Ed's cryoSPARC live guide 2019

v0.1 2019.09.20

Requirements: Web browser (Chrome recommended by cryoSPARC developers)

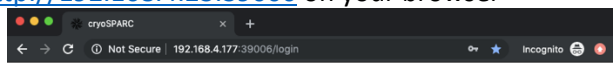
Issues: cryoSPARC live is in beta.

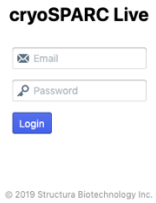
Instructions for setup and use

Accessing cryoSPARC live

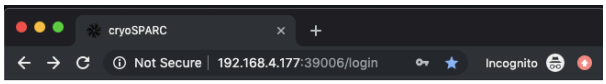
1. CryoSPARC (https://cryosparc.com/) is one of the software packages commonly used for single-particle cryoEM reconstruction. We have installed it on the Amazon web services cloud for this practical.

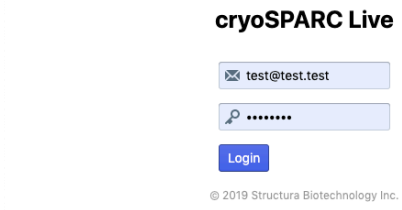
To access, go http://192.168.4.23:39006 on your browser



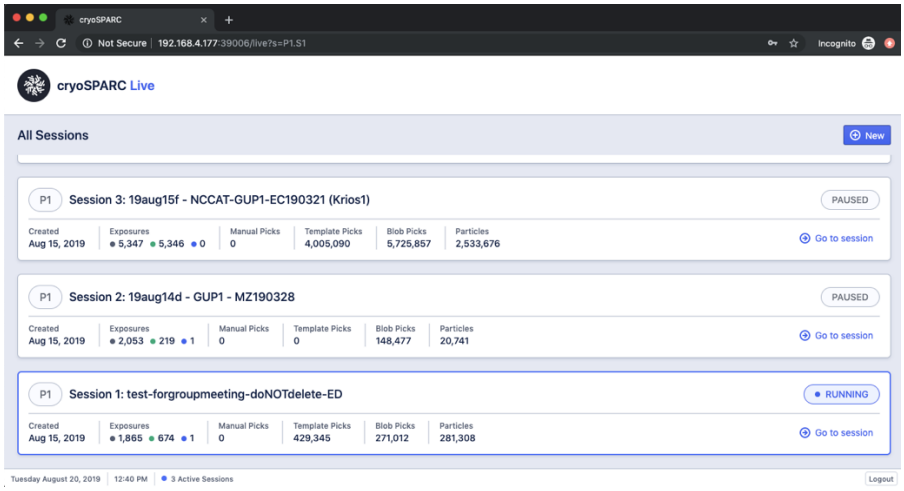


2. Login using the following credentials
   - Email: test@test.test
   - Password: testtest

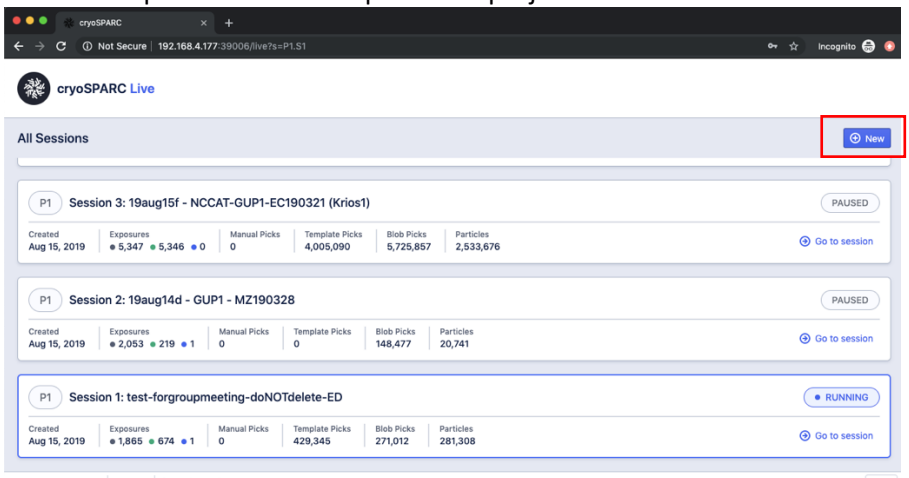


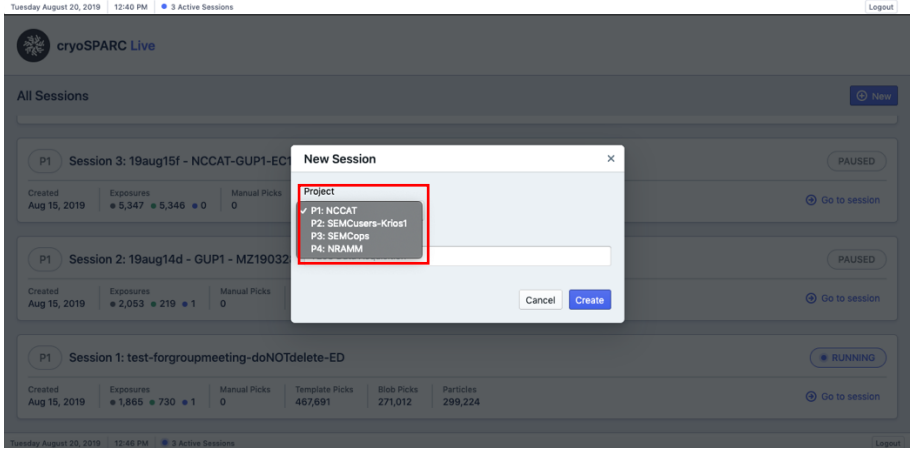


3. Once you sign in, you will be on the main page

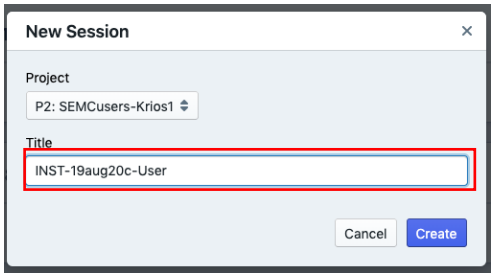
- 

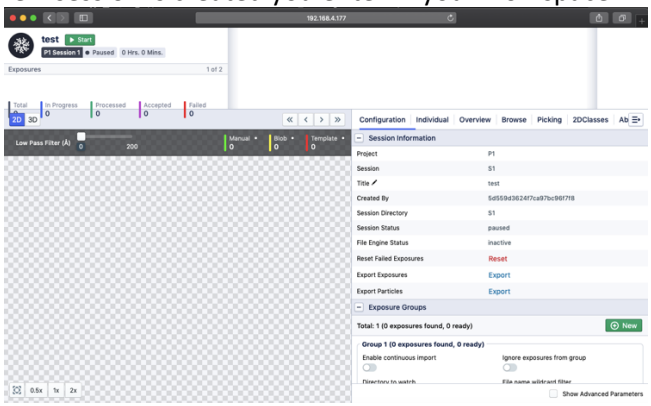
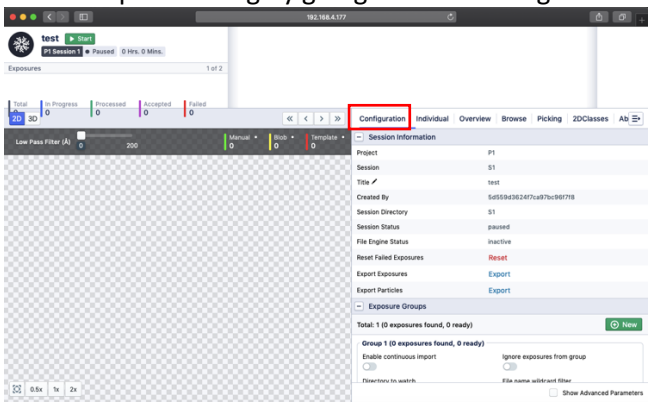
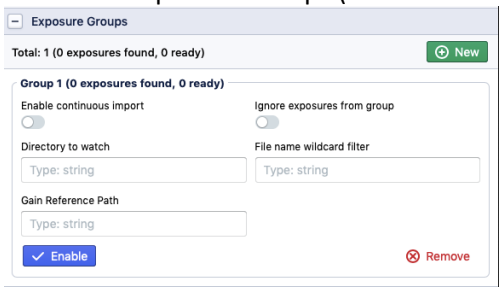
Making Your Project and Dataset

4. Currently all information is stored in /beegfs/appion/cryosparclive/
5. Create a new workspace in one of the pre-made projects.
   - 

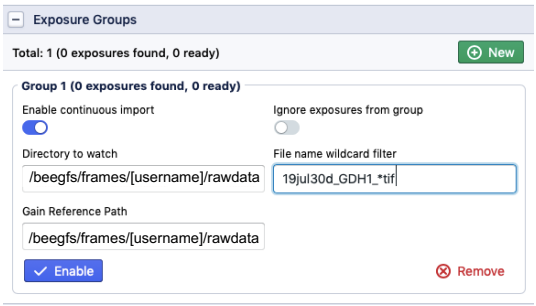
   - 

6. Label the session Institution code – Leginon session name – User name, e.g. “SEMC-19aug20c-mkopylov (Krios1)”

- 

7. Once a new session is created you enter in your workspace.
   - 
8. Now we can set up streaming by going into the Configuration tab.
   - 
   - 1st set the Exposure Groups (where to look for data)
   - 

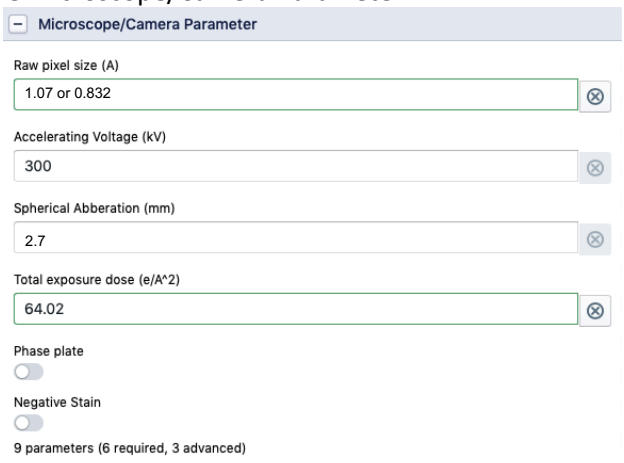
   - Input the following:
     - Enable continuous import: [toggle yes]
     - Directory to watch: /beegfs/frames/[username]/[leginon session]/rawdata/
     - File name wildcard filter: *tif or [session][Atlas name]*tif, e.g. 19jul30d_GDH1*tif
     - Gain Reference Path: /beegfs/frames/[username]/[leginon session]/rawdata/references/[filename]_norm_0.mrc (for Krios1)
     - Click Enable to lock in settings



- 2nd set the Compute Resources (how many gpus to use)

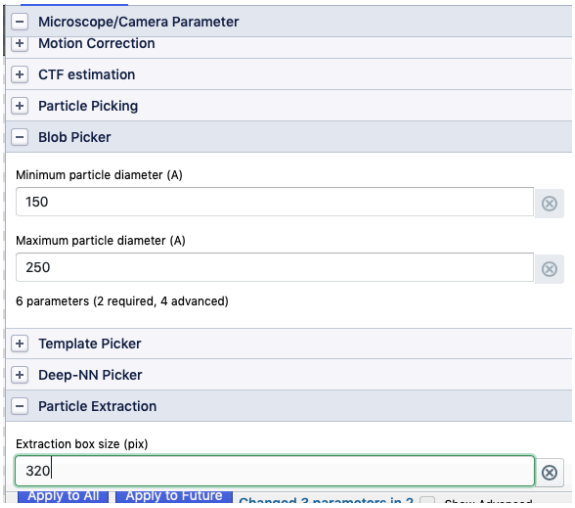
  - Use the default lanes for all and select nominally 2 gpus.

- 3rd set the Microscope/Camera Parameter



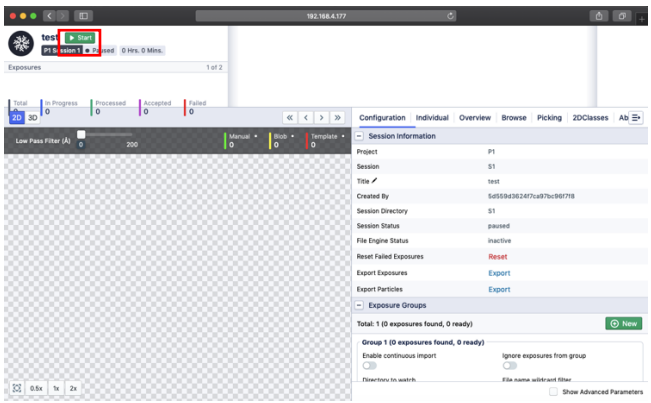
  - Raw pixel size (A): typically 1.1 for 81K or 0.83 for 105Kx.
  - Accelerating Voltage (kV): 300
  - Spherical Aberration (mm): 2.7
  - Total exposure dose (e/A^2):

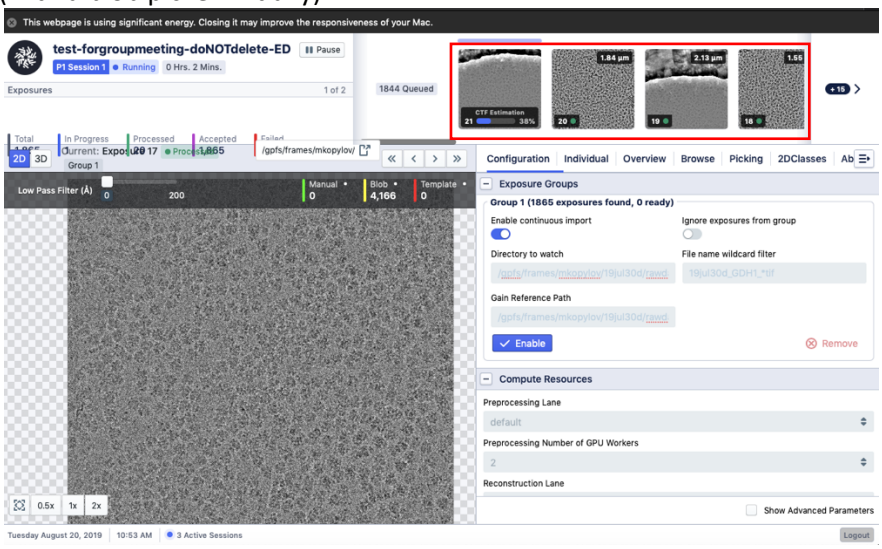
- 4th set Blob Picker and Particle Extraction size



- These are initial parameters and may be refined later.
- Note: default mask diameter is dependent on particle diameter.
- Lastly, select Apply to All to lock in the parameters.
-

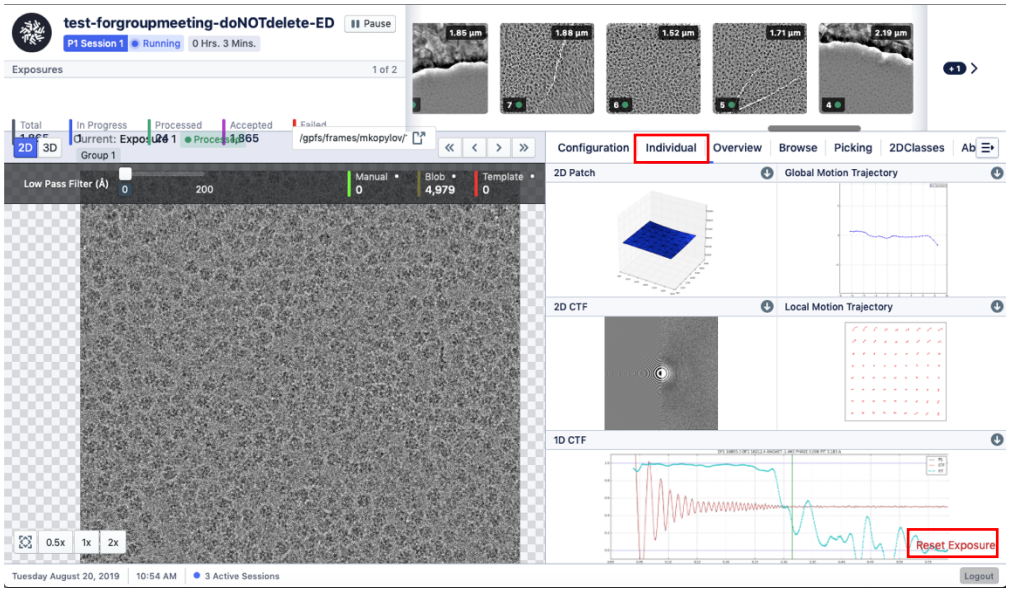
Begin live

9. Now you are ready to start cryoSPARC live
    - Press start
    - 

10. cryoSPARC live uses the processing lane to do Motion Correction, CTF estimation and Particle picking (with a blob picker initially).
    - 
    - Images processed will be noted on the top bar. Green means they have been processed if there is an issue there would be a red mark.

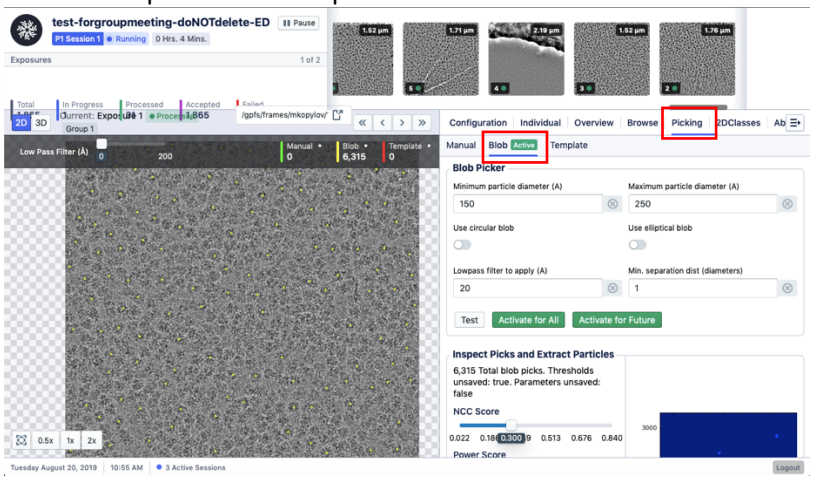
On the fly feedback

11. The pre-processing is displayed in visual format in the individual tab.

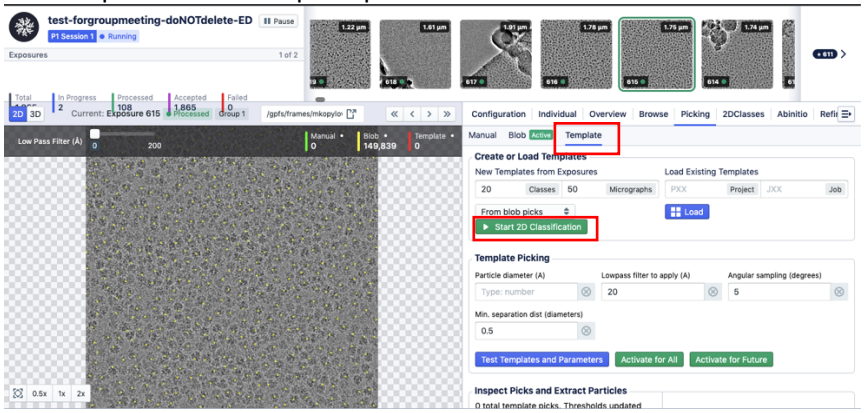


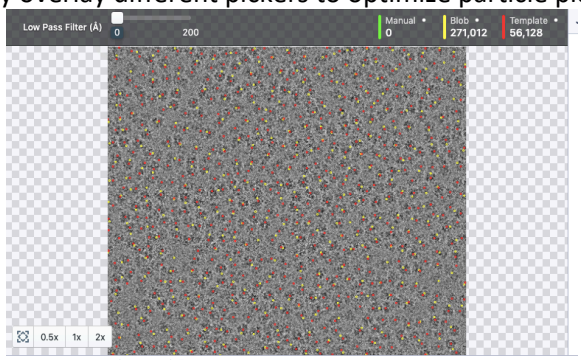
- If there is a failure or changes have been made the image may be reprocessed by clicking Reset Exposure.

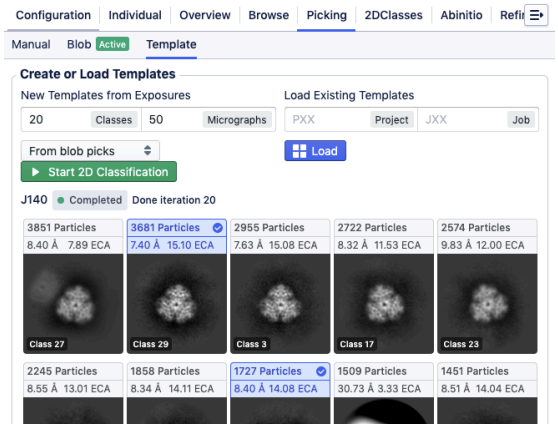
12. Optimize particle picking in the Picking tab.
    - The default picker is a Blob picker.

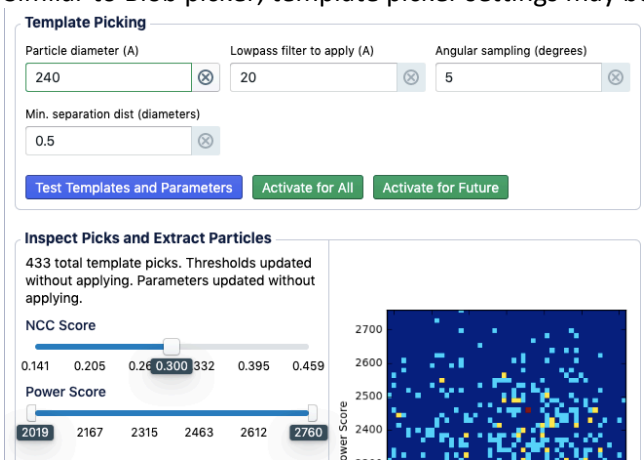


    - Blob pickers show up yellow and can be optimized by changing particle diameter or picking thresholds. Individual images may be tested, then new parameters applied to all or just the future images.
    - An issue that should be fixed in the next release is that one has to refresh the page to properly see new picks propagate to other micrographs.
    - Manual pickers and Template pickers are also available.

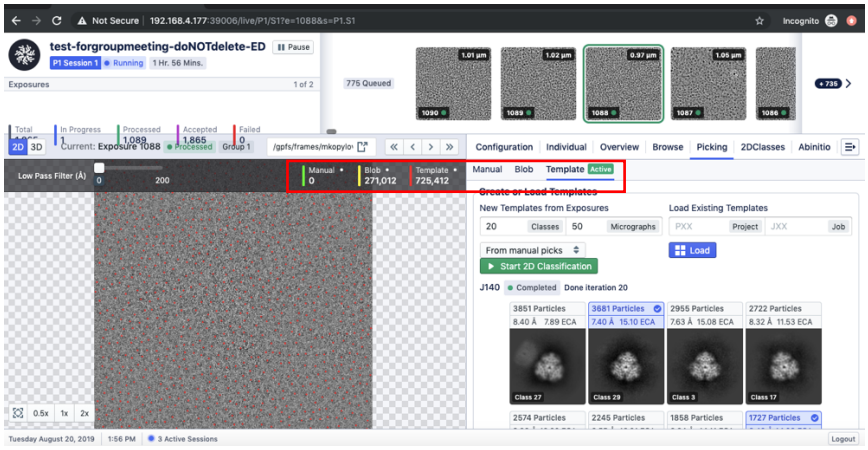


- Template pickers default to 20 classes and 50 micrographs. If one would like to start template picking earlier make note of how many micrographs have been acquired before starting 2D classification for template generation.
- You may overlay different pickers to optimize particle picking.
  - 

- After 20 iteration an interactive mode becomes available to select templates for future picking.
  - 

- In addition, existing templates may be loaded. Note, they need to be inputted as Project: “P1” and Jobs: “J140” including the letter.
- Similar to Blob picker, template picker settings may be tested, then set.
  - 

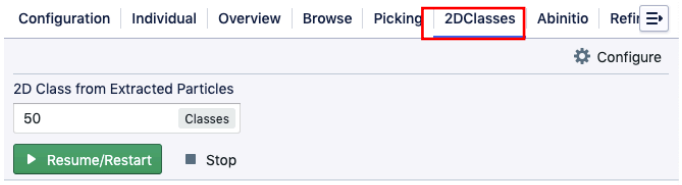
- Template picking shows up as red. Each picker may be toggled on and off on the micrograph by clicking on them. However, only 1 picker is active at a time.

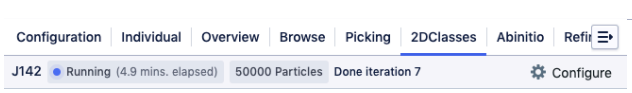


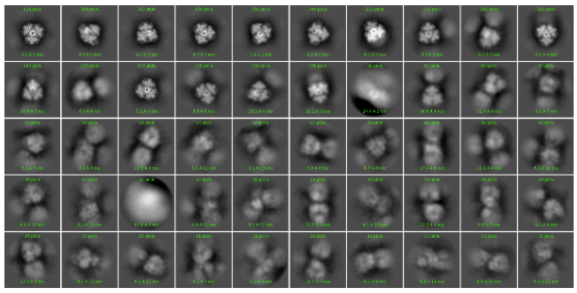
- Each process may take a while to queue up and propagate. When applying settings to all it will go back to each micrograph.

2D Classification

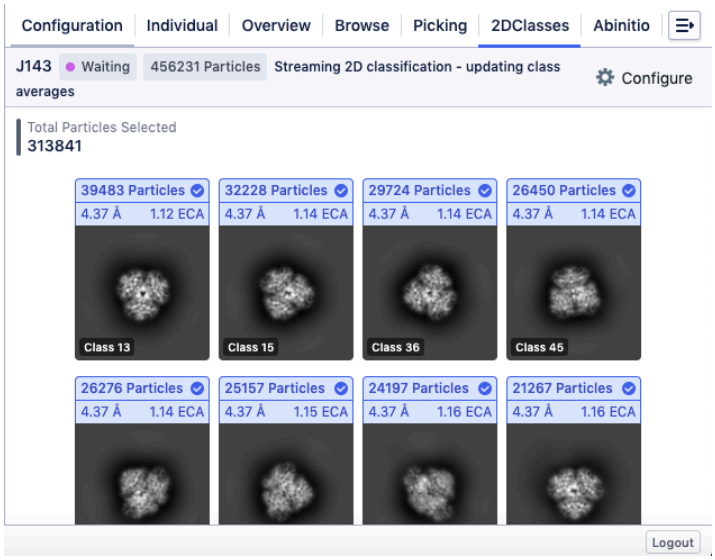
13. Now we can run 2D classification on the entire subset of blob/template picked particles. There is a delay as cryoSPARC live automatically extracts the boxes after particle picking.
    - Select 2DClasses tab and begin.







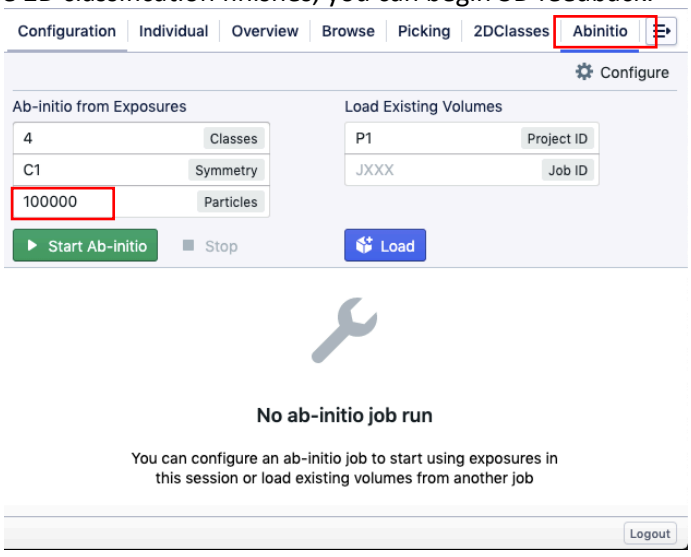
- When starting 2D classification the tab displays the output of the run.



- After 20 iterations an interactive picker becomes available. As new images are acquired it will be processed in the Streaming 2D classification.
- Select the good class average(s)
- Note: The processing requires a minimum number of micrographs so there may be a slight delay.

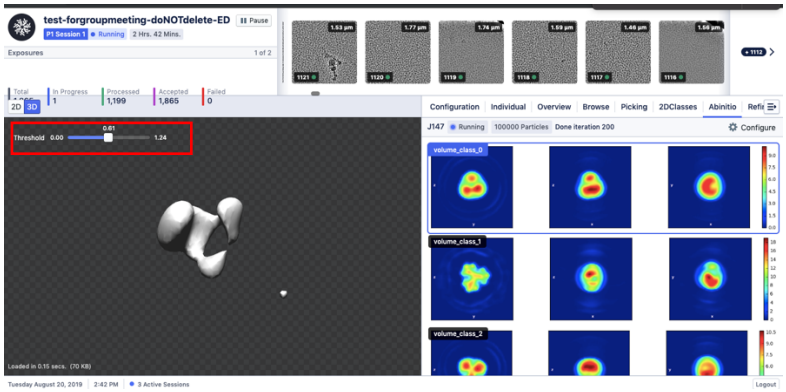
ab initio

14. Once the 2D classification finishes, you can begin 3D feedback.



- Note there is a default of 100,000 particles to begin and you must have selected 2D classes. If you have fewer particles, then adjust the value.

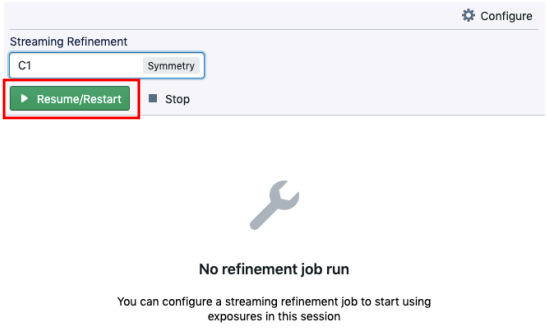
15. As ab initio run one may see the progress by selecting model and viewing the volume in the 3D viewer. The threshold may be dynamically changed as well.



16. Select good class(es) and then proceed to Refinement
    - As with 2D templates one may load an existing 3D volume to go straight into the next step.

Refinement

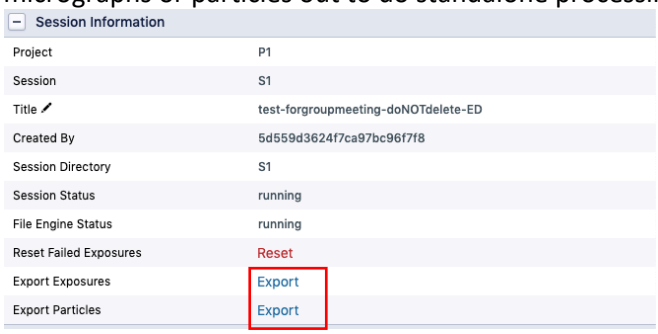
17. Start 3D refinement



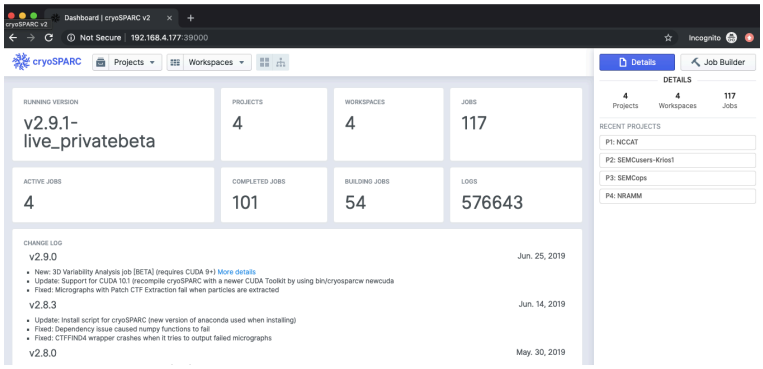
18. Similar to cryoSPARCv2 the refinement shows orthogonal 3D slides, FSC curves and Euler angle distribution plots.

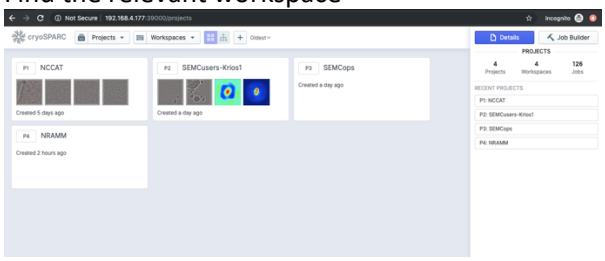
Doing more

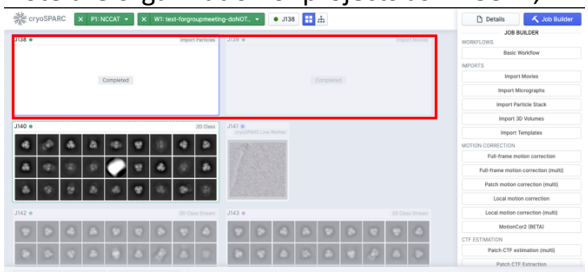
19. cryoSPARC live is built upon cryoSPARCv2 and you have additional features available to use.
20. Getting micrographs or particles out to do standalone processing.



    - In the initial Configuration tab you may Export Exposures or Export Particles
21. Accessing cryoSPARCv2
    - Goto 192.168.4.23:39000 and use the same login.

- 

- Find the relevant workspace
- 

- Note the organization of projects as: NCCAT, NRAMM, SEMC.
- 

- The exported micrographs or particles may be used for further processing. Please note the number of gpus available as to not impact streaming. Once cryoSPARC live is caught up with the latest image only 1 gpu would be needed in the default lane to keep up with data ingestion.

22. Done.