

Response to Comments

Direct visualization of solutes in laboratory ice samples

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Comments from H-W Jacobi (Referee):

The authors present an experimental study investigating the location of impurities in ice samples produced in the laboratory from aqueous solutions applying different freezing procedures. The ice samples containing either CsCl or 4,5,6,7-tetrachloro-2',4',5',7'- tetraiodofluorescein (or Rose Bengal) were analyzed using μ -computed tomography, where the presence of air bubbles and the concentrations of the impurities were related to the absorption of the applied x-ray radiation. The ice samples were generated by placing liquid solutions in a freezer leading to a freezing from the exterior to the interior or by freezing from the bottom to the top or by immersion freezing with liquid nitrogen. The presented results indicate important differences concerning the distribution of the impurities in different compartments of the ice samples. The authors distinguish for the condensed phase two compartments: the solid ice matrix with low impurity concentrations and liquid like regions (LLR) with aqueous solutions containing impurities at the solubility limit of 2.7 M in the case of CsCl. The μ -CT images allow further identifying if the LLRs are located inside the solid matrix or if they are located at an interface with air bubbles. The distribution of CsCl in the ice samples are not surprising: immersion freezing leads to larger fractions of the impurities incorporated in the solid ice matrix, while the slower freezing enables a more significant formation of LLRs. These experiments constitute the first experimental evidence of the impact of the freezing method on the impurity distribution in ice samples created in the laboratory. This technique has the potential to constitute a new standard for the characterization of ice samples containing impurities that are used for laboratory experiments concerning chemical reactions in ice and snow. Such reactions are important in polar regions, where they contribute to the formation of reactive nitrogen oxides and halogens inside the snow and the subsequent release to the atmosphere. However, the exact mechanisms of the reactions and how they are modified in the different compartments of the snow or ice grains are currently not well understood and can only be studied in laboratory experiments if the impurity distribution in the samples is known. Therefore, the manuscript reports important new findings and techniques that deserve publication in The Cryosphere. Nevertheless, before the publication of the manuscript I suggest addressing the comments described below.

Page 8: The authors propose that observed radio densities are composites of the densities of pure water ice and region with a solute ion concentration of 5.4 M, which is related to freezing point depression. This number directly affects all calculations using equation 1. How do uncertainties in the concentration translate into errors of the results? What about the error of the slope of the calibration curve shown in Fig. 1 and the errors of the radio densities for air and ice? I believe a more detailed discussion of the experimental and statistical errors and how they impact the results is needed.

We have expanded the discussion of the errors associated with uncertainties in the solute ion concentration the LLRs. Errors in the LLR solute concentration would scale the findings, changing V_{LLR}/V_{VOXEL} for our results. There is some uncertainty in the LLR solute ion concentration, perhaps as great as 20%. This error is considerably larger than the error in the slope of the calibration curve and the

radiodensities for air and ice, and we have therefore discussed it more extensively than the other sources of error.

The authors claim that the maximum concentration of 5.4 M in the LLR is well below the CsCl solubility. However, its solubility at -10 °C is not known. Are there any measurements of the temperature dependence of the CsCl solubility indicating that even at -10 °C it may not be below the assumed maximum concentration? If not, could the authors determine the solubility with the μ -CT using saturated solutions at different temperatures?

We were unable to locate data on the solubility of CsCl at -10 °C. However, one of the reasons we chose CsCl as our primary test compound was its high solubility in water. According to NIH (NIH, 2015), the saturation concentration of CsCl in water is approximately 9.6 M at 0 °C and 11.1 M at 20 °C. Assuming the saturation concentration change is linear with temperature, the saturation concentration at -10 °C would be 8.9 M, still well above the estimated LLR solute concentration of 2.7 M. Note that 5.4 M refers to the total solute concentration; not the concentration of CsCl.

Page 8: The authors distinguish regions with LLR volumes either smaller or larger than 10 % of the total volume. In my opinion, this is only a gradual difference. What is the reason to identify these two categories?

We agree with the opinion of the reviewer, as shown in Figure 3g, the LLR volume in a particular voxel can range from zero to above 90%, with no particular importance associated with fractions above or below 10%. We chose 10% as the dividing line because it allowed us to show how areas of highly concentrated solute (>10%) were more common in the freezer samples, and also spatially more likely to surround air bubbles; the choice of actual percentage was arbitrary.

Page 9f: According to the authors the degassing of the samples with helium leads to a reduction of the volume of the gas bubbles by 50 % compared to the air-saturated samples. I assume that degassing the aqueous samples with helium would only lead to replacing the dissolved nitrogen and oxygen by helium without modifying the gas volume. The degassing is a standard procedure for laboratory experiments. Is there any evidence in the literature that the volume of dissolved gas is actually reduced by a factor of 2 by degassing with helium? This may depend on the solubility of the gases nitrogen, oxygen, and helium. Did they author verify if the solubility of these different gases can explain such a difference in the dissolved gas volume?

There is some evidence in the literature that degassing with helium does reduce the volume of dissolved gas by around a factor of 2 (Snyder, 1983). The degree of degassing does depend on the solubility of gases involved. Assuming a solution in equilibrium with air at 25 C, the mole fraction solubility of air (assuming a composition of 20% oxygen and 80% nitrogen) is 1.4×10^{-5} , while for helium it is 7.0×10^{-6} , or almost exactly half the concentration of air in the solution. This difference does explain the observed reduction in bubble volume in the helium-degassed solution. We have added this calculation to the paper.

Page 13: The authors claim that the obtained results were robust and reproducible. However, Table 1 appears to show only results from one sample for each freezing method. I recommend including data of all experiments (for example in an additional table in the Supplement) and in Table 1 average numbers including some statistical information to provide information on the uncertainty of the experimental results.

Both generating the microCT imaging, and particularly segmenting those results into air, water ice, and LLRs, requires considerable effort. We were not able to evaluate as many samples as we would have liked, focusing instead on studying a range of sample conditions. We have included the histograms in

Supplemental Figure S10 as a way to provide some insight into sample reproducibility by comparing the raw (unsegmented) greyscale values from three identically-prepared samples.

Table 1: It remains unclear to me how the numbers for example of the CsCl Mass Fraction are calculated. According to my understanding the observed radio densities for each voxel can be translated into a CsCl concentration for each voxel using the calibration curve in Fig. 1. With the known voxel volume the total mass of CsCl for each volume can be calculated. The sum of the CsCl mass for the three material types (water ice, LLR 2-10%, LLR > 10 %) would give the total mass of CsCl present in each material type. In this way an absolute CsCl mass would be obtained that can then be used to calculate fractions if compared to the total CsCl mass in all three material types. Is that the procedure that was applied? If yes I recommend presenting absolute CsCl mass for each material type instead of mass fractions.

We used essentially the process described, with one change. We first segmented the imaged sample into four materials (including air bubbles), as described in the Methods section. For the two material types containing regions of concentrated LLRs (LLR 2-10% and LLR > 10%), we determined the total volume of the material type, as well as the average CsCl concentration in that volume. From these two pieces of information, we calculated the CsCl mass present in each material type. We could not directly measure CsCl present in the bulk ice, because the radiodensity for CsCl containing voxels overlapped with the radiodensity distribution for pure water ice. However, because we knew the volume and concentration of the initial solution, we knew the mass of CsCl present in the overall system. From that information, we calculated the CsCl mass present in the bulk ice by subtracting the overall original mass from the mass present in the other two materials.

Page 8, Fig. 2 and 3: The authors state that the radio density of air is 3996. Using this value and $RD_{ice} = 4848$ leads to a V_{LLR}/V_{Voxel} ratio of -0.034. How is it possible that a significant number of voxels can have V_{LLR}/V_{Voxel} ratios below this value? In fact, shouldn't be the segmentation: Radio density up to 3996: voxel with only air; radio density between 3996 and 4848: ice with air bubbles; radio density above 4848: ice with CsCl? What happens with voxels that include at the same time air bubbles and LLR and both smaller than the resolution of the voxel? Do they give an average radio density signal that let them appear as solid ice without impurities?

Visualization of a pure material using the micro-CT does not yield a single radiodensity value, but rather a distribution of values. While 3996 is the average radiodensity of air (page 8), some values are greater, some are less. This effect is visible in Figure 2c, where the air distribution (smaller left peak) overlaps the ice distribution (larger right peak). So, for pure air voxels, half should have V_{LLR}/V_{Voxel} values below -0.034, half greater. Deconvolution of these values is challenging; in fact, as the reviewer notes, a voxel containing an air bubble and LLR might well have an average radiodensity close to that of pure water, and be incorrectly categorized as such. We have attempted to discuss some of the uncertainties around this issue on pages 8 and 9. In addition, higher resolution imaging, such as Figure 4 and Supplemental Figure 7, can help resolve these “border” questions.

Page 14f: The authors describe some results of their experiments using plastic vials presenting only some videos and pictures in the supplementary material. If the authors do not present a quantitative analysis like for the experiments with the glass vials, this remains more or less anecdotal and can be deleted.

While we agree the results are qualitative, we do believe the plastic vial discussion could provide some useful guidance to other researchers who may not have considered the importance of sample container.

The authors present the concentrations using molarities (mol/L). Wouldn't it be better to use molalities (mol/kg) to avoid the impact of density changes on the concentrations?

Because the micro-CT imaging is done on a volumetric basis, we believe expressing concentrations in molarities was the most appropriate unit. We did our standard curve calibration knowing how much solute was present in a particular volume of solution. By then imaging an identical volume of pure water (or ice), we could determine how the radiodensity of that volume changed when various amounts of solute were present.

I think it should be mentioned in the abstract that μ -CT was used.

We agree, and have revised the abstract.

There is a series of publication by Heger et al. (e.g. J. Photoch. Photobio. A, 187, 275–284, 2007 or J. Phys. Chem. A, 109, 6702–6709, 2005) addressing also the location of impurities in ice samples, but using completely different techniques. I recommend discussing briefly these studies in the introduction.

We have discussed these studies in the introduction as suggested.

Page 3, lines 13ff: “As the snowpack consolidates, chemical compounds can remain at the surface of the crystals, or become trapped internally at grain boundaries or triple junctions”: I think this sentence is somewhat misleading because the mobility of the impurity is rather linked to processes at the snow grain scale and not at the snowpack scale. Thus, it rather depends on the metamorphism than on the compaction of the snowpack.

We have clarified the wording of the sentence to state “As the snowpack consolidates and snow grains metamorphose, chemical compounds can remain at the surface of the crystals, or become trapped internally at grain boundaries or triple junctions (Domine et al., 2008; Grannas et al., 2007).”

Page 3, lines 24ff: “photon fluxes can vary . . . possibly within crystals themselves.” Any evidence for this statement?

McFall and Anastasio (McFall and Anastasio, 2016) found slight enhancement of photon flux in ice crystals versus aqueous solutions, which suggests variation within the crystals may be possible. We have revised the text to include this reference.

Page 4, line 6f: “. . . with solutes in QLLs somewhat surprisingly having less mobility compared to solutes in LLRs.” Is that really surprising assuming that the QLL may be only a few layers of water molecules?

We agree and have removed the phrase “somewhat surprisingly”

I recommend include at least once the correct technical terminology for the compound “Rose Bengal” in the manuscript.

We have added the correct chemical name for Rose Bengal.

Comments from Anonymous Referee # 2

I found this paper interesting and easy to read, and agree with the authors that it is important that the effect of sample preparation method is considered when interpreting the results of analyses on laboratory samples. This article is relevant to The Cryosphere and its readership, demonstrating the results of a laboratory study on experimentally grown ice.

I would recommend that this article be published, after minor revision. I found several areas where I would have appreciated more information and/or discussion on both the choice of sample preparation methods, and the discussion of results. I have split my recommendations into General comments/Specific comments and Typographical comments.

General comments

Introduction/Methods

From the introduction as it is written, it is unclear to me why these three sample preparation methods have been chosen. The authors suggest that their results are relevant to solute positions in snowpack, but the sample preparation procedures here - freezing directly from a liquid solution to a (presumably crystalline) solid - do not seem to be immediately comparable with snowpack formation. Additional discussion of snowpack formation mechanisms, and/or justification of these sample preparation methods, would improve the paper, clarifying the reasoning behind these experiments. (Have these sample preparation methods been used in previous experimental studies? Does the resultant structure represent snowpack well?)

We have revised both the introduction and methods sections to explain the choice of freezing methods, and to refer to text in the results section discussing expected freezing behavior.

Why were CsCl and Rose Bengal chosen? Are these solutes present in real snowpack? If not, what is the reasoning behind using them, are they expected to behave like the real solutes? (And what is the evidence (with references) for this?)

CsCl was chosen because of its high aqueous solubility (suggesting all the material will be present in LLRs, rather than precipitated into the ice matrix) and large Xray absorption cross section (enabling ready detection and quantitation in the microCT). Rose Bengal was chosen for similar reasons. While these solutes are not commonly found in snowpacks, we chose them because they represent surrogates for some common compounds in snowpacks, such as sodium chloride (with CsCl as a surrogate) and polycyclic aromatic hydrocarbons (with Rose Bengal as a surrogate). Clearly, two model compounds cannot reflect the diversity of materials found in natural snowpacks. However, based on the structure of the materials, we believe they will behave in ways similar to other solutes in snow, and can be used to guide further research.

The samples frozen are of different sizes (Table 1). Is it possible to compare samples of different volume? Will the greater volume of the Freezer and LN2 samples not also affect the freezing behaviour?

Much of the behavior we have noted in our work, such as the association of solutes and air bubbles, seems to be independent of volume. While it is possible the greater volume of the Freezer and LN2 samples may have some minor effect on freezing behavior, we have seen no evidence to suggest sample volume would significantly affect sample morphology. In some cases, we conducted identical experiments in two different volumes, and saw similar patterns in freezing morphology independent of

sample volume.

If I understand correctly, you make the assumption with your calibration curve that the effect of CsCl on the radiodensity of water and ice is the same. And so all results based on this method are dependent on this assumption. What is the support behind this assumption? (Page 7).

Measured radiodensity is a function of the physical density of the material (as shown by the comparison of ice and water radiodensity on page 7) and the electron density of the atoms present in the material. Because electron density is an atomic-scale property, we believe CsCl electron density would remain the same in both ice and water. In the case of the physical density of either the CsCl-water or CsCl-ice mixtures, it is possible that the presence of CsCl could affect the density of these preparations differently. In order for such a difference to be noticeable in our work, however, the density change would have to be significant in both ice and water, and in the opposite direction (for example, with the addition of solute to ice making ice more dense, while solute-containing water were less dense). In addition, because of the low concentrations of solute in our experiments, we do not believe any density differences will have a noticeable impact on our results.

Discussion

Were there any measurements of the grain size/shape of the ice in these samples? And the position of the pores/solutes with respect to grain boundaries? Might the grain size (likely controlled by the freezing temperature and subsequent annealing) play a role in the position/size of solute pockets?

We did not attempt to measure grain size or shape, nor the location of pores or solutes relative to grain boundaries. We do believe grain size (and morphology) could play a role in the position and size of solute pockets. Unfortunately, we do not currently have the equipment to identify and study individual grains; we hope to address this question in the future.

The experiments are carried out with a high concentration of solutes (with respect to inland snows). Additional discussion as to the impact of this higher concentration on the results would be enlightening – will a lower concentration have similar results?

We have not yet investigated whether lower concentrations of solute will alter the freezing pattern. We agree such work would be enlightening, however. We chose our test concentration (1 mM) as a compromise between solute concentrations in natural snowpacks and the need to have enough solute present for easy visualization.

I find the result that solutes are often associated with air bubbles in the Freezer samples, but less so for Freeze Chamber samples, very interesting – do you have any ideas of potential mechanisms which would be working differently in the two situations?

We also find this an interesting observation. We do not have a clear idea why this is the case, but suspect it is related to freezing speed. In the Freezer samples, the ice matrix may form slowly enough to allow the ice matrix to exclude both gases and solute to the same compartments. In the more quickly frozen Freeze Chamber samples, the ice matrix may form in such a way to allow smaller inclusions, which may preferentially form for either solute or gas, but not for both.

Specific comments

Page 3 Line 27. Define PAH at the first usage.

Corrected.

Page 4 Line 4. “the cage effect at a given temperature”. It is unclear what the cage effect is, an extra sentence explaining this would be helpful.

We have added an explanation of the cage effect.

Line 9. Not clear what is meant by “compartments”

We have changed the wording to use the term “reservoir”, used elsewhere in the paper.

Page 5 Line 4. You have given example of qualitative results, (visual), is there an equivalent example for quantitative?

We have clarified that our quantitative results are given in both tabular and graphical formats.

Line 17. What is the (potential) effect of the elevated concentration of solutes?

It is difficult for us to assess the impact of the solute concentration. Our best guess is the morphology would generally be similar at different concentrations, but the number of inclusions would vary. Figure 2 provides some clues; when the amount of dissolved gas present was reduced, the size of the bubbles was the same, but there were fewer bubbles. Further research would help address the question of how solute concentration would affect sample morphology.

Page 6 Line 4 Did you consider the effect of the annealing time? “At least 1 hour” – were some samples annealed for much longer? What effect might this have/did this have on the solute/pore distribution?

Some samples were annealed for as many as four hours. We do not think annealing time would have a significant effect on our sample morphology. We annealed the samples to give them time to transition from amorphous ice, to cubic ice, and finally to hexagonal ice (Beine and Anastasio, 2011). At -10 °C, the speed of this transition should be on the order of minutes (Hobbs, 1974), so our minimum 1 hour annealing time should be more than adequate. Once the ice has transitioned to hexagonal ice, we do not expect any further changes to the matrix.

Line 5 - You specify the thickness of polypropylene vial walls, you should also specify the glass wall thickness. Is this the same thickness? If it isn't, any thermal consideration as to the differences between the samples frozen in glass and plastic is not only due to the difference in thermal conductivity between glass and plastic, but also the thickness. Any difference between glass and plastic vials (even though there likely will be) cannot be evidenced by these experiments if the thickness is not the same.

We have added the glass vial wall thickness (0.8 mm). While we agree the wall thickness is a contributing factor in how the vials will transmit heat and therefore may affect how the sample will freeze, we believe the small difference in wall thickness between glass and plastic vials (approximately 20%) compared to the large difference in thermal conductivity between the two materials suggests material choice will be a more significant factor.

Line 28 “small amounts of sample” – can you quantify this?

As the sample container is not a perfect geometric shape, we have to use radiodensity differences to determine where the sample ends and where the vial begins. Because segmentation requires analysis of very large numbers of voxels, we have developed a semi-automatic process to do this, rather than

individually inspecting each voxel. Therefore, we do not have a reliable way to quantify the impact of this process. We estimate the amount of sample excluded here would be well under 1% of the overall sample volume.

Line 29 “very little sample in contact with air..” – similarly, is this quantifiable?

Similarly to the response directly above, we believe the amount of air voxels incorrectly included in the sample is well under 1% of the overall sample volume.

Page 7 Line 25 – the process of solute rejection on freezing has been seen in several systems, including papers earlier than the referenced Cho paper. I would suggest further literature should be included here (and possibly in other places) to avoid overreliance on this one article.

E.g. starting points for the sea ice system: Lake and Lewis 1970 J Geophys Res 75 3; Wettlaufer et al J. Fluid Mech. (1997), vol. 344, pp. 291-316. Other systems (metals): Worster J Fluid Mech. (1992), vol. 237, p p . 649-669

We have added additional literature as suggested, and thank the reviewer for these references.

Page 8 Line 2 – what is the temperature effect on the solubility of CsCl? I would anticipate the solubility to decrease with a decrease in temperature – so the value you quote here at 20C would not be appropriate at -10C. A difference in solubility will change the calculated masses.

A quick literature search provided me with this, but there are probably other options in the literature: Jiang et al, 2003 Indian Journal of Chemical Technology Vol. 10, 391-395

The solubility limit of CsCl does decrease with a decrease in temperature, and is 9.6 M at 0° C (NIH, 2015); we have added this value to the paper. Jiang et al. (Jiang et al., 2003) shows the decrease in solubility with temperature is roughly linear from 20° C to -10° C, so we expect CsCl solubility would still be well above the expected concentration in the LLR of 2.7 M. Since masses were calculated based on the assumption of CsCl present in LLRs with a concentration of 2.7 M (well below the expected solubility limit for CsCl in the system), we do not believe the calculated masses would change.

Line 5 – are there no other effects on the radiodensity than the concentration of solute? What about temperature?

Radiodensity is a function of the electron density of the imaged material. So, the concentration of the solute and the background radiodensity of the solvent are the only significant factors in determining the radiodensity of the sample. Temperature can have a small effect, in that sample density, and therefore effective concentration, does depend on temperature. However, within the temperature range of our experiments, the impact of temperature on density changes of either water or ice is minimal.

Line 14 – reminder of the value used for pure ice radiodensity would be helpful here.

We have added this value (4948).

Line 18-21 I find this sentence complicated to understand – is there a better way of presenting the four domains – in particular the distinction between 2-10% and 10% LLRs is not clear to me without rereading several times.

We have rewritten the sentence to make it clearer.

Page 9 Line 2 – can you put a number on the amount of solute “lost” in this process? Quantitative idea of the effect that the threshold has?

We do include the solute present in the matrix we have labeled “water ice” in Table 1, and discuss solute in this compartment in the text. We have calculated the mass of solute present in the water ice compartment by subtracting the total mass in the sample from the mass in the other more concentrated LLR compartments.

We initially attempted to determine the mass in the water ice compartment directly, by separating the pure ice voxels from the voxels containing <2% LLRs. This proved to be statistically challenging and gave poor results, so we did not pursue this effort further. We have added additional explanation of this point in the text.

Line 4 – why only carry out this calculation for some samples? Why not all?

We have reworded this sentence to clarify – we only calculated CsCl mass for CsCl-containing samples, not gas-only samples or Rose Bengal samples.

Line 14-16 – The different thermal conductivities between water and glass has no impact here – even if you had two materials with the same thermal conductivities, the sample would still freeze from the outside in. Suggest suppressing the reference to thermal conductivity.

Agreed, we have eliminated the reference to thermal conductivity.

Suppl Fig 1 - The directionality of bubbles in your Supplementary Figure 1 also seems to support freezing from the exterior of the sample – e.g. Carte 1961 talks about direction of bubble formation in a temperature gradient (Proc Phys Soc 77, <http://iopscience.iop.org/article/10.1088/0370-1328/77/3/327/pdf>), I’m sure there are other references as well.

We thank the reviewer for the reference, and have modified the text to include this reference and further discussion of the bubble shape and morphology.

Line 21 can you quantify the size of the bubbles in the two figures?

We have quantified the approximate bubble size and added this information to the text.

Fig 2c – adding a vertical line and label at the central point of the air peak would make the histogram easier to interpret, with an equivalent label for the pure ice peak at $V_{LLR}/V_{VOXEL} = 0$.

We have added arrows to indicate the air and ice peaks, which seemed to be a bit clearer than adding additional vertical lines, but accomplishes the same goal.

Page 10 Line 2 – this would read better if the sentence was inverted to mention log scale before the “clearly...” comment. E.g. “Taking into account the log scale... the volume is clearly less...”

Agreed, we have made the suggested change.

Line 12 – What are you basing the expectations of freezing speed/direction of heat removal/position of solute inclusions on? Need a reference (or more explanation of the reasoning).

We have added additional description of our reasoning.

Line 21 – can you find a more technical term than “blow up”? –e.g. magnification/detail...

We have changed “blowup” to magnification, on Line 21 and in three other places.

Line 22 – do you have a mechanism/an idea for the link between solute pocket position and bubbles?

We speculate that the collocation is related to exclusion of gases and solutes by the forming ice matrix. The freezing front will tend to push the gases and LLRs ahead of it, with the LLR likely remaining at the freezing front and the gas furthest from the ice. As multiple freezing fronts meet, the gas bubbles will meet and enlarge, with the areas of concentrated solute encircling the bubbles.

Line 24 – The use of the word “identically” here is disingenuous, the samples are not produced identically (I agree the solutions may have been). But as the aim of the paper is to demonstrate the differences when samples are produced using nonidentical ls, it would bring this message home more convincingly if you avoid referring to differently frozen samples as “identically” produced. (There is another place this happens later on as well – Page 14, Line 25).

We agree the use of “identically” here can be confusing, and have changed the term to “similarly” to avoid confusion. We made the same change for Page 14, Line 25.

Line 29 – “blow up” – as above.

Replaced with “magnification”.

Page 11 Line 1 – “surprisingly different” – are these morphologies repeatable? To me it is not that “surprising” that a difference in freezing front gives a difference in solute distribution – maybe a different word than “surprisingly” could be used – I agree that it is interesting that this happens and that you have been able to observe it. Also, you are comparing samples of different size, this will cool/freeze differently regardless of environment.

The morphologies were repeatable. We have replaced “surprisingly” with “substantially”.

Line 10 – Why would a freezing front process only affect the solutes and not air bubbles? I would be interested in an expansion on a theory for the mechanism for this.

We are not sure why a freezing front would affect only solutes and not air bubbles, and do not have a working theory to explain the observations presented here. Nonetheless, we do see segregation of solute towards the surface of the sample.

Line 23 – “No air bubbles...” I don’t agree there is nothing in FigS6 – I see one inclusion/something in Fig S6.

We believe that single spot is a deformity in the wall of the sample container, which subsequently shows up on the video. We have added additional explanation to the figure caption. Compared to the results obtained using the previous two freezing methods, notable features are absent in the LN2 sample.

Fig 3g – (g) label appears twice. Labelling of individual curves is unclear.

We have removed the duplicate figure letter. Line identity is indicated both on the figure with labels next to the lines, and in the figure caption by reference to the line color. Which aspect of line labels remains unclear?

Line 28 – how does histogram show that voxels contain concentrated solutes?

In the inset graph, the orange line (LN2 sample) has some voxels at the right end of the curve with V_{LLR}/V_{voxel} greater than that in the pure water sample. The histogram indicates a higher radiodensity in these voxels, which in turn shows the presence of solute. Because solutes will be present in LLRs, these voxels contain concentrated solutes. We have changed the text to clarify this point.

Page 12 Line 5 – If the effect is barely visible, does that necessarily mean it is not there/definitely not important?

No, in fact we believe this is an important finding – even rapid freezing in liquid nitrogen can likely result in concentrated LLRs. We have revised the text to avoid diminishing this finding.

Line 12 – where are the concentrated solutes in Figure 3f? It is not clear in either Fig 3f or Fig S9 where these are – there seem to be only air bubbles.

Some small inclusions visible in other data (not presented) were smoothed away during image processing, but the text was not changed to reflect this. We have revised the text to match the figures.

Line 17-19 – this sentence is poorly worded (“much different than ...”). Can you expand on the reasons why the ice matrix would be modified? There needs to be a reference for this.

We agree the phrase beginning with “much different than...” is awkward and removed it. As we state in the text, the reasons behind the change in morphology is not clear to us, and we do not have a strong hypothesis for why the ice matrix would be modified. Therefore do not have any references to support this idea. We did, however, feel it appropriate to present two possible ideas as to why the ice matrix would form differently in the presence of different solutes.

Line 24 – precipitates in LLRs? – but these LLRs are not visible in Figure 3f or Supplementary Figure 6? So how could there be precipitates within them?

As discussed previously in the text (page 7 lines 1-3), mathematical smoothing can eliminate small (~80 μm in diameter) features, such as LLRs. While the LLRs are not visible in Figure 3f or Supplemental Figure 7, we believe they are present. The LN2 results in Figure 3e show no inclusions, while a higher-resolution version of that figure (Supplemental Figure 7) reveals small bubbles and solute inclusions. It is possible Rose Bengal could be present as a precipitate in small inclusions, yet not visible in our reconstructed images.

Line 25 – it would improve the flow of this section if the discussion about the histogram was combined with earlier discussion (Page 11, line 7-8) as the earlier part seems truncated and unfinished.

We agree and have made the suggested change.

Page 13 Line 22/Fig S10 – do you only have replicates for the freeze chamber? It would be interesting to have the equivalent histograms for each method.

Creating the histograms requires segmenting and then analyzing the imaging data, which is a time-consuming process. Therefore, we only created the comparison histograms for these three freeze chamber samples. Visual inspection of results for the other sample types (Freezer and LN2) suggests the differences between freezing methods are reproducible.

Line 24 – You cannot say that the “two variables [freezing method and solute] are the primary factors influencing ice morphology” as you only change these variables in your experiments, so of course they are the two primary influencing factors here.

We have revised the text to remove this implication these are the only factors at work in the system.

Page 14 Line 1 – what are the errors on the $V_{LLR}/V_{VOXEL} = 2-10\%$ for the two methods? These would be useful to determine whether the factor of 2 difference is reliable – as a difference between 0.003 and 0.006 doesn't seem large (but may still be significant).

Because of the effort required to determine V_{LLR}/V_{Voxel} for each sample replicate, we do not have enough replicates to adequately characterize the error of V_{LLR}/V_{Voxel} .

Line 24 – If I understand correctly, LLRs are present when there is solute extruded from the crystallising ice – so why do you have LLRs in this (pure ice) sample?

The legend on this figure mistakenly and confusingly includes reference to LLRs. We have removed that legend line.

Line 25 – use of “identical” confusing again.

We have changed the word to “similar”.

Line 29 – “while the reason for this morphology is unclear...” the morphology of bubbles, and the effect of a temperature gradient, has been studied previously – e.g. Proceedings of the Physical Society, Volume 77, Number 3 <http://iopscience.iop.org/article/10.1088/0370-1328/77/3/327/pdf>.

We thank the reviewer for the reference, and have changed the text accordingly.

Page 15 Line 15- Where are the “elongated solute inclusions” in Figure S14. They seem no more or less elongated than the air bubbles.

We have revised this sentence and eliminated reference to elongated solute inclusions.

Figure 5 – is this a vertical slice? Specify this in the caption. Also - “along the direction of the temperature gradient” – specify which direction – i.e. from colder to warmer.

We have clarified that the images are vertical slices and specified the direction of the temperature gradient.

Page 16 Line 16 – a reference for “melting into the bulk ice” is required (e.g. Movement of brine pockets by salt diffusion - Notz D and Worster MG (2009) Desalination processes of sea ice revisited. J. Geophys. Res. Ocean., 114(5), C05006 (doi:10.1029/2008JC004885))

We have added the reference as recommended.

Line 20 – what is the concentration of solutes in clean polar samples? This should be mentioned in the introduction as well.

Of course the concentration can vary considerably, but concentration of solutes in clean polar snow is on the order of 10 μM (Yang et al., 1996). We have added this information to the introduction.

Line 21 – “significant impacts” - I think significant should only be used in statistical context, so here “important” or similar would be more appropriate. There should also be more discussion on how this assumption that a lower concentration will have the same effect was made.

We have changed the word “significant” to “important”, and revised the sentence to state that we believe lower solute concentrations may also impact sample morphology.

Page 17 Line 3 – this sentence (“Cesium chloride..”) starts abruptly, would be improved with an introductory couple of words.

We have added several words to this sentence to bridge from the previous paragraph.

Line 14-15 “Our work here can help guide further investigations to understand the driving forces shaping snow and ice structures in the natural world, as well as the rate of chemical reactions in snow and ice.” – it is not clear to me how this work helps to understand the driving forces etc, nor how there is any (direct) impact on studies into the rate of chemical reactions.

We believe our findings suggest both freezing method and the nature of the solutes present are important to determining the morphology of ice matrices, both natural and artificial. For studying the rate of chemical reactions, the location of solute in laboratory samples may have significant impacts on the measured reaction rates, so knowing how freezing method can influence the solute location can directly impact these studies. We have revised the text to address the reviewer’s suggestion.

Line 16-19 “At the same time, our results suggest subtle changes in the preparation of laboratory ice samples can have significant impacts on the location of solutes in samples, requiring careful and consistent sample preparation to ensure meaningful results.” - this part is the important conclusion from this work. I would therefore suggest removing/reducing/rephrasing the first half of this paragraph, and expanding on the second half.

We have rearranged the paragraph as suggested, and rewritten parts of it to incorporate the reviewer’s suggestion.

Supplementary info

It would be more coherent in there was only one format for videos and one for images in the supplementary information.

We agree this would be desirable and more coherent. However, because of the various software packages used to generate the figures and videos in the supplemental material, the initial output formats will necessarily differ. While it is possible to standardize the format, we have used common formats available to users of standard computers, and therefore don’t believe the end users have been inconvenienced.

It would be useful to have brief captions/explanations of supplementary images and videos in the supplementary information, to avoid having to search through the main text for the information.

We appreciate the suggestion and have added a caption file describing each supplementary image or movie.

The colours marked “orange” in the text do not appear orange in the videos (at least on my screens), they are yellow.

We do agree the color can appear yellow on some monitors. Since the only other color in the videos is red, which seems to appear as red on all systems, the remaining color should not confuse the reader, even if it appears more yellowish than orange. We have indicated this caution in the captions file, and we have changed the legend in the videos to call the color yellow rather than orange.

Typographical errors

Pg 3 Line 6 “to form hydroxyl radicals” Pg 5 Line7 – missing word? “was produced from...” Pg 5 Line 10 - repetition of “its” Pg 8 Line 17 “VVOXEL” should be in capitals Pg 11 (22-23) “Results for a 1.0mM CsCl sample.. are shown in Fig. 3e”

We have corrected these errors.

Figure 4 – the blue arrows are not visible when printed in black and white.

We see the arrows when printed in black and white, and appreciate the reviewer’s observation. We have changed the arrows to yellow to provide better contrast.

Comments from S. Maus (Referee)

The paper presents and discusses results from X-ray tomographic imaging of aqueous solutions frozen in small containers in the laboratory. With two solutions, cesium chloride (CsCl) and Rose Bengal solution, three different freezing methods were used: (i) freezing by putting containers in a normal freezer, (ii) unidirectional bottom-up freezing with containers placed on a cold plate and (iii) putting small vials into liquid nitrogen. The frozen samples are imaged by X-ray microtomography, mostly at a voxel size of 16 μm , to obtain 3-d greyscale images of X-ray transmission. After segmentating the greyscale images into different classes that reflect solute and air or gas content, the authors discuss these images qualitatively in terms of distribution of solute inclusions and air bubbles. The authors also perform a quantitative analysis of the distribution and content of solute in liquid like regions (LLRs) versus solute incorporated in the solid ice matrix, as well as some observations on the movement of liquid inclusions. The authors conclude that the work shows that *the structure of laboratory ice samples, including the location of solutes, is sensitive to freezing method, sample container, and solute characteristics, requiring careful experimental design and interpretation of results.* The work is proposed to enhance our understanding of solute segregation in natural snow and ice, as well as of *the air-ice interface and liquid-like regions within the ice matrix.*

I agree with two other referees that the paper is relevant for ‘The Cryosphere’ and its readership, and also mostly with their comments. I would like to add comments on two major issues on which the paper in my opinion needs improvement. First, I encourage the authors to improve review and referencing of the published background on ice observations and solute redistribution during freezing, to be included in the introduction and discussion of their own observations. Second, I think that there is potential for improvement in the quantitative analysis of the 3-d images, and a

critical discussion of the method and results. In my comments I will give literature examples that I hope will help the authors to improve their analysis and presentation.

Main concerns I-III

I. Background - ice, freezing and solute inclusions

1. In the literature on ice physics and chemistry there are several books that include fundamental discussions of ice structure and solute distribution during freezing but none of these is mentioned. I recommend to have a look into the literature (e.g., Shumskii, 1955; Hobbs, 1974; Lock, 1990; Petrenko and Whitworth, 1999; Pruppacher and Klett, 1997), and possibly cite from there.

We thank the reviewer for these references, and have included them as appropriate.

2. It is well known that during the freezing of saline solutions most solute is rejected into the remaining mother solution and not incorporated into the solid ice matrix (e.g., Hobbs, 1974; Petrenko and Whitworth, 1999). This fact should be more clearly mentioned in the text. The authors for example write (P16, L13-16) *While the air bubbles remain stationary in the ice matrix, the CsCl moves, consistent with the idea that solutes are present as a concentrated liquid-like solution, which can migrate either along the boundaries between air bubbles and the bulk ice, or possibly by melting into the bulk ice itself.* Such formulation indicates that solute rejection during solidification of water is only an *idea* rather than a fact under most conditions.

We have revised the noted section, as well as the introduction and abstract, to state the exclusion as a well-established finding rather than an idea.

3. I am also missing background literature on observations of solute inclusions in ice. For example, already Quincke (1905) has described the morphology and distribution of liquid inclusions of ice grown from saline solutions, and there is much more information in the books on ice physics mentioned above. As an example, Shumskii (1955) describes the solute distribution in ice as (p.180): ‘The distance between neighbouring interlayers of inclusions in a crystal decreases with increasing concentration of impurities in the remainder mother solution; often this distance is as much as 35-45 μ with inclusions 8-15 μ thick’. Such information is certainly relevant for the discussion and interpretation of the results in the present paper.

We have included reference to previous work on solute inclusions, and tried to use them to guide interpretation of our results.

4. An idea of the expected microstructure and potential separation of solute inclusions and air bubbles may be obtained by consulting published work based on thin section analysis of frozen solution or pure water droplets (e.g., Hallett, 1964; Rohatgi and Adams, 1967). For example, a useful information from these studies is the dependence of dendrite or plate spacing on freezing velocity: The faster the freezing, the smaller the spacing of ice plates and solute inclusions, which normally implies smaller dimensions of solute inclusions. For the present study this may affect the detectability of solutes, especially for the samples frozen very rapidly in liquid nitrogen-based freezing.

We thank the reviewer for these references, and have incorporated them into our text.

5. Earlier basic work on solubility of ions in the solid matrix as well as solute partitioning at a freezing interface (e.g., Tiller, 1963; Gross et al., 1975, 1977) should be mentioned and discussed.

Such information is particularly important when it comes to the quantitative analysis and discussion - see my comments below.

We have incorporated ion solubility in the solid matrix as appropriate.

6. How is the freezing point depression the authors assume for CsCl (2.7 M CsCl at -10 °C) computed, or on which reference is it based? E.g. according to Pruppacher and Klett (1997) (p. 125, Fig. 4-12) one might expect that a value of 3.1 to 3.3 M is a more realistic value at -10 °C. While such a change in equilibrium concentration would affect the estimates of the volume of LLR from the greyscale images, it would not affect the solute content within liquid inclusions. However, it would decrease the maximum solute content in voxels in the histogram, and thus may give hints on the proper estimation and possible rescaling of equation (1).

Our use of 2.7 M is based on theoretical thermodynamic calculations, and corresponds to the ideal case given in Pruppacher and Klett (Pruppacher and Klett, 2010) Figure 4-12. Pruppacher and Klett and Haynes (Haynes, 2014) both present data for the freezing point depression of CsCl, but only up to a salt concentration of 1.8 M (Pruppacher and Klett) or 1.4 M (Haynes). Extrapolating their data to the concentrations expected in our samples (i.e., at -10 °C) suggest the CsCl concentration in LLRs would be somewhere between 3 and 3.2 M, i.e., 10 – 20% higher than our ideal case concentration, but neither source presents freezing point depression data measured at such a high concentration. In the absence of measured information for the actual composition of CsCl solutions under our experimental conditions, we have elected to stay with the theoretical prediction of salt concentration of 2.7 M, especially since the change to non-ideal conditions would be relatively small (i.e., 20% or less). However, we have altered the text to note the uncertainties in the CsCl concentration in LLRs at -10 °C and explain the potential impact of the non-ideal concentration on our findings.

7. X-ray tomography of solutions frozen in the laboratory has been performed earlier Miedaner (2007); Miedaner et al. (2007) and may be compared to the present results.

We have included these references both in the introduction and in the text as appropriate.

II. Image segmentation and quantitative analysis of solute content and locations

1. The proposed segmentation approach is based on equation (1) on page 8 assuming 2.7 M equilibrium concentration CsCl at -10 °C. Please have a closer look in the literature to evaluate the uncertainty of this estimate.

We have done so and expanded the text to better describe uncertainties of our estimate, as discussed in the response to point I.6. above.

2. The choice of a cutoff at $LLR = 2\%$ to estimate the solute content in liquid like regions seems somewhat arbitrary. As the pure ice histogram in Fig. 2c extends to slightly above $LLR = 4\%$, rather such a cutoff would be more consistent. At least would a $LLR = 4\%$ cutoff define a lower bound of the solute content in liquid inclusions.

We agree, the choice of cutoff is somewhat arbitrary, and that a cutoff of 4% would virtually eliminate the chance that a voxel with $LLR > 4\%$ actually is pure ice. However, such a choice could also incorrectly imply that many voxels with an $LLR < 4\%$ contains no solute. As stated on Page 8, Line 25ff, we chose 2% as the cutoff because it is three standard deviations above the mean for LLR in pure ice voxels. Therefore, the number of voxels that are actually pure ice but are mistakenly included as containing solute is quite small, and this value seemed a reasonable cutoff for voxels containing appreciable solute. Note, also, the graph may be visually misleading, as the Y axis is a log scale, imply a larger number of voxels

above 2% than are actually present.

3. As the authors correctly point out, the solute content will be underestimated due to the possibility of mixed air and solute pixels, which may then have radio densities between brine and air, and thus be classified as ice. Can this bias be estimated? An approach to place a bound on this bias could be to count the surface voxels of the air bubbles and assume that these contain CsCl brine and air. How would this affect the results in Table 1?

We considered various methods to address this issue quantitatively, but found no practical solution. Given the large number of voxels present, manual counting was not possible, and the software tools available to us did not have the capabilities to perform this analysis. We agree it represents an uncertainty in our analysis, and have added additional text to discuss the problem.

4. In the histogram in Fig. 3 the maximum volume fraction of liquid is roughly 0.9. However, if calibration and equation (1) would be correct I would expect that the maximum value should at least be 1 (due to expected noise even larger), provided that there are at least some liquid inclusions that exceed the volume of a voxel with side length 16 μ m. While this may not be the case, I would expect that it would very likely be the case for high resolution imaging with 2 μ m voxel size. How does such a histogram look like, and may it be used to improve the calibration in eq. (1)?

We agree such an analysis would be useful and interesting and useful. Unfortunately, to do such an analysis would require imaging aqueous solutions at 2 μ m resolution to create a standard curve, as was done at the 16 μ m resolution samples. We did not have the resources to do such a standard curve, although we hope to further investigate higher-resolution imaging in the future.

5. According to Table 1 the solute content classified in solute inclusions is 12-35 %, and the remainder is concluded to be incorporated in the ice matrix. How does this compare to expected solubility limits in the solid? E.g., Gross et al. (1975) suggested a solubility limit of 1-2 $\times 10^{-4}$ M for HCl in the solid ice matrix. The result from the authors calculations (65 to 88% of the initial 1 mM CsCl in the ice matrix) would roughly imply a 3-9 times larger solubility of CsCl in ice. Do any studies exist that support such a high solubility of CsCl in solid ice? If not, then this might be another indication that eq. (1) should be changed by a prefactor that gives larger liquid fractions of at least 1 at the higher end of the histogram. Again, it appears very important to present a similar analysis of high resolution images, that could solve this problem.

We agree with the estimate of 65% of CsCl present in “water ice”, as given in Table 1. However, we do not believe all the CsCl present in this water is present as solute in the solid ice matrix. Rather, most of this solute is likely present as LLRs where V_{LLR}/V_{VOXEL} is $< 2\%$. Due to resolution and background noise, our imaging system cannot reliably distinguish between voxels where, for example, $V_{LLR}/V_{VOXEL} = 1\%$, and a voxel containing only pure water ice. However, higher resolution imaging results, such as Figure 4 or Supplemental Figure 7, suggests smaller inclusions are indeed present.

The “missing” CsCl mass here is $0.65 * 126.3 \mu\text{g} = 82.1 \mu\text{g}$, or $0.49 \mu\text{mol}$. Assuming this solute is entirely present as LLRs with solute concentration of 2.7 M, this equates to a total LLR volume of 0.18 μL . The volume of pure ice (again from Table 1) is 716 μL . Therefore, assuming the remaining CsCl is distributed equally throughout the voxels labeled as pure ice in Table 1, the calculated average V_{LLR}/V_{VOXEL} for these voxels is 0.025%, indistinguishable from water ice in our system. While it is possible the CsCl is present (at least partially) as solutes in the solid ice matrix, we believe it is more likely to be present primarily as small LLR inclusions.

We have revised the text, incorporating also the ideas from I. 6. above, to more clearly address this issue.

6. The inset in Figure 3 compares the histogram envelope around the radiodensity of ice for the Milli-Q and solute samples. It indicates that the ice peak and envelope in the histogram is slightly shifted to the right for the frozen solutions with respect to frozen Milli-Q - which is particularly apparent for the LN2 samples. Such a shift would indeed be consistent with Cs and Cl incorporated in the solid ice matrix, where they act in the same way as strong X-ray absorbers as when in liquid solution. It would be very interesting to evaluate, if it is possible to estimate the solute content in the ice matrix from this shift.

We also noted that shift and agree it is an interesting finding. We agree it probably represents Cs and Cl incorporated into the ice matrix. We did attempt to estimate the solute content by subtracting the histogram of the solute-containing sample from a sample of pure MilliQ water. Unfortunately, this process was very sensitive to variability in the distributions of the background samples, and we were not successful. To understand this variability, we would have had to conduct a number of imaging studies on various days of both pure water ice and CsCl solutions, an effort we unfortunately did not have the resources to conduct. We do think it is a worthwhile activity, and hope to do this in the future.

7. A statistical analysis of size distribution of solute inclusions and air bubbles would be very helpful. Such a statistics would also justify to include the results from Rose Bengal solutions, that else is given too little weight in this study.

We agree that such an analysis would be worthwhile and useful. Such an analysis, however, would require considerable time and resources, and we were unable to complete it for this paper. We hope to revisit this issue in the future.

III. Results and Discussion

1. Every paragraph in the discussion contains a reference to supplementary material, that is the discussion is based very much on the latter (S1-S16). While it is helpful to provide such material, I regard it as inappropriate to build up the discussion of a research paper on that much supplementary information. Some of this information should become part of the paper and the discussion should be rewritten.

We understand and share the reviewer's concern, but are somewhat restricted by the image formats available to us in a standard scientific paper. Of the 16 Supplemental files, all but 3 are video files. Of the video files, most are represented as individual images in the paper itself. It is not possible to incorporate these videos into the paper as currently structured for The Cryosphere.

Specific comments

P 4, L 23-25 -> *But to our knowledge this method has not been used to investigate the structure and solute locations for laboratory samples prepared under controlled conditions with specific solutes - I would not call the freezing conditions controlled, as neither cooling rates or supercooling in the samples were controlled or measured.*

While we do no present measurements of cooling rates, the sample preparation methods were controlled in the sense that the freezing methods were repeatable. We have changed the word "controlled" to "reproducible", to avoid implying we cooled the sample at a standard rate.

P 4, L 29 -> *In this work we focus on cesium chloride (CsCl) as our solute. However, because a previous study (Cheng et al., 2010) found different solutes can affect freezing morphology and therefore may influence solute location, we also imaged ice containing the organic compound Rose Bengal. I suppose that CsCl was chosen because it warrants a high X-ray contrast between ice and*

solute. Why was Rose Bengal chosen? Also, as the results presented are, except for a histogram in Fig. 3 as well as supplementary material, for the CsCl solutions, I would rather suggest to remove the few Rose Bengal results and notes, and rather present a systematic and quantitative comparison elsewhere.

In addition to its high Xray absorption cross section as noted by the reviewer, CsCl was also selected because of its high aqueous solubility. Rose Bengal was chosen because of its relatively high aqueous solubility (for a chemical of its size) and large Xray absorption cross section. While we agree experimental results for CsCl are far more extensive than for Rose Bengal, we do think including this compound is essential to demonstrating the idea that solute choice can make a significant difference in sample morphology. We hope to present a more extensive and quantitative evaluation of solute effects in the future.

P 5 L 1 → Cheng et al., 2010 - this is a reference to a study based on a rather different method, that yields the surface distribution of solutes/ions. There exist other studies that have shown the influence of solute on freezing pattern, for example the mentioned work by Rohatgi and Adams (1967). I cannot see that the cited paper is an argument to use Rose Bengal as an alternative solution.

We offer the cited paper not as an argument to use Rose Bengal in particular, but as a motivation to study solutes other than CsCl. We have added the Rohatgi and Adams reference to this sentence.

P 16 L 13 → While the air bubbles remain stationary in the ice matrix, the CsCl moves, consistent with the idea that solutes are present as a concentrated liquid-like solution, which can migrate either along the boundaries between air bubbles and the bulk ice, or possibly by melting into the bulk ice itself - First, I find it surprising, that the air bubbles remain stationary, because it is well established that air bubbles migrate in a temperature gradient at similar rates as liquid inclusions (Dadic et al., 2010). Second, some reference on the process of brine pocket migration should be mentioned here, please have a look at Light et al. (2009) and the literature reviewed therein. Third, Light et al. (2009) also found migration for solid crystals, so the movement of solute is no proof for its liquid character.

“Stationary” here may be a relative term; we did not note migration of the large air bubbles in the sample shown, but we did not attempt to rigorously quantify this motion, either. We do note that Dadic et al. (2010) presents measured data of bubble migration in laboratory samples, and found rates at -10 C ranging from $1.5\text{-}3\ \mu\text{m h}^{-1}/(\text{K}^{-1}\ \text{cm}^{-1})$. Brine inclusions (original data from Light et al. 2009, and also cited in Figure 7 in Dadic et al. 2010), however, moved at approximately $10\ \mu\text{m h}^{-1}/(\text{K}^{-1}\ \text{cm}^{-1})$. While we agree with the reviewer these rates are roughly similar, these findings also suggest bubbles and brine inclusions may move at different rates, and are consistent with our observation that solute inclusions move faster than air bubbles. Additionally, we note that our system may not be accurately modeled by comparison to pure brine inclusions or air bubbles. Light et al. (2009) notes that “dissolved gases may play a role in the migration of brine inclusions” and further states that “The effect of included gas bubbles on brine migration has not been studied.”

We have revised the text to include these ideas and the other suggestions made by the reviewer.

P 16 L 23 → surprisingly - considering earlier studies on the freezing of saline solutions I would not rate this as surprising.

We have removed the word surprising.

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1 Direct visualization of solute locations in laboratory ice samples

2
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4
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6
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8 Abstract

9
10 Many important chemical reactions occur in polar snow, where solutes may be present in several
11 reservoirs, including at the air-ice interface and in liquid-like regions within the ice matrix. Some recent
12 laboratory studies suggest chemical reaction rates may differ in these two reservoirs. While
13 investigations have examined where solutes are found in natural snow and ice, ~~few studies have examined~~
14 ~~similar research has not identified~~ solute locations in laboratory samples, nor the possible factors
15 controlling solute segregation. To address this, we ~~used micro-computed tomography (microCT) to~~
16 ~~examine~~ ~~examined~~ solute locations in ice samples prepared from either aqueous cesium chloride (CsCl) or
17 Rose Bengal solutions that were frozen using several different methods. Samples frozen in a laboratory
18 freezer had the largest liquid-like inclusions and air bubbles, while samples frozen in a custom freeze
19 chamber had somewhat smaller air bubbles and inclusions; in contrast, samples frozen in liquid nitrogen
20 showed much smaller concentrated inclusions and air bubbles, only slightly larger than the resolution
21 limit of our images (~2 μm). Freezing solutions in plastic versus glass vials had significant impacts on
22 the sample structure, perhaps because the poor heat conductivity of plastic vials changes how heat is
23 removed from the sample as it cools. Similarly, the choice of solute had a significant impact on sample
24 structure, with Rose Bengal solutions yielding smaller inclusions and air bubbles compared to CsCl
25 solutions frozen using the same method. Additional experiments using higher-resolution imaging of an
26 ice sample show that CsCl moves in a thermal gradient, supporting the idea that the solutes in ice are
27 present in mobile liquid-like regions. Our work shows that the structure of laboratory ice samples,
28 including the location of solutes, is sensitive to freezing method, sample container, and solute
29 characteristics, requiring careful experimental design and interpretation of results.

30 31 1. Introduction

32 Snowpacks can be important locations for a variety of chemical reactions, particularly in polar
33 regions (Bartels-Rausch et al., 2014; Domine and Shepson, 2002). Because light can penetrate several
34 10s of cm into the snowpack, photochemical reactions are particularly important (Grannas et al., 2007),
35 including nitrate photolysis forming NO_x (Beine et al., 2002; Chu and Anastasio, 2003; Jacobi et al.,
36 2004), hydrogen peroxide photolysis ~~forming to form~~ hydroxyl radical (Chu and Anastasio, 2005; Jacobi
37 et al., 2006), and transformation of organics (Dibb and Arsenault, 2002; Sumner and Shepson, 1999).

38 A variety of potential chemical reactants have been identified in snowpacks ~~worldwide;~~
39 concentrations can vary considerably, with typical concentrations on the order of 10 μM in clean Arctic
40 snows (Yang et al., 1996). Impurities can integrate into snow crystals during formation, or be deposited
41 onto the surface of formed crystals. Reactants and products also partition between the snow crystals and
42 the overlying air; the large surface area of the snow crystals provides an extensive environment for
43 reactions to occur. As the snowpack consolidates and snow grains metamorphose, chemical compounds

44 can remain at the surface of the crystals, or become trapped internally at grain boundaries or triple
45 junctions (Bartels-Rausch et al., 2014; Domine et al., 2008; Grannas et al., 2007).

46 There appear to be three reservoirs for impurities in snow: a quasi-liquid layer (QLL) at the ice-
47 air interface; liquid-like regions (LLRs) within the ice (e.g., at grain boundaries); and in the bulk ice
48 matrix, i.e., between frozen water molecules (Barret et al., 2011; Grannas et al., 2007; Jacobi et al.,
49 2004). While the exact location of solutes in snow is not well understood (Bartels-Rausch et al., 2014),
50 the location is important for several reasons. First, chemicals ~~present~~ in a surface QLL can be more
51 readily released to the atmosphere compared to impurities segregated into an internal LLR; furthermore,
52 gas-phase oxidants and other species can readily partition from the air onto solutes at the air-ice interface.
53 Second, photon fluxes can vary considerably in various locations within the snowpack (Phillips and
54 Simpson, 2005), although there appear to be only small differences and possibly within crystals
55 themselves (McFall and Anastasio, 2016). Third, the rates of reactions of impurities appear to vary with
56 location. For example, photolysis rates of PAHs (polycyclic aromatic hydrocarbons) have been reported
57 to can be up to five times faster in surface QLLs compared to in whole ice samples (where PAHs are
58 likely in LLRs) or in aqueous solution (Kahan and Donaldson, 2007, 2010; Ram and Anastasio, 2009).
59 An investigation of reactions in frozen solutions (Kurkova et al., 2011) suggested the QLL and LLR
60 physical reaction environments are substantially different, with QLLs best represented by a 2D cage and
61 LLRs as a 3D cage. This work also found that the cage effect (i.e., the tendency for a compound to be
62 surrounded by solvent molecules, which can impede the ability of a compound to react) at a given
63 temperature was much more pronounced for reactions occurring in QLLs than LLRs, with solutes in
64 QLLs ~~somewhat surprisingly~~ having less mobility compared to solutes in LLRs.

65 Because of the potential reactivity differences between the reservoirs, understanding reaction
66 rates in different ~~compartments-reservoirs~~ requires knowing where solutes are located. Solute locations in
67 natural snow and ice samples have been studied using electron microscopy (Barnes et al., 2003;
68 Lomonaco et al., 2009; Rosenthal et al., 2007), and were found to preferentially segregate to grain
69 boundaries and triple junctions. Additional work has evaluated the nature of these compartments,
70 showing that solutes segregate and concentrate in LLRs (Heger et al., 2005; Heger et al., 2006). When an
71 aqueous solution is frozen, most solutes are excluded from the forming ice matrix (Hobbs, 1974; Petrenko
72 and Whitworth, 1999), often forming platelets of ice separated by brine or dendritic structures (Rohatgi
73 and Adams, 1967; Shumskii, 1964). Recently, some studies have used various techniques to directly
74 examine the location of solutes themselves in laboratory snow and ice samples (Cheng et al., 2010;
75 Miedaner, 2007; Miedaner et al., 2007) Nonetheless, solute location is poorly understood in many
76 experimental systems, and ~~However, we are aware of only one study (Cheng et al., 2010) which directly~~
77 ~~examined solute location in laboratory snow and ice samples. Instead, solute location~~ is most often
78 inferred from the way the sample is made (Kahan et al., 2010) or from chemical behavior (Kurkova et al.,
79 2011).

80 The main goal of this paper is to examine the location of solutes in laboratory-prepared frozen
81 solutions. In order to do this, we use X-ray computed tomography (CT), a technique that has been used to
82 create 3-dimensional images of a variety of biological and natural materials (Blanke et al., 2013; Evans et
83 al., 2008). High resolution microCT, which is capable of a spatial resolution of < 10 μm , has been used to
84 look at the structure of natural snow and ice (Chen and Baker, 2010; Heggli et al., 2011; Lomonaco et al.,
85 2011; Obbard et al., 2009). But to our knowledge this method has not been used to investigate the
86 structure and solute locations for laboratory samples prepared under ~~controlled-reproducible~~ conditions
87 with specific solutes.

88 Thus here we examine the locations of impurities in frozen aqueous solutions prepared in the
89 laboratory. We are primarily interested in the locations of solutes in ices prepared using different freezing
90 methods aimed at putting solutes in specific reservoirs within the ice; these methods, or similar ones, have
91 been used both in our previous research as well as by other investigators. In this work we focus on
92 cesium chloride (CsCl) as our solute. However, because a previous study-studies (Cheng et al., 2010;
93 Rohatgi and Adams, 1967) found different solutes can affect freezing morphology and therefore may
94 influence solute location, -we also imaged ice containing the organic compound Rose Bengal (4,5,6,7-
95 tetrachloro-2',4',5',7'-tetraiodofluorescein). For our samples we present both qualitative (visual) and semi-
96 quantitative (tabular and graphical) results.

97 98 2. Methods

99 We prepared samples by freezing 1.0 mM aqueous solutions of cesium chloride or, in a few
100 cases, 1.0 mM Rose Bengal. High purity water (“Milli-Q water”) was produced from house-treated
101 deionized water that was run through a Barnstead International DO813 activated carbon cartridge and
102 then a Millipore Milli-Q Plus system. We chose cesium chloride (Sigma-Aldrich, 99.9%) for our primary
103 solute because of its its-high solubility in water and high X-ray mass attenuation coefficient ($\sim 4.4 \text{ cm}^2 \text{ g}^{-1}$
104 at 70 keV (NIST, 2015)), enabling visualization of low concentrations in our microCT system. We also
105 used Rose Bengal to study the impacts of solute size and polarity on sample morphology. While 1.0 mM
106 of solute is higher than typical total solute concentrations in continental (inland) natural snows, it is
107 within the range of concentrations measured in coastal snowpacks (Beine et al., 2011; Douglas and Sturm,
108 2004; Yang et al., 1996). The chosen concentration allows easy visualization in our system and provides
109 enough material to evaluate spatial patterns in the sample.

110 We froze most samples as a 500 μl aliquot in a capped glass vial (approximately 3 cm high and 1
111 cm in diameter, 0.8 mm wall thickness, with a total vial volume of $\sim 2 \text{ ml}$) using one of three methods.
112 These methods were chosen because they had been used in our laboratory, as well as others, and also due
113 to differences in the speed of heat removal from the samples; we discuss later the expected morphologies
114 for the various sample types. In the first technique (“Freezer”), we placed samples upright on a plastic
115 plate in a laboratory freezer at —approximately -20°C ; freezing took approximately 1 hour. In the second
116 technique (“Freeze Chamber”), we froze samples upright in a custom-built freeze chamber (Hullar and
117 Anastasio, 2011) whose base was cooled to either -10 or -20°C . Typically, the sample sat directly on the
118 base of the freeze chamber surrounded by air. However, we also froze some samples surrounded by
119 drilled metal plates, effectively placing the sample in a metal “well”; the distance between the sample and
120 the surrounding plates was around 1 mm. In the third technique (“Liquid Nitrogen” or “LN2”) we froze
121 samples by putting the aqueous sample in a vial, capping it, then immersing it in a bath of liquid nitrogen
122 deeper than the height of the liquid in the vial; freezing time was ~ 30 seconds. We allowed all samples to
123 anneal at -10°C for at least 1 hour before imaging. We froze a small number of samples in either
124 polypropylene vials (wall thickness $\sim 1 \text{ mm}$) or with a larger sample volume (750 μl).

125 We imaged samples using a MicroXCT-200 (Zeiss Instruments) micro-computed tomography
126 (microCT) scanner. To maintain our samples at -10°C , samples were held in a custom cold stage for the
127 MicroXCT-200 (Hullar et al., 2014). The custom cold stage was placed on the scanner’s sample stage,
128 whose position is controlled by the scanner software to submicron precision. Scanning parameters were
129 set based on the manufacturer’s guidelines. For most imaging, we set source and detector distances to 40
130 and 130 mm respectively; voltage and power were set at 70 keV and 7.9 W, and the manufacturer’s LE3
131 custom filter was used for beam filtration. The microCT acquired 1600 projections over 360 degrees of

132 rotation, with an exposure time of 2 s. Images were reconstructed using the manufacturer's software on
133 an isotropic voxel grid with 15.9358 μm edge lengths. Some samples were analyzed at higher resolution,
134 with a voxel edge length of 2.1146 μm . For these samples, we set source and detector distances to 60 and
135 18 mm, used the LE5 beam filter, collected 2400 projections spanning 360 degrees, and set beam voltage,
136 power, and exposure time to 60 keV, 6 W, and 30 s respectively. The microCT scanner software outputs
137 slicewise TIFF images of the X-Y plane of the sample, with greyscale values corresponding to the
138 radiodensity of each voxel at that Z plane.

139 We imported digital TIFF images into the Amira software package (Visualization Sciences
140 Group, FEI) for reconstruction and segmentation. Our segmentation procedure used the Amira
141 segmentation tools to isolate the sample from surrounding materials; generally, our procedure should
142 include very little sample container at the expense of excluding some small amounts of sample in contact
143 with the vial wall. Similarly, the segmentation procedure excludes very little sample in contact with air
144 above the sample, while including small amounts of top air as sample. Some images presented here were
145 mathematically smoothed by the software, which sometimes resulted in small features ($< 80 \mu\text{m}$ in
146 diameter) being eliminated from movies and still images; however, smoothing did not substantially
147 change the interpretation of our results. In some cases we prepared histograms of the data, which were not
148 smoothed and include all sample data.

149 To quantitate CsCl concentration in each voxel, we first imaged samples of Milli-Q water, as both
150 liquid and ice, and measured the average radiodensity (image greyscale value) of a subvolume within
151 each sample. As expected, the average radiodensity of ice ($4948 \pm 160 (1\sigma)$) was less than that of liquid
152 water ($5372 \pm 194 (1\sigma)$) due to the lower density of ice. Our measured radiodensity ratio between ice (at -
153 10°C) and water (at 20°C) was 0.921, matching a calculated density ratio from literature values (Haynes,
154 2014) of 0.921. Next, we imaged 8 aqueous solutions of CsCl at varying concentrations (1.0 mM to 5.0
155 M) to construct a calibration curve. Plotting these points (Fig. 1) shows a linear relationship between
156 CsCl concentration and measured radiodensity, with a y-intercept value within the range of our measured
157 radiodensities for pure liquid water. Therefore, the measured radiodensity of a voxel within a sample
158 containing CsCl in solution (or ice) is linearly related to the amount of CsCl present in the voxel. We
159 assume the relationship between CsCl concentration and radiodensity is the same for ice and water. This
160 allows us to determine the amount of CsCl present in a sample voxel by subtracting the average greyscale
161 value of pure water (or ice) and then using the standard curve to calculate the CsCl mass.

162 When aqueous solutions are frozen, solutes are generally excluded from the forming ice matrix,
163 resulting in a two distinct components: pure (or nearly pure) water ice, and a concentrated solution of
164 solute (Cho et al., 2002; Lake and Lewis, 1970; Wettlaufer et al., 1997), which can be present at the air-
165 ice interface (i.e., as a QLL) and/or in LLRs within the sample. Freezing-point depression dictates that
166 the solute concentration in these regions is solely a function of the ice temperature (Cho et al., 2002) and
167 is independent of the solute concentration in the initial solution. For example, at -10°C , the predicted
168 total solute concentration in LLRs is 5.4 M of solute ions, or 2.7 M of a binary salt such as CsCl. This
169 LLR concentration is considerably lower than the solubility limit of CsCl (11.1 M at 20°C , 9.6 M at 0°C
170 (NIH, 2015)), but higher than the solubility limit of Rose Bengal (1 mM, temperature not given, (Neckers,
171 1989)). Therefore, we do not expect CsCl to precipitate, although Rose Bengal might.

172 As described earlier, we use the Fig. 1 calibration curve to convert microCT greyscale values of
173 radiodensity for each voxel to the mass of solute in each voxel. While this mass could be expressed as an
174 equivalent concentration in the voxel, we believe it is more accurate to consider each voxel as a mixture
175 of pure water ice (with zero solute) and LLRs (regions with a total solute ion concentration of 5.4 M at –

176 10° C, equivalent to a CsCl concentration of 2.7 M). Thus we express the composition of each voxel as
177 the fraction of voxel volume occupied by liquid-like regions, V_{LLR}/V_{VOXEL} :

178

$$179 \quad \frac{V_{LLR}}{V_{VOXEL}} = \frac{(RD_{MEAS} - RD_{ICE}) / Slope}{2.7 M} \quad (1)$$

180

181 where V_{LLR} is the LLR volume, V_{VOXEL} represents the volume of the entire voxel, RD_{MEAS} is the measured
182 radiodensity of the voxel, RD_{ICE} is the radiodensity of pure ice (4948), and Slope is the measured slope of
183 the standard curve line ($10409 M^{-1}$; Fig. 1). A voxel containing only pure ice has $V_{LLR}/V_{VOXEL} = 0$, while
184 a voxel composed entirely of 5.4 M total solute in water has $V_{LLR}/V_{VOXEL} = 1$. Our estimated
185 concentration of total solute ion concentration in LLRs is based on theoretical calculations and assumes
186 ideal behavior from the solution (Cho et al., 2002; Pruppacher and Klett, 2010). However, at higher
187 concentrations, solutions can deviate from ideal behavior. Pruppacher and Klett (Pruppacher and Klett,
188 2010) and Haynes (Haynes, 2014) both present data for the freezing point depression of CsCl, but only
189 up to a salt concentration of 1.8 M (Pruppacher and Klett) or 1.4 M (Haynes). Extrapolating their data to
190 the concentrations expected in our samples (i.e., at -10 °C) suggest the CsCl concentration in LLRs
191 would be somewhere between 3 and 3.2 M, i.e., 10 – 20% higher than our ideal case concentration, but
192 neither source presents freezing point depression data measured at such a high concentration. In the
193 absence of measured information for the actual composition of CsCl solutions under our experimental
194 conditions, we have elected to stay with the theoretical prediction of salt concentration of 2.7 M. If the
195 actual LLR solute concentration is higher (lower) than 2.7 M, the V_{LLR}/V_{VOXEL} values presented here
196 would be lower (higher); we estimate the largest magnitude of this error as approximately 20%.

197 For clarity, we ~~have use the measured V_{LLR}/V_{VOXEL} values to segment segmented~~ many of our
198 images into four domains: voxels containing only air (defined as $V_{LLR}/V_{VOXEL} < -3.4\%$), voxels
199 containing ice and little or no solute ($V_{LLR}/V_{VOXEL} = -3.4\%$ to 2%), voxels containing a moderate amount
200 of solute ($V_{LLR}/V_{VOXEL} =$ with 2-10% of volume) as LLRs, and voxels containing a substantial amount of
201 solute ($V_{LLR}/V_{VOXEL} >$ with more than 10%) of their volume occupied by LLRs. We define an “air” voxel
202 as having a radiodensity less than or equal to the average radiodensity of an imaged air sample, i.e., 3996.
203 As noted above, greyscale values from images of pure materials vary somewhat, meaning a clear
204 distinction between two materials with similar average greyscale values is not possible. We chose to set
205 the cutoff for segmenting LLRs at ~~2%~~, a greyscale value of 5507, ~~since this a~~ threshold ~~is~~ three standard
206 deviations greater than the average greyscale value for pure ice, which will essentially eliminate the
207 problem of identifying water ice as solute. However, bBecause of this high threshold it is quite likely that
208 solute is present in some voxels characterized as “ice”. On the other hand, voxels defined as having an
209 LLR percentage of 2% or greater almost certainly contain solute. For ~~some CsCl-containing~~ samples, we
210 calculated the mass of CsCl present in each domain. Because the statistical distributions of voxels
211 containing only pure water ice and those containing <2% LLR as well as pure water ice overlapped, we
212 could not determine the As the mass of CsCl present in the water ice”ice” domain could not be
213 determined directly. Therefore, w, we assumed any mass not present in either the LLR 2-10% or LLR
214 >10% domains is present in the “ice” domain.

215

216 **3. Results and Discussion**

217 We first present imaging results for samples prepared without added solute (frozen Milli-Q
218 water). Figure 2a shows a reconstructed image of a “pure” ice sample prepared by freezing air-saturated
219 Milli-Q in a glass vial in a laboratory freezer; the full movie, which shows the sample rotating, is in
220 Supplemental Fig. S1. Air bubbles are visible as light grey spheroids, and are generally located towards
221 the center of the sample, away from the vial walls and base. This is likely because the entire outer surface
222 of the vial was cooled and, ~~because glass has a higher thermal conductivity than liquid water,~~ the water
223 apparently froze from the outside inward. Supporting this idea, some of the bubbles appear to elongate
224 along the radial axes of the sample, similar to the bubble elongation seen by Carte (Carte, 1961) in a
225 temperature gradient. The isolation of bubbles within the middle of the sample seems to follow
226 Shumskii’s (Shumskii, 1964) model of the formation of the “central nucleus”, with impurities (in this
227 case, air bubbles) forced to the center of a freezing water mass.

228 Figure 2b shows a reconstruction of a similar Milli-Q sample, but now where the solution was
229 degassed with helium for 30 minutes before freezing; the full movie is in Supplemental Fig. S2. Because
230 He degassing replaces the more soluble nitrogen and oxygen in the air-saturated solution with less soluble
231 helium, fewer bubbles are present in Fig. 2b. The size of the bubbles, however, is roughly similar in the
232 two figures (approximately 150-300 μm), suggesting bubble size is a function of the freezing method, not
233 of the gas itself.

234 Figure 2c shows a histogram of the number of voxels containing various radiodensities,
235 represented here as the ratio $V_{\text{LLR}}/V_{\text{VOXEL}}$, in the two water ice samples. A ratio of zero represents the
236 average radiometric density for pure water ice, with values slightly greater or less than zero indicating
237 noise in the sample images and reconstruction. Voxels containing only air comprise the smaller, second
238 peak centered at approximately $V_{\text{LLR}}/V_{\text{VOXEL}} = -0.05$, which overlaps with the primary (pure ice) peak.
239 Taking into account that the Y axis (voxel count) is a log scale, ~~t~~ The two curves show the volume of gas
240 bubbles is clearly less for the helium degassed treatment; note the Y axis (voxel count) is a log scale.
241 Table 1 shows the estimated volumes of water ice and gas bubbles in the two samples, as determined by
242 our segmentation process (see the Methods section). The gas volume in ice made from air-saturated
243 water is approximately 1.4 %, while the ice made from helium-saturated Milli-Q has approximately half
244 the gas volume. Figures 2a and 2b appear to show a larger difference in gas volume between the two
245 samples, suggesting that many of the small bubbles in the sample imaged in Fig. 2b may have been
246 smoothed away and thus not visible. For a solution in equilibrium with air at 25 C, the mole fraction
247 solubility of air (assuming a composition of 20% oxygen and 80% nitrogen) is 1.4×10^{-5} , while the value
248 for helium is 7.0×10^{-6} (Haynes, 2014), i.e., half the concentration of air in the solution. The expected
249 volume of bubbles in the helium degassed treatment agrees well with the observed volume.

250 Next, we examined the effect of freezing method on both freezing morphology and solute
251 location. The Freezer, Freeze Chamber, and LN2 sample preparation methods are described in the
252 Methods section. Figure 3 shows the results of imaging several combinations of freezing method and
253 solute. We start with an image of the ice made by freezing 1.0 mM CsCl in a laboratory freezer. As
254 shown in Fig. 3a (and the Supplemental Fig. S3 movie), both air bubbles and concentrated CsCl LLRs are
255 relatively large, with the LLRs tending to wrap around the air bubbles. Figure 3b is a blowup
256 magnification of the red-bordered area in Fig. 3a, showing examples of large solute inclusions wrapped
257 around air bubbles (lighter gray spheroids).

258 Figure 3c (movie: Supplemental Fig. S4) shows an identically a similarly prepared sample as the
259 Freezer sample in Fig. 3a, but frozen in our Freeze Chamber. Compared to the Freezer sample, the Freeze
260 Chamber sample has smaller air bubbles and inclusions, more solute present near the top of the sample,

261 and the areas of concentrated solutes (LLRs) are less likely to be associated with the air bubbles. These
262 points are clearly shown in Fig. 3d, which is a blowup-magnification of the red bordered area of Fig. 3c.
263 Considering that these two samples were frozen at similar temperatures, the morphologies are surprisingly
264 substantially different. As seen in Table 1, the fraction of voxels containing a LLR fraction >10% is
265 about five-fold less in the Freeze Chamber sample than the Freezer sample, while the fraction of voxels
266 with an LLR concentration between 2 and 10% doubles. This finding indicates the freezing process in the
267 freeze chamber creates smaller-is less likely to create large-LLR inclusions than does the freezer, with
268 LLRs distributed more widely throughout the sample. Additionally, substantial amounts of solute were
269 segregated towards the surface of the Freeze Chamber sample; presumably, the sample froze from the
270 bottom and solutes were preferentially excluded from the advancing freezing front. However, the same
271 process did not affect the air bubbles, which are well distributed throughout the sample. We believe these
272 structural differences may be due to faster freezing in the Freeze Chamber sample, as the freeze chamber
273 removes heat more quickly than the freezer because of direct contact between the bottom of the vial and
274 the chilled base plate in the chamber. Previous work (Hallett, 1964; Rohatgi and Adams, 1967) has
275 shown faster freezing gives closer spacing of ice dendrites or plates in the sample as it freezes, which then
276 leads to smaller solute inclusions or bubbles, similar to our finding here. Supplemental Fig. S5 shows a
277 sample prepared in the same way as in Fig. 3c, but with the metal plates in place in the freeze chamber,
278 which surrounds the vial with metal rather than air. Here, we see similar bubble size and location as the
279 sample frozen in the freeze chamber without the metal plates. However, unlike the sample frozen without
280 plates in the freeze chamber, the solute distribution with plates shows no segregation towards the top of
281 the sample, probably because the close proximity of the conductive metal plates removed heat from the
282 sides and bottom of the sample simultaneously, similar to the Freezer case.

283 Results for a 1.0 mM CsCl sample prepared with the third freezing method – liquid nitrogen – is
284 are shown in Fig. 3e, with the full movie in Supplemental Fig. S6. No air bubbles or significant solute
285 inclusions are visible. However, as discussed earlier, some very small inclusions and air bubbles can be
286 removed by the mathematical smoothing done by the reconstruction software, so very small features (<
287 ~80 μm) may be present in the sample but lost in the reconstruction. A histogram of raw (i.e., not
288 smoothed) greyscale values from the LN2 sample image does show some voxels contain concentrated
289 solutes (Fig. 3g), as indicated by $V_{\text{LLR}}/V_{\text{VOXEL}}$ for some voxels towards the right-hand side of the graph
290 being greater than that of pure water ice. As a further test of the possibility of solute inclusions in LN2
291 samples, we examined unreconstructed cross-sections of a 1.0 mM CsCl sample frozen in liquid nitrogen
292 and imaged at ~2 μm voxel resolution. As illustrated in Supplemental Fig. S7, there are some light
293 (concentrated solute) and dark (air bubble) areas, suggesting some segregation of CsCl and air occurs
294 even with rapid freezing (~30 seconds). However, this effect is barely-less noticeablevisible in the
295 quickly frozen liquid nitrogen sample (Supplemental Fig. S7), and much more pronounced in the other
296 two freezing methods (Figs. 3a and 3c). Analogous findings, although using a very different experimental
297 system, were reported by Heger et al. (Heger et al., 2005), who found solutes were concentrated by as
298 many as six orders of magnitude with slow (several minutes) freezing, but only three orders of magnitude
299 when frozen in liquid nitrogen.

300 Figure 3g shows the histogram for the 1.0 mM CsCl solutions frozen using each of the three
301 freezing methods, as well as for Milli-Q water ice frozen in a laboratory freezer. Unlike the images seen
302 in Figs. 3a through 3f, where mathematical smoothing can eliminate small structures, the histograms
303 include all the voxels in the sample. As discussed in Fig 2c, water ice has two overlapping peaks,
304 corresponding to air bubbles (left peak) and ice (right peak). Some voxels, shown in the “saddle”

305 between the two peaks, contain both air bubbles and pure water ice, and will therefore have a greyscale
306 value between air and ice. The Fig. 3g histogram clearly shows how CsCl tends to be present in larger
307 LLR volumes in the Freezer sample, including some voxels that are almost completely composed of 2.7
308 M CsCl solution, with a maximum V_{LLR}/V_{VOXEL} of 0.9. This finding supports the idea of solutes
309 segregating to concentrated LLRs during freezing, since if solutes were precipitating and forming solid
310 inclusions in the bulk ice, the calculated ratio in a voxel could be higher than 1. The fact that the ratio
311 gets close to, but never exceeds, 1 is consistent with our tricomponent model of air, relatively pure ice,
312 and concentrated LLRs with a maximum concentration of 5.4 M total solute.

313 The increased number of air voxels on the left end of the curve for the 1.0 mM CsCl freezer
314 sample represents voxels composed entirely of air. This number is larger than in the water sample,
315 supporting the imaging findings that the presence of solute actually increases the size of air bubbles. For
316 the Freeze Chamber and LN2 samples, the number of ~~voxels containing only air-bubbles~~ is smaller, and
317 voxels containing air are more likely to contain at least some fraction of ice or solute. For the Freeze
318 Chamber sample, the histogram correlates with the images (2c and 2d), with fewer voxels containing a
319 large volume fraction of highly concentrated regions than in the Freezer sample. Finally, the liquid
320 nitrogen ~~histogram is~~ results are nearly identical to water ice, although a few voxels with concentrated
321 solute are present (also seen in Supplemental Fig. S7).

322 ~~-~~Next, we examined the impact of solute on freezing morphology and solute location, by
323 replacing CsCl with Rose Bengal, a large, organic molecule (see structure in Supplemental Fig. S8).
324 Figure 3f (movie: Supplemental Fig. S9) shows a sample containing 1.0 mM Rose Bengal frozen in our
325 freeze chamber. Using 1.0 mM Rose Bengal instead of 1.0 mM CsCl (Fig. 3c) gives a very different
326 freezing pattern, with only a few small bubbles and ~~a few no visible~~ areas of concentrated solute. While
327 mathematical smoothing has likely eliminated some of the smaller structures, the overall sample
328 morphology is quite different than that produced by the same concentration of CsCl. Miedaner and
329 Miedaner and co-workers (Miedaner, 2007; Miedaner et al., 2007), using different compounds, also found
330 that sample morphology was highly sensitive to solute identity. Interestingly, changing solute in our
331 system ~~this change in solute~~ alters not only the structure of solute inclusions, but also the size of the air
332 bubbles. The exact reason for the change in morphology is unclear. CsCl is more polar than Rose
333 Bengal, and could influence the movement of the polar water molecules into the forming ice matrix. As a
334 relatively large organic molecule, Rose Bengal ~~is also a relatively large organic molecule, much different~~
335 than the ions of CsCl, and could therefore ~~might~~ potentially modify the ice matrix due to its size. Finally,
336 we note the thermodynamically predicted final concentration of solute ions at -10°C is 5.4 M; at this
337 concentration CsCl should still be in solution, while a substantial portion of the Rose Bengal should have
338 precipitated. Whether precipitated Rose Bengal is present as solids incorporated into the ice matrix or as
339 precipitates in LLRs is not known.

340 ~~Figure 3g shows the histogram for the 1.0 mM CsCl solutions frozen using each of the three~~
341 ~~freezing methods, as well as Milli-Q water ice frozen in a laboratory freezer. Unlike the images seen in~~
342 ~~Figs. 3a through 3f, where mathematical smoothing can eliminate small structures, the histograms include~~
343 ~~all the voxels in the sample. As discussed in Fig 2c, water ice has two overlapping peaks, corresponding~~
344 ~~to air bubbles (left peak) and ice (right peak). Some voxels, shown in the “saddle” between the two~~
345 ~~peaks, contain both air bubbles and pure water ice, and will therefore have a greyscale value between air~~
346 ~~and ice. The Fig. 3g histogram clearly shows how CsCl tends to be present in larger LLR volumes in the~~
347 ~~Freezer sample, including some voxels that are almost completely composed of 2.7 M CsCl solution, with~~
348 ~~a maximum V_{LLR}/V_{VOXEL} of 0.9. This finding supports the idea of solutes segregating to concentrated~~

349 ~~LLRs during freezing, since if solutes were precipitating and forming solid inclusions in the bulk ice, the~~
350 ~~calculated ratio in a voxel could be higher than 1. The fact that the ratio gets close to, but never exceeds,~~
351 ~~1 is consistent with our tricomponent model of air, relatively pure ice, and concentrated LLRs with a~~
352 ~~maximum concentration of 5.4 M total solute.~~

353 ~~The increased number of air voxels on the left end of the curve for the 1.0 mM CsCl freezer~~
354 ~~sample represents voxels composed entirely of air. This number is larger than in the water sample,~~
355 ~~supporting the imaging findings that the presence of solute actually increases the size of air bubbles. For~~
356 ~~the Freeze Chamber and LN2 samples, the number of air bubbles is smaller, and voxels containing air are~~
357 ~~more likely to contain at least some fraction of ice or solute. For the Freeze Chamber sample, the~~
358 ~~histogram correlates with the images (2c and 2d), with fewer voxels containing a large volume fraction of~~
359 ~~highly concentrated regions than in the Freezer sample. Finally, the liquid nitrogen results are nearly~~
360 ~~identical to water ice, although a few voxels with concentrated solute are present (also seen in~~
361 ~~Supplemental Fig. S7).~~

362 The reproducibility of samples prepared on different days but using identical methods was quite
363 good, with similar patterns seen for each replicate (Supplemental Fig. S10). Each combination of
364 freezing method and solute gave a distinct distribution of solute and air bubbles, suggesting these two
365 variables have a significant impact on ice morphology in our experimental system. are the primary
366 factors influencing ice morphology.

367 Table 1 lists the calculated volume of each material domain and the total CsCl mass present,
368 including all sample voxels, based on segmentation described in the Methods section. As seen in the
369 images and histogram, the Freezer sample has the highest fraction (0.00019) of voxels containing 10% or
370 more LLR volume, approximately 5 times greater than the Freeze Chamber sample. In contrast, the
371 fraction of voxels with $V_{LLR}/V_{VOXEL} = 2\text{-}10\%$ in the Freezer sample (0.003) is about half that in the Freeze
372 Chamber sample, and the fraction of gas bubbles appears to be less than in the Freeze Chamber sample.
373 However, this may be a computational artifact; voxels containing LLR next to gas bubbles will have a
374 greyscale value somewhere between air and LLR, and therefore may be mistakenly counted as water ice
375 voxels. Unfortunately, determining the magnitude of this error is difficult -requiring estimating the
376 surface area of both air bubbles and any adjacent LLRs to identify suspect voxels - and is beyond the
377 scope of this study. Because LLRs in the Freezer samples are more concentrated and appear to be more
378 frequently found next to air bubbles (as seen in Fig. 3b), this effect may be more pronounced in the
379 Freezer samples than Freeze Chamber samples. However, the number of voxels mistakenly classified as
380 water (or less concentrated solute) is limited to boundaries between air and LLRs and therefore small, and
381 should not affect the overall interpretation of results. Examining the location of the CsCl mass, more than
382 10% of all CsCl present in the Freezer sample is found in voxels with LLRs >10%, while in the Freeze
383 Chamber sample only around 1% of the mass is found in these most concentrated LLRs. For both Freezer
384 and Freeze Chamber samples, about two-thirds of the CsCl mass is found in the ice compartment,
385 suggesting most solutes are present in very small LLR inclusions that are indistinguishable from water
386 ice. For the LN2 sample, only 12% of the mass is found in detectable LLRs, with the remainder
387 distributed throughout the water ice. It is also possible that the CsCl in the LN2 samples is present not as
388 liquid inclusions, but as solid solution within the water ice. However, the solubility of HCl in solid ice is
389 $(1\text{-}2) \times 10^{-4}$ M (Gross et al., 1975), while the CsCl solubility in solid ice would need to be 5-10 times
390 greater, assuming all the CsCl is present in solid solution. The “missing” CsCl mass here is $0.88 * 126.3$
391 $\mu\text{g} = 111.1 \mu\text{g}$, or $0.66 \mu\text{mol}$. Assuming this solute is entirely present as LLRs with solute concentration
392 of 2.7 M, this equates to a total LLR volume of 0.24 μL . The volume of pure ice (again from Table 1) is

393 725 μ L. Therefore, assuming the remaining CsCl is distributed equally throughout the voxels labeled as
394 pure ice in Table 1, the calculated average V_{LLR}/V_{VOXEL} for these voxels is 0.034%, indistinguishable
395 from water ice in our system. While it is possible the CsCl is present (at least partially) as solutes in the
396 solid ice matrix, we believe it is more likely to be present primarily as small LLR inclusions.
397 Additionally, we present evidence later in this paper supporting the idea that solutes are predominantly
398 present as LLR inclusions.

399 We next examined the impact of sample container on sample morphology and solute distribution
400 by imaging samples frozen in plastic vials instead of the glass vials we used above. While many of the
401 samples discussed thus far were frozen in the laboratory freezer, most of the samples prepared in plastic
402 vials were frozen in the freeze chamber. Therefore, to allow appropriate comparisons, we first present a
403 sample of water (no solute) frozen in the freeze chamber and compare this with previous samples frozen
404 in the freezer. Milli-Q water frozen in the freeze chamber in a glass vial (Supplemental Fig. S11) gives
405 similar spatial distribution and somewhat smaller air bubbles sizes as ~~an identical~~ a similar sample frozen
406 in a laboratory freezer (Fig. 2a and Supplemental Fig. S1). However, freezing water in a plastic vial
407 rather than glass can make a significant difference in ice morphology, as shown in Supplemental Fig. S12.
408 While ice in a glass vial forms many roughly spherical bubbles, water frozen in a plastic vial using our
409 freeze chamber forms long vertical channels; such directional growth of air bubbles in a freezing liquid
410 has previously been reported (Carte, 1961). While the reason for this morphology is not entirely clear,
411 unclear, we believe it is related to how heat is removed from the sample during freezing (~~Carte, 1961~~).
412 Because plastic conducts heat more poorly than water, ice, or glass, the vial walls act as insulators,
413 forcing heat to be primarily removed from the bottom of the sample where the plastic vial contacts the
414 chilled plate at the base of the freeze chamber. This may promote the formation of vertical air channels as
415 the ice freezes upwards through the sample, rather than from the walls towards the interior in the glass
416 vial sample.

417 We next examine the impact of freezing in plastic for a sample containing solutes. Supplemental
418 Fig. S13 shows a 1.0 mM CsCl solution frozen in the freezer in a plastic vial; compared to the similarly
419 treated sample frozen in a glass vial (Fig. 3a), the air bubbles and concentrated inclusions are smaller in
420 the plastic vial. Interestingly, the air bubbles in the plastic vial CsCl Freezer sample do not show any of
421 the elongation found when Milli-Q water is frozen in a plastic vial in the freeze chamber (Supplemental
422 Fig. S12), which may be related to the directional heat removal in the freeze chamber. Finally, once again
423 using the freeze chamber, Supplemental Fig. S14 shows 1.0 mM Rose Bengal frozen in plastic in the
424 freeze chamber. Here, we see substantial volumes of LLRs and more bubbles than seen in the sample
425 frozen in a glass vial, but without any elongation to bubbles or LLRs. ~~elongated solute inclusions;~~
426 however, the air bubbles are not elongated.

427 We also performed several other experiments to examine the nature of LLRs. Figure 4 shows a
428 cross-section of microCT images of the same sample (1.0 mM CsCl, frozen in ~~laboratory~~-freezer) at voxel
429 resolutions of 16 μ m (left) and 2 μ m (right); the corresponding movies are in Supplemental Fig. S15. The
430 areas of light grey in the lower resolution image (16 μ m voxel resolution), such as the area highlighted by
431 the arrow, are likely areas where CsCl is present in small areas of concentrated LLRs bordered by pure
432 water ice, although the voxel resolution does not show these features separately. As would be expected if
433 freezing water effectively excludes solutes from the forming bulk ice matrix, the right hand image shows
434 areas of concentrated LLRs adjacent to areas of pure water ice, supporting the idea discussed earlier that
435 during freezing solutes are preferentially excluded from the forming ice matrix into small areas of

436 concentrated solution. The higher resolution image in Fig. 4 also shows very clearly how the solutes in
437 LLRs often wrap around the bubbles in the Freezer CsCl samples.

438 Finally, Fig. 5 (and the accompanying movie in Supplemental Fig. S16) ~~gives further evidence supporting~~
439 supports the idea that CsCl is contained in liquid-like regions in our ice samples. We placed a 1.0 mM
440 CsCl sample (glass vial; Freezer) in the microCT sample holder set at -10 °C and took images of the
441 sample (2 μm voxel resolution, X-Z plane) at 0, 11, and 22 hours. The temperature gradient in the sample
442 holder was measured later by placing a thermocouple sensor between the glass vial and the holder wall at
443 various positions. The ~~vertical~~ temperature difference between the bottom and middle of the holder
444 (approximately 1.7 cm, extending above and below the 1 cm height of the frozen sample in the vial) was
445 2.2 °C, resulting in a temperature gradient of 0.13 °C mm⁻¹. As seen in the three images, over the 22
446 hours of this experiment the bright areas of CsCl move in the direction of the temperature gradient,
447 towards the warmer top of the vial, at a rate of approximately 10 μm 0.01 mm h⁻¹. (i.e., 7.7 μm h⁻¹/(K⁻¹
448 cm⁻¹)). In many cases, the solutes appear to be migrating around the surfaces of air bubbles, which are
449 visible as darker grey spheres. While the air bubbles appear to remain stationary in the ice matrix, with
450 an estimated maximum migration rate of 0.15 μm h⁻¹/(K⁻¹ cm⁻¹), the CsCl moves. Solutes are excluded
451 from, consistent with the idea the forming ice matrix during freezing (Hobbs, 1974; Petrenko and
452 Whitworth, 1999); here, it appears the that solutes are present as a concentrated liquid-like solution, which
453 can migrate either along the boundaries between air bubbles and the bulk ice, or possibly by melting into
454 the bulk ice itself (Notz and Worster, 2009). While we cannot rule out the possibility that the migrating
455 solutes might be present as solid salt crystals, as seen in other work for ice under a temperature gradient
456 (Light et al., 2009), the moving solutes in our images appear to be in liquid-like regions. (Light et al.,
457 2009). Previous studies have found bubbles migrate in a temperature gradient at rates of around 1.5-3 μm
458 h⁻¹/(K⁻¹ cm⁻¹) (Dadic et al., 2010), while brine inclusions move at around 10 μm h⁻¹/(K⁻¹ cm⁻¹) (Light et al.,
459 2009). While our results support the idea of brine moving faster than bubbles, the relative rates in our
460 experiments seem much different (with the bubbles moving slower and the brine moving faster) than
461 suggested by previous literature. However, the earlier studies were done in systems containing either
462 bubbles or brine inclusions, not both; as noted by Light (Light et al., 2009), “The effect of included gas
463 bubbles on brine migration has not been studied.”

464 **4. Implications and Conclusions**

466 Using microCT we directly visualized the locations of solute, gas, and bulk ice in laboratory-
467 prepared ice samples. While the chemical concentrations we used are higher than those in clean polar
468 samples, because of the substantial morphological differences seen between pure ice samples and solute-
469 containing samples, we expect that solutes in natural snow and ice might sometimes have similar,
470 significant-important impacts on sample morphology, including the location and sizes of liquid-like
471 regions and air bubbles.

472 Highlighting the sensitivity of ice structure to freezing conditions, we found a surprisingly large
473 difference between samples prepared at freezing temperatures in an upright freezer (where the sample was
474 surrounded by cold air) versus our custom-built freeze chamber (where the sample sat on a cold plate).
475 Samples frozen in liquid nitrogen, as expected, did not have the large air bubbles and LLR inclusions
476 found in Freezer or Freeze Chamber samples; nonetheless, we did find some evidence for the segregation
477 of solutes into LLRs, even with the fast freezing of liquid nitrogen.

478 In addition to freezing conditions, the choice of solute (either Cesium chloride and/or Rose
479 Bengal-) also impacted the ice sample structure differently; CsCl yielded larger air bubbles and solute
480 inclusions compared to Rose Bengal. While the observed variations in the locations and sizes of solute
481 inclusions might be expected for solutes of different polarity and size, the influence of solute on bubble
482 morphology is more surprising. CsCl samples frozen in our laboratory freezer showed large LLRs, often

483 wrapping around air bubbles. While QLLs at the surface ice-air interface of ice or snow are obviously in
484 contact with atmospheric oxidants, the preferential collocation of internal LLRs and air bubbles represents
485 a previously unrecognized air-ice interface. Depending on the chemistry occurring at this interface, the
486 bubbles might be a source of oxidants and other gas-phase chemicals to internal solutes, and might have
487 significant impacts for chemical transformations under certain conditions.

488 ~~Our work here can help guide further investigations to understand the driving forces shaping~~
489 ~~snow and ice structures in the natural world, as well as the rate of chemical reactions in snow and ice. At~~
490 ~~the same time, Our results here suggest that~~ subtle changes in the preparation of laboratory ice samples
491 can have significant impacts on the location of solutes in samples, requiring careful and consistent sample
492 preparation to ensure meaningful results. Ideally, researchers would directly evaluate the location of
493 solutes for each sample preparation method, as we have done here; we recognize, however, this is a
494 significant undertaking and not possible for every laboratory to do. Beyond the impacts on laboratory
495 science, our work here may be able to help ~~Our work here can help guide further investigations to~~
496 ~~understand the driving forces shaping snow and ice structures in the natural world, as well as~~
497 ~~investigations of~~ as the rate of chemical reactions in various compartments in snow and ice.

498

499 **Supplemental Information**

500 Supplemental information is available at <http://dx.doi.org/10.1594/PANGAEA.855461>.

501

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508

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Sample	Volume (mm ³) ^a						Volume Fraction ^{a, b}			CsCl Mass Fraction ^{a, c}			
	Initial solution volume (μL)	Total CsCl mass (μg)	Gas	Water ice	LLR 2-10%	LLR >10%	Gas	Water ice	LLR 2-10%	LLR >10%	Water ice	LLR 2-10%	LLR >10%
<u>MillQ water</u>													
Freezer	500	0	5.96	430	0	0	0.014	0.986	0	0	--	--	--
Freezer, degassed	500	0	3.23	432	0	0	0.007	0.993	0	0	--	--	--
<u>1 mM CsCl</u>													
Freezer	750	126.3	5.07	716	2.35	0.141	0.007	0.990	0.003	0.00019	0.651	0.233	0.116
Freeze chamber	500	84.2	5.55	473	2.67	0.0176	0.012	0.983	0.006	0.000037	0.640	0.346	0.014
Liquid nitrogen	750	126.3	0	725	1.50	0	0	0.998	0.002	0	0.879	0.121	0.000

701

702 Table 1. Sample volumes and fractions by material type.

703

704 ^a “Gas” is defined as having a greyscale value of < 3996, “Water ice” is defined as containing < 2% liquid-like region (LLR), “LLR 2-10%” is
705 water ice containing an LLR fraction of between 2 and 10%, and “LLR > 10%” is water ice containing > 10% LLR. The original sample volume
706 (either 500 or 750 μL) is not fully captured in the volumes reported here. The segmentation process eliminates some of the lower part of the
707 sample, reducing the reported volume somewhat.

708 ^b Fraction of imaged sample volume (not initial solution volume). See text for details.

709 ^c Fraction of total CsCl mass present in each domain. Because the mass of CsCl present in the water ice compartment could not be determined
710 directly, we assumed any mass not present in either the LLR 2-10% or LLR >10% domain is present in the water ice domain.

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714 **Figure Captions**

715
716 Figure 1. Radiodensity of pure water (red open squares, three data points) and of aqueous solutions
717 containing CsCl (blue triangles).
718

719 Figure 2. Reconstructed images (*a* and *b*) and histogram (*c*) of water ice samples frozen in a laboratory
720 freezer, imaged using microCT (~16 μm voxel size) and segmented to show air bubbles (light grey) and
721 the bulk ice matrix (darker grey). The glass sample vial is not shown. The ice in panel *a* was made using
722 air-saturated water, while that in *b* was made with water degassed with helium for 30 min before freezing.
723 Panel *c* shows the distributions of the radiodensities within the two samples, expressed as the fraction of
724 each voxel that would be occupied by a liquid-like region (LLR) assuming the total solute concentration
725 is determined by freezing point depression (i.e., 5.4 M at -10°C ; (Cho et al., 2002)).
726

727 Figure 3. Reconstructed images and histograms of ice samples frozen using three freezing methods and
728 with two different solutes. Samples were imaged using a ~16 μm voxel size and segmented to show air
729 bubbles (light grey), the bulk ice matrix (darker grey), voxels where $V_{\text{LLR}}/V_{\text{VOXEL}}$ is between 2 and 10%
730 (orange) and where $V_{\text{LLR}}/V_{\text{VOXEL}} > 10\%$ (red). The sample vial is not shown. a) 1.0 mM CsCl solution
731 frozen in freezer. b) blowup-magnification of the area in a) identified by the dashed red square. c) 1.0
732 mM CsCl solution frozen in freeze chamber (without metal plates). d) blowup-magnification of the
733 dashed-line area of c). e) 1.0 mM CsCl solution frozen in liquid nitrogen. No air bubbles or inclusions
734 are visible at this scale. f) 1.0 mM Rose Bengal solution frozen in freeze chamber. g) histogram showing
735 distribution of voxel counts for the CsCl and Milli-Q water ice samples shown above: water ice frozen in
736 freezer, black dotted line; 1.0 mM CsCl frozen in LN2, orange line; 1.0 mM CsCl frozen in freezer, blue
737 line; 1.0 mM CsCl, frozen in freeze chamber, green line. The inset shows an expanded view from
738 $V_{\text{LLR}}/V_{\text{VOXEL}} = -0.1$ to 0.1.
739

740 Figure 4. Side-by-side micro CT cross sections of the same sample (1.0 mM CsCl, frozen in laboratory
741 freezer) imaged at approximately 16 μm (panel a) and 2 μm (panel b) voxel sizes. Lighter tones indicate
742 areas of higher radiodensity, i.e., higher solute amounts. The scale bar applies to both images.
743

744 Figure 5. Vertically sliced X-ray images of a 1.0 mM CsCl ice (laboratory freezer, voxel resolution ~ 2
745 μm) after 0, 11, and 22 hours in the CT sample chamber. Lighter tones indicate areas of higher
746 radiodensity (e.g., greater CsCl amounts). Air bubbles are visible as darker gray spheres. The
747 temperature of the sample holder was set at -10°C , but the top of the sample was approximately 1.3°C
748 warmer than the bottom, corresponding to a temperature gradient of approximately $0.13^\circ\text{C mm}^{-1}$.
749 Arrows highlight two of the areas where CsCl moves along the direction of the temperature gradient,
750 from colder to warmer.
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