Point-by-point Response to Editorial Comments:

Editorial comments:

Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please refer to the instructions for authors.

We have proofread the manuscript and the re-submission should be corrected.
2. 3.2: Please explain how and where to create a side window for x-rays.

We have added a figure for this purpose, namely figure 3 in the new draft. More detail of the edits is provided in the response to reviewer 3.
3. 4.5: Where is the SmartMap setup; which software: SEM or EDX?

This step now reads: *"Adjust the SmartMap setup in the EDX software"*
4. Please cite all figures in order.

This has been corrected.
5. Please consider showing images (or in the video) the improvement in the map by modifying the process as indicated in the representative results (lines 322-324).

We replaced one figure (number 4 in the previous draft) with one that represents more clearly those improvements including shadowing that has almost completely been avoided and reduced beam damage by mapping at a lower voltage of 2kV.
6. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in Figure Legend, i.e. "This figure has been modified from [citation]."

The explicit copyright permissions for reuse of figures have been obtained prior to our initial submission. We have now included the official notifications and updated the citation in the figure legends.
7. Line 428: Please avoid the use of contractions (use "does not" instead of "doesn't").

This has been fixed throughout.
8. As we are a methods journal, please revise the Discussion (3-6 paragraphs) to also include the following in detail with citations:
a) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

Please refer to lines 678-711 in the revised draft. Two paragraphs were added to address these concerns, and the first paragraph from the original discussion section was eliminated and merged into the others to meet the 6 paragraph limit.

9. Please sort the Materials Table alphabetically by the name of the material.

This has been done.

Point-by-point Response to Reviewer 1

Manuscript Summary:
The authors present a tutorial of cryo-SEM and cryo-FIB techniques and their application for energy materials with solid/liquid interfaces. This will be a good resource for those working in this field. I have no issues with the content of the work and believe it to be high quality and impactful.

Major Concerns: None

Minor Concerns:
1. The title at present seems overly broad, and may imply cryo-TEM to many readers. I would suggest the title to instead specify cryo-SEM/FIB so that the scope of the article is more clear.

The title has been revised to "*Nanoscale characterization of liquid-solid interfaces in energy devices by coupling cryo-FIB with scanning electron microscopy and spectroscopy*"

2. Figures 3 and 4 could use some annotations to make clear which layers are the Li and electrolyte. At present, it is not obvious to readers without some experience in interpreting FIB cross-sections of Li metal deposits.

We thank the reviewer for the suggestion. Figures depicting cross sections of lithium metal batteries have been annotated as suggested.

Point-by-point Response to Reviewer 2

Reviewer #2:

Manuscript Summary:
This manuscript represents cryogenic microscopy techinques that have been in development for many years and as such it will serve as an invaluable guide for many upcoming cryo-EM researchers, especially in the field of energy storage research. I have a few minor questions and

suggestions that I think should be addressed in the manuscript. The authors did a nice job of detailing the process and results and I recommend the manuscript for publication with very minor revisions.

Major Concerns: N/A

Minor Concerns:
Line 179, Should a user wait for thermal equilibration before imaging begins? If so, approximately how long?

We have not observed any evidence that samples warm enough to warrant intentionally waiting for the system to re-equilibrate. However, it takes a few minutes to complete the loading process (the transfer rod has to the retracted, vacuum valves have to be closed, the slush station needs to be either emptied or covered etc.) and get set up at the controls of the FIB, turn on both beams and move the stage into position. We have added the following comment to the manuscript:

"*NOTE: the time required to set up to start imaging is usually sufficient to allow the sample to reach thermal equilibrium on the cryo-stage, especially if both stages in the prep-chamber and the SEM chamber are cooled to the same temperature and the transfer time of the shuttle from one stage to the other is minimized.*"

Line 211, Do you recommend condensation and then curing over curing while condensing? Please elaborate on why or why not.

In response to this comment and those from reviewer 3, we have elaborated on this and added two citations, namely Hayles et al. (Ref. 25) and Schreiber et al. (Ref. 13), which discuss the development of this method and describe it in more detail. Much of the elaboration was too involved for the protocol and is placed in the discussion section, but we have added the following sentence to step 2.9.: "This should produce a uniform layer of uncured organometallic platinum, and the user can briefly image the sample surface to confirm even coverage." The addition to the discussion section is as follows:

"FIB systems typically use an organometallic platinum gas to carry the platinum to the surface of the sample. Under cryogenic conditions this precursor condenses on the cold sample surface to form a non-conductive platinum-containing organic compound[27]. A curing process during which the layer is exposed to the ion beam then releases the organic component, allowing a conductive platinum layer to form. This step is critical for high-quality results as the platinum both dissipates charge and mitigates gallium implantation[13,27]. Orienting the sample so that the surface is normal to the GIS source is the best way to get a continuous layer, and the exact position will need to be adjusted for each system. FIB systems typically use an organometallic platinum gas to carry the platinum to the surface of the sample. Under cryogenic conditions this

precursor condenses on the cold sample surface to form a non-conductive platinum-containing organic compound[27]. A curing process during which the layer is exposed to the ion beam then releases the organic component, allowing a conductive platinum layer to form. This step is critical for high-quality results as the platinum both dissipates charge and mitigates gallium implantation[13,27]. Orienting the sample so that the surface is normal to the GIS source is the best way to get a continuous layer, and the exact position will need to be adjusted for each system. "

General question: I would assume that a battery/deposit would have a macroscope (hundreds of microns) electrolyte thickness but the electrolytes on the Li deposits look to be only a few microns in thickness, how is that thickness achieved? This may be useful information for users, especially since FIB milling is realistically limited to tens of microns sized features.

We have found that the electrolyte thickness varies widely between sample geometries. For example, a symmetric cell composed of two lithium foils and a membrane (celgard) separator will consistently produce an electrolyte layer a few microns thick after opening the cell and freezing in slush nitrogen, while a cell with an o-ring separator, a lithium foil on one electrode and a stainless steel substrate may produce an electrolyte layer hundreds of microns thick. The latter case was resolved by switching to a membrane separator. However, these observations are anecdotal, and users should adapt to their particular case.

Thank you for this question, as it inspired edits which will make the protocol more useful. Namely, we added to the discussion: *"Next, the technique is not immediately compatible with all sample geometries. For example, some battery samples tend to feature a thick electrolyte layer (30-100 μm) upon freezing which will require impractically long milling times when using a standard gallium ion FIB. Often slight modifications can be made to overcome this limitation. We have found that the electrolyte thickness can be reduced by switching from an O-ring separator to a membrane separator. However, the impacts of such modifications will vary between samples and should be done with careful consideration."*

General comment: The detection of Li is possible with EDS, but it is not straightforward for many EDS detectors. What detector was used for Li X-ray detection? Perhaps a small discussion on detecting Li would be beneficial. Please add the EDS detector model to the table of materials.

The reviewer is correct that detection of Li by EDX is not straight forward. The detector used in this work is an Oxford X-max 80 mm, which cannot practically be used to detect lithium. We have clarified this in the discussion section:

"While detection of lithium by EDX is possible[29], it requires the use of a detector specifically optimized for low energy X-rays which was not done in this work. More sensitive detectors will also improving the x-ray collection efficiency and thereby reduce the required electron dose for EDX mapping."

Point-by-point Response to Reviewer 3:

Reviewer #3:

Manuscript Summary:
This manuscript describes a general protocol for the preparation and cross-sectional imaging and x-ray compositional analysis of cryogenically frozen specimens containing liquid/solid interfaces. The authors provide sufficient background in the introduction to motivate their contribution. The manuscript is divided into specific sections describing 1) cryogenic specimen preparation and transfer into the SEM instrument; 2) general SEM imaging to locate a region of interest; 3) FIB milling for cross-sectional imaging, and; 4) EDX compositional mapping. SEM image figures are presented which support the description of the protocol.

Major Concerns:
The presented figures only partially support the explained protocol, where additional figures and schematics are needed. Specifically:
* The last paragraph of the introduction describes the use of a "workstation with a 'slush pot'". This is followed by a convoluted description of freezing and transfer of specimens into the SEM chamber. Please include either an annotated photograph or a schematic describing the various parts/features (e.g. "slush pot"). Additionally, please be more explicit when describing the specimen shuttle suitcase transfer device. Many (if not most) readers will not have a clue about the Quorum system, so jargon like "prep" chamber, and the description of a "transfer rod featuring a small airlock" should be avoided to minimize confusion. For example, describing of the "transfer rod" is confusing; what I suspect you are trying to describe is the specimen shuttle suitcase device with an integrated air lock and transfer rod manipulator. Please be explicit. Again, an annotated picture of schematic would be helpful.

Thank you for pointing out that such a critical portion of the protocol was unclear. A new figure has been added as you have requested (figure 1 in the new version) as well as a short description in the text. All portions of the cryogenic system are shown with annotations. In addition, all jargon or imprecise language has been either eliminated or properly explained throughout the protocol. We hope that these edits make the protocol clearer.

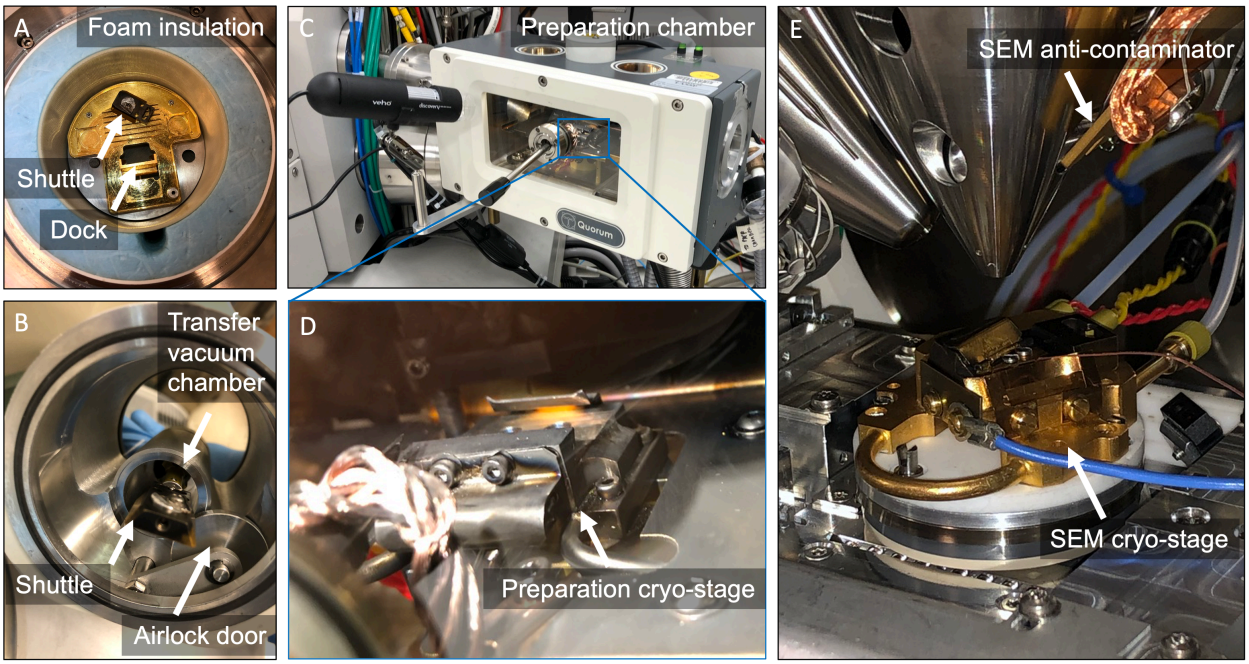

Figure 1: Components of the cryogenic FIB/SEM system used. (A) The slush pot for initial sample preparation. The main portion and a reservoir under the foam insulation are filled with liquid nitrogen, which is converted into slush nitrogen by reducing the pressure above the liquid nitrogen using a vacuum pump. Samples are plunge frozen in the slush nitrogen and attached to the shuttle before the vertical dock is used to lift the shuttle out on the transfer arm. **(B)** The inside of the transfer system. A small airlock holds the shuttle under weak vacuum during transfer to the preparation chamber, and the arm itself (not shown) allows users to move the sample onto the cryogenically cooled stage. **(C)** An outside view of the preparation chamber, where samples can be sputter-coated prior to imaging. **(D)** A closeup of the cryo-stage in the preparation chamber. **(E)** The cryo-system inside the SEM chamber, featuring the stage and the anticontaminator.

* Regarding section 3 (Prepare cross-sections), Please include a schematic illustration that describes the geometry, dimensions, and orientations of the specimen cuts relative to the beams and X-ray detector. Having to rely on the written description is quite challenging.

A figure has been added to this effect, and is the third figure in the revised draft.

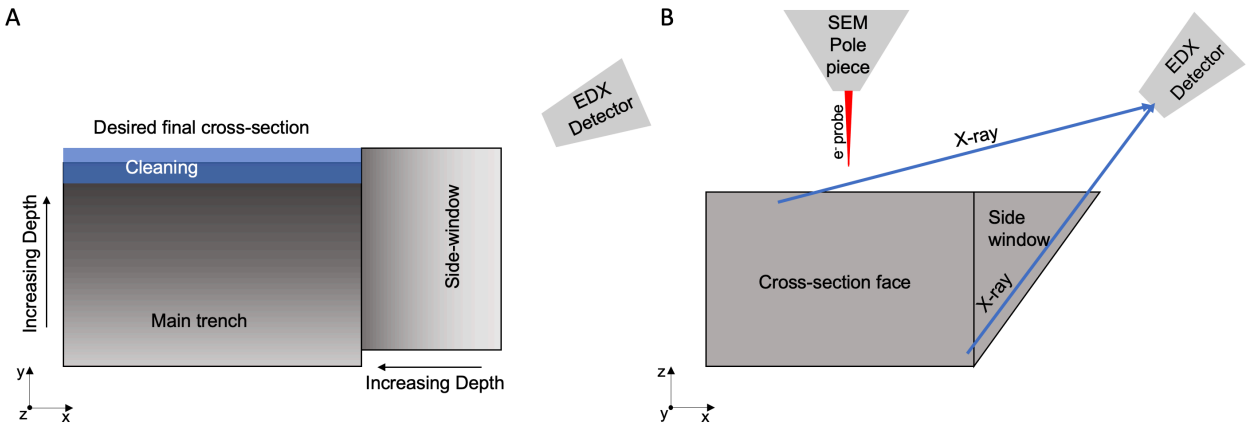

Figure 3: Setup of milling windows, including a side window for improved x-ray yield. (A) A schematic showing the key features of the milling process (placements are not exact). The main trench and side window are drawn showing the direction of increasing depth (indicated both by the labeled arrows and the gradient in shading), and the cleaning cross section (blue) is shown overlapping partially with the main trench. The side window is aligned relative to the position of the EDX detector to allow for detection of x-rays generated from the entire cross section. **(B)** A sketch demonstrating the benefit of the side window. As the electron probe scans the cross-section, electrons excite x-rays, which are measured by the EDX detector. Without a side window, shadow effects would result in parts of the cross-section (such as the bottom right here) to appear dark.

In the main text we have added: "*For EDX experiments, the FIB milling geometry should be optimized and the position of the EDX detector should be taken into account as shown schematically in Figure 3. Fig. 3A depicts the milling setup viewed from the direction of the ion beam: A main trench and side window are created first, with the side window rotated clockwise 270 degrees to produce the desired depth gradient with respect to the position of the EDX detector. Subsequently, a cleaning cross section is milled (blue box in Fig. 3A) to create the final face of the cross section. The side window is milled at least 1 µm past the end of the original main trench so that the cleaning cross section will be at least flush with the side of this trench. The side window establishes a line of sight from each point in the cross-section to the detector (Fig. 3B).*"

* Describe some of the limitations of the Quorum system, specifically lack of full rotation and how it affects the approach (maybe it doesn't affect it much).

Minor Concerns:
1. Line 50: please include after "energy devices" any recent relevant work by others, including the references below for corroded materials (Schreiber et al. 2018, Ultramicro 194, 89-99; Perea et al. 2020, npj Mater Degrad 4, 1-7; Li et al. 2020, Corr Sci 174, 108812.

The citations have been added.

2. Line 93: awkward use of hyphens. Consider using parentheses.

The hyphens were replaced with parentheses as suggested.

3. Line 105: provide statement about temperature control. You just say "held at liquid nitrogen temperature"

We have clarified that the stages should be held at -175°C throughout the protocol.

4. Line 105: Describe that metals other than Au-Pd can be sputter coated.

The line now reads *"…sputter coated with a conductive layer, such as a gold-palladium alloy."*

5. Lines 108-110: again having an annotated picture/schematic would be helpful in explaining this. Otherwise, those not familiar with the Quorum systems will not know what you are talking about. Consider that others may be using other cryo FIB/SEM systems by different companies.

As discussed above, we have followed the suggestion by the reviewer to provide details on our set up.

6. Lines 118-122: This is an important step. Provide more details as to how this is done in your specific case. I suspect since an FEI microscope is used, that this requires a loosening of the setscrew on the GIS, and clockwise rotation a specific number of turns. Please be explicit about this in your case and mention that this specific procedure may vary with different equipment.

We have added *"On the FEI Strata used here, this is done by loosening a set screw on the side of the GIS source and rotating the collar 3 turns clockwise."*

7. Line 128: Section 1.1.5 would benefit from having a section heading distinguishing "Set up the cryo prep station" from the above "set up the microscope"

This edit was made.

8. Line 131: provide more details as to what would be causing clogging. Might not be obvious to many.

The line has been edited to "*This flushes moisture out of the system to mitigate the formation of ice in the lines upon cooling, which can impede the flow of gas.*"

9. Line 138-140: again having a figure would be helpful for explaining this

See prior discussion of the added figures.

10. Line 155: again, the specimen shuttle suitcase device is not the "transfer rod"; it does have a transfer rod.

The term "transfer system" has been used to clarify this point.

11. Line 167: provide an estimated thickness of the Au-Pd film deposited using the described conditions

The step now reads: *"At this point, a ~5-10 nm thick gold-palladium layer can be sputtered onto the sample surface to mitigate charging. Typical starting values are 10 mA for 10 seconds, though these parameters should be adjusted for each system."*

12. Line 211-212: Provide an expatiation of this step. This procedure is very important to provide sufficient protective cap and was first describe by Hayles et al (JOM, 226, 3 263-269, 2007) and then further explained by Schrieber et al. (Ultramicro, 194, 89-99, 2018) as 'curing' the film into a denser protective cap.

In the protocol we simply changed this step to include: "*This should produce a uniform layer of uncured organometallic platinum, and the user can briefly image the sample surface to confirm even coverage."* However, we made extensive additions including both references to the discussion section to include details on this important step. The discussion now includes the following:

"FIB systems typically use an organometallic platinum gas to carry the platinum to the surface of the sample. Under cryogenic conditions this precursor condenses on the cold sample surface to form a non-conductive platinum-containing organic compound[27]. A curing process during which the layer is exposed to the ion beam then releases the organic component, allowing a conductive platinum layer to form. This step is critical for high-quality results as the platinum both dissipates charge and mitigates gallium implantation[13,27]. Orienting the sample so that the surface is normal to the GIS source is the best way to get a continuous layer, and the exact position will need to be adjusted for each system. FIB systems typically use an organometallic platinum gas to carry the platinum to the surface of the sample. Under cryogenic conditions this precursor condenses on the cold sample surface to form a non-conductive platinum-containing

organic compound[27]. A curing process during which the layer is exposed to the ion beam then releases the organic component, allowing a conductive platinum layer to form. This step is critical for high-quality results as the platinum both dissipates charge and mitigates gallium implantation[13,27]. Orienting the sample so that the surface is normal to the GIS source is the best way to get a continuous layer, and the exact position will need to be adjusted for each system. "

13. Line 389: unclear as to what you are referring to.

In the submitted version line 389 mentioned changing the position of the side window, and we are interpreting this comment to mean that it was unclear why one would have to move the side window. Figure 3 and the accompanying discussion should clarify that the side window is used to establish a direct line of sight from every point on the cross section face, and that users should change the geometry of this trench as needed.

14. Line 424-436: again having a schematic to describe the trenching geometry would be helpful when trying to understand your description of windows.

See the previous discussion of the figures.

Point-by-point Response to Reviewer 4:

Reviewer #4:

Manuscript Summary:
The liquid/solid interface plays an essential role in various fields and a thorough atomic-level understanding of it is on demand. The cryo-SEM used in this manuscript shows higher efficiency in the preparation of samples than cryo-STEM, which requires ultra-thin samples. The vitrification can preserve the native state of interface and cryo-FIB can access the internal interfaces buried between two bulk phases. In addition, the chemical characterization is possible by EDX mapping. This technique shows good potential in characterizing the liquid/solid interface at nanoscale.
The protocols introduced in this manuscript about sample preparation, structural and chemical characterization are detailed and clear. The sample prepared and transferred according to the protocols can avoid crystallization and ice contamination to a great extent. Coating the sample initially with a thin layer of gold-palladium can minimize the effects of sample charging and radiation damage. However, there are some issues the authors need to clarify before the recommendation for publication.

Minor Concerns:
Figure 1 a, b in manuscript show a well-preserved sample and a less-preserved sample, while the protocols used to prepare these two samples haven't been mentioned. What are the factors leading to the difference? What's more, the authors claimed that one should insert the

sample into nitrogen quickly. This is a key step in vitrification. It would be good to quantify the cooling rate.

We added clarification that "For Fig. 4, b*oth samples were nominally prepared according to the same procedure, however, brief exposure to air most likely resulted in surface reactions for the sample in Fig. 4B possibly due to a thinner electrolyte layer on the surface of the lithium electrode. Screening of each sample after loading into the cryo-FIB helps identify potential issues due to the vitrification process."* Additionally, the figure legend now says: "*The surface is far rougher, and deposits are not fully covered by electrolyte, suggesting sample reactions may have occurred due to prolonged air exposure during the preparation."* The intention of the figure is to demonstrate the effects of doing this step incorrectly and clarify that users must evaluate each sample. Thank you for pointing out that this was not sufficiently clear.

Our setup does not allow for quantification of the cooling rate for individual samples. However, we have noted in the discussion of cryogens the increase in cooling rates when using liquid nitrogen vs. slush nitrogen: *"To prevent crystallization, slush nitrogen is used in this procedure, as it reduces the Leidenfrost effect and accelerates cooling compared to liquid nitrogen*[8,23,24]*. We also note that compared to aqueous solutions many organic liquids require significantly lower cooling rates for vitrification*[25,26]*, which is beneficial for freezing of thicker organic electrolyte layers."*