## Review of "Development of the Droplet Ice Nuclei Counter Zürich (DRINCZ): Validation and application to field collected snow samples" by David et al.

### General comment

**In this manuscript the authors describe and characterise a large volume immersion mode drop assay (DRINCZ). The authors thoroughly characterise the horizontal temperature gradient across the 96 well plate in the system and recommend a correction which is of use to other instrumental setups. The authors report a ± 0.9 ˚C uncertainty for DRINCZ and go on to validate the instrument by comparing to literature data of NX-illite. A field study investigating snow melt samples is also undertaken which shows agreement (mostly) with previous snow melt measurements. The authors then relate the INP concentrations measured to airmass trajectories and propose scavenging of INP by precipitation led to the lowest INP concentrations measured.**

**This manuscript is well written and presents results which are of interest to the ice nucleation community. The manuscript is in the scope of AMT and I support its publication after the following comments have been properly addressed.**

We thank the reviewer for the positive recommendation and for raising several points that we now address individually below and in the revised manuscript to make the paper clearer. Reviewer comments reproduced in bold and author responses in regular typeface. All line numbers in authors' response refer to revised manuscript.

### Major comment

**Although I like the manuscript and find the results of use to the ice nucleation community, I am unclear on the novelty of this instrument compared to others that have already been presented. The authors have acknowledged that the technique is based on the design of previous instruments. Is the method used to characterise and correct the horizontal gradients in the plate the only novelty? If so, I suggest this is made clearer in the final manuscript or that the unique traits of this instrument are further clarified.**

We thank the reviewer for pointing this out. Indeed, the instrument is quite similar to previously developed drop freezing assays. New aspects are a method for determining horizontal gradients across the well plate and a fully automated data analysis, which only requires the user to enter a folder path name into a MATLAB function. We have now added the ease of data analysis as one of the benefits of DRINCZ by stating in the abstract (line 23): *"with a user friendly and fully automated analysis procedure."* Unfortunately, it is difficult to directly compare the ease of use and data analysis developed for DRINCZ relative to similar setups based on published papers, so we cannot be more specific.

**The horizontal gradients of the plate have been characterised but the vertical gradients within the wells have not been explored. These should be discussed in the text. A reference to Beall et al. (2017) would be appropriate as they characterise the gradient within 50 µL droplets within wells with a similar profile (PCR plate).**

It is very difficult to measure the vertical bias in a 50 µL well and this is also acknowledged in Beall et al. (2017). It is important also to note that in the setup of Beall et al. (2017), the polypropylene well tray is in contact with an aluminum block rather than the ethanol itself. This can lead to gradients due to the block, which would be negligible in our setup where the tray is in direct contact with the ethanol. Nevertheless, we have mentioned that the bath leveler can help reduce this issue on lines 176-180. We now reference Beall et al. (2017) in the revised manuscript to point out the possibility of vertical gradients in the wells and write that we attempt to avoid them by ensuring that the entire volume of solution inside the well is surrounded by ethanol. The addition to the text reads: *"It has been shown that large vertical gradients of up to 1.8 °C can exist between the bottom of a well and the air above it in block-based drop freezing setups (Beall et al., 2017). We anticipate vertical gradients to be reduced in DRINCZ due to the direct contact between the cooling medium (ethanol) and the well tray when the ethanol levels remains constant during cooling. Therefore, we incorporated a bath leveler composed of a level sensor and solenoid valve to ensure that the ethanol level remains constant."* (lines 176-180).

**Figure 9 displays data for NX-illite dilutions. I find the text a little misleading in presenting the data as though there are only a "few" outliers for the 0.01wt% dilution. The vast majority of data for all three triplicates for this dilution give higher freezing temperatures than higher weight percent suspensions (at the same value of ns). This is in contradiction to what we expect of ns. I believe the source of this error is different to the uncertainties characterised in previous sections as the data is consistently offset to higher freezing temperatures. This issue is not seen (in most cases) in the dilutions for the snow melt study and suggests this discrepancy may be material dependent and related to the distribution of particles. Although the authors do mention this issue, the extent of the discrepancy between dilutions is glossed over in the text. I must stress that I do not believe this inconsistency in the dilutions is a result of an error in the instrument but rather an error as a result of the material or sampling method. With this said, the results should be presented in the text to acknowledge the true extent of this discrepancy.**

The observed difference in the $n_{sBET}$ values at the lowest weight percent compared with the higher concentrations falls within the uncertainty of the instrument (± 0.9 °C). Moreover, there is considerable variability between the triplicates performed with the same suspension concentrations. This can be seen in Fig. A4, where the *FF* curves of all NX-illite DRINCZ experiments are shown. In addition, we have updated Fig. 9 so that the difference between the triplicates of the NX-illite suspensions can be seen more easily. Nevertheless, we acknowledge that $n_{sBET}$ of the 0.01 wt% NX-illite suspension is constantly above the $n_{sBET}$ of the 0.05 and 0.1 wt% NX-illite suspensions. One reason for this might be that very few random freezing events occurring at warm temperature in the higher diluted sample may constantly increase cumulative active site densities to lower temperatures. However, based on the available data, we cannot exclude an effect due to dilution of a single stock suspension, which can lead to a bias compared with preparing suspensions of each concentration separately. We now discuss both possibilities in section 4.2 and reference the Harrison et al. (2018) study where issues arising from diluting a single stock suspension are discussed in detail. Nonetheless, we need to emphasize, that more investigations would be needed to establish the significance of the increased $n_{sBET}$ observed at the lowest suspension concentration given the random variability between repetitions in such freezing experiments.

**Minor comments**

**Line 67-77: Dilution is not the only means of changing the measurable range of INP. Concentrating the particles per droplet can also extend the range. I suggest this is added to the discussion.**

This is a valid point. We add on line 71-73 after "aliquot": "*Alternatively, to explore freezing towards warmer temperatures, field samples (e.g. rain or snow samples) can be concentrated by evaporating a part of the sample water*."

**Line 116: At what temperature does the ethanol bath start at and what temperature does it end, i.e. 0 ˚C to -30 ˚C. In addition to this, if the sample is added to an ethanol bath at 0 ˚C (as suggested by line 165) is the sample allowed time to equilibrate? If not, this could lead to thermal gradients not just horizontally across the plate but vertically in the wells (see major comment). I suggest adding information on the cooling profile of the bath to this section.**

The wells are left to equilibrate to the bath temperature at 0°C for one minute before the experiment and cooling ramp is initiated. We have now added to the text on lines 107-108: *"The well tray is placed in the tray holder (Fig. A1) and left to rest for 1 min at 0°C before the experiment is started."*

**Section 2.1.2: This describes the detection of freezing events in wells. Is this similar to other methods, e.g. (Stopelli et al., 2014)? Clarify what is different.**

There is no appreciable difference in the detection method for identifying a freezing event relative to Stopelli et al. (2014). We have now added "*Similar to Stopelli et al, (2014)*" on line 123. However, rather than using a fixed intensity change as done by Beall et al, (2017), we use a normalized threshold of 0.6 as explained in the section. We have now clarified this difference on lines 168-170 by stating: "…*rather than relying on a fixed change in light transmission through the well as done by other drop freezing setups (Beall et al., 2017). This ensures that the initial freezing detection is independent of the absolute change in light transmission through a well*."

**Section 2.2: What is the error of the sensor? What will be the fluctuation in the ethanol level? Do the authors consider it negligible?**

There is no appreciable error in the sensor as it is a binary switch that either detects contact with or without the ethanol. As the sensor triggers the opening and closing of the solenoid valve to allow ethanol to flow into the bath, we expect fluctuations in the ethanol bath to be negligible.

**Line 182-183: K type thermocouples can have large uncertainties, commonly ± 2.2 ˚C, compared to other thermocouple types (e.g. T type). Were these K type thermocouples calibrated other than by the manufacturer? What is the error of these? I suggest showing these errors in the figures (or an example of the errors).**

Indeed, there can be differences between different thermocouples. That is why we did the temperature calibration using the same thermocouple in all five test locations. Therefore, the observed difference in the well temperature is based on the same thermocouple and not sensor dependent. As the temperature error reported is based on one thermocouple, the differences that we report can be attributed to the locations of the wells in the tray. We did not calibrate the thermocouple in-house but rather compare the thermocouple temperature to the bath temperature of the chiller which is measured by a PT-100 temperature sensor. As such we have clarified this in the text by adding: "*The same thermocouple was used for all the well temperature measurements to avoid biases between different thermocouples.*" to lines 196-197

**Line 183-184: Were the wells completely filled with ethanol or 50 µL? If completely filled does this represent the gradients that would be present in the wells and plate in a typical 50 µL experiment?**

The wells were filled with 50 µL of ethanol to reproduce the experimental procedure used for DRINCZ freezing runs. We have now added *"50 µL of ethanol …"* to the text on line 197.

**Section 3.2: The characterisation of the horizontal gradient across the plate is very useful for the community. However, has the vertical gradient within the wells been considered (see major comment)? This system uses a similar well profile to that used by Beall et al. (2017) who found that a vertical stratification of 0.5 ˚C can be found in wells in which the headspace (air above the wells) is ≥6 ˚C warmer. Please discuss this in the text and reference Beall et al. (2017) where appropriate.**

It is very difficult to measure the vertical bias in a 50 µL well and this is also acknowledged in Beall et al. (2017). It is important to note that in the setup of Beall et al. (2017), the polypropylene well tray is in contact with an aluminum block rather than the ethanol itself. This can lead to gradients due to the block, which would be negligible in our setup where the tray is in direct contact with the ethanol. Nevertheless, as we now state in the response to the major comments, the bath leveler helps to reduce this issue (see response in major comments and revised manuscript line 175-180).

**Also, you use the median freezing temperature of SA water to determine the offset in temperatures across the plate. Is there not a random probability of the SA water freezing at different temperatures without a temperature bias to start with? Would there also not be uncertainties due to accidental contamination in the individual wells during the setup of the experiment? Does this not create uncertainty in this experiment? What was the rationale for using SA water? If you were to use freezing temperatures (rather than direct measurements of the wells) then would something that froze more consistently at the same temperatures be a better standard to use, i.e. pollen has a narrow window for freezing.**

We completely agree that there is a stochastic component to the freezing of the SA water. However, by averaging several experiments, the random variations cancel out while systematic bias adds up. Moreover, we calculate the precision of the instrument using the standard deviation of the temperature required for 50 % of the wells to freeze (see Section 3.3), which is less affected by random variability. In addition to minimize the effect of stochasticity, the 50% FF should also reduce the influence of contamination. Therefore we have added a sentence to Section 3.3 on line 263-264 stating: *"Furthermore, by using the 50 % FF the influence of contamination and outliers is minimized."* We chose SA water as the bias and precision standard for two reasons. First, the SA water is used in the majority of the experiments to prepare or dilute the samples. Therefore, its freezing curve needs to be well known for background correction. Moreover, we use SA water as a reference sample to control the performance of DRINCZ and the constancy of the background. Therefore, we accumulated a large number of DRINCZ experiments with SA water available for closer analysis. Second, SA water has the lowest accessible freezing temperatures and at the lowest temperatures we expect the largest bias since the gradient between the air and the bath temperature is maximized.

**Section 3.3: It is unclear to me why you assess the uncertainty of the instrument and combine this with the variability in the freezing temperature of SA water at this point. The water baseline in other studies, e.g. field-based studies, could potentially be worse (or better) than what you have done in these experiments. Should the experimental error as a result of the water impurities not be considered separately in respect to the particular experiment/environment?**

Indeed, we use the freezing experiments performed with SA water for two purposes. First, in the laboratory we need to know the background freezing due to impurities present in the SA water for the samples that we prepare, collect or dilute with SA water (see Section 4.1). In the field, we also conduct background measurements with SA water in order to correct the observed IN concentrations. Second, we chose to use SA water as standard for quantifying instrument uncertainties because we accumulated a considerable number of SA water experiments performed by different users over a longer time period and therefore, we have the best statistics for this sample. In Sect. 3.3 we use the SA water experiments to establish instrumental uncertainties stemming from well-to-well temperature variations.

**Section 4.2: There is no mention on how these suspensions/dilutions are made, how are the particles suspended? This could be particularly important given the results for NX-illite. I recommend adding this information in this section or the methodology.**

Thank you for pointing this out. We have now added that: *An initial stock suspension of 0.1 wt % NX-illite was prepared and then diluted to produce additional mass concentrations of NX-illite of 0.05 and 0.01 wt%. The suspensions were manually shaken for 30 s, poured into a dispensing tray and then immediately pipetted into the well plate. Triplicates of each suspension concentration were investigated with DRINCZ..."* to lines 364-367 in Section 4.2.

**Figure 9: it looks like the temperature intervals where no freezing events were observed are displayed in this figure, i.e. when binning the data, temperature intervals where 0 events were observed are still shown in the cumulative plots. As there are triplicates in this figure it makes it hard to discern which data points are real freezing events and which are artefacts of the binning process. This makes it difficult to interpret the data and the extent of the discrepancy between dilutions. If this is the case, I suggest removing the data points from the cumulative plots where there were no freezing events within a temperature interval for clarity.**

Indeed, the plot shows triplicates for each weight percent but the values are not binned but just plotted at the observed temperature. We agree that the plot is a bit hard to interpret so we have remade the figure to help differentiate between the values reported in literature and shaded the triplicates to more clearly see the run-to-run variability at the different NX-illite concentrations. We have decided to keep all the observed $n_{sBET}$ values since cumulative active site densities indeed remain at a constant value when there is no freezing event in a given time interval. Moreover, constant values indicate that there is a poor data basis relying on rare freezing events.

**Line 355-357: I would not definitively say that the ns is extended as expected. All three triplicates for the 0.01wt% dilutions are giving warming freezing temperatures than the higher weight percent suspensions (at the same value of ns). See major comment.**

The purpose of the sentence is to state that by diluting the solution, we can observe freezing at lower temperatures and measure $n_s$ at higher values. This is true even if the 0.01 wt% is slightly higher in $n_s$ than the 0.05 and 0.1 wt% (within the instrumental uncertainty). Since we agree that we do not show a textbook case for the extension of $n_s$ range by dilution, we have removed “as expected” from the sentence (376-377).

**Line 357-358: In relation to the major comments, you state a few data points from the 0.01wt% suspension appear as outliers (and only at warmer temperatures), whereas all three runs for this dilution are shifted to warmer temperatures for the same value of ns. I suggest restructuring this paragraph to better represent the data and discuss the inconsistencies.**

We have removed “a few data points” from the sentence to more accurately represent the higher $n_{sBET}$ of the lowest weight percent solution at warm temperatures. As described in the response to the major comment, this discrepancy may be due to the presence of a few random active sites which lead to an increase in $n_{sBET}$ that extends to lower temperatures or issues arising from diluting from a stock suspension. We have reworded lines 377-382 to: *"Similar to the observations of Harrison et al. (2018), the data points from the 0.01 wt. % solution appear as outliers at the warmest temperatures. However, it is not possible to determine if these outliers are due to random freezing events that occur at high temperatures and therefore produce elevated cumulative* $n_{sBET}$ *values at lower temperatures or if they are due to an uneven distribution of the active sites in each aliquot that may result from diluting a single stock suspension rather than producing individual weight percent suspensions (Harrison et al., 2018)."* to offer an explanation for the observed divergence in the $n_{sBET}$ between the wt% suspensions.

**Line 358-360: In relation to the above, you reference that Harrison et al. (2018) used individually weighed suspensions rather than a single stock suspension to minimise the effect of uneven particle distributions. Why was this not done here if you believe this is the issue? This seems important as you are validating the instrument yet have inconsistent results on dilution.**

As the discrepancies in $n_{sBET}$ fall within the instrumental uncertainty of DRINCZ, looking for the true cause for the observed differences in the $n_{sBET}$ values after dilution might be an over-evaluation of the data. Moreover, in addition to instrument uncertainties, there is also random variability in *FF* curves obtained from drop freezing assays. Indeed, when examining the *FF* curves shown in Fig. A4, there is considerable variability between triplicates performed with the same suspension concentration. Additionally, there are very few freezing events that occur at the highest temperatures in the 0.01 wt% suspension. Therefore, these high temperature freezing events that are responsible for the high cumulative $n_{sBET}$ values of the 0.01 wt% suspension shown in Fig.9 can be random.

**Line 362-363: At temperatures colder than -15 ˚C this doesn't seem to be the case (especially if you look at the 0.1-0.05wt% suspensions). There is just as good agreement with BINARY at colder temperatures (Hiranuma et al., 2015) but no comparison is made to this instrument.**

We have now changed the sentence to state that the data falls between BINARY Leeds-NIPI and IR-NIPI as follows: *"Furthermore, considering the ± 0.9 ˚C uncertainty, depicted by the horizontal error bars, the differences between concentrations are not significant. They fall within the same range as the measurements of Beall et al, (2017) and between BINARY and Leeds-NIPI and IR-NIPI at colder temperatures (Fig. 9)."* (lines 384-385)

**Line 374-375: Were the samples analysed at this field location or in the lab where the background freezing has been characterised? Were blank (pure SA water) experiments run at the time of these experiments to check the background signal had not changed?**

Thank you for pointing this out. The samples were actually measured in the laboratory in Zurich and we have now clarified this by adding this information to lines (403-404): *"The samples were shipped and stored frozen until processed with DRINCZ at the Atmospheric Physics Laboratory at ETH Zurich to avoid any bacterial growth or changes due to liquid storage (Stopelli et al., 2014)."* Furthermore, we always ran an SA water blank before running DRINCZ on a measurement day to ensure that the background is the same and the system is working properly.

## Technical comments

**Line 41-43: This sentence needs restructuring/ re-wording as it is a bit clunky. E.g. an ice nucleating particle (singular) cannot get immersed in multiple cloud droplets.**

Thank you, we have now made the sentence singular

**Line 54: Should the word ‘or’ be in this sentence?**

We have removed “or”

**Line 57-59: No available technique can detect the lowest INP concentrations that are actually present in the atmosphere. I would suggest putting in a range of the INP concentrations detected with these techniques and rewording to say “to detect lower atmospheric INP concentrations”.**

We have now reworded the sentence to state: *“lower atmospheric INP concentrations.”* (line 60). We have decided not to include a range as the measurable INP concentrations depend on the sampling method (e.g. time of sampling, impinger, filter etc.) as well as the measurement technique.

**Line 102: What material is the 96-well plate made from? Polypropylene? I suggest adding here.**

Thank you, we have now added polypropylene to the text.

**Line 170-174: Suggest removing the terms ‘potential’ and ‘possible’ as adding 0 ˚C ethanol to ethanol at -30 ˚C will create a gradient, even if only small. Cooling the ethanol to 0 ˚C simply minimises this gradient.**

Done

**Line 201-201: Consider rephrasing this sentence.**

We have now added a reference to Fig. 3 to clarify that the spread is referring to the temperature calibration.

**Line 208-209: Consider rephrasing for ease of understanding.**

We have now clarified that the observed bias is referring to the freezing temperature bias across the well plate.

**Line 235-236: Harrison et al. 2018 is not a suitable reference in this instance. As I understand, they make individual temperature measurements for each well and as such, they take into account the horizontal gradient in temperature across the plate without the need for such a correction.**

The Harrison et al. (2018) citation here is just meant as an example that such gradients do exist in block-based systems, which are observed in the IR-NIPI. Therefore we have changed the sentence (lines 251-252) to reflect this by adding *“… have been observed or modelled*.”

**Figure 2c: Perhaps label the peak which signifies initial nucleation**

We have now clarified this in the figure caption by adding the sentence: *“The most intense peak corresponds to the ice nucleation temperature and the second most intense peak is due to the slow freezing of the solution after nucleation.”*

**Line 295: device not devices**

Done

**Line 307-309: This representation of the background you present is for DRINCZ in this particular lab environment. The baseline may change in field studies. I suggest rephrasing this section.**

We have now added the preceding sentence (lines 323-324): *"Furthermore, an SA water sample is run as a standard at the beginning of each measurement day to ensure the system is operating correctly*." as to further motivate the use of SA water as a background and to ensure that the instrument background is reproducible in other settings.

**Line 356: Suggest changing to "samples overlap to an extent"**

Done

**Line 445: missing bracket**

Thank you.

**Figure 9: the triangular symbols are hard to distinguish from one another. Suggest using different symbol shapes.**

Thank you for pointing this out. We have now updated the symbols for clarity.

## References:

Beall, C. M., Stokes, M. D., Hill, T. C., DeMott, P. J., DeWald, J. T. and Prather, K. A.: Automation and heat transfer characterization of immersion mode spectroscopy for analysis of ice nucleating particles, Atmos Meas Tech, 10(7), 2613–2626, doi:10.5194/amt-10-2613-2017, 2017.

Harrison, A. D., Whale, T. F., Rutledge, R., Lamb, S., Tarn, M. D., Porter, G. C. E., Adams, M. P., McQuaid, J. B., Morris, G. J. and Murray, B. J.: An instrument for quantifying heterogeneous ice nucleation in multiwell plates using infrared emissions to detect freezing, Atmospheric Meas. Tech., 11(10), 5629–5641, doi:https://doi.org/10.5194/amt-11-5629-2018, 2018.

Stopelli, E., Conen, F., Zimmermann, L., Alewell, C. and Morris, C. E.: Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation, Atmospheric Meas. Tech., 7(1), 129–134, doi:10.5194/amt-7-129-2014, 2014.