EMAN2 + FreAlign Tutorial

Using the Workflow

The FreAlign integration component to EMAN2 was added in release 2.0. This tutorial should not be used with versions of EMAN2 prior to release 2.0. FreAlign must also be installed properly as per the instructions that can be found in the EMAN2 wiki.

General EMAN2 Tips

• Make sure you have the latest available version of EMAN2 installed. We recommend using the current snapshot version, and only reverting to the last stable release if you experience problems.
➡ EMAN2 documentation is largely provided via the Wiki at: http://blake.bcm.edu If you wish to edit the Wiki, create an account for yourself, then send email to sludtke@bcm.edu and we will adjust permissions so you can edit. Previously this was an 'open' wiki, but we had serious spam problems, and now have to individually approve new users. You don't need an account to browse the current contents, of course.
➡ GUI Tips: Interactions with the interface in EMAN2 are quite similar to EMAN1 in many ways. EMAN2 will work best with a 3-button scroll mouse, though there are alternatives using keyboard modifiers for people using one button mice on Macs.
• In most display windows (plots, 2-D images and 3-D volume display), the middle mouse button will open a 'control panel' for the widget with many options to control the display
• The right mouse button is used for panning in 2-D or 3-D image windows, and can be used to zoom (by shift+dragging), and to reset the zoom (clicking) in plot windows.
• The left mouse button has various purposes in various contexts.
• The scroll-wheel will generally act as a zoom. Use the control-panel for more precise control
• If you have a one button mouse, one of the modifier keys (depending on platform) combined with a mouse click will serve the same role as a middle-click. You may need to try them (alt, command, ctrl, shift) to discover which works on your machine.
• In the control panels, and other places in the EMAN2 interface you may encounter 'ValSliders'. These are widgets where a slider is attached to a text-box with a number in it. Dragging the slider controls the number, and entering a number will change the slider. In addition, the text-box can be used to control the range of the slider and get more precise control. By typing '<value' or '>value' in the text box you can change the limits of the slider. Note that it is also possible to enter values which are outside the current slider range.

Running e2refinetofrealign.py

- Expand Single Particle Reconstruction, causing the familiar EMAN2 workflow tasks to appear.
- In the 3D Refinement section, expand the Frealign section. You should see 3 options: Run – e2refinetofrealign, Run – e2runfrealign, and Run – e2refinefromfrealign.
- Select the Run – e2refinetofrealign task. A window should appear with multiple options including fbeaut, fcref, fstat, etc.
- First we need to find a 3D reconstruction we want to use. Click the Browse To Add button and the EMAN2 browser will appear. Navigate into whichever refine_XX directory you want to use, click on "bdb", and select one of the threed_filt_XX files. It is almost always the best option to select the last threed_filt_XX file. Once you are satisfied, click on the one you want to use and click ok.
- Now the 3D map you have selected should appear in the "Starting Models" section. It will appear something similar to "bdb:refine_03#threed_filt_05". There are 14 options on this window and we

have done our best to choose sensible defaults for all of them as per the FreAlign documentation. If you wish to learn more, there are brief descriptions of each option/parameter in the help text in the top of the window. If you wish to learn even more about each parameter, please consult the FreAlign documentation that can be found at: emlab.rose2.brandeis.edu/sites/default/files/readme_frealign.txt

- You can leave most of the options as they are in order to run FreAlign but there is one option that makes a big difference. The rrec option represents the resolution in angstroms that frealign will do a reconstruction to. For the purposes of these demos, if you are working with GroEL or MMCPN, a value of 10 angstroms is what you should use. For the Ribosome data, a value of 14 is good.
- When are you finished entering parameters and are satisfied, click ok. The process takes very little time, at most 20 seconds.

Running e2runfrealign.py

- Select the Run – e2runfrealign command. This will open a window displaying all of the FreAlign directories that have been used to run FreAlign.
- Click on "Browse To Add" and a list of all of the EM types in the current directory are displayed. For this, you will want to select the frealign directory you want to run frealign on. These directories are numbered sequentially automatically, with the most recent run of e2refinetofrealign.py corresponding with the highest-numbered frealign directory. Select the directory you want to use and click ok.
- The directory you selected should have been added to the "FreAlign Dirs" section. Make sure it is selected and click ok. This process takes a while depending on how many particles are in your reconstruction, the speed of your machine, etc.

Running e2refinefromfrealign.py

- Select the Run – e2refinefromfrealign task. This will open a window similar to the e2runfrealign task that lists all of the frealign directories that are associated with this project.
- Select the frealign directory you wish to use, in this case it will be whichever directory you used in the e2runfrealign step, and click ok. This step should take hardly any time at all.

Examining the Output from FreAlign

e2refinefromfrealign.py produces several output files:

- It converts the FreAlign output 3D map into a map that is normalized so that it can be compared to the initial EMAN2 3D map. This 3Dmap will be called OutputMap_Normalized_XX.mrc. To view this 3D map, use the workflow's "Browse" task and select it from the appropriate frealign_XX directory. In that directory also exists the output from FreAlign. To learn more about this output, please go to the FreAlign doc: emlab.rose2.brandeis.edu/sites/default/files/readme_frealign.txt
- e2refinefromfrealign also generates a diff.txt file that contains, for each particle, the difference between the values for the euler angles and shifts entered into FreAlign and what it aligned them to.
- Also created by e2refinefromfrealign is the fourier shell correlation between the EMAN2 3D map and the FreAlign 3D map. e2refinefromfrealign also parses the FreAlign output and converts the FSC that FreAlign creates to the format and location that the EMAN2 workflow needs. To view these FSC's, select the Resolution task. It will open the Project Resolution Report which will list all directories that contain FSC curves. On this list you should see the frealign directory that you have been working with. Two columns over from the frealign name is the e2eotest column. This will list the resolution to which the 3D map from FreAlign was calculated to. Double click on the directory and a curve will appear. The thick line represents the FSC calculated by FreAlign which represents the FSC between even and odd particle reconstructions. The thin line is the FreAlign/EMAN2 FSC.