Response to Referee #2

We would like to thank the referee for their insightful comments and have responded below. The referee comments are highlighted in red with our responses in black.

This manuscript presents a new instrument to measure low concentrations of ice nucleating particles in the atmosphere. It relies on commercial multiwell plates to allot the samples extracted from filters. For cooling, it uses a Stirling engine chiller that can be easily operated in the field. Because the heat transfer between the chiller and the multiwell plate is not uniform, the temperature of each well is determined with an IR camera. The same camera is also used to detect freezing from the heat release. To retrieve the actual temperature of the wells, use is made of the temperature increase upon freezing. The plateau temperature reached during freezing is taken as 0°C and the whole temperature curve is adjusted to this reference value by applying an offset correction. Validation and calibration revealed a temperature uncertainty of ±0.9°C. The setup has been tested with K-feldspar chips, NX-illite and an aerosol sample taken from the City of Leeds. All these measurements show reasonable agreement with reference measurements from literature or performed with the µL-NIPI developed and available in the Leeds group. This manuscript is well suited for AMT and will be useful for other groups developing similar setups. For publication in AMT, the quality of presentation should be improved. Some descriptions remain vague and the figures are often not fully explained. In the introduction there is often only one reference given, while other relevant references to support the statements are lacking.

Comments

Lines 22 – 23: "and their temperature is determined by the ice-liquid equilibrium temperature…." This sentence should be improved because it is difficult to understand without having read through the manuscript.

We have amended the text to read as "In this paper we first present the calibration of the IR temperature measurement, which makes use of the fact that following ice nucleation aliquots of water warm to the ice-liquid equilibrium temperature (i.e. 0°C when water activity is ~1), which provides a point of calibration for each individual well in each experiment."

Line 46: literature referring to more recently developed CFDC's should be added.

References have been updated to: " There are several different methods of conducting ice nucleation experiments that include Continuous Flow Diffusion Chambers (CFDC's) e.g. (Salam et al., 2006; Rogers et al., 2001; Kanji and Abbatt, 2009; Stetzer et al., 2008; Garimella et al., 2016; Kohn et al., 2016),

Line 56: Vergara-Temprado et al. (2017) is not the best reference to support this statement. Other references need to be added, e.g. Kanji et al. (2017).

Kanji *et al.* (2017) added. Vergara-Temprado et al. 2017 was a modelling study in which field data was compiled and compared to the model. One conclusion from this study was that there was a lack of field data.

Line 61: Again, this statement needs to be based on more and more recent literature because different types of CFDC's are in use.

This has now been updated to include the following references "(Eidhammer et al., 2010; Al-Naimi and R., 1985; Prenni et al., 2009; DeMott et al., 2010)"

Line 61: the aerosol concentrator needs to be referenced.

Now referenced "This can be improved with aerosol concentrators (Prenni et al., 2013; Tobo et al., 2013),…"

Lines 64 – 66: "In principle, if the ice-nucleating properties of the aerosol particles in question are insensitive to mixing state, then increasing the amount of aerosol per droplet will scale with inverse proportionality to the INP concentration,…". This sentence needs to be improved. What is meant by "mixing state" in this context? What is meant by "increasing the amount of aerosol per droplet will scale with inverse proportionality to the INP concentration?" Do you really mean "aerosol per droplet" or not rather "particles per droplet"?

This has now been reworded to "In principle, increasing the number of particles per droplet, and therefore surface area of nucleator per droplet, will increase the sensitivity of the experiment to rarer INP. This enables quantification of lower INP concentrations."

Line 69: “volume of suspension used in each aliquot”: this formulation needs to be improved. The volume of suspension can be easily increased by diluting the suspension, but I guess this is not what is meant. Moreover, if filter samples are collected, it needs to be explained why extracting the filter with less water or evaporation of some of the water used for extraction is not an option.

We are not ruling out other methods of increasing the number of particles per aliquot of water, there may be other alternatives. We have focused on what we have done here. We have modified the section as follows: “The alternative approach is therefore to increase the number of particles within each aliquot of water. In principle, increasing the number of particles per droplet, and therefore the surface area of nucleator per droplet, will increase the sensitivity of the experiment to rarer INP. This enables quantification of lower INP concentrations” To increase the number of aerosol particles per volume of liquid the time period over which an atmospheric sample is collected can be extended, but in doing so temporal resolution would be lost. A method of increasing the sensitivity of an immersion mode technique is to increase the volume of the collected suspension used in each aliquot, while maintaining the concentration of particles per unit volume. This increases the number of particles per aliquot of liquid and therefore makes it more likely that rarer INP will be detected. The use of larger volume droplet suspensions has been exploited in the past e.g. (Vali, 1971; Bigg, 1953), and has been the strategy employed in the development of some recent instruments e.g. (Beall et al., 2017; Du et al., 2017; Stopelli et al., 2014; Conen et al., 2012).These large volume assays capture the rarer, more active INP but often miss the more abundant but less active INP. Hence they are frequently used alongside a smaller droplet instrument to achieve a complimentary dataset.”

Line 131: Where does the difference in set cooling rate and realized cooling rate come from? Why is this difference constant?

This cooling rate comes from a series of preliminary experiments with the justification being seen in the ramp rates for the IR-NIPI seen in the figures. The difference is likely constant as it is a lag between the temperature transfer from the cooled aluminium plate to the multiwell plate.

Line 152: What is meant by “aging of a sample in water”?

It has been demonstrated that for some materials ice-nucleating ability will change with time spent in water, sometimes over short periods of time. We have altered the text to clarify this and added references that demonstrate this behaviour. “This not only speeds up analysis, it also reduces the effect of any time-dependent aging processes such as the rapid deactivation of an albite sample suspended in water observed by Harrison et al. (2016).”

Lines 155 – 156: How representative is the surface temperature for the temperature distribution within the wells? Heat transfer simulations of Beall et al. (2017) performed for their multiwell setup showed temperature gradients within the wells. Please comment.

Yes, there are likely to be gradients in the wells, but our feldspar chip experiment suggest the gradients within the wells are not biasing results beyond the quoted uncertainty. The IR camera gave similar results to those of the µl-NIPI instrument where gradients in the droplet are thought to be negligible. In the IR-NIPI the feldspar chips are at the base of the well and our IR camera is reading the water air interface, yet we still see good agreement with the µl-NIPI system (within the T uncertainty).

It should also be noted that we did experiments with both polystyrene and polypropylene plates. Polypropylene is an order of magnitude greater thermal conductor than polystyrene. Between the two plates we saw no difference in freezing temperatures of NX-illite and the calculated offsets between the two experiments were similar (within the 0.6^{0}C standard deviation mentioned earlier). Hence, we again think that the gradients are minor in all cases.

We think this is discussed adequately in section 3.3.

Lines 164 – 165: The increase of the well temperature up to 0°C during freezing indicates a slow heat removal and limited thermal contact to the aluminum cold stage via the thermally conductive pad. From this, heating of adjacent wells is expected. Can you comment on this?

We expect to see a rise in temperature to equilibrium irrespective of thermal contact. The heat production during the initial crystal growth process is very rapid, much more rapid that the rate at which heat is dissipated for almost all droplet arrays even in µL droplets on an aluminium block. We do expect to see some heating of neighbouring wells but it is often minor and we do not have any quantitative data to describe the extent of this heating. However, you can see an example of the heating of neighbouring wells in Fig. 4A at ~2000 seconds where a well has frozen and the neighbouring wells increase in temperature. The use of individual temperature measurements of wells mitigates any impact this heating might have on freezing temperatures. In the caption we have added 'Note that the freezing events at ~2000s appear to cause some heating in the adjacent well.'

Line 168: how can you observe that the temperature maximum was reached within 1 s when you read out the IR camera only every 20 s? What do you mean by visual inspection?

We record an image every 20 seconds but the IR camera we use has a live screen display in which we can monitor the experiment. The text has been altered to reflect this "Visual inspection of the live screen display of the IR camera revealed that the temperature reached a maximum within 1 s. "

Line 173: Can you quantify the temperature differential between the cold stage and the aliquot?

We know that the cold plate is significantly colder than the water aliquots. This does not matter because we use the IR emissions to monitor temperature. As explained in the text the ramp rate is set to 1.3 °C min^{-1} (which results in a cooling rate of 1 °C min^{-1} in the aliquot) to account for a lag between the cold stage and aliquot. We do not think there is a need to quantify the offset of the coldstage and the aliquot any further as we measure temperature independently via the IR camera.

Lines 183 – 186: You mention several aluminum wells. How many did you test?

We used 16 in an experiment but only monitored 3 of them with thermocouples. This is now explained in the text: "We performed a number of experiments to test the IR temperature measurement calibrated using the above method. In the first instance we used highly conductive individual anodised aluminium wells for 50µL droplets. The temperatures of three of these wells were recorded independently using T type thermocouples embedded in the aluminium wells to give a representative temperature of the well and aliquot of water (see inset in **Error! Reference source not found.**)."

Lines 196 – 198: It would be helpful if you could add a figure illustrating the temperature gradient within the plate

The temperature gradient can be seen in figure 2a. This is now referenced in the text "As mentioned above there is a gradient across the entire plate (Fig. 2A)…"

Lines 204 – 205 and Fig. 4: From the scheme shown in the inset, results from 12 wells should have been measured, but in Fig. 4B results of only 6 wells are shown. Why? It would be helpful when you would label the wells shown in the inset with the numbers of the wells indicated in panel B.

Two wells measured with thermocouples were contrasted to three surrounding wells measured with the IR camera, so a total of six IR measurements were recorded. The schematic inset was to demonstrate that thermocouples were placed in the corners where the difference between the surrounding wells was at a minimum and that up to four of the surrounding wells could be used to compare to the thermocouple measured well. We accept the schematic is confusing and has been redrawn just to display the position of the wells observed.

Lines 215 – 216: By how many degrees was the freezing temperature reduced due to filtering of the Milli-Q water?

It looked to improve the baseline by ~2-3°C. This has been added to the text. "Filtering of the Milli-Q water to 0.2 µm reduced the temperature at which pure water droplets froze by 2-3°C. Sartorius Ministart, non-pyrogenic, single use filters were used for this (product code 17597-K)."

Section 3.3 and Fig. 6 (Feldspar chips): This section needs better explanations of the rationale and the execution of the experiment.

We have made section 3.3 clearer.

• You refer to Whale et al. (2015) for the setup but Whale et al. describes experiments with droplets containing a suspension of mineral particles and not with grains/chips. Please explain better the sample preparation of this study.

The reference in the text is for Whale *et al.* (2018) in which they describe this method. We later reference Whale *et al*. (2015) for the later stages of the experiment as the overall procedure of the experiment is explained better in this paper. This is clearer now in the revised section.

• How large were the grains/chips?

This is in the text as "each droplet contained a single ~100 µm sized grain of K-feldspar" but has been re-clarified later in the text as "A total of 19 grains were collected (~100 µm in size), assigned a number and their position tracked through the course of each experiment."

• How many droplets were deposited on a grain for the µL-NIPI experiment?

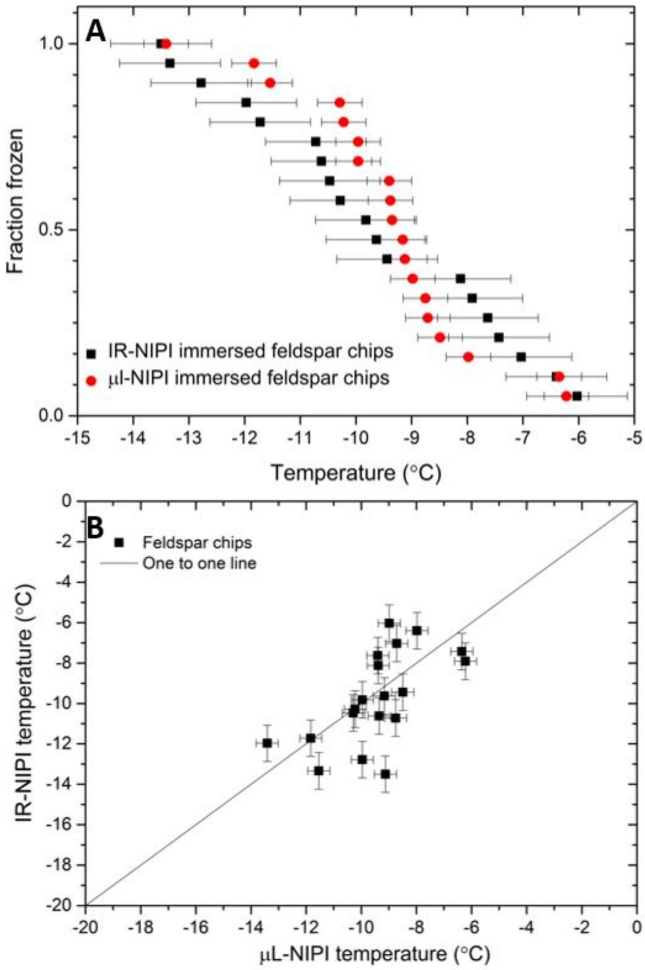
A single µL droplet was deposited on top of each grain. This has now been clarified in the revised text. "The grains were then used in the µL-NIPI experiment by placing the grains onto a glass cover slip atop a cold plate and pipetting a single 1 µL droplet onto each grain, before carrying out a standard µL-NIPI experiment."

• Are the results shown in Fig. 6 averages from all 20 selected grains? The "fraction frozen" label on the y-axis seems to indicate this. If yes: why did you select 20 individual grains if you lump all results together in the end?

The grains have not been averaged. Each droplet in the experiment contains a single grain and so there is a single freezing temperature recorded for each grain. The plot is showing the fraction frozen.

• You show 19 IR-NIPI (black squares) and 19 µL-NIPI (red circles) data points. Is there a correspondence with the individual chips? Is on each line of the plot a pair of IR-NIPI and µLNIPI results of the same chip? If this is the case, the labeling of the y-axis as fraction frozen is misleading. Rather, in this case, the y-axis should indicate a numbering of the grains.

We have include a separate plot in fig 6 to demonstrate this. We have now plotted IR-NIPI freezing temperature for a feldspar chip against the freezing temperature measured on the µl-NIPI. The one to one line is displayed along with the error in temperature for the two instruments. This can be seen in figure 6 below.



We have revised the text to the following in section 3.3: "The resulting fraction frozen plot for this experiment can be seen in Figure 2a and the corresponding correlation plot is shown in Figure 6b. The two instruments yielded similar fraction frozen curves and the individual feldspar grains nucleated ice at a similar temperature in both experiments. The correlation plot in Figure 6b shows that the freezing temperatures of a single grain were not identical in the two experiments, which is consistent with the stochastic nature of nucleation at active sites that have a characteristic freezing temperature (Vali, 2008; Vali, 2014). The agreement between the two instruments suggests that the temperature measurement and calibration of the IR-NIPI were robust and that there is no major temperature gradient within the aliquots in the multiwell plates."

The caption has also been updated to "**Figure 1. (A)** Plot of the fraction frozen curves for single feldspar particles per droplet in both the µL-NIPI (using 1 µL droplets) and IR-NIPI (using 50 µL droplets) experiments. The error bars display the error in temperature measurement on both instruments. **(B)** Shows the freezing temperature for the individual feldspar chips as measured by the IR-NIPI and µL-NIPI instruments. The one to one line is shown in bold and the error in temperature for the two instruments are represented by the error bars."

Why 19 and not 20 data points when you chose 20 grains?

This is a typo. We originally had 20 grains but one grain was dropped whilst transferring it over to the next experiment so only 19 grains were tested on both experiments. We have changed 20 to 19 in the text.

• What is the meaning of the error bars? Did you perform several freezing cycles and average? Did you average over several droplets on top of a grain in the case of µL-NIPI?

The Error bars are simply the errors associated with the temperature measurement. This has now been clarified in the figure caption. "**Figure 2.** Plot of the fraction frozen curves for single feldspar particles per droplet in both the µL-NIPI (using 1 µL droplets) and IR-NIPI (using 50 µL droplets) experiments. The error bars display the error in temperature measurement on both instruments."

• In some cases the error bars of IR-NIPI and µL-NIPI do not overlap. Any explanation?

This is consistent with the stochastic nature of nucleation at active sites. This is now discussed in the revised section 3.3.

• It is unclear why chips are taken and not suspensions with concentrations adjusted such that the mass of K-feldspar is the same for the 1 µl drops of the µL-NIPI and the 50 µl drops in the multiwell plate of the IR-NIPI setup.

We have improved the motivation of section 3.3. We have now added the following text "The purpose of this experiment was to have the same amount of material per droplet in each experiment and to have the material at the base of the droplet in order that the results from the two instruments could be directly compared. In doing so we could investigate the extent to which the gradient within the 50 µL wells might be a problem."

Line 259: ns(T) should be the same independent of concentration because it refers to one temperature and is per illite surface area present in a sample.

When we change the surface area of material in a droplet we change the n_s to which we are sensitive. We have modified the text to refer to surface area instead of wt% suspensions to help minimise confusion. "The results demonstrated good agreement with each other and exhibited the expected trend of the droplets containing smaller amounts of nucleant surface area freezing at lower temperatures and having higher $n_s(T)$ values than the droplets with higher amounts of surface area."

Line 300: 167 L: shouldn't 16.7 x100 yield 1670 L?

This was a typo and has now been changed to 1670

Line 300: Why is "filters" in plural? How many filters did you collect and measure?

One sample was collected and referred to here. Others were collected, but these will appear in a separate publication. The text has been altered to reflect this. "In order to demonstrate the utility of this approach for atmospheric aerosol samples, a filter sample was collected in Leeds as part of a field campaign held on the evening of the 5th November. A sample of atmospheric aerosol was collected using a Mesa PQ100 air sampler for 100 min. An inlet head with an upper cut-off of 10µm was utilised and air was sampled at 16.7 L min^{-1} on to a 0.4 µm polycarbonate track-etched Whatman filter, with a total of 1670 L of air sampled. The filter was then placed in to 6 mL of Milli-Q water and vortexed for 5 min to wash the particles from the filter and into suspension."

Line 310: specify what kind of modelling you mean here.

The text has been modified to "Since high-resolution regional modelling of the effect of INP on high latitude, cold sector-clouds suggests that 0.1 to 1 INP L^{-1} is a critical concentration and much lower concentrations still impact clouds (Vergara-Temprado et al., 2018), measurements with IR-NIPI will be extremely useful, particularly in environments with low INP concentrations."

Lines 317 – 318: this formulation should be improved

The text has been adjusted to "We demonstrate that IR thermometry is a sound method for determining the freezing temperature of 50 µL water droplets in multiwell plates. This method overcomes potential distorting influences such as thermal gradients across the plate, the effect of freezing wells warming surrounding wells and poor thermal contact to the underlying cold plate."

Line 333: Could you specify here how you intend to automate the system further.

We have ideas for the potential automation but would rather not specify them here. We realise this comment is vague and so have removed the following sentence from the text. "The use of the multiwell plates and the IR camera lends the IR-NIPI to the possibility of automating the system further and this is an objective for future work."

Figure 1: can you indicate in this figure how the Stirling engine chiller is connected to the aluminium cold stage?

The figure has been adjusted to show this. Please see below.

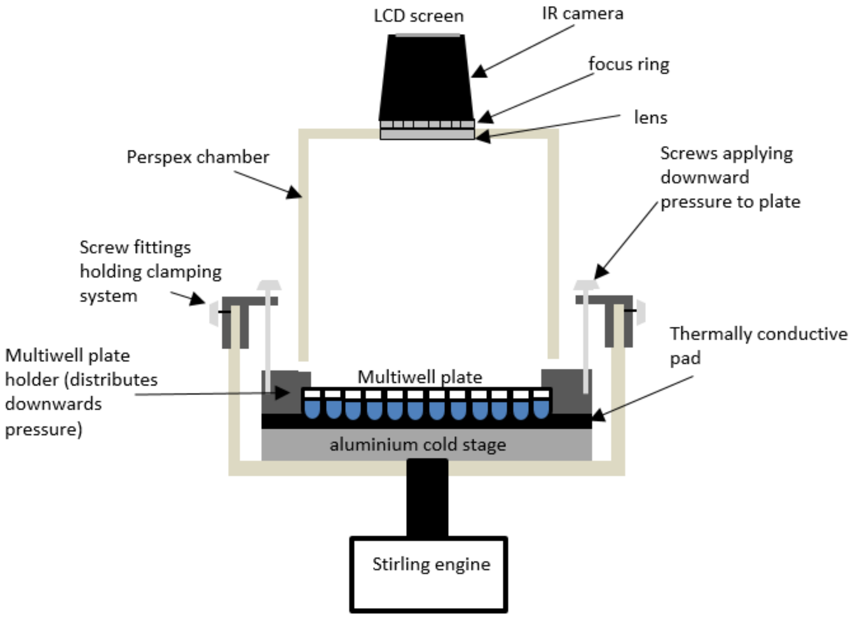


Figure 2B: one temperature ramp seems to be an outlier to warmer temperature. Do you know why?

We think the well associated with this temperature ramp had poor thermal contact with the underlying pad. An advantage of using the IR measurement of each well is that the influence of such issues is removed. The following has been added to the caption: "Note that one well had a higher temperature than the others, likely due to poor thermal contact with the aluminium substrate. By using IR thermometry to measure the temperature of each well individually such variability is accounted for."

Figure 3, inset: the water in the aluminum well is drawn as if it did not wet the well. Is this realistic? Was the aluminum coated?

The aluminium was anodised and was hydrophobic, hence this is realistic.

Figure 3, the figure caption of panel B needs to state explicitly whether T(thermocouple) – T(IR) is shown or the opposite. You might discuss the implications of the negative peaks observed in the residuals of panel B.

This has been clarified in the figure caption "The difference was calculated by IR(T) – Thermocouple(T). The negative spikes are a result of the IR camera directly reading the water temperature as it is heated by ice formation whereas the thermocouple measurement is reading the temperature of the aluminium well which is less affected by the latent heat release."

Figure 3, figure caption, line 523: "The point of freezing is highlighted in blue"… This statement is confusing since a whole area is indicated in blue. Do you mean "the area of freezing" or to which point do you refer?

This has now been corrected to "The range over which freezing occurs is highlighted with a blue rectangle as this is where the thermal properties of ice and the initiation of heat release affect the temperature readings."

Figure 4, figure caption, line 542: "A schematic diagram of the experiment is shown of the wells within a 96 well plate chosen for temperature checks." This sentence needs to be improved.

The text has been modified to read "A diagram of the wells within a 96 well plate chosen for the comparison of IR and thermocouple measurements is displayed as an inset."

Figure 4, the figure caption of panel B needs to be improved. Which wells are shown? Can you number the wells in the multiwell plate shown as an inset in panel A according to the numbering in panel B?

This is a good idea, we have adjusted the inset to correspond to the numbers in panel B and amended the figure caption accordingly. Please see below.

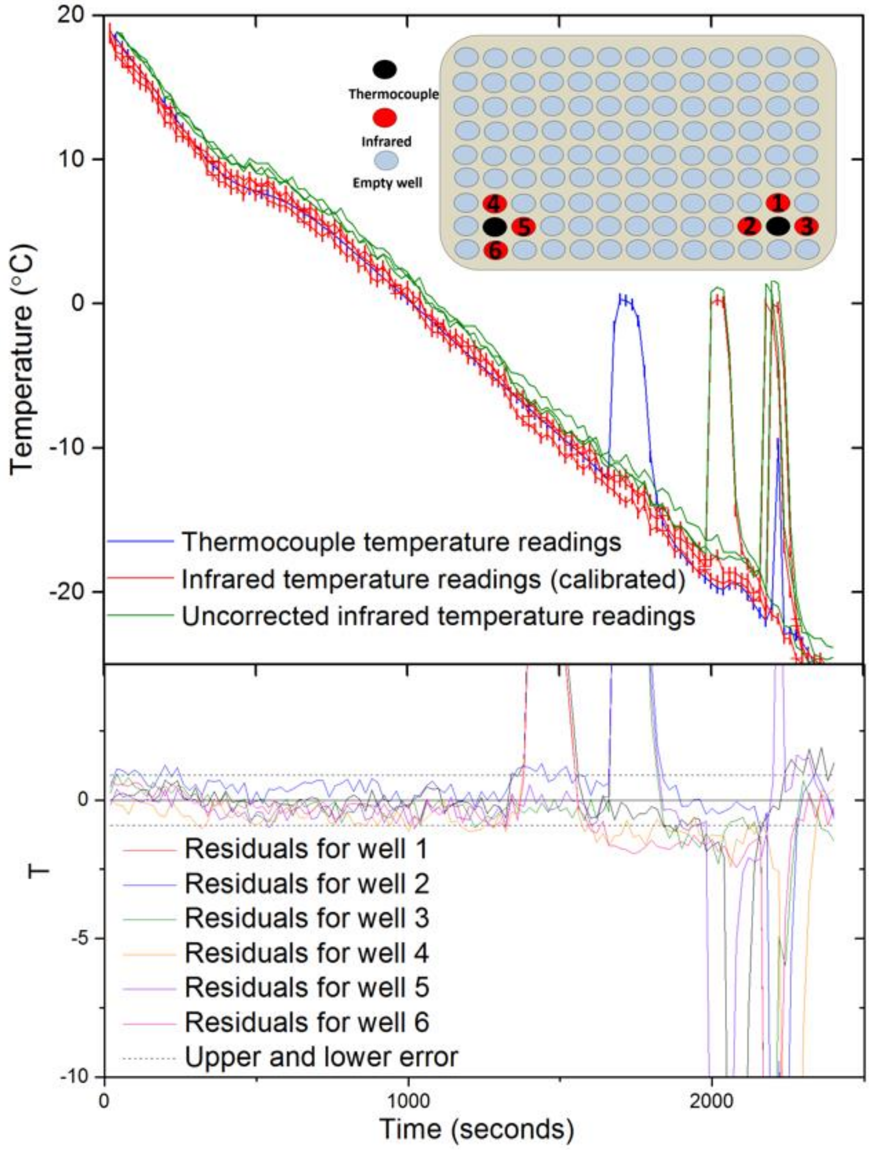


Figure 5: the y-axis has a strange scale with 5 digits between labels, implying a spacing of 0.083333333?!?! You might want to improve.

Thank you for pointing this out. It has now been adjusted to 0.1 increments.

Figure 5: the different blank curves should be given with different colors or symbols so that they can be discriminated from each other. For some blanks, the data points at low fraction frozen seem to be missing!?

We have created 3 separate panels for this figure to show the corresponding internal blanks with the relevant experiments. The main/original plot shows a culmination of representative blanks from this set up. The data points at low fraction frozen are not missing for the blanks. For the internal water blanks 12 wells were dedicated to a handling blank, hence the lower data density compared to blanks where we used all of the wells to test the water quality.

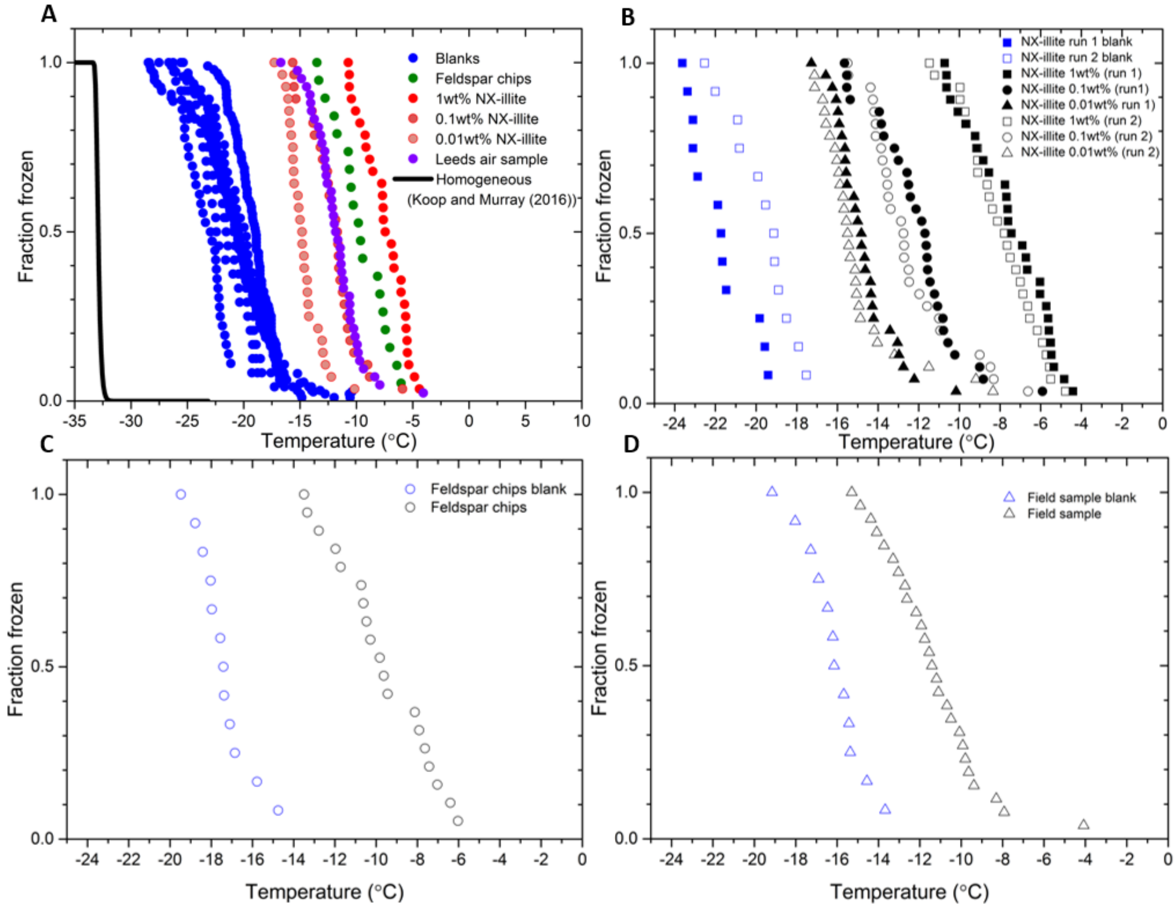


Figure 7A: The two darker blues and the two darker reds are difficult to discriminate. Please improve the color palette.

This has now been amended.

Figure 7A, figure caption: the error bars should be explained. Is this the error of ±0.9°C or the standard deviation between several freezing cycles?

The figure caption has been amended to address this. "The error bars represent the temperature error of ±0.9°C."

Technical comments

Line 49: "systems" should be replaced by "methods"

Done

Lines 73 – 75: "This instrument is part of the NIPI suite of instruments that includes the µL-NIPI and when used together these devices allow measurements to be taken over a very wide range of INP concentrations." Consider splitting this sentence into two

Changed to "This instrument is part of the NIPI suite of instruments that includes the µL-NIPI. When used together these devices allow measurements to be taken over a very wide range of INP concentrations."

Line 92: what is meant by "other expressions"?

Changed to "The fraction of the droplet population frozen throughout the explored temperature range can then be determined, from which the ice-nucleating active site density or INP concentration can be derived (Vali et al., 2015)."

Line 130: "Stirling engine chiller" would be more precise.

This has been changed to “The unique design, in combination with a Stirling engine-based chiller…”.

Lines 156 – 158: the wording of this sentence should be improved.

This has been adjusted to “This contrasts with the approaches adopted in other experiments where the temperature is recorded and assumed to be representative for all droplets, for example when employing a cold stage housing an embedded thermocouple whose reading is used to represent the temperature of the droplet array.”

Line 214: remove “see”.

Done

Line 220: The reference “Polen et al., 2018” is missing from the reference list.

Corrected

Line 269: delete “and is illustrated”

Done

Line 271: “takes advantage” sounds strange. Try rewording.

This has been reworded to “This material has also been investigated by Beall *et al*. (2017) using an instrument that also uses 50µL droplets: the Automated Ice Spectrometer (AIS).”

Line 274: “were”: you switch to past tense here, while before you used present time.

The text has been altered to be in present. “Both the IR-NIPI and AIS data are in good agreement with one another. It can be seen that the larger volume assays (IR-NIPI and AIS) give results towards the upper spread of literature data but are still consistent with other results (**Error! Reference source not found.**b). Dry dispersed techniques have also been plotted as unfilled blue squares in Fig. 7b, but none of these techniques are sensitive in the range of $n_s(T)$ seen by the large droplet instruments. The new data from the IR-NIPI has extended the dataset for NX-illite to warmer temperatures than in previous measurements, illustrating the utility of the technique.”

Line 281: “flowing”: do you mean “following”?

Yes, thank you. This has been changed.

Line 288: “suspended” instead of “suspend”.

Done

Line 300: “in to” should be one word.

Done

Line 305: in the equation it is “Nu(T)” not “Nυ(T)”. Please adjust.

Done

Lines 328 – 329: “an aerosol sample in the atmosphere of the city of Leeds” sounds strange. Improve formulation to e.g.: “an aerosol sample from the City of Leeds”.

This has been amended to “The utility of IR-NIPI for the analysis of atmospheric samples was also demonstrated by collecting and analysing an aerosol sample from the city of Leeds, England.”

Line 453 – 454: use abbreviation: Atmos. Chem. Phys.

Done

Legend of Fig. 6: “IR-NIPI” not “IR-NIP”

Thank you. This is now fixed