FIRT User Guide 1.5

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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 **FIRTPreProcess**, **FIRT** and **FIRTMask.**

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

For B shell,

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

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

For C shell,

setenv PATH <installation directory of FIRT>/bin:$PATH

setenv LD_LIBRARY_PATH <installation directory of FIRT>/lib:$ LD_LIBRARY_PATH

2. Usage of FIRT

✓ **Tilt series preprocess using FIRTPreProcess**

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*thickness/cos(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:

    ./FIRTPreProcess **-input** test.ali **-output** preprocessed_test.ali

    or

    ./FIRTPreProcess **-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 FIRT algorithm

This program is a MPI program and performs a full FIRT 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**, a folder named **reProjection** will be created. In the folder **reProjection**, three types of files will be created:

a. GroundTruth.mrc, the omitted projection image in the minimum tilt angle (the smallest abs value);

b. crossVReproj_iterXXX.mrc, the re-projection image of the reconstruction generated by cross validation process under the XXX-th iteration;

c. fullRecReproj_iterXXX.mrc, the re-projection image of the reconstruction generated by full FIRT reconstruction under the XXX-th iteration.

Besides, in the folder **crossValidation**, two types of files will also be created:

a. crossV_iterXXX.frc, the FRC calculated between GroundTruth.mrc and crossVReproj_iterXXX.mrc;

b. fullRec_iterXXX.frc, the FRC calculated between GroundTruth.mrc and fullRecReproj_iterXXX.mrc;

Attention: crossV_iterXXX.frc and fullRec_iterXXX.frc will be used in **FIRTMask**.

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 to generate the final 3D reconstruction by **FIRTMask** in the next step.

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

-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.

-FIRTIteration : the iteration number of FIRT = ND + ART, default as 20.

-ARTIteration : the iteration number of ART during one FIRT iteration, default as 1.

-ARTLambda : the constant coefficient to control the step of update in each ART iteration, default as 1.

-NDIteration : the iteration number of ND during one FIRT iteration, default as 200.

-NDLambda : the constant coefficient to control the step of update in each ND iteration, default as 0.2.

-NDK : the threshold value to control the diffusion intensity.

-help : for help

One example of running FIRT using 8 CPU cores:

   mpirun –n 8 ./FIRT **-input** preprocessed_test.ali **-tiltfile** test.tlt **-outputPath** testFolder **-slice** 0,511 **-FIRTIteration** 20 **-ARTIteration** 1 **-ARTLambda** 1 **-NDIteration** 200 **-NDLambda** 0.2 **– NDK** 200

   ✓   **Verification filtering based on cross validation FRC (FIRTMask)**

   This program generates the final verification filtered tomogram by combining all the 2D reconstruction slices from **FIRT** and masking out the unfaithful restored information in Fouried domain based on the crossV_iterXXX.frc and fullRec_iterXXX.frc, which are generated by **FIRT**.

   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 FIRT.

-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:

> ./FIRTMask **-inputPath** testFolder/reconstruction **-tiltfile** test.tlt **-output** masked_FIRTreconstruction.mrc **-slice** 0,511 **-thickness** 200 **-crossVfrc** testFolder/crossValidation/crossV_iter19.frc **-fullRecfrc** testFolder/crossValidation/fullRec_iter19.frc

3. Citation of FIRT

Chen Y., Zhang Y., Zhang K.*, Deng Y., Zhang F.* and **Sun F.*** (2016), FIRT: filtered iterative reconstruction technique with information restoration. *Journal of Structural Biology* XXX(X) : XXX-XXX. doi: 10.1016/j.jsb.2016.04.015.