

Anonymous Referee #2 RC3

The manuscript “Characterization of individual ice nuclei by the single droplet freezing method: a case study in the Asian dust outflow region” by Ayumi Iwata and Atsushi Matsuki describes an application of a droplet freezing apparatus to the characterization of the ice nucleating (IN) particles sampled during two Asian dust events. The authors report on the application of several microanalysis techniques (AFM, Micro-Raman, SEM, EDX) to reveal the chemical composition and morphology of the most IN active particle in a subset of a sample, identified by the droplet freezing technique. A combination of the single-particle microanalysis techniques with droplet freezing assay methods is a very promising development in the field of the ice nucleating particle research. An important advantage of the method described in the manuscript is the realization of a “single IN particle per water droplet” approach, which greatly reduces uncertainty associated with preparation of suspensions, characterization of particle size and surface distribution, and variability of particle properties across the droplet population, which are the common issues in a conventional cold stage experiment. However, before starting characterizing the most active ice nucleating particles with all the modern and expensive single particle techniques, one has to be completely sure that the particles identified as the most IN active are really such particles. To be honest, I am not convinced that this is the case. I would nevertheless support the publication of the manuscript provided that authors carefully address the multiple critical issues listed below. This might require a tedious re-evaluation of the results or even new experiments because some issues cannot be resolved without repeating the experiments.

*We appreciate all the positive comments and valuable feedbacks the reviewer provided us. We carefully examined and addressed the issues raised by the reviewer, which also involved thorough re-evaluation of the results and new experiments to validate our experimental method. We believe that the additional information will help improve the technical soundness of our paper. Please find our response to each of the comments below (in blue italic).*

1. I would like to draw the author’s attention to the both papers of Schrod at al., AMT 2016, and ACP 2017, who essentially describes a similar but much better characterized setup (FRIDGE) to study the deposition freezing of ice on the atmospheric IN particles collected with an unmanned aircraft over eastern Mediterranean. This group has gone a long way improving their instrument and achieving reliable results, you can learn a lot from them. These papers have to be mentioned and discussed.

*Thank you for reminding us of the works using FRIDGE. We have certainly learned a lot from FRIDGE. We added Ardon-Dryer and Levin, (2014), Schrod at al., (2016) and Schrod at al., (2017) as the references which also reported the measurements of the atmospheric ice nuclei using cold stage. Now*

they are discussed in the context of the limitations and the merits of IDFM, and cited in the result section as follows:

*'The advantage of IDFM is that we can keep track and be sure which of the collected single particle was actually nucleating ice. Thus the identified IN active particles by IDFM can be studied in detail by various particle analysis techniques. Comparing IDFM with the measurement methods of ice nuclei concentration number using FRIDGE (Ardon-Dryer and Levin, 2014; Schrod et al., 2016; Schrod et al., 2016), we have to sacrifice the accuracy, quantitative evaluation, and cooling rate of freezing temperatures and humidities based on the activated fraction since the evaporation of droplets around frozen particles by the Bergeron-Findeisen effect. By selected the higher cooling rate and -30 °C as the end cooling temperature, we also minimized the evaporation and the scavenging of the droplets around the rapidly growing ice crystals in the experiments of atmospheric particles. Then, most of atmospheric particle (excluding the very close droplets of ice crystals) were not completely dried the formed droplets until temperature reached -30 °C. Note that, however, the selected cooling rate is considerably faster when compared with the typical cooling rate in the convective cloud updraft.'*

2. The method description is incomplete. Many important details are missing, preventing the objective evaluation of method applicability. The only reference supposedly describing the setup (Akizawa et al., 2016), refers to an application of a Linkam stage for mineralogical samples. I wonder how is that related to the IN study discussed in the manuscript. Peckhaus et al., ACP 2016, also used a Linkam stage for a cold stage experiments.

*We added and changed the introduction and the method section as follows:*

*'Several laboratory studies used similar cold stage to test the ice nucleation activities of various atmospherically relevant standard particles (Fornea et al., 2009; Baustian et al., 2010; Mason et al., 2015; Whale et al., 2015; Knopf et al., 2014), but not enough studies have been made so far to investigate on the immersion-mode ice nucleation (mixed phase cloud) by the individual particles in the actual atmosphere.'*

*'The sample particles were deposited onto a Si wafer substrate with a hydrophobic coating (Glaco, Soft99 Corporation, Japan). Particles were observed for their position, size, and shape under an optical microscope with x50 magnification (Olympus, Japan) as shown in Fig. 1a. Subsequently, the substrate was transferred onto a cold stage in a closed cell (THMSG600, Linkam Scientific Instruments, UK). Since the cold stage used in this study is cooled by liquid nitrogen, the exposed tube through which the liquid nitrogen passes in the cold cell becomes a cold trap which can act as an additional sink for the water vapor. Therefore, in this study, all cooling parts except the cold stage surface were covered by insulating*

*material. The temperature measured at the cold stage was calibrated by the substances of known melting points (Akizawa et al., 2016). Furthermore, we confirmed that the temperature gap between the substrate and the cold stage was consistently smaller than 0.3 °C by observing the melting of water. During the ice nucleation experiment by atmospheric particles, the stage temperature and the dew point were recorded every 1 seconds. The temperature measurement and the images were synchronized with the PC internal clock.'*

3. The method described in the manuscript allows identifying the first particle within the field of view that initiated the freezing. This particle is then labeled the “ice nucleating” particle, and the others as “non-nucleating” particles. I wonder what happens with all other particles on the substrate? Do they initiate freezing too? Please explain this clearly in the manuscript.

*It is a very important point. We do not have uniform distribution of particles on the substrate, because impinging jet of the impactor concentrates particles closer to the nozzle center, and there is a hotspot where we see too many particles that are close to each other. We discard these areas with too many (right in the center) or too sparse particles (far from center), so we do not keep track on what happened to all other particles on the substrate outside the field of view. We do see cases with multiple particles freezing in the same field of view by monitoring the collected particles in a relatively wide area (Fig.1) and it is not necessarily only one particle that we are labeling as “ice nucleating”. We added this in the method section as follows:*

*‘By comparing the optical images before and after the ice nucleation experiments, the individual particles that formed ice crystals (excluding those coalescing with adjacent droplets or crystals) were identified and regarded as IN active particles. Most of the particles collected on the substrates were monitored under an optical microscope with x5 magnification (Fig. 1a). We did see multiple particles freezing in the same field of view. However, we did not cover all of the collected particles. Therefore we must note that the Non-active particles or IN active particles outside of our field of view are not included in our counts.’*

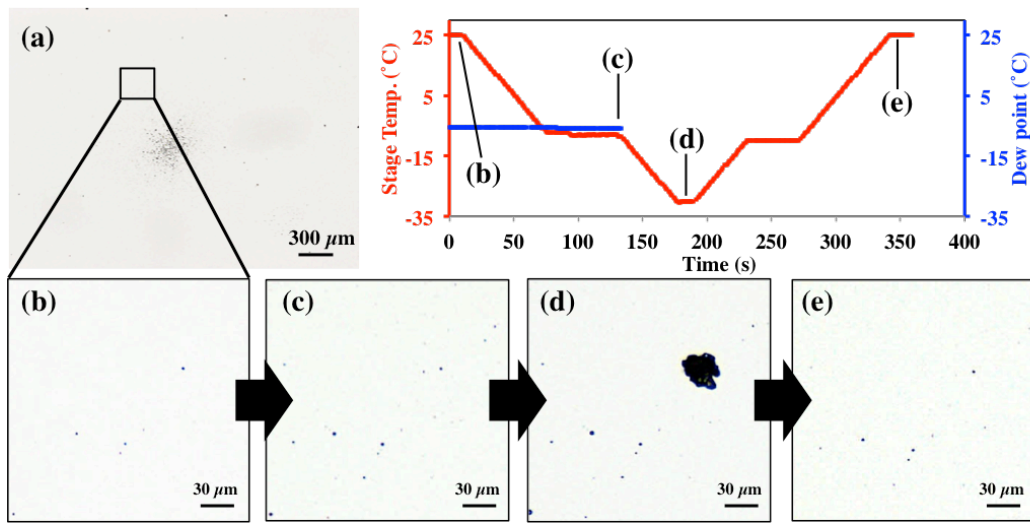


Figure 1: Optical images of sample particles deposited on Si wafer substrate before the freezing experiment (a, b), after exposure to water super saturation conditions at  $-9\text{ }^{\circ}\text{C}$  (c), after cooling to  $-30\text{ }^{\circ}\text{C}$  (d), and after sublimation and evaporation by dry air (e). The inset graph shows the stage temperature and the dew point of the wet air introduced into the cell before exposing the stage to water super saturation.

4. The setup description in the manuscript doesn't have it very clear, but if I understand correctly, the method has a very important limitation. In a closed cell with the humidified gas disconnected from the cell, the droplets are in equilibrium with the water vapor. The moment the first crystal appears, it grows very fast because the vapor is supersaturated with respect to the ice surface. Now that the system contains all three phases of water, droplets start evaporating and some of them would evaporate completely without having a chance to freeze. To prevent complete evaporation, you use the very fast cooling rate ( $30\text{ K/min}$ ), but this reduces the time remaining for the other droplets to freeze to less than 20 s (if the first droplet freeze at  $-30\text{ }^{\circ}\text{C}$  and the homogeneous freezing is over at  $-40\text{ }^{\circ}\text{C}$ ). Ice nucleation is a stochastic process described by a rate equation, so that the probability of freezing at given temperature is a function of time spent at this temperature. At such cooling rate, the freezing of "second-best" IN particles can be inhibited by this time-dependent issue and all the droplets will freeze homogeneously concealing the potential freezing activity of the IN particles. A chance of freezing is further reduced by droplets evaporation.

*It is a very important point. It is true that part of the reason we selected the higher cooling rate is to prevent the evaporation of the droplets. Another reason is to restrict the final size of the growing ice crystals so that they do not coagulate with, or dry many of the surrounding droplets. By selected the higher cooling rate and  $-30\text{ }^{\circ}\text{C}$  as the end cooling temperature, we also minimized the evaporation and*

*the scavenging of the droplets around the rapidly growing ice crystals in the experiments of atmospheric particles. Then, most of atmospheric particle (excluding the very close droplets of ice crystals) were not completely dried the formed droplets until temperature reached -30 °C. However, as the reviewer points out, we cannot fully rule out the possibility that droplets very close to an ice crystal may be fully evaporated. Therefore, it is possible that a moderately IN active particle may be miss-interpreted as non-active particles. We admit that the current method still has limitations and space for future improvements with respect to the quantification and accuracy of the IN counting. The fast cooling rate may also cause “second-best” IN to be overlooked in the current method. These limitations are now clearly explained in the section 3.1 (also as a response to the first comment). However, our primary objective here is to identify the properties of the individual ice nucleating particle in the actual atmosphere, by realizing a clear-cut identification of IN active particles. Therefore, we believe being able to identify even the most active fraction (with potential miss counting of the “second best” IN) from the actual atmosphere is still an important step forward towards our understanding of the IN behaviors of atmospheric aerosols, which are often found in complex mixtures (both internally and externally).*

5. In this way, only one ice nucleating particle can be identified per sample. The other, just very slightly less active ice nucleating particles would have no chance to initiate freezing and would be labeled as non-nucleating. This would result in a wrong statistics of the IN vs the non-IN particles, and as a consequence in a biased composition of non- IN active particles. If I am wrong, please show your results in form of “the number of frozen droplets as a function of the substrate temperature”. Such curves can be used to retrieve the so-called ice nucleating active site (INAS) densities, that can be better compared with the measurements of other groups.

*We have to admit that our technique is not a perfect method that can resolve all technical issues encountered in the ice nucleation experiments. The biggest advantage of course is that we can keep track and be sure which single particle was actually nucleating ice. In return, we cannot fully rule out the possibility that the “second-best” IN particles may had been overlooked due to the evaporation of droplets in the vicinity of the frozen particles. Therefore, accuracy and the quantitative evaluation of freezing temperatures based on the activated fraction and the INAS could be biased to some extent. The number of ice crystals formed are compromised especially at lower temperatures as the number and size of ice crystals increase and more droplets in the vicinity of the growing ice are subjected to evaporation. In this study, only a small number of particles collected in the atmosphere formed ice crystals in a limited temperature range between -26 °C and - 30 °C. so it is difficult to present the “the number of frozen droplets as a function of the substrate temperature”. Instead, we show INAS and activated fraction of*

*standard samples, and the validity of this experimental method is discussed by comparing these result with previous studies.*

*When comparing our results with those found in literature, we must also note that strict comparison is difficult because of the uncertainties and differences related to the size distribution and composition of dust particles used in different experiments. Assumptions made to calculate the surface area can also be a source of bias. The K-feldspar used in this study as a reference mineral dust was purchased from the same supplier, but the lot number is different (from different source rock) from the K-feldspar used in Atkinson et al (2013). Therefore, composition of K-feldspar used in the current study and that used in Atkinson et al. (2013) are not exactly the same. Nevertheless, we added the ice nucleation active sites (INAS) of the 4 standard mineral particles measured by the current IDFM (Fig. S-2).*

*The ice nucleation active sites (INAS) obtained in this study agreed within an order of magnitude difference compared to those found in the literature (despite many different experimental conditions). Further, we would like to emphasize that the relative order of the onset temperatures found for different particles is always consistent (i.e. K-feldspar > Na-feldspar > quartz > kaolinite >> pure water) and even the range of the freezing temperatures are not far off from the reported values.*

*It was probably not clearly stated in the manuscript, so the following change was made with regard to the onset temperatures:*

*‘Heterogeneous ice nucleation observed in all standard mineral samples tested in this study (K-feldspar, Na-feldspar, quartz, kaolinite) consistently occurred at higher temperatures than the homogeneous freezing temperature. To ensure large enough observation area for the IDFM, these reference mineral samples were milled from rock mass or coarse powder state, then were collected on three set of Si wafers by impactor in the clean booth. The total number of the particles monitored during the ice nucleation experiment by IDFM was 4,509, 2,271, 4,759, and 1,435 particles, respectively. In this study, the freezing onset temperature of the sample was defined as the temperature at which the ice IN active fraction of the total observed particles reached 0.01. As a result, the freezing onset temperatures for K-feldspar, Na-feldspar, quartz, and kaolinite ranged between -22.2 to -24.2 °C, -24.7 to -25.7°C, -24.8 to -26.8 °C and -27.2 to -29.2 °C, respectively (Fig. S-1). Therefore, the ice nucleation activity of K-feldspar was the highest and that of kaolinite was the lowest. The order and the range of observed onset temperatures for these minerals were consistent with the results found in literature (Atkinson et al., 2013; Marry et al., 2011).’*

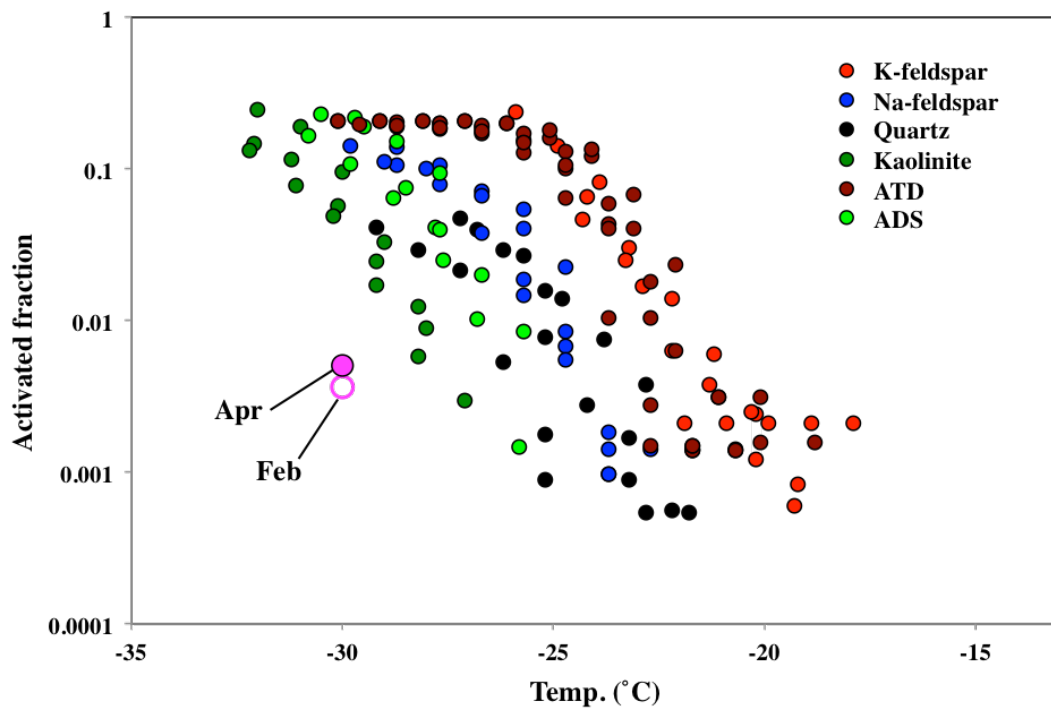


Figure S-1: Activated fractions of the reference mineral dust particles. Results of the atmospheric samples collected in February and April are also shown for comparison.

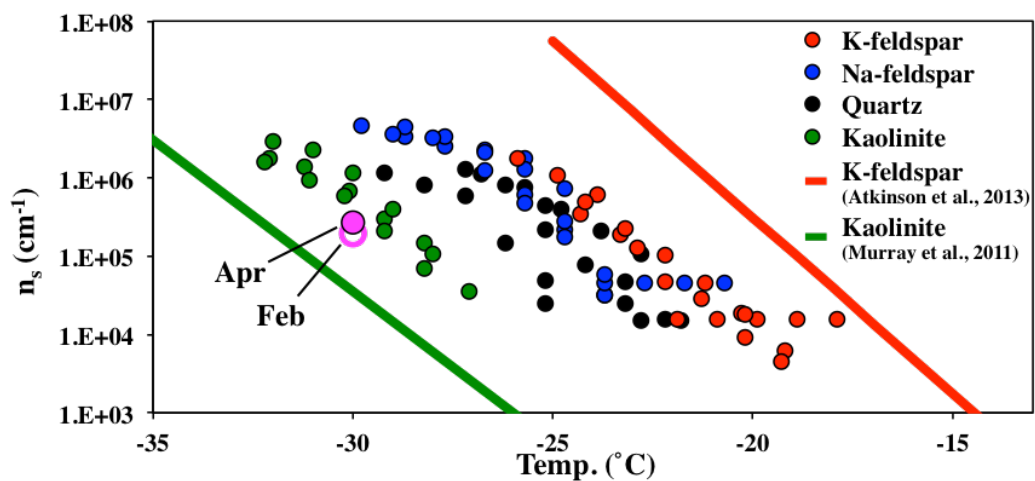


Figure S-2: the ice nucleation active site (INAS) densities for the reference single component mineral dust samples. These INAS densities were calculated from the activated fractions (Fig. S-1) and the averaged sphere equivalent surface areas obtained from the 2D silhouette of each particles in the microscopic image.

6. The critical issue discussed in the above comments can be also a consequence of the Linkam stage design. The standard cell of a Linkam stage has the metal coolant tubing exposed to the environment, making this tubing the coldest spot inside the cell. Water condensed on the tubing during the condensation step must freeze first during the cooling. As explained above, such ice surface would immediately become a strong sink for the water vapor. If the cooling rate is too slow, the liquid droplets condensed on the residual particles would evaporate before having a chance to freeze. I suspect that this is the reason for using a cooling rate of 30 K/min. If this is the case, it is a serious drawback of the system and has to be clearly stated in the manuscript. For the future work, I would recommend enclosing the substrate with the collected particles into a separate cell, so that just this area of the substrate is exposed to the supersaturated vapor.

*We are aware of this issue of the Linkam stage design. Before the experiment, we made a slight modification in the Linkam stage such that the metal coolant tube is covered by the insulating material and made sure that the condensation on this tube does not act as a strong sink for the water vapor. This is better explained in the revised manuscript as follows:*

*'Since the cold stage used in this study is cooled by liquid nitrogen, the tube through which the liquid nitrogen passes in the cold cell becomes a cold trap which can act as an additional sink for the water vapor. Therefore, in this study, all cooling parts except the cold stage surface were covered by insulating material.'*

7. At the cooling rate this high, a strong temperature gradient across the substrate can arise. The freezing temperature can be biased towards low values. Was temperature measured on the surface of a substrate or taken from the Linkam stage internal measurement? How was the temperature of the freezing onset determined? Have you been recording the microscope images? If yes, what was the image acquisition rate? Was it synchronized with temperature measurements? Since this is the first time you report the measurements with the new setup, you should convince the reader that the setup is well characterized.

*The temperature measured on the surface of a substrate and the Linkam stage internal measurement is calibrated based on the melting experiment of some materials as described in Akizawa et al., 2016. The temperature difference between the substrate surface and the stage, as well as the temperature gradient was determined to be less than 0.3 °C based on the observation of melting of pure ice.*

*The microscopic images were recorded with the maximum rate of (5 fps). During the ice nucleation experiment by atmospheric particles, the stage temperature and the dew point were recorded every 1 seconds. The temperature measurement and the images were synchronized with the PC internal clock.*



*This is better explained in the revised manuscript as follows*

*'The sample particles were deposited onto a Si wafer substrate with a hydrophobic coating (Glaco, Soft99 Corporation, Japan). Particles were observed for their position, size, and shape under an optical microscope with x50 magnification (Olympus, Japan) as shown in Fig. 1a. Subsequently, the substrate was transferred onto a cold stage in a closed cell (THMSG600, Linkam Scientific Instruments, UK). Since the cold stage used in this study is cooled by liquid nitrogen, the exposed tube through which the liquid nitrogen passes in the cold cell becomes a cold trap which can act as an additional sink for the water vapor. Therefore, in this study, all cooling parts except the cold stage surface were covered by insulating material. The temperature measured at the cold stage was calibrated by the substances of known melting points (Akizawa et al., 2016). Furthermore, we confirmed that the temperature gap between the substrate and the cold stage was consistently smaller than 0.3 °C by observing the melting of pure ice. During the ice nucleation experiment by atmospheric particles, the stage temperature and the dew point were recorded every 1 seconds. The temperature measurement and the images were synchronized with the PC internal clock.'*

8. What is the relationship between the field of view of the microscope and the sampling area containing droplets condensed on the aerosol particles? What if the first ice crystals are located outside of the view area? In this case, the initial ice crystals would not be detected but the local vapor pressure would be reduced due to the vapor deposition on the growing crystals, leading to evaporation of droplets and inhibition of freezing. Please give a detailed assessment of this effect. Given the uncertainty of the method of identifying the most active IN particles, I don't see the point of discussing the single particle microanalysis. If possible, give the maximum detail of the cold stage operation and detection techniques. If the measurements data permit, present your data not just in form of on-set freezing temperature, but in form of temperature dependent freezing curves. Otherwise, the measurements have to be repeated with a lower or variable cooling rate, and the results analyzed as discussed above.

*Monitoring of ice crystal formation by the optical microscope covers most of the sampled atmospheric particles (please refer to our response corresponding to the 3rd comment) By selecting the higher cooling rate, we minimized the evaporation and the scavenging of the droplets around the rapidly growing ice crystals. Then, most of atmospheric particle (excluding the very close droplets of ice crystals) were not completely dried the formed droplets until temperature reached -30 °C in this study. Therefore we believe that suppression of ice crystal formation by local vapor pressure reduction has been minimized at least down to -30 °C. However, as shown in temperature dependent freezing curve for the standard dust samples in Fig. S-1, the quantification of the activated fraction especially at lower temperatures is*

*compromised as a result of the drying of droplets by growing crystals. In return, this method enables direct identification of individual particles with high IN activity within the ambient samples.*

*We also added the following explanations of the both limitation for detection of particle and quantitative for ice nucleation in IDFM in the section 3.1 as follows:*

*“The advantage of IDFM is that we can keep track and be sure which of the collected single particle was actually nucleating ice. Thus the identified IN active particles by IDFM can be studied in detail by various particle analysis techniques. Comparing IDFM with the measurement methods of ice nuclei concentration number using FRIDGE (Ardon-Dryer and Levin, 2014; Schrod et al., 2016; Schrod et al., 2016), we have to sacrifice the accuracy, quantitative evaluation, and cooling rate of freezing temperatures and humidities based on the activated fraction since the evaporation of droplets around frozen particles by the Bergeron-Findeisen effect. By selected the higher cooling rate and -30 °C as the end cooling temperature, we also minimized the evaporation and the scavenging of the droplets around the rapidly growing ice crystals in the experiments of atmospheric particles. Then, most of atmospheric particle (excluding the very close droplets of ice crystals) were not completely dried the formed droplets until temperature reached -30 °C. Note that, however, the selected cooling rate is considerably faster when compared with the typical cooling rate in the convective cloud updraft. Also, we cannot fully rule out the possibility that droplets very close to an ice crystal may be fully evaporated.*

*With respect to the particle size detection/limit, further, the impactor already size segregate particles and limit the test particles in the super-micron range. The diameter of the collected atmospheric particles whose ice crystal formation could be monitored was ranged between 1.16 and 5.47 μm in the identification by the optical microscope. Meanwhile, the laser spot size (i.e. spatial resolution) of micro Raman spectroscopy approaches the diffraction limit of approximately 1 μm in diameter. All in all, the size of IN active particles that can be analyzed by this method is limited to super micron particles.’*

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