Alignment toolset for Fiji

Yan Chastagnier

October 18, 2024

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1 Summary

This toolset is based on TurboReg_ alignment plugin. Its purpose is to improve alignment by preprocessing the images and automatize the alignment of several stack, both intra (align the slices together inside a stack) and inter stacks (align stacks together).

2 Setup

Download TurboReg plugin from http://bigwww.epfl.ch/thevenaz/turboreg/ and put the .jar file into Fiji.app/plugins/. Alternatively, enable the *BIG-EPFL* update site, to get the plugin along with others from the EPFL group directly from Fiji.

Put Alignment_0.2.ijm file into the directory Fiji.app/macros/toolsets/ (on mac os, right click Fiji.app > Show content folder).

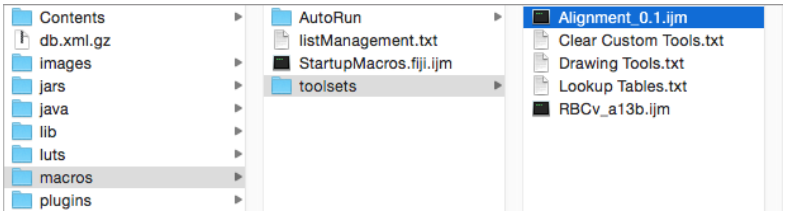

Figure 1 – Location to put .ijm file.

Click on » at the extreme right of the toolbar then select the toolset to display the tools.

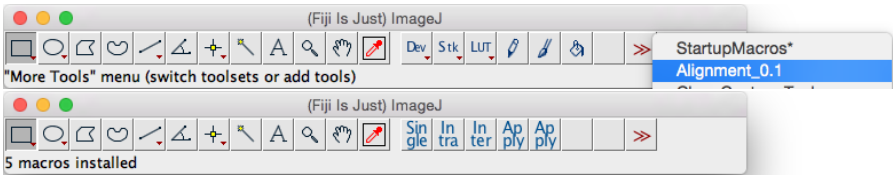

Figure 2 – Display the tools. Top: selection of the toolset. Bottom: the tools are installed.

3 Computation process

To align the slices of a stack, there are two possibilities : either sequentially by aligning each slice in the stack by the previous one or aligning everything on a reference slice.

In both cases, before using turboreg to find the shift, the images are preprocessed in order to improve the efficiency. First, a gaussian blur of sigma 6 px is applied to smoothen the image. Second the find edges function is applied to extract morphological specificities. Third, a median filter of radius 3 is applied to even out the characteristics. Fourth, the image is optionally rescaled. Finally the image is cropped to keep only the center part of the image in order to avoid artifacts due to edges of the field of view.

If sequentially is selected, image n+1 is aligned on image n for each image. This comes handy when there are huge variations either of intensity in the whole field, or of shapes.

If sequentially is not selected, either the user choose which frame to take as reference, or an additional step is to determine the best frame to use as a reference. First a substack containing one frame every *Extract step* is created (or the whole stack is copied if it has less than 20 frames). The median of that substack is computed and all slices are aligned on it. The frame which is the closest to that median is selected as reference frame for the stack. The idea behind this selection is to align everything on an existing slice that is the closest to "what most frames look like". Everything is not aligned on the median because it could contain artifacts if for example there

is a permanent drift, making it not looking like any of the frames. Finally, all frames are aligned on the reference frame.

Once the translations have been computed, the median translations in x and y are subtracted to minimize the part of the image that is lost when shifted.

4 Parameters

Single, intra and inter tool all start with a parameters dialog box. Most parameters are common to all tools.

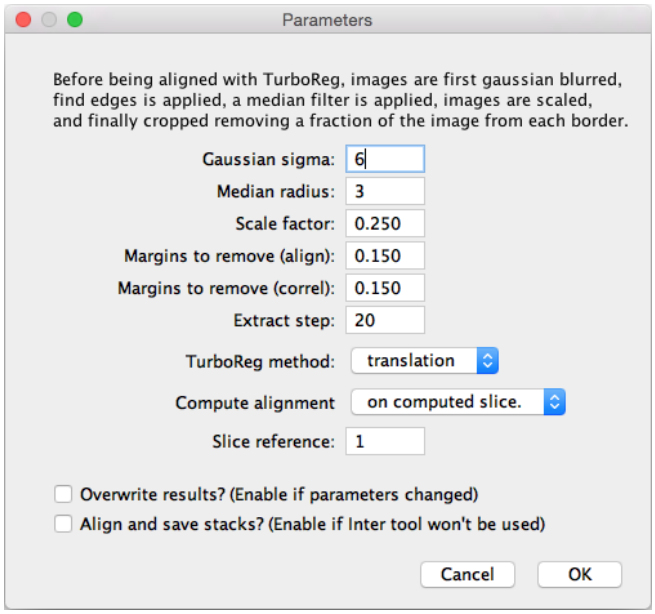

Figure 3 – Parameters.

- *Gaussian sigma*: sigma of the gaussian filter used as a first step to remove high frequency components of the images to align.

- *Median radius*: radius of the median filter used after find edges to remove outlier pixels and smoothen the shapes.

- *Scale factor*: multiplicative factor that will be applied to the size of the image. Alignment performs much faster with reduced size. Use for example the value 0.5 to divide the size of the images by a factor 2. It should be checked that reducing the size of the images doesn't alter the alignment. Or, if alignment is not good, try an higher value for this parameter to see if it does improve.

- *Margins to remove (align)*: portion of the image to remove from each side before running TurboReg. For example, it is necessary for fiber bundle images to remove the edges of the fiber bundle and only align a portion containing only the sample. In this case, 0.15 is a good value if the bundle is inscribed in the image. Another example is to reduce the effect of vignetting (dark circle around the image due to optics).

- *Margins to remove (correl)*: portion of the image to remove when computing correlation. After alignment, some borders of the image may become black because there was no value to assign those pixels. This could greatly alter the correlation value even if the alignment is good.

- *Extract step*: sets the period of the image extraction when not using sequential aligning and computing reference slice.

- *TurboReg method*: translation corresponds to a translation on x and y axis. rigidBody corresponds to translation with an additional rotation. Other TurboReg methods have not been implemented.

- *Compute alignment*: choose on which frames images should be aligned. 1) *sequentially* references each frame to the previous one. 2) *on computed slice* references each frame on a computed one (see method in previous section). 3) *on specified slice* references each frame on a frame enter by the user in the following parameter.

- *Slice reference*: frame used as reference in the case of *Compute alignment on specified slice*.

- *Overwrite results? (Enable if parameters changed)* (Intra only): if ticked, computes the alignment for all the stacks found. If not, computes it only for the stacks found that don't have a previously outputed shifts.txt file. If a parameter has changed, tick it to process all files. If the process was interrupted and needs to be resumed without changing parameters, do not tick it.

- *Align and save stacks? (Enable if Inter tool won't be used)* (Intra only): if ticked, directly align and save the stacks, along with computation of the correlation. This should be ticked only if Inter stack alignment is not needed. It is a tiny bit faster than doing Apply afterwards.

5 Tools

5.1 Single tool

This tool computes shifts and aligns currently opened image stack without saving anything.

First, select the stack, then click on the tool and choose the parameters. The stack will be aligned in place.

5.2 Intra tool

This tool computes intra stack shifts for all the .tif images found in a given directory. In each stack, all the slices will be aligned on the reference stack.

Click on the tool, choose the parameters, then select the directory containing the .tif stacks. Computed alignment will be saved in a file finishing with *-shifts.txt* for each stack.

5.3 Inter tool

This tool computes inter stack shifts for all the .tif images found in a given directory, by aligning the reference stack of each stack. Intra alignment tool must be run first.

Click on the tool, choose the parameters, then select the directory containing the .tif stacks. The *-shifts.txt* files will be updated by adding the movement between stacks to the movement inside stacks.

5.4 Apply tool

This tool opens a stack selected by the user and applies the shifts generated by Intra and Inter tools, without saving anything.

Click on the tool, then choose a stack to apply the alignments on it.

5.5 Apply tool

This tool applies the computed shifts and saves the aligned stacks, as well as computing correlations between the resulting images and the reference.

Click on the tool, choose to overwrite the results or not, select the directory containing tif stacks and the results of Intra and Inter tools.

6 Resulting files

In this section, a stack of image will be referenced as <img>.tif, where <img> is the name of the file without the extension.

When using the Intra and Inter tool, a text file named <img>-shifts.txt is generated containing the shifts values. The first line contains the reference slice, the horizontal shift from the inter stack alignment, the vertical shift for the inter stack alignment, the rotation for the inter stack alignment, and the correlation to other stacks. The following lines contain a value for each slice of the stack: the second line contains horizontal shift from the intra stack alignment, the third line contains the vertical shift from the intra stack alignment, the forth line contains the rotation from the intra stack alignment, and the fifth line contains the correlation to the reference slice of the stack.

When using the second Apply tool, the movements computed in the <img>-shifts.txt are applied to <img>.tif file, and the result is saved in a new image stack <img>-aligned.tif.