
ICON 1.7.2 User Guide

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1. Installation

Go into the installation directory and run `./install` to install ICON.

The script `install` firstly extracts the `fftw-3.3.4.tar.gz` and `nfft-3.2.3.tar.gz` in the directory of **supportLib**. And then, it generates three static link libraries `libfftw3.a`, `libfftw3f.a` and `libnfft3.a`, and copies them into the directory of **lib**. Finally, it generates 4 executable files in the directory of **bin**, including **ICONPreProcess**, **ICON**, **ICONMask1** and **ICONMask2**.

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 three steps. Firstly (optional), a dose dependent low-passing filter will be applied to the projection file. Secondly, subtracting the mode value of each projection image. Thirdly (optional), normalizing the variance of each tilt image to be $0.33 \cdot \text{thickness} / \cos(\text{tilt-angle})$.

The parameters are described as follows.

-input (-i) : the tilt series.

-tiltfile (-t) : 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 (-th) : the thickness of specimen in pixel. If this option is not used, then only subtract the mode value of projection images.

-output (-o) : the preProcessed projection file.

-apix (-a) : the pixel size of projection images. If this option is not used, then no dose dependent filtering will be executed.

-dosefile (-df) : the file containing dose information of each projection image (each line for one image orderly). If this option is not used, then no dose dependent filtering will be executed.

-help (-h) : 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: (1) 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. (2) The first step (dose dependent low-passing filter) is optional and will be executed only when ‘-dosefile’ and ‘-apix’ are not empty; the third step (normalization) is optional and will be executed only when ‘-thickness’ is not empty.

✓ 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: (1) crossV.frc and fullRec.frc will be used in **ICONMask1** or **ICONMask2**. (2) For ICON 1.7.2, ICON can only reconstruct square tilt series with $n_x = n_y$, please clip the tilt series into square first.

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 **ICONMask1** or **ICONMask2** in the next step.

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

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- input (-i)** : the aligned tilt series.
 - tiltfile (-t)** : the aligned tilt file.
 - outputPath (-o)** : the path of a folder saving the result, two folder named “crossValidation” and “reconstruction” will be created inside.
 - slice (-s)** : 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 (-iter)**: 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 (-d)** : 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 (-thr)** : the threshold used in ICON, default as 0.03
 - help (-h)** : 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 (ICONMask1 or ICONMask2)**

Two programs, **ICONMask1** and **ICONMask2**, can be chosen to combine all the 2D reconstruction slices from **ICON** and generate a final verification filtered tomogram by masking out the unfaithful restored information in Fourier domain. The radius of mask is calculated according to the files of `crossV.frc` and `fullRec.frc`, which are generated by **ICON**. Different filtering strategies are used in **ICONMask1** and **ICONMask2**. For **ICONMask1**, the filtering is operated in a large 3D volume with the same size of the final tomogram. For **ICONMask2**, the filtering is operated on a series of sub-volumes and then all these sub-volumes will be combined into the final tomogram. **ICONMask2** is more robust and memory efficient and it is always recommended, especially for a large tomogram.

Notice: ICONMask1 in **ICON 1.7.2** can only deal with a reconstruction of the same X Y Z, which means the number of slices should be the same as the X/Y size of a `midxxxxx.mrc`.

ICONMask1

The parameters of **ICONMask1** are described as followed:

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- inputPath** (-i) : the folder that contains all 2D reconstructed slices (named `midxxxxx.mrc`), normally corresponding to the **reconstruction** folder generated by **ICON**.
 - tiltfile** (-t) : the aligned tilt file.
 - output** (-o) : the masked 3D reconstruction.
 - slice** (-s) : 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** (-th) : the thickness of the final masked 3D reconstruction in pixel.
 - radius** (-r) : 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** (-cf) : the FRC curve from the cross validation process. If '`radius`' is used, this option is not used.
 - fullRecfrc** (-ff) : the FRC file from the full reconstruction process. If '`radius`' is used, this option is not used.
 - help** (-h) : for help
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For example:

```
./ICONMask1 -inputPath testFolder/reconstruction -tiltfile test.tlt -output
```

masked_ICONreconstruction.mrc **-slice** 0,511 **-thickness** 512 **-crossVfrc**
testFolder/crossValidation/crossV.frc **-fullRecfrc** testFolder/crossValidation/fullRec.frc

ICONMask2

The parameters of **ICONMask2** are described as followed:

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- inputPath** (-i) : the folder that contains all 2D reconstructed slices (named midxxxxx.mrc), normally corresponding to the **reconstruction** folder generated by ICON.
 - tiltfile** (-t) : the aligned tilt file.
 - output** (-o) : the masked 3D reconstruction.
 - slice** (-s) : 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** (-th) : the thickness of the final masked 3D reconstruction in pixel.
 - radius** (-r) : 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.
 - gaussWidth** (-gw) : the width of gaussian edge of the soft mask (in pixel). If '-crossVfrc' & '-fullRecVfrc' are used, the default value is calculated according to FRC0.3 - FRC0.5; if '-radius' is used, the default value is 10.
 - crossVfrc** (-cf) : the FRC curve from the cross validation process. If 'radius' is used, this option is not used.
 - fullRecfrc** (-ff) : the FRC file from the full reconstruction process. If 'radius' is used, this option is not used.
 - zshift** (-z) : the shift (in pixel) of sample in Z axis, default as 0.
 - nomask** (-nm) : if this value is set to 0 then a validation filtering mask will be executed; otherwise, no validation filtering mask will be executed, default as 0.
 - blockSize** (-bs) : the size of sub-volume (a cube mask), default as 150.
 - help** (-h) : for help
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For example:

```
./ICONMask2 -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* 195(1): 100-112. doi: 10.1016/j.jsb.2016.04.004.