

大学院等高度副プログラム：基礎理学計測学「先端的研究法：低温電子顕微鏡」

2020/08/31-2020/09/04



Tutorial of Relion 3.1 : Innexin-6 pre-processing

Innexin-6を題材とした実習

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Relionに必要なハードウェア・ソフトウェア

I am buying new GPUs, what do you recommend to run RELION on?

Our collaborator in Stockholm, Erik Lindahl, has made a useful [blog with GPU hardware recommendations](#). Briefly, you'll need an NVIDIA GPU with [a CUDA compute ability of at least 3.5](#), but you don't need the expensive double-precision NVIDIA cards, i.e. the high-end gamer cards will also do, but do see Erik's blog for details! Note that 3D auto-refine will benefit from 2 GPUs, while 2D and 3D classification can be run just as well with 1 GPU. Apart from your GPUs you'll need a decent amount of RAM on the CPU (at least 64Gb), and you may also benefit from a fast (e.g. a 400Gb SSD!) scratch disk, especially if your working directories will be mounted over the network connecting multiple machines.

OS : Linux。GPU用のライブラリとしてCUDAが必要。

GPU の種類: NVIDIA社製。ゲーム用の安価なカードでかまわない。GeForce 1080など。

高価な倍精度のGPU(例えば、Tesla)は必要ない。

GPUの枚数: 2D分類は1枚でも動く。3D auto-refineは2枚以上必要。

主メモリ: できるだけ多く。最低でも64Gbyte。

ディスク: scratch領域にSSDがあるとよい。

CPU: CPUだけしか動かないタスクもあるので、コア数も多いほうがよい



NVIDIA GeForce 1080 Ti

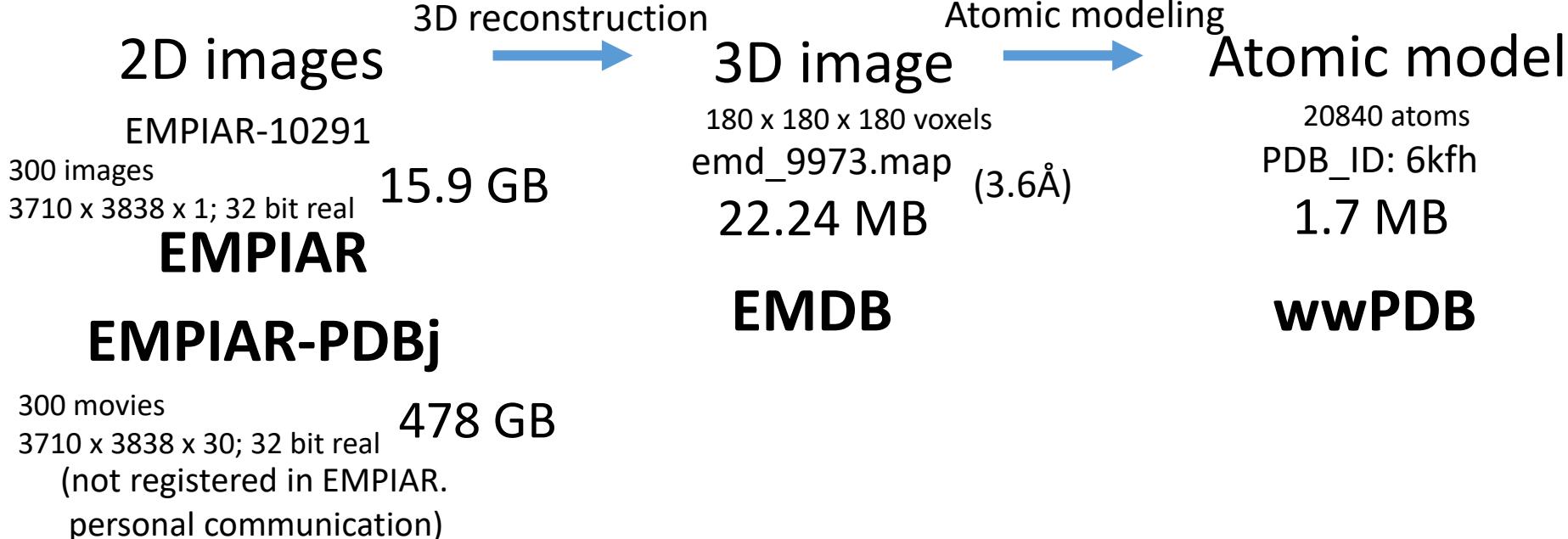
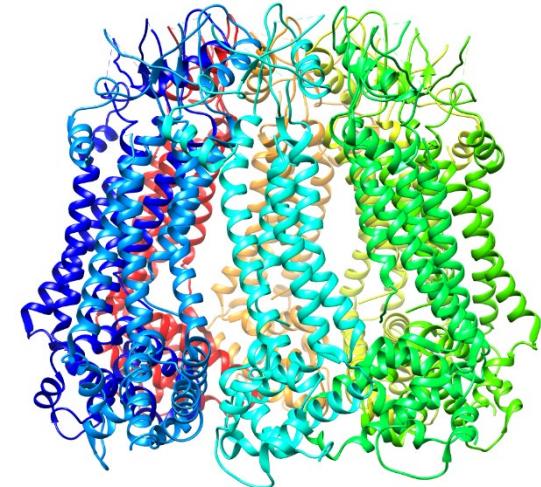
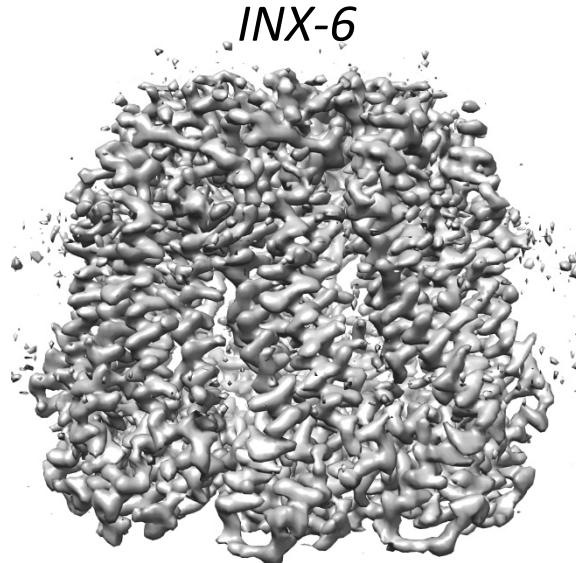
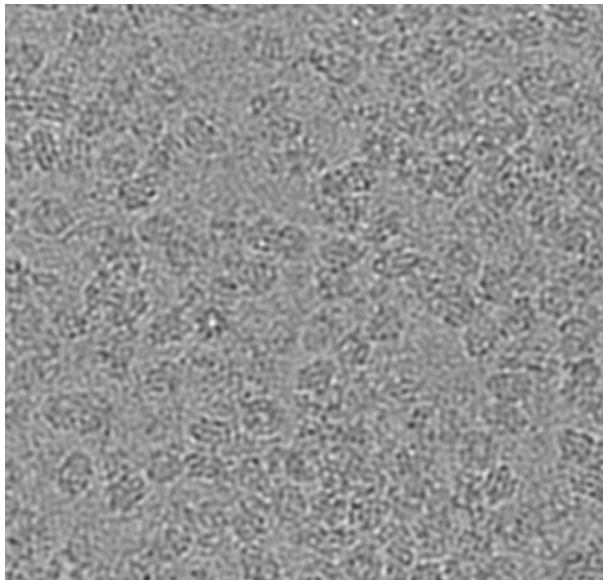
- 事前にインストールが必要なライブラリ
 - CUDA (GPU計算のためのライブラリ)
 - openmpi (ジョブ並列用のライブラリ)

- 以下のライブラリは無ければ、relionのコンパイラがインストール時にローカルにインストールする。
 - FFTW (高速フーリエ変換のライブラリ)
 - FLTK (GUIのライブラリ)



NVIDIA GeForce RTX™ 2080 Ti

Data processing for EM Single Particle Analysis



Summary of innexin-6 EM data

Batuujin Burendei, Ruriko Shinozaki, Masakatsu Watanabe, Tohru Terada, Kazutoshi Tani,*, Yoshinori Fujiyoshi and Atsunori Oshima.
Cryo-EM structures of undocked innexin-6 hemichannels in phospholipids. Science Advances 12 Feb 2020: Vol. 6, no. 7, eaax3157
DOI: 10.1126/sciadv.aax3157

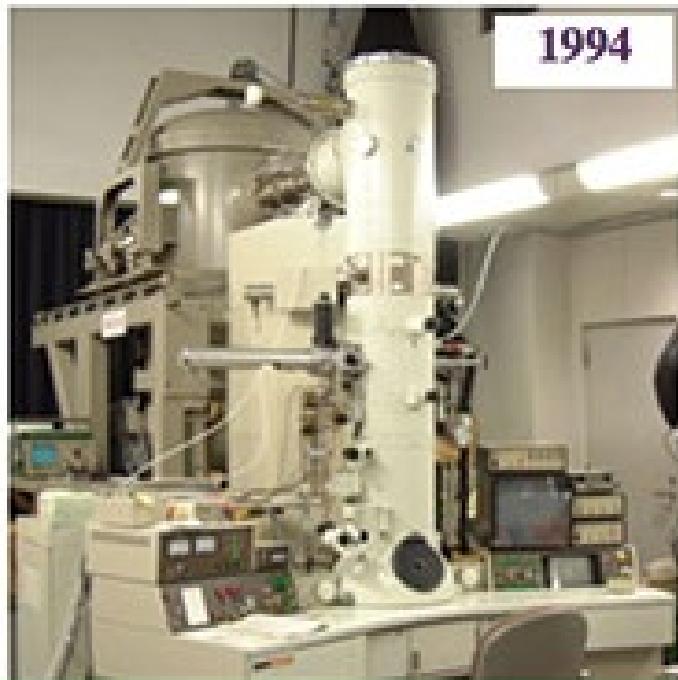
EMPIAR	EMDB	Resolution	PDB	molecules	Details of EMPIAR	Data size of EMPIAR entry
10289	9971	3.8 Å	6kff	WT INX-6 in a nanodisc	Motion corrected 2D projection images of innexin-6 gap junction hemichannels in nanodiscs.	49.5 GB. 933 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.
10290	9972	3.8 Å	6kfg	WT INX-6 in a detergent	Motion corrected 2D projection images of innexin-6 gap junction hemichannels in detergent.	26.4 GB. 497 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.
10291	9973	3.6 Å	6kfh	WT INX-6ΔN in a nanodisc	Motion corrected 2D projection images of N-terminal deleted innexin-6 gap junction hemichannels in nanodisc.	15.9 GB. 300 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.

- EMPIARに登録されているのはMotion Correction後の静止画像のみ
- undocked hemichannelはEMPIAR, EMDB, PDBで公開。Docked hemichannelは、EMDB (9570, 9571), PDB(5h1q, 5h1r)で公開されているが、EMPIARにはデータがない。

JEM-3000SFF(JEOL)

The data were collected using a JEM-3000SFF (JEOL) electron microscope at 300 kV equipped with a K2 summit direct electron detector camera (Gatan).

Burendei, B., Shinozaki, R., Watanabe, M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A. Sci Adv, 6:eaax3157-eaax3157, 2020



Flagellar filament @9Å (1995)
Flagellar filament @4.5Å (2003)

JEOL JEM-3000SFF (G3)
Liq-He cooled specimen stage
First FEG (Field Emission Gun)

+

Gatan社製K2 Summit直接検出型カメラ



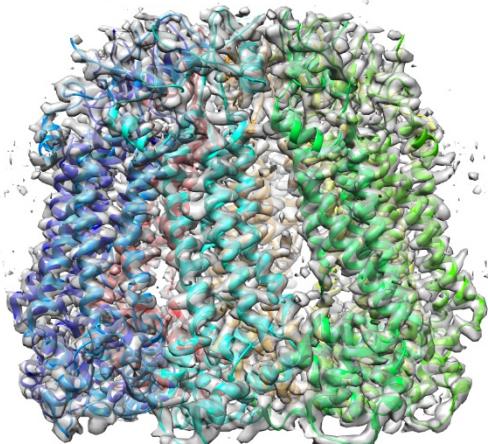
GATAN

Innixin-6 : docked and undocked states

Structural component of the gap junctions.

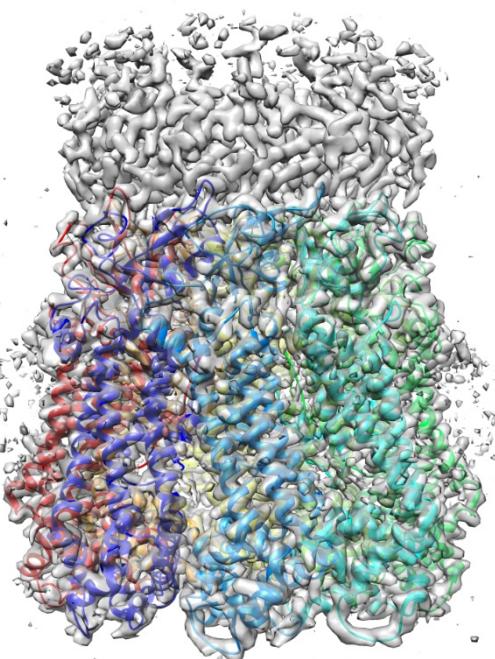
Undocked innixin-6
hemichannel

EMPIAR-10291



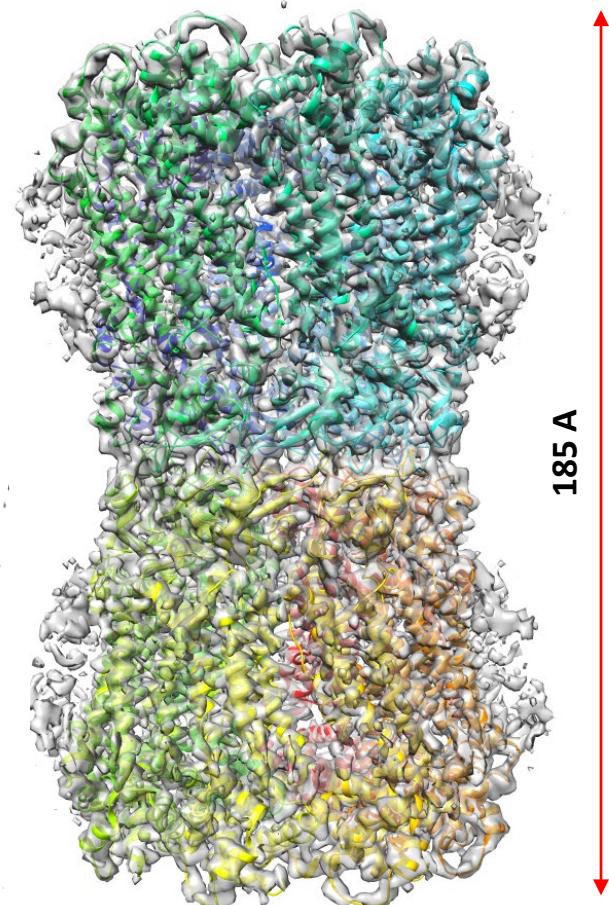
EMD-9973/PDB_ID:6kfh

Burendei, B., Shinozaki, R., Watanabe,
M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A.
Sci Adv, 6:eaax3157-eaax3157, 2020



EMD-9570/PDB_ID:5h1q

Docked innixin-6



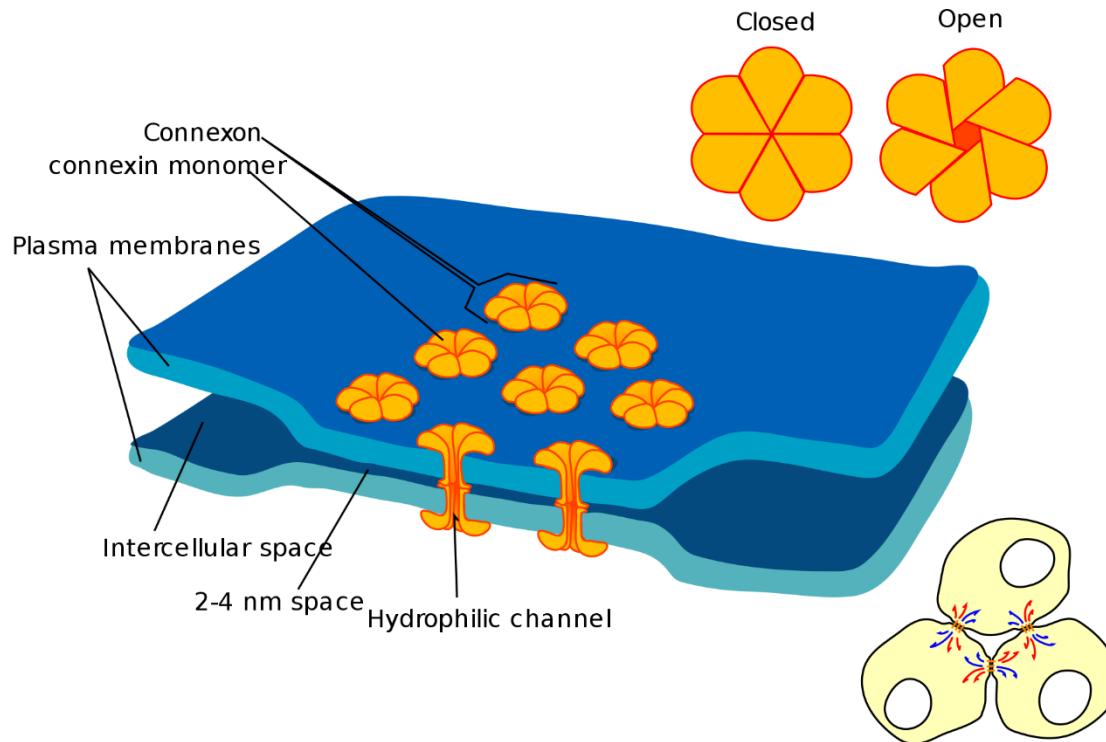
EMD-9571/PDB_ID:5h1r

Oshima, A., Tani, K., Fujiyoshi, Y. Nat Commun, 7:13681-13681,

ギャップ結合 (gap junction)

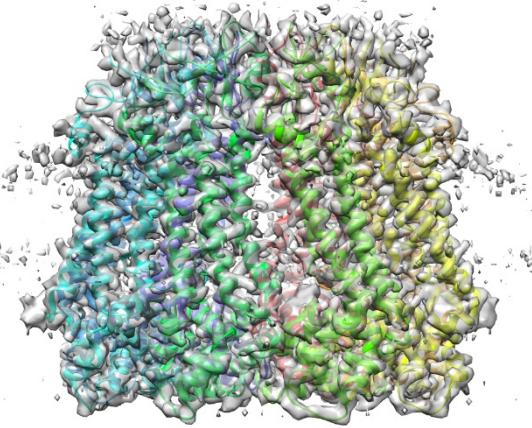
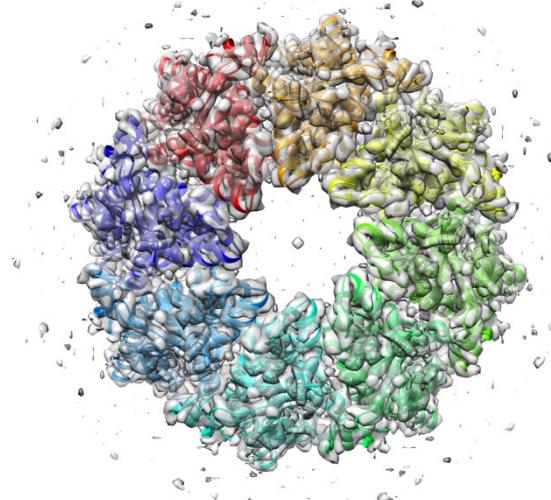
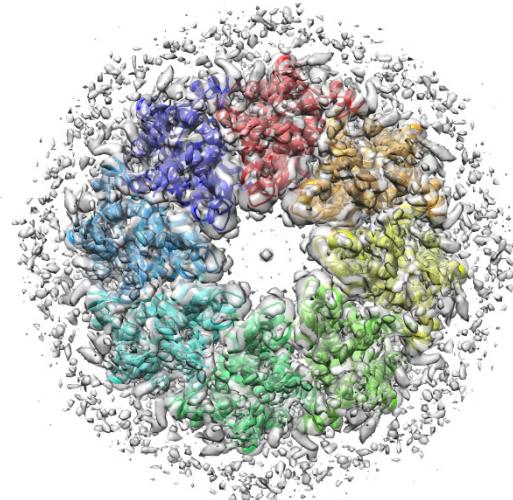
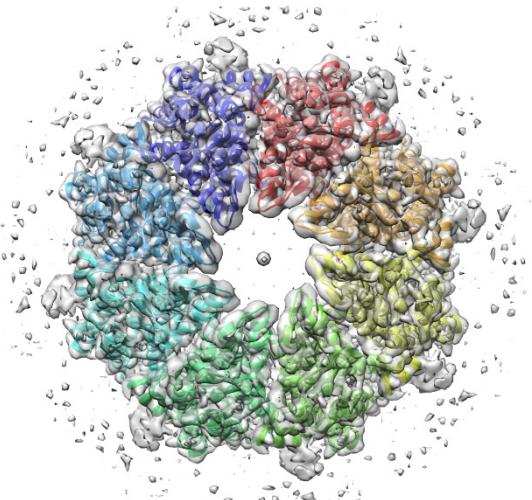
ギャップ結合(ギャップけつごう、英: Gap junction)は、隣り合う上皮細胞をつなぎ、水溶性の小さいイオンや分子を通過させる細胞間結合のこと。

並んだ2つの細胞の細胞膜にはコネクソンと呼ばれるタンパク複合体の末端が複数並んでおり、橋渡し構造をなしている。このコネクソンがチャネルとなり、ここを通って無機イオンや小さい水溶性分子が隣接細胞の細胞質から細胞質へと直接移動することができる。また、細胞同士を電気的に結合するため、心筋組織などの興奮伝播にも関わっている。

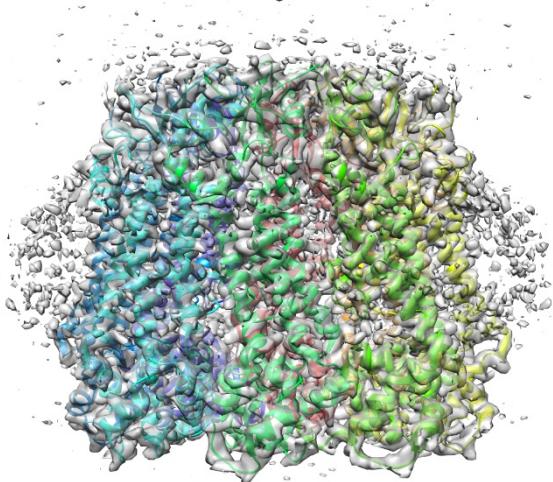


Wikipediaからの引用

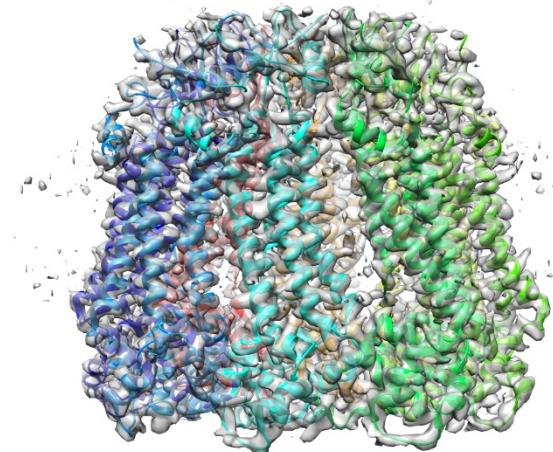
Undocked innexin-6 hemichannel



EMD-9971/PDB_ID:6kff
EMPIAR-10289
WT INX-6 in a nanodisc



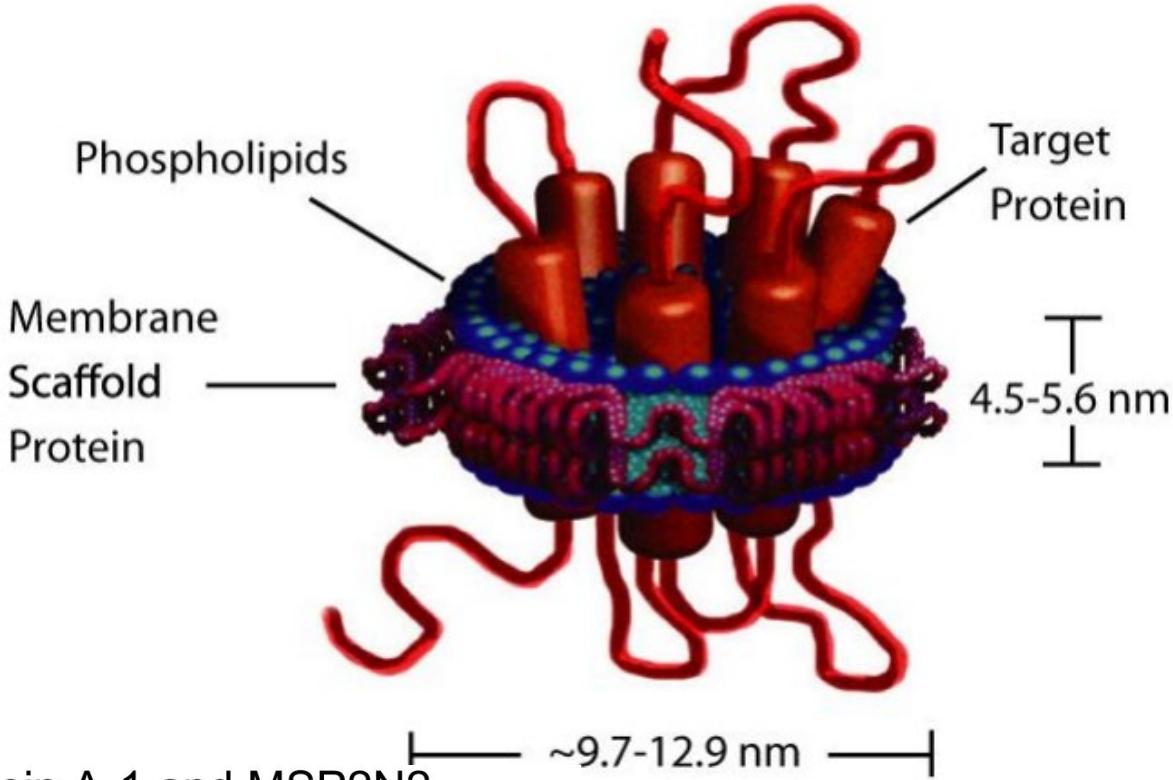
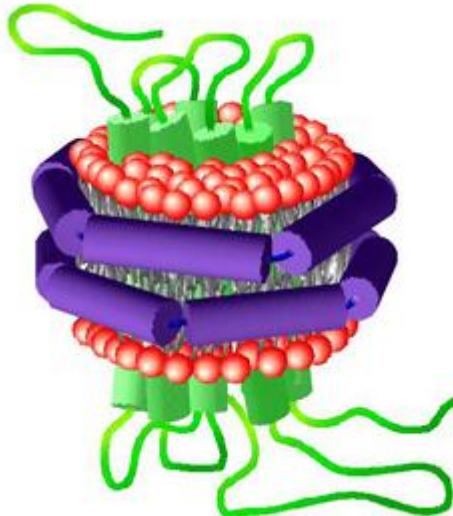
EMD-9972/PDB_ID:6kgf
EMPIAR-10290
WT INX-6 in a detergent



EMD-9973/PDB_ID:6kfh
EMPIAR-10291
WT INX-6 Δ N in a nanodisc

nanodisc

Example of a Nanodisc containing a 7-transmembrane protein

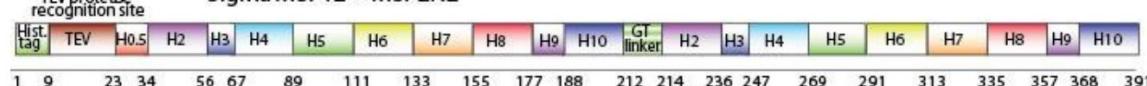


Protein Maps of Apolipoprotein A-1 and MSP2N2

Apolipoprotein A1



Sigma MSP12 = MSP2N2



PDB_ID: 1av1
Apolipoprotein A-1
(APOA1_HUMAN)

To investigate the structure in a lipid bilayer, we reconstituted undocked WT INX-6 hemichannels in nanodiscs using the membrane scaffold protein 2N2 (**MSP2N2**) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), as confirmed by Coomassie brilliant blue-stained gel and negatively stained electron micrographs (fig S1A).

Burendei, B., Shinozaki, R., Watanabe, M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A., 9
Sci Adv, 6:eaax3157-eaax3157, 2020

EMPIAR-10289
WT INX-6 in nanodisc
INX6hemiNano001

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

These long circles
are not proteins.
micelle ? Nanodiscs ?

EMPIAR-10290
WT INX-6 in detergent
INX6hemiDet002

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

EMPIAR-10291
WT INX-6 Δ N in nanodisc
INX6hemiDet001

Pixel_width: 1.232 Å

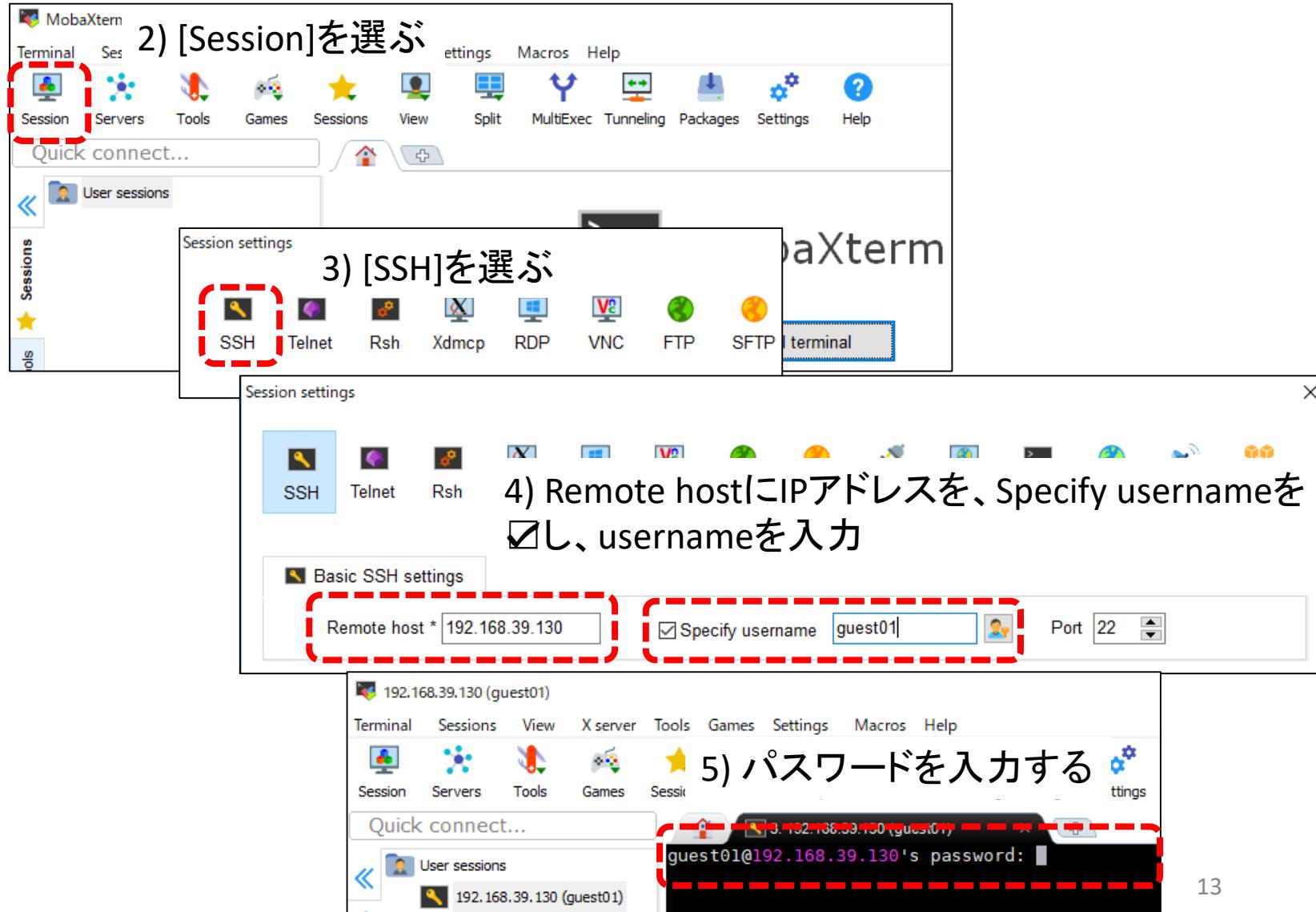
After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

MobaXtermによるログインの方法

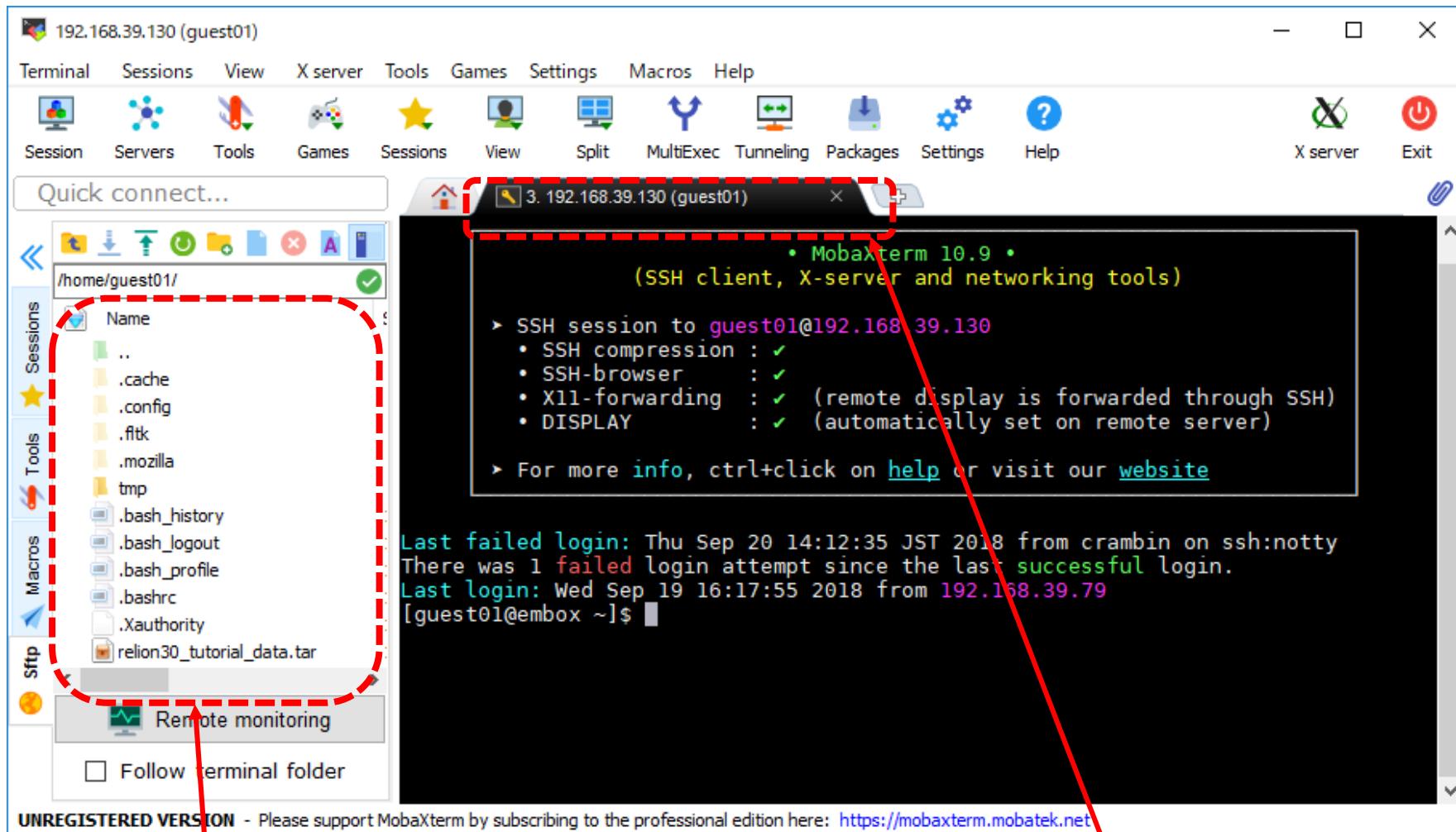
1) アイコン



をクリックして起動



MobaXtermによるログイン画面



画面左にログイン先のファイルのリストが表示されます。

複数のログイン画面もタブで分けて表示できます。

※[Ctrl]キー+マウスホイールで、フォントの大きさを変えることができます。

Basic Unix Commands (1)

↑	前に使ったコマンドを表示	Show the previous command
↓	後に使ったコマンドを表示	Show the next command
[Tab] key	ファイル名の補間 例) <code>ls relion[Tab]</code> <code>ls relion30_tutorial</code>	Compensate file name (example) <code>ls relion[Tab]</code> <code>ls relion30_tutorial</code>
[Ctrl]+[C]	コマンド実行の中止	Quit the command
[Ctrl]+[Z]	コマンド実行の一時停止	Pause the command
bg	一時停止したコマンドをバックグラウンドで実行	Execute the paused command in background
kill %%	バックグラウンドで実行させた直前のコマンドの実行停止	Stop executing the latest background job
pwd	現在のディレクトリを表示	Print the current directory
cd [directory]	[directory]に移動	Go to [directory]
cd ..	一つ上のディレクトリに移動	Go to the upper directory
cd	ホームディレクトリに移動	Go to your home directory
ls	ファイルのリストの表示	Show a list of files
ls -l	ファイルのリストの表示。日付やファイルサイズも表示。	Show a list of files with their updated dates and file sizes. ¹⁵

Basic Unix Commands (2)

cat [filename]	[filename]の内容を表示	Show the content of [filename]
less [filename]	[filename]の内容を表示 ↓:ダウン、↑:アップ、q:終了	Show the content of [filename] ↓:page down、↑ :page up、 q:exit
vi [filename]	[filename]を編集 [Esc]:コマンドモード [i]:入力 モード [x]:削除 [:q!]:保存し ないで終了 [Shift ZZ]:保存し て終了	Edit [filename] [Esc]:command mode, [i]:insert, [x]:delete [:q!]:quit without save [Shift ZZ]:quit after save
top	実行中のジョブのリストを表 示 [q]:終了 [1]:threadsを表 示	Show a list of jobs. [q]:exit, [1]:show threads
nvidia-smi	GPUで実行中のジョブを表示	Show a list of GPU jobs
kill [processID]	[processID]のプロセスの実行 中止	Stop executing the process [processID]

演習アカウントのホームディレクトリの内容

ホームディレクトリ /home/guest01の下に、以下のディレクトリがあります。

```
[guest01@embox2 ~]$ ls -lt  
drwxrwxr-x. 3 guest01 guest01  
[guest01@embox2 ~]$
```

演習用のデータの入ったディレクトリ

18 8月 26 19:48 EMPIAR-10291_100mic

```
[guest01@embox2 ~]$ ls -lt EMPIAR-10291_100mic
```

合計 16

```
-rw-rw-r--. 1 guest01 guest01 4501 8月 29 15:57 10291.xml  
drwxrwxr-x. 2 guest01 guest01 4096 8月 26 19:52 data ←empiarのメタデータファイル  
[guest01@embox2 ~]$
```

ディレクトリEMPIAR-10291_100micの下のディレクトリdata/に100個のmrcファイルがあるはず。

```
[guest01@embox2 ~]$ ls -lt EMPIAR-10291_100mic/data/
```

合計 5562400

```
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano206.mrc  
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano234.mrc  
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano275.mrc  
:  
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano233.mrc 3710 x 3838 x 1; 32 bit real  
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano248.mrc  
-rw-rw-r--. 1 guest01 guest01 56956944 8月 26 19:52 INX6Ndelnano249.mrc
```

ディレクトリEMPIAR-10291_5movieの下のディレクトリmovie/に5個のmrcファイルがあるはず。

```
[guest01@embox2 ~]$ ls -lt EMPIAR-10291_5movie/movie/
```

合計 8343160

```
-rwxrwxr-x. 1 guest01 guest01 1708678624 9月 1 21:02 INX6Ndelnano020.mrc 3710 x 3838 x 30; 32 bit real  
-rwxrwxr-x. 1 guest01 guest01 1708678624 9月 1 21:02 INX6Ndelnano019.mrc  
-rwxrwxr-x. 1 guest01 guest01 1708678624 9月 1 21:01 INX6Ndelnano010.mrc  
-rwxrwxr-x. 1 guest01 guest01 1708678624 9月 1 21:01 INX6Ndelnano007.mrc  
-rwxrwxr-x. 1 guest01 guest01 1708678624 9月 1 21:01 INX6Ndelnano006.mrc
```

1.1 Launch Relion

Launch Relion from the “project directory”.

In this tutorial, the project directory is `~/EMPIAR-10291_100mic`.

Type following commands to launch relion:

```
$ cd  
$ cd EMPIAR-10291_tutorial  
$ relion
```

* When you launch in the first time, you will be asked:
Only run the relion GUI from your ProjectDirectory. Do you want to start a new project here [y/n]?
answer ‘y’.

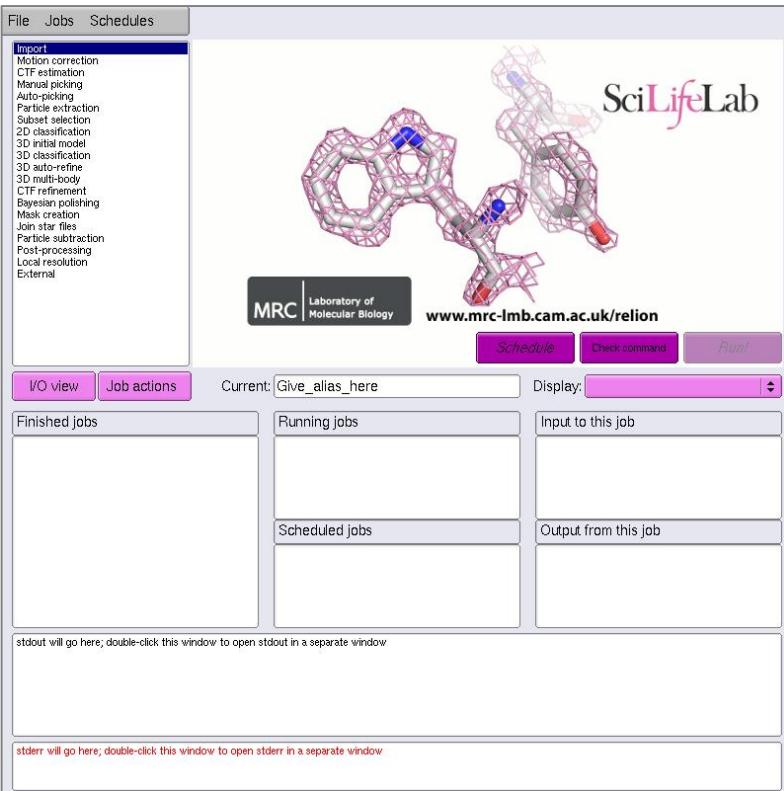
* The file “default_pipeline.star” is generated, which describes jobs you have done.

※ Relionを起動したターミナルで、以下のキーを押すと、Relionがバックグラウンドで起動され、ターミナルとしてコマンド入力に使うことができる。

```
[Ctrl] + [Z] キーを押す  
$ bg
```

※ Relionを二回目以降に起動する場合は、コマンドの最後に&をつけると、バックグラウンドでの起動となる。

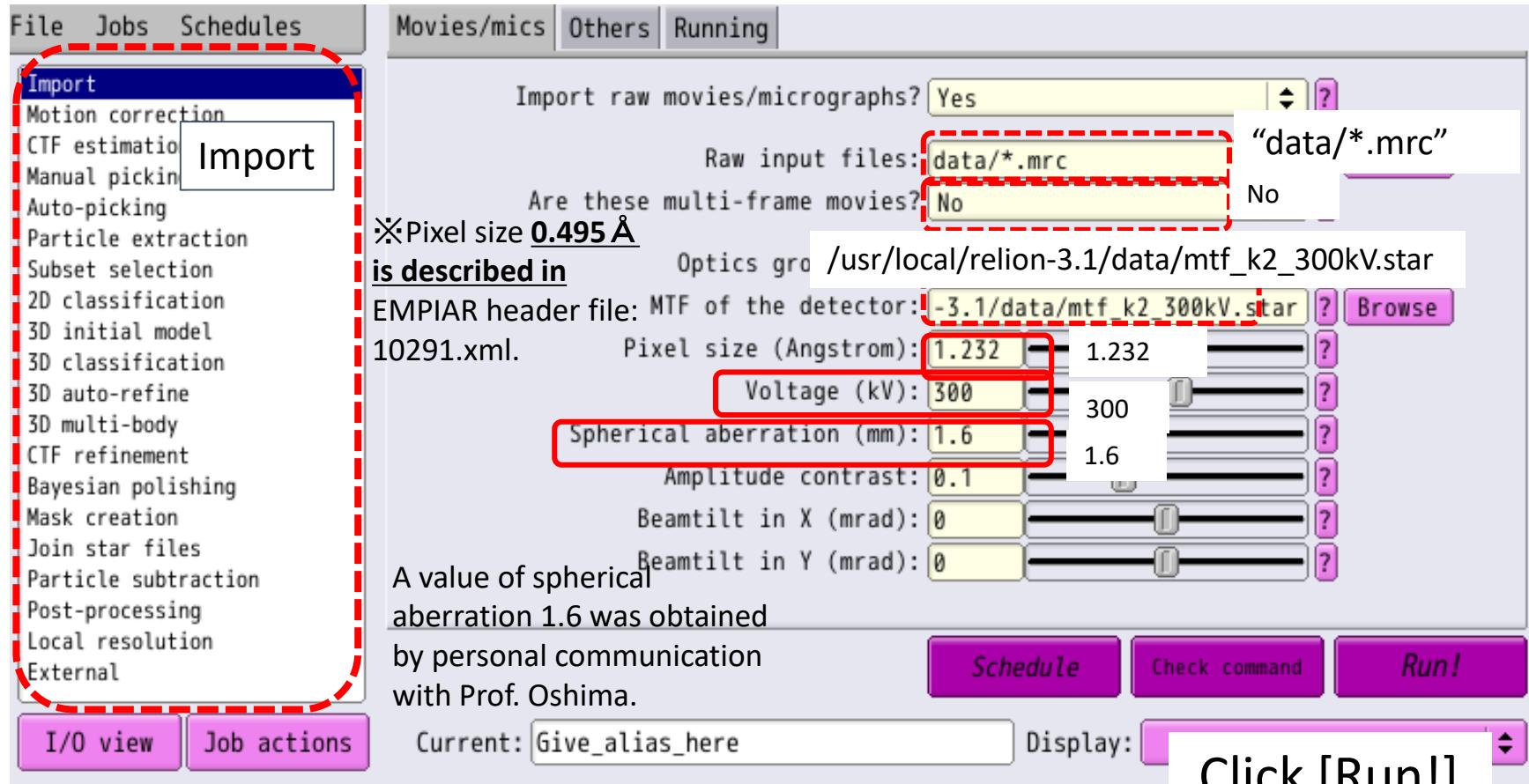
```
$ relion &
```



1.1 Import micrographs / movies

Select “Import” from Job type browser.

Job type browser



The directory “Import/job001” is generated. The file “movies.star” has a list of micrographs.

You can check it by the command: less Import/job001/movies.star

1.3 CTF estimation (< 1min)

The screenshot shows the Relion software interface for CTF estimation. The main window has a menu bar with File, Jobs, Schedules, I/O, CTFFIND-4.1, Gctf, and Running. The title bar indicates the current job is MotionCorr/job001/micrographs.star. A sidebar on the left lists various processing steps: Import, Motion correction, CTF estimation (which is selected), Manual picking, Auto-picking, 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, I/O view, Job actions, and Cur.

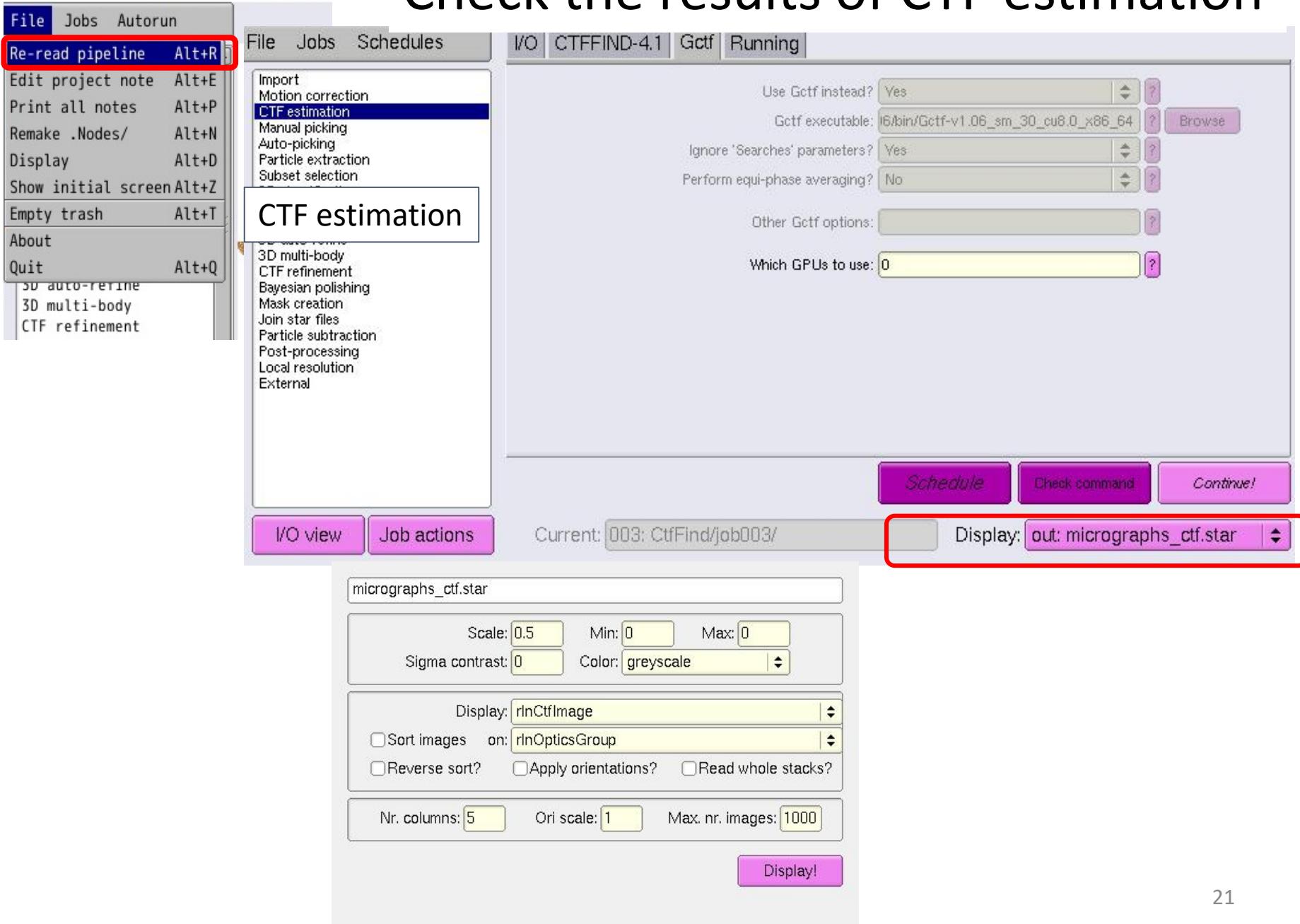
The main configuration area for CTF estimation includes:

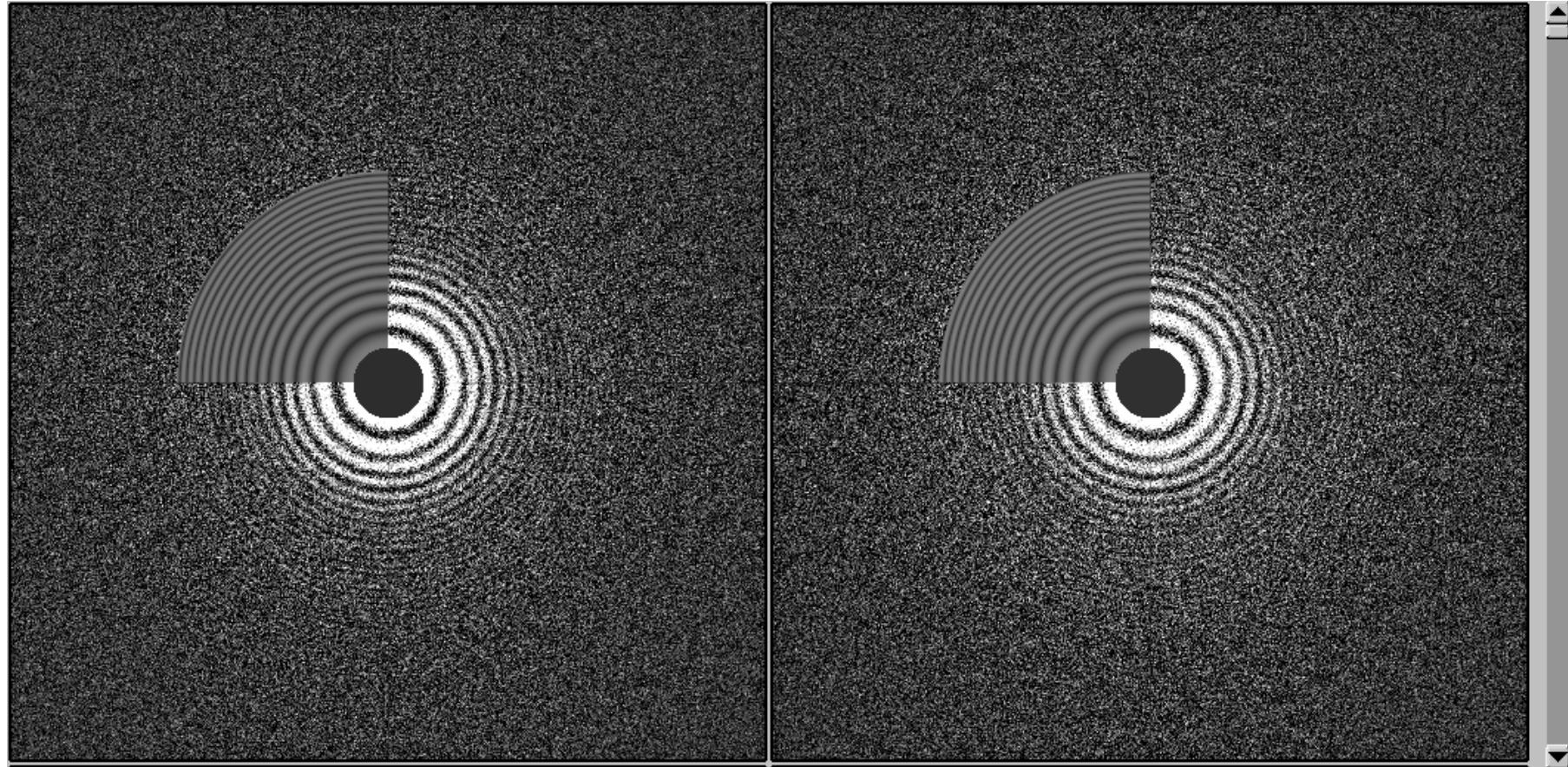
- Input micrographs STAR file: /path/to/job001/micrographs.star
- Use micrograph without dose-weighting? No
- Estimate phase shifts? No
- Phase shift - Min, Max, Step (deg): 0, 180, 10
- Use CTFFIND-4.1? Yes
- CTFFIND-4.1 executable: /ctffind-4.1.5/bin/ctffind
- Use power spectra from MotionCorr job? No
- Use exhaustive search? No
- Estimate CTF on window size (pix): -1
- FFT box size (pix): 512
- Minimum resolution (A): 30
- Maximum resolution (A): 5
- Minimum defocus value (A): 5000
- Number of MPI procs: 6
- 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: [empty input field]

At the bottom right, there is a pink button labeled "Click [Run !]" and a smaller "Run!" button.

RELION-3.0.5: /home/guest01/tmp/relion30_tut

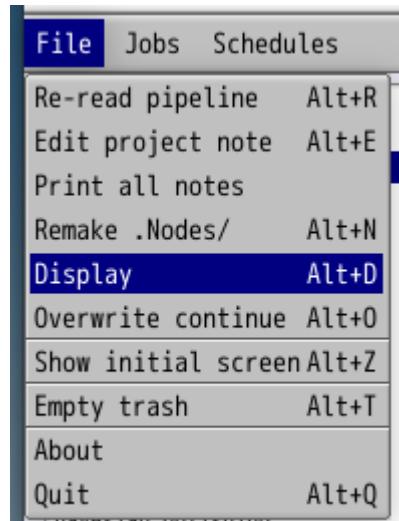
Check the results of CTF estimation



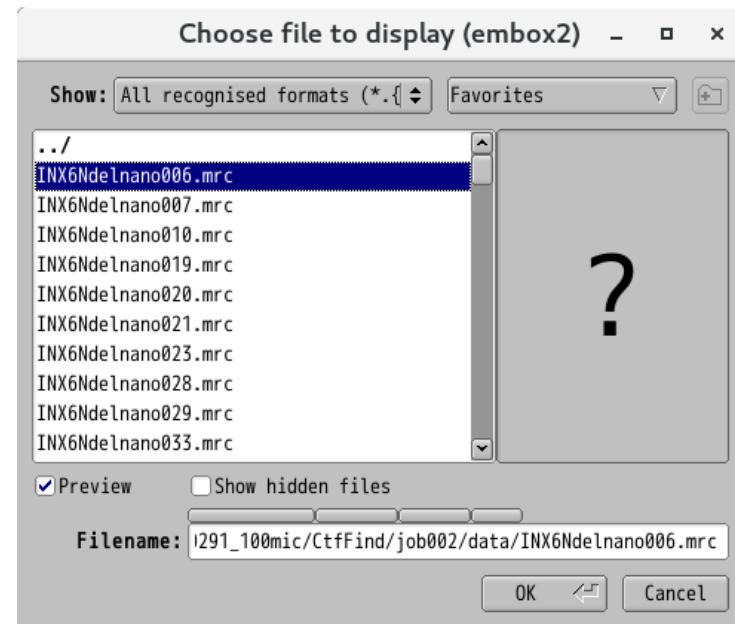


Check CTF-corrected micrographs

(1) Select [File] -> [Display]



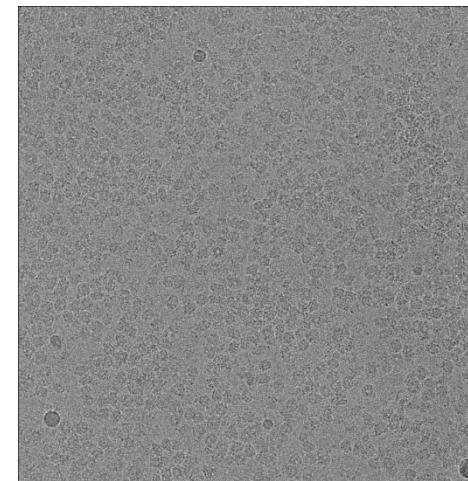
(2) Select one of the file in CtfFind/job002/data



(3) Input your favorite “scale” and “Low pass filter” values.

A screenshot of the 'Display!' dialog box. It contains several input fields: 'INX6Ndelnano006.mrc' in a text field, 'Scale: 1' in a red-bordered field, 'Min: 0' and 'Max: 0' in adjacent fields, 'Sigma contrast: 0' and 'Color: greyscale' dropdowns, 'Lowpass filter (A): 10' in a red-bordered field, 'Highpass: 0', 'Pixel size (A): 1', and a 'Display!' button.

(4) You can see the CTF-corrected micrograph



INX6Ndelnano006.mrc

After
CTF estimation

No filter

Lowpass filter:10 \AA

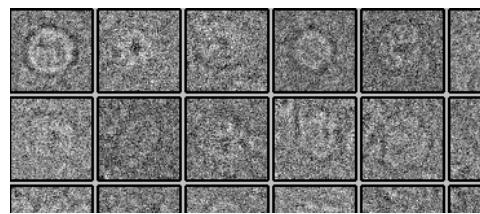
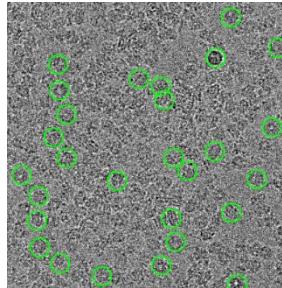
Lowpass filter:20 \AA

Lowpass filter:30 \AA

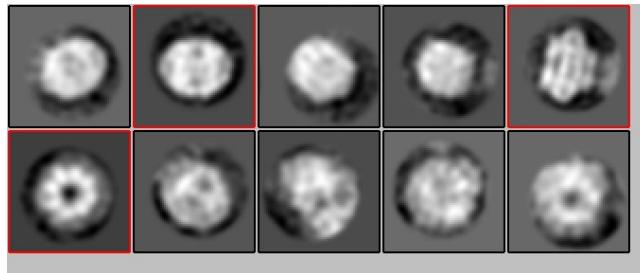
Picking by Relion

*For a few
micrographs*

Manual picking



Auto-picking: LoG-based

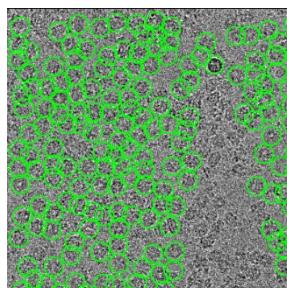


Class2D

Select Good Classes for reference

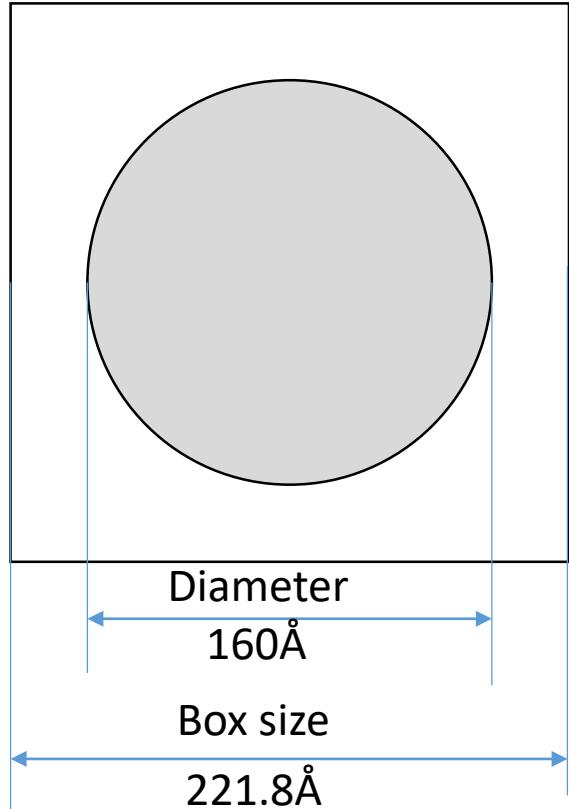
*For many
micrographs*

Auto-picking: reference-based



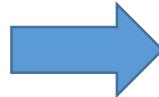
Box size of particles

Pixel size: 1.232Å

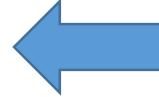


180 pixel

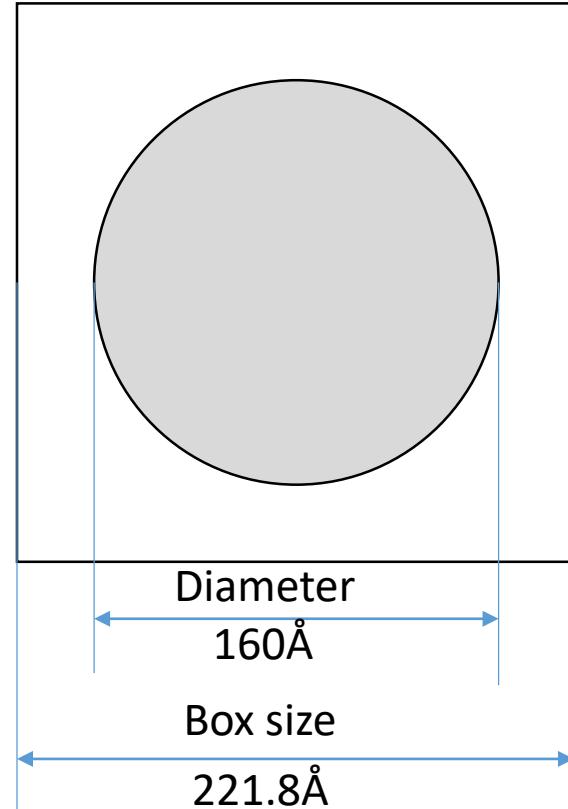
To reduce
Computation costs



Just before
Refine3D



Pixel size: 3.465Å



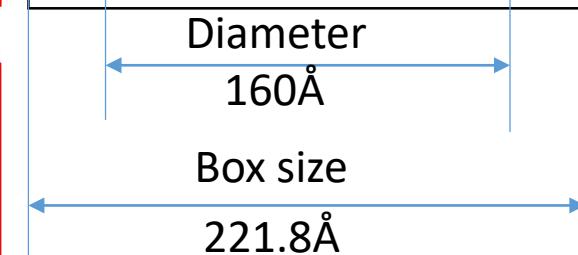
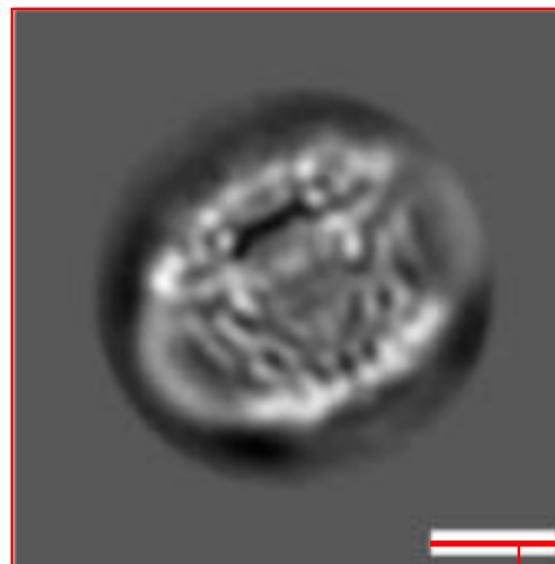
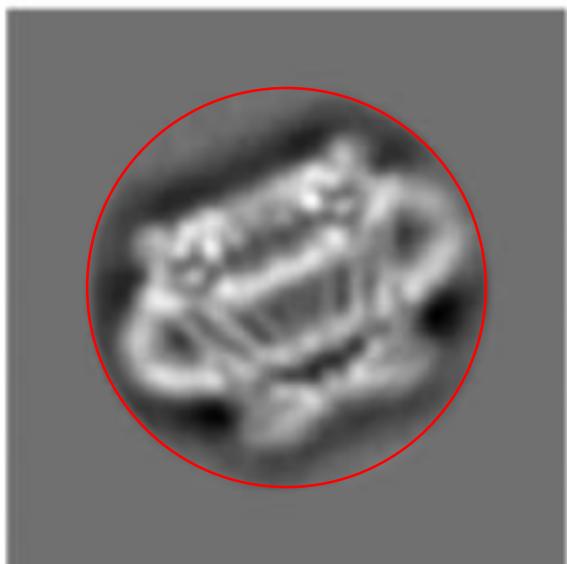
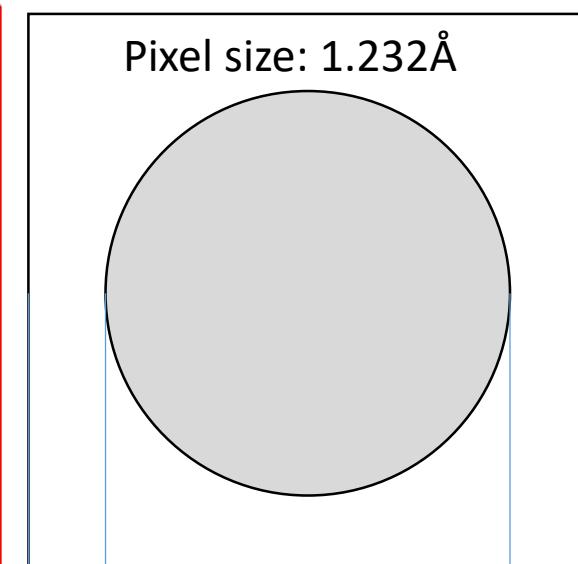
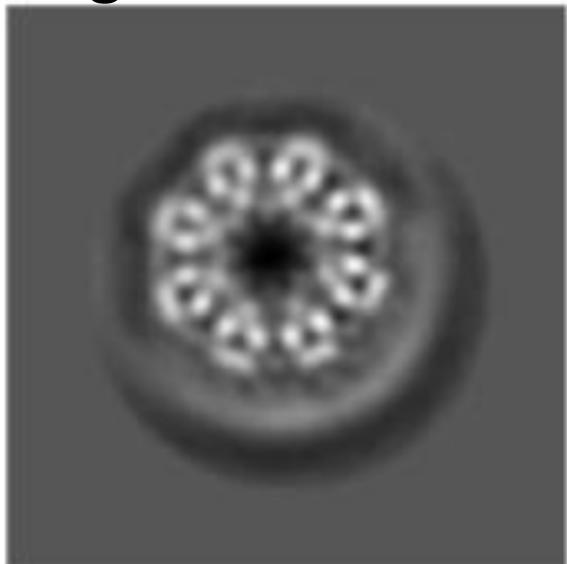
64 pixel

C

2D Class average images from the original article

Fig.S3c

Burendei et al. Science Advances. 2020. DOI: 10.1126/sciadv.aax3157



180 pixel

方形の1辺 $7.44\text{cm} = 222.75\text{\AA}$

マスク直径 $5.28\text{cm} = 158.08\text{\AA}$

1.67 cm : 50 Å

1.4 Manual Particle Picking

The screenshot shows the CtfFind software interface. At the top, there is a menu bar with File, Jobs, Schedules, I/O, Display, Colors, and Running. The I/O tab is highlighted with a red box. The title bar shows the path CtfFind/job003/micrographs_ctf.star. Below the menu, there is a sidebar with options: Import, Motion correction, CTF estimation, **Manual picking** (which is selected and highlighted with a blue box), Auto-picking, and P. The main area has tabs: I/O, **Display** (highlighted with a red box), Colors, and Running. In the Display tab, settings include Particle diameter (A) set to 160, Scale for micrographs set to 0.2, Sigma contrast set to 3, White value set to 0, Black value set to 0, Lowpass filter (A) set to 10, Highpass filter (A) set to -1, and Pixel size (A) set to -1. There is also a setting for Pick start-end coordinates helices? set to No. The Alias field is set to 5mic. Buttons for Schedule, Check command, Run, and a context menu are visible. A context menu is open over a grayscale micrograph showing particles, with options: Save STAR with coordinates, Load coordinates, Reload coordinates, Clear coordinates, Help, and Quit. The context menu is also highlighted with a red box.

File

data/INX6Ndelnano006.mrc **pick** 0 CTF 98

data/INX6Ndelnano007.mrc **pick** 0 CTF 192

data/INX6Ndelnano010.mrc **pick** 0 CTF 189

data/INX6Ndelnano019.mrc **pick** 0 CTF 211

data/INX6Ndelnano020.mrc **pick** 0 CTF 202

data/INX6Ndelnano021.mrc **pick** 0 CTF 199

data/INX6Ndelnano022.mrc **pick** 0 CTF 202

data/INX6Ndelnano023.mrc **pick** 0 CTF 181

上から順に[pick]を選び、粒子をピックする。

上から5つの画像に対して手動ピックを行う。

※[Shift]キーを押しながらクリックすると、○が消える！

I/O Display Colors Running CtfFind/job003/micrographs_ctf.star

Input micrographs: CtfFind/job003/micrographs_ctf.star ? Browse

I/O Display Colors Running

Particle diameter (A): 160 160

Scale for micrographs: 0.2 0.2

Sigma contrast: 3

White value: 0

Black value: 0

Lowpass filter (A) 10 10

Highpass filter (A) -1

Pixel size (A) -1

Pick start-end coordinates helices? No

Scale for CTF image: 1

Alias: 5mic

Schedule Check command Run

Current: 5mic

マウス左ボタンで粒子をクリックし50-100個ほど選択

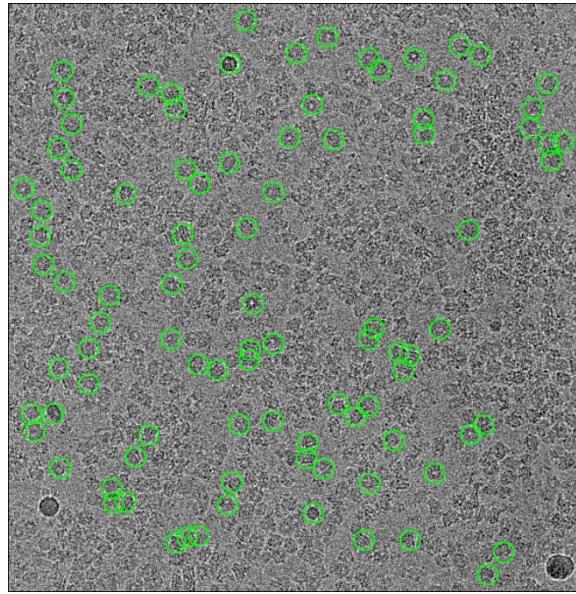
マウス右ボタンでポップアップメニューを表示させ、[Save STAR with coordinates] を選び、ピックした座標を保存。

ManualPick/Many/Movies/*_manualpick.starに保存される。

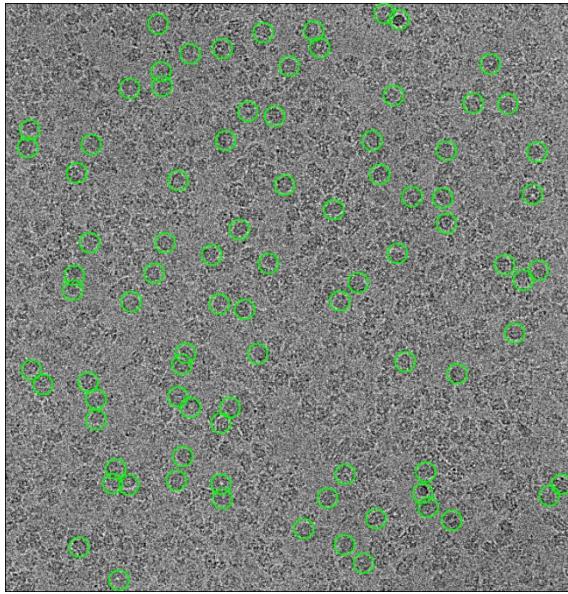
Save STAR with coordinates
Load coordinates
Reload coordinates
Clear coordinates
Help
Quit

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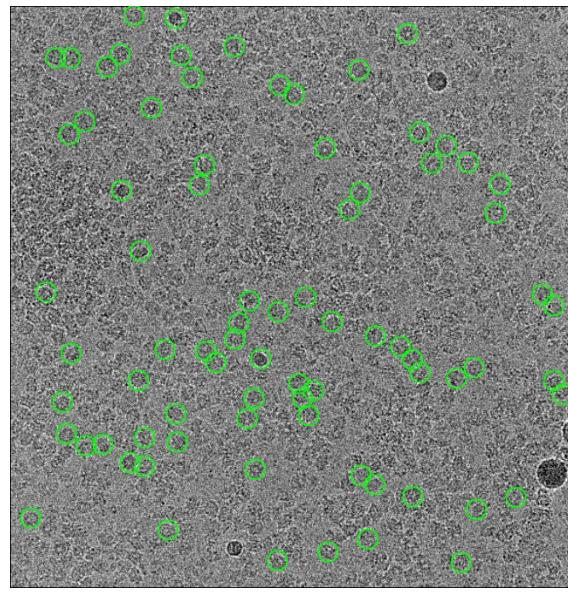
Manual Particle Picking



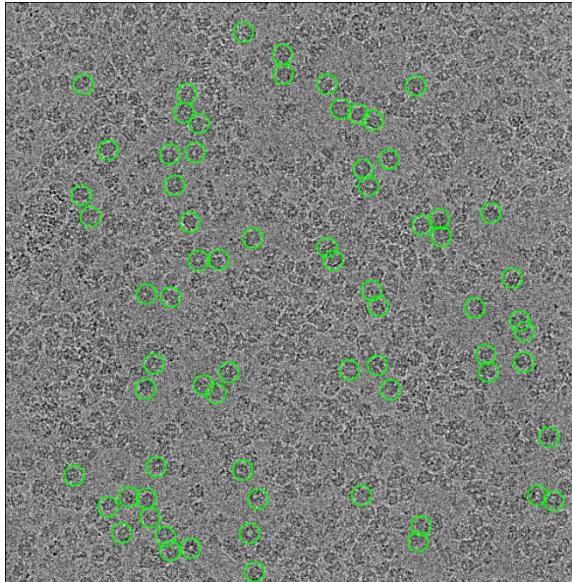
006 : 94 particles



007 : 87 particles



010 : 79 particles



0019 : 70 particles



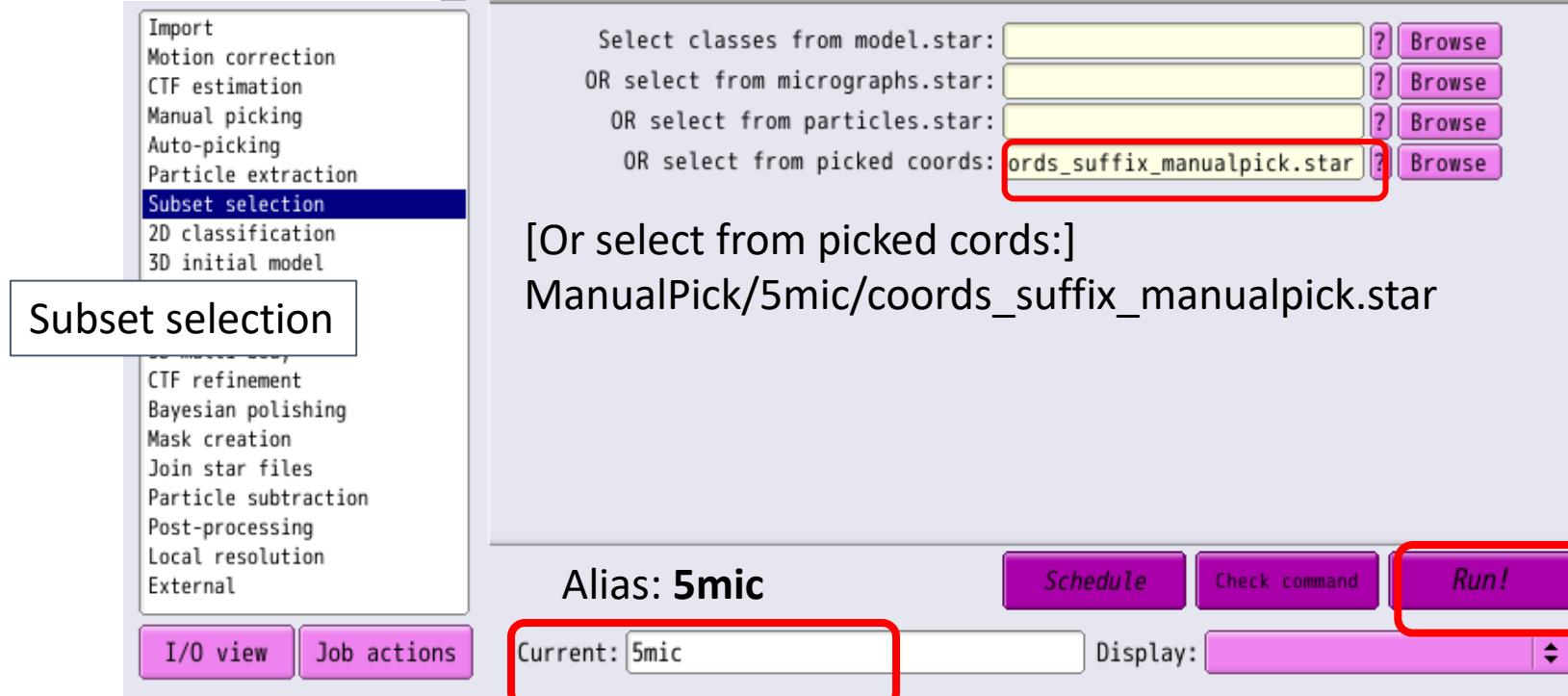
0020 : 80 particles

file	pick	CTB
data/INX6Ndelnano006.mrc	94	CTB
data/INX6Ndelnano007.mrc	87	CTB
data/INX6Ndelnano010.mrc	79	CTB
data/INX6Ndelnano019.mrc	70	CTB
data/INX6Ndelnano020.mrc	80	CTB
data/INX6Ndelnano021.mrc	0	CTB
data/INX6Ndelnano023.mrc	0	CTB
data/INX6Ndelnano028.mrc	0	CTB

Subset selection

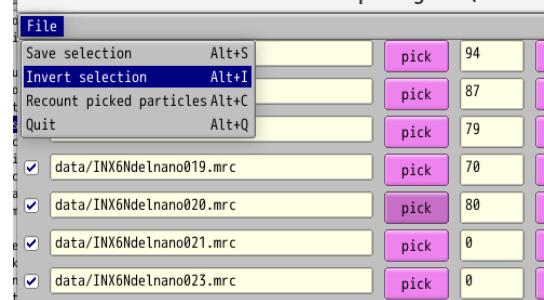
まず、最初の5枚の電顕画像だけを選択

Select "Subset selection"

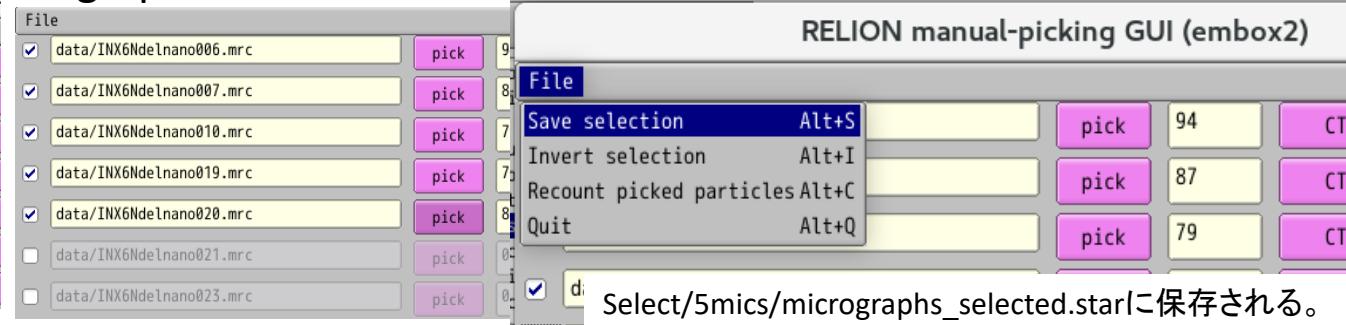


[File]⇒[Invert selection] Check for the top 5

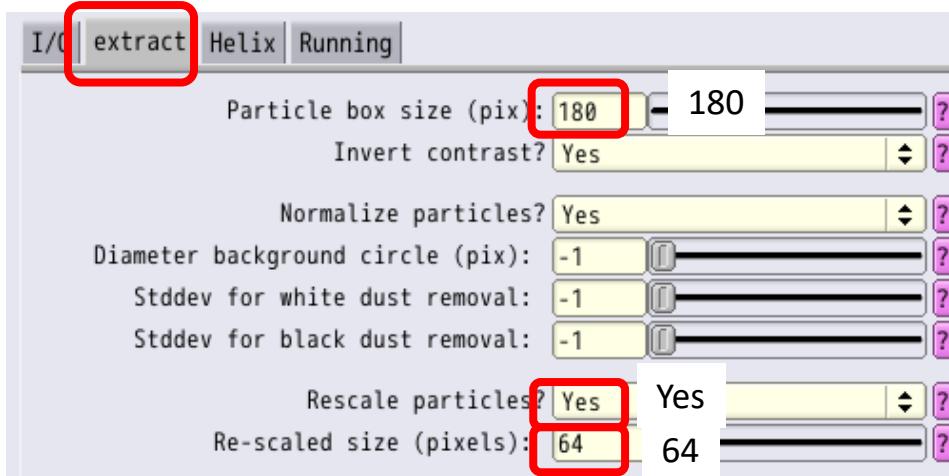
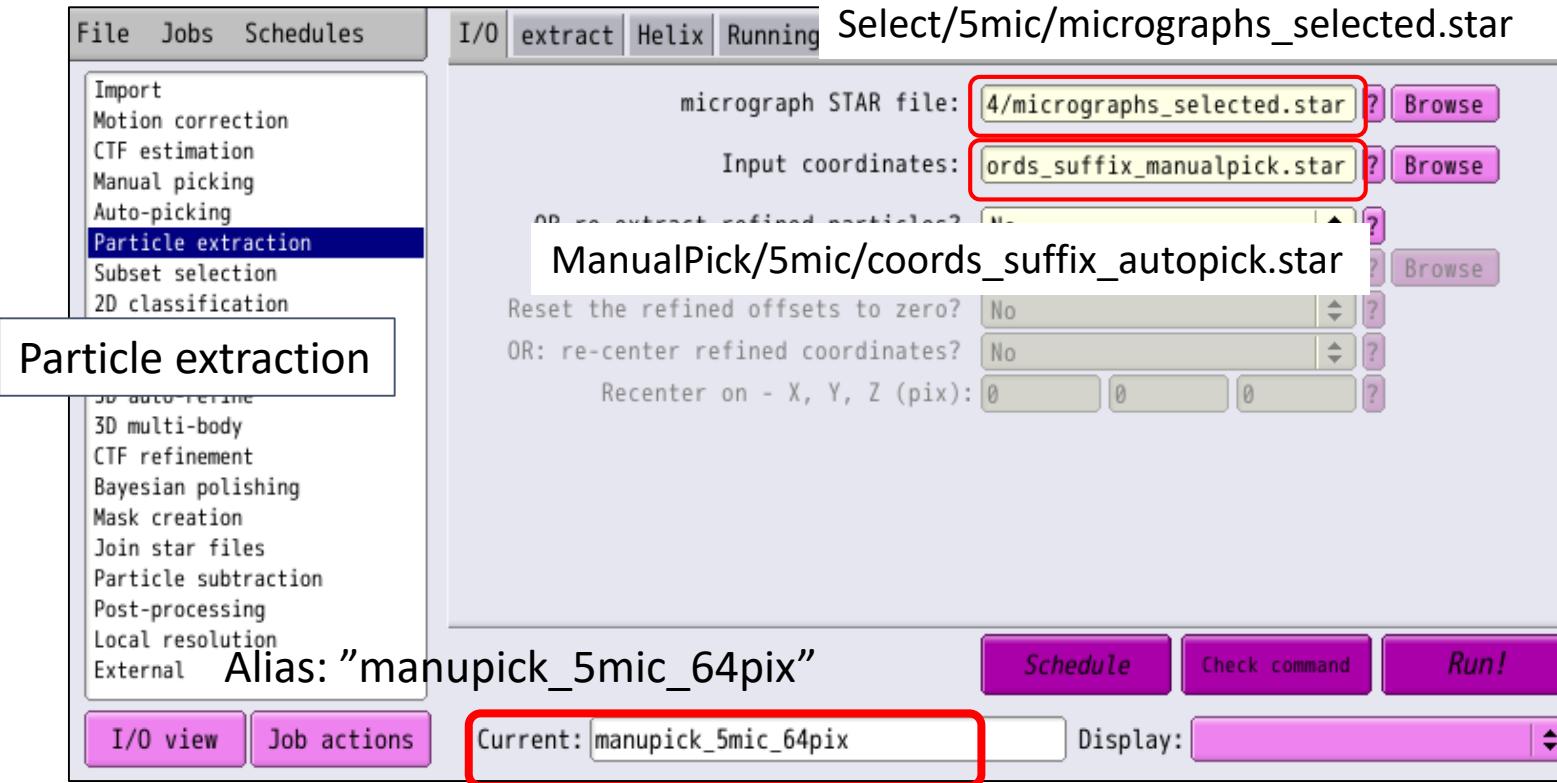
To uncheck Micrographs



[File]から、[Save selection]を選ぶ



1.6 Particle extraction



*Box size: 180 pixel \times 1.232 = 221.7 Å

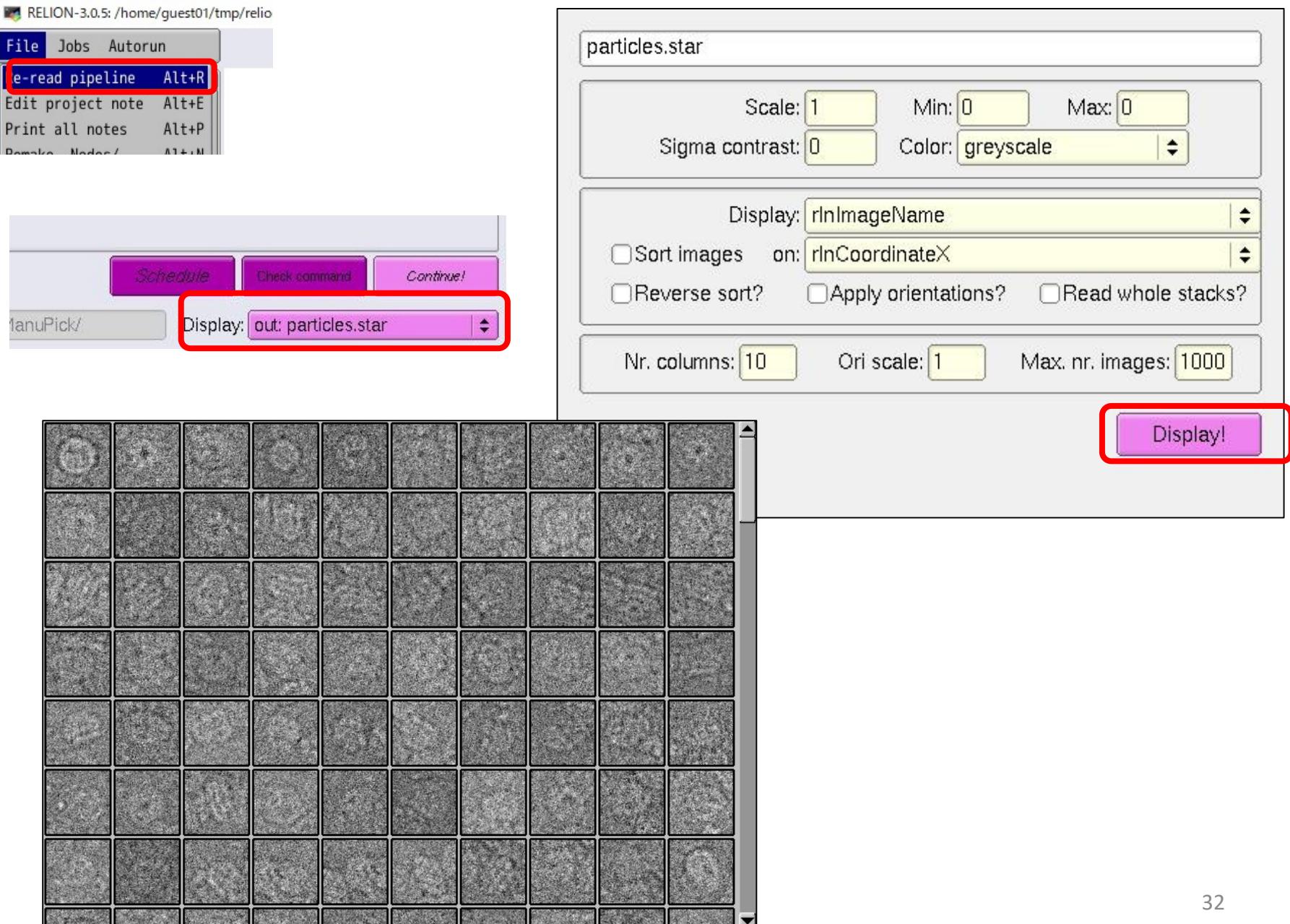
*Box size of the particle has to be even number.

*To reduce computational costs, we rescale 180 pixel into 64 pixel.

Click [Run !]

Run!

1.6 Particle extraction



1.7 2D Classification : to make templates for autopick

[1GPU, 1 min]

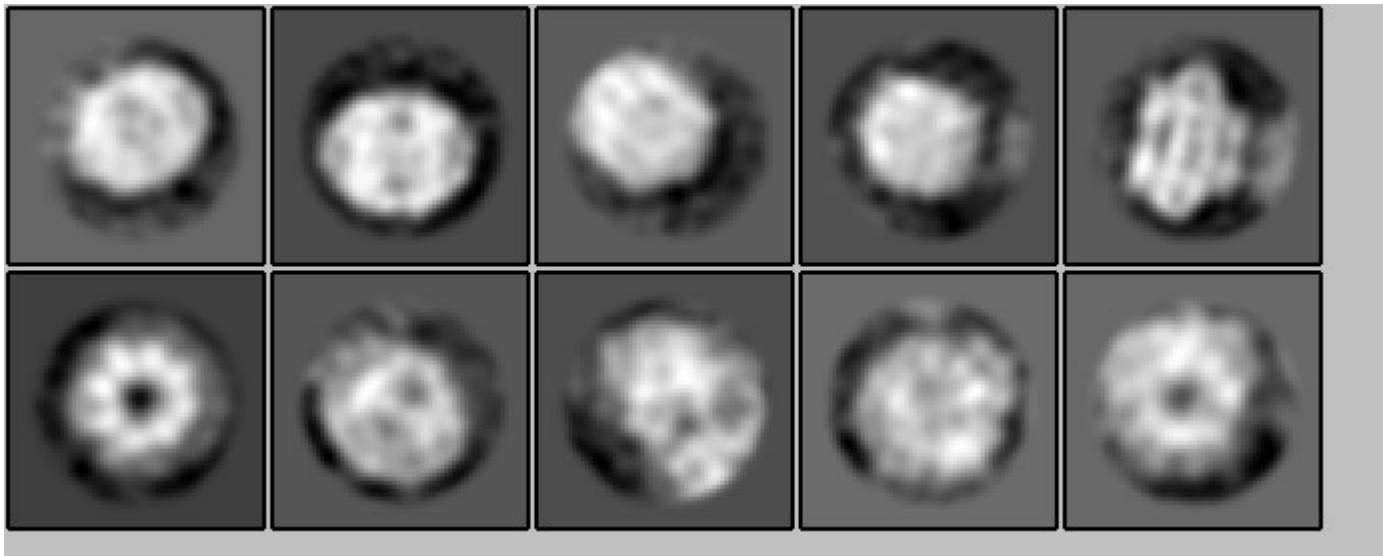
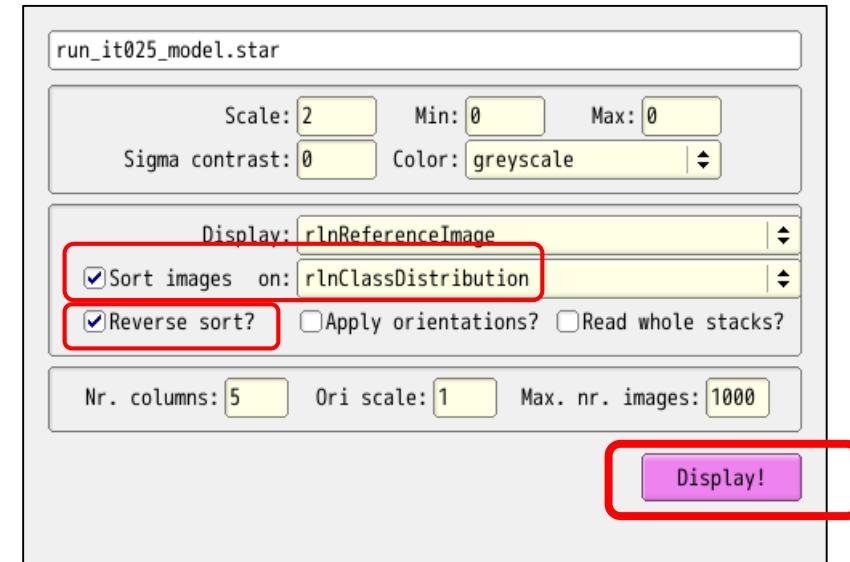
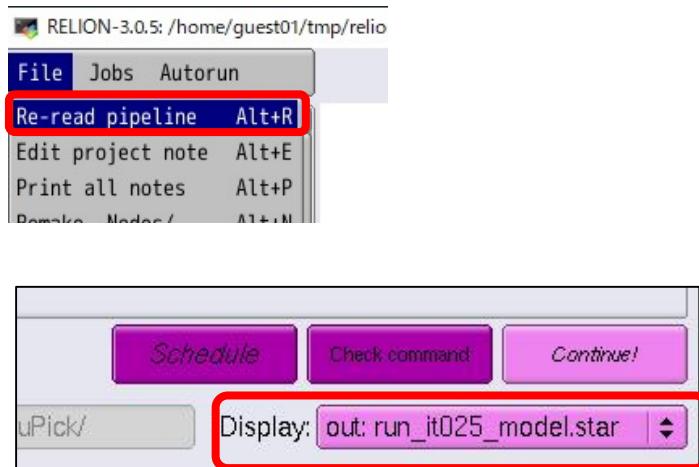
The screenshot shows the Relion software interface with the following configuration:

- File Jobs Schedules** (top left)
- I/O CTF Optimisation Sampling Helix Compute Running** (top right)
- Extract/manupick_5mic_64pix/particles.star** (Input images STAR file)
- Continue from here:** (disabled)
- Number of classes: 10** (highlighted with a red box)
- Regularisation parameter T: 2**
- Number of iterations: 25**
- Use fast subsets (for large data sets)? No**
- Mask diameter (A): 160** (highlighted with a red box)
- Mask individual particles with zeros? Yes**
- Limit resolution E-step to (A): -1**
- Schedule Check command Run!** (bottom right)
- Alias: "manupick_5mic_64pix"** (center bottom)
- Current: manupick_5mic_64pix** (center bottom)
- Display:** (center bottom)
- I/O view Job actions** (bottom left)
- Compute Running** (highlighted with a red box) (bottom left)
- Use parallel disc I/O? Yes**
- Number of pooled particles: 3**
- Pre-read all particles into RAM? No**
- Copy particles to scratch directory:**
- Combine iterations through disc? No**
- Yes GPU acceleration? Yes**
- Which GPUs to use: 0** (highlighted with a red box)
- 各受講者のGPU番号
(0,1,2,3のどれか)を入力** (Japanese note)
- I/O CTF Optimisation Sampling Helix Compute Running** (highlighted with a red box) (bottom right)
- Number of MPI procs: 1**
- Number of threads: 4** (highlighted with a red box)
- 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:**
- Run!** (bottom right)

***relion_refine**というプログラムが実行される。

1MPI, 4 threads, 1GPUでメモリ VIRT 47.6GB RES 492MB 使用。

1.7 2D Classification : to make templates for autopick



1.8 Selecting templates for auto-picking

I/O Class options Subsets Class2D/manupick_5mic_64pix/run_it025_model.star

Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection 2D classification 3D initial model

Subset selection

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

I/O view Job actions Current: manupick_5mic_64pix Display: Run!

Select classes from model.star: job006/run_it025_model.star ? Browse
OR select from micrographs.star: ? Browse
OR select from particles.star: ? Browse
OR select from picked coords: ord_ssuffix_manualpick.star ? Browse

Alias:"manupick_5mic_64pix"

Sort images on: rlnClassDistribution
Sorted by number of particles belong to the class

run_it025_model.star

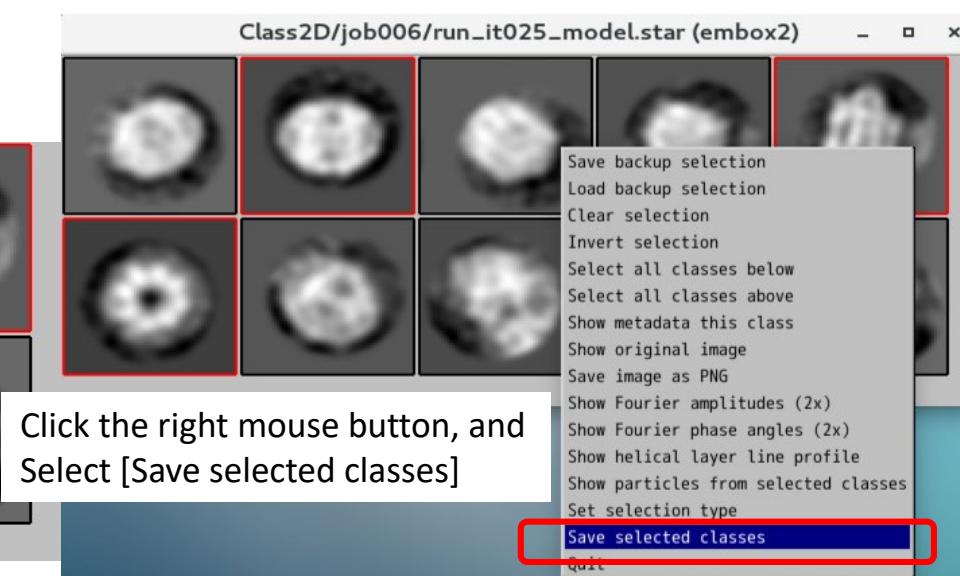
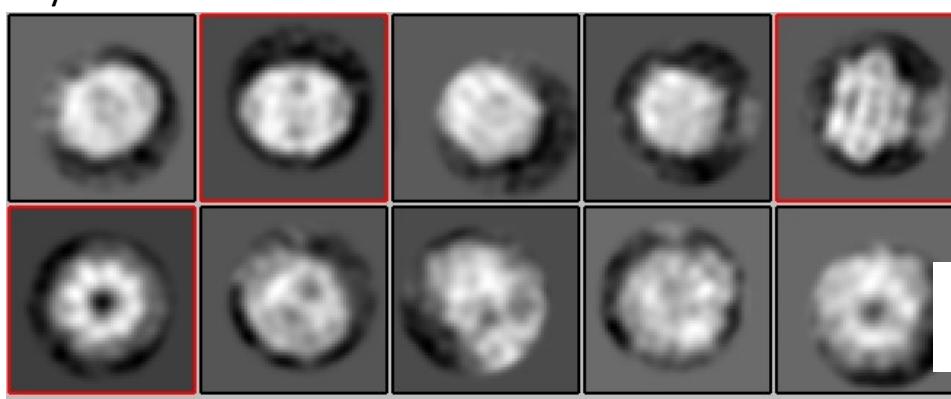
Scale: 1 Min: 0 Max: 0
Sigma contrast: 0 Color: greyscale

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

Nr. columns: 10 Ori scale: 1 Max. nr. images: 1000
Max nr selected parts per class: -1

Display

Among the 10 classes, select a few nice-looking classes, by the left button of the mouse.



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

Auto-picking;reference-based; for 5mic

Four parameters have to be optimized : 1. Picking threshold, 2. Min.inter-particle dis, 3. Max. stddev noise 4. Min. avg_noise. Repeating ref-based picking for the 5 micrographs help us to optimized them.

[1GPU, < 1min]

File Jobs Schedules I/O Laplacian Ref Select/5mic/micrographs_selected.star

Import Motion correction CTF estimation Manual picking **Auto-picking** Particle extraction Subset selection

Auto-picking

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

I/O view Job actions Current: manu_5mic_64pix Schedule Check command Run!

I/O Laplacian References **autopicking** Helix Running

Lowpass filter references (A) 20
Highpass filter (A) -1
Pixel size in references (A) 3.465
A) 160

3.465 Å
(=1.232 Å * 180 pixel /64 pixel)

Input your GPU number (0,1,2,3)

References have inverted contrast? Yes
Are References CTF corrected? Yes
Ignore CTFs until first peak? No

Picking threshold: 0.05
Minimum inter-particle distance (A): 100
Maximum stddev noise: 1.1
Minimum avg noise: -999

Write FOM maps? No
Read FOM maps? No

Shrink factor: 0
Use GPU acceleration? Yes
Which GPUs to use: 0

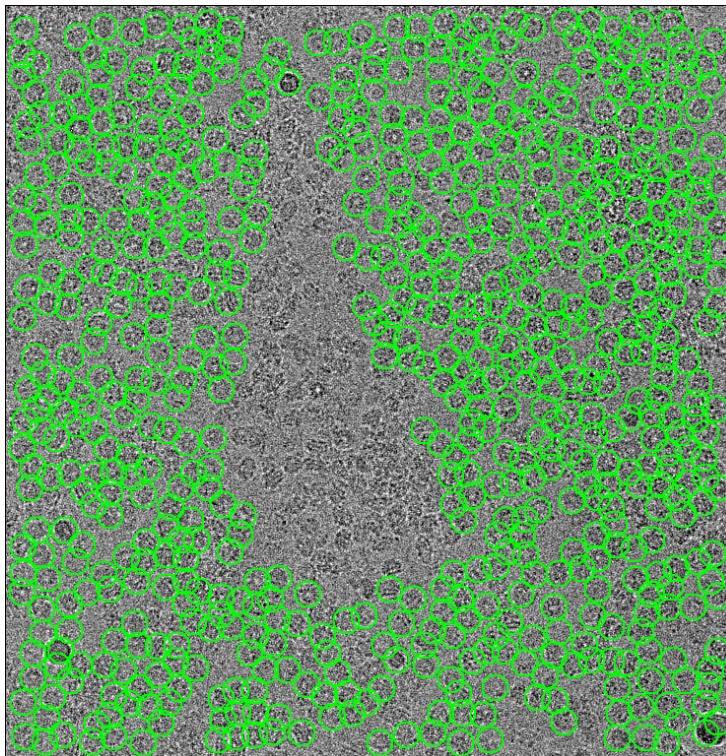
Run!

Auto-picking; reference-based; for 5mic

The screenshot shows the RELION-3.0.5 software interface. On the left, a menu bar includes 'File', 'Jobs', 'Aautorun', 'Re-read pipeline Alt+R' (highlighted with a red box), 'Edit project note Alt+E', 'Print all notes Alt+P', and 'Rescale Images Alt+M'. Below the menu are buttons for 'Schedule', 'Check command', and 'Continue!'. A 'params/' tab is selected. In the center, a table lists five micrographs with their respective particle counts and CTF values:

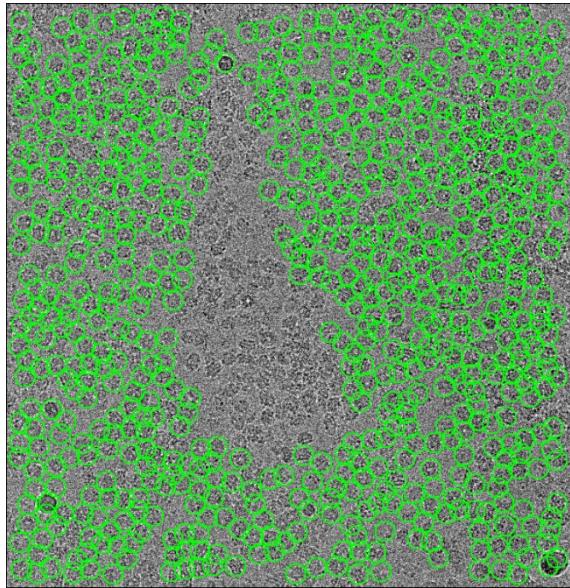
Micrograph	Pick	674	CTF	19895.3
data/INX6Ndelnano006.mrc	pick	638	CTF	19207.3
data/INX6Ndelnano007.mrc	pick	686	CTF	18995.4
data/INX6Ndelnano019.mrc	pick	655	CTF	21145.6
data/INX6Ndelnano020.mrc	pick	600	CTF	20288.3

A red box highlights the 'Display: out: coords_suffix_autopick.' dropdown menu.

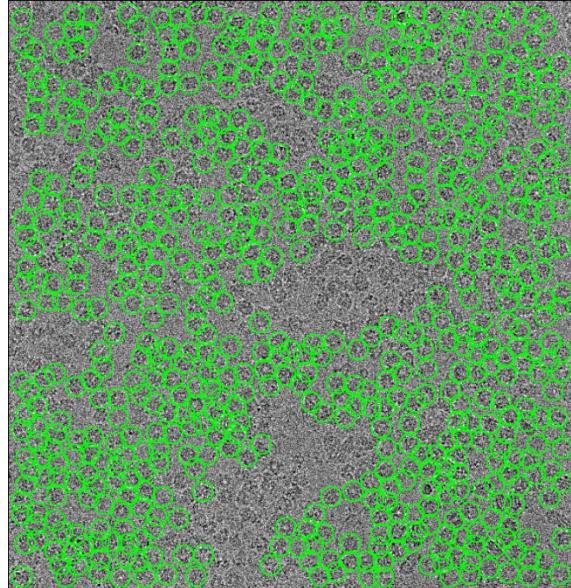


Total number of particles from 100 micrographs is 3253
i.e. on average there were 651 particles per micrograph.

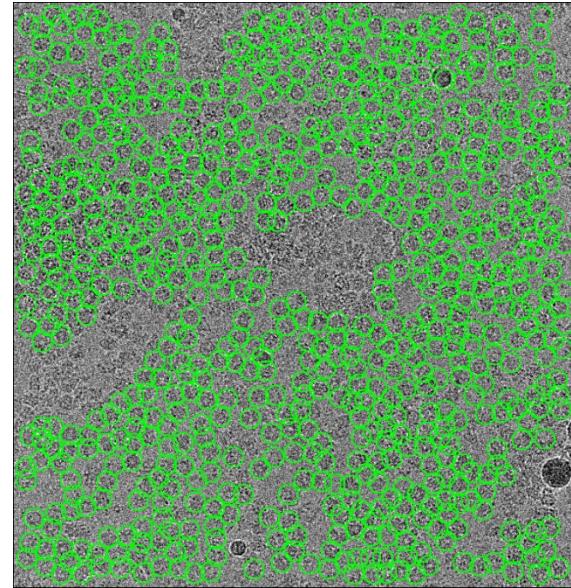
Auto Picking



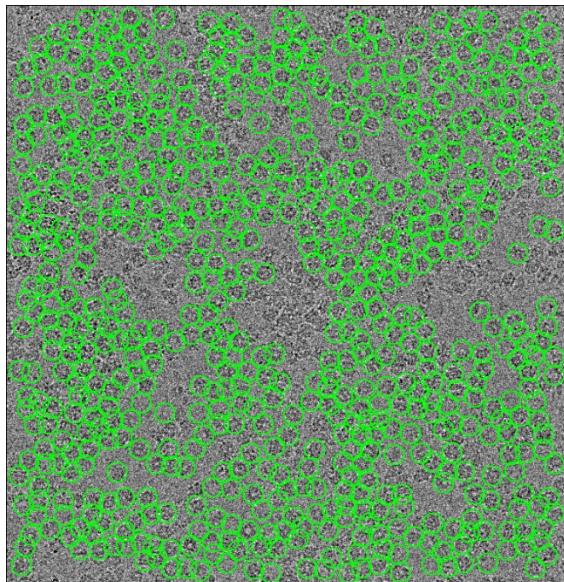
006 : 674 particles



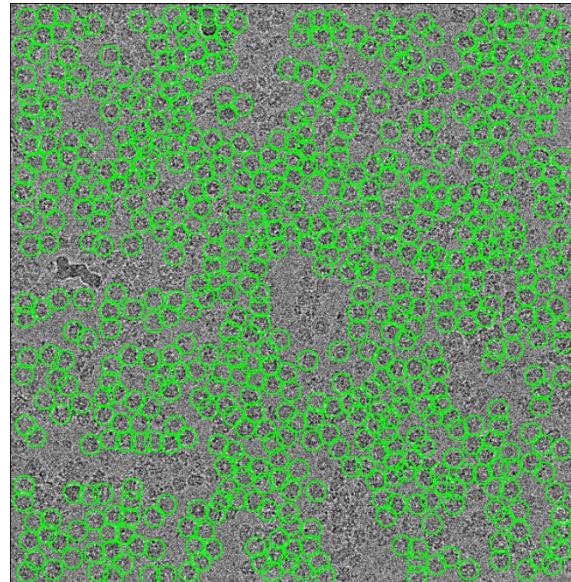
007 : 638 particles



010 : 686 particles



019 : 655 particles



020 : 600 particles

Ndelnano006.mrc	pick	674	CTF	19895.3
Ndelnano007.mrc	pick	638	CTF	19207.3
Ndelnano010.mrc	pick	686	CTF	18995.4
Ndelnano019.mrc	pick	655	CTF	21145.6
Ndelnano020.mrc	pick	600	CTF	20288.3

Auto-picking;reference-based; for 100mic

Four parameters have to be optimized : 1. Picking threshold, 2. Min.inter-particle dis, 3. Max. stddev noise 4. Min. avg_noise. Repeating ref-based picking for the 5 micrographs help us to optimized them.

[1GPU, < 1min]

File Jobs Schedules I/O Laplacian Ref CtfFind/job002/micrographs_ctf.star

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

Auto-picking

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

Input micrographs for autopick: job002/micrographs_ctf.star ? Browse

Pixel size in micrographs (A) -1

2D references: /job007/class_averages.star ? Browse

OR: provide a 3D reference? No

Select/manu_5mic_64pix/class_averages.star

3D angular sampling: 30 deg

OR: use Laplacian-of-Gaussian? No

Use the optimized four parameters.

Alias:"manu_100mic_64pix" Schedule Check command Run!

I/O view Job actions Current: manu_100mic_64pix

I/O Laplacian References autopicking Helix Running

I/O References autopicking Helix Running

Lowpass filter references (A) 20

Highpass filter (A) -1

Pixel size in references (A) 3.465

A) 160

3.465 Å
(=1.232 Å * 180 pixel /64 pixel)

Input your GPU number (0,1,2,3)

References have inverted contrast? Yes

Are References CTF corrected? Yes

Ignore CTFs until first peak? No

Picking threshold: 0.05

Minimum inter-particle distance (A): 100

Maximum stddev noise: 1.1

Minimum avg noise: -999

Write FOM maps? No

Read FOM maps? No

Shrink factor: 0

Use GPU acceleration? Yes

Which GPUs to use: 0

Run!

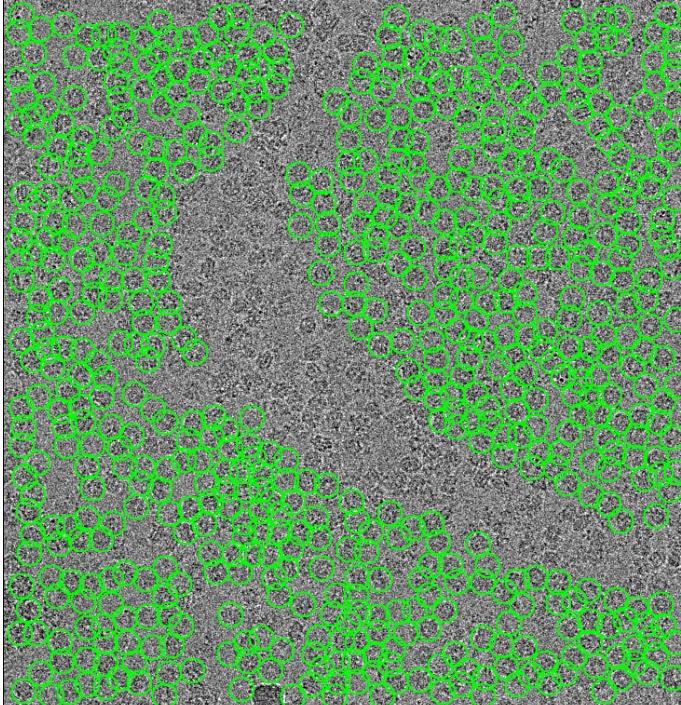
Auto-picking; reference-based; for 100mic

RELIION-3.0.5: /home/guest01/tmp/relio

File Jobs Autorun
Re-read pipeline Alt+R
Edit project note Alt+E
Print all notes Alt+P
Remove Nodes Alt+U

Schedule Check command Continue!

Display: out: coords_suffix_autopick.



File					
✓ data/INX6Ndelnano006.mrc	pick	674	CTF	19895.3	
✓ data/INX6Ndelnano007.mrc	pick	638	CTF	19207.3	
✓ data/INX6Ndelnano010.mrc	pick	686	CTF	18995.4	
✓ data/INX6Ndelnano019.mrc	pick	655	CTF	21145.6	
✓ data/INX6Ndelnano020.mrc	pick	600	CTF	20288.3	
✓ data/INX6Ndelnano021.mrc	pick	711	CTF	19941.8	
✓ data/INX6Ndelnano023.mrc	pick	655	CTF	20295.9	
✓ data/INX6Ndelnano028.mrc	pick	659	CTF	18192.1	
File					
✓ data/INX6Ndelnano248.mrc	pick	487	CTF	21312.2	
✓ data/INX6Ndelnano249.mrc	pick	541	CTF	20736.9	
✓ data/INX6Ndelnano261.mrc	pick	617	CTF	21737.5	
✓ data/INX6Ndelnano272.mrc	pick	587	CTF	22592.9	
✓ data/INX6Ndelnano275.mrc	pick	504	CTF	23313.2	
✓ data/INX6Ndelnano282.mrc	pick	512	CTF	19622.1	
✓ data/INX6Ndelnano283.mrc	pick	512	CTF	20057	
✓ data/INX6Ndelnano296.mrc	pick	625	CTF	18982	

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

1.9 Particle extraction

CtfFind/job002/micrographs_ctf.star

I/O extract Helix Running

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

micrograph STAR file job002/micrographs_ctf.star ? Browse

Input coordinates coords_suffix_autopick.star ? Browse

OR re-extract refined particles? No

Refined particles STAR file: ? Browse

Reset the refinement parameters

OR: re-center particles? Autopick/manu_100mic_64pix/coords_suffix_autopick.star

Recenter on - X, Y, Z (pix): 0 0 0 ?

Particle extraction

3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External Alias "refauto_manu_100mic_64pix"

I/O view Job actions Current: refauto_manu_100mic_64pix Disp

I/O extract Helix Running

Particle box size (pix) 180

Invert contrast? Yes

Normalize particles? Yes

Diameter background circle (pix): -1

Stddev for white dust removal: -1

Stddev for black dust removal: -1

Rescale particles? Yes

Re-scaled size (pixels): 64

Run!

Schedule Check command Continue!

Display: out: particles.star

60292 particles are stored in:
Extract/template_based/particles.starに保存

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Appendix: Motion Correction

Import movie/*.mrc

Launch “relion” in the directory “EMPIAR-10291_5movie/”.

Job type browser

The screenshot shows the Relion Job Type Browser interface. The top navigation bar has tabs: File, Jobs, Schedules, Movies/mics (which is selected), Others, and Running. A red dashed box highlights the "Jobs" tab. The left sidebar lists various processing steps under the "Import" category, with "Import" also highlighted by a red box. The main configuration area shows the following settings:

- Import raw movies/micrographs? Yes
- Raw input files: "movie/*.mrc" (highlighted by a red box)
- Are these multi-frame movies? Yes
- Optics: /usr/local/relion-3.1/data/mtf_k2_300kV.star
- MTF of the detector: /usr/local/relion-3.1/data/mtf_k2_300kV.star (highlighted by a red box)
- Pixel size (Angstrom): 1.232 (highlighted by a red box)
- Voltage (kV): 300 (highlighted by a red box)
- Spherical aberration (mm): 1.6 (highlighted by a red box)
- Amplitude contrast: 0.1
- Beamtilt in X (mrad): 0
- Beamtilt in Y (mrad): 0

A note in the center states: "※Pixel size **0.495 Å** is described in EMPIAR header file: 10291.xml." Below it, another note says: "A value of spherical aberration 1.6 was obtained by personal communication with Prof. Oshima."

At the bottom, there are buttons for Schedule, Check command, Run! (highlighted by a red box), I/O view, Job actions, Current: Give_alias_here, and Display:.

Click [Run!]

Motion Correction

No GPU, 2min for 5 movies

File Jobs Schedules

I/O Motion Running

Import/job001/movies.star

- 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

Motion correction

Input movies STAR file: Import/job001/movies.star

? Browse

First frame for corrected sum: 1

?

Last frame for corrected sum: -1

?

Dose per frame (e/A²): 0.2

?

Pre-exposure (e/A²): 0

?

Do dose-weighting? Yes

?

Save non-dose weighted as well? No

?

Save sum of power spectra? No

?

Sum power spectra every e/A²: 4

?

A value of “Dose per frame (e/A²)” 0.2 was obtained by personal communication with Prof. Oshima.

Schedule

Check command

Run!

I/O view

Job actions

Current: Give_alias_here

I/O Motion Running

Schedule

Check command

Run!

I/O Motion Running

Batch: 450
Number of patches X, Y: 5 5 5

Number of MPI procs: 1 1
Number of threads: 6 6

Submit to queue? No

Queue name: openmpi

Queue submit command: qsub

Standard submission script: /usr/local/reliion-3.1/scripts/qsub.csh

Browse

Minimum dedicated cores per node: 24

44

Additional arguments:

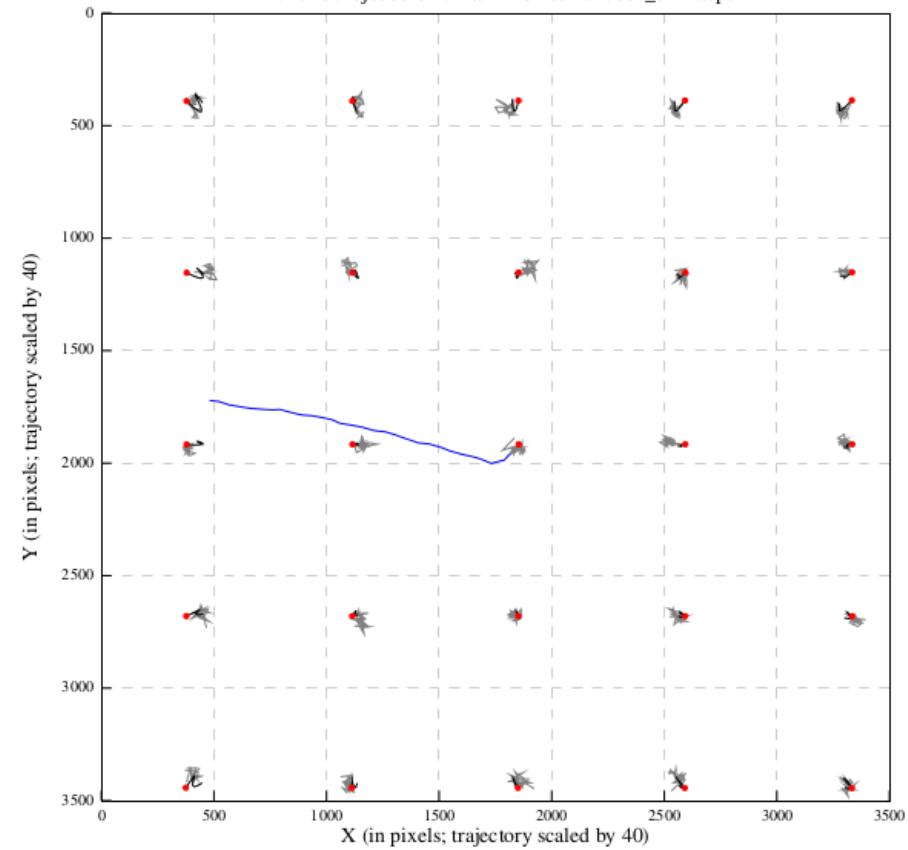
Use RELION's own implementation? Yes

Click [Run!]

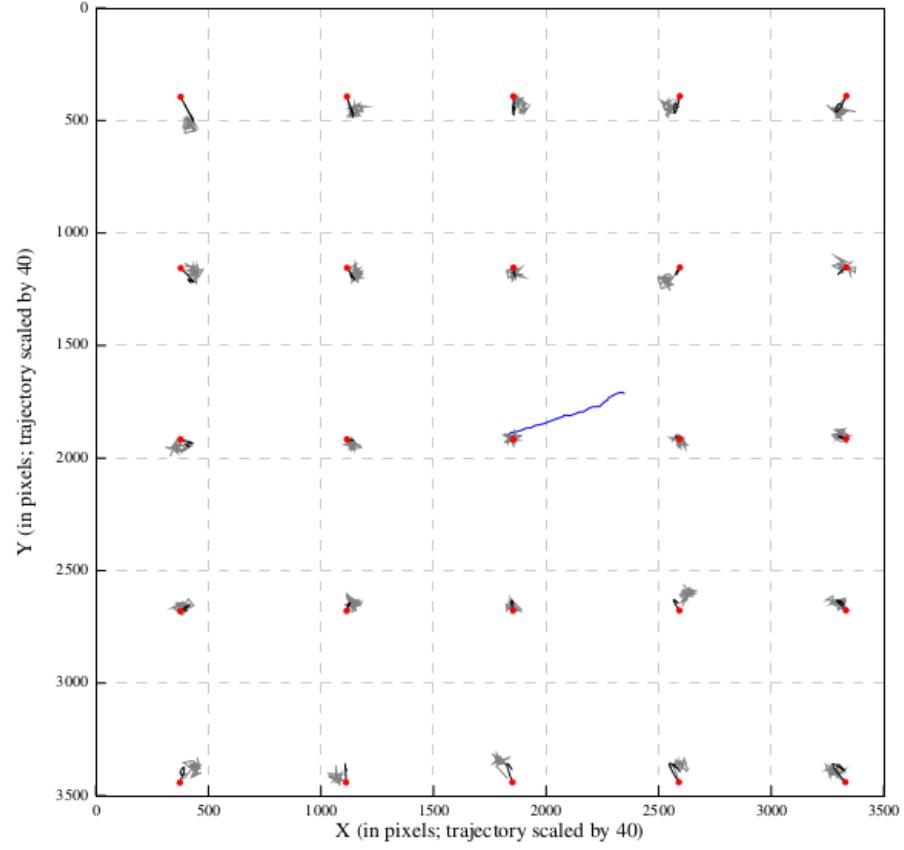
MOTIONCOR2 executable: inCor2-1.1.0/MotionCor2_1.1.0-Cuda80

Which GPUs to use: 0

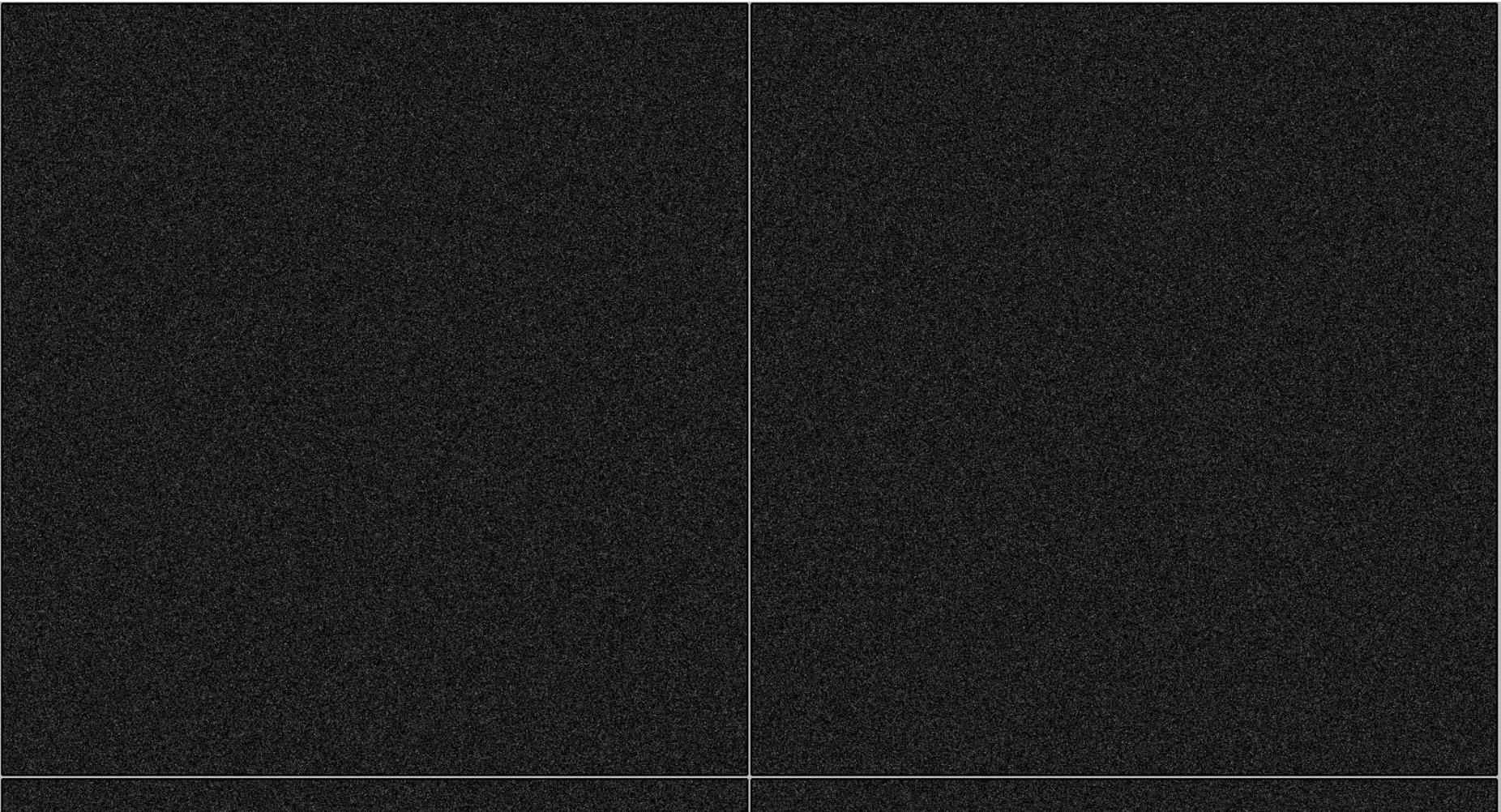
MotionCorr/job002/movie/INX6NdeInano006_shifts.eps



MotionCorr/job002/movie/INX6NdeInano007_shifts.eps

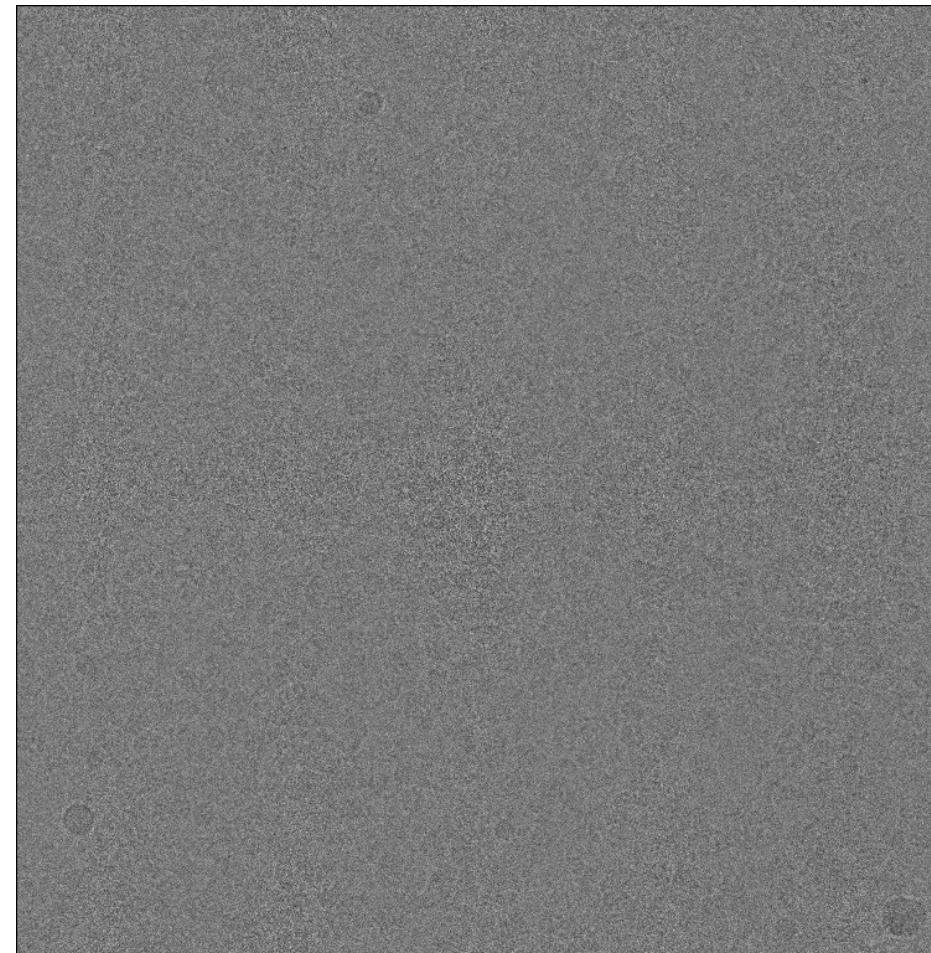


movie/INX6Ndelnano006.mrc

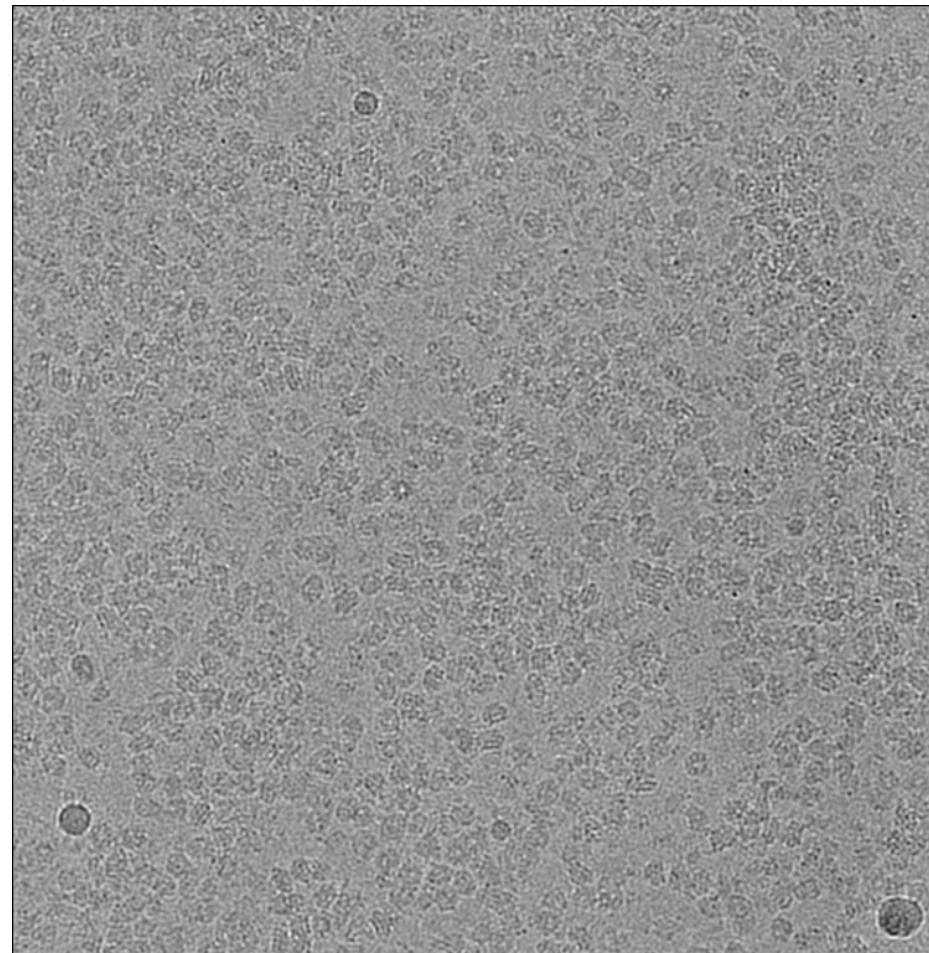


First two frames among 30 frames

MotionCorr/job002/movie/INX6Ndelnano006.mrc



Motion Corrected micrograph

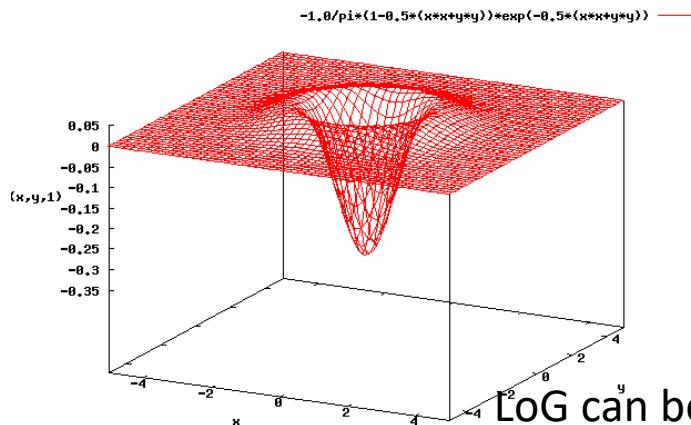


20 low pass filtered

Appendix: LoG-based autopicking

“Blob” detection using LoG filter

LoG : Laplacian of Gaussian



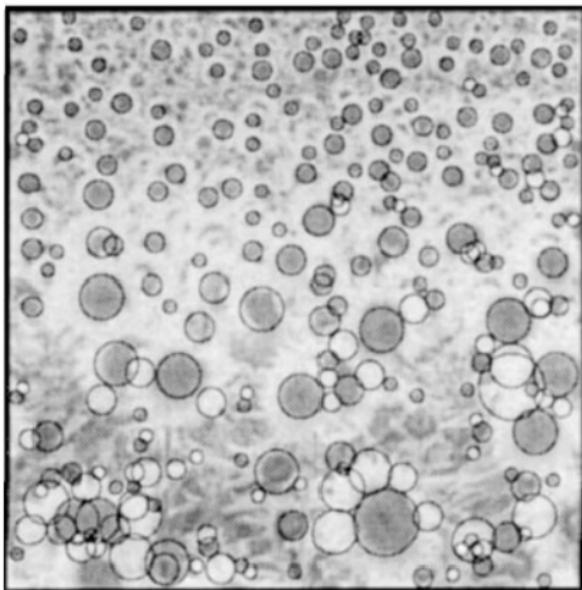
$$\phi(\mathbf{r}) = \frac{1}{2\pi\sigma^2} \exp\left[-\frac{\mathbf{r}^2}{2\sigma^2}\right]$$
$$\text{LoG}(\mathbf{r}) = \frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} = -\frac{2}{\sigma^2} \left(1 - \frac{x^2 + y^2}{2\sigma^2}\right) \phi(\mathbf{r})$$

[Big Gauss] – [normal Gauss]

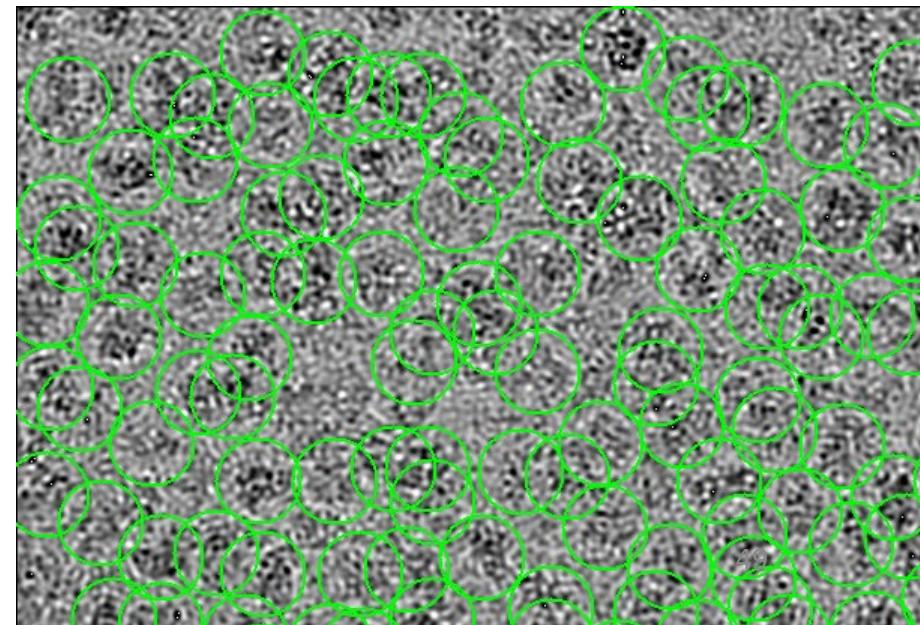
$$\text{LoG}(\mathbf{r}) \cong \text{DoG}(\mathbf{r}) = \phi(\mathbf{r}|k\sigma) - \phi(\mathbf{r}|\sigma)$$

LoG can be approximated as DoG (Difference of Gaussians)

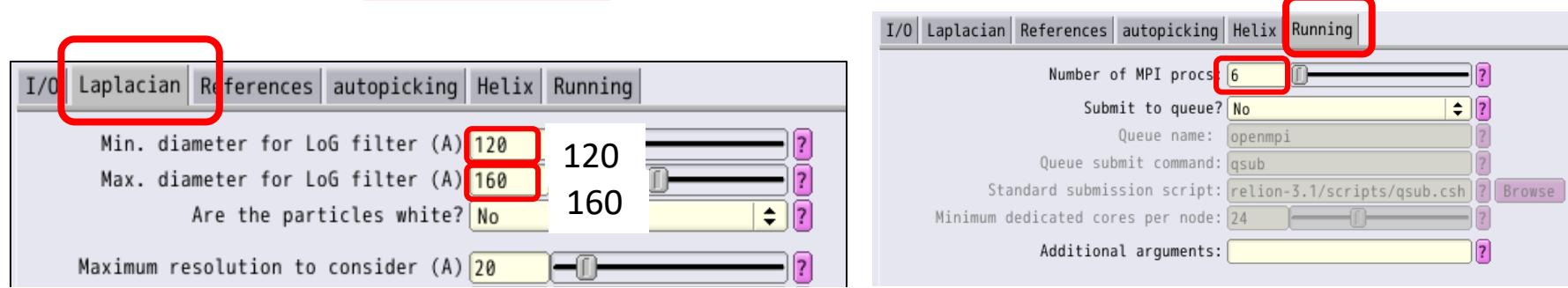
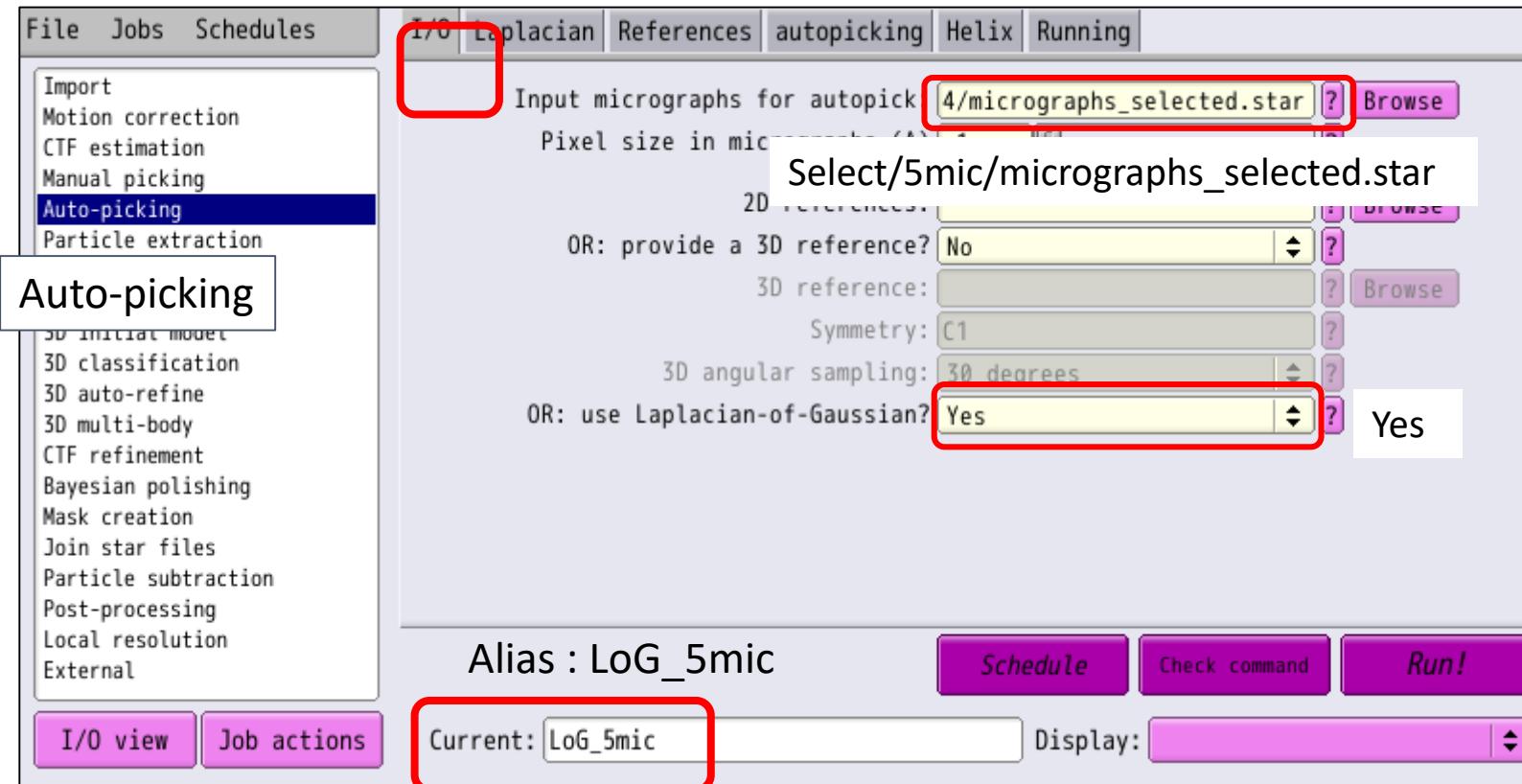
T. Lindeberg (1998). Feature detection with automatic scale selection. Int. J. Computer Vision. 30 (2): 77–116



CryoEM particle picking by Relion 3.0



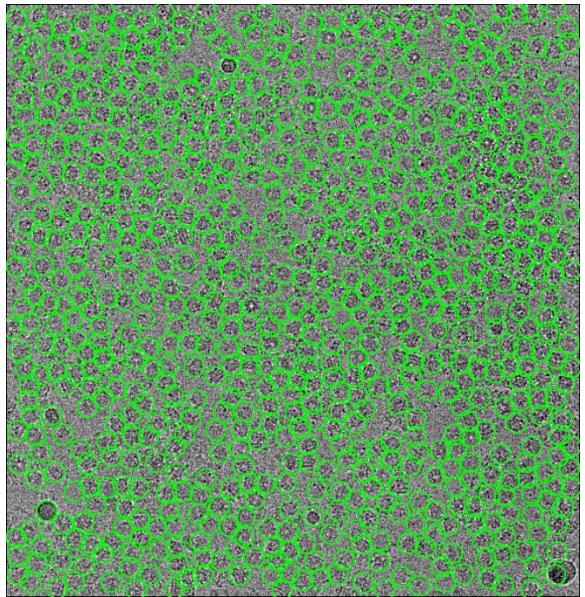
1.5 LoG-based autopicking (1分以下)



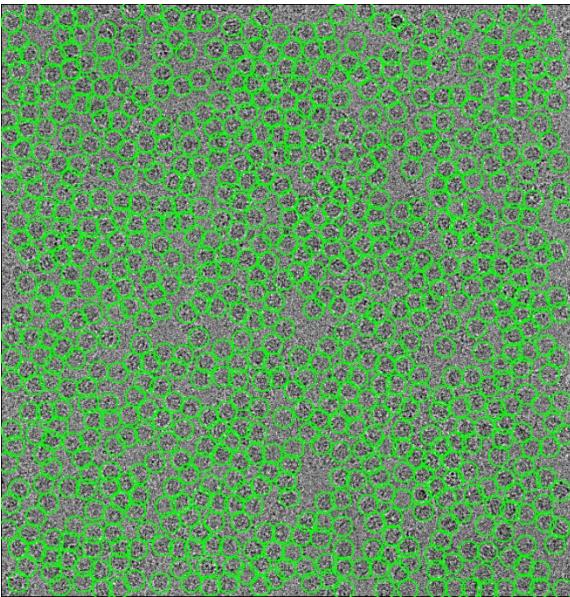
※dmin,dmaxをユーザーが指定。それらをもとに全部で $4+3+4=11$ 通りのdを試す。
dmin/5, dmin/4, dmin/3, dmin/2, dmin, (dmin+dmax)/2, dmax, 2dmax, 3dmax,
4dmax, 5dmax

Run!

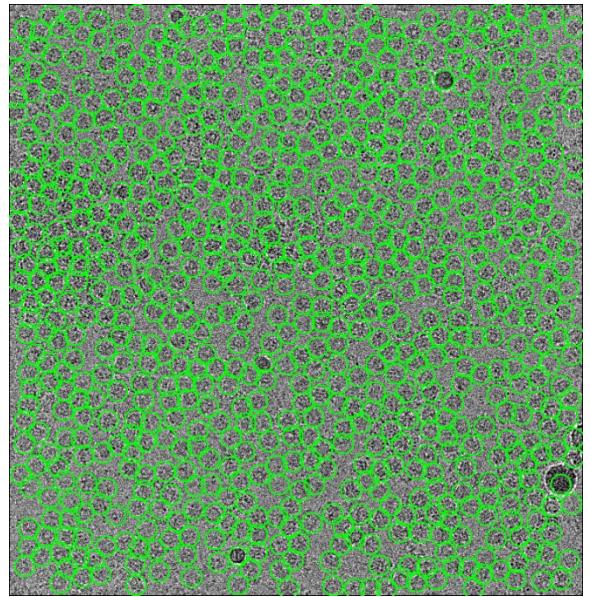
LoGbased Auto Picking



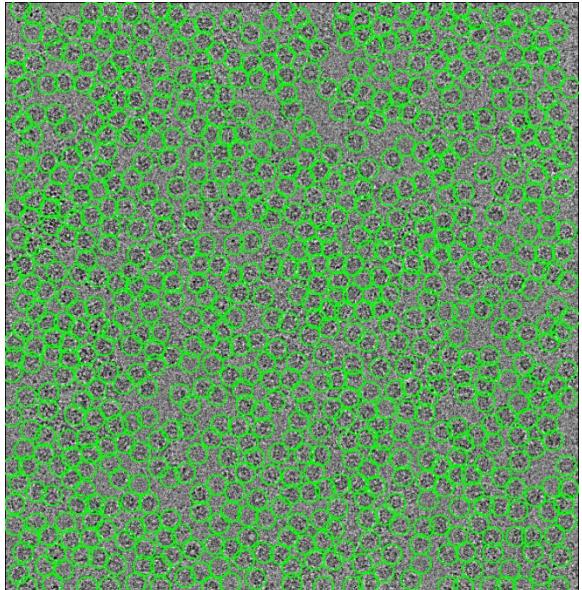
006 : 726 particles



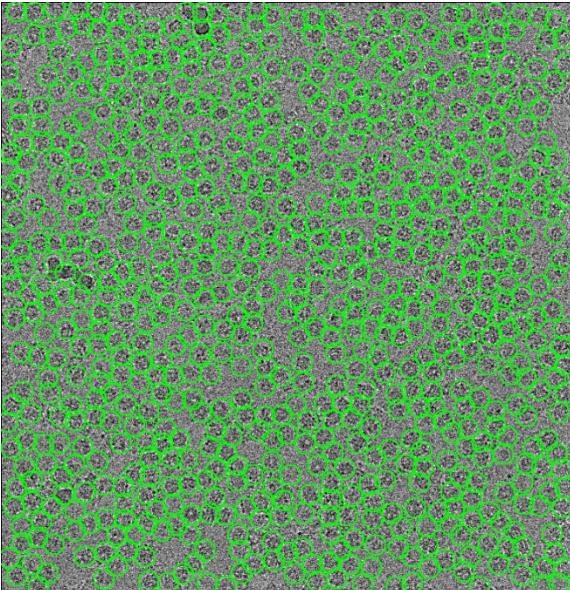
007 : 726 particles



0010 : 733 particles



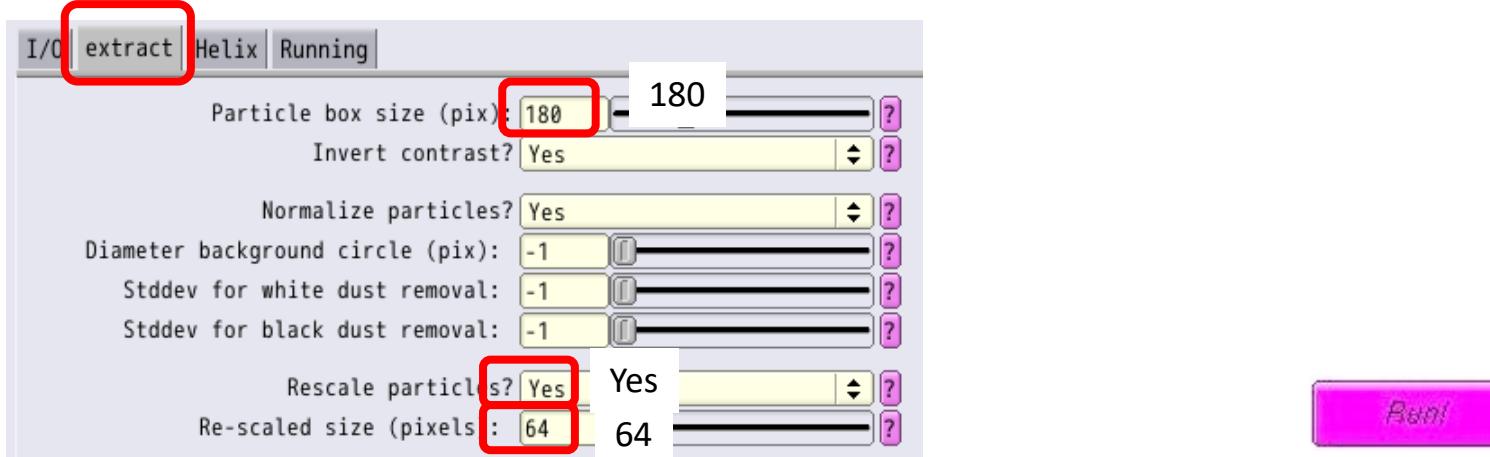
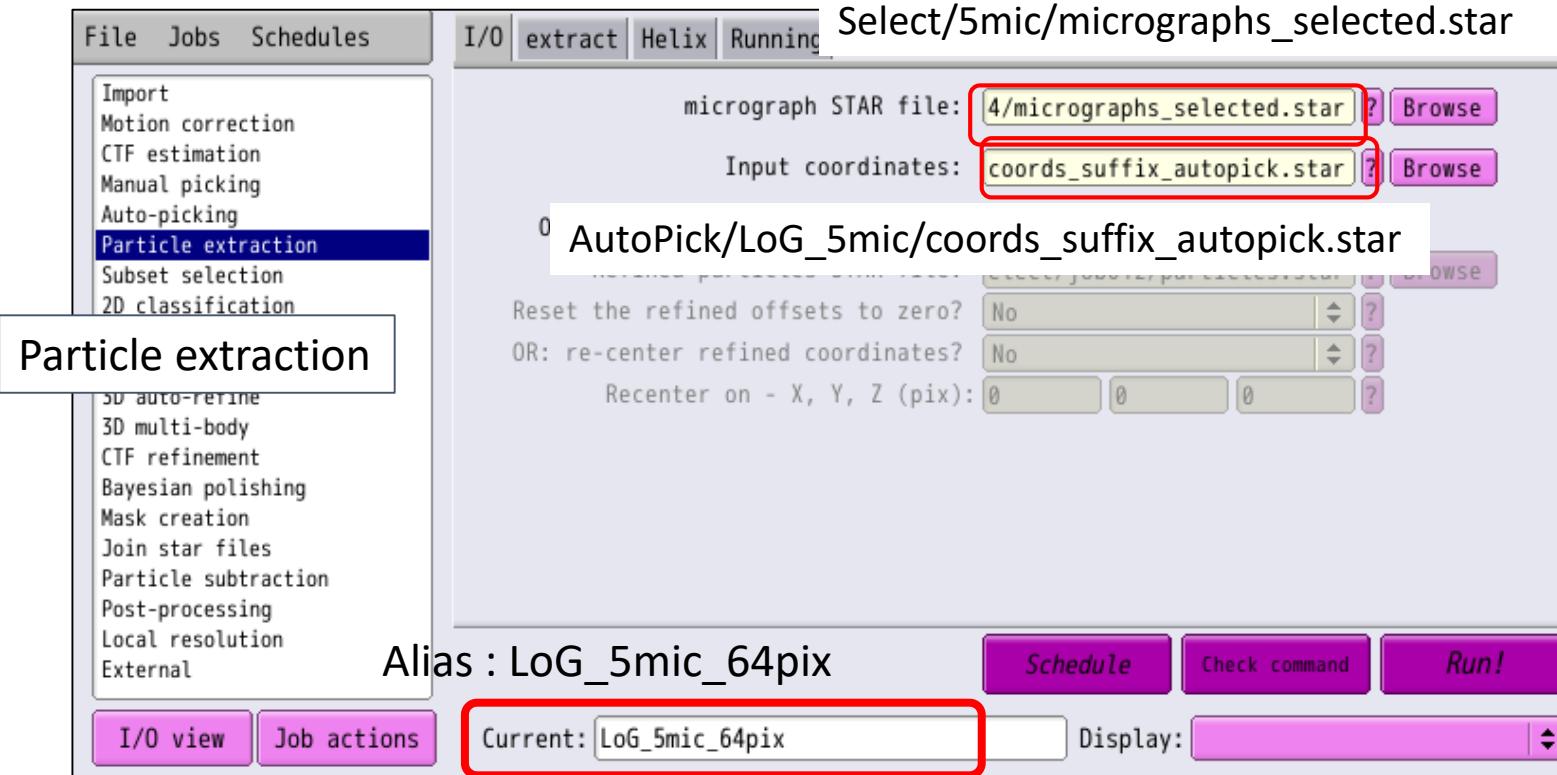
0019 : 713 particles



0020 : 708 particles

a/INX6Ndelnano006.mrc	pick	726	CTF	19895.3
a/INX6Ndelnano007.mrc	pick	726	CTF	19207.3
a/INX6Ndelnano010.mrc	pick	733	CTF	18995.4
a/INX6Ndelnano019.mrc	pick	715	CTF	21145.6
a/INX6Ndelnano020.mrc	pick	708	CTF	20288.3

1.6 Particle extraction



1.6 Particle extraction

RELIION-3.0.5: /home/guest01/tmp/relio

File Jobs Autorun
Re-read pipeline Alt+R
Edit project note Alt+E
Print all notes Alt+P
Remove Nodes / Alt+M

Schedule Check command Continue!

ianuPick/ Display: out: particles.star ▾

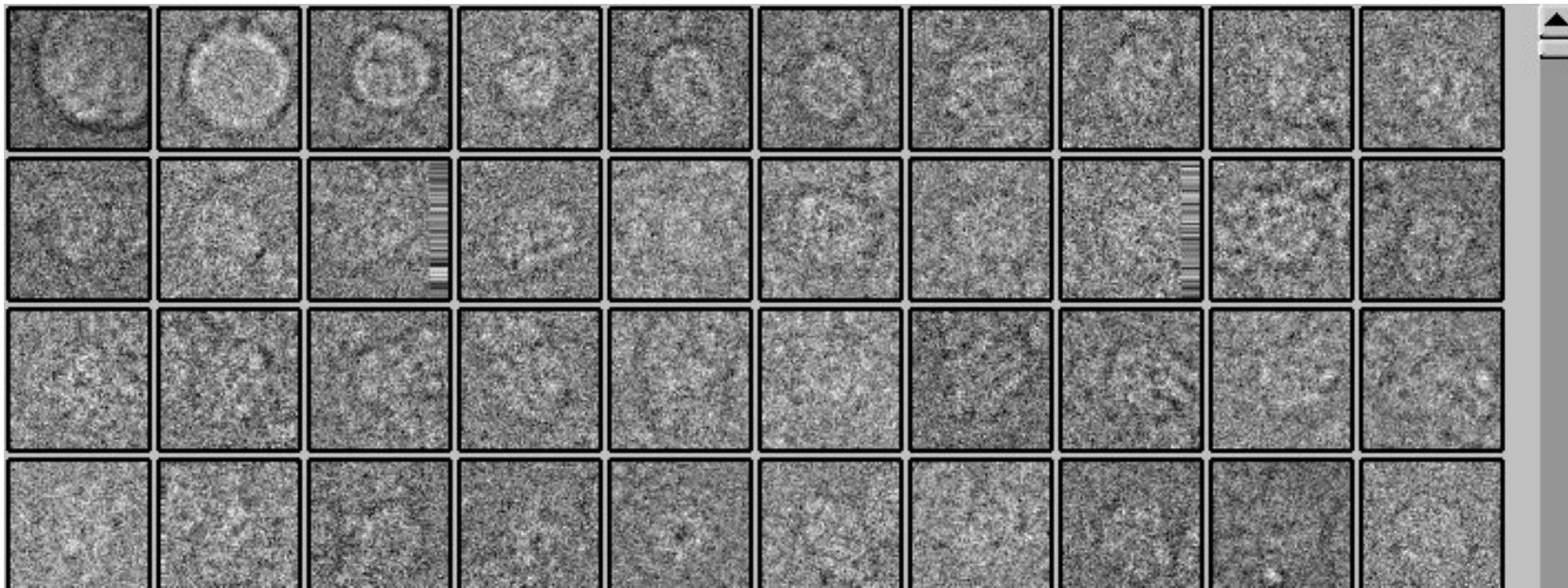
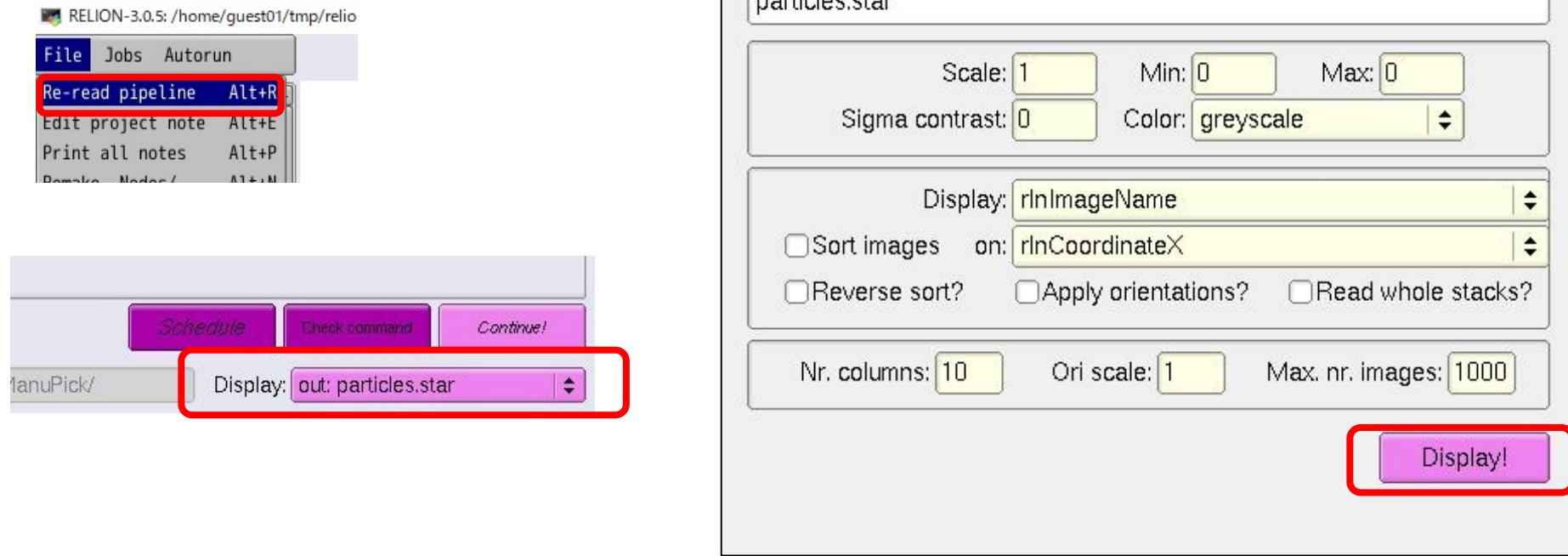
particles.star

Scale: 1 Min: 0 Max: 0
Sigma contrast: 0 Color: greyscale ▾

Display: rlnImageName ▾
 Sort images on: rlnCoordinateX ▾
 Reverse sort? Apply orientations? Read whole stacks?

Nr. columns: 10 Ori scale: 1 Max. nr. images: 1000

Display!



1.7 Making templates for autopicking (1分)

[2 GPU;]

The screenshot shows the Relion software interface for 2D classification. The main window title is "Extract/LOG_5mic_64pix/particles.star". The left sidebar has a menu with "2D classification" highlighted. The main panel shows the input STAR file path "tract/job023/particles.star" and the "Optimisation" tab selected. The "Number of classes" is set to 10, and the "Mask diameter (A)" is set to 160. The alias "Alias : LoG_5mic_64pix" is displayed at the bottom. A red box highlights the "Optimisation" tab and the "Number of classes" field.

The screenshot shows the "Compute" tab settings. It includes options like "Use parallel disc I/O?", "Number of pooled particles" (set to 3), "Pre-read all particles into RAM?", "Copy particles to scratch directory?", "Combine iterations through disc?", "Use GPU acceleration?" (set to Yes), and "Which GPUs to use" (set to 0:1). A red box highlights the "Compute" tab and the "Use GPU acceleration?" field.

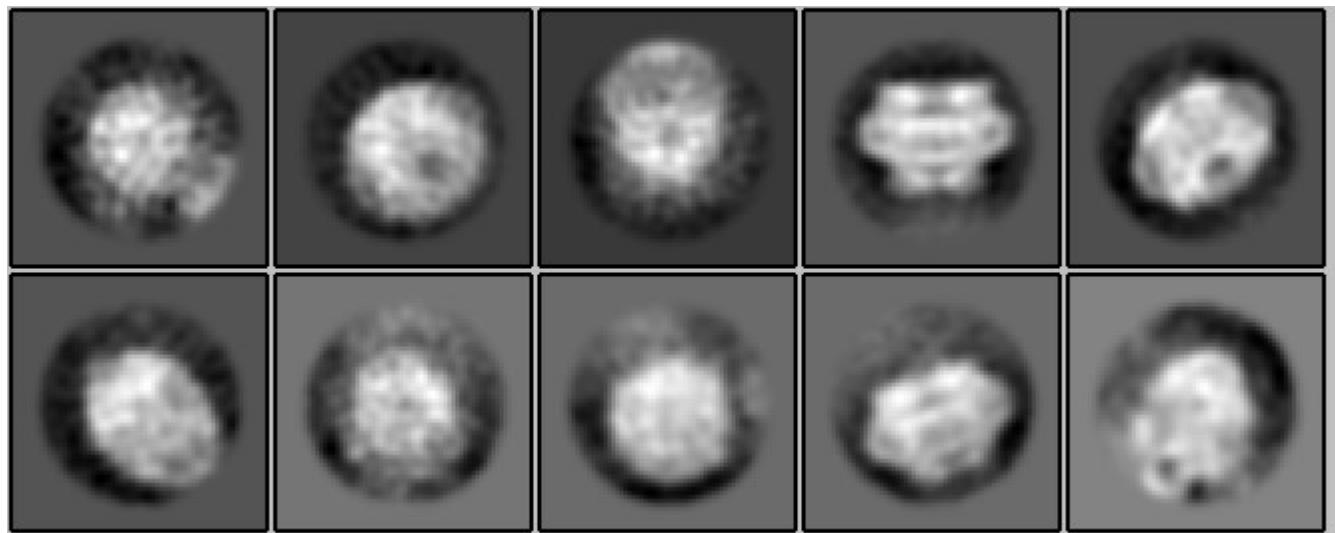
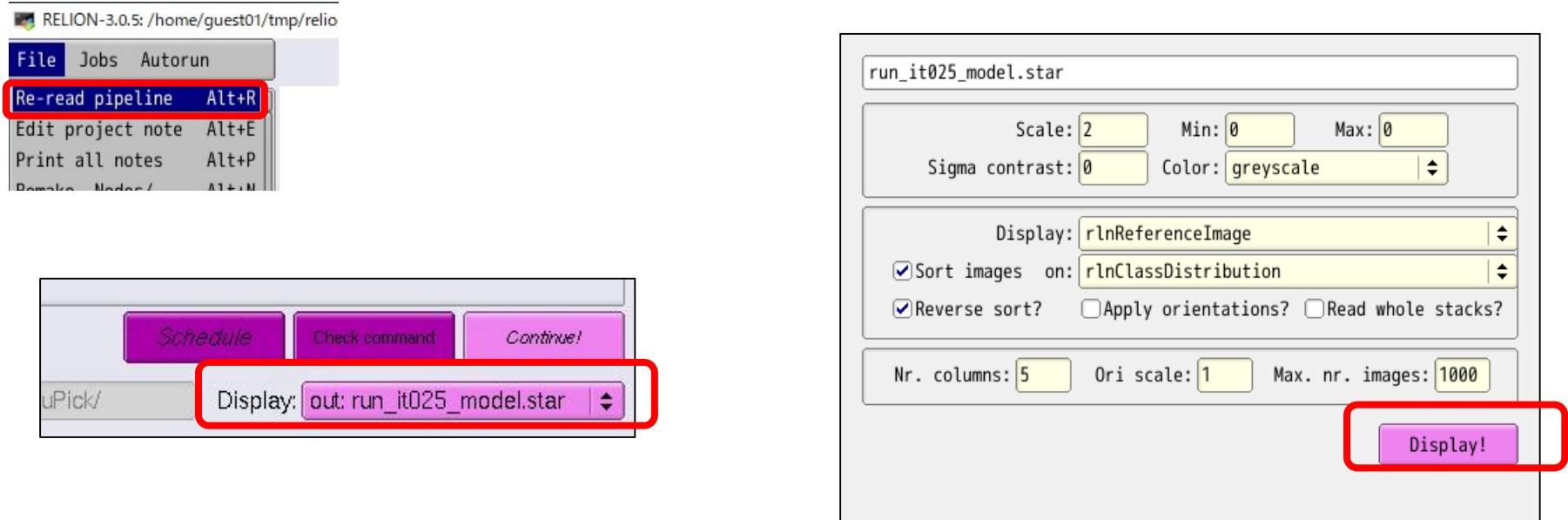
Input your GPU numbers.
Let's use 2 GPUs !

The screenshot shows the "Running" tab settings. It includes options like "Number of MPI procs" (set to 3), "Number of threads" (set to 12), "Submit to queue?", "Queue name" (set to openmpi), "Queue submit command" (set to qsub), "Standard submission script" (set to relion-3.1/scripts/qsub.csh), "Minimum dedicated cores per node" (set to 24), and "Additional arguments". A red box highlights the "Running" tab and the "Number of MPI procs" field.

Run!

54

1.7 Making templates for autopicking



No C8 top view....