

Local map sharpening with LocScale

LocScale is a program for map sharpening. LocScale uses prior information from a refined atomic model to improve contrast of cryo-EM density maps. It can be helpful in the common case of resolution variation that manifests in many cryo-EM 3D reconstructions, and it can be used as an alternative to other commonly applied global map sharpening methods.

In this example we will use the *R. norvegicus* TRPV1 channel (EMD-5778) from the EMDB map and model challenge [1].

In the typical workflow for LocScale you would start off with a globally sharpened map such as e.g. obtained from Relion and cryoSPARC, or post-processing routines from other refinement packages. This map serves as your initial template for model building and model refinement. The model refinement step is essential as it is the distribution of the atomic displacement parameters (ADPs, or B-factors) that serves as the basis for the local of sharpening or blurring operations performed by LocScale.

To save time we have already refined the atomic model and computed a mask. You will use:

```
Map:          emd5778_unfilt.mrc
Mask:         emd5778_mask.mrc
PDB model:    pdb3j5p_rsref.pdb
```

You can find these in the LocScale tutorial data.

For the LocScale run it is advised to use the unsharpened and unfiltered map resulting from the 3D reconstruction, i.e. before any post-processing has been applied.

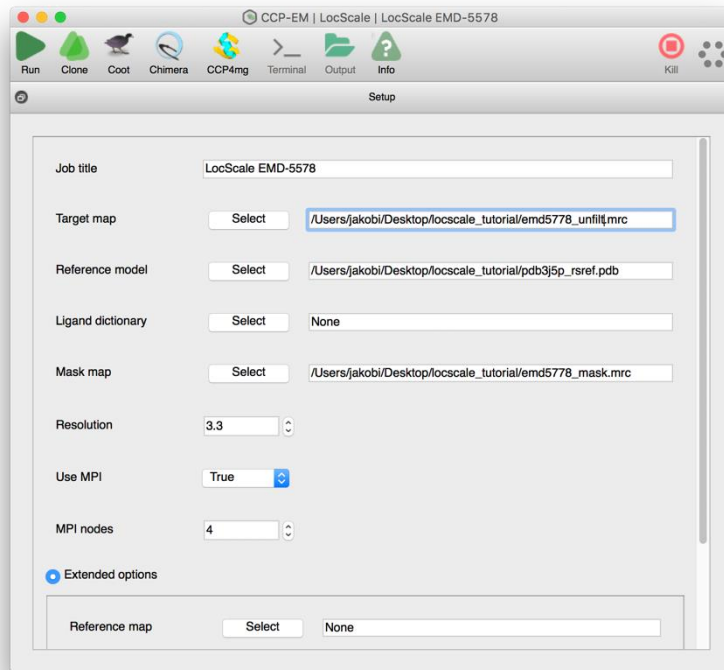
Part 1) Local sharpening using LocScale with default settings

1) From the main GUI launch the LocScale task window.

2) Enter the following parameters and hit run:

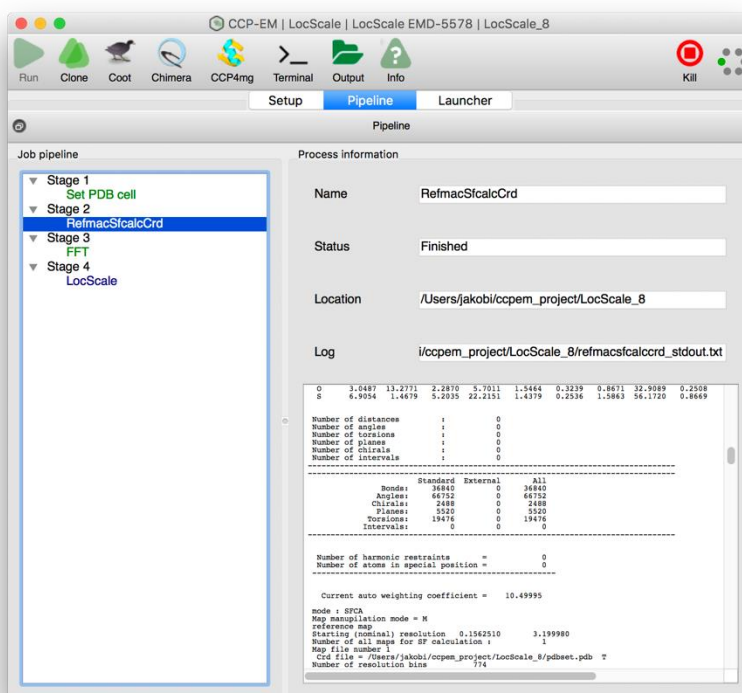
```
Target map:          emd5778_unfilt.mrc
Reference model:     pdb3j5p_rsref.pdb
Mask map:           emd5778_mask.mrc
Resolution:         3.2
Use MPI:             True
MPI nodes:          2
```

The computations can be sped up by using parallel processing via MPI. Choose this option if your computer has multiple processors available. It is not absolutely necessary to provide a mask, but using a mask will significantly speed up the computation.



Using a mask, the LocScale pipeline takes ~5 min (on MacBook Pro with two MPI threads) for this map.

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 log file for that stage. Double clicking on the log file window will launch your preferred text editor with the log file.

4) Inspection of the results. Click the the 'Launcher' to see the output from LocScale, or simply click the 'Chimera' icon in the process task bar, which will display all relevant files.

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

Part 2) Optimizing parameters for local sharpening

The LocScale pipeline applies a number of default settings that will provide the desired result in most cases. Nevertheless, there are several parameters in the LocScale pipeline that can be optimized to improve the sharpening operation. Let us try change two of these (the reference map resolution and the window size) and investigate their effect.

1) From the jobs list choose previous LocScale job. From the process task bar, choose clone.

2) Enter the following parameters, choose a resolution and hit run:

```
Target map:      emd5778_unfilt.mrc
Reference model: pdb3j5p_rsref.pdb
Mask map:       emd5778_mask.mrc
Resolution:    2.5
Use MPI:        True
MPI nodes:      2
```

3) From the process task bar, choose clone.

4) Enter the following parameters, choose a resolution and hit run:

```
Target map:      emd5778_unfilt.mrc
Reference model: pdb3j5p_rsref.pdb
Resolution:      3.2
Mask map:       emd5778_mask.mrc
Use MPI:        True
MPI nodes:      2
```

Check the box for "Extended Options" and choose a window size:

```
Window size:    40
```

5) Inspection of the results. Launch chimera, load all maps from the various runs and compare.

Which of the parallel runs with different input parameters gave you the best results? If you compare the results from the different runs what effect do the input parameters have?

Part 3) Dealing with incomplete models

Since LocScale uses model information, it is important to be aware of the effect and incomplete model or model errors can have on the result. We will consider two scenarios: One in which we only have a C-alpha trace of the the protein and one in which we have been able to build a model for only a part of the map.

1) From the main GUI launch the LocScale task window.

2) Enter the following parameters and hit run:

Input map: emd5778_unfilt.mrc
Resolution: 2.5
Reference model: pdb3j5p_ca.pdb
Mask map: emd5778_mask.mrc
Use MPI: True
MPI nodes: 2

3) From the process task bar, choose clone.

4) Enter the following parameters and hit run:

Input map: emd5778_unfilt.mrc
Resolution: 2.5
Reference model: pdb3j5p_incompl.pdb
Mask map: emd5778_mask.mrc
Use MPI: True
MPI nodes: 2

5) Inspection of the results. Launch chimera, load all maps from the various runs and compare.

How do these results compare to your previous runs? Does it make a big difference if including side chains or not? What could you modify to improve your results with an incomplete model?

LocScale references:

Jakobi et al., Model-based local density sharpening of cryo-EM maps. eLife 6:e27131, 2017
Gitlab repository: <https://gitlab.tudelft.nl/aj-lab/locscale>

CCP-EM reference:

Burnley, T., Palmer, C.M. & Winn, M. Recent developments in the CCP-EM software suite. *Acta Cryst D* 73, 469-47, 2017.

EMDB model challenge 2016 reference:

Lawson & Chiu. Comparing cryo-EM structures. *J Struct Biol* 204(3):523-526, 2018
https://challenges.emdataresource.org/?q=model_challenge

Contact:

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

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