Author's response to the reviewers and the revised paper:

Response to Reviewer #1

The authors are grateful to the reviewer for the important comments that helped them clarify the manuscript.

In black and bold are quotations that the reviewer cites from the manuscript.

Reicher et al. describe the calibration and operation of a cold-stage for measurement of ice nucleation activity. The instrument used a microfluidic device for generation of monodisperse droplets inside an oil phase. The device is operated in batch mode. Droplets are prepared in a continuous flow system. Then the flow is stopped and the device is placed on a cold-stage/microscope for nucleation experiments. Characterization experiments using pure water, eutectic solutions, and several test dusts are described. Application of the technique to ambient aerosol is demonstrated. This is a solid paper that describes the application of a microfluidic device for ice nucleation studies. The characterization and validation experiments are of high quality. Specifically, the temperature characterization and comparison with the various test dusts at water activity of one and less than unity are thorough and convincing. The main conclusion of the paper that "WISDOM is a suitable tool for studying atmospheric ice nucleation, both in homogeneous and heterogeneous immersion freezing" is well justified. However, the paper should be viewed in perspective of the current literature. At least 10+ similar cold-stages have been built, characterized, and described in the last few years. This is also not the first cold-stage that uses a microfluidic device. In fact the predecessor work by Stan et al. (2009) managed to operate the instrument in continuous flow mode, rather than batch mode, and thus is more technologically advanced than the work here. In many places the manuscript tries to 'sell' or 'justify' the microfluidic technique presented here as an advance in technological capabilities. For examples, the microfluidic technique is 'cheap', 'easy to prepare', 'easy to operate', 'production of droplets is fast', 'a range of droplet volumes can be used', 'cooling down to homogenous temperature range allows an extensive investigation of atmospheric particles'. A list is provided that suggests that WISDOM solves 'some critical issues inherent in other currently used instruments', including 'fast production of droplets', high statistical power' due to 'fast analysis of thousands of droplets', 'droplets are monodisperse and individually analyzed', 'the use of oil minimizes possible artefacts', 'small droplet volumes decreases freezing artefacts by impurities, 'static array opens the possibility to investigate several freezing cycles for the same droplets', and 'microfluidics method and the small droplet volumes enable working with small volumes'. The statements above are either misleading or wrong or have not been demonstrated in this paper (see further below). It indicates that the authors have not critically reflected on the differences and similarity of WISDOM with the current technology and some of the necessary tradeoffs that are being made when designing cold stages. The WISDOM instrument does not demonstrate any aspects that has not been also addressed in other designs. A blunt assessment of the technique is that it is on par with the current state of cold-stage designs. However, although the technique is certainly valuable (and cool!), it does not represent an advance in either science or technology relative to the

published literature. A revised manuscript must better reflect the techniques ability and limitations relative to other existing techniques. Issues with justification. Quotes from the manuscript are in bold font

Authors' Reply: We have addressed the comments and re-wrote parts of the manuscript to adhere to the comments made by the referee. We believe that it now better reflects the abilities and limitations of WISDOM relative to other existing techniques.

The microfluidic chips are cheap and easy to prepare and to operate. Production of droplets is fast and a range of droplet volumes can be used within the same microfluidic device. These statements are highly subjective. Please elaborate. What does cheap mean? The chips are custom made, there is cost for that equipment and labor involved, which is not free. On top the system requires a microscope, liquid flow control, and more complex cooling setup relative to a regular cold-stage. What does fast mean? If cheap and fast are a 'selling' argument for the technique, then it should be compared to other cold-stage techniques. The real metric that need to be compared relative to other techniques. Total cost of equipment, total cost per 'experiment' in terms of equipment and consumables. (2) Number of drops and total volume that can be studied in a reasonable 'experiment' (e.g. working with a dust sample for one morning). Related to this number is the lowest and highest INP concentrations that can be captured in that experiment. Factors such as channel clogging, or chip damage due to expansion of water upon freezing should be accounted for here fairly. (3) Ease of use relative to a regular cold-stage technique. These are the key pieces of information that are needed to weigh whether one should adopt this technique or not (other than personal preference, which may well justify the route of microfluidics).

Authors' Reply: The descriptions "cheap and easy" referred to the microfluidic chips (or devices) and not to the entire WISDOM system. However, the authors understand the reviewer's concern and in order to avoid any subjective declarations this statement is removed from the manuscript.

In more detail:

(1) The authors agree that a statement about the microfluidic system cost is subjective and will differ from lab to lab. However, in the manuscript we only claimed that the microfluidic devices themselves are cheap and do not refer to the entire system. Because cost is subjective, and we could not find this information in any other paper from the relevant fields, we could not provide a comparison of the costs with different cold stages. Hence, we removed this statement from the text, from the introduction and from the summary. We evaluate our devices at 1 euro per each device, the cost consists of the cost of a microscope glass slide and the cost of a PDMS layer on top. We do not expect this to change much from place to place.

(2) Fast: Time to produce the droplets is about 30-40 seconds and then about 10 seconds to disconnect the device from the tubes and to transfer it to the cooling stage.

We cannot provide any specific information about clogging of the devices, as we did not study this in detail. Clogging will depend on the particles' properties and concentration and maybe on the flows used. Hence if channels are clogged or damaged (normally rare and we do recycle each device for a few experiments) we do not use the device since it is unknown how this

affects the concentration of the material inside the droplets or the change in the temperature equilibration in the device if the channels are damaged. In section 3.6 we state these issues where we discuss disadvantages of this system.

(3) The authors agree here too, a statement about ease of use in comparison with other cold stages is subjective. In the quoted statement it was claimed that the preparation of the microfluidic device is easy and not that WISDOM is easier than the other cold stages.

The authors would like to clarify that the cooling stage is not more complex relative to the other stage-based instrument as the reviewer pointed out and similar cooling stages are in use within the ice nucleation community. We did not find in the text where it was presented as complex.

A range of droplet volumes can be used other cold-stages have worked with the same range of droplet volumes and sample statistics

Authors' Reply: The statement above introduces the versatility of the microfluidic device to study ice nucleation in droplets of varying volumes. For example, device with 100-µm diameter can be used to produce droplets with any diameter that is smaller than 100 microns.

Cooling down to homogenous temperature range allows an extensive investigation of atmospheric particles Furthermore, supercooling is limited due to the presence of impurities, which increases with the volume of the droplet. Hence, to allow comprehensive studies down to the homogeneous region, low volumes are used and generation of these volumes is not trivial and may cause further complications. The authors should elaborate on the point they are trying to convey here. Presumably, this is meant to convey that a technique like WISDOM is needed? Three points. First, may authors have accomplished studying homogeneous freezing using small drops successfully in past and present studies. Clearly it is doable and the alluded to 'complications' have been solved in some way by these authors. Second, one might be inclined to believe that going to small drop volume solves some problems. If done correctly, small volumes do allow generation of drops with no impurities and thus studying homogeneous nucleation (as demonstrated widely in the literature). However, the impurities are still present. Simply subdividing the sample into more droplets will not help raising the lower limit of detection of ice nucleation activity for that sample. Specifically, if a sample has 105 nuclei per liter of liquid, studying a large number of small droplets or a smaller number of large droplets will produce the same result. This study has not demonstrated any advance in purifying water. The achievement of pure water freezing has been managed before by others when working with small volumes. Third, the number of drops studied per experiment here is quite small (500). While this means that homogeneous freezing temperature can be reached, it also means that the total volume studied is quite small, and thus the lower limit of detection for ice nuclei is much larger than in devices that use larger droplets.

Authors' Reply: The authors made this statement to explain what the considerations to employ low volumes of droplets were.

(1) The authors did not claim that homogeneous freezing in low volumes was not studied successfully before. In fact, Figure 5 and Figure 6 present some of these studies which are used as benchmarks for the WISDOM studies.

(2) With all due respect, the authors do not agree here with the referee. How the active sites are distributed as a function of temperature is critical here. It is indeed correct that ice nucleation of materials can be studied well by using higher volumes of droplets, if the nucleation sites are active at warmer temperatures than the freezing of the impurities that are present in the sample (normally between ~-20 to -25C (Hiranuma et al. 2015, ACP)). A good example for this is *snomax*. In the atmosphere, supercooling is observed down to -37C and mineral dust have also active sites in colder regions than -25C. Therefore, low volumes are more sensitive to the active sites that are less rare and that are activated between -25C and down to the homogenous region. This was our consideration. In contrast, WISDOM is not sensitive to the rare active sites if they are active at warmer temperatures. In some cases, it is impossible to increase the surface area in the droplets (if there is not enough sample or if the high particle concentration disables the droplets production), then it will be possible to use a larger sample of droplets. This important point is now added to section 3.6:

"…. the small droplets' volumes reduce the sensitivity to rare active sites. This may be solved by performing many experiments or by using larger droplets with more surface area within the droplets".

(3) 500 droplets are normally not small for the concentrations and the materials that we use. The considerations regarding the effect of overall volume are given in (2) above.

WISDOM solved some critical issues inherent in other currently used instruments including (1) fast production of droplets minimizes sample sedimentation or other aging process that may occur in a suspension, leading to higher reliability of the measurements and to a better estimation of the surface area of the material that is exposed in the droplets. This has not been shown here. First, it is unclear how fast fast is. No times are given in the paper. Second, it has not been compared to how fast others can perform an experiment. Third, the paper does not demonstrate that this technique is more reliable than others. There is no metric for reliability. This statement is clearly not justified. (2) **The high statistical power that can be achieved easily by fast analysis of thousands of droplets.** Again, fast is subjective. The number of drops given here is that approximately 550 forty-microns and 120 hundred-micron droplets can be monitored per experiment. The duration of an experiment is unclear, but it includes chip production, chip loading, and post processing. So how many experiments can one person do in a week? Is the statistical power really higher than in other studies? For example, Hader et al. (2014, ACP) generate and analyze 500-800 drops per experiment in the 80-100-micron size range. Another example Peckhaus et al. (2016, ACP) generate and analyze 1200- 1500 drops drops per experiment om 100-micron size range. Both studies also use oil immersion. Does WISDOM have really faster analysis and higher statistical power than these studies? Perhaps so, but it must be proven.

Authors' Reply:

(1) We agree that "fast" is subjective - removed from the text.
(2) High statistical power - removed from the text.

(3) the droplets are monodispersed and individually analysed, in contrast to other emulsion techniques See above for examples that also use individual analysis.

Authors' Reply: In Hader et al (2014) the droplets are not monodispersed and in Peckhaus et al (2016) do not have an emulsion. The authors refer here to few other studies that used emulsion in the DSC for example and recorded the average freezing behaviour. This meant to clarify that there are some more parameters as frozen fraction and freezing rate that can be obtained with individual droplets, which cannot be obtained by bulk methods. We removed the sentence "in contrast with other emulsion techniques".

(4) the use of oil minimizes possible artefacts from droplets' evaporation, neighbour seeding or vapour . . . See above cited studies and several other systems that do the same.

Authors' Reply: As was explained earlier, these are advantages in WISDOM that the authors find important and maybe this adds to the variability between the different techniques. It is important to clarify that it is not stated that WISDOM is the only cold stage setup for ice nucleation studies that has these properties.

(5) the small droplet volumes decrease freezing artefacts by impurities, and in the absence of INPs the water freezes below the homogenous freezing threshold (-37°C), in comparison with instruments that employ droplets with larger volumes which limit the workable temperature range. This point is incorrect as stated above. Working with small droplet volumes allows studying dilute solutions and ice nuclei that have high concentration in liquid. The high concentrations are the result of the overall low liquid volume that is analysed. Working with large droplets is a choice to increase the detection limit. Furthermore, many investigators work with small droplets before, so this is not a distinguishing feature of WISDOM.

Authors' Reply: (5) Similar points are explained before.

(6) using a static array opens the possibility to investigate several freezing cycles for the same droplets Re-freeze experiments have been performed with static droplet arrays since the pioneering work by Vali in the 1960's. This is not a distinguishing feature of WISDOM.

Authors' Reply: (6) the authors did not claim that this is a distinguishing feature of WISDOM. The text only describes other possible experiments in WISDOM.

(7) the microfluidics method and the small droplet volumes enables working with small volumes which is an advantage when working with atmospheric samples. First, working with small volumes and atmospheric samples has been also done by previous investigators. Second, it is incorrect to cite the small droplet volume as an advantage. It may be an advantage under some circumstances, but not others. Specifically, the poor lower limit of detection for small droplets is a clear disadvantage. To summarize the critique of this section. The text is prefaced with "WISDOM's largest advantage is the use of microfluidics technology which solves some critical issues inherent in other currently used instruments". The issues that WISDOM solves have been solved in some form or another in previous studies. WISDOM shares the advantages and disadvantages of the design choices involving the selection of oil, droplet size, batch mode, and optical detection. Claims regarding high statistical power, ease of use, and cost need to be proven. The paper should give an honest assessment of whether a new investigator should pursue the microfluidics route or the setup or something else. WISDOM and the nanoliter droplet freezing assay have similar control over drop volume, automated drop generation, drop detection, refreeze capabilities, etc. Perhaps telling is that the entire section in the draft manuscript is devoid of any references to the literature to back up their claims.

Authors' Reply: (7) we explained the considerations for choosing the small volume in the previous replies. The authors do not claim that it was not done before, and we clearly discuss the advantages and disadvantages of the volume range chosen. In any case, microfluidics allows the use of higher volumes. The authors of this manuscript made every effort to describe the system in an objective way, and to cover existing literature.

Other comments

However, in the atmospheric heterogeneous ice nucleation field, microfluidics techniques are not widely adopted, despite many potential advantages.

What are these potential advantages? Please be specific. Also, there are clearly potential disadvantages to microfluidics. These should be discussed as well.

Authors' Reply: These points are clearly stated in section 3.6, the authors corrected section 3.6 in order to represent better the advantages and disadvantages of WISDOM, considering the points that the reviewer raised:

"WISDOM uses of microfluidics technology which solves some critical issues inherent in other currently used instruments: (1) good control of size number of monodisperse droplets, (2) fast production of hundreds of nearly monodisperse droplets minimizes sample sedimentation or agglomeration that may occur in a suspension, leading to a good estimation of the surface area of the suspended material. Moreover, several droplet diameters can be employed in the same device without its modification, (3) good statistics achieved by individual analysis of thousands of droplets, (4) monodisperse droplets individually analyzed, in contrast to some emulsion techniques as the Differential Scanning Calorimeter (DSC) experiments, allow to obtain the frozen fraction at each temperature, and to achieve detailed information about active sites

and freezing rates, (5) the use of oil minimizes possible artefacts from droplets' evaporation, neighbor seeding or vapor transfer due to the Wegener–Bergeron–Findeisen processes, (6) the small droplets' volume decreases freezing artefacts by impurities, allows to reach the homogenous freezing threshold (-37°C), (7) possible investigation of several freezing cycles for the same droplets, (8) the microfluidics method and the small droplet volumes enable working with small sample volumes which can be an advantage when working with atmospheric samples.

WISDOM has a very accurate temperature calibration that spans a wide temperature range, using the eutectic freezing method. WISDOM most resembles the instrument used by in Edd et al. (2009). However, it seems that issues with temperature calibration in Edd et al. (2009) led to a temperature offset, and hence different freezing rates. Stan et al. (2009) achieved better temperature accuracy and high statistics. However, the freezing experiment was conducted in a flow mode, which is more complicated than in the WISDOM setup and requires complicated modeling. In addition, the cooling rates that were used were very fast, which induces additional errors. Riechers et al. (2013) had high temperature accuracy as they also used a DSC. However, they had to collect the droplets from the device as there was no static array option and this may add further complication and contamination.

It is noted also that use of microfluidics technology has a few disadvantages. These may include: (1) oil may interact with some of the analyzed particles, possibly leading to biased data, (2) the microchannels are susceptible to clogging, (3) it is not possible to perform any post analysis to the droplets content after the experiment, (4) because of the small droplets' volumes, and there is less chance to characterize rare active sites".

Figure 2 needs better explanation. The y-axis is not clearly defined. Presumably, Delta GL stands for change in grey level observed during warming or cooling? These quantities should be defined in the caption and text. Is the scale 0-255 or 0-1 or 0-100? What is std mean on the y-axis? Are the curves the population mean, or are they for a single droplet? The thermodynamic prediction for the eutectic melting point for the NaCl and pure water should be added to the graph.

Authors' Reply: More detailed explanation is added to the text in section 2.3:

"The optical brightness of a droplet changes during a phase transition (freezing or melting) due to the different interaction of light with the liquid and the solids. For phase transition detection, an in-house image processing LabVIEW program monitors automatically the optical brightness change. The program detects the droplets using a spherical shape criterion and sets a square surrounding the droplet that defines an array of pixels that are attributed to that specific droplet. A change in the optical brightness is represented by the gray level value of the image's pixels, ranging from 0 to 255. Freezing is calculated per movie frame and is defined as the subtraction of the brightness mean value for each droplet in two consecutive frames (ΔGL), thus allowing derivation of freezing rates. At the beginning of the analysis, the first 15 frames are used to identify the noise level of the signal by calculating its standard deviation (std(ΔGL)). The program then searches for the maximal freezing signal that is also greater than 5 times the noise level. The temperature associated with this freezing signal is assigned as the freezing temperature for that droplet.

In this algorithm, the program can distinguish successfully between a phase transition event and noise that arises from the camera signal, droplet movement or any other interruption. Figure 2 presents a spectral analysis for different types of phase transitions observed in WISDOM. Since WISDOM operates in transmission microscopy mode, the light is scattered more efficiently by ice crystals in comparison with a liquid droplet and a freezing event involves droplet darkening and a negative signal. Example for the negative signal of a freezing event of a single droplet can be seen in Figure 2a. In comparison, during melting, the droplet becomes brighter until all the crystals melt, and the signal is positive. In Figure 2b+c the analysis of melting signal and eutectic melting signal are presented for the whole frame".

And to the figure 2 caption:

"Spectra of different phase transition events as observed in WISDOM. a) freezing, b) eutectic melting, and c) melting onset and clear point (liquefaction) are the mean of all sampled droplets in a single experiment. The phase transition is defined optically by the brightness information obtained by the gray level of the image pixels. Std(ΔGL) describes the standard error of the difference in mean GL for two consecutive frames. At the beginning of the experiment the noise level is studied and freezing or melting is detected only if std(ΔGL) is as least 5 times greater than the noise std level. Freezing and melting examples are for pure water droplets and the eutectic melting example is for aqueous solution droplets of NaCl. Eutectic melting point of NaCl and pure water melting point are marked by the yellow and red lines in b and c, correspondingly. In all cases the droplets diameter is 100 μm."

melting points were added to the graph as well.

Figure 3. The C and H (presumably cooling and heating) should be explained in the caption. The Delta T is a temperature and should have units of K. The freezing temperature of pure water should be given here. The text states that the delta T is evaluated against an extrapolated temperature at equilibrium conditions. Does that mean that the equilibrium conditions T for freezing and melting are not constant in the plot?

Authors' Reply: Explanation is added to figures 3. The extrapolation results in equilibration are the values that we calibrate against.

Section 3.2 provides statistics for the T50 for several devices and repeats for individual devices. However, no spectra are shown. How is the repeatability vis-a-vis early freeze events? The authors should show an overlay of the temperature spectra for all of these samples to convince the reader of the repeatability across the full range of temperatures.

Authors' Reply: Per the Reviewer's request, a graph of the full range is now added in appendix A.

Conclusions → homogenous should be homogeneous. **Authors' Reply:** Corrected in the text, thank you!

Response to Reviewer #2

The authors appreciate the thorough review and would like to thank the reviewer for taking the time and for helping to improve our manuscript, especially it's qualitative nature.

This work attempts to build on the extensive past literature on droplet freezing assays, by leveraging microfluidic technologies. Several calibrations experiments are performed on the stage in an attempt to quantify the accuracy and precision of measurements of ice nucleation rates and ice active site densities. It is noteworthy that assessing the absolute values of these quantities is a particularly challenging endeavor, owing to the lack of 'primary standards' whose nucleation behaviours are known to a high degree of certainty. Indeed, past intercomparing studies have yielded considerable spreads in rates and ice active site densities for homogeneous nucleation and the heterogeneous nucleators, and deviations between instruments has not been satisfactorily addressed. While the current work could definitely be of interest to the community of researchers interested in atmospheric ice nucleation processes, there are serious gaps in the paper which I feel need to be addressed prior to consideration for publication. The majority of these gaps revolve around the qualitative nature of the comparisons performed, and lack of error analysis on the rates and ice active site densities determined.

Major issues

- In figures 5, 7, 8 & 9, there are no vertical error bars presented to represent the uncertainty in the measurements. What is for example, the uncertainty stemming from the stochastic nature of nucleation in the homogeneous freezing experiments? What is the effects of the uncertainty stemming from the random sampling of ice nuclei into droplets on the heterogeneous ice active site densities in figures 7, 8, 9. Without statistically sound error bars and confidence intervals on the certainty of the data, comparisons are rendered almost meaningless.

Authors' Reply: In figure 5 error bars have been added for WISDOM and for the rest of the used data. In figure 7 the errors were confined in the marker itself, both for x and y axis. We preferred different marker sizes because visually it was hard to place the error bars in a clear way. This is now mentioned in the figure caption:

"Accumulated active site density spectra (n_s) of K-feldspar and Illite-NX particles as a function of temperature from validation experiments of immersion freezing in WISDOM. Frozen fraction values are represented by a color bar, for few surface area values that are exposed in 40 and 100 μm droplets. The dependence of the nucleation site density on the surface area is illustrated here. WISDOM uncertainties are included within the size of the markers. The uncertainty in n_s is propagated from the error in the surface area and the error in the frozen fraction. For validation, previous immersion freezing measurements are also presented (Hiranuma et al. (2015) and Atkinson et al. (2013)). For the Hiranuma et. al. fit, the maximum deviation between maxima and minima in the vertical axis are shown by the error bars for the relevant temperature range."

Moreover, new graphs with the error bars for the illite and for the k-feldspar data is now added in appendix B.

Authors' Reply: In figure 8 and 9 error bars are added.

- The authors note in the abstract (L16), that the method produces excellent 'statistics'. To what quantity are the authors referring to here? Nucleation rates? Ice active densities? If so, what is the effects of sampling hundreds of droplets over say 50? By what kind of factors are uncertainties reduced? If this is purported to be a major advantage of the technique, surely the improvement in these 'statistics' by this method should be quantified?

Authors' Reply: Due to the concern of both reviewers regarding the term "excellent statistics", this was removed from the manuscript:

"Frozen fraction, ice nucleation active surface site (INAS) densities and freezing kinetics can be obtained from WISDOM measurements using hundreds of individual droplets in a single freezing experiment. Extensive calibration experiments using eutectic solutions and previously studied materials are described".

- in this direction, some of the benefits of this technique over past techniques should be elaborated upon and clarified; the discussion of past issues, without acknowledging how they have been successfully dealt with in the past is rather peculiar. For instance, many cold stage instruments do not suffer from issues surrounding the Bergeron-Findeison process, and do not need oil to be placed on the droplets. At line 53, it is said that generation of 1 uL volumes is not trivial. To my understanding, this can be done with a pipette, which would seem rather trivial to me.

Authors' Reply: In section 3.6 the advantages and disadvantages of the WISDOM are discussed. Also, the considerations for choosing the microfluidics approach are described. We further show the importance of a thorough calibration of temperature and temperature equilibration properties. Clarification: in line 53, it is claimed that smaller volumes are not trivial to generate and not 1 microliter droplets, which is indeed trivial.

- In section 2.3, on the automated detection of phase transitions, it is noted that the algorithm can 'successfully distinguish between a phase transition event and noise' (L 150). Whilst this statement may well be correct, I see no mention of to what accuracy the algorithm can successfully distinguish between phase transitions. Is this 100% accuracy? How many experiments were performed manually to determine this?

Authors' Reply: This is monitored for each freezing event and done separately for each experiment since the noise level varies between experiments. The freezing causes larger reduction of the grey level than the noise, and hence it is possible to

separate it from the noise. So, a threshold is being set to differentiate the noise. This leads to very high success rate in identifying the freezing events.

- In the quoted value of ±0.25 K for the Linkam cryostage temperature sensor, which is subsequently quoted in the captions for figures such as 5 and 7, how was this value determined? Knowing this would certainly be useful for the reader. By what procedure was this value obtained?

Authors' Reply: This value (<±0.25K) is the uncertainty of the Pt100 temperature sensor in the temperature range. Using this value with our calibration results we propagate the total temperature uncertainty (±0.3K). This was not clear in the text and we thank the Reviewer for this comment which is now better explained in the text.

- In figure 7, the data for NX illite appear to be at the extreme lower end of the spread, based on the error bars used for the Hiranuma et al. data. Surely this should be discussed in the text?

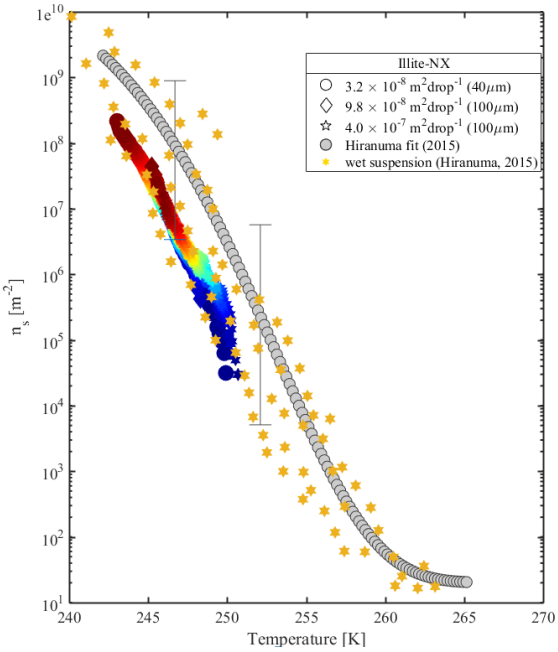
Authors' Reply: The comprehensive data of Hiranuma et al. covers different instruments and several immersion freezing techniques. Hiranuma et al. also mention the spread of the results, up to three orders of magnitude. In the effective WISDOM's temperature range, the fit of the results from only two instruments (CU-RMCS and NC-State-CS) that are relatively different from each other, in comparison to the spread among most of the other instruments. WISDOM and CU-RMCS agree well within the same order of magnitude on the number of active sites. Investigating the reasons for the spread in the results of all instrumentations is beyond the scope of this paper and is thoroughly discussed in Hiranuma et al. The Binary and Leeds data were also fixed to n_s that is based on BET measurement, instead of the data that was n_s based on geometry. Our results are also normalised to the BET surface area and now the agreement with Binary and Leeds are clearer.

- In lines 268-269, it is said that ns is in best agreement with the Leeds-NIPI for NX-illite. Yet close inspection (the subtle shades of grey used here in the graph make this a bit difficult to see), shows that there is in fact no overlap in the temperature range between the measurements presented here, and those of the Leeds NIPI μL.

Authors' Reply: Explanation is united with the reply to the next comment.

- In addition, in comparison to the binary instrument in figure 7, the data are up to an order of magnitude or greater off, which is not immediately obvious as the authors have chosen to only label the scale for every factor of 100 increase. It is noted that this is within the uncertainty of the instruments, but what is the uncertainty of the quoted values for the WISDOM (see my first point above…). Does the uncertainty really cover 2 orders of magnitude? With what degree of statistical certainty are you sure that these two measurements are in agreement?

Authors' Reply: The conclusion that the Illite-NX data is in good agreement with the BINARY rely on the fact that there is less than one order of magnitude between the two data sets and this difference is justified by uncertainties of both instruments. The temperature at which there is more than an order of magnitude difference (~250K) is where the variation between our measurements are the highest and for < 0.01% of the droplets. This is where very rare active sites exist (in the used surface area) and is less representative of the material. Moreover, if the slope of the Leeds NIPI is extrapolated, it would nicely overlap with WISDOM data. Here it is also expected that the data is of higher significance as there are many more freezing events. Examination of the Hiranuma et al data and our data, it seems that the data converge to the WISDOM and CU-RMCS results and slope:



*The labelling of the y axis is changed to every factor of 10 increase to avoid confusion of the readers.

*The figure is now replaced with a new figure that contains data from several instruments from Hiranuma et al. (2015) and the data from different instruments is now represented by colours instead of the grey shades.

- Lines 136-137: If the chips are being clogged by larger particles, then you may be severely altering the size-dependent particle composition of the samples as they pass through. How is this dealt with and accounted for?

Authors' Reply: This is indeed a concern and we do not work with clogged devices or with materials that tend to clog the devices. Moreover, we repeat experiments with the same device for few times (with fresh droplets), and do not observe evidence of critical alteration in the size distribution. We would expect that such alterations will increase with increasing number of repetitions and that we will see a reduction in the ice formation efficiency. For materials with particles >5 micron

in diameter, we experienced immediate clogging. From our experience, these particles readily settle in the suspension, even before the droplet production and also in cases where it was stirred during the droplets production. We also expect that for materials that tend to clog the device, it will be difficult to quantify that as it might change with the flow used or concentration of the material in the suspension and the material's size distributions. Hence, we do not work with suspensions of larger particles or when we observe evidence for clogging.

Other issues

- Line 28: INP should be INPs

INP changed in the text to INPS. Thank you!

- Line 34: Why is this only 'possibly' in future climates?

How can we be certain that this will affect climate?

We have performed further changes in the revised version of the manuscript:

- The Illite-NX n_s values used in figure 7, were n_s-geo instead of ns-BET, and now it is corrected.
- The authors have realized that the n_s values given in figures 8+9 is over estimation of n_s due to a mistake in the surface area estimation. The graph is now updated with the correct values. The revised manuscript contains examples of three MOUDI's stages, collected in one intensive Saharan dust event, that were comprise of a very small amount of dust.

Changes in the manuscript are highlighted in yellow, and sentences that were cut are crossed with a line.

The WeIzmann Supercooled Droplets Observation (WISDOM) on a Microarray and application for ambient dust

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Abstract. The WeIzmann Supercooled Droplets Observation on Microarray (WISDOM) is a new setup for studying ice nucleation in an array of monodisperse droplets for atmospheric implications. WISDOM combines microfluidics techniques for droplets production and a cryo-optic stage for observation and characterization of freezing events of individual droplets. This setup is designed to explore heterogeneous ice nucleation in the immersion freezing mode, down to the homogeneous freezing of water (235 K) in various cooling rates (typically 0.1-10 K min^{-1}). It can also be used for studying homogeneous freezing of aqueous solutions in colder temperatures. Frozen fraction, ice nucleation active surface site (INAS) densities and freezing kinetics can be obtained from WISDOM measurements for hundreds of individual droplets in a single freezing experiment. Calibration experiments using eutectic solutions and previously studied materials are described. WISDOM also allows repeatable cycles of cooling and heating for the same array of droplets. This paper describes the WISDOM setup, its temperature calibration, validation experiments and measurement uncertainties. Finally, application of WISDOM to study the INP properties of size-selected ambient Saharan dust particles is presented.

1 Introduction

In mixed phase clouds, water droplets remain stable in a supercooled state below 273 K and ice nucleates spontaneously as droplets reach the homogeneous freezing temperature, below 236 K (Pruppacher et al., 1998). At warmer temperatures, ice particles may coexist with supercooled droplets, due to heterogeneous nucleation facilitated by the presence of ice nuclei particles (INPs)(Cantrell and Heymsfield, 2005). In cases where INPs are immersed in the droplet before supercooling, referred to as immersion freezing mechanism, the droplets first grow to supercritical size before freezing occurs (de Boer et al., 2011). Observations and modeling studies suggest that immersion freezing is the prominent mechanism for heterogeneous ice formation in mixed phase clouds (Ansmann et al., 2008; Field et al., 2012; Nagare et al., 2016; Possner et al., 2017; Rosenfeld and Woodley, 2000).

Ice particles affect the radiative and microphysical properties of mixed phase clouds and Earth's hydrological cycle. Therefore, they can influence present and possibly future climate (Hoose and Möhler, 2012; IPCC, 2013). Studying ice formation in clouds is hence important, and yet, due to its complexity, this process is still not fully understood and presents a great challenge

to laboratory and field researchers as well as for clouds and climate modelers (DeMott et al., 2010; Schnaiter et al., 2016; Ullrich et al., 2017).

Offline studies of immersion freezing often use cold stage techniques (Budke and Koop, 2015). The basic idea is to place an array of droplets over a cold stage and cool continuously until all are frozen, to obtain a quantitative measurement of their corresponding freezing temperatures (Vali, 1971). The droplets may be microliter-sized and observed with a simple camera. Smaller droplets, down to the pico-liter range, are usually observed under a microscope. In both cases, freezing events are identified by optical changes in the droplets when they crystalize (Atkinson et al., 2013; Hiranuma et al., 2015; Knopf and Lopez, 2009; Murray et al., 2011).

Cold stage techniques may suffer from technical issues such as droplets evaporation and vapor transfer due to the Wegener–Bergeron–Findeisen process, where ice grows on the expense of supercooled droplets or from seeding of neighboring droplets by formation and surface growth of frost halos (Budke and Koop, 2015). Some cold stages instruments place oil over the droplets or use droplet in oil emulsions to prevent these effects (Murray et al., 2012). Still, results from cold stage experiments may be biased by effects of inhomogeneous temperature of the substrate and the surroundings or by various contaminations caused during droplets' preparation and measurement (Hiranuma et al., 2015). Furthermore, supercooling is limited due to the presence of impurities, which increases with the volume of the droplet. Hence, to allow comprehensive studies down to the homogeneous region, low volumes (<1 µL) are used and generation of these volumes is not trivial and may cause further complications.

Microfluidics is a technology of fluids manipulation in micro-channels array on a small device. Microfluidics is widely used in a range of fields, such as physics, chemistry, biology, life sciences and the food industry (Neethirajan et al., 2011; Sackmann et al., 2014; Whitesides, 2006). Recent studies used microfluidic apparatus to study ice nucleation processes. Riechers et al. (2013) used a microfluidics device to produce and collect monodisperse droplets of water in various sizes, which were subsequently observed under a microscope to study their homogeneous freezing. Stan et al. (2009) recorded nucleation in water droplets and silver iodide seeded droplets, while droplets were flowing during cooling. Schmitz et al. (2009) established 'Dropspots', a static microfluidic array of droplets, later used by Edd et al. (2009) to measure nucleation kinetics. However, in the atmospheric heterogeneous ice nucleation field, microfluidics techniques are not widely adopted, despite many potential advantages.

The WeIzmann Supercooled Droplets Observation on Microarray (WISDOM) is a new instrument combining the cold stage technique with microfluidics technology, and is designed to study immersion freezing of micrometer-sized droplets, while addressing most of the technical issues listed above. The WISDOM setup introduces several advantages of microfluidics to the atmospheric ice nucleation field. ~~The microfluidic chips are cheap and easy to prepare and to operate. Production of droplets is fast and a range of droplet volumes can be used within the same microfluidic device.~~ WISDOM is based on the 'Dropsopts' static array (Schmitz et al., 2009), which enables the separation and the fixation of the droplets, so that each individual droplet is recorded and studied, and also can be used for repetition of freezing cycles and further exploration of the nucleation process of a specific sample.

In this paper, we present the WISDOM setup, its calibration and validation procedures. For validation experiments, homogeneous and heterogeneous freezing were examined by following homogeneous freezing rates of pure aqueous or solutions or deriving the efficiencies of three types of mineral dust surrogates and collected ambient Saharan dust to validate heterogeneous freezing experiments.

Homogeneous nucleation rates are described stochastically using the volume-dependent ice nucleation rate ($J_v(T)$) in supercooled droplets, given by the frozen fraction (f_{ice}) of droplets with volume V at a certain temperature (T) and time intervals (Δt) (Alpert et al., 2011; Murray et al., 2010; Riechers et al., 2013),

$$J_v(T) = \frac{-\ln(1 - f_{ice}(T))}{V\Delta t}, \quad (1)$$

Heterogeneous freezing is described by a singular approach that assumes that nucleation occurs at a certain temperature due to special nucleation site. Hence, a cumulative number of nucleation sites per unit surface area, n_s, is used to describe the heterogeneous nucleation efficiency at a certain temperature,

$$n_s(T) = \frac{-\ln(1-f_{ice}(T))}{A}, \quad (2)$$

Where f_{ice} is the fraction of frozen droplets at temperature T, and A is the specific surface area of the immersed particles in each droplet (Vali, 1971; Vali et al., 2015; Whale et al., 2015).

Validation of WISDOM was further extended below the homogeneous nucleation temperature of pure water, using aqueous solutions as the freezing temperatures of solutions decrease as a function of the solution water activity.

2 Experimental setup

2.1 Droplets production and trapping

The WISDOM setup, shown in Figure 1, is made of a microfluidic setup which include a pressure controlled pump with 4 independent flow channels (OB1 MK3 by Elveflow), a stereoscope (SMZ-171 by Motic) that permits a full view of all channels and inlets, and a CCD camera (GS3 by Point Grey) that enable real time monitoring of the droplets production. The flows in the channels are continuous and controlled by the pressure pump. One channel is connected to the continuous (oil) phase, and a second channel contains the sample (aqueous solution that can contain INPs). The two phases meet in a narrow junction where monodisperse droplets are generated due to the pressure exerted by one phase over the other. The ratio between the flows determines the size of the emerging droplets; the volume increases with increasing flow rate of the sample. In this setup, droplets are suspended in an oil mixture, consisting of mineral oil (Sigma Aldrich) and a 2 weight percent (wt%) nonionic surfactant (span80®, Sigma Aldrich), added for droplets stabilization (Riechers et al., 2013). Hence, an array of picoliter (micrometer-size) droplets is generated directly on a device.

The principle of the Schmitz et al. (2009) design is that the droplets flow into round chambers that are connected by constriction channel. At a certain flow, the droplets are squeezed through the constriction channel and the array fills up with droplets. When the flow is too weak or stopped, the constriction channel stops the droplets' movement and they are trapped in the

chambers. The droplets are isolated and stable in the chambers and it is safe to move the device from the generation stage to the cold stage for the freezing experiments.

Devices are fabricated following the Schmitz et al. (2009) protocol. Briefly, the device pattern is imprinted on a polydimethylsiloxane (PDMS) polymer, later glued to a 1mm thick microscope glass slide using air plasma treatment. After the plasma treatment the PDMS surfaces are hydrophilic (Eddings, 2008). Therefore, the devices were used only in the following day, after their surfaces became hydrophobic, following their exposure to the atmosphere, or after their annealing at 60°C for half an hour.

2.2 Freezing experiments and detection

The droplets array is placed in a commercial cryostage (Linkam, THMS600) coupled to an optical microscope (Olympus, BX-51 with 10X magnification, transmission mode). Experiments are monitored by a microscope mounted CCD camera (Allied Vision Technologies, Oscar F-510C) for automatic identification of droplets and their freezing events. Both the device and the cooling stage are cleaned with 2-propanol. Then the device is placed over the stage together with a thin layer of oil on its bottom to provide good thermal conductivity. Each freezing experiment starts with dry N_2 purging to replace the moist atmosphere inside the cryostage to prevent condensation. During the experiment, N_2 flow prevents water condensation on the cryostage window. Freezing experiments are conducted with a cooling rate of 1 K min^{-1} which is relevant for atmospheric conditions and also allows good thermalization of the droplets, as will be shown in the calibration section (section 3.1). Each cooling cycle is followed by a heating cycle, where melting is observed. Analysis of the melting onset is then used to verify that the thermal conductivity is good and thus validate the measurement.

In-house LabVIEW software is used to record a freezing experiment movie file and analyze it offline. The temperature readings by the Linkam cryostage temperature sensor (<±0.25 K for the operated temperature range) and the movie frames are synchronized and integrated. In most cases, 1 second (or 0.017 K at 1 K min^{-1}) per frame is used. Currently, the WISDOM setup operates with two types of devices that differ in their droplets' trap diameter: 40 µm and 100 µm. Approximately 550 and 120 droplets can be monitored per experiment in the smaller and larger diameter devices, respectively. Statistically, for the same sample, larger droplets encompass more INP surface area within each droplet, which can be more sensitive for detecting rare active sites. The device can be reused for the same sample, if it is not clogged or destroyed during the experiment. However, because the channels of the 40 µm device are smaller they tend to clog faster (for instance by large particles).

2.3 Automatic detection of phase transitions

The optical brightness of a droplet changes during a phase transition (freezing or melting) due to the different interaction of light with the liquid and the solids. For phase transition detection, an in-house image processing LabVIEW program monitors automatically the optical brightness change. The program detects the droplets using a spherical shape criterion and sets a square surrounding the droplet that defines an array of pixels that are attributed to that specific droplet. A change in the optical brightness is represented by the gray level value of the image's pixels, ranging from 0 to 255. Freezing is calculated per movie

frame and is defined as the subtraction of the brightness mean value for each droplet in two consecutive frames (ΔGL), thus allowing derivation of freezing rates. At the beginning of the analysis, the first 15 frames are used to identify the noise level of the signal by calculating its standard deviation std(ΔGL). The program then searches for the maximal freezing signal that is also greater than 5 times the noise level. The temperature associated with this freezing signal is assigned as the freezing temperature for that droplet.

In this algorithm, the program can distinguish successfully between a phase transition event and noise that arises from the camera signal, droplet movement or any other interruption. Figure 2 presents a spectral analysis for different types of phase transitions observed in WISDOM. Since WISDOM operates in transmission microscopy mode, the light is scattered more efficiently by ice crystals in comparison with a liquid droplet and a freezing event involves droplet darkening and a negative signal. The negative signal of a freezing event of a single droplet is shown in Figure 2a. In comparison, during melting, the droplet becomes brighter until all the crystals melt, and the signal is positive. In Figure 2b+c the analysis of a melting signal and a eutectic melting signal are presented for the entire frame.

3 Results and WISDOM validation

3.1 Temperature calibration

Temperature accuracy is a most important parameter in ice nucleation experiments. An error propagation analysis by Riechers et al. (2013) demonstrated how the temperature uncertainty may lead to a distribution of temperatures between different instruments. Therefore, we performed a thorough temperature calibration using the known eutectic melting points and the melting points of several aqueous solutions as calibration reference points. Although ice nucleation experiments are performed while cooling, the calibration experiments were done while heating to improve the calibration precision and to avoid biases associated with supercooling of the liquids (Budke and Koop, 2015).

3.1.1 Droplets thermalization

The temperature of the Linkam stage was measured at the upper center part of the cooling stage and hence may differ from the actual temperature of the droplets in the device due to thermal effects such as temperature gradients and temperature lag. During cooling or heating, a vertical temperature gradient may develop between the top of the device, in contact with the inner ambient of the cryostage, and the bottom of the device, which is in contact with the cooling silver block. This gradient is expected to increase in magnitude, as the temperature of the stage decreases or increases below or above ambient temperature. Edd et al. (2009) used a similar setup and found a difference between the top temperature and the bottom temperature of about 2 K around 237 K and 3 K around 227 K. Stan et al. (2009) also reported a vertical gradient of 1-2 K, that was reduced to 0.5 K with a flow of cooled N_2 over their device. In addition, a thermal lag may arise during cooling or heating as the rate of temperature change is high and precludes proper temperature equilibration. Hence, a more accurate measurement of the droplet temperature is taken as a sum of the stage temperature with the contributions of both thermal gradient and lag.

Figure 3 demonstrates the combined effects of temperature change rate and device properties on the thermalization of pure water droplets (double distilled, 18.2 MΩ cm). Specifically, freezing and melting experiments at different rates were performed. The temperature difference (ΔT) is the difference between the measured values and the extrapolated temperature at equilibrium conditions (0 K min^{-1}). As expected, at slower temperature cooling (heating) rates, the droplets are more equilibrated with the stage temperature and ΔT is negligible. However, ΔT increases at higher temperature cooling (heating) rates (e.g.; 10 K min^{-1}). We observed that during cooling (heating) the droplet is warmer (colder) than the stage and will freeze (melt) at colder (warmer) temperature at higher cooling (heating) rates. We also found that because ΔT is higher, in absolute value, for devices of thicker PDMS and/or in devices which hold larger droplets, it should be considered in the final temperature calibration for these scenarios. Furthermore, ΔT was found to be almost symmetric for higher temperature cooling (heating) rates. However, for 1 K min^{-1}, ΔT during cooling is higher than that for heating. Our conjecture is that this can be an effect of the higher thermal gradient that develops as the temperature decreases well below ambient (236 K).

3.1.2 Melting of aqueous solutions

Figure 4 presents the measured melting points of NaCl solutions with different water activities. Reported melting points represent the temperature in which all ice crystals in the droplets completely melted, in contrast with melting temperatures reported for pure liquids such as water, where the onset of melting is defined as the melting point. Melting temperature results were consistent with theoretical melting temperatures reported in Koop and Zobrist (2009). This provides support to our conclusion that droplets thermalize with the cooling stage when using a heating rate of 0.1-1 K min^{-1}. For faster heating rates (i.e. 10 K min^{-1}), the thermal lag was more pronounced, leading to a melting point shift of about 2-3 K. For more concentrated solutions, faster heating rates shifted the melting points more.

3.1.3 Melting of eutectic solutions

Some aqueous solutions, such as NaCl and $MgCl_2$, arrange in a super-lattice at a certain wt% to form a solid with a well-defined melting point (eutectic) (252.05 K for NaCl and at 239.95 K for $MgCl_2$) (Borgognoni et al., 2009; Farnam et al., 2016). Interestingly, this type of melting has a smaller optical signature compared to that of melting points of pure substances, as can be seen in Figure 2b. We have set a specific water activity for a solution by determining its quantitative composition using the extended aerosol inorganic model (E-AIM) (Clegg et al., 1998) at room temperature (298 K). For calibration purposes, because eutectic melting had a negligible variation for different water activities used in the range of 0.99 to 0.95, we decided to take their average to achieve a single melting value. These eutectic melting temperatures are colder than the melting point of pure water and, therefore, are used for expanding WISDOM calibration range.

The final calibration is obtained for a device with a specific PDMS thickness and at a specific cooling (heating) rate. For example, devices with 100 μm diameter sized droplet and of 4mm PDMS thickness have a linear calibration curve of $T_{drop}=0.97\times T_{stage}-0.46$ at 0.1 K min^{-1}.

3.2 Measurement reproducibility and device variability

Device's inter-variability was determined from 20 devices by comparing their corresponding homogeneous freezing temperatures of pure water. Specifically, each device was recycled three times with freshly prepared droplets. Our results showed high reproducibility in the median freezing temperature, where 50% of the probed droplets froze (T_{50}), and high reproducibility in the melting point temperature. Variation within devices was always smaller than ±0.2 K at 1 K min^{-1} and 0.1 K min^{-1} (variation within the devices over the whole freezing range is presented in Appendix A).

3.3 Homogeneous freezing rates of pure water

Homogeneous nucleation in supercooled water occurs in WISDOM between 238 and 237 K for a cooling rate of 1 K min^{-1} and droplets diameter of 100 μm. Figure 5 shows WISDOM nucleation rates in comparison with other similar instruments. It is seen that the slope of the rate and temperatures are similar to the slopes reported for other instruments. The temperature where 50% of droplets froze (T_{50}) is also in the expected range according to model results of Hoffer (1961). WISDOM rates are slightly slower, but within the uncertainty of the instruments used by Riechers et al. (2013) and Stan et al. (2009). Stöckel et al. (2005) show a higher nucleation rate. This discrepancy can be explained by a decrease in the number of surface nucleation events due to the oil phase surrounding our droplets, whereas in Stöckel et al. (2005), droplets are suspended in air which allows surface nucleation may occur.

3.4 Homogeneous and heterogeneous freezing of aqueous solutions

The water-activity-based ice nucleation theory by Koop et al. (2000) describes the dependence of the freezing temperature depression on the water activity (a_w) of the solution, regardless of the solute nature. Figure 6 presents the theoretical freezing and melting temperature curves from Koop et al. (2000) with homogeneous ice nucleation results measured in WISDOM, for four solutions with atmospheric relevance. Water activities for NaCl, ammonium sulfate (AS), glucose and levoglucosan mixtures were derived from the AIM model and were corrected for glucose and levoglucosan, for which water activity is temperature dependent (Knopf and Lopez, 2009; Zobrist et al., 2008). The experiments were conducted at 1 K min^{-1} for 40 and 100 μm droplet diameters. The results follow the theoretical curves of the water-activity-based ice nucleation, and the dependence of the homogeneous freezing on the droplet volume is as expected (Hoffer, 1961; Kuan-Ting and Wood, 2016) as the curve of the smaller diameter droplets (green curve) is slightly colder compared with the larger volume droplets (dark green curve).

Similar experiments were conducted for 0.1 wt% of Arizona Test Dust particles (ATD, Powder Technology Inc.) immersed in glucose solution droplets. The ATD particles facilitate the ice nucleation at warmer temperatures, in agreement with similar studies (Hartmann et al., 2011; Niedermeier et al., 2010), and the freezing depression follow the water-activity-based ice nucleation curves. Here, the dependence of the freezing point on the droplet volume is more pronounced, as the surface area

of the immersed particles is higher, hence they contain higher number of nucleation sites (Marcolli et al., 2007) as will be shown in the next section for two more types of dust.

Below 223 K, ice nucleation occurs at slightly lower temperatures than expected by the theoretical freezing curve. As the WISDOM temperature calibration is not valid in this temperature range, we cannot conclude if this is due to a change of the thermal conductivity of the device or an effect of the high concentration of the solute in the water.

3.5 Heterogeneous nucleation and n_s spectra of INP in pure water

3.5.1 Standard dust powder

Heterogeneous freezing efficiencies of suspended mineral dusts K-Feldspar and Illite-NX in supercooled water droplets are presented in Figure 7 and summarized in Table 1, and are compared to recent published data. The particles are suspended at different wt% and the frozen fraction of each suspension is derived as a function of temperature as represented by the color bar. To examine the freezing efficiency and compare the different mineral dust types, the results are normalized to the surface area within each droplet. Experiments were performed at 1 K min^{-1} for 40 and 100 µm droplets diameters. Suspension preparation and evaluation of the surface area are described in the appendix B.

The results demonstrate the effect of dust surface area immersed in the droplets on the freezing parameters. The freezing temperatures increase with increasing surface area and are also reflected in the warming of the median frozen fraction (T_{50}) colored in yellow. The spectra of the number of nucleation sites per unit surface area (n_s) also support surface area dependence because all spectra converge to a single line. The n_s results show the increase of nucleation sites at colder temperatures. Results from WISDOM are in good agreement with similar analyses from other instruments. In particular, n_s is in best agreement with the Leeds-NIPI (Broadley et al., 2012; Murray et al., 2011) results both for K-Feldspar and for Illite-NX particles. Results of Illite-NX particles are also in good agreement with the Binary instrument (Budke and Koop, 2015) and reside within the uncertainty of both instruments. The linear trend of few wt% support the assumption that particles in suspension are uniformly distributed and the droplets contain approximately the same surface area.

3.5.2 Ambient mineral dust

WISDOM can also be used for analyzing collected ambient particles. Mineral dust particles were collected in Rehovot, Israel (31.9N, 34.8E about 80m AMSL), during dust storm event on 12-13 March 2017). The dust was transported from the Sahara Desert and North Africa. Size-segregated ambient dust particles were collected on cyclopore polycarbonate filters using a Micro-orifice Uniform deposit Impactor (MOUDI; MSP Corporation model 110-R, (Marple et al., 1991)), that operated at 30 L min^{-1} and for 24 hrs, similarly to Huffman et al. (2013) and Mason et al. (2015). MOUDI has eleven stages with cut points (D_{50}) of 0.056, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10, and 18 µm. The size distribution of the particles was obtained by

Optical Particle Counter (OPC; GRIMM Technologies model 1.109) in the range of 0.25-32 µm, and used for estimations of surface area immersed in the droplets (further details in Appendix C).

For heterogeneous freezing experiments, a quarter of each filter is placed with 300 µL DDW in 1.5 ml Eppendorf vial and particles were extracted by intensive dry sonication (Hielcher; model UP200St VialTweeter). In Figure 8, the spectra of the nucleation sites per unit surface area (n_s) of three super-micron stages (D_{50} of 1.0, 1.8, 3.2 µm) are presented and summarized in T1. It is also seen that there are slightly more active sites for the larger particles (3.2 µm), as their surface area is higher and there is a higher probability to contain an active site. In Figure 9, n_s curves of the collected dust is compared to references of K-feldspar standard particles, analyzed in different instruments (the Leeds-NIPI (Atkinson et al., 2013), LACIS (Niedermeier et al., 2015)) and to measurements of ambient dust samples, from different locations around the world, including Israeli settled dust, that was analyzed in the AIDA chamber. Moreover, the freezing of the size resolved mineral dust analyzed in this study by WISDOM (slope in the temperature range) is consistent with the (grey) polygon that represents the estimated freezing efficiency for natural concentrations of K-feldspar in internally mixed mineral types (Atkinson et al., 2013). The results are also in agreement with Niemand et al. (2012), especially between 243 and 249 K. At warmer temperatures, n_s of ambient dust in this study showed lower efficiency than in Niemand et al. (2012). This difference can extend to one order of magnitude in n_s, and is more pronounced at smaller particles that were analyzed (around 1-1.8µm diameter). For the larger particles, more nucleating sites are observed. Both the current study and Niemand et al. (2012), suggest that K-feldspar is involved with the warmer part of their data (>248 K) as the results are consistent with the Atkinson et al. (2013) scale for ambient samples. The slope of the n_s derived in this study is similar to the slope from Atkinson et al. (2013) at warmer temperatures. For the colder regime, the slope is similar to the slope presented by standard K-feldspar particles in Niedermeier et al., (2015).

3.6 WISDOM in comparison to other cold stage instruments

The microfluidics technology used in WISDOM solves some substantial issues inherent in other currently used instruments: (1) good control of the size and number of monodisperse droplets, (2) fast production of hundreds of nearly monodisperse droplets minimizes sample sedimentation or agglomeration that may occur in a suspension, leading to a good estimation of the surface area of the suspended material. Moreover, several droplet diameters can be employed in the same device without its modification, (3) good statistics achieved by individual analysis of hundreds of droplets, (4) monodisperse droplets individually analyzed, in contrast to some emulsion techniques (such as Differential Scanning Calorimeter (DSC)), allow to obtain the frozen fraction at each temperature, and to achieve detailed information about active sites and freezing rates, (5) the use of oil minimizes possible artefacts from droplets' evaporation, neighbor seeding or vapor transfer due to the Wegener–Bergeron–Findeisen processes, (6) the small volumes decrease freezing artefacts by impurities, thus allowing to reach the homogeneous freezing threshold (-37ºC), (7) possible investigation of several freezing cycles for the same droplets,

(8) the microfluidics method and the small droplet volumes enable working with small sample volumes which can be an advantage when working with atmospheric samples.

WISDOM has a very accurate temperature calibration that spans a wide temperature range, using the eutectic freezing method. WISDOM most resembles the instrument used by in Edd et al. (2009). However, it seems that issues with temperature calibration in Edd et al. (2009) led to a temperature offset, and hence different freezing rates. Stan et al. (2009) achieved better temperature accuracy and high statistics. However, the freezing experiment was conducted in a flow mode, which is more complicated than in the WISDOM setup and requires complicated modeling. In addition, the cooling rates that were used were very fast, which induces additional errors. Riechers et al. (2013) had high temperature accuracy as they also used a DSC. However, they had to collect the droplets from the device as there was no static array option and this may add further complication and contamination.

The microfluidics technology has also disadvantages. These may include: (1) oil may interact with some of the analyzed particles, possibly leading to biased data, (2) the microchannels are susceptible to clogging, (3) it is not possible to perform any post analysis to the droplets content after the experiment, (4) the small droplets' volumes reduce the sensitivity to rare active sites. This may be solved by performing many experiments or by using larger droplets with more surface area within the droplets.

4 Summary and conclusions

The new setup WISDOM is based on microfluidics technology and its detailed validation is presented. Based on a set of validation measurements and a good agreement with other instruments, we conclude that WISDOM is a suitable tool for studying atmospheric ice nucleation, both in homogeneous and heterogeneous immersion freezing modes. Results of homogeneous freezing correspond to water-activity-based nucleation theory in supercooled droplets and represent well volume nucleation rates. Heterogeneous freezing in supercooled droplets also agrees well with literature data. Furthermore, freezing efficiency dependence on the particles surface area within the droplets is clearly observed. Using microfluidics allows a mass production of picoliter monodisperse droplets using low volumes of suspensions, which can be beneficial for immersion freezing studies over a wide range of supercooling down to homogenous temperature region. The good reproducibility of the devices, proved using pure water freezing cycles, enables the recycling of the same device for few freezing cycles. It is also shown that the temperature uncertainty can be reduced if the temperature calibration includes the microfluidic devices properties in the working temperature change rates, especially for melting experiments. In this work we have also demonstrated how WISDOM can be applied for studying the ice nucleation properties of ambient samples that contain very small quantity of sample. The particles were collected using the MOUDI during Saharan dust storm event. Results are in correspondence with literature data of ambient dust and further support Atkinson et al. (2013) and the possible importance of K-feldspar for ice nucleation in clouds, but further analysis of the mineralogy is still needed in order to verify that.

The authors declare that they have no conflict of interest.

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Appendix A: Device inter variability over the whole freezing range

Figure A1 presents the reproducibility of the microfluidic devices. For each device, the temperature variation between different freezing cycles of pure water is presented for the entire range of the freezing at various frozen fractions (0.1 to 1). These experiments were conducted for three cooling rates, 0.1 K min^{-1}, 1 K min^{-1} and 10 K min^{-1}. For 1 K min^{-1} the device's variability was the smallest, and the deviation in the results between different cycles was < 0.2 K for most cases. While in some cycles the temperature was reproducible in <0.05 K, in other cycles the temperature varied in <0.2 K. This is not valid for frozen fractions <0.2, where the variability was the highest, as was the case for the other two cooling rates. This may be due to contaminants that exist in the water or in the devices themselves. The preparation of the devices is mostly inside a hood, but ambient particles may be trapped during the process. For cooling rate of 0.1 K min^{-1}, the variability between the different cycles was also <0.2 K, but the variability was higher in comparison to the variability seen at 1 K min^{-1}. This can be explained stochastically and also may be attributed to better resolution of temperature reading during slower cooling rates. For 10 K min^{-1} the variability was between 0.2K and 0.3K. The faster cooling rate may slow the equilibration of droplets' temperature with respect to the stage (as demonstrated in this work), and also low resolution of temperature reading due to the fast cooling rate. The variability presented here is also probably affected by the uncertainty of the temperature sensor of the Linkam stage (<0.25 K).

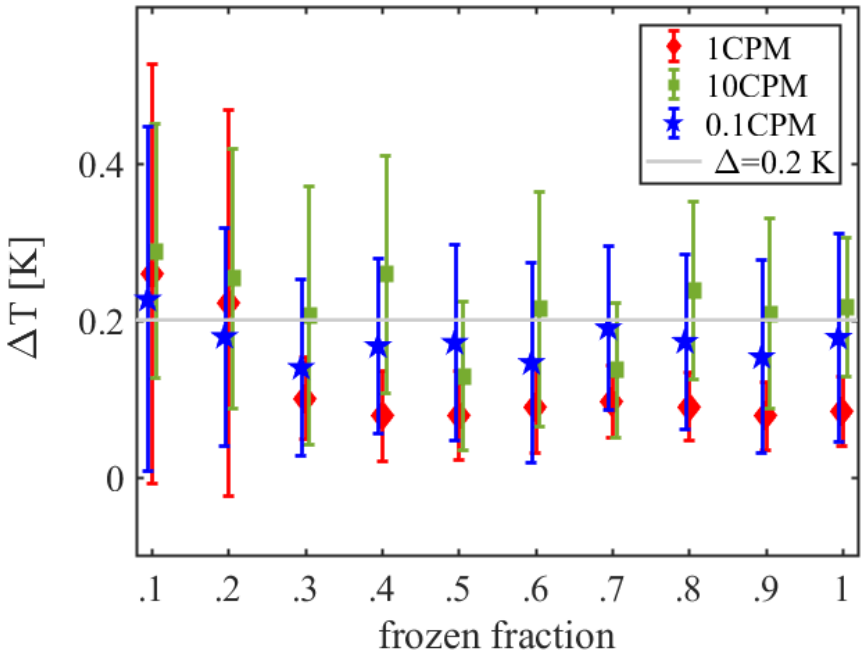

Figure A1. Variability of the WISDOM devices for three cooling rates. The markers present the average temperature variability for all the devices and the error bars represent one standard deviation. A line is placed at Δ=0.2 K, the upper value which explains the variability in the results of different freezing cycles at 0.1 and 1 K min^{-1}.

Appendix B: Suspension preparation and characterization

Illite-NX, ATD and K-feldspar powders were suspended in double distilled water and sonicated twice for 30 seconds with a 20 second pause, using Hielcher up200St ViralTweeter, adjusted especially for Eppendorf vials. K-feldspar suspensions were additionally stirred overnight as sonication alone was not enough to achieve a good suspension and intensive sedimentation was observed. For validation experiments, suspensions of 0.1 to 1 wt% were used. Figure B1 presents nucleation site densities for Illite-NX and K-feldspar particles, and the freezing efficiencies as function of the surface area in the droplets. Characterization of the powders can be found in Hiranuma et al. (2015), Marcolli et al. (2007) and in Atkinson et al. (2013) and quantification the powders specific surface area was based on N_2 adsorption analysis of Brunauer–Emmett–Teller (BET) (Brunauer et al., 1938) using Quantachrome Instruments Nova 2200e and resulted in 1.9±0.6 $m^2 g^{-1}$ for the K-feldspar powder, 108.6±2.8 $m^2 g^{-1}$ for the Illite-NX powder and 37.1±1.4 $m^2 g^{-1}$ for the ATD powder. In order to ensure a proper analysis of the surface area, and avoid possible surface contaminants as water, surface cleaning was done by degassing the powders at 60°C for 3 hours ahead of the BET analysis. Evaluation of the surface area in each droplet was then calculated by the wt% which was used, knowing the approximate surface area per mass and assuming that the mass is distributed uniformly inside the droplet with the same volume

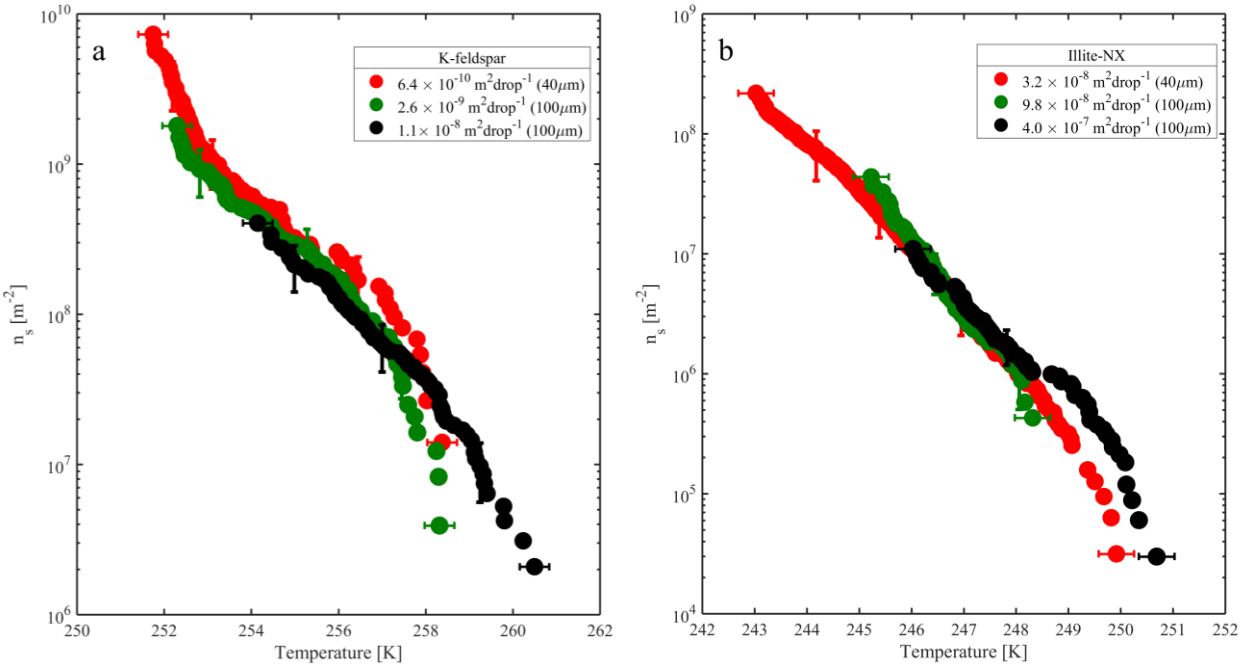

Figure B1. Accumulated active site density spectra (n_s) of K-feldspar (a) and Illite-NX (b) particles with different surface areas suspended in water at cooling rate of 1°C per minute. The error bars are located at three representative frozen fractions, 0.1, 0.5 and 0.9.

Appendix C: Collection of ambient particles during dust storm events in Rehovot

The GRIMM measurement was synchronized to the MOUDI stages for the estimation of the total surface area that was collected on the filter for droplets surface area estimation. For that, two base assumptions were made: (1) all the particles that were collected are extracted to the water later used for the freezing experiments, (2) sphericity of the particles. The GRIMM bins are synchronized to the MOUDI stages based on collection efficiency of the MOUDI, obtained from Marple et al. (1991). For example, on certain MOUDI stage, all the particles that own diameter that is larger than the D_{50} have high chance to be impacted on that stage. All the rest of the sizes, that are smaller in their diameter, will continue to the next stage and will have high chance to deposit there. Hence, the GRIMM's bins were synchronized to the MOUDI D_{50} stages. For the n_s calculations, the surface area was based on the number of particles that were measured in a certain bin and their total surface area. To calculate the surface area of a particle (assuming sphericity) in a certain bin, the midpoint of that bin was used as a radius. To calculate the total mass of the particles in each filter, dust density of Quartz was used (2.65 g cm^{-3}), as this is usually the dominant mineral (Mahowald et al., 2014). The error of the n_s data is propagated from the error in the frozen fraction, the error of the droplet's volume and the error of the MOUDI's collection efficiency in the different stages, the later was the dominant one.

For control, analysis of blank filter was done. The blanks were sonicated before analysing them and freezing was mostly colder than the freezing temperatures that are presented here and hence no special reduction of the final active sites was done.

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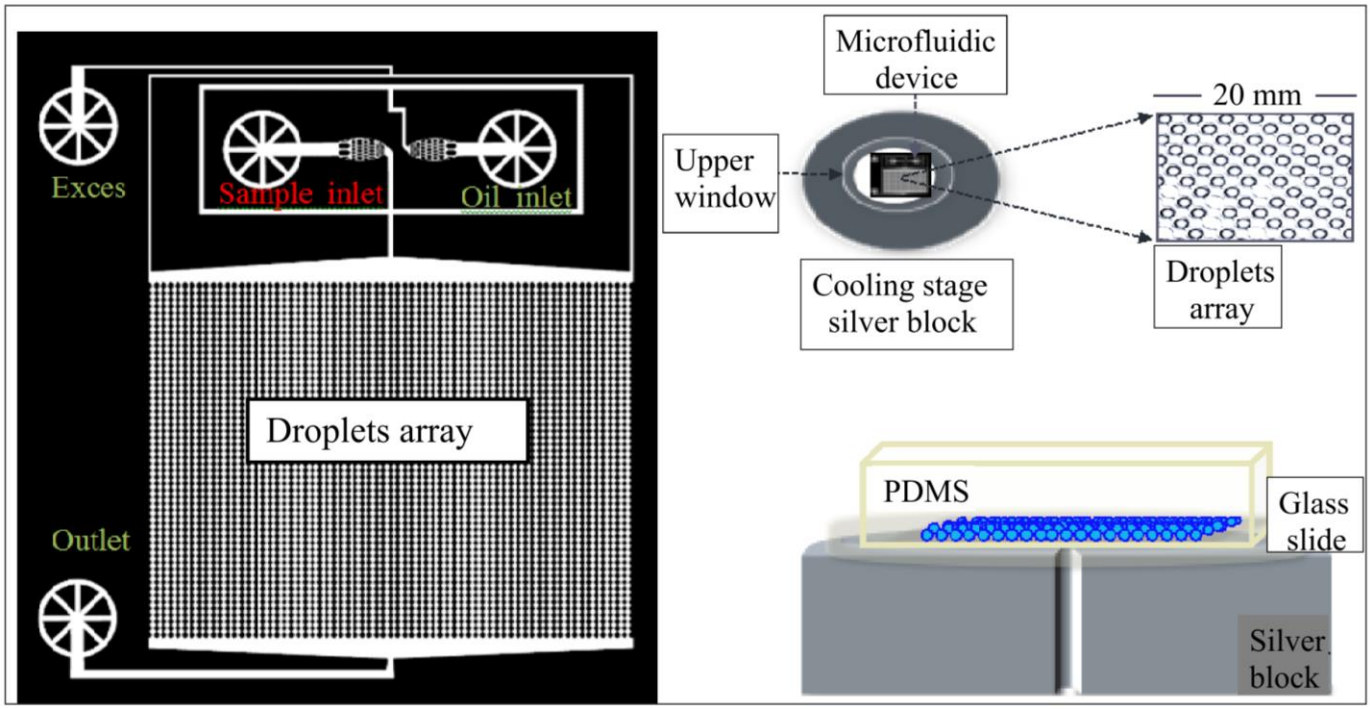

Figure 1: The WISDOM setup. a) The design of the microfluidic device is based on Schmitz et al. (2009). Aqueous solutions (including the sample) and oil are connected through the inlets and merge in a junction to generate monodisperse droplets. Subsequently, droplets flow into a trap array and settle in them as the flow is stopped. The device is transferred into a cooling stage for subsequent freezing experiments. b) upper and c) side views of the device, which is made of PDMS, plasma glued to a microscope glass slide, placed over the cooling silver block.

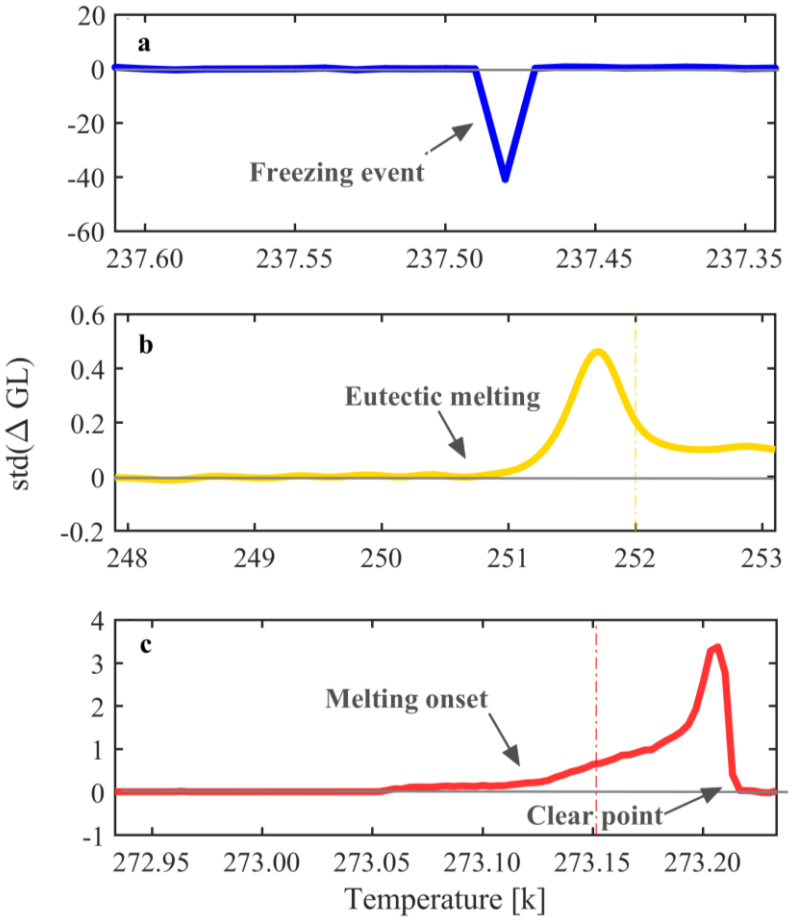

Figure 2: Spectra of different phase transition events as observed in WISDOM. a) freezing, b) eutectic melting, and c) melting onset and clear point (liquefaction) are the mean of all sampled droplets in a single experiment. The phase transition is defined optically by the brightness information obtained by the gray level of the image pixels. std(ΔGL) describes the standard error of the difference in mean GL for two consecutive frames. At the beginning of the experiment the noise level is studied and freezing, or melting are detected only if std(ΔGL) is as least 5 times greater than the noise std level. Freezing and melting examples are for pure water droplets and the eutectic melting example is for aqueous solution droplets of NaCl. Eutectic melting point of NaCl and pure water melting point are marked by the yellow and red lines in b and c, correspondingly. In all cases the droplets diameter was 100 μm.

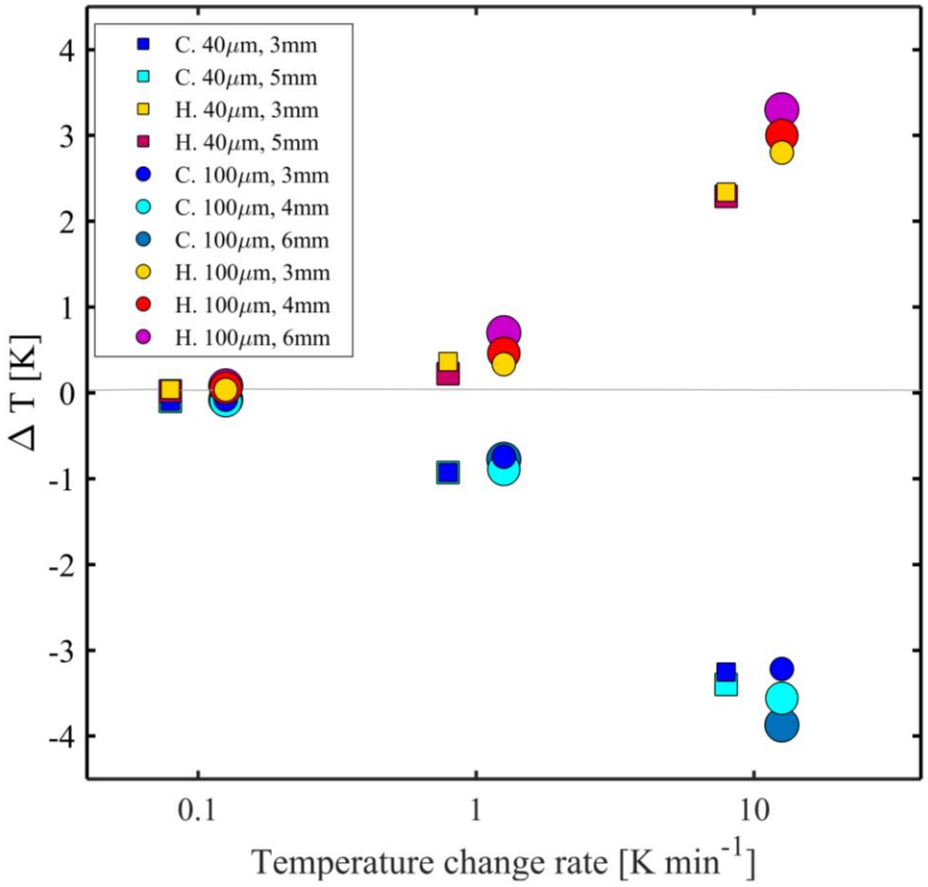

Figure 3. The temperature difference (ΔT), defined as the temperature difference between the stage temperature and the droplet extrapolated temperature at equilibrium conditions at different cooling (heating) rates. Freezing and melting points of pure water are represented by circles and squares (40 and 100 μm droplet diameter, in correspondence) for different PDMS thicknesses and are represented by different colors. C denotes cooling and H denotes heating. Droplets are close to equilibrium with the stage temperature at rates <0.1 K min^{-1} and ΔT increases with increasing temperature change rate and with the PDMS height.

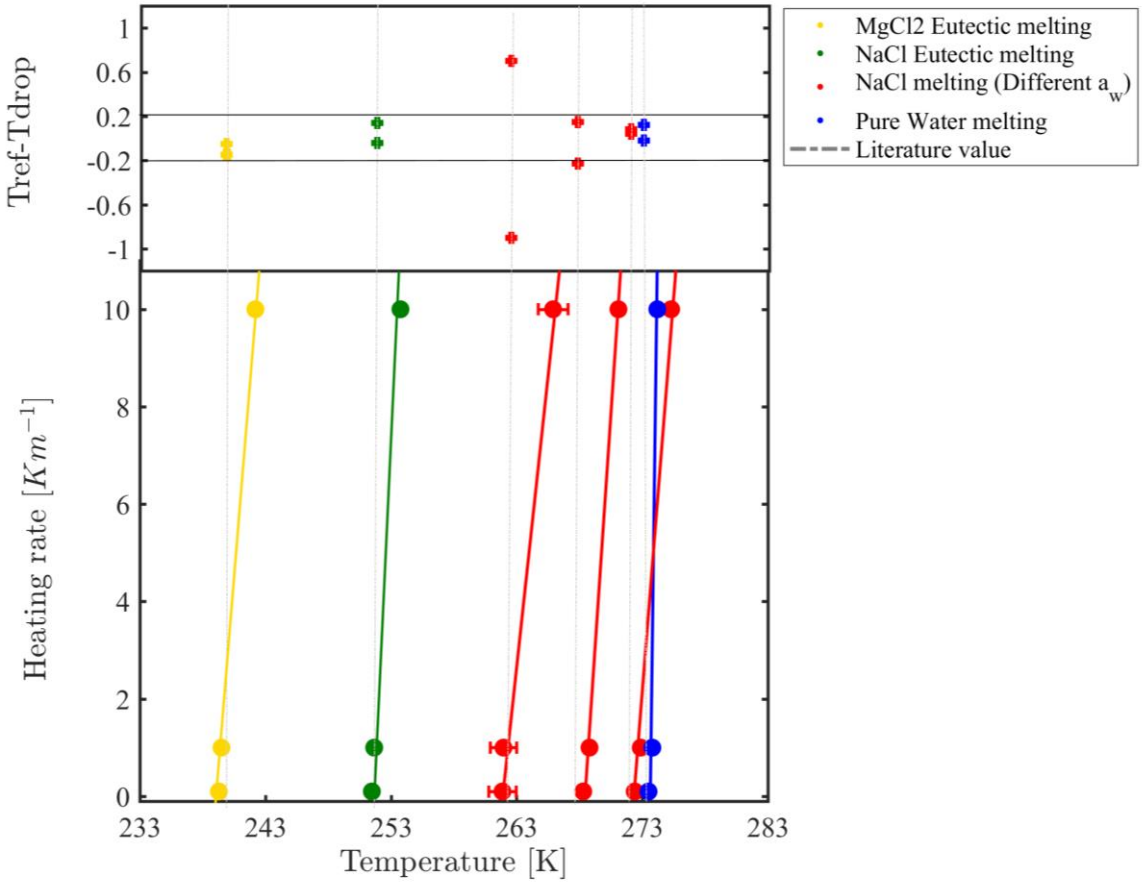

Figure 4. Temperature calibration by melting points of eutectic solutions and pure water droplets for different heating rates. Calibration is presented for 100 µm droplets with 4 mm PDMS thickness. The onsets of pure water droplets are also considered. Eutectic melting is used for the colder temperatures range (<253 K) while clear point (liquefaction) at various water activities is taken for the warmer temperature range. The upper panel presents the temperature difference between the reference value and the cooling stage temperature after calibration. Most of the differences are within the range ±0.2 K.

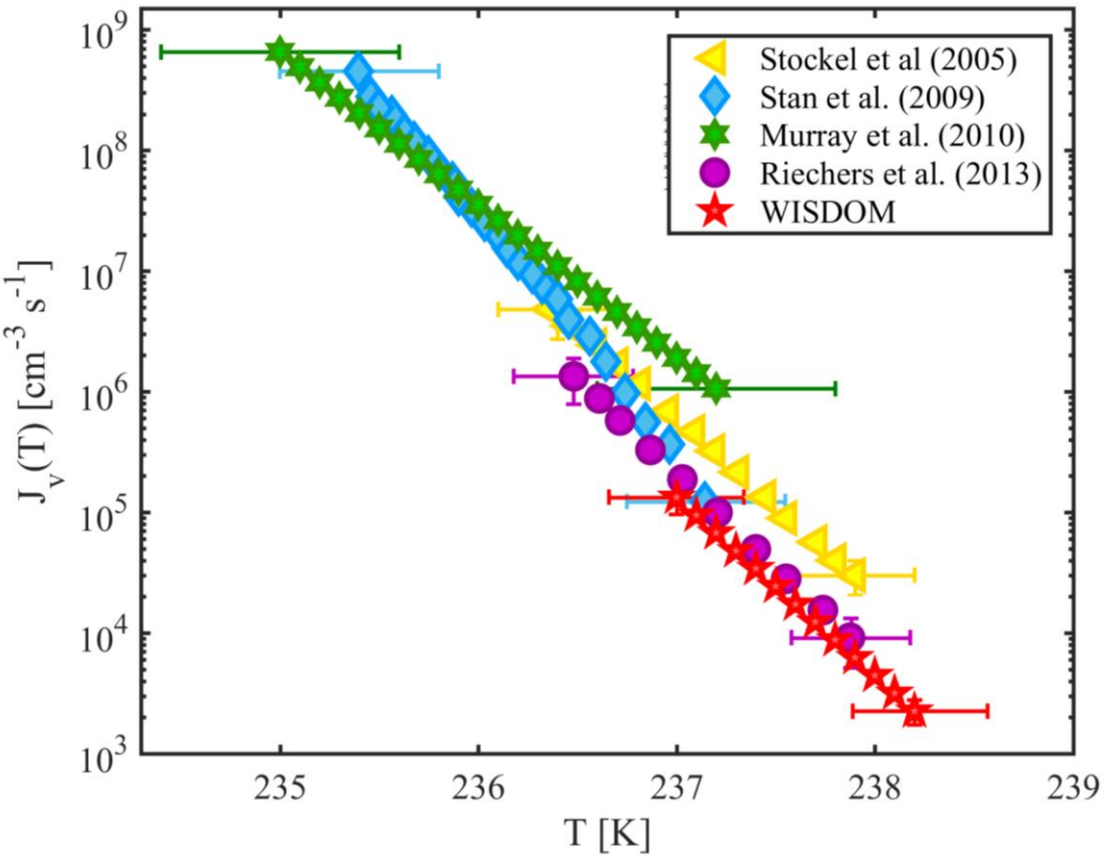

Figure 5. The volume-dependent homogeneous freezing of pure water, derived for 100 μm droplets with 4 mm PDMS height. WISDOM rates are compared to relevant literature data. The obtained fit from WISDOM is $J_{v(T)}$=exp(-3.4T+817.6). Temperature uncertainty for WISDOM is ±0.3 K.

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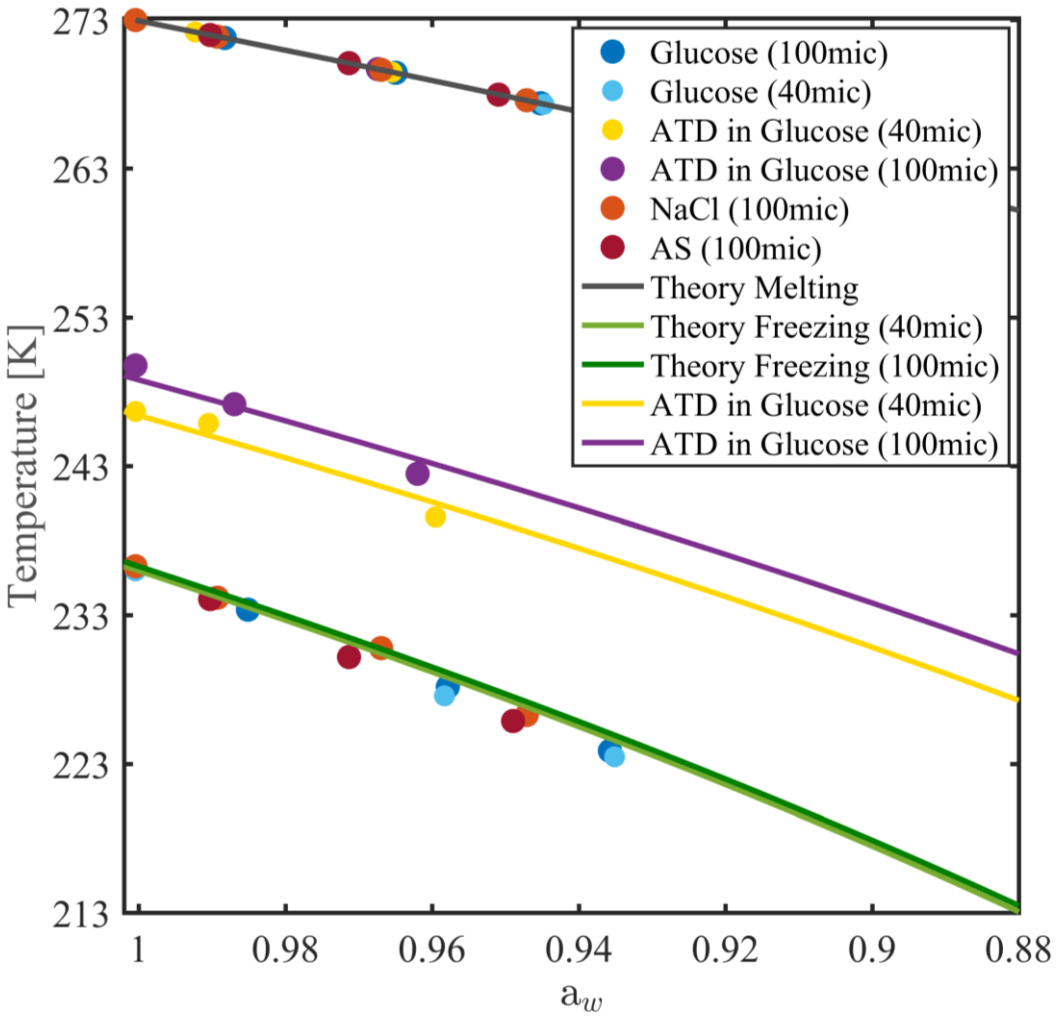

Figure 6. Homogeneous and heterogeneous ice nucleation temperatures for 40 and 100 μm aqueous solution droplets as a function of solution water activity. Freezing and melting curves are derived from Koop et al. (2000). Heterogeneous ice nucleation is performed with 0.1 wt % ATD particles immersed in the droplets.

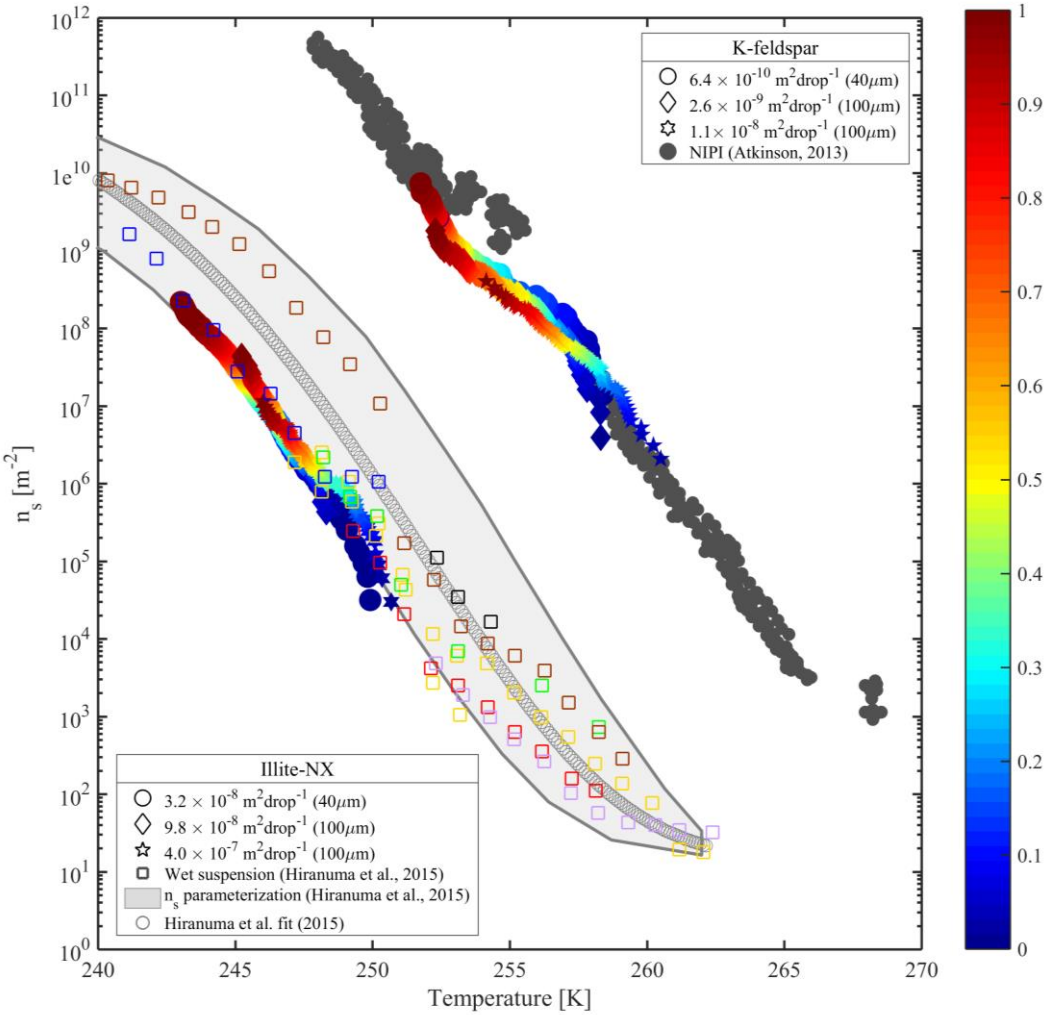

Figure 7. Accumulated active site density spectra (n_s) of K-feldspar and Illite-NX particles as a function of temperature from validation experiments of immersion freezing in WISDOM. Frozen fraction values are represented by a color bar, for few surface area values that are exposed in 40 and 100 μm droplets. The dependence of the nucleation site density on the surface area is illustrated here. WISDOM uncertainties, propagated from surface area estimation and measured frozen fraction errors, are included within the size of the markers. For validation, previous immersion freezing measurements are also presented (Hiranuma et al. (2015) and Atkinson et al. (2013)). T-binned data (1°C) normalized by the BET surface area from Hiranuma et al. (2015) is presented in the color squares, only for wet suspension analysis (Binary (red), CSU-IS (orange), Leeds-NIPI (purple), M-AL (green), M-WT (black), NC-State-CS (brown) and CU-RMCS (blue)). Hiranuma et. al. log fit and n_s (BET) parameterization are also presented.

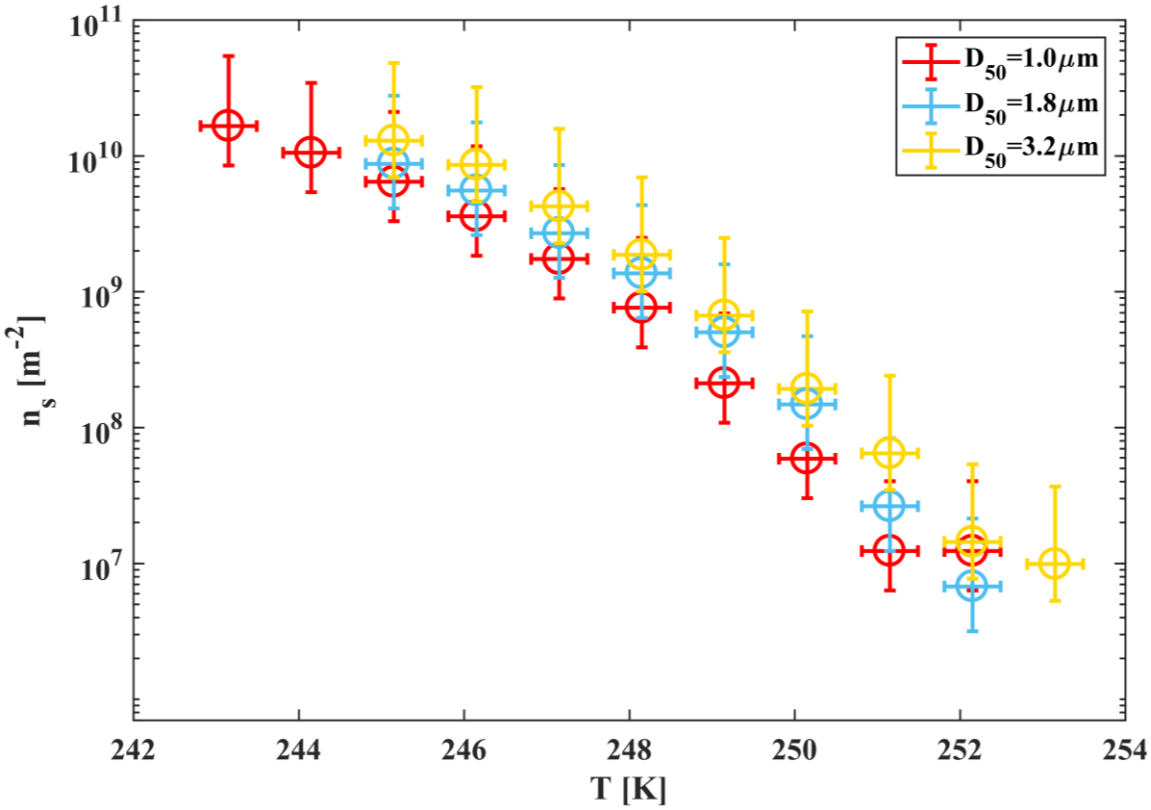

Figure 8. Accumulated active site density spectra (n_s) of ambient super-micron mineral dust particles collected in Israel during Saharan dust events in 2017, for three different sampling stages of the MOUDI; D_{50} of 1, 1.8 and 3.2 µm.

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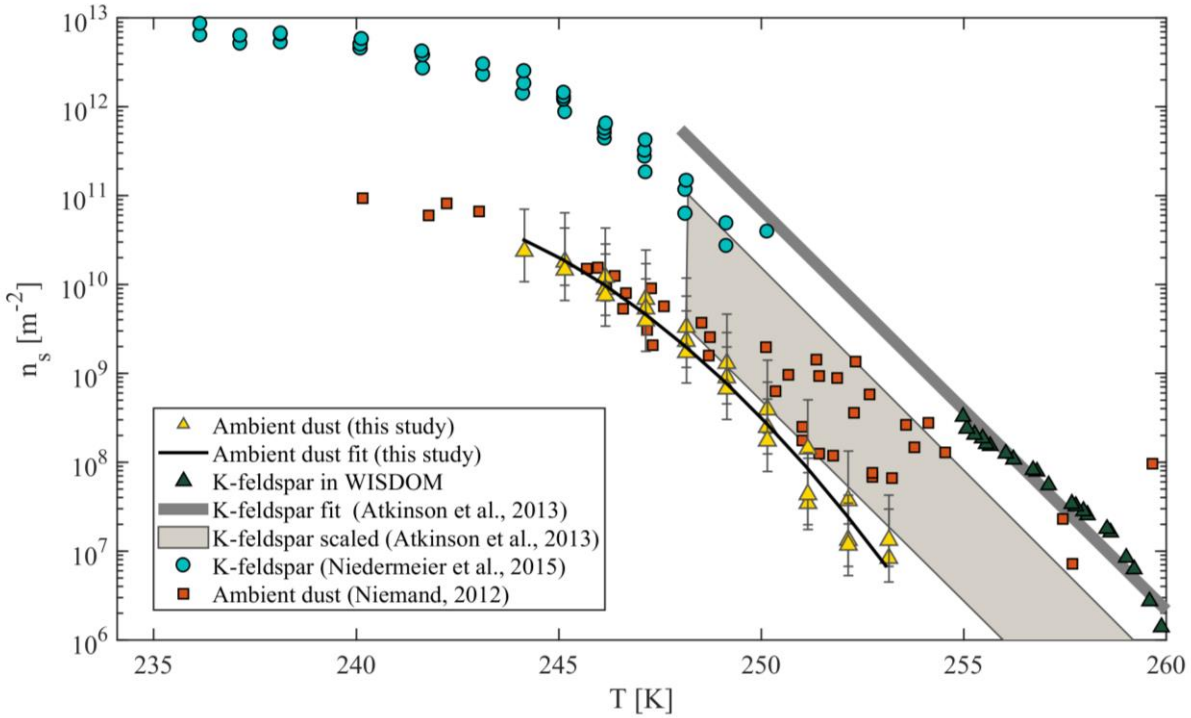

Figure 9. Accumulated active site density spectra (n_s) of ambient super-micron mineral dust particles collected in Israel during dust event in 2017 for three MOUDI stages that were analyzed with D_{50} of 1, 1.8 and 3.2 µm. The fit $\boldsymbol{ln(n_s) = -0.05T^2 + 24.68T - 2.93}$ (R^2=0.98) is also presented. References of K-feldspar standard particles activated in WISDOM, Leeds-NIPI (Atkinson et al., 2013) and LACIS (Niedermeier et al., 2015) instruments are presented, as well as ambient dust particles that were analyzed in AIDA and included Israeli dust (Niemand et al., 2012).

Table 1. Summary of immersion freezing experiments performed for WISDOM validation.

	Droplets diameter [µm]	SA [m^2 $drop^{-1}$]	T_{50} [K]	BET [m^2g^{-1}]
Illite-NX				
0.2wt%	95.1±3.6	3.2×10^{-10}	246.4	108.6±2.8
0.8wt%	96.1±2.9	9.8×10^{-08}	247.8	
1wt%	38.2±2.4	4.0×10^{-07}	245.4	
K-feldspar				
0.2wt%	99.6±2.8	6.4×10^{-10}	255.3	1.9±0.6
0.8wt%	98.2±2.6	2.6×10^{-09}	257.0	
1wt%	39.8±2.4	1.1×10^{-08}	253.3	
0.1 wt% ATD in glucose				
$a_{w=1}$	98.1±3.8	1.8×10^{-08}	250.0	37.1±1.4
$a_{w=0.987}$	101.2±2.9	2.0×10^{-08}	247.5	
$a_{w=0.962}$	99.1±4.6	1.9×10^{-08}	242.9	
$a_{w=1}$	38.3±3.2	1.1×10^{-09}	246.2	
$a_{w=0.991}$	39.9±3.3	1.2×10^{-09}	240.2	
$a_{w=0.959}$	37.3±2.8	1.0×10^{-09}	236.3	
DS 12-13/03/17				
D_{50} 1.0	89.9±4.3	3.6×10^{-10}	247.5	-
[µm] 1.8	95.6±9.6	6.0×10^{-10}	248.8	
3.2	89.4±10.8	2.6×10^{-10}	248.7	