

IMAGIC to Frealign

HANDS-ON

In the following you will find a work flow describing how to get all image and parameter files so that images created in IMAGIC can be used in Frealign

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A square box containing the text "IMAGIC to Frealign" in a bold, sans-serif font. "IMAGIC" is on the top line, "to" is on the second line, and "Frealign" is on the third line.

1. Last Multi-Reference Alignment

Look for the latest images, which were multi-reference aligned (with a file name like: [particles_mra_best](#), or any other name you used).

NOTE:

This file **MUST** contain **ALL** images, which you once picked from the micrograph patches (command **PICK-ALL-PARTICLES**).

The related MRA references are forward projections (all typical views) created from an (intermediate) 3-D reconstruction, which means that their Euler angles are known. During the **MULTI-REFERENCE-ALIGNMENT** each aligned image gets the Euler angles of the reference to which it correlates best.

NOTE:

Each multi-reference alignment in **IMAGIC** is **PROJECTION-MATCHING** if the references are forward projections of a 3-D reconstruction (command **THREED-FORWARD**).

Note that all important parameters are stored in the headers of these aligned images: Euler angles, CTF and alignment parameters.

2. Micrograph Identification Number

Frealign needs to know the (identification) number of each micrograph from which each single particle was picked.

1. Check if the micrograph numbers are available in the headers of your micrographs ([micrograph_filt_coarse](#)). Use the **READ** option in the command **HEADER**:

```
IMAGIC-COMMAND: header
Specify option           : read
Read options available   : micrograph_id
Source of header values  : file
Input (header) file, image loc#s : micrograph_filt_coarse
```

Unfortunately, the distributed micrographs ([micrograph_filt_coarse](#)) do not contain micrograph numbers.

2. So you will simply write the location numbers into the headers. Create a file ([micrograph_number.plt](#)) with your text editor and write the numbers (1 ... 300), one number per line. If you do not want to type in 300 numbers you can copy the printout of the above **HEADER** command and globally remove "not specified").
3. Write the micrograph numbers into the header of the micrographs:

```
IMAGIC-COMMAND: header
Specify option           : write
Options available       : micrograph_id
Source of header values  : file
Plt input file with header values : micrograph_number.plt
Input (header) file, image loc#s : micrograph_filt_coarse
```

3. Create (Untreated) Patches

1. Cut the micrographs ([micrograph_filt_coarse](#)) into patches using the command **CUT-IMAGE**:

```
IMAGIC-COMMAND: cut-im
Input file, image loc#s       : micrograph_filt_coarse
Output file, image loc#s     : patches_frealign
```

```

Mode of operation           : best
Output image dimensions X,Y : 288,288
Overlap between fields     : 90

```

4. Extract your Particles from the Untreated Patches

Frealign only works with the original images, which are not CTF corrected and not aligned. Therefore you have to re-do your particle extraction using the non CTF corrected micrographs. Of course, you can use the old coordinates file (`ccf_coord.plt`) created by the command **PICK-ALL-PARTICLES**.

Call the command **CUT-IMAGE**:

```

IMAGIC-COMMAND: cut-im

Input file, image loc#s      : patches_frealign
Output file, image loc#s    : particles_frealign
Mode of operation           : aperiodic
Output image dimensions X,Y  : 160,160
                             large -images are not CTF corrected (point spread)
Coordinates (plt) file      : ccf_coord

```

5. Take over the Micrograph Numbers

As mentioned above, **Frealign** needs the micrograph numbers, which are not available in the headers of the aligned images (`particles_mra_best`). Use **HEADER** to take over these header values:

```

IMAGIC-COMMAND: header

Specify option               : takeover
Takeover option              : index
How to specify index         : label

```

```
Howmany indeces wanted           : 1
Give label #1                     : mident
Input (header) file, image loc#s  : particles_frealign
Input=output (header) file        : particles_mra_best
```

6. Check Micrograph Numbers

Check if the particles are sorted by their micrograph identification number:

```
IMAGIC-COMMAND: header
Specify option                    : look
Options available                 : micrograph_id
Input (header) file, image loc#s  : particles_frealign
```

If nothing went wrong during your practical your images are ordered correctly.

NOTE:

If the images are not sorted by their micrograph numbers you have to sort both, the particles file ([particles_frealign](#)) and the aligned image file ([particles_mra_best](#)). Use the command **EXCLUSIVE-COPY** and options **SORT**, **LABEL**, and **MIDENT**.

7. Export to Frealign

The command **IMAGIC-TO-FREALIGN** converts the un-treated images into a **Frealign** MRC file and will create a **Frealign** parameter file.

```
IMAGIC-COMMAND: imagic-to-frealign
Option used for current command      : CONVERT
Input file with ORIGINAL images      : particles_frealign
Header file of PROCESSED images      : particles_mra_best
Invert contrast/densities            : zero_float_after_invert
Source of Euler angles               : mra_header
Source of magnification values       : interactive_constant
Image magnification                  : 59000           you have to know
Source of micrograph numbers         : header
Source of defocus values             : header
```

Now all files are created, which you need to work with **Frealign** (**particles_frealign.mrc** and **particles_frealign.par**).

YOUR NOTES:

Frealign Download

<http://emlab.rose2.brandeis.edu/frealign>

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