Electronic Supplementary Information (ESI)

On-chip analysis of atmospheric ice-nucleating particles in continuous flow

Mark D. Tarn,*a,b Sebastien N. F. Sikora,a Grace C. E. Porter,a,b Bethany V. Wyld,a Matan Alayof,c Naama Reicher,c Alexander D. Harrison,a Yinon Rudich,c Jung-uk Shim*b and Benjamin J. Murray*a

a. School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK.
b. School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, UK.
c. Department of Earth and Planetary Sciences, Weizmann Institute of Science, Rehovot 76100, Israel.

* Email: m.d.tarn@leeds.ac.uk; Tel: +44 (0) 113 343 5605.
* Email: j.shim@leeds.ac.uk; Tel: +44 (0) 113 343 3903.
* Email: b.j.murray@leeds.ac.uk; Tel: +44 (0) 113 343 2887.

Contents
1. Thermocouple calibration  Page S2
2. Experimental setup (Figs. S1-S2)  Page S3
3. Cold plate characterisation (Fig. S3-S4, Table S1)  Page S5
4. Aerosol sampling in the Eastern Mediterranean (Table S2)  Page S10
5. Discussion of the operational characteristics of the LOC-NIPI  Page S11
6. References  Page S12
1. Thermocouple calibration

The three thermocouples (0.5 mm Ø x 150 mm long, 406-532 series K-type, TC Direct, Uxbridge, UK) that were connected to the multichannel temperature controller and inserted into the aluminium cold/warm plates of the cold stage platform were calibrated against a platinum resistance thermometer (PRT; Netushin NR-141-N L10, RS Components, Northants, UK). Likewise, the thermocouples (80 μm Ø, ±2.2 °C, 5SRTC-TT-KI-40-1M series K-type, Omega Engineering Ltd., Manchester, UK) connected to a data logger (TC-08, ±0.025 °C, Pico Technology, St. Neots, UK) that would be inserted into the temperature reference channel of the microfluidic device for measuring the flowing oil temperature were calibrated against the same PRT. This PRT was connected to a custom electronics package that utilised an Arduino Nano microcontroller (purchased from RS Components) with a resistance temperature detector (RTD) converter board (Model 3328, Adafruit Industries, USA) to log the temperature readout. The PRT had previously been calibrated against a precision PRT probe (Model 5608, ±0.0013 °C, Fluke Corporation, USA) connected to a digital readout stack (Model 1560, Fluke Corporation, USA) that had itself been calibrated by the National Physical Laboratory (NPL, Teddington, UK).

The thermocouples and the calibrated PRT were inserted into a narrow-bore hole in a metal cylinder, and the hole was filled with methanol. The metal cylinder was wrapped in copper tubing that was connected to a liquid nitrogen dewar at one end and an exhaust vent at the other, and the entire assembly was encased in insulating foam cladding. The liquid nitrogen dewar was opened, allowing nitrogen to flow through the copper tubing and so to cool the metal cylinder with the probes inside. Once the temperature had reached below −45 °C, the dewar was closed and the setup allowed to slowly return to room temperature over the course of about 1 day. The temperature reading of each probe was logged the entire time, and the slow rise in temperature ensured that the probes were all at equilibrium with the temperature of the methanol in the metal cylinder.

The thermocouple readouts were plotted against the PRT readout and correction factors for each thermocouple were calculated that, when applied, would correct the original thermocouple temperature readings to those of the calibrated PRT. The thermocouples (5SRTC-TT-KI-40-1M series) connected to the TC-08 logger were corrected using linear fits, whilst the thermocouples (406-532 series) connected to the multichannel temperature controller were corrected using 2nd order polynomial fits. Following calibration, the temperature errors associated with the thermocouple (5SRTC-TT-KI-40-1M series) inserted into the chip were estimated to be ±0.03 °C, while the errors of the three thermocouples (406-532 series) inserted into the aluminium plates of the cold stage were estimated to range from ±0.02 °C to ±0.04 °C.
2. Experimental setup

Fig. S1 Photograph of the inside of the temperature control unit. Temperature control was achieved by means of a custom microcontroller-based controller working in tandem in a ‘call-response’ fashion with a Python program running on an attached personal computer (PC), to form a closed-loop proportional-integral-derivative (PID) control system. The microcontroller continuously modulated the duty cycle and direction of heat flow (drive direction) through each Peltier element using bi-directional (dual H-bridge) DC motor drivers under pulse width modulation (PWM) control. Plate temperature feedback was provided by K-type thermocouples embedded into each aluminium plate on the cold stage platform, connected to the microcontroller by thermocouple amplifiers read by an analog-to-digital converter. At 5 Hz, the attached PC requested the current aluminium plate temperatures from the microcontroller, determined the required duty cycle and drive direction for each Peltier element as a function of the difference between the current temperature and desired temperature set point in each case, and then implemented these via a command issued to the microcontroller. A switching DC-DC power converter was used to step-down the 12 V DC supply to the required voltage to drive the three Peltier elements (6 V), which was further stepped-down to 5 V via a low-dropout voltage linear regulator to power the microcontroller.
Fig. S2 Photograph of the experimental setup, showing the cold stage platform connected to the custom-built temperature control unit. The microfluidic device was placed in the chamber of the cold stage platform and fluids were introduced using syringe pumps. Visualisation of droplets in the microfluidic channel was achieved using a zoom lens with a high-speed camera (*camera not shown*), and lighting provided by a coaxial LED connected to the zoom lens assembly. Cooling of the Peltier elements in the cold stage platform was achieved by pumping a solution of polyethylene glycol in water through liquid heat exchangers in the platform, which was achieved using a refrigerated circulating chiller (*not shown*). The temperature inside the microfluidic device was measured via a thermocouple that was connected to a temperature data logger (Pico Logger).
3. Cold plate characterisation

Following the calibration of the thermocouples, the on-chip temperature was characterised in relation to the position of the chip on the cold plate (an 8.4 mm x 14 mm block of aluminium) of the cold stage platform in order to determine any temperature differences between the temperature reference channel and the droplet channel, and to estimate the temperature uncertainties during INP analysis. Two 5SRTC-TT-KI-40-1M series thermocouples (80 μm diameter) were each inserted into blunt 21 G x 7/8” syringe needles (B. Braun Sterican) that had been removed from the plastic syringe connectors, such that the tips of the thermocouples extended around 3 mm from the end of the blunt needles. The thermocouples were then glued into the syringe needles in order to seal them. Holes were punched into the temperature reference channel and the droplet channel of a microfluidic device at an angle of ~45° prior to bonding to a glass microscope slide. This allowed the thermocouples to be inserted into both channels, with the tips of the thermocouples extending into the microchannels (Fig. S3). The angle of the access holes ensured that the thermocouples could slide easily into the microchannel, rather than experiencing a 90° turn if the holes had been punched perpendicular to the channel as is usually done for inlet and outlet tubing access holes. The rigidity and diameter of the blunt needles made the thermocouples easy to handle and to insert into the access holes of the chip, taking only a matter of seconds to insert and position them, and with a sufficient seal between the PDMS of the chip and the syringe needle to prevent any leakage of fluids. The presence of a somewhat bulky thermocouple (with 80 μm diameter wires, and a tip that was estimated to be ~160 μm wide and ~90-100 μm in height) did not appear to negatively affect the fluid flow in the wider and deeper channel (300 μm × 140 μm cross-sectional area), i.e. the fluid passed freely through the temperature reference channel and exited via the outlet tubing.

Given the relatively large cross-sectional area that the thermocouple tip occupied in the microchannel, we assumed that the thermocouple gave a “bulk” temperature measurement near to the centre of the microchannel, the region in which the hydrodynamic fluid flow is fastest and which the droplets (80-100 μm diameter in a 140 μm tall channel) are largely expected to occupy (Fig. S3b). Stan et al. [1] had previously used an approach in which thermometers were micropatterned onto the upper and lower surfaces of their microchannel in order to measure the in-channel temperature, but had found that the gradients between the centre of the channel and its boundaries were large, hence the droplet temperature had to be modelled. Obtaining measurements using a thermocouple that was on a similar same size scale to the microchannel meant that modelling was not required. Further to this, the use of the thermocouple meant that there was a greater degree of flexibility in the flow rates and droplet velocities that could be applied, since thermocouple readings would take this into account whilst modelling would need to be performed for each given flow rate.
In order to perform temperature characterisation measurements, Novec™ 7500 Engineered Fluid was pumped into the temperature reference channel and the droplet channel at a flow rate of 24 $\mu$L min$^{-1}$, whilst an additional 0.05 $\mu$L min$^{-1}$ of Novec™ 7500 was pumped into the droplet channel to represent the additional flow velocity provided by an aqueous suspension during droplet production. These flow rates were selected since they represented the typical flow rates that were applied during the bulk of the LOC-NIPI droplet experiments. The chip was placed onto the cold plate of the cold stage platform (Fig. S3a) with a thin layer of silicone oil between the two to provide better thermal contact compared to placing the chip onto the stage without oil.

**Fig. S3** (a) Photograph showing thermocouples inserted into the temperature reference channel and droplet channel of a microfluidic device for characterisation of the on-chip temperatures on the cold stage platform. The thermocouples were glued into blunt syringe needles to make them easy to handle and to insert into the access holes of the chip whilst forming a seal. The chip is shown placed onto the cold stage platform such that the tips of the thermocouples are located in the centre of the cold plate in x-direction, ~4 mm from the left edge of the cold plate. (b) Photograph of a thermocouple inside a microchannel within the microfluidic device. The shadowed region at the right-hand side of the image shows the sleeved part of the thermocouple passing through the thickness of the PDMS.

Thermocouple readings were recorded simultaneously in the temperature reference channel and the droplet channel with flowing oil at cold plate temperatures of +10 °C, 0 °C, −10 °C, −20 °C, −30 °C, and −39 °C. Temperature measurements were taken with the chip being moved to multiple positions in the x-direction across the cold plate, from the left edge of the plate (designated as 0 mm) to the...
right edge of the plate (at 8.4 mm). An example of the results is shown in Fig. S4a, illustrating the measurements of both the temperature reference channel and the droplet channel at the various cold plate temperatures, and this entire process was repeated three times.

**Fig. S4** (a) Plot showing the on-chip temperatures inside the temperature reference channel (■ with solid lines) and the droplet channel (○ with dashed lines) of the microfluidic device at varying cold plate temperatures. In-channel temperatures were measured at various positions across the cold plate in the x-direction, and the droplet channel temperature measurements also represent the temperatures that a droplet will experience as it passes through the channel. (b) The temperature in the droplet channel at different cold plate temperatures, with the dashed black line representing how a 1:1 relationship would appear. The droplet channel temperatures at the centre of the cold plate (~3.5-5.5 mm) were closest to the cold plate set temperature, whilst those at the edges of the cold plate (0.0 mm or 8.1 mm) were furthest from the cold plate set temperature.

The LOC-NIPI was designed such that the droplets would experience an initial rapid cooling to reach an isothermal region over the centre of the cold plate, as demonstrated by the results in Fig. S4a, where the droplet freezing assay measurements take place. As the droplets entered the cold plate region, they experienced estimated cooling rates ranging from 200 °C min⁻¹ (~0.3 °C mm⁻¹) at a cold plate temperature of 0 °C to nearly 2,400 °C min⁻¹ (~3.3 °C mm⁻¹) at a cold plate temperature of −39 °C. The isothermal region was defined as having a variability of ±0.2 °C, and was estimated to be from ~3.5 mm to ~5.5 mm in the x-direction, with the droplets spending ~0.2 s at the given temperature.
The differences between the cold plate set temperature and the droplet channel temperature are illustrated in Fig. S4b. This shows that the largest differences between the two are experienced at the very edges of the cold plate (shown as 0.0 mm and 8.1 mm in the x-direction in the figure) while the smallest differences are found at the centre of the cold plate (~4-5 mm in the x-direction) where the experimental droplet measurements were taken. The differences between the temperature reference channel and the droplet channel at the coldest part of the cold plate (i.e. the centre, ~4 mm in the x-direction) were used to generate a calibration curve that was used to correct the temperature reference channel readings to give the estimated droplet channel temperature during droplet freezing experiments. Further, the droplet channel measurements represent the “temperature trajectory” that a droplet would follow as it moves over the cold plate. The data collected for a cold plate temperature of −39 °C was also used to estimate the time that droplets passed over the isothermal region of the cold plate (covering a region of up to ~2 mm), where the temperature was reasonably consistent (±0.2 °C), for calculation of the homogeneous volume ice nucleation rate coefficient, $J_V(T)$, of purified water.

The placement of the microfluidic device onto the cold plate such that the thermocouples were in at the central position of the plate (~4 mm in the x-direction), where droplet measurements would take place, was repeated several times and the variability in the placement was determined. The variability in the x-direction was ±120 μm between placements, and the variability in the y-direction was ±290 μm. Based on the multiple readings in the centre of the chip, temperature uncertainties for droplet experiments were estimated for different temperature ranges, as shown in Table S1. These temperature errors could be improved in future iterations of the platform by including alignment marks in the microfluidic design, such that the chip and thermocouple could be even more accurately and reproducibly aligned with the cold plate.
**Table S1** Temperature uncertainties in the on-chip temperature readings during LOC-NIPI experiments, determined from multiple temperature measurements at different locations in the centre of the cold plate, in addition to temperature probe errors and temperature variations during experiments.

| On-chip temperature range (°C) | Temperature error (°C) |
|--------------------------------|------------------------|
| +10 to 0                       | ±0.2                   |
| 0 to −10                       | ±0.4                   |
| −10 to −19                     | ±0.5                   |
| −19 to −28                     | ±0.6                   |
| < −28                          | ±0.7                   |
4. Aerosol sampling in the Eastern Mediterranean

Table S2 Details of the aerosol samples collected during the field campaign in Rehovot, Israel, in October-November 2018. Samples were either collected using a filter-based platform (BGI PQ100 Air Sampling System with a PM$_{10}$ inlet, Mesa Laboratories), or using an impinger (Coriolis® Micro, Bertin Technologies).

| Sample       | Date                              | Start time | End time | Sampling method       | Sampling rate (L min$^{-1}$) | Volume of sampled air (L) |
|--------------|-----------------------------------|------------|----------|-----------------------|----------------------------|--------------------------|
| 3 h sample   | 31st October 2018                 | 14:28      | 17:28    | Filter-based (PQ100)  | 16.67                      | 3,000                    |
| 24 h sample  | 25th October 2018 to 26th October | 11:45      | 11:45    | Filter-based (PQ100)  | 16.67                      | 24,000                   |
| 20 min sample| 3rd November 2018                 | (1) 15:09  | (1) 15:19| Impinger (Coriolis)   | 300                        | 6,000                    |
5. **Discussion of the operational characteristics of the LOC-NIPI**

The LOC-NIPI is versatile in terms of the temperature increments that can be probed and the number of droplets that can be analysed at each temperature, depending on the user’s requirements; the main compromise is the length of time the user is willing to take to perform a full set of experiments. For example, the user could investigate a narrow temperature range but with high resolution (e.g. 0.1 °C temperature increments) and a high number of droplets per temperature increment. However, in the same amount of time the user could probe the 0 to −37 °C range in the chip with a lower temperature resolution (e.g. 0.5-1 °C increments) and fewer droplets analysed.

The length of time required for the temperature of the flowing liquid to stabilise in the chip after changing the cold plate set temperature depended on the set temperature, i.e. colder temperatures took longer than warmer temperatures. At the coldest temperatures, it could take several minutes for the temperature to stabilise, though this timeframe could be shortened by lowering the temperature of the refrigerant recirculated by the chiller to cool the Peltier elements via the liquid heat exchangers (set to +5 °C during these experiments). To provide some context of timeframes that experiments could take, performing an analysis between 0 and −40 °C in increments of 5 °C, with 200 droplets analysed per temperature (at 1.5 droplet s⁻¹), would take around 40 min, and the amount of aqueous sample analysed would be around 2-4 μL at the typical flow rates employed.

Increasing the resolution to 2 °C temperature increments would increase this time to around an hour and 45 min, with around 6-11 μL of aqueous sample being analysed. Likewise, keeping the temperature resolution to increments of 5 °C but increasing the number of droplets per temperature increment to 1,000 would take a similar time of around an hour and 50 min. Clearly, the parameters chosen by the user will be a compromise between the temperature increment, the number of droplets desired per temperature increment, and the total experimental time, all of which will depend on the type of information required for a given series of experiments.

The LOC-NIPI allows a great deal of flexibility in the type, quantity, and quality of data that the user can obtain in terms of droplet numbers, temperature ranges and temperature increments. However, the compromise for better quality data is the time taken to acquire it: the user will need to weigh up the amount and type of data they desire against the amount of time they are willing to spend on it. The automation of the procedure will help this somewhat, but a compromise will always be present given the way in which the platform operates, i.e. by stepping through a range of temperatures.
6. References

[1] C. A. Stan, G. F. Schneider, S. S. Shevkoplyas, M. Hashimoto, M. Ibanescu, B. J. Wiley and G. M. Whitesides, Lab Chip, 2009, 9, 2293-2305.