Micro-kelvin temperature-stable system for biocalorimetry applications

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ABSTRACT
Achieving micro-kelvin (μK) temperature stability is critical for many calorimetric applications. For example, sub-nanowatt resolution biocalorimetry requires stabilization of the temperature of the calorimeter to μK levels. Here, we describe how μK temperature stability can be accomplished in a prototypical calorimetric system consisting of two nested shields and a suspended capillary tube, which is well suited for biocalorimetry applications. Specifically, we show that by employing nested shields with μTorr-levels of vacuum in the space between them as well as precise feedback control of the temperature of the shields (performed using high-resolution temperature sensors), the effect of ambient temperature fluctuations on the inner shield and the capillary tube can be attenuated by ∼100 dB. We also show that this attenuation is key to achieving temperature stabilities within ±1 and ±3 μK (amplitude of oscillations) for the inner shield and the capillary tube sensor, respectively, measured in a bandwidth of 1 mHz over a period of 10 h at room temperature (∼20.9 ± 0.2 °C). We expect that the methods described here will play a key role in advancing biocalorimetry.

I. INTRODUCTION

Most biological processes have an associated thermal signature as they involve the generation of heat due to inevitable inefficiencies that are associated with all physiological processes. One illustrative and ubiquitous example of this is metabolism that is common to all cells and living organisms and plays a central role in the creation and homeostasis of life and involves complex biochemical networks to generate and consume high energy carriers resulting in a characteristic heat signature. Direct calorimetry, i.e., measuring the heat released in response to physical, chemical, or biological processes, is one of the most efficient techniques to quantitatively measure the metabolic output of biological samples. However, it is technically challenging to perform such calorimetric measurements on small model organisms and single cells because the absolute heat output from these samples is very small, typically in the nanowatt range and lower. Consequently, one key technical requirement toward attaining high calorimetric resolution is to achieve high-resolution thermometry to sense small temperature changes in the calorimeter caused by the metabolism of the organisms.

Resistance thermometry is a well-established technique for high-resolution micro- and nanoscale temperature sensing applications that can typically offer a temperature resolution of 10–50 μK over a measurement bandwidth of 1–20 mHz, as reported by several works. Drift in the ambient temperature of a typical laboratory is much larger and will, therefore, limit the thermometric resolution of resistive, high-resolution calorimeters. Hence, it is necessary to develop a highly temperature-stable environment to be able to perform high-resolution, precision calorimetry. The existing literature describes some methods for designing and building general, temperature-stable systems (i.e., thermostats) for various applications. However, there are specific challenges associated with developing temperature-stable thermostats for implementing precision calorimetry of live biological samples because they need to be biocompatible to maintain the necessary physiological conditions and allow optical access for live imaging of the organisms or biological samples. Moreover, the behavior of living organisms
varies with time, depending on their sleep cycle, eating habits, and movement.\textsuperscript{17,18} For example, the investigation of the metabolic activity of \textit{Drosophila melanogaster} (Fruit flies) associated with their circadian clocks will require calorimetric measurements over a day, covering their entire sleep cycle.\textsuperscript{19,20} Similarly, studying the metabolic activity of \textit{Danio rerio} (Zebra fish) embryos requires multiple days of calorimetric measurements as the embryos develop through various stages.\textsuperscript{21} Hence, achieving long-term temperature stability of biological calorimetry systems is critical and will enable a broad range of investigations on the metabolic outputs associated with numerous interesting biological processes of model organisms and cells.

Over the past few decades, several researchers have developed temperature-stable systems for biological and biochemical applications. Recently, Hur et al.\textsuperscript{15} presented a three-shield system with a stability of ±15 μK in the inner-most shield over 40 h, which was implemented to study the metabolic activity of \textit{C. elegans} worms. Bae et al.\textsuperscript{22} developed a system stable up to 55 μK over 11 h and implemented it to measure the heat output of liquid biochemical samples. Hong et al.\textsuperscript{23} studied the metabolic activity of \textit{Tetrahymena thermophila} with a calorimetric system having a thermal stability of ±80 μK over 10 h. Maskow et al.\textsuperscript{24} and Fiorino et al.\textsuperscript{25} also developed systems with thermal stabilities of ±100 μK. In addition, several other researchers have developed systems with temperature stabilities ranging from 200 μK to 2 mK, with applications in measurements of heat outputs of brown fat cells,\textsuperscript{26} \textit{C. elegans},\textsuperscript{27} \textit{Reuber H35 Hepatoma cells},\textsuperscript{28} and biofilms of bacteria such as \textit{P. pudita}.\textsuperscript{29}

In this article, we describe an in-house developed thermostat consisting of two nested shields, mechanically connected to each other through weak thermal links and maintained under vacuum conditions of <10 μTorr. With this system, we achieve a temperature stability within ±1 μK for the inner shield and a temperature stability within ±3 μK for a suspended capillary tube that can be used for biological calorimetry. The above-mentioned stability is measured as the amplitude of temperature oscillations in a bandwidth of 1 mHz over 10 h at room temperature (∼20.9 ± 0.2°C). As described in more detail below, this high stability is accomplished using a combination of AC-driven resistance thermometry and common-mode signal cancellation to sense the temperature fluctuations of the metal shields and the capillary tube, followed by active proportional–integral–differential (PID)-based feedback control of the shields to eliminate long term (hours to days) temperature drift. Below, we describe in detail the mechanical and thermal design of this system along with a thermal model, and the approach we took to implement high-resolution thermometry and feedback control of temperature. Subsequently, we discuss the temperature stability achieved in the nested shields and the suspended capillary tube. We analyze the dominant factors affecting the stability, including room-temperature fluctuations, contributions of thermal radiation through the optical window, disturbances due to the open capillary tube and the presence of a physiological buffer medium, as well as the electronic drift in the circuitry and in the voltage excitation sources. Finally, we conclude by discussing approaches to further improve the temperature stability of thermostats employed in biocalorimetric applications.

II. MECHANICAL AND THERMAL DESIGN

The system employed in this work (Fig. 1) consists of two nested shields, a sensing and a matching capillary tube, a fluidic pumping system and a reservoir connected to the sensing capillary tube, and an optical imaging system. To achieve high-temperature stability on the capillary tube (where biological samples are loaded), the system must be designed such that fluctuations in the ambient temperature do not perturb the temperature of the capillary tube. Toward this goal, our system (Fig. 1) consists of two nested shields (an outer shield (OS) and an inner shield (IS)), which are thermally

![FIG. 1. (a) Schematic showing the developed calorimeter system that consists of capillary tubes integrated with a sensing thermistor and two nested shields, the outer shield (OS) and the inner shield (IS). The shields are equipped with polyimide heaters for temperature control. The IS is supported via four borosilicate spheres within the OS. Weak thermal coupling between the shields and sensors is achieved through the excellent thermal insulation provided by the approximate line contacts of the spheres, and due to the vacuum in the enclosed space. The schematic also shows the location of the specimen for biological measurements. A stopper is integrated into the sensing capillary for locating the specimen near the temperature sensor in the capillary. The system also includes a flow system with a reservoir and a pump for inserting a suitable buffer medium through the capillary, an imaging system, and an optical window to allow visual access to the specimen. (b) Detailed isometric expanded view of the OS, the IS, the sensing capillary and the matching capillary placed in proximity to each other, and the optical windows integrated into the OS.](image-url)
weakly coupled with each other and with the ambient, which minimizes the effects of environmental perturbations. In our system, the OS (12 × 12 × 8 cm$^3$) is a 1.2 cm thick copper enclosure that houses the IS and two capillary tubes. The IS (7 × 7 × 3.5 cm$^3$) is supported within the OS by four borosilicate spheres (6.35 mm in diameter), which are located in conical pockets machined on the surfaces of the OS as well as the IS. The spheres are steadily positioned in the pockets between the OS and the IS and no adhesive is needed at the copper-sphere contacts. Each of these borosilicate spheres [see Fig. 1(a)] makes an approximate line contact with their respective conical pockets resulting in a weak thermal link (∼36 mW/K) while providing robust mechanical support to the IS. The IS is U-shaped, i.e., enclosed on five sides, but open on the bottom side [see Fig. 1(b)], creating an internal pocket that is 3 × 3 × 1.2 cm$^3$ in dimension. The sensing and matching capillary tubes [made of Teflon perfluoroalkoxy alkane (PFA) resin] are attached to the IS on the bottom side allowing optical access of the sensing capillary tube through windows integrated in the OS [see Figs. 1(a) and 1(b)]. An IR reflective glass (Hot Mirror, Edmund Optics) and an IR absorptive glass (KG5 Schott filter) are used in series to make the windows.

The sensing capillary tube is designed to allow sensitive thermometric measurements consistent with the demands for biological calorimetry applications. For example, the central region of the sensing capillary tube is capable of housing a biological sample keeping the sample optically accessible through the window integrated into the OS [see Figs. 1(a) and 1(b)] similar to what was presented in a recent study. The sensing capillary tube is anchored thermally to the IS and extends out of the OS through hermetically sealed microfluidic feedthroughs. Furthermore, it is connected to a microfluidic system that enables the loading and unloading of biological samples and supplying a physiological buffer solution through the tube during biological experiments. A matching capillary tube is also mounted on the IS to enable cancellation of the common-mode thermal drifts in the sensing and matching capillary tubes. We note that these capillary tubes are in excellent thermal contact with the IS and are in close proximity to each other and, therefore, experience near-identical thermal background drift [see Fig. 1(b)]. However, the sensing and the matching capillary tubes differ in two aspects: first, the sensing tube extends out of the OS, whereas the matching tube does not, and second, the sensing tube has a stopper extending to its midpoint that can be used to localize a biological specimen to a region close to where a thermostor is mounted on the sensing tube [see Fig. 1(a) and Ref. 8] to measure the heat output of the sample.

As mentioned above, the shields are made of copper, which features a high thermal conductivity (∼400 W/m K at 300 K) and, hence, helps reduce thermal gradients across the shields. Furthermore, the shields have relatively large masses (the OS and the IS weigh ∼5.6 and ∼1.2 kg, respectively) resulting in long thermal time constants that mitigate the impact of high-frequency temperature fluctuations. Furthermore, the OS is hermetically sealed to maintain a relatively high vacuum (<10 µTorr) via a combination of a turbo molecular pump (Agilent TwisTorr 84 FS) and a dry scroll pump (Agilent IDP-10), which significantly suppresses the contribution of air to the heat transfer between the OS, the IS, and the capillary tube sensor. For example, in past work, Lee et al. showed that the thermal conductance due to the convective heat transfer from air is reduced to <15 µW/K at vacuum levels of under 1 mTorr. The radiative heat transfer between the OS and the IS is minimized by covering the relatively high emissivity copper surfaces (as high as 0.7 when oxidized) of the IS and OS with low emissivity (∼0.07) aluminum sheets.

Furthermore, the IS is externally covered with a 3 mm-thick Polyethylene foam to reduce conductive and convective heat transfer from the surrounding environment. Finally, the entire thermostat (OS + IS + capillary tubes) is placed on an elevated aluminum stage mounted to an inverted optical microscope (Zeiss Axiovert 200) and enclosed in a box covered with reflective foam insulation to prevent air currents in the room from perturbing the system.

In Fig. 2, we present a thermal model corresponding to the system shown in Fig. 1, which includes the heat capacities of the shields as well as the various thermal pathways connecting the ambient to the nested shields and the sensing capillary tube. This model offers a convenient way for understanding how changes in the ambient temperature perturb the temperature of the shields and the sensing capillary tube and was used as a guide to design the thermostat system. The shields are designed to have high thermal capacitances. The OS weighs ∼5.6 kg, while the IS weighs ∼1.2 kg, and the thermal capacitances of the OS (C$_{OS}$) and the IS (C$_{IS}$) are ∼2.156 and ∼0.462 kJ/K, respectively (specific heat capacity of copper is ∼0.385 kJ/kg K$^{-1}$). The high thermal capacitances of the shields help attenuate the effects of high-frequency ambient temperature fluctuations when they reach the OS and the IS. The temperature fluctuations when they reach the OS and the IS. The temperature fluctuations when they reach the OS and the IS.
fluctuations of the OS are linked to the ambient through a thermal conductance $G_{\text{Amb-OS}}$, which primarily accounts for the heat transfer to the OS by conduction through the microscope’s aluminum stage and due to ambient air drifts. The key pathway for temperature fluctuations in the IS is the conductive heat transfer through the weak thermal links that connect it to the OS and is given by $G_{\text{OS-IS}}$ and estimated to be $\sim 36 \text{ mW/K}$ (based on the contact area and thermal conductivity of the borosilicate glass spheres). As described above, the convective heat transfer between the OS, the IS, and the capillary tube is greatly reduced in the presence of $\mu$Torr levels of vacuum, and the radiative heat transfer between the walls of the OS and the IS is suppressed using reflective aluminum sheets. There are multiple factors contributing to the temperature fluctuations in the sensing capillary tube, including the conductive heat transfer from the IS along the capillary tube (by $G_{\text{IS-Cap}}$) and estimated to be $\sim 30 \mu\text{W/K}$, based on the dimensions of the capillary tube and the thermal conductivities of the used PFA tubing and water.

Furthermore, the thermal capacitance of the sensing capillary tube ($C_{\text{Cap}}$) is $\sim 7$ J/kgK (specific heat capacity of PFA is $\sim 1050 \text{ J/kgK}^\circ$), which is low compared to $C_{\text{OS}}$ and $C_{\text{IS}}$ and, hence, makes the capillary tube more susceptible to high-frequency temperature fluctuations.

Table I enlists the estimated thermal capacitances and thermal conductances shown in the thermal model (Fig. 2).

<table>
<thead>
<tr>
<th>$C_{\text{OS}}$ (kJ/K)</th>
<th>$C_{\text{IS}}$ (kJ/K)</th>
<th>$C_{\text{Cap}}$ (J/K)</th>
<th>$G_{\text{OS-IS}}$ (mW/K)</th>
<th>$G_{\text{IS-Cap}}$ (μW/K)</th>
<th>$G_{\text{Amb-Cap}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sim 2.156$</td>
<td>$\sim 0.462$</td>
<td>$\sim 7$</td>
<td>$\sim 36$</td>
<td>$\sim 30$</td>
<td></td>
</tr>
</tbody>
</table>

III. THERMOMETRY AND FEEDBACK CONTROL

The temperature of the shields is feedback-controlled by precisely measuring their temperature using high-resolution resistance thermometry, followed by an active proportional–integral–differential (PID) feedback scheme. This approach helps reduce low-frequency temperature fluctuations and results in improved long-term temperature stability as quantified below. Each shield is integrated with two sets of resistance (PID) feedback schemes. These schemes independently monitor the temperature stability of each shield.

An AC-driven Wheatstone bridge configuration is used for the resistance thermometry [Fig. 3(a)]. Furthermore, the sensing and the matching capillary tubes also feature AC-driven Wheatstone bridge circuits for temperature sensing. We note that AC-modulation helps eliminate drift in the sensor circuits due to thermally induced sources ($1/f$ noise) and other DC offsets. The sensing thermistors [labeled $R_{\text{sensing}}$ in Fig. 3(a)] have a resistance of $10 \text{ kΩ}$ ($\pm 1\%$) at $25^\circ$C. The sensing thermistors used in the shields were obtained from US Sensor Corp. (USP 12838), while the miniature thermistors used in the sensing and matching capillary tubes were obtained from Ametherm NTC (SM06103395). The former has a temperature coefficient of resistance (TCR $\text{ranging}$) of $\sim 4.4/\text{K}$, while the latter has a TCR $\text{ranging}$ of $\sim 3.95/\text{K}$. Furthermore, we employed $10 \text{ kΩ}$ resistors ($R_{\text{matching}}$) in the matching legs of the Wheatstone bridge circuits to balance the bridge. The upper half of the bridge consists of $40 \text{ kΩ}$ resistors ($R_{\text{upper}}$) on either branch with an additional potentiometer ($R_{\text{mat, pot}}$) on the left (matching) branch for fine balancing of the bridge. A $40 \text{ kΩ}$ resistance is chosen for the upper branches to minimize the effects of voltage source fluctuations on the sensing thermistor. All fixed resistors used in the Wheatstone bridges are high precision resistors with ultra-low temperature coefficient of resistance (Vishay Z201 Series Z-Foil resistors, $\pm 0.2 \text{ ppm/K}$).

The bridge is excited with a sinusoidal voltage ($V_{\text{in}}$, 1–3 V peak-to-peak) using a waveform function generator (Agilent 33120A) at frequencies in a range of $10$–$50 \text{ Hz}$ (a different frequency was used for each circuit to prevent signal cross-coupling). The signals from the sensing and the matching (right and left) branches of the Wheatstone bridges are fed into an instrumentation amplifier (Analog Devices AD524) to perform common-mode cancellation, reducing the contribution of thermal drifts in the sensed temperature signal. The differential signal is then amplified (Gain = $100$, Gain drift $< 25 \text{ ppm/K}$) and measured (see below). By analyzing the circuit in Fig. 3(a), the voltage output ($\Delta V_{\text{out}}$) can be related to the change in the resistance ($\Delta R_{\text{sensing}}$) of the sensing resistor by

$$
\Delta V_{\text{out}} = V_{\text{in}} \times \text{Gain} \times \left( \frac{R_{\text{sensing}} + \Delta R_{\text{sensing}}}{R_{\text{upper}} + R_{\text{sensing}} + \Delta R_{\text{sensing}}} \right) \left( \frac{R_{\text{matching}}}{R_{\text{matching}} + R_{\text{mat, pot}}} \right).
$$

Furthermore, the temperature change ($\Delta T$) in the shields and the capillaries can be obtained from the measured $\Delta V_{\text{out}}$ by

$$
\Delta T = \left( \frac{\Delta V_{\text{out}}}{V_{\text{in}} \times \text{Gain}} \right) \times R_{\text{sensing}} \times \text{TCR}_{\text{sensing}}.
$$

The $\Delta V_{\text{out}}$ from the sensors employed in the feedback loops of the shield is measured using a lock-in amplifier (SR 530, Stanford Research Systems) and recorded using a PCI-6014 data acquisition card (NI) in the LabVIEW environment at a 1 Hz sampling rate. The measured voltage output is converted into a temperature change using Eq. (2) and fed into a PID-algorithm, which controls the current supplied to the polyimide heaters (Omega KH series) attached uniformly over the outer surfaces of the OS and the IS [see Fig. 1(a)].
FIG. 3. (a) Circuit diagram for the AC-driven Wheatstone bridge circuits used for high-resolution sensing of the temperature changes in the shields and the capillary tubes. Resistance thermometry is implemented using high TCR (temperature coefficient of resistance) thermistors as sensors. The resistance of the sensor (\(R_{\text{sensing}}\)) changes due to temperature changes, leading to an AC voltage difference at the output of the bridge, which is amplified using an instrumentation amplifier (Analog Devices, AD 524) before being recorded. (b) Diagram of the circuit employed to supply currents to the heaters for feedback-control of the shield temperatures. The voltage control has two inputs: a manual input (\(V_{\text{offset}}\)) to provide for a large heating offset and a PID input (\(V_{\text{PID}}\)) to precisely maintain the shield temperature at the desired set point. (c) Schematic description of the PID-based feedback control loop implemented to stabilize the temperature of the two shields. The output from the temperature sensing circuits (\(V_{\text{sens}}\)) provides feedback to the PID controller to modulate the voltage (\(V_{\text{PID}}\)) supplied to the heater circuits [shown in (b)], which, in turn, controls the heating power provided to the shields and maintain the shields at the desired temperature set points.

We note that uniform heating is crucial to avoid temperature gradients in the shields.

In contrast to the temperature sensors for feedback control, output from all other temperature sensors (independent sensing circuits of the shields and the circuits of the sensing and the matching capillary tubes) was recorded using a NI USB-4431 data acquisition card in the LabVIEW environment at a 1000 Hz sampling rate. Subsequently, the desired signal was obtained via a Fast-Fourier transform analysis of the acquired data to retrieve the signal at the modulation frequency.

IV. PERFORMANCE CHARACTERIZATION AND DISCUSSION

We conducted measurements to quantify the improvement in temperature stability that can be accomplished via the measures described above. We performed experiments in a temperature-stabilized room where the temperature set point was chosen between 20 and 22 °C and the temperature drift was within \(\pm 0.2\) K over 10 h.
The entire microfluidic system including the sensing capillary was filled with water during the measurements to mimic the conditions under which biocalorimetric measurements are usually performed. Below, we describe our experiments to quantify: (1) the temperature stability of the system accomplished passively (i.e., without feedback control and vacuum conditions), (2) the micro-kelvin ($\mu$K) temperature stability accomplished with feedback control and vacuum conditions, and (3) the noise sources that currently limit the achievable temperature stability.

A. Thermostat performance without active feedback control

First, we performed experiments to identify the temperature stability of the shields and the capillary tube in the absence of both active feedback control and a vacuum in the outer shield. From the model presented in Fig. 2, one expects that in these measurements the temperature fluctuations in the thermostat are substantially attenuated when compared to the ambient as the thermal resistance and capacitance network acts as a low pass filter. The results of this experiment are shown in Fig. 4. Specifically, Fig. 4(a) shows the temperature drift of the room, which is $\sim 100$ mK over a 10-h period. Next, Fig. 4(b) shows the measured amplitude of temperature fluctuations of the OS and the IS in the same 10-h period. Then, Fig. 4(c) shows the measured temperature fluctuations of the sensing and the matching capillary tubes, while Fig. 4(d) shows the differential capillary signal (obtained by subtracting the temperature drift in the matching capillary tube from the temperature drift in the sensing capillary tube) over the same 10-h period and measured in a 10 mHz bandwidth (obtained by performing FFT on the raw data with bins of 100 s).

We performed three 10 h-long measurements (including the measurement described in Fig. 4) and report here the amplitude of the fluctuations with the mean and the standard deviation of this value in the three measurements. From these measurements, we concluded that the amplitude of fluctuations in the ambient temperature was $\pm 101$ mK with a standard deviation of 9.1 mK, while the fluctuations in the temperature of the OS was $\pm 8.1$ mK with a standard deviation of 2.3 mK, and that of the IS was $\pm 4.9$ mK with a standard deviation of 0.13 mK. The temperature oscillations of the OS and the IS show similar trends (albeit attenuated) and are correlated with the fluctuations seen in the ambient temperature [Figs. 4(a) and 4(b)]. The transient sharp rise and decline in the temperatures of the OS and the IS around the $\sim$ fifth hour is triggered by a sudden perturbation in the ambient temperature. Furthermore, as expected qualitatively from the model shown in Fig. 2, the OS is more susceptible to the changes in the ambient temperature as compared to the IS as the IS is isolated from the ambient and the OS through careful thermal design, including the use of weak thermal links.

Our measurements also revealed that the mean thermal stability of the sensing capillary was $\pm 5.2$ mK with a standard deviation of 0.55 mK, while the mean thermal stability of the matching capillary was $\pm 4.9$ mK with a standard deviation of 0.59 mK. As expected, since the sensing and the matching capillaries are both in excellent thermal contact with the IS, their temperature fluctuations follow...
each other closely throughout the 10-h period [Fig. 4(c)] revealing a large common mode signal. The oscillations in the sensing capillary are slightly higher than the matching capillary, likely because the sensing capillary tube is conductively coupled to the external environment through water and the microfluidic feedthroughs connecting it to the external reservoir. The differential capillary signal had a much-improved thermal stability of ±0.29 mK with a standard deviation of 0.079 mK. These data show that, under the passive temperature control conditions described here, the use of two identical capillary tubes and a common-mode cancellation approach significantly reduces the impact of large ambient thermal drifts.

B. Thermostat performance with active feedback control

Next, we explored how the temperature stability can be improved by employing feedback-control as well as the introduction of a vacuum in the OS to improve the thermal isolation between the OS, the IS, and the capillary tubes. In these measurements, the temperature set points of the OS and the IS were maintained to be ∼1 and ∼1.25 K higher than the room temperature, respectively. The temperature set point of the shields was chosen to be ∼1 K above room temperature as the drift in the room temperature was ∼5 times smaller (∼0.2 K), enabling the control of the shield temperature by simply controlling the joule heating in the shields. We also note that the temperature of the IS was set close to that of the OS to avoid significant thermal gradients between the shields and along the capillary tubes. Furthermore, a vacuum level of <10 μTorr was maintained in the OS.

In Fig. 5, we show representative results for the measured temperature drift of the ambient [Fig. 5(a)], the OS [Fig. 5(b)], the IS [Fig. 5(c)], the sensing [Fig. 5(d)] and the matching [Fig. 5(e)] capillary tubes, and the differential capillary signal [Fig. 5(f)] over a period of 10 h, measured at both 1 and 10 mHz bandwidths. We
also incorporate data in 1 mHz bandwidths (measured by further performing a 10 s moving average on the 10 mHz data) as such data are relevant for long-term measurements performed, for example, over a period of several hours to a day. Here, we report the actual temperature fluctuations separately in the two different bandwidths rather than the temperature fluctuations normalized over the frequency bandwidth as the noise is strongly dependent on frequency at low frequencies. In total, we performed six 10 h-long measurements including the measurement shown in Fig. 5 to characterize and demonstrate the thermal stability performance of the developed system. From these measurements, we concluded that the drift in the ambient temperature was ±132 μK (1 mHz) with a standard deviation of 18.9 μK, while the mean thermal stability of the OS was ±390 μK (1 mHz) and ±460 μK (10 mHz) with a standard deviation of 48 μK (1 mHz) and 42 μK (10 mHz). The mean thermal stability of the IS was ±0.85 μK (1 mHz) and ±1.85 μK (10 mHz) with a standard deviation of 0.13 μK (1 mHz) and 0.2 μK (10 mHz). The mean thermal stability of the sensing capillary was ±2.6 μK (1 mHz) and ±5.2 μK (10 mHz) with a standard deviation of 0.68 μK (1 mHz) and 0.89 μK (10 mHz), while the mean thermal stability of the matching capillary was ±20 μK (1 mHz) and ±5.3 μK (10 mHz) with a standard deviation of 0.66 μK (1 mHz) and 0.69 μK (10 mHz). The differential capillary signal had a mean thermal stability of ±2.4 μK (1 mHz) and ±0.3 μK (10 mHz) with a standard deviation of 0.38 μK (1 mHz) and 0.70 μK (10 mHz).

These data unambiguously show that by combining active feedback control with the nested shield design in the presence of μTorr levels of vacuum conditions, the impact of ambient fluctuations can be attenuated by ~105 dB. These data demonstrate the remarkable capability of the developed thermostat to maintain a very high level of thermal stability over an extended period. We note that the effect of ambient temperature fluctuations was attenuated by ~52 dB in the OS due to the large thermal capacitance of the copper shield and the PID-based feedback control of its temperature. The temperature fluctuations in the IS were attenuated further by ~53 dB compared to the OS. This attenuation is attributed to the presence of both feedback-control and vacuum conditions that serve to thermally decouple the IS and the OS. The sensing and the matching capillary tubes have comparable thermal stabilities, while, on average, the matching capillary is slightly more stable than the sensing capillary. This is because the sensing capillary is filled with water and is conductively connected to the external environment through microfluidic feedthroughs. Nonetheless, the room temperature fluctuations in the sensing capillary tube and the differential capillary signal are attenuated by ~90–95 dB in the above measurements due to the exceptional capability of the two-shield system developed here to isolate the capillary tubes from the room temperature fluctuations and the excellent thermal contact of the capillaries to the temperature-controlled IS.

We note that the thermal stability of the differential signal was only slightly improved as compared to the sensing capillary tube’s thermal stability in a 1 mHz bandwidth, while it was slightly worse in the 10 mHz bandwidth. This is likely because common-mode cancellation is more effective in canceling out low-frequency thermal drifts. Furthermore, given the fact that, under these conditions, the sensing and matching capillary tubes are already very stable, the common-mode cancellation has only a limited impact on temperature drift. This observation is in strong contrast to the observation that the differential signal features a ~20-fold improvement in stability in the absence of vacuum and feedback control as discussed in Sec. IV A. These data indicate that common-mode cancellation is likely of great importance only in the presence of large ambient thermal drifts, which are prevalent during long-term measurements over a day or more (necessary for certain studies) or in the absence of feedback control.

Interestingly, we find that while the thermal stability of the capillary temperature signal is very high (~< ±3 μK in 10 h as discussed above), it is lower than the stability of the IS (~< ±1 μK in 10 h). This is because the sensing capillary tube has a relatively small thermal mass and is susceptible to additional perturbations through thermal pathways that do not directly affect the IS, as suggested by the model shown in Fig. 2. For example, the capillary tubes are designed to be optically accessible through the optical window integrated into the OS (essential for using this system in biological applications) and, hence, are coupled to the ambient and experience perturbations through radiation via the optical window. Furthermore, the sensing capillary tube is connected to an external reservoir via microfluidic feedthroughs with water in it, which acts as a thermally conductive pathway between the ambient and the capillary tube sensor.

While the above measurements were performed without a biological sample, the thermal stability of the system is not affected due to the presence of a small biological organism, sample, or a cell in the sensing capillary tube because such a sample will not significantly change the thermal conductance of the capillary tube sensor. Furthermore, in previously published work, we clearly established that the capillary tubes, such as those used in this work, are fully biocompatible. Therefore, the system presented here is expected to measure true metabolic rates from novel biological samples within its resolution limits.

### C. Effect of electronic drift on the thermal stability performance

Following the characterization of the thermal stability of the system, measurements were performed to characterize the stability of the electronics used and their effect on the thermal stability of the system. Electronic components used in the Wheatstone bridge temperature sensing circuits, such as the Vishay Z201 Series Z-Foil precision resistors as well as the potentiometers and the instrumentation amplifier, tend to drift due to fluctuations in the room temperature. As mentioned in Sec. III B, the Vishay Z201 Series Z-Foil precision resistors used in the circuits drift up to ±0.2 ppm/K, the Vishay Spectrol 534 series potentiometers drift up to ±20 ppm/K, and the gain (set as 100) of the AD524 instrumentation amplifier drifts up to 25 ppm/K. We note that, in our experiments, these circuits are placed in a room that is stable within ±200 mK over 10 h. The electronic components have a tendency to heat up while in use. For example, the AD 524 instrumentation amplifier has a power dissipation of ~450 mW (at ±15 V) and can heat up significantly above room temperature. As the temperature of the electronic components drifts, their resistances and gains do change, leading to noise and drift in the measured signal. To quantify the impact of the electronic noise, we performed an experiment where the sensing thermistor was replaced with a fixed precision resistor. The data from this experiment is shown in Fig. 6(a). As can be seen, the noise equivalent voltage drift in this experiment is ±5.4 nV, while the corresponding noise equivalent temperature drift is ±0.55 μK over 10 h.
FIG. 6. Characterization of the noise and drift associated with electronics. (a) The drift in the output voltage and the corresponding temperature change of an AC-driven Wheatstone bridge circuit used for the temperature sensing of the shields and the capillaries. The measurement was performed using a precision resistor in place of the temperature sensing thermistor, over a period of 10 h. The drift in the output voltage is likely caused due to thermal drift in the electronic components used in the circuits, such as the drift in the gain of the instrumentation amplifier (AD 524), the potentiometer resistance, as well as the resistance of the precision resistors, and also due to drifts in the excitation voltage. (b) The drift in the voltage excitation source (Agilent 33120A) used to excite the AC-driven Wheatstone bridge circuits, measured over a period of 10 h. All measurements are shown in bandwidths of 1 mHz as well as 10 mHz. The drift in the output of the Wheatstone bridge circuits is ±0.55 μK, measured as temperature fluctuations about a set point, which is of the same order of magnitude as the temperature drifts in the IS and the capillary tubes, showing that the electronic circuit drifts are a major factor limiting the thermal stability of the system.

V. CONCLUSION

We present the design and implementation of a high-temperature stability thermostat that is suitable for sensitive biocalorimetric applications. The developed system offers a thermal stability of ±1 μK in the inner thermal shield and ±3 μK in the capillary tube that is designed to be used for biocalorimetry, as measured in a 1 mHz bandwidth over a period of 10 h. This stability is achieved through careful thermal engineering of nested shields, the introduction of vacuum conditions, and the implementation of high-resolution thermometry and temperature feedback control, which enabled attenuation of the temperature fluctuations of the inner shield by over 100 dB relative to the ambient. Moreover, temperature fluctuations of the differential capillary sensor relative to the ambient attenuated to a similar level, reaching ~95 dB. We note that the presented thermostat also has a channel connected to an external reservoir that can be used to load and unload biological samples, as well as to maintain a steady supply of a physiological buffer medium carrying nutrients and oxygen to the sample. Furthermore, it features an optical window for visual access with a high-resolution light microscope. The excellent long-term thermal stability of the system along with the presence of the above-mentioned features is likely to make this system a practical tool for sensitive biological calorimetry and thermometry applications on model organisms and cells.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

K.P. and R.M. contributed equally to this paper.
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DATA AVAILABILITY

The data that support the findings of this study are available within the article.

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