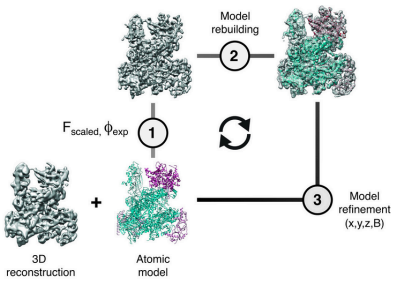
A brief introduction to the use of LocScale

What is LocScale?

LocScale is a reference-based local amplitude scaling tool that used prior model information to improve contrast of cryo-EM density maps.

To reliably reflect the required level of local sharpening and local resolution variation in the target map, the model used for scaling should have refined B-factors using appropriate protocols. The method is described in detail in a bioRxiv preprint [1].



LocRes - local resolution estimation based on windowed FSC calculation

LocScale - local sharpening based on reference-based amplitude scaling

The program is available here for Download .

[1] Jakobi et al. bioRxiv 121913 (2017)

LocScale performs local amplitude scaling based on a atomic reference structure.

Requires `sparx` , `EMAN2` , (`mpi4py`)

```
usage: locscale.py [-h] -em EM_MAP -mm MODEL_MAP -p APIX [-ma MASK]
                   [-w WINDOW_SIZE] -o OUTFILE [-mpi]

*** Performs local amplitude scaling against atomic reference ***

Optional arguments:
  -h, --help            show this help message and exit
  -em EM_MAP, --em_map EM_MAP
                        Input filename EM map
  -mm MODEL_MAP, --model_map MODEL_MAP
                        Input filename PDB map
  -p APIX, --apix APIX  pixel size in Angstrom
  -ma MASK, --mask MASK
                        Input filename mask
  -w WINDOW_SIZE, --window_size WINDOW_SIZE
                        window size in pixel
  -o OUTFILE, --outfile OUTFILE
                        Output filename
  -mpi, --mpi           MPI version call by: "mpirun -np 4 python locscale.py
                        -em map1.mrc -mm map2.mrc -ma mask.mrc -p 1.0 -w 10
                        -mpi -o scaled.mrc"
```

Link to source

Please follow these instructions to generate your model map with B-factor weighted structure factors and electron form factors.

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Model map generation from a PDB coordinate model

Produce locally contrast-optimized maps using LocScale requires a reference model map that accurately reflects the variation in resolution and contrast expressed in atomic B-factors. We therefore highly recommend to first perform coordinate and ADP refinement against a globally sharpened and locally filtered map as described in our bioRxiv preprint [1].

The model map can be generated by any procedure that computes the model map from B-factor weighted structure factors using electron scattering atomic form factors. We here provide a recommended procedure to generate the model map and check consistency with the experimental data using the provided `prepare_locscale_input.py` script.

In the following the procedure is illustrated for TRPV1 channel [2] from the EMDB Model Challenge [3]. All required files can be found here.

```
phenix.python prepare_locscale_input.py -mc pdb3j5p.pdb -em emd_5778_full.map
```

This procedure generates three files:

- pdb3j5p_4locscale.pdb (shifted) PDB model with same P1 unit cell as map
- `pdb3j5p_4locscale.mrc` **Reference model map**
- `emd_5778_full_4locscale.mrc` **(shifted) original reconstruction**

The two MRC maps are to be used for input in the LocScale procedure.

By default structure factors from the model are computed to Nyquist frequency. This is the preferred procedure for sharpening using LocScale.
A different resolution cut-off can be supplied using the `-dmin` flag. See

```
phenix.python prepare_locscale_input.py -mc pdb3j5p.pdb -em emd_5778_full.map -dmin 4.0
```

See `prepare_locscale_input.py` script for further options.

LocScale maps can be computed using any source of reference maps for scaling. If you prefer to use your own workflow to generate reference model maps, we do recommend to make sure your map complies with the following procedure.

Note:

CCTBX/CCP4 follow the ZXY convention for map axis order, whereas most EM programs follow XYZ. This will mostly not have any consequences for visualization, but it may cause issues in further processing. If you prefer to process in the XYZ convention please see here.

References:
[1] Jakobi et al. bioRxiv 121913 (2017)
[2] Liao et al., *Nature* **504**:107-112 (2013)
[3] EMDB Model Challenge 2015/2016

If using a reference map not generated by `prepare_locscale_input.py` please make sure the reference map complies to the following.

(1) The EM map should be zero-based, i.e. the map origin should be located at (0,0,0). The `check_and_set_ori_zero.py` script is a quick way to ascertain the map is zero-based.

```
% python check_and_set_ori_zero.py -m emd_5778.mrc
```

(2) If the map was already zero-based this step can be skipped. If the map was shifted in (1), the PDB model should be shifted by the same amount in real coordinates (i.e. voxel_shift*pixel_size). In the present case the shift vector is [128 128 128] voxels. With a pixel size of 1.2156 Å therefore the model translationa vector is t = [155.5968 155.5968 155.5968] Å.

- Open model and map in Chimera (`chimera emd_5778.mrc pdb3j5p.pdb &`)
- Shift model to fit with zero-based map (`move 155.5968,155.5968,155.5968 model #1 coord #1`)
- Save model relative to map coordinates (`write format pdb relative #1 #0 pdb3j5p_4locscale.pdb`)

(3) Check if PDB symmetry matches that of EM map.

```
% e2iminfo.py -H emd_5778.map | grep -E 'MRC.xlen|MRC.ylen|MRC.zlen|MRC.alpha|MRC.beta|MRC.gamma'
```

```
MRC.alpha: 90.0
MRC.beta: 90.0
MRC.gamma: 90.0
MRC.xlen: 311.193603516
MRC.ylen: 311.193603516
MRC.zlen: 311.193603516
```

```
% cat pdb3j5p_4locscale.pdb | grep -E 'CRYST1|SCALE'
```

```
CRYST1    1.000    1.000    1.000  90.00  90.00  90.00 P 1           1
SCALE1      1.000000  0.000000  0.000000        0.00000
SCALE2      0.000000  1.000000  0.000000        0.00000
SCALE3      0.000000  0.000000  1.000000        0.00000
```

The symmetry mismatch will most likely cause problems in map generation. Reference map and experimental map should be sampled on the same grid spacing. The correct CRYST1 record in this case should be:

```
CRYST1  311.194  311.194  311.194  90.00  90.00  90.00 P 1
SCALE1      0.003213  0.000000  0.000000        0.00000
SCALE2      0.000000  0.003213  0.000000        0.00000
SCALE3      0.000000  0.000000  0.003213        0.00000
```