

CiCLE 単粒子解析リモート講習会

2021/06/24-25

**CiCLE**

Cyclic Innovation for Clinical Empowerment

# Tutorial of cryoSPARC : Innexin-6 using Blob picker

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2021/06/24-25



Following five steps are the same.

Step 0: Log-in

Step 1: Create a Project

Step 2: Create a Workspace

Step 4: J1:Import Micrographs

Step 6: J2:CTF estimation

# J3: Curate Exposures to chose 5 micrographs

Details Job Builder

JOB BUILDER

Search...

Exposure Curation (1)

Manually Curate Exposures

J2 Patch CTF (M)

J3 (Manually Curate Exposures) BUILDING

New Job J3

INPUTS

exposures (exposure) 1  
Min: 1, Max: Infinity, Repeats: no

Group 1 → J2.exposures

particles (particle) 0  
Min: 0, Max: Infinity, Repeats: no

PARAMETERS

Cancel Queue Create

Outputs

exposures

exposures (exposure) 0  
Min: 1, Max: Infinity, Repeats: no

particles (particle) 0  
Min: 0, Max: Infinity, Repeats: no

PARAMETERS

exposure Count: 50

exposures\_incomplete

exposure Count: 0

# J3: Curate Exposures to chose 5 micrographs



P9: Blob\_picker\_EMPIAR-10291-5..

W1: Test Workspace

J3



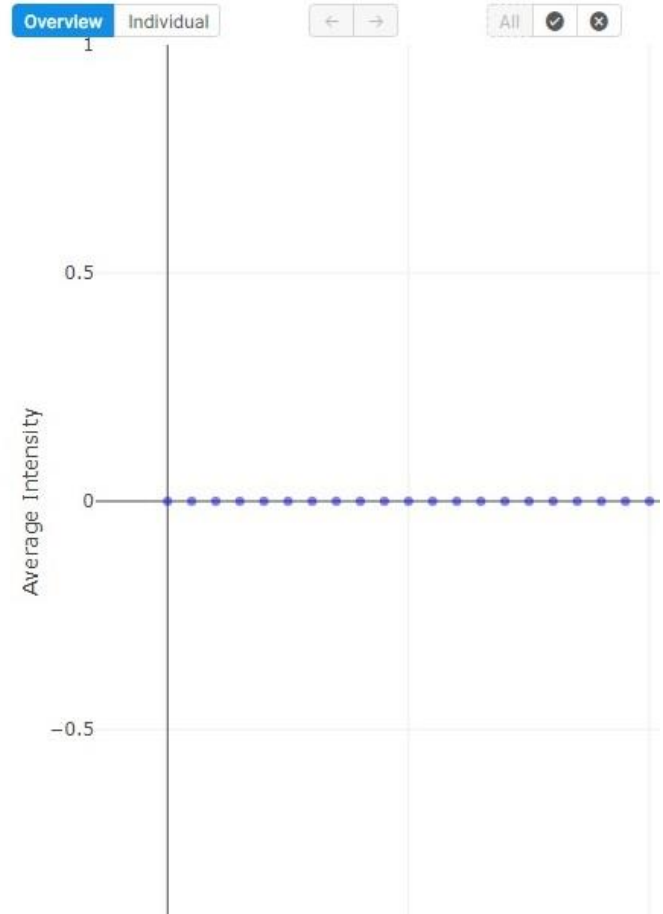
Interactive Overview Inputs and Parameters Outputs Metadata

Click [CTF Fit] to sort micrographs by CTF Fit resolution

Interactive Overview Inputs and Parameters Outputs Metadata

Total: 50 Accepted: 0 Rejected: 0 Selected: 50

#	Avg Inten.	DF Avg	Astig	Phase	CTF Fit	CTF CC	DF Range	Tilt Angle	Rel Ice Thick.
0	0.00	18859.27	286.56	0.00	3.36	0.00	200.42	1.07	0.99
1	0.00	19764.39	295.24	0.00	3.29	0.00	185.50	1.16	1.00
2	0.00	18040.11	315.57	0.00	3.01	0.00	225.43	2.32	0.99
3	0.00	19384.49	279.55	0.00	3.15	0.00	62.59	0.38	0.99
4	0.00	20762.72	301.92	0.00	3.19	0.00	131.86	0.62	0.99
5	0.00	19490.10	321.89	0.00	3.25	0.00	235.66	1.35	0.99
6	0.00	20822.42	324.41	0.00	3.08	0.00	202.37	1.99	0.99
7	0.00	19057.53	325.82	0.00	3.24	0.00	129.13	0.69	0.99
8	0.00	20459.54	317.62	0.00	3.31	0.00	135.21	0.20	0.99
9	0.00	20948.03	300.11	0.00	3.29	0.00	124.79	0.38	0.99
10	0.00	19271.78	315.20	0.00	3.18	0.00	121.07	0.83	0.99
11	0.00	22158.61	270.35	0.00	3.41	0.00	238.85	1.26	1.00
12	0.00	17263.65	301.90	0.00	3.17	0.00	140.22	1.65	1.00
13	0.00	15184.32	292.43	0.00	3.10	0.00	105.76	0.95	1.00
14	0.00	16868.62	269.51	0.00	2.92	0.00	138.43	1.66	0.99
15	0.00	20916.81	332.67	0.00	3.13	0.00	173.83	1.59	1.00
16	0.00	23697.07	298.17	0.00	3.13	0.00	126.59	1.56	0.99
17	0.00	22361.74	280.86	0.00	3.12	0.00	264.55	1.94	1.00
18	0.00	22391.86	301.64	0.00	3.08	0.00	119.96	0.87	1.00
19	0.00	16804.89	347.21	0.00	3.10	0.00	171.93	1.02	1.00
20	0.00	19811.17	317.46	0.00	3.26	0.00	113.30	1.16	1.00
21	0.00	17550.73	291.34	0.00	3.00	0.00	142.76	1.48	1.00
22	0.00	18949.22	272.69	0.00	3.04	0.00	187.88	1.32	1.00
23	0.00	17797.35	313.50	0.00	3.09	0.00	142.80	1.44	1.00
24	0.00	16653.01	316.37	0.00	3.22	0.00	186.46	1.65	0.99
25	0.00	22337.41	302.95	0.00	3.22	0.00	228.69	2.23	0.99
26	0.00	22946.32	327.75	0.00	3.18	0.00	135.70	1.65	1.00
27	0.00	17908.13	280.29	0.00	3.08	0.00	141.50	1.18	1.00
28	0.00	15813.52	303.95	0.00	3.00	0.00	150.72	1.51	1.00
29	0.00	20521.20	288.42	0.00	3.04	0.00	168.46	1.96	0.99
30	0.00	19087.72	294.05	0.00	3.38	0.00	139.64	0.75	1.00
31	0.00	19212.50	297.14	0.00	3.12	0.00	92.40	1.01	1.00



# J3: Curate Exposures to chose 5 micrographs



P9: Blob\_picker\_EMPIAR-10291-5..

W1: Test Workspace

J3



Interactive

Overview

Inputs and Parameters

Outputs

Metadata

Interactive

Overview

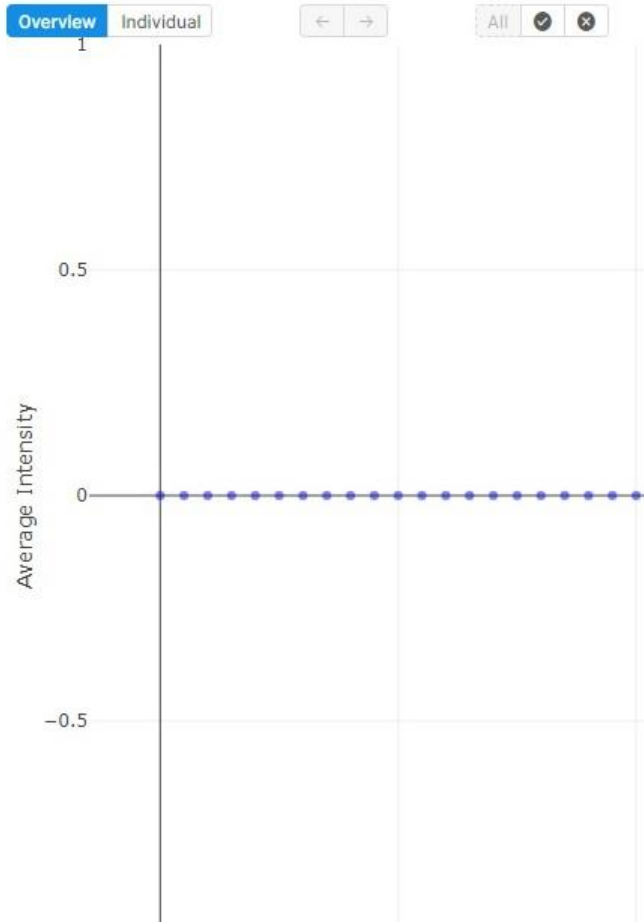
Inputs and Parameters

Outputs

Metadata

Total: 50 Accepted: 0 Rejected: 0 Selected: 50

#	Avg Inten.	DF Avg	Astig	Phase	CTF Fit▲	CTF CC	DF Range	Tilt Angle	Rel Ice Thick.
14	0.00	16868.62	269.51	0.00	2.92	0.00	138.43	1.66	0.99
35	0.00	18555.86	292.92	0.00	2.94	0.00	146.25	1.17	1.00
21	0.00	17550.73	291.34	0.00	3.00	0.00	142.76	1.48	1.00
28	0.00	15813.52	303.95	0.00	3.00	0.00	150.72	1.51	1.00
2	0.00	18040.11	315.57	0.00	3.01	0.00	225.43	2.32	0.99
29	0.00	20521.20	288.42	0.00	3.04	0.00	168.46	1.96	0.99
38	0.00	20991.48	269.37	0.00	3.04	0.00	232.51	1.13	1.00
22	0.00	18949.22	272.69	0.00	3.04	0.00	187.88	1.32	1.00
43	0.00	15628.46	312.99	0.00	3.05	0.00	275.49	0.72	1.00
36	0.00	18959.45	286.61	0.00	3.06	0.00	103.17	1.05	0.99
39	0.00	21215.71	352.12	0.00	3.06	0.00	150.36	1.27	1.00
18	0.00	22391.86	301.64	0.00	3.08	0.00	119.96	0.87	1.00
27	0.00	17908.13	280.29	0.00	3.08	0.00	141.50	1.18	1.00
6	0.00	20822.42	324.41	0.00	3.08	0.00	202.37	1.99	0.99
33	0.00	19702.57	305.75	0.00	3.09	0.00	135.91	0.93	1.00
23	0.00	17797.35	313.50	0.00	3.09	0.00	142.80	1.44	1.00
41	0.00	14115.84	297.99	0.00	3.10	0.00	214.86	1.63	0.99
19	0.00	16804.89	347.21	0.00	3.10	0.00	171.93	1.02	1.00
44	0.00	16594.49	290.11	0.00	3.10	0.00	167.82	0.67	0.99
13	0.00	15184.32	292.43	0.00	3.10	0.00	105.76	0.95	1.00
17	0.00	22361.74	280.86	0.00	3.12	0.00	264.55	1.94	1.00
31	0.00	19212.50	297.14	0.00	3.12	0.00	92.40	1.01	1.00
15	0.00	20916.81	332.67	0.00	3.13	0.00	173.83	1.59	1.00
16	0.00	23697.07	298.17	0.00	3.13	0.00	126.59	1.56	0.99
46	0.00	21178.86	304.74	0.00	3.14	0.00	233.15	1.37	1.00
3	0.00	19384.49	279.55	0.00	3.15	0.00	62.59	0.38	0.99
12	0.00	17263.65	301.90	0.00	3.17	0.00	140.22	1.65	1.00
49	0.00	18821.46	279.25	0.00	3.17	0.00	138.60	1.35	0.99
10	0.00	19271.78	315.20	0.00	3.18	0.00	121.07	0.83	0.99
26	0.00	22946.32	327.75	0.00	3.18	0.00	135.70	1.65	1.00
4	0.00	20762.72	301.92	0.00	3.19	0.00	131.86	0.62	0.99
24	0.00	16653.01	316.37	0.00	3.22	0.00	186.46	1.65	0.99
25	0.00	22337.41	302.95	0.00	3.22	0.00	228.69	2.23	0.99



# J3: Curate Exposures to chose 5 micrographs



P9: Blob\_picker\_EMPIAR-10291-5.. W1: Test Workspace J3

Interactive Overview Inputs and Parameters Outputs Metadata

Select each micrographs in the top 5, and click Individual

Interactive Overview Inputs and Parameters Outputs Metadata

Total: 50 Accepted: 0 Rejected: 0 Selected: 50

#	Avg Toton	DF Avg	Astig	Phase	CTF Fit	CTF CC	DF Range	Tilt Angle	Rel Ice Thick
14	0.00	16868.62	269.51	0.00	2.92	0.00	138.43	1.66	0.99
35	0.00	18555.86	292.92	0.00	2.94	0.00	146.25	1.17	1.00
21	0.00	17550.73	291.34	0.00	3.00	0.00	142.76	1.48	1.00
28	0.00	15813.52	303.95	0.00	3.00	0.00	150.72	1.51	1.00
2	0.00	18040.11	315.57	0.00	3.01	0.00	225.43	2.32	0.99
29	0.00	20521.20	288.42	0.00	3.04	0.00	168.46	1.96	0.99
38	0.00	20991.48	269.37	0.00	3.04	0.00	232.51	1.13	1.00
22	0.00	18949.22	272.69	0.00	3.04	0.00	187.88	1.32	1.00
43	0.00	15628.46	312.99	0.00	3.05	0.00	275.49	0.72	1.00
36	0.00	18959.45	286.61	0.00	3.06	0.00	103.17	1.05	0.99
39	0.00	21215.71	352.12	0.00	3.06	0.00	150.36	1.27	1.00
18	0.00	22391.86	301.64	0.00	3.08	0.00	119.96	0.87	1.00
27	0.00	17908.13	280.29	0.00	3.08	0.00	141.50	1.18	1.00
6	0.00	20822.42	324.41	0.00	3.08	0.00	202.37	1.99	0.99
33	0.00	19702.57	305.75	0.00	3.09	0.00	135.91	0.93	1.00
23	0.00	17797.35	313.50	0.00	3.09	0.00	142.80	1.44	1.00
41	0.00	14115.84	297.99	0.00	3.10	0.00	214.86	1.63	0.99
19	0.00	16804.89	347.21	0.00	3.10	0.00	171.93	1.02	1.00
44	0.00	16594.49	290.11	0.00	3.10	0.00	167.82	0.67	0.99
13	0.00	15184.32	292.43	0.00	3.10	0.00	105.76	0.95	1.00
17	0.00	22361.74	280.86	0.00	3.12	0.00	264.55	1.94	1.00
31	0.00	19212.50	297.14	0.00	3.12	0.00	92.40	1.01	1.00
15	0.00	20916.81	332.67	0.00	3.13	0.00	173.83	1.59	1.00
16	0.00	23697.07	298.17	0.00	3.13	0.00	126.59	1.56	0.99
46	0.00	21178.86	304.74	0.00	3.14	0.00	233.15	1.37	1.00
3	0.00	19384.49	279.55	0.00	3.15	0.00	62.59	0.38	0.99
12	0.00	17263.65	301.90	0.00	3.17	0.00	140.22	1.65	1.00
49	0.00	18821.46	279.25	0.00	3.17	0.00	138.60	1.35	0.99
10	0.00	19271.78	315.20	0.00	3.18	0.00	121.07	0.83	0.99
26	0.00	22946.32	327.75	0.00	3.18	0.00	135.70	1.65	1.00

Overview Individual

Current: 2

File: 010449528331665084267\_INX6NdeInano028.mrc

Individual

# J3: Curate Exposures to chose 5 micrographs



P9: Blob\_picker\_EMPIAR-10291-5..

W1: Test Workspace

J3



Interactive

Overview

Inputs and Parameters

Outputs

Metadata

Click [Save] and [Done]

Interactive Overview Inputs and Parameters Outputs Metadata

Total: 50 Accepted: 0 Rejected: 0 Selected: 50

#	Avg Inten.	DF Avg	Astig	Phase	CTF Fit▲	CTF CC	DF Range	Tilt Angle	Rel Ice Thick.
14	0.00	16868.62	269.51	0.00	2.92	0.00	138.43	1.66	0.99
35	0.00	18555.86	292.92	0.00	2.94	0.00	146.25	1.17	1.00
21	0.00	17550.73	291.34	0.00	3.00	0.00	142.76	1.48	1.00
28	0.00	15813.52	303.95	0.00	3.00	0.00	150.72	1.51	1.00
2	0.00	18040.11	315.57	0.00	3.01	0.00	225.43	2.32	0.99
29	0.00	20521.20	288.42	0.00	3.04	0.00	168.46	1.96	0.99
38	0.00	20991.48	269.37	0.00	3.04	0.00	232.51	1.13	1.00
22	0.00	18949.22	272.69	0.00	3.04	0.00	187.88	1.32	1.00
43	0.00	15628.46	312.99	0.00	3.05	0.00	275.49	0.72	1.00
36	0.00	18959.45	286.61	0.00	3.06	0.00	103.17	1.05	0.99
39	0.00	21215.71	352.12	0.00	3.06	0.00	150.36	1.27	1.00
18	0.00	22391.86	301.64	0.00	3.08	0.00	119.96	0.87	1.00
27	0.00	17908.13	280.29	0.00	3.08	0.00	141.50	1.18	1.00
6	0.00	20822.42	324.41	0.00	3.08	0.00	202.37	1.99	0.99
33	0.00	19702.57	205.75	0.00	3.00	0.00	135.01	0.92	1.00

Overview Individual

Current: 2  
File: 010449528331665084267\_INX6NdeInano028.mrc

Individual

Save Done

# J4: Blob picker

JOB BUILDER

Search...

Particle Picking (8)

- Manual picker
- Blob picker**
- Template picker
- Filament tracer (BETA)
- Inspect particle picks
- Extract from Micrographs
- Extract From Micrographs (CPU)
- Downsample Particles

J3 ● Curate Exposures

Completed

J4 (Blob picker) BUILDING

New Job J4

INPUTS

micrographs (exposure) 1  
Min: 1, Max: Infinity, Repeats: no  
Group 1 → J3.exposures\_accepted

PARAMETERS

Blob Picking

- Minimum particle diameter (A): 120
- Maximum particle diameter (A): 160
- Use circular blob:
- Use elliptical blob:
- Use ring blob:
- Lowpass filter to apply (A): 20
- Angular sampling (degrees): 5
- Min. separation dist (diameters): 1
- Number of mics to process: NONE
- Number of mics to plot: 10

JOB BUILDER

J4 (Blob picker) BUILDING

New Job J4

INPUTS

micrographs (exposure) 0  
Min: 1, Max: Infinity, Repeats: no

PARAMETERS

Blob Picking

- Minimum particle diameter (A): NONE

Outputs

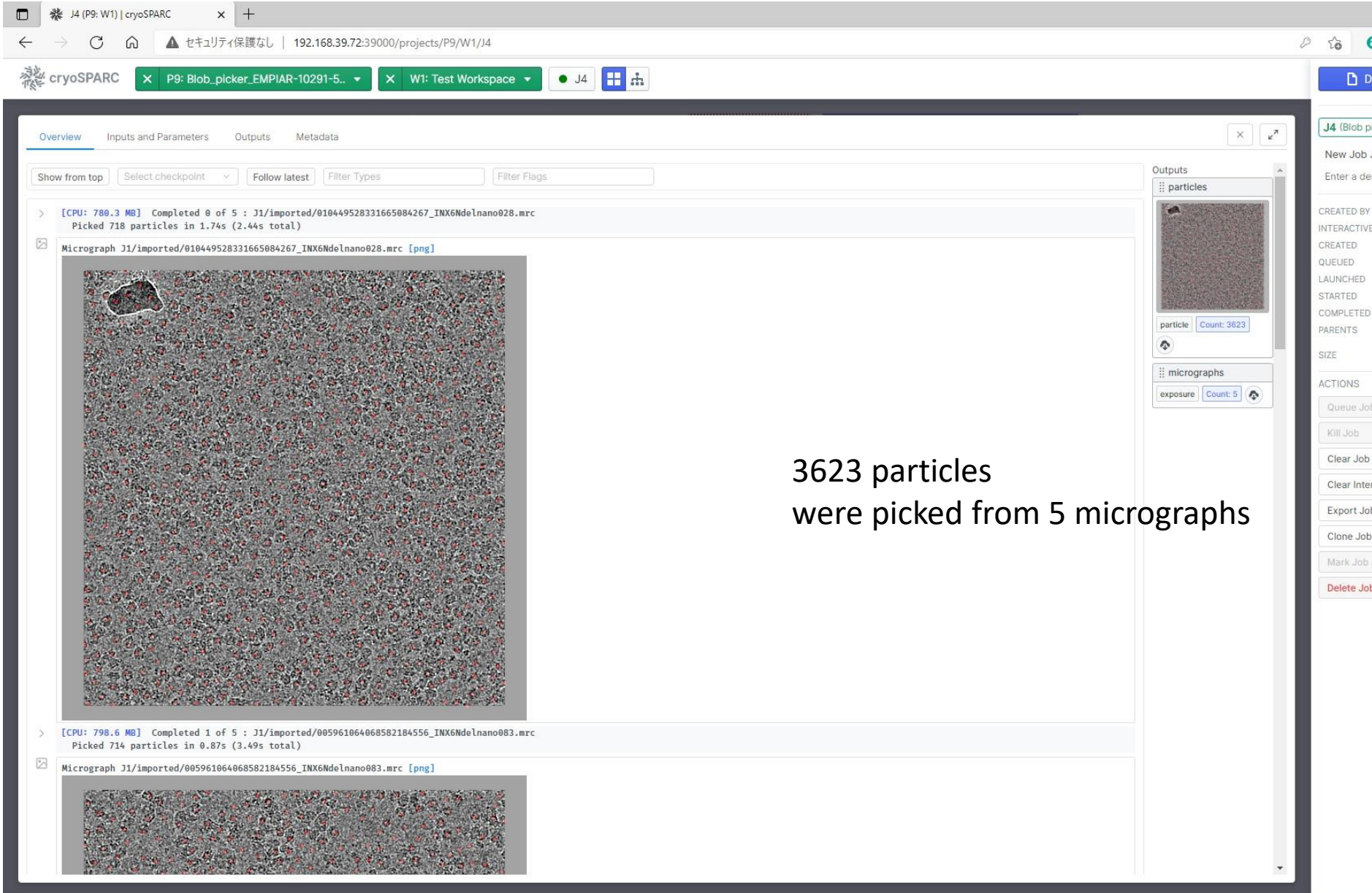
- exposures\_accepted (exposure) Count: 5
- exposures\_rejected (exposure) Count: 45
- exposures\_uncategorized (exposure) Count: 0

Create

Cancel Queue



# J4: Blob picker



The screenshot shows the cryoSPARC web interface for a job named "J4". The browser address bar shows the URL "192.168.39.72:39000/projects/P9/W1/J4". The interface includes tabs for "Overview", "Inputs and Parameters", "Outputs", and "Metadata". Below the tabs, there are filters for "Show from top", "Select checkpoint", "Follow latest", "Filter Types", and "Filter Flags". The main content area displays a list of jobs. The first job is "J1/imported/010449528331665084267\_INX6NdeInano028.mrc", which is completed and shows "Picked 718 particles in 1.74s (2.44s total)". Below this job, a large micrograph is displayed with a white outline around a specific region. The second job is "J1/imported/005961064068582184556\_INX6NdeInano083.mrc", which is also completed and shows "Picked 714 particles in 0.87s (3.49s total)". Below this job, a smaller micrograph is displayed. On the right side, the "Outputs" panel shows a list of "particles" with a count of 3623 and a "micrographs" section with a count of 5. The right sidebar contains a list of job actions, including "Queue Job", "Kill Job", "Clear Job", "Clear Intern", "Export Job", "Clone Job", "Mark Job", and "Delete Job".

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

> [CPU: 789.3 MB] Completed 0 of 5 : J1/imported/010449528331665084267\_INX6NdeInano028.mrc  
Picked 718 particles in 1.74s (2.44s total)

Micrograph J1/imported/010449528331665084267\_INX6NdeInano028.mrc [png]

> [CPU: 798.6 MB] Completed 1 of 5 : J1/imported/005961064068582184556\_INX6NdeInano083.mrc  
Picked 714 particles in 0.87s (3.49s total)

Micrograph J1/imported/005961064068582184556\_INX6NdeInano083.mrc [png]

Outputs

particles  
particle Count: 3623

micrographs  
exposure Count: 5

J4 (Blob picker)

New Job J4

Enter a description

CREATED BY INTERACTIVE

CREATED QUEUED

LAUNCHED STARTED

COMPLETED PARENTS

SIZE

ACTIONS

Queue Job

Kill Job

Clear Job

Clear Intern

Export Job

Clone Job

Mark Job

Delete Job

3623 particles  
were picked from 5 micrographs

# J5: Extract From Micrographs

**JOB BUILDER**

Search...

- Particle Picking (8)
- Manual picker
- Blob picker
- Template picker
- Filament tracer (BETA)
- Inspect particle picks
- Extract from Micrographs**
- Extract From Micrographs (CPU)
- Downsample Particles

**J3** Curate Exposures

Completed

**J4** Blob picker

**J5 (Extract from Micrographs)** BUILDING

New Job J5

**INPUTS**

- micrographs (exposure) 1  
Min: 1, Max: Infinity, Repeats: no  
Group 1 → J3.exposures\_accepted
- particles (particle) 1  
Min: 0, Max: Infinity, Repeats: no  
Group 1 → J4.particles

**PARAMETERS**

- Compute settings
  - Number of GPUs to parallelize (0 for CPU-only): 1
- Particle Extraction
  - Extraction box size (pix): 180
  - Fourier crop to box size (pix): NONE
  - Recenter using aligned shifts:
  - Number of mics to extract: NONE
  - Flip mic. in x before extract?:
  - Flip mic. in y before extract?:

**Outputs**

- exposures\_accepted (exposure) Count: 5
- exposures\_rejected (exposure) Count: 45
- exposures\_uncategorized (exposure) Count: 0
- particles (particle) Count: 3623
- micrographs (exposure) Count: 5

**PARAMETERS**

- Number of GPUs to parallelize (0 for CPU-only):

Cancel Queue Create

# J5: Extract From Micrographs

Browser window: J5 (P9: W1) | cryoSPARC

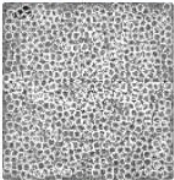
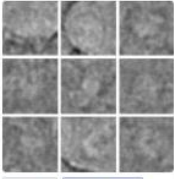
URL: 192.168.39.72:39000/projects/P9/W1/J5

Navigation: Overview | Inputs and Parameters | Outputs | Metadata

Filters: Show from top | Select checkpoint | Follow latest | Filter Types | Filter Flags

```
> [CPU: 233.2 MB] Starting multithreaded pipeline ...
> [CPU: 233.4 MB] Started pipeline
> [CPU: 271.3 MB] GPU 0 using a batch size of 2048
> [CPU: 271.4 MB] - 0.0: processing J1/imported/010449528331665084267_INX6Ndelnano028.mrc
  Writing to /shared_data_SSD4TB/cryosparc_projects/guest09/P9/J5/extract/010449528331665084267_INX6Ndelnano028_particles.mrc
> [CPU: 271.4 MB] - 0.1: processing J1/imported/005961064068582184556_INX6Ndelnano083.mrc
  Writing to /shared_data_SSD4TB/cryosparc_projects/guest09/P9/J5/extract/005961064068582184556_INX6Ndelnano083_particles.mrc
> [CPU: 1.01 GB] - 0.0: processing J1/imported/005110174331643817134_INX6Ndelnano103.mrc
  Writing to /shared_data_SSD4TB/cryosparc_projects/guest09/P9/J5/extract/005110174331643817134_INX6Ndelnano103_particles.mrc
> [CPU: 438.3 MB] (1 of 5) Finished processing micrograph 0.
> [CPU: 839.4 MB] - 0.1: processing J1/imported/017090782055260799699_INX6Ndelnano123.mrc
  Writing to /shared_data_SSD4TB/cryosparc_projects/guest09/P9/J5/extract/017090782055260799699_INX6Ndelnano123_particles.mrc
> [CPU: 438.4 MB] (2 of 5) Finished processing micrograph 1.
> [CPU: 823.4 MB] - 0.0: processing J1/imported/001443482135254543153_INX6Ndelnano161.mrc
  Writing to /shared_data_SSD4TB/cryosparc_projects/guest09/P9/J5/extract/001443482135254543153_INX6Ndelnano161_particles.mrc
> [CPU: 438.4 MB] (3 of 5) Finished processing micrograph 2.
> [CPU: 438.4 MB] (4 of 5) Finished processing micrograph 3.
> [CPU: 438.7 MB] (5 of 5) Finished processing micrograph 4.
> [CPU: 438.8 MB] ---- Completed. Extracted 3237 particles in 11.75s.
> [CPU: 250.8 MB] -----
> [CPU: 250.8 MB] Compiling job outputs ...
> [CPU: 250.8 MB] Passing through outputs for output group micrographs from input group micrographs
> [CPU: 250.8 MB] This job outputted results ['micrograph_blob']
> [CPU: 250.8 MB]   Loaded output dset with 5 items
> [CPU: 250.8 MB] Passthrough results ['mscope_params', 'ctf', 'ctf_stats', 'micrograph_blob_non_dw']
> [CPU: 250.8 MB]   Loaded passthrough dset with 5 items
> [CPU: 251.4 MB]   Intersection of output and passthrough has 5 items
> [CPU: 251.4 MB] Passing through outputs for output group particles from input group particles
> [CPU: 252.3 MB] This job outputted results ['blob']
> [CPU: 252.3 MB]   Loaded output dset with 3237 items
> [CPU: 252.3 MB] Passthrough results ['location', 'ctf', 'pick_stats']
> [CPU: 254.0 MB]   Loaded passthrough dset with 3623 items
> [CPU: 254.0 MB]   Intersection of output and passthrough has 3237 items
> [CPU: 254.0 MB] Checking outputs for output group micrographs
> [CPU: 254.0 MB] Checking outputs for output group particles
> [CPU: 254.0 MB] Updating job size ...
> [CPU: 254.0 MB] Exporting job and creating csg files ...
> [CPU: 254.0 MB] *****
> [CPU: 254.0 MB] Job complete. Total time 12.26s
```

Outputs

- micrographs:  exposure Count: 5
- particles:  particle Count: 3237

JOB DETAILS

J5 (Extract from Micrographs) COMPLETED

New Job J5

Enter a description.

CREATED BY: guest09

INTERACTIVE: No

CREATED: Wed, Jun 23 2021 7:15:07 PM

QUEUED: Wed, Jun 23 2021 7:19:48 PM

LAUNCHED: Wed, Jun 23 2021 7:19:49 PM

STARTED: Wed, Jun 23 2021 7:19:50 PM

COMPLETED: Wed, Jun 23 2021 7:20:06 PM

PARENTS: J3, J4

SIZE: 401.35 MB

ACTIONS

- Queue Job on default
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

# J6: 2D Classification

JOB BUILDER

Search...

Particle Curation (5)

- 2D Classification
- Select 2D classes
- Class Probability Filter
- Rebalance 2D Classes (BETA)
- Create Templates

J5 ● Extract From Micrographs



J6 (2D Classification) BUILDING

New Job J6

INPUTS

particles (particle) 1  
Min: 1, Max: Infinity, Repeats: no  
Group 1 → J5.particles

PARAMETERS

Particle preprocessing  
Window dataset (real-space):

2D Classification  
Number of 2D classes:  
10

Maximum resolution (Å):  
6  
Initial classification uncertainty factor:  
2  
Circular mask diameter (Å):  
NONE

Re-center 2D classes:  
  
Show plots from intermediate steps:

Random Seeds

Compute settings  
Cache particle images on SSD:  
  
Number of GPUs to parallelize:  
2

Cancel Queue

Create

JOB BUILDER

J6 (2D Classification) BUILDING

New Job J6

INPUTS

particles (particle) 0  
Min: 1, Max: Infinity, Repeats: no

PARAMETERS

Particle preprocessing

Window dataset (real-space):

2D Classification

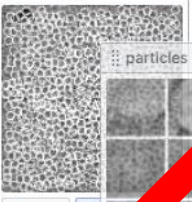
Number of 2D classes:  
50

Maximum resolution (Å):  
6


Initial classification uncertainty factor:  
2

Outputs


micrographs



particles



particle Count: 3237



particle Count: 3237

# J6: 2D Classification

Browser tabs: J6 (P9: W1) | cryoSPARC

Address bar: 192.168.39.72:39000/projects/P9/W1/J6

Navigation: Overview | Inputs and Parameters | Outputs | Metadata

Summary: Iteration 20  
[CPU: 1.37 GB] — Effective number of classes per image: min 1.00 | 25-pct 1.00 | median 1.16 | 75-pct 3.02 | max 9.03  
[CPU: 1.37 GB] — Probability of best class per image: min 0.00 | 25-pct 0.43 | median 0.93 | 75-pct 1.00 | max 1.00

20 classes for iteration 20 [png] [pdf]

Class	ptcls	ess
1	902	13.0 Å
2	779	11.7 Å
3	769	13.3 Å
4	638	10.5 Å
5	69	3.5 Å
6	54	3.5 Å
7	14	3.5 Å
8	5	3.5 Å
9	4	3.5 Å
10	3	3.5 Å

Noise Model for iteration 20 [png] [pdf]

Effective number of assigned classes for iteration 20 [png] [pdf]

Class	Number of Images
1	~2000
2	~100
3	~100
4	~100
5	~100
6	~100
7	~100
8	~100
9	~100
10	~100

Outputs: particles (Count: 3237), class\_averages (Count: 10)

Job Details: J6 (2D Classification) COMPLETED

Job Information: Created by guest09, Interactive No, Created Wed, Jun 23 2021 7:26:16 PM, Queued Wed, Jun 23 2021 7:29:43 PM, Launched Wed, Jun 23 2021 7:29:43 PM, Started Wed, Jun 23 2021 7:29:45 PM, Completed Wed, Jun 23 2021 7:31:15 PM, Parents J5, Size 30.86 MB

Actions: Queue Job on default, Kill Job, Clear Job, Clear Intermediate Results, Export Job, Clone Job, Mark Job as Complete, Delete Job

# Comparison with 2D Classification of manually picked particles. Step 8: Template Picking – J4:2D classification

The screenshot displays the cryoSPARC web interface for job J4 (2D Classification). The main content area shows the results for Iteration 20, including a row of 10 class averages and a noise model plot.

**Iteration 20 Summary:**

- Effective number of classes per image: min 1.00 | 25-pct 1.00 | median 1.00 | 75-pct 1.00 | max 1.00
- Probability of best class per image: min 1.00 | 25-pct 1.00 | median 1.00 | 75-pct 1.00 | max 1.00

**2D classes for iteration 20:**

Class	Particles	Resolution (Å)
1	19 ptcls	23.9 A 1 ess
2	19 ptcls	30.4 A 1 ess
3	13 ptcls	35.3 A 1 ess
4	10 ptcls	33.0 A 1 ess
5	10 ptcls	37.1 A 1 ess
6	9 ptcls	88.7 A 1 ess
7	9 ptcls	45.1 A 1 ess
8	7 ptcls	88.7 A 1 ess
9	4 ptcls	88.7 A 1 ess
10	0 ptcls	nan A nan ess

**Noise Model for iteration 20:**

The plot shows the noise model for iteration 20. The x-axis represents resolution in Å (DC, 22Å, 11Å, 7.4Å, 5.5Å, 4.4Å, 3.7Å). The y-axis represents the noise level (0.0 to 3.0). Three curves are shown: current sigma (blue), current noise (orange), and initial sigma (green). The current noise curve shows a peak at approximately 22Å and then decreases, while the current sigma curve drops to zero at 5.5Å.

**Effective number of assigned classes for iteration 20:**

The bar chart shows the effective number of assigned classes for iteration 20. The x-axis represents the number of images (30, 40, 50, 60). The y-axis represents the number of images (30, 40, 50, 60). The bar at 40 images is the highest, reaching approximately 60.

**Outputs:**

- particles: 100
- class\_averages: 10

**Job Details:**

Job J4 (2D Classification) is COMPLETED. The job was created on Sat, Jun 19 2021 5:51:41 P. The job was completed on Sat, Jun 19 2021 6:09:40 P. The size of the job is 13.75 M.

**Actions:**

- Queue Job on default
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

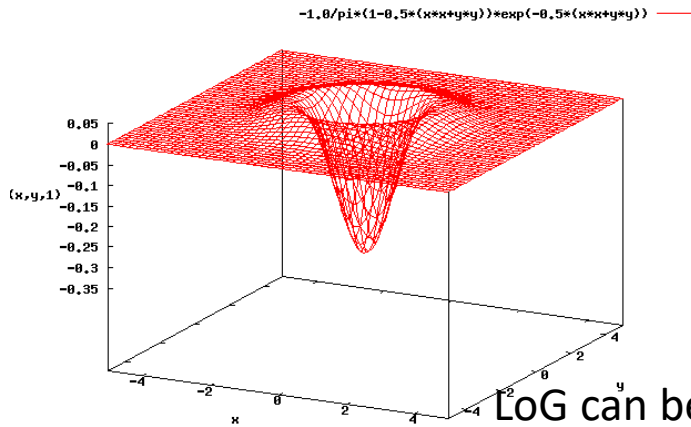
**Navigation:**

- Dashboard
- Projects
- Resource Manager

# Relion 3.1: LoG-based autopicking

# “Blob” detection using LoG filter

LoG : Laplacian of Gaussian



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

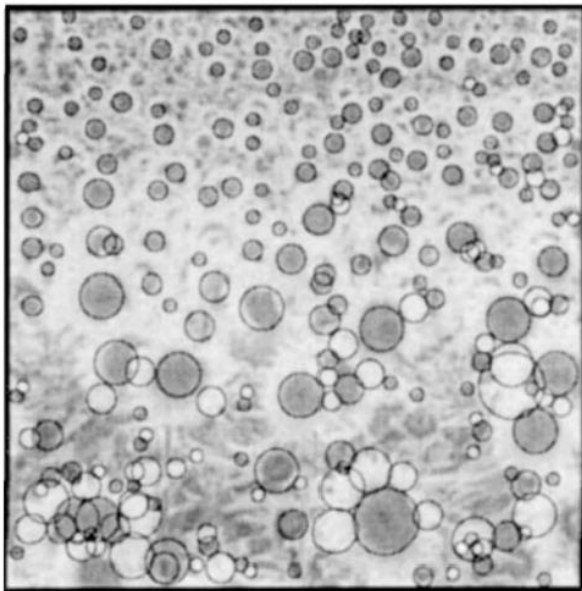
$$\text{LoG}(\mathbf{r}) = \frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} = -\frac{2}{\sigma^2} \left(1 - \frac{x^2 + y^2}{2\sigma^2}\right) \phi(\mathbf{r})$$

[Big Gauss] – [normal Gauss]

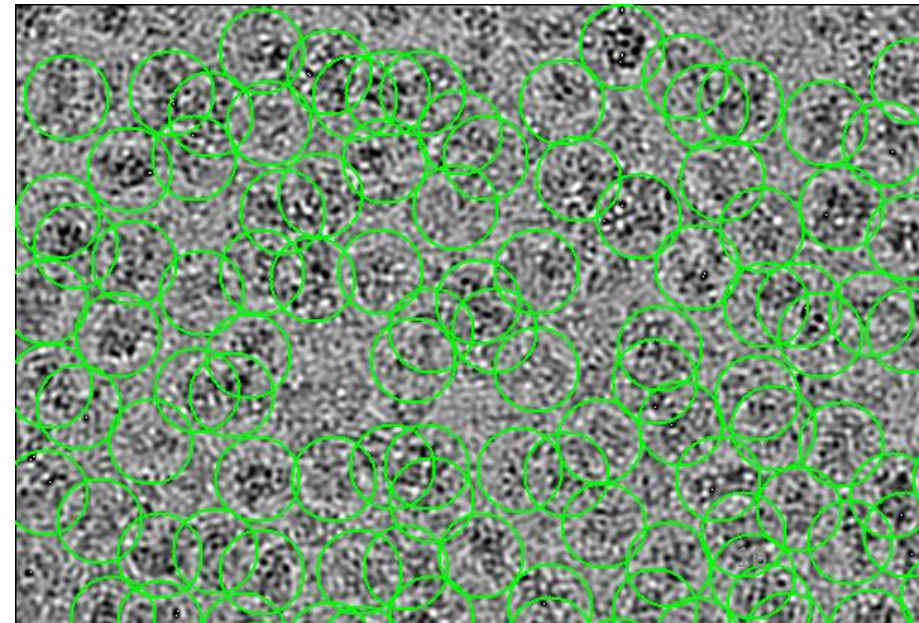
$$\text{LoG}(\mathbf{r}) \cong \text{DoG}(\mathbf{r}) = \phi(\mathbf{r}|k\sigma) - \phi(\mathbf{r}|\sigma)$$

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)

File Jobs Schedules I/O Laplacian References autopicking Helix Running

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

Input micrographs for autopick: 4/micrographs\_selected.star [?] Browse

Pixel size in micrometers: 5.0 [?] [?]

2D references: [?] Browse

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!

I/O view Job actions Current: LoG\_5mic Display: [?]

I/O Laplacian References autopicking Helix Running

Min. diameter for LoG filter (A) 120 120 [?]

Max. diameter for LoG filter (A) 160 160 [?]

Are the particles white? No [?]

Maximum resolution to consider (A) 20 [?]

I/O Laplacian References autopicking Helix Running

Number of MPI processes: 6 [?]

Submit to queue? No [?]

Queue name: openmpi [?]

Queue submit command: qsub [?]

Standard submission script: relion-3.1/scripts/qsub.csh [?] Browse

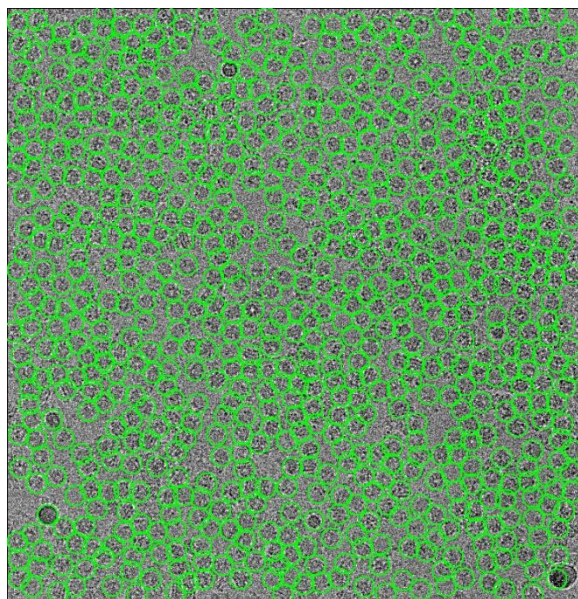
Minimum dedicated cores per node: 24 [?]

Additional arguments: [?]

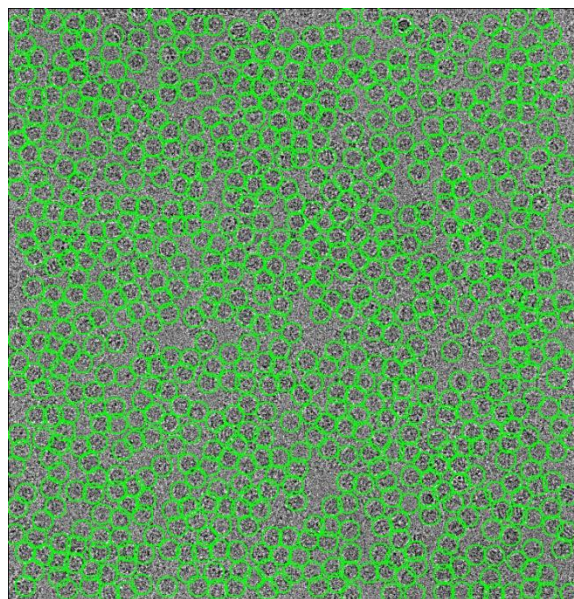
※dmin,dmaxをユーザーが指定。それらをもとに全部で4+3+4=11通りのdを試す。  
 dmin/5, dmin/4, dmin/3, dmin/2, dmin, (dmin+dmax)/2, dmax, 2dmax, 3dmax,  
 4dmax, 5dmax



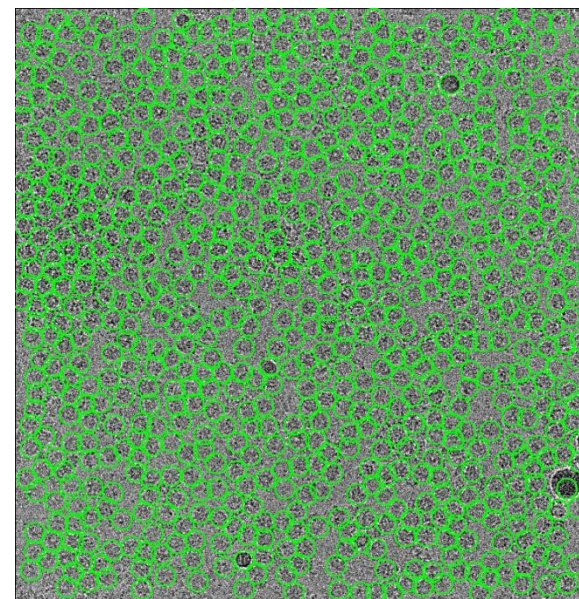
# LoGbased Auto Picking



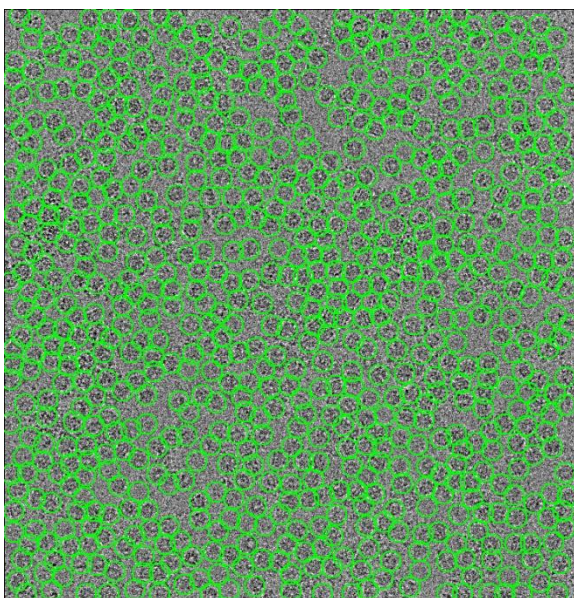
006 : 726 particles



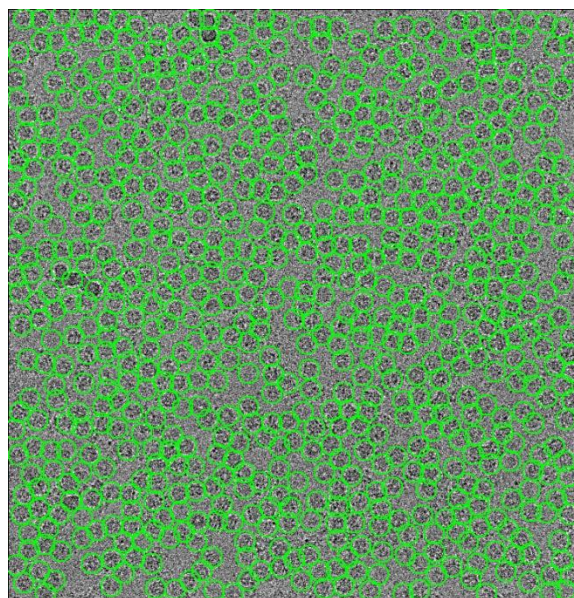
007 : 726 particles



0010 : 733 particles



0019 : 713 particles



0020 : 708 particles

a/INX6NdeInano006.mrc	pick	726	CTF	19895.3
a/INX6NdeInano007.mrc	pick	726	CTF	19207.3
a/INX6NdeInano010.mrc	pick	733	CTF	18995.4
a/INX6NdeInano019.mrc	pick	713	CTF	21145.6
a/INX6NdeInano020.mrc	pick	708	CTF	20288.3

# 1.6 Particle extraction

File Jobs Schedules I/O extract Helix Running Select/5mic/micrographs\_selected.star

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

micrograph STAR file: 4/micrographs\_selected.star ? Browse  
Input coordinates: coords\_suffix\_autopick.star ? Browse

0 AutoPick/LoG\_5mic/coords\_suffix\_autopick.star

Reset the refined offsets to zero? No ?  
OR: re-center refined coordinates? No ?  
Recenter on - X, Y, Z (pix): 0 0 0 ?

Alias : LoG\_5mic\_64pix

I/O view Job actions Current: LoG\_5mic\_64pix Display:

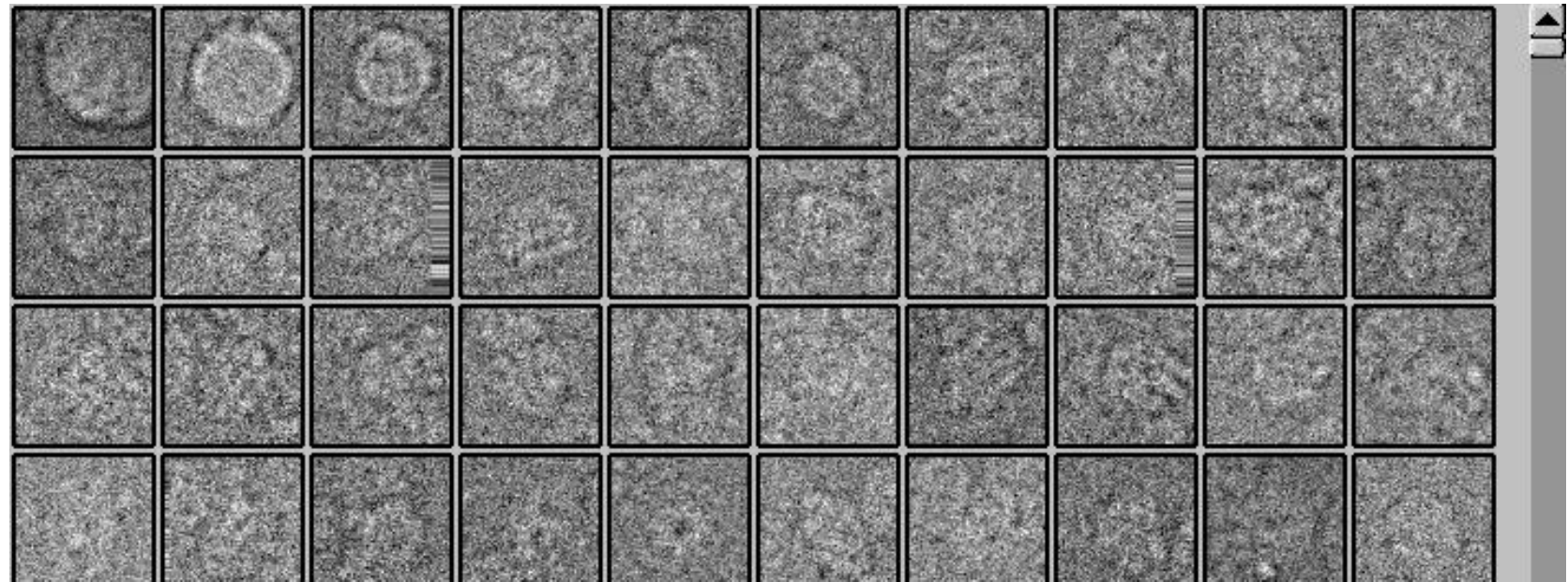
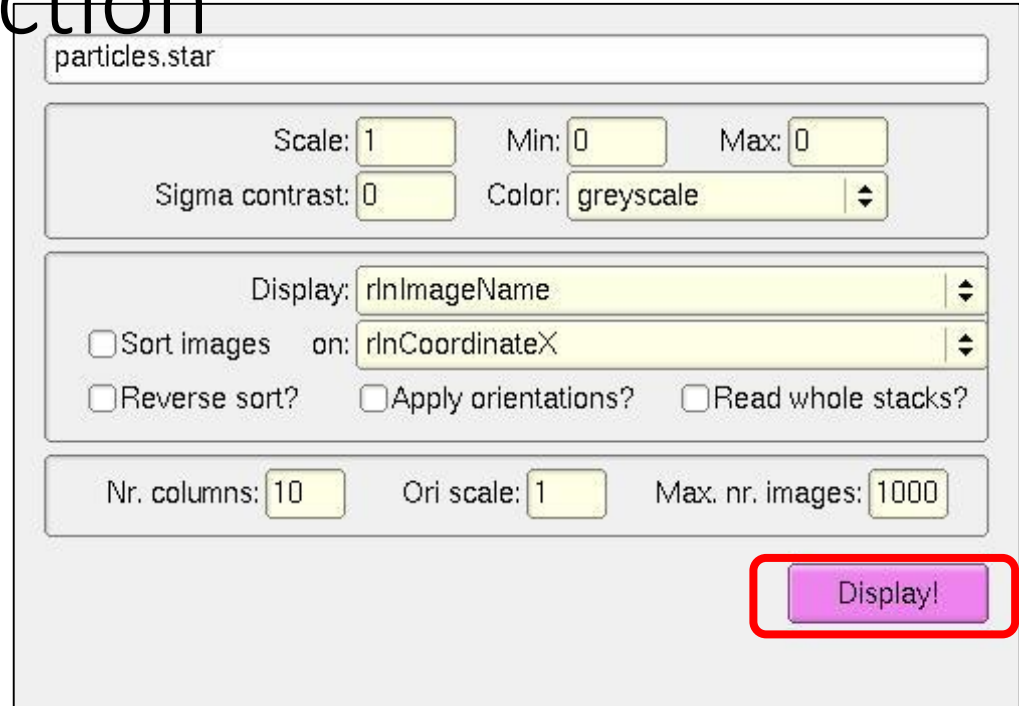
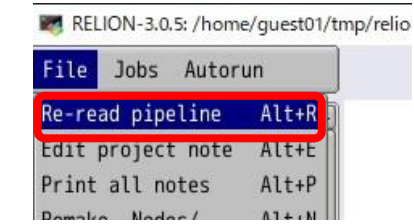
Schedule Check command Run!

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!

# 1.6 Particle extraction



# 1.7 Making templates for autopicking (1min)

[2 GPU;]

File Jobs Schedules I/O CTF Optimisation Sa Extract/LoG\_5mic\_64pix/particles.star

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

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

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

Alias : LoG\_5mic\_64pix  
Current: **LoG\_5mic\_64pix** Display:

Schedule Check command Run!

I/O view Job actions

2D classification

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  
Which GPUs to use: **0:1**

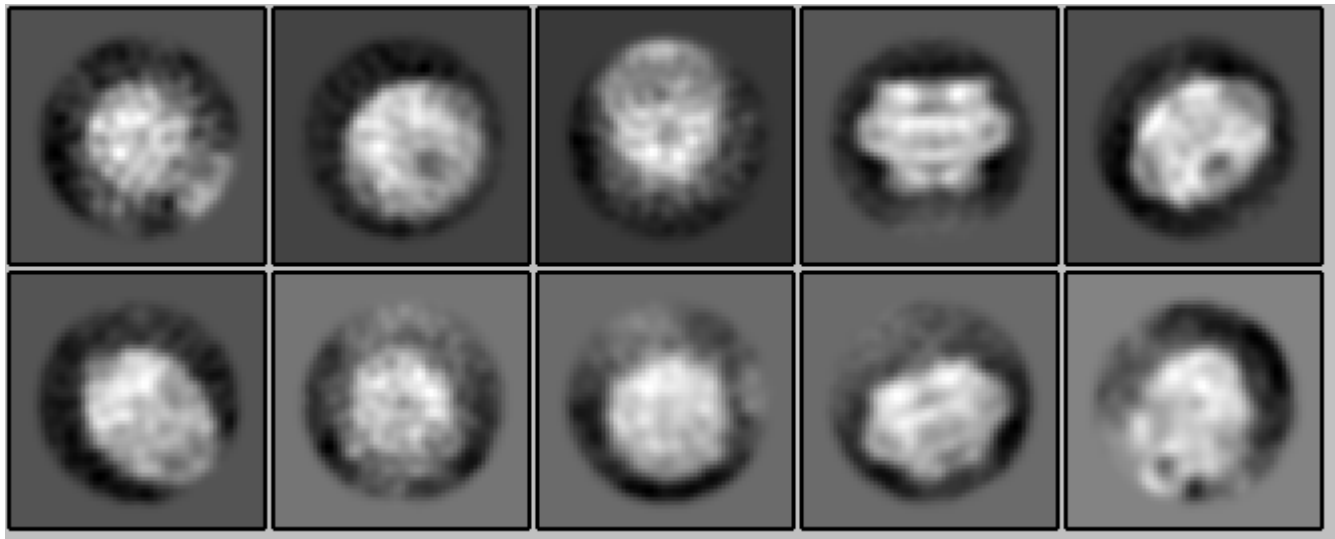
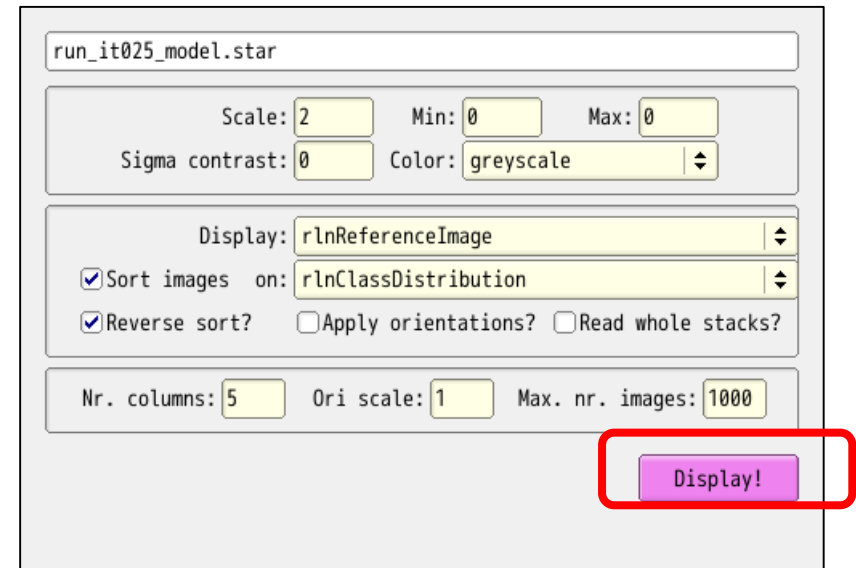
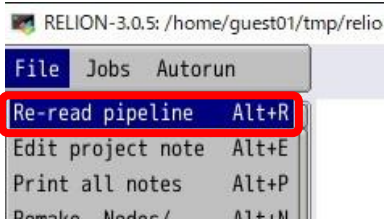
I/O CTF Optimisation Sampling Helix Compute **Running**

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

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

Run!

# 1.7 Making templates for autopicking



No C8 top view....