DS4H Image Alignment – user manual

https://github.com/UniBoDS4H/IA



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1. BRIEF DESCRIPTION

Our innovative tool is a powerful image alignment software designed to streamline the alignment process both automatically and manually. With its intuitive and user-friendly interface, you can achieve precise and professional results in just a few simple steps.

The distinctive feature of this tool is its flexibility in offering various options for image alignment. Let's start with the automatic functionality, which utilizes advanced algorithms to detect salient features of the images and align them optimally. This option is particularly useful for aligning large sets of images quickly while maintaining high accuracy.

However, we understand that in some cases, greater customization may be necessary. That's why our tool also provides the option to align images manually. Users have the freedom to adjust positions and angles, allowing total control over image alignment to ensure accurate results in complex or specific situations.

To make the workflow even more efficient and organized, the tool introduces the concept of projects. Users can create dedicated projects for each set of related images, making data management easy and allowing them to easily pick up where they left off at a later time. This feature significantly increases productivity and reduces the risk of errors.

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Furthermore, our tool is at the forefront when it comes to semi-automatic corrections. By using the "reuse of images" technique, it allows automatically applying alignment corrections that have already been performed on other similar images. This process further simplifies the workflow and saves valuable time.

Another exciting aspect of this tool is the ability to perform elastic deformations using *bUnwarpJ*. This advanced option enables even more precise and sophisticated corrections, adapting image alignment based on local deformations, resulting in natural and realistic outcomes.

In summary, our image alignment tool offers a wide range of functionalities, combining the efficiency of automatic alignment with the precision of manual alignment. Project management and image reuse simplify the workflow, while elastic deformations with *bUnwarpJ* add a dimension of customization and perfection to the final results. Whether you're a professional image editor or an enthusiast, this tool will help you achieve the best alignment results for your needs.

2. LICENSE

The software and all the materials are available at the **https://github.com/UniBoDS4H/IA** website and are copyright protected.

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3. SYSTEM REQUIREMENTS

DS4H-IA is written in *Java* 8. The standalone version requires *the OpenJDK-8* installed into the computer. The source code requires *ImageJ/Fiji*. *DS4H-IA* works with MAC, Linux, and Windows. However, it was tested using Fiji version Windows 64-bit "ImageJ 1.54f", release 2023 August 01.

NOTE: Currently, the only macOS devices supported are those with Apple M1 ARM processors. In addition, for macOS devices, the automatic alignment based on the SIFT algorithm is currently not supported.

4. INSTALLATION

Starting from a **compiled version** of *DS4H-IA*:

- Download the *plugin* compiled version for your operating system (*e.g., Windows, Linux*, and OS X environments) from: https://github.com/UniBoDS4H/IA/releases
- 2. Put the "DS4H_ImageAlignment-X.Y.Z.jar" file inside the "plugin" folder in ImageJ/Fiji.
- 3. Enjoy!

Starting from the **source files** of *TDSFT*:

- 1. Download the source files from https://github.com/UniBoDS4H/IA/releases
- 2. Extract the files from "SourceCode.zip" to a folder whose path is without any white space.
- 3. Open the "SourceCode" folder.
- 4. Launch a terminal and put the code "make create_release".
- When the command has finished its work, go to the "target" folder and copy the "DS4H_ImageAlignment-X.Y.Z.jar"
- 6. Paste the jar file in to the "plugin" folder of ImageJ/Fiji

5. USAGE

The plugin features a graphical interface composed of modal windows displayed according to the required functionality. The most informative windows are:

- Main Window represents the area where all the images in use are displayed. It allows viewing the images and provides several functionalities, including editing automatic and manual alignment algorithms, configuring parameters for the bUnwarpJ plugin, managing the entire working project through "import," "export," or "clear" options, and the ability to select the target among all images and remove any unnecessary images;
- *Preview Window* allows displaying a single image and provides the ability to add or remove alignment points, as well as managing the size and color of the specified points;
- Output Window displays the final result of the alignment, offering the possibility to save the images, reuse them for a subsequent alignment, or apply the bUnwarpJ elastic deformation algorithm to all the images.

5.1. How to use DS4H

Below are described all the available features for users. The screenshots shown in this section were captured using a Lenovo computer with the Ubuntu operating system.

5.1.1. Loading the images

After launching our plugin, the first window that will open is the main window (Figure 1.1). You can observe the structure of this window. To load the images, you can rely on two options present in the top menu:

- "File"
- "Project"



Figure 5.1: Main GUI.

5.1.2. Load the images with File

After selecting the "File" option in the menu bar, another window for loading images will open (Figure 1.2). You will be able to browse through the system's folders and select a group of images to load into the plugin.

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⊟ Videos 🖻 Fiji.app	test_32_bit.tif	367,1 MB	Image	16 mag
+ Other Locations				

Figure 1.2: Multiple image loading using "File".

5.1.3. Loading images with Project

After clicking on "Project," a submenu will be displayed, from which you will need to select "Import." Subsequently, a window will open (Figure 1.3), from which you can choose a folder. All the images present in the selected folder will be imported automatically.

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Files of Type:	All Files									\sim
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Figure 1.3: Loading using "Project > Import".

5.1.4. Plugin settings

In our software, we also give the user the possibility to customize e settings of the automatic, manual and elastic alignment. If you click on *"Settings"* option menu bar, a submenu will open with three options:

- 1) **"Manual**" by selecting this option, the manual alignment menu will open (Figure 1.4), giving the user the possibility to configure:
 - "Algorithm," to choose the type of manual alignment algorithm to be used, ranging from "Translative," "Affine," to "Projective";
 - a) **"Translative**": aligning images through horizontal and vertical shifts to bring corresponding features into spatial correspondence, at least **one corner points**;
 - b) "Affine": aligning images with translation, rotation, scaling, and shear transformations to achieve spatial correspondence between corresponding features, at least three corner points;
 - c) "Projective": aligning images with perspective transformations, including translation, rotation, scaling, shear, and skew, to establish spatial correspondence between corresponding features, at least four corner points.
 - You can choose to apply "Translation," "Scaling," and "Rotation";
 - "Point Overload," selecting how to handle any excess points in case they are not necessary, choosing between "First point available," "RANSAC," or "Minimum Least Square.

Manual	alignment algorithm 😑	×		
Algorithm:	Translational	\sim		
Algorithm info:	At least 1 corresponding point(s) for each image is required.			
✓ Translation	Scaling Rotation			
Point Overload:	First points available	~		

Figure 1.4: Menù di configurazione algoritmo manuale.

- 2) "Automatic" by clicking on this option, a menu will open (Figure 1.5) where you can set:
 - "Detector", the type of automatic algorithm to automatically detect points for alignment;
 - "Threshold Factor", to select any "non-optimal" points;
 - *"Scaling Factor"*, allows you to choose by how much each individual image should be scaled

Automatic alignment algorithm 😑 🜸							×	
Detector:	รเ	SURF					\sim	
Threshold factor:	 0	11	5	1	0	' ' 15	 ;	20
Scaling factor:		 2	- 3	- 4	- 5	 6	 7	- 8
Algorithm:	Translational				~			
Algorithm info:	At least 1 corresponding point(s) for each image is required.							
✓ Translation □ Scaling □ Rotation								

Figure 1.5: Menù di configurazione algoritmo automatico.

- 3) "bUnwarpJ": by selecting this option, it will be possible to configure the parameters to use during the elastic deformation algorithm (Figure 1.6). Among the many parameters that can be set, I will focus on the most relevant one, "Mode," specifically, this allows us to choose between three different options:
 - "Mono", causes the program to perform only unidirectional registration, i.e., from source to destination;
 - "Accurate" and "Fast" involve bidirectional registration and affect the stopping criteria used internally by the program.

bUnwaprJ settings 🕞 🙁					
Mode :	FAST ~				
Initial_Deformation :	Very Coarse ~				
Final_Deformation :	Very Coarse ~				
Image Sample Factor :	• • • • • • • • • • • • • • • • • • •				
Divergence Weight :	0.0				
Curl Weight :	0.0				
Landmark Weight :	0.0				
Image Weight :	0.1				
Consistency Weight :	10.0				
Stop Threshold :	0.01				

Figura 1.6: Menù di configurazione bUnwarpJ.

5.1.5. Target selection

After successfully loading the images into our plugin, to perform manual and automatic alignment, it is necessary to select the "Target" image from all the loaded images. This image serves as the reference on which the alignments will be performed. Specifically, all the images will be adjusted based on this image. To set the "Target," simply click on the "TARGET" button (Figure 1.7)

DS4H Image Alignment 😑 💿 🙁
File Project Settings About
20190311_Mosaic_Cy3_NoAligned.tif
20190311_Mosaic_DAPI_NoAligned.tif
2 TARGET
20190311_Mosaic_FITC_NoAligned.tif
3 TARGET 🗵
Automatic Alignment
Manual Alignment

Figura 1.7: Finestra principale con immagini caricate.

5.1.6. Remove an image

In case the user realizes that they have inadvertently loaded an unnecessary image after successfully loading the images into our plugin, they can remove it from the project by clicking on the red trash bin icon next to the "TARGET" button (Figure 1.7). This way, the image will be completely removed from the project.

5.1.7. Manage corner points

After successfully loading the images into our plugin, to perform manual alignment, it is necessary to indicate the same number of corner points on each loaded image. The minimum number of points required varies depending on the selected algorithm. In those algorithms that require more than a corner point, they must be numbered in the same order in each image. To set the corner points on each image, you will need to click on the desired image in the main window. Subsequently, the preview window will open (Figure 1.8), where you can work on the selected image.



Figura 1.8: Finestra di preview.

5.1.8. Add new corner points

If you press the left mouse button twice within the boundaries of the current image, a corner point will be created at the exact position of the cursor (Figure 1.9). You can see the result of this operation with the creation of three corner points. The order of point creation is highlighted by automatic numbering.



Figure 1.9: Finestra di preview in cui sono stati aggiunti 3 punti.

5.1.9. Single or multiple corner points selection

As mentioned earlier, it is possible to insert multiple corners within the same image. You can select them individually (Figure 1.10) or in multiple selection mode (Figure 1.11). When multiple corners are selected, you can move them all together. The movement of corner points is limited within the boundaries of the image, preventing the user from placing corner points outside the image's boundaries that they are working on.



Figure 1.10: Single corner point selection.



Figure 5.11: Multiple selection.

5.1.10. Copy of corner points

To facilitate the use of our plugin, we have also implemented the functionality of copying the inserted corner points from one image and pasting them onto another. To begin, it is necessary to

select at least one corner point within the image. Subsequently, you can choose the destination image where you want to paste the corner points, selecting it from the drop-down menu under the "copy to" option (Figure 1.12). Finally, by clicking the "Copy" button, the operation will be completed. In case some of the copied corner points end up outside the destination image, the user will be notified (as shown in Figure 1.13), and the corner point will not be inserted into the destination image.



Figure 5.12: Selection of corner point and copy the points into image "3".



Figure 1.13: Copy the corner point out the image.

5.1.11. Delete the corner points

After selecting one or more corner points, you can completely remove them from the image by pressing the "Delete" button (Figure 1.14) with the cursor. This will result in the complete removal of the selected corner points from the image (Figure 1.15), and the corner point indices will be automatically scaled to fit the new configuration.



Figure 1.14: Selection corner "1" and "2".



Figure 5.15: Result after removing corner "1" and "2".

5.1.12. Modify corner points

If the color of the corner points provides little significant contrast or if you want to change the order between two inserted corner points, you can make adjustments to both the appearance and the order of each corner point. This can be done by clicking on the settings icon, which will open the settings window for the inserted corner points (Figure 1.16). Through this window, you can:

- Vary the corner color;
- Change the corner selection color;
- Alter the corner index color;
- Adjust the corner size;
- Replace the index number a corner point with that of another inserted corner.



Figure 5.16: Corner point settings window.

5.1.13. Project export

Once the user has loaded the images, they can perform the "Project > Export" operation to export the entire working project. Specifically, using this function, the user can save all the images with their respective set points on each image, along with the selected target, to any folder on their system. When the "Export" option is clicked, the user will be prompted (Figure 1.17) to choose the path destination for saving the project. Automatically, а folder named "YYYYMMDD_HHMM_DS4H_Project" (where YYYYMMDD indicates the year, month, and day of the save, and HHMM indicates the hour and minute) will be created, containing all the images and a single JSON file containing all the information about the "target" and the corner points.

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Files							
Folder name:	/home/matteo/Desktop						
Files of Type:	All Files						~
			Oper	1	С	ance	el

Figure 5.17: Export the images inside "Desktop".

5.1.14. Manual Alignment

Once corner points have been specified on each image, it will be possible to align them by clicking the "Manual Alignment" button in the main window. Now that the alignment process has started, a window will be displayed to show the progress of the alignment (Figure 1.18)



Figure 1.18: Loading window.

When the alignment is complete, the output window will be displayed, which contains a scrollable stack with all the images aligned according to the selected target (Figure 5.19).



Figure 5.19: Output window.

If we want to see our aligned images overlaid after aligning them, we can use ImageJ/Fiji. By doing so, we can obtain the various aligned images overlaid within the same stack (Figure 1.20).



Figure 5.20: Output window with overlay images.

5.1.15. Save the images

Once we have obtained our aligned stack, we can perform a series of operations, including saving the images. To do this, simply click on the "Save" option in the menu bar and select which of the output images you want to save (Figure 1.21). After choosing which images to save, you will be prompted to choose the directory where you want to save the images. This way, in the specified path, you will find a folder named "*YYYYMMDD_HHMM_DS4H_AlignedImages*".



Figure 5.21: Save images window.

5.1.16. Reuse the images

After aligning the images, in addition to saving, you can perform another operation, which involves using all or some of the images for further alignments using the "Reuse" option in the menu bar. Once this option is selected, you will be prompted to choose the images you want to use for this operation (Figure 1.22). Through this procedure, it is possible to correct alignment errors obtained during the automatic phase, allowing for a manual alignment on the images obtained during the automatic process

Reuse 🗕 🗆 🗙
20190311_Mosaic_Cy3_NoAligned.tif
20190311_Mosaic_FITC_NoAligned.tif
20190311_Mosaic_DAPI_NoAligned.tif
3 INCLUDED
Reuse

Figure 5.22: Reuse the images window.

5.1.17. Apply elastic deformation algorithm

After aligning the images, you can perform the final operation, which involves applying the *bUnwarpJ* elastic deformation algorithm. To do this, simply click on the "Elastic" option in the menu. Automatically, our program will use the settings configured in the main window as input parameters for the algorithm.

5.1.18. Automatic alignment

After you have successfully loaded your images into our plugin, you can easily use the automatic alignment feature by clicking on the "Automatic Alignment" button. Various algorithms are available. The main ones are *SURF* (available for all the systems) and *SIFT* (available just for Windows systems). A loading window will immediately appear showing the progress of the alignment (Figure 1.18). Once the alignment is successfully completed, the loading window will be shown output (Figure 1.19).

6. CONTACTS

If you have any question about DS4H-IA, write an email to:

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