

1. Although they focus on insoluble particles, as indicated in the title, I believe the authors measured sea salt, soluble particles. The composition shown in Figure 7 shows a strong presence of Na and Cl. The result may indicate that they measured crystallized particles that were once soluble in the hailstones or their precursors. I believe that the result is still good, but some discussion needs to be clarified including the title.

Thank you for your insightful observation. This paper briefly mentioned that during sublimation, dissolved components may undergo precipitation. For instance, in cases where the original hailstone possessed a brine-like composition, the sublimation process could result in the precipitation of once-soluble salt particles.

However, in convective systems, sodium chloride and other crystalline inorganic salts injected into an updraft have the potential to remain undissolved in the surrounding water, acting as ice-nucleating particles under certain conditions. Research has highlighted the ability of deliquescent salts to serve as INPs in the atmosphere (1,2). Additionally, soluble salt particles like NaCl, NaI, KI, and KCl have been found to induce contact freezing at specific temperatures, further supporting their role as INPs (3).

The behavior of salt crystals in a convective system is influenced by multiple factors, such as solution properties and convective transport processes (e.g., updraft speed). There's a possibility that salt crystals may act as INPs without dissolving in the surrounding water. However, the unique contribution of this paper lies in its ability to analyze insoluble particles, acknowledging that soluble particles are not explicitly ruled out. Nonetheless, the focus remains on insoluble particles, and this distinction will be discussed further in the text without the need to change the title.

(1) <https://acp.copernicus.org/articles/21/13903/2021/>

(2) <https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2017JD027864>

(3) <https://journals.ametsoc.org/view/journals/amsm/58/1/amsmonographs-d-16-0006.1.xml>

Additional discussion was included in lines 178 through 183 of the revised manuscript.

2. I think Figure 9 is the most important result in this study, but they simply classify the particles into three categories: C-, Cl-, and Si-based. I suggest they discuss their particle categories more. I assume they are carbonaceous, sea salt, and dust particles. With particle shape analysis and more detailed compositional analyses, it would be possible to identify them. At least for simple classifications such as those in Fig. 9, there is no need to discuss SEM-EDS conditions, but a rough qualitative measurement can classify them.

We acknowledge the importance of Figure 9 in our study and appreciate the suggestion to provide more detailed discussions on particle categories. In categorizing unknown particles within hailstones, we employed k-means clustering and silhouette scores for statistical clustering to reveal particle similarities (Demšar et al., 2013) (1). Subsequently, we identified three main

clustered groups based on the predominant elements C, Si, and Cl. These elements were determined by analyzing each particle cluster and determining the dominant element in each cluster.

The reviewer's assumption aligns with our findings, as the predominant elements (C, Si, and Cl) generally correspond to carbonaceous, dust, and salt particles, respectively. A similar correlation was previously observed by Lata et al. in 2021 (2). Their paper inspired our approach to create a decision flow chart to set elemental percentage thresholds to separate the particles into the categories found through the k-means clustering approach. However, it's important to note that carbonaceous particles may also originate from sources like soil dust, referred to as soil organic carbons, which are particularly common in arid and semi-arid areas (3). Furthermore, our analysis found instances where both silicates and carbonaceous particles exhibited the presence of heavier metals. Therefore, we further distinguished the clusters dominated by C that contained heavy metals with those that did not, as well as Si-based particles also containing heavy metals. These additional categories are now also included in this revised manuscript.

We've included an illustrative example in the figure below to show a representative particle from each of the three major identified categories, allowing for more precise differentiation under CLSM and SEM. Hu et al. (2022) (4) is an example of a study that used the SEM to examine dust particles. By comparing the morphology of the dust particles in their study, for example, with images obtained through our study's method, we can draw comparisons with other particles previously studied using SEM. To emphasize the analytical capabilities of the methods employed in our study, including studying dust morphology, we will include the figure below in the revised text showing the confocal topography and SEM images for our different particle types.

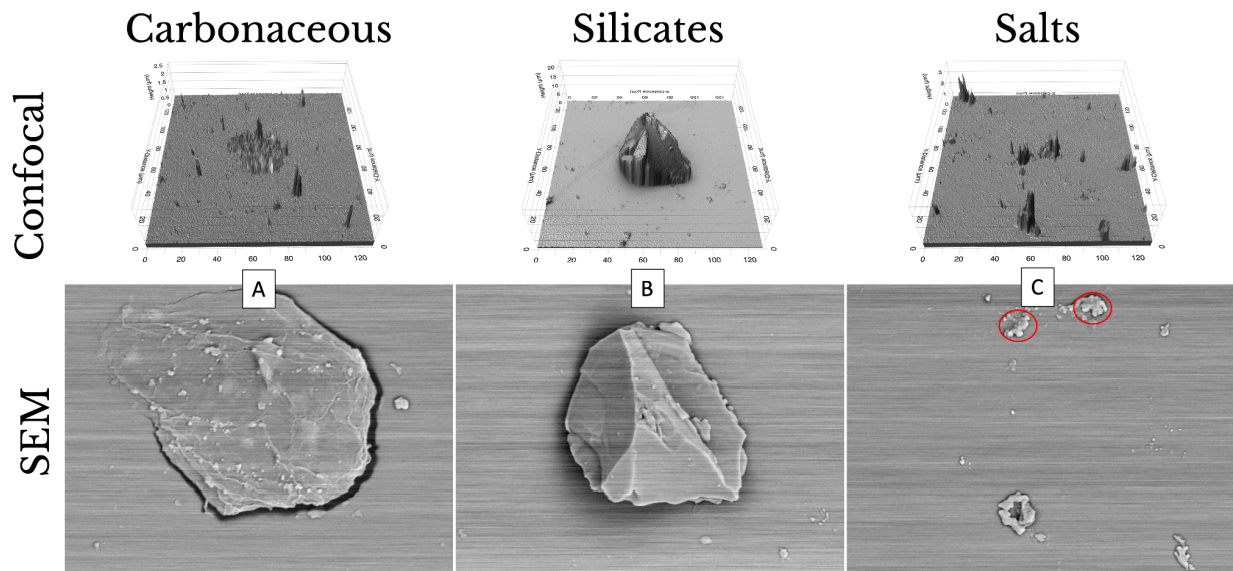


Figure 11: Examples from each of the primary particle categories are presented, along with their ProFilmOnline topographical output obtained through a Confocal Laser Scanning Microscope (CLSM, top), accompanied by their corresponding Scanning Electron Microscopy (SEM, bottom) image.

(1) <https://orangedatamining.com/>

(2) <https://pubs.acs.org/doi/10.1021/acsearthspacechem.1c00315>

(3) <https://www.mdpi.com/2073-445X/11/2/176>

(4) <https://www.sciencedirect.com/science/article/pii/S0048969722024081>

Discussion and the figure have been added to the revised text from lines 287 through 304.

3. In the later discussion, there is very limited discussion of the results from the CLSM. I suggest more discussion using the results.

We acknowledge the need for a more comprehensive discussion regarding the results obtained from the CLSM. We emphasized how we used it to locate the particle and ensure it was the same particle being analyzed in the SEM. However, CLSM also provides topographical and shape information about the analyzed particles, as seen in the figure in our previous response (which is included in the updated version of the manuscript). While a detailed analysis of the topography and shape of the particles is for a future publication focusing on additional applications of this method, this information does have implications for understanding ice nucleation processes in the cloud.

Surface topography is an active site for ice nucleation, influencing nucleation modes and the energy barrier for ice formation. This aligns with findings from Holden et al. in 2021 (1), suggesting that surface topography plays a significant role in nucleation. Additionally, other laboratory studies (2) have demonstrated that particle shape, size, and coating can impact the ice nucleation ability of particles, including soot. This paper shows that coatings or internal mixing have resulted in different ice nucleation abilities compared to bare particles. Importantly, these studies emphasize that particle size is not the sole determinant, highlighting the critical role of surface topography and particle shape in influencing atmospheric ice nucleation.

In our manuscript revision, we have incorporated a more thorough discussion of the CLSM results within the context of the figure discussed in the previous answer.

(1) <https://www.pnas.org/doi/full/10.1073/pnas.2022859118>

(2) <https://acp.copernicus.org/articles/22/5331/2022/>

The discussion has been added in lines 277 through 286 of the revised manuscript.

4. Although the authors have many discussions about the influence of glass plates, I suggest using a metal plate or a substrate consisting of single elements (e.g., Cu, Al, and

C). A glass plate contains so many elements that it interferes with the particle composition. By using other substrates, you will reduce such interferences.

We appreciate your comment. Initially, we considered using a nickel base, as we anticipated finding elements in the hailstone, such as Al and C, based on previous studies (e.g., Michaud et al. 2014). However, using a glass base, we can examine the sample using transmitted light microscopes (i.e., CLSM), which would not be possible with a metal substrate, as glass is the only substrate transparent to visible light. Additionally, we encountered challenges with adhering hailstones to a metal surface, as we have yet to experiment to assess the adherence of ice to the nickel or other metal bases and the potential reactions of formvar during the sublimation process.

Given our need for using the CLSM, plus our exploration of various methods to prevent contamination, including methods to remove/subtract such contamination from spectral results, we have found that the current approach using glass is the most suitable for extracting the information we seek from the hailstones.

Additional text has been added to the revised manuscript to further support the use of glass as the substrate in lines 156 through 157.

5. Did formvar coatings also contribute to the C signal? If so, C-based particles may need to be reconsidered.

Good question. We compared potential C signals in clear glass sections (i.e., not containing visible particles) with and without formvar. Additionally, we examined C signals associated with a particle categorized as a carbonaceous to discern any significant differences in C presence. Our findings confirm minor C contributions from the formvar, as illustrated in the figure below. Due to its minimal thickness, the formvar layer's impact on the C signal is deemed negligible. We value this observation and have incorporated this figure and accompanying discussion into the revised text.

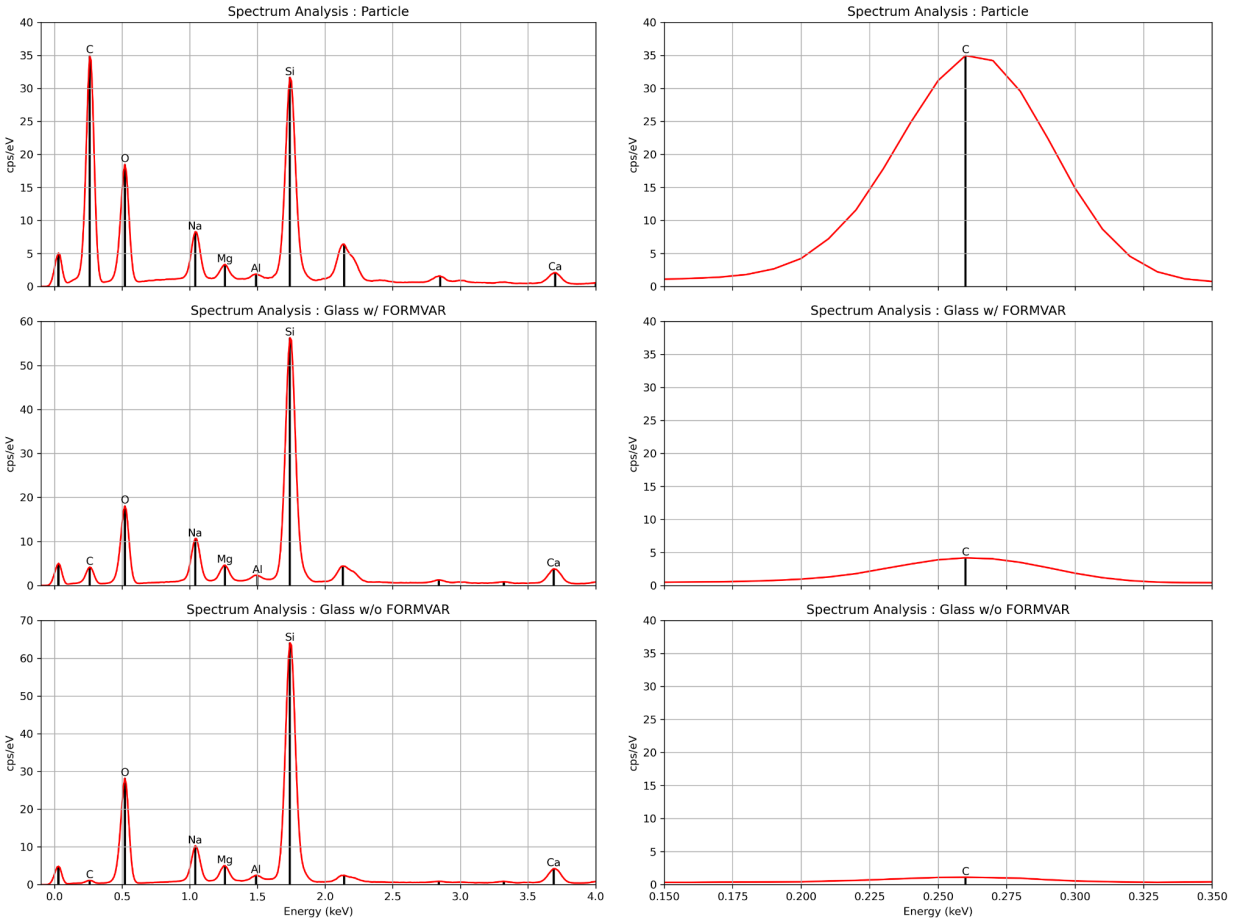


Figure 15: The left figure displays a single-point spectral analysis of a C-based particle with formvar (top panel), a clear area with formvar (middle panel), and a clear area without formvar (bottom panel). Each panel in the figure to the right is the corresponding panel providing a closer view of the C peak. Due to the formvar's thickness and the results in this figure, the formvar layer's impact on the C peak is considered negligible.

Additional text that includes this discussion was added in lines 345 through 350 of the revised manuscript.

6. In the conclusion section, I do not think it is good to introduce other techniques such as Raman spectroscopy and STXM. They could be placed in the recommendation or other sections.

Thank you for your recommendation. I agree with the suggestion, which has been addressed in the revised version of this paper.

The text was moved to lines 351 through 367 in the revised manuscript.

7. Could biological particles be identified by SEM using their specific morphology and composition, such as a tracer of P, S, and Cl?

The SEM is a valuable tool for identifying biological particles based on their unique morphology and elemental composition. This imaging technique provides high-resolution, three-dimensional images of specimen surfaces, enabling the visual characterization of known biological structures such as cells, bacteria, and viruses. This can be coupled with EDS to analyze elemental tracers like phosphorus, sulfur, and chlorine.

However, a known biological particle must be used as a control for accurate comparisons with potential traces found in unknown particles within a hailstone. Our hailstone analysis method was initially developed using a hailstone sample that exhibited relatively weak traces of phosphorus and sulfur, rendering these elements unsuitable as references for distinguishing biological particles.

EDS, in general, cannot distinguish organic from inorganic carbon; this method encourages the exploration of supplementary methods (e.g., Kirchstetter et al., 2004 (1), Moffet et al., 2011 (2), Orlando et al., 2021 (3)) to enhance the overall understanding of biological particles, especially in scenarios involving subtle traces of specific elements.

(1) <https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2004jd004999>

(2) <https://digital.library.unt.edu/ark:/67531/metadc831481/>

(3) <https://doi.org/10.3390/chemosensors9090262>

No additional text was added for this response.

1. Line 80: "Energy dispersive spectroscopy" should read "Energy dispersive X-ray spectroscopy".

Thank you for this comment. This has been addressed in the revised manuscript.

This has been addressed in lines 79 and 80 of the revised manuscript.

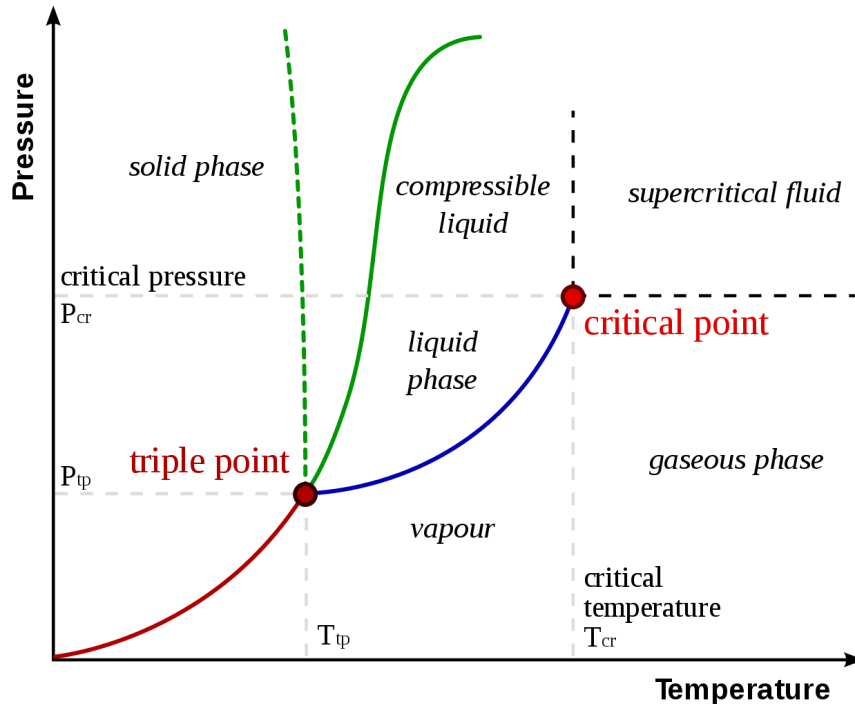
2. Line 83: SEM works by scanning a focused beam of electrons.

To address this point, we changed the text from "SEM works by focusing a beam of electrons onto the sample, which causes the emission of secondary electrons and backscattered electrons." to "SEM works by scanning a focused beam of electrons onto a sample, inducing the emission of secondary electrons and backscattered electrons."

This has been addressed in lines 82 through 83 of the revised manuscript.

3. Line 108: I think a sublimation point depends on both humidity and temperature, whereas a melting point depends only on temperature.

Thank you for your comment. Both sublimation and the melting point of water depend on temperature and partial vapor pressure (which is proportional to humidity), as shown in the following figure. For this reason, we consider that no change is needed to the text.



No additional text was added for this response.

4. Line 170: I was not sure if sublimation in dry air can occur with silica gel. Although it can be determined by a detailed calculation, I think silica gel may not achieve the low humidity needed for sublimation. I am not sure about the current conditions, but it is better to check.

We argue that the silica gel can achieve the low humidities required for sublimation because the hailstone sample from Central Argentina used in this case successfully underwent sublimation within a 48-hour timeframe. We are unsure what “current conditions” means and are open to discussing this point further.

No additional text was added for this response.

5. Line 197-200: Although a high vacuum SEM has a better SEM image than a low vacuum SEM, a low vacuum SEM has sufficient EDS capability for the purpose used in this study.

Thank you for highlighting this aspect. Not all SEM models incorporate low vacuum environment capability, and during the development of our method, the SEM at our disposal lacked this feature.

Many SEMs with this capability to analyze samples with low vacuum conditions introduce a known gas, like nitrogen, or allow some oxygen from the environment to enter the chamber. However, this will raise potential concerns. One concern is contamination from the surrounding air. Without a clean oxygen source entering the chamber or a filter for purification (because SEMs do not have built-in air filtration systems), contaminants from the surrounding atmosphere could compromise the chamber. The hailstone consists of ultra-pure water, so it is susceptible to absorbing impurities in the air, which could impact the sample's integrity.

Another consideration under low vacuum conditions is atmospheric skirting, wherein a gaseous environment modifies the primary electron beam profile. The electron beam is typically divided into two fractions: an un-scattered beam with the original distribution profile and diameter and a scattered beam forming a "beam skirting" around it (1,2,3). This beam alteration occurs before reaching the particle surface, impacting the resolution of high-resolution imagery and spectral analysis through the EDS.

Therefore, due to potential issues related to contamination, lack of control over the beam's trajectory, and the resulting impact on resolution and spectral analysis, we respectfully disagree with the notion that low vacuum conditions would suffice for our method. We appreciate this observation; this discussion has been included in the revised version.

(1) <https://www.sciencedirect.com/science/article/pii/S0065253908609026>

(2) <https://onlinelibrary.wiley.com/doi/abs/10.1002/sca.4950230505>

(3) <https://onlinelibrary.wiley.com/doi/abs/10.1002/sca.4950220304>

Additional discussion was included in lines 204 through 217 of the revised manuscript.

6. Line 204: Is "sigma" OK?

Thank you for this observation; it has been updated in the revised version to "Sigma."

The word has been corrected in line 222.

7. Line 208-209: A high voltage does not always improve the spatial resolution of SEM images due to its expansion in the materials. Please check again.

Thank you for your comment. While the statement in question is supported by literature (Goldstein et al., 2017) (1), I agree with the reviewer that there are instances where excessively high voltages may lead to the expansion or penetration of electrons into the materials, potentially affecting the resolution negatively. We, therefore rewrote the sentence in the following way:

"The accelerating voltage of the primary beam determines the wavelength of electrons, and higher voltages are generally advised for enhanced spatial resolution in electron imaging

(Goldstein et al., 2017). However, it's important to consider that this principle may not universally apply to all materials. In some cases, excessively high voltages could result in electron expansion or penetration into the materials, potentially diminishing resolution. Therefore, it is essential to determine the optimal voltage, potentially opting for a lower one, when analyzing specific samples to ensure optimal imaging resolution.”

(1) <https://link.springer.com/book/10.1007/978-1-4939-6676-9>

Correction was replaced in lines 226 through 231.

8. Line 214: I believe that a working distance does not affect the beam diameter. Please check it.

The relationship between working distance and beam diameter in SEM systems is well-established in existing literature (1,2). The working distance, representing the distance between the final lens of the SEM column and the specimen, influences the beam diameter, which is the diameter of the electron beam at the specimen. The electron optics within the SEM column governs this relationship.

As detailed in literature reference (1), the increase in working distance results in a proportional increase in the beam diameter. The geometric spreading of the electron beam over a greater distance from the final lens to the specimen contributes to this phenomenon.

Moreover, studies on the design and fabrication parameters for optical systems, including SEM (2), emphasize the interdependence of working distance and beam diameter. Specifically, variations in the working distance directly impact the beam diameter, with an increase in working distance corresponding to a larger beam diameter. This reinforces our statement in the paper, aligning with established literature on SEM systems.

(1) <https://www.sciencedirect.com/topics/engineering/lateral-resolution>

(2) <https://www.mdpi.com/1424-8220/18/12/4150>

No additional text was added for this response.

9. Line 225: A 15 kV can measure higher than Fe. Please check it.

The sentence reads: "The choice of 15 kV ensures that heavier elements are included in the EDS analysis by exciting the K lines of elements up to Fe." However, the intention was not to imply that we can measure up to Fe at a 15 kV accelerating voltage; instead, the emphasis is on the capability to measure heavier elements such as Fe at this voltage. It has been addressed in the revised version.

Correction was done in lines 244 through 245.

10. Line 237: There is no Figure 7D.

Thank you for pointing out this discrepancy. The reference has been corrected to "7-B," aligning with the intended figure for citation in this text.

Text was replaced in line 257.

11. Line 239: I agree. Please see my major comment

Thank you for your feedback. We acknowledge your agreement and have considered your major comment.

12. Line 258-260: If the particles have been classified according to these criteria, there is no need to use the cluster analysis (line 253-257).

Thank you for the opportunity to clarify our classification approach. Going into the study, we did not know what types of particles we would find in our hailstone, so we started with a cluster analysis to see which elemental clustering was dominant in our sample. Utilizing k-means and silhouette scores analysis allowed us to identify particle similarities based on statistical clustering. Subsequently, with the knowledge of each particle's cluster, we determined the predominant element within each category (i.e., carbonaceous, silicates, salts.). Applying thresholds from the data, particles included in the C-based group had a C abundance greater than 10% weight, with this abundance being higher than that of Cl and Si. Those categorized in the C-heavy group met the same criteria as the C-based group but also had an abundance greater than 1% weight of heavier metals such as Ti, Cr, Fe, Ni, Zn, Br, and Mo. Particles categorized in the Si-based group had an Si abundance greater than 10% weight, with this abundance being higher than that of C and Cl. The Si-heavy group met the same criteria but had an abundance greater than 1% weight of heavier metals such as Ti, Cr, Fe, Ni, Zn, Br, and Mo. Finally, particles with a Cl abundance greater than 10% weight, with this abundance being higher than that of C or Si, were categorized in the Cl-based group.

Determining how the data should be categorized without this initial clustering would be challenging. Therefore, cluster analysis was necessary to ensure an appropriate classification of particles. We have emphasized this point in the revised manuscript.

Discussion and the figure have been added to the text from lines 287 through 304.

13. Line 275: Is it true that CCSEM can load samples automatically?

Based on the information provided by SEM manufacturers, computer-controlled scanning electron microscopes offer automated sample-loading capabilities (1,2,3,4,5). For instance, the ZEISS EVO scanning electron microscope incorporates Automated Intelligent Imaging, a feature that enhances sample throughput. Additionally, it provides tools for relocating regions of interest and ensuring the integrity of collected data, thus facilitating automated and efficient

sample handling. Similarly, the JEOL FE-SEM has an AI system called NeoEngine, which tracks electron beam trajectories, streamlining operations with minimal user intervention. We added a reference to the revised paper to describe this capability.

(1) <https://www.zeiss.com/microscopy/en/products/sem-fib-sem/sem/evo.html>

(2) <https://www.azom.com/article.aspx?ArticleID=19595>

(3) <https://www.thermofisher.com/us/en/home/electron-microscopy/products/desktop-scanning-electron-microscopes.html>

(4) <https://www.semtechsolutions.com/blog/better-performance-from-scanning-electron-microscope-use/>

(5) https://www.hitachi-hightech.com/global/en/sinews/technical_explanation/130301/

No additional text was added for this response.

14. Line 276-277. I do not believe that CCSEM can measure thousands of particle compositions in less than an hour. EDS needs an acquisition time of at least several seconds.

After reviewing your comment and cross-referencing it with my notes, I agree with your observation. More precisely, when we focused on analyzing individual particles, as described in this methods paper, we assigned a 2-minute acquisition time. Considering this time constraint, our analysis permitted the theoretical examination of approximately 30 single-point analyses per hour. These analyses could be for a single particle or encompass multiple spots within a single particle. To accommodate your observation, the sentence has been rewritten: "A CCSEM-EDS software can be programmed to autonomously analyze multiple particles consecutively, without requiring human intervention (Vander Wood, 1994)."

The text was replaced in lines 327 through 328.

15. Line 280: There is no section 2.4.2.

Thank you for identifying this inconsistency; this has been updated in the revised version to "section 3.3.2."

The text was replaced in lines 330 and 332.

16. Line 285-288. I do not think changing the acceleration voltage is effective. First, when using low voltage first, you will not see heavy elements. Second, it is very time-consuming, as suggested in line 278.

Adjusting the voltage, although time-consuming, becomes especially relevant when analyzing particles that are 1 micron or smaller in size. This is because the SEM has a higher resolution compared to the CLSM. This higher resolution in the SEM gives us a distinct advantage,

allowing for a more detailed examination of smaller particles. This prevents any elemental contribution from the base due to a large activation volume, as illustrated in Figure 8 and discussed in depth surrounding this figure.

No additional text was added for this response.

17. Figure 7. There is a missing peak identification around 2.1 keV. Why is this?

The spectral peak corresponds to gold, as it was used in coating the sample and is consequently excluded from the analysis. I have incorporated this clarification into the figure caption.

Additional text was added to Figure 7's caption.

18. Figure 9. I cannot see the right images. Are they from Figure 6?

Thank you for identifying this inconsistency; this has been updated in the revised version to "... Figure 5."

This inconsistency was addressed in Figure 9.