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Temperature regulation during ultrasonic manipulation for long-term cell handling in a microfluidic chip

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Abstract

We demonstrate simultaneous micromanipulation and temperature regulation by the use of ultrasonic standing wave technology in a microfluidic chip. The system is based on a microfabricated silicon structure sandwiched between two glass layers, and an external ultrasonic transducer using a refractive wedge placed on top of the chip for efficient coupling of ultrasound into the microchannel. The chip is fully transparent and compatible with any kind of high-resolution optical microscopy. The temperature regulation method uses calibration data of the temperature increase due to the ultrasonic actuation for determining the temperature of the surrounding air and microscope table, controlled by a warm-air heating unit and a heatable mounting frame. The heating methods are independent of each other, resulting in a flexible choice of ultrasonic actuation voltage and flow rate for different cell and particle manipulation purposes. Our results indicate that it is possible to perform stable temperature regulation with an accuracy of the order of ± 0.1 °C around any physiologically relevant temperature (e.g., 37 °C) with high temporal stability and repeatability. The purpose is to use ultrasound for long-term cell and/or particle handling in a microfluidic chip while controlling and maintaining the biocompatibility of the system.

1. Introduction

Ultrasonic standing wave (USW) technology is a suitable tool for gentle manipulation of cells and other bioparticles in various biotechnology applications [1]. In macro-scaled systems (with typical active volumes >100 μl), cells can be handled by MHz-frequency ultrasound on the time scale of days to months without any detectable damage or loss in viability [2], and without any cavitation or acoustic-streaming-generated shear forces of significance from the surrounding fluid [3]. However, when downscaling the system dimensions the acoustic energy is deposited into smaller volumes, which may lead to an increase in temperature that is not compatible with careful cell-biological experiments. In the present paper, we use ultrasound for simultaneous manipulation of particles and temperature regulation in a silicon-based microfluidic chip for long-term gentle handling of sub-μl-volume cell samples.

Several cell-based applications in biotechnology are dependent on the development of non-intrusive tools for manipulation of cells or other bioparticles in microfluidic chips. In comparison to available techniques for contactless manipulation in miniaturized systems (e.g., dielectrophoresis [4] and optical tweezers [5]), USW technology is a promising alternative for such long-term cell handling in terms of flexibility, cost-effectiveness and gentleness [6, 7]. In an USW, suspended particles are driven to the pressure nodes found in half-wavelength intervals, due to the primary acoustic radiation force, which is proportional to the particle volume, sound frequency and pressure amplitude squared. The nodes are formed at specific frequencies fulfilling the resonance condition of the cavity hosting the standing wave [8]. We have previously shown that ultrasound may be used for retention of cells during perfusion in a relatively small ($\sim$30 μl) PDMS-based chip for more than 1 h without any losses in cell viability

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[1]. However, when downscaling the system size even further, e.g., by the use of a microfabricated silicon chip with sub-μl-volume fluidic channels, a temperature increase in the active fluid volume cannot be avoided. Furthermore, since the fluid channel has a high surface-to-volume ratio and only constitutes ∼2% of the highly heat-conductive silicon structure, it is difficult to regulate the fluid temperature externally (e.g., by regulating the fluid temperature outside the channel inlet of the chip). Therefore, the temperature in the chip itself must be regulated in order to retain a biocompatible environment.

In macro-scaled USW systems for high-power particle manipulation, the temperature is most often controlled by a loop of cooling water close to the active chamber [9, 10]. Such a cooling system is not needed for low-power acoustic particle manipulation given that the sample volume is large enough (typically >100 μl) [3]. As an example, the estimated temperature increase in the latter system is at most 0.5 °C during 30 min of operation at 0.54 MPa pressure amplitude. In silicon-chip-based USW applications, little effort has been made to investigate the temperature development. A possible reason is that most of the reported USW applications in such systems aim for high-throughput continuous separation of particles or cells [11, 12]. Thus, particles are only manipulated for a few seconds, making temperature control less important in terms of biocompatibility. However, in USW applications aiming for long-term manipulation, retention and cultivation of cells in microfluidic chips, a temperature regulation system is necessary.

In the present paper, the origin of the heat deposition is investigated and the temperature increase is measured as a function of the applied transducer voltage and manipulation time in a miniaturized USW system operated in both non-flowing and flow-through mode. The system is based on a glass-silicon-glass microfluidic chip and a transducer with a refractive element for oblique coupling of ultrasound into the fluid channel, which has been described elsewhere [6]. The developed transducer-chip system is compatible with any kind of high-resolution optical microscopy and condenser illumination, suitable for detailed characterization of individual cells. We demonstrate a novel regulation procedure where temperature-versus-voltage calibration data can be used as feedback to the external temperature control systems (in our case a warm-air heating unit and a heatable mounting frame) for defining the temperature of the media in contact with the transducer-chip assembly. By the use of on-line temperature monitoring, we demonstrate regulation around an arbitrary physiological temperature with a long-term stability below ±0.1 °C, independently on both transducer actuation voltage and flow rate.

2. Experimental arrangements

The temperature measurements were performed with a micro thermocouple inserted into the fluid channel of the chip. The chip (GeSim, Dresden, Germany) and its channel structure are illustrated in figure 1(*a*). The layer dimensions of the chip were 200/250/1000 μm (bottom glass, silicon, upper glass), respectively. The channel width of 375 μm was close to half the wavelength at the employed ultrasonic frequency of 2.0 MHz. The chip was excited using an obliquely coupled ultrasonic transducer (depicted in figure 1(*b*)) consisting of a piezoceramic plate (PZ-26, Ferroperm, Denmark) attached by conductive glue to an aluminum wedge with an angle, θ_i,

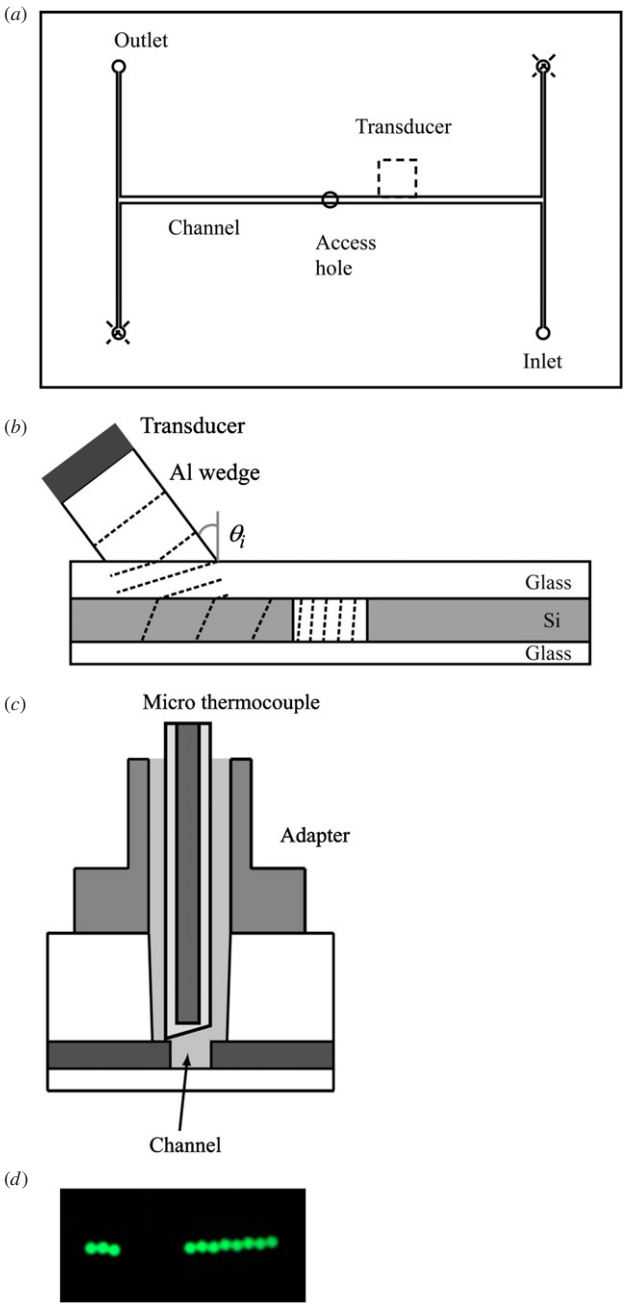

Figure 1. (*a*) Top-view of the chip showing the channel design, access hole and transducer position. (*b*) Cross section of the chip showing the obliquely coupled USW transducer (not to scale). (*c*) Cross section of the chip showing the T type (copper-constantan) and Teflon-insulated micro thermocouple with a total tip diameter (sensor and sheath layer) of 0.41 mm. The micro thermocouple was threaded down into the fluidic channel through the access hole (diameter ∼0.70 mm) in the upper glass layer approximately 3 mm downstream from the transducer, thus measuring the local temperature of the fluid inside the microchannel. (*d*) One-dimensional USW manipulation and aggregation of 10 μm beads at the resonance frequency of 2.0 MHz.

(This figure is in colour only in the electronic version)

of 48° relative to the surface normal, in order to efficiently couple a horizontal standing wave into the fluid channel via wave refraction (cf dotted lines in figure 1(*b*)). The transducer was attached on top of the chip by a quick-drying and water-soluble electrode gel (Parker Laboratories, USA) and driven by a function generator (Stanford DS345, Stanford, USA) coupled to a RF amplifier (75A250, Amplifier Research, USA). The fluidic system connected to the channel consisted of adapters, valves and Teflon tubing. The flow rate in the chip was controlled by a syringe pump and the chip-transducer system was mounted on an inverted fluorescence microscope (Axiovert 135, Zeiss, Germany). 10 μm green-fluorescent polystyrene beads (Bangs Laboratories, USA) diluted in water (10^6 beads per ml) were used as a cell model (i.e., having similar size and acoustic properties) in the present experiments simulating long-term manipulation of cells.

The absolute temperature measurements were performed with a T-type (copper-constantan) and Teflon-insulated micro thermocouple with a total tip diameter (sensor and sheath layer) of 0.41 mm (IT-21, Physitemp Instruments, USA). The micro thermocouple was threaded down into the fluidic channel through an access hole (diameter ∼0.70 mm) in the upper glass layer approximately 3 mm downstream from the transducer, thus measuring the local temperature of the fluid inside the microchannel (cf figure 1(*c*)). The ambient temperature outside the chip was measured by a second non-insulated micro thermocouple (MT-4, Physitemp Instruments, USA) with a tip diameter of 0.31 mm. Real-time automatic monitoring of temperature data with an accuracy of ±0.2 °C was performed using a thermo control unit (P655-LOG, Dostmann electronic, Germany) connected to a standard PC where the data were stored and further processed using MS Excel and Matlab software. Continuous measurements of both internal channel temperature and external room temperature were performed during all experiments. In this way, any fluctuations in room temperature could be taken into account. Finally, in the experiments for 37 °C regulation of the microchannel temperature, a warm-air heating unit with temperature control (Tempcontrol 37-2, Zeiss, Germany) was used to connect to an in-house built plastic hood placed on top of the microscope stage combined with a heatable mounting frame (Heatable mounting frame K-H, Zeiss, Germany) in direct contact with the chip. For practical reasons, the hood did not cover the syringe pump and the fluidic components.

In order to establish stable one-dimensional USW manipulation and aggregation of beads, the transducer was first positioned and aligned. Thereafter, the frequency was tuned into resonance by observing when stable and rapid bead aggregation occurred without flow at a transducer voltage of 10 V_{pp} (cf figure 1(*d*)). This optimum frequency was 2.00 MHz, and was not changed during the experiments. Then, the ultrasound was turned off in order to let the chip reach thermal equilibrium with its surroundings before starting any measurement. The initial channel temperature without ultrasound was logged, followed by near-continuous (2 min sample) temperature measurements during 60 min for the applied transducer voltage. The temperature measurement series started at 1 V_{pp} and was thereafter repeated for stepwise increasing voltages up to a maximum of 25 V_{pp}. During the long-term experiments, the manipulation robustness in the chip was visually checked in the beginning and at the end of the period, in order to verify that all beads in the aggregates were retained and the aggregates were kept in their initial positions during the whole measurement.

To investigate possible sources of energy deposition, the surface temperatures of both the PZT element on the transducer, and of the chip surface were measured by attaching two microthermocouples to the PZT element on the transducer and the upper chip surface, respectively, using a thermoconducting gel. The chip surface temperature was recorded at four different positions: close to the transducer (approximately 2–3 mm away) and 1 cm away in two different and orthogonal directions. The measurements were performed at a resonance frequency of 2.00 MHz (with 10 V_{pp} applied voltage) using both the previously described transducer with an aluminum wedge and a similar transducer but with a PMMA (Polymethyl methacrylate) wedge.

3. Results

Several experimental parameters might influence the channel temperature such as the USW exposure time, the applied transducer voltage, the flow rate and the surrounding temperature. Four experiments were performed to evaluate the effects of each of these parameters.

In the first experiment, the temperature stability and repeatability during 12 h of particle manipulation at a constant transducer voltage of 10 V_{pp} without any flow were evaluated. This voltage is typically the maximum level needed for stable manipulation of cells in medium-ranged flows (typically a few tens of μl min^{-1}) [6, 7] and, thus, an approximate value of the upper voltage level in a future long-term cell handling application. The experiment was performed with the external heating system consisting of the sealed warm-air heating unit (above the chip) combined with the heatable mounting frame (in contact with the chip). Figure 2 shows the channel temperature from three independent measurements, starting with an ambient (externally regulated) temperature close to 36 °C. Typically, the fluid temperature inside the microchannel stabilized at a level ∼0.5–1 °C below the ambient temperature, possibly due to a small non-vanishing thermal gradient across the chip. As indicated in the diagram, for each of the three measurements the temperature rises during the first few minutes, and then remains constant with averages and standard deviations of 36.66 ± 0.05 °C, 36.20 ± 0.08 °C and 36.12 ± 0.07 °C for the whole period, respectively. The repeatability can be quantified by the average and standard deviation of the data from all three measurements, resulting in 36.3 ± 0.3 °C.

In a control experiment without any temperature regulation the temperature in the channel was measured after one hour of sound application for a total of 14 voltage steps applied to the transducer ranging from 0 to 25 V_{pp}. The experiment, presented in figure 3, was performed without flow and with an ambient (non-regulated) room temperature close to 21 °C. Here, we first note that there is an immediate increase (a 'bias') in temperature when the transducer is turned on at 1 V_{pp}, which indicates that the mechanism of heat deposition in the fluid channel is of a more complex nature. However, the temperature dependence to the applied voltage above 1 V_{pp} is near quadratic; for example, if a 'biased' power series

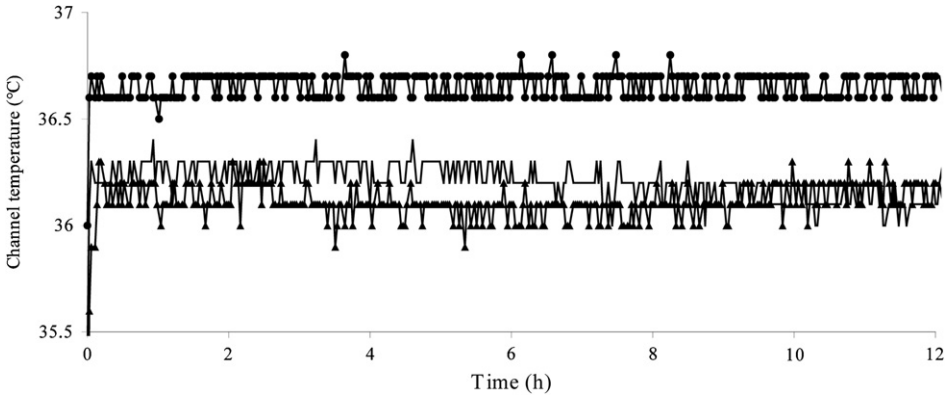

Figure 2. The temperature stability during 12 h of particle manipulation is shown in three experiments at a constant transducer voltage of 10 V_{pp} without any flow and with an ambient temperature of 36 °C.

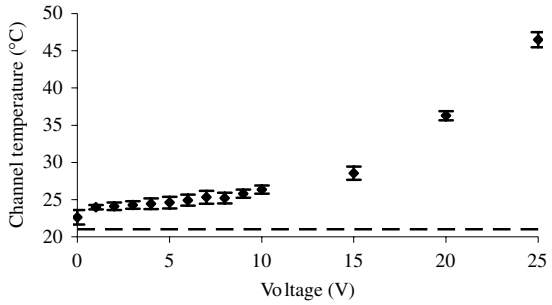

Figure 3. The channel temperature measured as a function of an applied transducer voltage ranging from 0 to 25 V_{pp} for an ambient temperature close to 21 °C (indicated by the dotted line). The temperature data are averaged from the three measurements at 2.0 MHz and the error bars correspond to two standard deviations.

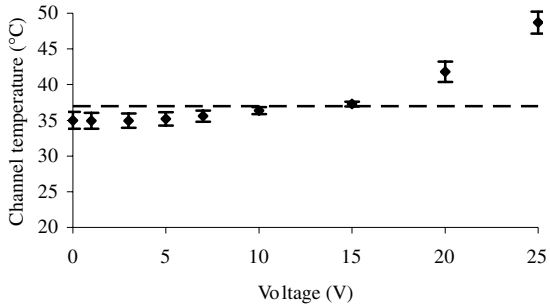

Figure 4. The channel temperature measured as a function of an applied transducer voltage ranging from 0 to 25 V_{pp} for an ambient temperature close to 36 °C using the external heating system. The temperature data are averaged from three measurements and the error bars correspond to two standard deviations. The dotted line indicates the physiologically important temperature 37 °C.

of type $T = aU^b + c$ (where T is the temperature increase, U is the applied voltage and a, b, c are constants) is fitted to the measured data, we obtain $b \approx 2.04$ for the bias level $c = 2.75$ °C.

In order to investigate the possibilities to regulate the channel temperature around an arbitrary physiological temperature (e.g., 37 °C), we performed a similar experiment as presented in figure 3 but now combined with external heating of the chip surroundings (sealed warm-air system and heatable mounting frame system). The results are presented in figure 4, where the initial external heating was adjusted to 36 °C prior to ultrasonic actuation. In contrast to the experiment without external heating (cf figure 3), we note that there is almost no 'bias' level of the temperature curve in figure 4. Here, a similar power series fit gives a power $b \approx 2.31$.

It is also of interest to investigate to what extent the flow rate influences the channel temperature. Therefore, measurements were performed at different flow rates ranging from 0 to 500 μl min^{-1} with a constant transducer voltage (7 V_{pp}) and with no external heating (ambient temperature close to 21 °C). The results are presented in figure 5. As can be seen, there is no significant change in temperature at different flow rates, although a minor decrease can be noted at the highest measured flow rate (500 μl min^{-1}, corresponding to a mean flow velocity ~90 mm s^{-1}).

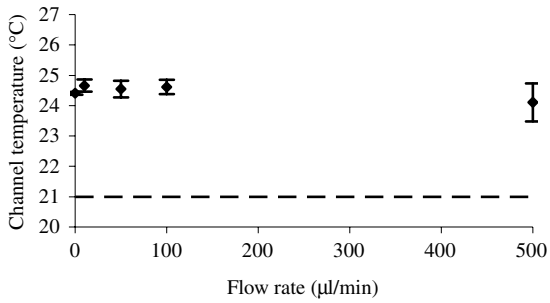

Figure 5. The effect on the channel temperature from the flow rate investigated at a transducer voltage of 7 V and with an ambient temperature of 21 °C (indicated by the dotted line). The data are averaged from the three measurements and the error bars correspond to two standard deviations.

Finally, figure 6 presents the absolute increase in channel temperature at three different levels of external heating when the transducer is turned on at constant voltage amplitude of 10 V_{pp}. The investigated external heating levels were ~22 °C (no heating) and 32 °C and 36 °C (by the use of the sealed warm-air system and the heatable mounting frame system). Here, for the chosen voltage amplitude 10 V_{pp}, the temperature increase is approximately 1–1.5 °C for all three initial temperatures (reached within a few minutes and then remains stable).

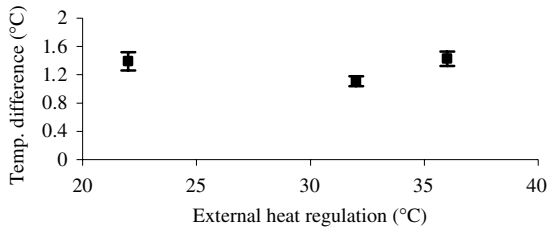

Figure 6. The absolute increase in channel temperature evaluated at three different levels of external heating when the transducer is turned on at a constant voltage amplitude of 10 V_{pp}.

4. Discussion and conclusion

In this section, we will consider temperature regulation strategies for use with ultrasonic actuation for standing-wave manipulation in a microfluidic chip. The purpose is to use ultrasound for long-term cell and/or particle handling in a microfluidic chip while controlling and maintaining the biocompatibility of the system. In addition, the origin of the ultrasound-generated heat is investigated and discussed.

As seen in figure 2, the standard deviation for each of the three measurements over the whole time period of 12 h is less than 0.1 °C. This deviation is actually less than the accuracy of the thermocouple probe (±0.2 °C). Furthermore, the accuracy of the thermocouple is also comparable to the standard deviation of the data from all three measurements in figure 2 (0.3 °C), indicating the accuracy in terms of repeatability. However, considering all available experimental data (including the measurements at different voltages and flow rates), the total accuracy is within ±1 °C, especially for low or high actuation voltages (cf figure 4).

The temperature increase due to the applied ultrasound is at most a few °C for the actuation voltages needed in cell manipulation applications (<10 V_{pp}). Typically, the threshold for manipulation of biological cells is 2–3 V_{pp} in similar chip structures (with slightly varying channel layouts) [6, 7]. Thus, the voltage interval 2–10 V_{pp} results in acoustic forces of sufficient magnitude for retention of cells in chips operated in flow-through mode at medium flow rates (∼5–10 μl min^{-1}). For such flow rates, we also note that the temperature is independent on the flow (cf figure 5). Overall, the temperature increase has a near quadratic relationship to the applied transducer voltage (and, thus, to the acoustic pressure).

To investigate possible sources of energy deposition, the surface temperatures of both the PZT element on the transducer, and of various positions on the upper chip surface were measured, as described in the experimental arrangement section. Starting with an initial temperature of 21.0 °C, the PZT element on the Al-wedge transducer reaches a surface temperature of 26.1 °C compared to 32.1 °C for the PMMA-wedge transducer. However, for both transducers the temperature of the upper surface of the chip stabilizes at 24.4 ± 0.3 °C close to the wedge, and thereafter decreases with increasing distance (typically 0.08 ± 0.02 °C/mm). Considering these data, we conclude that the major source of heat deposition into the fluid channel is due to the electromechanical losses in the PZT element (k_{33} = 0.68 for the PZT elements). Other possible sources, such as absorption of sound in the different layers in the chip, seem to be of less significance. For example, the higher PZT-element temperature of the PMMA-wedge transducer can be explained by the lower thermal conductivity of PMMA (∼0.17–0.19 W m^{-1} K at room temperature), compared with aluminum (235 W m^{-1} K at room temperature). Thus, less heat is transported away from the PZT element through the wedge down to the chip. Furthermore, the greater sound absorption in PMMA (relative to the absorption in aluminum) seems to be of minor importance.

In order to incorporate the ultrasonic actuation in a temperature regulation system and take into account the benefits from the USW-induced heating, we suggest using the following procedure. First, the chip is calibrated by measuring the temperature response as a function of the applied transducer voltage. Then, the calibration data can be used to define the externally regulated temperature. For example, the dotted line (37 °C) in the diagram in figure 4 reveals an actuation voltage of approximately 13 V_{pp} for the external regulation temperature 36 °C. If a lower actuation voltage is needed, the externally regulated temperature can be increased in order to retain the channel temperature of 37 °C. As seen in figure 6, the temperature increase is relatively independent of the initial channel temperature (within the accuracy of the measurement), which makes it possible to predict the final temperature in the channel even when both the actuation voltage and the externally regulated temperature are changed. If the long-term standard deviations are taken into account, this procedure is suitable in applications requiring temperature regulation accuracy of the order of ±1 °C, which is sufficient for most cell biological experiments [13]. However, it is possible to obtain a regulation accuracy of ±0.1 °C by continuous monitoring of the temperature.

Besides the biological aspects, another advantage of temperature regulation is the improved maintenance of the system resonance frequency. A difficulty with the long-term USW manipulation is the drift in resonance frequency due to shifts in temperature. This can be corrected for using an electronic feedback loop, which has the drawback of increased complexity. Our described manipulation system with temperature control offers both improved biocompatibility and improved resonance frequency stability.

To conclude, we have demonstrated that ultrasound-generated heating can be combined with external heating in order to regulate the temperature in a microfluidic channel independent of the ultrasonic actuation voltage and the flow rate, and with high temporal stability and repeatability. The regulation temperature may be chosen arbitrarily within the physiologically relevant interval (e.g., from 20–37 °C). Our system is designed for ultrasonic standing wave (USW) manipulation of cells or other bio-active particles (e.g., functionalized beads) in applications requiring stable and gentle handling of cells during long terms (hours–days). In future work, we aim for handling, positioning, retention, on-chip cultivation and optical characterization of delicate animal cells in a temperature-regulated chip.

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