FIRT User Guide 1.5.3

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1.   Installation

Go into the installation directory and run **./install** to install FIRT.

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 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   (-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.
-help	(-h):	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 the 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 at 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 (in Fourier domain) to generate the final 3D reconstruction by **FIRTMask** in the next step.

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

-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.

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

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

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

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

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

-NDK   (-nk):   the threshold value to control the diffusion intensity.

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

-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

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* 195(1): 49-61. doi: 10.1016/j.jsb.2016.04.015.