We thank referee #1 for his comments, questions, and suggestions, which are highly appreciated and have been taken into account upon revision of our manuscript. The comments and our answers are listed below.

Referee comment: The authors should stress what is the scientific innovation in their instrument given the very recent paper of Harrison et al. (2018), which was mentioned shortly in the end of the introduction section.

Author’s response: TINA studies ice nucleation in 960 microliter range droplets in one experiment, which enables the analysis of many samples or dilution series with good statistics in a short period of time. The cooling system allows a fast and high-precision temperature control over a wide temperature range at variable cooling rates. The infrared detection is an efficient method to doubtlessly determine freezing events, which was first applied to droplet freezing assays by Zaragotas et al. 2016. As discussed by Grothe 2018 (doi:10.5194/amt-2018-177-RC3, 2018), the authors of Harrison et al. 2018 attended several workshops and conferences and also have been the organizers for one conference, where our Twin-plate ice nucleation assay (TINA) with infrared detection was presented and discussed, so that our new setup was well-known to them.

Referee comment: Also, why infrared detector enables improved detection over other methods?

Author’s response: The infrared detector monitors the temperature of each droplet during cooling. As soon as a droplet freezes, latent heat is released and a sharp signal can be detected. For clarification, we modified the last sentence of paragraph 2 in section 1, where we replaced “improved” by “efficient”.

Referee comment: The ability of high-throughput experiments was mentioned repeatedly in the manuscript, and it will be valuable contribution if the authors could use their existing data to show if this ability is important.

Author’s response: TINA is suitable for high-throughput experiments because the instrument enables the study of ice nucleation in 960 microliter range droplets in one experiment, which enables the analysis of many samples or dilution series with good statistics in a short period of time. This is demonstrated in Figure 10, for which aqueous extracts of two aliquots of an atmospheric aerosol filter sample were treated in three different ways. All treated samples and untreated controls were measured in five different dilutions to provide the full ice nucleation spectrum for each sample. Each dilution was measured in 96 droplets, and every sample consisted of two aliquots. All in all, 4608 droplets were measured for Figure 10, which correspond to six experiments performed by TINA. For each freezing experiment down to -30 °C, TINA takes about 45 min, which means 4.5 h of operation of TINA for Figure 10.

Referee comment: I also wonder why error bars are lacking from all data and figures.

Author’s response: The uncertainty of the temperature sensor was used as the error of the temperature and was added into the figure captions. The error of the IN number concentrations was calculated using the counting error and the Gaussian error propagation, and error bars of the IN number concentrations were added into all figures.

The section 2.4 was optimized and the following paragraph was included:
"Assuming ice nucleation as a time-independent (singular) process, the number concentration of IN ($\frac{\Delta N_m}{\Delta T}$) active at a certain temperature ($T$) per unit mass of material is given by Eq. (1) (Vali, 1971a).

$$\frac{\Delta N_m}{\Delta T} (T) = -\ln \left(1 - \frac{s}{a - \sum_{i=0}^{s}}\right) \cdot \frac{c}{\Delta T}; \ 0 \leq j \leq a$$

(1)

where $s$ is the number of freezing events in 0.1 K bins ($\Delta T$), $a$ is the number of all droplets, $m$ is the mass of the particles in the initial suspension, $V_{\text{wash}}$ is the volume of the initial suspension, $V_{\text{drop}}$ is the droplet volume, and $d$ is the dilution factor of the droplets relative to $m$. The measurement uncertainty ($\delta \frac{\Delta N_m}{\Delta T} (T)$) was calculated using the counting error of $s$ plus one digit and the Gaussian error propagation (Eq. (3)).

$$\delta \frac{\Delta N_m}{\Delta T} (T) = \sqrt{\left(\frac{1}{s - \sum_{i=0}^{s}} \cdot \frac{c}{\Delta T} \cdot \sqrt{s+1} \cdot \frac{\sqrt{s^2+1}}{s}\right)^2 + \left(\frac{1}{s - \sum_{i=0}^{s}} \cdot \frac{c}{\Delta T} \cdot \frac{s \cdot \sqrt{\sum_{i=0}^{s+1}}}{a - \sum_{i=0}^{s}}\right)^2}$$

(3)

The cumulative IN number concentration ($N_m(T)$) is given by Eq. (4).

$$N_m(T) = -\ln \left(1 - \frac{\sum_{i=0}^{s}}{a}\right) \cdot c; \ 0 \leq j \leq a$$

(4)

The error of the cumulative IN number concentration ($\delta N_m(T)$) was calculated using Eq. (5).

$$\delta N_m(T) = \sqrt{\left(\frac{c}{1 - \frac{\sum_{i=0}^{s}}{a}} \cdot \frac{\sqrt{s^2+1}}{a}\right)^2}$$

(5)

According to the above equations, the uncertainty is proportional to the number of frozen droplets per temperature bin. In the freezing experiments described below, the lowest number of freezing events and largest uncertainties were obtained at the lower and higher end of each dilution series (Poisson distribution). Data points with uncertainties $\geq 100\%$ were excluded (overall less than 6% of the measurement data)."

Specific comments:

Referee comment: Line #26: It is stated that there is a good agreement with literature data. Where was this shown or detailed in the manuscript?

Author’s response: In section 3.2, we discussed the results of our experiments with Snomax®, which are shown in Figures 7 and S4. “These findings are in accordance with the results of Budke and Koop (2015).“ Here, we replaced “in accordance” with “in good agreement”.

In the same section, we also discussed the results of our experiments with Mortierella alpina, which are shown in Figures 8 and S5. “The cumulative number of IN and the initial freezing temperature of 268 K (-5 °C) are in good agreement with the literature (Fröhlich-Nowoisky et al., 2015; Pummer et al., 2015).”

Referee comment: Line #76: I think it is confusing: up to 10 K min-1 or more?

Author’s response: We tested our setup with continuous cooling rates of up to 10 K min⁻¹, but it is possible to run the setup at higher cooling rates. But it has to be considered, that for each cooling rate a new correction matrix has to be generated. For clarification, we deleted “or more”.

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Referee comment: Line #83: Is this the correct place to introduce the similar approach by Harrison et al.?

Author’s response: We deleted the sentence “Very recently, a similar approach for droplet freezing experiments with IR detection has been presented by Harrison et al. (2018), investigating K-feldspar, NX-illite, and atmospheric aerosol samples.” at the end of section 1, and we modified in section 1: “Infrared (IR) detectors enable efficient detection of droplet freezing (Harrison et al., 2018; Zaragotas et al., 2016).”

Referee comment: Line #94: Here it is not clear if the plates are commercial product or self-designed? If commercial, manufacture details should be specified.

Author’s response: We added the following sentence: “For each experiment, new sterile multiwell plates are used (96-well: Axon Labortechnik Kaiserslautern, Germany, 384-well: Eppendorf, Hamburg, Germany).”

Referee comment: Line #137: It confused me that it was cooled to 218.2 K and heated from 220.7 K?

Author’s response: To clarify this, we modified the sentence: “The temperature within the bath was cooled down from 303.2 K to 218.2 K (30.0 °C to -55.0 °C) in 5 K steps, warmed to 220.7 K (-52.5 °C), and raised again from 220.7 K to 300.7 K (-52.5 °C to 27.5 °C) in 5 K steps.”

Referee comment: Section 2.2: So what is the temperature uncertainty of TINA and how was it propagated?

Author’s response: The temperature uncertainty of TINA is 0.2 K. We added the following sentence at the end of section 2.2: “From the calibration measurements, we obtained a total uncertainty estimate of $\delta_{\text{total}} < 0.2$ K ($\delta_{\text{total}} = \delta_{\text{Thermistor}} + \delta_{\text{TC}} + \delta_{\text{Morti}}$).”

Referee comment: Line 144: I think it is still not clear at this point what is the temperature gradient you refer to. I would first defined that.

Author’s response: We included thermocouple measurements in the individual wells of the sample holder blocks to correct for a temperature gradient within the two blocks. We added the following paragraph at the end of section 2.2, and included three new figures (Figure 4, S1, S2, S3) while renaming the existing. “To determine a potential temperature gradient of the sample holder blocks, two thermocouples (K type, 0.08 mm diameter, Omega) were positioned in various wells of multiwell plates (Figure S1a/b), each filled with 30 µL pure water (see Sect. 3.1). These thermocouples were connected to the thermocouple in the elevation of each sample holder block, and the temperature offset between sample holder block and wells was measured for a continuous cooling rate of 1 K min$^{-1}$ (Figure S1c). Below -2 °C, the temperature offset between sample holder block and wells is nearly constant, in this example ~0.16 K and ~0.19 K. The measurement was performed in duplicates for all observed wells. Figure S2 shows the temperature gradient exemplarily for the 384-well sample holder block in a 2D interpolation based on all measurements.

To characterize the uncertainty of this measurement, the two thermocouples were placed in an ice water bath, and the sample holder block was cooled down to 2 °C, 1 °C, 0 °C, -1 °C, and -2 °C ($T_{\text{block}}$), while the difference between the ice water and the block temperature was monitored by the thermocouples ($T_{\text{diffTC}}$) (Figure S3). From these experiments, we obtained thermocouple uncertainties $\delta_{\text{TC}} < 0.05$ K ($\delta_{\text{TC}} = T_{\text{block}} + T_{\text{diffTC}}$). Additionally, we used undiluted IN filtrate of Mortierella alpina 13A (see Sect. 3.2) as calibration substance, and a freezing experiment was performed as described for the biological reference materials (see Sect. 3.2). These results were used to compensate for the temperature
gradient, and the thermocouple measurements were used to correct the temperature offset between gradient-corrected wells and thermistors. A correction matrix was calculated, and this matrix was used to correct subsequent freezing experiments. Figure 4 shows the results of the fungal IN filtrate measurement (a) before and (b) after correction. After correction, all fungal IN filtrate measurements showed a standard deviation of < 0.06 K ($\delta_{\text{Morti}}$). From the calibration measurements, we obtained a total uncertainty estimate of $\delta_{\text{total}} < 0.2$ K ($\delta_{\text{total}} = \delta_{\text{Thermistor}} + \delta_{\text{TC}} + \delta_{\text{Morti}}$)."

Referee comment: Line #151: please clarify why do you mention here Zaragotas et al. (2016).
Author’s response: We deleted the sentence “In contrast, Zaragotas et al. (2016) used infrared camera, which was calibrated only once by the company, to measure the accurate temperature of each droplet.”

Referee comment: Line #152: I think it would be nice if you will add the plate temperature at the different images.
Author’s response: We thank the referee for this suggestion, and we added the plate temperature at the different images.

Referee comment: Line #157: what is the resolution in which images are taken?
Author’s response: We added the information about the resolution of the images to section 2.3 to specify the method: “The camera has a resolution of 206 x 156 pixels, and it takes ten pictures per second. These pictures are averaged to one picture per second.”

Referee comment: Line #182: Are those new plates? or the same plates described earlier in the text?
Author’s response: We changed the sentence as follows: “For background measurements, 3 µL aliquots of autoclaved and filtered pure water were pipetted into new sterile multiwell plates by a liquid handling station.”
Moreover, we added this information in section 2.1.: “For each experiment, new sterile multiwell plates are used (96-well: Axon Labortechnik Kaiserslautern, Germany, 384-well: Eppendorf, Hamburg, Germany).”

Referee comment: Line #209: Please add a reference to this claim.
Author’s response: We assume that different plates from different manufacturers can lead to differences in freezing because of the production process and well shape, but cannot add a specific reference. For clarification we changed the sentence as follows: “The 96-well plates were obtained from a different manufacturer than the 384-well plates.”

Referee comment: Line #235: Is this correct? Class A only seen for high suspension concentrations.
Author’s response: We thank the referee for this comment. We removed the following text: “These differences result from three different classes of IN with different activation temperatures as described by Turner et al. (1990). Based on this classification, the Snomax® sample contains a large number of class A and C IN, but only a few IN of class B. These findings are in accordance with the results of Budke and Koop (2015). Below 259 K (-14 °C), a flat plateau arises where no IN are active.” and we included the following sentence: “These findings are in good agreement with the results of Budke and Koop (2015)”

Referee comment: Line #302: per liter air? Or liter water.

Author’s response: The IN concentration was calculated per liter air, which passed the filter during sampling. We included the following sentence: “All IN concentrations were calculated per liter air.”

Technical corrections:

Referee comment: Line #97: Fig. 1b should be describes before Fig. 1c.
Author’s response: Changed as suggested.

Referee comment: Line #165: add “is” after Vdrop, and m, and etc..
Author’s response: We modified the sentence.

Referee comment: Line #206: You can remove ‘respectively’.
Author’s response: This has been removed.

Referee comment: Line #209: ’showed’ and not ‘show’. Also found in other places in the text.
Author’s response: We replaced it in several places in the text.