Reviewer's comments on the manuscript by Thomas Häusler, Lorenz Witek, Laura Felgitsch, Regina Hitzenberger and Hinrich Grothe "Heterogeneous freezing of super cooled water droplets in micrometre range- freezing on a chip"

The manuscript of Häusler et al. describes a droplet-freezing assay designed for characterization of ice nucleating particles (INP) in immersion mode. The novelty of the instrument is the method of substrate preparation and small droplets size from 20 to 80 µm. The small droplet size could potentially result in a large assay dimension, allowing for simultaneous observation of several hundreds of droplets and therefore providing excellent counting statistics. Furthermore, the authors claim that the cavities can be filled with aqueous suspension in a single sweep, which altogether could make the apparatus very attractive for routine measurements in the field of atmospheric science and cell biology.

A freezing assay is undoubtedly the simplest and cheapest technique allowing for characterization of INPs, compared to cloud chambers and continuous flow diffusion channels (CFDC). It has, however, quite a number of caveats that has to be taken into account when planning the experiment and analyzing the results. Recently, a number of assay-based setups have been described in the literature in great detail, including several instrument intercomparison studies (see Hiranuma, 2015, Wex, 2015). The main problem identified in these intercomparison studies is the discrepancy between the dry particle methods (where a droplet was condensing on an INP prior to freezing) and suspension methods, where INPs were placed in water suspension and then cooled. On top of this problem come various issues of water purity, influence of the substrate, cooling rate dependency, effect of immersion oil, droplet size issues, concentration issues, time dependency, etc. Given the recent amount of efforts in this field, one would expect a thorough analysis of the expected caveats pertaining to a new instrument. I do not find such analysis or mere discussion of the caveats in the manuscript. Instead, while reviewing the previous studies, the authors focus their attention on the technical drawbacks of oil-suspended droplet assays, ignoring all other studies where freezing of droplets ensembles has been analyzed using classical nucleation theory and statistical analysis.

Lacking such characterization of the experimental setup, the manuscript gives nothing more than a technical description of the instrument and a few example measurements. As such, this manuscript obviously belongs into the "Atmospheric Measurements Techniques" and I would strongly suggest resubmitting the manuscript there (after addressing the critical issues). Alternatively, the authors might choose to provide missing details and instrument characterization according to the specific comments given below. To my opinion, the manuscript is not acceptable for ACP in its current form.

General remarks:

1. Several recent studies addressed freezing of INP suspension droplets placed either in individual compartments (Budke, 2015), or placed on a substrate (Whale, 2015, Harrison, 2016, Peckhaus, 2016). These studies used suspensions of INPs prepared with different concentrations to cover the broad range of freezing temperature. In all these studies, it was clearly shown that freezing temperature is a function of cooling rate and average number of INP particle per droplet. Other studies (Hader, 2013, Wright, 2013a, Wright, 2013b) have shown significant effect of cooling rate on the freezing curves, and Herbert, 2014, and Peckhaus, 2016 have shown that this behavior is consistent with the stochastic theory of ice nucleation. None of these studies is mentioned in this manuscript. The conclusion of instrument "accuracy" (Abstract, Conclusion, and elsewhere) is drawn based on the comparison of median freezing temperatures, but the data used for this comparison has been obtained with droplets of different sizes and different concentrations of INP (Figure 9 of the manuscript)! It is also not clear how accuracy can be derived by comparison to other instruments? These issues have to be fixed.

2. Peckhaus (2016) has used up to 1000 identical 0.2 nL droplets deposited on a silicon substrate and coated with oil. It was shown there that pure water droplets froze homogeneously and no effect of substrate on ice

nucleation has been found. The setup used by Peckhaus (2016) has much in common with the instrument described in this manuscript (droplet size, automatic detection of freezing events, use of a substrate and oil coating), and a comparison might be instructive for the reader. It would be interesting to explore the role of etching on the heterogeneous freezing, as no effect on the monocrystalline silicon substrate was observed in their study (I am referring to your statement "*Tests of an uncoated silicon plate indicated that the silicon itself is IN active.*" (page 5 line 25). Consider using their SBM-based parameterization scheme to compare your measurements with the literature data.

3. The whole discussion of droplet volume dependence is a mystery to me. Clearly, changing the size of the droplet containing suspended INPs would change the total surface available for critical ice embryo formation. The same effect can be achieved by changing the concentration of INPs. The median freezing temperature measured with two droplet assays can only be compared if droplets of the same size and the same concentration have been cooled down with the same cooling rate until freezing. To compare results obtained with different setups and under different conditions, the ice nucleation community came up with the notion of ice nucleating active surface (INAS) site density (ns), which authors introduce (equation 1), but don't use. By carrying out the freezing experiments with the different concentration of INPs and using the INAS density parameterization a much wider range of temperature can be accessed (see Budke, 2015, or Wex, 2015). Is there any reason for not using this approach? Is that because the volume of the droplets has not been measured? The only information that is provided is the cavity diameter (20 to 80 µm) which is not sufficient since the cavity depth and geometry is not given.

4. Give the reader more information on the method of spreading of particle suspension. Can you be sure that no particle residuals are left on the surface between the etch pits? What is the variability of INP concentration inside the etch pit introduced by spreading? Have you tried to analyze the residuals in the pits: is the distribution homogeneous? If not, what is the mass distribution? These issues have to be addressed if you plan to resubmit the manuscript.

5. The size of a single etch pit would allow for tremendous assay dimensions. Taking the pits center-to-center separation distance of 0.1 mm (as estimated from the figure 6), one would be able to create 10.000 etch pits on a 10 by 10 mm chip. I see, however, only 24 cavities in the video frame shown in figure 5 and this is appr. The number of individual data points on the freezing curves. What is the reason for not using more of the cavities? Is that because of the limited field of view of the microscope or difficulties with the freezing detection at lover magnification? Please discuss these issues.

6. Provide the value of the cooling rate for all presented experiments. I estimate the cooling rate to be around ~ 8K/min (from the temperature and time readings of the supplemental video). Such cooling rate can be responsible for up to 1 K difference in T50 (Wright et al. (2013) and Herbert et. al. (2014), Peckhaus (2015).

7. The claim of automatic detection of the freezing events is not very convincing. Indeed, the liquid and frozen droplets shown in figure 6 are distinctively different. Looking at the video record of the freezing droplets available in the Supplement, I doubt that an automatic routine is capable of capturing the freezing based on the change of brightness, at least not in the given example. If I compare frames 1 and 2 captured from the Supplemental video (see my figure 1 below), the difference in individual droplets is hardly detectable by eye, and yet all of them must have been frozen at this temperature. If I am wrong, please provide in the supplement the "contrast graphs" for each droplet from the supplemental video to convince the reader that the automatic software is indeed capable of capturing the individual freezing times.

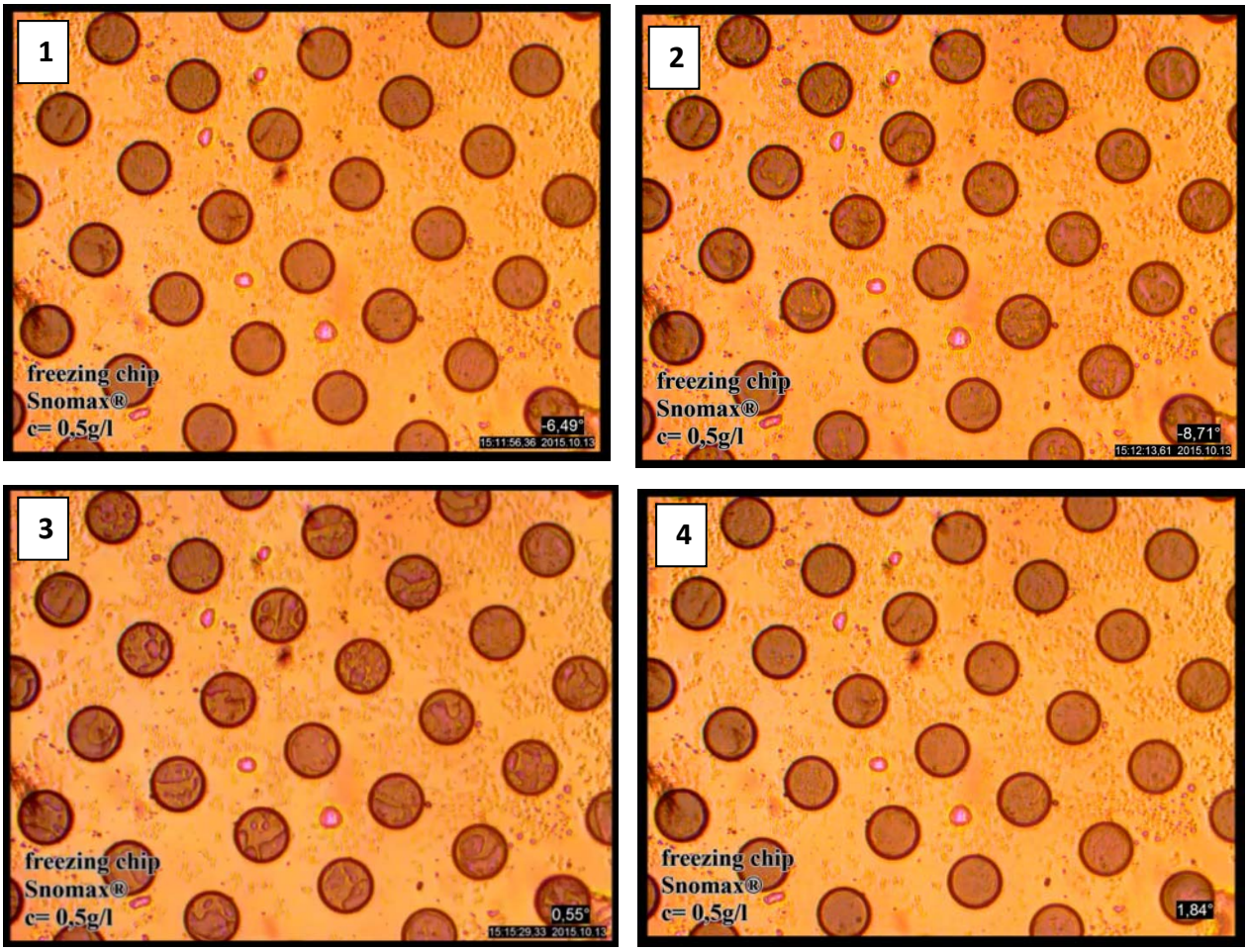

Figure 1. Individual frames captured from the supplemental video.

8. Such high cooling rate can be responsible for false temperature reading. The frame 3 of my Figure 1 (above) recorded at 0.55°C still shows some structures in the etch pits, which are obviously absent in the frame 4 recorded at 1.84°C, where all droplets are frozen. There must be a time lag in the temperature measurement, caused by thermal inertia of temperature sensor, thermal contact between the sensor and the chip, or response time of the readout electronics. Please check carefully your data.

Specific comments:

1. The authors put such a strong emphasis on not using the oil matrix that it is rather surprising to see some solid inclusions in their own oil layer covering the chip (Supplemental video, particulate movement on the right side of the frame starting from 15:12:07). I suppose these are particles left on the chip surface after spreading the suspension?

2. What would be the cleaning procedure for the chip between the measurements? How durable is the gold coating?

3. Abstract, page 1: “Finally, it opens a temperature window down to -37°C for freezing experiments which was not accessible with many former approaches and allows determination of IN also with weak nucleation activity”. Contrary to your statement, the freezing curves you show occupy a very narrow temperature range

(within 2 K, except for Juniper pollen), indicating a very concentrated solution and a high cooling rate. To demonstrate applicability of the instrument for measurements of the INPs with weak nucleation activity you would have to do measurements with diluted samples and present your results in form of INAS surface site densities.

4. Page 3, lines13-14: “INP distribution and surface site location are random events which do not involve time sequences and therefore behave according to the singular model”. I do not understand this statement. Neither distribution of INPs between the droplets nor the distribution of active sites on the total surface of INPs in the droplets are “events”, that is, they are not distributed along the time axis. Anyway, the singular hypothesis is not about that, it is about prescribing certain fixed freezing temperature to each individual active site. By the way, singular hypothesis does not predict cooling rate dependence of median freezing temperature.

5. Page 3, line 26. Is the reference to Stan et al., 2009, correct here? There is nothing about free-falling droplets in this work. Please check.

6. Page 4, lines 3 to 9. I count only four main problems with the oil emulsions, a) to d).

7. Page 6, line 14. Could you provide more information on the microcline used in your study? Any microcline contains a certain amount of Na-rich feldspar, and the microstructure can be very different. Harrison (2016) compared several feldspars and found a tremendous spread of freezing efficiency. Any additional information would be useful, like place of origin, composition, crystal structure (via powder diffractometry) etc. How the grain size has been determined? Have you measured the effective surface, e.g. via BET? Otherwise, comparison with another “microcline” is rather useless.

8. Page 8, lines 16 – 18: “Different milling parameters result in variable surface textures (as e.g. cracks, edges and steps) which play an important role in the INA of microcline. The higher freezing temperature found in our experiment might be due to varied milling parameters.” This is a speculation unless you compare the freezing efficiencies in terms of n_s(T). The difference may result from the fact that this was a different “microcline”.

9. Page 8, lines 26-27: “Wex et al. (2015) worked with Snomax® concentrations of about 0,5 g/L (the same as here), but they generated droplets with diameters about 1200µm…” Wex et al., (2015) has derived the n_m(T), a number of ice active entities per mass of dry Snomax, which is the mass-based analogue of n_s(T). In the intercomparison study of Wex et al. (2015) the data of seven different instruments fit perfectly into a very narrow range disregarding all different experimental methods and measurements conditions. Their work is a good example of how the experimental data should be treated and I strongly recommend using their approach to compare your data to experiments of other groups.

10. Page 9, lines 22 -23: “We were able to show the efficiency and accuracy of our setup by comparing the measurements of freezing temperatures of different INPs with already published results (Figure 9).” Again, accuracy cannot be demonstrated by comparing your results with the literature data. Accuracy (or more precisely, “uncertainty”) is a measure of your confidence in the reported results, obtained from the measurements according to certain mathematical rules. This reminds me of the fact that there is no discussion of the measurement uncertainty in the whole manuscript, apart from the error bars that are visible in the figure 9. The values of median freezing temperature, however, are given with a precision of 0.1K (see Conclusion section). This has to be changed.

References:

Hiranuma, N., et al. (2015). A comprehensive laboratory study on the immersion freezing behavior of illite NX particles: a comparison of 17 ice nucleation measurement techniques, Atm. Chem. Phys **15**(5): 2489.
Wex, H., et al. (2015). Intercomparing different devices for the investigation of ice nucleating particles using Snomax (R) as test substance, Atm. Chem. Phys **15**(3): 1463.

Budke, C., et al. (2015). BINARY: an optical freezing array for assessing temperature and time dependence of heterogeneous ice nucleation, Atmos. Meas. Tech. **8**(2): 689.

Whale, T.F., et al. (2015). A technique for quantifying heterogeneous ice nucleation in microlitre supercooled water droplets, Atmos. Meas. Tech. **8**(6): 2437.

Harrison, A.D., et al. (2016). Not all feldspars are equal: a survey of ice nucleating properties across the feldspar group of minerals, Atmos. Chem. Phys. **16**(17): 10927.

Peckhaus, A., et al. (2016). A comparative study of K-rich and Na/Ca-rich feldspar ice nucleating particles in a nanoliter droplet freezing assay, Atmos. Chem. Phys. Discuss. **2016**: 1.

Hader, J.D., et al. (2013). Contribution of pollen to atmospheric ice nuclei concentrations, Atmospheric Chemistry and Physics Discussions **13**(12): 31673.

Wright, T.P., et al. (2013a). The role of time in heterogeneous freezing nucleation, J. Geo. Res. A. **118**(9): 3731.

Wright, T.P., et al. (2013b). Minimal cooling rate dependence of ice nuclei activity in the immersion mode, J. Geo. Res. A. **118**(18): 10.

Herbert, R.J., et al. (2014). Representing time-dependent freezing behaviour in immersion mode ice nucleation, Atmos. Chem. Phys. **14**(16): 8501.