

Authors' response to reviewers' comments for "The Microfluidic Ice Nuclei Counter Zürich (MINCZ): A platform for homogeneous and heterogeneous ice nucleation" by Florin N. Isenrich, Nadia Shardt, Michael Rösch, Julia Nette, Stavros Stavrakis, Claudia Marcolli, Zamin A. Kanji, Andrew J. deMello, and Ulrike Lohmann

We are grateful for Gabor Vali's comments and constructive suggestions that improved our manuscript. Below we outline our point-by-point replies and revisions to the manuscript. Page and line numbers refer to the uploaded document with tracked changes.

Reviewer #1: Gabor Vali

Comment

The instrument described in the paper is based on a good idea and it was built with care. The paper presents a thorough description in clear language appropriate for an AMT publication. The main novelty of this instrument is to separate the droplet production microfluidic device from the testing section where the cooling of the sample and the observation of freezing events takes place. The advantage derived is a better control of the sample temperature, minimizing internal temperature gradients that are the limiting factor to accuracy in some other droplet freezing devices.

On the production section, the choice of materials is crucial. This is well described in the paper but would find it helpful to clarify two things: Why is a surfactant (line 216) needed for a water in oil suspension? How are air bubble introduced (line 221) and why? In the end, are the water droplets in contact with the tubing and air, or also with some oil? How particle-free is the air? Is the surfactant likely to be covering the droplets in the test section?

Authors' response

A surfactant is needed to aid droplet formation and prevent the droplets from coalescing, especially at the outlet of the microfluidic device where the tubing is inserted. Surfactants are widely used (and needed) to stabilize the aqueous phase in microfluidic settings (e.g., Reicher et al. *Atmos. Meas. Tech.*, 11(1), 233, 2018; Tarn et al. *Micromachines*, 12(2), 1, 2021). One alternative to the use of surfactants is to physically restrict droplet motion, as reported by Brubaker et al. (*Aerosol Sci. Technol.*, 54(1), 79, 2019), but this physical restriction is not possible in the commercial PFA tubing that we use.

Regarding the air bubble, each syringe filled with support fluid (HFE or water) is pressed to first infuse the inlet PTFE tubing with the support fluid, and then the syringe plunger is withdrawn to take up a small volume of air. Each syringe plunger is withdrawn further to take up the primary fluid (either the surfactant–oil mixture or the aqueous sample). The air bubble only serves as a barrier between the support fluid and the primary fluid in the PTFE tubing. The air bubble remains in the inlet PTFE tubing and does not enter the microfluidic chip. In the end, the water droplets are in contact with the surfactant–oil continuous phase.

Change to manuscript

Page 8, lines 237–238: added "The air bubble remains in the inlet tubing and does not enter the microfluidic chip."

Comment

The precision of droplet sizes is indicated in Tables 1 and 2 in terms of the estimated variation in droplet diameters. The $\pm 5 \mu\text{m}$ amounts to about 6.5%. This translates into a volume variation of about 20% which is not negligible in the evaluation of the results. This is a greater limitation to the overall performance of the instrument than is acknowledged in the paper. The authors' comment on this would be helpful.

Authors' response

We need to clarify that the $\pm 5 \mu\text{m}$ uncertainty that we report is a measurement uncertainty, instead of a physical variation in droplet diameter. This measurement uncertainty arises from the resolution of the CMOS camera and the magnification of the stereoscope, with an uncertainty in droplet radius of 2 pixels equating to our reported $\pm 5 \mu\text{m}$ in droplet diameter. To more precisely investigate the droplet size distribution, we have now observed the droplet sizes during production on an inverted bright field microscope (Ti-E, Nikon, Switzerland) equipped with a $20\times 0.4 \text{ NA}$ objective lens and a high-speed camera (Phantom Miro M310, Vision Research, USA). The standard deviation in one droplet population was $0.5 \mu\text{m}$ around the mean droplet diameter based on measurements obtained using ImageJ (Schneider et al. Nat. Methods, 9(7), 671, 2012), corresponding to a variation in droplet volume of 2%.

Change to manuscript

Page 11, lines 300–310: “The accuracy of mean diameter measurements is estimated to be $\pm 5 \mu\text{m}$. This measurement uncertainty arises from the resolution of the CMOS camera and the magnification of the stereoscope, with an uncertainty in droplet radius of 2 pixels equating to our reported $\pm 5 \mu\text{m}$ in droplet diameter. However, the physical variability in droplet diameter for one droplet population is far less than this measurement accuracy. We independently monitored droplet generation on an inverted bright field microscope (Ti-E, Nikon, Switzerland) equipped with a $20\times 0.4 \text{ NA}$ objective lens and a high-speed camera (Phantom Miro M310, Vision Research, USA). We used flow rates of $Q_{\text{water}} = 1.0 \mu\text{L min}^{-1}$, $Q_{\text{surfactant}} = 1.5 \mu\text{L min}^{-1}$, and $Q_{\text{spacer oil}} = 2.0 \mu\text{L min}^{-1}$, the same as those used for the water experiment on day 1 (Table 1). The standard deviation of droplet diameter in one droplet population was $0.5 \mu\text{m}$ around the mean based on measurements obtained using ImageJ (Schneider et al., 2012), corresponding to a variation in droplet volume of 2%.”

Comment

The small droplet size and the immersion of the tubing in a liquid are the main features regarding temperature accuracy. However, mention is made of a stack of glass slips (line 163) being placed below the tube. How does this limit the flow of the cooling liquid around the tube and to what extent does it introduce further temperature gradients. Could this be clarified?

Authors' response

The ethanol in the cooling bath does not actively flow around the tubing, but rather, heat is removed by the Peltier element located below the aluminium container. As the thickness of the glass slides placed at the bottom of the bath is uniform, we would not expect any horizontal temperature gradients where the tubing is placed (as confirmed by the fact that the freezing temperature is not affected by the location of the droplets in the array, as shown in the Appendix). However, regardless of the presence of glass slides, a vertical temperature gradient will develop within the bath upon cooling. Therefore, it is crucial to place the thermocouples in the same plane as the tubing (see Fig. 1c) to ensure

that the measured temperature is representative of the temperature of the droplets in the tubing. The position of the thermocouples in the same plane as the PFA tubing is ensured by the use of grooves in the PEEK holder that keep the thermocouples in place.

Changes to manuscript

Page 10, lines 266–267: added “During cooling of the ethanol bath, a vertical temperature gradient develops from the bottom to the top of the bath.”

Page 10, lines 271–272: added “There are no horizontal temperature gradients, as confirmed by the fact that there is no spatial bias in freezing temperature (Appendix B).”

Comment

The spatial uniformity of temperature is demonstrated in Figs. B1 and B2. This display in terms of x and y coordinates` is somewhat unclear. Do both the x and y coordinates of all droplets in a sample are included? Probably yes. Also, is the x and y coordinate system given with respect to the internal dimension of the test chamber? A simple change to using the distance from the walls would be easier to understand.

Authors' response

We thank the reviewer for this suggestion, and we have therefore made the following changes to the manuscript.

Changes to manuscript

Pages 20–21: changed the x-axes of Figs. B1 and B2 to illustrate distance in millimeters instead of pixel coordinates. Additionally, we have reduced the size of the symbols to better discriminate between droplet locations.

Page 6: modified the schematic of the ethanol bath in Fig. 1b to include an outline of the field of view to help orient the reader.

Comment

The results and comparisons to other works are presented as the fraction frozen versus temperature. This is a straightforward manner of showing the results. However, it is specific to the volumes of the sample in the experiment. For even slightly polydisperse populations of drops the function loses generality and makes the calculation of the nucleation rate J for homogeneous freezing contain an error. It also influences the comparison of the three runs with microcline, as, according to Table 2, the drop volumes were about 20% larger for run 1 than for runs 2 and 3. The volume-dependence makes the $FF(T)$ functions inadequate for comparisons with other experiments. It is not clear if any adjustments were made in Fig. 5 to overcome the problem.

In any case, this problem with the volume-dependence is not critical for this AMT paper. It would be more important for a science paper. To fully account for the volume variations in the samples is not a trivial matter. For the comparisons with literature results an appropriate caveat regarding the constant-volume assumption is probably sufficient. A more thorough step to bring results of different experiment on a comparable basis is conversion of the FF data into spectra (eq. 4 in Vali, G.: Revisiting the differential freezing nucleus spectra derived from drop-freezing experiments: methods of calculation, applications, and confidence limits, **Atmos. Meas. Tech.**, 12, 1219-1231, doi: 10.5194/amt-12-1219-2019, 2019.).

Authors' response

We agree that the effect of volume on the frozen fraction of droplets should be considered when frozen fractions are converted to nucleation rates. In a forthcoming publication, we show that the effect of small variations in volume is negligible for the homogeneous nucleation rate. For heterogeneous nucleation, we assume that the variability in the particle surface area per droplet most probably exceeds the aforementioned effect expected from variations in droplet volume. However, this effect could vary from one ice-nucleating particle type to another depending on its size distribution, and it needs to be assessed for the specific particle in question. Thus, we agree with the reviewer that the effect of volume variations warrants further investigation for heterogeneous nucleation rates, which we will consider in future work. As suggested, we add a short note for the reader to be aware of this effect when interpreting the reported frozen fractions.

Change to manuscript

Page 16, lines 462–464: added “We note that further interpretation of the frozen fraction and detailed theoretical analysis, such as calculation of particle surface area per droplet, may require considering the potential influence of variation in droplet volume, as outlined in, for example, Vali et al. (2019).”

Comment

Regarding the image analysis process. As described it is a demanding process. Is there some future improvement possible so that the apparatus would really become as user-friendly as it aims to be (line 134)?

Authors' response

For the present version of the apparatus, image analysis is indeed demanding, but future work is planned to tackle this problem. Because the difference in contrast between a liquid droplet and a frozen droplet depends on the polycrystallinity of the emerging ice phase, in very pure water, the ice phase has very few grain boundaries that scatter light. The contrast improves markedly when the water contains solutes (i.e., the droplet becomes much brighter). We aim to develop a fully automated image analysis algorithm based on the semi-automated approach described in this manuscript.