Frealign: Running Frealign

Once all the necessary parameters and files are set up, Frealign can be run in two different modes: refinement or search. To run a refinement (local search of alignment parameters to improve a previous alignment), type

```
frealign_run_refine
```

This will launch the refinement scripts to run the cycles indicated in `mparameters`, starting with "`start_process`" and finishing with "`end_process`". Frealign can be run on a local Linux workstation or a cluster. For clusters, the type (e.g. SGE, LSF) must be indicated in `mparameters`. **IMPORTANT: To run on a cluster, the refinement has to be launched from the head node.** The `mparameters` file can be changed "on the fly" without stopping a Frealign run. New parameters, including new values for end_process will be read in after a cycle finishes.

To run a brute-force search (for initial parameter determination), use Frealign's mode 3 by setting "`MODE`" in `mparameters` to 3, "`DANG`" to the desired angular step width and "`res_high_refinement to the desired resolution`". A value of 5 deg for "`DANG`" usually yields good results but users can also try larger values to speed up computation. For the initial resolution limit ("`DANG`"), a value between 25 and 35 Å is recommended. Also set "`start_process`" and "`end_process`" appropriately. Normally, one or two rounds of brute-force search are sufficient to align most particles correctly. Again, to start the brute-force search, type

```
frealign_run_refine
```

To speed up the brute-force search, one can change the pixel size of the particle images to a larger size using pixel binning. This can be accomplished using "`resample.exe`" (or "`resample_mp.exe`" for multi-CPU machines), which is included with the Frealign distribution. The binning will produce a new particle parameter stack with the new pixel size. Please note that the `mparameters` file has to be updated with the new pixel size. Furthermore, the "`dstep`" parameter has to be updated since the binning changes the effective detector pixel size by the same factor as the pixel size on the specimen. For example, if the unbinned values for "`pix_size`" and "`dstep`" were 1.2 and 5 and 2 x 2 binning is applied, the new values will be 2.4 and 10, respectively. Please remember to update these values also when returning to the unbinned particle stack at a later stage of the processing when higher resolution details are to be reconstructed. The x,y shifts in the alignment parameter files do not have to be changed in any way when working with binning as they are given in units of Angstroms.

Additional details of how to run Frealign can also be found in this PDF document. Please note that the command "`frealign_run_search`" is not supported anymore. Use "`MODE 3`" instead as described above.

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