In the following, the reviewer comments are written in bold and our answers in italics. Text passages from the revised manuscript are in quotation marks, modified or newly added passages are marked in green.

Author's response to Reviewer 3

First of all, we would like to thank the reviewer for reading our revised manuscript and providing feedback on ambiguities, which helped us to improve the new sections and figures of the revised manuscript.

Dear authors,
Thank you for this new version of the manuscript and for the replies to my questions. In my opinion, this revised version is easier to follow, particularly due to the numerous explanations added to your methodology. I also appreciate the inclusion of the Monte Carlo simulation, which provides a statistical understanding of the search radius, and the use of bootstrapping to evaluate the relevance of particles' compositions during the campaign.
One overall aspect I still find difficult to understand is how the center of the search radius is chosen. From L212: "the software identifies the image with the highest number of ice crystals for each measurement cycle and tags the ice crystal positions in the images as the center of an area exceeding a size threshold of 30 pixels with a brightness threshold of 30 of 256 on a scale stretching from the darkest to the brightest recorded signal (Schrod et al., 2016)" I understand that the center is determined based on the 30 adjacent pixels. However, in the schematic for your simulation in Fig. 4, the center (red cross) is clearly positioned in an area that is not surrounded by 30 pixels above the brightness threshold of 30. Then, in Fig. 5, it seems that the center of the search radius corresponds to the center of the ice crystal. I find these descriptions contradictory and would appreciate further clarification, especially that the center of search radius is a key step for your method.

The software of FRIDGE determines the position of the INP as the center of the ice crystal it detects, as you cited from the manuscript. That is symbolized as the blue circle marker in the schematic in Fig. 4, which is in the center of the grey shaded area. The center of the search radius (red cross) is the location reported later to the SEM analysis. It is not necessarily the true position of the INP, as it includes the various errors as described in the text.
To clarify that, we have modified the caption of Fig. 4.

„Figure 4: Conceptual model for the simulation of the INP-recovery from FRIDGE in SEM. The blue circle is the true location of the INP, the yellow circles are non-INPs. The red cross is the search origin position in SEM. It deviates from the true INP position due to the errors by INP shift, unprecise ice crystal center determination and the re-positioning uncertainty due to calibration mismatch and mechanical backlash. The circles show search radii of 25, 50 and 75 µm from the red search origin position. The grid shows the approximate pixel size of the optical camera of FRIDGE."

We have added the relevant information on the search radius origin to the caption of Fig. 5, too.

Figure 5: Model simulation results. Cumulative fraction of unrecovered particles $frac_{miss}$ (pink line), fraction of ambiguous locations $frac_{ambig}$ (blue), and fraction of correctly identified INPs $prob_{INP}$ (thin black) as function of search radius – originating from the reported INP position including errors - for different INP fraction $frac_{INP}$ and total particle numbers n. For this figure, a standard deviation of the positioning uncertainty of 25 µm is used. The total number of INPs n_{INP} is shown for each plot. The search radius where 20% (blue), 10% (red), 5% (orange) and 1% (green) fraction / probability is reached is marked by vertical lines for $frac_{miss}$ (broken line) and for $frac_{ambig}$ (solid line). The lowest possible error probability range is indicated by a shaded area for below 20% (blue), below 10% (red), below 5% (orange) and below 1% (green). If no shaded area is visible, the error probability is greater than 20%.

Figure 6 shows the search radius of 50 µm around the identified ice crystal center point (blue point in Fig.4), without the error calculations.

Specific comments

-L155: "However, even after thorough cleaning a small amount of ice formation can regularly be observed at temperatures at or below -30°C, constituting the background concentration and defining the limit of detection, which is in the order of 0.1 L-1 of atmospheric air for a collection volume of 100 L." It appears that ice formation on silicon wafer only appears at -30°C, so is the background concentration is only for particles that nucleate ice at this temperature or below?

In general, ice nucleation can occur on cleaned substrates at warmer temperatures. Typically, however, the number of ice crystals on the wafer increases as the temperature decreases. In the present study, the lowest operating temperature of FRIDGE was -30°C. Since the highest number of ice crystals is typically observed at this temperature, the results at -30°C are affected the most by background concentration. The limit value was specified in relation to a specific volume. For warmer temperatures, the limit value is significantly lower.
Thus, this limit is based on the typical number of counts on cleaned wafers at -30°C, calculated on a sample volume. The limit can vary with the cleaning procedure, the sampling volume etc. and should be seen as a guideline.

-L234: "can be caused" here you employ a modal verb which suggests that condensation is only a possible explanation for the higher particle counts observed with FRIDGE compared to SEM. Is there any evidence or analysis that could strengthen this claim? In other words, is it not possible to determine with greater certainty whether condensation is indeed the cause?

The choice of words may be somewhat misleading.
Condensation is undoubtedly the cause of the higher number of positions identified for SEM. We observed this quite frequently in higher amounts during the initial phase of the development of the method. There may be other reasons, but they are rare (and therefore of minor importance). Condensation, however, cannot always be avoided, and it is sometimes visible though the whole picture series, especially at a relative humidity close to 100%. A compromise must then be made between the visibility of condensation and the size of the ice crystals.
We changed the wording as follows.

"Higher numbers of ice crystal positions identified for SEM are mainly caused by misclassifying areas with condensation, which may occur while working close to RH = 100 %."

-L259: "mechanical movement" can you add an explanation?

The sample stage in the SEM can be moved mechanically by actuators in orthogonal directions. By positioning the wafer accordingly on the stage, the x- and y-axis directions of movement correlate with the x- and y-axes of the coordinate system on the wafer.

"This is necessary because the internal Cartesian SEM coordinate system is determined by the axes of the mechanical movement of the stage, whose position is encoded by high-precision encoders. The SEM coordinate system is centered in the middle of the stage (Fig. 3c)."

-Fig. 3b: There are 3 pictures (top left, bottom left and bottom right) with each calibration marks and 1 picture (top right) of the entire calibration system incorporation the 3 marks? Please add explanation.

We have added an explanation to the figure caption and the heading in b). In addition, we have changed figures 3a) and 3c) to clarify the coordinate transformation procedure and make it easier for the reader to follow.

„Figure 3: a) pixel coordinate system from the FRIDGE image (blue) and wafer internal normalized Cartesian coordinate system(orange) to locate ice crystal positions (white circle) from the FRDIGE images: the normalized coordinate system (orange) is defined by two normalized vectors (n01, n10 with a length of 100) identifying a position by its x- and y-coordinate; b) calibration image showing the marked calibration points for the coordinate system from the ice crystal identification step and a picture showing the entire calibration system (top right); c) wafer on the SEM stage showing the SEM internal cartesian coordinate system aligned to the directions of the mechanical movement in millimeters (mm); d) SEM picture of a calibration cross on the wafer surface."

-Fig.4: Did you think about integrating the pixel size of this schematic? Looking at the radius of the inner circle (25 µm) and the area around, I don't see how there could be 30 adjacent pixels with brightness above the threshold in that area. Perhaps you can increase the size of ice crystal?

Changed as requested.

-L297: "The direction of shift is randomly chosen; the distance is randomly sampled from a mirrored normal distribution with a standard deviation of some typical uncertainty assumptions." Could you further explain what you mean by typical uncertainty assumption?

As outlined, there are errors leading to the uncertainty of the reported INP position. For instance, the INP may no longer be at the center of the crystal due to a particle shift (see Chapter 2.3), or asymmetric crystal growth could have caused an inaccurate determination of the center point (see Chapter 2.4). The limited resolution of the FRIDGE images compared to the SEM also introduces uncertainty (see Chapters 2.4 and 2.5.1).
Charrier (2016) defines the uncertainty of retrieving defined points on a wafer in the SEM with an average standard deviation of 15 µm. Taking a conservative approach, our estimation is based on the higher values from the experiment, as the inaccuracy due to the resolution of the FRIDGE images must also be considered.
As we do not know the uncertainties of the particle shift and the potentially asymmetric ice crystal growth, these errors were not taken into account for the simulation.

The paragraph reads now:

„The direction of shift is randomly chosen, the distance is randomly sampled from a mirrored normal distribution with a standard deviation of some typical uncertainty assumptions for the accuracy of relocating a defined point on the wafer in the electron microscope. The uncertainties associated with the particle shift and the potentially asymmetric ice crystal growth are not incorporated into the simulation, as their values are not yet sufficiently established."

-L304: "(total number of 20000 / 50000 / 100000 particles on the wafer with different INP fractions of 0.0005 / 0.001 / 0.002)" Please discuss this further and add references.

The number of particles refers to the typical atmospheric aerosol concentrations and number we had in our samples.
We have added two references, dealing with atmospheric INP fractions.

"Figure 5 shows the results of a selection of parameters corresponding to typical application conditions (total number of 20000 / 50000 / 100000 particles on the wafer with INP fractions of 0.0005 / 0.001 / 0.002 (DeMott et al., 2017; Ren et al., 2023))."

-L305:" In this case the standard deviation of the position uncertainty of 25 µm was assumed." Why did you choose this one?

See our answer on comment L297.

-Fig 5: I am having difficulty understanding how the probability of INP correctly identified (thin black line) is equal to 1 for the smallest search radii, but at the same time the fraction of INP missed is increasing as search radii are decreasing.

With a very small search radius around the calculated coordinate, there is a high probability that the particle found here is the true INP, as the chance that a non-INP is very close to that position is the lower, the smaller the search radius is.
But taking the position uncertainty into account, also the chance of missing the INP increases with decreasing search radius, as in this case even small position uncertainties lead to an empty position (i.e., ´miss´).
I.e., at a low search radius, due to the position uncertainty many INP are missed, but the ones that are found, have a high probability of having been the actual INP. Increasing the search radius leads to higher recovery rates, but also to higher uncertainties with respect to the particle being the actual INP.
To find an optimal radius, a compromise must be found between all parameters.

-Fig.4: Why don't you integrate the pixel size of this schematic? Looking at the radius of the inner circle (25 um) and the area around, I don't believe there are 30 adjacent pixels with brightness above the threshold in that area.

Scheme modified as requested.

-L395: "From the statistical calculation in Tab. S1 with a 95% confidence level, a limit of approximately 10 particles per group can be derived to make a reliable quantitative statement, for groups with less particles the uncertainties become large" do you mean that since the 95% confidence level for less than 10 particles spans reaches 0 as lower limit, it is not possible to make a reliable quantitative statement?

Yes, that is what we meant.
If the lower and upper limits of the confidence interval are both above 0, it is certain that the corresponding group contributes to the ice formation process and you can give value with a corresponding confidence interval. However, if the lower limit of the confidence interval is 0, we cannot exclude that the group did not participate in the ice formation process within the specified uncertainty.
Our statement is only based on the calculations from the case study, and should be seen a guide line. As we stated in the manuscript, further considerations of limits or statements should employ common statistical approaches for counting statistics and compositional data and must be evaluated in the respective context.
However, the results with the given confidence intervals are still the best estimated value even if the uncertainties may be very large for small particle numbers. We indicate uncertainties for all values so that the reader can form his or her own opinion.

Technical corrections

-Fig.3: "polar coordinatesystem" change to "polar coordinate system"

For a better understanding, the whole figure 3a) is revised (see Fig. 3). The polar coordinate system is no longer part of the figure.

-Fig 3: in the cation is mentioned "d10" but not in the figure.

For a better understanding, the whole figure 3a) is revised (see Fig. 3). The polar coordinate system is no longer part of the figure.

-Fig 3: please add in the caption that white circle is an ice crystal.

Changed as requested.

„Figure 3: a) pixel coordinate system from the FRIDGE image (blue) and wafer internal normalized Cartesian coordinate system(orange) to locate ice crystal positions (white circle) from the FRDIGE images: the normalized coordinate system (orange) is defined by two normalized vectors (n01, n10 with a length of 100) identifying a position by its x- and y-coordinate; b) calibration image showing the marked calibration points for the coordinate system from the ice crystal identification step and a picture showing the entire calibration system (top right); c) wafer on the SEM stage showing the SEM internal cartesian coordinate system aligned to the directions of the mechanical movement in millimeters (mm); d) SEM picture of a calibration cross on the wafer surface."

-Fig. 4: "die" replace by "due"

Changed as requested.

„ Figure 4: Conceptual model for the simulation of the INP-recovery from FRIDGE in SEM. The blue circle is the true location of the INP, the yellow circles are non-INPs. The red cross is the search origin position in SEM. It deviates from the true INP position due to the errors by INP shift, unprecise ice crystal center determination and the re-positioning uncertainty due to calibration mismatch and mechanical backlash. The circles show search radii of 25, 50 and 75 µm from the red search origin position. The grid shows the approximate pixel size of the optical camera of FRIDGE."

-Fig. 4: "For this plot" there are several plots here, which one are you referring to?

We do not refer to a single plot, we refer to the whole figure. We have changed the wording accordingly.

Figure 5: Model simulation results. Cumulative fraction of unrecovered particles $frac_{miss}$ (pink line), fraction of ambiguous locations $frac_{ambig}$ (blue), and fraction of correctly identified INPs $prob_{INP}$ (thin black) as function of search radius – originating from the reported INP position including errors - for different INP fraction $frac_{INP}$ and total particle numbers n. For this figure, a standard deviation of the positioning uncertainty of 25 µm is used. The total number of INPs n_{INP} is shown for each plot. The search radius where 20% (blue), 10% (red), 5% (orange) and 1% (green) fraction / probability is reached is marked by vertical lines for $frac_{miss}$ (broken line) and for $frac_{ambig}$ (solid line). The lowest possible error probability range is indicated by a shaded area for below 20% (blue), below 10% (red), below 5% (orange) and below 1% (green). If no shaded area is visible, the error probability is greater than 20%.

-L.280: "orange" it is yellow, no?

As the color was ambiguous, we now changed it clearly yellow and adapted the plot and text.

-L361: "Based on the modeling, we would consider a value of around 100,000 particles on the wafer to be a good starting point, as the proportion of incorrectly identified particles increases significantly with higher particle numbers." Consider changing "good starting point" as this can be understand that 100, 000 particles minimum are suitable, while higher number of particles increases uncertainty.

We adjusted the words. The section now reads as follows.

"Based on the modeling, we would consider a collection of around 100,000 particles on the wafer as a good number for meaningful measurements under typical free-tropospheric conditions. Of course, it has to be adapted to the actual conditions, e.g., when the fraction of INP is significantly different. Therefore, we recommend to determine the particle concentration in the atmosphere in parallel to the collection and then perform a quick analysis of the wafers in FRIDGE to calculate the proportion of INPs in the total aerosol. Based on this proportion the sampling parameter can be adjusted to balance a sufficient number of INPs on the wafer but prevent an overload."

Author's Response to Reviewer 4

First of all, we would like to thank the reviewer for reading our manuscript and pointing out some issues which might raise concerns by the reader.

This manuscript presented the details of a method for the INP or IR analysis by coupling a SEM and an offline ice nucleation diffusion chamber. The method has been applied in a few peer-reviewed papers without detail information by the authors. This manuscript is attempted to provide a full description on this method. The topic of this study fits the scope of this journal. There are several issues need to be addressed before it can be considered for publication.

Major comments:

1. There are other groups used a similar SEM/EDX method for INP and IR analysis which the authors didn't mention in the manuscript. Please see the review by Knopf et al. which shows a few studies that have been used the SEM/EDX analysis that coupled with ice nucleation measurements (https://pubs.acs.org/doi/10.1021/acsearthspacechem.7b00120). Although the novelty for this method is not fully described in this manuscript or is still in question, but I do think it may be worth for a publication as it provides the details for the method for their previous publication and future manuscripts.

We have added additional references to studies using SEM to analyze INPs.

"Even though the method cannot provide high temporal resolution measurements due to longer sampling times, it can provide detailed information on morphology in addition to chemistry and size of individual INPs and IRs (China et al., 2017; Cziczo et al., 2009, 2013; Ebert et al., 2011; Eriksen Hammer et al., 2018; Knopf et al., 2014; McCluskey et al., 2014; Mertes et al., 2007; Prenni et al., 2009; Wang et al., 2012; Worringen et al., 2015)."

As you have recognized, only papers showing results using this method have been published so far. But these papers do not describe the method or only describe it very briefly. The novelties of our manuscript have already been described and explained in our answers during the previous review discussion.

2. One major concern I have is on the misidentification of INPs. It is mainly due to the low spatial resolution of FRIDGE images during ice nucleation experiments. The successful rate is only 13% to 50%, 30% on average. That limits the number of INPs analyzed. If the authors can identify the INP one by one, then the interpretation on the chemical characterization of INPs remain valid even for a small number of identified INPs. However, that will require improve the spatial resolution of FRIDGE images significantly.

As already stated in the conclusions, we agree with the referee that a higher camera resolution in FRIDGE would improve the method a lot (see the following quote). However, we still are confident in the presented results of our methodology, provided that the uncertainties described are sufficiently addressed.

"Following the uncertainty analysis presented here, we identified the FRIDGE camera resolution as one important area of improvement. A higher camera resolution to document the ice crystal growth in FRIDGE would improve the accuracy of the ice crystal center point identification and make it easier to find the calibration point in the SEM. This could significantly reduce a substantial part of the uncertainties."

3. For the INP classification scheme, if I understood it right, the authors used only the information that whether an element is presented in the INP. The EDX should have provided relative content of each element. Why not use this quantitative information?

It is true that relative proportions of the individual elements can be obtained using EDX analysis. However, for our rather rough classification of the particle classes (silicates, carbonates, C-rich, etc.) due to low counting statistics, the classification based on the presence of the elements is sufficient. If the focus lies on subdividing the aluminosilicates into different groups (e.g., K-feldspar, etc.), we would have to do this on the basis of the percentages.

4. Details for the ice nucleation experiment are not sufficiently presented. What is the temperature uncertainty across the 45mm wide Si wafer? This is very important for ice nucleation experiments. In L185, -186, it is not clear. How was the ice nucleation conducted? How long did it allow for ice to nucleate after introducing the water vapor?

Several details of the ice nucleation experiment FRIDGE are published in previous papers (Schrod et al., 2016; DeMott et al., 2018; DeMott et al., 2025). The uncertainty of the nucleation temperature is estimated to be below 0.5°C for the wafer as a whole (DeMott et al., 2025). This was derived from spot measurements at the surface, as infrared-measurements are too inaccurate. We are confident that this 0.5°C upper bound is valid for the FRIDGE measurements, due to intercomparison of FRIDGE INP data with those from independent methods of other laboratories on test aerosols like ATD, cellulose, Snomax, Saharan dust as well as on ambient aerosol.
We added the temperature information and the reference to the manuscript.

"A small amount of silicon oil is applied on the bottom of the wafer as well as on the temperature sensor to ensure good thermal contact and a homogeneous temperature distribution. The temperature variance is estimated to be below 0.5°C across the entire wafer (DeMott et al., 2025). "

During the initial phase of the coupling procedure, the ice typically grew for 100 seconds before sublimating again (the evaluation of ice as function of time is given by Schrod et al., 2016 in Fig. 2). This time frame for was adopted from the pure FRIDGE experiments, where it was chosen to ensure that as many INPs as possible were activated and have grown significantly.
However, as described in the manuscript, large ice crystals introduce some difficulties and inaccuracies for coupling the activation procedure with INP identification experiments in SEM. As stated in the manuscript, the accuracy of INP recovery can be increased if the ice crystals do not grow to large ("are at a stage close to activation"), as particle shifts and possible asymmetries in ice crystal growth are limited. Therefore, the typical growth time of an ice crystal in FRIDGE is currently about 40 seconds, which is enough to activate most of the INPs.

5. The contribution of ice nucleation on the blank Si wafer. L346-352, Ice crystal can also form on the blank Si wafer. If you can not find an INP within a certain area, that could mean it formed ice on the blank. Then, increasing the search radius is not helping to increase the number of identified INPs but increase the misidentified INPs. This is also critical to the calculation of INP concentration. In L516, Section 3.2, does the INP concentration calculation consider the blank correction?

There are two different aspects, which have to be distinguished:
(1) Even on a cleaned wafer, a weak ice crystal growth can be observed in FRIDGE, which becomes stronger with decreasing temperature (for details see Section 2.1 in the manuscript and our answer on comment on L155 from Reviewer 3). These grown ice crystals are called background counts. Reasons for background counts can be residues or particles remaining on the wafer after cleaning, but defects of the wafer surface can also contribute to background counts. Ice crystal growth on surface defects may appear at temperatures primarily below -30°C and contamination particles on the wafer are more likely for the temperature range used in our studies. The number

of ice crystals counted in an atmospheric sample in FRIDGE is always corrected by the background counts when calculating the concentration.
Certainly, these particles can also be counted as INPs in the real sample, which means that contamination particles can be included in the characterization of the atmospheric aerosol. However, since the wafers are cleaned in advance to keep the background freezing at a low level, the influence of potential contamination particles can be assumed to be minimal.

(2) *During the observation of the individual ice crystal positions in the SEM, it is possible that no particle is found at an identified position (within the selected radius). We refer to such a situation as "blank". There are many potential reasons for this: possible particle shift during the processing in FRIDGE, erroneous position of the ice crystal origin and incorrectly selected search radius (see chapter 2.5.3). The reasons for a blank position in the SEM are mainly due to reasons not related to the FRIDGE part of the method, it is therefore not necessary to correct the concentration obtained from FRIDGE by these "blank" positions.*

Author's Response to the Editor

Dear Authors,

I have received two more reviews of your revised manuscript. After going through the reviews and the manuscript, I agree that there have been substantial improvements to the manuscript. Yet some concerns remain and these need to be addressed as indicated in the reviews you have received. I think these can be easily addressed and will improve the manuscript further.

Upon reading the reviews and paper, one aspect that stood out to me as raised by a previous round of reviews that I think you need to sufficiently address or justify is evaluating the efficiency of the coupling method (FRIDGE and SEM) with known reference aerosol. i.e. how sure are you that the SEM analysis only detects the particles that nucleated ice based on the co-ordinate system and the distance between the particles. If a lab controlled study of different aerosol types were used, then this can be verified by the particle composition obtained. However, with ambient particles, you can have a variety of compositions nucleating ice because of internal mixing. Could you also address this in your reviews or as a separate response to my comment.

I consider this round to be a mix between minor and major revisions. As such a step forward towards a final version. However, I will indicate major revisions so I can get at least one opinion from an external review in addition to my own review after you re-submit a revised version.

Best,
Zamin A. Kanji

We can understand the editor's point of view, that laboratory experiments with test aerosol may be a good chance to evaluate a method.
However, the method is used for identification of ambient atmospheric particles with complex mixing state which is more complicated and it is not certain that the method, if verified with pure test aerosol, will work the same way as with ambient aerosol. Samples from an atmospheric mixture of particles with a variety of compositions and probably a high excess of acids and volatile compounds can behave different from pure compounds. Since the individual steps had been verified separately (see list below), our aim for the coupling was to develop and evaluate the method under the more complex situation to show the real uncertainties that apply to the atmospheric situation. Consequently, the whole coupling procedure with the ESEM was tested using ambient particles only.

As mentioned above, the individual steps of the FRIDGE-ESEM method were verified separately and independently.
-Sampling and analysis in FRIDGE were tested with AgI, Fluorescein aerosol and haematite (Schrod et al. 2016).
-The identification of ice crystal origins on the wafer and the coordinate calculation by our counting algorithm was also repeated. However, as this is an automated process, it always provides the same output with the same input, in this case the same coordinates.
-To locate the identified ice crystal center coordinates from the wafer internal coordinate system in SEM, the recovery of the calibration points is essential. For this step, we provide a value of 20 µm for the inaccuracy due to the limited resolution of the FRIDGE images. Repeated calibration on the basis of the calibration picture resulted in a deviation of ± 10 µm. As this deviation does not exceed the inaccuracy due to image quality, it was not considered further in the manuscript.
-For the uncertainty of recovering defined points in the SEM a mean deviation of 15 µm was determined.

Laboratory experiments conducted during the early phase of the method have proven that if you deposit good ice nuclei, such as AgI, onto the wafer surface, you will only find/identify these good INP at the approached positions.