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

CiCLE
Cyclic Innovation for Clinical Empowerment

Tutorial of Relion 3.1 : Innexin-6 pre-processing

Innexin-6を題材とした実習

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2020/09/02

大阪大学

INSTITUTE for PROTEIN RESEARCH

Relionに必要なハードウエア・ソフトウエア

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 of 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。

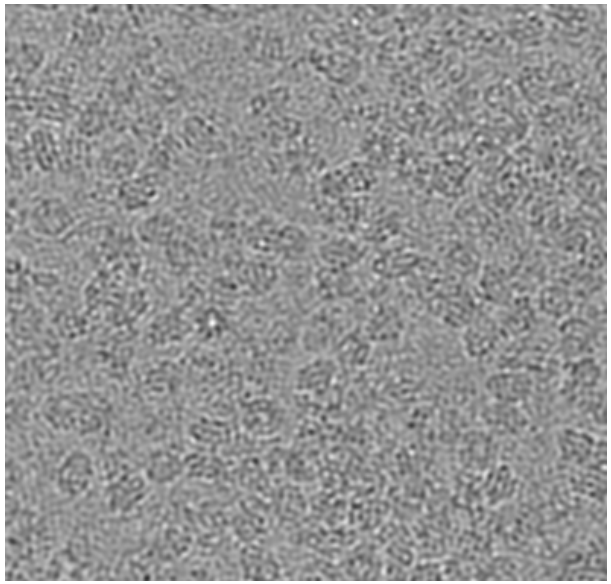
・事前にインストールが必要なライブラリ
　・CUDA (GPU計算のためのライブラリ)
　・openmpi (ジョブ並列用のライブラリ)

・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)

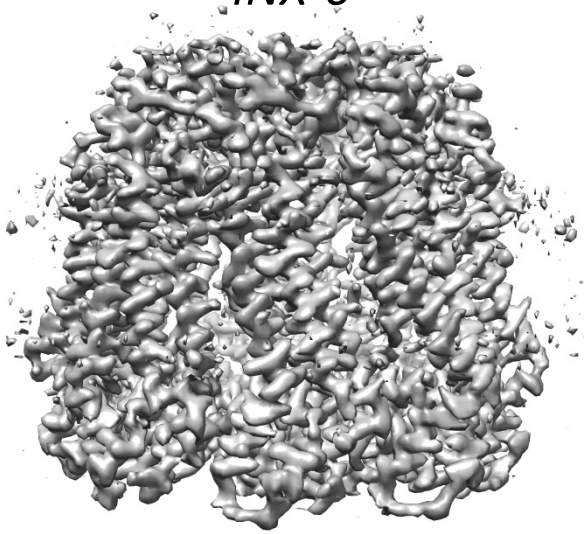
NVIDIA GeForce 1080 Ti

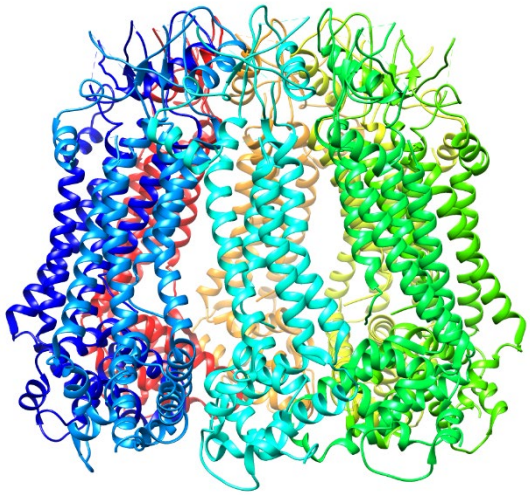
NVIDIA GeForce RTX™ 2080 Ti

Data processing for EM Single Particle Analysis



INX-6





2D images

EMPIAR-10291

300 images

3710 x 3838 x 1; 32 bit real

15.9 GB

EMPIAR

EMPIAR-PDBj

300 movies

3710 x 3838 x 30; 32 bit real

478 GB

(not registered in EMPIAR.
personal communication)

3D reconstruction →

3D image

180 x 180 x 180 voxels

emd_9973.map

(3.6Å)

22.24 MB

EMDB

Atomic modeling →

Atomic model

20840 atoms

PDB_ID: 6kfh

1.7 MB

wwPDB

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MotionCorrection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

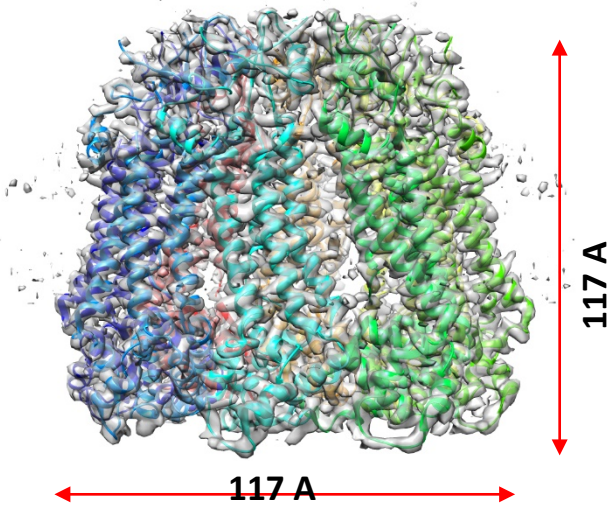
Innexin-6 : docked and undocked states

Structural component of the gap junctions.

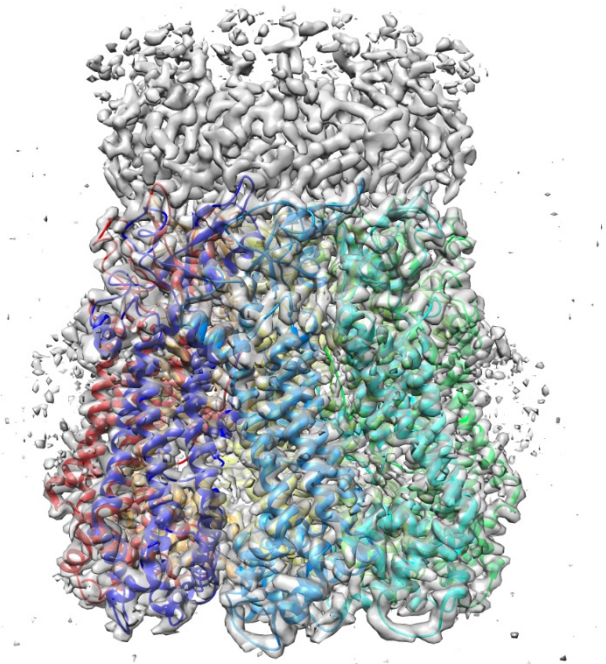
Docked innexin-6

Undocked innexin-6 hemichannel

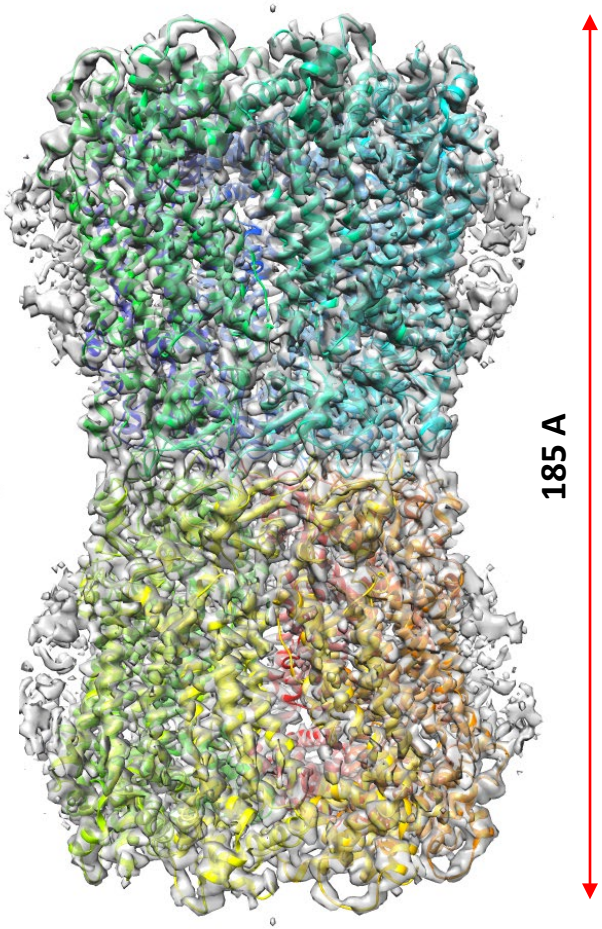
EMPIAR-10291



EMD-9973/PDB_ID:6kfh



EMD-9570/PDB_ID:5h1q



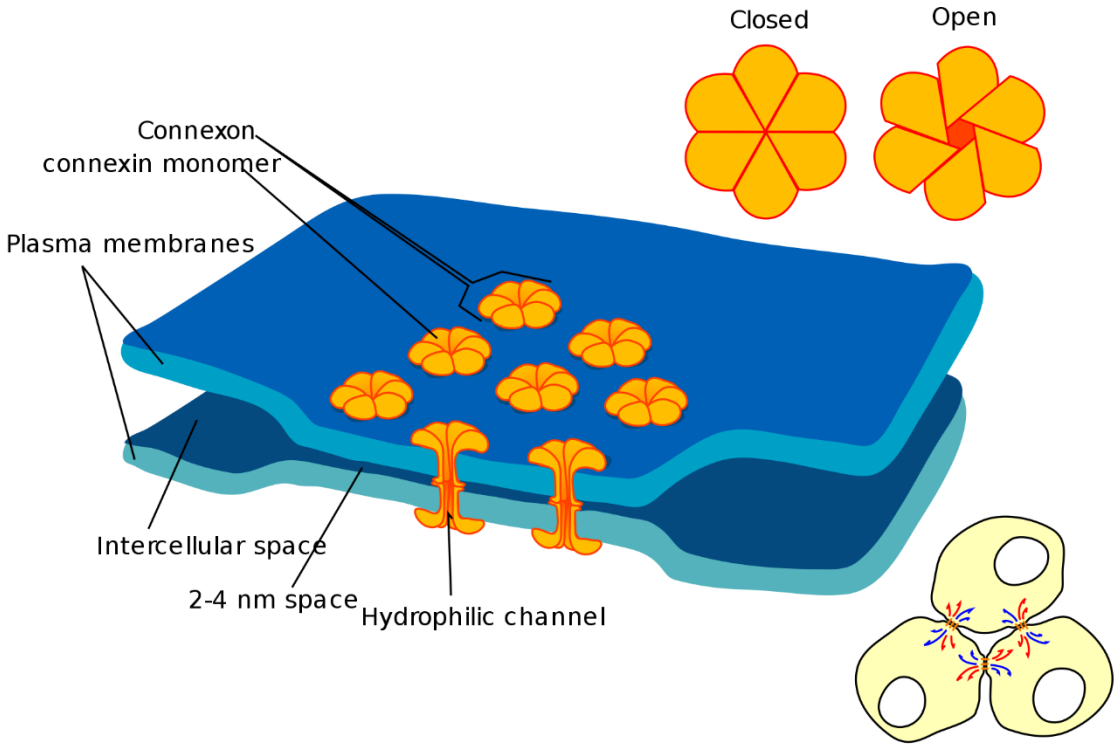
EMD-9571/PDB_ID:5h1r

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

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

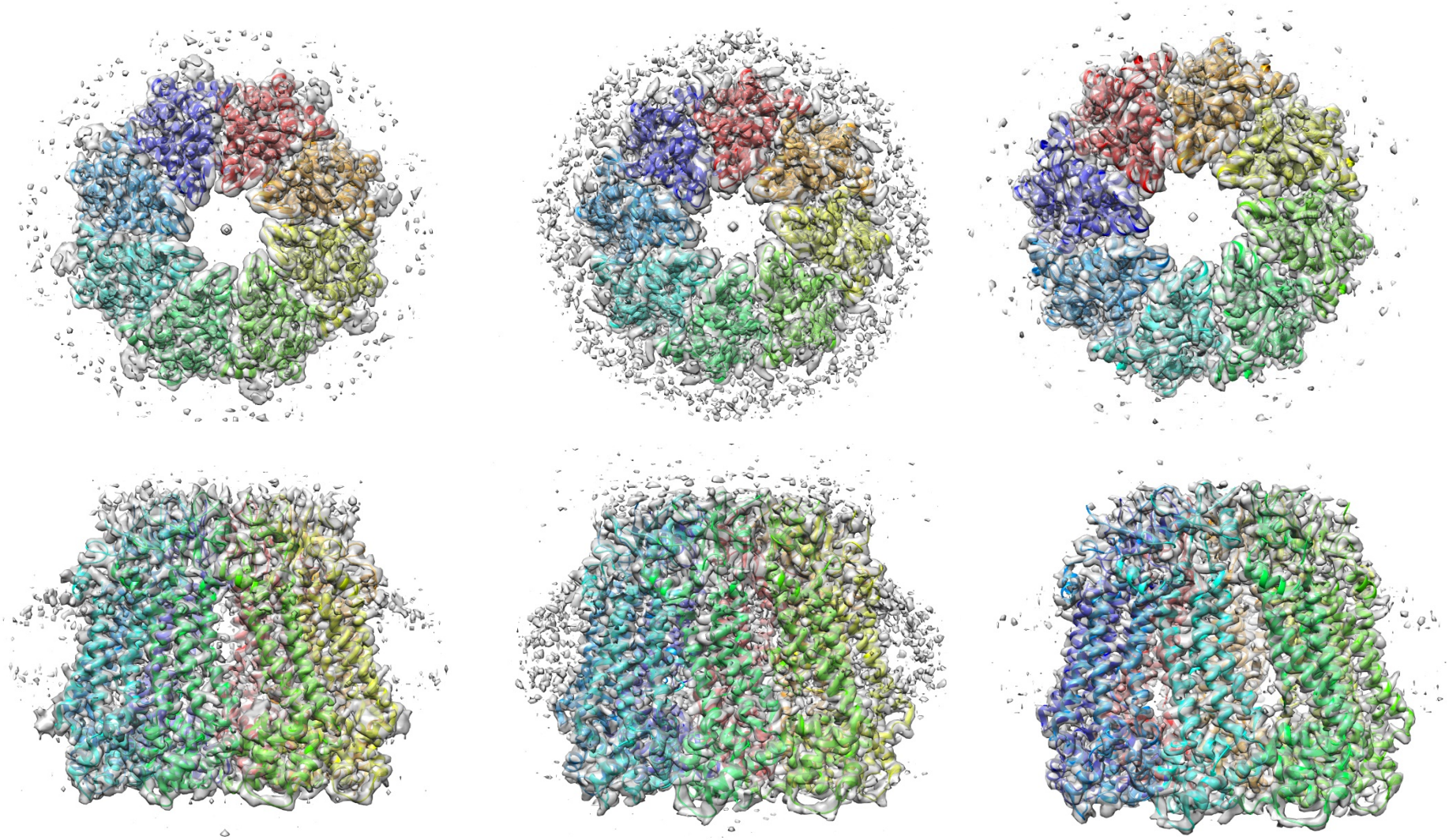
ギャップ結合 (gap junction)

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

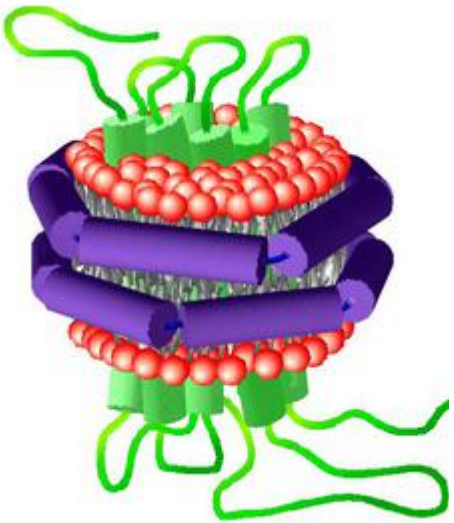


EMD-9971/PDB_ID:6kff
EMPIAR-10289
WT INX-6 in a nanodisc

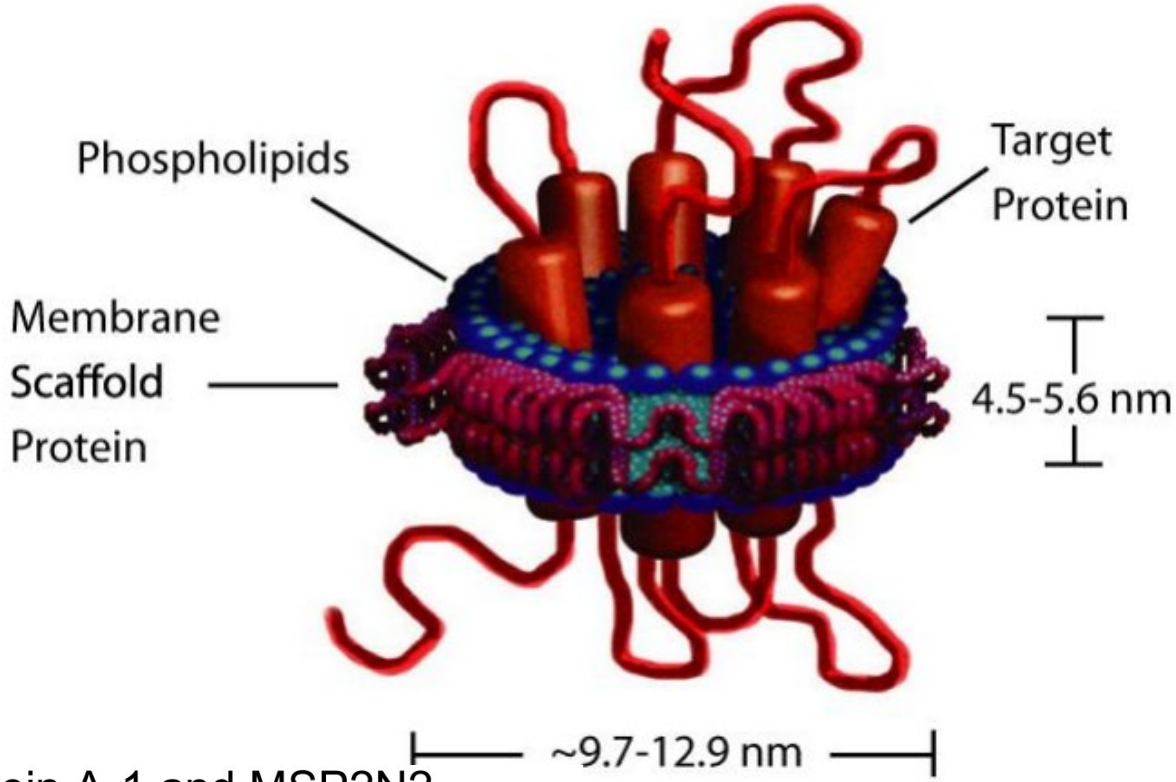
EMD-9972/PDB_ID:6kfg
EMPIAR-10290
WT INX-6 in a detergent

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

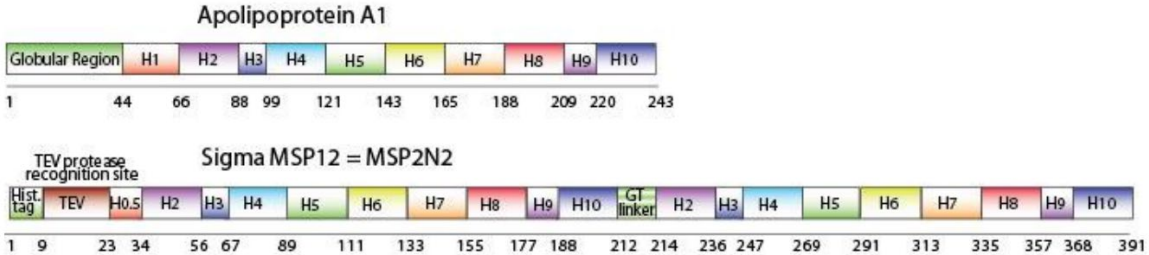
nanodisc



Example of a Nanodisc containing a 7-transmembrane protein



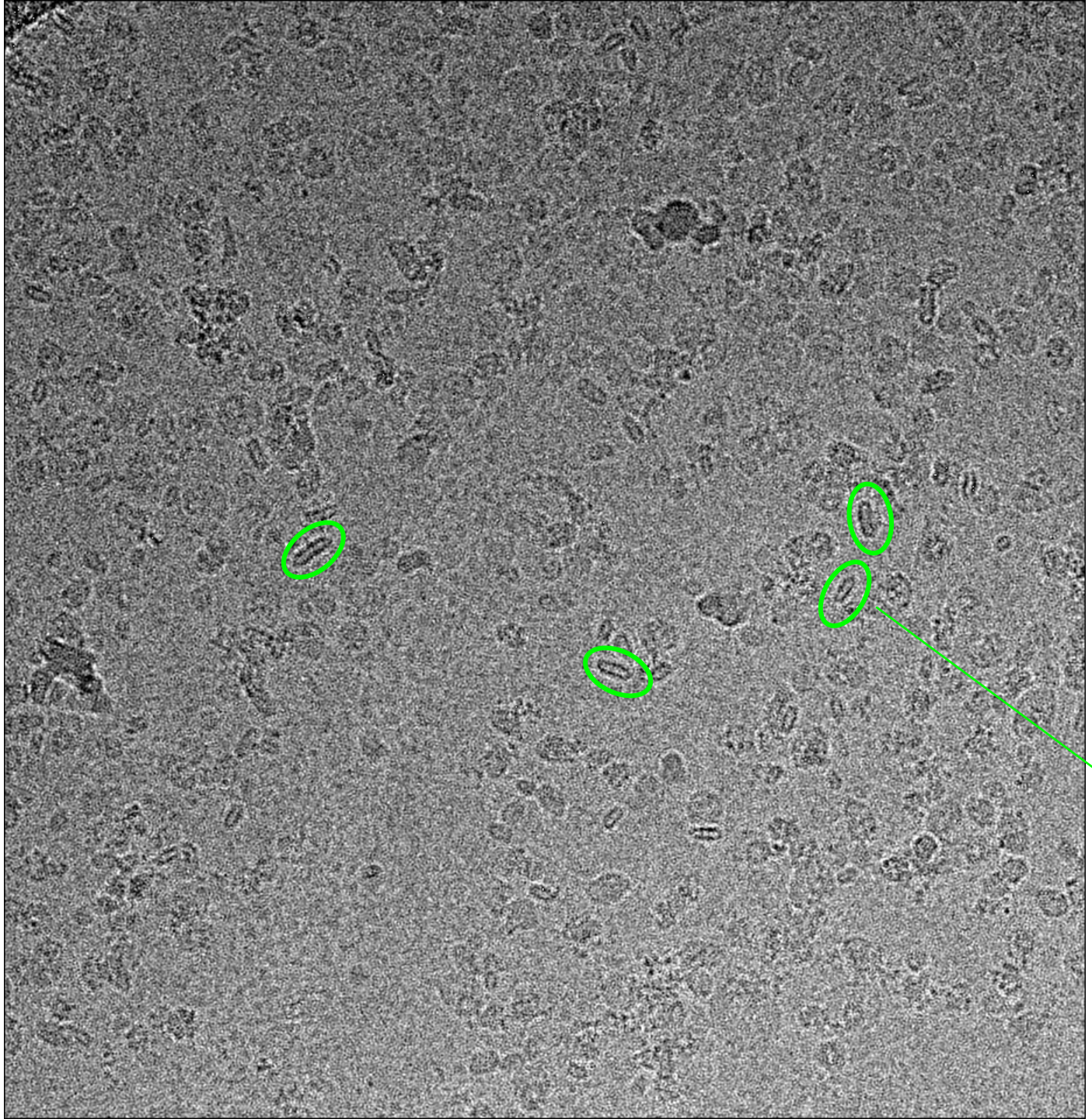
Protein Maps of Apolipoprotein A-1 and MSP2N2



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.
Sci Adv, 6:eaax3157-eaax3157, **2020**



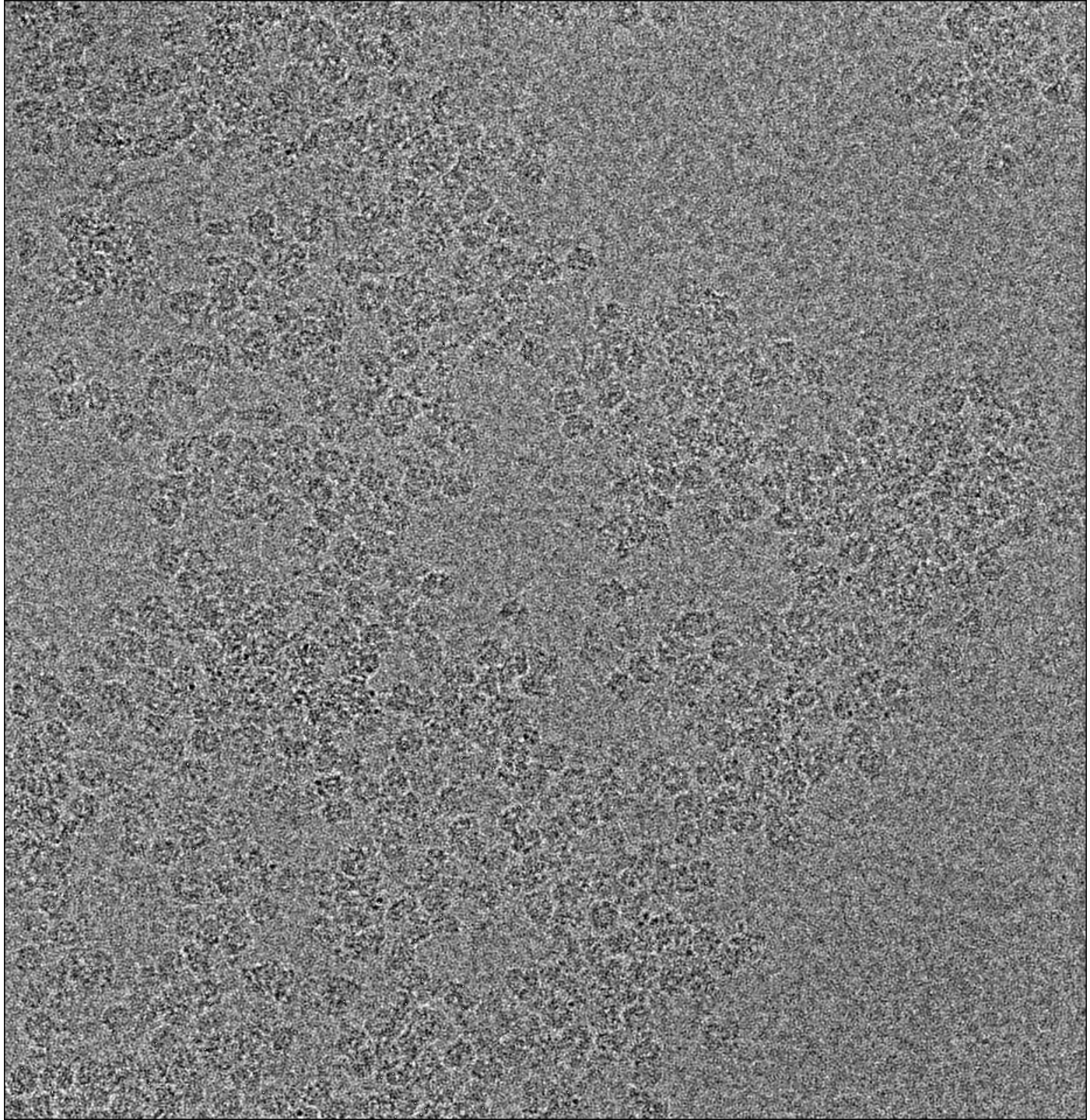
EMPIAR-10289
WT INX-6 in nanodisc

INX6hemiNano001

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

These long circles are not proteins. micelle ? Nanodiscs ?

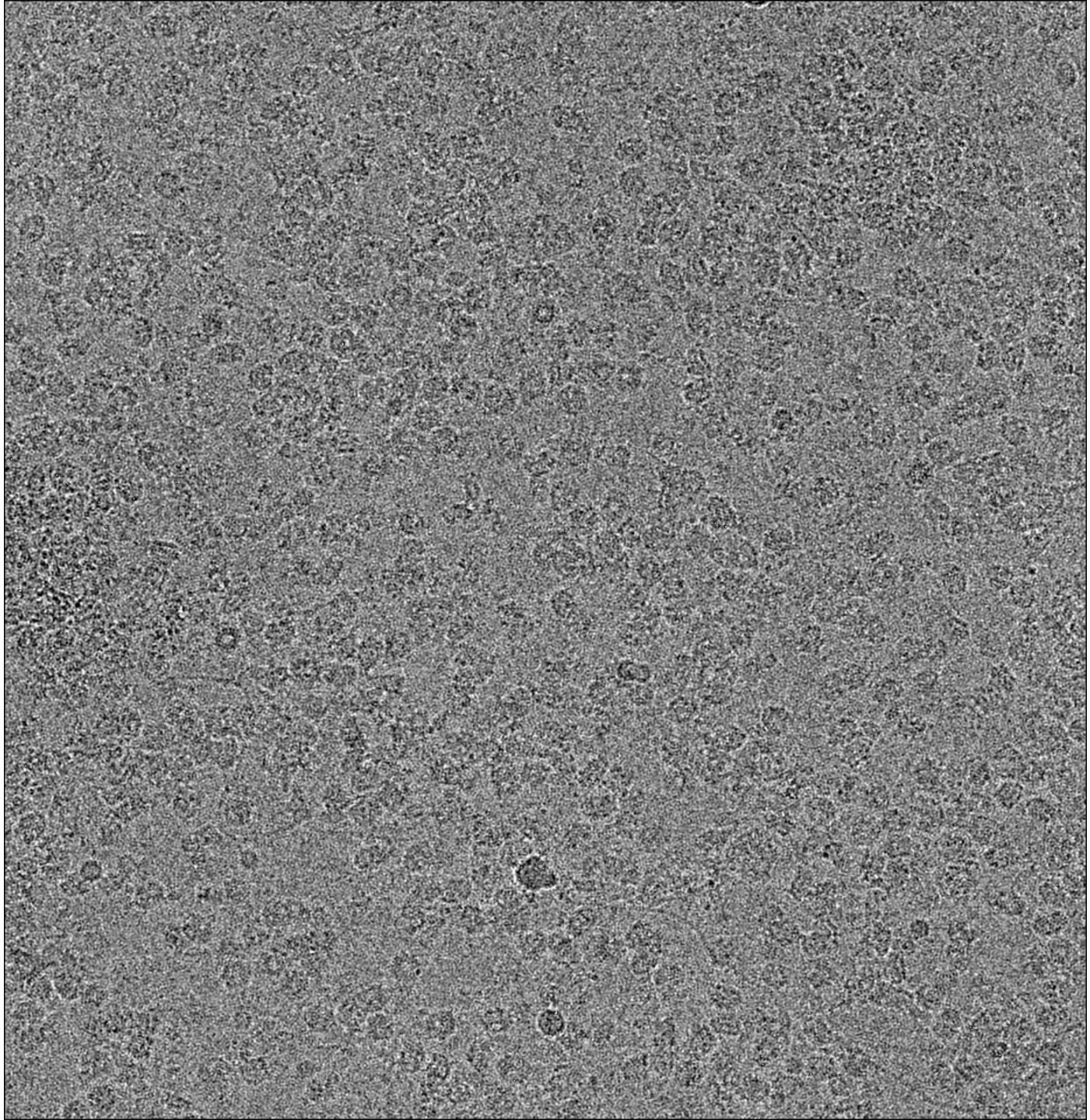


EMPIAR-10290
WT INX-6 in detergent

INX6hemiDet002

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å



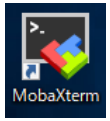
EMPIAR-10291
WT INX-6ΔN in nanodisc

INX6hemiDet001

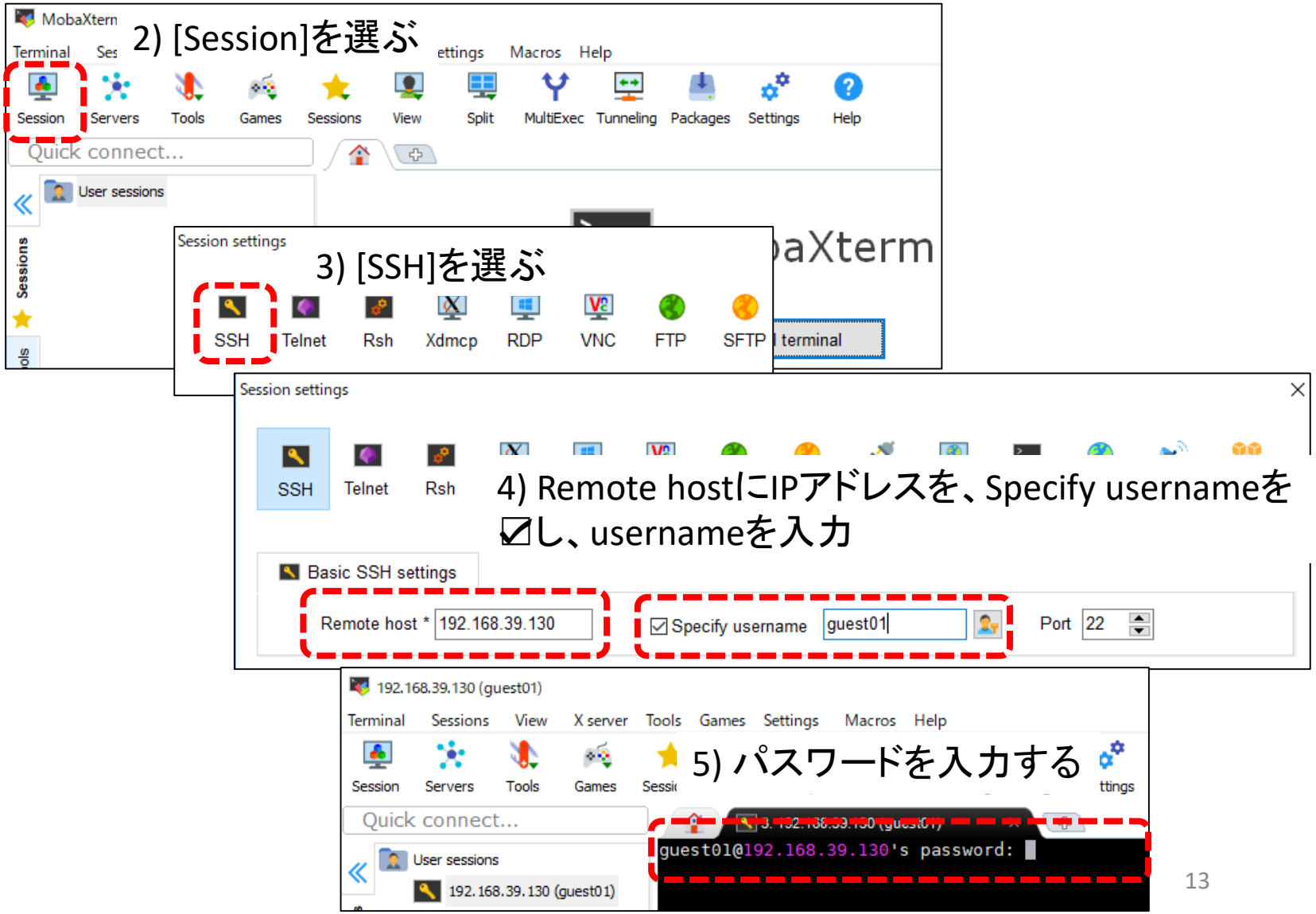
Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

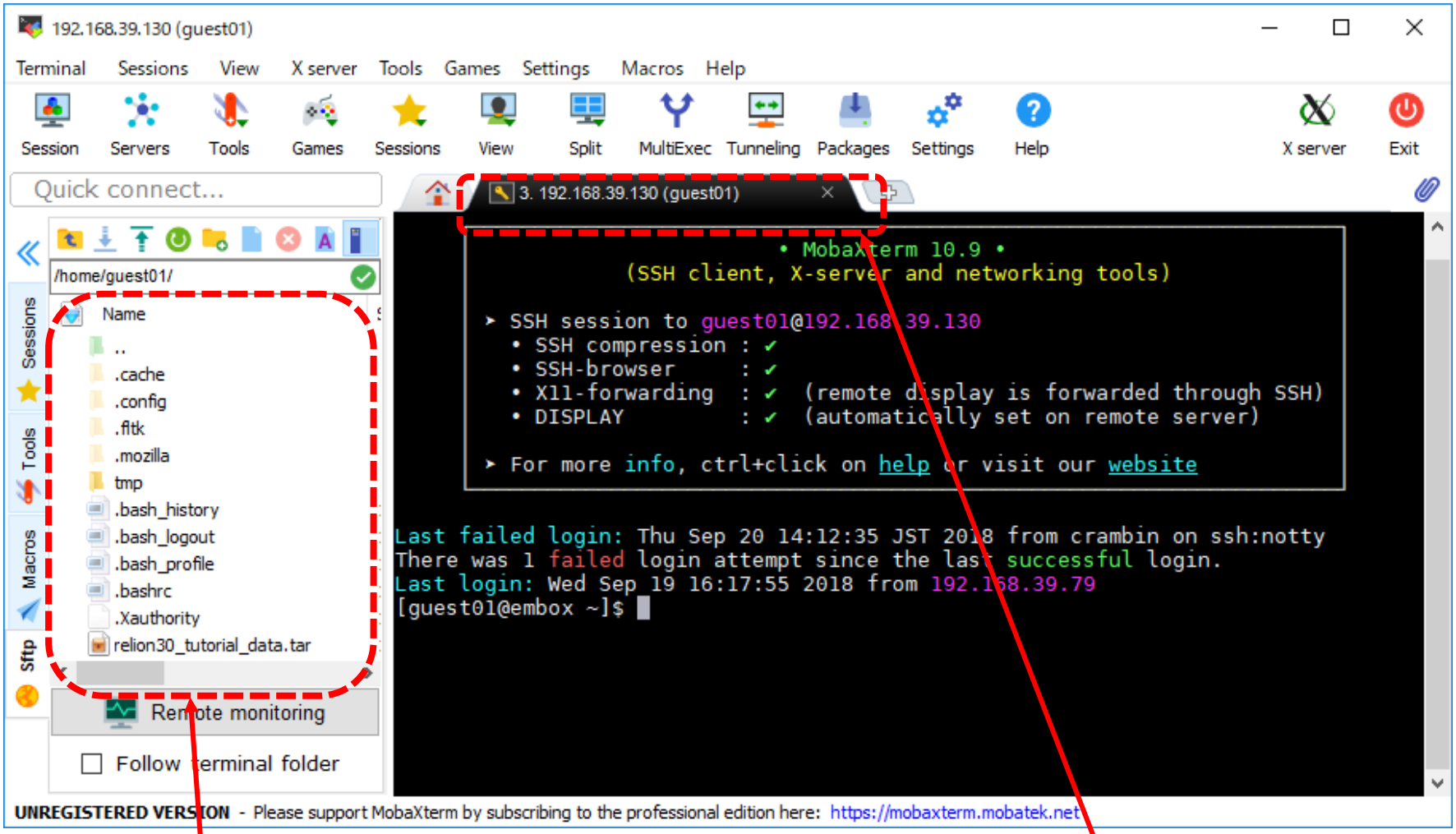
MobaXtermによるログインの方法

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) **ls relion[Tab]** **ls relion30_tutorial**	Compensate file name (example) **ls relion[Tab]** **ls relion30_tutorial**
[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.

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 [ShiftZZ]: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        18  8月 26 19:48 EMPIAR-10291_100mic
[guest01@embox2 ~]$
```

演習用のデータの入ったディレクトリ

```
[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
[guest01@embox2 ~]$
```

←empiarのメタデータファイル

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
-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
```

3710 x 3838 x 1; 32 bit real

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
-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
```

3710 x 3838 x 30; 32 bit real

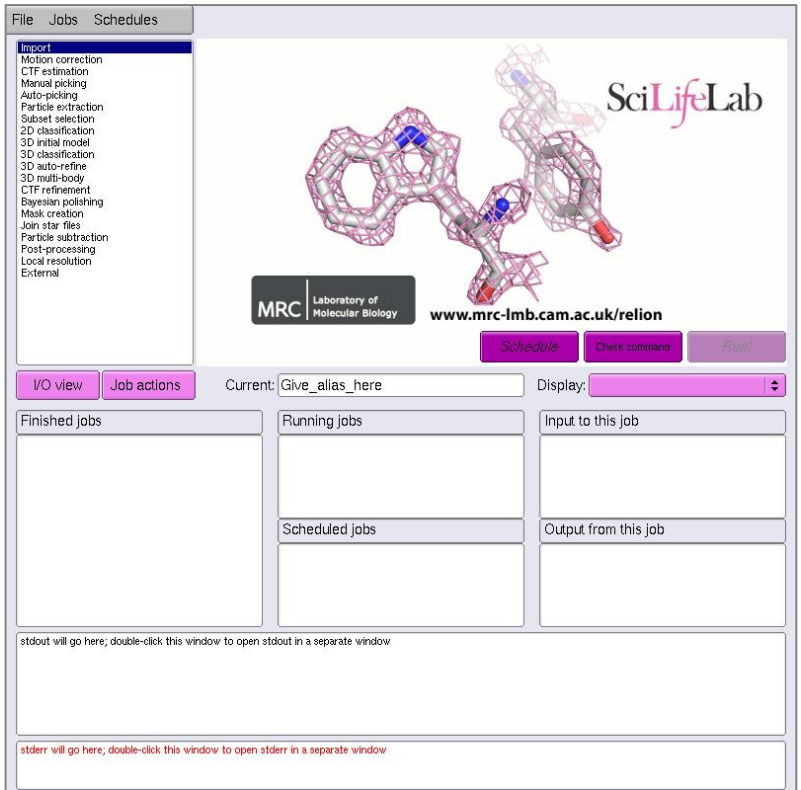
1.1 Launch Relion

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
```

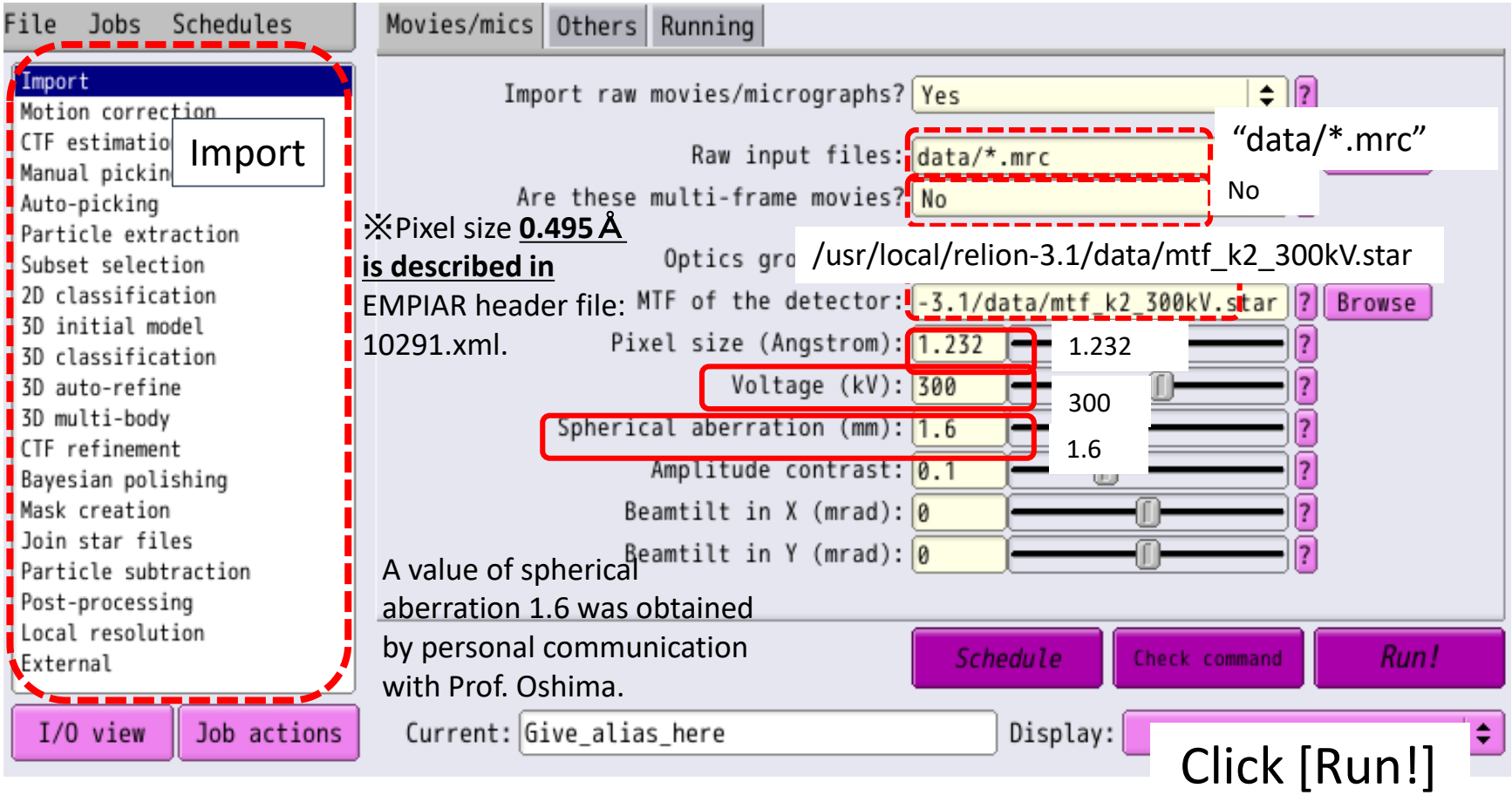
※Relionを二回目以降に起動する場合は、コマンドの最後に&をつけると、バックグラウンドでの起動となる。

```
$ relion &
```

1.1 Import micrographs / movies

Select “Import” from Job type browser.

Job type browser



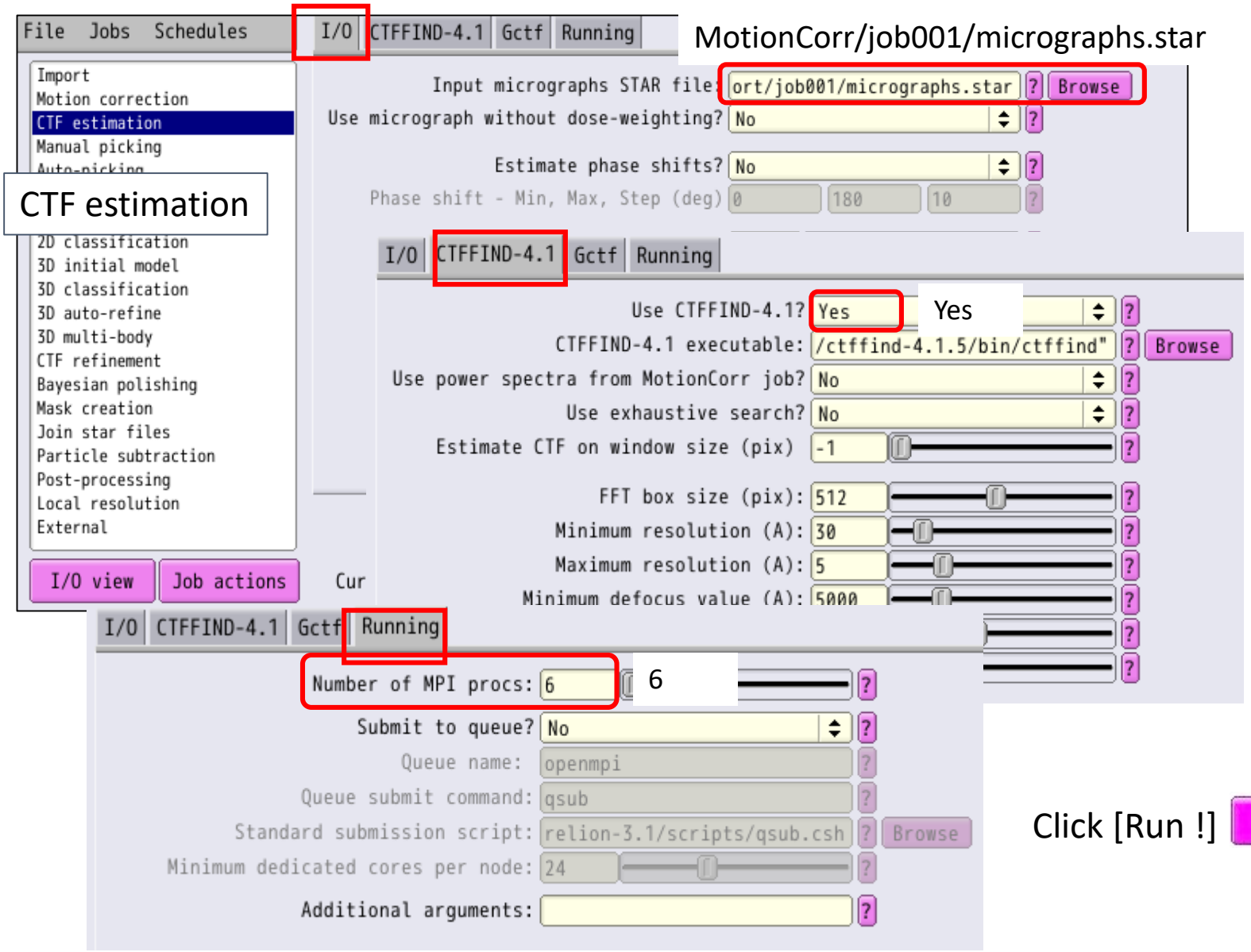
※Pixel size **0.495 Å is described in** EMPIAR header file: 10291.xml.

A value of spherical aberration 1.6 was obtained by personal communication with Prof. Oshima.

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

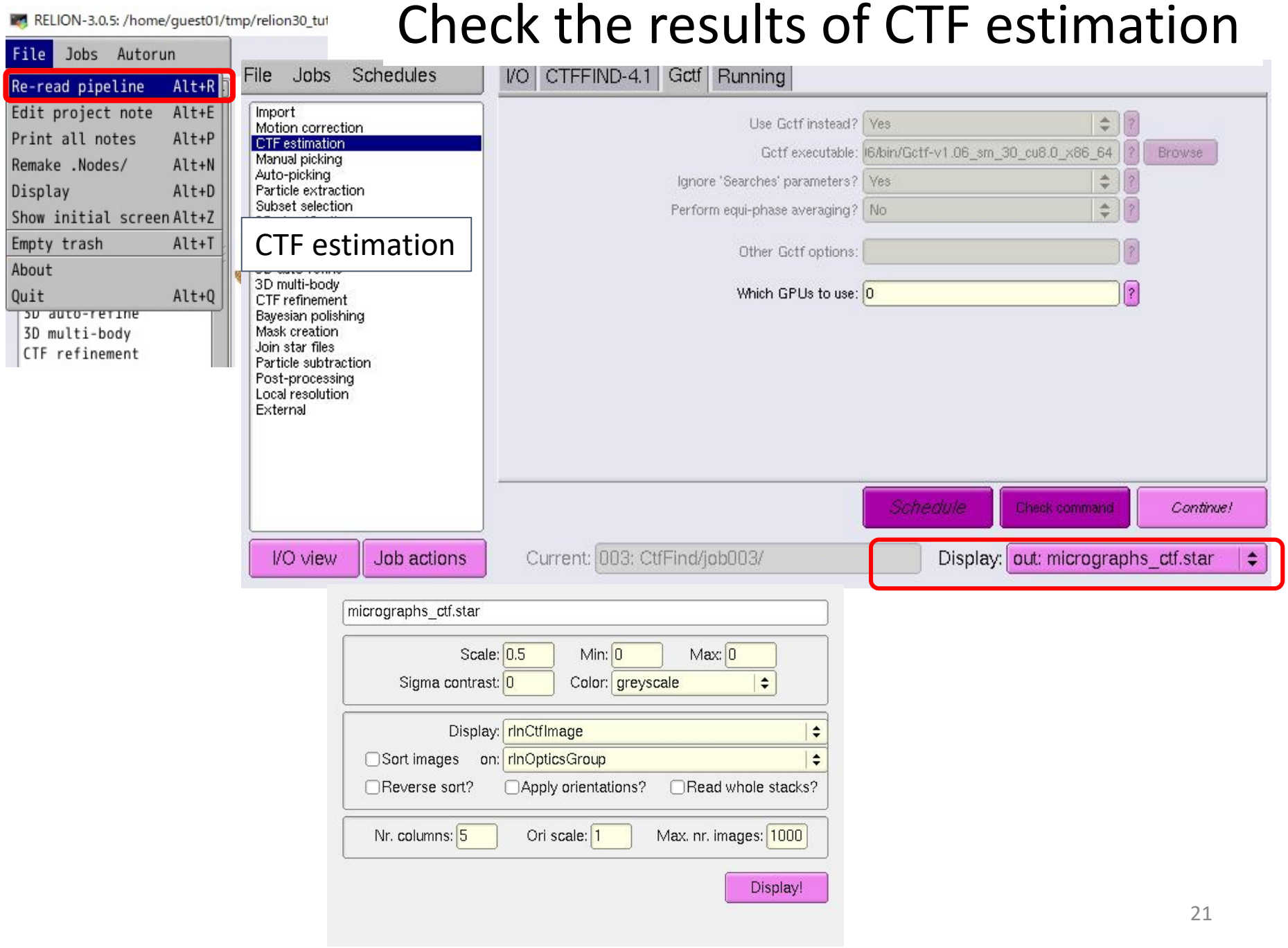
You can check it by the command: `less Import/job001/movies.star`

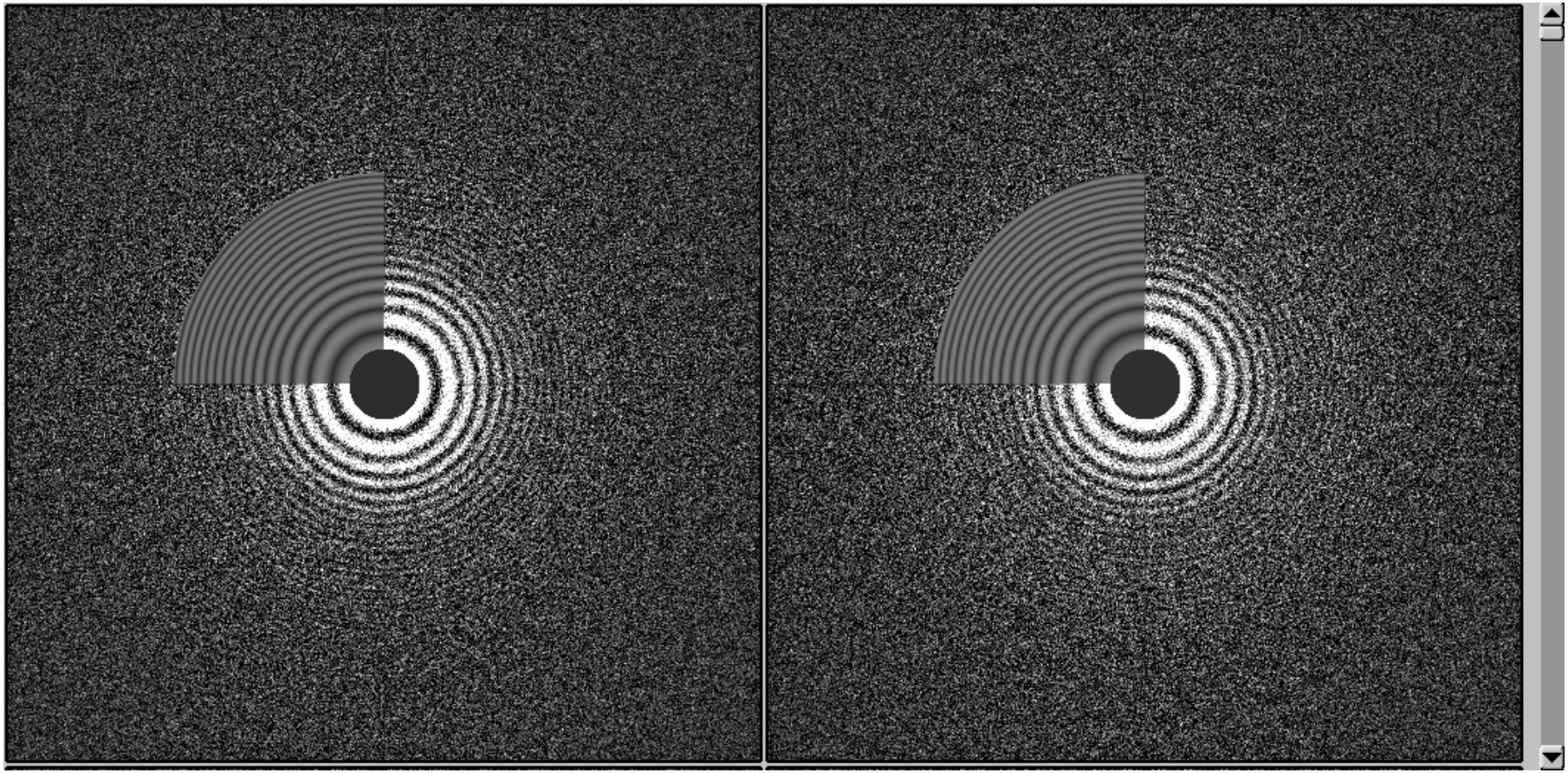
1.3 CTF estimation (< 1min)



Click [Run !]

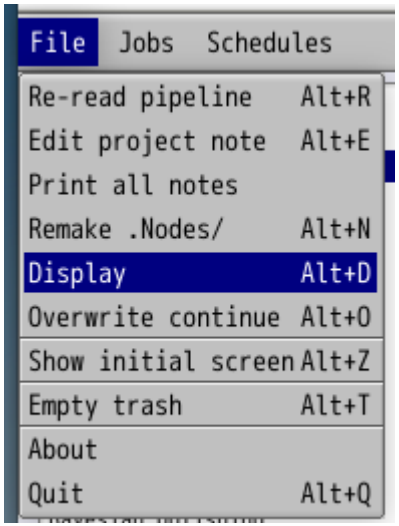
Check the results of CTF estimation



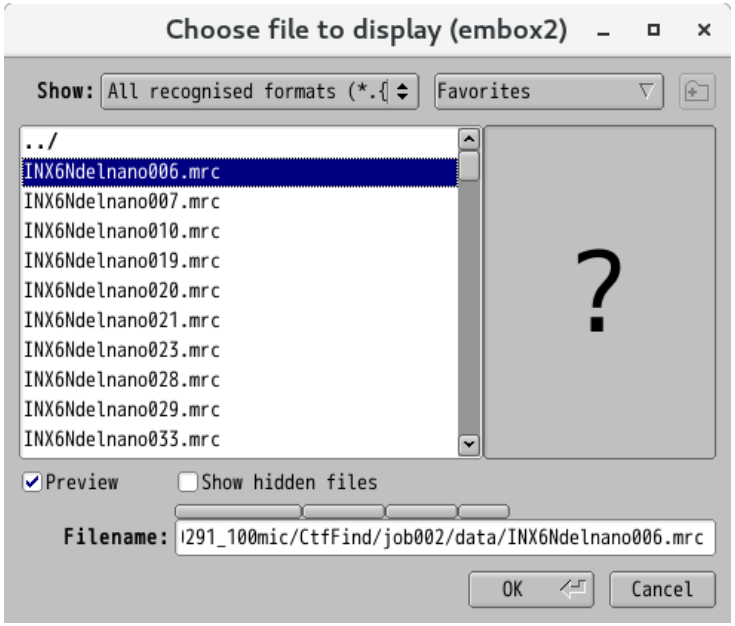


Check CTF-corrected micrographs

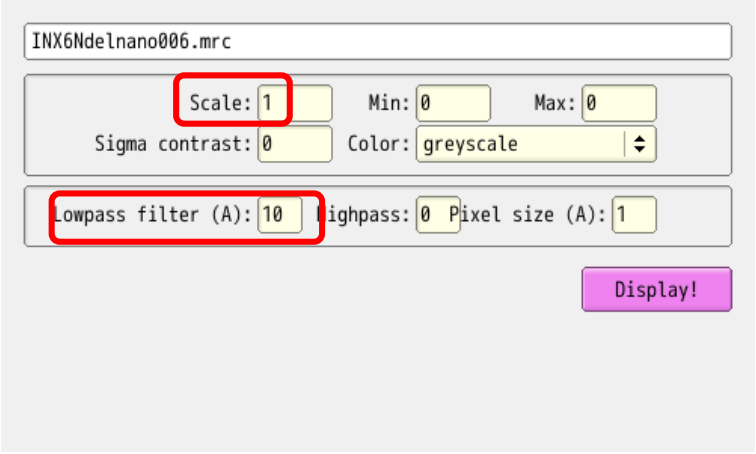
(1) Select [File] -> [Display]



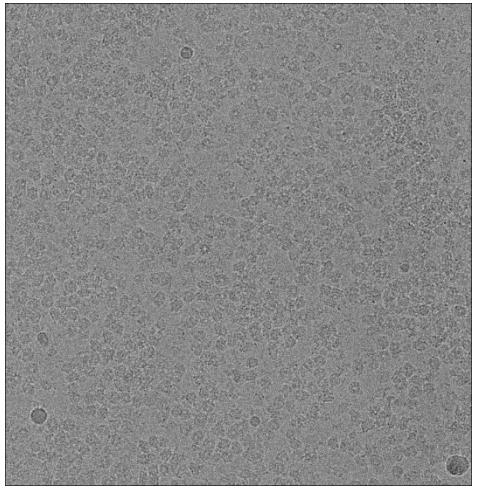
(2) Select one of the file in CtfFind/job002/data



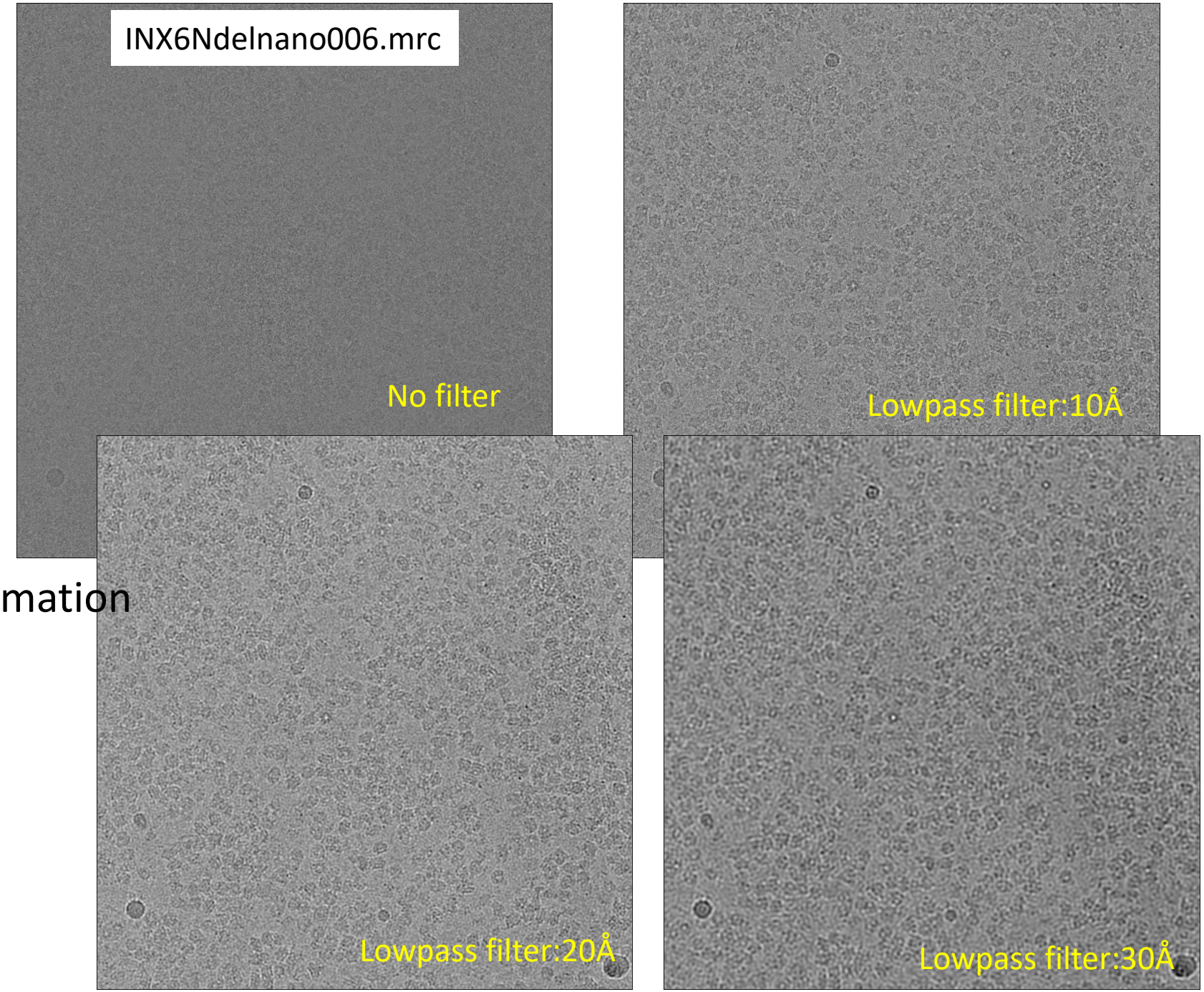
(3) Input your favorite “scale” and “Low pass filter” values.



(4) You can see the CTF-corrected micrograph

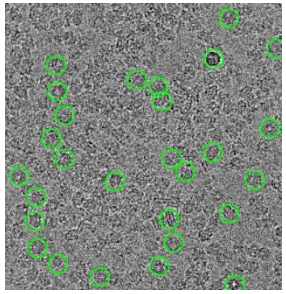


After
CTF estimation

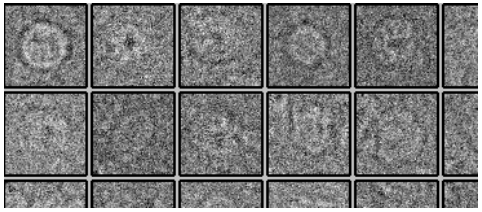


Picking by Relion

For a few micrographs

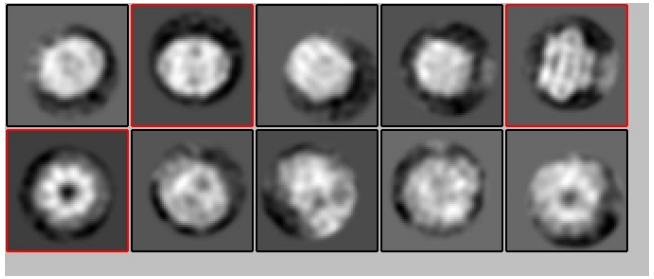


Manual picking

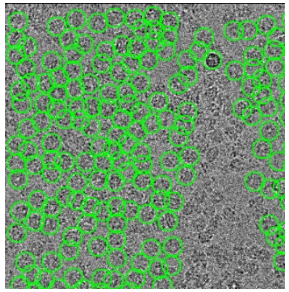


Auto-picking: LoG-based

Class2D



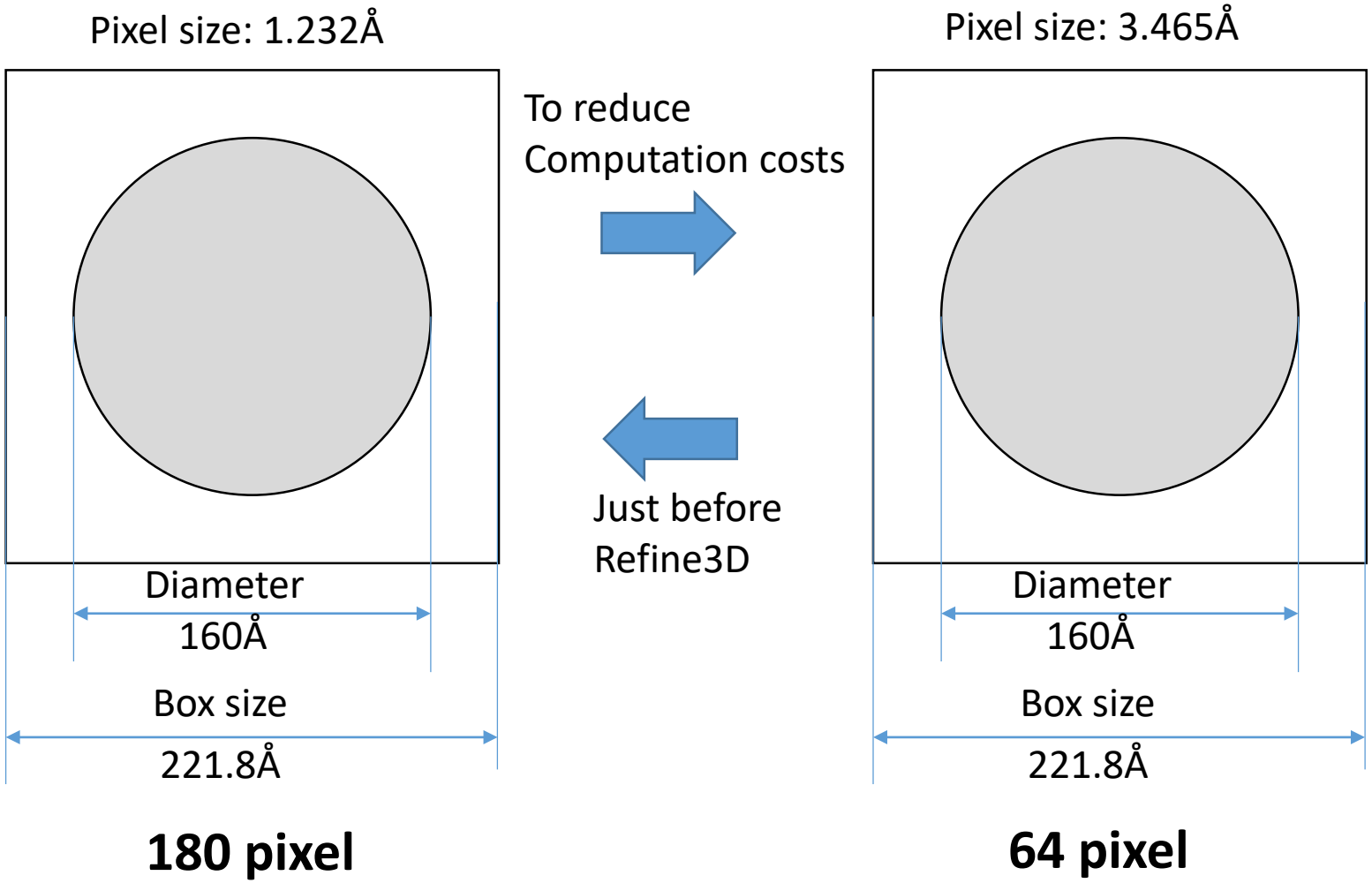
Select Good Classes for reference



For many micrographs

Auto-picking: reference-based

Box size of particles

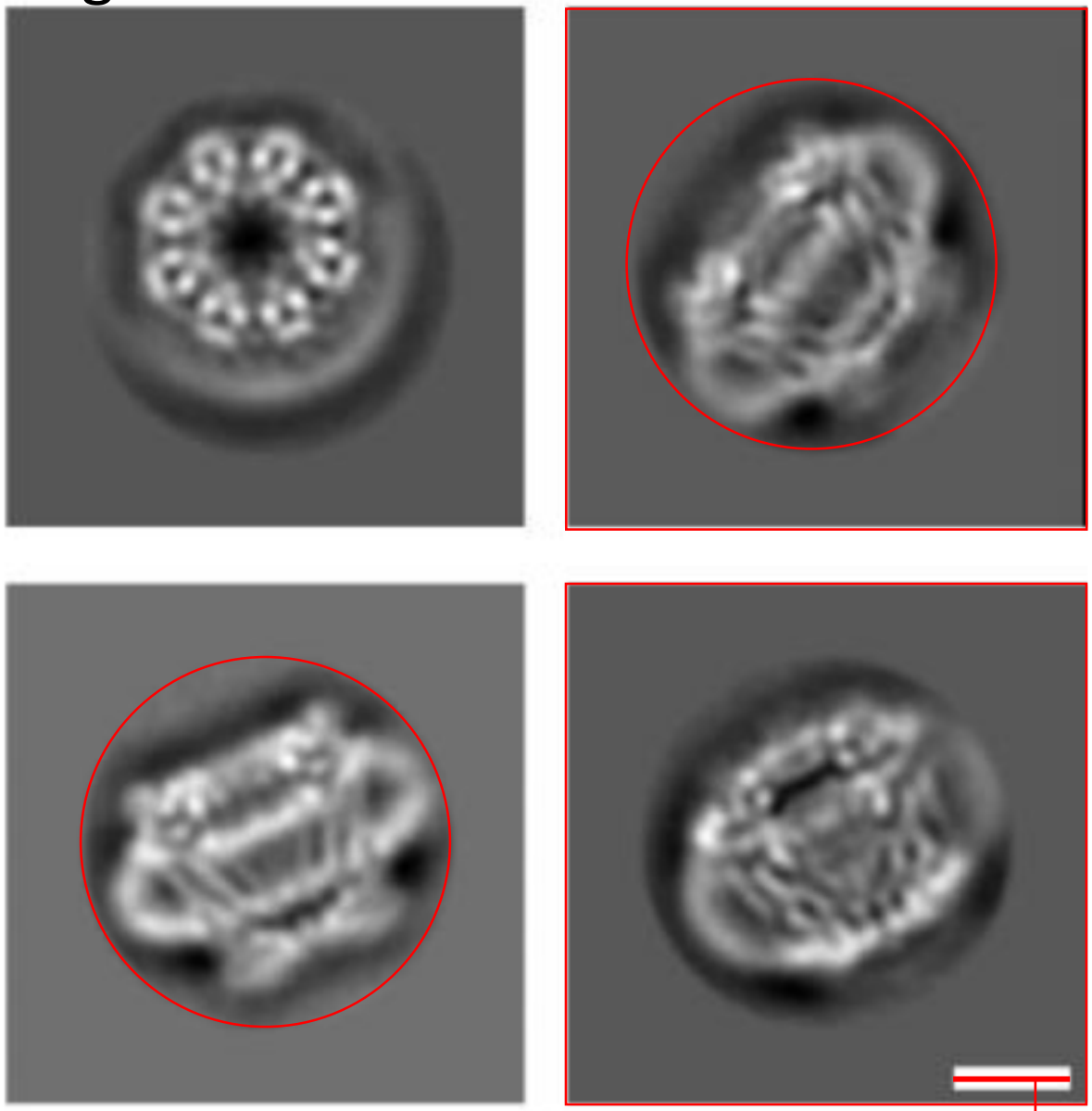


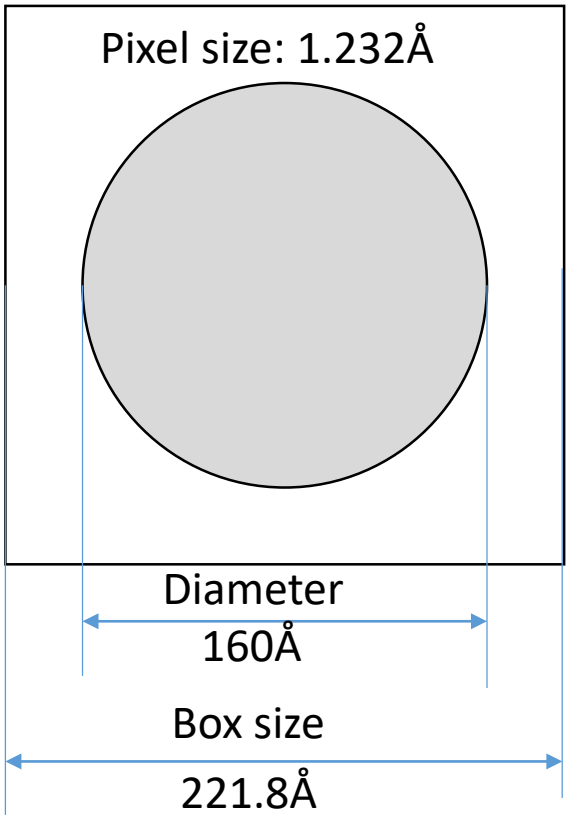
C

2D Class average images from the original article

Fig.S3c

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





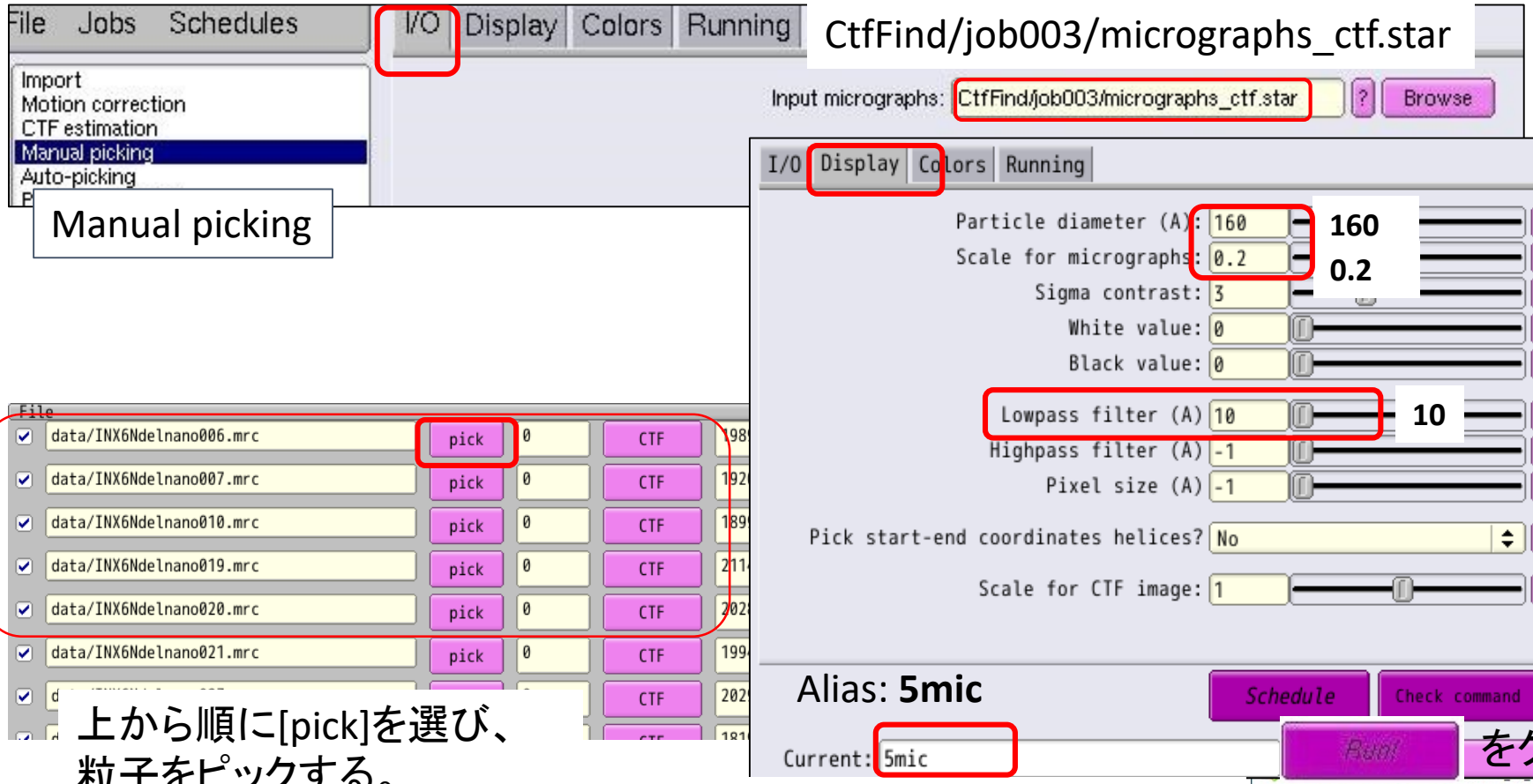
180 pixel

方形の１辺7.44cm = 222.75 Å

マスク直径5.28cm = 158.08 Å

1.67 cm : 50 Å

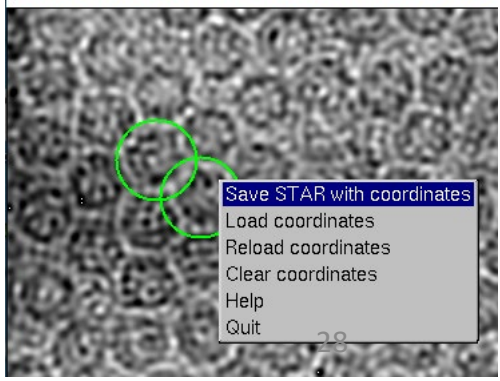
1.4 Manual Particle Picking



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。

をクリック！

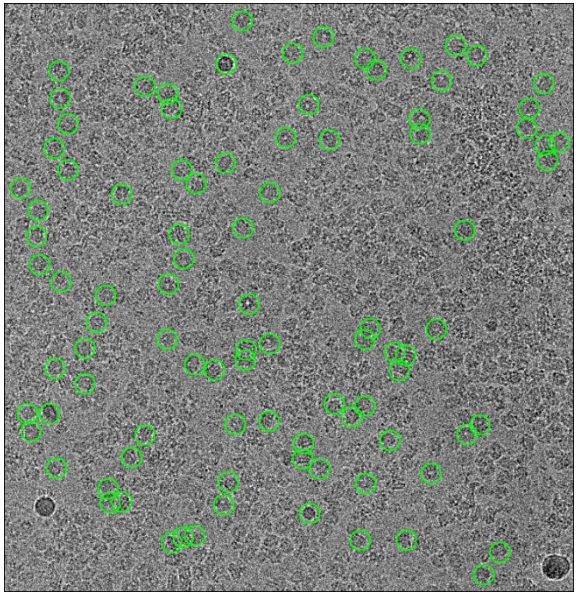
マウス左ボタンで粒子をクリックし
50-100個ほど選択
マウス右ボタンでポップアップメ
ニューを表示させ、
[Save STAR with coordinates]
を選び、ピックした座標を保存。



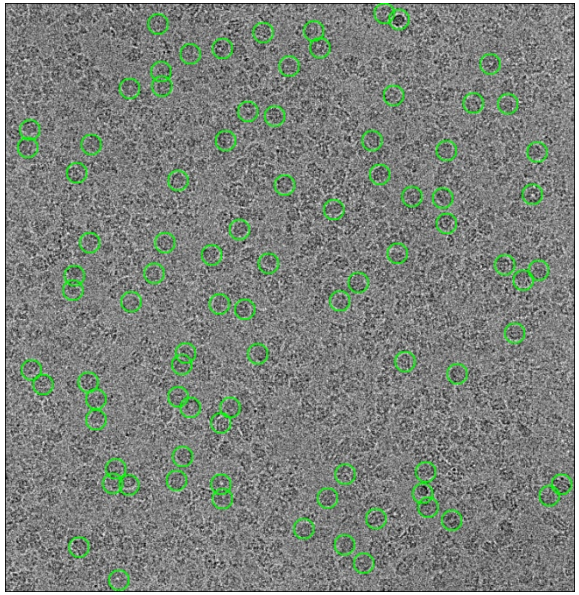
※[Shift]キーを押しながら
クリックすると、〇が消える！

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

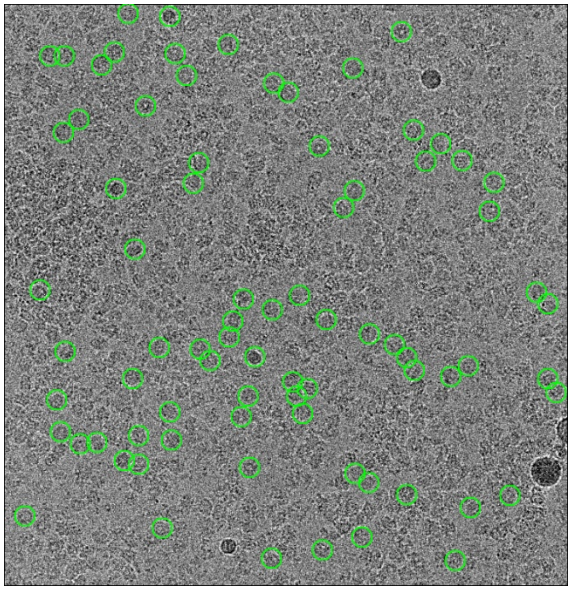
Manual Particle Picking



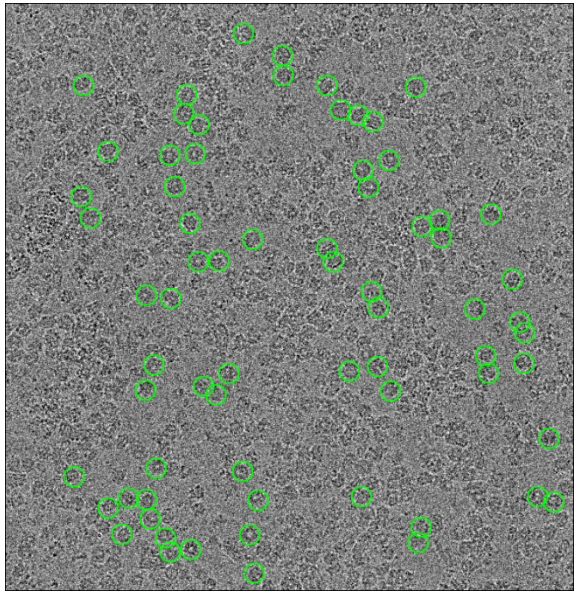
006 : 94 particles



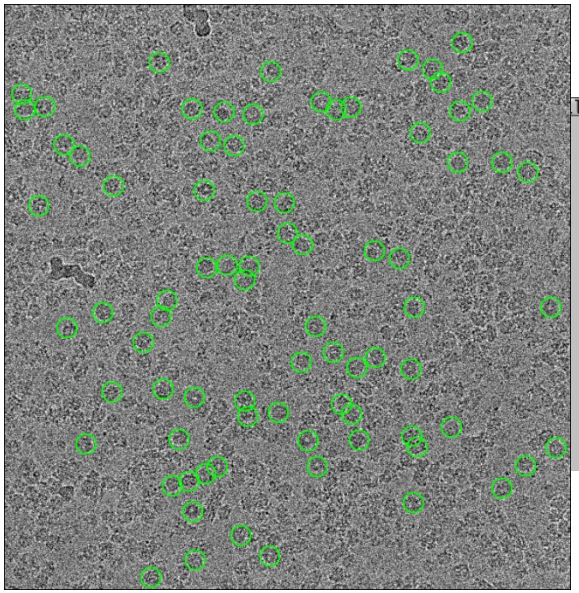
007 : 87 particles



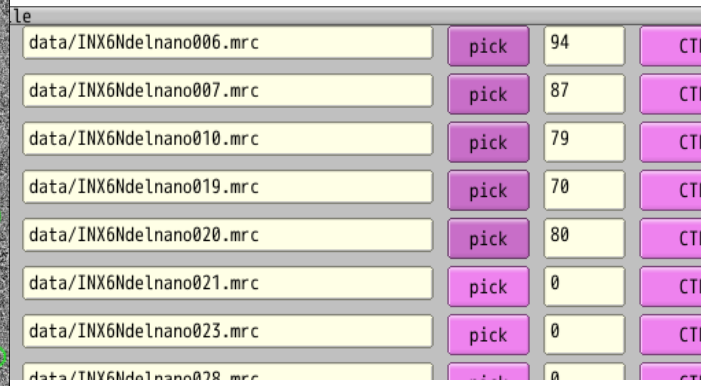
010 : 79 particles



0019 : 70 particles



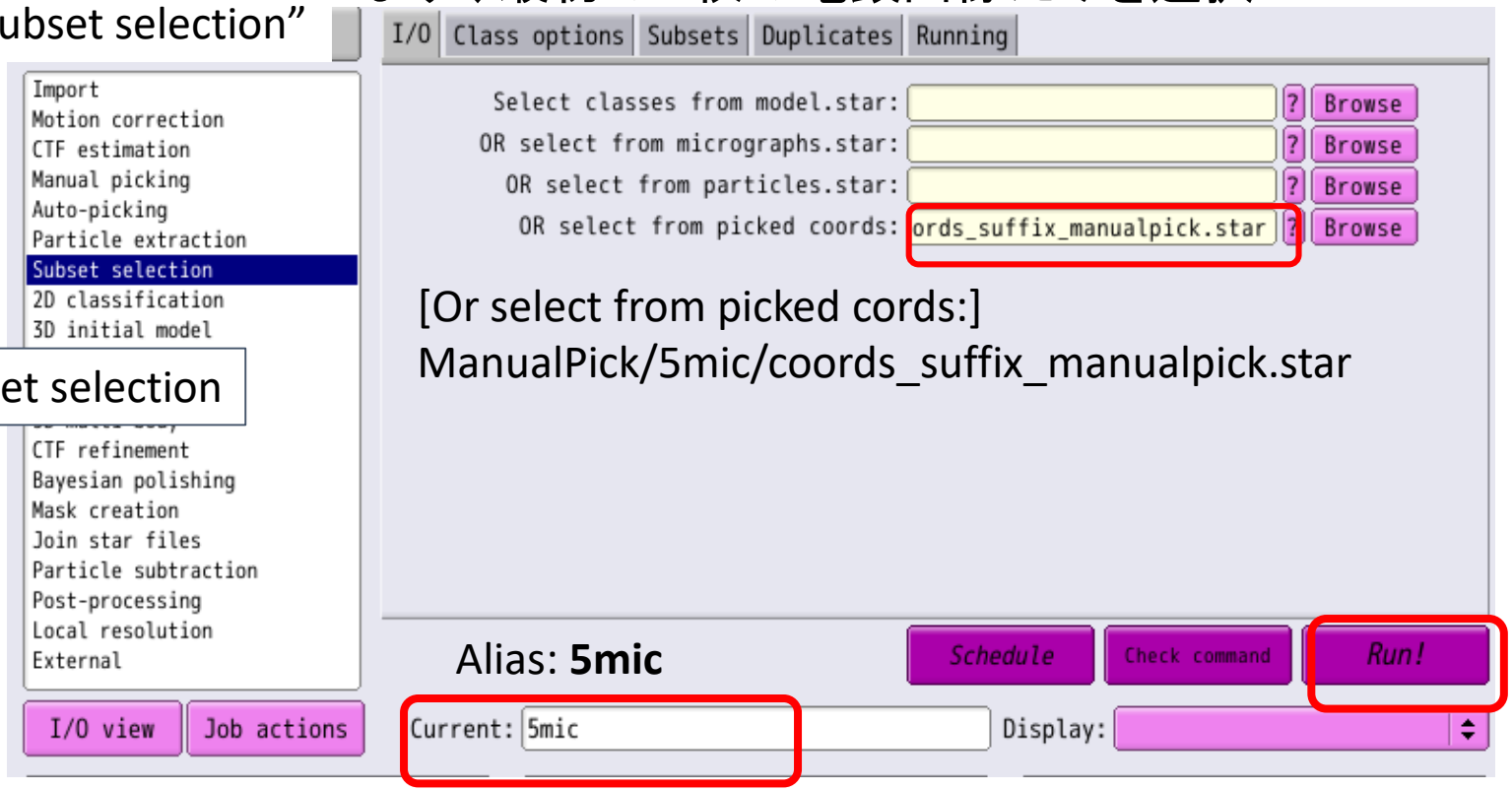
0020 : 80 particles



Subset selection

まず、最初の5枚の電顕画像だけを選択

Select ”Subset selection”



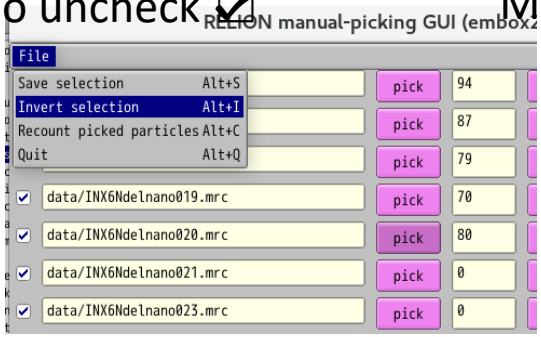
Subset selection

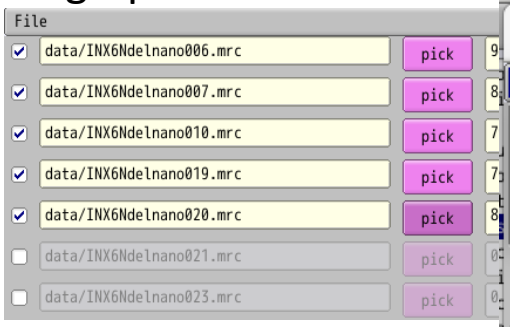
[Or select from picked cords:]
ManualPick/5mic/coords_suffix_manualpick.star

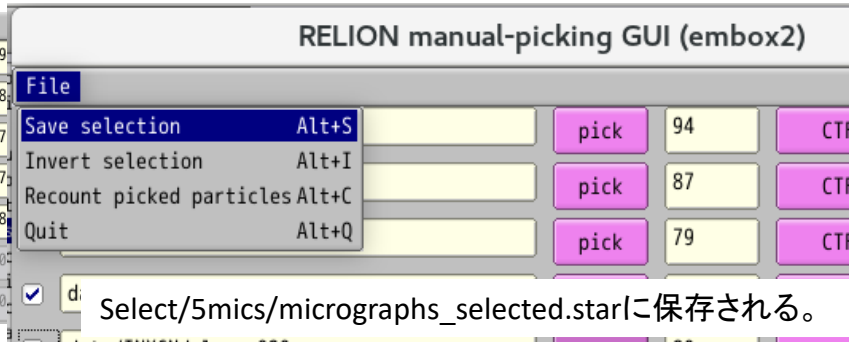
[File]⇒[Invert selection]
To uncheck ☑

Check ☑ for the top 5 Micrographs

[File]から、[Save selection]を選ぶ

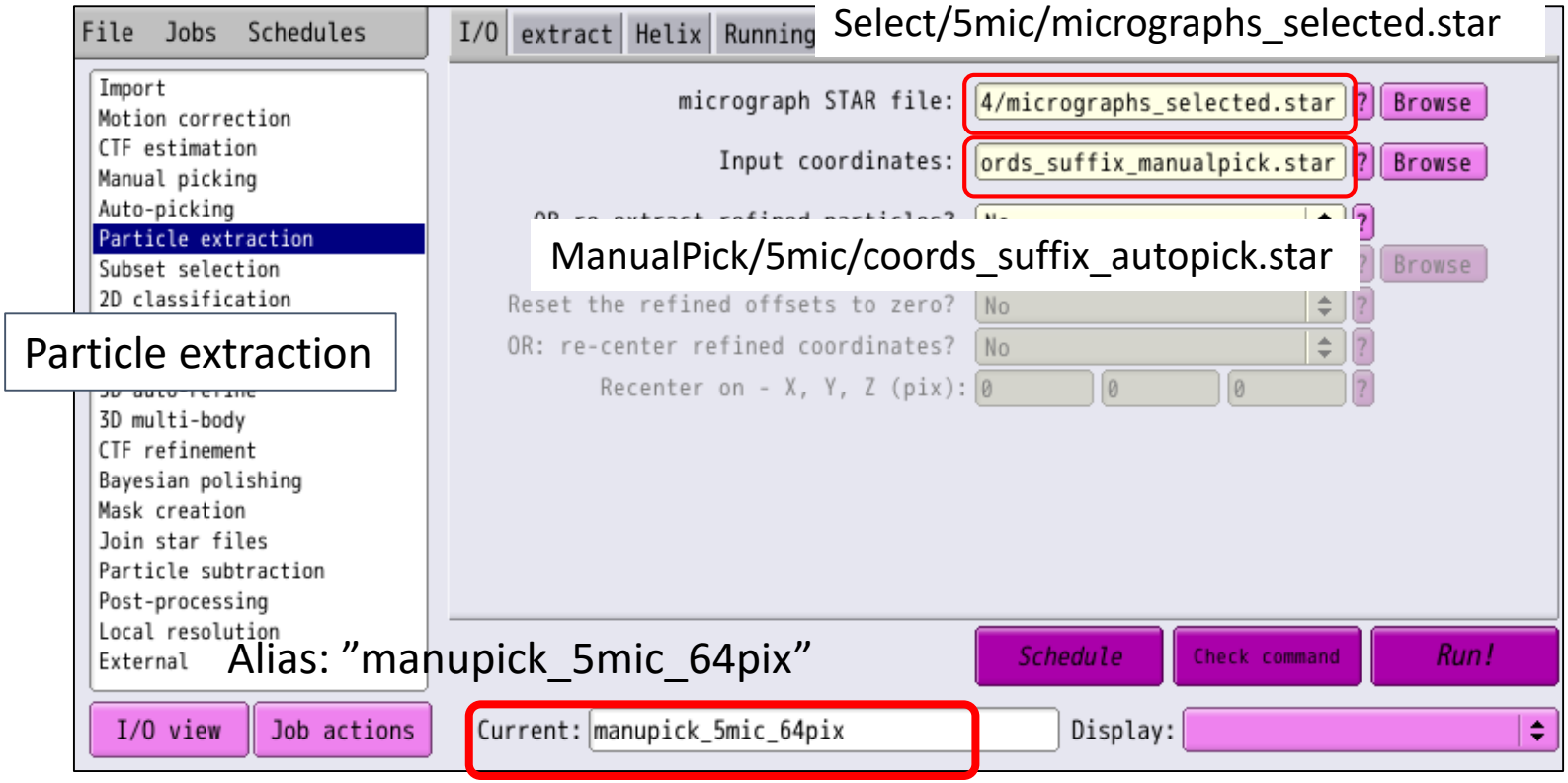


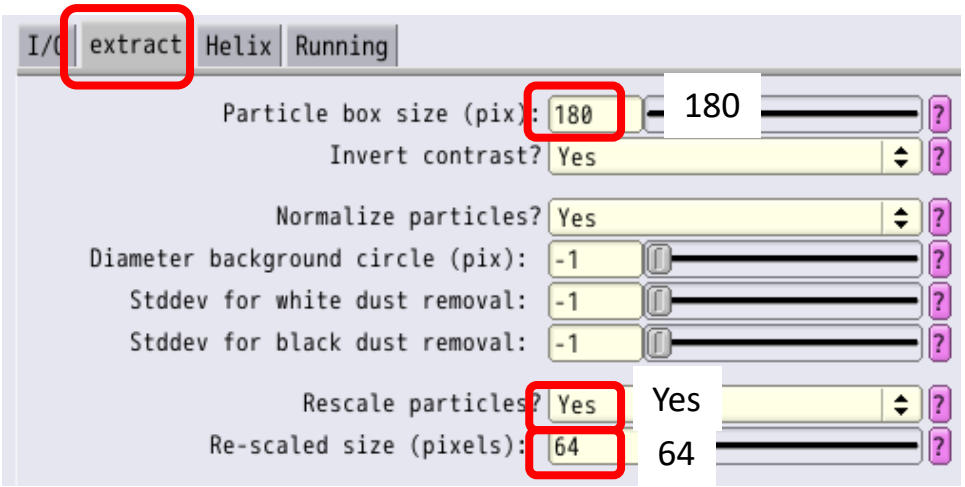




Select/5mics/micrographs_selected.starに保存される。

1.6 Particle extraction





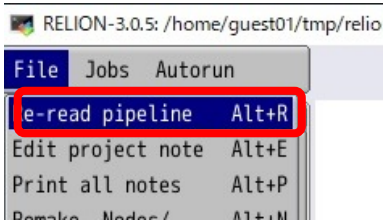
*Box size: 180 pixel x 1.232=221.7 Å

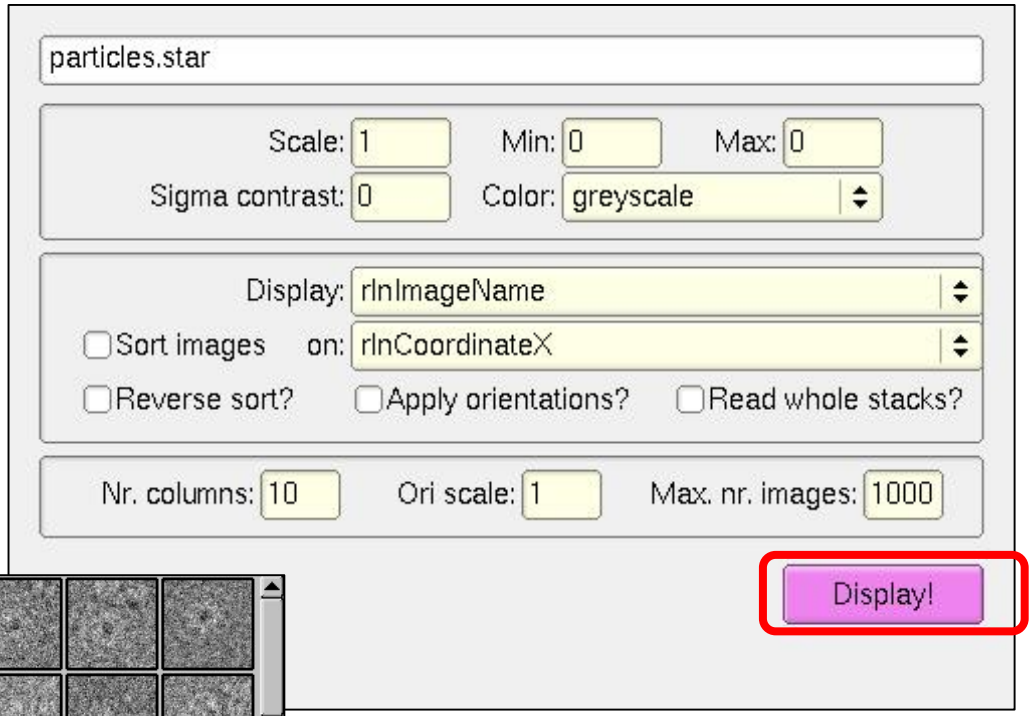
*Box size of the particle has to be even number.

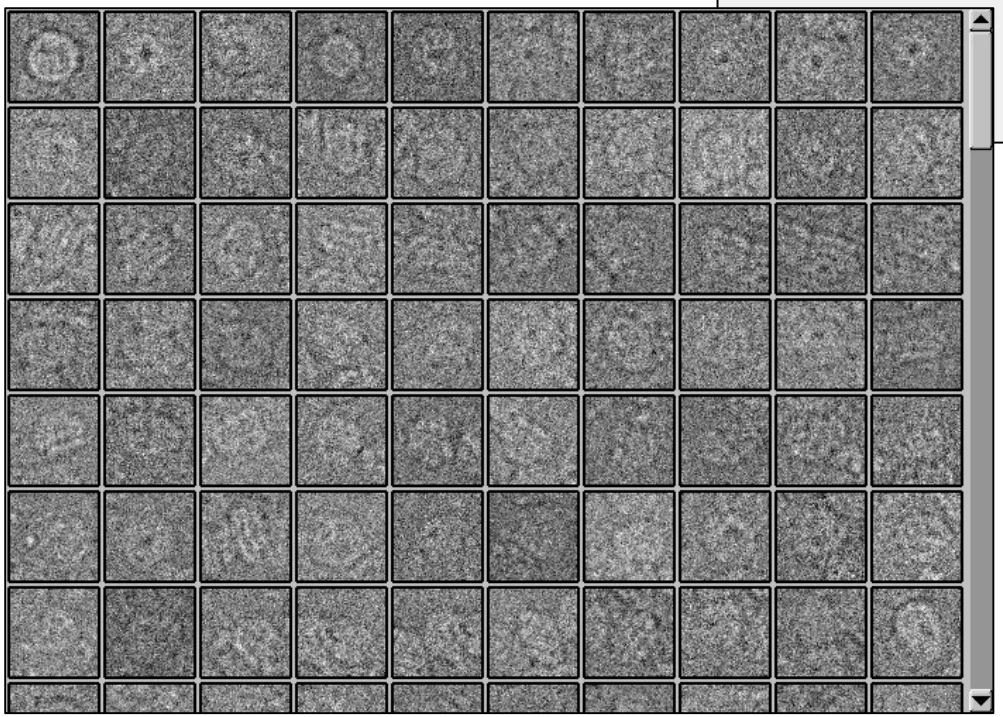
*To reduce computational costs, we rescale 180 pixel into 64 pixel.

Click [Run !] 

1.6 Particle extraction







1.7 2D Classification : to make templates for autopick

[1GPU, 1 min]

Extract/manupick_5mic_64pix/particles.star

2D classification

Alias:"manupick_5mic_64pix"

File Jobs Schedules

Import
Motion correction
CTF estimation
Manual picking
Auto-picking
Particle extraction
Subset selection
2D classification
3D initial model
3D multi-body
CTF refinement
Bayesian polishing
Mask creation
Join star files
Particle subtraction
Post-processing
Local resolution
External

I/O view | Job actions

I/O | CTF | Optimisation

Input images STAR file: tract/job005/particles.star | Browse
Continue from here: | Browse

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

Number of classes: 10 (10)
Regularisation parameter T: 2
Number of iterations: 25
Use fast subsets (for large data sets)? No
Mask diameter (A): 160 (160)
Mask individual particles with zeros? Yes
Limit resolution E-step to (A): -1

Schedule | Check command | Run!

Current: manupick_5mic_64pix | Display:

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

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 PU acceleration? Yes
Which GPUs to use: 0

各受講者のGPU番号
(0,1,2,3のどれか)を入力

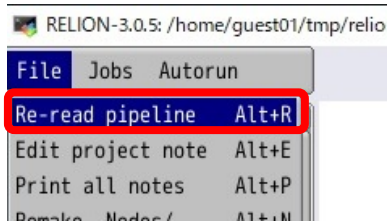
I/O | CTF | Optimisation | Sampling | Helix | Compute | Running

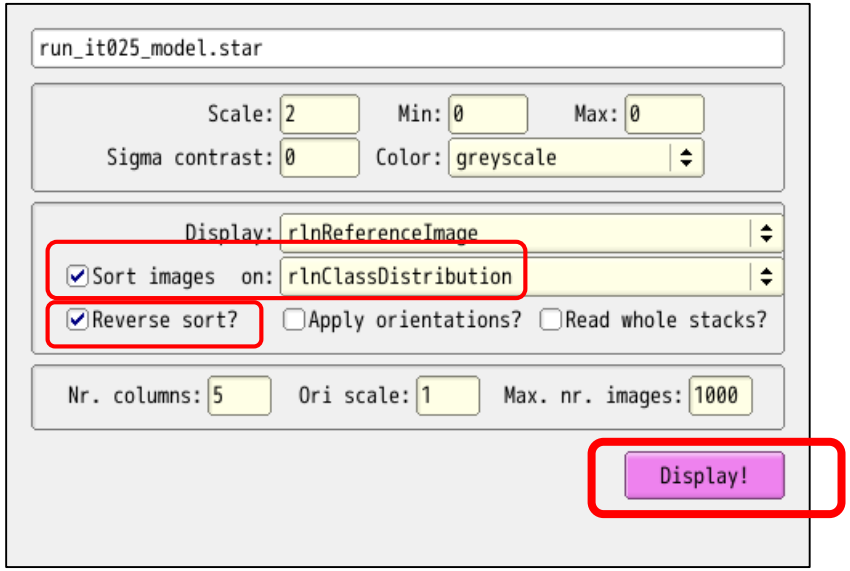
Number of MPI procs: 1
4 ber of threads: 4
submit to queue? No
Queue name: openmpi
Queue submit command: qsub
Standard submission script: relion-3.1/scripts/qsub.csh
Minimum dedicated cores per node: 24
Additional arguments:

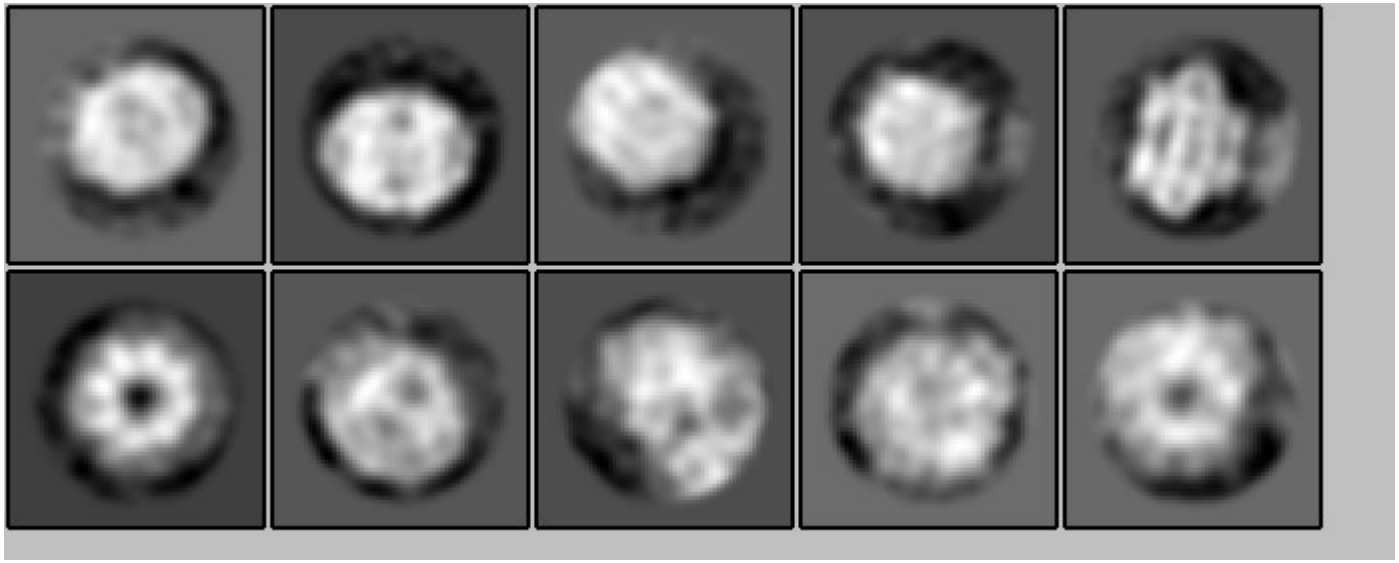
Run!

※*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

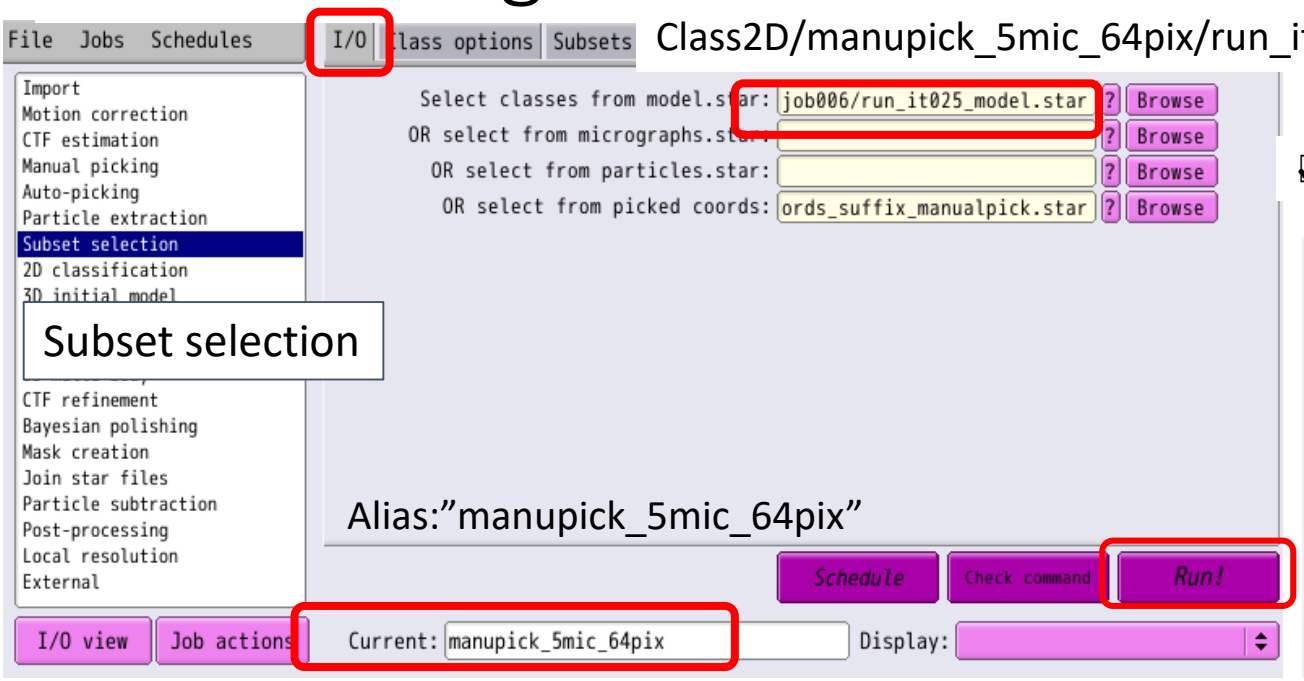






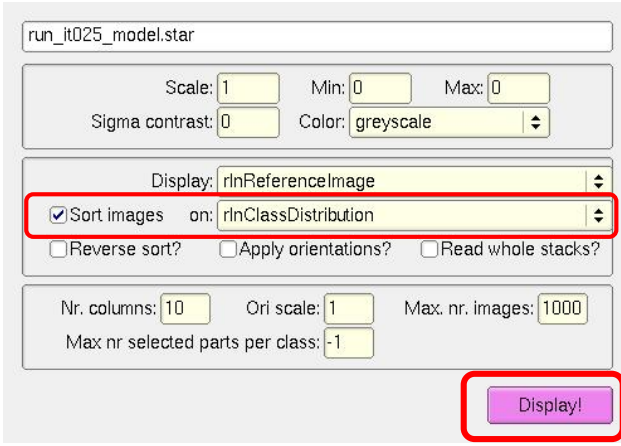
1.8 Selecting templates for auto-picking

Class2D/manupick_5mic_64pix/run_it025_model.star

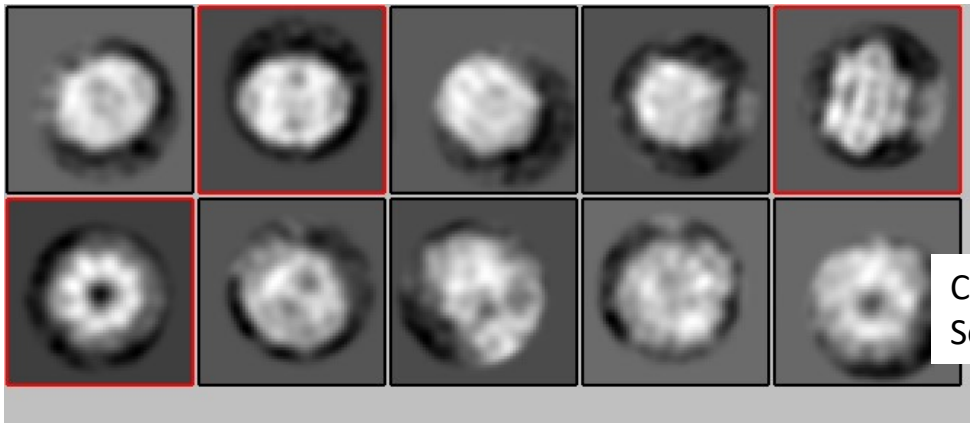


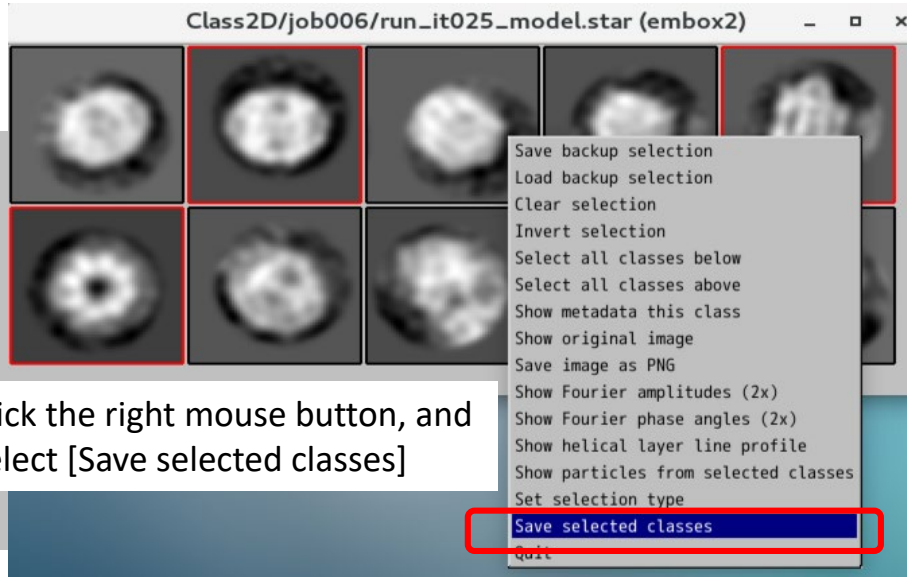
☑Sort images on: rlnClassDistribution

Sorted by number of particles belong to the cl



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

Input micrographs for autopick: 4/micrographs_selected.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 de ?
OR: use Laplacian-of-Gaussian? No ?

No

Alias:"manu_5mic_64pix"

Schedule | Check command | Run!

I/O view | Job actions | Current: manu_5mic_64pix

These four parameters have to be optimized.

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

Picking threshold: 0.05 — 0.0
Minimum inter-particle distance (A): 100 — 100
Maximum stddev noise: 1.1 — 1.1
Minimum avg noise: -999 — -999
Write FOM maps? No
Read FOM maps? No
Shrink factor: 0
Use GPU acceleration? Yes — Yes
Which GPUs to use: 0

Run!

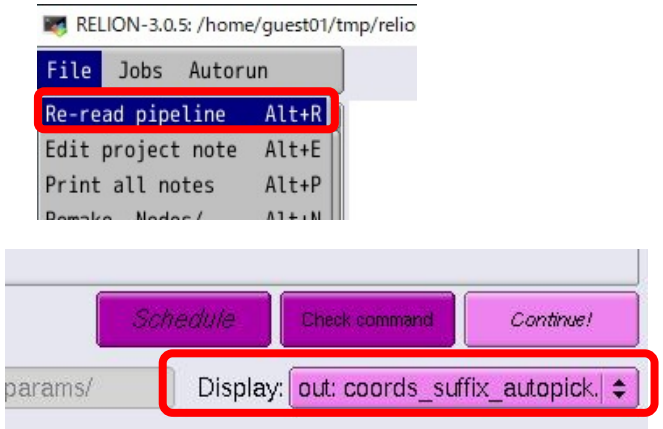
I/O | Laplacian | References | autopicking | Helix | Running

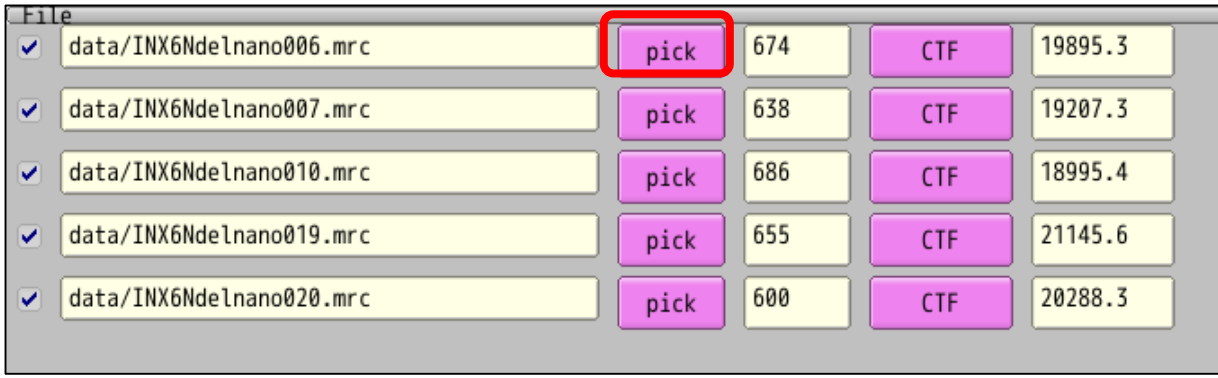
Lowpass filter references (A) 20
Highpass filter (A) -1
Pixel size in references (A) 3.465
A) 160
g) 5
References have inverted contrast? Yes
Are References CTF corrected? Yes
Ignore CTFs until first peak? No

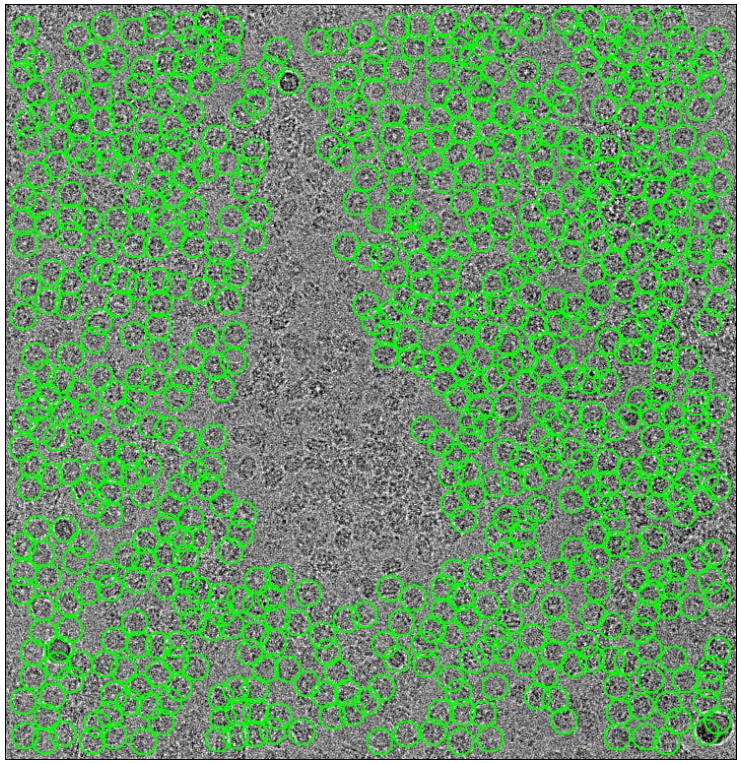
3.465 Å
(=1.232 Å * 180 pixel /64 pixel)

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

Auto-picking;reference-based; for 5mic

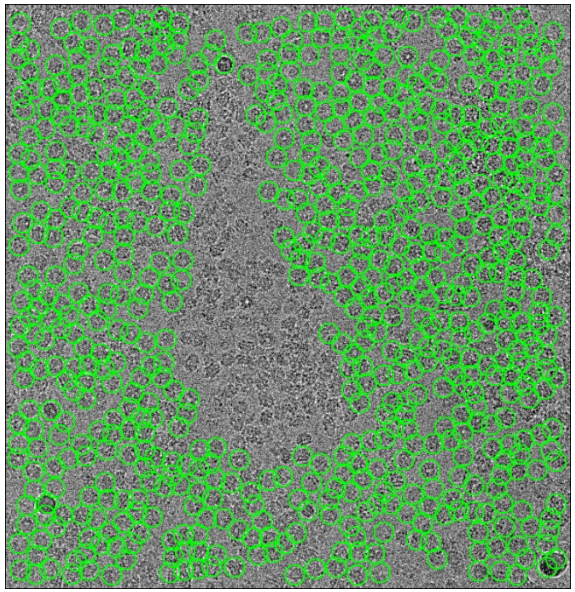




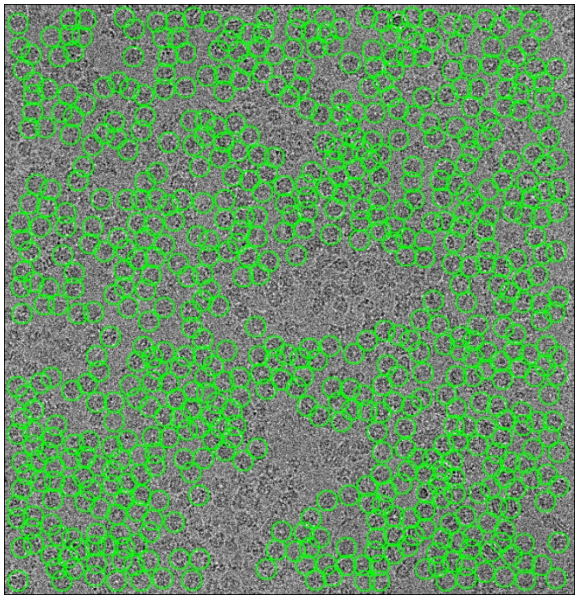


Total number of particles from 100 micrographs is 3253
i.e. on average there were 651 particles per micrograph.

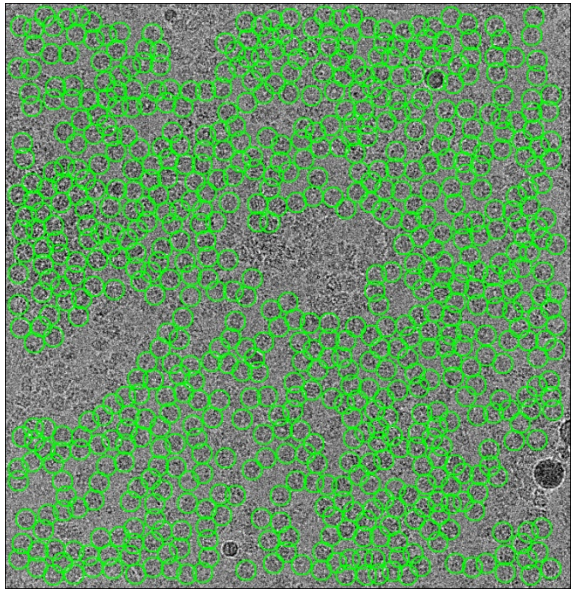
Auto Picking



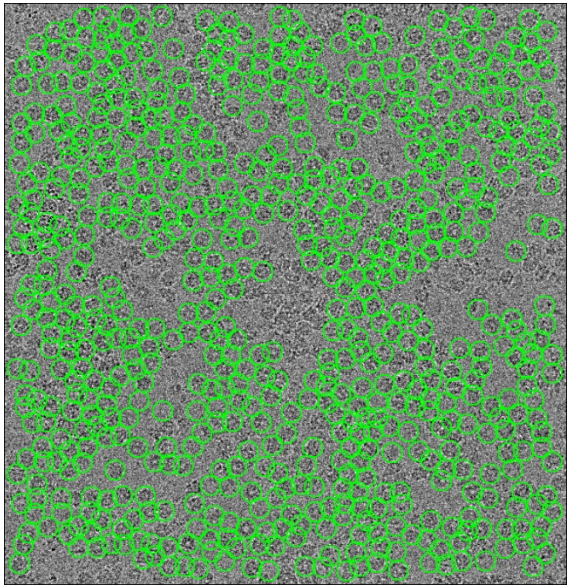
006 : 674 particles



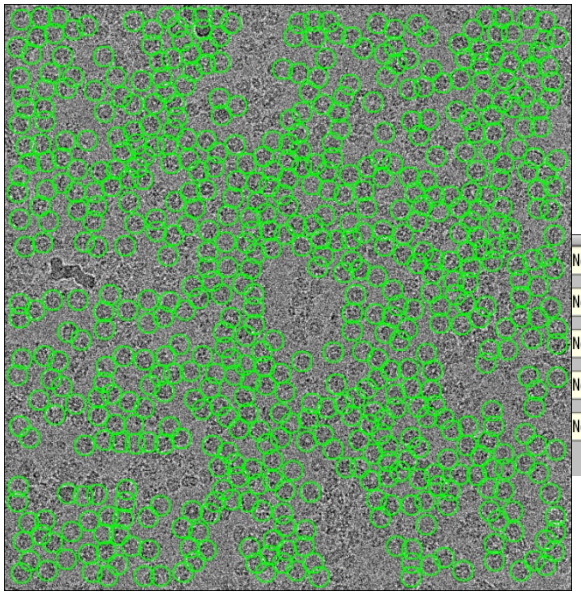
007 : 638 particles



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 de ?
OR: use Laplacian-of-Gaussian? No ?

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

Lowpass filter references (A) 20 ?
Highpass filter (A) -1 ?
Pixel size in references (A) 3.465 ?
A) 160 ?
g) 5
References have inverted contrast? Yes ?
Are References CTF corrected? Yes ?
Ignore CTFs until first peak? No ?

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

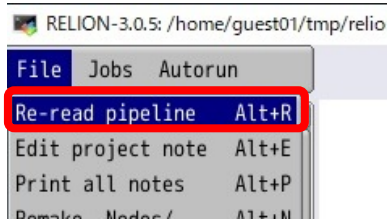
I/O | Laplacian | References | autopicking | Helix | Running

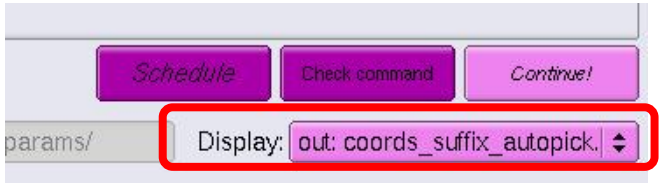
Picking threshold: 0.05 — 0.0
Minimum inter-particle distance (A): 100 — 100
Maximum stddev noise: 1.1 — 1.1
Minimum avg noise: -999 — -999
Write FOM maps? No
Read FOM maps? No
Shrink factor: 0
Use GPU acceleration? Yes — Yes
Which GPUs to use: 0

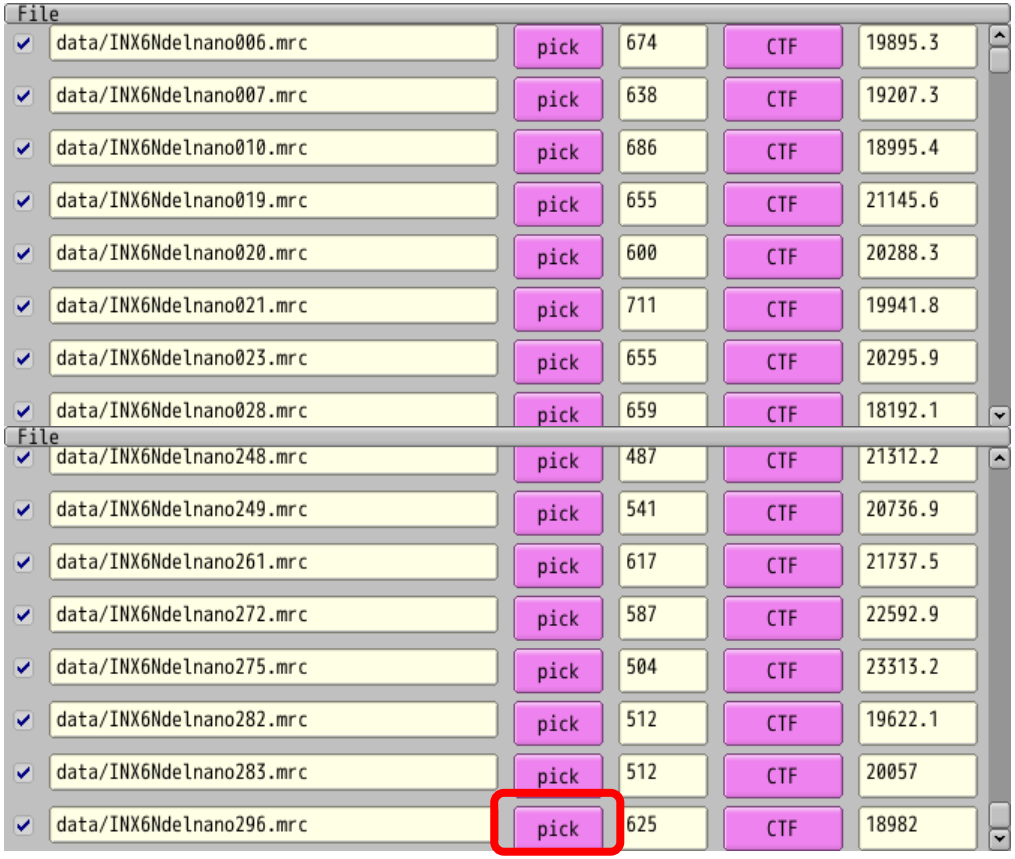
Input your GPU number (0,1,2,3)

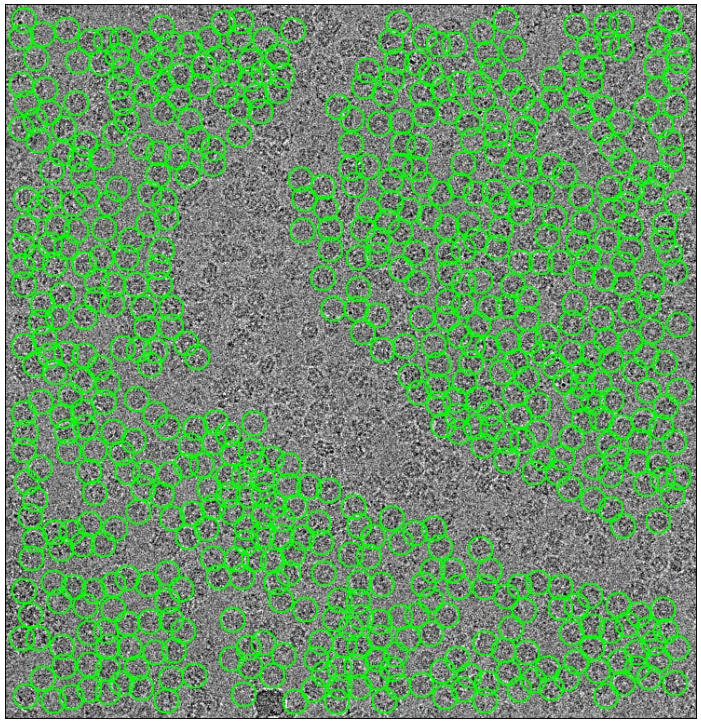
Run!

Auto-picking;reference-based; for 100mic









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

File Jobs Schedules

I/O extract Helix Running

Import
Motion correction
CTF estimation
Manual picking
Auto-picking
Particle extraction
Subset selection
2D classification
3D multi-body
CTF refinement
Bayesian polishing
Mask creation
Join star files
Particle subtraction
Post-processing
Local resolution
External

Particle extraction

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 th

OR: re-c

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

Autopick/manu_100mic_64pix/coords_suffix_autopick.star

Alias"refauto_manu_100mic_64pix"

Schedule

I/O view Job actions Current: refauto_manu_100mic_64pix Disp

I/O extract Helix Running

Particle box size (pix): 180 ?

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 ?

Yes

Re-scaled size (pixels): 64 ?

64

Run!

Schedule Check command Continue!

Display: out: particles.star

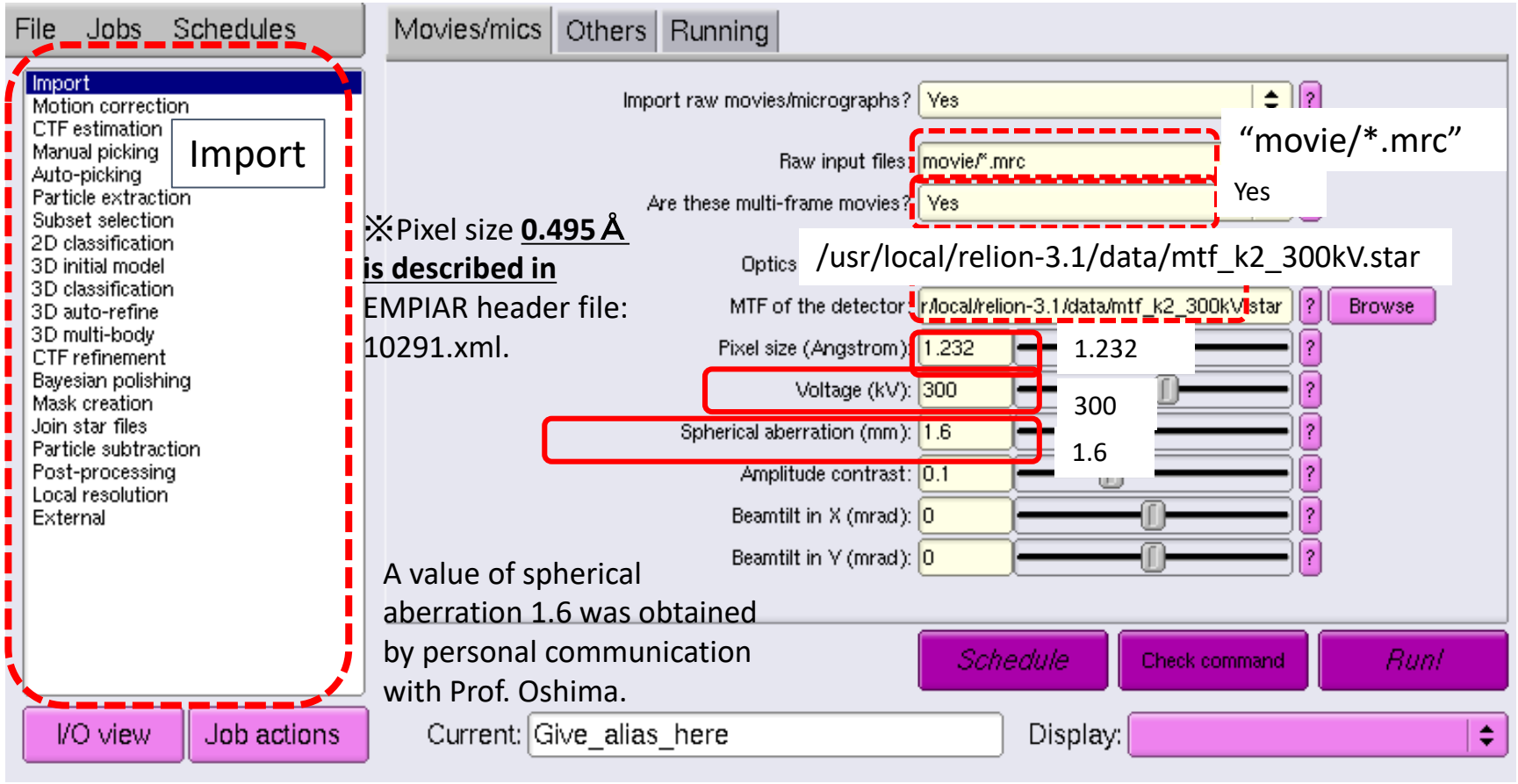
60292 particles are stored in:
Extract/template_based/particles.starに保存

Appendix:
Motion Correction

Import movie/*.mrc

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

Job type browser



※Pixel size **0.495 Å is described in** EMPIAR header file: 10291.xml.

A value of spherical aberration 1.6 was obtained by personal communication with Prof. Oshima.

Click [Run!]

Motion Correction

No GPU, 2min for 5 movies

File Jobs Schedules

- 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

I/O view | Job actions

I/O | Motion | Running

Import/job001/movies.star

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 — 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!

Current: Give_alias_here

Display:

I/O | Motion | Running

Number of patches X, Y: 5 — 5 | 5 — 5

Group frames: 1

Binning factor: 1

Gain-reference image: | Browse

Gain rotation: No rotation (0)

Gain flip: No flipping (0)

Defect file: | Browse

Use RELION's own implementation? Yes — Yes

MOTIONCOR2 executable: mCor2-1.1.0/MotionCor2_1.1.0-Cuda80 | Browse

Which GPUs to use: 0

I/O | Motion | Running

Number of MPI procs: 1 — 1

Number of threads: 6 — 6

Submit to queue? No

Queue name: openmpi

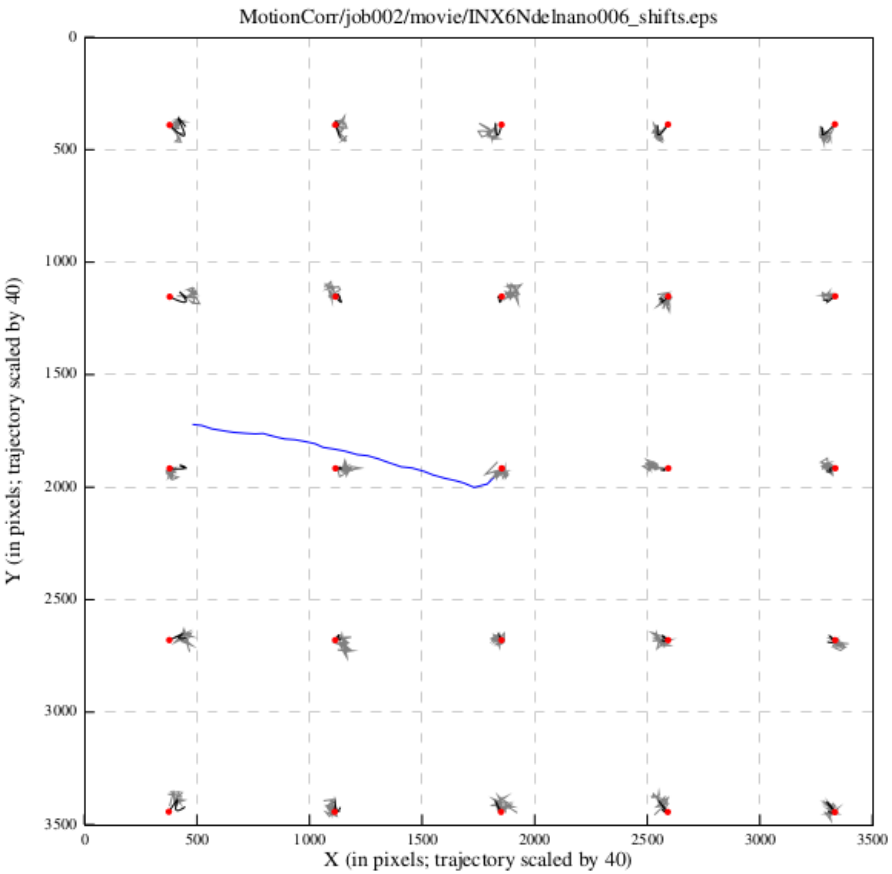
Queue submit command: qsub

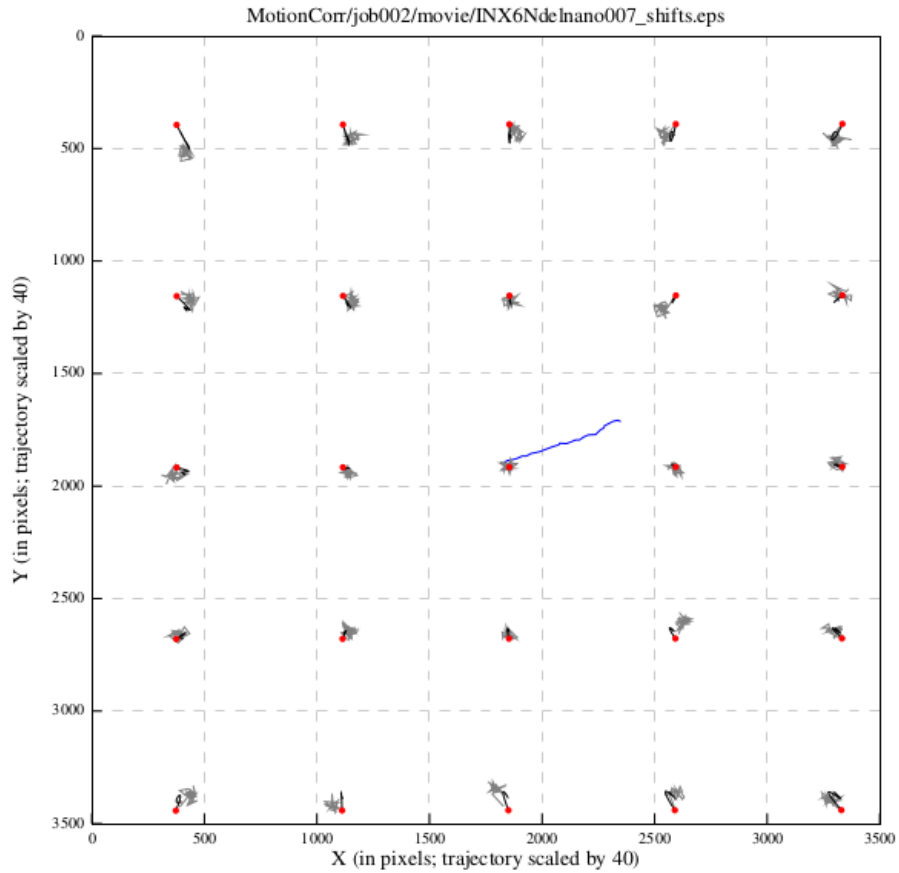
Standard submission script: /usr/local/relion-3.1/scripts/qsub.csh | Browse

Minimum dedicated cores per node: 24

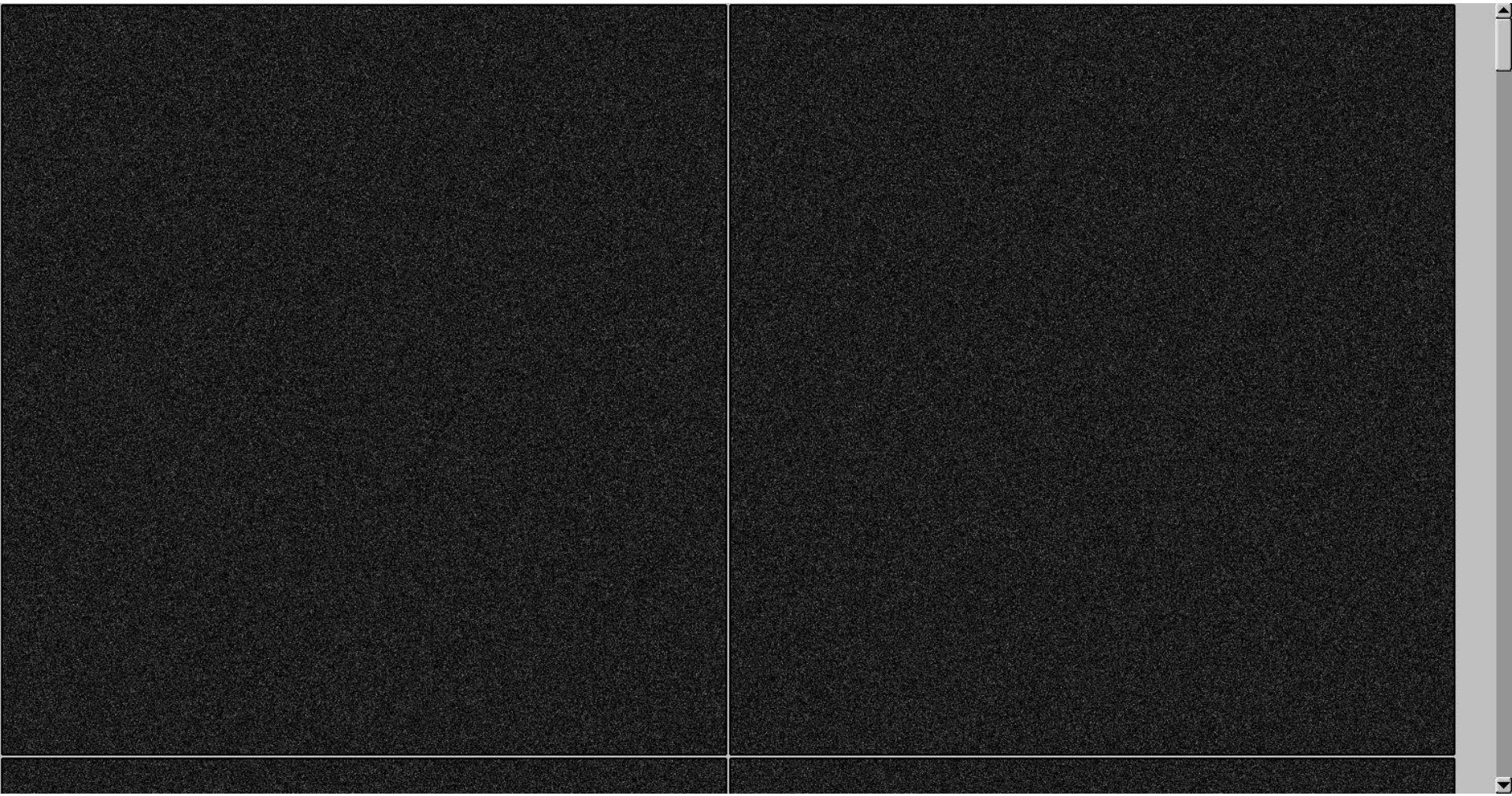
Additional arguments:

Click [Run!]


MotionCorr/job002/movie/INX6Ndelnano006_shifts.eps
Y (in pixels; trajectory scaled by 40)
X (in pixels; trajectory scaled by 40)
0
500
1000
1500
2000
2500
3000
3500

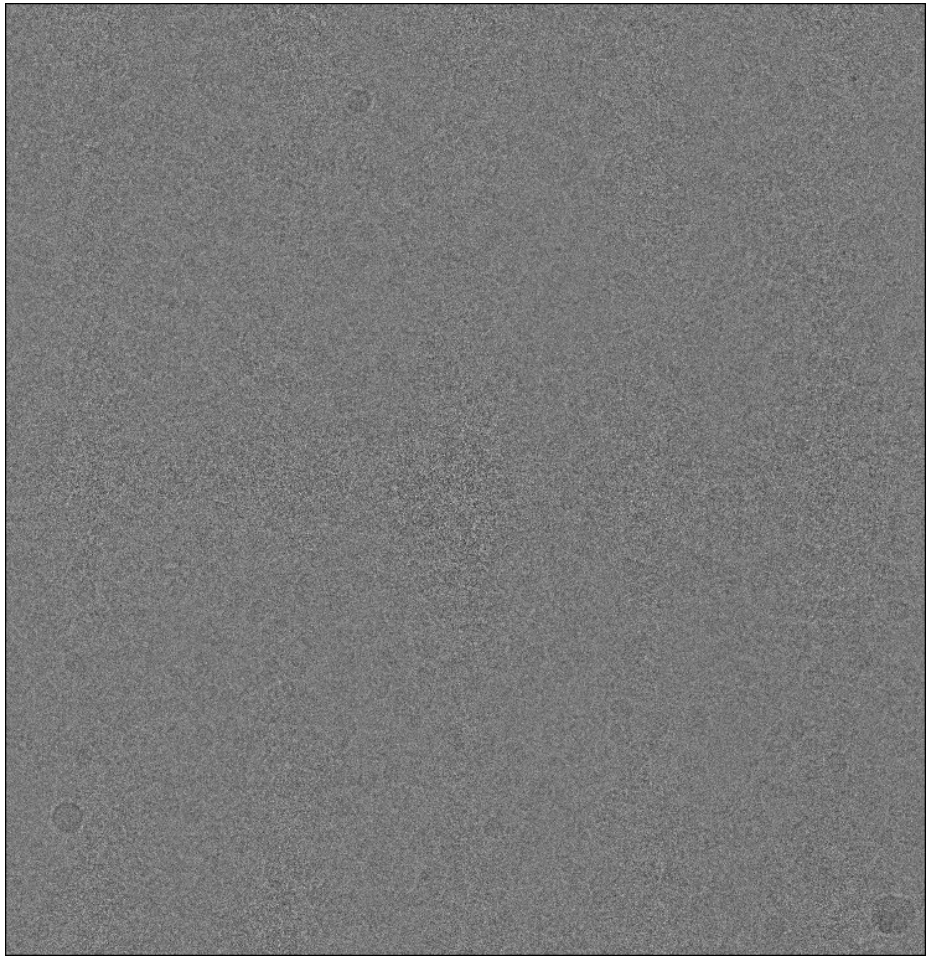

MotionCorr/job002/movie/INX6Ndelnano007_shifts.eps
Y (in pixels; trajectory scaled by 40)
X (in pixels; trajectory scaled by 40)
0
500
1000
1500
2000
2500
3000
3500

movie/INX6Ndelnano006.mrc

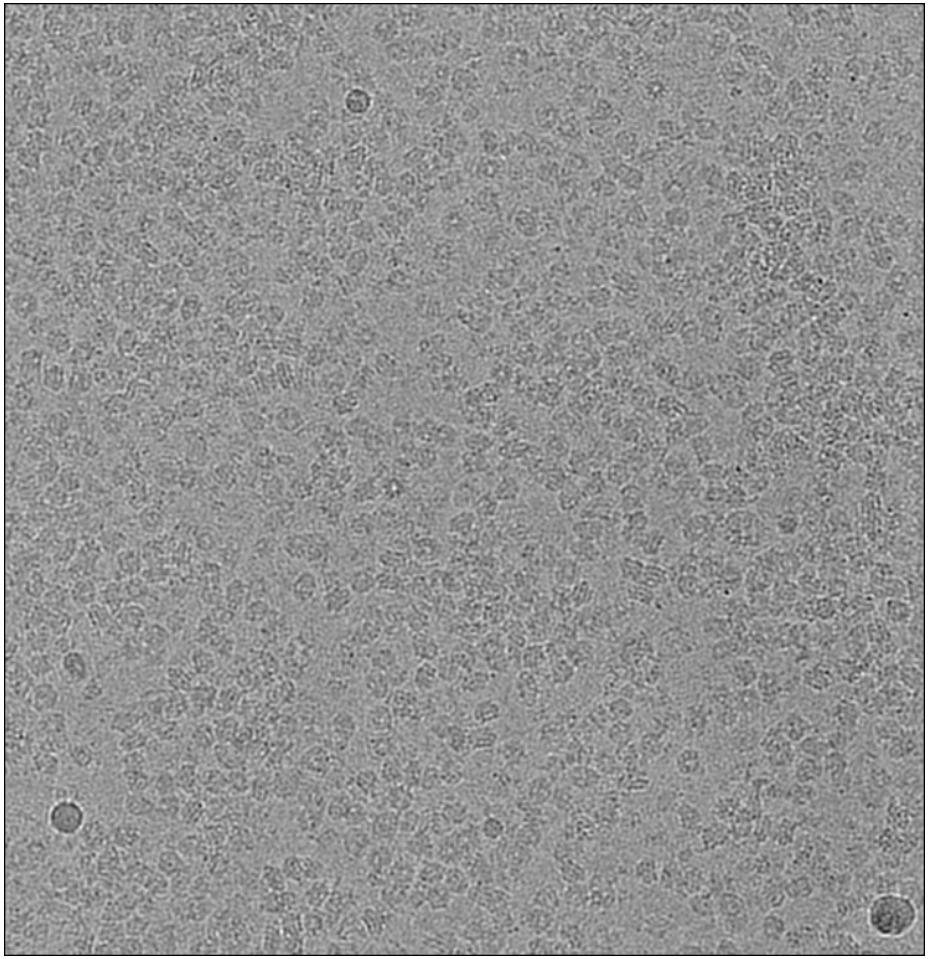


First two frames among 30 frames

MotionCorr/job002/movie/INX6Ndelnano006.mrc



Motion Corrected microraph

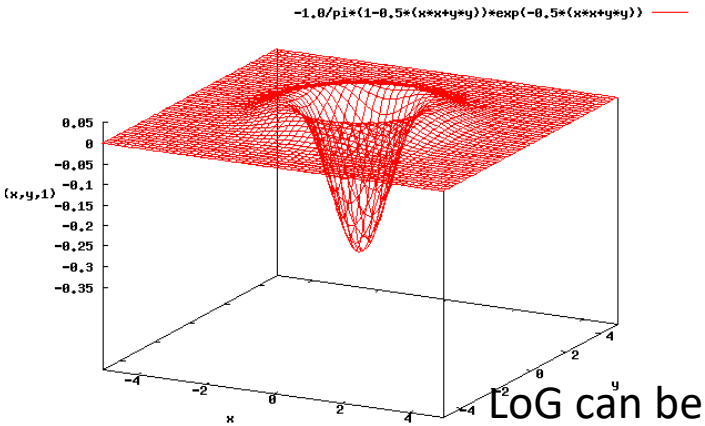


20 low pass filtered

Appendix:
LoG-based autopicking

“Blob” detection using LoG filter

LoG : Laplacian of Gaussian



$$\phi(\boldsymbol{r}) = \frac{1}{2\pi\sigma^2}\exp\left[-\frac{\boldsymbol{r}^2}{2\sigma^2}\right]$$

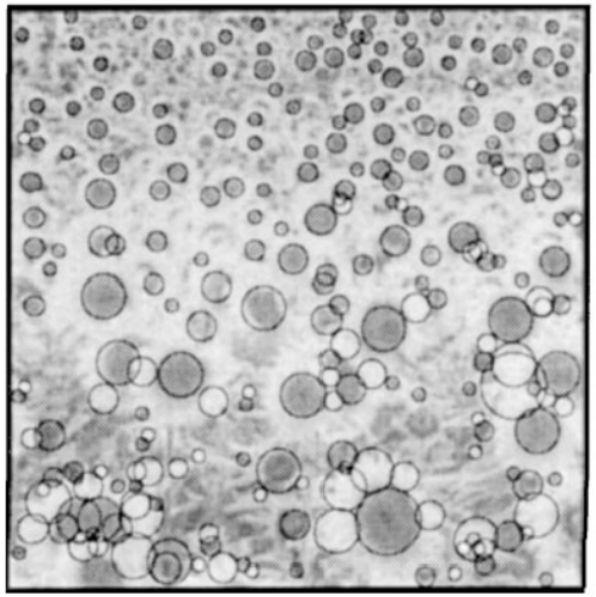
$$\mathrm{LoG}(\boldsymbol{r}) = \frac{\partial^2\phi}{\partial x^2} + \frac{\partial^2\phi}{\partial y^2} = -\frac{2}{\sigma^2}\left(1 - \frac{x^2+y^2}{2\sigma^2}\right)\phi(\boldsymbol{r})$$

[Big Gauss] – [normal Gauss]

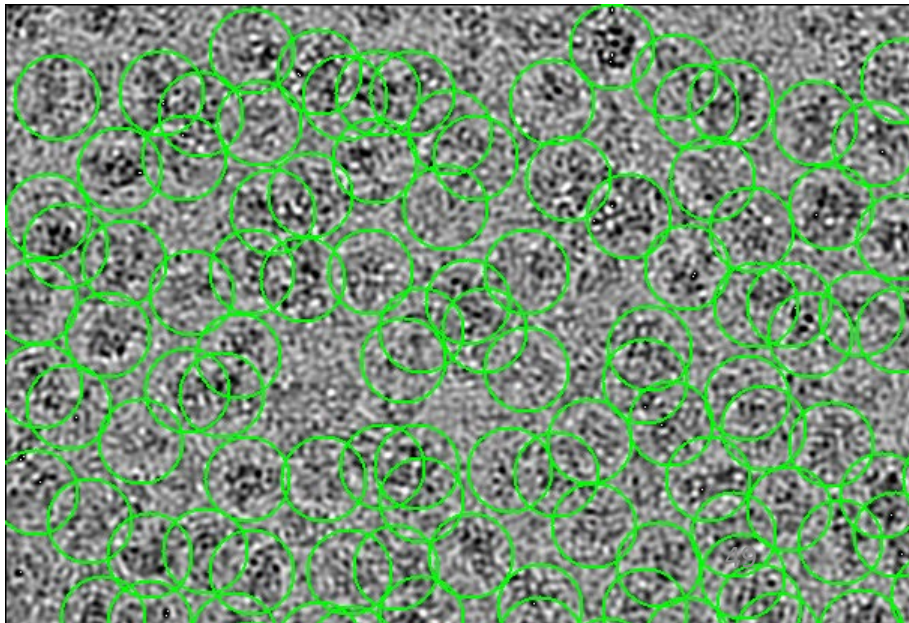
$$\mathrm{LoG}(\boldsymbol{r}) \cong \mathrm{DoG}(\boldsymbol{r}) = \phi(\boldsymbol{r}|k\sigma) - \phi(\boldsymbol{r}|\sigma)$$

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)

File Jobs Schedules

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

Auto-picking

I/O view | Job actions

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

Input micrographs for autopick: 4/micrographs_selected.star ? Browse

Select/5mic/micrographs_selected.star

Pixel size in mic

2D references:

OR: provide a 3D reference? No ?

3D reference: ? Browse

Symmetry: C1 ?

3D angular sampling: 30 degrees ?

OR: use Laplacian-of-Gaussian? Yes ?

Yes

Alias : LoG_5mic

Schedule | Check command | Run!

Current: LoG_5mic

Display:

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

Min. diameter for LoG filter (A): 120 ?

Max. diameter for LoG filter (A): 160 ?

Are the particles white? No ?

Maximum resolution to consider (A): 20 ?

120
160

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

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 ? Browse

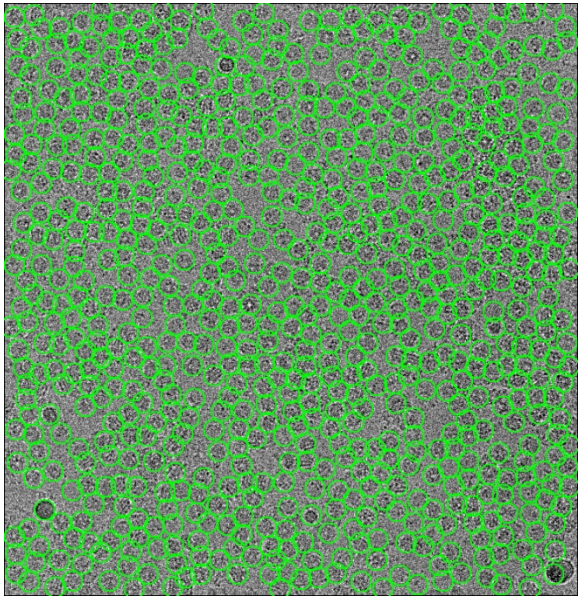
Minimum dedicated cores per node: 24 ?

Additional arguments: ?

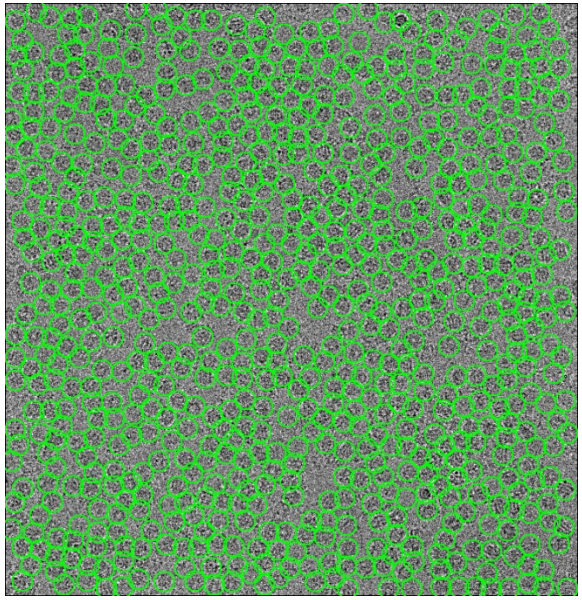
※dmin,dmaxをユーザーが指定。それらをもとに全部で4+3+4=11通りのdを試す。
dmin/5, dmin/4, dmin/3, dmin/2, dmin, (dmin+dmax)/2, dmax, 2dmax, 3dmax, 4dmax, 5dmax

Run!

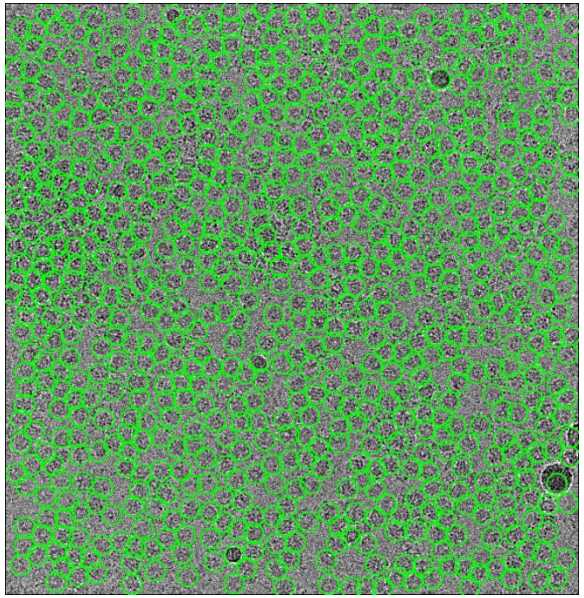
LoGbased Auto Picking



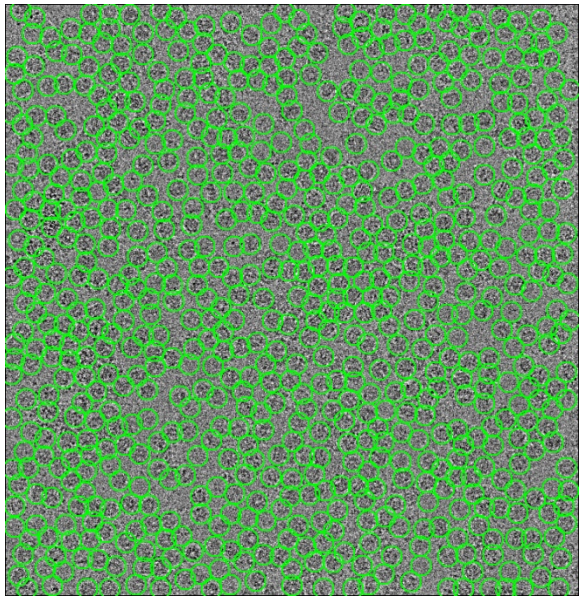
006 : 726 particles



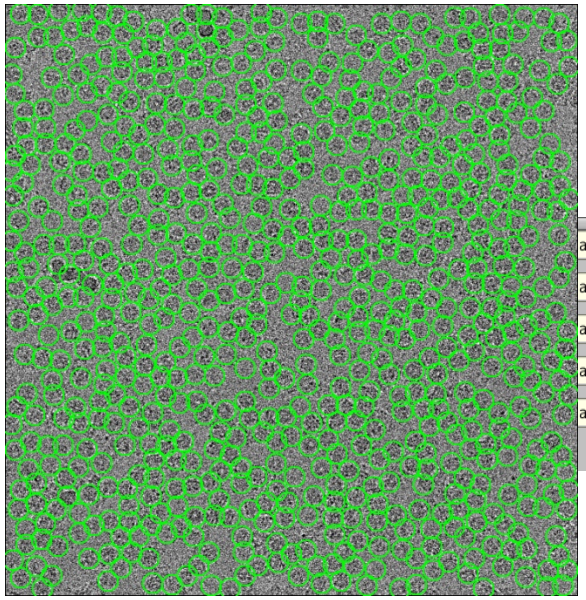
007 : 726 particles



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	713	CTF	21145.6
a/INX6Ndelnano020.mrc	pick	708	CTF	20288.3

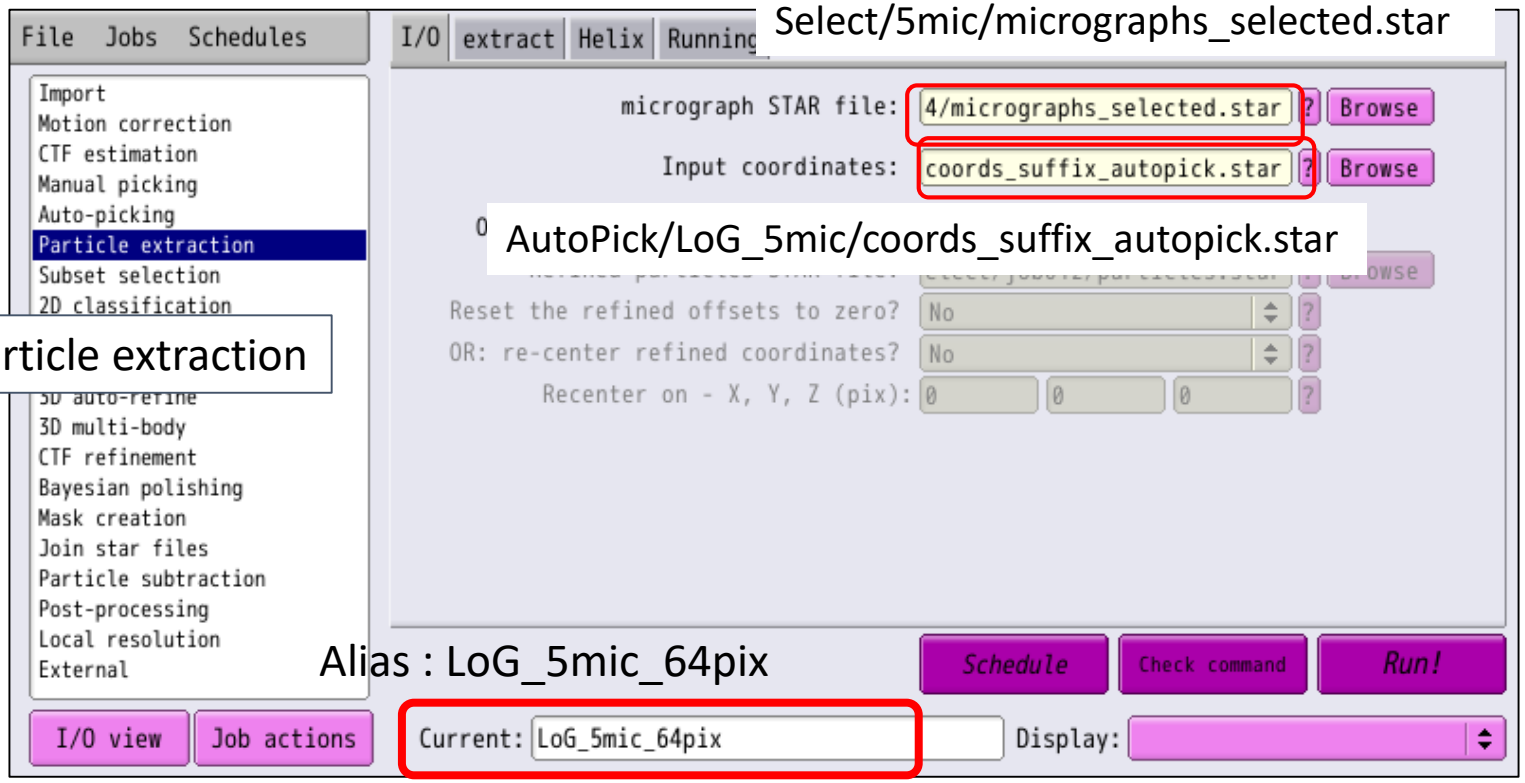
1.6 Particle extraction

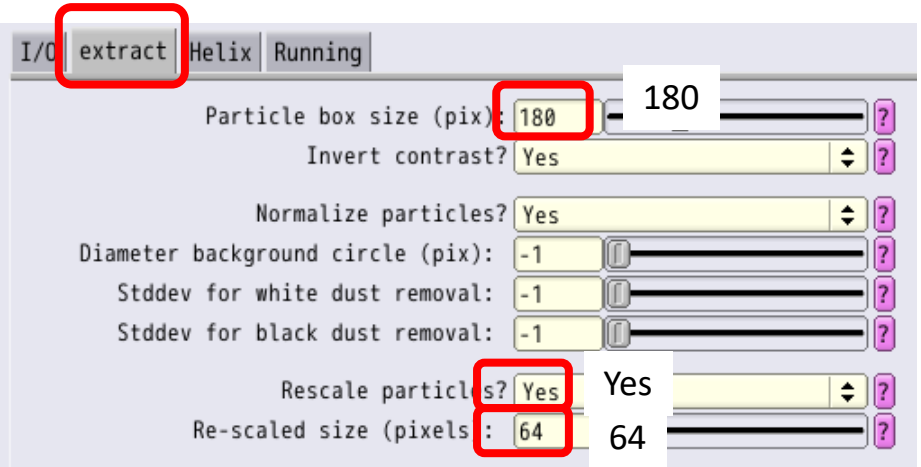
Select/5mic/micrographs_selected.star

AutoPick/LoG_5mic/coords_suffix_autopick.star

Particle extraction

Alias : LoG_5mic_64pix



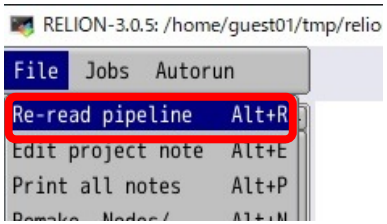


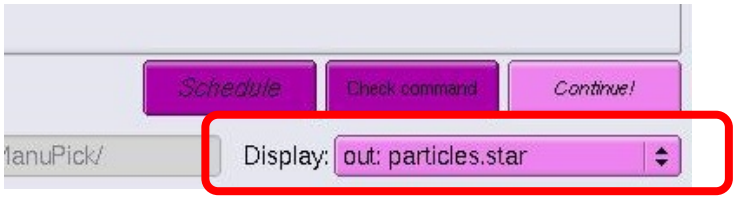
180

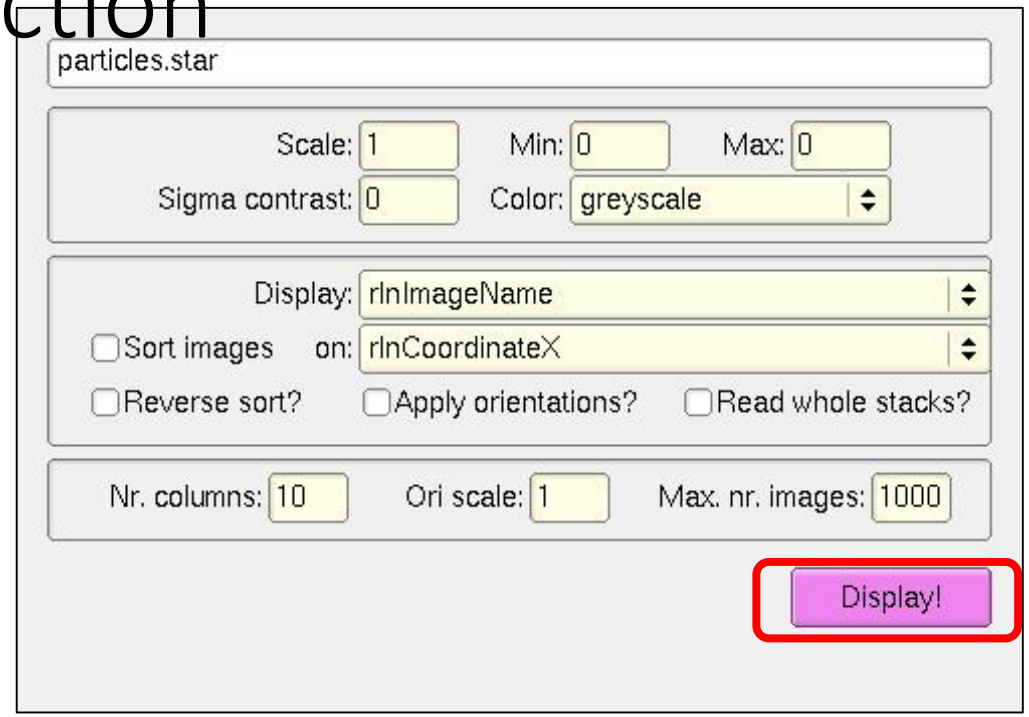
Yes

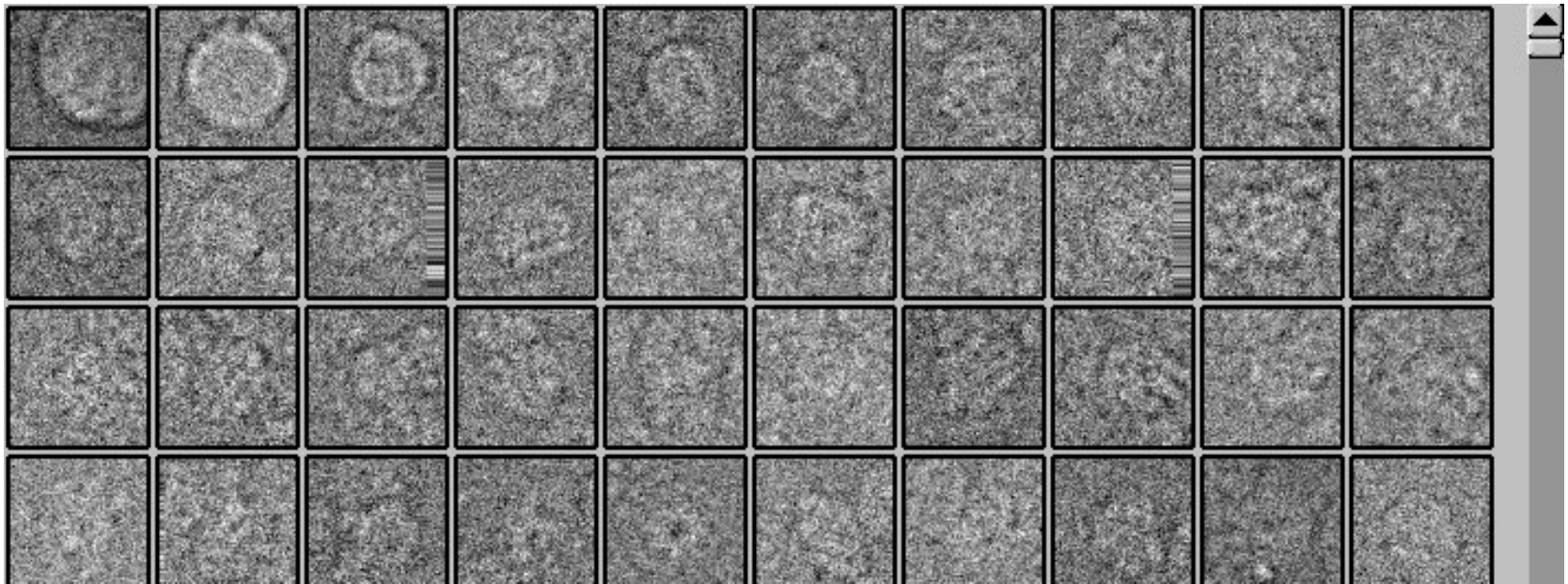
64

1.6 Particle extraction









1.7 Making templates for autopicking（1分）

[2 GPU;]

Extract/LoG_5mic_64pix/particles.star

File Jobs Schedules

Import
Motion correction
CTF estimation
Manual picking
Auto-picking
Particle extraction
Subset selection
2D classification
3D initial model
CTF refinement
Bayesian polishing
Mask creation
Join star files
Particle subtraction
Post-processing
Local resolution
External

2D classification

I/O view | Job actions

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

Input images STAR file: tract/job023/particles.star ? Browse
Continue from here: ? Browse

Number of classes: 10 (10)
Regularisation parameter T: 2
Number of iterations: 25
Use fast subsets (for large data sets)? No
Mask diameter (A): 160 (160)
Mask individual particles with zeros? Yes
Limit resolution E-step to (A): -1

Alias : LoG_5mic_64pix

Schedule | Check command | Run!

Current: LoG_5mic_64pix
Display:

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

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
Use GPU acceleration? Yes (Yes)
Which GPUs to use: 0:1

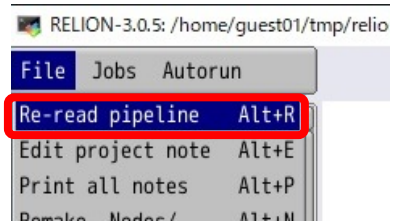
I/O | CTF | Optimisation | Sampling | Helix | Compute | Running

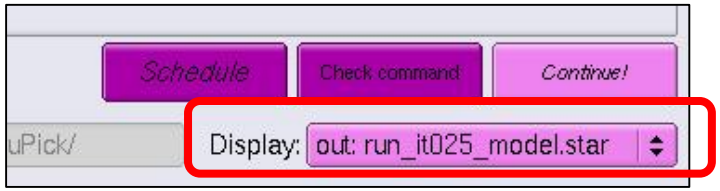
Number of MPI procs: 3 (3)
Number of threads: 2 (2)
Submit to queue? No
Queue name: openmpi
Queue submit command: qsub
Standard submission script: relion-3.1/scripts/qsub.csh ? Browse
Minimum dedicated cores per node: 24
Additional arguments:

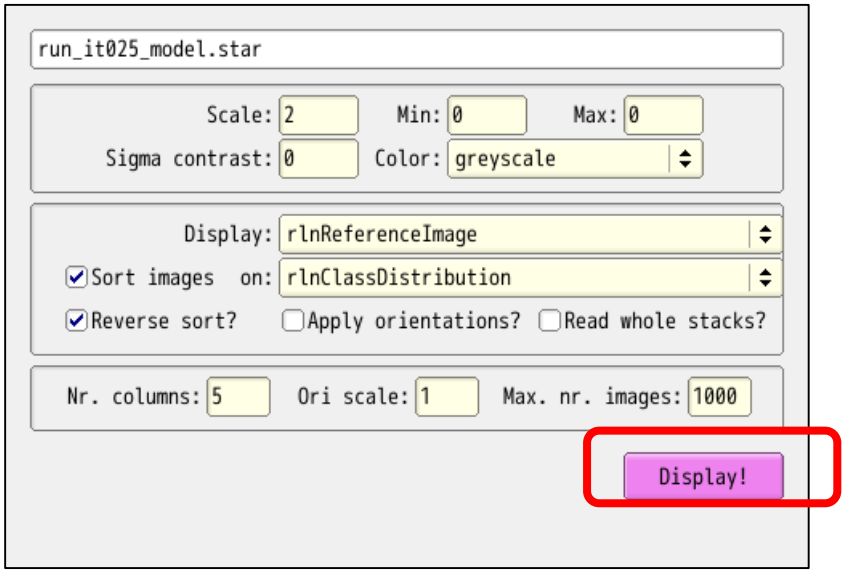
Run!

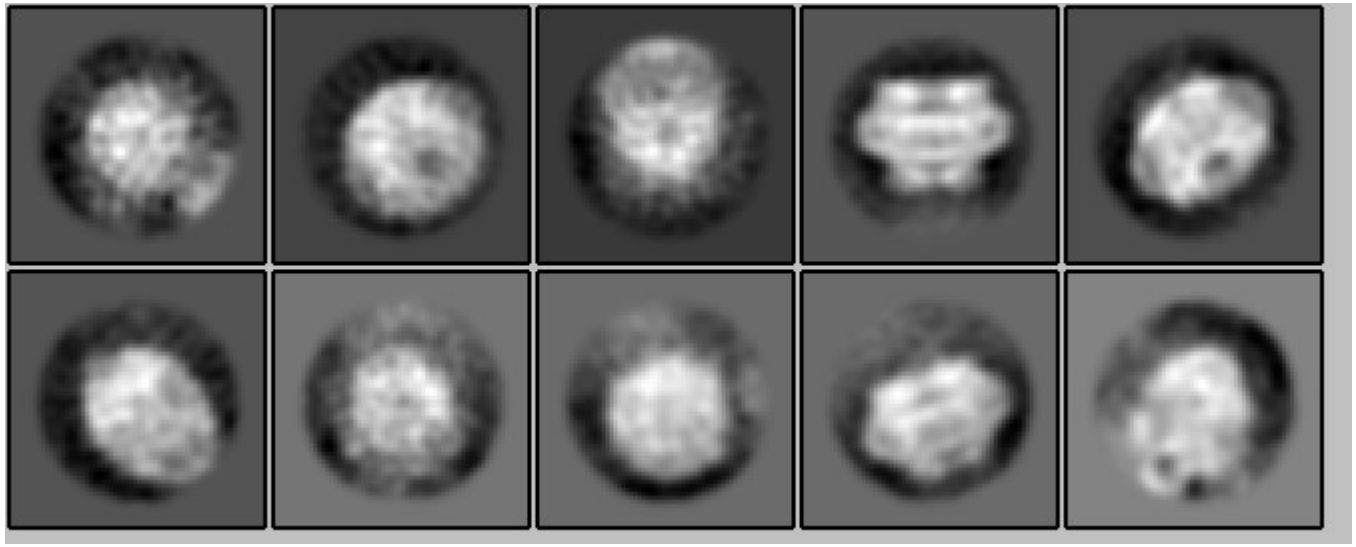
Input your GPU numbers.
Let's use 2 GPUs !

1.7 Making templates for autopicking









No C8 top view….