Reviewer comments on "**Development of the DRoplet Ice Nuclei Counter Zürich (DRINCZ):Validation and application to field collected snow samples**" by Robert O. David, Maria Cascajo Castresana, Killian P. Brennan, Michael Rösch, Nora Els, Julia Werz, Vera Weichlinger, Lin S. Boynton, Sophie Bogler, Nadine Borduas-Dedekind, Claudia Marcolli, Zamin A. Kanji
By Gabor Vali

General:
This paper is a good addition to the literature on INP measurements. Not fundamentally new, but every implementation of the drop-freezing technique, or of any other method, brings new challenges and new ways of solving them. The authors deal with those challenges reasonably well. This paper stands out with its focus on evaluating instrument-caused uncertainties. There are some parts of that evaluation that would benefit from a second look.

We would like to thank Gabor Vali for his positive and very helpful comments and respond to the individual concerns below. Reviewer comments have been reproduced in bold typeface, and author responses are in regular font. All line numbers in authors' response refer to the revised manuscript.

Detailed comments:

41-57 In describing different approaches to INP detection it is useful to separate those that examine air samples and those that take water samples.

In lines 41-56, we are describing single particle methods, which use aerosolized samples that can either be dry dispersed or atomized from a suspension. We now clarify this in lines 51-52. Thus, they can be used to investigate air samples, suspensions or seawater samples. Furthermore, we add "*immerse the samples in water and*" to line 60 to show that bulk methods use samples suspended in solution.

81 Does Bigg (1953) contain data and hail and snow samples? Please check.

Thank you for pointing this out. Indeed, Bigg, (1953) does not use or describe a bulk method to investigate INPs. The citation has been removed from line 83 (revised manuscript).

88 - 89 The goals are stated in overly broad terms. The proposed measurement are expected to be relevant to MPC clouds but no claim should be made that they examine those responsible for ice formation in those cloud. There are other elements to that story beyond the INP measurements. Also, to what degree can these measurements illuminate 'fundamental understanding' of ice nucleation?

We have reformulated the sentence to more explicitly describe the motivation behind the development of DRINCZ. The sentence now reads (90-94): *"In order to further quantify the variability of ambient INP concentration relevant for ice formation in MPCs and increase the understanding of the ice nucleation ability of laboratory and field collected samples, we developed and characterized the DRoplet Ice Nuclei Counter Zurich (DRINCZ). DRINCZ is a drop freezing instrument to investigate ice nucleation at temperature conditions between -25 ˚C and 0 ˚C, representative for MPCs."*

97 In addition to those cited, a design much like the one in this paper was described by Vali (1995; Principles of ice nucleation. Chapter 1 in: "Biological Ice Nucleation and Its Applications", R. E. Lee Jr, G. J. Warren, and L. V. Gusta, Eds., APS Press, The American Phytopathological Society., St. Paul, Minnesota, USA. 370 pp.; ISBN: 0-89054-172-8).

Thank you, we have now added the citation to the text (line 101).

103 Is the foil seal enough to exclude ethanol vapors from getting into the samples and thereby producing a freezing point depression?

This is a valid concern but we believe is not an issue in our experiments. The comparison with literature values of NX-Illite as shown in Fig. 9 do not show a significant bias when compared to techniques that use a sealed cooling block where exposure to ethanol vapors is not an issue. Furthermore, the foil seals used with DRINCZ are non-permeable, suggesting that the probability of ethanol vapors entering the wells would be negligible. To clarify this, we have added “non-permeable” to the description of the foil (line 107).

123 -> Since the position of the sample tray is fixed, and so is its dimension, why is such an elaborate process necessary for identifying the well locations?

As DRINCZ is meant to be a field deployable instrument, the camera mounting location is variable. Even if it is mounted as reproducibly as possible, there are still some variations that can lead to issues when using a fixed well location. Therefore, the code looks for the wells instead of hard coding their locations. This justification is provided at the start of section 2.1.1, lines 129-132 (124-127 in original manuscript).

> **Also, if done this way, to what extent does perspective from the camera lens distorts the circular shape of the wells near the edges of the tray?**
>
> This is a valid point and some edge effects can be seen in Fig. 2a. Nevertheless, the change in light transmission through the well is still significant enough to be detected by the camera to overcome the distortion and shading from edge effects and therefore provide an accurate freezing temperature.

131-132 The meaning of " .... centered at the edge ... as the well center." could probably be clarified better.

Thank you for pointing out that this is confusing. We have therefore reworded the sentences and the preceding sentence to state:” *The CHT first identifies pixels along regions of large gradients in brightness to identify pixels at the edge of the well. To determine the center of each well, the algorithm draws circles of varying diameter (ranging between 15 and 30 pixels in diameter, which corresponds to the observed diameters of a well in terms of pixel number) around these edge pixels and classifies the pixel intersecting the largest number of circles as the well center.”* (lines 135-138)

135 Random order and sorting seem unnecessary with the fixed geometrical arrangement of this setup. What is the rationale here?

As described on lines 129-132 (lines 123-125 in original manuscript), the location of the camera is not fixed in the current setup and can be removed for easier packing and shipping. Therefore, the well locations are not hard coded into the software but rather identified using the CHT. However, future versions of DRINCZ could attempt to have a perfectly reproducible camera location and mounting system so that this would not be needed anymore.

152 Isn't the first instant of intensity change over a threshold magnitude sufficient to detect nucleation? If not so, why not? What possible reason exists for a significant peak in the signal, comparable to that caused by nucleation, prior to nucleation?

The threshold proved to be necessary to account for fluctuations in the light transmission through a well due to turbulence and air entering the ethanol bath. The noise arising due to these fluctuations can be seen in panels b and c of Fig. 2.

157 What is meant by "all recorded images"?

We simply mean all images, and have removed “recorded” from the revised manuscript so as to simply mean all of the images of a well (see line 165)

Fig 2. It is unclear to me what mean intensity and normalized intensity refer to. Is it an average within the circle for a given well? Are they for a given well over repeated trials? Are the averages over many wells?

The mean intensity (I_t) is just the average value of all of the pixels in a single well and is explained in lines 157-158. Therefore, there is a mean intensity for each well at every temperature (every image). Similarly, there is a normalized value Z_t' for each well at every temperature (each image, see lines 163-168).

To clarify this, we have now added “of *a single well*” to the caption of Figure 2b and “*for the same well as in b*” to the explanation of the caption for Figure 2c.

190 What is 'maximum standard deviation'?

We have now removed maximum standard deviation from the sentence and added a new sentence to explain the maximum standard deviation which reads: *“The maximum standard deviation taken as the temperature difference between the temperature fit and the individual well temperature was ±0.6 ˚C.”* on lines 203-205.

196 -> The work here described in Section 3.2 is certainly well directed and quite extensive. However, it is surprising that nucleation temperatures of SA water are used instead of direct temperature measurements. It is the temperature of the water before, and at the instant, of nucleation that is most relevant. Direct temperature measurement of the water in the wells is not without its own difficulties (locating the sensors in the wells, sensor lead errors, etc.) but the variations in nucleation temperatures from well to well, even for SA water or other similar sample, are bound to be adding uncertainty to the calibration. What governed the decision to use nucleation temperatures to evaluate bias across the well-plate?

To directly measure the temperature of each well during an experiment, 96 thermocouples would be needed, which all can vary in temperature by about ± 2°C if they are not calibrated accurately. Alternatively, if the same thermocouple were used for all wells, 96 freezing runs would be needed to obtain only one temperature measurement for each well. Such a procedure is not feasible. Therefore, freezing runs performed with SA water were averaged, such that random variability cancelled out and systematic bias added up.

> **To assess the quality of this approach it would be useful to know how much variation in nucleation temperatures was observed for any given well within the 20 repeat tests. The two sources of variations - within a given well and among different wells - should be both presented and the sufficiency of the use of the median for each well thus evaluated.**

We completely agree. The spread in the freezing temperature of the SA water in individual wells was indeed included in the original manuscript in the Appendix as Fig. A3, but was not mentioned in the main text. Although the distribution of freezing in the wells varied, the median was chosen as the most representative due to its definition as the center value of the distribution. Thus, it should be less sensitive to outliers than the mean. We have now added a reference to this figure on line 218 and reordered the Appendix figures accordingly, as such it is now Fig. A2.

226 Section numbering is off.

Thank you for pointing this out. We have now renumbered the section to be consistent with the rest of the manuscript (see line 242).

244 This standard deviation refers to the distribution of observed freezing temperatures among wells? Again, please distinguish between single well repetitions and variations among wells.

We have now added (lines 259-261) that the standard deviation here refers to "*the standard deviation in the observed freezing temperatures of the SA water experiments across all wells"*

246 The 50% fraction corresponds to the steepest point on the FF curve for SA water. But this is not a general result; other samples may have no such correspondence between the two measures.

This is absolutely true, but we chose the 50 % FF here as this is the most probable temperature at which the SA water freezes and therefore represents the best estimate of the bulk freezing properties of the water with a reduced influence from outliers and contamination. To clarify the reviewer concern we have now added the sentence (lines 263-264): *"Furthermore, by using the 50 % FF the influence of contamination and outliers is reduced."*

258 - 259 Fig. 4a shows, as expected, that the standard deviation varies according to the slope of the FF curve (sample size effect). Assigning this patters to the influence of ethanol circulation is likely to be incorrect.

Figure 4 shows that the deviation (bias) of each well in freezing temperatures from the median (panel a) and mean value (panel b) exhibit a non-random pattern. We see the ethanol circulation as the most likely explanation for such a pattern as the cooled ethanol circulates around the tray in a clockwise direction as indicated by the arrows in Fig. 4. We now clarify this on lines 222-224 of the revised manuscript.

More general point: to what extent in ethanol circulation predictable? This is a valid question in light of the flow being turbulent with the level control adding pulses of liquid.

In regards to the impact of the ethanol pulses on the ethanol circulation, it is important to point out that the pulses add very small volumes of ethanol and therefore likely have little impact on the circulation. In contrast, the change in bath level due to contraction during cooling likely has larger impacts on the flow in the bath due to changes of the exposed internal surface area of the bath. Therefore, the addition of ethanol likely makes the bath circulation more consistent. Regardless, no impact on the circulation was observed when observing the bath by eye with or without the use of the bath leveler.

304 -> The background correction via Eq. (10) is valid, but it is surprising that the correction is finally presented in terms of FF, via Eq. (11). Fitting a correction equation to $k_{bg}(T)$ would be more direct and more readily applicable to a variety of samples with different volumes and/or dilutions.

Indeed we use the method described by the reviewer, i.e. $k_{bg}(T)$ is used when correcting for the freezing background. The conversion to *FF* is just used to demonstrate how the background influences the *FF.*

342 -> Section 4.2 is well done. It is a good demonstration of the DRINCZ's capabilities. Was background correction applied?

Thank you for pointing this out. The results presented in section 4.2 are background corrected so we have added: *"and background corrected (using Eq. 11)"* to the sentence (lines 373-374).

373 -> Sections 4.3 to 4.5 introduce a topic beyond the description of the instrument. As has been amply shown in the extensive literature on the topic, analyses of snow samples are valid tools as inputs to the analyses of cloud processes, but with the attendant complicating factors partially discussed here. That current results vary within the range reported for other such measurements is due does demonstrate that the sampling techniques were adequate and that the atmosphere is relatively conservative in the range of INP contents of snow. They do not substantially reinforce the validation of the instrument per se; that validation is more clearly supported by the calibrations and by the illite sample results. It is not stated (or it escaped me) whether the freezing analyses were done in the field or in the laboratory. This would be relevant to possibly show that the instrument is rugged enough for field use and that different setups do (or do not) effect the results.

We acknowledge that the measurements of field collected samples do not act as a validation of the technique. Rather they are added to the manuscript to show that the technique can be applied to field collected samples while providing the scientific community with additional observations of INP concentrations collected in snow samples.

In this case DRINCZ was not deployed in the field but the samples were shipped frozen to the laboratory in Zurich where they were stored frozen until the experiments were conducted. We have now adapted lines 403-404 to clarify that the samples were shipped frozen and the measurements with DRINCZ were conducted in Zurich by changing the sentence to read as: *"The samples were shipped and stored frozen until processed with DRINCZ at the Atmospheric Physics laboratory at ETH Zurich, to minimize any bacterial growth or changes due to liquid storage* (Stopelli et al., 2014)*."*

References:

Bigg, E. K.: The formation of atmospheric ice crystals by the freezing of droplets, Q. J. R. Meteorol. Soc., 79(342), 510–519, doi:10.1002/qj.49707934207, 1953.

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.