



ISGC 2017 WORKSHOP

# RELION INTRODUCTION

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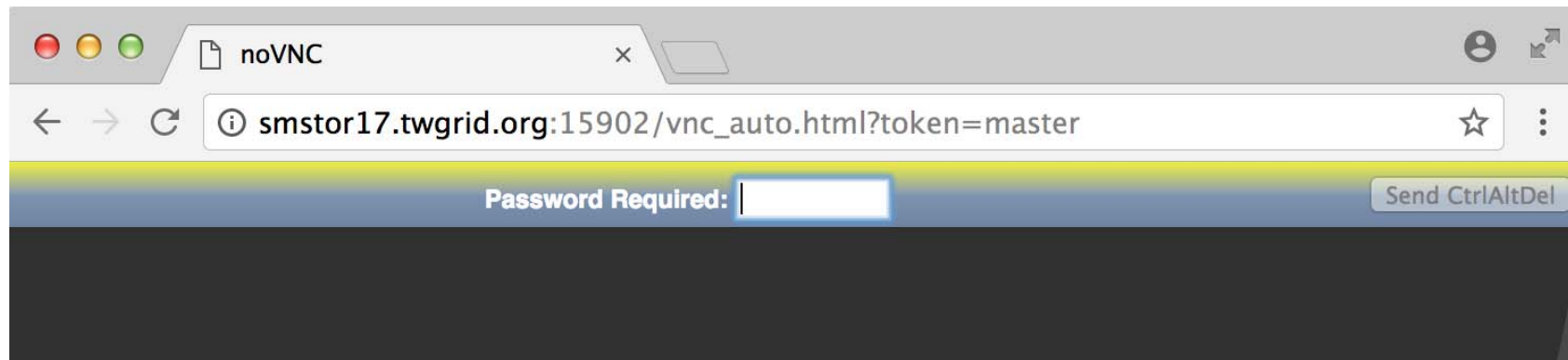
# Outline

- Environment for Installation
- GUI Interface
- Import Micrographs
- Particle Extraction
- 2D Classification
- 3D Classification

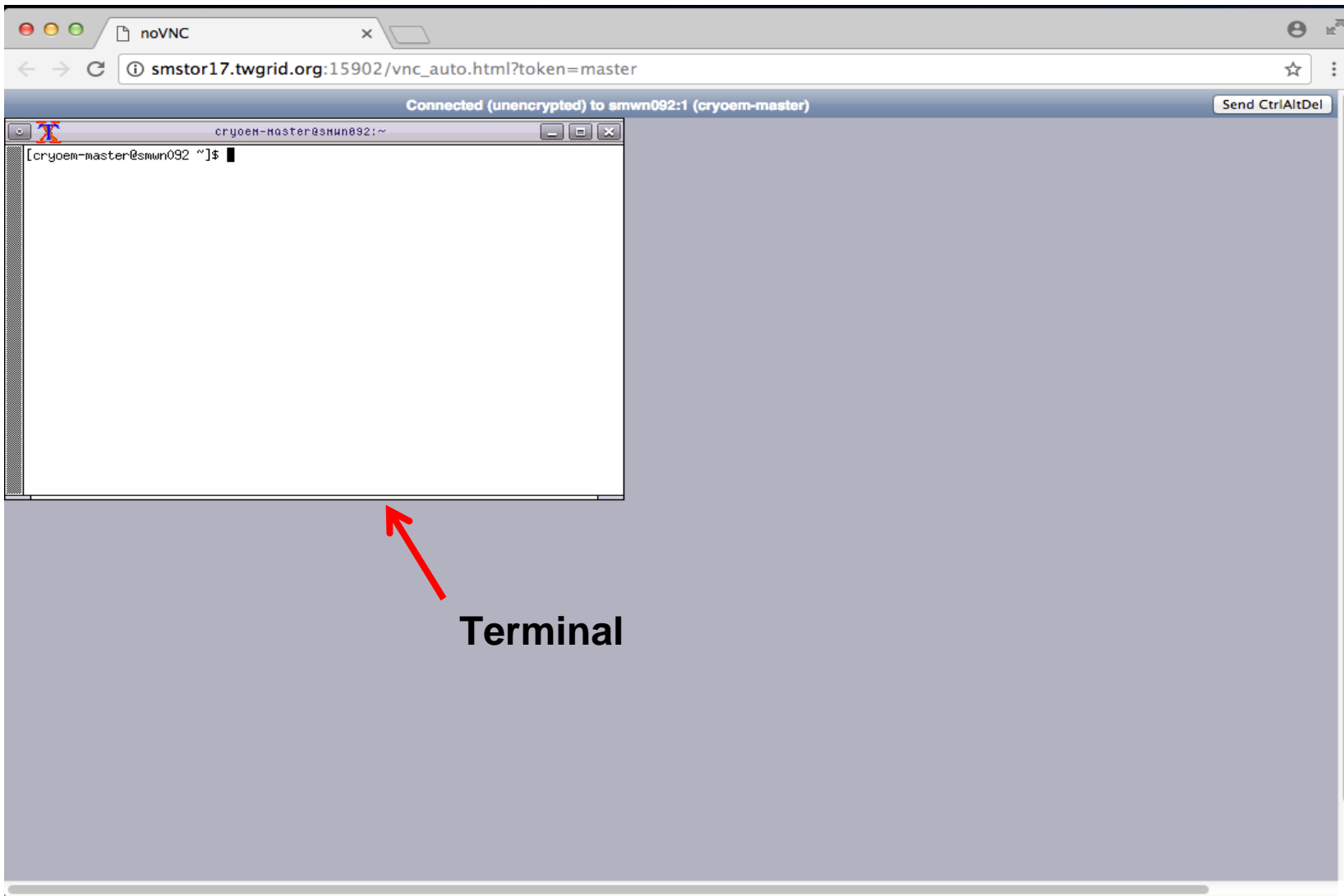


# Login Web VNC

- Use FireFox, Chrome or some other browser supporting HTML5
- Please link to the address on the paper and login with the password



# Web VNC Desktop



Terminal

# Environment for Installation

# RELION



# Bash Shell Setting (for reference)

- Set up all environment variables ( OpenMPI, Relion, even CUDA )

```
[cryoem-master@smwn092 ~]$  
[cryoem-master@smwn092 ~]$ vi ~/.bashrc
```

In the file : `~/.bashrc`

```
# OpenMPI  
export PATH=/usr/lib64/openmpi/bin:$PATH  
  
# Relion  
export LD_LIBRARY_PATH=/cryoem_home/cryoem-master/Documents/relion/build/lib:$LD_LIBRARY_PATH  
export PATH=/cryoem_home/cryoem-master/Documents/relion/build/bin:$PATH  
  
# EMAN2  
source /cryoem_home/cryoem-master/Documents/EMAN2/eman2.bashrc
```

**All these part are completed on your account !!**



# Relion Execution

Step 1 : Use : **cd** to change to the ProjectDirectory

```
[cryoem-master@smwn092 ~]$  
[cryoem-master@smwn092 ~]$ cd Desktop/practice/
```

Step 2 : Type : **relion** to start

```
[cryoem-master@smwn092 practice]$  
[cryoem-master@smwn092 practice]$ relion
```

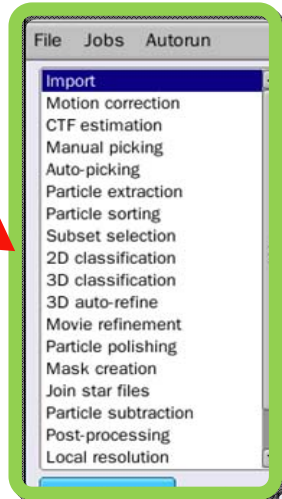
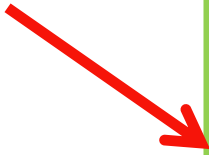
Step 3 : Type : **y** for yes to start the project under this directory

```
Only run the relion GUI from your ProjectDirectory. Do you want to start a new  
project here [y/n]? █
```

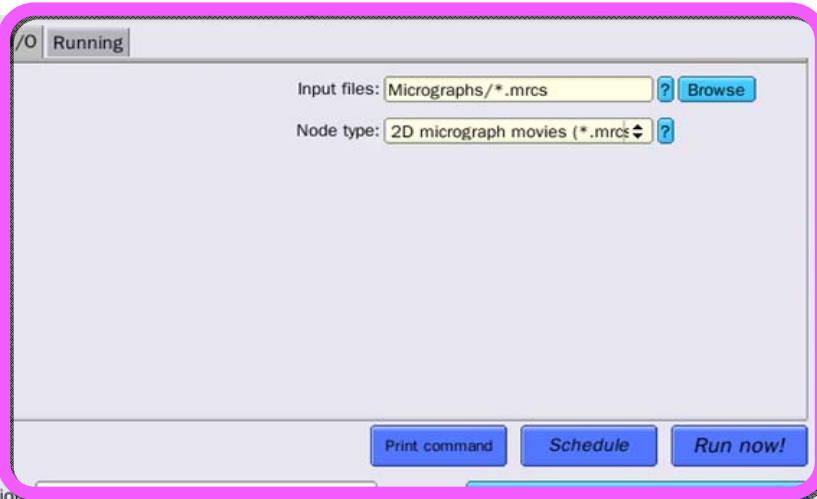


# GUI Introduction

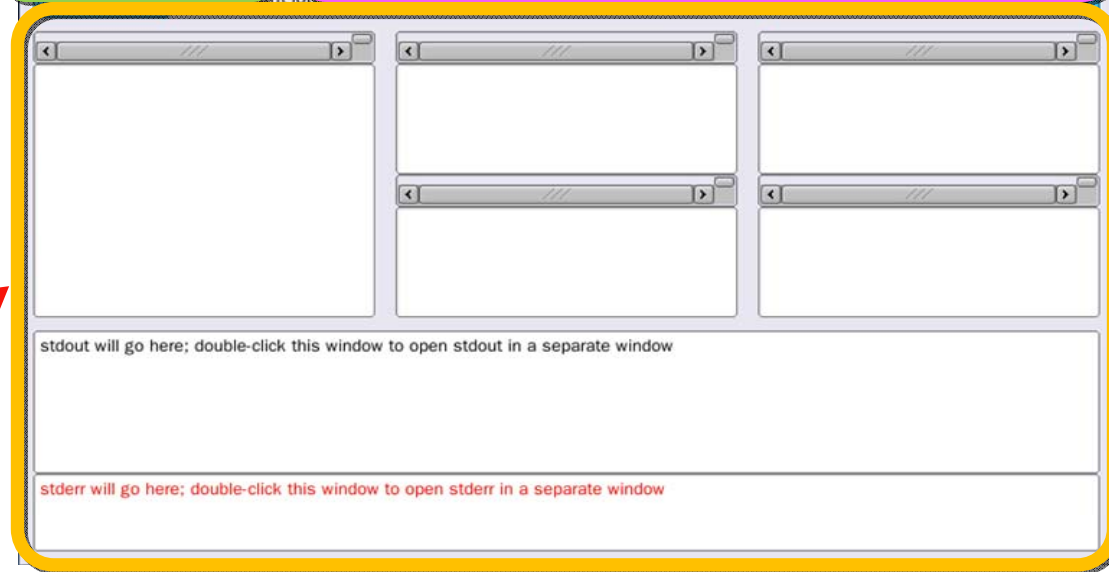
Process Section



Calculation Parameter



Job Monitor





# RELION DEMO

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## Import Micrographs



# Import Micrographs

- Import MRC Images into the the project

『\*』 in linux means : all

Explanation

Browse file

Input files: Frame\_Corrected/\*.mrc ? Browse

Node type: 2D micrograph movies (\*.mrcs)  
**2D micrographs/tomograms (\*.mrc)**  
2D/3D particle coordinates (\*.box, \*\_pick.star)  
Particles STAR file (.star)  
Movie-particles STAR file (.star)  
2D references (.star or .mrcs)  
Micrographs STAR file (.star)  
3D reference (.mrc)  
3D mask (.mrc)  
Unfiltered half-map (unfil.mrc)

Print command Schedule Run now!

Run the job



# RELION DEMO

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## Particles Extraction



# Particles Extraction – Step 1

- I/O setting

The screenshot displays a software interface for particle extraction. The main window has a menu bar with 'File', 'Jobs', and 'Autorun'. Below the menu bar, there are tabs for 'I/O', 'extract', 'Helix', and 'Running'. The 'I/O' tab is active, showing the following settings:

- micrograph STAR file: Import/job001/micrographs.star [?] Browse
- Input coordinates: .box [?] Browse
- OR re-extract refined particles?: No [?]
- Refined particles STAR file: [?] Browse
- Re-center refined coordinates?: Yes [?]
- Manually set pixel size?: No [?]
- Pixel size (A): 1 [?]

At the bottom of the main window, there are three buttons: 'Print command', 'Schedule', and 'Run now!'.

Two file selection windows are overlaid on the main window. The top window shows the directory structure: '../' and 'Import/'. The bottom window shows the directory structure: '../' and 'job001/'. The text 'Fool-proof~!!' is overlaid on the bottom window. The filename field in the bottom window is set to '/home/test\_R/work/practice/.Nodes/1/Import/'.



# Particles Extraction – Step 1

Particle Coordinates File

I/O extract Helix Running

micrograph STAR file: Import/job001/micrographs.star ? Browse

Input coordinates: .box ? Browse

OR re-extract refined particles? No ?

Refined particles STAR file: ? Browse

Re-center refined coordinates? Yes ?

Manually set pixel size? No ?

Pixel size (A) 1

Print command Schedule Run now!

Show: Input coords\_suffix file {{coord;}} Favorites

Preview Show hidden files

Filename: /home/test\_R/work/practice/.Nodes/2/

OK Cancel

In Relion 2.0: .box file from EMAN2  
can't be catch directly



# Particles Extraction – Step 1

- **.box file path – Tricky step to cheat the program**

Generate a directory under the Project Directory (practice)

```
[cryoem-master@smwn092 Frame_Corrected]$ pwd  
/cryoem_home/cryoem-master/Desktop/practice/.box/Frame_Corrected
```

Put all .box files generated by EMAN2 under this directory

```
[cryoem-master@smwn092 Frame_Corrected]$ ls  
Falcon_2012_06_12-14_33_35_0_movie.box  Falcon_2012_06_12-16_55_40_0_movie.box  
Falcon_2012_06_12-14_57_34_0_movie.box  Falcon_2012_06_12-16_59_12_0_movie.box  
Falcon_2012_06_12-15_14_01_0_movie.box  Falcon_2012_06_12-17_02_43_0_movie.box  
Falcon_2012_06_12-15_41_22_0_movie.box  Falcon_2012_06_12-17_14_17_0_movie.box  
Falcon_2012_06_12-15_53_09_0_movie.box  Falcon_2012_06_12-17_17_05_0_movie.box  
Falcon_2012_06_12-15_56_10_0_movie.box  Falcon_2012_06_12-17_23_32_0_movie.box  
Falcon_2012_06_12-16_26_22_0_movie.box  Falcon_2012_06_12-17_26_54_0_movie.box  
Falcon_2012_06_12-16_44_07_0_movie.box
```

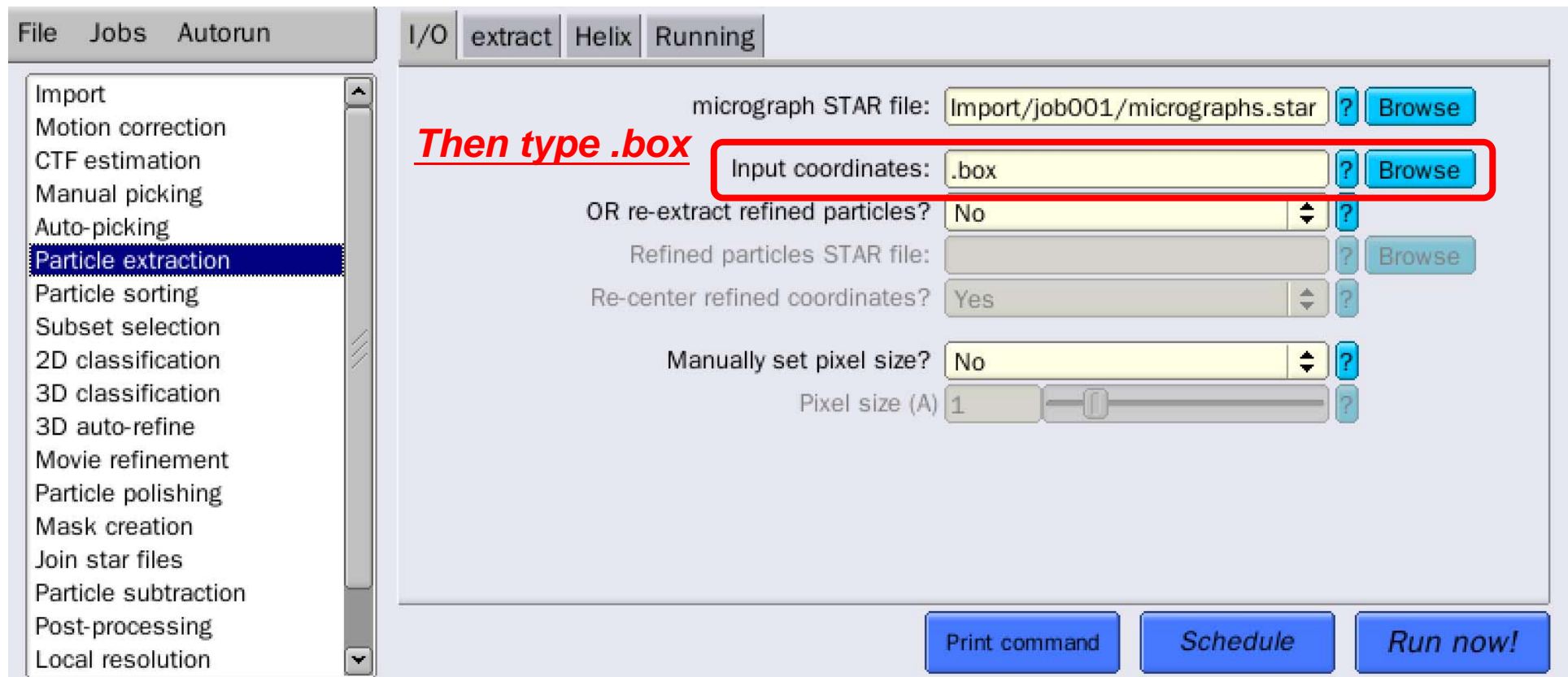
**All things have been done!!**

**Could just check it by “Show hidden files”**



# Particles Extraction – Step 1

Now we can combine Relion 2.0 with EMAN2 Boxing again!!



# Particles Extraction – Step 2

- extract

The screenshot shows a software interface with a top navigation bar containing tabs for 'I/O', 'extract', 'Helix', and 'Running'. The 'extract' tab is active. The main area contains several settings:

- Particle box size (pix): 100 (with a slider and a help icon)
- Invert contrast?: Yes (with a dropdown arrow and a help icon)
- Normalize particles?: Yes (with a dropdown arrow and a help icon)
- Diameter background circle (pix): 60 (with a slider and a help icon)
- Stddev for white dust removal: -1 (with a slider and a help icon)
- Stddev for black dust removal: -1 (with a slider and a help icon)
- Rescale particles?: No (with a dropdown arrow and a help icon)
- Re-scaled size (pixels): 128 (with a slider and a help icon)

Annotations with red arrows point to specific elements:

- 'For testing data' points to the 'Particle box size' field.
- 'Particle : white' and 'Background : Black' point to the 'Invert contrast?' dropdown.
- 'Normalize each parti' points to the 'Normalize particles?' dropdown.
- 'Finish the setting & Run for extraction' points to the 'Run now!' button.

At the bottom, there are three buttons: 'Print command', 'Schedule', and 'Run now!'.





# RELION DEMO

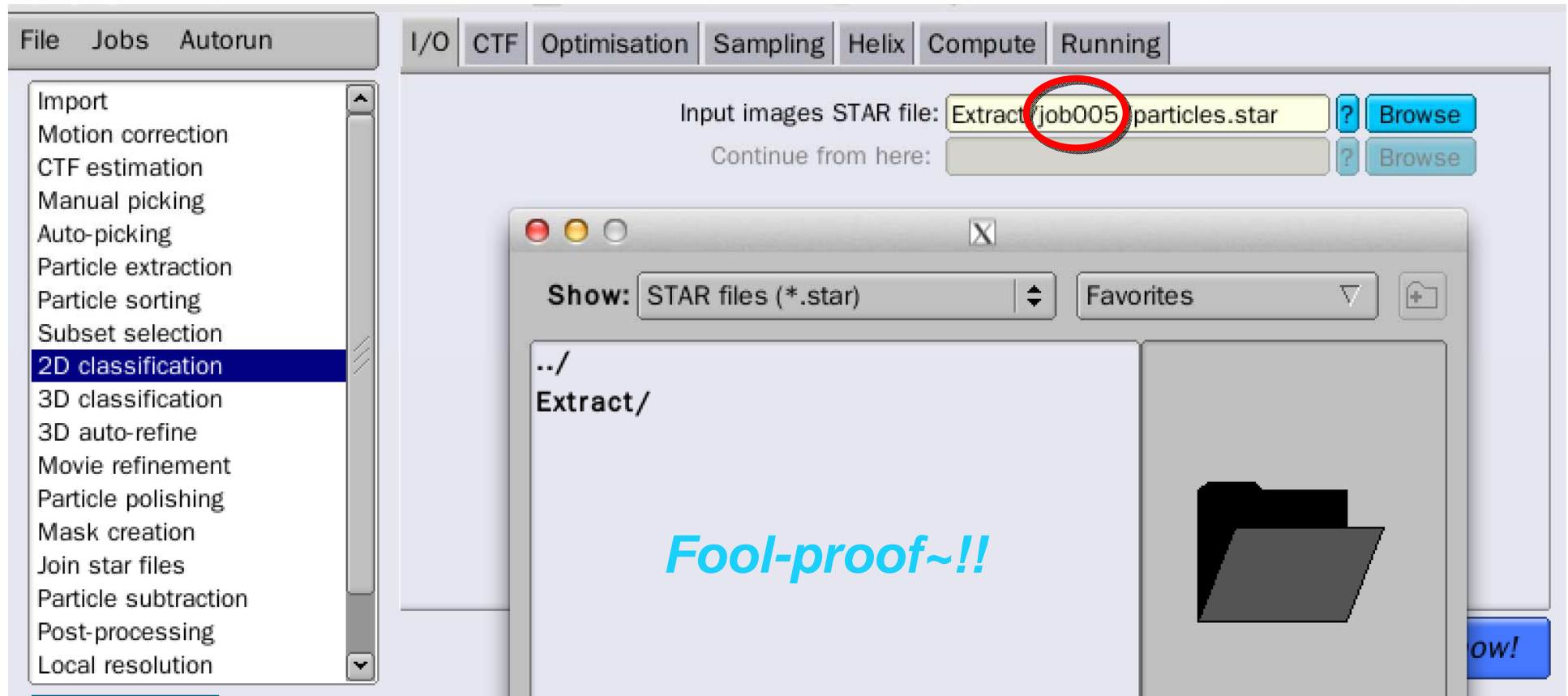
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## 2D Classification



# 2D Classification – Step 1

- I/O setting



# 2D Classification – Step 2

I/O CTF Optimisation Sampling Helix Compute Running

Do CTF-correction? No

Have data been phase-flipped? No

Ignore CTFs until first peak? No

**Due to jumping out the part of CTF measurement  
Just set CTF-correction as “ NO ”**

Print command Schedule Run now!



# 2D Classification – Step 3

For today's calculation

Initial setting

I/O CTF Optimisation Sampling Helix Compute Running

Number of classes: 20

Number of iterations: 25

Regularisation parameter T: 2

Mask diameter (A): 200

Mask individual particles with zeros? Yes

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

Print command Schedule Run now!



# 2D Classification – Step 4

I/O CTF Optimisation Sampling Helix Compute Running

Perform image alignment? Yes  ?

In-plane angular sampling: 5  ?

Offset search range (pix): 5  ?

Offset search step (pix): 1  ?

Print command Schedule Run now!



# 2D Classification – Step 5

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

**Physical core** →

\*\* TWgrid support  
24 physical core  
in each account!!

Number of MPI procs: 24

Number of threads: 1

Submit to queue? No

Queue name: openmpi

Queue submit command: qsub

Standard submission script: /EM/RELION/relion/bin/qsub.csh [Browse](#)

Minimum dedicated cores per node: 1

Additional arguments: **--angpix 3.54**

**Define pixel size for algorithm**  
Add a parameter : **--angpix 3.54**  
(3.54 angstrom per pixel)

**Run the job**

[Print command](#) [Schedule](#) [Run now!](#)



# RELION DEMO

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## Subset Selection



# Subset Selection

model.star : class-merged view file

The screenshot displays a software interface for subset selection. On the left is a menu with options: Import, Motion correction, CTF estimation, Manual picking, Auto-picking, Particle extraction, Particle sorting, **Subset selection**, 2D classification, 3D classification, 3D auto-refine, Movie refinement, Particle polishing, Mas..., Join..., Parti..., Post..., and Loca... Below the menu is a 'Job' button. The main window has tabs for 'I/O', 'Class options', and 'Running'. A red arrow points to the 'I/O' tab, which contains the following text and controls:

- Select classes from model.star: ?D/job049/run\_it025\_model.star ? Browse
- OR select from micrographs.star: ? Browse
- OR select from particles.star: ? Browse
- OR select from picked coords: ? Browse

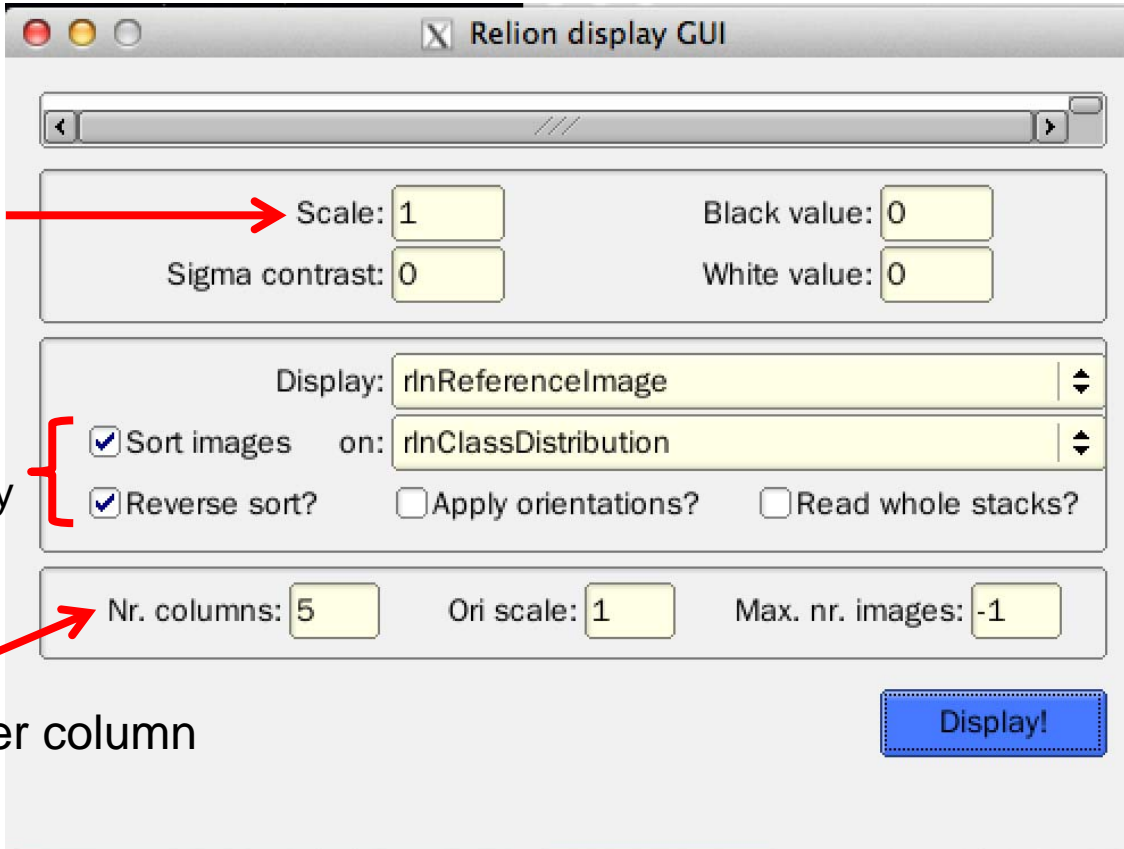
Two file browser windows are open. The top window shows the current directory as `../Class2D/` and contains a folder icon. The bottom window shows the current directory as `../` and lists the file `run_it025_model.star`. The right pane of this window is labeled `<empty file>`. Both windows have a 'Show: STAR files (\*.star)' dropdown and a 'Favorites' section.





# Subset Selection

- After run Subset Selection



The screenshot shows the 'Relion display GUI' window. It features several control panels:

- Ratio of display:** A red arrow points to the 'Scale' input field, which is set to 1. Other fields include 'Sigma contrast' (0), 'Black value' (0), and 'White value' (0).
- Click this two for reasonable display:** A red bracket groups the 'Sort images' and 'Reverse sort?' checkboxes, both of which are checked. Other options include 'on: rlnClassDistribution', 'Apply orientations?' (unchecked), and 'Read whole stacks?' (unchecked).
- How many class per column:** A red arrow points to the 'Nr. columns' input field, which is set to 5. Other fields include 'Ori scale' (1) and 'Max. nr. images' (-1).

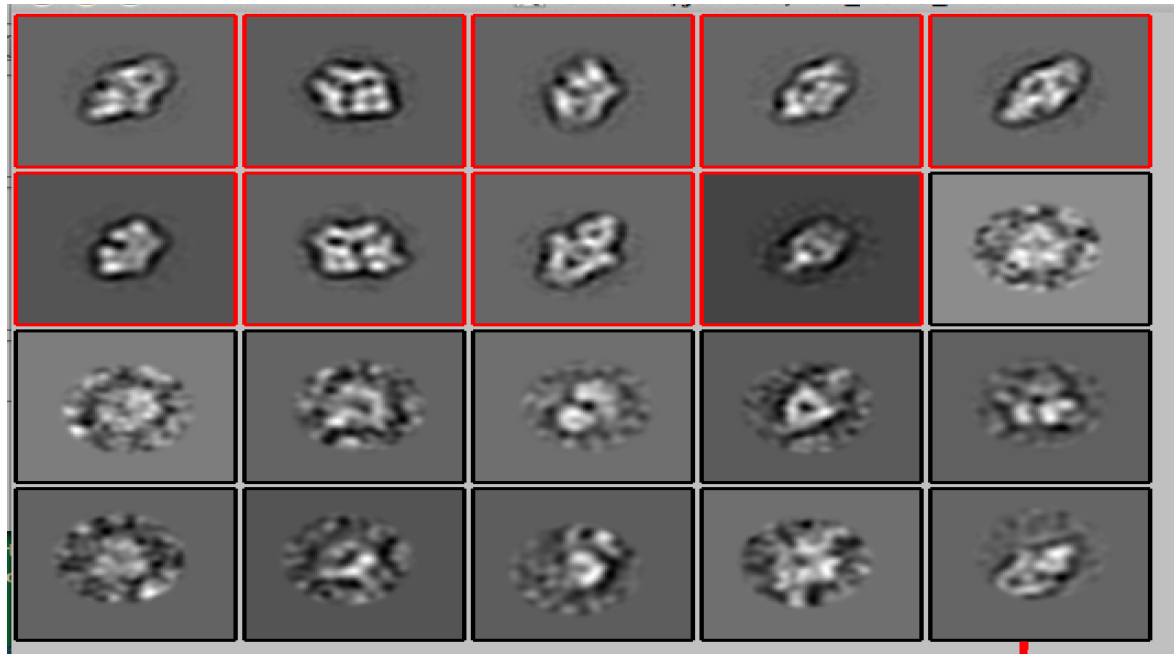
A blue 'Display!' button is located at the bottom right of the GUI.



# Subset Selection

- One image represents one class average.
- Based on biological knowledge, pick up those possible class containing structural information.

Largest amount



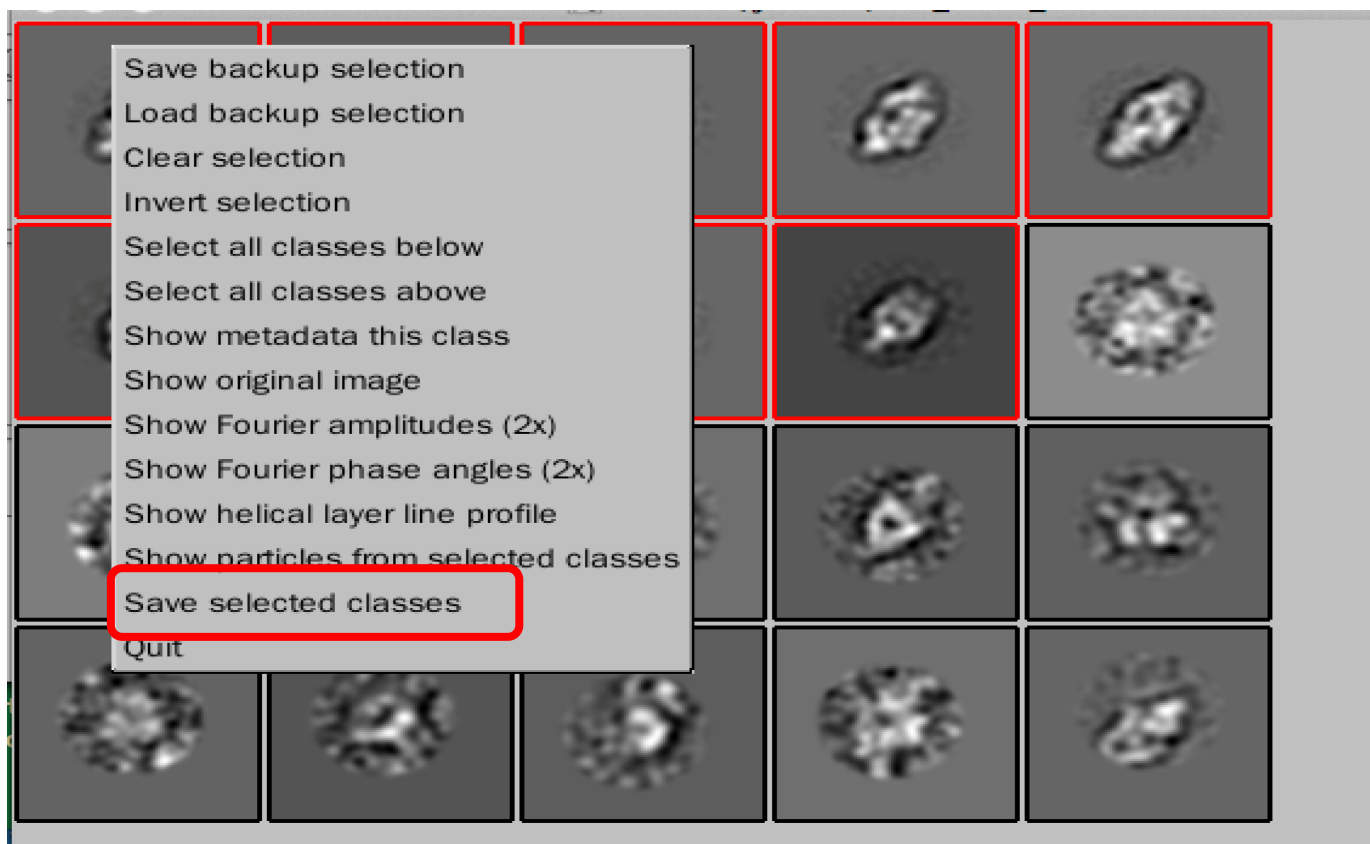
smallest amount



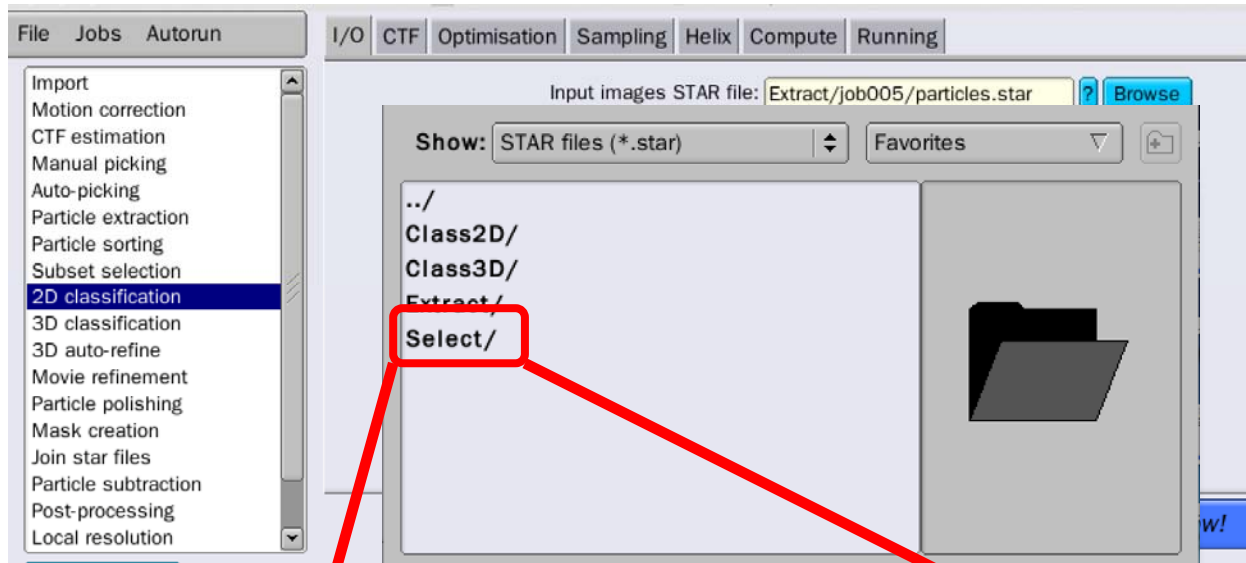
RELION

# Subset Selection

- Click **Right Button** of mouse.
- Choose **Save selected classes** to save all the classes you want



# 2 Choices or ..... More !!



Go Back !?

Go Forward !?

2D Classification

3D Classification



# RELION DEMO

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## 3D Classification



# 3D Classification


I/O Reference CTF Optimisation Sampling Helix Compute Running

Input images STAR file:  ?

Continue from here:  ?

Reference map:  ?

Reference mask (optional):  ?



Give a reference model for 3D reconstruction

I/O Reference CTF Optimisation Sampling Helix Compute Running

Number of MPI procs:   ?

Number of threads:   ?

Submit to queue?  ?

Queue name:  ?

Queue submit command:  ?

Standard submission script:  ?

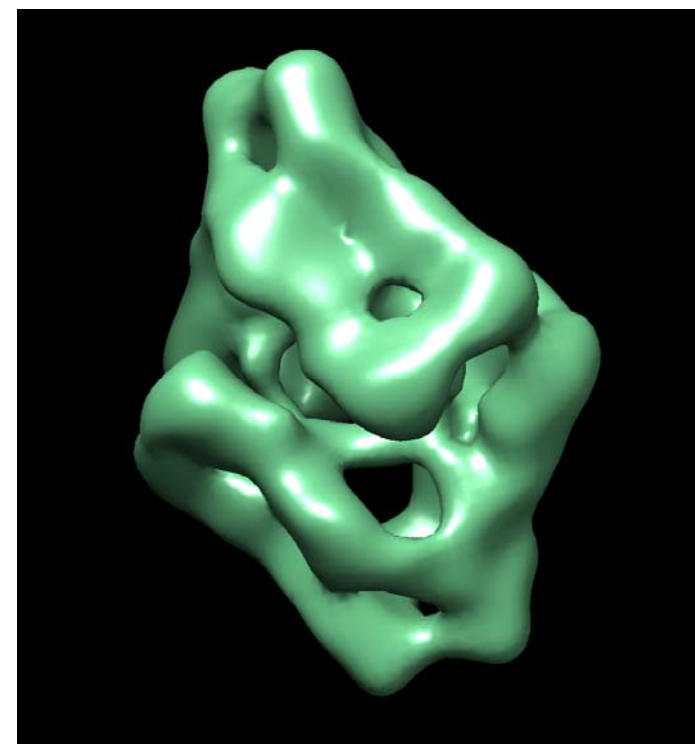
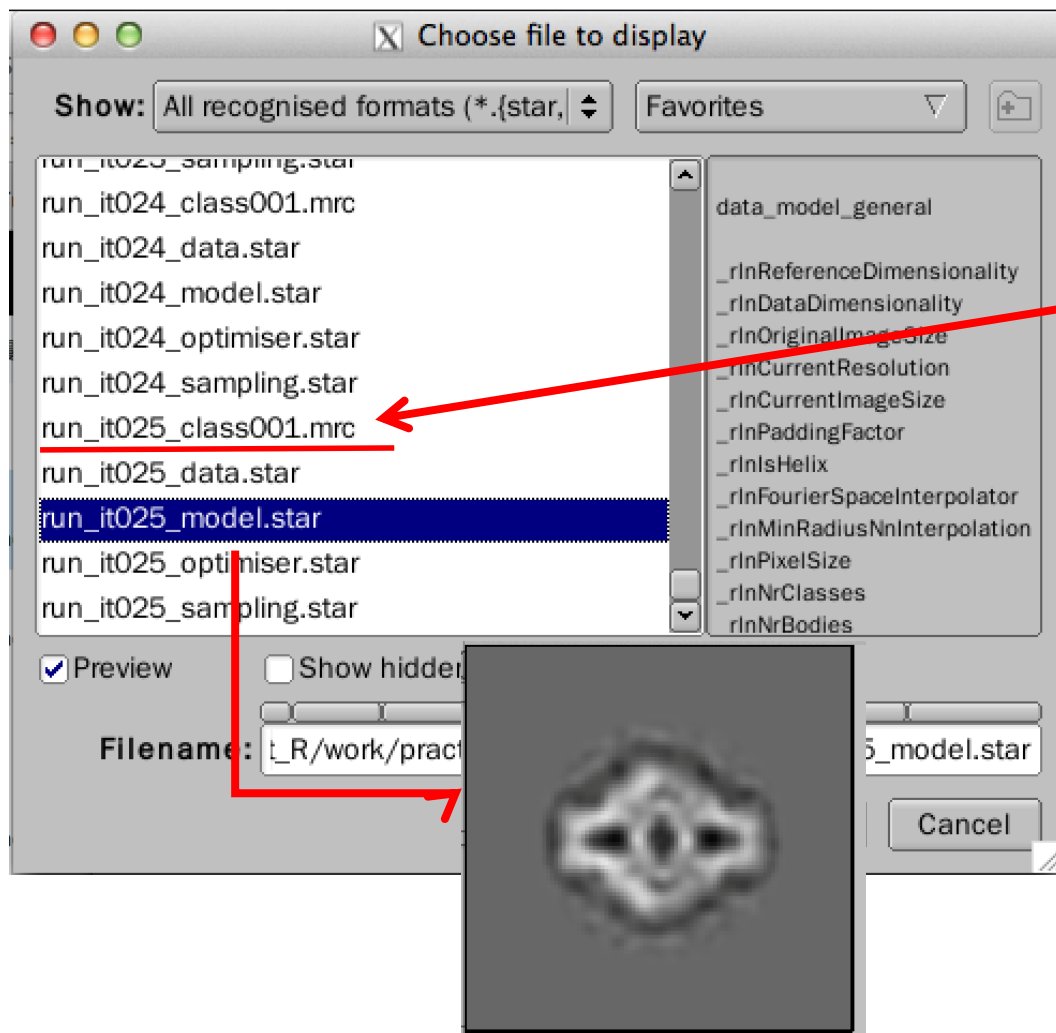
Minimum dedicated cores per node:   ?

Additional arguments:  ?

**Define pixel size of 2D image**



# 3D Classification



Display 3D model with Chimera



# FTP to Account

General | Advanced | Transfer Settings | Charset

Host:  Port:

Protocol:

Logon Type:

User:

Password:

Example:  
User : cryoem001  
Password : isgc2017-]crYOem001

Connect OK Cancel





# THANK YOU

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**END**

**Reference: Relion official website**

**[http://www2.mrc-lmb.cam.ac.uk/relion/index.php/Main\\_Page](http://www2.mrc-lmb.cam.ac.uk/relion/index.php/Main_Page)**



**RELION**