

imodtkalign

What is imodtkalign?

imodtkalign is a graphical user interface for the alignment of serial sections with the IMOD (<http://bio3d.colorado.edu/imod/>) software package from the Boulder Laboratory for 3D Electron Microscopy of Cells (<http://bio3d.colorado.edu/>). The GUI uses perl/Tk to control image several of the IMOD programs for the alignment and the `convert` program from ImageMagick (<http://www.imagemagick.org/>) for image manipulation.

The aim of imodtkalign is to provide a simple pipeline for the registration of images from serial sections for light or electron microscopy. The GUI enables user to align several hundreds of images and large amounts of image data up to 30gb. The pipeline works with rgb or grayscale data, images with alpha channel are currently not supported.

Install imodtkalign

Due to problems with commandline-tools, imodtkalign currently only runs on Linux derivatives. The following software is required:

- Perl/Tk, Image::ExifTool for perl
- ImageMagick
- IMOD

In most Linux distribution by Perl/Tk and Image::ExifTool can be installed by using the package manager. In Ubuntu the packages are in: perl-tk and libimage-exiftool-perl, thus running:

```
sudo apt-get install perl-tk libimage-exiftool-perl
```

in a terminal installs them. ImageMagick is also in most package repositories, in Ubuntu use:

```
sudo apt-get install imagemagick
```

to install it.

IMOD must be obtained from <http://bio3d.colorado.edu/imod/>, follow the appropriate installation instructions on the website to install it on your system.

After installing all prerequisites download the latest version of ImodTkalign.pl from <http://www.q-terra.de/biowelt/3drekon/>. Extract the zip-archive and change to its location in the terminal. Then copy the .pl-file to /usr/local/bin:

```
sudo cp imodtkalignXX.pl /usr/local/bin/
```

where XX is the current version number. Make it executable for all users:

```
sudo chmod ugo+x /usr/local/bin/imodtkalignXX.pl
```

and set a symbolic link to it:

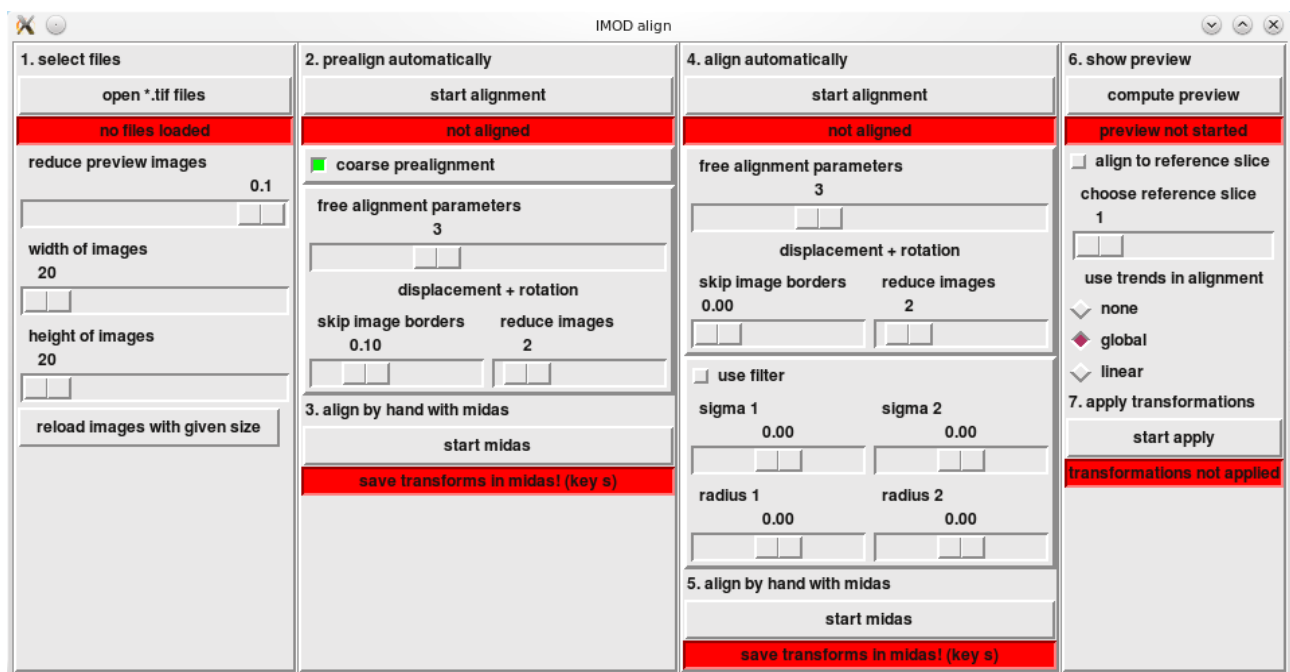
```
sudo ln -s /usr/local/bin/imodtkalignXX.pl /usr/local/bin/imodalign
```

Now all users can start the software by typing `imodalign` into a terminal. Running `imodtkalign` from a terminal is highly recommended, since otherwise Ubuntu has some trouble with the paths to the IMOD-programs. Additionally, the terminal provides useful information if something does not work.

Usage of imodtkalign

Loading images

As mentioned before, start `imodtkalignXX.pl` from a terminal by just typing `imodalign`. The graphical user interface is used from the left to the right and from top to bottom. Main buttons for each alignment-step are underlined by a red text field, that changes to yellow while the required program is working and to green when the step is finished. Warning: Do not try to load images with white-spaces or other non-alphanumeric characters in their path. This may causes a lot of problems, often when a lot of work is just done.



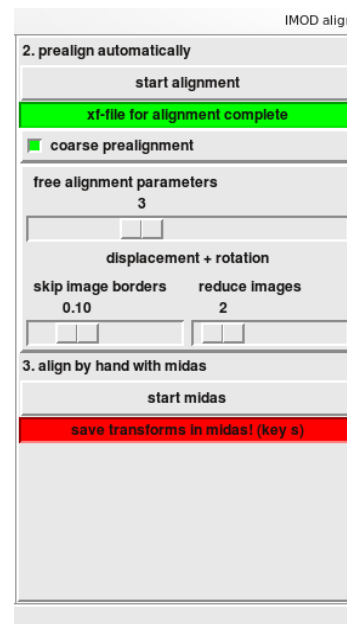
The first button opens a file dialog, where the user can choose the files to load. currently only tif-files with grayscale or rgb are supported. Files can be selected by choosing the first file, hold the shift-key and klick on the last file or by choosing separate files with the ctrl-key pressed. The program copies and down-samples the original images to one 10th of their size. All images are extended to the width and height of the largest image(s). These default parameters can be changed either before loading the images or by pressing **reload images with given size** when the parameters are

changed after the first load. The yellow text field and a progress bar occur during the loading process.

Automated alignment

After loading the images the user can start the first automated alignment. The following parameters can be changed:

- Checking the **coarse prealignment** button will cause the program to search for coarse misalignments first. When the images are rotated to each other this is highly recommended.
- **Free alignment parameters** controls what kind of transformations are allowed in the alignment process. Possible values are:
2: displacement only
3: displacement and rotation
4: displacement rotation and magnification
5: displacement rotation, magnification and distortion
- The **skip image border** parameter is useful when images of different sizes are loaded, because smaller images are extended by a gray border. To prevent the program from using this border for the alignment, one can choose how much of the image border is ignored during the fine alignment
- **reduce images** enables further down-sampling of the images during alignment but will not touch the copied images for the manual alignment in the next step



Manual alignment with Midas

The **start midas** button evokes the Midas-program from IMOD, that provides an easy to use tool for manual alignment. The manual alignment step is needed because the automated alignment often went into local optima and thus erroneous alignments. In Midas always two successive sections can be aligned to each other. The software later calculates the needed transformations to align the complete stack by the list of single transforms from the midas program.

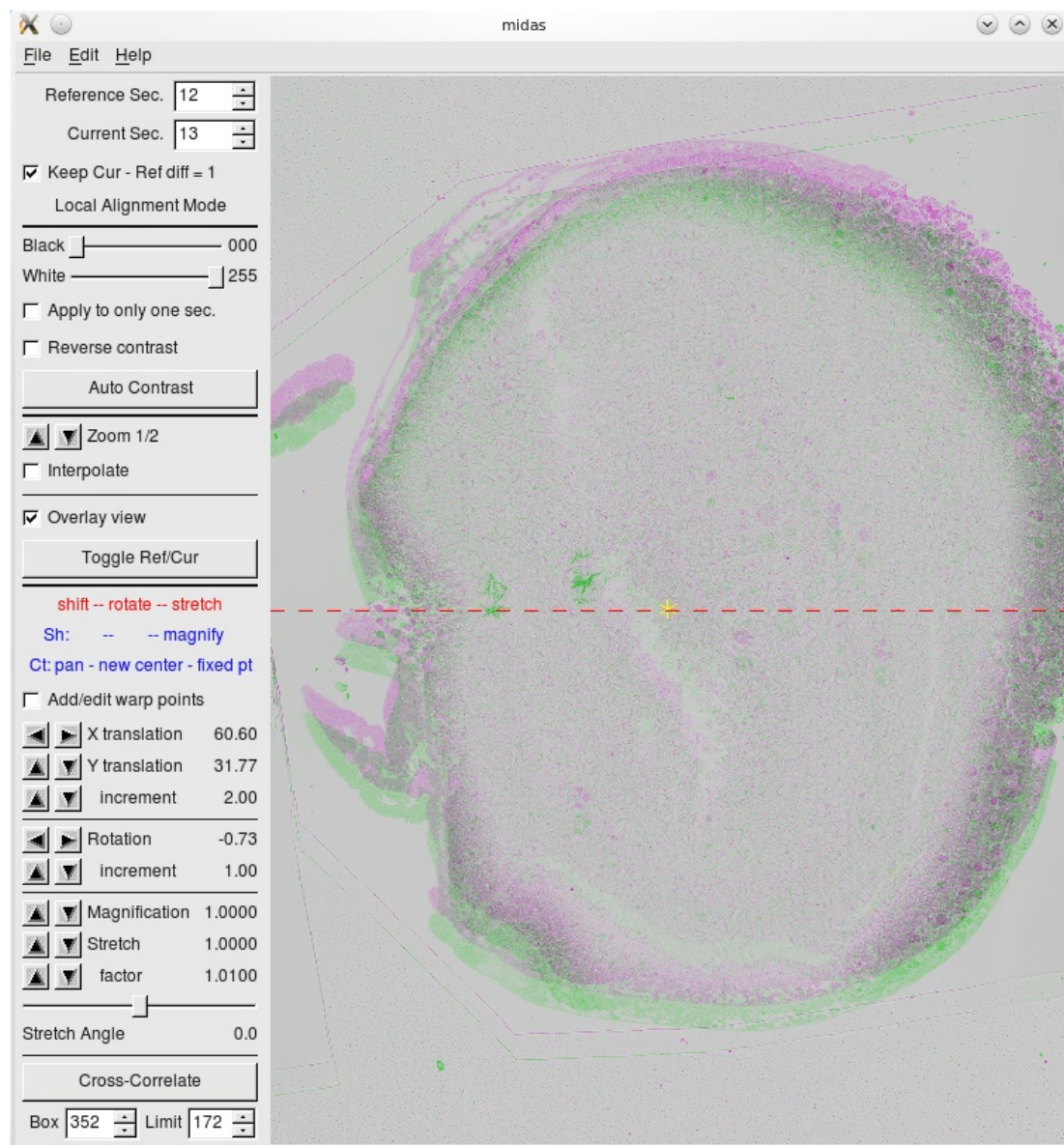
By default the Midas program shows an overlay view of two sections, colored red and green. When they are well aligned, corresponding areas will sum to gray pixels. The user can do transformations by:

- left mouse button = move current section
- middle mouse button = rotate current section
- right mouse button = stretch current section (always save (press “s”-key) before using it!)
- Shift + right mouse button = scale current section
- Ctrl + left mouse button = moves current and reference image in the window
- Ctrl + middle mouse button = moves the center of rotation
- Ctrl + right mouse button = set an additional fixed point for stretching of the current section

Most important keys for the usage of Midas are:

- a = move to next pair of reference / current section
- b = move to previous pair of reference / current section
- s = save transformation

Stretching images with midas is not easy and often results in unusable alignments. To avoid problems with mistaken stretches the user should store the transformation as often as possible by just typing the “s”-key. If done the user can easily go back to the last good transformations in the menu **Edit, Revert to stored transform**. Mirrored images can be corrected by using **Edit, Mirror around X-axis**. When finished it is most important not to forget to store the alignment with the “s” key. The Midas-program can be closed than. During the manual alignment one should have a look if there is



a well orientated sections (as reference section) in the stack. This section can later be used as reference for the whole stack, thus, its number should be kept in mind.

Refinement of the alignment

In the next step the manual alignment can be refined by an additional automated and manual alignment. Thus, the previous manual alignment need not to be perfect but only should prevent the second automated alignment from running into local optima again. Since in large datasets there are always some problematic sections that tend to be misaligned even after all these alignments, the second manual alignment can be used to correct them by hand. The parameters are as in the previous sections, but in the automated alignment the check button for coarse prealignment is lacking. Additionally there is an option to use filters to help the automated alignment. If one wants to use these filters please refer to the description at: <http://bio3d.colorado.edu/imod/doc/man/xfalign.html>.

Preview

After the first alignment step the user can always preview the alignment by pressing the **compute preview** button. This opens the 3dmod program, that shows the down sampled and calculated image stack. With the Z-Slider or page down / page up keys it is possible to move through the sections. Pressing the middle or right mouse button will cause the program to run through the sections. Clicking one of these mouse-buttons again will stop the run through the series. The preview computation is aware of the parameters given in the next section and thus does the same as the **start apply** step will do with the original images.

Apply to original images

In the last step the transformations are applied to copies with the same size and color mode than the original images. These resulting images can be found in the same directory as the original images, named with the prefix `imodal_`. Both, the **start apply** and **compute preview** routines accept the parameters given above the **start apply** button. The parameters are:

- **align to reference slice**: if this button is checked, the slice chosen by the slider is taken as reference slice for the stack.
- **use trends in alignment**: none means that the transformations are used as they are, to compute the alignment of each image to the previous one. global and linear try to compute trends in the alignment, as for example shifts of the sections or overall rotations.

The `imodtkalign`-skript uses temporary files stored in a directory `imodalalign_tmp` within the directory of the original images. To have access to the temporary calculations if something went wrong, the temporary directory is not removed by the program. This should be done by hand, when the alignment was finished successfully.

Bugs / drawbacks:

- no support for colored images other than rgb
- leaves temporary data in image directory
- no support for paths with whitespaces or other non alphanumeric characters

License

The software and its documentation is distributed under the GNU Public License

Acknowledgments

This small little Perl-script would not exist or make any sense without the powerful IMOD-software developed by David Mastronarde and co-authors at the Boulder Lab for 3D Electron Microscopy of Cells. I am thankful that people develop such software and that there are organizations founding such projects. Without that we would depend on commercial tools only, most of them not allowing to develop our own extentions and solutions.