

Relion

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Outline

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② relion 操作說明

③ 流程

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- Motion Correction
- CTF Estimation
- Manual Picking
- Particle Extraction
- 2D Classification

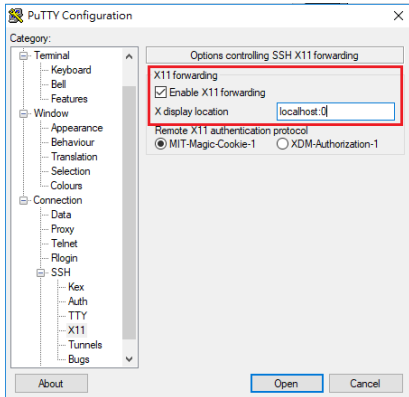
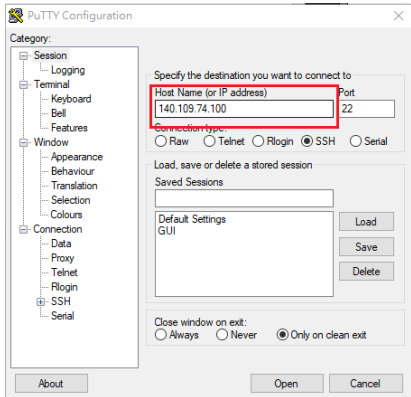
- Subset selection
- Auto Picking
- Particle Extraction
- 2D Classification
- Subset Selection
- 3D Initial Model
- 3D Classification
- Chimera

④ 資料來源

開啟 GUI 介面 (Windows)

以 140.109.74.100 這個 sever 為例，

● Windows 記得打開 Xming !!



開啟 relion

輸入帳號

```
hueilun@ubuntu16: ~/relion21_tutorial/betagal
login as: hueilun
hueilun@140.109.74.100's password: 輸入密碼

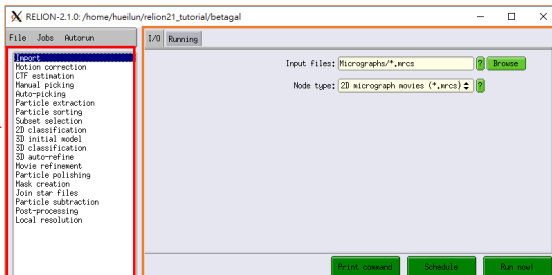
.~~. .~~.   Wednesday, 10 January 2018, 10:53:50 AM
'. \ ' ' / .'   Linux 4.4.0-104-generic x86_64 GNU/Linux
.~ .~~~.~.~.
: ~.'~'.~.~. :   Uptime.....: 5 days, 11h13m35s
~ ( ) ( ) ~   Memory.....: 436036496kB (Free) / 528287728kB (Total)
( : '~'.~'.~' : )   Load Averages.....: 0.33, 1.39, 1.31 (1, 5, 15 min)
~ .~ ( ) ~.~   Running Processes...: 967
( : '~' : )   IP Addresses.....: 140.109.74.100 and 140.109.74.100
'~ .~~~.~'
'~'

Last login: Wed Jan 10 10:41:27 2018 from 140.109.73.17
hueilun@ubuntu16:~$ cd /home/hueilun/relion21_tutorial/betagal/ 輸入資料檔案位置
hueilun@ubuntu16:~/relion21_tutorial/betagal$ relion
█
```

在開啟 relion

操作介面

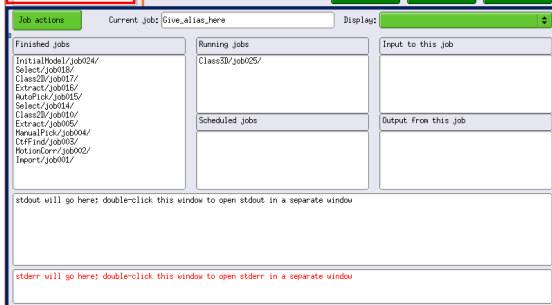
工作類型



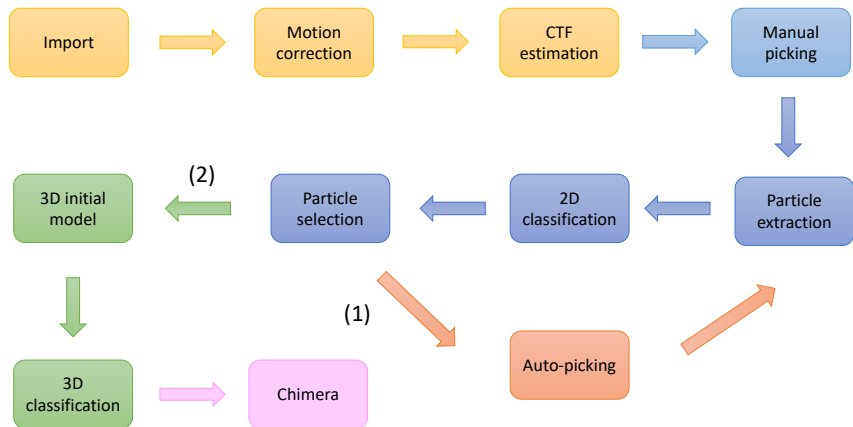
計算參數



工作監控



流程



Import

I/O

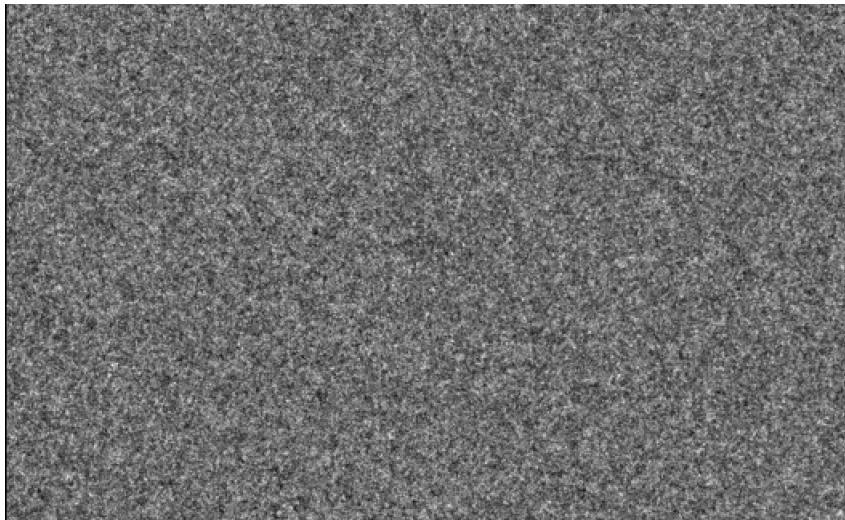
- **Input files:** Micrographs/*.mrcs

(* 表示讀入所有檔案。)

- **Node type:** 2D micrograph movies (*.mrcs)

(如果檔案類型是 mrc，則選擇 2D micrograph /tomograms (*.mrc))

Import-Output



Motion Correction

I/O

- **Input movies STAR file:** Import/job001/micrographs.star
- **Last frame for corrected sum:** 16
- **Pixel size (A):** 3.54

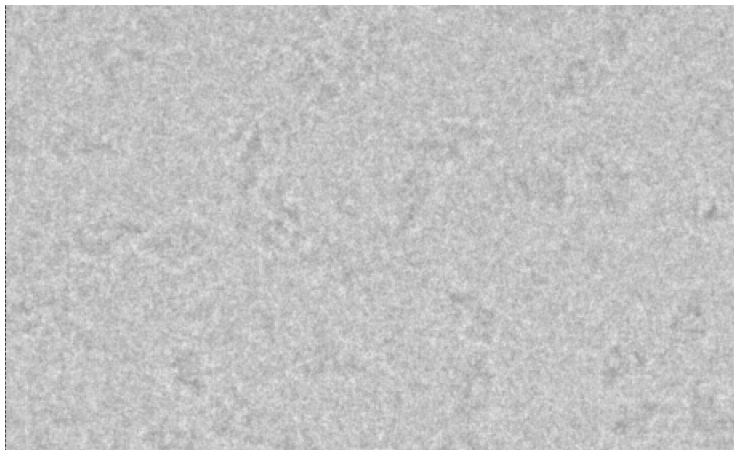
Montioncor2

- **Use MOTIONCOR2?** Yes
- **MOTIONCOR2 executable:** MotionCor2
(路徑位置: /home/tlchen/MotionCor2/MotionCor2)
- **Number of patches X,Y :** 5, 5

Dose-weight

- **Do dose-weighting?** Yes

Motion Correction-Output



CTF Estimation

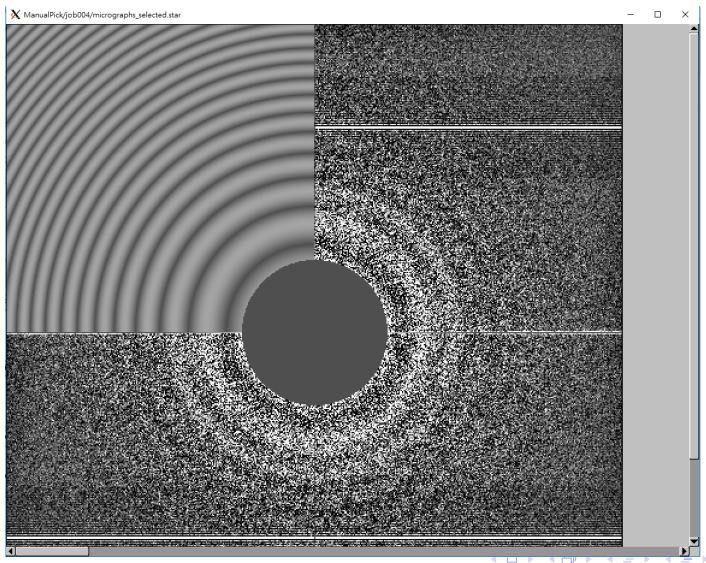
I/O

- **Input micrographs STAR file:** MotionCorr/job002/corrected_micrographs.star
- **Magnified pixel size (Å):** 3.54

ctffind4.1

- **Use CTFFIND-4.1?** Yes
- **CTFFIND-4.1 executable:** ctffind
(路徑位置: /home/tlchen/ctffind/bin/ctffind)

CTF Estimation-Output



Manual Picking -Step1

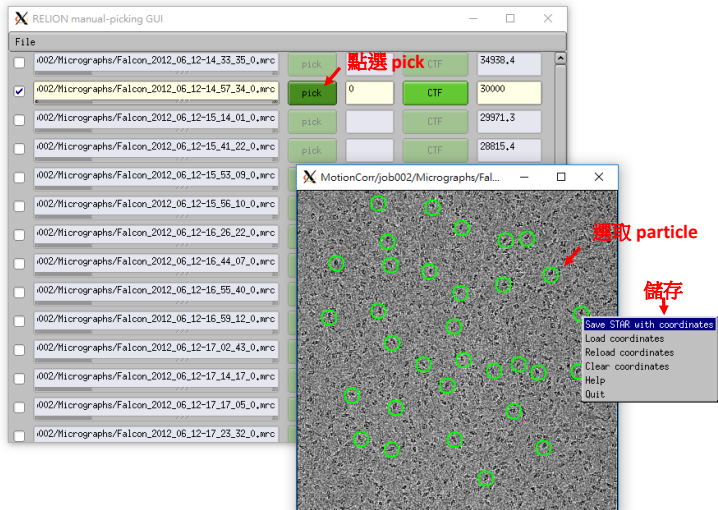
I/O

- **Input micrographs** CtfFind/job003/micrographs_ctf.star

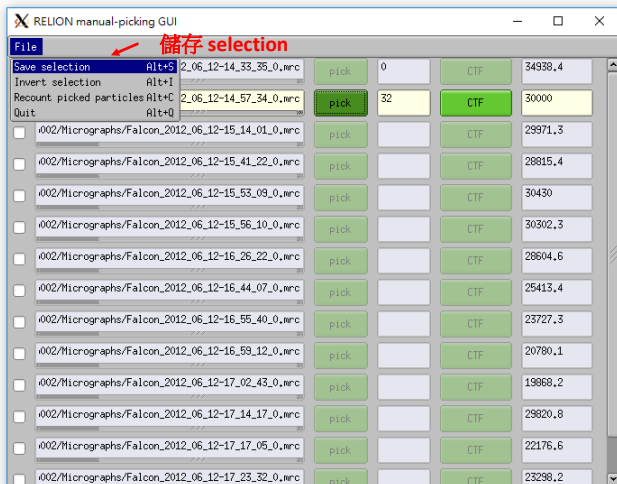
Display

- **Particle diameter (A):** 320
- **Pixel size:** 3.54

Manual Picking -Step2



Manual Picking -Step3



Particle Extraction

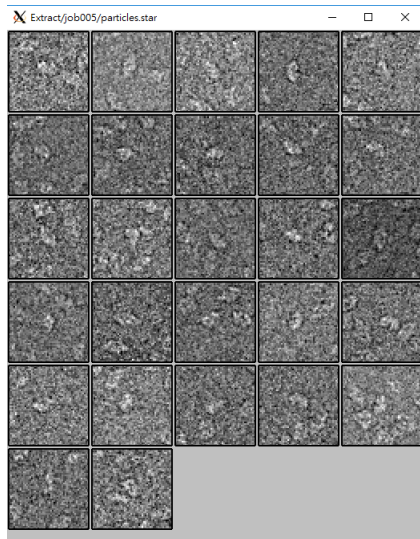
I/O

- **Micrograph STAR file:** ManualPick/job004/
micrographs_selected.star
- **Input coordinates:** ManualPick/job004/
coords_suffix_manualpick.star
- **Pixel size(A):** 3.54

extract

- **Particle box size (pix):** 150
- **rescaled partical?** Yes
- **re-scaled sized size (pixels):** 50

Particle Extraction-Output



2D Classification

I/O

- **Input images STAR file:** Extract/job005/particles.star

CTF

- **Do CTF-correction?** Yes

Sampling

- **Number of classes:** 2 (若是要找 reference 只要設 1 就足夠了。)
- **Mask diameter (A):** 320

Compute

- **Use GPU acceleration:** Yes

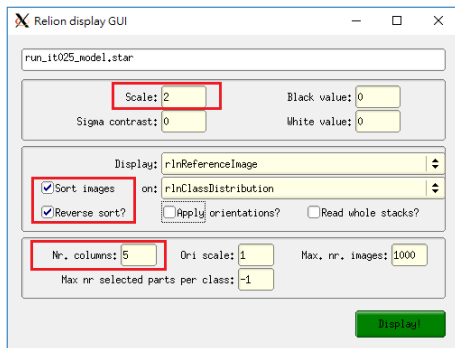
2D Classification-Output



Subset Selection-Step1

I/O

- **Select classes from model.star:** Class2D/job010/
run_it025_model.star

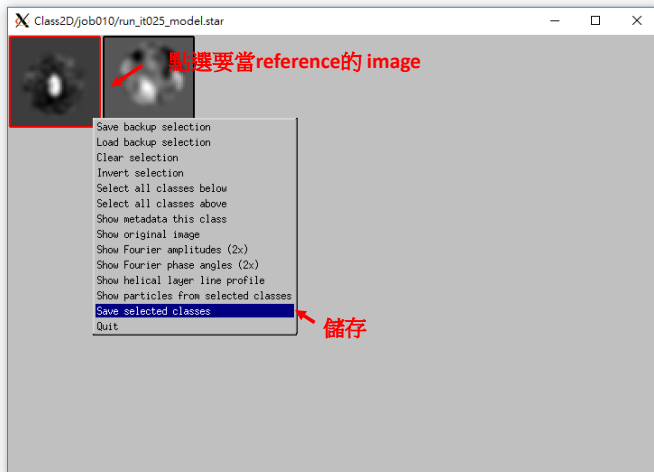


The screenshot shows the 'Relion display GUI' window. The file path 'run_it025_model.star' is entered in the top text box. Below this, there are several groups of controls:

- A group containing 'Scale: 2' (highlighted with a red box), 'Black value: 0', 'Sigma contrast: 0', and 'White value: 0'.
- A 'Display:' dropdown menu set to 'rlnReferenceImage'.
- A group containing two checked checkboxes: 'Sort images' and 'Reverse sort?' (both highlighted with a red box), followed by an 'on:' dropdown set to 'rlnClassDistribution', and two unchecked checkboxes: 'Apply orientations?' and 'Read whole stacks?'.
- A group containing 'Nr. columns: 5' (highlighted with a red box), 'Ori scale: 1', 'Max. nr. images: 1000', and 'Max nr selected parts per class: -1'.

A green 'Display!' button is located at the bottom right of the window.

Subset Selection-Step2



Auto Picking

I/O

- **Input micrographs for autopick** CtfFind/job003/
micrographs_ctf.star
- **References:** Select/job014/class__averages.star
- **Pixel size in micrographs(A):** 3.54

Reference

- **Pixel size in references (A):** 10.62 ($3.54 \times 3 = 10.62$)

autopicking

- **Picking threshold :** 0.5
- **Use GPU acceleration:** Yes

Auto Picking-Output

The screenshot displays the RELION manual-picking GUI. The main window, titled "RELION manual-picking GUI", contains a "File" section with a list of micrographs. Each row includes a checkbox, the file path, a "pick" button, a numerical value, a "CTF" button, and a numerical value. The list shows 16 micrographs, all of which are checked. To the right, a smaller window titled "MotionCorr/job002/Micrographs/Fal..." displays a motion correction image, which is a noisy, green-tinted micrograph.

File	pick		CTF	
✓ 002/Micrographs/Falcon_2012_06_12-14_33_35_0.mrc	pick	1509	CTF	34938.4
✓ 002/Micrographs/Falcon_2012_06_12-14_57_34_0.mrc	pick	2022	CTF	30000
✓ 002/Micrographs/Falcon_2012_06_12-15_14_01_0.mrc	pick	1842	CTF	29971.3
✓ 002/Micrographs/Falcon_2012_06_12-15_41_22_0.mrc	pick	1883	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-15_53_09_0.mrc	pick	1808	CTF	34938.4
✓ 002/Micrographs/Falcon_2012_06_12-15_56_10_0.mrc	pick	2005	CTF	34938.4
✓ 002/Micrographs/Falcon_2012_06_12-16_26_22_0.mrc	pick	1675	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-16_44_07_0.mrc	pick	2045	CTF	29971.3
✓ 002/Micrographs/Falcon_2012_06_12-16_55_40_0.mrc	pick	1847	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-16_59_12_0.mrc	pick	1720	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-17_02_43_0.mrc	pick	1897	CTF	18815.4
✓ 002/Micrographs/Falcon_2012_06_12-17_14_17_0.mrc	pick	2097	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-17_17_05_0.mrc	pick	1999	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-17_23_32_0.mrc	pick	2056	CTF	28815.4
✓ 002/Micrographs/Falcon_2012_06_12-17_26_54_0.mrc	pick	2076	CTF	34938.4

Particle Extraction

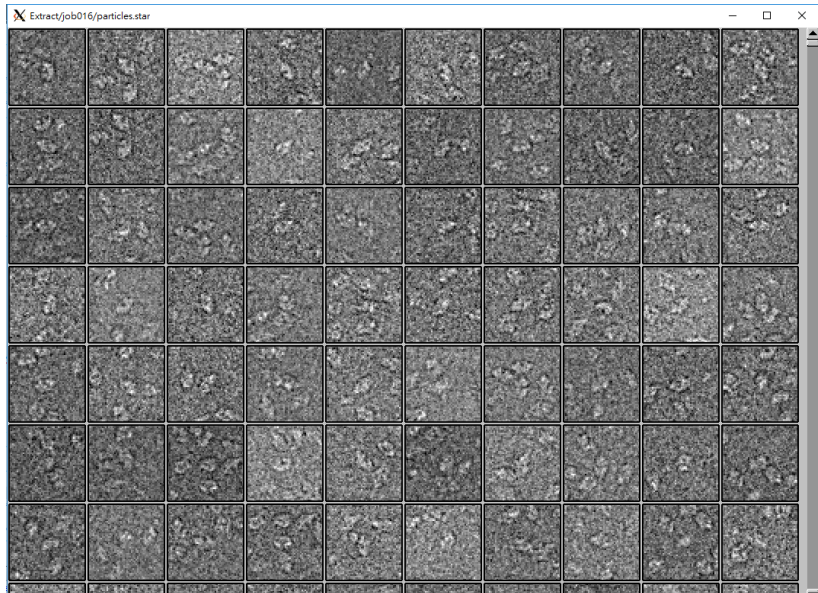
I/O

- **Micrograph STAR file:** CtfFind/job003/micrographs_ctf.star
- **Input coordinates:** AutoPick/job015/coords_suffix_autopick.star
- **Pixel size(A):** 3.54

extract

- **Particle box size (pix):** 150
- **rescaled partical?** Yes
- **re-scaled sized size (pixels):** 50

Particle Extraction-Output



2D Classification

I/O

- **Input images STAR file:** Extract/job016/particles.star

CTF

- **Do CTF-correction?** Yes

Sampling

- **Number of classes:** 20 (猜測可能的 classes 數量)
- **Mask diameter (A):** 320

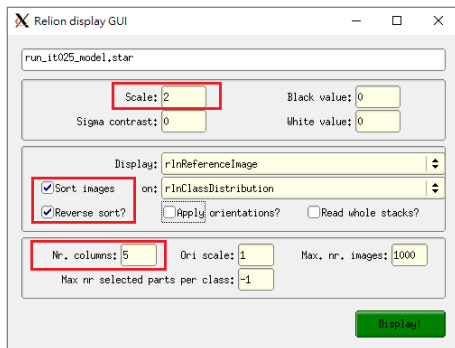
Compute

- **Use GPU acceleration:** Yes

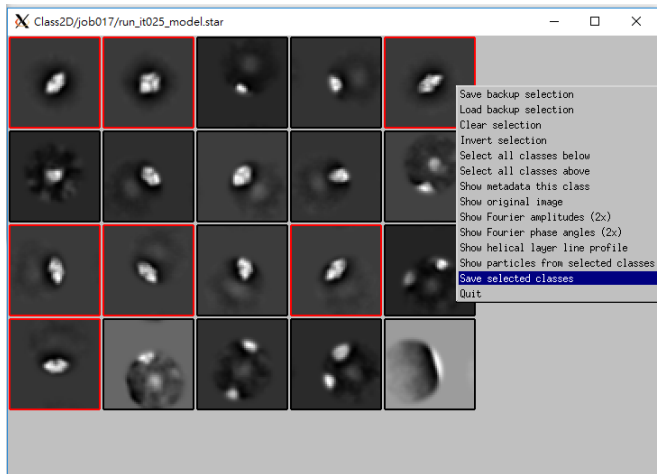
Subset Selection-Step1

I/O

- **Select classes from model.star:** Class2D/job017/
run_it025_model.star



Subset Selection-Step2



3D Initial Model

I/O

- Input images **STAR** file : Select/job018/particles.star

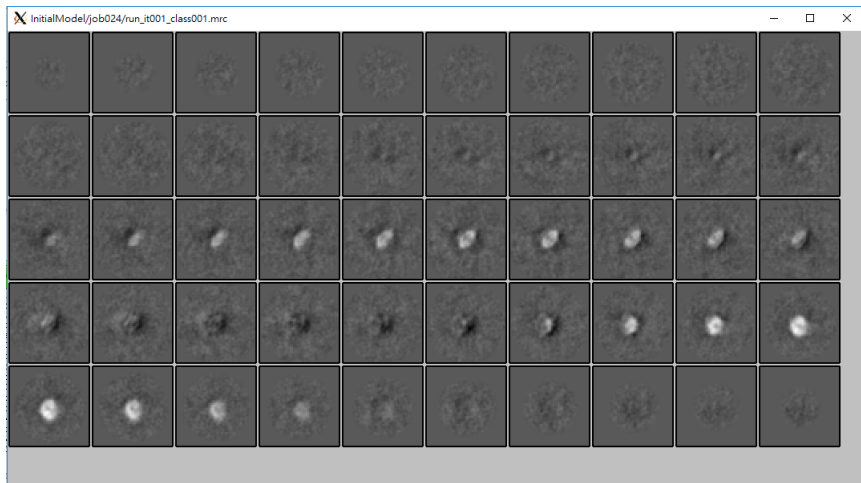
SGD

- Mask diameter (**A**): 320
- Symmetry: C1
- Number of iterations: 1

Compute

- Use **GPU** acceleration: Yes

3D Initial Model-Output



3D Classification

I/O

- **Select classes from model.star:** Select/job018/particles.star
- **Reference map:** InitialModel/job024/run_it001_class001.mrc

CTF

- **Has reference been CTF-corrected?** Yes

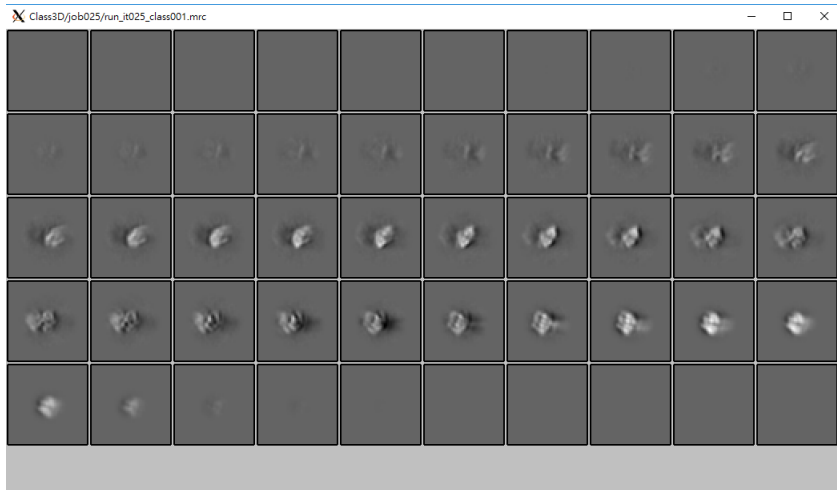
Optimisation

- **Mask diameter (Å):** 320

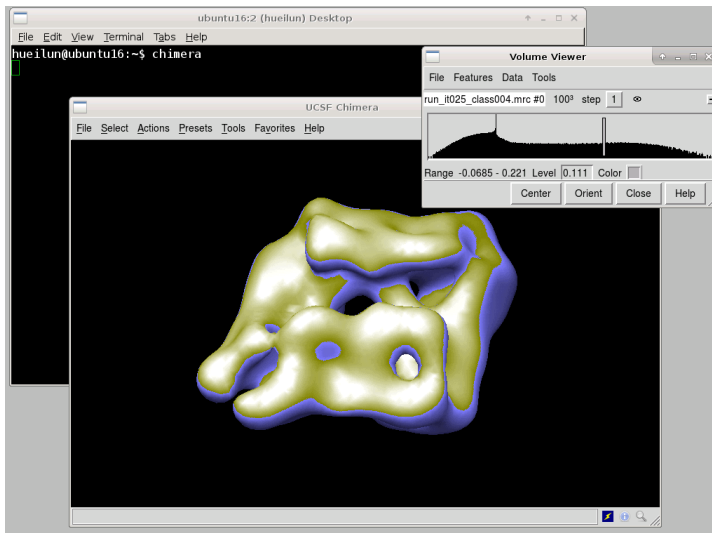
Compute

- **Use GPU acceleration:** Yes

3D Classification-Output



Chimera



VNC

Chimera 需要使用 VNC Viewer 開啟。

```

hueilun@ubuntu16:~$
login as: hueilun
hueilun@140.109.74.100's password:

.~. .~.      Thursday, 11 January 2018, 05:51:29 PM
.' \ ' ' / .'  Linux 4.4.0-104-generic x86_64 GNU/Linux
.~ .~.~.~.~.
: '~.'~.'~.'~.: Uptime.....: 6 days, 18h11m14s
~ ( ) ( ) ~ Memory.....: 339973388kB (Free) / 528287728kB (Total)
( : '~.'~.'~.'~.: Load Averages.....: 0.05, 0.08, 0.15 (1, 5, 15 min)
~ '~.'~.'~.'~.: Running Processes..: 967
( : '~.'~.'~.'~.: IP Addresses.....: 140.109.74.100 and 140.109.74.100
'~.'~.'~.'~.'
'~.'

Last login: Thu Jan 11 17:48:33 2018 from 140.109.73.17
hueilun@ubuntu16:~$ vncpasswd
Password:
Verify:
hueilun@ubuntu16:~$ cp /home/tlchen/xstartup ~/./vnc
cp: cannot stat '/home/tlchen/xstartup': No such file or directory
hueilun@ubuntu16:~$ vncserver ← 輸入 vncserver

New 'ubuntu16:2 (hueilun)' desktop is ubuntu16:2 ← 由這裡可知
Starting applications specified in /home/hueilun/.vnc/xstartup ip:140.109.73.17:2
Log file is /home/hueilun/.vnc/ubuntu16:2.log

hueilun@ubuntu16:~$ vncserver -kill :2 ← 使用完，請關閉 session。
Killing Xvnc4 process ID 31597
hueilun@ubuntu16:~$ █

```

第一次使用需要設定

1.輸入vncpassed -> 設定密碼

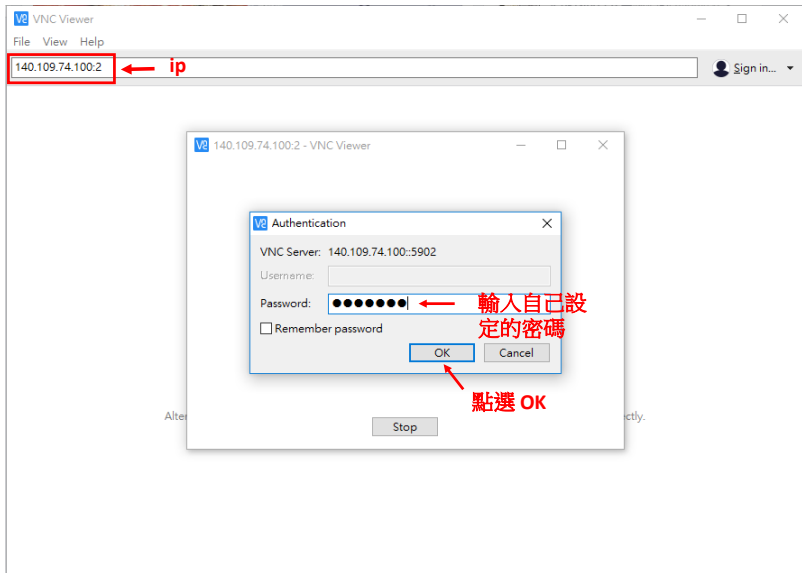
2. 輸入 `cp /home/tlchen/xstarup ~/.vnc`

輸入 vncserver

← 由這裡可知

ip :140.109.73.17:2

使用完，請關閉 session。



資料來源

- Relion 操作手冊

`ftp://ftp.mrc-lmb.cam.ac.uk/pub/scheres/relion21_tutorial.pdf`

- Relion 範例資料 (relion21_tutorial)

`ftp://ftp.mrc-lmb.cam.ac.uk/pub/scheres/`