

ICON User Guide 1.6

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1. Installation

Go into the installation directory and run `./install` to generate 3 executable files in the directory of `bin`, including **ICONPreProcess**, **ICON** and **ICONMask**.

Please remember to add the following lines into your environment setting.

For B shell,

```
export PATH=<installation directory of ICON>/bin:$PATH
```

```
export LD_LIBRARY_PATH=<installation directory of ICON>/lib:$ LD_LIBRARY_PATH
```

For C shell,

```
setenv PATH <installation directory of ICON>/bin:$PATH
```

```
setenv LD_LIBRARY_PATH <installation directory of ICON>/lib:$ LD_LIBRARY_PATH
```

2. Usage of ICON

✓ Tilt series preprocess using **ICONPreProcess**

This program preprocesses the projection file by two steps. Firstly, subtracting the mode value of each projection image. Secondly (optional), normalizing the variance of each tilt image to be $0.33 \times \text{thickness} / \cos(\text{tilt-angle})$.

The parameters are described as follows.

-input	:	the tilt series.
-tiltfile	:	the file containing aligned tilt angle of each projection image. If this option is not used, then only subtract the mode value of projection images.
-thickness	:	the thickness of specimen in pixel. If this option is not used, then only subtract the mode value of projection images.
-output	:	the preProcessed projection file.
-help	:	for help.

For example:

```
./ICONPreProcess -input test.ali -output preprocessed_test.ali
```

or

```
./ICONPreProcess -input test.ali -tiltfile test.tlt -thickness 100 -output preprocessed_test.ali
```

Attention: It is recommended to run this step against the original tilt series before alignment but using the aligned tilt file. After preprocessing, you can run newstack in IMOD to generate a preprocessed and aligned tilt series.

✓ 3D reconstruction using ICON algorithm

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 the folder **crossValidation**, five files will be created including:

a. GroundTruth.mrc, the omitted projection image at the minimum 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.

Attention: crossV.frc and fullrec.frc will be used in **ICONMask**.

In the 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 (in Fourier domain) to generate the final 3D reconstruction by **ICONMask** in the next step.

The parameters of **ICON** are described as below:

-input : the aligned tilt series.

-tiltfile : the aligned tilt file.

- outputPath** : the path of a folder saving the result, two folder named “crossValidation” and “reconstruction” will be created inside.
- slice** : the slices of reconstruction that include 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 using INFR for 5 iterations to generate a stable initial value, and then reconstruct using ICON for 50 iterations, and finally reconstruct using INFR for 10 iterations for fidelity.
- dataType** : the type of dataset. There are two options: 1 for cryoET or plastic embedded ET (signal in black and background in white); 2 for negatively stained ET (signal in white and background in black); default as 1.
- threshold** : the threshold used in ICON, default as 0.3
- help** : for help
-
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One example of running ICON using 8 CPU cores:

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

✓ Verification filtering based on cross validation FRC (ICONMask)

This program generates the final verification filtered tomogram by combining all the 2D reconstruction slices from **ICON** and masking out the unfaithful restored information in Fourier domain based on the crossV.frc and fullrec.frc, which are 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 aligned 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).

- thickness** : the thickness of the final masked 3D reconstruction in pixel.
- radius** : the mask radius (in pixel) used in the Fourier domain of the combined 3D reconstruction. If this option is used, 'crossVfrc' and 'fullRecfrc' are not used.
- crossVfrc** : the FRC curve from the cross validation process. If 'radius' is used, this option is not used.
- fullRecfrc** : the FRC file from the full reconstruction process. If 'radius' is used, 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
```

3. Citation of ICON

Deng Y., Chen Y., Zhang Y., Wang S., Zhang F.* and Sun F.* (2016), ICON: 3D reconstruction with 'missing-information' restoration in biological electron tomography. *Journal of Structural Biology*XXX(X) : XXX-XXX. doi: 10.1016/j.jsb.2016.04.004.