Author's response to Anonymous Referee #2
Below we provide in blue-colored font a point-by-point reply to each comment.

The authors have assembled an experimental setup to grow ice crystals on a sample surface and developed a method to locate where ice forms to investigate the topographical features on the underlying surface by atomic-force microscopy. While the setup could prove useful to study ice growth on surfaces, the intended use to study ice nucleation mechanisms requires a higher vertical resolution to detect small ice crystals and pinpoint the location of ice active sites. In addition, much better control of temperature and relative humidity in the mixing chamber is needed. I think such could be achieved and encourage the authors to improve the setup towards this direction.

We thank the reviewer for their detailed review of our manuscript and helpful comments.

We would like to clarify that our manuscript is intended to detail our approach of collocating ice formation/growth locations observed optically to the sample surface structure observed by AFM. If the interpretation is that we are monitoring ice formation with AFM, we apologize for the lack of clarity and emphasize that we only observe ice optically. We agree that with optical observation, which is ultimately wavelength-limited to at least a micron, it is impossible to pinpoint ice nucleation sites with the necessary resolution of less than 10 nm. Other approaches in the literature that have ostensibly studied ice nucleation have a similar resolution limit due to the risk of beam damage. The ESEM approach also does not control the humidity but infers the local humidity around a growing ice crystal through relating the ice growth rate to the local supersaturation. This calculation can be carried out in our work as well, but due to the network of ice which rapidly forms on the surface, the humidity varies substantially across the surface and with time. Determining a representative humidity at these length-scales is not trivial and is obviously not a number but a landscape. We respectfully contend that the main message of the paper stands without investigating the humidity in great detail at this stage in development. Afterall, we are not studying kinetics of ice nucleation or crystal growth where the exact supersaturation is necessary for drawing conclusions, but instead we are relating sample surface structure to a pathway of water vapor condensation and ice growth.

<u>Specific comments</u>
Page 1 line 16 Heterogeneous ice nucleation is not limited to temperatures above -36°C. Deposition ice nucleation relevant for cirrus cloud formation occurs at lower temperatures and below water saturation. As the experimental setup described in this manuscript might become useful to investigate deposition ice nucleation, I recommend mentioning it here in the introduction.

We have included this point in the discussion and outlook, "The setup described here is also applicable to studying deposition mode nucleation at sub -36 °C and sub-saturation (relevant to cirrus cloud formation), and optical observations of immersion mode nucleation on substrates."

Page 1 line 24 The parametrization by DeMott et al., 2010 is not based on size as an ice nucleation property, but simply relates the concentration of INP to the concentration of particles above a threshold size, not implying that only these particles act as INP. This is often misinterpreted, please revise.

We changed "based on macroscopic properties like aerosol size" to "relating the concentration of INPs to the concentration of particles above a threshold size".

Page 1 line 26 Useful parametrizations should capture various situations. Please elaborate and provide references supporting the claim that the mentioned parametrizations are not accurate outside the conditions for which they were developed. Also, surface site density of ice active sites derived from field measurements and laboratory studies have been used to parameterize ice formation in models eg., Vergara-Temprado et al., 2017. This could be mentioned.

We changed this sentence to "However, as is true for extrapolations in general, such models are expected to be less accurate when applied to conditions outside the range of measured values used to fine tune these models,", and included the reference to Vergara-Temprado et al.

Page 1 line 31 Please specify what kind of information microscopy can provide to distinguish mechanisms of ice nucleation.

By imaging the surface structure at an ice nucleation site, microscopy can provide additional information regarding condensation versus direct vapor deposition mechanisms if pores, cracks, or stepped structures are resolved or not. To further clarify this point we have modified the end of this sentence to read, "innovative microscopy aimed at uncovering the local structural and chemical properties of ice nucleation sites is needed.".

Page 2 line 3f It is unclear how the 10nm size is derived. Given the resolution of light microscopy, pixel size etc., used in the current setup it seems unrealistic to detect such small objects, making the discussion of framerate and its dependence on temperature and humidity conditions irrelevant. What is the smallest detectable size in the current setup and what is the limiting component?

This part of the paper presents an introduction to the challenges faced by using high resolution microscopy to study ice nucleation events. It is not a discussion of our approach, or our results. The 10 nm size is an order-of-magnitude benchmark based on the discussion in the first paragraph relating to critical-nucleus size. The value is used to exemplify the scale which non-optical microscopy techniques must be able to resolve, and at high frame rates, to truly observe ice nucleation at a definitive site.

Page 2 line 15 Clarify how this estimate was made. The resolution is 1.6um? This seems not to be high enough to see growth of 1um crystals. In addition, I calculate at least 10-times longer growth needed to reach this size at this conditions. The mentioned growth rate indicates a RH>>100% and questions the control of relative humidity in the experiment. Ice growth can be used to infer humidity in the specimen chamber (see S3 in Kiselev et al., 2016). I highly recommend a comparison of relative humidity based on ice growth rates and the method used by the authors to determine humidity.

We give an estimate of growth velocity for these structures, treating the projected area as an effective circle, only to serve as an example of the speeds involved in our observations of ice growth. Our growth rate is based on measurements made when the crystal is resolved optically (greater than 1um) then extrapolated to represent small length-scales. The growth habits we observe are polycrystalline, highly branched crystal clusters, whereas the velocity equation used by Kiselev et al., 2016 assumes an atomically flat spherical surface.

Page 2 line 16f Please elaborate how high-speed AFM can advance heterogeneous nucleation research.

HS-AFM can advance heterogeneous nucleation research by directly observing nucleating crystals with 10 nm resolution, or less, by way of high frame-rate AFM. This has the potential to yield the best nucleation site location accuracy of the techniques currently available. We added to the manuscript: "… if emerging ice crystals could be observed directly with ~10 nm resolution, thus improving significantly the accuracy of locating nucleation sites."

Page 2 line 20 How accurate can the site of ice formation be located with this setup? It is mentioned on page 2 line 1 that the spatial resolution must be on the order of nanometers to locate the ice nucleation site. Please derive the minimum resolved distance for your camera system and verify with a resolution target. A discussion of what accuracy would be desirable in contrast to what can be achieved would be helpful to clarify down to what scale the setup can be sensitive.

In our setup, we are ultimately limited by the wavelength of visible light used for optical microscopy. No light-based microscopy – including our own – would be sufficient to specify a nucleation site with any accuracy.

Page 2 line 22 Surface features on a feldspar specimen of the size used in this study might not be present on micrometre sized dust particles found at mixed-phase cloud level, and therefore be not relevant for ice nucleation on these particles. I recommend not to emphasize atmospheric relevance.

The morphological features we analyze in this paper are simple surface steps, which are ubiquitous and expected to be abundant on most if not all feldspar dust particles. However, the small size and the complex morphology of these aerosol particles makes it impossible to acquire high-resolution AFM data and isolate the role of individual surface steps. Also in response to reviewer #1, we added text to the manuscript intended to clarify the atmospheric relevance:

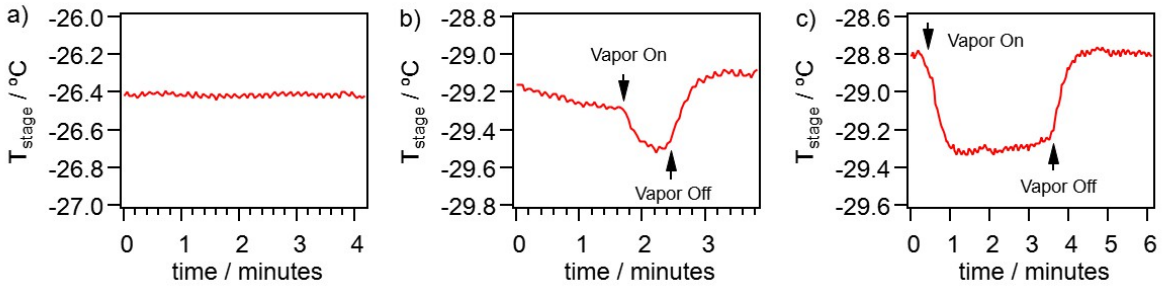
Before the last sentence of the introduction we added: "*While studying aerosol particles collected from the atmosphere would provide a more direct connection to atmospheric conditions. The typically complex structure and chemistry of these particles often precludes identifying the individual nanoscale processes that are important. For this study, instead, we choose extended flat substrates of known composition on which the role of individual topographic features can be examined.*"

And to the discussion and outlook section we added: "*In the discussed example of ice formation, the step-height analysis is used to corroborate the involvement of the liquid phase of water during the observed rapid formation and propagation of ice on feldspar…"*

Page 3 line 1ff Provide a temperature calibration to demonstrate the stability (1°C/hr mentioned in Sec.2.2.), accuracy of temperature control and homogeneity in the mixing chamber. Temperature control is crucial to study ice nucleation and therefore the interpretation of observations made with the setup. Please clarify if temperature is actively controlled or only monitored with the TC-720. Active temperature control is desirable for this type of setup.

The temperature control in this work is passive and monitored by the TC-720 controller. Typical experiments last less than 60 seconds (vapor flow on-to-off time). While we agree that strict temperature control is important when quantifying nucleation kinetics, we are not engaging those experiments here. The 1°C/hr stability figure was regularly observed during setup of experiments,

however it was not recorded. In lieu of this data we show the stability of the stage temperature over the minutes timescale for three cases: a) without vapor flow, b) during a short vapor flow experiment, and c) a long vapor flow experiment. In section 2.2 we have revised the paper to remove the mention of 1°C/hr, and instead include a discussion regarding the temperature variation during an experiment, "During the course of an icing experiment the temperature measured at the sample stage decreases by approximately 0.5 °C due to the diverting of a portion of cold gas back to the stage when the vapor flow is turned on."



Page 3 line 11 Ice and mixed-phase clouds form at a variety of conditions. Ice clouds do not require water saturated conditions. Specify conditions that can be crated in the mixing chamber.

Because the final humidity in the chamber is a mixture of vapor and dry flows, in principle the full range of humidities below saturation can be explored by adjusting the relative flow rates.

Page 3 line 17f How is frost formation in the mixing column prevented?

Over repeated experiments we do eventually form ice in the small mixing column portion of the cell. The small size of the column leads it to become clogged quickly once ice forms. This then leads to no flow into the cell. We remedied this by clearing out the cell with warm dry nitrogen between experiments.

Page 3 line 20f Advantages compared to what other technique? What can be learned from using different flow rates?

Control over total flow rate is useful in practice for various reasons. Large flow velocities can disrupt growing ice crystals that may be weakly bound to a substrate. On the other hand, if one were limited to slow flow rates this would cause long delays when changing cell conditions from, for example, low humidity to a desired humidity. The flow rates one might require will be substrate-dependent and also will depend on the conditions one is attempting to create within the cell (e.g. turbulent, laminar, etc.).

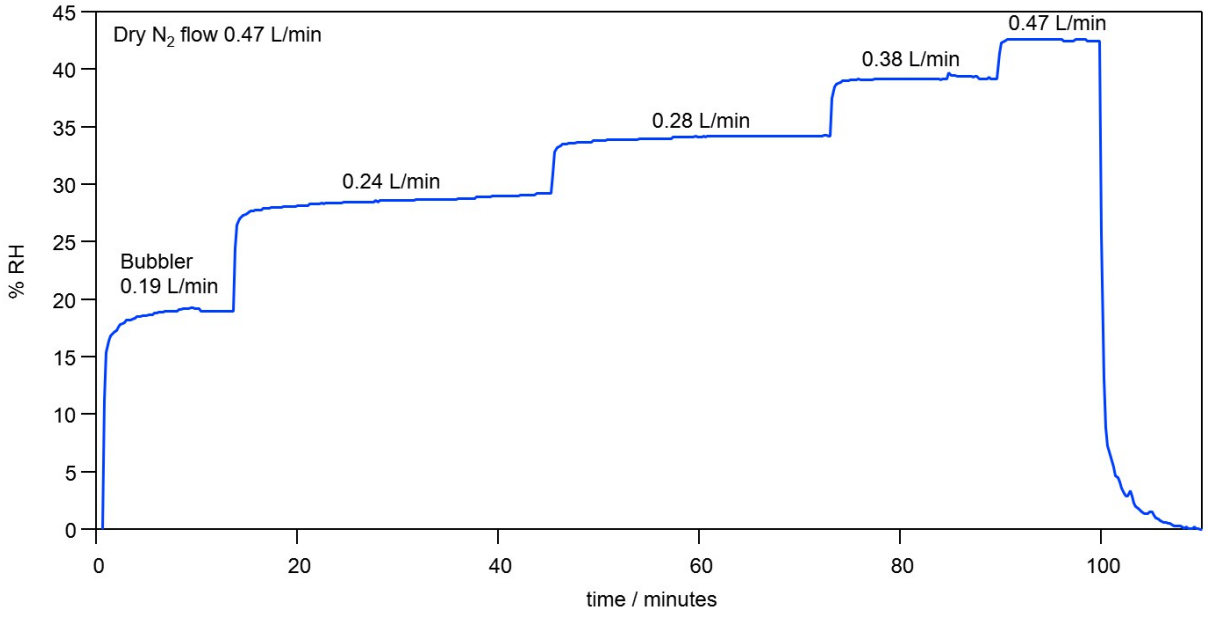
Page 3 line 23f Please explain why thermal gradients are minimized by that.

In a cold-stage only setup the gas above the sample is cooled by the stage. This results in a gradient in temperature from the gas-sample interface upwards. Here we have minimized this gradient by also flowing cold gas into the chamber so the sample is cooled from above and below. To make this clearer we modified this sentence to "*In our setup, a cold atmosphere flows into the small ~ 30 µL volume cooling the sample from above and below, thus minimizing temperature differences laterally and from the sample-gas interface upwards.*"

Page 3 line 32 Please provide exemplary time series of temperature and relative humidity during an experiment. What is the purpose of switching the wet flow on and off? Could the humidity sensor be used to measure humidity in the outlet flow to verify the humidity in the chamber?

Page 4 line 1 How long does it take to reach steady-state humidity? To vary the humidity in the mixing chamber the flow through the bubbler is adjusted. Does this change steady state? Provide measured humidity after the bubbler as function of flow rate. Another strategy to adjust humidity in the wet flow might be to change the temperature of the bubbler.

The vapor flow is turned on and off to control the generation of ice during an experiment. We did not use the outlet to measure humidity because the humidity sensor time to steady state is long compared to the experiment. This is partly due to the capacitive sensor used for RH measurement as well as the volume around the sensor that must be exchanged. The humidity exiting the chamber over time is also convoluted by the ice growth within the small chamber volume, further compounding the inaccuracy of the final RH measurement. As an illustration, we include here the measured humidity at the outlet of the sample chamber at room temperature under similar conditions to typical experiments. Varying the flow rate of the bubbler changes the proportion of vapor mixed into the constant dry nitrogen flow. Changing the flow rate also changes the time with which steady state is reached. For example, in the data shown below, at the lowest bubbler flow rate the effective sensor time constant is approximately 45 seconds, while at the highest bubbler flow the time constant is about 20 seconds. These times are far too long for the typical duration of an icing experiment which is less than 1 minute.



Page 4 line 9ff Knowing and controlling the relative humidity (RH) in the experiment is essential for interpretation of results and to infer the ice nucleation mechanism. Calibration of relative humidity should be done much more carefully by eg., using a dew point mirror to measure humidity in the outflow of the chamber. While AH might be useful to determine flow rates of the wet flow, chamber conditions should be reported as relative humidity and temperature. Convert AH to RH throughout the manuscript.

We opted to provide the estimated AH input to the cell rather than a RH because the RH is unknown at the surface location where we observe the ice formation/growth. Since we are not calculating quantitative kinetic parameters related to ice nucleation we show here the trend for increasing humidity without declaring that we have accurate knowledge of the actual humidity where the events take place.

Page 4 line 19 Converting the error in AHin of 0.08g/m3 to RH gives +/- 18% which is a very high uncertainty for ice nucleation experiments.

Please see above.

Page 5 line 1 AHin reported here and considering the uncertainty given on the last page, relative humidity is equal to RHw= 85% +/- 18%. Conditions above water saturation are within the experimental accuracy, making the interpretation of the data as purely deposition ice nucleation imprecise. This underlines the point made in the comment above, that control of the experimental conditions is insufficient for ice nucleation experiments. Compare estimated saturation conditions against calculation based on ice crystal growth rate or measure the humidity at the chamber outlet.

Please see above.

Page 5 line 3 Couldn't AFM detect pores on the substrate? What is the horizontal resolution of AFM used here?

The AFM can routinely detect features down to sub-10 nm in size. However, the optical and temporal resolution of our camera is not adequate to observe an ice nucleation event – it can only record the subsequent growth of the ice crystals. Therefore, any attempt to associate a point on an AFM image with an ice crystal's nucleation site will have an error of several microns.

Page 5 line 5 "Ice formation" instead of "ice nucleation" would be more accurate.

We agree with the referee and we have changed this in the text.

Page 5 line 12ff What is discussed here is ice growth and not ice nucleation. Inferring ice nucleation mode from this observation seems over-reaching. The two processes (ice growth and ice nucleation) should be separated more clearly throughout the manuscript.

We agree and have changed this from "mode" to "pathway".

Page 6 line 14 All four humidities applied are high above water saturation (RH=134%, 167%, 201%, 234%). It is surprising to see sensitivity of ice formation on the amount of supersaturation in this high humidity regime other than a change in growth rate. As pointed out in the discussion, different grow rates are a more plausible explanation for the observation than the probability of ice nucleation. The context in which the experimental results are interpreted should be clarified. Is it about ice growth or ice nucleation mechanisms?

The RH at the surface is clearly not given by the numbers mentioned above, otherwise the cell would be full of water. This is why we provide the input AH to convey the trend in humidity without claiming precise knowledge of the actual humidity at the surface. The benefit of using humidity greater than

saturation is that it reveals very clearly the ability of surface steps to provide pore-like condensation channels. If we limited our experiments to RH at saturation, the limited water content in the channels would be quickly dehydrated once ice is formed nearby, making it difficult to observe the ubiquitous nature of the water channels on the surface. The larger context of our results is that by combining surface topography with optical microscopy we can better define the pathway to ice formation on surfaces.

Page 7 line 5 Please provide the resolution of the current setup. Is the CCD pixel size limiting the resolution?

We are limited to about 1.6 um resolution, which is partly a function of the NA of our long-working distance objective. We are not limited by the CCD pixel density.

Page 10 Fig.2 check if there is a mix-up between e), d). The description in the figure caption seems to be switched. Images show a scale bar of 5um and this seems to be a typical scale how accurate ice formation can be located. In the introduction it is correctly mentioned that ice nucleation occurs on structures with a scale of few nanometres. Features in eg. e) are on a 1000-times larger scale, questioning the interpretation as ice nucleating sites.

The scale bars here are correct. We modified the caption to "… *(e) Expanded view of a portion of panel (d)…".* We did not intend to claim that we are able to pinpoint ice nucleation sites. We circle the area within which nucleation occurs and subsequently and ice crystal emerges. We do not claim to have isolated the precise site.

Page 10 Fig. 3 replace AH with RH (=167% +/- 18%).
Page 12 Fig. 6 b) replace AH with RH (=134%, 167%, 201%, 234% +/-18%) .

Please note the discussion above. We are currently unable to precisely define the RH at the surface and throughout the experiment, and therefore have opted to provide the approximate AHin for each run.

References

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Kiselev, A., Bachmann, F., Pedevilla, P., Cox, S. J., Michaelides, A., Gerthsen, D., and Leisner, T.: Active sites in heterogeneous ice nucleation-the example of K-rich feldspars, Science, 355, 367–371, https://doi.org/10.1126/science.aai8034, 2016.
Vergara-Temprado, J., Murray, B. J., Wilson, T. W., O'Sullivan, D., Browse, J., Pringle, K. J., Ardon-Dryer, K., Bertram, A. K., Burrows, S. M., Ceburnis, D., DeMott, P. J., Mason, R. H., O'Dowd, C. D., Rinaldi, M., and Carslaw, K. S.: Contribution of feldspar and marine organic aerosols to global ice nucleating particle concentrations, Atmos. Chem. Phys., 17, 3637–3658, https://doi.org/10.5194/acp-17-3637-2017, 2017.