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

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大阪大学

INSTITUTE for PROTEIN RESEARCH

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.

[Nprocess] × [threads for each process] <= [Total Nthreads in machine]

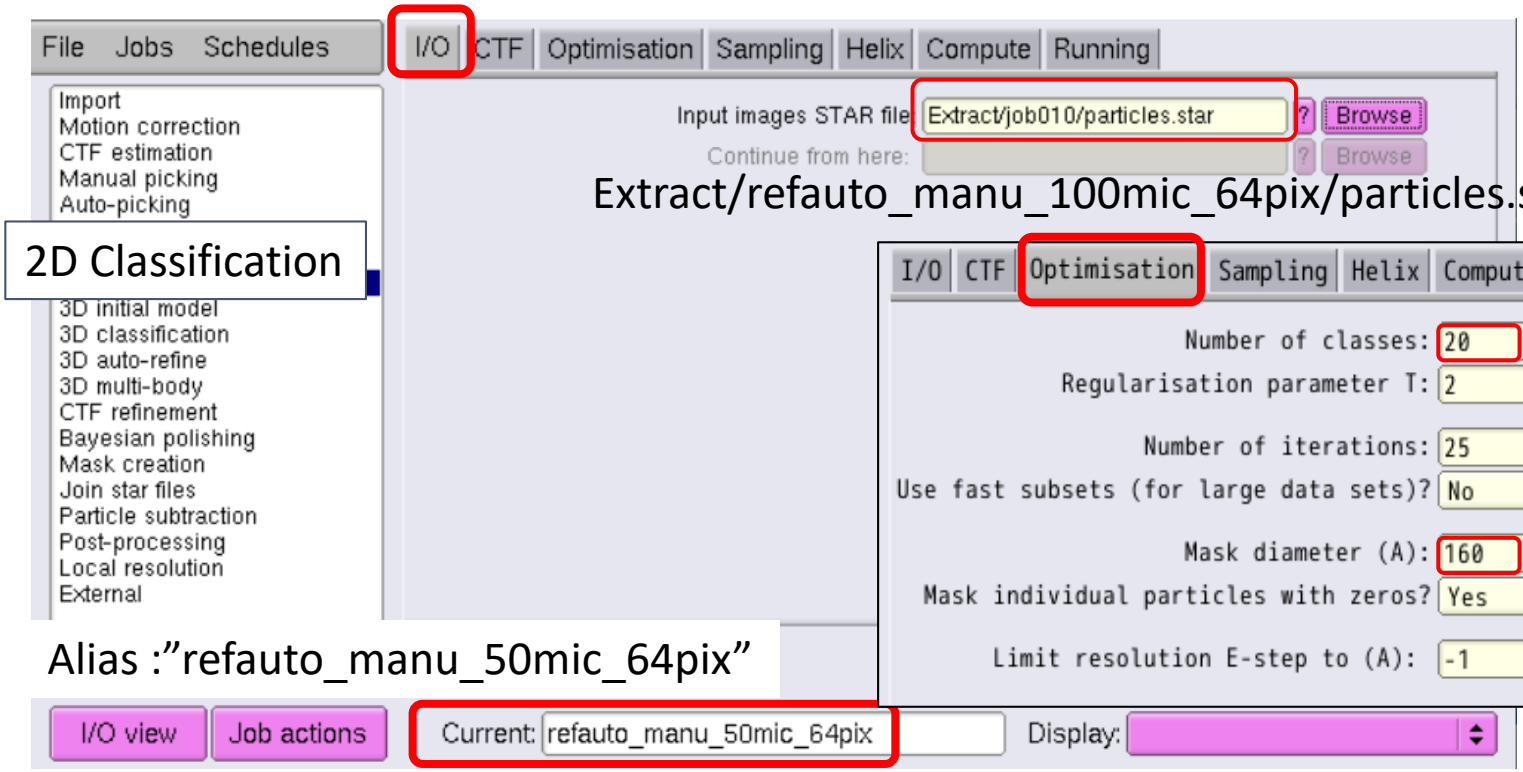
- Each process use GPU (not thread). Many processes can share one GPU.

[Nprocess] >= [N_GPU]

Refine3D requires

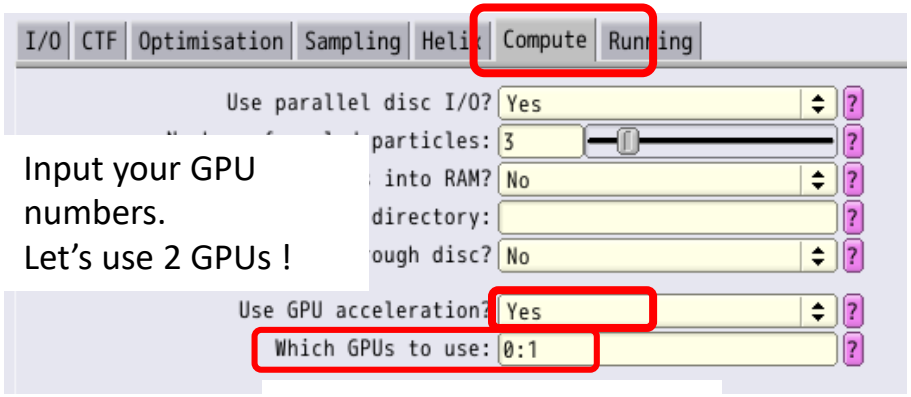
Even number of GPUs,
[Nprocess] = [N_GPU] + 1

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

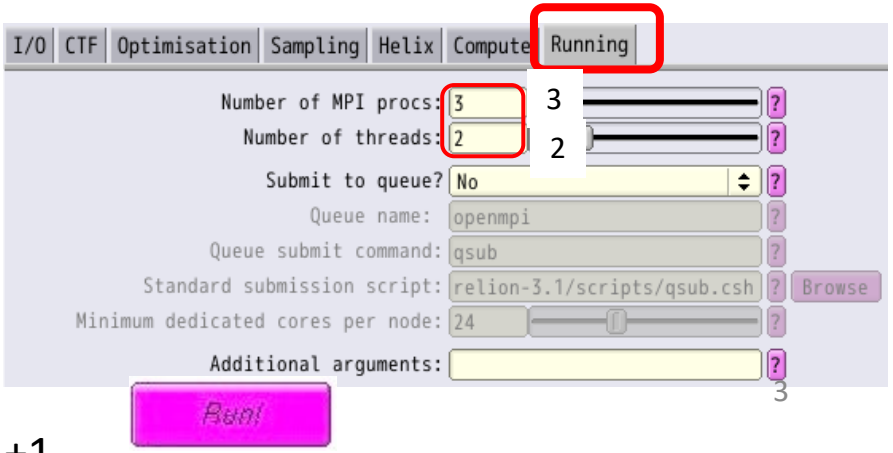


Extract/refauto_manu_100mic_64pix/particles.star

Alias :"refauto_manu_50mic_64pix"



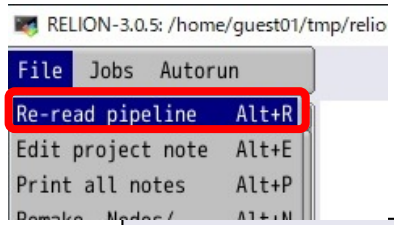
Input your GPU numbers.
Let's use 2 GPUs !

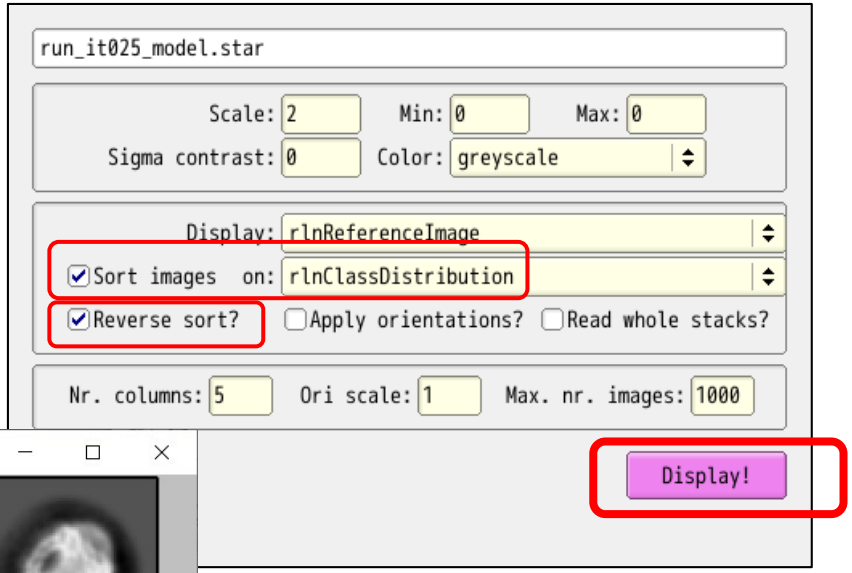


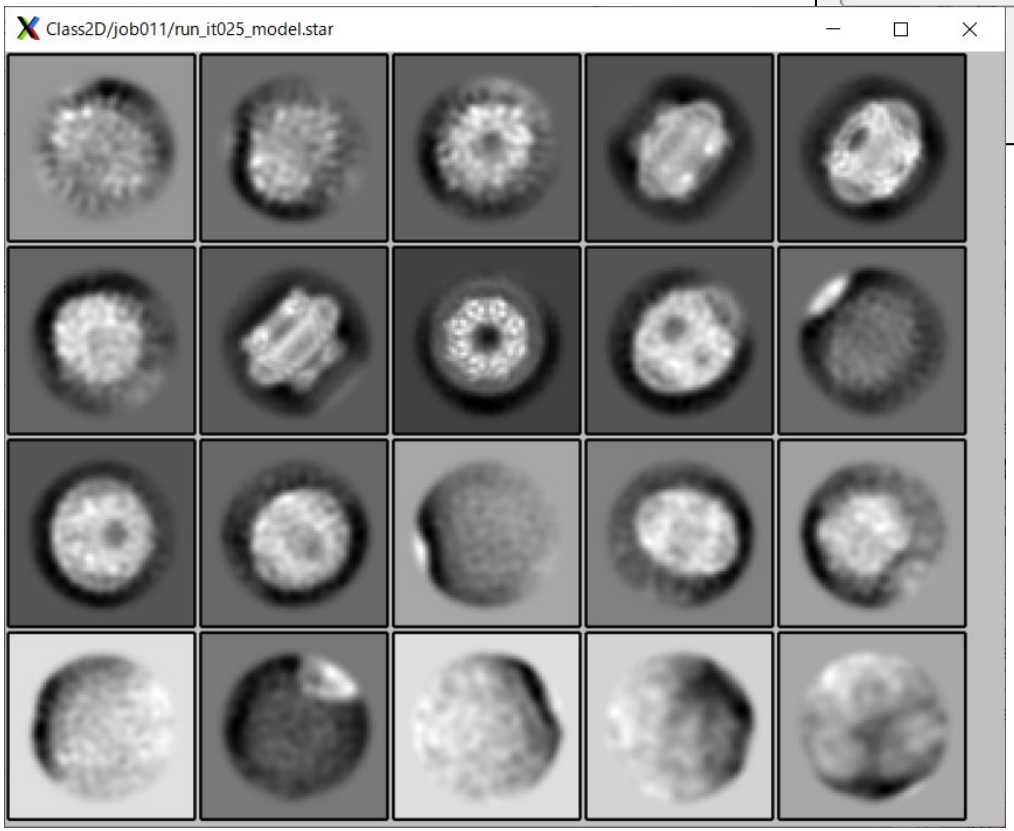
Input your GPU numbers.
Let's use 2 GPUs !

[#MPI] = [#GPU] +1

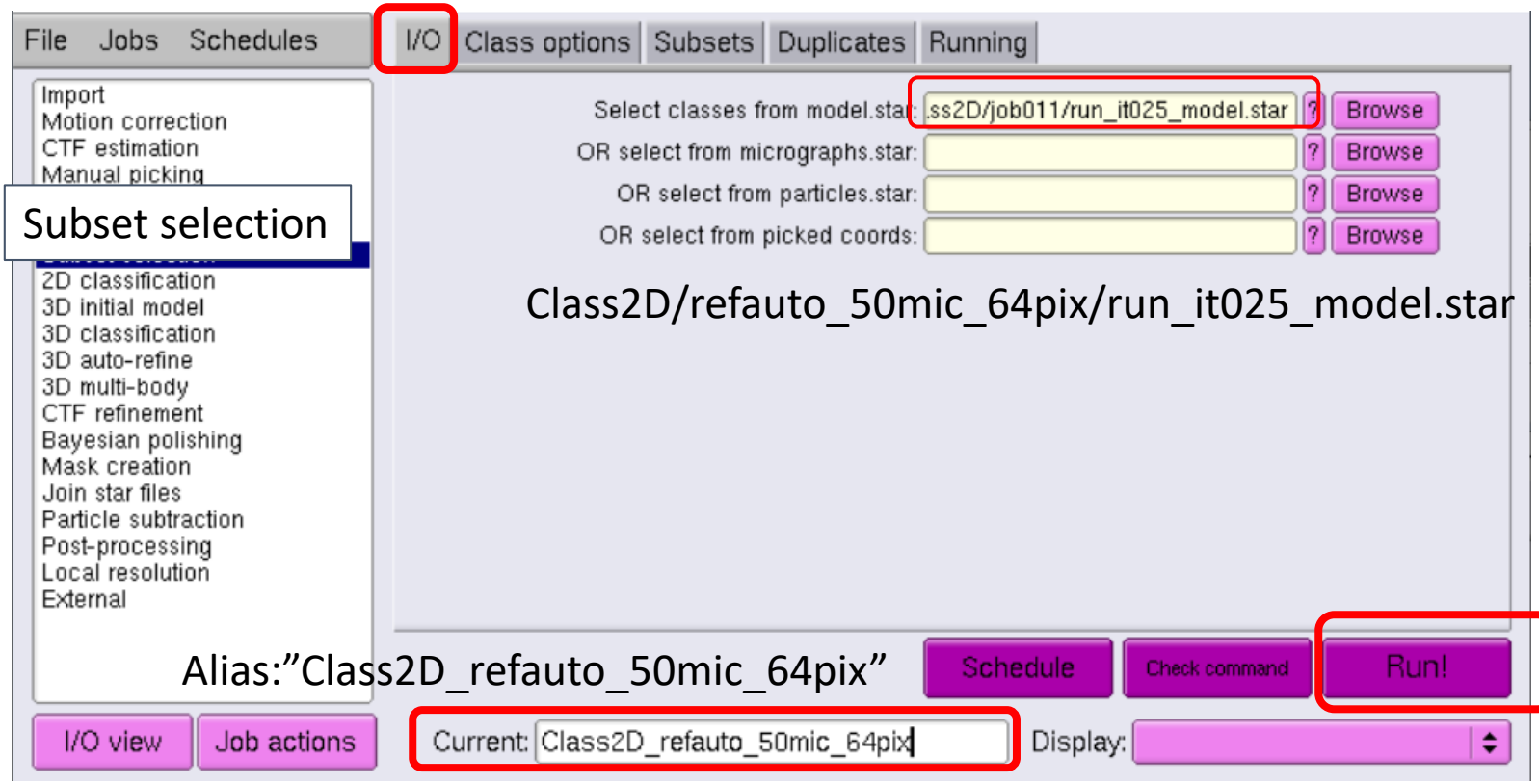
011:Reference-free 2D class averaging

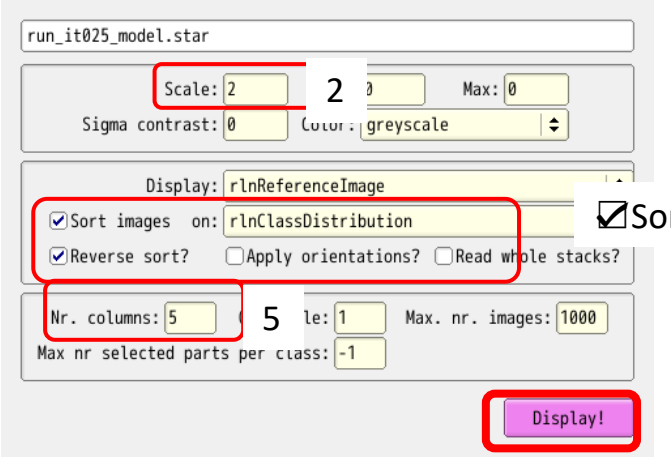






012: Subset selection from 2D classes

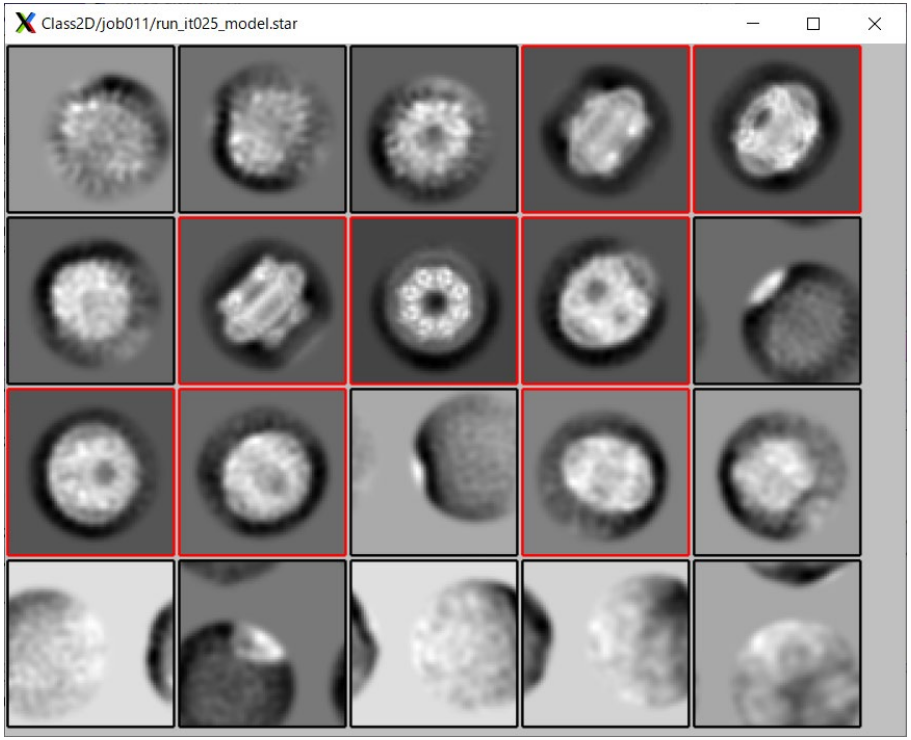




☑Sort images on: rlnClassDistribution

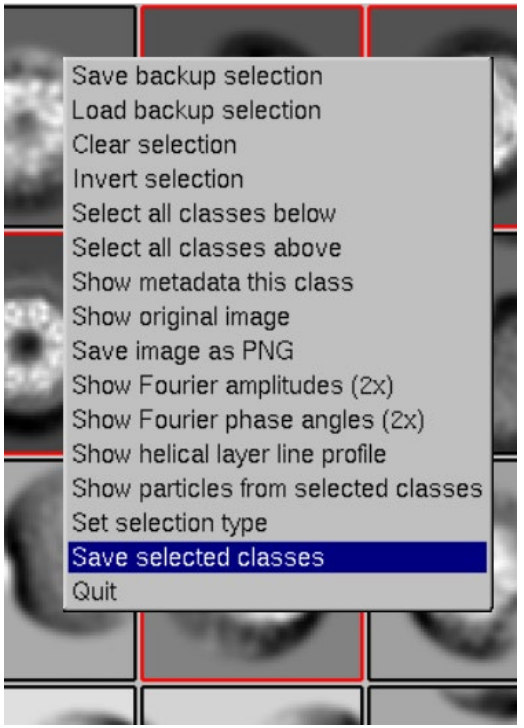
012: Subset selection from 2D classes

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




```
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.
```

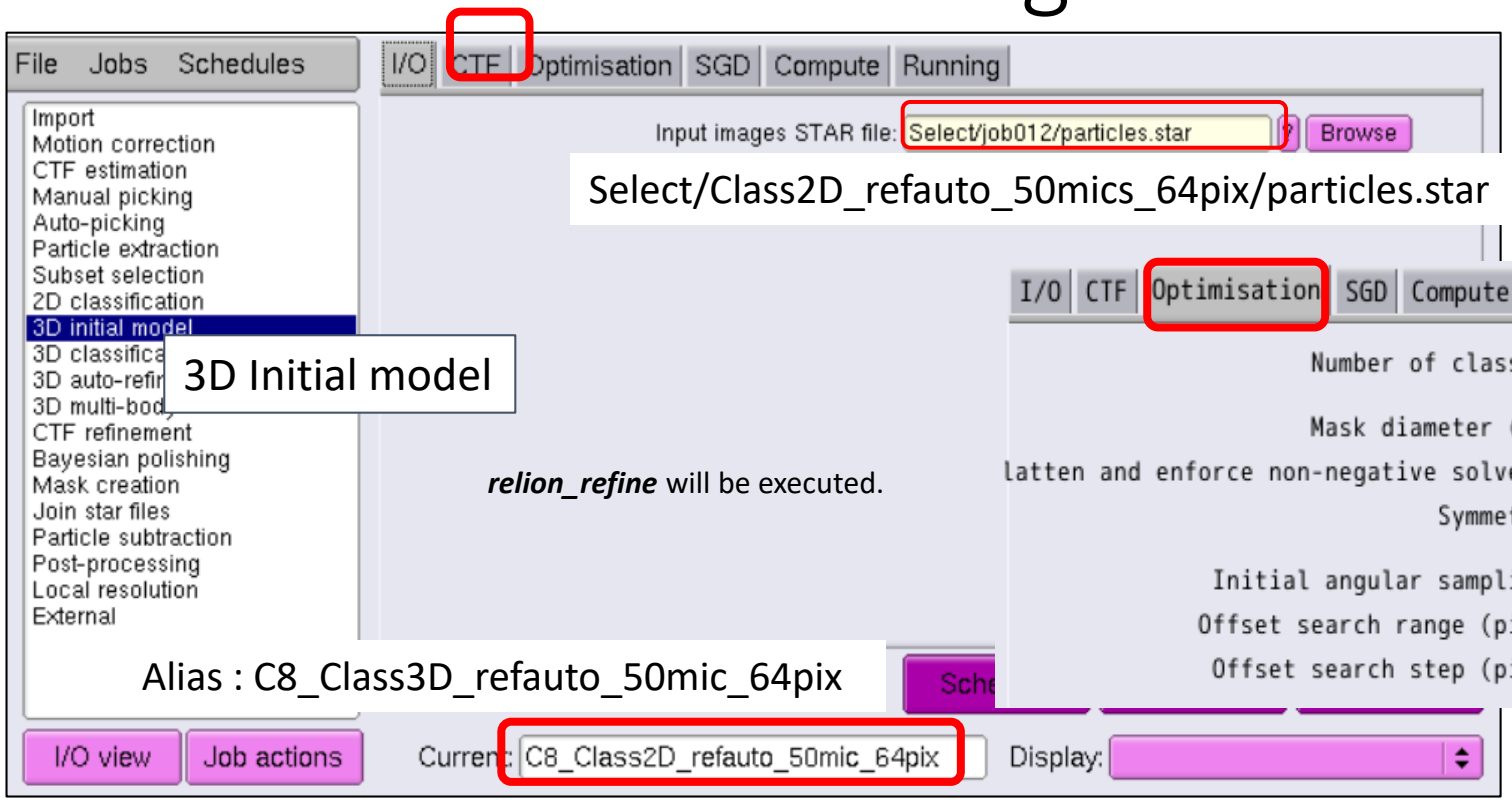
Click the right mouse button, and Select [Save selected classes]

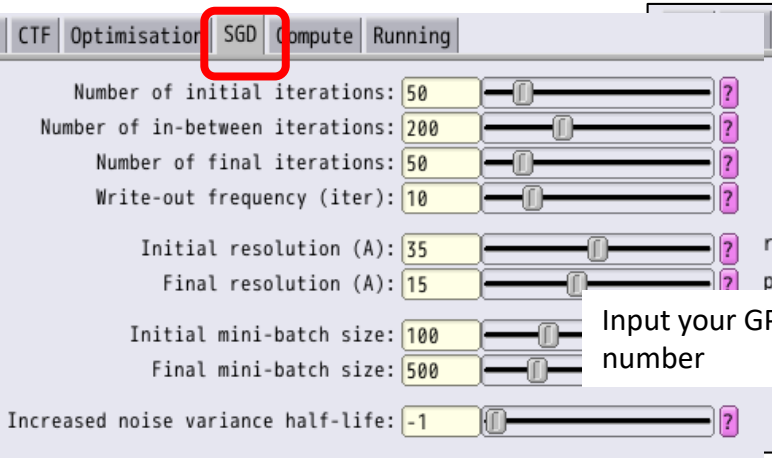


Saved Select/job012/class_averages.star with **8** selected images.
Saved Select/job012/particles.star with **14932** selected particles.

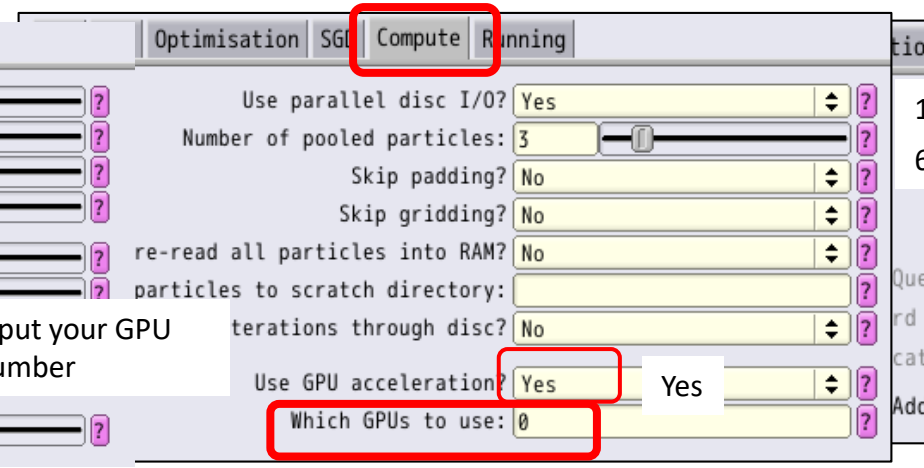
013:De novo 3D model generation

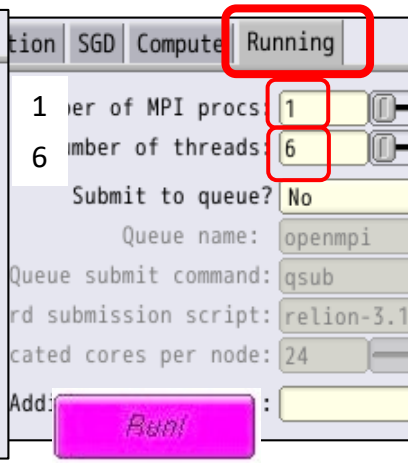
[1GPU, 10 min]





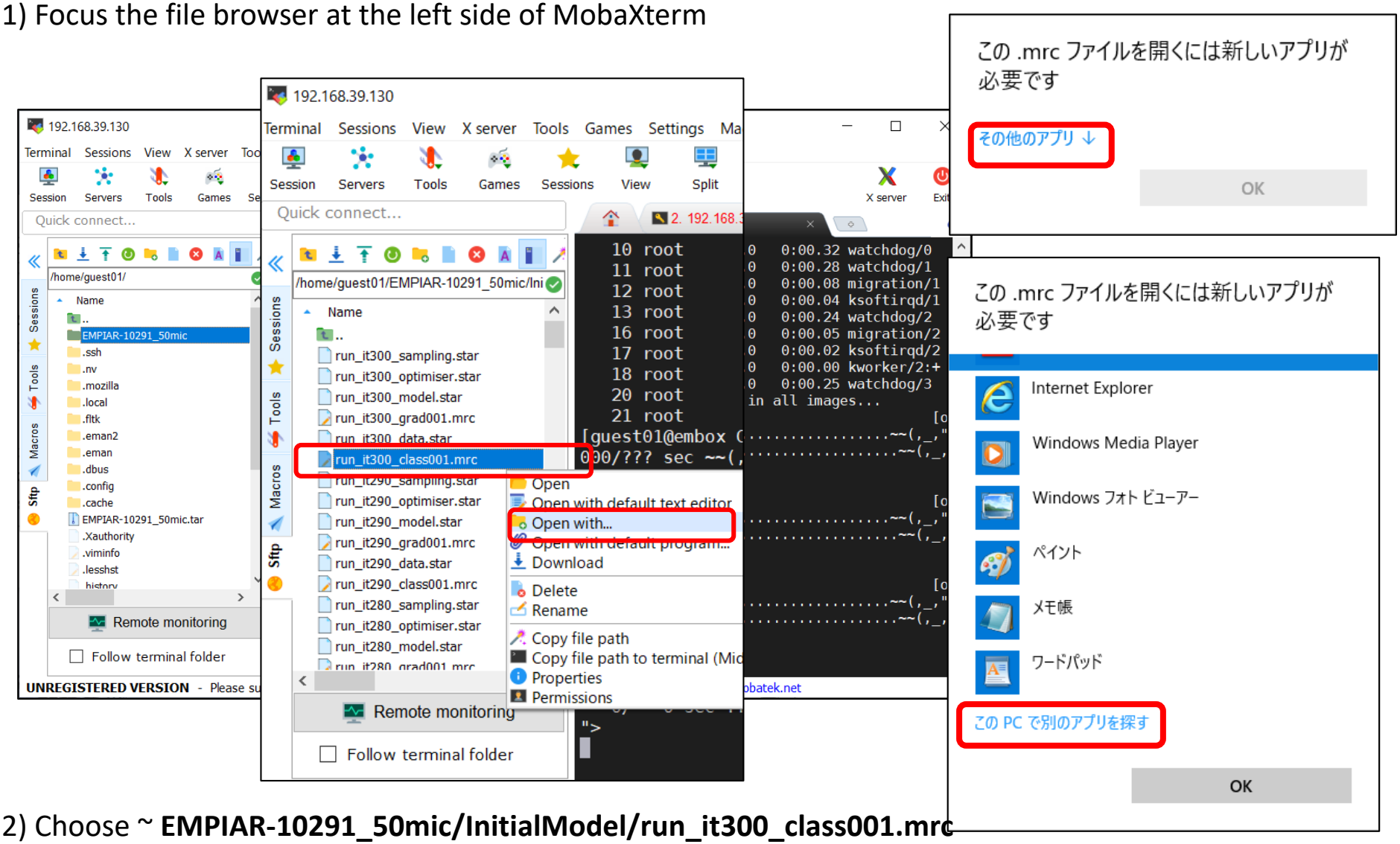
Input your GPU number





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

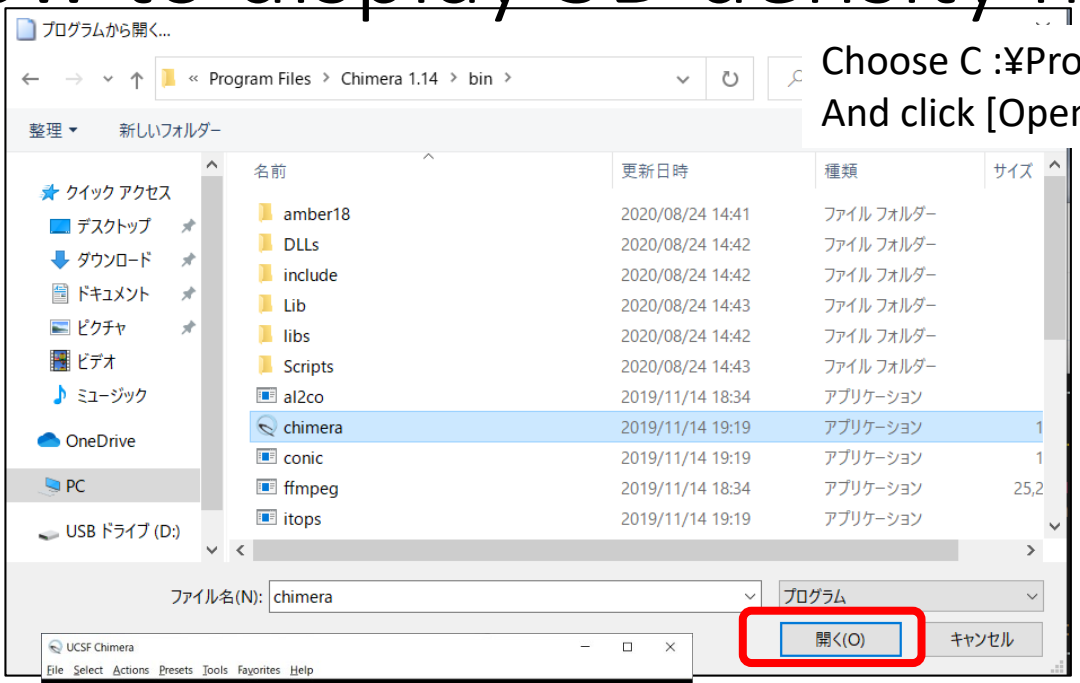
1) Focus the file browser at the left side of MobaXterm



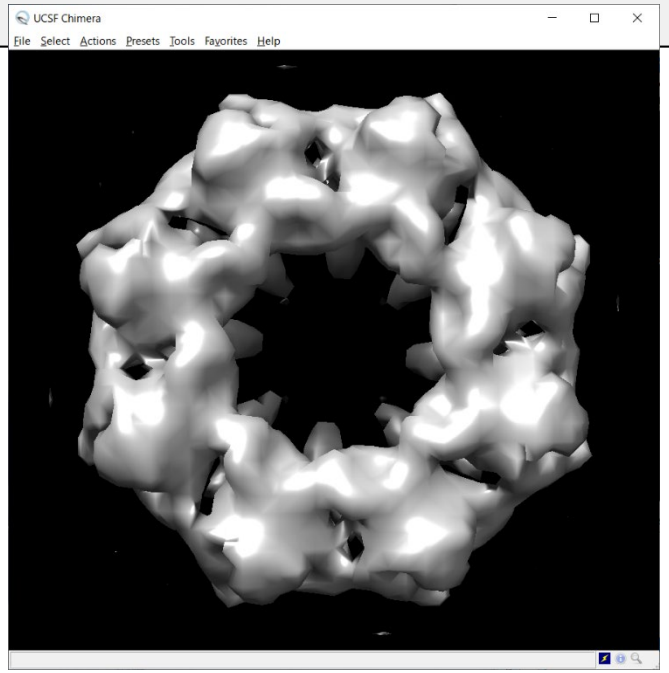
2) Choose ~ **EMPIAR-10291_50mic/InitialModel/run_it300_class001.mrc**
Click the right mouse button and chose [Open with…]

3) Setup application to open *.mrc file

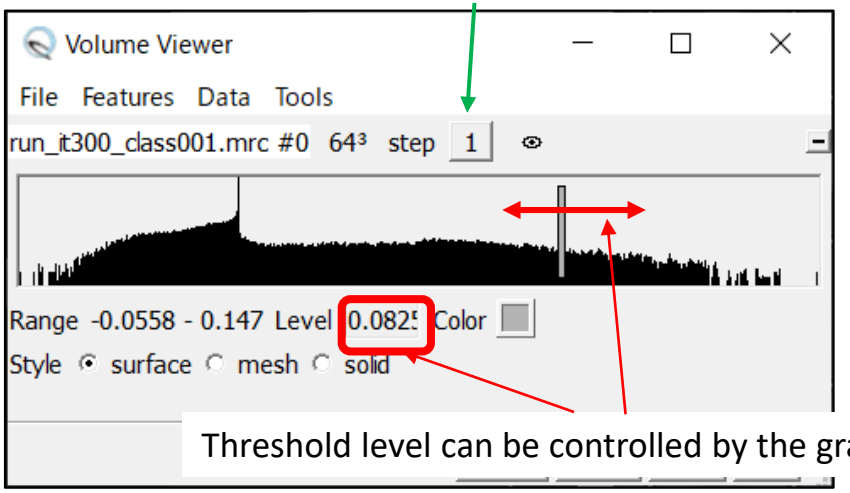
How to display 3D density map(*.mrc)



Choose C :¥Program Files ¥ Chimera1.14¥bin¥chimera
And click [Open]



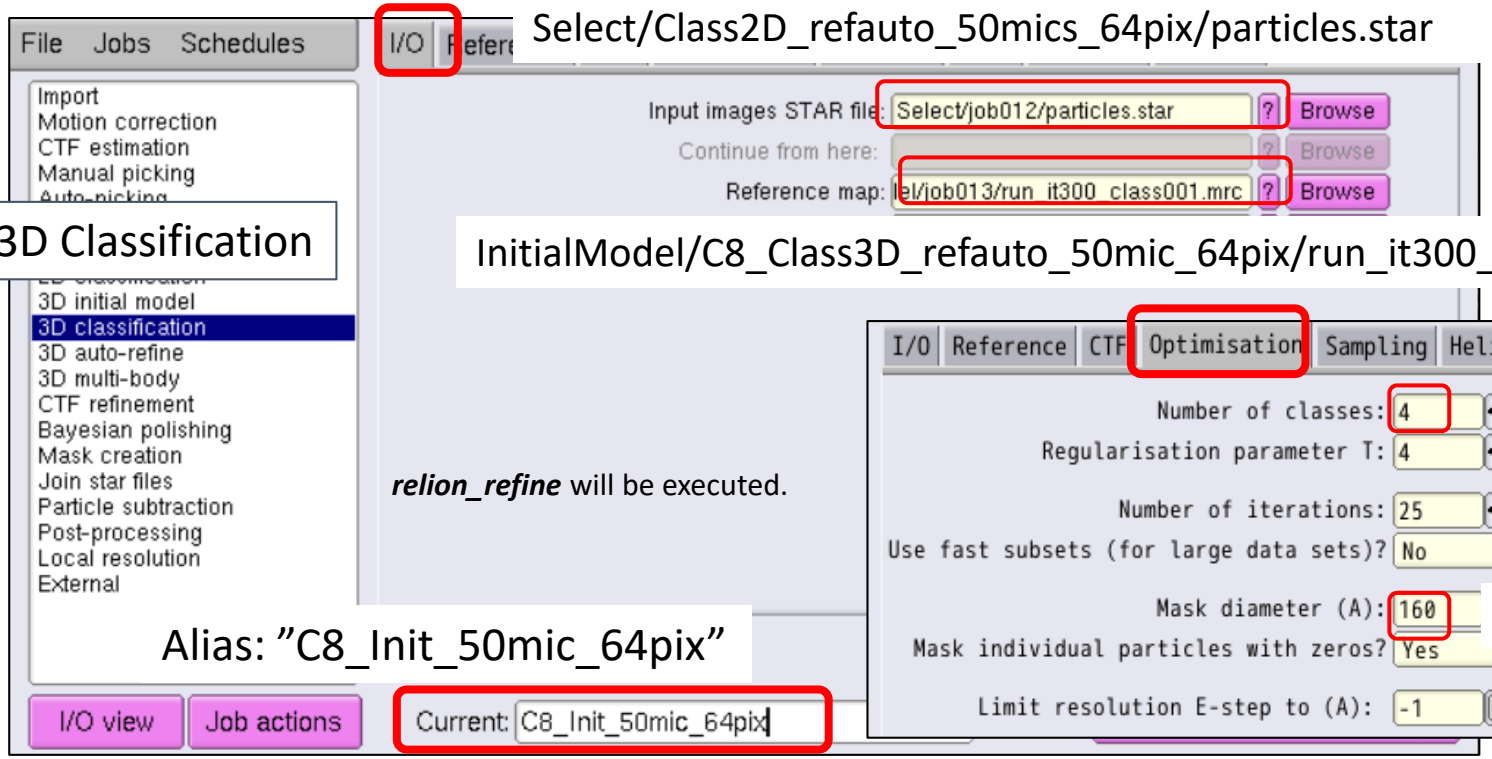
[step] controls number of minimum voxels for display.
If step=2, 2x2x2 voxels are unified into one voxel.



Threshold level can be controlled by the gray bar.

014: 3D classification

[2GPUs, 12 min]



Select/Class2D_refauto_50mics_64pix/particles.star

3D Classification

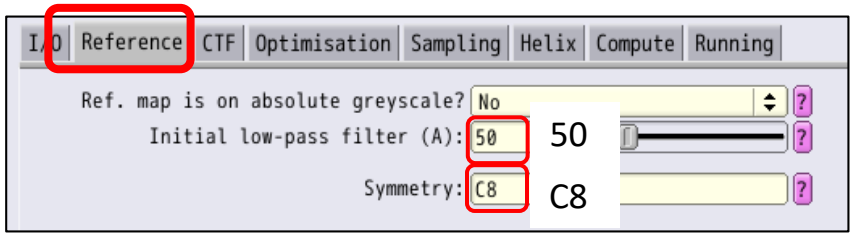
InitialModel/C8_Class3D_refauto_50mic_64pix/run_it300_class001.mrc

relion_refine will be executed.

4

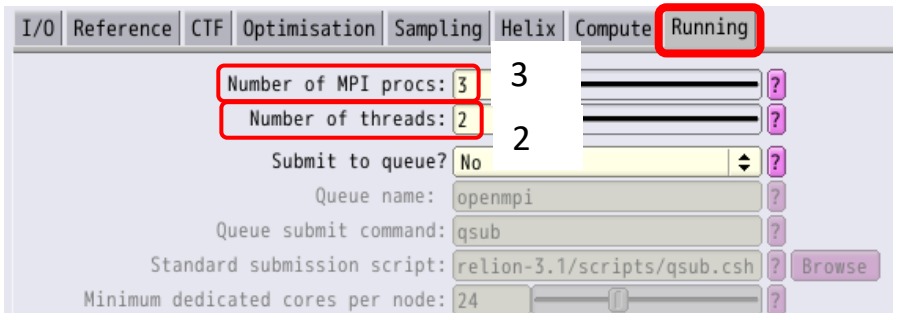
160

Alias: ”C8_Init_50mic_64pix”



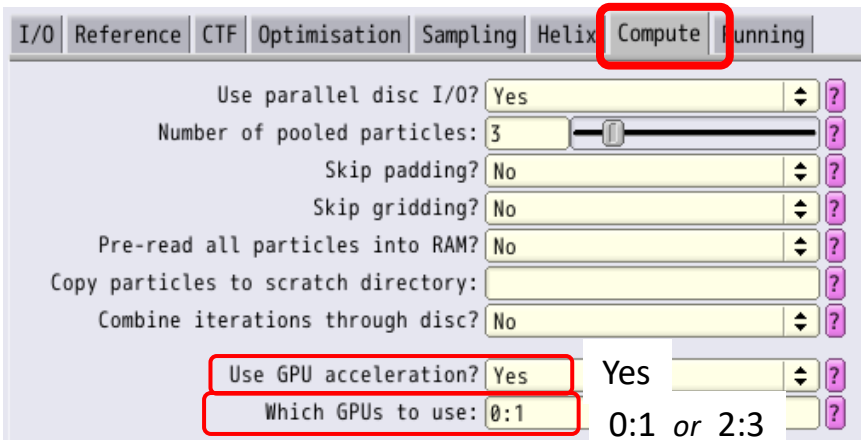
50

C8



3

2



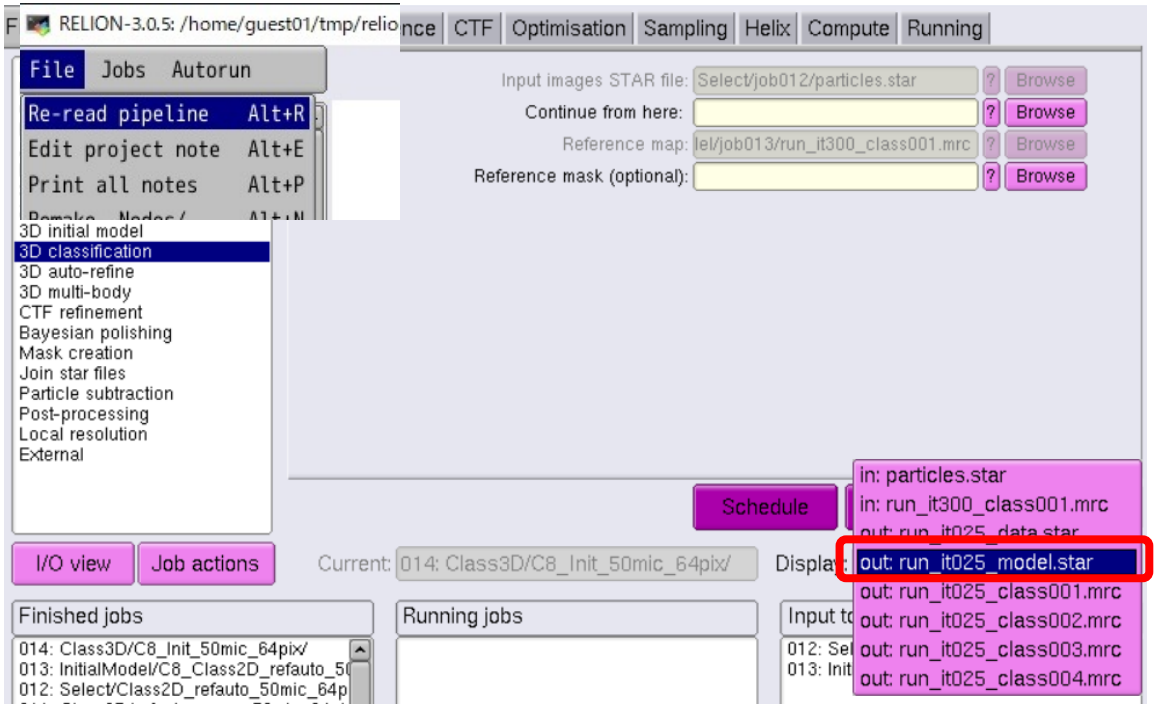
Yes

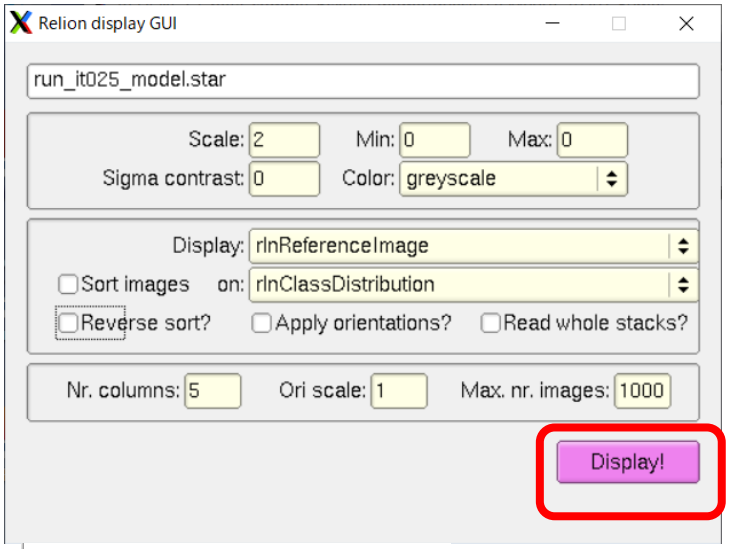
0:1 *or* 2:3

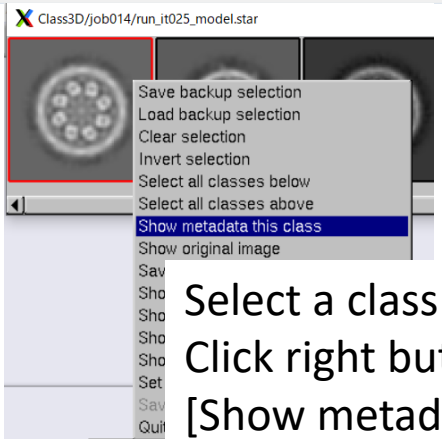
Run!

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

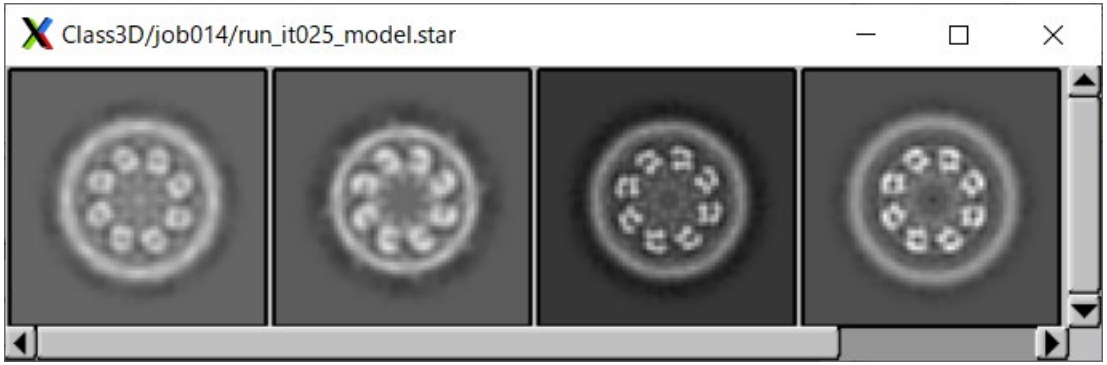
014: 3D classification







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

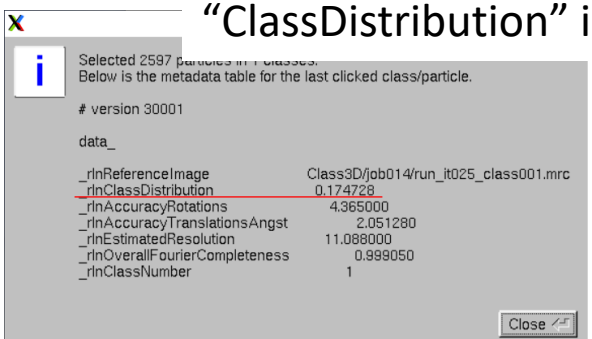


Class001
0.174728

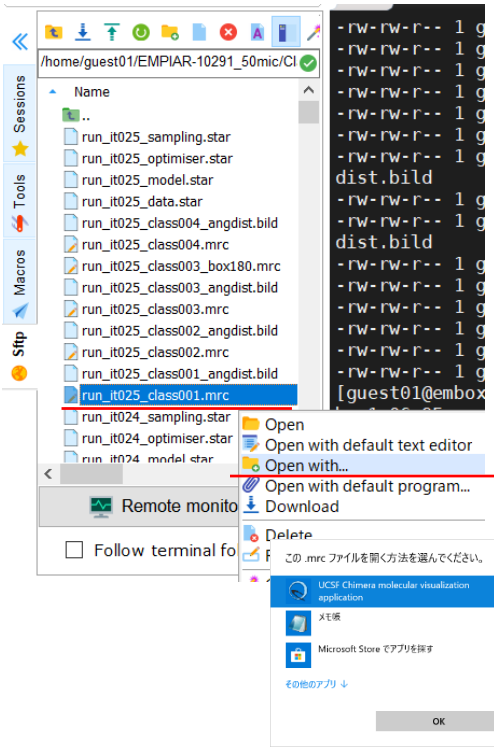
Class002
0.198948

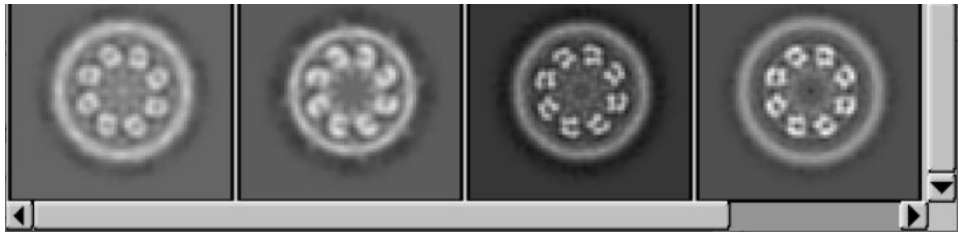
Class003
0.286835

Class004
0.339489



014: 3D classification



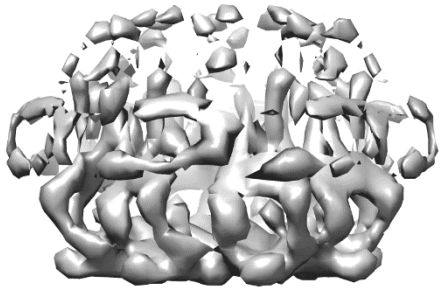


Class001 0.174728 | Class002 0.198948 | Class003 0.286835 | Class004 0.339489

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

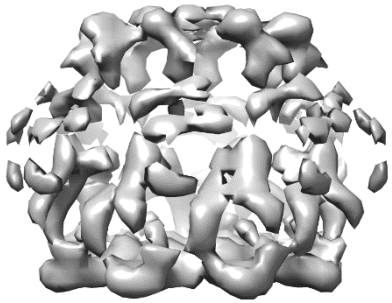
Directory: **EMPIAR-10291_50mic/Class3D/C8_Init_50mic_64pix/**

3D density maps: **run_it025_class001.mrc, run_it025_class002.mrc, run_it025_class003.mrc, run_it025_class004.mrc**



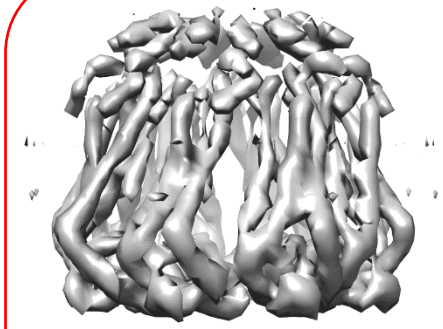
run_it025_class001.mrc

ClassDistribution 0.174728



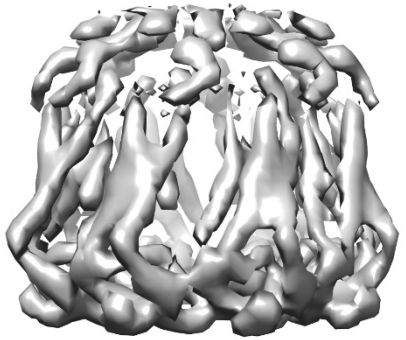
run_it025_class002.mrc

ClassDistribution 0.198948



run_it025_class003.mrc

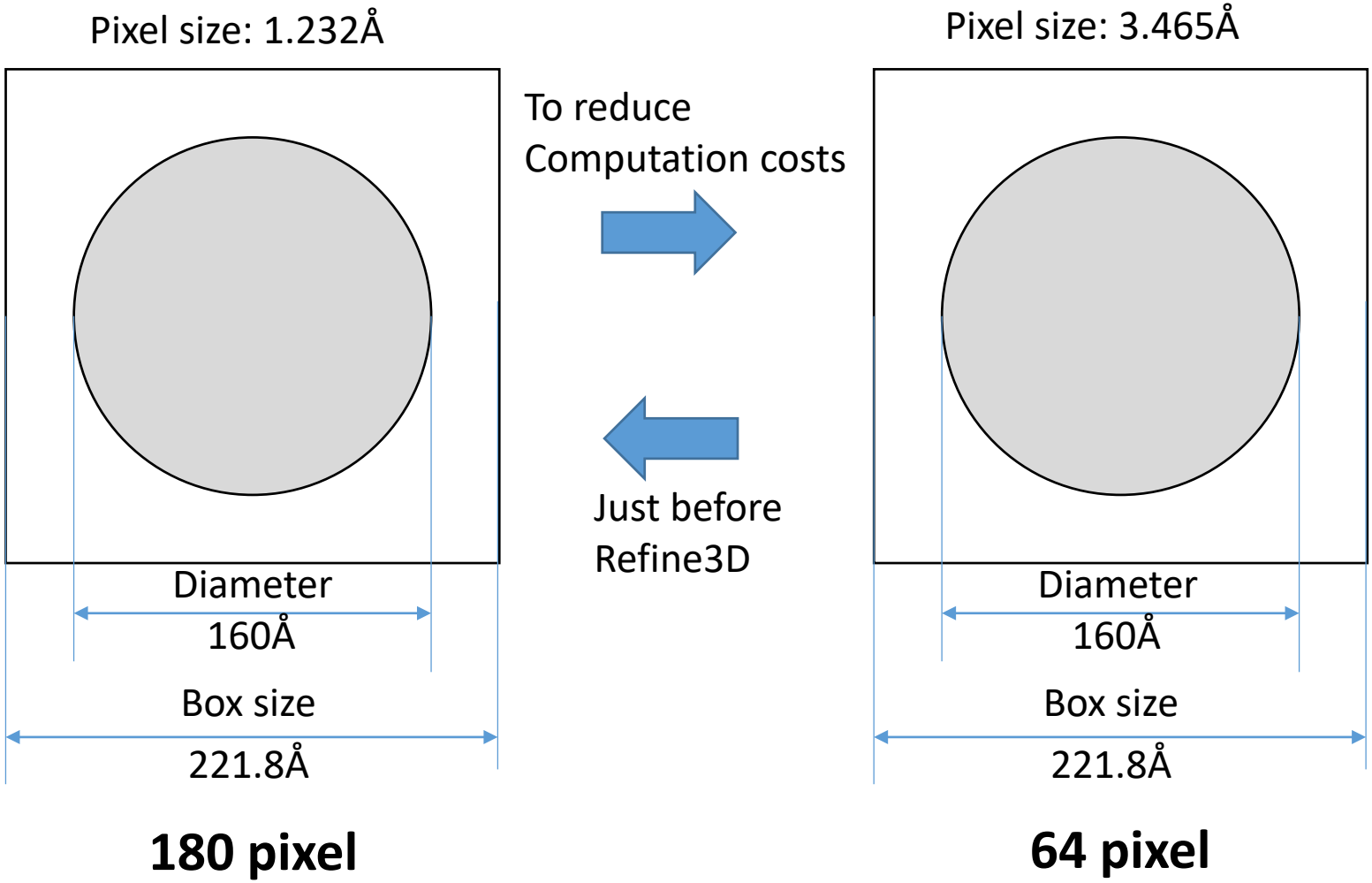
ClassDistribution 0.286835



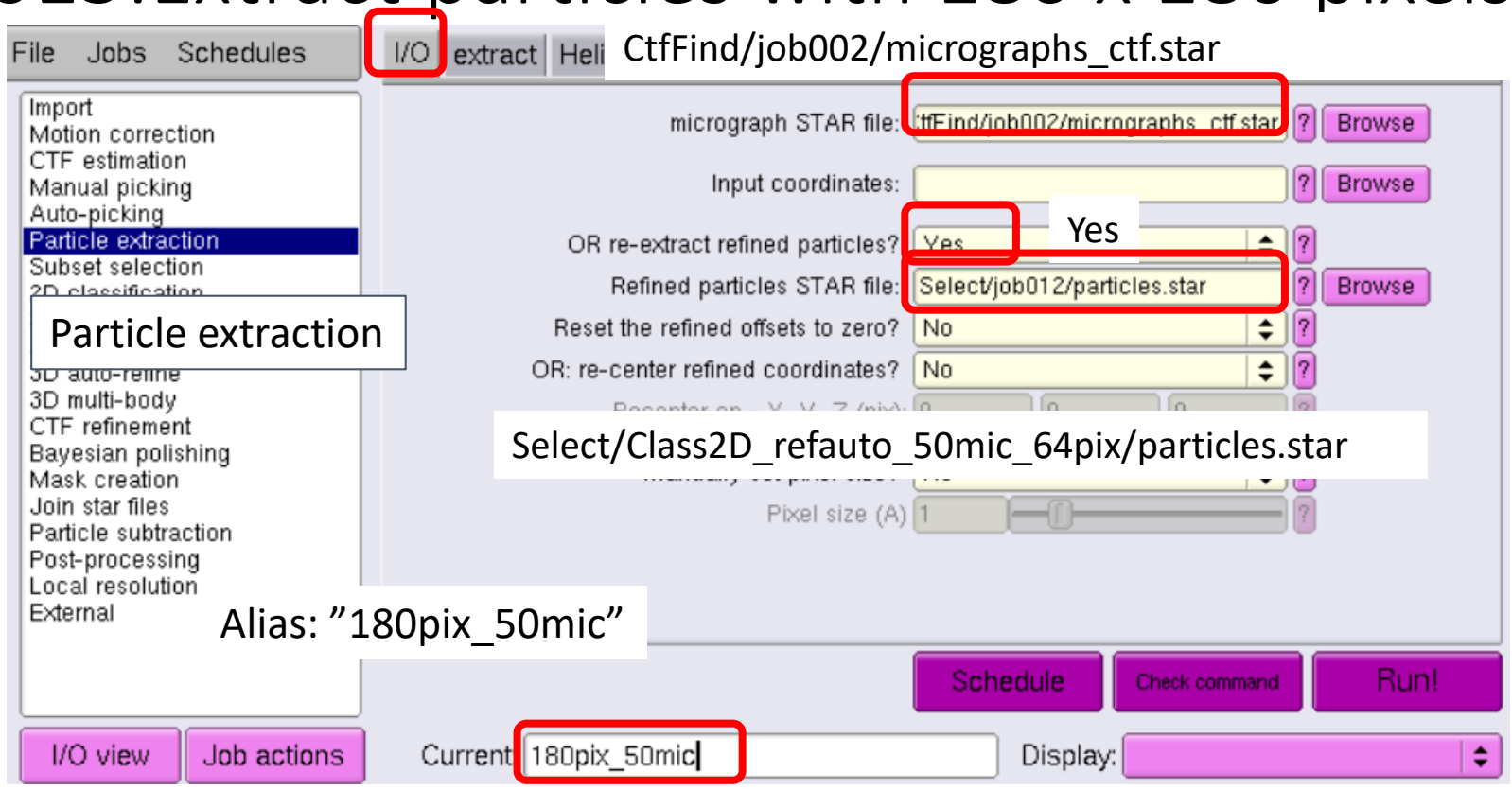
run_it025_class004.mrc

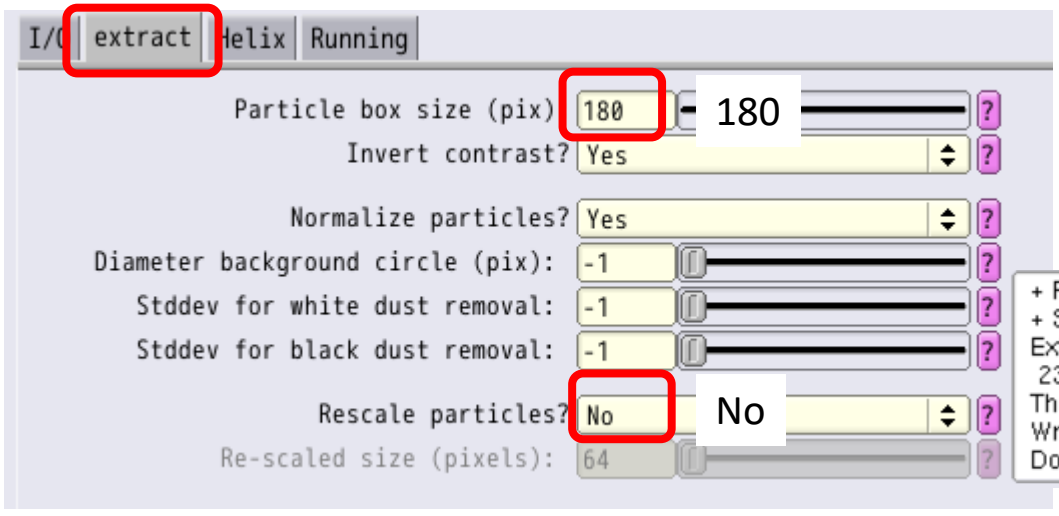
ClassDistribution 0.339489

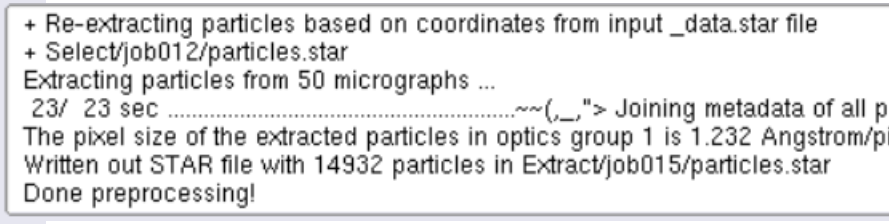
Box size of particles



015:Extract particles with 180 x 180 pixels







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

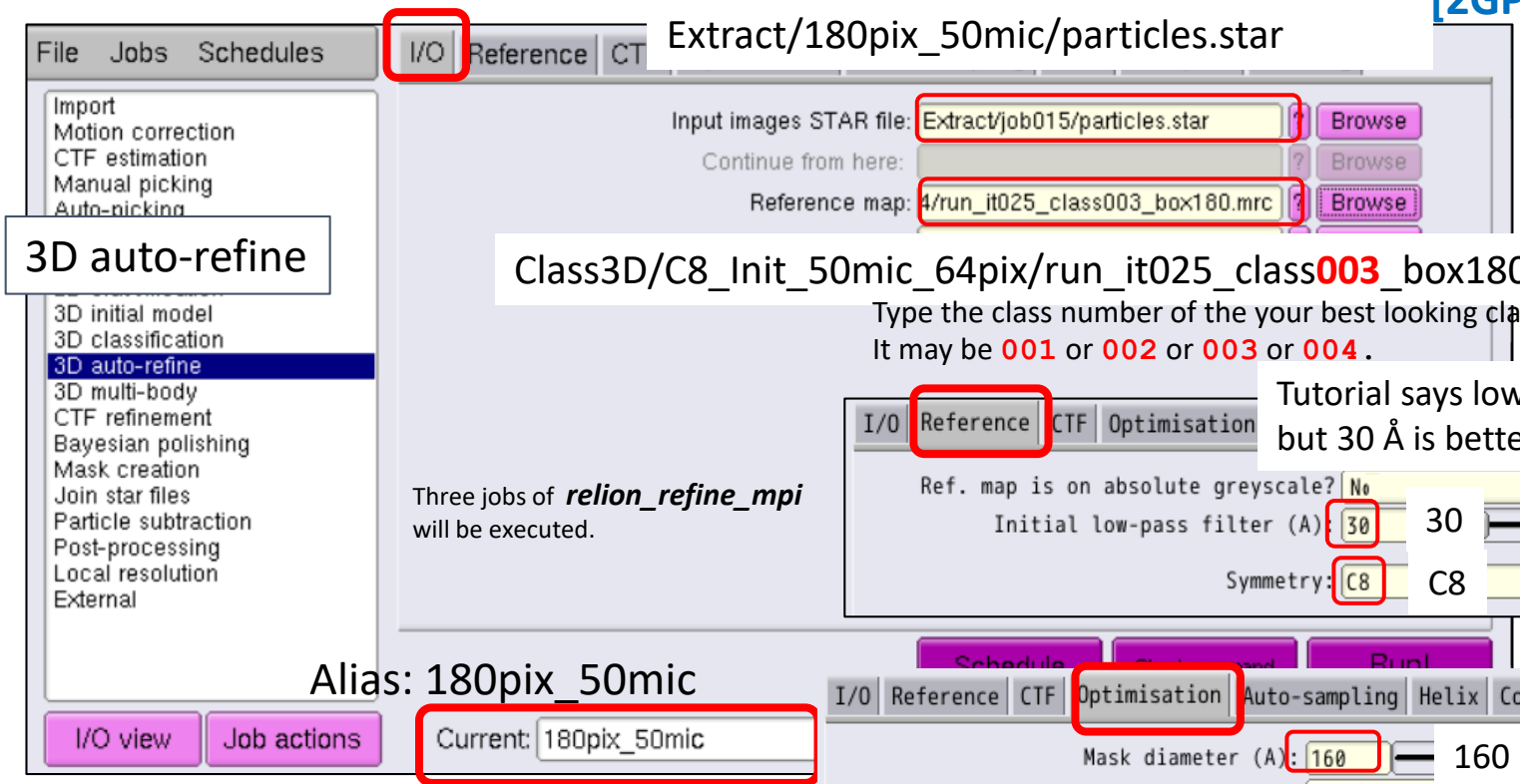
Type following UNIX commands:

```
[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]



3D auto-refine

Extract/180pix_50mic/particles.star

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** .

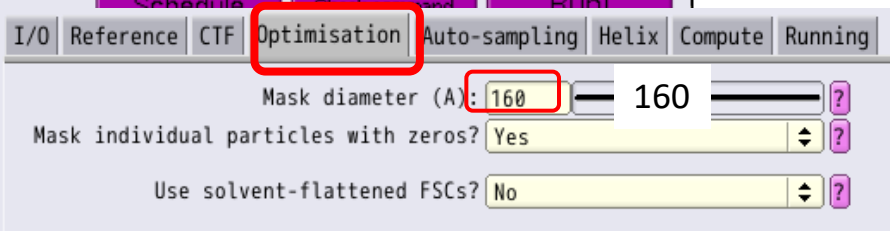
Three jobs of ***relion_refine_mpi*** will be executed.

Alias: 180pix_50mic

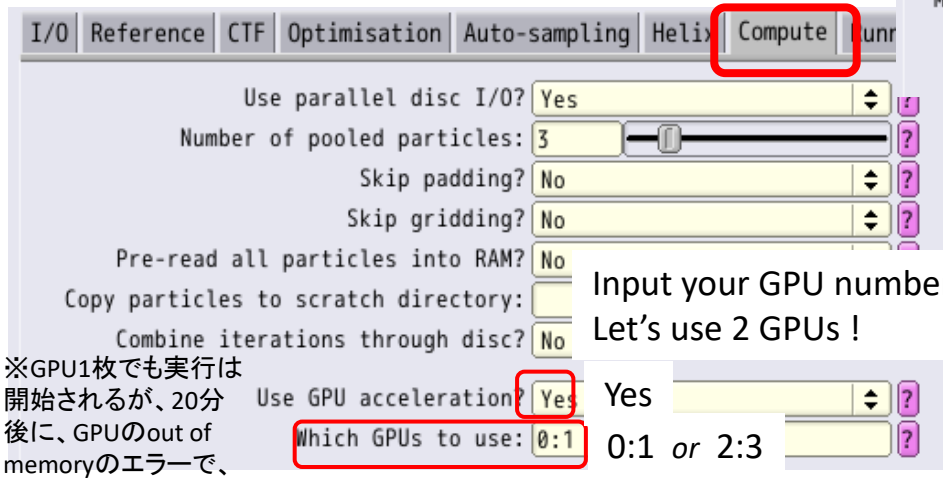
Tutorial says low-pass filter is 50Å, but 30 Å is better for this case.

30

C8



160

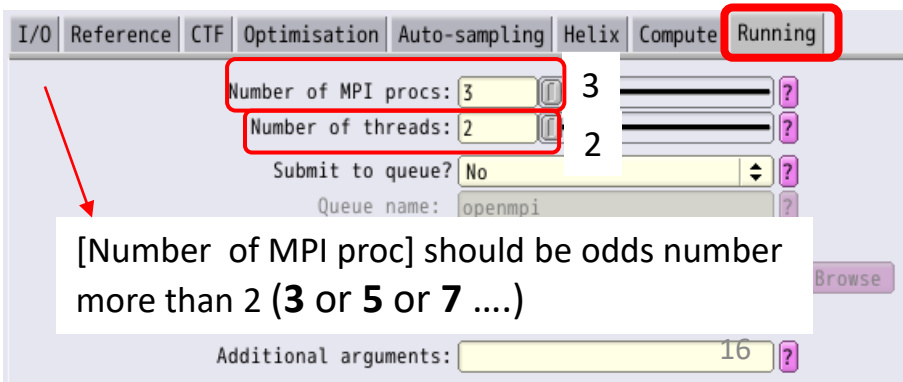


Input your GPU numbers.
Let's use 2 GPUs !

Yes

0:1 *or* 2:3

※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。



3

2

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

016:High resolution 3D refinement

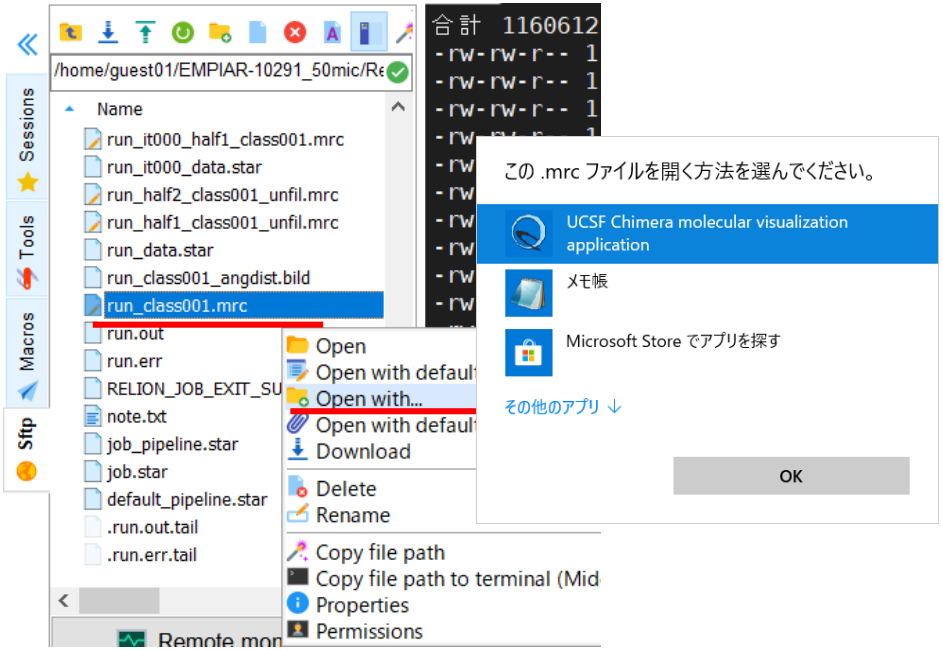
You can know the resolution from the message from Relion GUI.

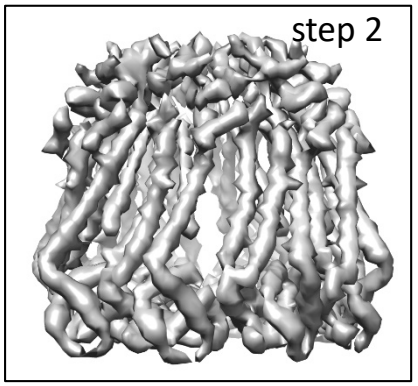
```
1.18/1.18 min .....~~(,_,">
Auto-refine: Refinement has converged, stopping now...
Auto-refine: + Final reconstruction from all particles is saved as: Refin
Auto-refine: + Final model parameters are stored in: Refine3D/job016/r
Auto-refine: + Final data parameters are stored in: Refine3D/job016/run_data.star
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
```

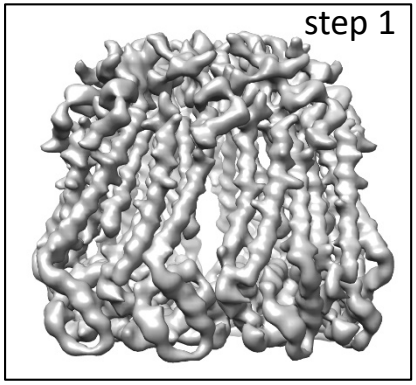
or type a following command:
`tail Refine3D/180pix_50mic/run.out`

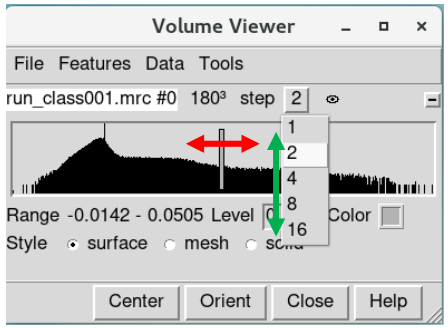
MobaXtermのファイルブラウザで、**Refine3D/180pix_100mic/run_class001.mrc**を選び、
右クリック・メニューから、[Open with…]でUCSF Chimeraで開いて確認する。

Refine3D/first3dref/run_class001.mrc

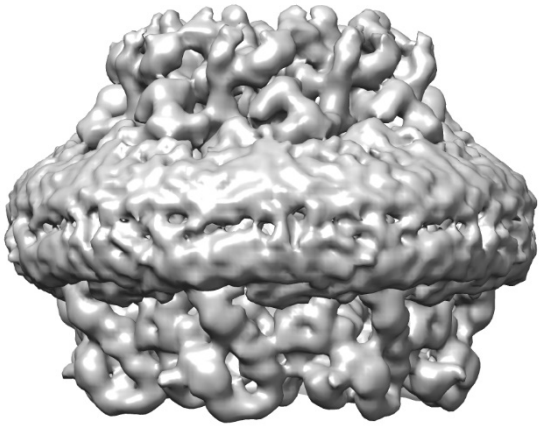




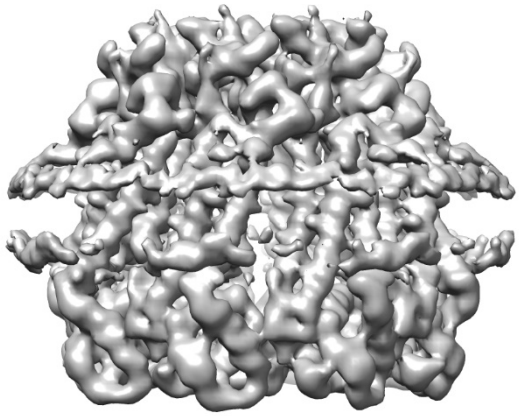




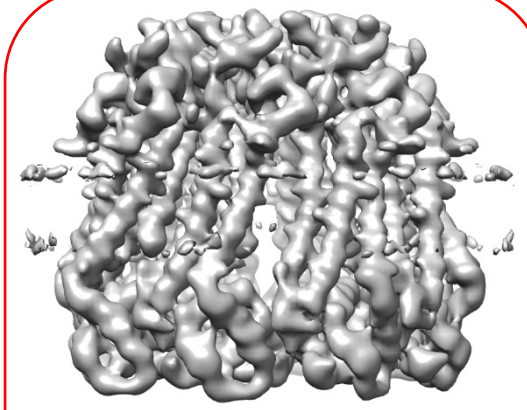
Level threshold for Refined3d map



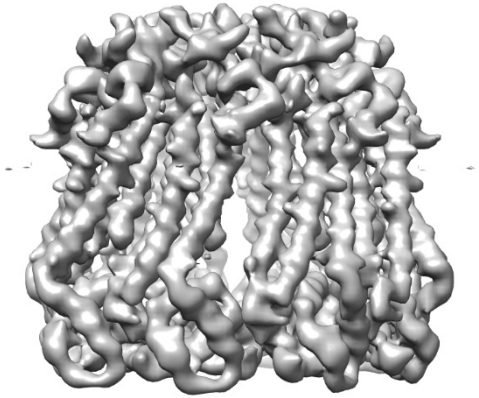
Level = 0.01



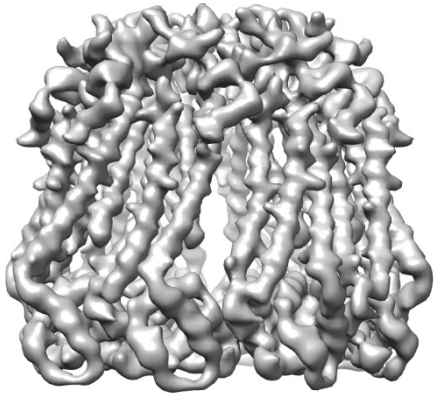
Level = 0.014



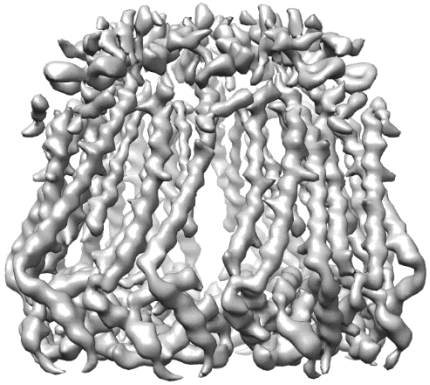
Level = 0.016



Level = 0.018

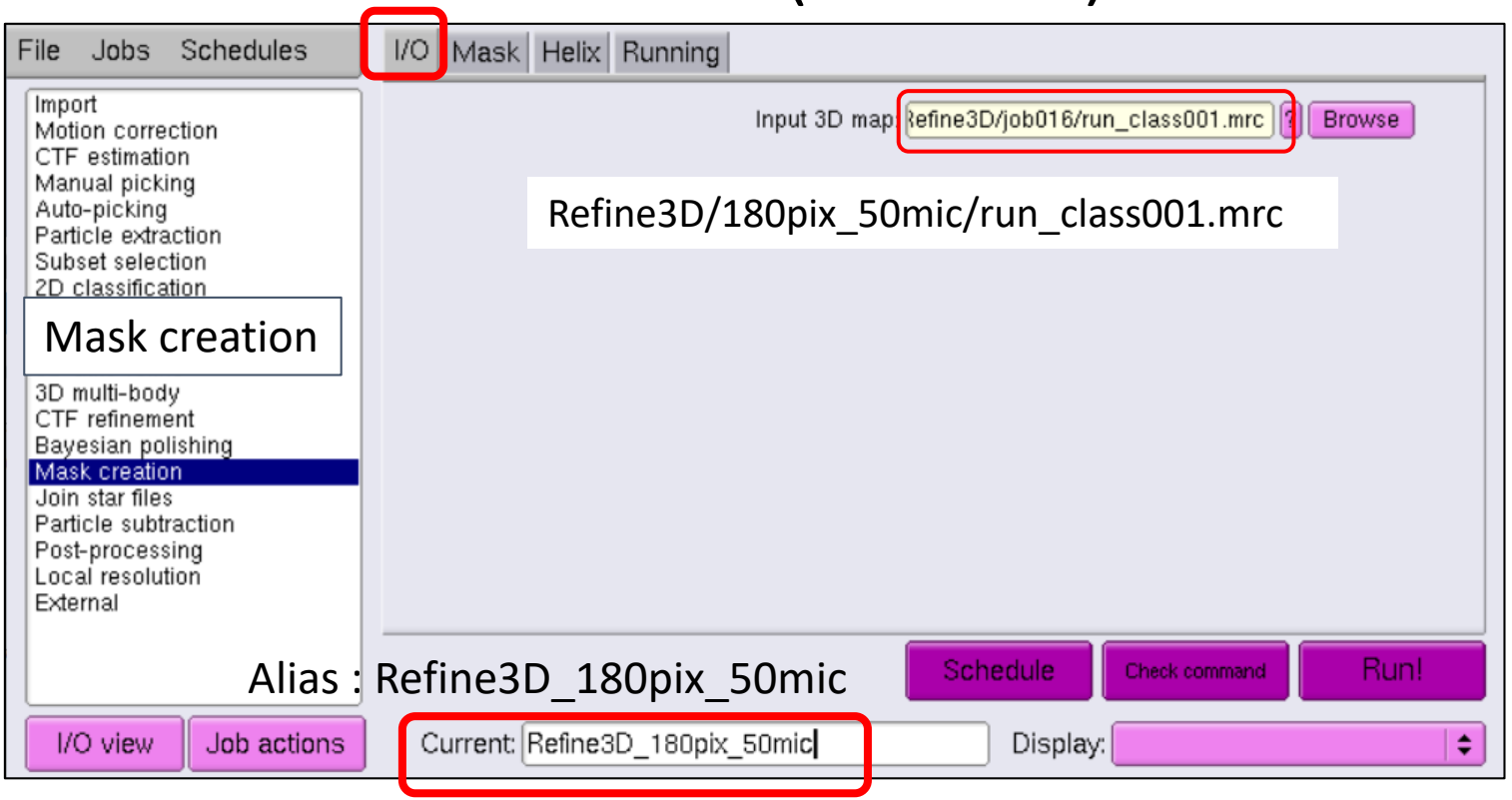


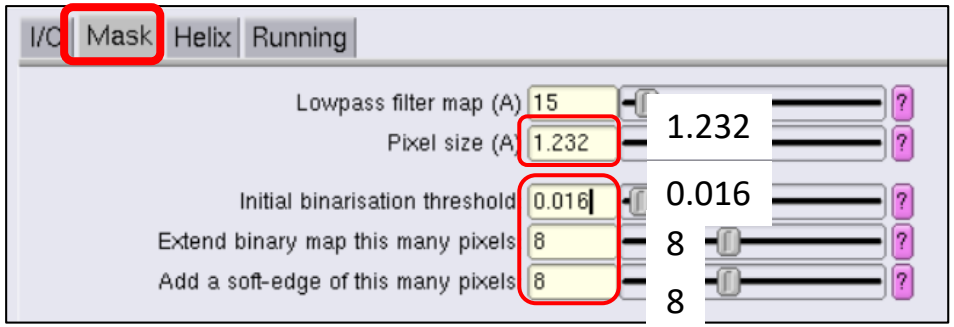
Level = 0.020



Level = 0.025

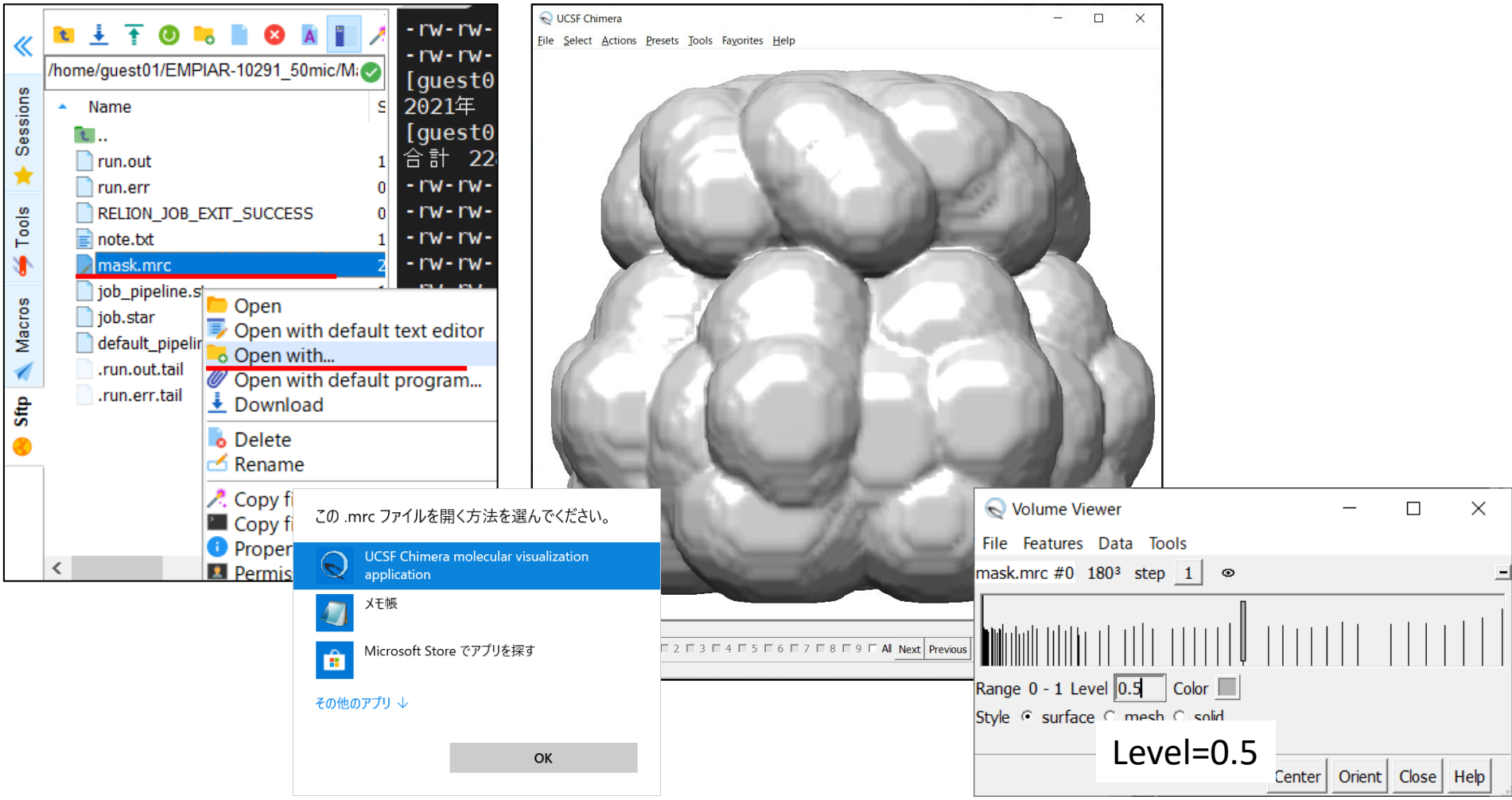
017:Mask Creation (<1min)





017:Mask Creation : Checking mask.mrc

select **MaskCreate/Refine3D_180pix_50mic/mask.mrc**
from MobaXterm and open by UCSF Chimera using [Open with…] menu

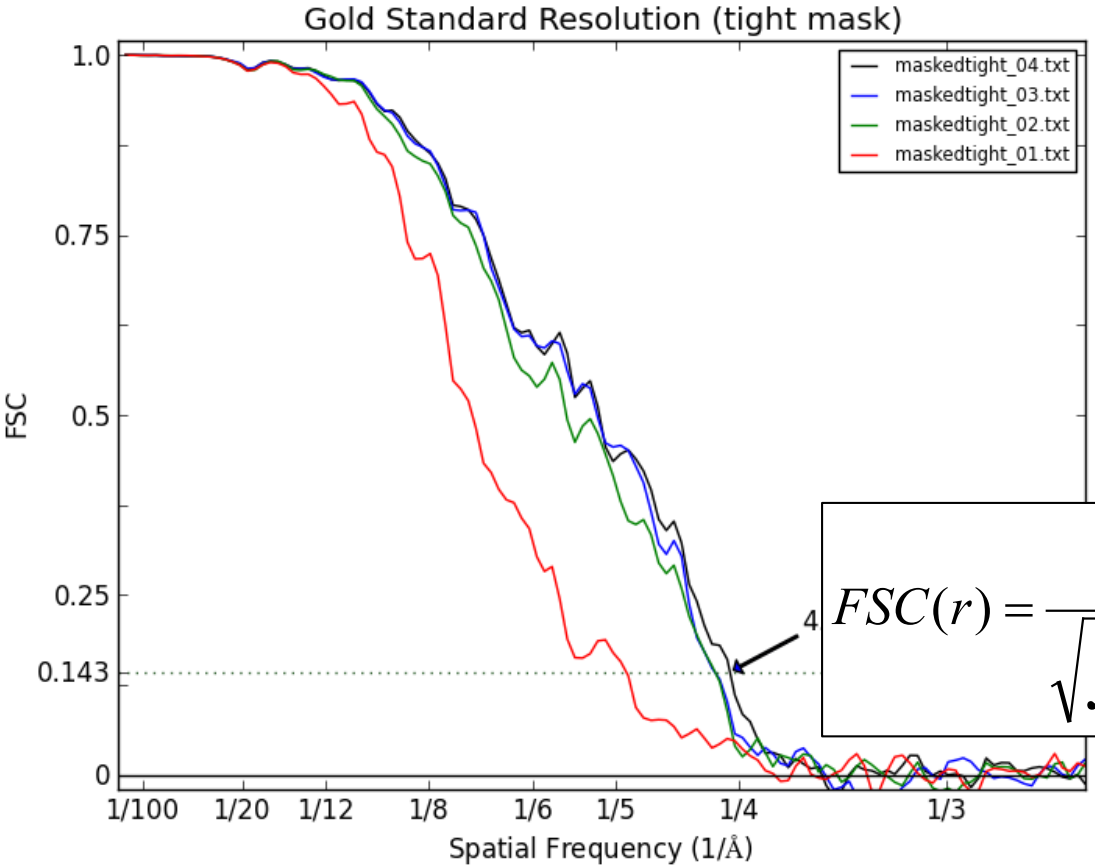


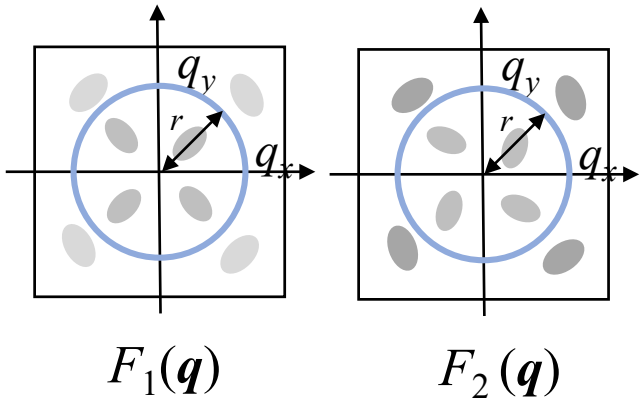
Resolution from Fourier Shell Correlation (FSC)

1)Divide 2D particle images into two sets, and reconstruct two 3D maps $f_1(\boldsymbol{x})$ and $f_2(\boldsymbol{x})$. Their Fourier transferred maps are called $F_1(\boldsymbol{q})$ and $F_2(\boldsymbol{q})$.

2)Calculate correlation $FSC(r)$ on a spherical shell with spatial frequency $r=|\boldsymbol{q}|$.

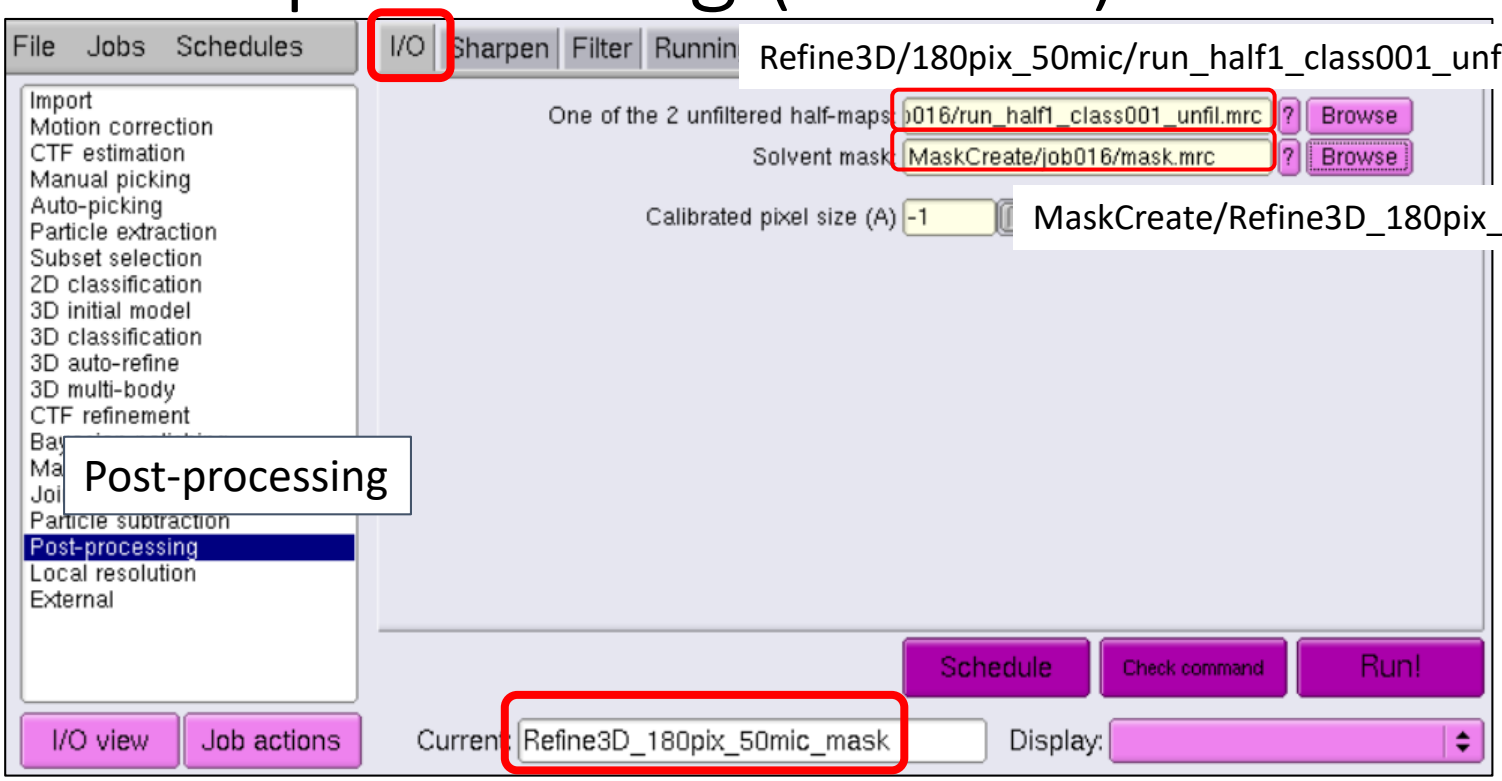
3) Plot $FSC(r)$ versus spatial frequency r. Resolution is defined spatial frequency with $FSC(r)=0.143$ $(=1/7)$.



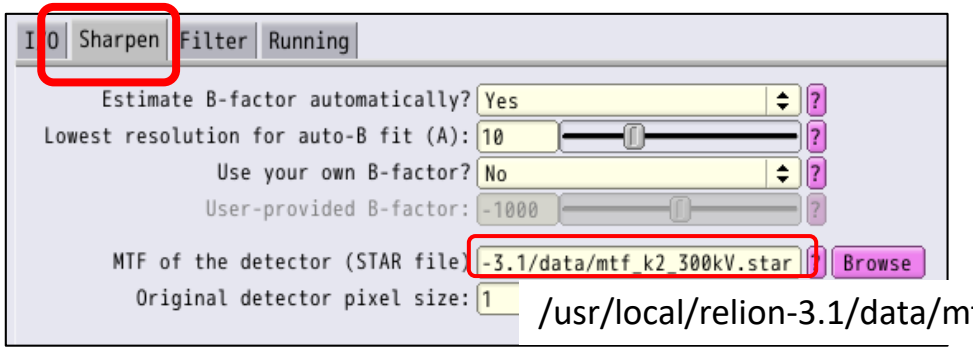


$$FSC(r)=\frac{\int_{|\boldsymbol{q}|=r}\mathrm{Re}\left[F_1(\boldsymbol{q})\cdot F_2(\boldsymbol{q})^*\right]d\boldsymbol{q}}{\sqrt{\int_{|\boldsymbol{q}|=r}|F_1(\boldsymbol{q})|^2\,d\boldsymbol{q}\cdot\int_{|\boldsymbol{q}|=r}|F_2(\boldsymbol{q})|^2\,d\boldsymbol{q}}}$$

018: Postprocessing (< 1min)



Alias:Refine3D_180pix_50mic_mask

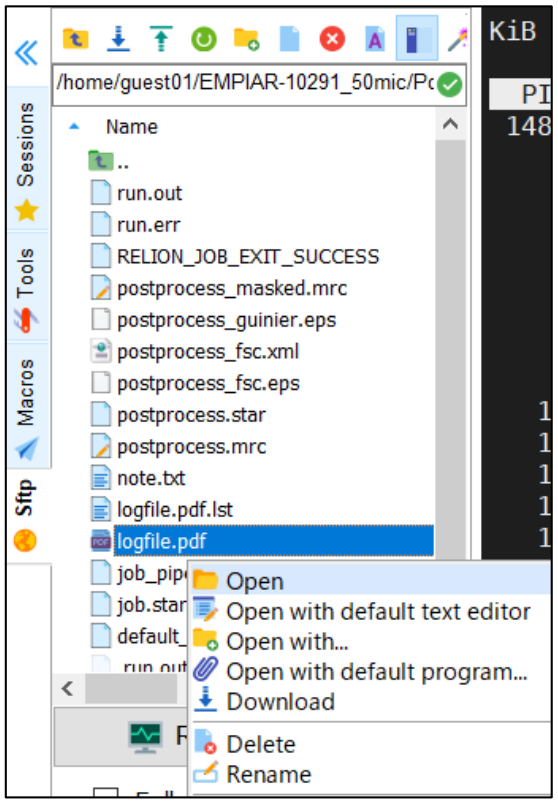


/usr/local/relion-3.1/data/mtf_k2_300kV.star

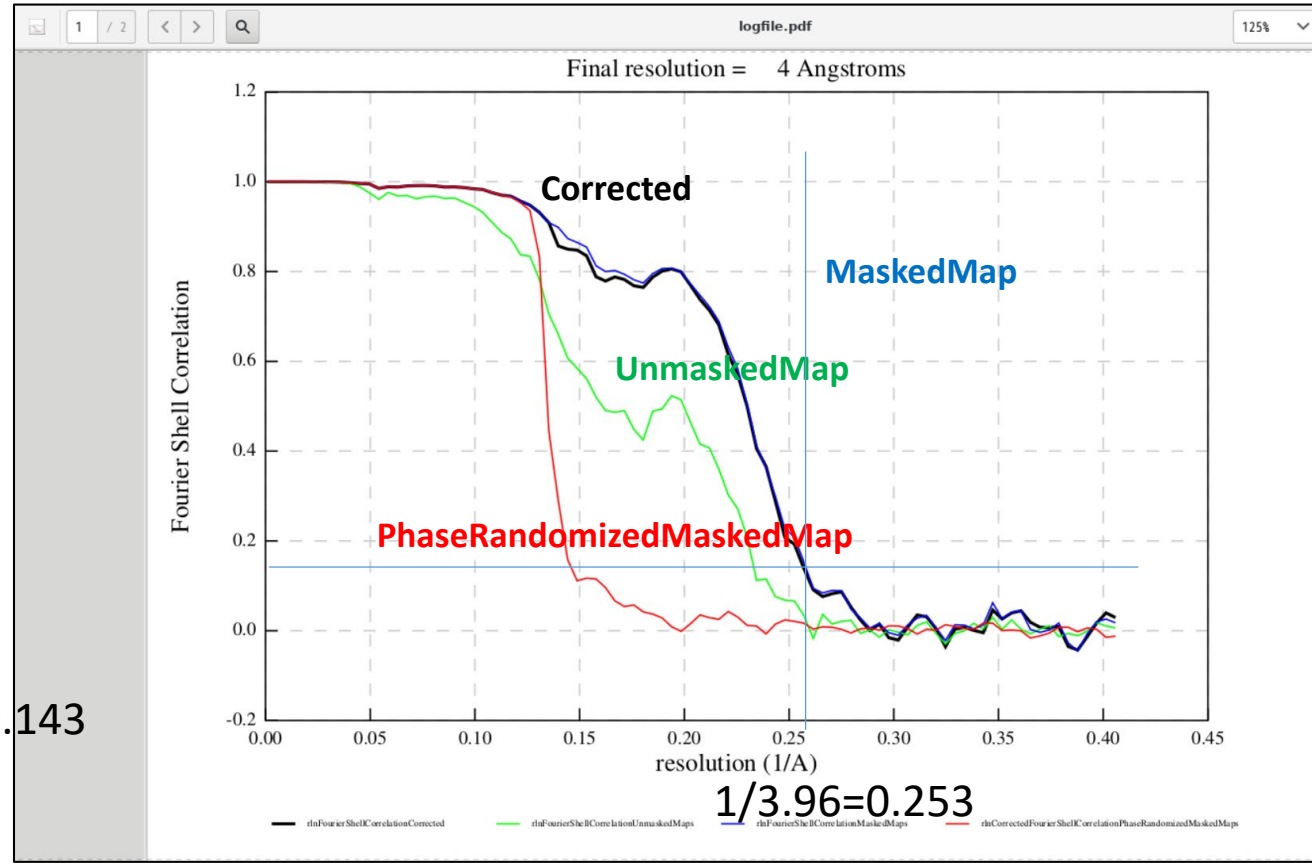
018:Check FSC

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
```



+ FINAL RESOLUTION: 3.96



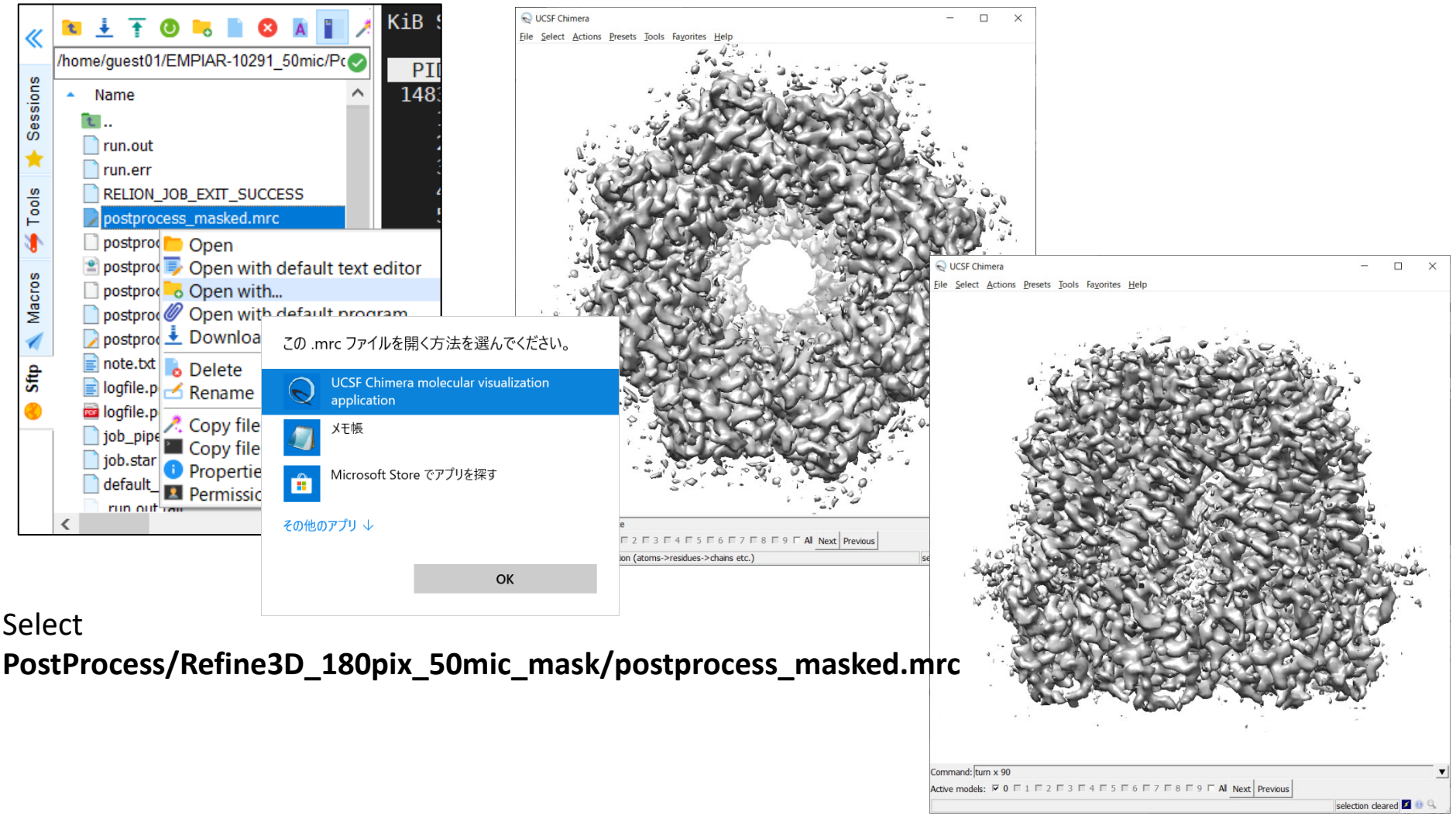
1/7=0.143

1/3.96=0.253

018:Check 3D map

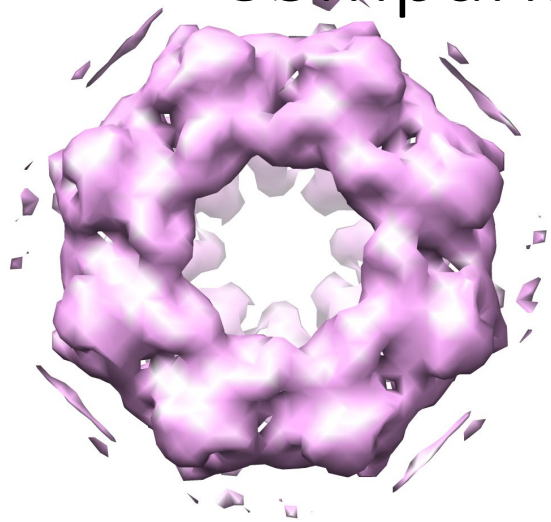
```
  + correlation of fit:          0.890162
  + apply b-factor of:           -195.197
== Writing output files ...
  + Processed map:               PostProcess/job017/postprocess.mrc
  + Processed masked map:        PostProcess/job017/postprocess_masked.mrc
  + Metadata file:               PostProcess/job017/postprocess.star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

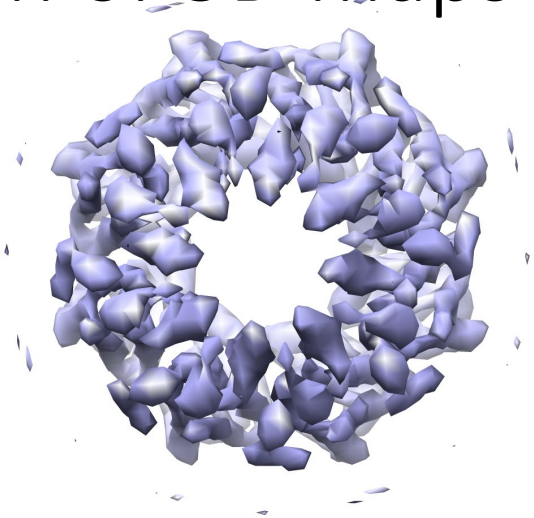
```
+ FINAL RESOLUTION:     3.96
```

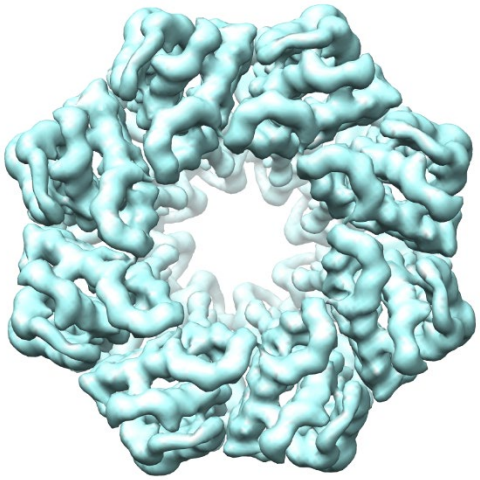


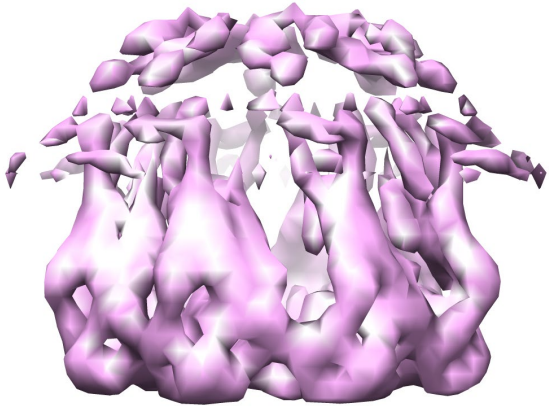
Select
PostProcess/Refine3D_180pix_50mic_mask/postprocess_masked.mrc

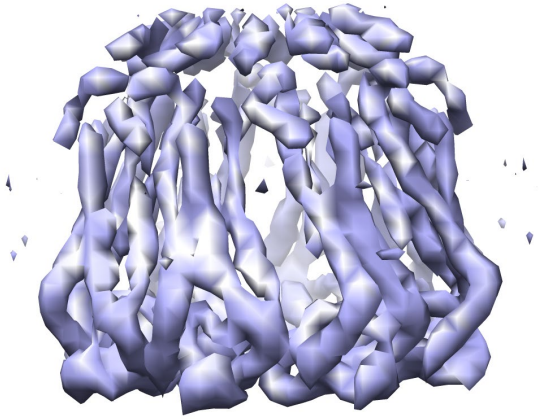
Comparison of 3D maps of INX-6

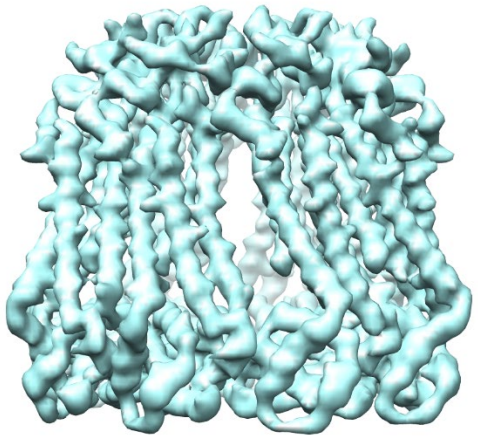










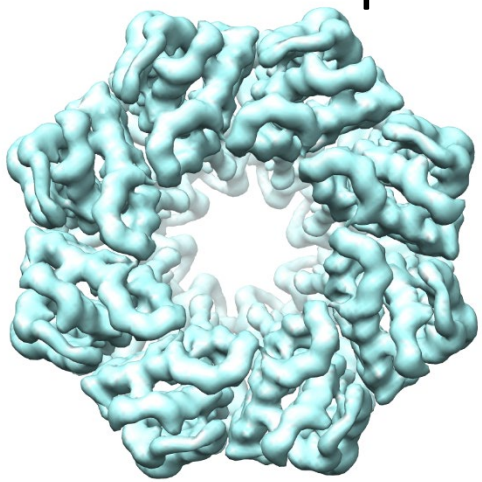


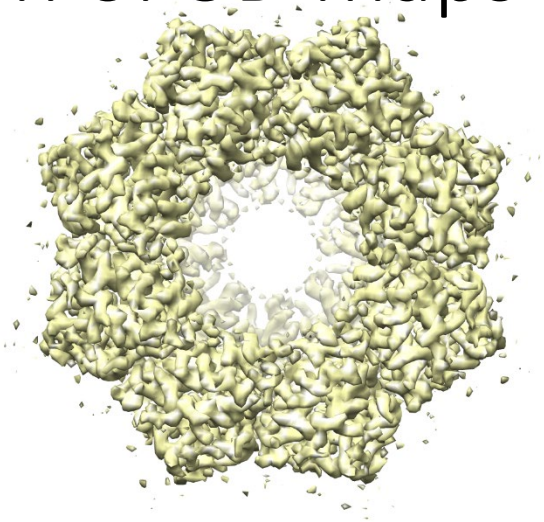
InitialModel
64^3 voxels
from 50 micrographs
14932 particles

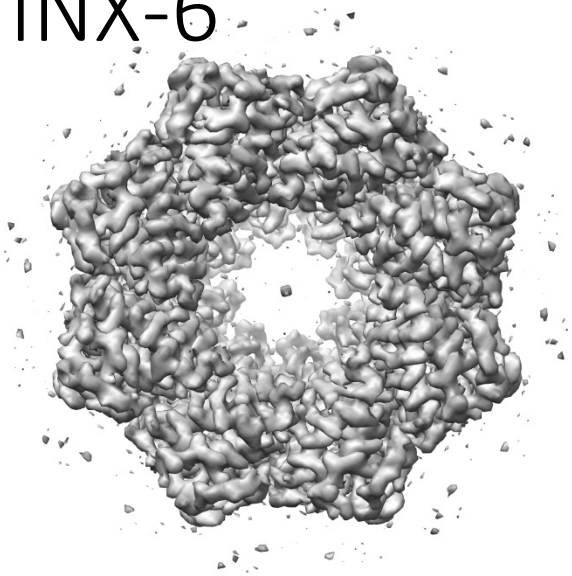
Class3D, best class (003)
64^3 voxels
From 50 micrographs
14932 particles

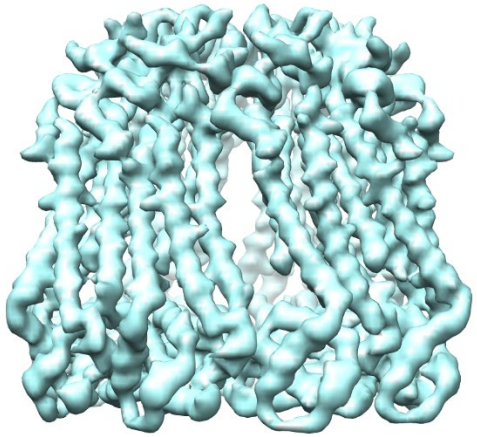
Refine3D
180^3 voxels
Resolution: 4.26 Å
from 50 micrographs

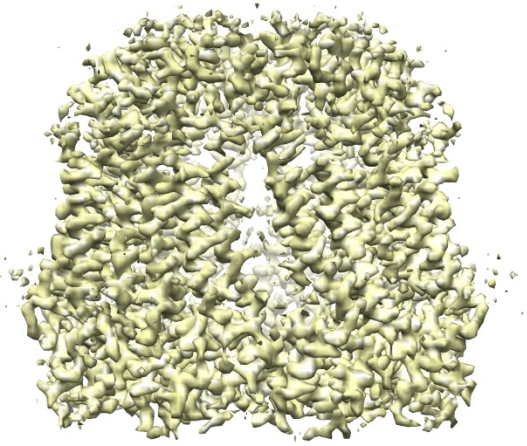
Comparison of 3D maps of INX-6

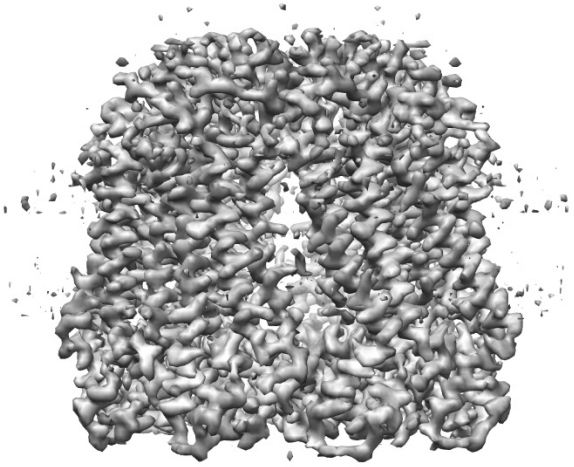












Refine3D
180^3 voxels
Resolution: 4.26 Å
from 50 micrographs

Postprocess with mask
180^3 voxels
Resolution: 3.96 Å
from 50 micrographs

EMD-9973.
180^3 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.