[6] "Extract"*to draw a rectangle have the stack width & height (the rectangle location doesn't matter)
   c. **"Fixed Width/Height"** impose manually the size of the rectangle.
4) **"Single Z (XYt)"** will extract per time point the Z(t) plane, whereas ***"Full Z (XYZt)"*** will extract all the Z stack (then Z(t) doesn't matter).
5) **"Registration"** apply StackReg (http://bigwww.epfl.ch/thevenaz/stackreg/) with rigid body.
6) **"Suffix"** will rename the newly substack with Original_Name_Sample-1. This name will be used for saving (si below)
7) **"Saving"**. You can decide of at the end you want do nothing, or automatically save the image and/or close it.

II-D. Semi Auto Plot [5]

- After Initializing the variables (*[0] "Load & Init"*), start *[5] "SemiAutoPlot "*.
- Moving the Z (wheel alone), you can change the Z plan.

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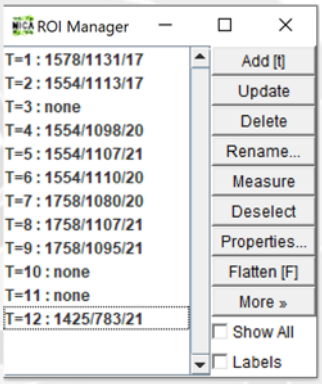
- [Left Click] in the middle of the structure to be tracked
  The macro will store/update the x,y,z location of the current time-point and open the subsequent frame
- You can skip some timepoint (Either with [Alt]+[Wheel] or with the slider), they will be interpolated.
- [Space bar] to stop the process.
- When last time-point is defined, it automatically run the *[6] "Extract"*

II-E. Extract [6]

- Based on the defined 3D location per time-point, the macro interpolates the potential missing coordinates.
- If the user defined the ROI as **"Manual Rect"**, the macro waits until a rectangle ROI is drawn.
- The macro then extract per time-point the XY image either full size image, or a rectangle centered on the x,y coordinates.
- If selected, then the macro perform a StackReg>Rigid body
- If "8-bit" has been selected, the macro will convert according the initial Gray-limits defined in *Brightness&Contrast*
- At the end, if selected, the macro save automatically the results based on the original name wth its suffix as defined in *"Parameters [9]"*.

II-F. Fine update

- *"PreviousT [1]"* resp. *"NextT [2]"* will show you the previsou (resp. next) time-point with the x,y location and the selected Z plan.
  You can also select in the ROI-Manager the time-point you want to reach directly. The ROI name tell the time-point, and x,y,z coordinate. If "none", the time-point will be interpolated



- *"StoreZ [3]"*. Creating or moving the current yellow cross in an updated Z-plan, you can store it pressing [3].
- *"ClearT [4]"*. Remove the current x,y,z coordinate and store it as "non" in the ROI-Manager.

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