1. Install

Go into folder **ICONv1.5** and use command **./install** to generate 3 executable files, including **ICONPreProcess**, **ICON** and **ICONMask** which can be found in folder **bin.**

2. ICONPreProcess

This program preprocesses the projection file by subtracting the mode values of each projection image.

The parameters are described as followed:

##

-input : the projection file

-tiltfile : the tilt file

-output : the preProcessed projection file

-help : for help

##

For example:

./ICONPreProcess **-input** test.ali **-tiltfile** test.tlt **-output** preprocessed_test.ali

3. ICON

This program is a mpi program and performs a full ICON reconstruction and a cross validation process at the same time. Two folders named **crossValidation** and **reconstruction** will be created in the "**-outputPath**" (a parameter defined by user, see parameters description).

In folder **crossValidation**, five files will be created including: **a. GroundTruth.mrc** the omitted projection image in the lowest tilt angle (the smallest abs value); **b. crossV_reProjection.mrc** the re-projection image of the reconstruction generated by cross validation process; **c. fullRec_reProjection.mrc** the re-projection image of the reconstruction generated by full ICON reconstruction; **d. crossV.frc** the FRC calculated between GroundTruth.mrc and crossV_reProjection.mrc; **e. fullrec.frc** the FRC calculated between GroundTruth.mrc and fullRec_reProjection.mrc**. Notice:** crossV.frc and fullrec.frc will be used in **ICONMask**.

In folder **reconstruction,** a series of 2D full reconstruction slices (without mask) named **minxxxxx.mrc** will be generated. Such mrc files will be combined and masked to generate the final 3D reconstruction by **ICONMask**.

The parameters are described as followed:

##

-input : the projection file

-tiltfile : the tilt file

-outputPath : the path of a folder saving the result, two folder named "crossValidation" and "reconstruction" will be created inside.

-slice : the slices for reconstruction including 2 parts split by ',' . For example, 0,511 means that reconstruct 512 slices ranging from slice 0 to slice 511

-ICONIteration : the iteration number including 3 parts split by ',' . For example, 5,50,10 means that, firstly, reconstruct with INFR for 5 iterations to generate a stable initial value, and then reconstruct with ICON for 50 iterations, and at last reconstruct with INFR for 10 iterations for fidelity

-dataType : the type of dataset. There are two options : 1 for cryoET or plastic embedded ET; 2 for negatively stained ET ; default as 1

-threshold : the threshold used in ICON, default as 0

-help : for help

##

For example:

mpirun –n 8 ./ICON **-input** preprocessed_test.ali **-tiltfile** test.tlt **-outputPath** testFolder **-slice** 0,511 **-ICONIteration** 10,50,10 **-dataType** 1 **-threshold** 0

4. ICONMask

This program combines all the 2D reconstruction slices generated by **ICON** under a full reconstruction and masks out the unfaithful restored information based on the crossV.frc and fullRec.frc, which are also generated by **ICON**.

The parameters are described as followed:

##

-inputPath : the folder that contains all 2D reconstructed slices (named midxxxxx.mrc), normally corresponding to the **reconstruction** folder generated by ICON

-tiltfile : the tilt file

-output : the masked 3d reconstruction

-slice : the reconstructed slices for combination including 2 parts split by ','. For example, 0,511 means that combining 512 slices ranging from slice 0 (mid00000.mrc) to slice 511 (mid00511.mrc)

-thikness : the thickness of the final masked 3D reconstruction

-radius : the mask radius (in pixel) used to mask out the fourier space of the combined 3D reconstruction, if this option is used, then 'crossVfrc' and 'fullRecfrc' are not used

-crossVfrc : the frc file of cross validation process, if 'radius' is used, then this option is not used

-fullRecfrc : the frc file of full reconstruction process, if 'radius' is used, then this option is not used

-help : for help

##

For example:

./ICONMask **-inputPath** testFolder/reconstruction **-tiltfile** test.tlt **-output** masked_ICONreconstruction.mrc **-slice** 0,511 **-thickness** 200 **-crossVfrc** testFolder/crossValidation/crossV.frc **-fullRecfrc** testFolder/crossValidation/fullRec.frc