User manual for TYGRESS

Tomography-Guided 3D Reconstruction of Subcellular Structures (TYGRESS) is a hybrid method, which combines the advantageous features of both single particle cryo-EM and cryo-ET/subtomogram averaging to provide high-resolution structures of macromolecular complexes within their native, cellular environment. In this method, one typical single particle cryo-EM micrograph is acquired (i.e., with an electron dose that is higher than for individual tilt series images) just prior to a traditional low dose cryo-ET tilt series. The cryo-ET data are processed, including subtomogram averaging of the particle of interest. The parameters, i.e. the particle position and alignment, are then used to guide the particle picking in the initial single particle cryo-EM data set and to initialize the angular alignment parameters. This guiding information from ET, allows the application of the single particle reconstruction technique to complex subcellular samples (despite of the superposition of many structures in the single-particle projection image).

Requirements/computational environment:
Before installation of TYGRESS, please ensure that the following software packages are installed.

MATLAB: Major software for running TYGRESS
IMOD & PEET: Tilt series alignment, tomogram generation & Subtomogram averaging
(http://bio3d.colorado.edu/imod/ & http://bio3d.colorado.edu/PEET/)
FREALIGN: 3D reconstruction
CTFFIND3: Detect defocus value
Bfactor: Apply a B-factor value for the final reconstruction (http://grigorifflab.janelia.org/frealign)
EMAN2: 'normalization' function (http://blake.bcm.tmc.edu/EMAN2/)

After downloading the TYGRESS program, assign its path to Matlab. Modify the parameters in the file named **'TYGRESS_input.txt'** (as seen above) before running TYGRESS.

How to use TYGRESS:
Step 1. Download TYGRESS package and unzip it.
Step 2. Prepare two TYGRESS text files (please see example file for details):
  A) One file is named '**TYGRESS_input.txt**', which contains all the parameters to run TYGRESS, such as the file path, file name, 3D reconstruction parameter, etc…
  B) The other file is named **'DataList.txt'**, which contains the data set names ('main name' + 'sub name') and the total model number.
Step 3. Modify the 'ctffind3.csh' file in the TYGRESS code folder (file used to determine the CTF) (please see example file for more details; about how to modify this file please visit http://grigorifflab.janelia.org/sites/default/files/readme_ctf.txt).
Step 4. Replace the path of your Frealign installation in 'mreconstruct_noMask.com'.
Step 5. Open the Matlab interface, then in Matlab open the TYGRESS_GUI; input the path of the TYGRESS parameter file (TYGRESS_input.txt) and DataList (DataList.txt) files. Click yes on the corresponding step to have TYGRESS run that step (please see figure 1). All steps should be run in order if it is the first time you are running your data through TYGRESS. Final 3D reconstructions are performed using FREALIGN and all outputs are saved into the 'Reconstruction' folder. (For more information about FREALIGN, please visit http://grigorifflab.janelia.org/frealign).
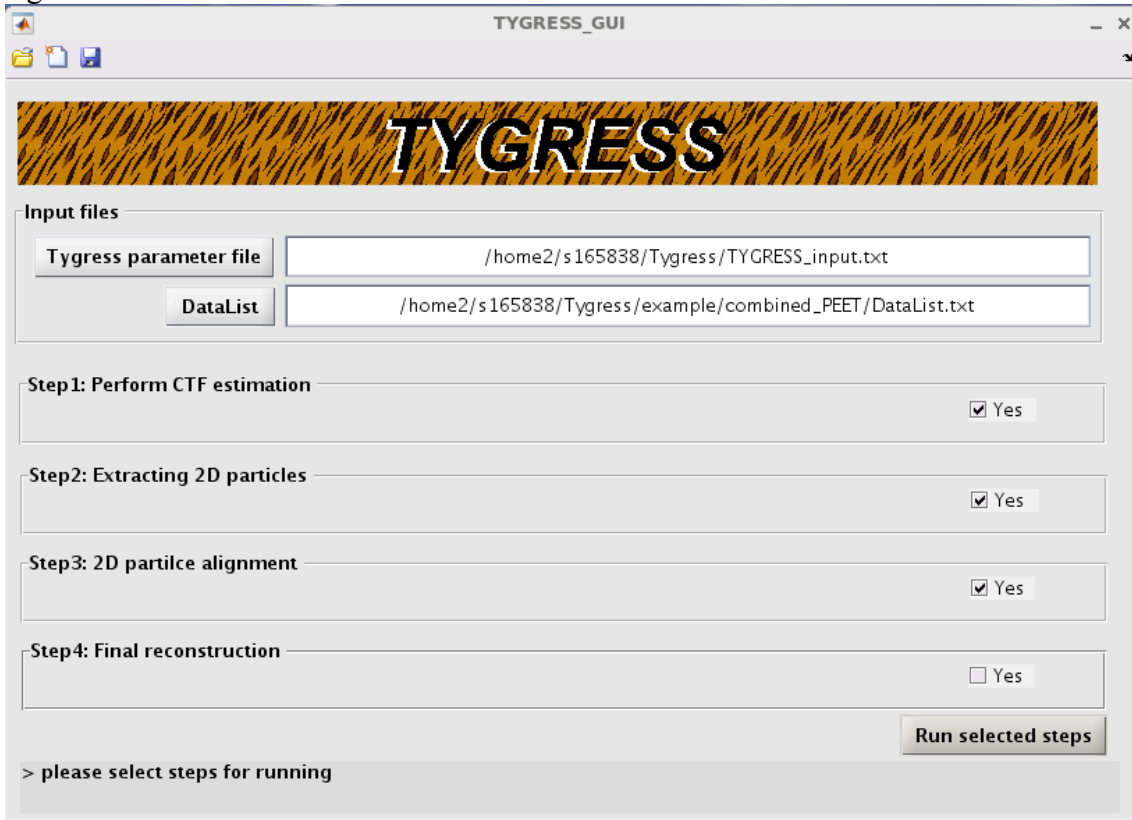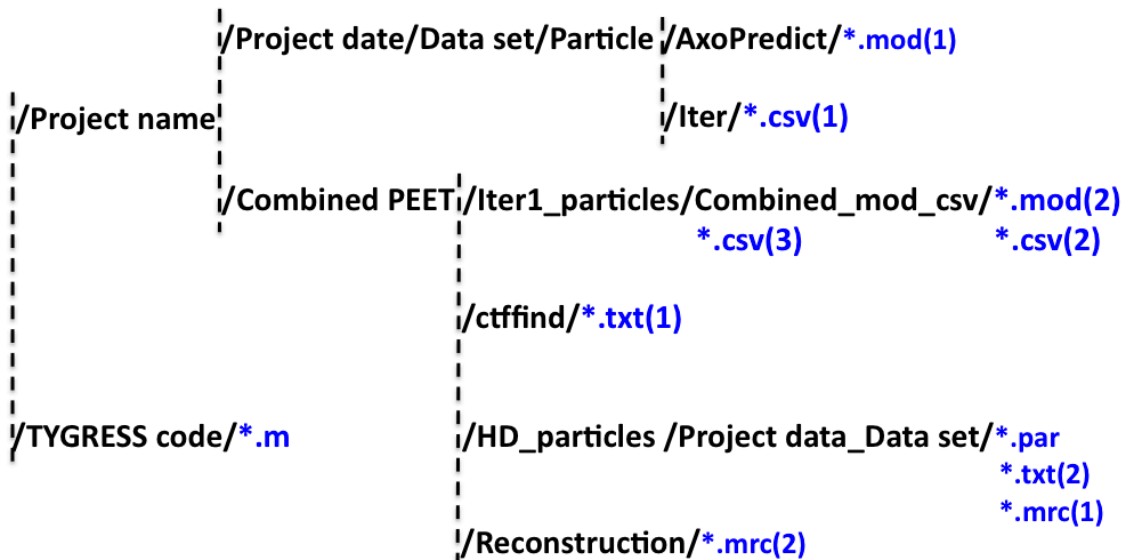
Figure 1: TYGRESS GUI

Figure 2: Directory tree of TYGRESS

Please note:
- .mod(1) and .csv(1) is parameter after running PEET for individual tilt series
- .mod(2) and .csv(2) is parameter for running PEET of entire data, and .csv(3) is the output
- .csv(3) and mod(2) are used for TYGRESS alignment and generate .par file
- .mrc(1) is 2D particle data set and .mrc(2) is final output 3D sturcutre
- .txt(1) is the parameter of defocus and .txt(2) is coordinates parameter of each particle

- .m is code for running TYGRESS
- <u>Project date</u> folder contains all tilt series data; each tilt series is processed (alignment and reconstruction in IMOD) in its own folder; particles folder including AxoPredict and Iter folder (alignment results from individual PEET runs).
- <u>CombinedPEET</u> folder includes combined alignment for all 3D subtomogram particles in PEET (Iter1_particles); Combined_mod_csv contains the file for running PEET for entire data set; 2D HD particles are picked in each data set under HD_particles folder. The defocus detection for HD images is put in ctffind folder.
- <u>TYGRESS code</u> folder stores all codes needed for running TYGRESS. The extension 'm' are Matlab scripts, which can be only executed in Matlab. The structure is output as a '.mrc' file.
- <u>Reconstruction</u> folder stores all output results after reconstruction with Frealign.

Additional useful tools for preparing files used for running PEET on the whole data set;

If you need the Matlab code to prepare your parameter files for running subtomogram averaging, please do the following:

In a Matlab window, go to your TYGRESS code directory and input command 'TYGRESS_scripts(TYGRESS_input.txt, DataList.txt,[1 0 0 0 0])'

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Please send an email to zhiguo.shang@utsouthwestern.edu if you have any question about running TYGRESS

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