Response to Reviewer #2

In this manuscript Polen et al. describe a study of non-homogeneous ice nucleation in 'pure' water samples and a variety of substrates. They then go on to make a sequence of suggestions for groups using droplet freezing assays. This is a highly valuable manuscript and it will help both newcomers to the field and established groups improve their droplet freezing techniques. I agree with the authors statement that individual groups have a great deal of knowledge on this subject, which isn't necessarily made publically available. The comments I have made below are made in this spirit. I support the manuscript's publication and list a few comments which I hope the authors will use to further strengthen their manuscript:

We thank the referee Ben Murray for his positive response to our manuscript and for his detailed comments that we have used to further improve the quality and clarity of our manuscript. Below we respond to each comment in turn and describe any revisions made accordingly.

Handling blank experiments vs. 'pure' water control experiments. In the list of recommendations, I encourage the authors to include a recommendation that experimentalists conduct full handling blank experiments in addition to 'pure' water control experiments. By handling blank experiment I mean putting the water used in the experiment through the full process that the water containing a sample had been through. In Vergara-Temprado et al. (1) we demonstrated that this was critical. The 'pure' water control experiments were lower than the handling blanks (see Figure 2). The experiments where droplets were loaded with black carbon froze at a similar temperature to the handling blanks, but were above the pure water control. We consequently reported limiting values. In Fig 12, is the control experiment a handling blank?

This is a very useful and important point. We agree that the use of handling blanks (also referred to as a "method blank" or "field blank") is important and have added this to our list of recommendations under number 7. We have also added a brief discussion of the importance of method blanks as recently reported by Vergara-Temprado et al. In Figure 12 we are using a control, not a handling or method blank, but method blanks will be tested and reported for future experiments of this nature.

Abstract: The word 'plagued' is perhaps a bit strong. It is a limitation.

We agree, the wording has been changed to: "These droplet freezing experiments are often limited by contamination"

The authors refer to homogeneous nucleation at -38 C and the 'homogeneous nucleation limit'. This creates the impression that homogeneous nucleation has a well-defined limit where it occurs. It is volume and time dependent and this matters. For example, a 1 um droplet cooled at 1 K min-1 will have a freezing probability of 0.5 at -33.5C (according to Koop and Murray (2)). Furthermore Herbert et al.(3) found that enough cloud droplets started to freeze in a cloud to start

to affect cloud properties at around -33 C or so (depending on the homogeneous parameterisation), even though 50% of droplets would only freeze homogeneously at around -38 C. I would like to see the introductory sentences adjusted to be less definitive about the when homogeneous nucleation becomes important. Also, at ln 51, define the 'homogeneous nucleation limit' as, for example, the T at which 50% of 10 um droplets are expected to freeze on cooling at 1 K min-1.

We agree and appreciate the suggestion. We have discussed the homogeneous freezing temperature in an artificially simplistic binary manner. We have changed the term "limit" to "regime" and cite Koop and Murray (2016) for a detailed discussion of the homogeneous freezing rate and thus freezing probability versus temperature and the relevant equations. This seems more appropriate for the purpose of our manuscript. This has been changed throughout the manuscript, such as in the Introduction as follows:

"Water contamination or substrate interferences can also induce freezing well above the homogeneous freezing temperature regime that ensues in the temperature range of -35 to -40 °C (Koop and Murray, 2016), restricting the heterogeneous temperature regime accessible by DFTs."

Ln 34-35. Vergara-Temprado et al. (4) could be cited here, this paper clearly shows a sensitivity of clouds to INP.

This citation has been added.

Ln78. Replace 'steals' is not the best choice of words.

Phrasing has been changed to: "The WBF process occurs when one droplet freezes and takes up water vapor at the expense of unfrozen droplets…"

Ln93-97: Tarn et al. (5) also used microfluidic technology to study heterogeneous freezing. One of the objectives was to see if the oil and surfactant influenced nucleation. They measured ice nucleation (ns) for a range of materials and compared to literature. The results suggest that these technologies can be used to make droplets and study heterogeneous nucleation.

Thank you for suggesting this very recent relevant publication. This citation has been added and we briefly discuss their relevant findings regarding microfluidic droplet generation for ice nucleation measurements as follows:

"Some recent microfluidic ice nucleation techniques use fluorinated oils and/or large concentrations of surfactant to stabilize the emulsified droplets (Reicher et al., 2018; Stan et al., 2009; Tarn et al., 2018). Their measured homogeneous freezing temperatures are typically within the expected range (-35 to -37 °C), but the surfactant may have

unrecognized influences on heterogeneous freezing processes since freezing is enhanced via contact between the immersed particle and droplet interface (Durant and Shaw, 2005; Futuka, 1975; Gurganus et al., 2014; Tabazadeh et al., 2002)**. However, Tarn et al. (2018) concluded that surfactants seemed to have little effect on the observed heterogeneous freezing temperatures for a number of particle types examined using droplets prepared by microfluidics with added surfactant."**

Ln 214. 'correct for', replace with 'account for'. This isn't a correction.

Phrasing has been changed.

Fig 2 and discussion. These images are similar to those shown in Fig 4 or Whale et al. (6), mention that the new findings are consistent with what was previously found. Also, note that it is relatively easy to see if this is a problem.
Also, just for this discussion: we have found that this becomes a more significant problem when doing experiments in a humid environment. We solved this problem by improving the design of our ul-NIPI chamber to make it more air tight.

Yes we also see that this issue is exacerbated in the summer months when humidity is much higher. We have discussed this in the manuscript so that other groups are aware of this issue and can design their systems to reduce it, as mentioned above. The citation has been added and we discuss the similarity of their results as follows:

"Frost growth similar to this has been reported previously by Whale et al. (Whale et al., 2015) **in their cold stage system. This suggests that our system is not air tight enough to perform this type of experiment when ambient humidity levels are elevated such as during summer."**

'Particle sedimentation out of the droplet'. I don't think this is very likely. They may sediment to the bottom of a droplet, but are unlikely to sediment across an interface. Emersic et al. provide no evidence that particles can sediment out of a droplet.

This statement has been changed as not to suggest that particles are likely to cross the surface layer of the droplet. Until direct measurements of this phenomenon are reported we feel that we cannot be certain that no sedimentation out of the water droplet occurs, especially since there is a layer of oil beneath the water droplets in our microfluidic device. We have changed this as follows:

"…and any time-dependent effects such as particle sedimentation or aggregation."

P 9-10. We have also compared HPLC water and compared to water from MiliQ systems. We have found that the quality of HPLC water is also variable. I would recommend that whatever the source of water, the experimenter should demonstrate its quality and do the experiments to test the quality at sufficient intervals. I also agree that water from MiliQ machines can be highly variable, but in our experiments if the machine is well-maintained then the quality is systematically high, although we too have had periods when the quality was much lower.

This is an important perspective and a major reason why we believe more open discussion should be had within the ice nucleation community on the sources of water and effective strategies to reduce water contamination and related effects. We have added this insight to point 4 in the list as follows:

"Interestingly, we have also heard that other research groups found bottled water is not as consistent as their MilliQ-produced water. This demonstrates the inconsistencies and variabilities that are common between research groups and suppliers, further emphasizing the importance of routinely assessing and reporting the water background freezing spectrum that each group and method observes. We suggest that no matter what source of water is used that researchers regularly test it and report their findings in all publications when possible."

Fraction frozen plots throughout. I think it would be valuable to show the theoretical homogeneous nucleation curve. I would suggest using Koop and Murray (2) since in this paper the authors attempted to constrain classical theory in a physically plausible way, which gives more confidence in the values at higher temperatures (relevant for ul sized droplets) where there is very little or no data.

We have added homogeneous nucleation curves to each of the droplet freezing spectra for the relevant droplet volumes using equations A9a and A9b from (Koop and Murray, 2016) **to calculate $J_{homogeneous}$ and a freezing probability derived similarly to that calculated using Equation 9 from Beydoun et al. (2016):**

$$P_f = 1 - exp(-\left(\frac{V}{\dot{T}}\right) * \int_{T_0}^{T_{hom}} JdT$$

where $\dot{T}$ is the cooling rate, V is the droplet volume, and T_{hom} and T_0 are the bounds that encompass the full freezing probability used here of 250 K to 220 K.

We describe this in Section 3:

"We include the theoretical homogeneous freezing spectrum for our droplet sizes in all our droplet freezing temperature spectra below. This was produced using the parameterization of Koop and Murray (2016) to calculate the freezing rate, $J(T)$, and Eqn. 9 from Beydoun et al. (2016) to determine the frozen fraction, P_f, using $J(T)$."

Substrate dependent nucleation: Mention the result of Price et al. (7) (Figure 4) where it was shown that a Teflon substrate produced lower freezing temperatures when compared to a salinized glass surface.

This is nice additional information that further supports our arguments regarding the importance of substrate effects on initiating freezing at artificially warmer temperatures. We have added a brief discussion of this paper's results as follows:

"Price et al. (Price et al., 2018) **reported observing lower freezing temperatures when droplets were placed on a Teflon substrate compared to on a standard silanized hydrophobic glass surface. This provides further support for the important role that substrate choice can have on the freezing temperature spectrum observed in droplet freezing techniques."**

Ln 713: On the topic of K-feldspar being sensitive to water. We explored this in Harrison et al. (8) and showed that BCS 376 (a standard feldspar which is available for anyone to buy) only degraded by _1 C over 16 months in water. Other feldspars, in particular those exhibiting hyperactivity, are more sensitive to time in water.

Thank you for the suggestion. We have added the citation and brief discussion of these findings, as follows:

"The very ice-active K-feldspar minerals are especially subject to degradation in water due to surface ion etching, particularly for those displaying hyperactive ice-activity (Banfield and Eggleton, 1990; Holdren and Berner, 1979; Kumar et al., 2018; Peckhaus et al., 2016). Harrison et al. (Harrison et al., 2016) **found that a particular and common type of feldspar that does not display hyperactivity, BCS 376, was able to maintain its IN activity over many months in water. Engineered nanoparticles from inert metal oxides with reproducible particles sizes, surface properties, and pore sizes may be the most reliable type of INP standard, though this has not yet been evaluated and may be restricted to a narrow freezing temperature range (Alstadt et al., 2017; Archuleta et al., 2005; Findenegg et al., 2008; Marcolli et al., 2016)."**

Also, I think Vali's comment on this paper is really valuable, and it would be useful to record this approach in the literature (either in Polen et al. or Vali could consider a short note in AMT setting this out formally?).

We absolutely agree that Gabor Vali's comment provides important insight and should be shared more widely with the ice nucleation community. We have added a discussion of his suggestions regarding correcting the c_INP(T) or K(T) spectrum using 'pure' water control data through an iterative (k(T)) versus cumulative (K(T)) approach, and cited his comment on our manuscript (doi: 10.5194/amt-2018-134-SC1). We have also had extensive discussions with Gabor Vali regarding the k(T) approach. Through this he realized there

are some important but not obvious details involved in properly using k(T), such as the width of the temperature step ΔT used. He plans to write a tutorial on this, using some of our droplet freezing data to illustrate the correct application of the analysis. We will also demonstrate the use of k(T) in a forthcoming paper that describes the design and evaluates the performance of our new microfluidic droplet freezing approach.

The following discussion was added to the manuscript:

"Alternatively, retrieval of the differential nucleus concentration, referred to as k(θ) in Vali (1971), is also recommended to assess the INP concentration in the sample versus that caused by background freezing. This approach can be used as a means of quantitatively attributing the INP signal to the sample versus the background for each droplet over the entire freezing spectrum. The differential nucleus concentration can be calculated using:

$$k(T) = -1/(V_d*\Delta T)*\ln[1-\Delta N/N(T)] \quad (2)$$

where k(T) is the differential ice nucleus concentration, V_d is the droplet volume, ΔT is a temperature step that must be prescribed in the analysis, ΔN is the number of droplets that froze in that ΔT temperature step, and N(T) is the total number of unfrozen droplets at T. An important aspect is that ΔT is <u>not</u> the temperature step of the actual measurements, such as from the frequency at which images are acquired. To produce meaningful k(T) spectra the ΔT should be large enough such than more than one droplet typically freezes in a given temperature step. In our initial k(T) analysis we found a ΔT interval of 0.05 or 0.1 °C to work well for our experimental conditions. ΔT should be varied until a reasonable representation of the droplet freezing spectrum is produced that displays the important features of the spectrum and allows the sample to be distinguished from the background freezing of a control. Realizing that this is an important and nuanced detail, Gabor Vali is planning to produce a tutorial explaining the use of k(T) and selection of ΔT, using some of our data to illustrate this method. Referring back to Eq. (2), as an example, given an array of 100 droplets and a specified ΔT of 0.1 °C intervals, if the first 2 droplets freeze within one measurement interval, ΔT = 0.1 °C, ΔN = 2, and N(T) = 98. Using this metric, each freezing event in the interval ΔT is the result of at least one active INP, but given a small ΔT and a large N the interval can be approximately attributed to a single active INP.

Inherent to all droplet freezing methods is the assumption that the freezing of any droplet at a given temperature interval is caused by the combination of INPs present from the sample plus any background freezing due to impurities and substrate artifacts. The differential ice nucleus method, k(T), provides a quantitative assessment of the sample versus the background INP concentration at each temperature interval. k(T) is an alternative approach to the more commonly used method of just subtracting the cumulative K(T) or c_{INP} background spectrum from the cumulative sample spectrum. This k(T) analysis method is discussed in detail by Gabor Vali in the comment (doi: 10.5194/amt-2018-134-SC1) he provided on the discussion version of this manuscript (https://www.atmos-meas-tech-discuss.net/amt-2018-134/amt-2018-134-SC1-supplement.pdf), based on the framework originally laid out in Vali (1971)."

References

Alstadt, V. J., Dawson, J. N., Losey, D. J., Sihvonen, S. K. and Freedman, M. A.: Heterogeneous Freezing of Carbon Nanotubes: A Model System for Pore Condensation and Freezing in the Atmosphere, J. Phys. Chem. A, acs.jpca.7b06359, doi:10.1021/acs.jpca.7b06359, 2017.

Archuleta, C. M., DeMott, P. J. and Kreidenweis, S. M.: Ice nucleation by surrogates for atmospheric mineral dust and mineral dust/sulfate particles at cirrus temperatures, Atmos. Chem. Phys., 5, 2617–2634, 2005.

Banfield, J. F. and Eggleton, R. A.: Analytical transmission electron microscope studies of plagioclase, muscovite, and K-feldspar weathering, Clays Clay Miner., 38(1), 77–89, 1990.

Beydoun, H., Polen, M. and Sullivan, R. C.: Effect of particle surface area on ice active site densities retrieved from droplet freezing spectra, Atmos. Chem. Phys., 16(20), 13359–13378, doi:10.5194/acp-16-13359-2016, 2016.

Durant, A. J. and Shaw, R. A.: Evaporation freezing by contact nucleation inside-out, Geophys. Res. Lett., 32(20), L20814, doi:10.1029/2005GL024175, 2005.

Findenegg, G. H., Jähnert, S., Akcakayiran, D. and Schreiber, A.: Freezing and Melting of Water Confined in Silica Nanopores, ChemPhysChem, 9(18), 2651–2659, doi:10.1002/cphc.200800616, 2008.

Futuka, N.: A Study of the Mechanism of Contact Ice Nucleation., J. Atmos. Sci., 32, 1597–1603, 1975.

Gurganus, C. W., Charnawskas, J. C., Kostinski, A. B. and Shaw, R. A.: Nucleation at the Contact Line Observed on Nanotextured Surfaces, Phys. Rev. Lett., 113(23), 235701, doi:10.1103/PhysRevLett.113.235701, 2014.

Harrison, A. D., Whale, T. F., Carpenter, M. A., Holden, M. A., Neve, L., O'Sullivan, D., Vergara Temprado, J. and Murray, B. J.: Not all feldspars are equal: A survey of ice nucleating properties across the feldspar group of minerals, Atmos. Chem. Phys., doi:10.5194/acp-16-10927-2016, 2016.

Holdren, G. R. and Berner, R. A.: Mechanism of feldspar weathering—I. Experimental studies, Geochim. Cosmochim. Acta, 43(8), 1161–1171, doi:10.1016/0016-7037(79)90109-1, 1979.

Koop, T. and Murray, B. J.: A physically constrained classical description of the homogeneous nucleation of ice in water, J. Chem. Phys., 145(21), 211915, doi:10.1063/1.4962355, 2016.

Kumar, A., Marcolli, C., Luo, B. and Peter, T.: Ice nucleation activity of silicates and aluminosilicates in pure water and aqueous solutions – Part 1: The K-feldspar microcline, Atmos. Chem. Phys., 18(10), 7057–7079, doi:10.5194/acp-18-7057-2018, 2018.

Marcolli, C., Nagare, B., Welti, A. and Lohmann, U.: Ice nucleation efficiency of AgI: review and new insights, Atmos. Chem. Phys., 16(14), 8915–8937, doi:10.5194/acp-16-8915-2016,

2016.

Peckhaus, A., Kiselev, A., Hiron, T., Ebert, M. and Leisner, T.: A comparative study of K-rich and Na/Ca-rich feldspar ice-nucleating particles in a nanoliter droplet freezing assay, Atmos. Chem. Phys., 16(18), 11477–11496, doi:10.5194/acp-16-11477-2016, 2016.

Price, H. C., Baustian, K. J., McQuaid, J. B., Blyth, A., Bower, K. N., Choularton, T., Cotton, R. J., Cui, Z., Field, P. R., Gallagher, M., Hawker, R., Merrington, A., Miltenberger, A., Neely, R. R., Parker, S. T., Rosenberg, P. D., Taylor, J. W., Trembath, J., Vergara-Temprado, J., Whale, T. F., Wilson, T. W., Young, G. and Murray, B. J.: Atmospheric Ice-Nucleating Particles in the Dusty Tropical Atlantic, J. Geophys. Res. Atmos., doi:10.1002/2017JD027560, 2018.

Reicher, N., Segev, L. and Rudich, Y.: The WeIzmann Supercooled Droplets Observation on a Microarray (WISDOM) and application for ambient dust, Atmos. Meas. Tech., 11(1), 233–248, doi:10.5194/amt-11-233-2018, 2018.

Stan, C. A., Schneider, G. F., Shevkoplyas, S. S., Hashimoto, M., Ibanescu, M., Wiley, B. J. and Whitesides, G. M.: A microfluidic apparatus for the study of ice nucleation in supercooled water drops., Lab Chip, 9(16), 2293–2305, doi:10.1039/b906198c, 2009.

Tabazadeh, A., Djikaev, Y. S. and Reiss, H.: Surface crystallization of supercooled water in clouds, Proc. Natl. Acad. Sci. U. S. A., 99(25), 15873–15878, 2002.

Tarn, M. D., Sikora, S. N. F., Porter, G. C. E., O'Sullivan, D., Adams, M., Whale, T. F., Harrison, A. D., Vergara-Temprado, J., Wilson, T. W., Shim, J. and Murray, B. J.: The study of atmospheric ice-nucleating particles via microfluidically generated droplets, Microfluid. Nanofluidics, 22(5), 52, doi:10.1007/s10404-018-2069-x, 2018.

Vali, G.: Quantitative Evaluation of Experimental Results an the Heterogeneous Freezing Nucleation of Supercooled Liquids, J. Atmos. Sci., 28(3), 402–409, doi:doi.org/10.1175/1520-0469(1971)028<0402:QEOERA>2.0.CO;2, 1971.

Whale, T. F., Murray, B. J., O'Sullivan, D., Wilson, T. W., Umo, N. S., Baustian, K. J., Atkinson, J. D., Workneh, D. A. and Morris, G. J.: A technique for quantifying heterogeneous ice nucleation in microlitre supercooled water droplets, Atmos. Meas. Tech., 8(6), 2437–2447, doi:10.5194/amt-8-2437-2015, 2015.