BiDiFuse: a FIJI plugin for fusing bi-directionally recorded microscopic image volumes

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BiDiFuse Manual

1. Introduction

Deep tissue imaging is increasingly used for non-destructive interrogation of intact organs and small model organisms. Although methodological developments, including advances in tissue clearing and high-speed volumetric imaging, have tremendously extended the imaging depth, various limitations still exist. An intuitive approach to increase the imaging depth by almost a factor 2, is to record a sample from two sides and fuse both image stacks. However, imperfect three-dimensional alignment of both stacks presents a computational challenge.

BiDiFuse is a FIJI plugin which merges bi-directionally recorded image stacks. This is done on the basis of three user-selected landmark points, from which 3D rigid transformation are calculated and applied to register both images. The transition point of both datasets can subsequently be selected on the basis of intensity- or edge-based parameters, or set manually. A smooth transition between both image stacks can be done by taking the weighted sum of both image stacks of 10 planes surrounding the transition point.

2. Overview of plugin

2.1 Plugin install

The plugin is written as a plugin for FIJI, a free Java-based image processing program. Fiji can be downloaded from www.fiji.sc . **Please note that BiDiFuse is compatible with ImageJ 1.50g and higher.**

The plugin requires that TransformJ (part of ImageScience) is installed in FIJI. This plugin can directly be downloaded in FIJI using the update tool **Help > Update > Mange update sites > ImageScience**

The plugin can be downloaded from: https://github.com/JanDetrez/BiDiFuse/

The demo datasets can be downloaded from: http://www.limid.ugent.be/downloads.htm

To install the plugin, drag and drop the BiDiFuse.JAR file on FIJI and restart FIJI to complete the plugin installation.

The plugin can then be called from **Plugins > BiDiFuse**.

2.2 BiDiFuse Registration

BiDiFuse Registration initialisation

To initialise the registration process open two bi-directionally recorded images in FIJI that require fusion. Once open, choose **Plugins > BiDiFuse > BiDiFuseRegistration** to start the registration process.

When **Help** is enabled, the plugin will display extra dialogs to guide the landmark selection process.

In a first step, the orientation of the image stacks should be set before starting BiDiFuse. This can be done with the buttons in the GUI. The orientation should be as follows:

- The slices in each stack should have the order.
- The image stacks should NOT be mirrored with respect to each other (left = left, right = right)

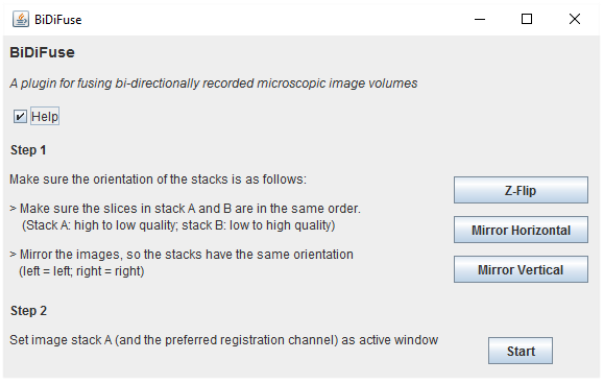

Fig. 1: GUI to initialise BiDiFuse Registration

In a second step, the user is asked to set Stack A as active windows and choose the channel that will be used to identify three landmarks on. Selecting the channel can be done with the sliders that appear below each multi-dimensional image in FIJI.

Upon proceeding, a duplicate of Stack A and B will be generated to start the landmark identification process. A copy of the correctly oriented image stacks will be saved in the image path on the hard disk. This copy can be useful when the user wants to fuse the images later, without going through the orientation process again.

Landmark identification

If **Help** is enabled, three additional dialogs will be displayed during the landmark identification process. Landmarks can be selected by a mouse-click, and must be added to the ImageJ ROI manager by the hotkey [T]. Three landmarks should be identified per image stack.

To start, two landmarks (P1, P2) can freely be chosen in one image of stack A. Then, a line will be drawn on the vector P1-P3, orthogonal to P1-P2. The user should select the third point (P3) and this axis.

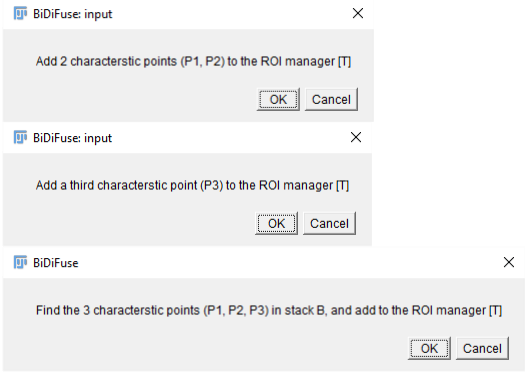

Fig. 2: Optional dialogs that guide users through the BiDiFuse Registration process

The landmarks in stack A should then be localised on stack B, in the same order.

Additional hotkeys: '+' and '-' can be used for zooming
'space' can be used to select the wand tool, for panning through the image.

After registration, the registration variables are written to a text file in the directory of the images. The user can then choose to directly proceed to the fusion process, or start fusion later. This can be useful to run the memory-intensive fusion process on a remote server.

2.3 BiDiFuse Fusion

After registration, the user can immediately continue with the fusion process. Alternatively, the process can be called from **Plugins > BiDiFuse > BiDiFuseFusion**.

The user can select the **transition point** between both stacks based on the difference in intensity and edge content between both image stacks. If there is no clear intensity- or edge content difference between both stacks, the detection algorithms do not return a result.

The **blending range** determines the number of slices that will be fused with the selected **blending method** (Linear weighted sum (eq. 4 in manuscript), maximum, minimum, mean, median, average, sum) surrounding the transition point. In this way, a smooth transition from Stack A to B can be achieved. When set to 0, stacks will simply be concatenated at the transition point after registration.

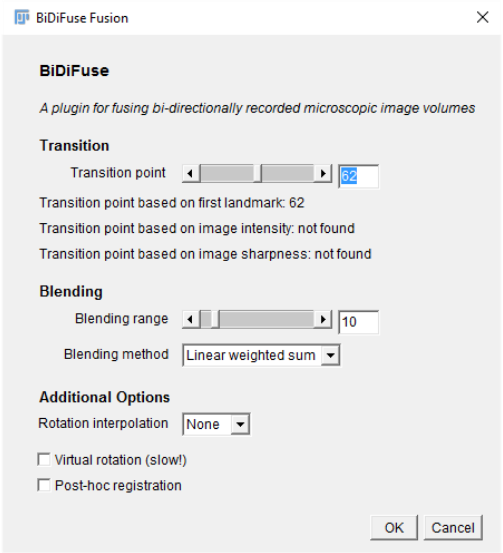

Fig. 3: GUI to initialise the BiDiFuse Fusion

Additional options can be selected in the BiDiFuse Fusion GUI:

- The **interpolation method** for image rotation can changed from Nearest Neighbours (no interpolation) to Linear Interpolation. Linear interpolation generates better results, but is slower and more memory-intensive.
- To save RAM, a checkbox is available to enable **virtual rotation**. When checked, individual planes will be rotated (in X, Y and Z) and written to the hard disk between successive rotations. Although this considerably slows down the fusion process, stacks larger than the size of size of the RAM can be rotated.
- A **post-hoc registration** can be done based on scale invariant feature transform (SIFT). When checked, one overlapping plane will be extracted after the rotation process (the plane containing P1 in stack A, and the plane containing P1 in stack B). These planes will then be registered using SIFT. If the registration can be optimised, an additional rigid transformation based on the SIFT output will be applied to all planes in stack B. A log message will appear when no additional registration optimisation can be performed.

The fusion process will generate a single fused image stack. As a result of image translation and rotation, the edges of the fused stack will contain non-overlapping data. To remove these edges, the fused stack can be cropped to only retain the overlapping data.

3. Results

The images below show the landmark identification process in BiDiFuse on the demo dataset "BrainBloodvessels". After selecting three landmark points in Stack A, the corresponding landmarks are indicated in Stack B. Subsequent fusion of the data shows the alignment of both stacks in a XZ-view.

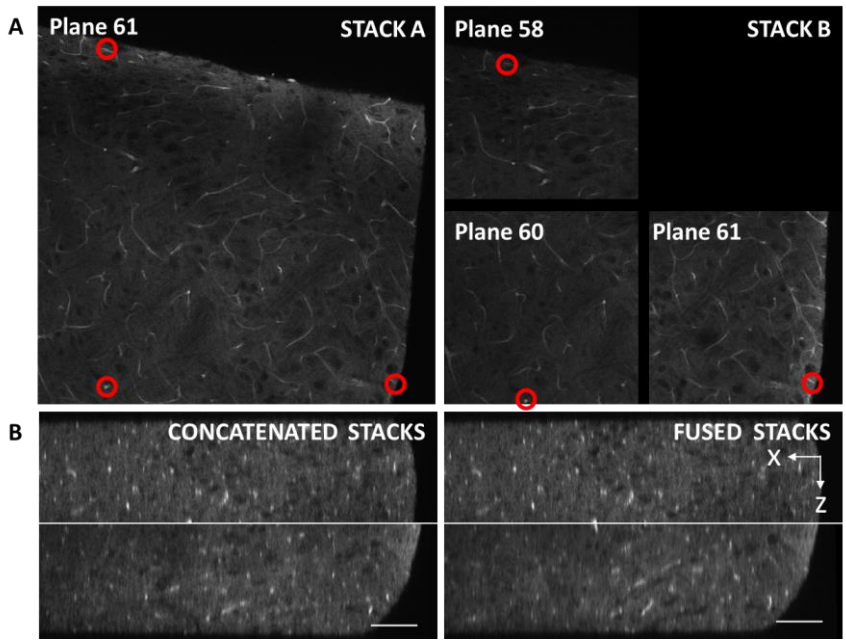

Fig. 4: Registration and fusion of demo dataset
(A) Registration of cleared brain slices recorded on Zeiss LSM780 confocal microscope (demo image "BrainBloodvessels"). Three landmark points are indicated in Stack A, after which the same landmarks are indicated in Stack B. (B) Concatenating Stack A and B (mirrored) on the transition point, shows clearly how fusing corrects for the misalignment between both stacks. Scale bars 100 µm.

The BiDiFuse workflow is independent of the specimen type, fluorescent label, and imaging modality. The images below show the fusion results of different specimens recorded with different microscopic imaging modalities.

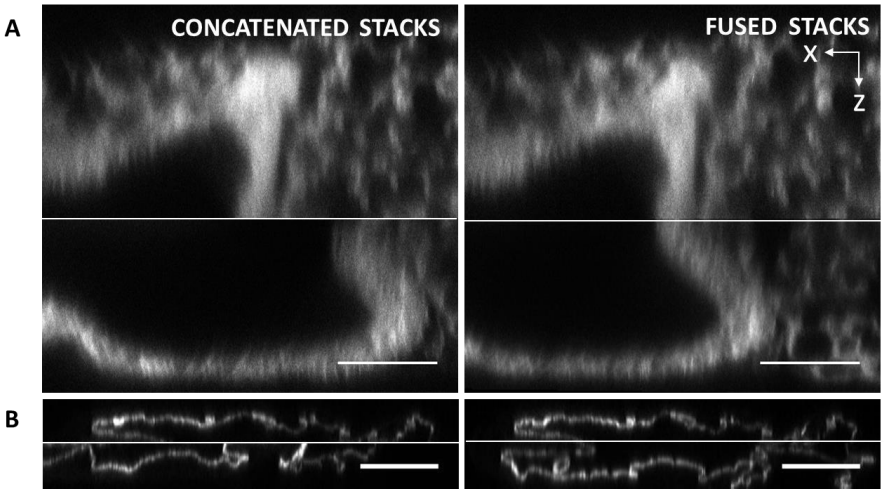

Fig. 5: Fusion of different samples
(A) Registration of tdTomato-signal in cleared mouse lung (demo image "Lung"). (B) Registration of autofluorescent signal from the gizzard of a termite. Scale bars 100 µm.

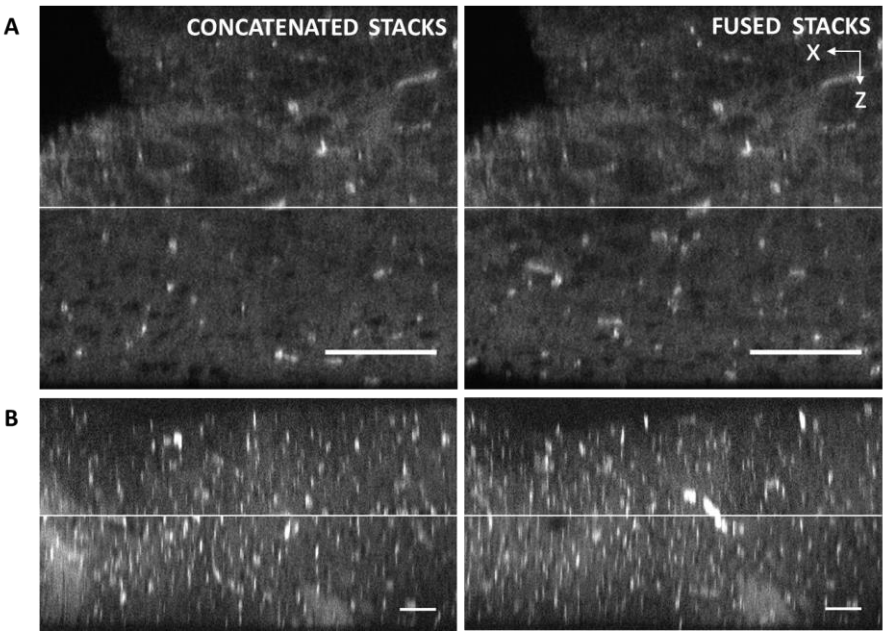

Fig. 6 Fusion of images from different microscopic imaging modalities
Fusion of cleared brain sample recorded on (A) a two-photon microscope (Zeiss LSM780), and (B) an Apotome.2 structured illumination microscope (Zeiss). Scale bars 100 µm.

4. Demo images

Demo images are available from http://www.limid.ugent.be/downloads.htm

- "BrainBloodvessels"
  - Specimen: Cleared 1 mm brain slice
  - Label: ROSA$^{mT/mG}$ mouse with strong expression of tdTomato in the endothelium
  - Imaging: Zeiss LSM780 confocal on AxioExaminer Z1 upright microscope, Objective C-Apochromat 10x/0.45 W
- "Lung"
  - Specimen: Cleared 1 mm lung slice
  - Label: ROSA$^{mT/mG}$ mouse with strong expression of tdTomato in the endothelium
  - Imaging: Zeiss LSM780 confocal on AxioObserver Z1 inverted microscope, Objective Plan-Apochromat 20x/0.8 (air)
- "BrainGFAP"
  - Specimen: Cleared 1 mm brain slice
  - Label: DAPI (channel 1), GFAP antibody (channel 2)
  - Imaging: PerkinElmer UltraVIEW VoX 3D Live Cell Imaging System, Objective 10x/0.4 (air)

5. Problems and Solutions

No overlapping landmarks can be found in the image stacks

- The original datasets should be recorded in such a way that they have a maximally overlapping field of view, sufficient contrast in the overlapping planes to identify landmarks, and without undersampling the Z-axis.

The datasets are poorly aligned at the transition point

- To increase the precision of the rigid transformation, the distance between the landmarks in XY should be as long as possible. In this way, the coordinates for calculating the angles using the atan2 function are more reliable.
- BiDiFuse only accounts for rigid transformations (translations and rotations). Therefore, the sample should be immobilised for microscopy for optimal results. This can be done by mounting the sample between coverslips, in imaging chambers for mounting cleared samples with variable thickness (eg. Easy-Mount®, Chamlide), or on a rotatable stage.

Calculations for the intensity- or edge-based parameters returns 'not found'

- To guide the user which z-plane is the best choice for switching from Stack A to Stack B, the intensity and sharpness (derived from Sobel filtering) of each overlapping plane is measured. These values are subtracted with increasing plane number, and the plane which returns the first negative result is considered as the suggested plane. 'Not found' is returned when this value is not found on a plane within 10-90% of all overlapping planes. This can happen when the intensity or sharpness of one of the image stacks is a lot higher relative to the other. A user-based transition point should be provided in this case.

The plugin runs out of memory

- Although TransformJ is a convenient and fast solution for this task, it requires that the point of origin (in our case P1) is set in the centre of the image. To fulfil this criterion, we have to enlarge image stack B in 3D before rotation, which increases the RAM usage generally between 4-6x the size of stack B. To circumvent this, check the virtual rotation option in the BiDiFuse Fusion GUI to perform a virtual rotation. When checked, individual planes will be rotated (in X, Y and Z) and written to the hard disk between successive rotations. Although this considerably slows down the fusion process, stacks larger than the size of size of the RAM can be rotated.
- Use **Edit > Options > Memory** to increase the maximum amount of memory available for FIJI. Changes are applied after restarting FIJI. It is recommended to set the maximum memory not higher than 75% of the total RAM available.
- Use **File > Input > Tiff Virtual Stack** to load the original images as virtual stacks to save memory for fusing.

Known errors

- There was a problem with the class ij.plugin.frame.RoiManager .
    - BiDiFuse is compatible with ImageJ 1.50g and higher. This error is known to be related to using ImageJ versions below this version number.