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3D spatial alignment of multiple similar objects extracted from FIB-SEM data using Fijiyama

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We use this protocol and it's working

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Abstract

Procedure for the use of Fijiyama ("Yet Another Matching and Alignment tool for Fiji") to spatially align a series of similar cellular structures detected from 3D FIB-SEM data.

Materials

FIJI/ImageJ
The Fijiyama plugin for imagej - **https://github.com/Rocsg/Fijiyama**

Before start

Fijiyama ("*Yet Another Matching and Alignment tool for Fiji*") is a plugin for FIJI/ImageJ that allows volumetric datasets to be spatially registered in three dimensions [DOI: 10.1093/bioinformatics/btaa846]. We have previously used Fijiyama to spatially align several nuclear pores into a conserved orientation, for structural comparisons under various degrees of pre-processing. In the interest of broader accessibility, this protocol has been generalized so that it can be applied to other cellular structures, provided that those structures are sufficiently similar that they are nearly indistinguishable from one another (just like a nuclear pore). In the current protocol, these structures will be referred to as **SOI (Structures Of Interest)**.

Ensure that Fijiyama is installed before proceeding.

Data preparation

1

Note

Even if all SOIs are located within one 3D dataset ("source dataset"), a separate file for each SOI needs to be prepared for Fijiyama alignment.

Open FIJI/ImageJ.

2 Load the source file (dataset containing your SOIs).

3 Crop the stack so that the image only encapsulates one SOI.

Note

As a useful rule of thumb, crop the SOI so that it is as central as possible, and set the final image dimensions so that they are about 50% wider than the SOI.

4 Save the cropped stack as a new file, into a shared directory for all SOIs.

Note

Add a numerical suffix to the end of each filename to ensure that they always stay in order. Ensure that their prefixes are identical though, as this will save you time later.

5 Repeat these stages until you have the desired number of SOI in one folder.

Spatial alignment

6 Open FIJI/ImageJ.

7 Run the Fijiyama plugin.

8 Select "Series registration (N-times and/or N-modalities)".

9 Select the destination directory, where the output image transforms and results will be stored.

10 Select the directory where you have stored your SOIs.

11 A new dialog window will appear;
- In the ***"Times="*** box, enter the number of SOIs you intend to align (*by writing 1-n, where n is the total number of SOIs*).
- In the ***"Modalities="*** box, leave the dialog box blank.
- In the ***"Generic expression"*** box, enter the filename structure you used to name your SOIs (*for example, if you named your files "SOI1.tif", "SOI2.tif", etc. You would need to enter "SOI{Time}.tif" in this box*.)

12 Press OK, and a new dialog will appear.

13 Under the "Reference time" option, select the filenumber of the SOI that is already in the most desirable orientation; This will be the final global orientation that all other SOIs will be aligned to.

Note

If none of the SOIs are in a particularly desirable orientation, you can also try using a "mock" reference SOI by duplicating one of the SOIs, then rotating/transforming it into a desirable 3D orientation; Alignment of the first SOI to this mock reference will ensure that all subsequent SOIs have the same desirable orientation. Remember to discard the mock reference SOI after use.

There will also be one last chance to assign the global alignment at the very end of this protocol.

14 Press OK, and a new dialog will appear with instructions.

15 The next stages can be configured as required by the user, but we recommend starting with the default configuration first:
- Ensure "Step 0" is configured to use "Manual Registration" with rigid transformations.

- Ensure "Step 1" is configured to use "Automatic Registration" with rigid transformations, via 3D Block-matching. Set registration display to visualize only at end.

Note

Additional alignment stages and settings can also be configured here. Feel free to experiment with these configurations to suit your data.

16 Press the "Approve inter-time pipeline" button, which will lock the alignment configuration.

17 Press the "Start this action" button in the "Fijiyama registration manager" window.
A new window will appear with 3D depictions of the first two datasets; One dataset will be shown in green, the other will be shown in red. Using the instructions provided by Fijiyama, manually align the SOIs with one another.

When the SOIs are sufficiently aligned, press the "Position ok" button in the "Fijiyama registration manager" window. The 3D viewer window will now close.

Note

The alignment does not need to be absolutely perfect. This stage only serves to provide an approximate initial alignment to assist the subsequent automatic alignment stage.

18 Repeat the previous stage (go to step #17) until all pairings between SOIs have been manually aligned.

19 To save the alignments, run the next stage in the registration pipeline (*press the "Start this action" button in the "Fijiyama registration manager" window*).

20 To view a TIFF stack with the initial alignments, run the next stage in the registration pipeline (*press the "Start this action" button in the "Fijiyama registration manager" window*).

21 The next stage in the registration pipeline should be automatic registration, which is based on the preliminary manual alignments you have provided.

Press "Chain-run automatic steps" to run these automated stages without user intervention.

22 Once the automatic registration stages are complete, a new dialog will appear regarding the global orientation of your final alignments (which must be completed manually).

Press the "Start this action" button, and a new 3D window will appear depicting data over a 3D grid depicting each axes of the dataset.

Align the data into the most desirable orientation relative to the spatial axes, then press *"Axis ok"*.

Note

This is your last chance to adjust the global orientation of the final registered data.

23 Export the final results by pressing the "Start this action" button.

This concludes the registration process.

Note

Multiple successive image transformations normally diminish image quality, but Fijiyama compiles any successive operations that occur for an image into a singular global transform.

Note

If you've already completed the above processes, but find that you need another round of alignments, do a new alignment using the outputs from the process described; the new transformations can then be combined with the old transformations to create new global alignments; this function can be accessed by pressing the "Compose successive transformations into a single one" button in the Fijiyama start window.

Note

If you have multiple different versions of the same SOIs (i.e. exact same structure, but preprocessed in different ways), Fijiyama allows you to apply the global transformations (computed above) to other images. This function can be accessed by pressing the "Apply a computed transform to another image" button in the Fijiyama start window.