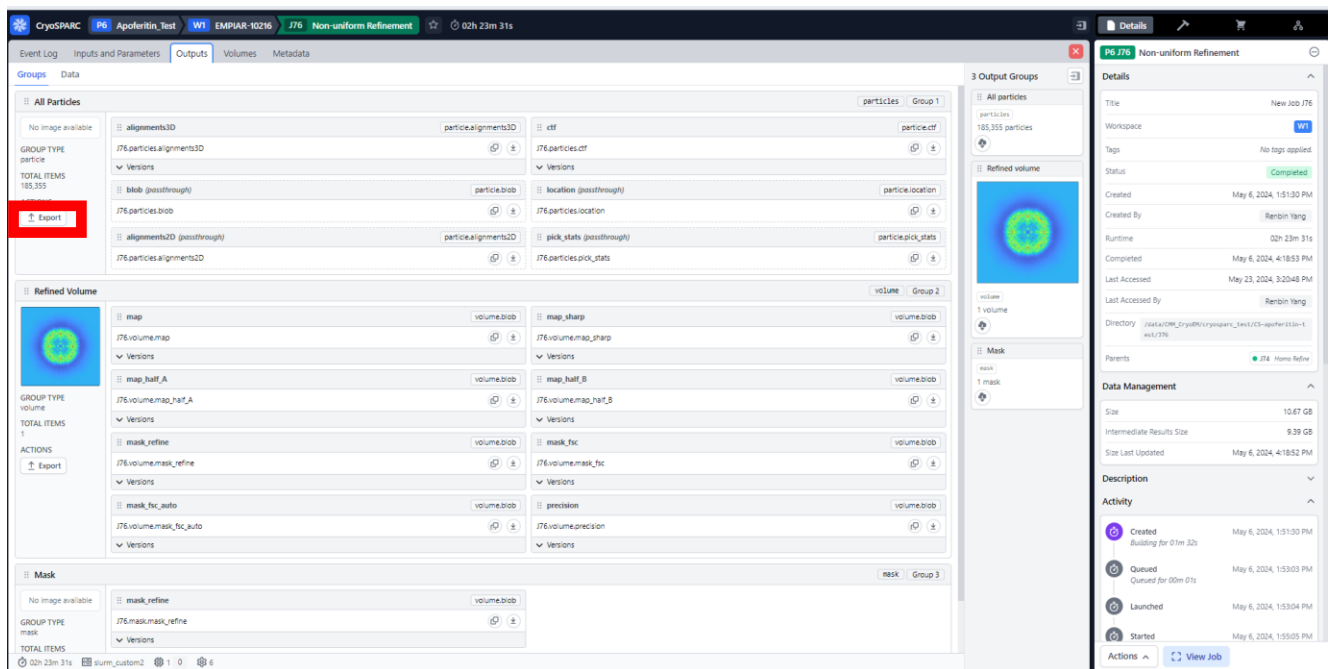


1. Importing particles image files and meta data files to Cryosparc:

Re-extract particles before importing particles to Cryosparc. In Cryosparc create Import Particle Stack job: the input particle meta data path is pointing to the particle.star file under Relion: /Relion/working/directory/Extract/jobID/particle.star, the input particle data path can be given vaguely with absolute directory for example: /Relion/working/directory/Extract/jobID

2. Exporting refined particles and volumes from Cryosparc

Once done with non-uniform (NU) refinement jobs in cryosparc, open the job, click “Outputs” tab, then click “Export” button in the Particles module. This will create .cs files under the Cryosparc project “exports/groups” subdirectory (in this case exports/groups/J76_particles, but your job number will be different).



3. Copy the .cs particle file from Cryosaprc

Find cryosparc Project folder (directory that was set when creating your project like P6 in this case /data/\$USER/cryosparc_test/CS-apoferitin-test/), copy the particles metadata file in .cs (J76_particles_exported.cs) format from particle folder (YOUR_PROJECT_DIRECTORY/exports/groups/J76_particles) to your RELION working directory on Biowulf HPC compute node:

```
cp J76_particles_exported.cs /Your/Relion/Working/Directory
```

4. Transform particle meta data from .cs format to .star format with pyem on Biowulf:

Open an interactive session at Biowulf HPC terminal:

```
sinteractive
module load pyem
csparc2star.py J76_particles_exported.cs csparc_P6_J76_particles_exported.star
```

Check if the format transformation is successful from the last n lines of the star file:

```
tail csparc_P6_J76_particles_exported.star -n 1
```

```
000032@J18/imported/015707174519121359351_CMM0371A_GO_100x_0_5S_11284.
mracs 22.059727 94.775558 -72.293991 6.181313 14.173312 25398.292969
25059.755859 -74.989311 2.599612 0.000000 2 2 1
```

Find the original particle.star file (the one you previously imported to Cryosparc) under the RELION working directory Extraction jobs. Check the last few lines and compare with transformed .star file:

```
tail JoinStar/job053/join_particles.star -n 2
```

```
000032@Extract/job051/Movies2/CMM0371A_GO_100x_0_5S_11284.mracs
000032@Extract/job051/Movies2/CMM0371A_GO_100x_0_5S_11284.mracs
MotionCorr/job017/Movies2/CMM0371A_GO_100x_0_5S_11284.mrc 1664.000000
3240.000000 15.775474 93.985748 167.664629 44.861935 4.901935
25364.966797 25101.199219 -66.30721 0.000000 0.000000 1 1
71 0.382277 65804.577874 0.913370 2 group_1
```

By comparison, it is obvious that we need to substitute the mracs file path from “J18/imported/015707174519121359351_” (random serial number from cryosparc database) to “Extract/job051/Movies2/” if you only have one data directory. We could achieve this via sed command:

```
sed -i -E 's$J18/imported\[0-9\]*\_Extract/job051/Movies2/$g' csparc_P6_J76_particles_exported.star
```

Now the mracs files path has been changed and served as a “label” to match the path in original .star file under RELION Extraction jobs, if you only have one data directory, you may proceed with next step to copy Micrograph path and particle Coordinates columns, however if you have multiple sub-datasets, you may need to change each sub-dataset accordingly. In this case, we have two sub-datasets, use following command to check the first sub-dataset:

```
head csparc_P6_J76_particles_exported.star -n 120 | tail -n 1
```

```
000001@Extract/job051/Movies2/CMM0371A_GO_100x_0_5S_00135.mracs -0.920194
173.523712 -77.108940 -9.927563 -2.684812 27939.923828 26555.974609 -60.830788
2.599612 0.000000 2 1 1
```

```
head JoinStar/job053/join_particles.star -n 120 | tail -n 1
```

```
000004@Extract/job050/Movies1/CMM0371A_GO_100x_0_5S_00114.mracs
000004@Extract/job050/Movies1/CMM0371A_GO_100x_0_5S_00114.mracs
MotionCorr/job006/Movies1/CMM0371A_GO_100x_0_5S_00114.mrc 2192.000000
232.000000 111.803734 171.106094 -0.33537 3.236935 -11.74807
```

```
15005.814453 14340.095703 -88.48279 0.000000 0.000000 1 1
12 0.384891 65798.354529 0.999249 2 group_1
```

Notice that my first 5000 movies are belonging to the first sub-dataset under `Extract/job050/Movies1/`, to correct that, we could use `sed` command as well:

```
sed -i -E 's$Extract\job051\Movies2\CMM0371A_GO_100x_0_5S_0([0-4])(.)(.)$Extract\job050\Movies1\CMM0371A_GO_100x_0_5S_04\1\2\3\4$g' csparc_P6_J76_particles_exported.star
```

5. Copy the Micrograph path and particle coordinate with pyem star.py script:

Now we could copy Micrograph path and particle coordinate from JoinStar/job053/join_particles.star file via another pyem script: **star.py** with a different version of pyem on Biowulf (the latest version has a bug to create unwanted columns which may cause trouble in RELION, **please use pyem/230315**).

```
module load pyem/230315
```

```
star.py --copy-micrograph-coordinates JoinStar/job053/join_particles.star \
csparc_P6_J76_particles_exported.star \
csparc_P6_J76_particles_exported_Micrograph_Added.star
```

Check the result via:

```
tail csparc_P6_J76_particles_exported_Micrograph_Added.star -n 1
```

000032@Extract/job051/Movies2/CMM0371A_GO_100x_0_5S_11284.mrcs
000032@Extract/job051/Movies2/CMM0371A_GO_100x_0_5S_11284.mrcs
MotionCorr/job017/Movies2/CMM0371A_GO_100x_0_5S_11284.mrc 1664.000000
3240.000000 22.059727 94.775558 -72.293991 6.181313 14.173312 25398.292969
25059.755859 -74.989311 2.599612 0.000000 2 2 1

We have the extra mrc file path pointing to the corresponding MotionCorr job in Relion. Now the csparc_P6_J76_particles_exported_micrograph_added.star with non-uniform (NU) refined parameters is ready for Bayesian polishing under your Relion project working directory.

6. Export half maps and mask for Bayesian Polishing:

For Bayesian polishing job in Relion, we need half maps from cryosparc NU-refinement for postprocessing. We could either download half maps (both) or similar to step one, export the **Refined Volumes** in the Cryosparc GUI, then under the Cryosparc project directory on Biowulf (for example after clicking Export, the volume files will be located at: /data/\$USER/cryosparc_test/CS-apoferitin-test/exports/groups/J76_volume/J76); then copy half maps (named with halfA/B) and mask_fsc_auto.mrc to Relion working directory. Change halfA to half1 and halfB to half2 for each of the two half maps. Use the three files for RELION Post-processing job. Then all is ready for RELION Bayesian polishing.