

## Ice Nucleation Spectrometer (INS) Instrument Handbook

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February 2022



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# **Ice Nucleation Spectrometer (INS) Instrument Handbook**

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February 2022

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Office of Science, Office of Biological and Environmental Research

## **Acronyms and Abbreviations**

AOS	Aerosol Observing System
AMF3	third ARM Mobile Facility
ARM	Atmospheric Radiation Measurement
CACTI	Cloud, Aerosol, and Complex Terrain Interactions
CCD	charge-coupled device
CCN	cloud condensation nuclei
CI	confidence interval(s)
CSU	Colorado State University
DI	deionized
DOE	U.S. Department of Energy
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HEPA	high-efficiency particulate absorbing
IN	ice nuclei
INP	ice nucleation particle
INS	Ice Nucleation Spectrometer
IOP	intensive operational period
IS	Ice Spectrometer
kPa	kilopascal
L <sup>-1</sup>	per liter
mL	milliliter
N <sub>2</sub>	nitrogen gas
nm	nanometer
PCR	polymerase chain reaction
SAIL	Surface Atmosphere Integrated Field Laboratory
SGP	Southern Great Plains
Slpm	standard liters per minute
STP	standard temperature and pressure
μL	microliter
μm	micrometer
UVB	ultraviolet B

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## **1.0 Instrument Title**

Colorado State University (CSU) Ice Nucleation Spectrometer (INS; also commonly called the Ice Spectrometer)

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## **3.0 Vendor/Developer Contact Information**

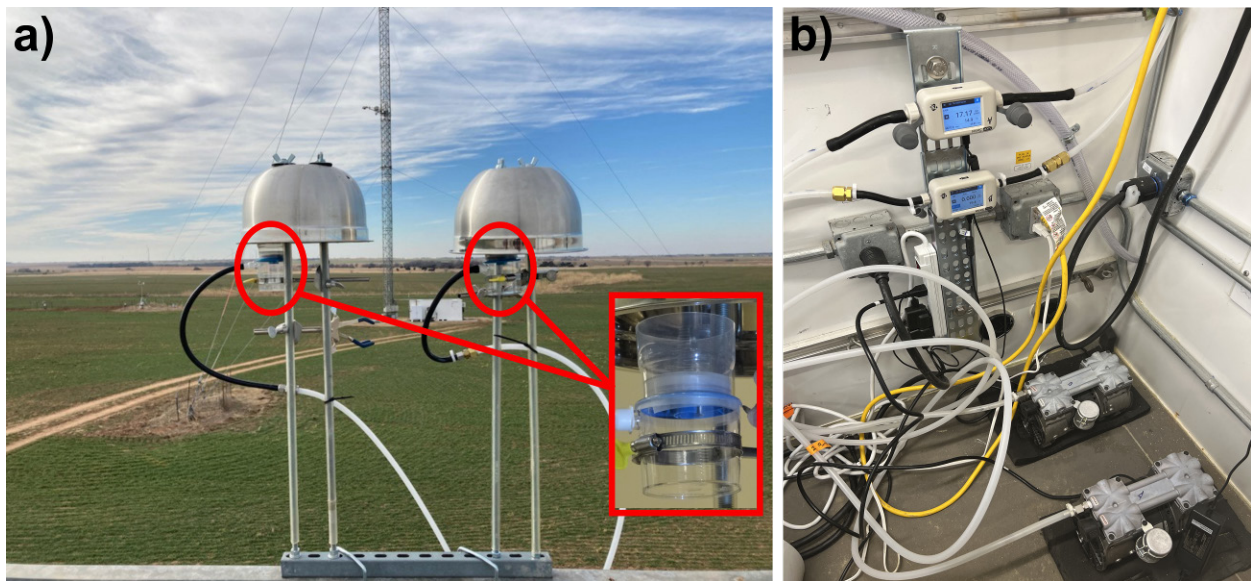
Flow meter vendor:  
TSI Incorporated  
500 Cardigan Road  
Shoreview, Minnesota 55126  
USA  
Tel: (651) 490-2811

Vacuum pump vendor:  
USA Thomas  
3810 Prospect Avenue, Unit A  
Yorba Linda, California 92886  
USA  
Tel: (714) 312-5531

The INS was built in-house at CSU.

## 4.0 Instrument Description

The Ice Nucleation Spectrometer (INS) is an offline analytical measurement system used to process filter samples for freezing temperature spectra of immersion-mode ice nucleating particle (INP) number concentrations. It is almost identical to the CSU Ice Spectrometer (IS) design. While basic immersion freezing methods have been applied for decades, recent intercomparison studies (DeMott et al. 2017, 2018b) with other methods for sampling ambient INPs support the method's utility. Use of the INS with filters produces spectra spanning wide dynamic ranges of temperature and, hence, INP concentration (e.g., six orders of magnitude). The INS is supported with well-established experimental protocols and has been applied in many diverse scenarios (e.g., Beall et al. 2017, DeMott et al. 2017, Hill et al. 2016, Hiranuma et al. 2015, McCluskey et al. 2014, 2017, 2018a, 2018b, Suski et al. 2018). See section 10.0 for details regarding the operational procedures and specifications of the INS processing protocol.



**Figure 1.** Filter unit sampling apparatuses, including a) single-use filter units under precipitation shields that are connected via tubing to b) the mass flow meters and vacuum pumps. The inset in a) is a magnified photo of a filter unit. Photos in a) and b) are from the Southern Great Plains (SGP) and Surface Atmosphere Integrated Field Laboratory (SAIL) sites, respectively.

Filter samples are collected routinely at select U.S. Department of Energy (DOE) Atmospheric Radiation Measurement (ARM) facilities. Each filter unit sampling apparatus consists of sterile single-use filter units prepared at CSU, a totalizing mass flow meter (TSI Mass Flow Meter 5200-1, TSI, Inc.), vacuum



pump (2688CE44 Oil-less Piston Compressor/Vacuum Pump, Thomas), tubing, and precipitation shields (Figure 1). Two identical apparatuses are operated in tandem to collect primary filters for INP analyses and duplicate filters that serve as backup or as archival samples for users to obtain for their own analytical ventures. The filter units are open-faced, secured outside to the Aerosol Observing System (AOS) railing, and shielded from precipitation. Vacuum line tubing connects each filter unit to the flow meter followed by vacuum pump, both of which are housed inside the AOS container.

## **5.0 Measurements Taken**

The primary measurement output of the INS is freezing temperature spectra of cumulative immersion-mode INP number concentrations in aerosols resuspended from individual filter samples, calculated in the units of # per L of air. Note that INPs are also interchangeably called ice nuclei (IN).

Output data from the INS include freezing temperature (°C), INP number concentration (L<sup>-1</sup> STP), upper and lower 95% CI values, and treatment flag. The treatment flag indicates if the suspension was untreated to obtain total INP number concentrations, heat-treated to deactivate biological (e.g., proteinaceous) INPs, or H<sub>2</sub>O<sub>2</sub> treated to remove all organic INPs. These are measured and/or calculated from preliminary output data files that contain date and time of processing, freezing temperature, and number of wells frozen (typically out of an array of 32, each containing a 50-uL aliquot of re-suspended aerosol) per 0.5 °C interval. Images of the unfrozen and frozen wells acquired throughout INS runs are obtained for visual quality control of the output data files.

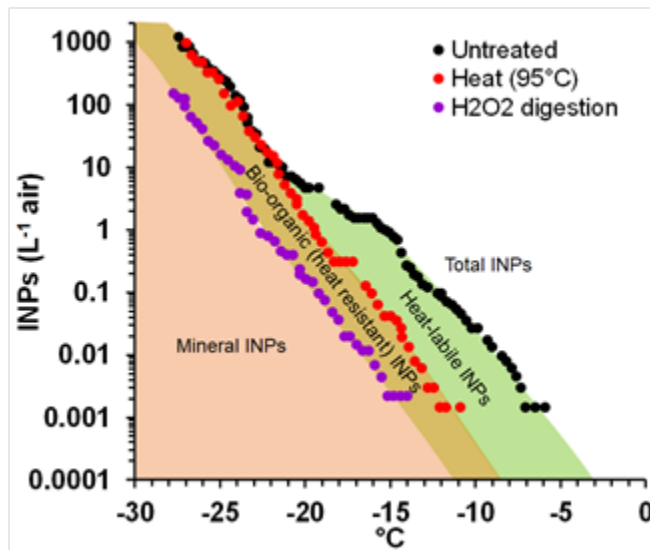
## **6.0 Links to Definitions and Relevant Information**

### **6.1 Data Ordering**

INP number concentration data from the INS sample processing can be accessed and ordered on the [ARM website](#). Data can be found by searching for the measurement “ice nuclei” or data source of “ice nucleation spectrometer”. “ice spectrometer” is also commonly used as an alternative to “ice nucleation spectrometer” for the data product description. INS data available on the ARM website include intensive operational period (IOP) data from previous campaigns in addition to routine INP mentor data.

### **6.2 Data Plots**

A plot containing representative INS output data is shown in Figure 2. These data include cumulative freezing spectra of untreated (total) INP number concentrations, heat-treated (e.g., proteinaceous) INP number concentrations, and organic INP number concentrations from a peroxide treatment. INPs left after peroxide digestion are inorganic (e.g., mineral) INPs. Data are from the ARM Cloud, Aerosol, and Complex Terrain Interactions (CACTI) campaign in Argentina.



**Figure 2.** Cumulative INP spectra from a filter collected during the ARM CACTI campaign. Spectra include total INPs in addition to unique treatments that differentiate heat-labile (proteinaceous), heat-resistant (bio-organic), and mineral INP concentrations.

## 6.3 Data Quality

### 6.3.1 Filter Sample Collections

Collection of filter samples for offline INS processing are quality controlled through monitoring the in-line pressure (kPa) and flow rate (Slpm) at the start and end times of filter collection. These values are used to determine if sampling errors occurred during collection (e.g., a significant change in pressure and/or flow rate may be indicative of a leak in the filter unit, tubing, or other connections throughout the system). To ensure accurate measurement of total volumes filtered, because flow rates decrease over the sampling period, we use a totalizing mass flow meter (TSI 5200-1, accuracy  $\pm 2\%$ ) updating every second.

### 6.3.2 INS Processing

To minimize contamination of the filters with INPs on lab surfaces or in consumables (e.g., tubes used for filter processing, pipet tips, polymerase chain reaction [PCR] plates), and to ensure that the deionized (DI) water used for re-suspension of particles from filters is as INP-free as possible, we apply a comprehensive protocol during preparation of the samples for measurement of INPs in the INS (Barry et al. 2021). Pipets are calibrated annually. A 0.1- $\mu\text{m}$  filtered DI water blank is included in each INS run, to correct for INPs present in PCR trays and the DI water used for resuspension. Blanks (using DI water in place of the sample resuspension) are also run for  $\text{H}_2\text{O}_2$  digestions to check for any INP contamination in the hydrogen peroxide and catalase used in the procedure.

For accurate temperature measurement in the INS, thermocouples are inserted just below the wells, via a horizontally drilled hole plugged with thermal grease. There is one thermocouple in each of the four PCR blocks. For each set of double blocks, readings from the two thermocouples are averaged. The HEPA-filtered  $\text{N}_2$  that purges the headspace above the PCR trays is precooled to a few degrees above the block temperature to prevent it warming the aliquots dispensed into the trays.

In addition to automated detection of well freezing, camera images are taken every 20 seconds, or approximately every 0.1 °C, to check for program errors. Checking images against automated detection output is performed on every IS run.

### **6.3.3 Control Blank Sample Collection and Processing**

Field filter unit blanks are prepared identically to the sampling filters and exposed briefly to air at the sampling position to monitor possible contamination during filter sample preparation and handling. These samples are preserved and processed in the same manner as the collected aerosol filter samples to obtain a mean background INP spectrum.

## **7.0 Technical Specification**

### **7.1 Units**

The calculated measurement quantity produced from the INS is INP number concentration per liter of air adjusted to standard temperature and pressure ( $L^{-1}$  STP, with STP being 0 °C and 101.32 kPa) at 0.5 °C-increments for freezing temperatures between 0 and typically  $-27$  to  $-29$  °C (the lower temperature limit is reached when more than 15 of the 32 wells freeze in the DI water blank).

### **7.2 Range**

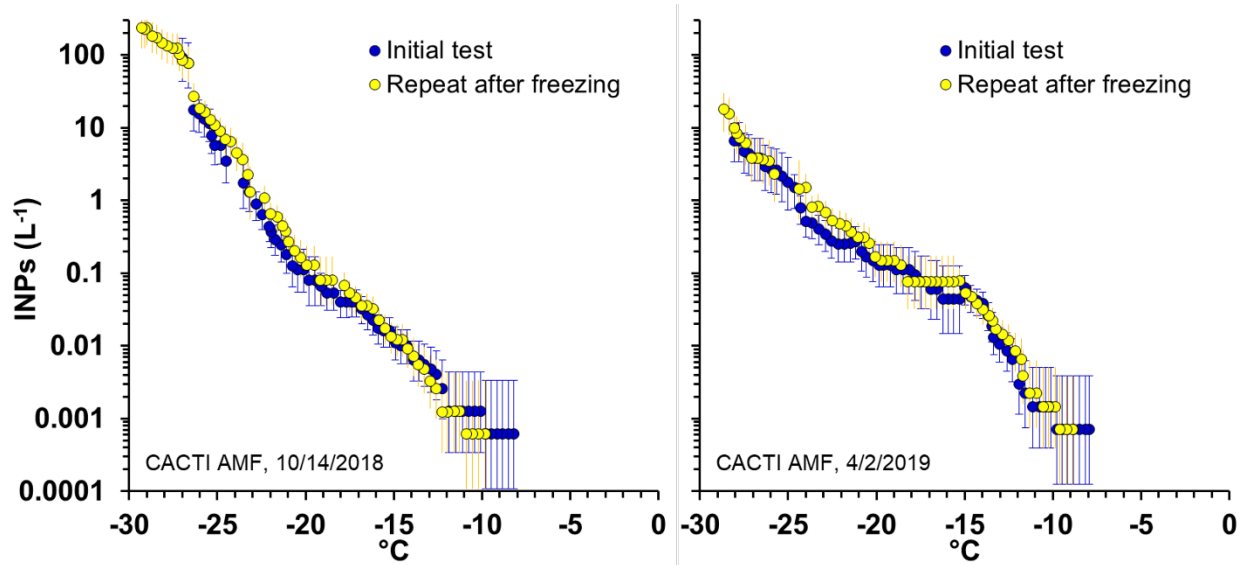
The temperature measurement range of the INS is between 0 and approximately  $-27$  to  $-29$  °C. The range of INP concentrations measurable depends mainly on total volume of air filtered. For a sampling period of 24 hours, and a total volume filtered of  $27 m^3$  (current average at SGP), the detection limit is  $0.0002 INPs L^{-1}$ . There is no upper limit, since we use serial dilutions (typically 11-fold) to bring suspensions within range. INP concentrations may exceed  $100 L^{-1}$  at  $-28$  °C (e.g., at SGP).

### **7.3 Accuracy**

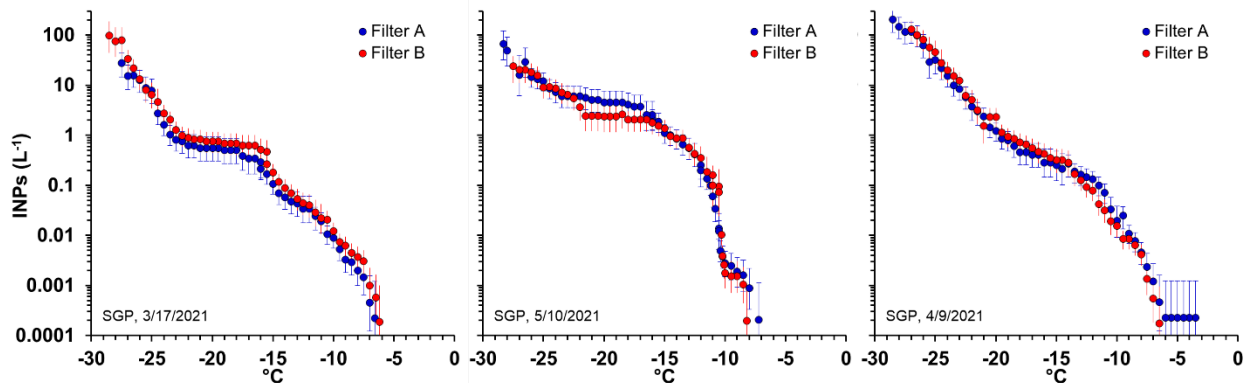
The temperature uncertainty in the INS technique is  $\pm 0.2$  °C (a combination of thermocouple uncertainty and temperature variation across the blocks due to gradients in cooling). Accuracy is also controlled by the inherent uncertainty in using count data of frozen wells. Binomial sampling confidence intervals (CI, 95%) are derived following Agresti and Coull (1998) to estimate the uncertainty in INP number concentrations, and their ranges vary according to the proportion of wells frozen. For a single well frozen (out of 32), the 95% CI ranges from  $\sim 0.2$  to  $\sim 5.5$  times the estimated INP concentration, while for 16 of the 32 wells frozen it ranges from  $\sim 0.6$  to  $\sim 1.6$  times.

### **7.4 Repeatability**

On several occasions in previous campaigns, we have confirmed the repeatability of measures within the range inherent to the method (see section 7.3). Two examples are given in Figure 3, from filter samples taken in the CACTI field campaign in Argentina. We have also tested replicate filters to confirm their comparability (Figure 4).



**Figure 3.** Repeatability of INP tests using suspensions that were tested immediately after resuspension of particles from filters, then retested after frozen storage of the suspension (tubes were briefly pulse-vortexed after thawing). Vertical bars are 95% confidence intervals.



**Figure 4.** Comparability of INP spectra obtained from replicate filters taken at SGP. Vertical bars are 95% confidence intervals.

## 7.5 Sensitivity

As mentioned in section 7.2, the sensitivity of the method is typically around  $0.0002 \text{ INPs L}^{-1}$ . Expressed in terms of lower limit of temperature of operation, the sensitivity of the INS is dependent on the background concentrations of contaminant INPs in the DI water blank controls. Concentrations of such artifacts are typically very low, at  $< 1.5 \text{ INPs mL}^{-1}$  at  $-25 \text{ }^\circ\text{C}$ .

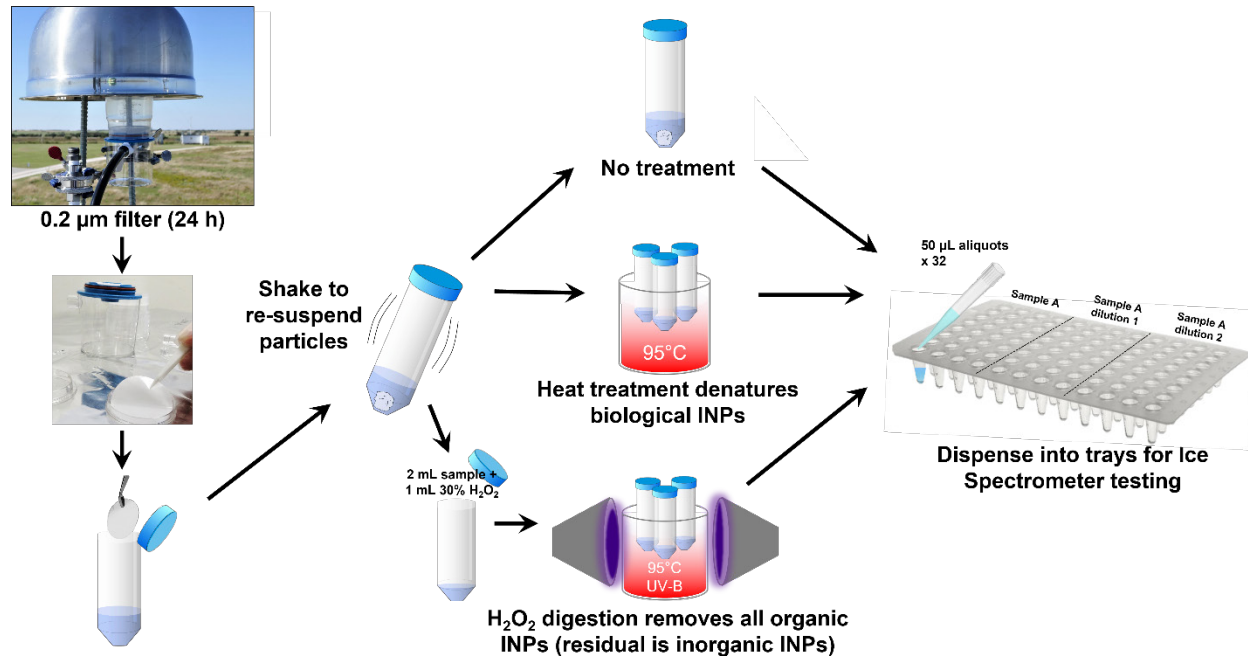
## 7.6 Input Values

The INS produces data containing the number of wells frozen, out of a total of 32, every  $0.5 \text{ }^\circ\text{C}$ , which are used to calculate final cumulative INP number concentrations.

## 7.7 Output Values

Output data from the INS include values of freezing temperature ( $^{\circ}\text{C}$ ), INP number concentration ( $\text{L}^{-1}$  STP), and upper and lower 95% CI values.

## 8.0 Instrument System Functional Diagram



**Figure 5.** Schematic of workflow used to prepare samples for INP measurement in the INS.

## 9.0 Instrument/Measurement Theory

The formation and microphysical modulation of cloud droplets and ice crystals are highly dependent upon aerosols that serve as cloud condensation nuclei (CCN) and INPs. In general, INP observations are limited relative to other aerosol properties, yet central to elucidating the role of aerosols in cloud formation, and subsequent cloud microphysical and radiative properties. Immersion freezing, whereby an INP first serves as a CCN, then freezes at temperatures above homogeneous freezing ( $-38^{\circ}\text{C}$ ) (Kanji et al. 2017, Murray et al. 2012), is a particularly important glaciation process for the formation and impacts of mixed-phase clouds. The INS mimics immersion freezing of cloud ice through ambient aerosols serving as INPs by way of heterogeneous ice nucleation. This technique provides quantitative information on the population of ambient aerosols that can facilitate cloud ice formation at a wide range of subzero temperatures.

The efficacy of an aerosol to serve as an INP largely depends on temperature and vapor saturation with respect to water and ice, in addition to its composition (chemical, mineral, or biological makeup), morphology, and size, and thus, its source (Hoose and Möhler 2012). Aerosols such as mineral dust, soil dust, sea spray, volcanic ash, black carbon, and biologically derived particles (e.g., intact or fragmented bacteria, pollen, fungal spores, lichens, algae, diatoms, soil organic matter, fatty acids, proteins, and other macromolecules) have been shown to serve as INPs (Conen et al. 2011, Cziczo et al. 2017, DeMott et al. 1999, 2018a, Hill et al. 2016, Hoose and Möhler 2012, McCluskey et al. 2014, Murray et al. 2012,

O'Sullivan et al. 2015, 2018, Petters et al. 2009). Among the natural sources, mineral dust and biologically derived particles are arguably the most crucial INPs found in the atmosphere. Mineral dust is an abundant INP, forming ice primarily at temperatures  $< -15$  °C, while classes of biological particles such as certain bacteria are capable of initiating freezing up to  $-1.5$  °C (Creamean et al. 2013, Despres et al. 2012, Frohlich-Nowoisky et al. 2016, Hoose and Möhler 2012, Kim et al. 1987, Murray et al. 2012). Measuring total INPs, in addition to their biological and mineral components, yields critical information on INP abundances and sources.

## **10.0 Setup and Operation of Instrument**

### **10.1 Filter Preparation**

The single-use Nalgene™ Sterile Analytical Filter Units are prepared by replacing their cellulose nitrate filters with 0.2- $\mu$ m polycarbonate filters backed with 10- $\mu$ m polycarbonate filters (each are 47-mm-diameter Whatman® Nuclepore™ Track-Etched Membranes), both pre-cleaned in-house (Barry et al. 2021). Filter units are disassembled and reassembled under ultraclean conditions in a laminar flow cabinet with near-zero ambient particle concentrations. The modified filter units are then capped and individually stored in clean airtight bags until use.

### **10.2 Filter Use and Preservation**

Once sampling is complete, typically after 24 hours (option to collect between 2 to 72 hours, depending on total aerosol loadings at each site), the 0.2- $\mu$ m filters containing the collected aerosol loadings are removed from the single-use filter unit and preserved frozen at approximately  $-20$  °C on site in individual sterile Petri dishes. Batches of samples preserved in Petri dishes are transported frozen to CSU where they are further preserved frozen until processing.

### **10.3 INS Sample Processing**

For re-suspension of particles prior to measurement of INPs in the INS, filters are placed in sterile 50-mL polypropylene tubes, 7-10 mL (smaller amounts for filters from “cleaner” environments, to increase sensitivity) of 0.1  $\mu$ m-filtered DI water added, and particles resuspended by tumbling end over end on a rotator for 20 min. Each INS is constructed using two 96-well aluminum incubation blocks, designed for incubating PCR plates, placed end to end and encased on their sides and base by cold plates (Figure 6a). There are two INS instruments placed side by side to double sample processing capacity (Figure 6b).

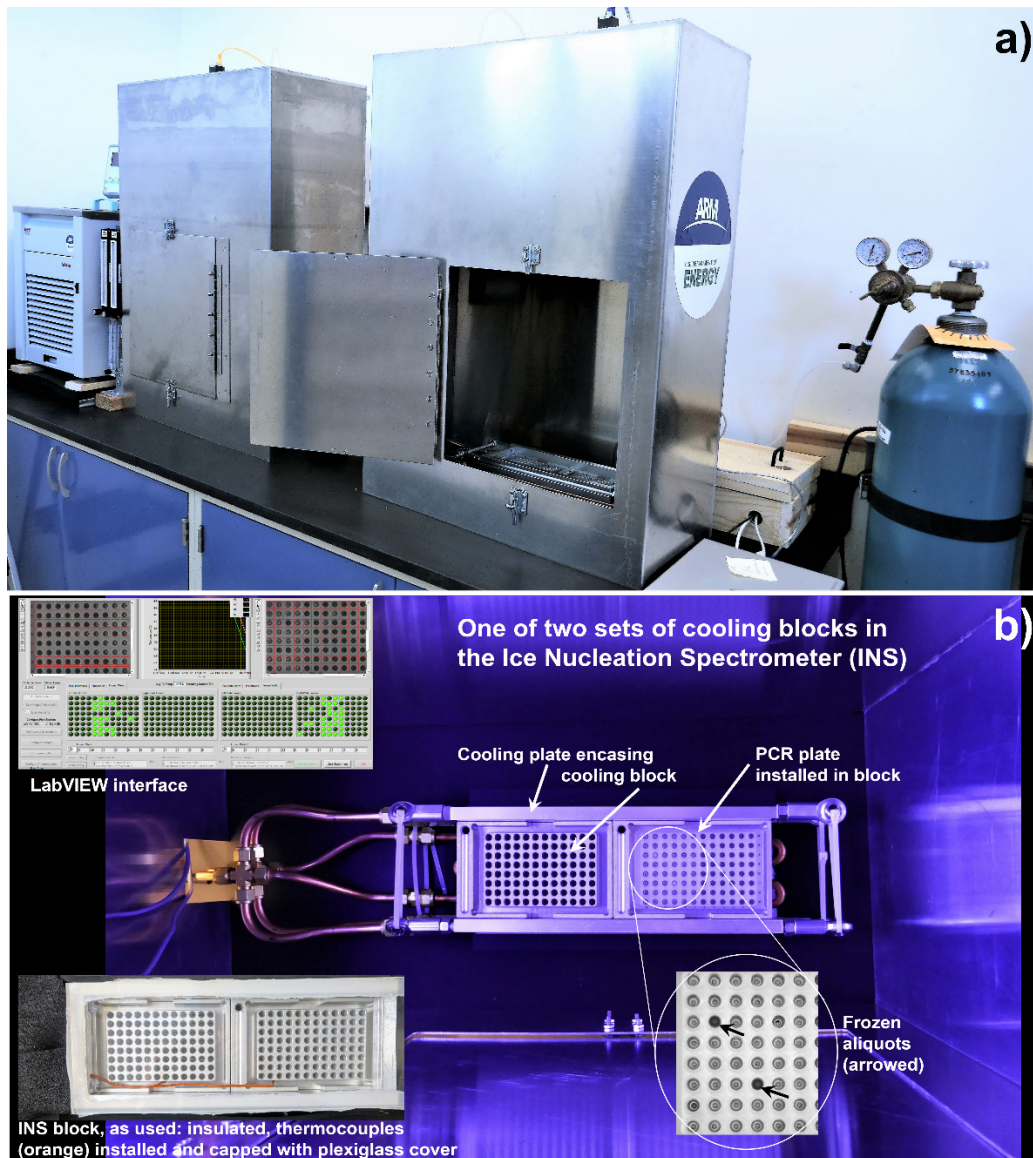
Immersion freezing temperature spectra are obtained by dispensing 50- $\mu$ L aliquots of aerosol suspensions into four sterile, 96-well PCR trays in a laminar flow cabinet. One INS run, therefore, processes  $384 \times 50$   $\mu$ L aliquots of suspension. There are typically 32 aliquots per level of dilution, and we use up to five 11- to 15-fold serial dilutions to cover the full temperature range/INP concentration. PCR plates are then placed into the blocks of the INS, the device covered with a plexiglass window, and the headspace purged with 750 mL  $\text{min}^{-1}$  of cooled and HEPA-filtered  $\text{N}_2$ . The device is cooled at  $0.33$  °C  $\text{min}^{-1}$  using a recirculating low temperature bath, and the freezing of wells is recorded every  $0.5$  °C automatically through an interface with a charge-coupled device (CCD) camera system. Limit of measurement is between  $-27$  and  $-29$  °C, depending on INPs in the DI water used for resuspension (this baseline is subtracted before subsequent calculations).

## 10.4 INP Number Concentration Calculation

From the fraction of drops frozen and the known total volume of air filtered at each temperature interval, we can calculate INP concentration with a universally used equation (Vali 1971):

$$K(\theta) (L^{-1}) = \frac{\ln(1-f)}{V_{drop}} \times \frac{V_{suspension}}{V_{air}}$$

where  $f$  is the proportion of droplets frozen,  $V_{drop}$  is the volume of each drop,  $V_{suspension}$  is the volume of the suspension, and  $V_{air}$  is the volume of air per sample (liters at STP: 0 °C and 101.32 kPa).



**Figure 6.** The INS, including: a) a photo of the exterior showing both cooling systems, one with the door open exposing the cooling blocks for scale and b) interior view of one system. PCR trays are loaded into the cooling blocks, the headspace purged with cooled and HEPA-filtered air, blocks cooled at 0.33 °C min<sup>-1</sup>, and freezing events logged automatically through a LabVIEW interface with a camera.

## 10.5 Sample Treatments

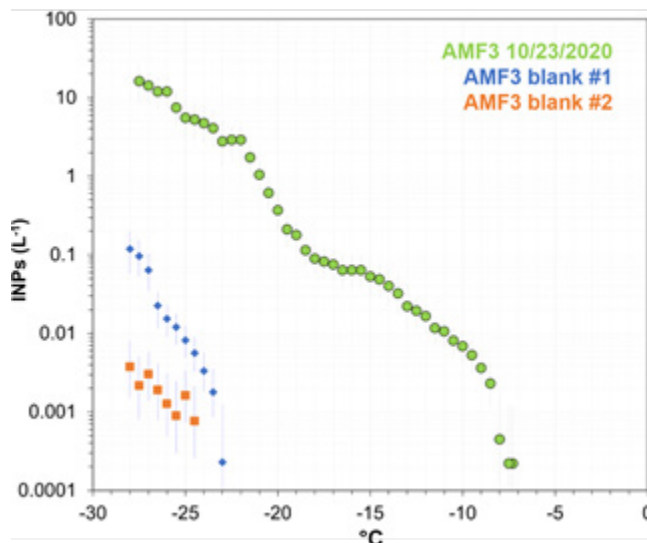
Thermal treatments and peroxide digestions provide valuable insights into INP composition (see Figure 2). Heat treatments are performed by heating 2.5 mL of sample suspension to 95 °C for 20 min to denature heat-labile INPs, such as proteins. Peroxide digestions are performed on a further 2 mL of suspension in a solution containing 10% H<sub>2</sub>O<sub>2</sub> (by adding 1 mL of 30% H<sub>2</sub>O<sub>2</sub> in water, Sigma-Aldrich®) and heating to 95 °C for 20 min while illuminating with ultraviolet B (UVB) fluorescent bulbs to generate hydroxyl radicals. Following digestion, residual H<sub>2</sub>O<sub>2</sub> is removed using catalase (MP Biomedicals™ catalase from bovine liver). This digestion removes all bio-organic INPs as described in detail in McCluskey et al. (2018b), Suski et al. (2018), and Testa et al. (2021). The difference in the INP temperature spectra after both treatments determines the influence of that INP type in the original sample, and the residual spectrum gives the inorganic/mineral INP component. Thus, this processing provides four key measures from each sample: total, heat-labile (i.e., biological), bio-organic, and inorganic (i.e., mineral) INP concentration. Treatments are typically applied to 1/3 of the total samples collected at any given location or during a given IOP.

## 11.0 Software

INS operation and data acquisition are controlled with custom LabVIEW software.

## 12.0 Calibration

Filter unit and DI water control blank tests are conducted routinely to obtain background INP spectra for calibration of the measured INP spectra from the aerosol filter samples (Figure 7).



**Figure 7.** Example of a 24-hour sample from the third ARM Mobile Facility (AMF3; Oliktok Point, Alaska) on 10/23/2020 and two blank filters collected at the same site.

TSI 5200 mass flow meters are checked against a TSI 5230 meter, which has enhanced 1.7% accuracy, and is reserved for this purpose. If TSI 5200 units are more than 5% adrift, they are returned to TSI for servicing and calibration.



## 13.0 Maintenance

The following maintenance procedures are required for the filter unit apparatuses:

- Check in-line temperature, pressure, and flow rate at the start of sample collection.
- Check in-line temperature, pressure, and flow rate at the end of sample collection.
- Clean and clean precipitation shields, as needed.
- Check for leaks in single-use filter units before use.
- Check for leaks/obstructions in vacuum tubing, and connection points, as needed.
- Check TSI 5200 mass flow meters (against TSI 5230, which has enhanced 1.7% accuracy, and is reserved for this purpose) annually, and return for servicing if required.
- Check performance of Thomas 2688CE44 pumps using TSI flow meters. When operating efficiently these pumps will be capable of producing a 0.5 kPa vacuum.

The following maintenance procedures are required for the INS:

- Clean plexiglass lids with Windex and DI water every two weeks.
- Deep clean lab space with Windex and Kimwipes once per month.
- Check copper piping for SYLTHERM™ XLT heat transfer fluid leaks.
- Watch rate of N<sub>2</sub> tank depletion for leaks.

## 14.0 Safety

Chemicals including 30% H<sub>2</sub>O<sub>2</sub> and catalase for the peroxide treatment processing, methanol for preparing sterile filters, and SYLTHERM™ XLT heat transfer fluid for the INS coolant are all used during filter collection and processing. These chemicals can be flammable and/or toxic.

## 15.0 Citable References

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