

Peter J. Peters Unanswered Q&A

Q Scribing need a jetting instrument I guess if I don't have access what is the best method to apply sample

A We use a pin to deposit sample on the grid. 12 pins are provided in the VitroJet. Through scribing we create an instant thin layer so there is no need for blotting.

Q the grids have to be clipped before jetting?

A The grids are pre-clipped before processing them. Our gripper handles autogrids.

Q when the big droplet of sample was delivered in the instrument?

A Right before deposition we pick up 0.5ul of sample using the automated pipette.

Q what is the process for changing different samples?

A This is guided through the user interface providing an option to change sample before each deposition.

Q How thin sample film can you presently scribe in the Vitrojet?

A The thinnest that's been measured was about 10-20 nm.

Q while the small droplet was taken out of the surface of bigger droplet, isn't the part suffering from the air water interface as well?

A The pin moves into the big droplet and doesn't pick up from the surface of the big droplet.

Q is it possible to write multiple times on the grid to increase the number of holes to work with in data collection?

A This is usually not needed since we have enough holes for data collection. However, in the in-house machine this is being tested to be provided in a later stage.

Q was liquid ethan jetted to the grids after writing? Can you please explain the advantages of using jetting than plunging?

A Yes, the grids are jet-frozen after the sample's been deposited using pin printing. Advantages include higher cooling rates and the ability to vitrify pre-clipped autogrids.

Q Does the jetting process not perturb the samples on the grid? Is the force not too harsh for samples that are being applied? Thank you, this is cool

A No, the jetting doesn't perturb the samples. The samples are frozen before the jet touches them. Glad you find it cool!

Q Has this been commercialized and if so is it available to purchase from what vendor?

A Yes, this is being commercialized with first products being delivered. The US distributor is Nanoscience Instruments, but you can always reach out to CryoSol directly.

Q What is the dead-volume of Cryosol - what is the minimum volume of the sample required?

A We introduce 0.5ul of sample with the pipette. From this, about 1nl is used per grid in VitroJet. Multiple grids can be made with the stock volume of 0.5ul. The sample can also be exchanged before each grid.

Q can you talk more about the type of grids used by vitrojett?

A You can use any type of grid in the VitroJet as long as it's pre-clipped.

Q if you don't mind, would you mind sharing who here in the US has the cryoSOL VitroJet equipment either purchased or installed? Thanks!

A The first US installation is taking place in a couple of weeks at OHSU on the West Coast.

Q would vitrojett also be suitable for cryoET (tomography) sample prep?

A We are currently testing pin printing and vitrification of thicker samples, such as cells in suspension.

Q How does the addition of detergent affect the vitrojett, in terms of sample thickness and deposition

A That's a good question, detergents will play a role in the surface tension of the sample. By tuning pin printing parameters the thickness can be adjusted.

Many of us will not be able to purchase the vitroject for several years, in the mean time what are the ideal room temperature/humidity conditions for oursing and performing standard plunge freezing of grids using a Leica or a Vitrobot instrument?

A The process is very different between blotting compared to pin printing, so we'll avoid making claims on what the best temperature and humidity conditions are for the mentioned instruments.

Q How fast would this scribing (writing) process be? Would you think there could be issues with degradation on the air-water interface during this sample application process?

A Scribing takes a few seconds depending on the settings you use. Particles with the air-water interface within a millisecond, we expect that particles will interact with the air-water interface in all available deposition methods.

Q Is there anything in development for the freezing of cells grown directly on grids?

A Yes, there is a device that is being developed.

Q The ethane jet seems somewhat "violent". Is this not affecting the deposited sample's thickness, (while it's still liquid)?

A The vitrification takes place prior to the jet physically making contact with the sample. We haven't seen any deterioration or thickness variation of sample due to the jetting.

Q Is it possible to pipette more than one buffer solution (in addition to the sample solution) onto the grid using the Vitrojet?

A No, this isn't possible at the moment. If this is a need, we can always look further into it.

Q does Vitrojet have any limitation on the sample size?

A No, we've tested a range of samples with varying size on the machine.

other additives like alcohols, DMSO, and lipids can dramatically change the viscosity of the fluid, and can at times make the fluid extremely non-newtonian. Does this also affect the deposition of the sample on the grids?

A Viscosity will influence the fluid properties of the sample in deposition. By tuning the pin printing parameters the layer thickness can be adjusted.

Q Would the jet verification damage the grids?

A No, jet vitrification does not damage the grids.

Q It looks all the steps are automatic. Is there any step in Vitrojet you would suggest to optimise for users? For example, when we use vitrobot, we tend to change blotting time and other parameters.

A The system is automatic but you are able to set your parameters before you start processing the grid. These parameters include plasma cleaning and pin printing.

Q can the pin size be varied base on particle size?

A The pin is much bigger than the particle size. Pin diameter can be varied primarily based on the amount of grid coverage.

I think it would be more interesting and easier to follow if it was presented all steps currently used for sample preparation and then compare with the cool new developments shown in this seminar (sample volume, time, temperature, advantages, disadvantages...)

A Thank you for the suggestion, we'll take it into consideration for the future.

Q I am a little bit concerned with the force with which the vitrojet jets out the liquid ethane onto the grid.

Q Could this affect sample adherence to the grid? Or perhaps the forces on both sides cancel out this effect?

A The vitrification takes place prior to the jet physically making contact with the sample. Jetting from both sides ensures cancellation of the forces on the grid.

Q what is the coverage rate of the entire grid surface by jetting? How the homogeneous is the ice thickness across the grid?

A The jet covers the deposited area completely, leaving homogeneous amorphous ice.

- Q how much of the grid area can be covered at each application with the pin? Can you apply more than one? (same or different samples?)
- A We can cover 1 x 1 mm, which can be evaluated with the camera in real time. Currently we are working on multiple applications.
- Q can you provide a reference for that publication about the type 3 secretion system?
- A Berger C, Ravelli RBG, López-Iglesias C, Kudryashev M, Diepold A, Peters PJ. Structure of the Yersinia injectisome in intracellular host cell phagosomes revealed by cryo FIB electron tomography. J Struct Biol. 2021 Feb 4;213(1):107701. doi: 10.1016/j.jsb.2021.107701. Epub ahead of print. PMID: 33549695.
- Q does jetting work with all types of grids?
- A Yes
- Q can the jet blow away the cells on the grids?
- A No, they're vitrified before the ethane jets make contact with the sample.
- Q why do you use ethane in jetting?
- A We have investigated different cryogenes (see VitroJet publication) and ethane gave the highest cooling rate.
- Q I understand why ethane is advantageous during plunging. Why are nitrogen streams not an option like the ones used in X-ray data collection.
- A Ethane is at its melting point when jetting. Nitrogen would be at its boiling point, giving slower cooling rates.
- Q Do you think cooling rates can change protein conformations and sample heterogeneity?
- A This would have to be explored as we haven't tested this yet.
- Q If the vitrification happens before the ethane jets hit the sample as the theoretical physicist claims, could you not just aim the ethane jet parallel to the surface of the grid to freeze the sample without the sample ever touching the ethane?
- A We could, but then the ethane would first hit the rim of the grid, cooling the sample through the gridbars rather than directly, resulting in slower cooling.
- Q For freezing cells grown on grids, blotting is required to remove the cell culture media. A clipped grid would probably retain even more cell culture media than an unclipped grid. How is this handled in your system?
- A In the current VitroJet, we are testing pin printing of cells in suspension. We are developing a system to vitrify cells grown on grids.
- Q how does the environment hold cryo conditions after the ethane jet? Before ethane jet sample was in liquid-state temp
- A After the ethane jet, the grid is transported through cold nitrogen vapor to be placed under liquid nitrogen.
- Q is SLAC going to get the cryojet?
- A Let's hope so!
- Q How fast can you jet right after printing sample on grid? Have you characterized the sample distribution in between the two air-water interfaces as a function of delay of jetting?
- A After pin printing, it takes about 100 ms to vitrify the sample. We haven't systematically checked particle distribution in the layer.
- Q does the scribing require the auto-wicking grids like the droplet-based methods?
- A No, no self-wicking grids are required. Scribing works on any grid as long as it's pre-clipped.
- Q Do you anticipate that, eventually, jetting can be used to vitrify much thicker samples -100s of microns?
- A Yes, this is why we are investigating a VitroJet for cells.
- Q can you vitro jet on grids with an additional continuous support film (carbon or GO)?

A Yes, we can work with grids with additional support films.

Q What's going to happen to all the training I did on sample preparation and vitrification? Is a robot going to replace me in the lab?

A Never! You'll be able to focus on the sample and on your imaging.

Q Can you put more than one sample on the grid? Can you mix samples on the grid?

A We're looking into this, in the current implementation we work with one sample per grid.

Q do we have to optimize vitrification condition like vitrobot?

A You are able to optimize settings for plasma cleaning and pinprinting. We implemented a camera such that you can evaluate the layer as it is being deposited, and once you are happy you can start vitrification.

Q Why you don't need to fill the whole grid? Why just in the middle is enough?

For a good reconstruction you do not need to fill the whole grid, typically only a number of squares are

A used. We implemented a camera to evaluate the deposition, and only write in the field of view of this camera which is 0.8 x 1 mm

Q what would be the minimum concentration of the sample? If the concentration is low, would the nonspecific absorption of the nanotip decrease the particles scribing to the grid?

A That is dependent on the sample itself, we use concentrations that are similar or a bit higher than the vitrobot. We have not seen particles aggregating on the tip.

Q grids are also not flat, so we choose a specific region, usually centered. Therefore not across a grid

A

Q in the slide where you are suggesting new MEMs device to replace TEM grid, is it mainly relying on creating microfluidic channels?

A This work is still under development.

Q if you think that the sample is vitrified before the ethane touches it, why don't use liquid nitrogen jet?

A Ethane is at its melting point when jetting. Nitrogen would be at its boiling point, giving slower cooling rates.

Q can one bypass the glow discharge part of the operation? I'm thinking of GO Grids which I don't want to glow discharge once created

A Yes, the machine is flexible and the plasma cleaning can be skipped.

Q Is there any difference on the effect of water-air interface on the sample when using cryojet instead of using vitrobot?

A This still needs to be systematically checked and we are planning on performing these tests.