Map denoising using LAFTER

LAFTER (Local Agreement Filter for Transmission EM Reconstructions) is a program for denoising of single particle reconstructions from cryoEM. Single particle analysis (SPA) entails the reconstruction of high-resolution volumes from tens of thousands of particle images with low individual signal-to-noise. Imperfections in this process result in substantial variations in the local signal-to-noise ratio within the resulting reconstruction, complicating the interpretation of molecular structure. LAFTER uses a local denoising filter which can improve the interpretability of cryo-EM maps.

LAFTER is based on a pair of serial real-space filters. It compares independent half-set reconstructions to identify and retain shared features that have power greater than the noise. It is capable of recovering features across a wide range of **signal-to-noise** ratios.

In this example we will use a structure of the human gamma-secretase complex at 3.4 Å resolution (EMD-3061)*.

In the typical workflow for LAFTER you require the two independent half maps from the SPA reconstruction. These maps should be unsharpened and unfiltered. Ideally the mask used in the SPA process should be used. The half maps for EMD-3061 were deposited and are available for download from the EMDB, and they are provided here. We have also include the original map and the PDB model for reference:

> Required
> Half Map 1:   EMD-3061-half-1.map
> Half Map 2:   EMD-3061-half-2.map
>
> For reference
> Full map: EMD-3061-full.map
> Model: 5a63.pdb

You can find these in the LAFTER tutorial data.

Part 1) Creating a mask using RELION

In this case no mask is available from the EMDB so we will generate one from the deposited full map using *relion_mask_create*. We will do this via the command line. Maps in the common MRC format (https://www.ccpem.ac.uk/mrc_format/mrc2014.php) often have either .mrc or .map extensions. However, *relion_mask_create* requires maps to have a .mrc extension. To make a copy with the correct extension, go to the LAFTER tutorial data directory in a command line terminal and run:

```
cp EMD-3061-full.map EMD-3061-full.mrc
```

The mask should not be too tight or sharp as this can introduce high-resolution artefacts in denoising. To avoid this we will low pass filter the map to 15Å, extend the mask by two pixels and add a soft edge of five pixels with the following command:

```
relion_mask_create --i EMD-3061-full.mrc --lowpass 15
--extend_inimask 2 --width_soft_edge 5
```
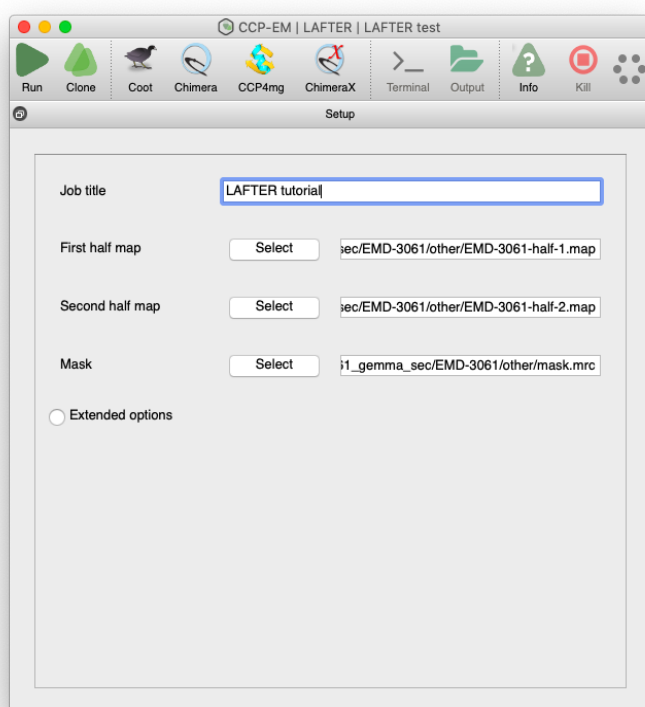
This will create the mask: mask.mrc. Use Chimera to compare the full map and the mask, looking to make sure no connected map density appears through the mask surface.

Part 2) Denoising using LAFTER

```
1) From the main GUI launch the LAFTER task window.

2) Enter the following parameters and hit run:
        First half map:       EMD-3061-half-1.map
        Second half map:      EMD-3061-half-2.map
        Mask:                 mask.mrc
```
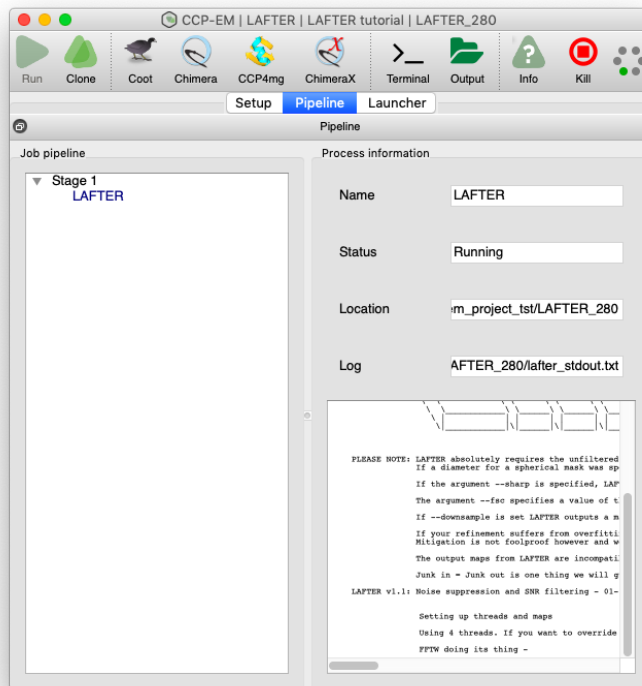

Note: LAFTER is capable of running without a mask if you input the particle diameter in the "Extended options" section, but this is strongly discouraged. If you do this, LAFTER will simply use a soft spherical mask instead. This will normally include a substantial amount of the solvent region surrounding the particle, which will degrade LAFTER's performance by making the noise estimates less accurate and causing early termination of the algorithm due to reduced half-map FSC measurements.

```
3) Monitoring job progress in 'Pipeline' tab
```
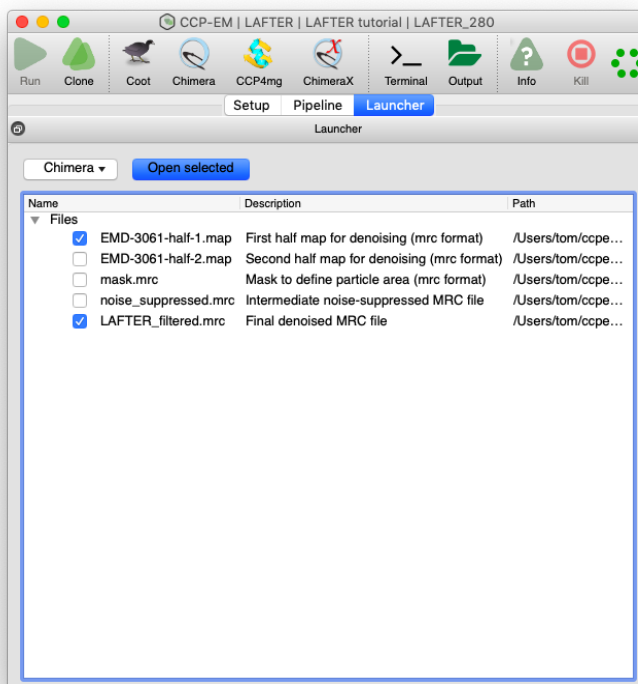

Whilst the job is running the green hexagon in the top right will rotate.

You can see the progression through the various stages in the pipeline on the left. Green is finished, blue running, grey yet to start (red failed).

Clicking on the stage will display the log file for that stage. Double clicking on the log file window will launch your default text editor to show the log file.

The computation is fast and should take less than 5 minutes on a modern laptop (e.g. 2 min on a MacBook Air).

```
4) Inspection of the results.
```


Click the 'Launcher' tab to see the output files from LAFTER, or simply click the 'Chimera' icon in the process task bar which will display the relevant files from the job. For further comparison manually load (File -> Open) the full map (EMD-3061-full.map) and the atomic model (5a63.pdb).

Which map looks best to you? Is there any improvement in the LAFTER_filtered map compared to the full map, and if so what are those?

How would this help for manual model building, e.g. using Coot or Chimera?

For downstream usage of the LAFTER filtered maps, we recommend using them for interactive model building and visual structure interpretation. However for automated refinement (e.g. REFMAC or phenix.real_space_refine) the original unfiltered maps should be used. This ensures correct calculation of the atomic displacement parameters (ADPs or B-factors).

Part 3) Second example of denoising using LAFTER - Beta-galactosidase

In this example we will use the beta galactosidase precalculated dataset from the RELION 3.1 tutorial. We have provided the following files from the tutorial:

        Required
        Half Map 1:   run_half1_class001_unfil.mrc
        Half Map 2:   run_half2_class001_unfil.mrc
        Mask: mask.mrc

        Reference
        Full map: postprocess.mrc
        Model: 5a1a_fitted.pdb (fitted using molrep)

You can find these in the LAFTER tutorial data.

```
1) From the main GUI launch the LAFTER task window.

2) Enter the following parameters and hit run:
     First half map:        run_half1_class001_unfil.mrc
     Second half map:       run_half1_class001_unfil.mrc
     Mask:            mask.mrc

     In "Extended options", select the "No oversampling" box.
```
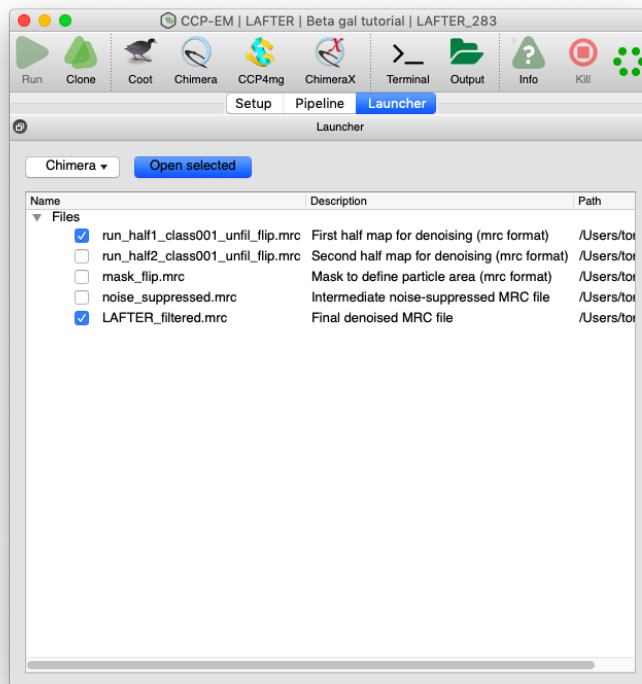
Note: by default, LAFTER oversamples the filtered maps by 2: the output box size is twice the input size, and the pixel size is halved. This improved the map smoothness for visualisation, but does create large output files. With larger maps, it is often more pragmatic to turn off the oversampling – but remember this means some useful map detail could be obscured by pixelation artefacts.

```
3) Inspection of results
```


Click the 'Launcher' to see the output from LAFTER, or simply click the 'Chimera' icon in the process task bar, which will display the relevant files from the job.

For further comparison manually load (File -> Open) the full map (postprocess_flip.mrc) and the atomic model (5a1a_fitted.pdb).

Which map looks best to you?
Are there any improvements in the LAFTER_filtered map compared to the globally sharpened map, and if so what are those?
How does this differ from the gemma secretase example and why?
Look at areas of weak density - for example try chain A around residue 732, how do the maps compare here and what is the cause of the differences?

LAFTER reference:
Ramlaul *et al.* (2019). A Local Agreement Filtering Algorithm for Transmission EM Reconstructions. *J Struct Biol* 205:30–40.

CCP-EM reference:
Burnley T, Palmer CM & Winn M (2017). Recent developments in the CCP-EM software suite. *Acta Cryst* D73:469–477.

RELION tutorial:
ftp://ftp.mrc-lmb.cam.ac.uk/pub/scheres/relion31_tutorial.pdf

Contact:

Do please report any issues or bugs.... it's much appreciated and helps us make the software better:

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