

2020/08/31-2020/09/04

**CiCLE**

Cyclic Innovation for Clinical Empowerment

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

Innexin-6を題材とした実習

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

川端 猛 (大阪大学 蛋白質研究所)

2020/09/02



# MPI process, threads and GPU

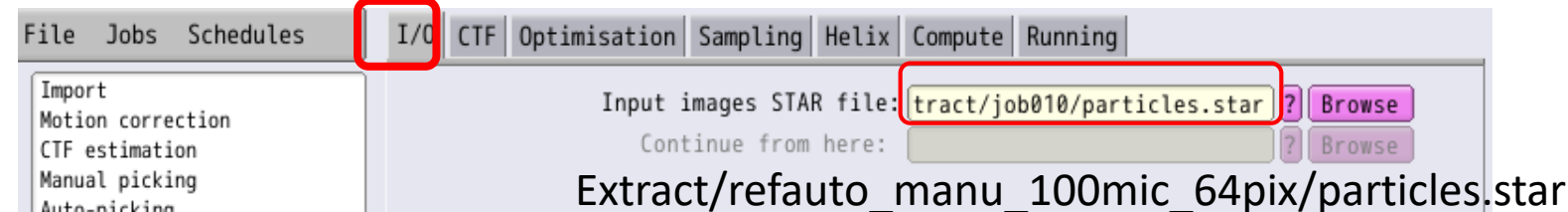
Number of MPI process: プロセスの数

Number of threadsの数: スレッドの数

- Unixでは、いくつかのプロセスが同時に動いています。(topで確認)
  - 各プロセスはそれぞれいくつかのスレッドを使います。
  - 同じプロセスのスレッドは同じメモリを共有します。
- $[\text{全プロセス数}] \times [\text{1プロセスが使うスレッド数}] \leq [\text{CPUのスレッド総数}]$
- 各プロセスがGPUを使います。  $[\text{プロセス数}] \geq [\text{GPUの枚数}]$   
複数のプロセスが一つのGPUを使うことも可能。
  - Refine3Dでは、必ず偶数個のGPU,  $[\text{プロセス数}] = [\text{GPUの枚数}] + 1$

# Reference-free 2D class averaging

[2GPUs, 35-50 min]



## 2D Classification

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

※1GPU, 3threadsで  
VIRT 47.7GBだが、  
RES 600MBIほど。4枚  
同時でも負荷はそれ  
ほど高くない。  
44分ほど計算時間  
がかかる。

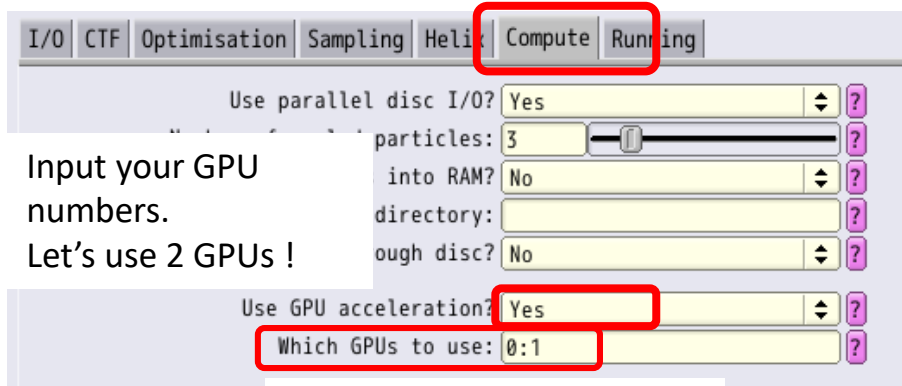
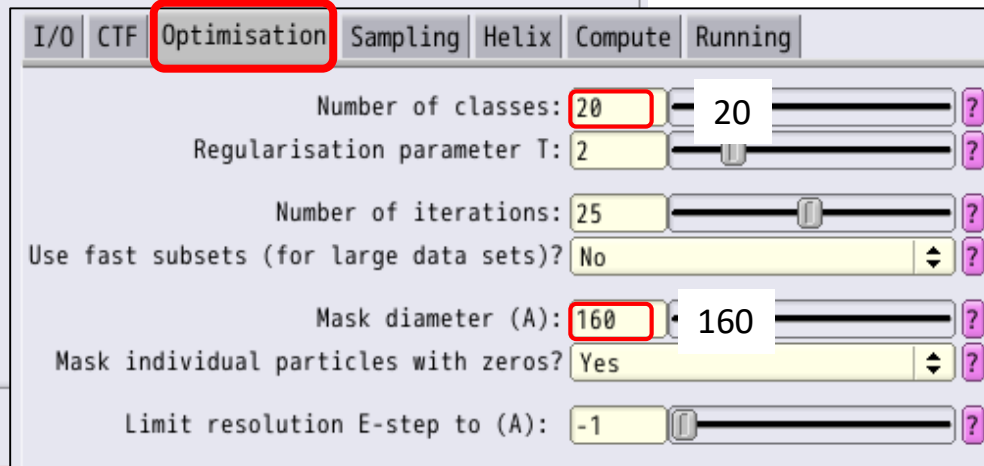
Alias : "refauto\_manu\_100mic\_64pix"

I/O view

Job actions

Current: refauto\_manu\_100mic\_64pix

Display:

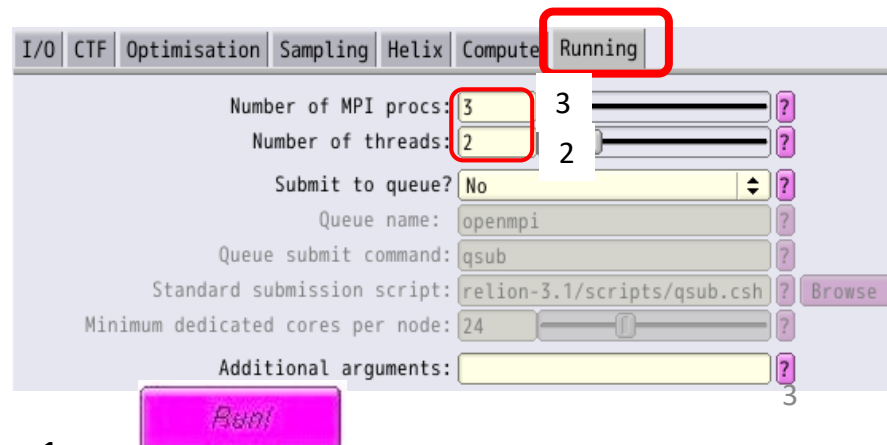


Input your GPU  
numbers.  
Let's use 2 GPUs !

Use GPU acceleration? Yes

Which GPUs to use: 0:1

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



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

# Reference-free 2D class averaging

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

Break Mode ALT+N

Schedule

Check command

Continue!

uPick/

Display: out: run\_it025\_model.star

run\_it025\_model.star

Scale: 2

Min: 0

Max: 0

Sigma contrast: 0

Color: greyscale

Display: rlnReferenceImage

☒ Sort images on: rlnClassDistribution

☒ Reverse sort?

☐ Apply orientations?

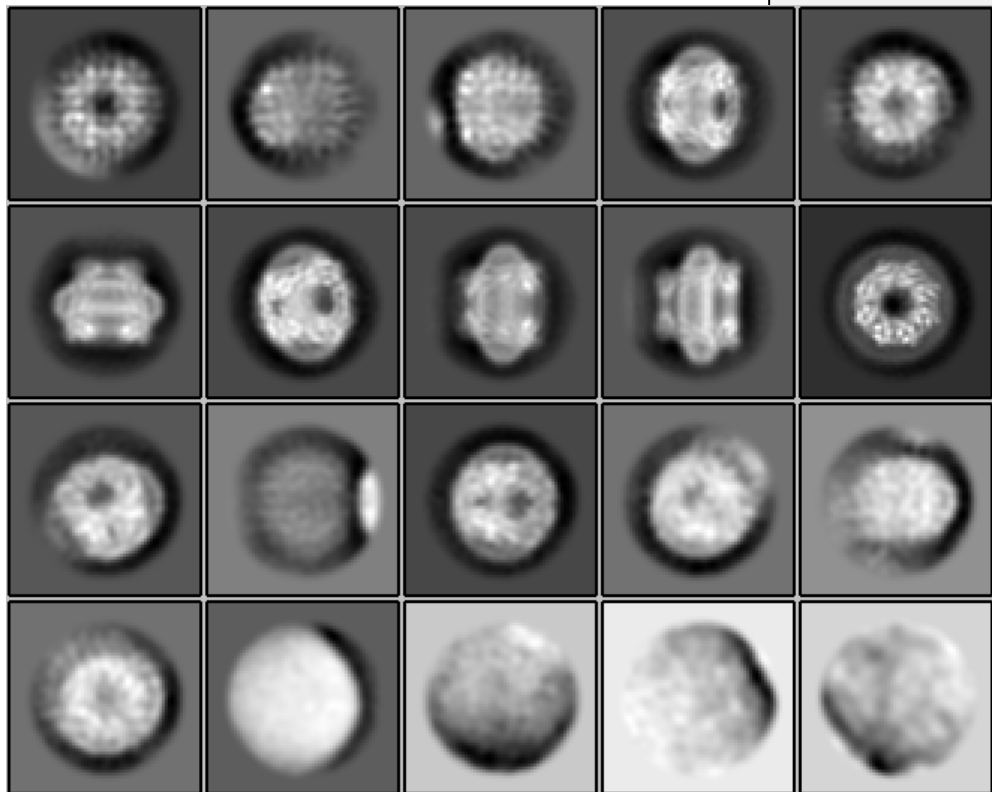
☐ Read whole stacks?

Nr. columns: 5

Ori scale: 1

Max. nr. images: 1000

Display!



## 2. Reference-free 2D class averaging: Subset selection

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: **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\_100mic\_64pix/run\_it025\_model.star

Alias: "Class2D\_refauto\_100mic\_64pix" Schedule Check command **Run!**

I/O view Job actions **Current:** Class2D\_refauto\_100mic\_64pix Display:

run\_it025\_model.star

Scale: **2** 2 Max: 0  
Sigma contrast: 0 Color: greyscale

Display: rlnReferenceImage

☒ 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

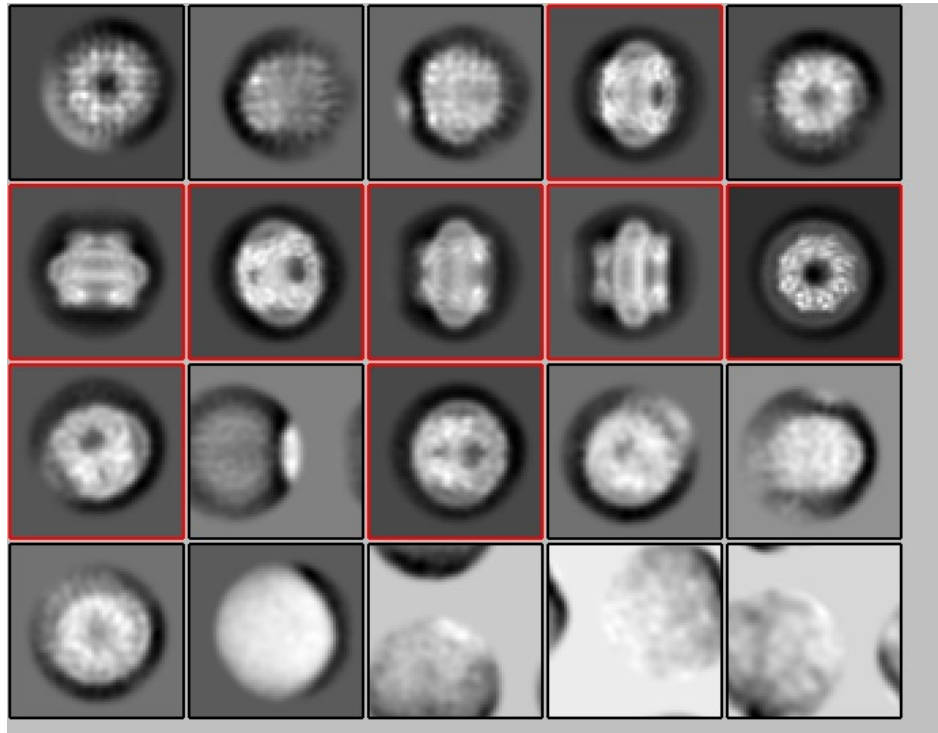
**Display!**

☒ Sort images on: rlnClassDistribution

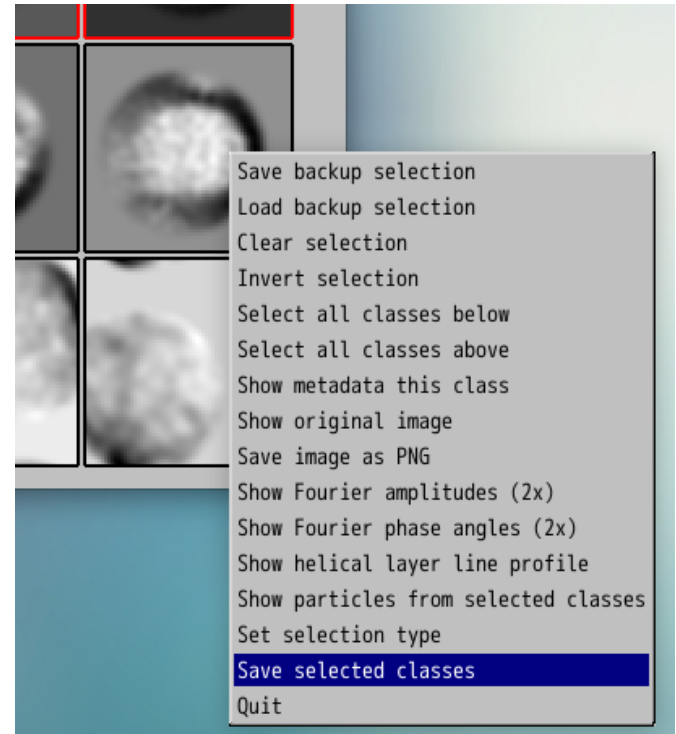
クラスに属する粒子数の数でソート

## 2. Reference-free 2D class averaging

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]



Total number of particles from 100 micrographs is **60292**  
i.e. on average there were 603 particles per micrograph.

Saved Select/job012/class\_averages.star with 8 selected images.  
Saved Select/job012/particles.star with **25617** selected particles.

# 3. De novo 3D model generation

[1GPU, 13 min]

File Jobs Schedules I/O **CTF** Optimisation SGD Compute Running

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking  
Particle extraction  
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 re  
External

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

Select/Class2D\_refauto\_100mics\_64pix/particles.star

**3D Initial model**

*relion\_refine*が実行される。  
VIRT 47.4GB  
RES 482 MB  
計算時間は6分ほど。

Alias : C8\_Class3D\_refauto\_100mics\_64pix

I/O view Job actions Current: **C8\_Class2D\_refauto\_100mics\_64pix** Display:

I/O **CTF** **Optimisation** SGD Compute Running

Number of classes: **1** 1

Mask diameter (A): **160** 160

latten and enforce non-negative solvent? Yes

Symmetry: **C8** C8

Initial angular sampling: 15 degrees

Offset search range (pix): 6

Offset search step (pix): 2

CTF Optimisation **SGD** Compute Running

Number of initial iterations: 50

Number of in-between iterations: 200

Number of final iterations: 50

Write-out frequency (iter): 10

Initial resolution (A): 35

Final resolution (A): 15

Initial mini-batch size: 100

Final mini-batch size: 500

Increased noise variance half-life: -1

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** Yes

Which GPUs to use: **0**

Optimisation SGD **Compute** Running

Number of MPI procs: **1**

Number of threads: **6**

Submit to queue? No

Queue name: openmpi

Queue submit command: qsub

rd submission script: relion-3.1

icated cores per node: 24

Add:

Run

# 3. 3D密度マップ(\*.mrc)の表示方法

1) MobaXtermの左のファイルブラウザの中に  
ログイン先のマシンのファイルが見える。

The image is a composite of three screenshots from the MobaXterm application, illustrating the process of opening a 3D density map file (.mrc).

**Top Screenshot:** Shows the MobaXterm interface. The left sidebar contains a file browser for the remote host 192.168.39.89. The file browser shows the directory `/home/guest01/` with various folders and files. The file `run_it300_class001.mrc` is selected and highlighted with a red box.

**Middle Screenshot:** Shows a context menu that appears after right-clicking the selected file. The menu options include "Open", "Open with default text editor", "Open with...", "Open with default program...", "Download", "Delete", "Rename", "Copy file path", "Copy file path to terminal (Middle)", and "Properties". The "Open with..." option is highlighted with a red box.

**Bottom Screenshot:** Shows a dialog box titled "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 needed to open this .mrc file). The dialog box has two buttons: "その他のアプリ ↓" (Other applications ↓) and "OK". The "その他のアプリ ↓" button is highlighted with a red box.

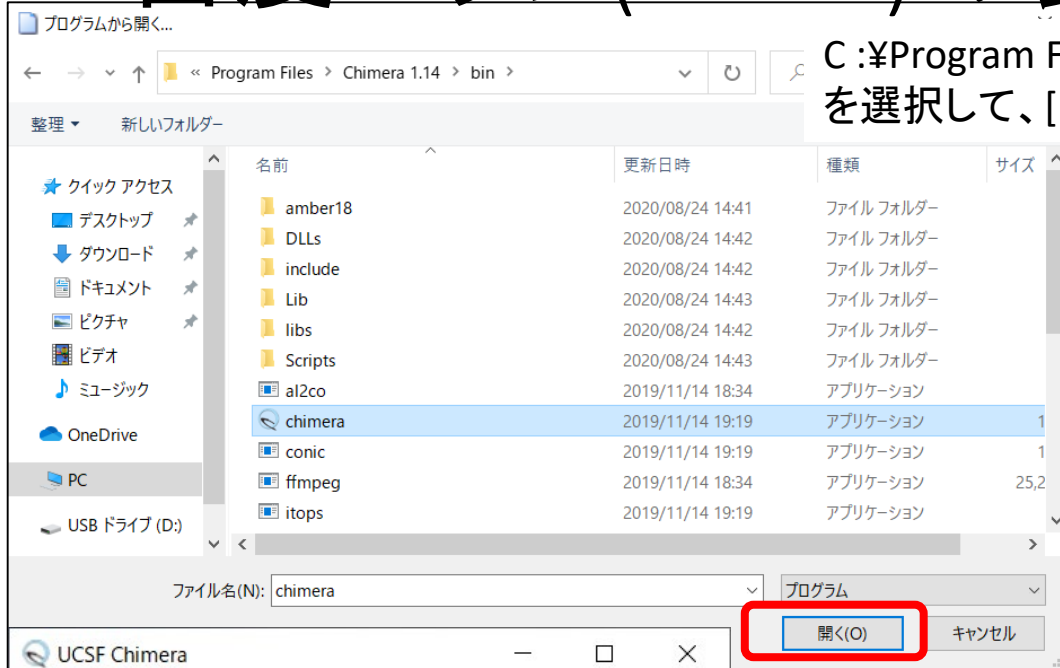
**Right Screenshot:** Shows a second dialog box titled "この .mrc ファイルを開くには新しいアプリが必要です" (A new application is needed to open this .mrc file). This dialog box lists several applications: Internet Explorer, Windows Media Player, Windows フォト ビューアー, ペイント (Paint), メモ帳 (Notepad), and ワードパッド (WordPad). The "この PC で別のアプリを探す" (Search for another application on this PC) button is highlighted with a red box.

2) ~EMPIAR-10291\_100mic/InitialModel/C8/run\_it300\_class001.mrc  
を選択し、マウス右クリックのポップアップで、[Open with...]を選択

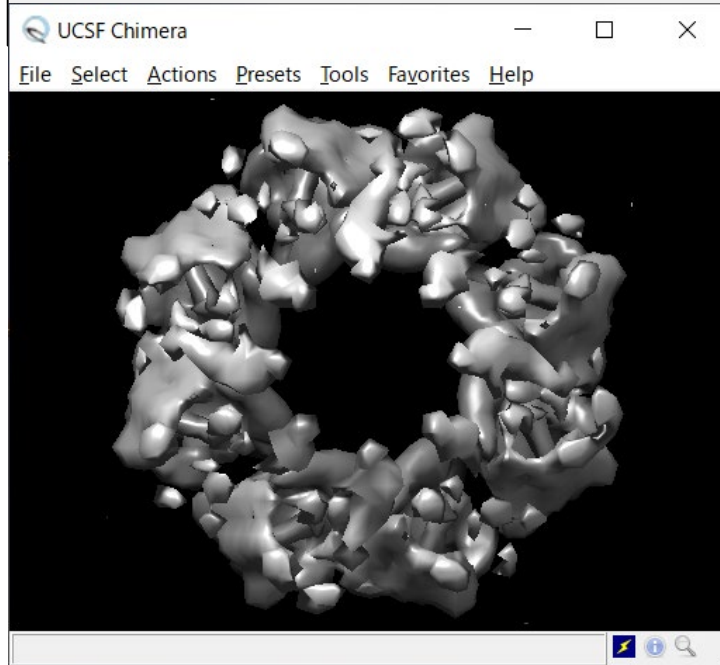
3) .mrcファイルを開くアプリの設定を行う



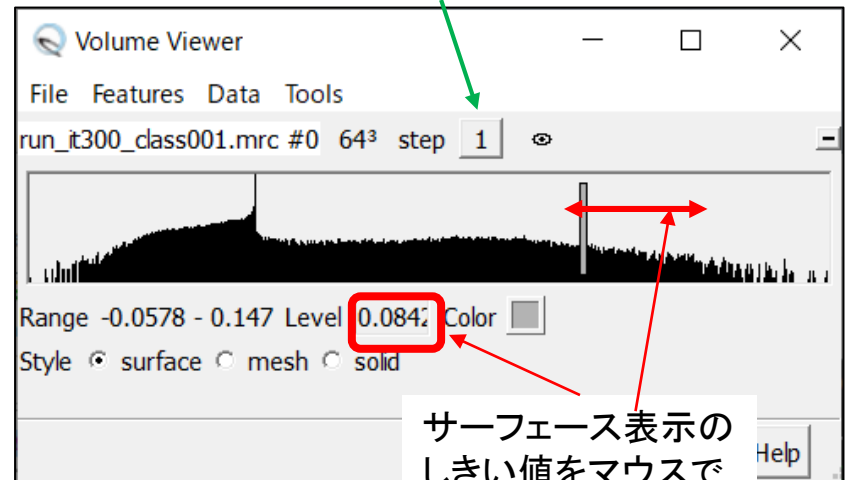
# 3. 3D密度マップ(\*.mrc)の表示方法



C :¥Program Files ¥ Chimera1.14¥bin¥chimera  
を選択して、[開く]



格子点のステップを調節可能。  
2の場合2x2x2の格子点を一つにまとめて表示



サーフェース表示の  
しきい値をマウスで  
調節可能

# 4.1 Unsupervised 3D classification

[2GPUs, 45 min]

The screenshot displays the cryoSPARC software interface with several key components highlighted:

- Left Panel (3D Classification):** A list of processing steps where "3D classification" is selected. Other options include Import, Motion correction, CTF estimation, Manual picking, 2D classification, 3D initial model, 3D auto-refine, 3D multi-body, CTF refinement, Bayesian polishing, Mask creation, Join star files, Particle subtraction, Post-processing, Local res, and External.
- I/O Tab:** The "I/O" tab is selected, showing the "Input images STAR file" set to `/job011/run_it025_data.star` and the "Reference map" set to `b013/run_it300_class001.mrc`. A text box indicates the initial model is `InitialModel/symC1/run_it300_class001.mrc`.
- Optimisation Tab:** The "Optimisation" tab is selected, showing settings for "Number of classes" (4), "Regularisation parameter T" (4), "Number of iterations" (25), "Mask diameter (A)" (160), and "Limit resolution E-step to (A)" (-1).
- Reference Tab:** The "Reference" tab is selected, showing settings for "Ref. map is on absolute greyscale?" (No), "Initial low-pass filter (A)" (50), and "Symmetry" (C8).
- Running Tab:** The "Running" tab is selected, showing settings for "Number of MPI procs" (3), "Number of threads" (2), "Submit to queue?" (No), "Queue name" (openmpi), "Queue submit command" (qsub), "Standard submission script" (relion-3.1/scripts/qsub.csh), and "Minimum dedicated cores per node" (24).
- Compute Tab:** The "Compute" tab is selected, showing settings for "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?" (No), "Combine iterations through disc?" (No), "Use GPU acceleration?" (Yes), and "Which GPUs to use?" (0:1).

Additional annotations and text boxes provide further context:

- A text box states: "(選択した11個のクラスに属する1156個の粒子)" (1156 particles belonging to the 11 selected classes).
- A text box states: *relion\_refine*が実行される。 VIRT 47.6GB RES 775 MB 計算時間は15分ほど。 (relion\_refine is executed. VIRT 47.6GB RES 775 MB Calculation time is about 15 minutes).
- A text box states: Alias: "C8\_Init\_100mic\_64pix".
- A text box states: Current: C8\_Init\_100mic\_64pix.
- A text box states: Input your GPU numbers. Let's use 2 GPUs!

# 4.1 Unsupervised 3D classification

RELION-3.1-beta-commit-9575df: /home/guest01/EMPIAR-10291\_100mic (embox2) - x

File Jobs Schedules I/O Reference CTF Optimisation Sampling Helix Compute Running

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking  
Particle extraction  
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

Number of MPI procs: 3  
Number of threads: 2  
Submit to queue? No  
Queue name: openmpi  
Queue submit command: qsub  
Standard submission script: relion-3.1/scripts/qsub.csh  
Minimum dedicated cores per node: 24  
Additional arguments:

Schedule Check command Continue!

Current: 014: Class3D/C8\_Init\_100mic\_64pix/ Display:

Finished jobs  
014: Class3D/C8\_Init\_100mic\_64pix/  
013: InitialModel/C8\_Class2D\_refauto\_1  
012: Select/Class2D\_refauto\_100mic\_64p  
011: Class2D/refauto\_manu\_100mic\_64pix  
010: Extract/refauto\_manu\_100mic\_64pix  
009: AutoPick/manu\_100mic\_64pix/  
008: AutoPick/manu\_5mic\_64pix/  
007: Select/manupick\_5mic\_64pix/  
006: Class2D/manupick\_5mic\_64pix/

Running jobs

Scheduled jobs

Input  
011: C  
013: n  
Output  
in: run\_it025\_data.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

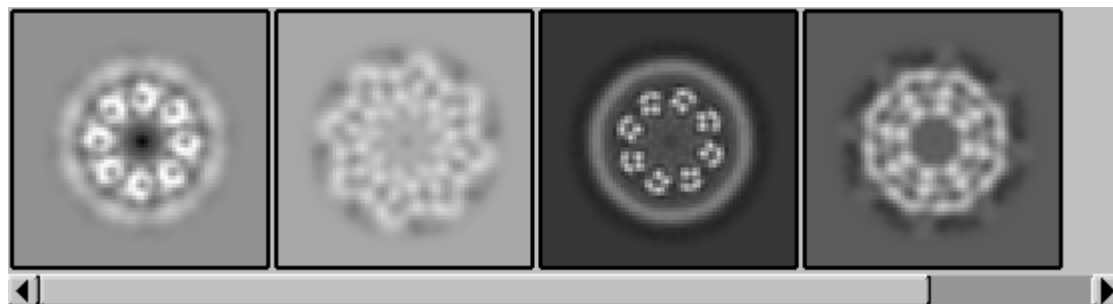
run\_it025\_model.star

Scale: 2 Min: 0 Max: 0  
Sigma contrast: 0 Color: greyscale

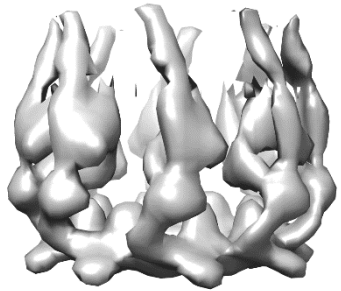
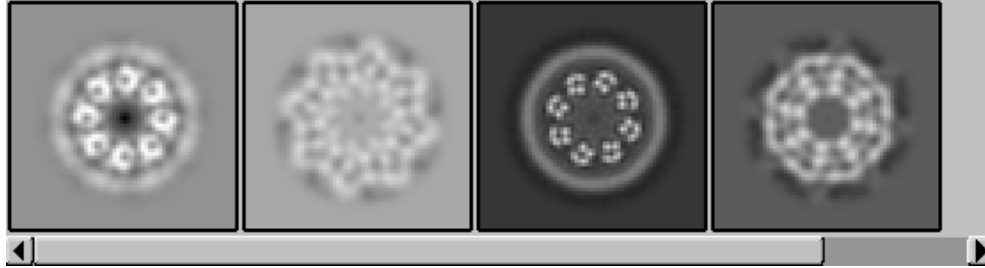
Display: rlnReferenceImage  
☐ Sort images on: rlnClassDistribution  
☐ Reverse sort? ☐ Apply orientations? ☐ Read whole stacks?

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

**Display!**

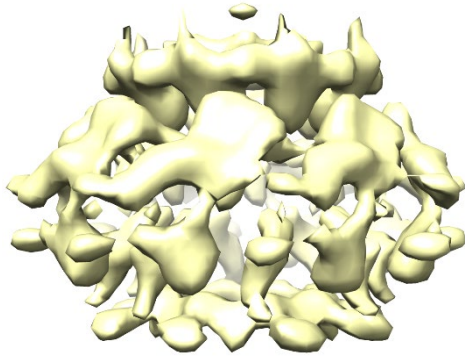


# 4.1 Unsupervised 3D classification



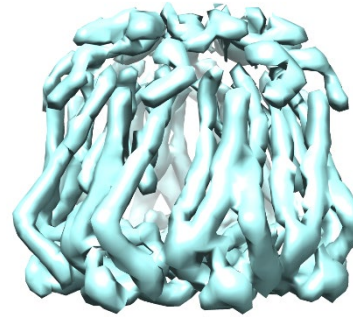
run\_it025\_class001.mrc

ClassDistribution  
0.240319



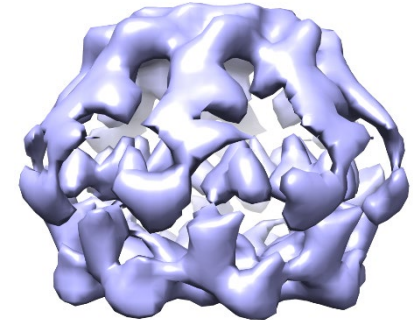
run\_it025\_class002.mrc

ClassDistribution  
0.198251



run\_it025\_class003.mrc

ClassDistribution  
0.349774



run\_it025\_class004.mrc

ClassDistribution  
0.211656

# 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

3D 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?  Yes ?

Refined particles STAR file:  ? Browse

Reset the refined offsets to zero?  ?

OR: re-center refined coordinates?  ?

Recenter on - X, Y, Z (pix):    ?

Select/Class2D\_refauto\_100mics\_64pix/particles.star

Alias: "180pix\_100mic"

**Schedule** **Check command** **Run!**

I/O view Job actions  Display:

**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?  No ?

Re-scaled size (pixels):  ?

# Convert 64<sup>3</sup>map into 128<sup>3</sup> map

Type following UNIX commands:

```
[guest01@embox~]$ cd  
[guest01@embox~]$ cd EMPIAR-10291_100mic  
[guest01@embox EMPIAR-10290_100mic]$ cd Class3D/C8_Init_100mic_64pix  
[guest01@embox C8_Init_100mic_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**.



# 5. High resolution 3D refinement

[2GPUs, 26 min]

The screenshot displays the cryo-EM software interface with several key components highlighted by red boxes and annotated with text:

- 3D auto-refine**: A callout box pointing to the '3D auto-refine' option in the left-hand menu.
- I/O**: A red box highlights the 'I/O' tab at the top of the main window.
- Input images STAR file**: A red box highlights the field containing 'tract/job015/particles.star'.
- Continue from here**: A red box highlights the empty field for continuing from a previous job.
- Reference map**: A red box highlights the field containing 'n\_it025\_class003\_box180.mrc'.
- Class3D/C8\_Init\_100mic\_64pix/run\_it025\_class003\_box180.mrc**: A text box showing the full path to the reference map.
- Type the class number of the your best looking class !!**: A text box explaining that the class number (001, 002, 003, or 004) should be entered.
- Tutorial says low-pass filter is 50Å, but 30 Å is better for this case.**: A text box providing a tip on the low-pass filter setting.
- Initial low-pass filter (Å)**: A red box highlights the field set to '30'.
- Symmetry**: A red box highlights the field set to 'C8'.
- Optimisation**: A red box highlights the 'Optimisation' tab at the top.
- Mask diameter (Å)**: A red box highlights the field set to '160'.
- Mask individual particles with zeros?**: A red box highlights the 'Yes' option.
- Use solvent-flattened FSCs?**: A red box highlights the 'No' option.
- Compute**: A red box highlights the 'Compute' tab at the top.
- Number of MPI procs**: A red box highlights the field set to '3'.
- Number of threads**: A red box highlights the field set to '2'.
- Submit to queue?**: A red box highlights the 'No' option.
- Queue name**: A red box highlights the field set to 'openmpi'.
- Use GPU acceleration?**: A red box highlights the 'Yes' option.
- Which GPUs to use**: A red box highlights the field set to '0:1'.

Additional text annotations include:

- Alias: 180pix\_100mic**: A text box indicating the alias for the current job.
- Current: 180pix\_100mic**: A text box showing the current job name.
- ※3つのrelion\_refine\_mpiのジョブが実行される。そのうち2つのジョブがVIRT 34.5GB、RES 1.5GBずつメモリを使う。計算時間は35分ほど。**: A note explaining that three jobs are executed, with two using 34.5GB of virtual memory and 1.5GB of resident memory, and a total computation time of approximately 35 minutes.
- ※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。**: A note warning that execution will start with one GPU but may end prematurely due to an out-of-memory error after 20 minutes.
- Input your GPU numbers. Let's use 2 GPUs !**: A text box suggesting the use of two GPUs.
- [Number of MPI proc] should be odds number more than 2 (3 or 5 or 7 ...)**: A text box explaining that the number of MPI processes should be an odd number greater than 2.

# 5. High resolution 3D refinement

```
011: Class2D/refauto_manu_100mic_64pix
010: Extract/refauto_manu_100mic_64pix
009: AutoPick/manu_100mic_64pix/
60/ 60 sec .....
Auto-refine: Refinement has converged, stopping now...
Auto-refine: + Final reconstruction from all particles is saved as: Refine3D/job019/run_class001.mrc
Auto-refine: + Final model parameters are stored in: Refine3D/job019/run_model.star
Auto-refine: + Final data parameters are stored in: Refine3D/job019/run_data.star
Auto-refine: + Final resolution (without masking) is: 4.10667
```

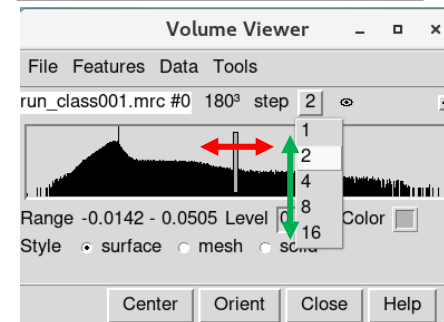
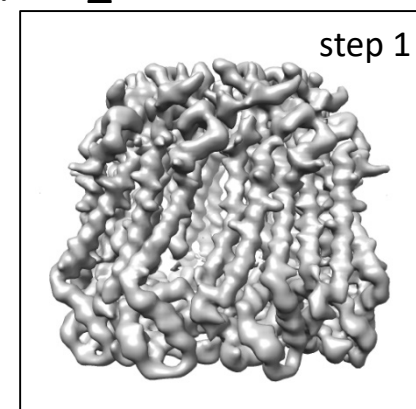
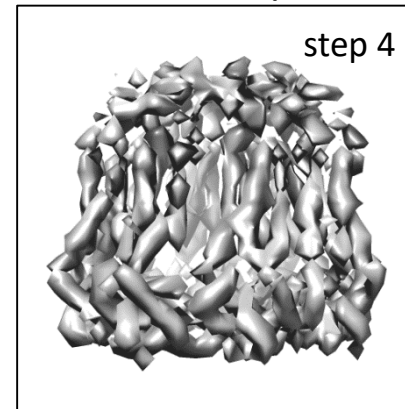
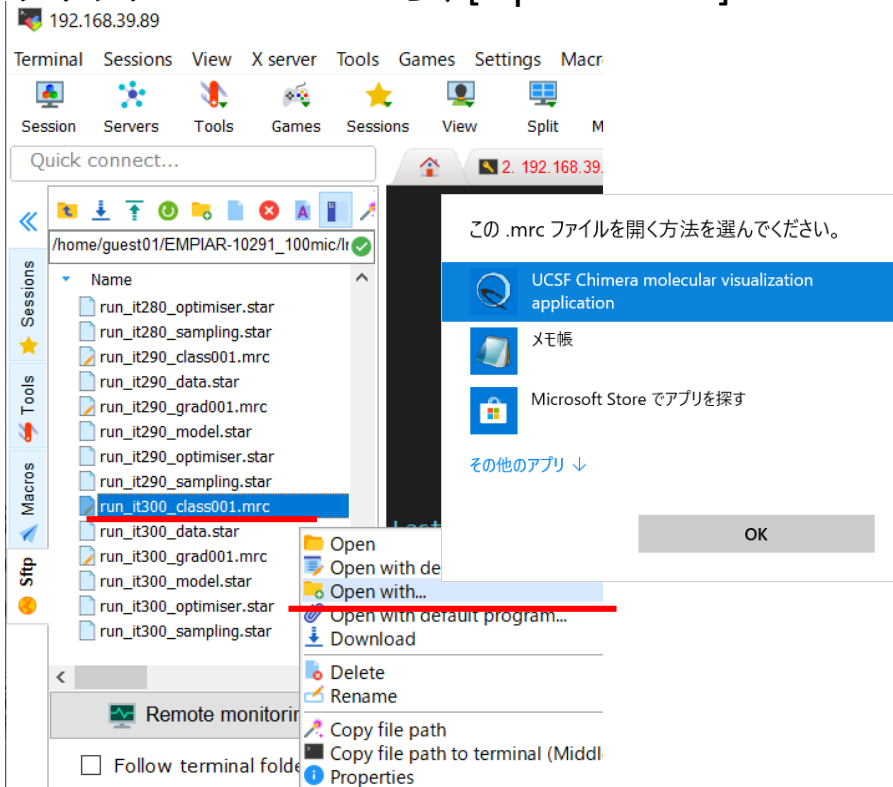
You can know the resolution from the message from Relion GUI.

or type a following command:

```
tail Refine3D/180pix_100mic/run.out
```

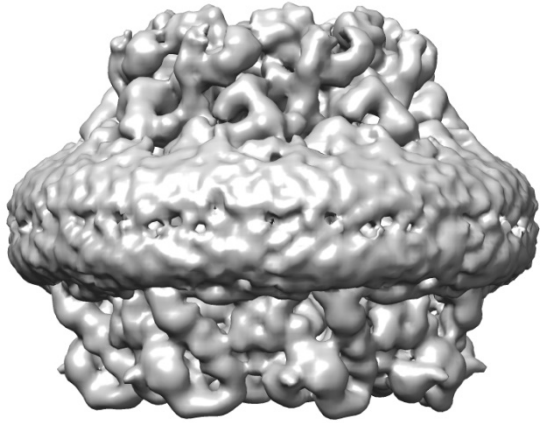
MobaXtermのファイルブラウザで、Refine3D/180pix\_100mic/run\_class001.mrcを選び、右クリック・メニューから、[Open with...]でUCSF Chimeraで開いて確認する。

Refine3D/first3dref/run\_class001.mrc

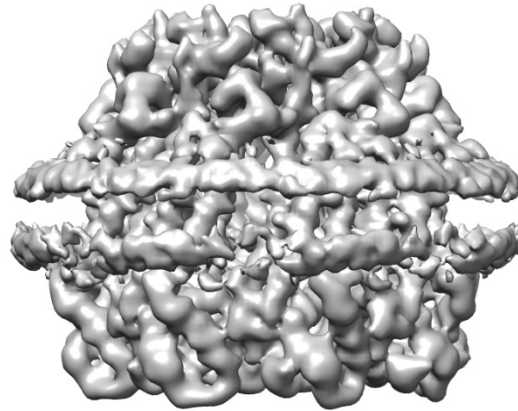




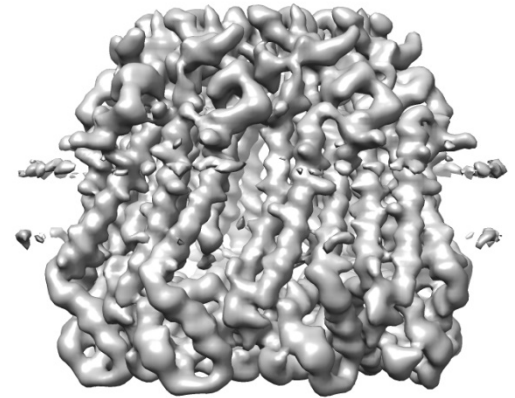
# Level threshold for Refined3d map



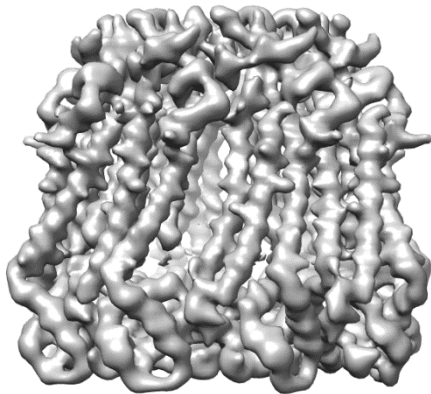
Level = 0.01



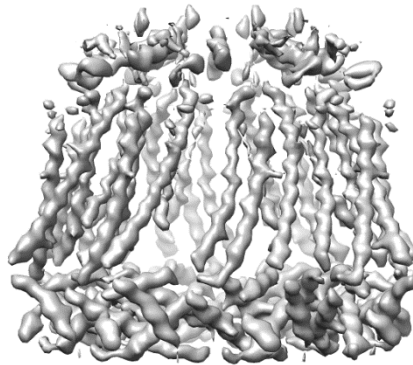
Level = 0.013



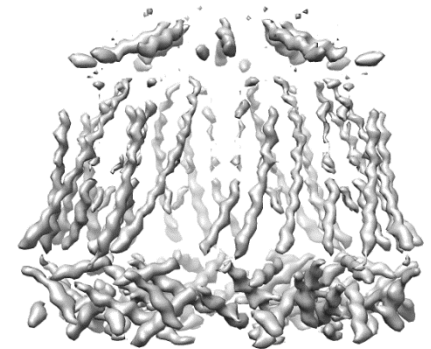
Level = 0.016



Level = 0.018

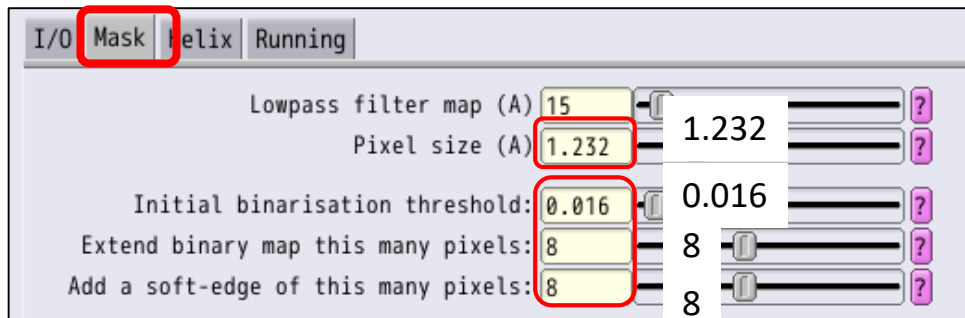
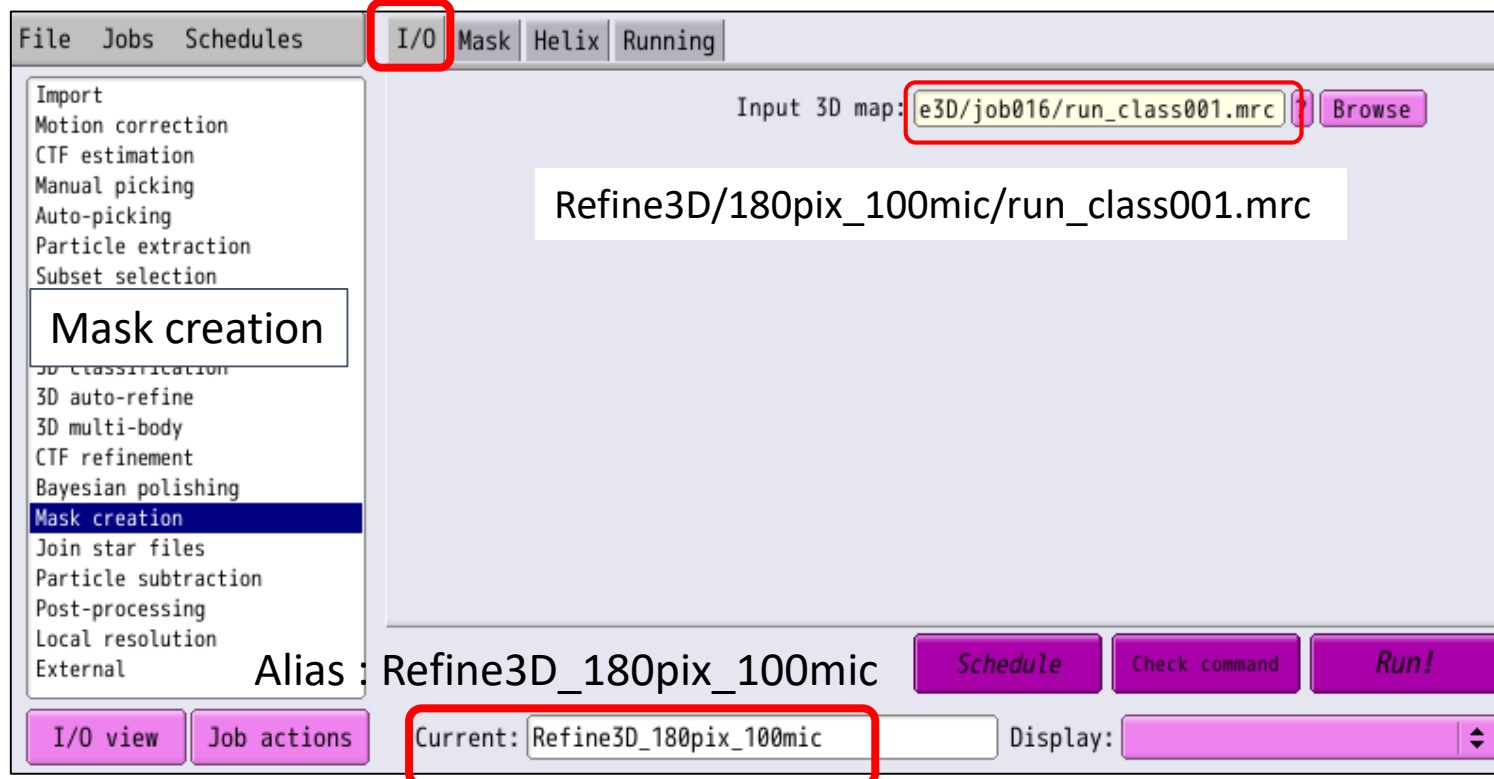


Level = 0.025



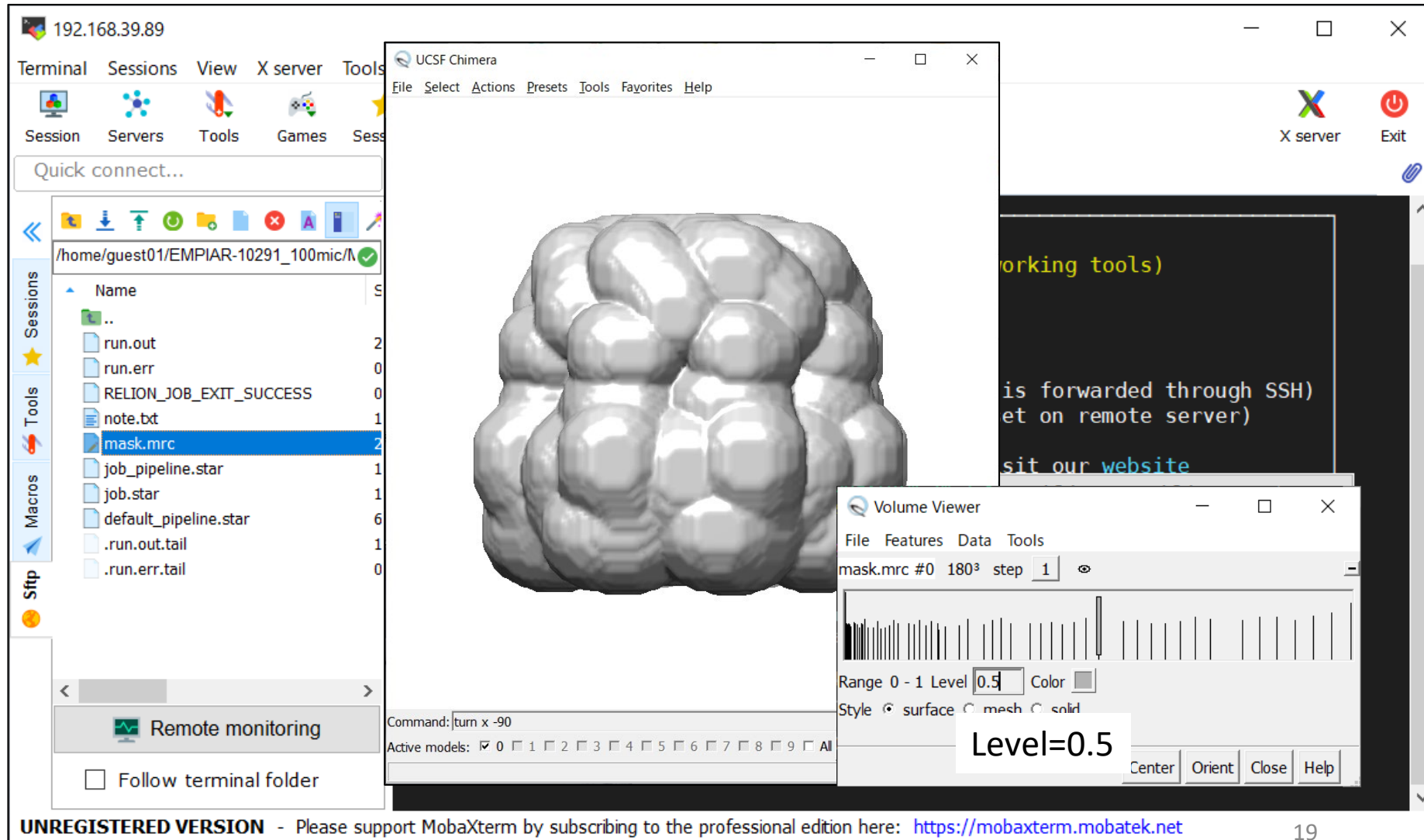
Level = 0.035

# 6.1 Making a Mask (1分)



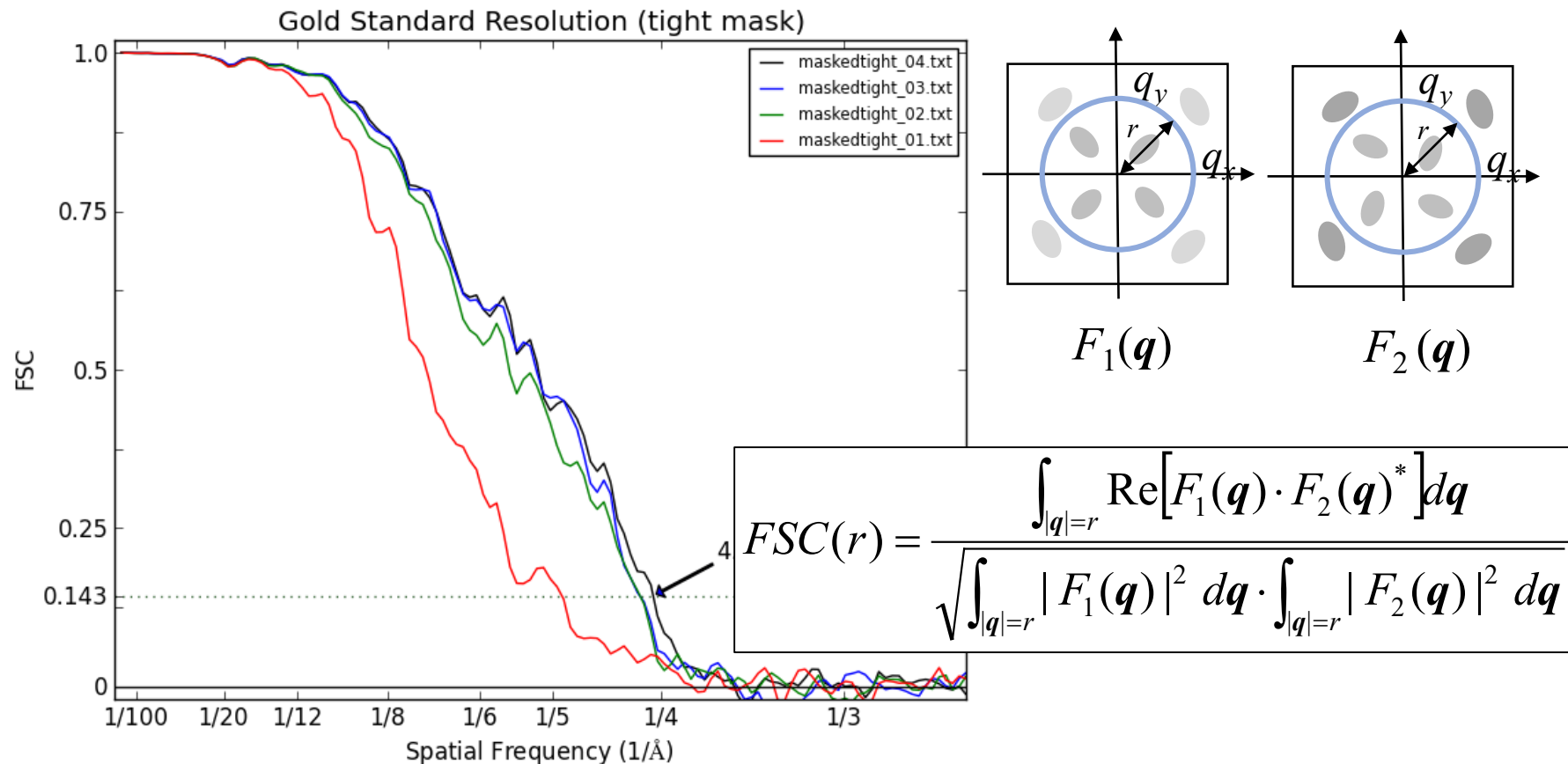
# 6.1 Making a Mask : Checking mask.mrc

MobaXtermのファイルブラウザで、MaskCreate/Refine3D\_180pix\_100mic/mask.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。

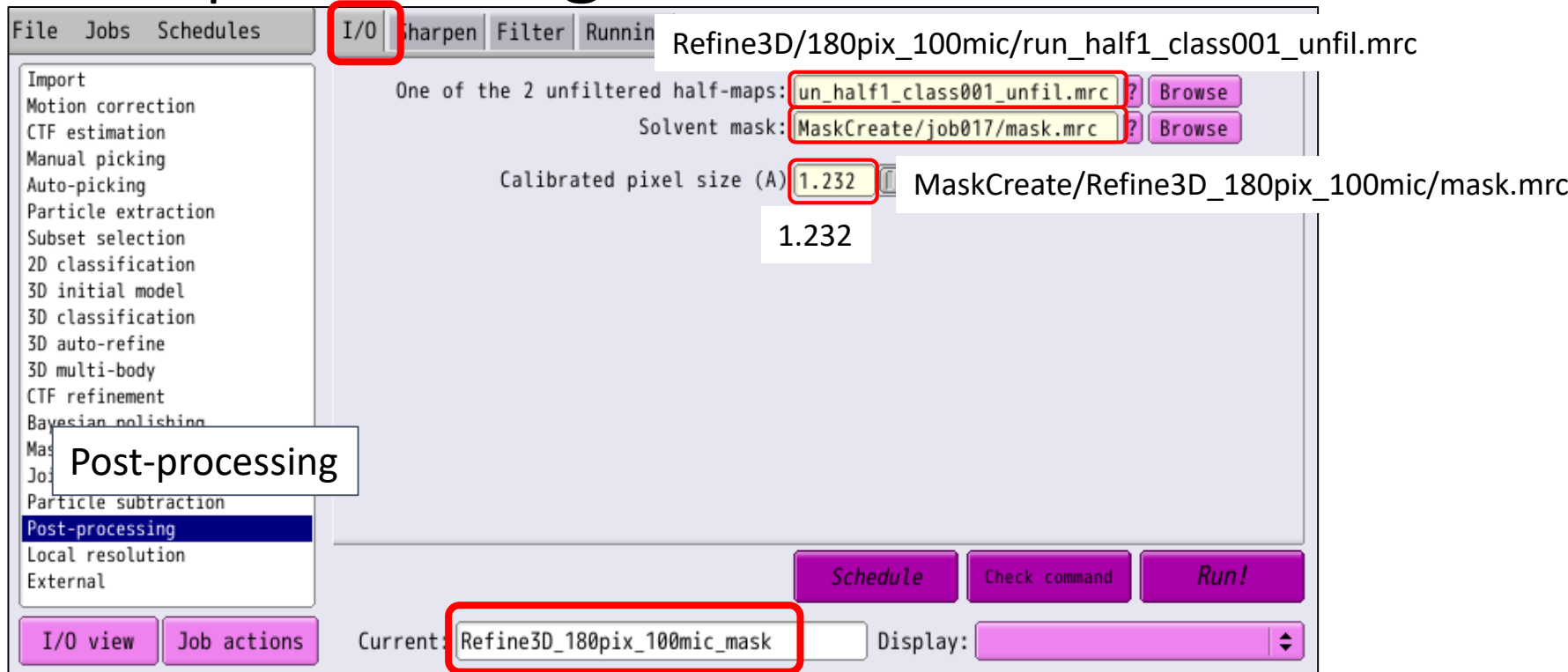


# Fourier Shell Correlation (FSC)による解像度

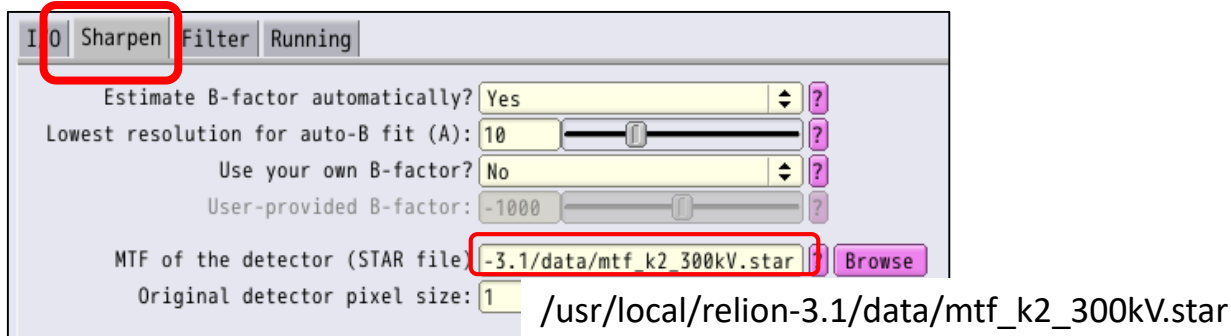
- 1) 2D粒子画像群を二つに分け、それぞれ独立に3Dマップ $f_1(x), f_2(x)$ を作成する。それをフーリエ変換し $F_1(q), F_2(q)$ とする。
- 2) ある周波数  $r=|q|$  の球殻(spherical shell)上で、の相関係数 $FSC(r)$ を計算する。
- 3) 相関係数 $FSC(r)$ を横軸を空間周波数に対して図示。FSC=0.5あるいは0.143(=1/7)のときの周波数の値を解像度とする。



# 6.2 Postprocessing

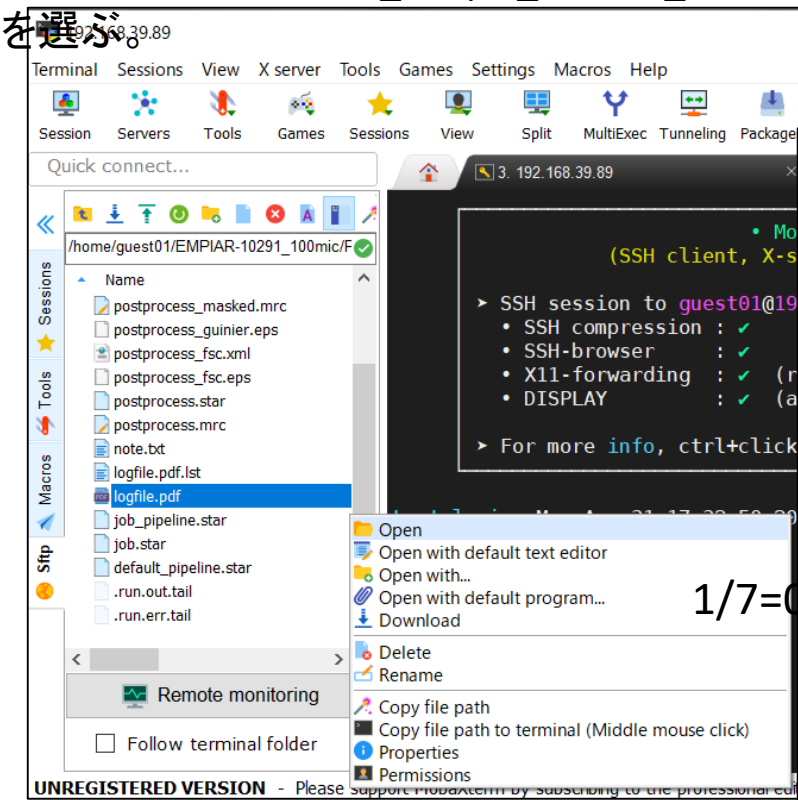


Alias:Refine3D\_180pix\_100mic\_mask



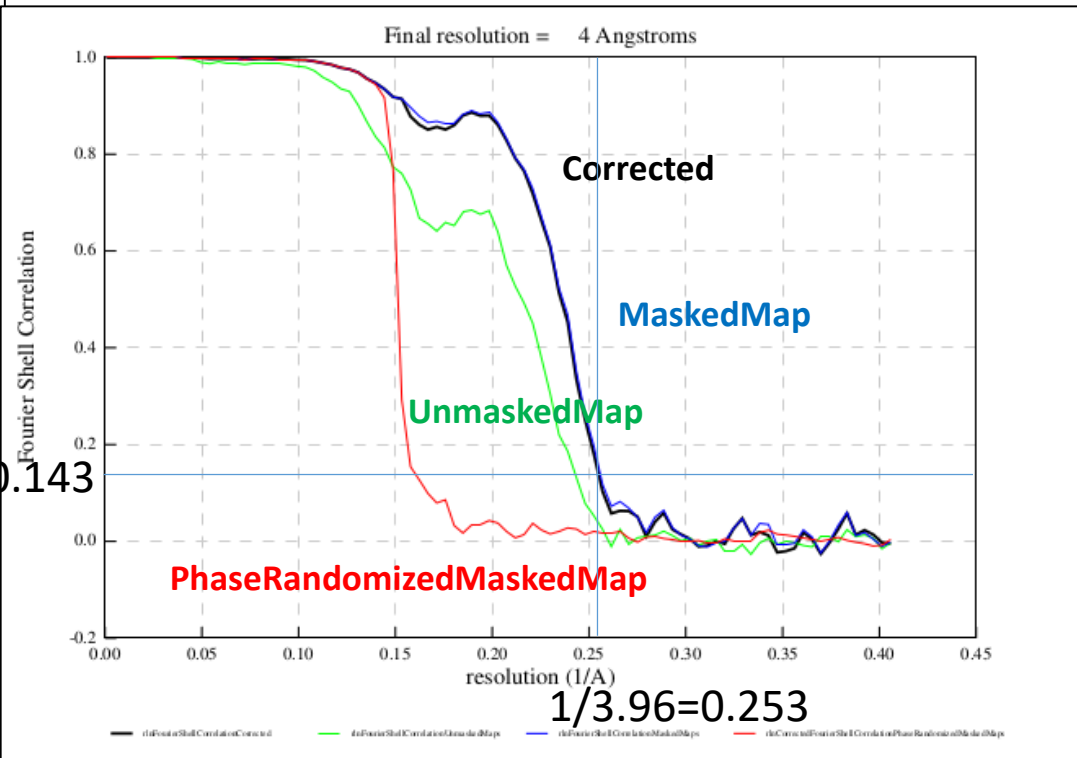
# 6.2 結果の確認

MobaXtermのファイルブラウザで、  
PostProcess/Refine3D\_180pix\_100mic\_mask/logfile.pdf  
を選ぶ。



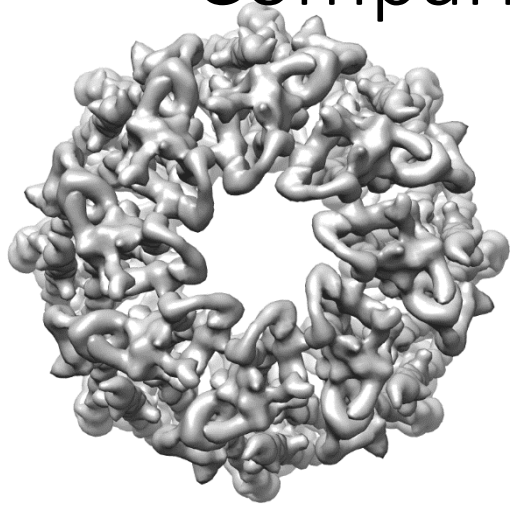
+ intercept of fit:	-11.6428
+ correlation of fit:	0.955742
+ apply b-factor of:	-198.981
== Writing output files ...	
+ Processed map:	PostProcess/job018/postprocess.mrc
+ Processed masked map:	PostProcess/job018/postprocess_masked.mrc
+ Metadata file:	PostProcess/job018/postprocess.star
+ FINAL RESOLUTION:	3.96

+ FINAL RESOLUTION: 3.96

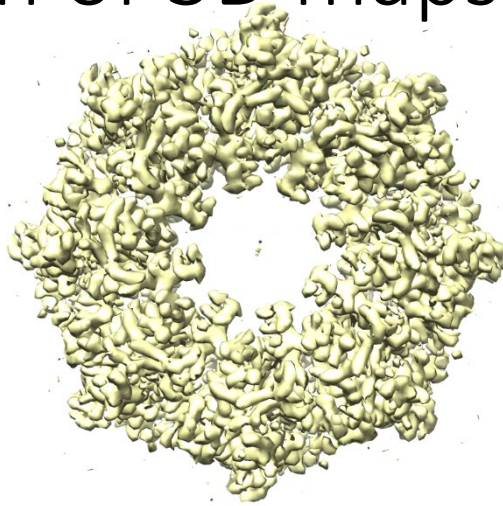




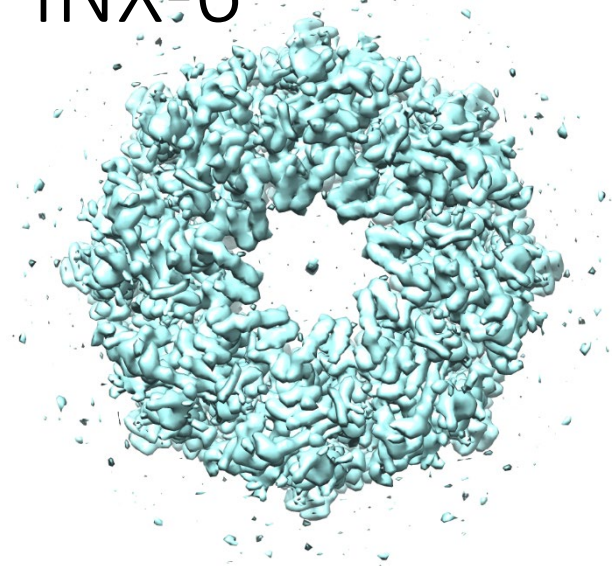
# Comparison of 3D maps of INX-6



Refine3D  
Resolution: 4.11 Å  
from 100 micrographs



Postprocess with mask  
Resolution: 3.96 Å  
from 100 micrographs



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