CryoEM Q&A Report - June 10, 2020

Webinar ID 948 6863 3490

Q		Is the origin related to the focus?
	A	*Not really; the origin is the point where spatial freqency is zero
Q		Is there any correlation between no. of origin and spots.
	A	*"Spots" are due to a repeating object, whose Fourier transform has information about the repeating object. Non-repeating information (noise?) is between these spots.
Q		is the maximum dose a function of dose rate?
	A	*Dose rate does not seem to be as important as total cumulative dose, at least for the dose rate that is practically achievable in the microscope
Q		why reciprocal dimension is 1/delta x not 1/N*delta x
	A	*the 1/delta x is the distance the the outside EDGE of the FT; the 1/N*delta x is the size of ONE (each) pixel in Fourier space
Q		Why are there less elastically scattedred electrons when the sample is so thin? is that beacuse the electrons have less sample to scatter from/with?
	A	*Yes, I think you've got it.
Q		What is ideal thickness of Vitreous Ice?
	A	*Relative to size of Object - Live Answered
Q		How the scattering differs in terms of different atoms? And what difference do we expect to see in the electron density of the map for these different atoms?
	A	*Heavier atoms scatter more strongly than lighter ones (so for instance, Ti or Au scatter more strongly than H or N), hence "stronger" scatterers are "easier" to see in the density map, or become more apparent, than lighter ones (in essence it's easier to see something heavy like gold or another metal than say, a water molecule). In the normal case, there is not much differ4ence between the scattering of protein vs water, so of course there is not much contraast, but that's what is really there.
Q		Why evenif thon ring is quite perfect and completely match with theoretical values, but the corresponding micrographs are not all good qualities
	A	*The Thon rings measure the quality of the micrograph, not the partcles in the micrographs.
Q		Can software correct over-focussed image?
	A	*Not as easily as correcting under-focusing
Q		Is the scattering cross-section related to the nuclear mass? Would it be possible to use heavy isotopes to increase the scattering in organic samples with C,N,O, etc?
	A	*Not really. The increase in scattering with MV (or isotope weight) is not big enough with electrons to justify using heavy isotopes.
Q		Can different stains(say, Uranyl formate or acetate) can help in better imaging in case of Negative Stain?

	A	*Different stains also have different pH values, if the protein is sensitive to pH then changing the stain can help sometimes eg. Use of Na-molybdate in place of highly acidic Ur-formate or acetate. live answered
Q		what are some common reasons for seeing positive staining?
	A	*Usually it is direct binding of the stain to regions of the protein
Q		What are the MW limits for neg stain?
	A	*Tungsten or molybdenum are the lightest MV stains I know of.
Q		Can negative stain gives the user an overview of the sample quality before goiing for Cryo-EM?
	A	*Generally speaking yes. But sometimes a component in your buffer (e.g., ATP) may react w/ the stain solution. This will give you an impression that you sample is bad, but actually it's good.
Q		i saw some people use negative TEM to do 2D classficaition. Can we genrate 3D reconstrction like useing EMAN2 or Relion
	A	*Almost all the programs assume the images are white particles on dark background (same as protein particles in negative stain images). All the cryoEM images are dark particles on light background, so you need to invert the contrast for cryo particle images. Therefore, the only difference between NS and cryo image processing is that you don't need to invert the constrast for NS particle images. live answered
Q		Hi, question on negative staining: could a bad or not good indicative staining prevent in going ahead with the sample analysis.. or maybe change conditions that in cryoEM would have given good results? Thanks!
	A	*Sometimes there is a correlation between negative stain information (or quality) and cryoEM; sometimes not.
Q		If there is any review article on negative stain options and approaches that you would recommend, please do share with us.
	A	*I have followed with good results protocols from a recently-ish review by Neil Ranson and Rebecca Thompson. Also a good review of neg stain for viruses from Audray K Harris at NIH. Becky and Neil are from the Astbury Centre in the UK.
Q		Do you recommend Neg stain as an initial work flow as a preliminary step for sample prep? Or not needed
	A	*I do in my facility, so as to not bog down the fancier instruments and leave them for cryo only work. But it depends on the PI/lab. We require students and PIs to screen their sample first by TEM negative stain. Gives us an idea of homogeneity, purity and concentration
Q		Negative staining used for phasing?
	A	*EM images have both amplitude and phase, so you don't need to do phasing like in X-ray crystallography. The EM map (both NS and cryo) can be Fourier transformed and used for phasing in X-ray crystal structure determination. live answered
Q		Would you do in parallel, more than as a prep step (negative stain)?
	A	*It really depends on the lab's preference. Many labs go directly to cryo, if there is scope time available for screening.
Q		During image classification, do we need to select those that have higher number of Thon rings?
	A	*One important considerartion is how far out in resolution the Thon rings go, not how many of them there are. The number of Thon ring out to a given resolution is a function of the defocus, as David said.
Q		how to determine the optimum range of underfocussing?
	A	*It is a balancing act, as David is saying
Q		after fourier transform a 512x512 real-space image, we get a 512x512 fourier-space image. Which point is the center? All four points in the middle?

	A	*depends on the particular FT program; the usual “zero” of the FT is a single pixel at the origin.
Q		Does the frequency of thon rings depends upon the wavelength of the scattering beam, or is it some other factor?
	A	*Wavelength is one of the factors; defocus is another; spherical abberation is another.
Q		Can you still be able to measure the size of your particle accurately if you apply defocus phase contrast?
	A	*If the contrast transfer function is corrected for properly, the size of the particle will be accurately portrayed.
Q		Are there any simulators that calculate Thon rings given a certain set of experimental parameters in order to prepare a data collection session?
	A	*It used to be possible in CTFit from Steve Ludtke. I don't remember if it migrated to be part of EMAN. But one used to be able to get the radius curve to do fitting and you could model how defocus, Cs, kV, etc impacted the curve and thus the look and amount of Thon rings https://jiang.bio.purdue.edu/software/
Q		what is the relationship between the defocus range and the resolution of image collected? how defocusing provide more information about low resolution data?
	A	*When you plot the Constrast Transfer Function with different defoci, the answer will be obvious to you.
Q		Why is the VPP so discredited now? How does it deal with instabilities in voltage?
	A	*It is not discredited; it is simply a bit more tricky to use, and it lowers throughput. For some things it may be worth it.
Q		In what conditions, phase plate is needed
	A	*small proteins only - Sometimes; also in tomography
Q		Can you explain why 90 degree for phase plate?
	A	*After 90° phase shift, a sine function becomes a cosine funciton. you get more info at low resolution in a cosine function.
Q		is the defocus and phase plate used in microED also?
	A	*microED only uses electron diffraction, which does not involve defocus, or Thon rings, at all.
Q		For CTF estimation of phase plate data you need to figure out defocus and phase shift. How can you distinguish if the advance in the function is coming from defocus or the phase shift of the phase plate?
	A	*Because of how these different terms come into the CTF equation, if there are enough Thon rings at high enough resolution, these can be separated.
Q		what about getting just black boxes for 2D averages from NS image. What does that indicate especially for helical reconstrutions. Is that a CTF issue?
Q		When do we have to warry about Ewald curvature?
	A	*The higher the resolution desired, and the thicker the object, the greater is the effect, as David showed.
Q		can the phase difference due to Ewald sphere be taken care by modulating the wavelength of electron beam?
	A	*The smaller the wavelength the bigger (1/wavelength) the sphere, and thus the flatter the Ewald sphere will look!
Q		how to recover the deleted job
	A	*if you have deleted a job and the associated data (without a backup somewhere) then unfortunately you cannot recover the job. However, if you have the raw data or other “parent” jobs in cryosparc still existing, then you can run the job again. For any specific questions relating to this, feel free to email us feedback@structura.bio and we will try to help
Q		can we get details for remote server login
	A	*https://guide.cryosparc.com/setup-configuration-and-management/how-to-download-install-and-configure/accessing-cryosparc

Q		for T20S datadownload, which specific files I need? There are multiple options
	A	*If you're using cryosparc, you can use the function `cryosparcm downloadtest` which will download a compressed folder of all the files required. Extract it by running the command `tar -xf <filename>.tar.gz` and you can then import the .tif files in cryoSPARC. Ali will cover all this soon!
Q		naive question here what is the bit depth recommended for TIFF 8,16,32, etc. for examp if we are processing neg stain data
	A	*For neg. stain, generally the details do not matter too much because you will be aiming at 15A at best. If your electron dose per frame is low enough, 8 bit depth is fine, but when the TIFF is compressed in LZW compression, the bit depth doesn't matter much since the compression removes all the extra wasted bitrate
Q		where should I place the project directory in my single user workstation? is it a good idea to always place it in the scratch ssd?
	A	*Normally, your SSD won't be as large as your raw storage server- data processing produces a lot of data, and you might run out of space fairly quickly on your SSD. It's best to keep your project on spinning disks; cryoSPARC will intelligently cache particle images on your SSD whenever you need them.
Q		how many tiff files are in the dataset? I tried to process and but only found 21 images
	A	*if you are referring to the T20S dataset available here https://cryosparc.com/docs/tutorials/t20s, it has 20 movies and 1 gain reference file.
Q		How do we redo this setting? The SSD setting.
	A	*To reset the project-level SSD caching open, choose the 'No project-level default' option. This is the default value for new projects.
Q		Is it possible to come back later and disable ssd caching option?
	A	*Yes, at the project level, it applies to all new jobs that are created in the project. You can also set this per-job
Q		can we import .tiff format as well
	A	*Yes, in fact the T20S movied we are importing now, are .tif format
Q		I tried to add the wild card, but it doesn't respond to my typing at all
	A	*For the list to update, press the enter key when you're focused on the path input or click away from the path input. (You must open the file browser, you can do this by clicking on the folder icon next to the path parameter input in the job builder sidebar)
Q		Is it possible to modify the job parameter while its running , like pausing it?
	A	*This is not possible - you will have to kill the job in progress, 'clear' the job, edit the parameters when it is in building status, then queue the job again.
Q		no, the problem is when I put pointer there and try to type, it didn't type anything. The typing works in other spaces apart from movies data path and gain references path.
	A	*Please click on the button with a folder icon to the right of the path input. The dialog that opens is where you can enter the path/blob and list matching files. When that is set correctly, press the 'select' button and you will see the path parameter value update in the sidebar
Q		Can you run cryosparc in command mode? or interactive mode only?
	A	*It's possible to build and run cryoSPARC job using the command-line interface. We've documented our CLI functions here: https://guide.cryosparc.com/setup-configuration-and-management/management-and-monitoring/cli
Q		Where can we check an estimation for how long a job will take?

	A	*No jobs post their estimated completion time except Ab-Initio. You can always see the general progress of any job by opening the job card and looking at the Overview tab, which will contain events from the job process itself.
Q		when we have a problem to run motion cor2 with k3 data, how do we fix it ?
	A	*Please check the discussion forum at discuss.cryosparc.com or send us an email at feedback@structura.bio with any screenshots, error logs, etc. Thanks!
Q		where can I find the parametes of pixel size, sperical aberration and exposure dose?
	A	*Your data collection facility/microscope operator should be able to provide you with the pixel size and exposure dose. The spherical aberration is generally known for most microscope types, e.g. for a 300kV Titan Krios it is 2.7
Q		Are these parameters are microscopy dependent, refineable?
	A	*Yes the exposure dose, pixel size, are microscopy dependant.
Q		Hi there! I was trying to do cryospac in the workshop but with my data. For some reason my import job is not working and the message is this: Traceback (most recent call last): File "cryosparc2_master/cryosparc2_compute/run.py", line 82, in cryosparc2_compute.run.main File "cryosparc2_compute/jobs/imports/run.py", line 467, in run_import_movies_or_micrographs assert params['cs_mm'] is not None, "cs_mm must be set in parameters" AssertionError: cs_mm must be set in parameters. Not sure if you can give me guidance. Thanks!
	A	*Please ensure you set 'Spherical Abberation (mm)' to 2.7
Q		so you are looking at the trajectories of the particles before they're picked?
	A	*Please look at our tutorial re: Patch Motion Correction for more details: https://cryosparc.com/docs/tutorials/patch-motion-ctf
Q		what types of GPU can cryosparc use? NVIDIA versus AMR Radeon...
	A	*cryoSPARC supports only NVIDIA GPUs with CUDA. Details: https://guide.cryosparc.com/setup-configuration-and-management/hardware-and-system-requirements
Q		can one import CTF estimations done in other softwares?
	A	*If the CTF metadata is for particles, and you have a .star file containing these values, you can use the Import Particles job to import CTF Estimations completed in other softwares.
Q		Can any motion correction job work for the patch CTF estimation? Do we need to use CTFFIND4 for local/ full motion correction?
	A	*The only required input for a Patch CTF Estimation job in cryoSPARC, are a set of motion-corrected movies regardless of how they were motion-corrected.
Q		Why I cannot see other changable parametres in the CTF estimation section?
	A	*Directly underneath the pruple 'building' tag in the sidebar, there's a toggle to turn on 'advanced parameters'. Turn that toggle on to view all the parameters Ali can see
Q		Sorry, I failed to follow how to load the Job2 outcomes to Job3 for CTf?
	A	*First make sure J3 is building and the sidebar shows the list of parameters to edit. If not, click on the 'building' tag on the job card. Next, click on the J2 card, press spacebar, then drag 'micrographs group' on the right side of dialog into the 'exposures' input box in the sidebar. You should see 'Group 1 → J2.micrographs' if this is successful.
Q		Are there any keyboard shortcuts for the Curration job?

	A	*Not in cryoSPARC v2.15 but it is being added for a future version!
Q		How do I run the patch curate exposure?
	A	*Did you mean exposure curation? Here's the full tutorial: https://cryosparc.com/docs/tutorials/exposure-curation
Q		Sorry, I did not find how to connet Job2 to Job3?
	A	*Please see this image: https://cryosparc.com/docs/tutorials/job-builder#overview-output-groups - you may need to scroll to the right if your screen width is small
Q		Wherre does that # figure in calcularions?
	A	*The '#' column in exposure curation refers to the exposure number in the order that it was imported by cryoSPARC. Exposures are given an ID from 1 to x number of exposures
Q		how to delete a job which in purple color
	A	*If you see the job builder (list of parameters) in the sidebar, click on the 'cancel' button at the bottom left of the sidebar. You'll then see the normal job details view on the sidebar and can click 'delete job'
Q		sorry, I still had difficulties in finding the ourput of motion correction for CTF? I asked for help many times. but did not get any reply. what should I do?
	A	*No problem, it would be more clear if I could paste an image in the answer! When you select the Patch Motion job card, inspect it by clicking the spacebar. Once the dialog is open (on the 'Overview' tab), the right side of the dialog shows 'micrographs' under 'outputs'. This square can be dragged onto the input field for the Patch CTF job using your mouse.
Q		how can i see the thon rings in the "patch CTF estimation" job?
	A	*You can open up the job and click the "Show from Top" button, which will enable the Overview tab to show all events; you can then search for the Radial plots there.
Q		I select the Patch Motion job card, then I clicked spacebar. It showed the details of processing. I did not see outputs. Anything wrong?
	A	*please look at the right hand side of the Overview tab. You should see a small box with the movie outputs
Q		Can we use 2D or 3D classes for Deep Neural Network based picking?
	A	*Current popular methods do not use 2D or 3D classes for deep neural network based picking. They rely on particle locations instead. There is potential for 2D classes and 3D classes to play a part in deep particle picking.
Q		where could I find the output ? I can not display as the toutorial showed
	A	*unfortunately it's difficult to diagnose without being able to see your screen. Do you mind sending a screenshot to feedback@structura.bio?
Q		Step 3 in particle picking
	A	*we are going to do this right now, in an Extract from Micrographs job. The Inspect Picks does not extract particles, only outputs their location.
Q		How to run the inspect particle picks job? It says "waiting"
	A	*'Waiting' in cryoSPARC means 'waiting for user input'. If you are already looking at the job dialog (spacebar after clicking on the job card), click on the 'Interactive' tab at the top left. You should see the interface with the picks then
Q		what did you use for input into the extract? the inspoect particles? or the pick particles?
	A	*You would use the outputs from the Inspect Picks job, specifically the micrographs and the particles.

Q		In the 2D clasification some time I get classes were it aligns on monomer of my hexamer and the results looks like a dodecamer with the “common monomer” with high intensity and the rest with lower intensity. How can I solve this problem?
	A	*we will try to answer this but in case we don’t have time today, it would be a great question to post on the discussion forum (discuss.cryosparc.com) so other users can also comment!
Q		I have this error: [CPU: 90.9 MB] Traceback (most recent call last): File "cryosparc2_worker/cryosparc2_compute/run.py", line 52, in cryosparc2_compute.run.main File "cryosparc2_compute/jobs/runcommon.py", line 823, in check_default_inputs assert False, 'Non-optional inputs from the following input groups and their slots are not connected: ' + missing_inputs + '. Please connect all required inputs.' AssertionError: Non-optional inputs from the following input groups and their slots are not connected: particles.blob. Please connect all required inputs.
	A	*Hi, this indicates you didn’t run the “Extract From Micrographs” job to extract the particles. See Step 3 under Particle picking on the left side of the screen share for details.
Q		Is it ever a problem to choose an enourmous number of classes to classify?
	A	*CryoSPARC 2D classification is fast even for large datasets and large numbers of classes - some benchmarks being shown now.
Q		how did you solve the SSD error?
	A	*If your job failed, clear the job, then press the “Building” tag on the job card to bring up the job builder on the right. Scroll down the the “Cache on SSD” parameter, and turn this off.
Q		can we manually remove few unwanted particles?
	A	*It’s not possible to manually remove particle picks from the blob or template picker at the moment.
Q		Sorry, I missed the inputs for the latest Inspect particle picks job
	A	*Inspect particle picks will take the ‘particles’ and ‘micrographs’ from the Blob Picker or Template Picker job
Q		ok, do I have to give all the 2D classes to the selected 2D classes?
	A	*If you are at the step where you are selecting 2D Classes to feed to the template picker, you only have to select two classes - a top view class and a side view class.
Q		Can you please show where is the “then press the “Building” tag on the job card to bring up the job builder on the right. Scroll down the the “Cache on SSD” parameter, and turn this off.”?
	A	*The ‘building’ tag appears in the center of the job card on the card view. Newly created jobs are displayed at the bottom of the screen, you may need to scroll down
Q		sorry I missed what two parameters you used for two separate 2D classification?
	A	*The first one had 50 classes and the second one had 100 classes
Q		Do you use patch motion also for larger datas sets: 2000-5000 movies? In that case, is it normal that the job is stucked in “launched” or do I need to revise something?
	A	*Yes, we’ve seen it be used on 20K+ exposures. There is most likely something wrong if the job is stuck- feel free to reach out to us with some logs on the Discussion Forum!

Q		I've encountered a CUDA-related error when running the "Patch motion correction(multi)" job. A similar question was answered on the cryosparc forum in 2018, but the versions that figure in my case are different and i was wondering if anythign's changed and what the problem is?
	A	*thanks for your question, if possible do you mind posting on the forum and we can try to answer there? Any screenshots, error logs, etc are appreciated!
Q		Can the number of iterations be changed in 2D classification?
	A	*Yes, it can. See the “Number of online-EM iterations” parameter.
Q		do you need to input the number of 3D classes you want to produce?
	A	*Yes, you need to specify the number of classes you want to find in Ab-Initio Reconstruction. The default is 1
Q		What resolution did you receive at the ab-initio reconstruction? did you use the 50 or 100 2d classes?
	A	*We used the 50-class 2D classification job
Q		Also, if I want to run a job again, changing the parameters, do I nee to clone it?
	A	*If you don’t want to keep the results, you can clear the job - that will set it back to a building state
Q		ok thanks. And before the ab initio step, how many classes do I need to select? as much as possible with clear features?
	A	*Yes, ideally in your Select 2D job, you should choose all the classes that look like your particle.
Q		What’s the result if you run 2 class ab-initio reconstruction job?
	A	*Ali is mentioning this now - what will happen if you run a 2-class abiintio on the T20S dataset
Q		How does the 3D reconstruction algorithm deal with preferred orientation problem?
	A	*We will cover this in more detail tomorrow.
Q		Do you have “jobs” for cryoET, tilt series, subtomogram averages, and tomograms at cryoSPARC?
	A	*CryoSPARC doesn’t have support for tomography data unfortunately
Q		Maybe you should define D7 for the participant. It is 7-fold symmetry with a perpendicular 2-fold.
	A	*I am moving this to the answered section based on your response so other participants can see this too.

LIVE ANSWERED QUESTIONS

Is the origin referring to a layer line?
How do we define the origin?
Can you please explain the Nyquist limit again in the practical manner. For 1.5 Å resolution, what do we need to measure?
Can the intensity of the electron beam affect the maximum resolution possible?
Does having more uniform spots gives a better resolution than having spots of varying size?
Does buffer change the elastic scattering ratio
Is there a relationship between preferred orientations in samples and sample thickness?
which one is structurally important elastic scattering or inelastic.
Does the grid material (ie gold vs. carbon) change the scattering ration?
does the unscattered has any contribution of the image
Will too thin of ice cause squeezing out of the particle?
Can going to thicker part of the ice give better orientations, in case there is an preferential orientation problem?

How to avoid protein denaturation in thin vitrous ice layer by Air water interface
For proteins having disordered or unstructured region affects on the scattering ? and how can this be solved?
Does the number of Thon rings vary in individual images?
Is there any phsyical intuition of a FT?
Does the number of thon rings indicate anything about resolution
Does selenomethionine labelling of proteins can help to get contrast?
If any protein uses a heavy metal as a cofactor, does it help in negative staining?
Is cryo-negative staining still considered an effective technique nowadays?
Why are overfocused beams not used?
Is the fewer tone rings the better?
how do you get different images of TEM to see tha particles vs electron scatrering to see the thon rings? Is that by using detectors in a different position? can you please explain this? Is one of the images a back scattering image?
could you correct that envelope decay by mathematical methods?
Can you explain the envelope function a little more?
When to use VPP? And does it really work for small proteins? (A)*People are getting structures of proteins with molecular weight of <50 kDa at resolution ~3.0 Å.
What factors limit the envelop function, which showing damaged higher resolution info? How to improve it?
*many factors: sample thickness, drifting, microscope lens quality (beam coherence, astigmatism, coma), etc. Live answered
Practically, is it always “better” to use the phase plate? What are some key pros/cons?
Does plase plate perfer certain biolgical sample, or general good for all kinds samples? What is disadvantage of phase plate?
How multiple users work on the same projects?
Is there a way to export the tree diagram of your procedures?
Can you please explain data sharing among projects or workspaces or jobs
how do we import and export data from or to relion
Does cryosparc have a prefered minimum dose per frame for motion correction? and how does it handle if one frame (for instance the last one) has a large dose than the others?
can you import data from a remote cluster without downloading it to the machine where cryo-spark is running?
Can you process different datasets in same project but in different workspaces?
Don't you have any problem to upload k3 data ?
what is flip gain ref in X ,y why would one need those options.
what about pixel size, spher abe paramer input are incorrect? where are these parameters coming from?: 0.6575, 2.7?
difference between patch and full frame motion correction?
How do we decide on the type of motion correction needed; patch, local or full motion correction?
What is the difference between Patch Motion correction vs Full-Frame motion correction jobs? Is one better than other?
does cryosparc read dm4?

Can the Total exposure dose for an import job be edited later without reimporting data?
Gain Reference data can be found from Data Collection parameters?
why not cryosparc read the pixel size from header
Formats acceptable for gain reference files?
what is meant by flip gain in X & Y? when to use this option?
Is this motion correction program developed by crosparc ? Is there option to thorow away any frame as motioncor2?
what pixel size should i use if the movies were binned during motion correction. e.g. data collected at 0.55A superresolution and binned by 2 in motion correction. What does binning do?
There are 5 different types of motion corretion in job builder. What is the difference between them?Thanks
how does no. of patches relate to quality of motion correction quality? and how about overlapping patches
What is the effect or consequence of a wrong electron dose?
When I collected the data, I noticed that each micrograph has its own gain-ref file. When processing the data, should we just use one of those gain ref files?
what are the units on x and y for the motion correction plot?
How does the use of carbon holey grids vs. gold grids affect motion correction? Is the use of one type of grid computationally limited (ie. it's always worse)?
can we apply more than one type of motion correction to the data set (frame-patch-local) is it required to apply all
Motioncorrection Trajectory of which path is shown in case of multiple patches And can we figure out where is the start point, perhaps colored rainbow in stead of one color?
can you import already motion corrected images? and how would you do it? yes you can use the Import Micrographs job.
Should motion correction be preformed over all frames or a subset of the first frames?
Can you correct for particle movement in the Z axis? Thanks!
does more number of frames improve the motion correction?
can this motion correction consider only in X and Y axis and not z axis?
Is Patch-motion job better than Full-frame and Local motion jobs combined? Has this been tested?
So, when do you use patch-based motion corr and when is better to do the full-frame followed by local motion correction?
what are the units on motion trajectory (X,Y)?
is the patch motioncorr superior to doing full-frame followed by local (after particle selection)?
How does the patch motion correction, is then applied to a movie in details? Patches are moved individually? Particle which happened to be at borders of patches, do they get corrupted?
Patch sizes for k2 or k3 appropriate time X y divisions
Can ctf estimate at a later stage be used to assess what is the best motioncor strategy?
Can we do per particle CTF prediction? Thanks
should we discard micrographs with ctf resolution estimation higuer than X being X the resolution we look for?

Is the CTF radial plot rescaled in some way? In my experience, the high frequency Thon rings are much smaller than the ones shown in the plot.
If we have data collected with VPP what do we have to take into acount for CTF correction?
what is the green Fit line in the ctf graph
is there a way to reject automatically images with strong ice ring in the exposure cuaration job?
EPU outputs gains for every movie file (at least EPU1 did)
what combination of factors you need to apply during exposure curation tool to get rid of bad particles ?
Can setting Max resolution in Patch CTF job to 4A effect the CTF estimation? If higher resolution is used, can the estimation become better?
what the particle picking techniques would be better to pick a very crowded micrograph?
Does reducing the box size remove some structural features of a protein in the final structure?
how to choose max and min size of particle?
how to choose box size when size of protein is not known
WIll you please repeat the 'rules of thumb' for extraction box size and for fourier crop box size, both in units of pixels? I assume that at this point we are using the original pixel size (in Angstrom) to arrive at a box size?
At what point, or using what output, would a user think "I need to make particle picking better" and so generate a template from an ab-initio reconstruction?
Can someone explain again the SSD not available fix? It was just barely too fast…
Does the resolution in the 2D classification reflect the final resolution of the structure?
Does it matter or not if the particle is not well centered after 2D classification?
"scales well with data set size" is this O(n log n)? 2D classification
if the 2D classes are not well centered will this impact the final resolution and how to fix it
How to solve off-centered problem although using template based?
"scales well with data set size" is this O(n log n)? 2D classification. says this was answered live but i did not hear it asked
is the a resolution limit that we should be using in ab inito reconstration
If we know that a structure is symmetric, does it help to input this information for abinitio model generation? for example icosahedral symmetry in viruses?
perhaps this is too specific for the workshop, but how would ab-initio work for a heterogeneous protein cage wherein you might have some cage samples with 1 or 2 subunits missing? presumably those particles would be weeded out during particle pickign since it would look different in the 2D class relative to a complete cage, correct?
Is there a strategy if you don't know the heterogeneity of your data how many classes you should specify for ab-initio? How long does a 5-class ab initio reconstruction take?
Why did we do the 2D classes if we don't use them in Ab-Initio reconstruction?
Can you explain why you did 2 2D classification jobs with different numbers of classes?
Why D7 symmetry?
Do we need to set symmetry at Ab-inital reconstruction?

How forgiving is the ab-initio reconstruction? We did a 2D class selection to help and then get a refinement to 2.92 Å reconstruction. If we use all particles from our 2D classification in ab-initio reconstruction and refine that, how bad would it be?
Is it possible use a external volume, with a different pixel size, as a template for refinement?
Is there a way to increase the number of iterations in the refinement step?
I created two homogenous refinement jobs applying: 1) C1 symmetry, which yielded in 12.86 Å model, and 2) D7 symmetry that resulted in 2.92 Å. I am wondering what's the reason of such siginificant difference between them?

NO ANSWERS NOTED IN TRANCSCRIPT

where I can select scratch/training/projects as the project directory?
what does that mean if see another center in the thon ring?
Could you describe, briefly and intuitively, the advancement of the electron wavefronts in terms of discrete electrons, which is how they are modeled at the camera
Can we get any useful/guiding imformation as model for Cryo-EM 3D re-construction of protein structure?
Typically it has to do with the shape and MW, so say a rod-like particle will probably be visible vs a tiny dot if both are of the same MW. I usually don't go below 100kDa, which would still be pushing it. On the bigger side, typically no limit, but I usually say to keep it <300nm
How exactly does the software (any really, but I'm using CryoSPARC) know what particles to average over the course of a movie? Or maybe a better question is how exactly does it track a particular particles movement?
How efficient is motion correction through cryosparc/RELION, in rectifiying the beam induced motion?
How does the number of frames and each frame time contribute to the blurring effect?
I've seen this honeycomb pattern in my 2D class averages! I wonder now if it's an aliasing problem. How do we correct for that if that's the case??
When you say 'finely sampled image', what is the parameter regarding data collection that we can play with?
Regarding the different phenomena limiting the resolution, David was mostly talking about applying corrections to the image. Is it possible that those 'resolution limitation values' reported in the several slides are changing with hardware improvements?
For microED how does the beam coherence limit your unit cell sizes that are able to be analyzed? Do you have guidance for LaB6 source vs FEG?
How do you match the tilted image with non-tilted ones?
what should be the regularisation parameter value(T) valuefor 2D & 3D classification when we face very low signal to noise containing images in relion?
How do you deal with orientation preference dataset?
how do you distinguish a junk particle ?
Similar question: Does the preferred orientations also depends on particle concentration?
can you predict the symmetry from just the data
it says: Job to import must be located inside the project directory. Please move the job folder into /scratch/training/projects/P2/imports
why I can't type on the movies data path?

How is the movie correction performed? What is the functional model for the deformation and which interpolation is used to produce images?
Can we bin data in patch motion correction?
why bin since it cutting resolution by half ?
how about per particle motion correction?
What is the minimum electron dose per frame that can be reasonably aligned with CS2 protocols?
how to distingsh the grid motion or particle motion?
Do you think there is any benefit in going a step further and trying to implement correciton for possible particle rotation during imaging?
When in Relion, I usually import 9 optic groups for each of the nine shots of data separatelly and the join before motion correction. Is it the same here?
is the CTF a correction because of the defocus?
why is it necessary to mask the center of FT to estimate CTF?
How much farther out the Thon rings need to be seen? Is there any cutoff for the number of rings?
What is your experience with ctf estimation for phase plate data?
what determines how far the thon rings will be spaced out ? or in other words how we can make thon rings to space out more to get a high resolution information?
what does it mean when the 2d landscape is curved and not flat as shown for this data set
how to interpret the 3D graph from ctf estimation? how to tell if it the results good or bad from that graph?
Some of the earlier papers on CTF estimation of movies suggested that CTF calculated per frame and averaged together is better than CTFs calculated from aligned movie. Have you played around with this?
I can not find the output of Motioncorretion? anyone can help, thanks
What is the patch size (or box size) in the patch motion correction job? How is it fitted to all different size or shape of particles?
Does ctf estimation work for zernike phase plates too?
For CTF estimation, did you use dose weighted images, or non-dose weighted images? Does it have the option to choose the non-weighted images for CTF estimation?
can he re-import data into the patch cft estimation?
During CTFfit, does the envelope function/dumping is used as weighting? Like the lower resolution rings used with more weight during fitting, that dampened higher resolution rings
will cryosparc support the EER file format soon?
How do you specify to pick particles from Dose weighted vs non dose weighted micrographs?
is it trecomended to collect high defocus and low defocus, so that i can get more low res and high res data?
How do you distinguish for certain samples between aggregates Vs those forming higher order complexes?
Extract Micrographs: Does the fourier crop step fail if the particles are asymmetric within the extraction box?
What do I need to do if I have the error before generating the 2D classes? thx
can we use 3D data developed from negative staining and use as a template?

Hi, about particle picking, What are the options to import particles picked elsewhere? I know you can import e.g. one particle.star file from Relion (stack or coordinates), but what about importing .box files or .star files with coordinates for each micrograph such as the output of many auto-pickers (gautomatch or crYOLO)? If this is not an option, is something that will be implemented soon?
do we have to reextract later for improved resolution with the correct box size if we down grade
How do you recenter your templates in case they are a bit off? Thanks!
How would you decide which class averages to pick in the case of unknown protein?
what is the angular sample degree? Should we change this parameter when picking particles?
on particle picking, for much smaller objects (say 20 nm diameter), would you proceed just the same way? Or are there any special tricks?
How well do the NCC and PS parameters work for lacey carbon grids?
should there be any perticular relation between extraction box size and fourier crop to box size? eg. 50% or 75%?
Is there a reason for not using a mask for the 2D classification steps? Are there advantages for using one vs. not using one?
misc: is a stack the same thing as an image, which is the same as a micrograph?
What can be learned from noise model figure?
can we create a mask to hide other particle in crowded dataset ?
Is it useful to re-extract your particles after 2D or 3D classification? to have your particles re-centered?
can you explain the bases of the space of 3D structures?
at what point do we unbin the data?
If we do a 3D classification using only one abinitio model in a dataset that contains several particles size, like the ones you resolved with several classes in the abinitio job, can we expect that the 3D classification job will sort out those different states?
HOW DO YOU ESTIMATE THE QUALITY OF YOUR AB INITIO RECONSTRUCTION?
Can we skip 2D classifications all together and use ab inito models directly
what to do if you see many doubled particles in the 2d classes?
Re: 2D classification, at some point, could you explain when to use or not to use Force max over poses/shift option, which is ON by default? I have heard from others that turning that off or on has made huge difference in whether or not they discern particles in their 2D class averages for their relatively small particles.
Is it correct to select 2 classes (selected 2D classes) before the Ab-initio?
could you comment on (or point to literature comparisions) the major differences between cryoSPARC vs. Relion vs. Focus-EM?
How did you define the resolution limit? From the tight line or the loose line?
At what point can you break symmetry? This has to do with identifying different states that might be present as you showed.
How does the masking work?
when to use a mask in refinement and where to get it from?
When you ab-initio classify into several classes taking the initial particle stack, what similarity do you choose between the classes?
If there is a mixture of liganded and unliganded protein in the sample, will a hetero refinement yield better results than the homogeneous refinement?
if you enforce wrong symmetry one can get yet higher resolution