A universally applicable method of calculating confidence bands for ice nucleation spectra derived from droplet freezing experiments

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10   **Abstract.** A suite of generally applicable statistical methods based on empirical bootstrapping is presented for calculating uncertainty and testing the significance of quantitative differences in temperature and/or ice active site densities between ice nucleation temperature spectra derived from droplet freezing experiments. Such experiments are widely used to determine the heterogeneous ice nucleation properties and ice nucleation particle concentration spectra of different particle samples, as well as in studies of homogeneous freezing. Our methods avoid most of the assumptions and approximations inherent to existing

15   approaches and if used properly can capture the full range of random variability and error in ice nucleation spectra. Applications include calculation of accurate confidence intervals and confidence bands, quantitative statistical testing of differences between observed freezing spectra, accurate subtraction of the background filtered water freezing signal, and calculation of a range of statistical parameters using data from a single droplet array freezing experiment if necessary. By improving the statistical tools available, this work will improve the quality and accuracy of future ice nucleation research and

20   will allow quantitative comparisons of the ice nucleation ability of different particles and surfaces.

1 Introduction

Ice nucleation (IN) is a complex process with significant implications for cloud properties in atmospheric science (Gettelman et al., 2012; Mülmenstädt et al., 2015; Froyd et al., 2022). Heterogeneous ice nucleation, where a separate phase or substance assists the nucleation of ice above the homogeneous freezing limit, is particularly difficult to study as the length

25   and time scales at play in nucleation cannot be directly observed (Fletcher, 1969; Kiselev et al., 2017; Holden et al., 2019). Most researchers resort to macroscopic measurements of this nanoscale process by creating droplets containing suspensions of the ice active material and observing freezing events as time passes or temperature changes (Vali, 2014). The most common technique is a variation on the droplet-on-substrate apparatus, where droplets of known sizes are created by manual pipetting, condensation, or microfluidic means (Brubaker et al., 2020; Budke and Koop, 2015; Stan et al., 2009; Whale et al., 2015;

30   Reicher et al., 2018; Gute and Abbatt, 2020; Chen et al., 2018; Roy et al., 2021; Polen et al., 2018). These droplets are usually

exposed to a negative temperature ramp and the freezing temperatures of each droplet are recorded to produce an ice nucleation rate or active site density spectrum as a function of temperature. Other procedures can be used to test the effects of time and other variables on IN activity (Wright and Petters, 2013). Because these experiments only indirectly measure IN activity, results can have high natural variability, even when measuring the same sample on the same instrument. This is no fault of the
35 experimentalist; it is simply a fact of the ice nucleation process that it can be affected by small variations in droplet sizes, suspension inhomogeneities, droplet environments, ice active site heterogeneity within the suspended sample, and temperature variations (Polen et al., 2018). Even in a perfect experimental setup, the stochastic nature of nucleation causes variation in the measured temperature dependence of a material's IN activity using a singular model (Vali, 2014, 2019). Combined with the large variations in IN activity observed between different ice nucleating substances and particles, this inherent uncertainty
40 creates difficulties in reliably assessing whether differences in observed IN spectra indicate a statistically significant difference in IN activity.

If we cannot eliminate experimental error, it must instead be accounted for and reported, usually in the form of a standard error or a confidence interval of the mean measurement recorded. To date there is no widely accepted or implemented approach to reporting uncertainty in IN temperature spectra derived from freezing experiments. This is due in part to the
45 complexity of ice nucleation – elementary statistical tests, methods, and approximations are markedly inaccurate, and practitioners of advanced statistical methods in this field are few. Yet, without reliable expressions of experimental uncertainty and variability, single IN spectra are less meaningful, and comparisons between IN spectra are imprecise at best and misleading at worst. To remedy these shortcomings, this study presents a set of generalized and statistically rigorous methods for representing experimentally determined IN spectra, calculating confidence bands and statistics across the range of the spectra,
50 and testing differences between spectra at any confidence level. We also address some remaining questions on droplet freezing experimental parameters to direct the field towards more rigorous and repeatable methods of experimentation and data analysis. An implementation of these approaches along with documentation and instructions for its use is provided freely for use or reference to assist in future research and improve the statistical treatment of ice nucleation data in the field.

2. Sample data and preprocessing

55 To demonstrate the statistical methods described here, we selected a realistic and complex toy IN dataset shown in Fig. 1. Volcanic ash from the 2015 eruption of Volcan de Fuego in Guatemala (Jahn et al., 2019) was tested for ice nucleation activity before and after being exposed to water in a 1 wt% suspension and allowed to dry under a constant 1 Lpm flow of pre-dried lab air similarly to Fahy et al. (2022). In both cases, a 0.1 wt% suspension of unaged or aged ash was created in HPLC water filtered through a 0.02 micron pore size Anatop syringe filter. These suspensions were then tested for IN activity on the
60 CMU-CS droplet-on-substrate system described in detail by Polen et al. (2018) and are compared to a background freezing spectrum obtained from the filtered water used to create the suspensions. Approximately 50 100 nL droplets were tested per

array with a cooling rate of 1 °C per minute. Two separate suspensions were tested for the unaged ash sample, and three
suspensions were tested from ash exposed to water in two separate experiments for the aged ash sample.
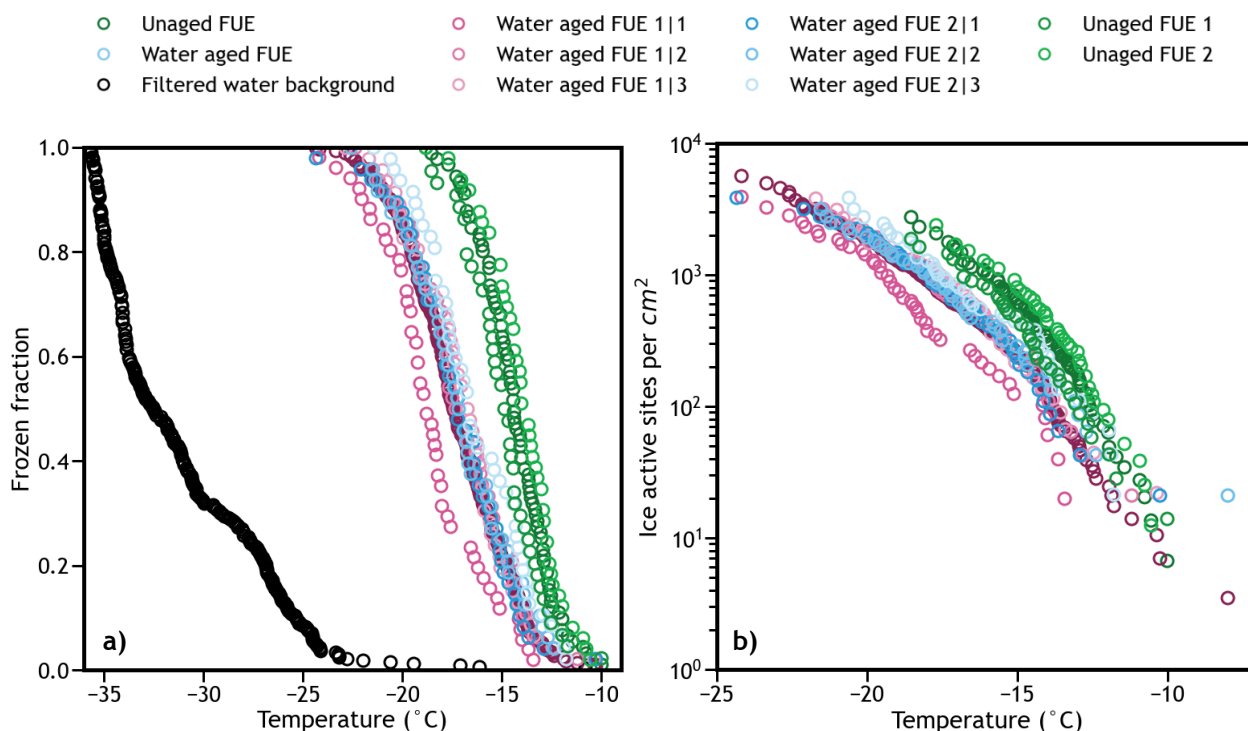
Figure 1. Raw (not interpolated or binned) and combined raw a) frozen fraction and b) surface area normalized ice nucleation
active site density spectra for sample droplet freezing data used in this study. Water aged spectra are from two separate aging
experiments with three freezing experiments each.

65   Since multiple freezing experiments were performed on nominally identical samples (e.g. the replicate suspensions
of the same ash or aging experiment), these spectra were combined by merging the lists of freezing events that occurred in
each experiment. The frozen fractions and ice nucleation active site density spectra were then recalculated as if the combined
freezing events occurred in a single experiment. The validity of this approach depends on the assumption that the suspensions
being combined have the same IN activity, and thus that drawing droplets from each of the suspensions individually is identical
70   (or at least insignificantly different) from drawing droplets from the rest of the suspensions. This is only true when the IN
spectrum of a given suspension is insignificantly different from the combined spectra of all other suspensions and the physical
and chemical properties (e.g., suspension concentration, sample type, water purity, background freezing) are identical between
suspensions. The second condition can easily be tested in the laboratory, while the first condition can be evaluated using
statistical tests described in this paper (see Section 5.2).

75   The ice active site density spectra (k and K) were calculated directly based on Eqs. 1 and 2 (Vali et al., 2015; Vali,
1971, 2019), where k is the differential spectrum, K is the cumulative spectrum, $f(T)$ is the raw fraction of droplets frozen at
temperature T, N_0 is the total number of droplets in the array, N is the number of droplets that have frozen up to a given point,

and ΔN is the number of droplets that have frozen in a given measurement interval. The variable ΔT is the size of the

measurement interval, the choice of which is discussed below. The normalization factor X can be average droplet volume,

80   mass of sample suspended per droplet, or as is used here, specific surface area of sample suspended per droplet, giving the

number of ice active sites at temperature T per unit of suspension volume (usually denoted K), sample mass (denoted n_m), or

sample surface area (denoted n_s) respectively. For simplicity and generality, in this work K and k will be used to describe any

cumulative or differential IN spectrum.

$$k(T) = -\frac{1}{X\Delta T} ln\left(1 - \frac{\Delta N(T)}{N(T)}\right) \tag{1}$$

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$$K(T) = \frac{1}{X} ln\frac{N_0}{N(T)} = -\frac{1}{X}ln(1 - f(T)) = \sum_{T_i}^{T_f} k(T)\,\Delta T \tag{2}$$

Differential IN spectra have only recently come into common use because most interpretations of the formulation of

k require high-quality data (e.g., many uniformly sized droplets with low background freezing activity) for the coarse binning

process used to ensure the data are not too sparse. See Vali (2019) for an in-depth discussion of this approach based on

90   improvements in ice nucleation data quality obtained using droplet microfluidics by Polen et al. (2018) and Brubaker et al.

(2020) that make the application of differential IN spectra feasible. However, differential spectra are extremely useful both for

visual interpretation of data and for quantitative comparison of IN spectra. Specifically, they can provide information on how

many IN sites become active at a given temperature, which is not immediately obvious from examining a cumulative spectrum.

For a more generally useful method of directly calculating raw differential spectra, ΔT was chosen such that the endpoints of

95   the temperature interval for a datapoint were the midpoints of the distance between the nearest neighbors on either side of the

datapoint, and ΔN is the number of droplets that froze at that datapoint. Vali (2019) stated that this approach loses the

quantitative significance of k because the value of k will vary based on the size of ΔT, however, we contend that it is this

variation in the size of ΔT that maintains the quantitative aspect of k, as the magnitude will be inversely proportional to the

temperature density of freezing events as expected. This results in noisy data, but when coupled with the interpolation

00   techniques presented in Section 3, this problem can be resolved using a smoothing algorithm. This approach has the advantage

of ensuring that every 'bin' has at least one freezing event in it while maintaining the advantages of differential IN spectra

compared to cumulative spectra, even for relatively low-quality data. However, as will be shown in Sections 4 and 5, high-

resolution data (e.g., from many droplets or freezing experiments) are still required for statistical comparisons of differential

IN spectra.

3. Interpolating freezing curves and calculation of continuous spectra

The most common style of reporting ice nucleation activity is using the cumulative ice nucleation active site density

curves (K) calculated directly from raw data as shown in the previous section, but there is an important limitation to this type

of data treatment. While it represents the data exactly as measured, there is no way to quantitatively compare one raw freezing spectrum with another without some type of interpolation. This is because even if a droplet freezes at a particular temperature

10   in one experiment, there is no guarantee that a droplet will freeze at or near that temperature in another experiment. Often the approximate difference between spectra is just compared by eye for lack of a better method. This presents issues when trying to subtract a background spectrum or when quantifying uncertainty and testing statistical difference between spectra and leads to a need for effective interpolation methods for comparing IN spectra.

3.1 Binning and its shortcomings

15   One common method for interpolating IN spectra is through temperature binning, where a temperature interval is represented by a single value of IN activity that is treated as constant throughout the interval. This approach is appealing, as it aligns with the discrete nature of IN experiments and allows straightforward calculation of differential IN spectra by using the bin width as ΔT (Vali, 2019). However, binning is widely accepted as an inefficient interpolation method for measurements of continuous variables such as ice nucleation activity and has been shown to reduce statistical power and bias statistical results

20   in data from a variety of disciplines (Selvin, 1987; Gehlke and Biehl, 1934; Manley, 2014; Altman and Royston, 2006; MacCallum et al., 2002; van Leeuwen et al., 2019; Virkar and Clauset, 2014). While ice nucleation activity is measured discretely, it is a continuous property – any given ice nucleation site has a theoretical ice nucleation rate over the entire continuous temperature range and combining many ice nucleation sites together results in a complex continuous curve (Beydoun et al., 2016). It is therefore desirable to transform the discrete measurements into continuous space to accurately

25   represent IN activity rather than further discretizing them as in binning.

3.2 Previous methods of continuous functional interpretation

To make a discrete variable continuous, some type of functional interpolation is required. Many studies approximate IN spectra as exponential polynomials or similar simple functions (Atkinson et al., 2013; Harrison et al., 2019, 2016; Vergara-Temprado et al., 2017; Kanji et al., 2013; Price et al., 2018; Niedermeier et al., 2015; Peckhaus et al., 2016). Exponential

30   polynomials can capture the overall exponential shape of cumulative IN spectra in most cases, however, they impose explicit assumptions about the shape (and therefore physical basis) of the IN spectra through their closed-form expressions. Particularly in complex samples that contain mixtures of different types of ice nucleation sites (e.g. Beydoun et al., 2017), simple polynomials are likely to be insufficient for accurate interpolation of IN spectra.

Instead, the ideal interpolation method would take a series of measured datapoints from a droplet freezing experiment

35   and would output a continuous IN parameterization that could predict the IN activity of the sample at any temperature. A physically-based parameterization such as a contact angle scheme (Beydoun et al., 2016; Ickes et al., 2017; Chen et al., 2008) or the singular-stochastic formulation of ice nucleation (Vali, 2014; Barahona, 2012; Niedermeier et al., 2011) would be preferred, however, these parameterizations require preexisting knowledge or assumptions about of the nature of the sample

being tested. For data analysis in laboratory or field studies, this information is often not available, and we must look elsewhere
40   for an interpolation method.

3.3 Piecewise interpolation for ice nucleation spectra

For a generally applicable interpolation scheme, piecewise fitting algorithms such as spline fits are the best option.
Spline fits provide accurate interpolations of arbitrarily complex data by fitting a series of polynomials to small portions of the
45   available data. The resulting piecewise functions are continuous and differentiable, meaning that only one or the other of the
cumulative or differential IN spectrum must be directly fit from the data – the other spectrum can be calculated by either
computing the negative derivative of the cumulative freezing curve or the negative antiderivative of the differential spectrum.
To find the best fitting method, a variety of algorithms available in the Python Scipy library (Virtanen et al., 2020) were
modified and tested for their ability to faithfully interpolate the combined water aged volcanic ash ice nucleation spectrum.
50   Splinederiv uses a cubic spline fit of the cumulative spectrum, splineint uses a cubic spline fit of the differential spectrum,
PCHIP uses the piecewise cubic Hermite interpolated polynomial algorithm of the cumulative spectrum, and smoothedPCHIP
is the PCHIP curve followed by a cubic spline fit with a smoothing factor.

Figure 2a (cumulative n_s) and 2b (differential n_s) compare these methods to a binning approach and the raw data from
Fig. 1 using the water aged FUE ash spectrum. On initial inspection, basic spline fits perform well at higher ice active site
55   densities. However, the splinederiv algorithm does not always maintain the monotonicity requirement intrinsic to the
cumulative spectrum (and correspondingly are not strictly positive in the differential spectrum). The splineint algorithm
corrects for this, but performs relatively poorly in capturing the behavior of early freezers, overestimating the IN activity
between –10 and –14 °C. The solution to these two problems is to interpolate the cumulative spectrum with the monotonicity
constraint offered by the PCHIP fitting algorithm and to take the derivative of this interpolation for the differential spectrum.
60   This method faithfully reproduces the cumulative IN spectrum because it calculates an exact interpolation, but as a result is
extremely noisy in the differential spectrum. By adding an additional smoothing step after the PCHIP interpolation (using a
simple smoothed cubic spline fit after the PCHIP algorithm), a smooth, interpretable, and realistic interpolated ice nucleation
spectrum can be derived from the raw data without losing the complexity present at the low temperature end of the spectrum
visible in the differential plot as shown in the smoothedPCHIP spectrum. This algorithm is used in Fig. 2c and 2d to compare
65   the interpolations of the unaged and water aged volcanic ash IN spectra with their corresponding raw datapoints. Interpolations
of individual spectra are shown in Fig. S1 in the Supplemental Information (SI). The smoothedPCHIP interpolation can be
seen to be monotonic and accurate to the observed data in the cumulative spectrum as well as smooth and readable in the
differential spectrum and will be used for the remainder of this work.
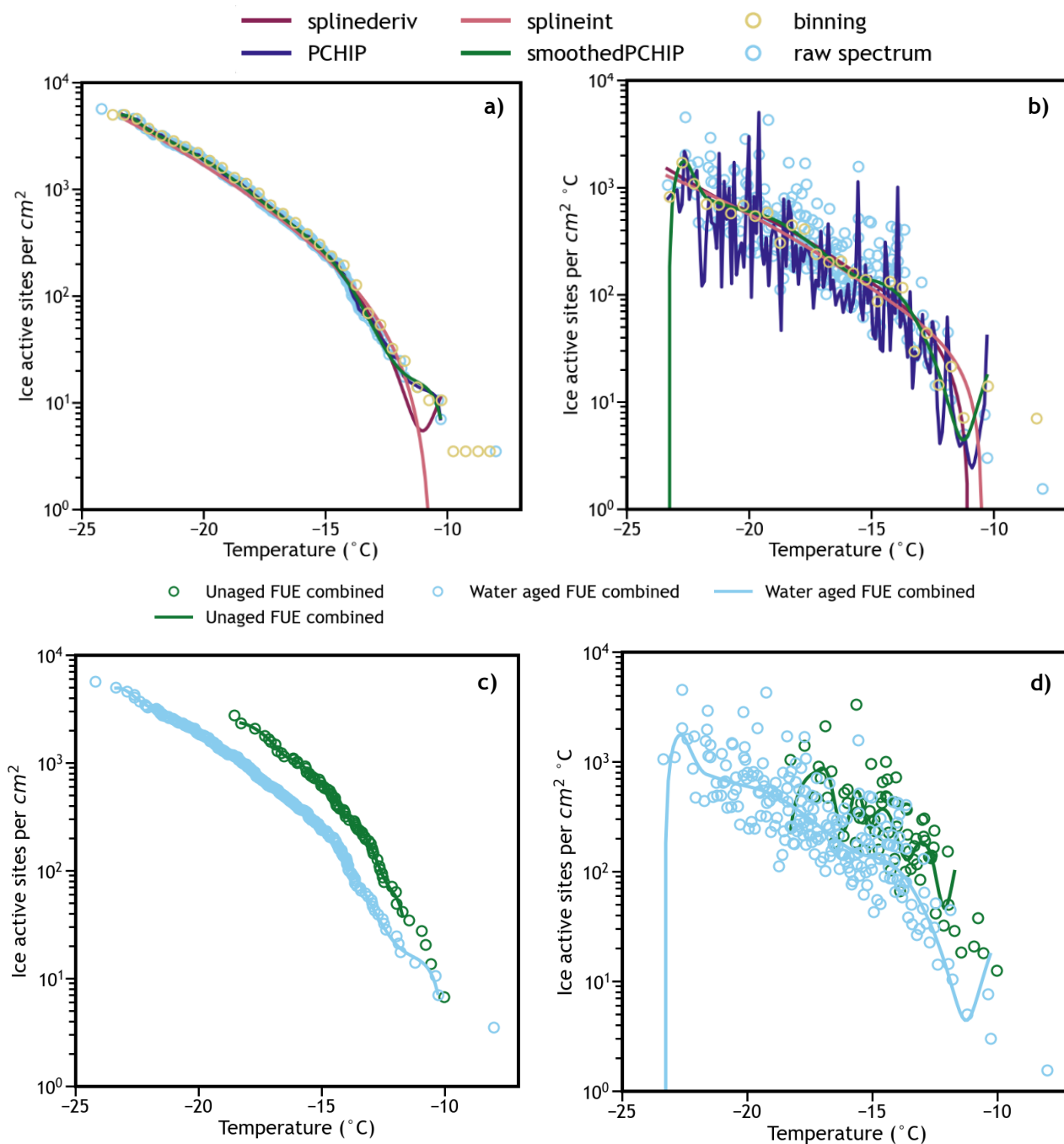
Figure 2. Comparison of interpolation methods on the water aged ash sample above in a) cumulative and b) differential spectra. Binning is accomplished by separating the temperature axis into 'bins', summing the number of freezing events in each bin, and then treating each bin as a single data point in Eqs. 1 and 2. The best available interpolation method, smoothedPCHIP, is used to interpolate the combined aged and unaged volcanic ash IN spectra and are compared to the raw data in cumulative and differential form in c) and d) respectively.

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4 Calculating confidence intervals and bands

4.1 Elementary statistical methods

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The question of how to calculate confidence intervals for IN spectra derived from droplet freezing experiments has been addressed several times in the IN literature. In some cases, a normal distribution about the frozen fraction curves is assumed. Where multiple freezing experiments are available and are interpolated such that means and standard deviations can be calculated for a collection of freezing spectra, a Z-interval or student's t-interval can be constructed (Polen et al., 2018; Jahn et al., 2019; Worthy et al., 2021) or standard deviations and standard errors are sometimes reported as-is (Chong et al., 2021; Roy et al., 2021; Losey et al., 2018). Other studies (e.g. Gong et al., 2020, 2019; McCluskey et al., 2018; Suski et al., 2018; Wex et al., 2019) have calculated approximate confidence intervals for frozen fraction values by treating them as binomial ratios and using the adjusted Wald interval suggested by Agresti and Coull (1998). In the latter case, calculating uncertainty for derived ice active site density spectra requires propagation of error through Equations 1 and 2, followed by an assumption of normality when the confidence intervals are calculated. There is, however, no reason to believe that the spread of freezing events in droplets should even approach a normal distribution, so techniques that use this assumption are unreliable. A better approximation for the variability in droplet freezing experiments is the Poisson distribution, in part because the widely used ice active site density spectra are based in Poisson statistics (Vali, 1971), but also because droplet freezing resembles a Poisson point process where freezing events occur approximately continuously and independently at a given rate. Koop et al. (1997) suggested the use of Poisson fiducial limits to calculate uncertainty in a variety of types of freezing experiments, and this approach has been used by several studies since (Yun et al., 2021; Alpert and Knopf, 2016; Knopf et al., 2020; Kaufmann et al., 2017). However, the distributions of IN sites across particles, distributions of these particles among droplets, distributions of freezing abilities of individual IN sites, distributions of freezing events that occur based on the aggregate freezing ability in a droplet, and temperature distribution between the droplets could all serve to skew or otherwise change the distribution of droplet freezing events measured. Using a Poisson distribution corrects for only some of these random factors, and because ice active site spectra are based on the Poisson process, these are the variables that most need to be considered when calculating experimental uncertainty. Thus, while these closed-form confidence limits are convenient, they are not likely to be accurate.

4.2 Parametric bootstrapping and its shortcomings

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Another class of methods of calculating confidence intervals for freezing spectra relies on a technique known as bootstrapping, where artificial freezing experiments are generated from a measurement using Monte Carlo simulations (Davison and Hinkley, 1997). When the simulations are based on an existing ice nucleation theory (e.g., when simulated experiments are produced using a parameterization of ice nucleation), this technique is known as parametric bootstrapping, and given enough simulations, the artificial experiments represent the full range of possible variability around the measured result that could be observed in the theoretical framework used.

For example, based on Wright and Petters (2013), Harrison et al. (2016) and subsequent publications simulate a number distribution of ice active sites in a collection of theoretical droplets based on the ice active site densities calculated from the original experiment. This model can be used to simulate freezing spectra by sampling these theoretical droplets and

105   assuming that freezing events occur when the number of ice active sites in each droplet is greater than or equal to one. When repeated enough times, this distribution of freezing spectra can be used to calculate confidence intervals for the measured data either by assuming that the quantiles of the distribution of simulated freezing spectra approximate the confidence intervals or by calculating simple Z-intervals from the distribution of simulated freezing spectra (although the latter invokes an assumption of normality).

110   An alternative method of parametric bootstrapping for confidence intervals of IN spectra models individual droplets freezing as a Poisson point process (again the same assumption used in deriving ice active site density spectra as $k(T)$ or $K(T)$) as shown in Vali (2019) and applied in Jahl et al. (2021) and Fahy et al. (2022). In this approach, the number of droplets that freeze in each temperature interval (or equivalently, the rate of droplet freezing) is used as the mean value of a discrete Poisson distribution. Then, for each temperature interval, a new number of droplets freezing in the interval is selected from the

115   distribution. When this is done for all temperature intervals, the simulated values are combined into a simulated experiment. Once ice active site density spectra are calculated from these simulations, and this process is repeated 100s to 1000s of times, the quantiles of the distribution of simulated ice active site densities for each temperature bin can be used as an approximation of confidence intervals.

Both parametric bootstrapping approaches described here rely on the parameterization to produce accurate results,

120   meaning that if the parameterizations are approximate or inaccurate, they may produce misleading or incorrect statistics. An in-depth analysis of the accuracy of the assumptions of each of these parameterizations is beyond the scope of this paper, but there are major concerns for each model. The calculations based on particle distributions in droplets (Wright and Petters, 2013; Harrison et al., 2016) assume that ice active sites are distributed evenly across the surface of a material, that the material is suspended evenly throughout the droplet, and possibly (depending on the specific approach) that the material is composed of

125   uniform spheres and that ice nucleation is time-independent or the characteristic temperatures for each given ice nucleation site are normally distributed. The first assumption is known to be false for some materials; minerals often have higher concentrations of and/or more ice active IN sites near or in specific nanoscale defects, cracks, pores, or other specific regions such as the perthitic textures in some feldspar minerals (Kiselev et al., 2017; Holden et al., 2019; Friddle and Thürmer, 2020; Whale et al., 2017). The second assumption may or may not be true, especially at higher suspension concentrations (Beydoun

130   et al., 2016). The third assumption depends on the material in question. The fourth assumption ignores time, one of the most important factors introducing uncertainty and randomness into droplet freezing experiments (Herbert et al., 2014; Wright and Petters, 2013; Knopf et al., 2020; Vali, 2014), and the fifth assumption does not have a theoretical basis and requires additional experimentation to determine the parameters of the normal distribution (Wright and Petters, 2013). Regardless of the specific approach used, these techniques either require extensive experimentation to determine the nature of the ice nucleation material

135   being studied or rely on assumptions that produce an incomplete and potentially inaccurate parameterization.

The calculations based on the Poisson distribution (Vali, 2019; Fahy et al., 2022b; Jahl et al., 2021) have very different assumptions. Stochasticity and IN site variability are accounted for in the process of simulation from the measured IN spectrum, however, this method requires coarse binning, as ideally multiple freezing events will occur within each bin. As discussed before, binning continuous data is inefficient. It also assumes that in these bins, the nucleation rate does not change

540   with temperature. For coarse temperature bins especially, this assumption will break down, as ice nucleation spectra are strong exponential functions of temperature (Fletcher, 1969). While the Poisson parametric bootstrapping method makes fewer assumptions and captures more variability than other parametric methods, it relies on risky and/or false assumptions, contributing systematic error to the confidence intervals.

4.3 Empirical bootstrapping as a superior approach

545   The most powerful (and originally proposed) bootstrapping method does not rely on any parameterizations. Instead, this non-parametric bootstrapping (known as empirical bootstrapping) takes the original experimental data and samples with replacement (a form of Monte Carlo simulation) to produce artificial datasets (Efron, 1979; Davison and Hinkley, 1997; Efron and Tibshirani, 1994; Shalizi, 2022). This method is remarkably well-suited to the problem of ice nucleation statistics, as droplet freezing experiments result in a list of freezing temperatures that can be easily sampled from to create new simulated

550   droplet freezing experiments. The large droplet numbers coupled with a limited freezing temperature range ensure that the empirical data covers most of the possible variability within each experiment. If multiple freezing experiments are performed on identically prepared samples, this method will even capture the variability in sample preparation and other aspects of the experiments being performed. Empirical bootstrapping requires no physical model of ice nucleation and so captures the stochastic nature of ice nucleation, inhomogeneities in ice active site distributions, and even any potential unknown sources of

555   variability within IN active materials. Thus, empirical bootstrapping is both universally applicable and unambiguous in its implementation, making it an ideal candidate for a standard statistical method for analyzing ice nucleation experiments. The only assumption required (which has already been made when deriving ice nucleation spectra) is that all datapoints must be statistically independent, meaning that no droplet can affect any other droplet's freezing temperature. This condition is already required for accurate ice nucleation measurements and is already implemented in most laboratories by physically isolating

560   droplets using an inert oil or gas or by separation of droplets in wells or microwells. Empirical bootstrapping is only otherwise limited bye the computational time available to draw many statistical simulations of an ice nucleation spectrum and the quality of the observed data itself, both of which are addressed in later sections as they become relevant. We suspect it has not yet been employed for this purpose simply because it is not commonly taught outside of advanced statistics (Hesterberg, 2015), although a lack of information accessible to those unfamiliar with computational statistics on how to generalize statistics

565   generated from a bootstrapping technique is likely to also have played a role.

Figure 3a and 3b show the application of empirical bootstrapping to simulate cumulative and differential spectra for the combined and interpolated volcanic ash ice nucleation data previously introduced in Figs. 1 and 2. Each spectrum is statistically simulated by randomly sampling with replacement n times from the list of freezing temperatures in the original

270 experiment using the choices function in the built-in 'random' library in Python, where n is the number of droplets in the original experiment. Where multiple droplets froze at a given temperature, that temperature is added multiple times to the 'observed' list. This process is repeated to create new 'sampled' freezing temperature lists until the desired number of simulated experiments is achieved. Each 'sampled' list is then sorted and analyzed as distinct freezing assays, each with its own IN spectra that can be interpolated as usual. The simulated spectra are distributed around the true data due to variations in which droplets are randomly sampled for each simulation, and the width of this distribution provides an estimate of how uncertain

275 the experimental value is at that temperature. Summary statistics of this distribution such as mean, standard deviation, and quantiles can be calculated by dividing the temperature range of each interpolated spectrum into a dense grid of evenly spaced points (e.g. ~10 points per degree Celsius) and calculating each statistic as usual using the distribution at each point. The resulting statistics as a function of temperature can then be re-interpolated extremely accurately due to the high density of data available, providing interpolated continuous functions for each summary statistic. This process is detailed further in the SI.

280 While the mathematical theory behind empirical bootstrapping is complex, Fig. 3 provides some evidence that this approach has successfully captured the possible variability in the ice nucleation spectra. Using the interpolated quantiles as a measurement of the spread of the simulated spectra, the magnitude of the variability in each spectrum largely follows the trends that would be expected. For example, the simulated cumulative spectra have much less relative variability than the simulated differential spectra and both types are less variable at intermediate temperatures where more droplets froze in the actual
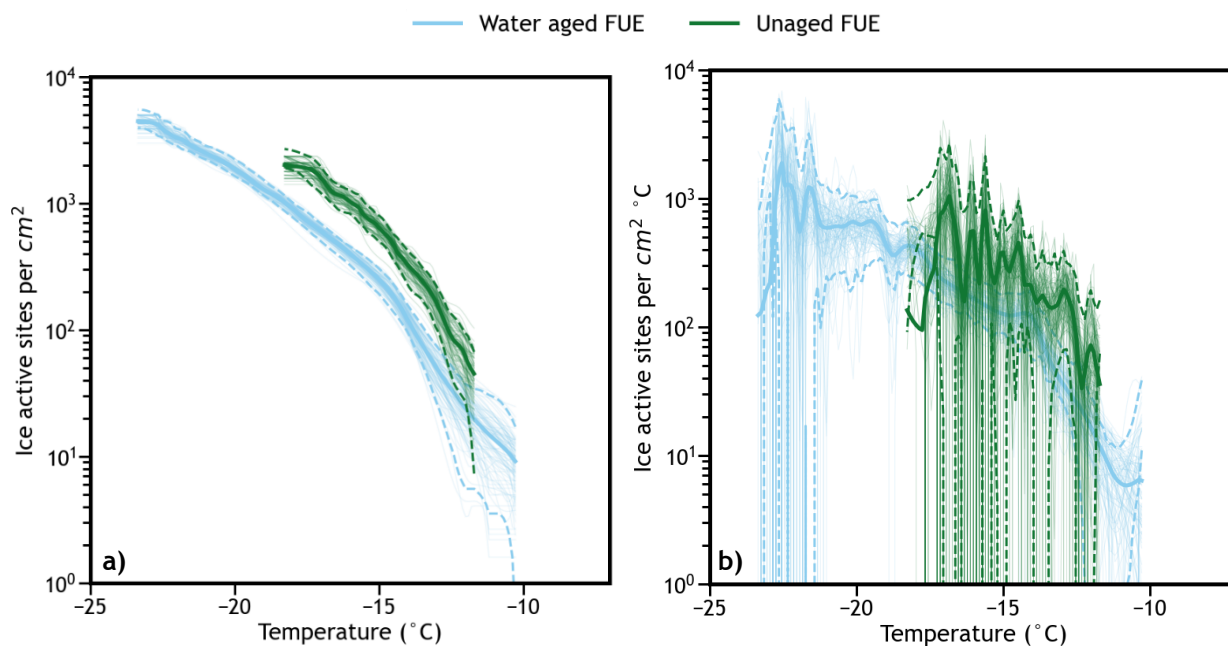
Figure 3. Interpolated combined data (bold line), interpolated 2.5th and 97.5th quantiles (dashed lines), and interpolated individual simulations (faint lines; N=100) of the a) cumulative n_s and b) differential n_s spectrum for each set of combined ash data from Figure 1. The simulated data (lines) using empirical bootstrapping provide a realistic estimate of the distribution of how the spectra could vary based on stochasticity, variations in the individual droplet freezing experiments comprising the combined experimental spectrum, and other factors contributing to experimental uncertainty.

experiments. This reflects the fact that increased sample sizes tend to reduce uncertainty as cumulative spectra represent a sum of all previous datapoints and most droplets tend to freeze at intermediate temperatures in a droplet freezing assay. The noisiness of the differential spectra indicates large uncertainty, meaning the differential spectrum for the unaged volcanic ash is largely uninterpretable, while the differential spectrum for the aged volcanic ash and both cumulative spectra are much more descriptive – for example, it can clearly be seen that the two cumulative spectra do not overlap significantly below –13 °C, a fact that will be further quantified in Section 5. The large variability (even to k values of zero) observed at the extrema of both differential spectra represent relatively rare freezing events for the samples, such that a given simulation may or may not observe freezing events at that temperature. These areas are more common in the unaged volcanic ash IN spectrum because that spectrum consists of fewer droplets than the aged volcanic ash IN spectrum, highlighting the importance of high-resolution data for meaningful interpretations of differential spectra. Also note that in the last degree of each differential spectrum, the measured results lie outside of the quantiles calculated because 100 simulations are not sufficient to fully estimate the variability in the measured spectrum; this problem would be remedied with additional simulations that fully sample that region of the IN spectrum.

4.4 Basic bootstrapped confidence bands and their limitations

Using this new method to simulate data that capture the variability inherent to freezing experiments, bootstrapped summary statistics describing the experimental measurement can be calculated. Values such as the bootstrapped standard error of the mean approximate the true standard error of the mean remarkably accurately when large numbers ($n \geq 200$) of bootstrap simulations are employed, a fact known as the 'plug-in-principle' (Efron and Tibshirani, 1994). Bootstrapped confidence intervals, however, are a more complex subject. The previous studies that used parametric bootstrapping methods assumed that the $\alpha/2$th and $1-\alpha/2$th quantiles of the simulations correspond to the lower and upper limits of the $1-\alpha$ level confidence interval respectively (Harrison et al., 2016; Vali, 2019). This assumption is common, and while it can work well for many applications, this 'quantile interval' has little basis in statistical theory. It is also strongly affected by bias, only partially corrects for skewed distributions (ice nucleation spectra are likely to be skewed upward based on the Poisson statistics they are derived from) and can be too narrow when applied to some distributions (Hesterberg, 2015; Efron, 1987). The strong bias that quantile intervals exhibit is particularly concerning when using potentially inaccurate parametric bootstrapping or when a small sample results in poor sample coverage in empirical bootstrapping.

Fortunately, other bootstrap confidence intervals exist. For a simple interval rooted in statistical theory, we can construct the reverse percentile interval, also known as the pivotal interval, where the upper and lower quantiles are subtracted from twice the sample mean for the lower and upper confidence intervals respectively. However, in skewed distributions such as uncertainty in ice nucleation spectra, the pivotal interval tends to be inaccurate. For a more traditional interval, we can construct a Z-interval around the measured spectrum with a bootstrapped estimation of standard error, but this assumes a normal variance – obviously a poor approximation of the complexity inherent to ice nucleation. A bootstrapped T-interval

(tboot) using the number of droplets in the original experiment as the number of degrees of freedom is a slightly better estimate, but still suffers from error from bias (including narrowness bias) and skewness (Hesterberg, 2015; Efron, 1987).

4.5 Better bootstrapped confidence bands

20   Significant work has gone into correcting these problems with basic bootstrapped confidence intervals. The tboot interval can be corrected for skewness to the tskew interval by including a second-order skewness term in the tboot calculation as shown by Johnson (1978). The quantile interval can be expanded by changing the quantile bounds by a factor related to the t-statistic to remove narrowness bias, called the expanded quantile interval or the BCa confidence interval (Efron, 1987; Hesterberg, 2015). However, by far the most accurate method is the studentized confidence interval, referred to as the

25   'bootstrap T' or 'confidence intervals based on bootstrap tables' elsewhere (Efron and Tibshirani, 1994; Hesterberg, 2015; Diciccio and Efron, 1996; Efron, 1979). This method corrects the errors of the t-interval by estimating the actual distribution of the t statistic for through bootstrapping. Specifically, the standard error of each simulated spectrum is calculated and is used to normalize the difference of each simulated spectrum from the mean of all simulated spectra. These normalized values are compiled into another distribution and the desired quantiles of this distribution are multiplied by the standard error of the

30   collective of simulated spectra to produce the final confidence intervals. To obtain the standard error of each individual simulated spectrum a second round of bootstrapping is needed using the simulated spectrum as the 'true' measurement resulting in 'resimulated' spectra. Further descriptions and equations for calculating this and all previously mentioned confidence intervals are provided in the SI. The process is computationally intensive, but it results in confidence intervals that are unaffected by bias or skewness, even for small sample sizes. Statistical theory calls such intervals 'second-order accurate', and

35   this property sets the 'bootstrap T' apart as a standard to compare other confidence intervals against.

The above methods were used to calculate confidence bands (continuous confidence intervals) for the cumulative and differential IN spectra of the unaged and water aged combined volcanic ash sample. Like the summary statistics, to create confidence bands confidence intervals were calculated at every 0.1 °C within each spectrum and were interpolated using a smoothed cubic spline fit. The different methods of calculating confidence bands for the combined unaged spectra representing

40   91 droplets are compared in Fig. 4. In general, the best-performing confidence bands (determined by being the closest to the studentized bands) are calculated using the quantile or expanded quantile methods and the skew-corrected t-interval method – the other approaches tend to be less accurate. In contrast, in the combined water aged spectra representing 286 droplets, the different methods of calculating confidence bands are in good agreement over most of the IN spectra (Fig. S2), although the studentized confidence bands show slightly different behavior at high temperatures where very few droplets are observed to

45   freeze. As in Fig. 3, the variability in the differential spectrum for this relatively low-resolution data is significant as shown by the wide confidence bands in Fig. 4b, although most confidence bands overestimate this variability compared to the studentized bands.
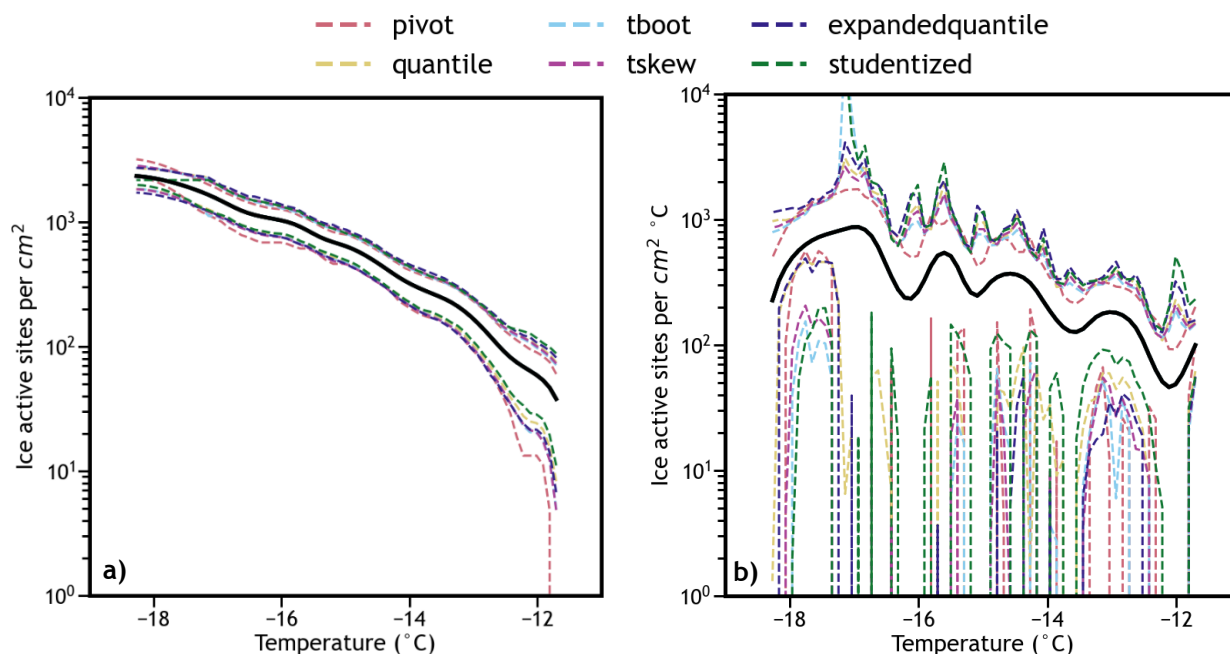
Figure 4. Comparison of methods to calculate confidence bands (shown as different-colored dashed lines) for a) cumulative and b) differential unaged volcanic ash n_s spectra representing 91 droplets. Experimental spectra are shown in black. Most calculation techniques are in good agreement across the range of the cumulative spectrum, but all techniques except the studentized intervals (green dashed line) overestimate the variability in the differential spectra, resulting in confidence bands that are too wide across most of the spectrum.

4.6 Recommendations for use of empirical bootstrapping methods

450   If accurate confidence bands on both the cumulative and differential spectra are required from low-resolution data, studentized intervals should always be used. Ideally, the studentized confidence bands should be used in all cases, but the computation time required for calculation of these confidence bands can be excessive. For most use cases then, the tskew bands are a good choice for somewhat conservative confidence bands rooted in theory, and we will use them in the remaining examples below. Expanded quantile or quantile bands are also an appropriate choice when empirical bootstrapping is used but

455   should be tested against the studentized bands for each system to check for potential biases in the data collection process. Quantile bands should be avoided when using small numbers of droplets (>5 per degree Celsius measured seems to be acceptable) or when using a parametric bootstrap, as the biases inherent to the parameterization will be amplified by the quantile bands. The pivot and tboot bands seem to be poor choices in the context of ice nucleation.

More droplets or more freezing arrays of the same sample will improve the accuracy and reduce the width of the

460   confidence bands, especially in differential IN spectra, further motivating the many recently developed microfluidic techniques (Brubaker et al., 2020; Reicher et al., 2018; Stan et al., 2009; Tarn et al., 2018; Weng et al., 2016; Roy et al., 2021). Fig. S3a

displays interpolations and resulting confidence bands for the aged volcanic ash when one, two, four, and all six experiments are combined, resulting in different numbers of droplets, and there are striking changes in the width and shape of the confidence bands. Additionally, the number of simulations (and resimulations if using studentized confidence bands) should be chosen carefully to ensure the full variability present in IN spectra is represented. In Fig. S3b, the tskew confidence bands of the water aged volcanic ash IN spectra are compared when the number of simulations (*nSim*) is 100, 1000, and 10000. Based on these simulations, we recommend at least 200 simulations per degree Celsius covered by the IN spectrum, although this might vary depending on the number of measurements in the dataset being used.

Finally, it is notable that Figs. 3 and 4 provide evidence that the interpolation technique used is not overfitting the differential data, as the quantiles and other confidence bands follow the general shape of the experimental spectrum. Since these statistics are calculated from an aggregate of 100 or 1000 samples, they would be expected to smooth out random variation present in a single measured spectrum that could be causing the complex interpolated curve observed. Because the aggregated data maintains the same complex shape, it can be assumed that it is at least somewhat meaningful, and that the interpolation technique is using an appropriate smoothing factor, however, this should be tested regularly to minimize potential overfitting.

5 Comparing IN spectra, testing statistical significance, and background subtraction

Confidence bands provide useful information about the variability of a single dataset – in the case of droplet freezing assays, 95% confidence intervals contain the true population mean ice nucleation activity of the suspension being sampled from in 19 out of 20 analyses (that is, either the true spectrum is within the confidence interval, or an event of probability at most 5% happened during data collection). All ice nucleation data should be reported with some form of confidence interval or quantification of the distribution of the measurements (e.g., standard error bars). These statistics must be calculated using a method such as empirical bootstrapping rooted in statistical theory to minimize assumptions about the ice nucleation experiment and accurately represent the uncertainty inherent to the experiment.

Another key application of statistics that quantify the variability within a dataset is in comparing measurements of different samples to assess the degree of similarity of their INA. In general terms, confidence bands can be used to compare two IN spectra by determining whether they could reasonably have been drawn from the same population. Often confidence intervals or bands are interpreted based on whether they overlap: If confidence intervals of two spectra do not overlap, they are statistically significantly different. However, it is not necessarily true that if the confidence bands overlap the two measurements are statistically the same at a given confidence level. This common misconception is based on the difference between error bars calculated using the standard error of the mean and confidence intervals (Barde and Barde, 2012; Belia et al., 2005).

For a more quantitative (and interpretable) method to compare two spectra, the ratio or difference between the two can be plotted as a function of temperature with its own confidence bands. If the confidence bands of these metrics contain

zero or one (as appropriate), then those two spectra are not statistically significantly different at the temperature examined and

95 confidence level used. More precisely, confidence intervals on difference and ratio plots can be used to test the null hypothesis that the difference or ratio of two ice active site density spectra is zero or one, respectively, a much more rigorous approach to comparing IN spectra.

5.1 Confidence bands for difference and ratio plots

Calculating confidence bands for differences or ratios of continuous variables is nontrivial, but for these metrics to

00 have any meaning, confidence bands are necessary. Subtracting or dividing the confidence bands of the compared spectra is not accurate. Elementary propagation of error formulas assume that the variability within both spectra (and of the difference or ratio) is normally distributed. Again, bootstrapping offers a superior solution. To simulate the variability in the difference spectra, individual simulations of each measurement can be subtracted or divided from each other pairwise, until a collection of simulated difference or ratio spectra combining the variability inherent to each measurement is produced. From these

05 simulations of the difference or ratio spectra, confidence bands can be produced using any of the methods in Section 4.

Figure 5a and 5b show the difference and ratio IN spectra comparing the water aged volcanic ash to the unaged volcanic ash. Suspension of minerals and volcanic ash in water can cause alteration of the ice-active surface sites due to a variety of complex processes as shown in recent literature (Maters et al., 2020; Kumar et al., 2019; Harrison et al., 2019; Perkins et al., 2020; Fahy
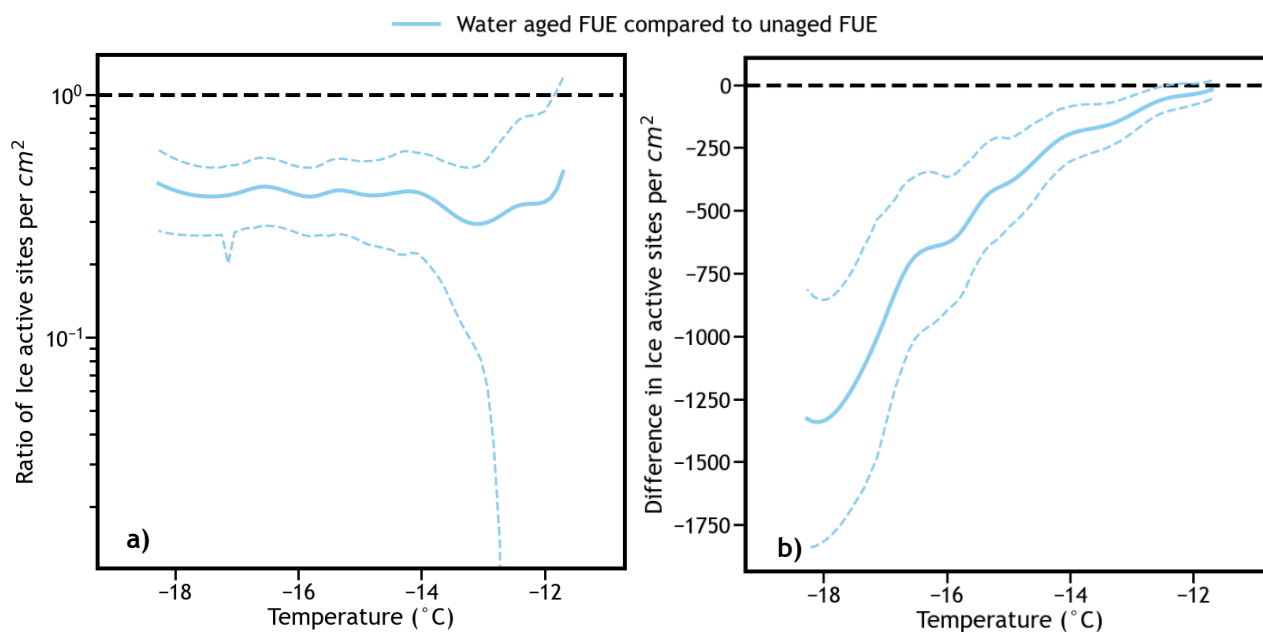
Figure 5. Comparison of the water aged n_s spectrum to the unaged n_s spectrum a) by dividing, and b) by subtracting. The dotted line appears at $\Delta n_s = 1$ in the ratio plot and at $\Delta n_s = 0$ in the difference plot, signifying no difference between the two spectra. Confidence bands are calculated using interpolated tskew bootstrapped ratio/difference simulations as discussed above. Based on the confidence bands, there is a statistically significant difference between the water aged and unaged ash spectra for temperatures < -12 ºC for both plots, and the magnitude of this difference is available as a function of temperature.

et al., 2022b; Jahn et al., 2019). Based on the confidence bands of either metric, it can easily be seen that below approximately –12 °C, there is a statistically significant difference between the IN activity of the aged ash and unaged ash with p<0.05, confirming that in this experiment there is an alteration of the IN activity of volcanic ash due to suspension in water. The magnitude of this difference can also be easily determined either in relative or absolute terms, providing a quantitative measurement of the change in IN activity due to chemical processing of this sample. In this case, the IN activity of water aged ash is reduced by a factor of 2-3 below –12 °C and is reduced by between 0 and 1500 ice active sites per square centimeter of ash surface area as a function of temperature. As before, only ~200 simulations per shared degree Celsius seems to be necessary for consistent confidence bands for each difference plot.

5.2 Applications of difference and ratio plots

Difference and ratio spectra have a variety of useful applications within the context of ice nucleation. The first has already been shown, as two spectra can be easily tested to determine whether there is a statistically significant difference between them. This is particularly useful in studies of chemical aging, where the change in IN activity after a given chemical treatment can be quantitatively measured using the difference or ratio metrics. Another application is in background freezing subtraction for IN spectra. All droplet-on-substrate methods have some level of background freezing activity measured as the ice active site density normalized to the volume of water in each droplet that can change day-to-day depending on the system (Polen et al., 2018; Vali, 2019). For accurate measurements and to compare between instruments, the instrumental background must be subtracted from any measured IN spectrum. This can be readily accomplished by calculating the difference between the IN spectrum of interest and the background freezing spectrum. Where there is no background, the difference is equal to the sample spectrum. By saving the subtracted simulations used to calculate the variability in this difference spectrum, the background-subtracted data can be compared further via another ratio or difference spectrum if desired. This can also be useful in determining whether a sample's IN activity is distinguishable from the instrumental background in weak IN materials. For all use cases, accurate confidence bands based on the bootstrapping procedures presented here are integral to ensuring rigorous and correct analysis and interpretation of the data, as simply subtracting $K(T)$ or $k(T)$ without accurate confidence intervals or other statistics does not fully represent the background-subtracted spectrum.
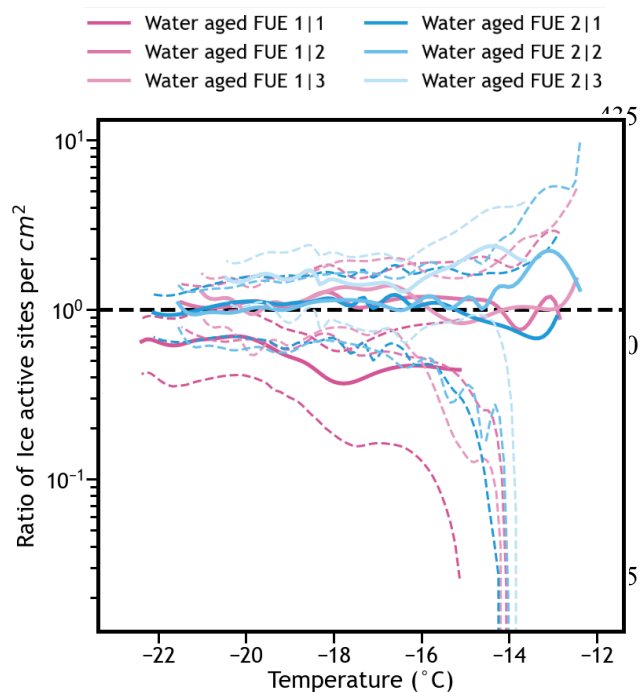
Figure 6. Comparison of individual water aged cumulative n_s spectra to the remaining combined water aged cumulative n_s spectra. Each curve is a ratio spectrum of one of the six experiments divided by the remaining five experiments combined (solid lines) with bootstrapped tskew confidence intervals (dotted lines). The experiment shown in purple (1|1) is statistically significantly different from the other five experiments and is therefore deemed an outlier.

A third application of ratio and difference spectra in IN activity is in locating outliers. Droplet-on-substrate IN measurements are extremely sensitive to contamination and human error, even when great care is taken during the sample preparation process. When two measurements of the same sample disagree, additional replicate measurements are taken to determine if a measurement is an outlier, usually visually. Ideally, a more quantitative measurement of outlier status would be used, such as the Grubbs Test (Grubbs, 1969), Tukey's Fences (Tukey, 1977), or the Modified Thomson Tau Test (Thompson, 1985). However, the usefulness of these common techniques and the assumptions they require for IN spectra is questionable. Instead, we propose that for a quantitative measurement of whether a sample is an outlier, the ratio spectrum comparing the sample in question with the combined spectrum of the remaining measurements of the same sample can be used. An example of this analysis is shown in Figure 6, where the various water aged ash freezing experiments are compared using a ratio plot to combinations of the remaining measurements. It can be clearly seen that only the spectrum shown in purple is statistically significantly different (in this case lower) at the 95% confidence level based on the bootstrapped tskew confidence bands. Therefore, this experiment could be treated as an outlier at that confidence level and excluded from future analysis. Even still, great care should be taken when dealing with potential outliers, and the confidence level required to exclude outliers should be carefully considered so as not to remove valid data. Whenever possible, decisions about whether to exclude a potential outlier should combine this statistical method with observations or lack thereof of specific experimental errors in the laboratory.

6 Summary and Conclusions

We have presented a rigorous and generalized set of methods for interpolating raw data, calculating confidence bands and other statistics, and quantitatively comparing IN spectra derived from droplet freezing assays. The interpolation methods discussed use ice nucleation data far more efficiently than previous binning methods and allow continuous quantitative comparison of IN spectra without losing the complexity of the original data. Empirical bootstrapping is introduced as an improvement on the elementary statistical methods and parametric bootstrapping previously used by capturing the full

65   variability present in each IN spectrum or collection of IN spectra with no assumptions about the nature of ice nucleation for the material being tested. Enhanced continuous confidence bands are calculated using rigorous and modern algorithms to replace the quantile intervals or z-intervals previously used. Finally, the ability to interpolate and simulate IN spectra is used to develop difference and quotient spectra with accurate confidence bands for quantitative comparison and statistical testing of ice nucleation activities between materials and background subtraction.

70   These approaches can be used to help answer many important research questions in the field related to statistically assessing observed changes or differences in IN activities and can be applied to any experimental setup using arrays of droplets freezing over time or at varying temperatures. They are supported by statistical theory and use widely accepted methodologies from the statistics literature. The universality, simplicity, and accuracy of this approach makes it an ideal candidate to be a standard statistical method by which to compare datasets from different instruments and groups. The bootstrapping approach

75   could be particularly useful for incorporating uncertainty in IN activity into advanced atmospheric models, as a full distribution of IN activity at each temperature can be easily estimated from simulations. To facilitate adoption of these statistics, all code developed for this project along with documentation and data to recreate the figures in this paper is available in archived form as was used at the time of writing at KiltHub (doi: 10.1184/R1/19494188; Fahy et al., 2022a) or in a living GitHub repository where updates or additional information may be added in the future (https://github.com/wdfahy/CMU-INstats).

80   Further refinement of these methods by optimizing code runtime, improving confidence interval coverage, adding simulation methods, and implementing different statistics may be accomplished in the future if necessary. Extension of the procedures described here may be possible to describe uncertainty in instruments that measure ice nucleation in the aerosol phase such as CFDC-type instruments and expansion chambers and are not limited to heterogeneous ice nucleation. This may lead to applications describing uncertainty in experiments analyzing a variety of nucleation processes under varying conditions. If

85   widely adopted, the quality and consistency of statistical treatment of nucleation data will improve, leading to enhanced representation and communication of results and interpretations within those fields.

Code availability

All code used in this project can be accessed in its archived form at: doi:10.1184/R1/19494188, with any updates or further work posted to https://github.com/wdfahy/CMU-INstats.

90   **Data availability**

All data used in this project can be accessed at doi:10.1184/R1/19494188.

Supplement link

See additional document.

Author Contribution

WDF and RCS conceptualized the paper. CRS contributed statistical methods. WDF wrote the script; collected, analyzed, and visualized data; and wrote the initial manuscript draft. All authors provided input into the methods developed and edited the manuscript.

Competing interests

There are no competing interests.

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