Electronic Supporting Information

Hydrodynamic Thermal Confinement: Creating Thermo-Chemical Microenvironments on Surfaces

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ES1. Experimental Setup and MFP head

The microfluidic probe (MFP) head is mounted on a motorized Z-stage for controlling the apexto-surface distance. The sample is placed on a motorized X-Y stages that confers the scanning capabilities of the setup.



Figure ES1.1. Photograph of the set-up showing a probe head with fluidic and electrical connections.

Microchannels and vias were etched on a 380-µm-thick, double-side-polished silicon wafer using standard photolithography and deep reactive-ion etching (DRIE). Electrical circuits were patterned using a lift-off process on the backside of the silicon wafer. The microchannels were sealed by anodically bonding a 500-µm-thick borosilicate wafer. The glass side of the MFP heads is anodically bonded to the silicon to create closed microchannels. Using silicon (high thermal conductivity) as the substrate material for the heating element rather than glass or plastic (low thermal conductivity) helps reduce the power required to heat the processing liquid. A probe made of glass or plastic would require higher power to heat up the processing liquid to the desired temperature, thus leading to an increased heat loss in the immersion liquid. Off-chip implementation was not considered as the temperature of the liquid at the exit of the MFP head cannot be precisely controlled because of heat losses along the channel. Placing the heating element in close vicinity to the probe apex is not preferable as it will result in the direct contact between the heater and the immersion liquid. This is problematic because of the heat dissipation to the immersion liquid (with the immersion liquid acting as a heat sink), potential electrolysis, and the need for off-plane microfabrication steps, which are difficult on such a small scale. We therefore placed the resistive element ~ 14 mm above the apex, where it is not in contact with the immersion liquid, but in the vicinity of the apex so that the temperature of the processing liquid remains constant along the flow path.



Figure ES1.2. Microfabricated MFP head. Photograph of the front- and back-side of an MFP head with microchannels and integrated resistive heater and RTD for temperature control.

ES2. Finite Element Method (FEM) Simulations and electrical analogy of heat transfer

Steady-state modeling of heat transfer in laminar flow was performed using COMSOL Multiphysics version 4.2 by coupling the *laminar flow* and the *heat transfer in liquid* physics. Water was used in all simulations (Newtonian incompressible, 998 kg m⁻³ density, 0.001 N s m⁻² viscosity, and 4185.5 J kg⁻¹ K⁻¹ heat capacity).

We conceptually divided the MFP head into two zones (Figure ES2.1): zone 1 corresponds to where the processing liquid is heated while flowing through a microchannel, and zone 2 is where the processing liquid flows downstream towards the apex. For optimal control of the temperature of the flow confinement, the liquid should exit zone 1 at the set point temperature and lose as little heat as possible while flowing through zone 2. The FEM results show that for flow rates between 1 and 10 μ L min⁻¹, the heat dissipation from the processing liquid is minimal (Figure ES2.1). As it can be observed: lower flow rates are favorable in zone 1 so that the liquid reaches the desired temperatures, and higher flow rates are preferable in zone 2 to prevent the temperature of the liquid from decreasing.



Figure ES2.1 Finite-element simulation of the temperature of the processing liquid while it flows from the inlet (left) to the apex (right). A unit volume of processing liquid is heated in zone 1 to the set point temperature. In the typical flow rate range (1-10 μ l/min), the processing liquid loses up to 20° C upon reaching the apex.

Using the electrical analogy, the heat flux q'' can be expressed as a function of the total resistance and the temperature difference between room temperature and the heating element^[1]:

$$q^{\prime\prime} = \frac{\Delta T}{R_{tot}}$$

Thermal resistances are calculated differently depending on the conductive or convective nature of heat transfer:

- Conductive: $R^d = \frac{t}{\kappa}$ with t the material layer thickness and κ the thermal conductivity Convective: $R^c = \frac{1}{h}$ with h the convection heat transfer coefficient



Figure ES2.2 Schematic and first order electrical equivalent of Zone 1.

For zone 1, the total equivalent resistance is given by:

$$R_{tot} = \frac{(R_{air}^{v} + R_{glass}^{d}) \cdot (R_{air}^{v} + R_{Si}^{d} + R_{water}^{v})}{2 \cdot R_{air}^{v} + R_{glass}^{d} + R_{Si}^{d} + R_{water}^{v}}$$

Which gives a heat flux $q'' \cong 5000$ W m⁻² to heat the processing liquid to 80° C when considering a 200 µm wide and 50 µm deep microchannel.

ES3. Analytical model of heat transfer

The dimensions of the heating element can be obtained by using the flow rate, the target temperature of the fluid and the channel dimensions as inputs. For flow in a sealed channel, we can apply the energy conservation to determine the mean temperature $T_m(x)$ inside a heated channel with a surface temperature of T_s .



Figure ES3.1 Schematic of analytical model of heating of liquid flowing through a microchannel with defined parameters and variables.

In this model, heat transfer due to conduction in the axial direction is neglected, hence energy conservation can be expressed as^[28]

$$q_{\rm conv} = \dot{m}c_p (T_{m,o} - T_{m,i}), \qquad (1)$$

where q_{conv} is the heat transfer rate, \dot{m} and c_p are the mass rate and the specific heat of the liquid flowing through the channel, and $T_{m,o}$ and $T_{m,i}$ the mean temperature of the liquid at the channel outlet and inlet, respectively.

Differentiating (1) on a control volume, we obtain:

$$dq_{\rm conv} = \dot{m}c_p dT_m \tag{2}$$

An equivalent expression for dq_{conv} is given by:

$$dq_{\rm conv} = q_s^{\prime\prime} P dx \,, \tag{3}$$

where q_s'' is the heat flux between the heater and the processing liquid and *P* the perimeter of the channel.

Substituting Eq. (3) into (2) we obtain:

$$\frac{dT_m}{dx} = \frac{q_s''P}{mc_p} \tag{4}$$

Using Newton's cooling law expressing the heat flux,

$$q_s'' = h(T_s - T_m),$$
 (5)

where *h* denotes the heat-transfer coefficient, we finally obtain:

$$\frac{dT_m}{dx} = \frac{Ph}{\dot{m}c_p}(T_s - T_m) \tag{6}$$

Solutions to Eq. (6) depend on the boundary condition applied to the channel surface. Here, we consider a constant surface temperature T_s and its difference to the mean temperature inside the channel: $\Delta T = T_s - T_m$. Eq. (6) then becomes:

$$-\frac{d(\Delta T)}{dx} = \frac{Ph}{mc_n} \Delta T \tag{7}$$

 $T_m(x)$ can now be obtained by separating variables and integrating from 0 to a position x:

$$\frac{T_s - T_m(x)}{T_s - T_{m,i}} = \exp\left(-\frac{Px}{\dot{m}c_p}\overline{h}\right)$$
(8)

Because the thermal entrance is relatively short, the average heat-transfer coefficient \overline{h} can be replaced by the fully developed heat-transfer coefficient h:

$$\overline{h} \approx h = N u \frac{k}{D_h},\tag{9}$$

with *Nu*, the *Nusselt* number, *k* the thermal conductivity and D_h , the hydraulic diameter of the channel. The *Nusselt* number for fully developed laminar flows and constant surface temperature is a function of the width/height aspect ratio (w/h) of the channel and is listed elsewhere. ^[29] In our probe head, we have w/h = 4, which gives Nu = 4.44.

We use Eq. (8) to determine the minimal length of the heater for heating the liquid up to 80° C. Solving Eq. (8) for *x*, gives

$$x = \frac{\dot{m}c_p}{Ph} \ln\left(\frac{T_s - T_{m,i}}{T_s - T_m(x)}\right) \tag{10}$$

Using $T_s = 80^{\circ}$ C, a flow rate of 1 µL min⁻¹ and a channel of 200 µm × 50 µm, we obtain a heater length of $x \approx 30$ µm. From this, we can establish that the heater should be at least 30 µm long to heat the processing liquid to 80 °C at 1 µL min⁻¹, which is in agreement with the results observed in the FE simulations (Figure ES2.1).



ES4. Hydrodynamic Thermal Confinement calibration and control

Figure ES4.1. Experimental validation of FE simulations and time response of the thermal system. (a) Comparison between experimental and simulation results for an injection flow rate of 2 μ l min⁻¹. The top left inset shows a photograph of a microfabricated glass slide with an integrated resistance temperature detector (RTD, inset bottom right) to measure the temperature of the flow confinement. (b) Graph of the time response of the system for 10° C steps. The top left inset shows the transition between 50° C and 60° C. The inset at the bottom shows the closed-loop control scheme with a PID controller.

The RTD was calibrated using a cold plate through which a liquid at a controlled temperature circulates. The calibration was performed in a 4-point probe configuration with an activation current of 1 mA. The temperature of the liquid was increases from 25° C to 90° C in six steps during which the voltage drop across the RTD is measured (Figure ES4.1).



Figure ES4.2 Calibration graph of the RTD patterned on the MFP head.

The current sourced vs. temperature relation of the heater was then established using the calibrated RTD to measure the temperature of the heater placed next to it. As expected, we observe a quadratic dependence between the current sourced and the temperature ($P = RI^2$) (Figure ES4.3).



Figure ES4.3 Calibration curve of the heater patterned on the MFP head. Error bars show the standard deviation for n = 4 experiments.

ES5. Relative importance of convection and diffusion of heat in the immersion liquid

Heat transport using from HTC to a surface should be primarily convection. However due to the presence of an immersion liquid, heat will diffuse from the HTC to the surrounding liquid and result in an alteration of the heat localization. Here, we discuss the relative importance of convection and convection.

For this, we assume that a flow particle travels a distance 2d at a velocity v between the moment it exits the apex and reenters it, which results in a residence time in the immersion liquid. We calculate the diffusion length R_d of this particle during the time t:

$$t = \frac{2d}{v} \tag{11}$$

$$t = \frac{R_d^2}{\alpha} \tag{12}$$

With $\alpha = \frac{k}{\rho c_p}$ the heat diffusivity. Equating S1 and S2, we can isolate the diffusion length R_d :

$$R_d = \sqrt{\frac{2dk}{v\rho c_p}} \tag{13}$$

When using typical operating parameters, we obtain a diffusion length $R_d \sim 34 \ \mu\text{m}$, which relatively small compared to the dimensions of the flow confinement (~100 × 50 μm)

ES6. Design and test of the DNA probes in the microarray

Sickle cell disease is a genetic disease which manifests itself as a single mutation in the β -globin (HBB) gene from nucleotide A to T resulting in an amino acid change from Glutamic acid to Valine.^[2] In its homozygote form, the mutation leads to a change in the shape of red blood cells, which become rigid and adopt a sickle shape. This induces many complications such as cerebrovascular diseases or susceptibility to infection leading to a high mortality at young ages in particular.

Sickle cell disease is usually diagnosed in adults and children through hemoglobin analysis using protein chromatography but DNA-based testing is increasingly used. In particular DNA analysis is applied to prenatal diagnostics and it is believed that systematic neonatal screening could reduce significantly child mortality.^[2] We apply selective DNA retrieval to the sickle cell mutation probe 3'-GA GGA CAC TTC AGA CG-5'.^[3]

The melting temperature of the sickle cell primer probes was measured experimentally in order to set the temperature of the flow confinement for the local denaturation experiment.

For this, 20 μ L of several DNA and SYBR concentrations were loaded into a q-PCR machine to perform a melting experiment.

The melting curve of the sickle cell primer (Figure ES6.1) shows the signal of both double- and single-stranded DNA at 100 nM concentration. Two intensity drops can be observed on the melting curve of the double-stranded DNA. The first drops occurs at the same temperature than the drop on the single-stranded curves, which indicates self-folding of the DNA strand due to a self-complementary structure. For this reason, probes hybridization on the microarray is performed at 45° C.

The second intensity drop shows the actual melting temperature of the double-stranded DNA which is close to 60° C.



Figure ES6.1 DNA melting curve for sickle cell primer probes for single- and double-stranded DNA. The Y-axis represents the fluorescence variation with temperature (-dF/dT), hence curve peaks correspond to fluorescence intensity drops.

DNA hybridization on microarray: We used custom 40K DNA microarrays (Mycroarray, Ann Arbor, MI) with the probe sequence 5'- CTCCTGTGGAGAAGTCTGCTTTTTTTTT – 3' and a labeled complementary strand (Integrated DNA Technologies Inc., Coralville, IA). 100 μ L of a 5 μ M solution of labeled probes in hybridization buffer (5× SSC, 0.1% SDS, 1% BSA) were incubated for 18 h at 45° C using a PDMS gasket and a cover slip. After incubation, the microarray was rinsed using TE Buffer (10 mM Tris, 1 mM EDTA) and gently dried using a nitrogen gun.

Local DNA denaturation with the MFP: We used a solution of 15% formamide (Sigma Aldrich, Saint-Louis, MO) in TE Buffer (10 mM Tris, 1 mM EDTA, pH 8) as processing liquid and TE buffer as immersion liquid. Flow rates were 2.5 and 7 μ L min⁻¹ for injection and aspiration, respectively. Flows were generated using a MFCS pressure-based flow controller (Fluigent, Paris, France). The apex-to-surface distance was set to 30 μ m for the entire course of the experiments. The heater and the RTD were connected to three Keithley 2440 (Keithley Instruments Inc., Solon, OH) using a custom-made holder. The exposure time of each DNA spot on the microarray was 3 min after the processing liquid had been heated to 60° C.



Figure ES6.2 Fluorescence graph of DNA spots after Formamide only and heat + buffer experiment.



Figure ES6.3 Fluorescence graph of spots 1-7 after three minutes' exposure to three different conditions: 15% formamide, 60° C TE buffer and 15% formamide at 60° C. Error bars represent the standard deviation (n = 5).

References:

- [1] F. P. Incropera, D. P. DeWitt, T. L. Bergman, A. S. Lavine, *Fundamentals of Heat and Mass Transfer*, **2007**.
- [2] D. C. Rees, T. N. Williams, M. T. Gladwin, *Lancet* **2010**, *376*, 2018.
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