Supplementary Material (ESI) for Lab on a Chip

On-chip Fabrication to Add Temperature Control to a Microfluidic Solution Exchange System

Helen Bridle, Maria Millingen and Aldo Jesorka*

Department of Chemistry and Bioscience, and Microtechnology Centre, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

* Corresponding author Aldo Jesorka e-mail: aldo@chembio.chalmers.se

Phone + 46 (0)31 772 61 12

Fax + 46 (0)31 772 61 20

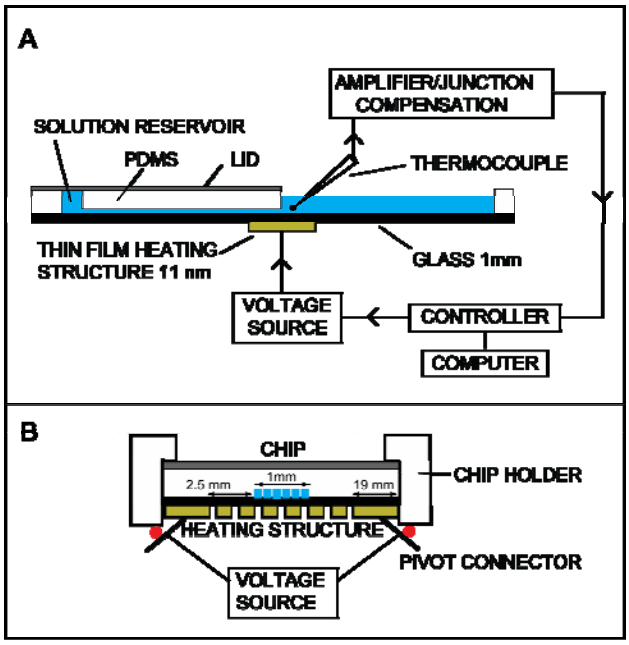
Supplementary Information

HARDWARE DESCRIPTION AND DESIGN:

Description of the microfluidic system: The system comprises 16 solution reservoirs individually connected to an open volume by microchannels. The microchannels exit as a tightly packed array. The channels have a centre-to-centre distance of 72 μm (channel width 50 and wall width 22), a height of 60 μm and are of equal length (50 mm). The open volume of the chip is filled with buffer solution so that the flows from the channels enter a solution filled bath. Flow from the solution reservoirs through the microchannels to the open volume is driven by the application of pressure to all solution reservoirs simultaneously. Flows exiting the microchannels viscously couple to form a patterned laminar flow, consisting of separate solution environments[1]. A cell, scanned outside the channel exits, is thus sequentially exposed to the different solution environments with solution exchange times of tens of ms. For this system, the solution exchange time for a 10 μm cell scanned at several mm/s has been estimated to be 10-12 ms[2]. The system could be used with many nano- or micro-sized probes but is most often used with cells since it was optimized for patch-clamp studies. Thus, we often refer to cells in the text rather than using the term probe. The microfluidic chip is

mounted in an in house built holder on an inverted microscope stage (Leica DM IRB, Wetzlar, Germany) equipped with a scanning stage. The motorised scanning stage we employed (Scan IM 120x100, Martzhauser Wetzlar GmbH & Co. KG, Wetzlar-Steindorf, Germany) has a travel distance of 120x100 mm and a maximum translation velocity of 180 mm/s.

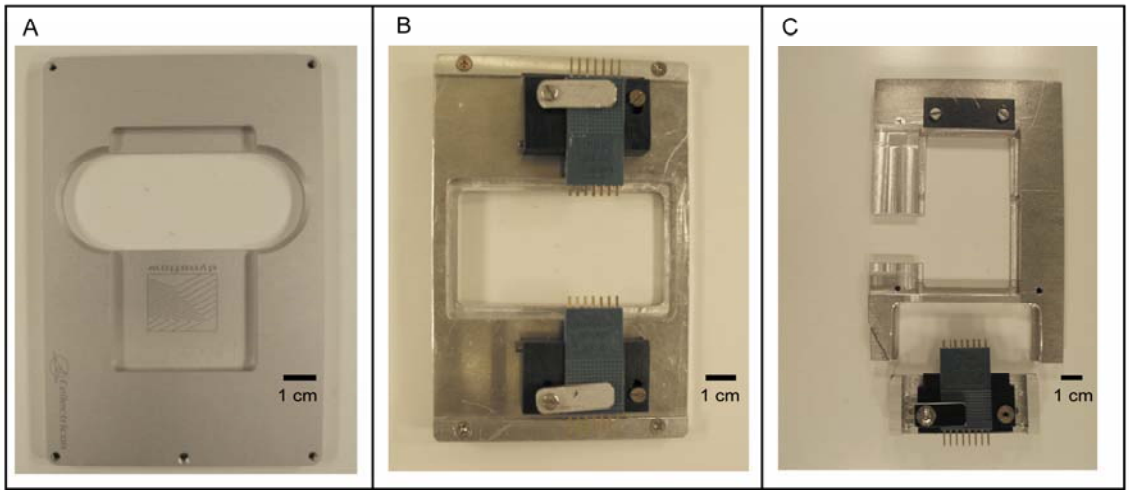
Figure S1. Schematic of the temperature system set-up.



A) A side cross section of the chip including a schematic of the set-up. The arrows indicate the direction of information flow.

B) A front cross section, at the channel outlets, of the chip placed in the holder. Not to scale.

Figure S2. Design of the microscope chip holder with electrical connectors.

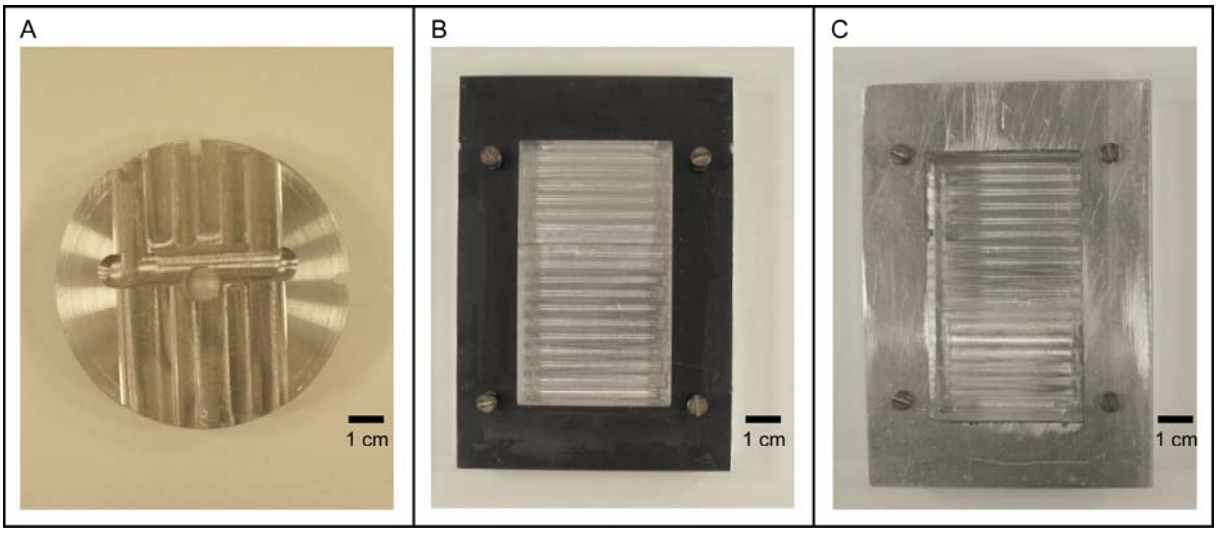


A) The commercially available Cellectricon Dynaflow® holder.

B) Bottom view of the new modified holder. The chip is mounted from underneath and clamped in place with the pivot connectors.

C) Bottom view of an alternative design of the new modified holder. The chip is mounted from underneath and clamped in place with one pivot connector protruding from the short side. This allows for the same orientation of the chip as in the original commercially available holder. The two different designs can be used on the same chip layout, since the gold strips emanating from the heating structure extend both to long sides and one of the short sides of the chip. Thus, both orientations of the chip on the microscope stage are possible, making access to the open volume more flexible.

Figure S3. Design of the equipment for microfabrication.



A) Modified holder for the UV mask aligner.

B) Enclosure for the photolithography development. The chip is placed face down, the black top screwed into place and developer added to the exposed glass of the chip. The black top covers the edges of the chip and presses gently into the PDMS between the chip edge and the glass.

C) Enclosure for the lift off. The design is the same as in B but the top is thicker since a greater volume of lift-off solution is applied.

CHARACTERISATION MEASUREMENTS:

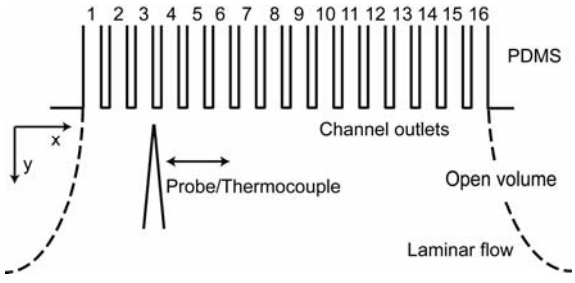
Experimental methods:

In-situ Thermometry: An in-house built microprobe[3, 4], with a spherical thermocouple having a diameter of 50 µm was employed. The E-type thermocouple was obtained from Omega Engineering, Inc. (Stamford, USA), and the bare leads encapsulated in a plastic holder. It was connected to a low noise thermocouple amplifier designed with Linear Technologies LTK01 instrumentation amplifier and LT1025 junction compensation ICs. The amplified signal was digitized with a 16 bit ADA16-8/2(CB)L AD converter (Contec, Japan), and interfaced to a personal computer (see Figure S1). The interface was also used to control a current limiting standard power supply used to apply voltage to the resistive heaters.

Prior to each experiment the thermocouple was externally calibrated to the temperature range between 22 and 50 ºC (by a 2 point calibration) using a reference thermometer with a 0.1 °C graduation. It had previously been confirmed that the thermocouple response was linear for the temperature range used (data not shown). The thermocouple manufacturer's accuracy specification is ±0.5°C over the full temperature range[5]. However, by calibrating and connecting to the amplifier we improve the accuracy in our setup to ±0.2°C.[3]

In order to characterize the temperature distribution at the channel outlets, the thermocouple was scanned from channel 1 to 16 while the temperature was sampled. Before the scans, the thermocouple was positioned at a distance of 25 µm and a height of 30 µm (the standard position) outside channel 5 using micromanipulators. When the desired temperature was reached, the voltage was clamped (see discussion below under Description of the different modes of operation). Subsequently, the thermocouple was scanned, perpendicular to the flow emanating from the channel outlets, by moving the microchip relative to the thermocouple with the motorised scanning stage (see Figure 4). The movement was programmed using Hyperterminal (Microsoft).

Figure S4. A schematic showing the channel outlets.



Flows exiting the channel outlets viscously couple to form a laminar flow in the open volume. The probe and/or thermocouple are placed within this laminar flow close to and perpendicular to the channel exits. Scanning of the probe occurs in the x direction at both a constant y and a constant z (where z is the height above the glass bottom of the chip). The heating structures are not shown.

Amperometry: The microfluidic chip was loaded with 100 μM of hexaammineruthenium (III) chloride in a buffer of 25mM KH_2PO_4, 25mM K_2HPO_4 and 0.1M KCl in channels 2, 4, 6, 8, 10, 12, and 14 and buffer in the other channels. A carbon fibre electrode having a cylindrical electroactive area (5 μm in diameter and 30 μm in length) (ProCFE, Dagan Corporation, Minneapolis, MN) was positioned outside the channel outlets with micromanipulators. The symmetry axis of the electrode was aligned in the flow direction in order to optimise the spatial resolution of the measurements. A potential of -0.3V *vs* Ag/AgCl was applied to the electrode using a Heka EPC10 triple patch-clamp amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany). The current resulting from reduction of the hexaammineruthenium (III) complex is proportional to the concentration of hexaammineruthenium (III) chloride and this was measured using the same amplifier. The electrode was scanned along a trajectory, perpendicular to the flow emanating from the channel outlets, by moving the microchip relative to the electrode with the motorised scanning stage, using Hyperterminal. Scans were performed at different temperatures and before the start of a scan the electrode was aligned 25 μm outside channel 1 and the thermocouple outside channel 5. The scans occurred in a plane 30 μm above the bottom of the chip and at different distances from the channel exits. The maximum scan speed utilised was 100 μm/s and the sampling frequency was 200 Hz. Due to electrode fouling during the measurements, the current corresponding to a specific concentration slowly decreases over time and to account for this decrease, the electrode traces were normalised.

Supplementary Material (ESI) for Lab on a Chip

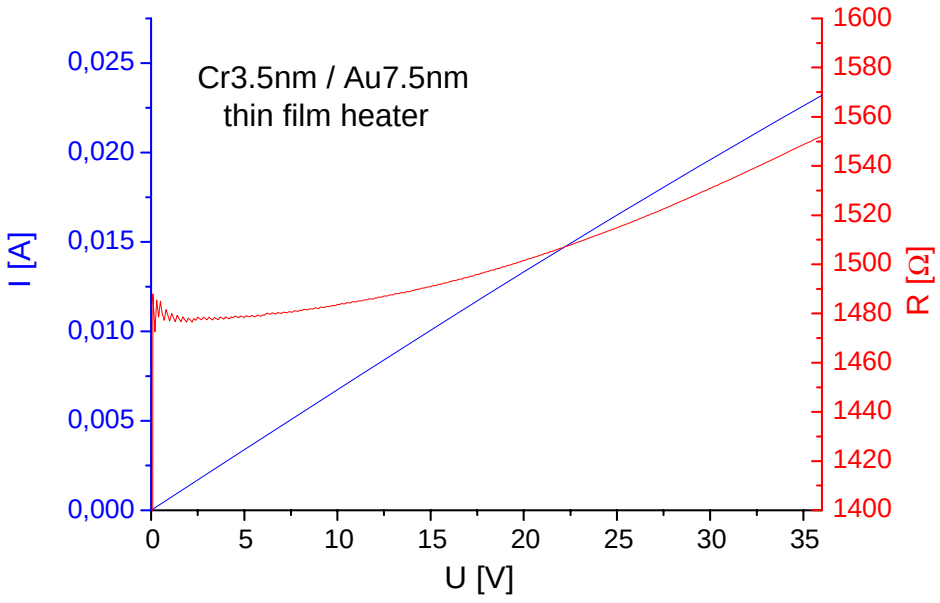
On-Chip Fabrication:

Table S1.

Heating Structure Number	Resistance (Ohm)
1	1489.9
2	1506.1
3	1503.7
4	1561.6
5	1553.2
6	1559.9

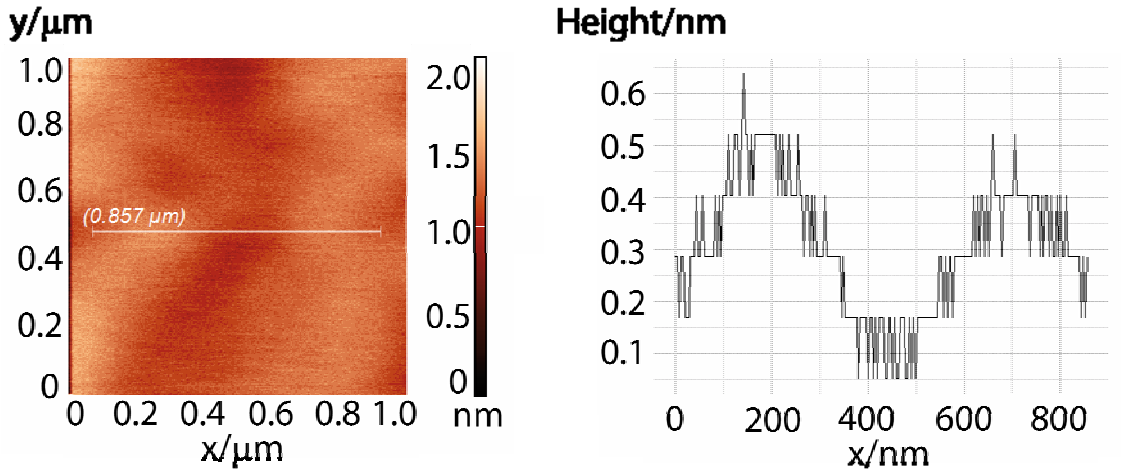
Mean Resistance: 1529.1. Standard deviation: 29.7.

Figure S5. U-I-R Characteristics of the thin film heater.



Typical current (resistance) vs. voltage characteristics of a thin film heater over the typical voltage range from 0 to 36V. For reproducible scan conditions, the resistive heater was placed with the glass side on a solid metal heat sink of 1 cm thickness. The measurements were carried out with a Keithley 4200-SCS parameter analyzer.

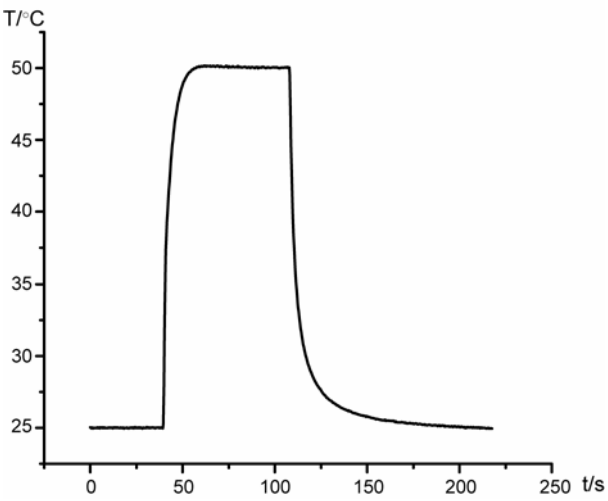
Figure S6, AFM surface characterization of the thin film heater.



Left image: Atomic force micrograph of a 1x1µm area of the Cr/Au surface of the evaporated thin film heating structure. Right image: Height profile along the line shown in the left image. This image shows that the heating structures have a uniform surface with a height variation of less than 1 nm. The measurements were performed on a NT-MDT Integra system in semicontact (tapping) mode.

Heating and Cooling:

Figure S7. Controlled heating and cooling.



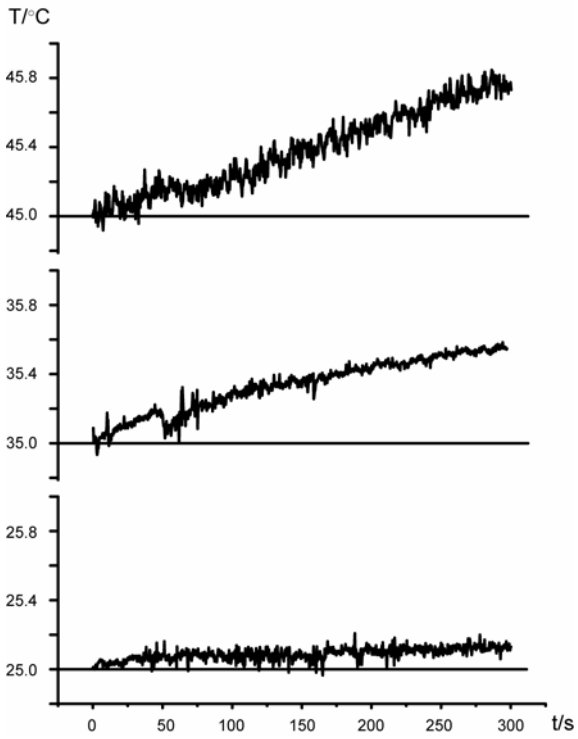
The heating rate is not limited by the performance of the system but by the desire for a controllable, smooth temperature change. The PI parameters were optimised to avoid overshoots and oscillations. The thermocouple was placed 25 μm outside channel 5, at a height of 30 μm and not moved for the duration of the experiment. Using the PI feedback, the temperature was increased from 25˚C to 50˚C. The time required for controlled heating of the system by 25˚C is 21s. There is no method of cooling, so any decrease in temperature relies on heat dissipation, but as can be seen in the figure the cooling rate is still quite fast. Cooling from 50˚C to 25˚C takes 84s; however, the initial cooling rate is much higher and it takes only 10s for the temperature to decrease from 50˚C to 30˚C.

Description of the different modes of operation: The system can be operated in two different modes; with or without a proportional/integral (PI) feedback loop. When PI feedback is enabled (feedback mode) the system maintains a given input temperature at the location of the thermocouple. Alternatively, when a desired temperature is reached one can apply the hold mode. The feedback will then be turned off and the voltage will be clamped to the value it had when the hold mode was selected. The hold mode is used when a probe, such as a patch-clamped cell, is scanned across all the channel outlets, *e.g.* in a dose-response experiment. When the cell is placed outside channel 1, the closest position for the thermocouple is ~300 μm away, outside channel 5, and therefore when the cell reaches channel 16 the thermocouple will be 300 μm outside of the flow, where the temperature is

lower than in the flow (see Figure S10). If the feedback mode was employed, PI regulated heating would compensate for this difference and the cell would thus experience a non-uniform temperature profile across the channels. The disadvantage of the hold mode is that no adjustment will be made for any local differences in temperature across the channel outlets or for the slight upward drift of the temperature as the system is continually heated.

Applying the hold mode fixes the voltage applied to the system. Prolonged heating will mean that the whole system slowly increases in temperature over time, and without the feedback this will not be adjusted for by decreasing the applied voltage. We have studied this upward drift over time for three different temperatures (see Figure S8). The maximum drift observed over 5 minutes is less than 0.15˚C at 25˚C and less than 0.80˚C at 45˚C. In all cases, the temperature is stable within ±0.2 ˚C for 60 seconds. While this time is likely to be sufficient for a scan across the chip to obtain a dose-response curve, it is clearly important to reset the temperature between scans, using the feedback mode.

Figure S8. Scans obtained using the thermocouple to demonstrate the temperature stability of the system over time when operated in the hold mode.

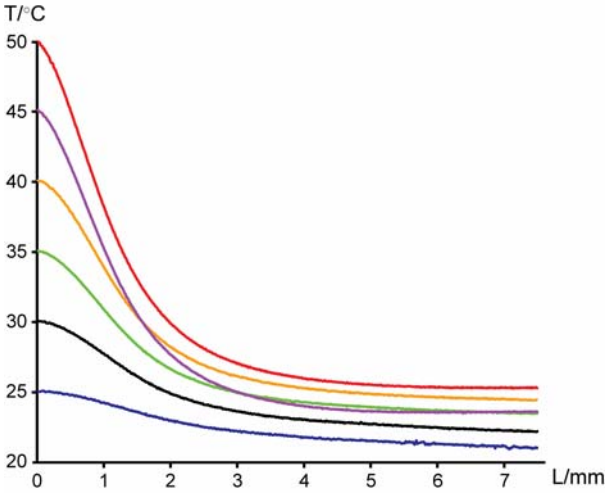


The thermocouple was placed 25 μm outside channel 5, at a height of 30 μm, and the desired temperature was reached using the PI feedback. Subsequently, hold was selected to fix the applied voltage and the thermocouple recorded the temperature over a period of 300 seconds. In the hold mode there is no compensation for the increase in temperature of the whole system during prolonged heating. Thus, an upward drift in temperature is observed. Note that the y axis is discontinuous.

In order to characterise the temperature distribution across the channel outlets when applying the hold mode, we used a scanning probe thermocouple as described in the experimental section. The temperature was set using the feedback mode while the thermocouple was outside channel 5, since this is where it would be placed initially in *e.g.* a dose-response experiment, and after that the hold mode was applied for the scan across all channel outlets. The results for at the standard position, of 25 μm from the channel outlets and a height of 30 μm (chosen since this is standard for a cell during patch-clamp experiments) are shown in the article (see Figure 2A). Additionally, we have used the thermocouple to map out the temperature in the open volume. Scans out to a distance of 7.5 mm in the open volume

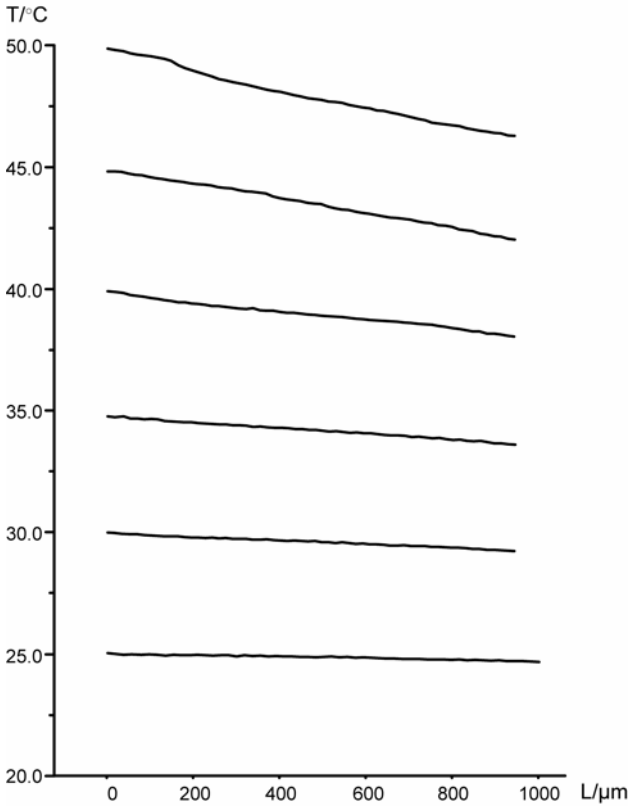
confirm that the heating effect is local since already at a distance of 2 mm the temperature is below 30˚C even when the temperature at the channel outlets is 50˚C. Thus, cells kept further out in the open volume are not exposed to high temperatures prior to experiments (see Figure S9). Scans out sideways from the open volume confirmed that the temperature was lower here as discussed above (see Figure S10).

Figure S9. Temperature profile out in the open volume.



The thermocouple was placed 25 µm outside channel 5, at a height of 30 µm, and the desired temperature was reached using the PI feedback. Subsequently, hold was selected to fix the applied voltage and then the thermocouple was scanned 7.5 mm out into the open volume. The temperature drops rapidly and by 2 mm outside the channel outlets it is under 30˚C regardless of the starting temperature. This means that cells placed out in the open volume will not be exposed to high temperatures prior to experiments.

Figure S10. Temperature profile outside the laminar flow pattern



The thermocouple was placed 25 μm outside channel 5, at a height of 30 μm, and the desired temperature was reached using the PI feedback. Subsequently, hold was selected to fix the applied voltage and then the thermocouple was scanned from the midpoint of channel 1 out of the laminar flow 1000 μm (at the constant y value of 25 μm). Outside of the laminar flow the temperature drops.

CALCULATIONS:

The diffusion coefficient can be calculated using $D = \frac{kT}{6\pi\eta a}$ where k is the Boltzman constant, T temperature, η viscosity and a is the effective hydrodynamic radius. In addition to the explicit inclusion of temperature in the numerator, the viscosity of water is very dependent on temperature. Both of these factors contribute to an increase in the diffusion coefficient as the temperature increases, and hence an increase in the diffusion zone width. Taking into account the effect of temperature based viscosity changes on the diffusion coefficient, we calculated that the diffusion zone width (w) between solution segments, given by $w = \sqrt{(2Dt)}$

where *t* is the time in seconds allowed for diffusion, will increase by 34% between 25˚C and 50˚C at 25μm from the channel outlets.

REFERENCES:

(1) Olofsson, J.; Pihl, J.; Sinclair, J.; Sahlin, E.; Karlsson, M.; Orwar, O. *Anal Chem* **2004**, *76*, 4968-4976.
(2) Granfeldt, D.; Sinclair, J.; Millingen, M.; Farre, C.; Lincoln, P.; Orwar, O. *Anal Chem* **2006**, *78*, 7947-7953.
(3) Nash, J. D.; Caldwell, D. R.; Zelman, M. J. *Journal of Atmospheric and Oceanic Technology* **1999**, *16*, 1474-1482.
(4) Markström, M.; Gunnarsson, A.; Orwar, O.; Jesorka, A. *Soft Matter* **2007**, *3*, 587 - 595.
(5) *Omega Temperature Measurement Handbook*; Stamford, Connecticut, Omega Engineering.