

CiCLE講習会・蛋白研セミナー「単粒子解析リモート講習会」

2021/01/18-2021/01/19

**CiCLE**

Cyclic Innovation for Clinical Empowerment

# Tutorial of Relion 3.1 : Innexin-6 2D-3D classification

Takeshi Kawabata (Institute for Protein Research, Osaka Univ.)

川端 猛 (大阪大学 蛋白質研究所)

2021/01/19



# MPI process, threads and GPU

What is **Number of MPI process** ?

What is **Number of threads** ?

- Many **processes** are running at the same time in Unix.  
(You can check by top command)
- Each process uses one or more **threads**.
- Threads for the same process can access the same memory space.

$$[\text{Nprocess}] \times [\text{threads for each process}] \leq [\text{Total Nthreads in machine}]$$

- Each process use GPU (not thread). Many processes can share one GPU.

$$[\text{Nprocess}] \geq [\text{N\_GPU}]$$

Refine3D requires

$$\text{Even number of GPUs,} \\ [\text{Nprocess}] = [\text{N\_GPU}] + 1$$

# 011: 2D classification of many particles [2GPUs, 23 min]

**I/O** CTF Optimisation Sampling Helix Compute Running

Input images STAR file:

Continue from here:

**2D Classification**

- 3D initial model
- 3D classification
- 3D auto-refine
- 3D multi-body
- CTF refinement
- Bayesian polishing
- Mask creation
- Join star files
- Particle subtraction
- Post-processing
- Local resolution
- External

Alias: "refauto\_manu\_50mic\_64pix"

**I/O** CTF **Optimisation** Sampling Helix Compute Running

Number of classes:  20

Regularisation parameter T:

Number of iterations:

Use fast subsets (for large data sets)?

Mask diameter (A):  160

Mask individual particles with zeros?

Limit resolution E-step to (A):

**I/O** CTF Optimisation Sampling Helix **Compute** **Running**

Use parallel disc I/O?

particles:

into RAM?

directory:

ough disc?

Use GPU acceleration?

Which GPUs to use:

Input your GPU numbers.  
Let's use 2 GPUs !

Input your GPU numbers.  
Let's use 2 GPUs !

**I/O** CTF Optimisation Sampling Helix Compute **Running**

Number of MPI procs:  3

Number of threads:  2

Submit to queue?

Queue name:

Queue submit command:

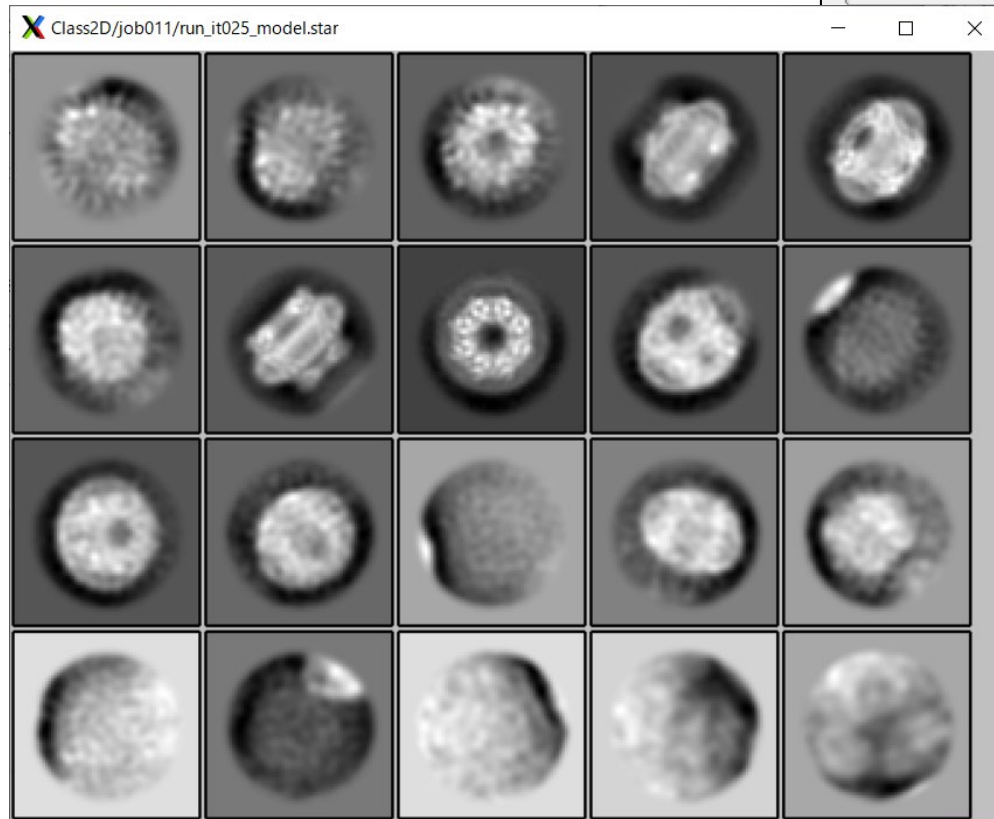
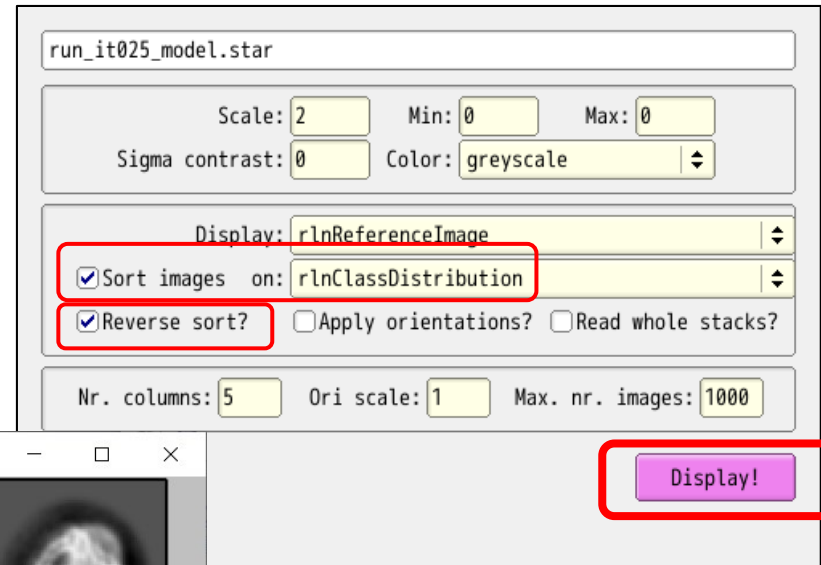
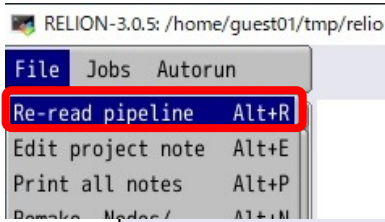
Standard submission script:

Minimum dedicated cores per node:

Additional arguments:

$$[\#MPI] = [\#GPU] + 1$$

# 011:Reference-free 2D class averaging



# 012: Subset selection from 2D classes

The screenshot shows the 'I/O' tab of a software interface. A red box highlights the 'I/O' tab label. Below it, a 'Subset selection' box lists various processing steps, with '2D classification' selected. The main area contains input fields for selecting classes from a model.star file, with the path `..ss2D/job011/run_it025_model.star` entered. Below this, the path `Class2D/refauto_50mic_64pix/run_it025_model.star` is displayed. At the bottom, a 'Current:' field contains `Class2D_refauto_50mic_64pix`. A 'Run!' button is highlighted with a red box. A text label 'Alias: "Class2D\_refauto\_50mic\_64pix"' is positioned above the 'Run!' button.

File Jobs Schedules **I/O** Class options Subsets Duplicates Running

Import  
Motion correction  
CTF estimation  
Manual picking

**Subset selection**

2D classification  
3D initial model  
3D classification  
3D auto-refine  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

Select classes from model.star: `..ss2D/job011/run_it025_model.star` ? Browse

OR select from micrographs.star: ? Browse

OR select from particles.star: ? Browse

OR select from picked coords: ? Browse

Class2D/refauto\_50mic\_64pix/run\_it025\_model.star

Alias: "Class2D\_refauto\_50mic\_64pix"

Schedule Check command **Run!**

I/O view Job actions Current: `Class2D_refauto_50mic_64pix` Display:

The screenshot shows the configuration dialog for `run_it025_model.star`. A red box highlights the 'Scale' field with the value '2'. Another red box highlights the 'Sort images on:' dropdown menu, which is set to 'rlnClassDistribution'. A third red box highlights the 'Nr. columns:' field with the value '5'. A 'Display!' button is also highlighted with a red box.

run\_it025\_model.star

Scale: 2 2 Max: 0

Sigma contrast: 0 Color: greyscale

Display: rlnReferenceImage

Sort images on: rlnClassDistribution  Sort images on: rlnClassDistribution

Reverse sort?  Apply orientations?  Read whole stacks?

Nr. columns: 5 5 le: 1 Max. nr. images: 1000

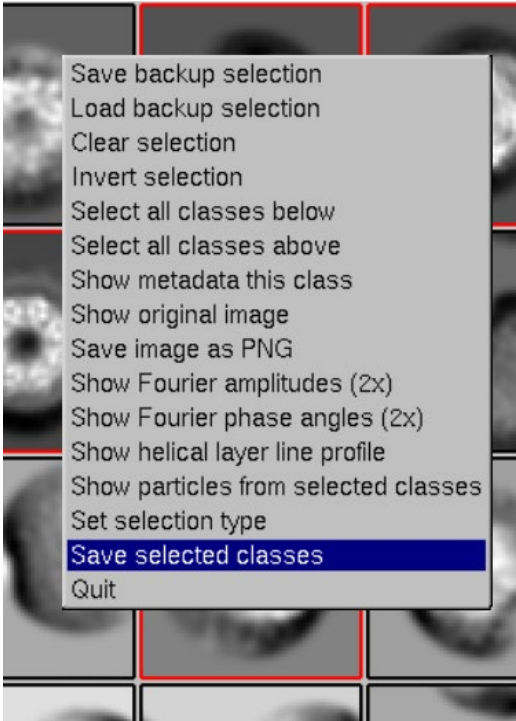
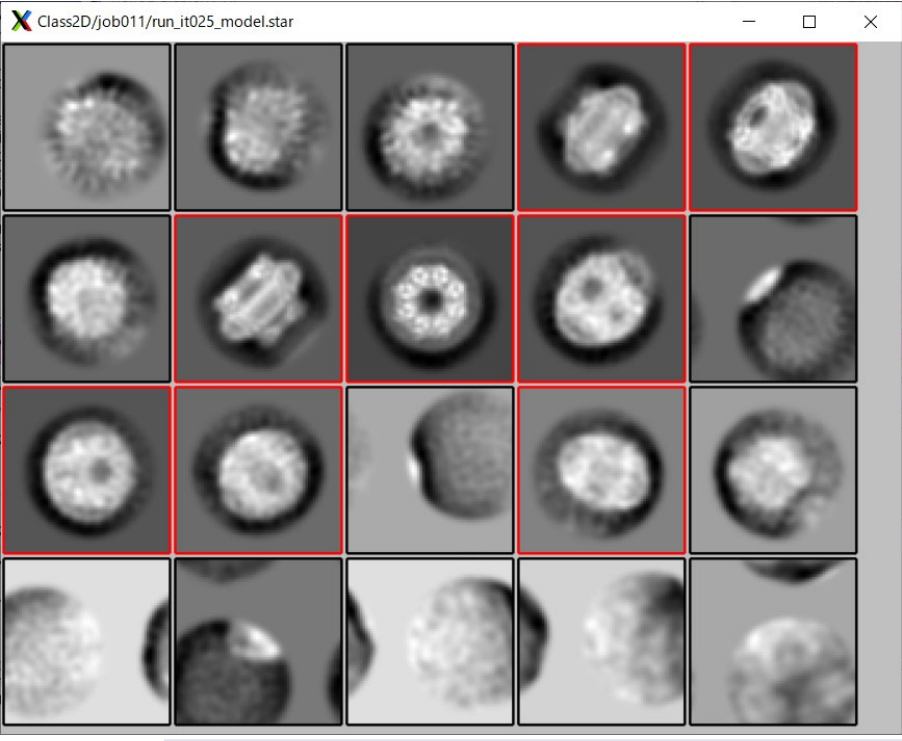
Max nr selected parts per class: -1

**Display!**

# 012: Subset selection from 2D classes

Among the 20 classes, select a few nice-looking classes, by the left button of the mouse. Selected classes are indicated by red boxes.

Click the right mouse button, and Select [Save selected classes]



```
Reading in all images...
0/ 0 sec .....~(,">
Written out Select/job012/backup_selection.star
Saved Select/job012/class_averages.star with 8 selected images.
Saved Select/job012/particles.star with 14932 selected particles.
```

Saved Select/job012/class\_averages.star with **8** selected images.  
Saved Select/job012/particles.star with **14932** selected particles.

# 013:De novo 3D model generation

[1GPU, 10 min]

The screenshot shows the 'I/O' tab of the software interface. The '3D initial model' option is selected in the left-hand menu. A text box points to this option with the label '3D Initial model'. The main area shows the 'Input images STAR file:' field with the path 'Select/job012/particles.star' highlighted by a red box. Below it, another text box contains the path 'Select/Class2D\_refauto\_50mics\_64pix/particles.star'. The 'Optimisation' tab is also visible and highlighted with a red box. At the bottom, the 'Current:' field shows 'C8\_Class2D\_refauto\_50mic\_64pix' highlighted with a red box. A text box at the bottom left contains the alias 'Alias : C8\_Class3D\_refauto\_50mic\_64pix'.

*reliion\_refine* will be executed.

The screenshot shows the 'Optimisation' tab of the software interface. The 'Number of classes:' is set to 1, 'Mask diameter (A):' is set to 160, and 'Symmetry:' is set to C8. Other parameters include 'Initial angular sampling: 15 degrees', 'Offset search range (pix): 6', and 'Offset search step (pix): 2'. The 'I/O' and 'CTF' tabs are also visible.

The screenshot shows the 'SGD' and 'Compute' tabs of the software interface. The 'SGD' tab is highlighted with a red box. Parameters include 'Number of initial iterations: 50', 'Number of in-between iterations: 200', 'Number of final iterations: 50', and 'Write-out frequency (iter): 10'. The 'Compute' tab is also highlighted with a red box. Parameters include 'Use parallel disc I/O?: Yes', 'Number of pooled particles: 3', 'Skip padding?: No', 'Skip gridding?: No', 're-read all particles into RAM?: No', 'particles to scratch directory:', 'iterations through disc?: No', 'Use GPU acceleration?: Yes', and 'Which GPUs to use: 0'. A text box points to the 'Use GPU acceleration?' field with the label 'Input your GPU number'. The 'Running' tab is also visible and highlighted with a red box. Parameters include 'Number of MPI procs: 1', 'Number of threads: 6', 'Submit to queue?: No', 'Queue name: openmpi', 'Queue submit command: qsub', 'Job submission script: reliion-3.1', and 'Allocated cores per node: 24'. A 'Run!' button is visible at the bottom right.



# How to display 3D density map(\*.mrc)

1) Focus the file browser at the left side of MobaXterm

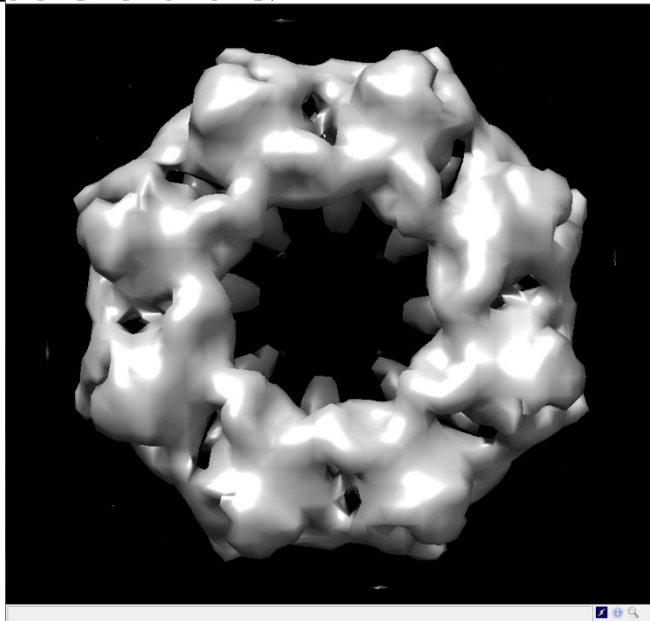
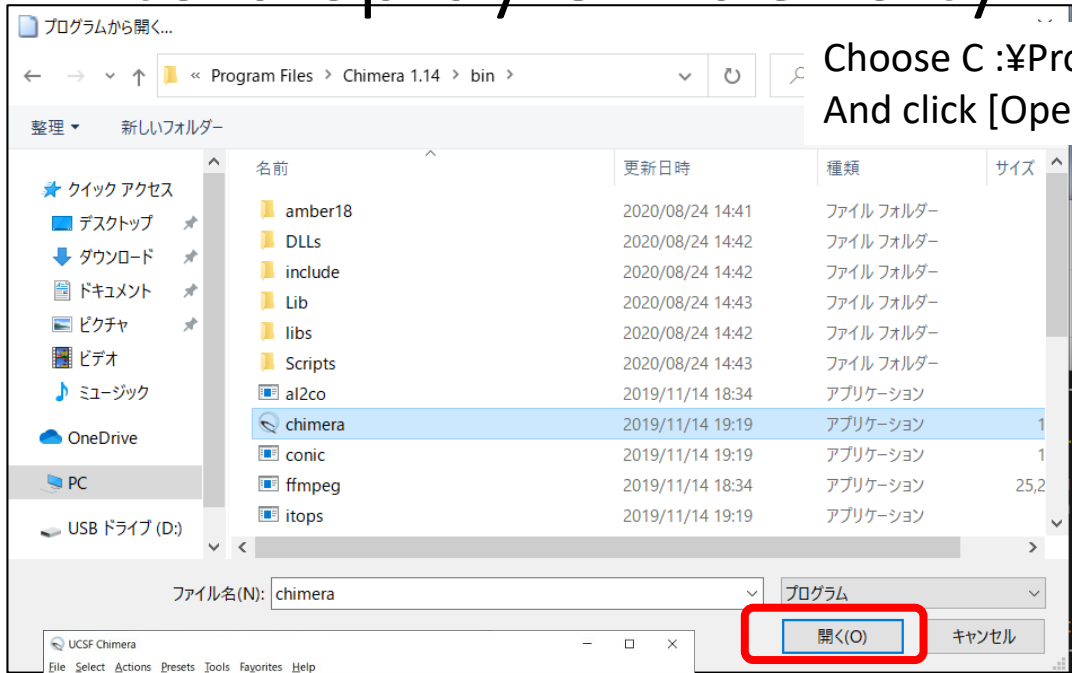
The screenshot shows the MobaXterm interface with a file browser on the left and a terminal window in the center. The file browser is displaying the directory `/home/guest01/EMPIAR-10291_50mic/Ini`. A file named `run_it300_class001.mrc` is selected and highlighted with a red box. A right-click context menu is open over this file, with the `Open with...` option highlighted by a red box. To the right, a dialog box is displayed with the message "この .mrc ファイルを開くには新しいアプリが必要です" (A new application is required to open this .mrc file). The dialog has a button labeled "その他のアプリ ↓" (Other applications ↓) highlighted with a red box. Below this, another dialog box shows a list of applications: Internet Explorer, Windows Media Player, Windows フォト ビューアー, ペイント, メモ帳, and ワードパッド. The button "この PC で別のアプリを探す" (Search for another application on this PC) is highlighted with a red box.

2) Choose `~EMPIAR-10291_50mic/InitialModel/run_it300_class001.mrc`  
Click the right mouse button and chose [Open with...]

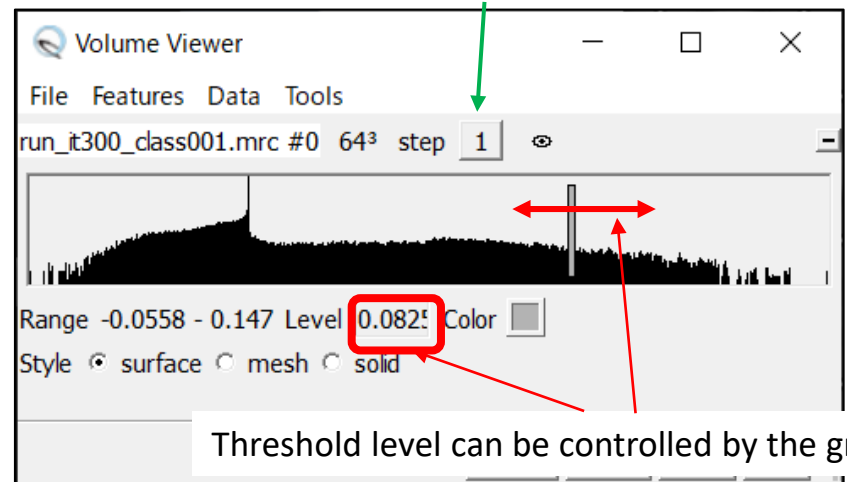
3) Setup application to open \*.mrc file



# How to display 3D density map(\*.mrc)



[step] controls number of minimum voxels for display.  
If step=2, 2x2x2 voxels are unified into one voxel.



# 014: 3D classification

[2GPUs, 12 min]

I/O Reference Select/Class2D\_refauto\_50mics\_64pix/particles.star

Input images STAR file: Select/job012/particles.star Browse

Continue from here: Browse

Reference map: rel/job013/run\_it300\_class001.mrc Browse

InitialModel/C8\_Class3D\_refauto\_50mic\_64pix/run\_it300\_class001.mrc

**3D Classification**

relion\_refine will be executed.

Alias: "C8\_Init\_50mic\_64pix"

I/O view Job actions Current: C8\_Init\_50mic\_64pix

I/O Reference CTF Optimisation Sampling Helix Compute Running

Number of classes: 4 4

Regularisation parameter T: 4

Number of iterations: 25

Use fast subsets (for large data sets)? No

Mask diameter (A): 160 160

Mask individual particles with zeros? Yes

Limit resolution E-step to (A): -1

I/O Reference CTF Optimisation Sampling Helix Compute Running

Ref. map is on absolute greyscale? No

Initial low-pass filter (A): 50 50

Symmetry: C8 C8

I/O Reference CTF Optimisation Sampling Helix Compute Running

Use parallel disc I/O? Yes

Number of pooled particles: 3

Skip padding? No

Skip gridding? No

Pre-read all particles into RAM? No

Copy particles to scratch directory:

Combine iterations through disc? No

Use GPU acceleration? Yes Yes

Which GPUs to use: 0:1 0:1 or 2:3

I/O Reference CTF Optimisation Sampling Helix Compute Running

Number of MPI procs: 3 3

Number of threads: 2 2

Submit to queue? No

Queue name: openmpi

Queue submit command: qsub

Standard submission script: relion-3.1/scripts/qsub.csh Browse

Minimum dedicated cores per node: 24

Run!

Input your GPU numbers.  
Let's use 2 GPUs !

# 014: 3D classification

RELION-3.0.5: /home/guest01/tmp/relion

File Jobs Autorun

Re-read pipeline Alt+R

Edit project note Alt+E

Print all notes Alt+P

3D initial model

**3D classification**

3D auto-refine

3D multi-body

CTF refinement

Bayesian polishing

Mask creation

Join star files

Particle subtraction

Post-processing

Local resolution

External

Input images STAR file: Select/job012/particles.star ? Browse

Continue from here: ? Browse

Reference map: rel/job013/run\_it300\_class001.mrc ? Browse

Reference mask (optional): ? Browse

Schedule

in: particles.star

in: run\_it300\_class001.mrc

out: run\_it025\_data.star

out: **run\_it025\_model.star**

out: run\_it025\_class001.mrc

out: run\_it025\_class002.mrc

out: run\_it025\_class003.mrc

out: run\_it025\_class004.mrc

Current: 014: Class3D/C8\_Init\_50mic\_64pix/ Display: 014: Class3D/C8\_Init\_50mic\_64pix/

Finished jobs

Running jobs

Input to

014: Class3D/C8\_Init\_50mic\_64pix/

013: InitialModel/C8\_Class2D\_refauto\_50mic\_64pix/

012: Select/Class2D\_refauto\_50mic\_64pix/

Relion display GUI

run\_it025\_model.star

Scale: 2 Min: 0 Max: 0

Sigma contrast: 0 Color: greyscale

Display: rnReferenceImage

Sort images on: rnClassDistribution

Reverse sort?  Apply orientations?  Read whole stacks?

Nr. columns: 5 Ori scale: 1 Max. nr. images: 1000

**Display!**

Class3D/job014/run\_it025\_model.star

Save backup selection

Load backup selection

Clear selection

Invert selection

Select all classes below

Select all classes above

**Show metadata this class**

Show original image

Save

Show

Show

Show

Show

Save

Set

Quit

Select a class and  
 Click right button and select  
 [Show metadata this class],  
 "ClassDistribution" is shown.

Class3D/job014/run\_it025\_model.star

Class001

Class002

Class003

Class004

0.174728      0.198948      0.286835      0.339489

Selected 2597 particles in 1 classes.  
 Below is the metadata table for the last clicked class/particle.

# version 30001

data\_

\_rnReferenceImage Class3D/job014/run\_it025\_class001.mrc

\_rnClassDistribution 0.174728

\_rnAccuracyRotations 4.365000

\_rnAccuracyTranslationsAngst 2.051280

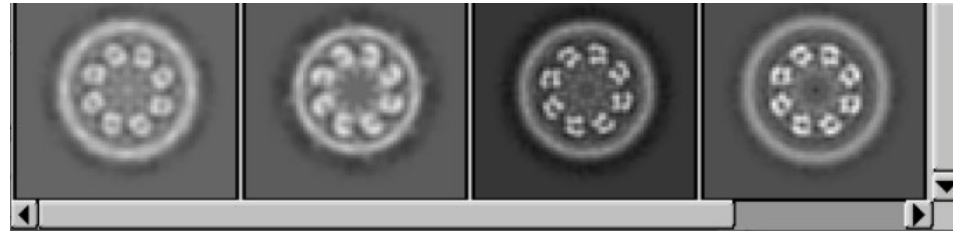
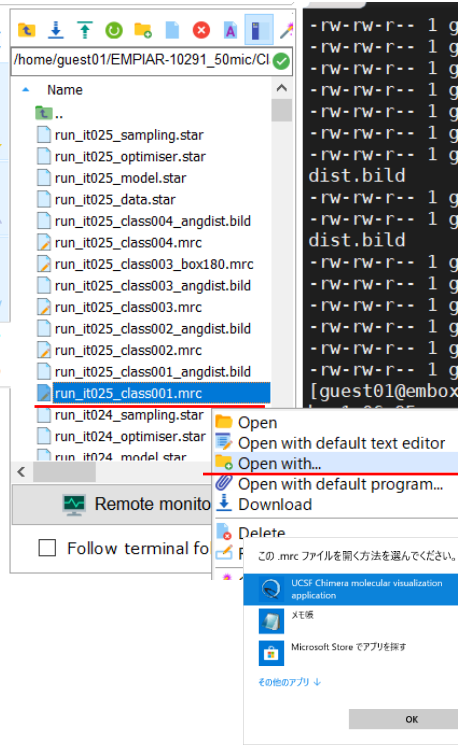
\_rnEstimatedResolution 11.088000

\_rnOverallFourierCompleteness 0.999050

\_rnClassNumber 1

Close

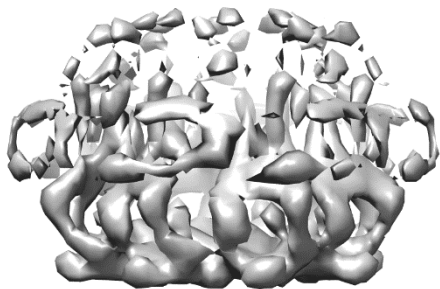
# 014: 3D classification



Class001 0.174728    Class002 0.198948    Class003 0.286835    Class004 0.339489

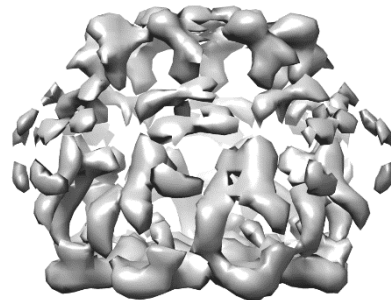
Open following four 3D density maps using UCSF Chimera (through MobaXterm).

Directory: **EMPIAR-10291\_50mic/Class3D/C8\_Init\_50mic\_64pix/**  
3D density maps: **run\_it025\_class001.mrc, run\_it025\_class002.mrc, run\_it025\_class003.mrc, run\_it025\_class004.mrc**



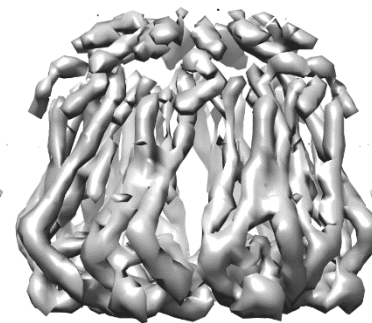
run\_it025\_class001.mrc

ClassDistribution  
0.174728



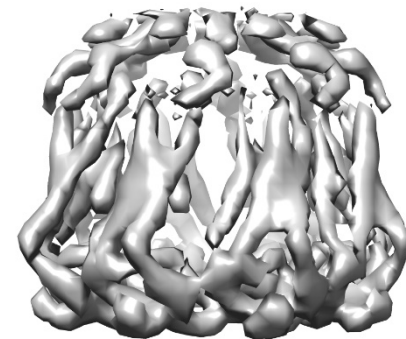
run\_it025\_class002.mrc

ClassDistribution  
0.198948



run\_it025\_class003.mrc

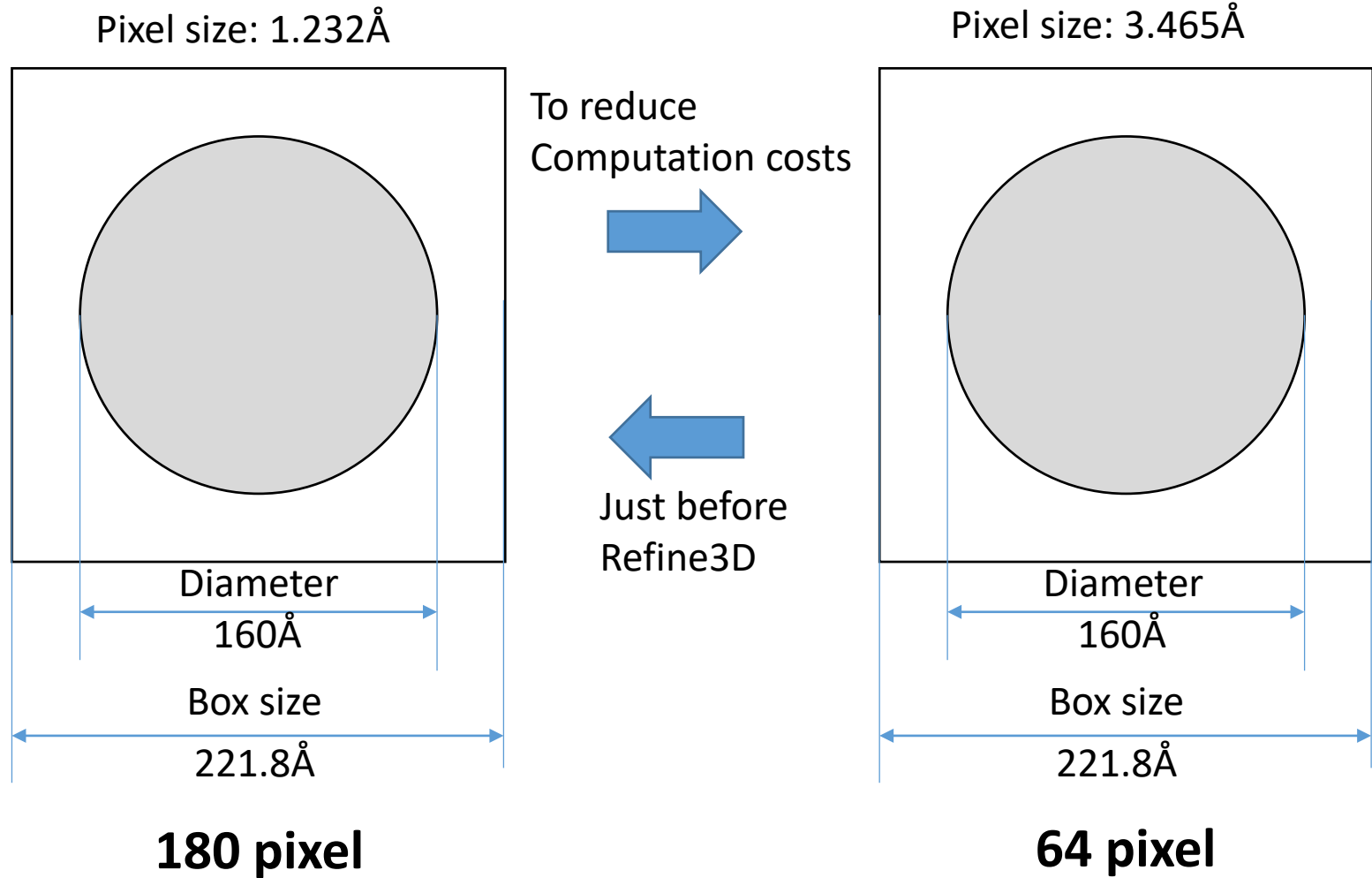
ClassDistribution  
0.286835



run\_it025\_class004.mrc

ClassDistribution  
0.339489

# Box size of particles



# 015: Extract particles with 180 x 180 pixels

File Jobs Schedules **I/O** extract Helix CtfFind/job002/micrographs\_ctf.star

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking  
**Particle extraction**  
Subset selection  
2D classification  
3D auto-refine  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

micrograph STAR file:  ? Browse

Input coordinates:  ? Browse

OR re-extract refined particles?  ?

Refined particles STAR file:  ? Browse

Reset the refined offsets to zero?  ?

OR: re-center refined coordinates?  ?

Pixel size (A)  ?

Schedule Check command Run!

I/O view Job actions Current  Display:

Alias: "180pix\_50mic"

Select/Class2D\_refauto\_50mic\_64pix/particles.star

Particle extraction

I/O **extract** Helix Running

Particle box size (pix)  - 180 ?

Invert contrast?  ?

Normalize particles?  ?

Diameter background circle (pix):  ?

Stddev for white dust removal:  ?

Stddev for black dust removal:  ?

Rescale particles?  ?

Re-scaled size (pixels):  ?

```
+ Re-extracting particles based on coordinates from input_data.star file
+ Select/job012/particles.star
Extracting particles from 50 micrographs ...
23/ 23 sec .....~~(,_"> Joining metadata of all p
The pixel size of the extracted particles in optics group 1 is 1.232 Angstrom/p
Written out STAR file with 14932 particles in Extract/job015/particles.star
Done preprocessing!
```

# Convert 3D map: $64^3$ into $128^3$

Type following UNIX commands:

```
[guest01@embox ~]$ cd
[guest01@embox ~]$ cd EMPIAR-10291_50mic/
[guest01@embox EMPIAR-10291_50mic]$ cd Class3D/C8_Init_50mic_64pix/
[guest01@embox C8_Init_50mic_64pix]$ relion_image_handler --i
run_it025_class003.mrc --o run_it025_class003_box180.mrc --new_box 180
--angpix 3.465 --rescale_angpix 1.232
```

Type the class number of the your best looking class !!

It may be **class001** or **class002** or **class003** or **class004**.



# 016:High resolution 3D refinement

[2GPUs, 25 min]

File Jobs Schedules **I/O** Reference CTF Extract/180pix\_50mic/particles.star

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking

3D auto-refine

3D initial model  
3D classification  
**3D auto-refine**  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

Input images STAR file: Extract/job015/particles.star

Continue from here:

Reference map: 4/run\_it025\_class003\_box180.mrc

Class3D/C8\_Init\_50mic\_64pix/run\_it025\_class**003**\_box180.mrc

Type the class number of the your best looking class !!  
It may be **001** or **002** or **003** or **004**.

Three jobs of *relion\_refine\_mpi* will be executed.

I/O **Reference** CTF Optimisation

Ref. map is on absolute greyscale?  No

Initial low-pass filter (Å):  30

Symmetry:  C8

Schedule

I/O view Job actions **Current: 180pix\_50mic**

Tutorial says low-pass filter is 50Å, but 30 Å is better for this case.

I/O Reference CTF Optimisation Auto-sampling Helix **Compute** Running

Use parallel disc I/O?  Yes

Number of pooled particles:  3

Skip padding?  No

Skip gridding?  No

Pre-read all particles into RAM?  No

Copy particles to scratch directory:

Combine iterations through disc?  No

Use GPU acceleration?  Yes Yes

Which GPUs to use:  0:1 or 2:3

Input your GPU numbers.  
Let's use 2 GPUs !

I/O Reference CTF **Optimisation** Auto-sampling Helix Compute Running

Mask diameter (Å):  160

Mask individual particles with zeros?  Yes

Use solvent-flattened FSCs?  No

I/O Reference CTF Optimisation Auto-sampling Helix Compute **Running**

Number of MPI procs:  3

Number of threads:  2

Submit to queue?  No

Queue name: openmpi

Additional arguments:  16

[Number of MPI proc] should be odds number more than 2 (3 or 5 or 7 ...)

※GPU1枚でも実行は開始されるが、20分後に、GPUのout of memoryのエラーで、不正終了する。

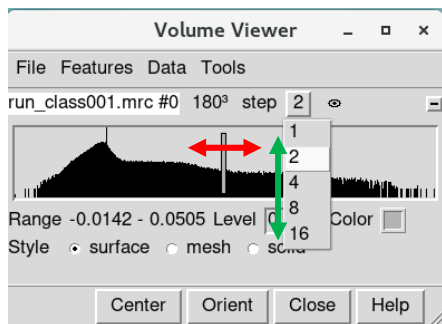
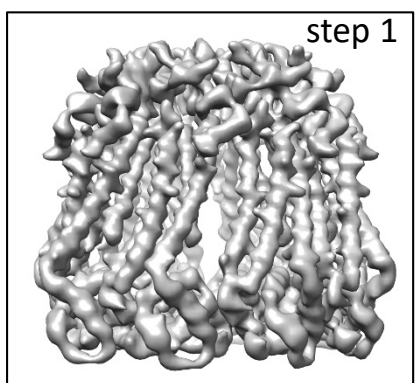
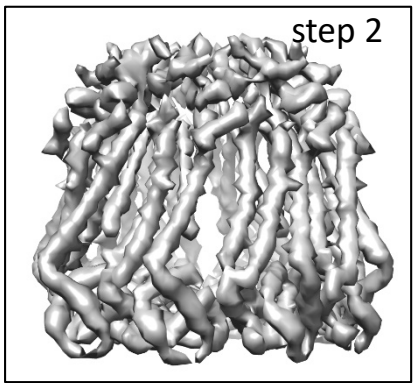
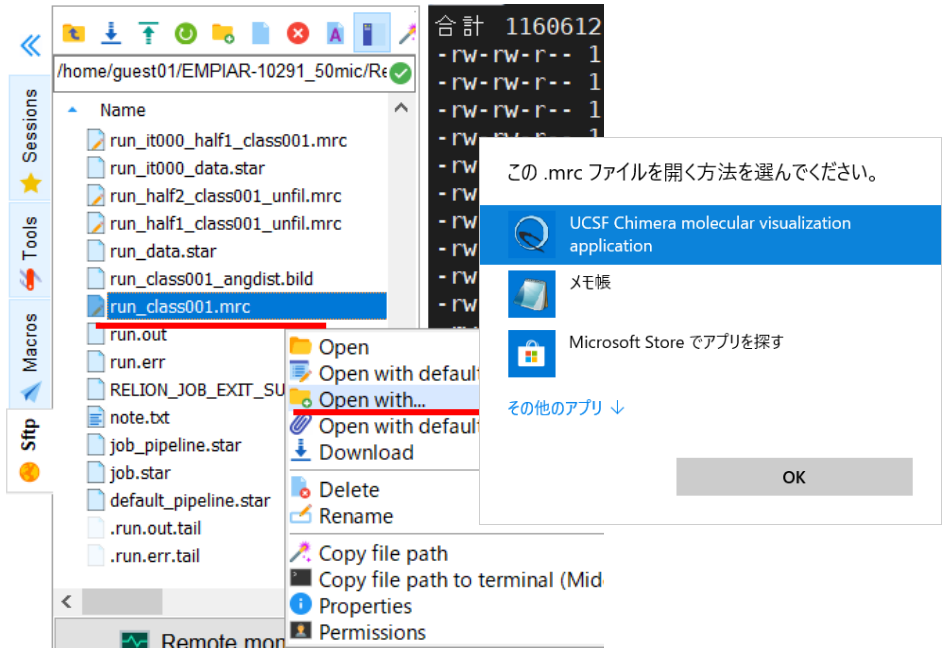
# 016:High resolution 3D refinement

You can know the resolution from the message from Relion GUI.

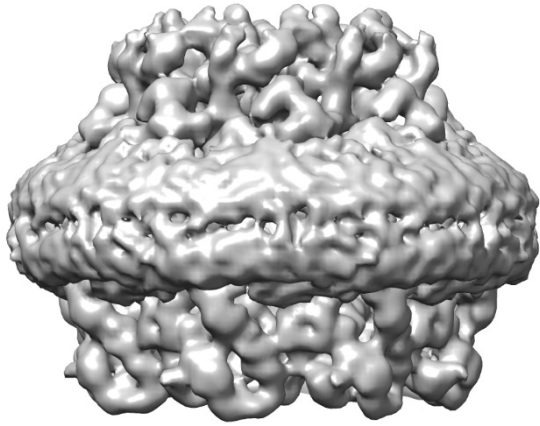
```
1.18/1.18 min .....~(,">
Auto-refine: Refinement has converged, stopping now...
Auto-refine: + Final reconstruction from all particles is saved as: Refine3D/job016/
Auto-refine: + Final model parameters are stored in: Refine3D/job016/
Auto-refine: + Final data parameters are stored in: Refine3D/job016/run_data.star
Auto-refine: + Final resolution (without masking) is: 4.26462
Auto-refine: + But you may want to run relion_postprocess to mask the unfil.mrc maps and calculate a higher resolution FSC
```

or type a following command:  
`tail Refine3D/180pix_50mic/run.out`

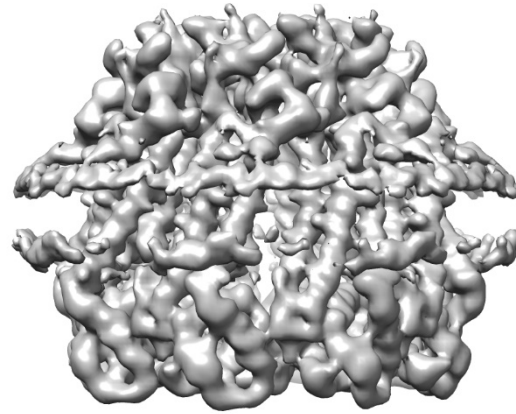
MobaXtermのファイルブラウザで、**Refine3D/180pix\_100mic/run\_class001.mrc**を選び、  
右クリック・メニューから、[Open with...]でUCSF Chimeraで開いて確認する。  
Refine3D/first3dref/run\_class001.mrc



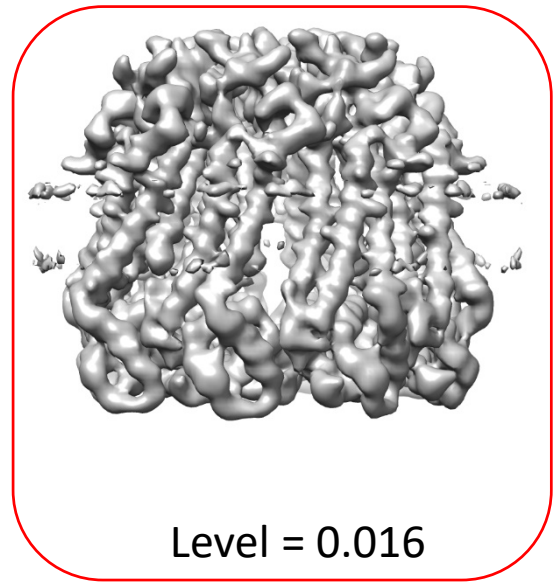
# Level threshold for Refined3d map



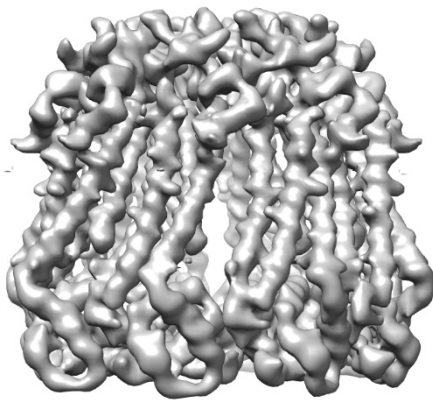
Level = 0.01



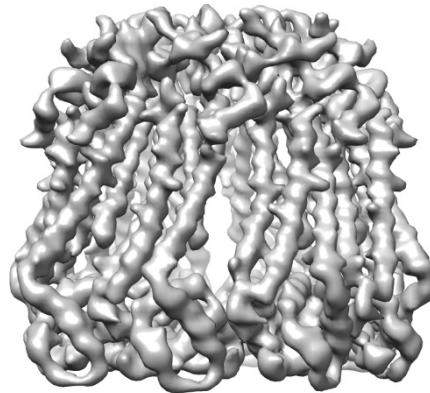
Level = 0.014



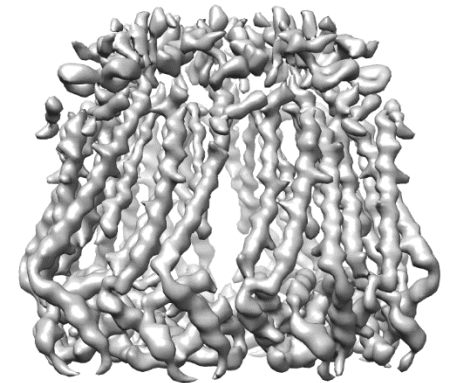
Level = 0.016



Level = 0.018



Level = 0.020



Level = 0.025

# 017:Mask Creation (<1min)

File Jobs Schedules **I/O** Mask Helix Running

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking  
Particle extraction  
Subset selection  
2D classification

**Mask creation**

3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

Input 3D map: refine3D/job016/run\_class001.mrc

Refine3D/180pix\_50mic/run\_class001.mrc

Alias: Refine3D\_180pix\_50mic   

**Current: Refine3D\_180pix\_50mic**

**I/O** Mask Helix Running

Lowpass filter map (A) 15  

Pixel size (A) **1.232**  

Initial binarisation threshold 0.016   

Extend binary map this many pixels 8   

Add a soft-edge of this many pixels 8

# 017:Mask Creation : Checking mask.mrc

select **MaskCreate/Refine3D\_180pix\_50mic/mask.mrc**

from MobaXterm and open by UCSF Chimera using [Open with...] menu

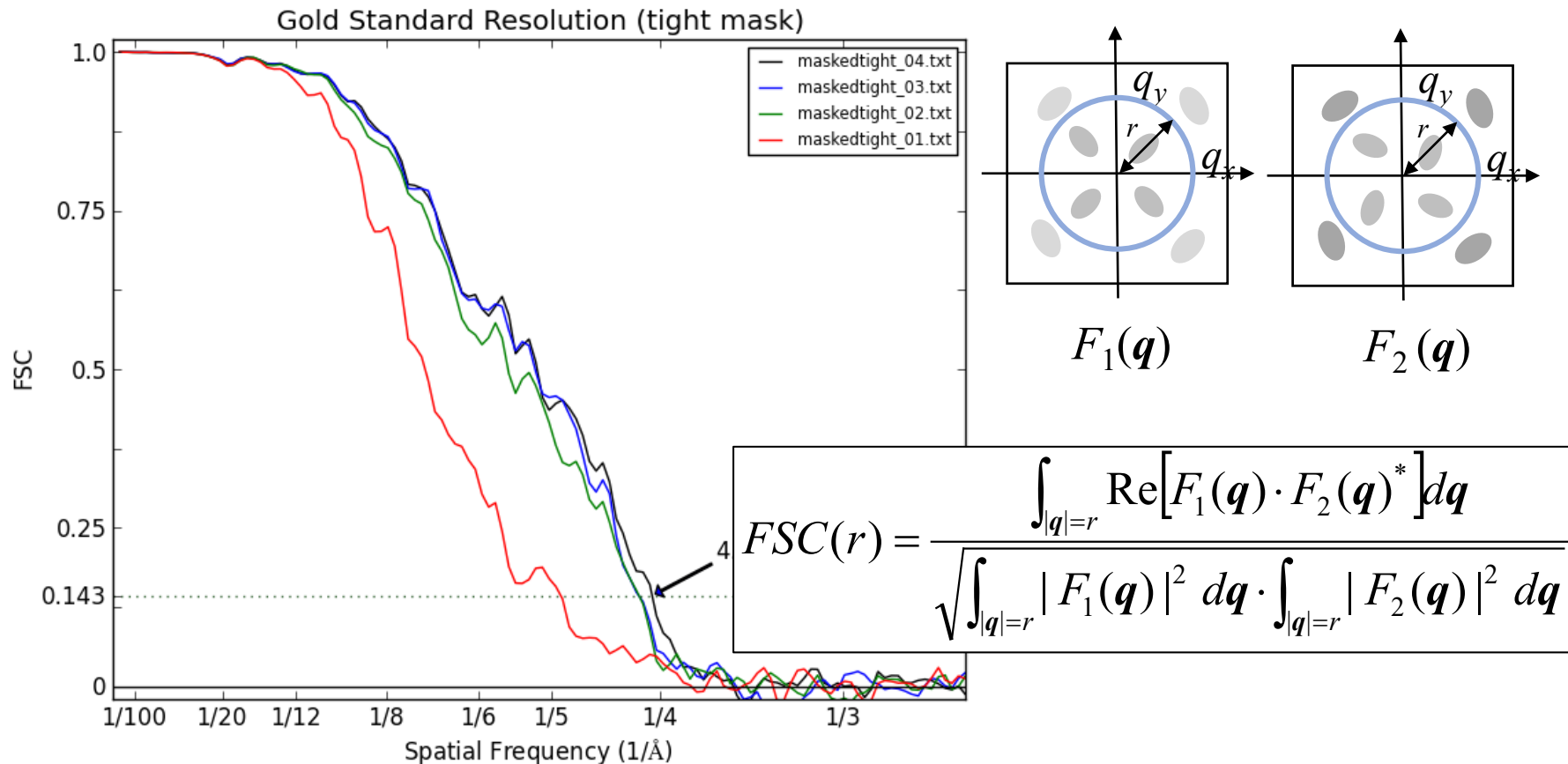
The image shows a multi-step process for opening a mask file in UCSF Chimera:

- MobaXterm File Manager:** A file browser window showing the directory `/home/guest01/EMPIAR-10291_50mic/M:`. The file `mask.mrc` is selected, and the "Open with..." option is chosen from the context menu.
- Terminal:** A terminal window in the background displays system permissions and user information, including `[guest0 2021年 [guest0 合計 22`.
- UCSF Chimera:** The main application window displays a 3D surface model of a protein complex, rendered in a light gray color.
- Volume Viewer:** A sub-window titled "Volume Viewer" shows the loaded file `mask.mrc #0` with a resolution of `1803` and `step 1`. It includes a histogram of the volume data and a control for the `Level`, which is set to `0.5`. The style is set to `surface`. Buttons for `Center`, `Orient`, `Close`, and `Help` are visible at the bottom.
- Application Selection Dialog:** A dialog box prompts the user to select an application to open the `.mrc` file. The "UCSF Chimera molecular visualization application" is selected.

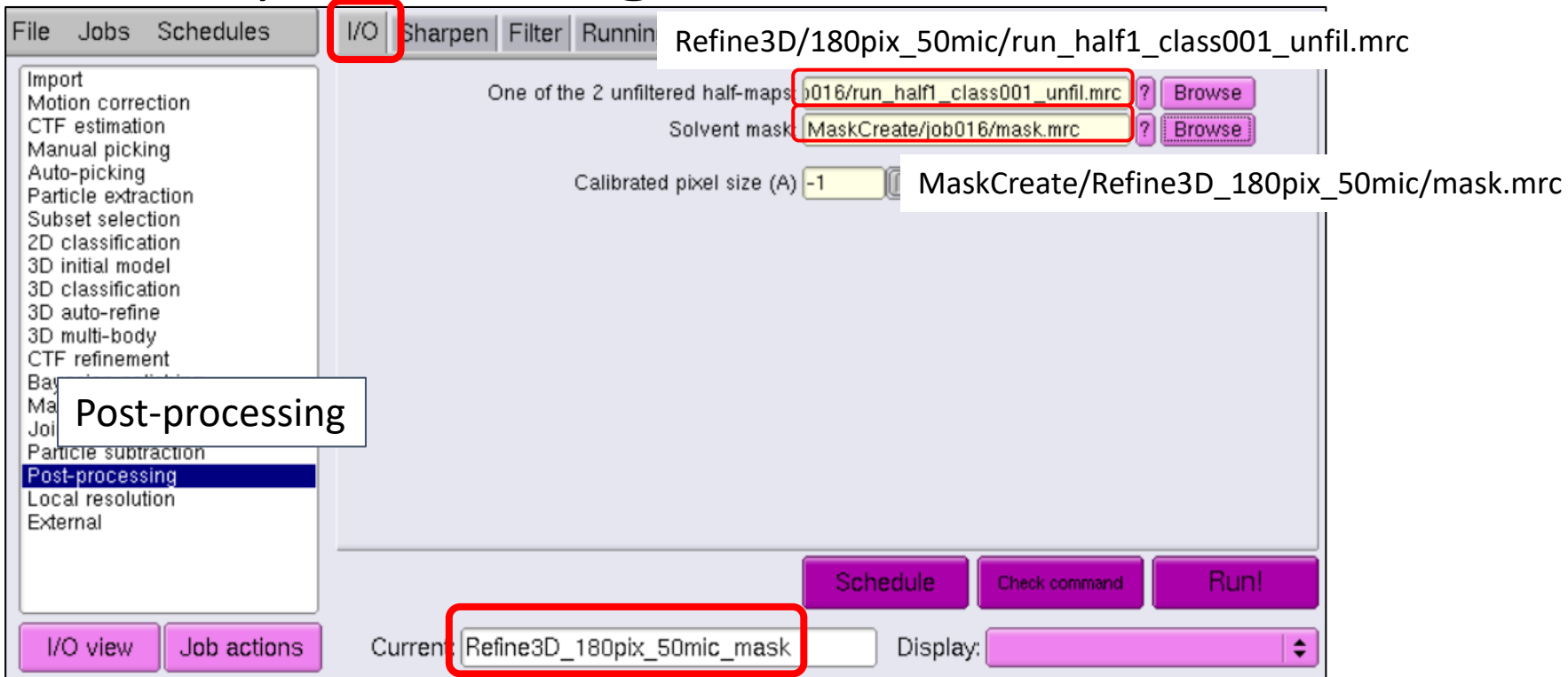


# Resolution from Fourier Shell Correlation (FSC)

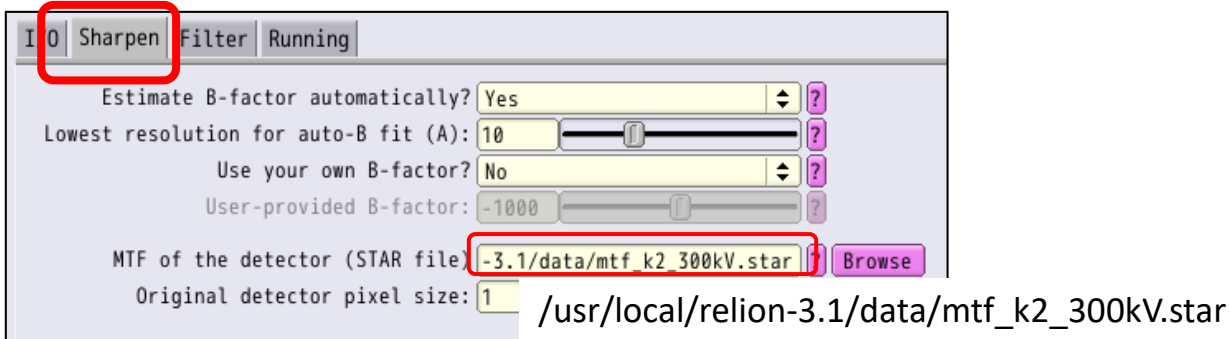
- 1) Divide 2D particle images into two sets, and reconstruct two 3D maps  $f_1(\mathbf{x})$  and  $f_2(\mathbf{x})$ . Their Fourier transferred maps are called  $F_1(\mathbf{q})$  and  $F_2(\mathbf{q})$ .
- 2) Calculate correlation  $FSC(r)$  on a spherical shell with spatial frequency  $r=|\mathbf{q}|$ .
- 3) Plot  $FSC(r)$  versus spatial frequency  $r$ . Resolution is defined spatial frequency with  $FSC(r) = 0.143$  ( $=1/7$ ).



# 018: Postprocessing (< 1min)



Alias:Refine3D\_180pix\_50mic\_mask

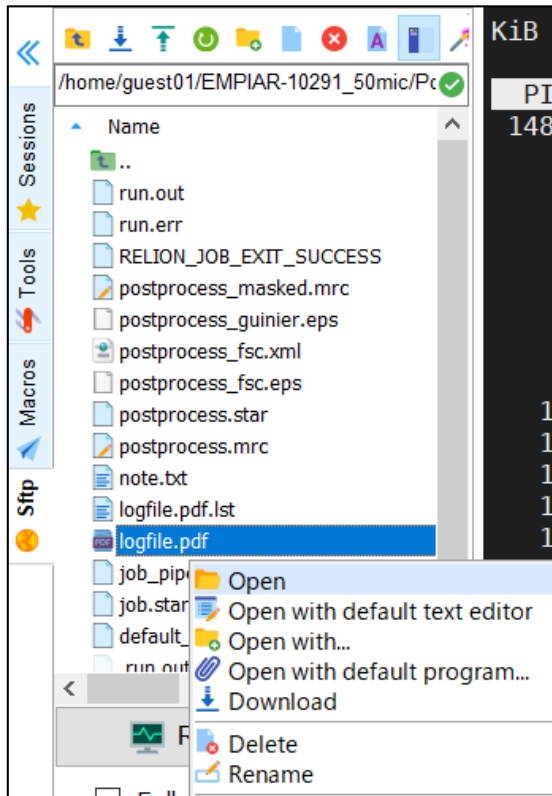




# 018:Check FSC

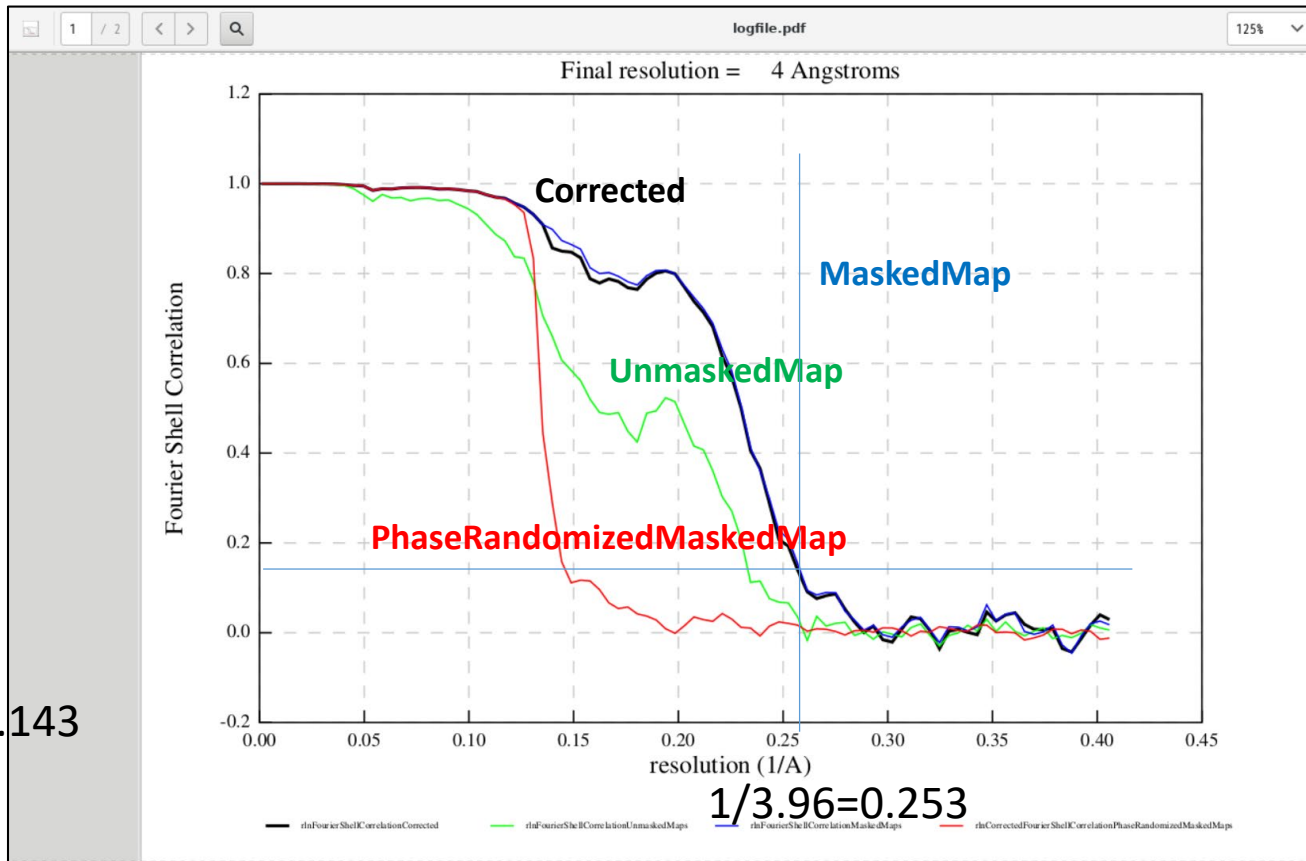
Choose,  
PostProcess/Refine3D\_180pix\_50mic\_mask/logfile.pdf

```
+ correlation of fit: 0.890162
+ apply b-factor of: -195.197
== Writing output files ...
+ Processed map: PostProcess/job017/postprocess.mrc
+ Processed masked map: PostProcess/job017/postprocess_masked.mrc
+ Metadata file: PostProcess/job017/postprocess.star
+ FINAL RESOLUTION: 3.96
```



$1/7=0.143$

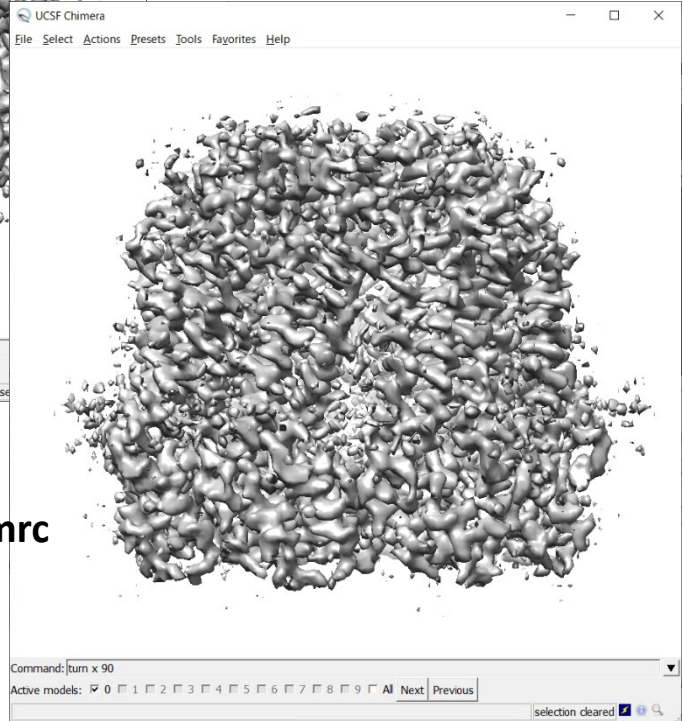
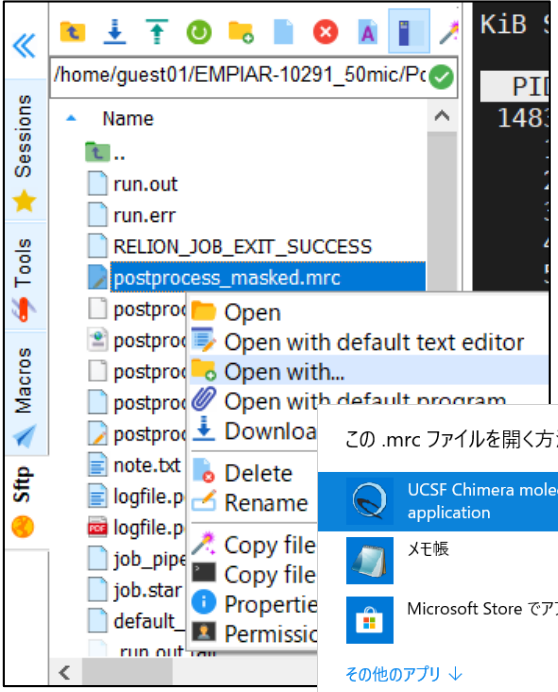
+ FINAL RESOLUTION: 3.96



# 018:Check 3D map

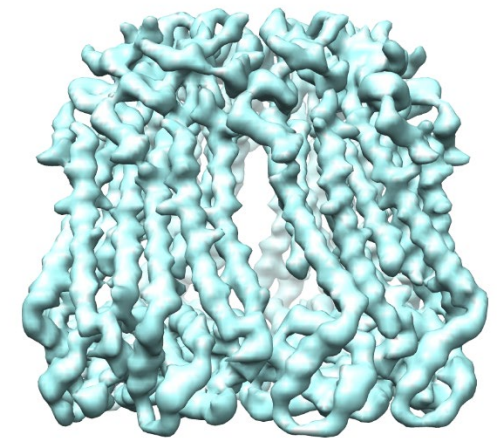
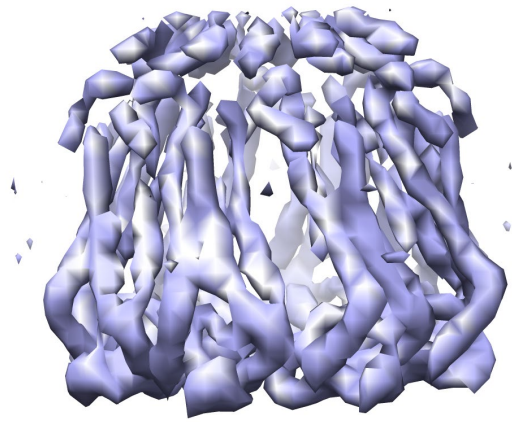
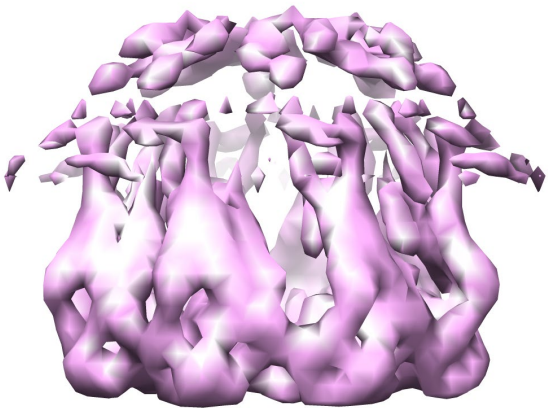
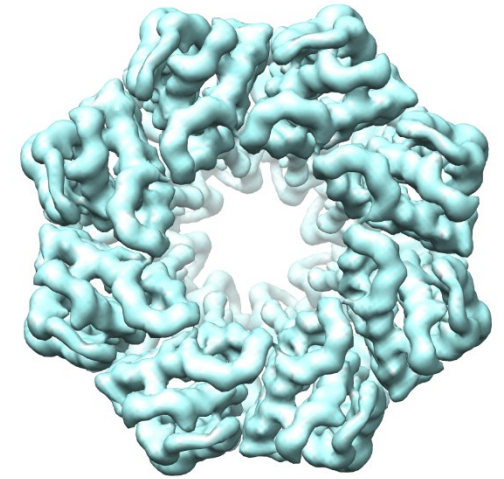
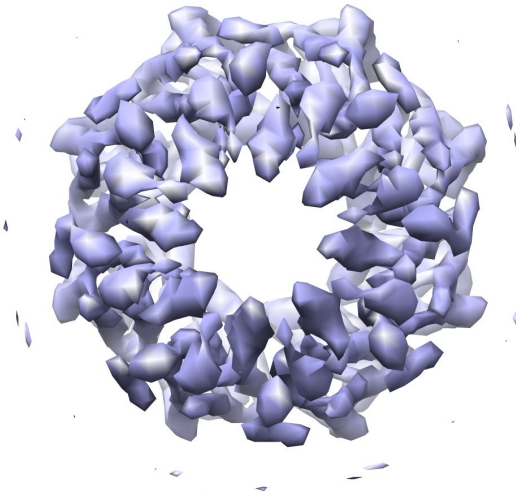
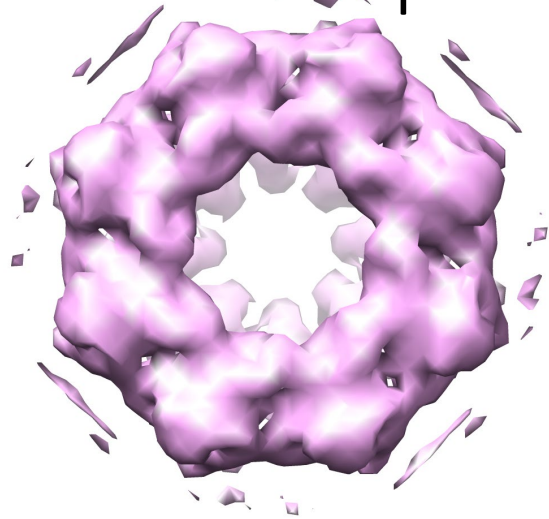
```
+ correlation of fit: 0.890162
+ apply b-factor of: -195.197
== Writing output files ...
+ Processed map: PostProcess/job017/postprocess.mrc
+ Processed masked map: PostProcess/job017/postprocess_masked.mrc
+ Metadata file: PostProcess/job017/postprocess.star
+ FINAL RESOLUTION: 3.96
```

+ FINAL RESOLUTION: 3.96



Select  
PostProcess/Refine3D\_180pix\_50mic\_mask/postprocess\_masked.mrc

# Comparison of 3D maps of INX-6



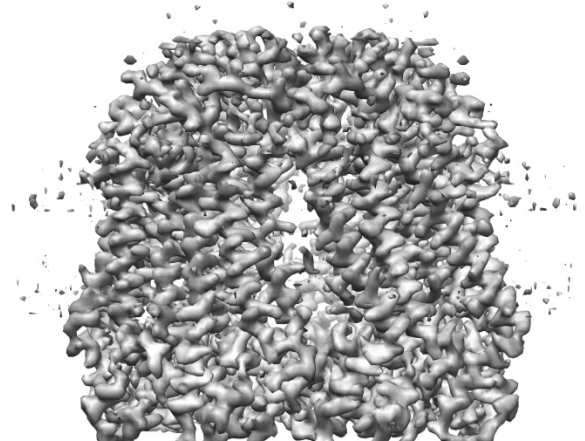
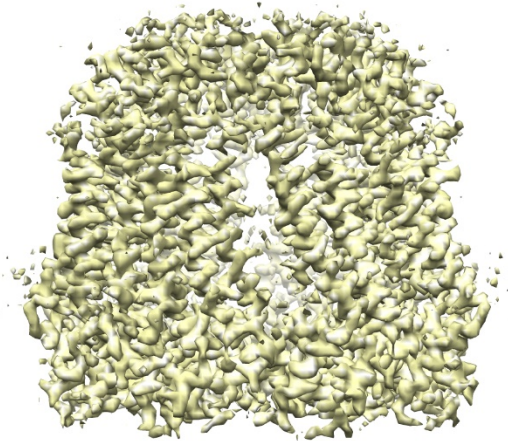
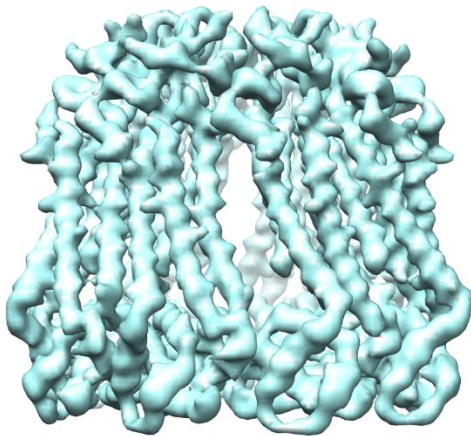
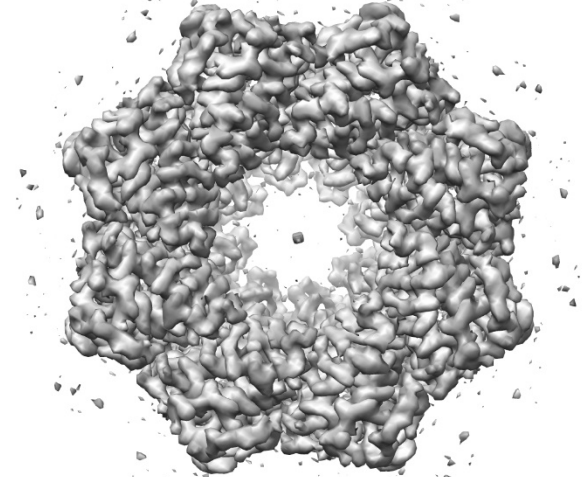
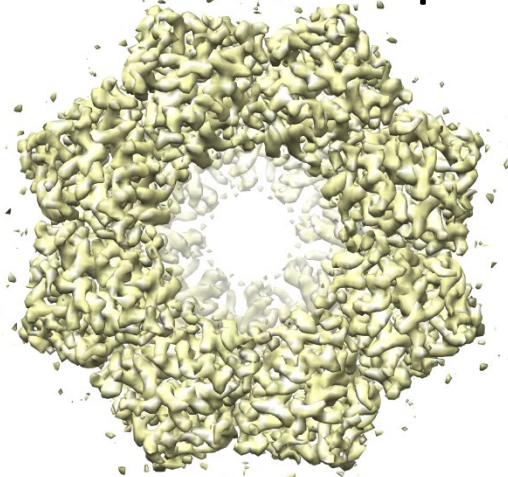
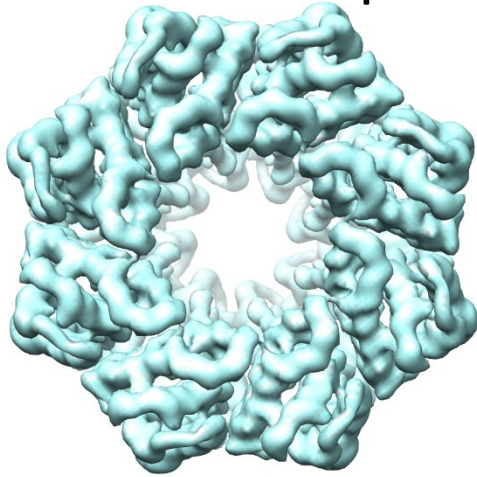
InitialModel  
64<sup>3</sup> voxels  
from 50 micrographs  
14932 particles

Class3D, best class (003)  
64<sup>3</sup> voxels  
From 50 micrographs  
14932 particles

Refine3D  
180<sup>3</sup> voxels  
Resolution: 4.26 Å  
from 50 micrographs



# Comparison of 3D maps of INX-6



Refine3D  
180<sup>3</sup> voxels  
Resolution: 4.26 Å  
from 50 micrographs

Postprocess with mask  
180<sup>3</sup> voxels  
Resolution: 3.96 Å  
from 50 micrographs

EMD-9973.  
180<sup>3</sup> voxels  
Resolution: 3.6 Å  
From 300 micrographs

# Acknowledgements

We thank following people:

- **Dr. Sjors Scheres and his lab members** for developing Relion.
- **Prof. Atsunori Oshima** for providing his EM data and helpful advices.