Supplementary information - Technical Note Ref.: B812443D

# 1 Thermocouple calibration

For our microchip we use thin metal films for both resistive heating and temperature sensing, as described in previous work [1]. The hybridization efficiency in FISH is strongly affected by temperature; therefore, calibration of the chamber temperature was necessary for optimized FISH. The thin-film element used (4.5 mm diameter) is in close proximity (1 mm) with the FISH chamber (2.5 mm diameter) and allows for accurate temperature control. Here we used a platinum heater, discussed in previous work [2], primarily because its resistivity exhibits a strong linear dependence on temperature and the temperature coefficient of resistance is relatively high. Additionally, we placed a spring-loaded thermocouple device, shown in figure S.1 and S.2, (40 gauge, 5TC-TT-K-40-36, Omega Engineering, Laval, Quebec, Canada) in contact with the top of the chip, centered directly above the chamber. We were able to control temperature reliability (and with redundancy) by monitoring both the heater temperature (via resistive sensing [1]) and the surface temperature (via thermocouple, described below).

To keep the system and microchip clean for observing FISH-labelled cells in fluorescence microscopy, we choose not to use heat paste between the top thermocouple and chip surface. However, without paste there is only one contact point between thermocouple and the chip surface, which might lead to inaccuracy. To solve this, we created a custom machined plastic fitting thermocouple device that was used to measure the temperature in a chamber near the top of the chip, figure S.1. The thermocouple was recessed within the plastic fixture, suspended 1 mm above the chip surface in a sealed air chamber (please figure S.1 and S.2). The fixture was spring-loaded and maintained contact with the surface while minimizing additional thermal mass to avoid disturbing the measurement.

Since the chamber temperature is difficult to measure without perturbing the thermal conditions, we used two distinct approaches for calibrating and accessing the accuracy of the top thermocouple relative to the chamber temperature. First, we bonded the chip with another embedded thin wire thermocouple (40 gauge, 5TC-TT-K-40-36, Omega Engineering, Laval, Quebec, Canada) placed directly in the chamber. The thin wire thermocouple (approx. 80 microns) was small enough that it deformed the 254 micron PDMS layer and permitted successful bonding, while adding minimal disturbance to the thermal conditions. Second, we used thermochromic liquid crystals (TLCs) to verify chamber temperatures in the steady-state. Previous work has demonstrated that TLCs provide a compact and effective method for measuring temperature and uniformity at the microscale [3]. Four sets of TLCs (R37C1W-37ºC, R58C3W-58ºC, R75C1W-75ºC, R93C3W-93ºC, Hallcrest Glenview, IL, USA) were used, each custom synthesized to change reflected colour with a bandwidth of 1-3ºC around one of the typical desired chamber temperatures for each FISH stage. The reaction chamber was filled with the stock TLC suspension and the color shift of the TLCs was observed as suggested by the manufacturer using the microscope mentioned below in brightfield mode.

Figure S.3 is a plot of the temperature measured in the air chamber (at the top of the chip), in the chamber (thermocouple), and at the heater (resistive) versus the current through the heater. From the data, we derived empirical fitting functions for calculating the chamber temperature, also shown in figure S.3. The TLC results were also consistent with the embedded thermocouple measurements and allowed the top spring loaded thermocouple to be accurately calibrated to the chamber (within 1ºC).

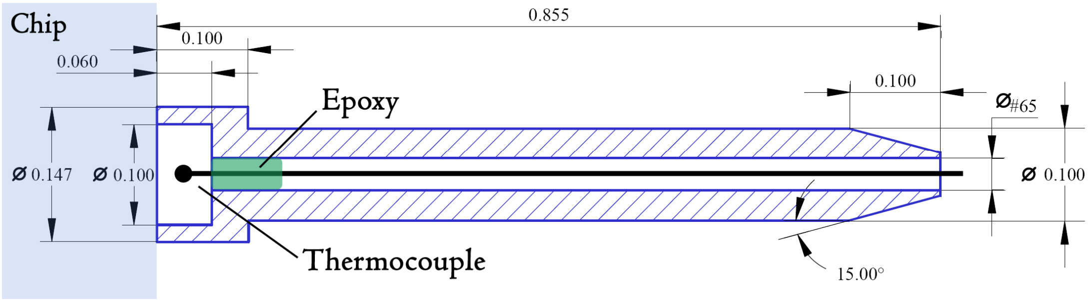


Figure S.1: Drawings for the thermocouple spring loaded pin (the spring slides on the outside and his held in place with a gantry that lowers over the top of the chip). The thermocouple is suspended in a sealed chamber that makes contact with the chip directly over the FISH chamber. The temperature of the air chamber above the surface allowed the prediction of the temperature in the FISH chamber to within 1ºC.

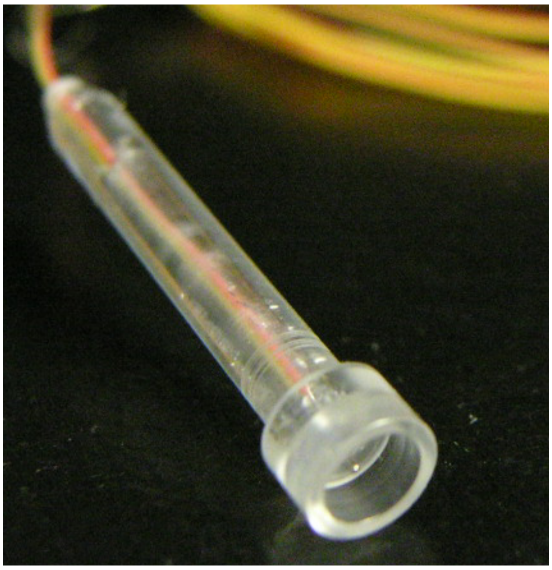

Figure S.2: Image of the thermocouple plastic fixture.

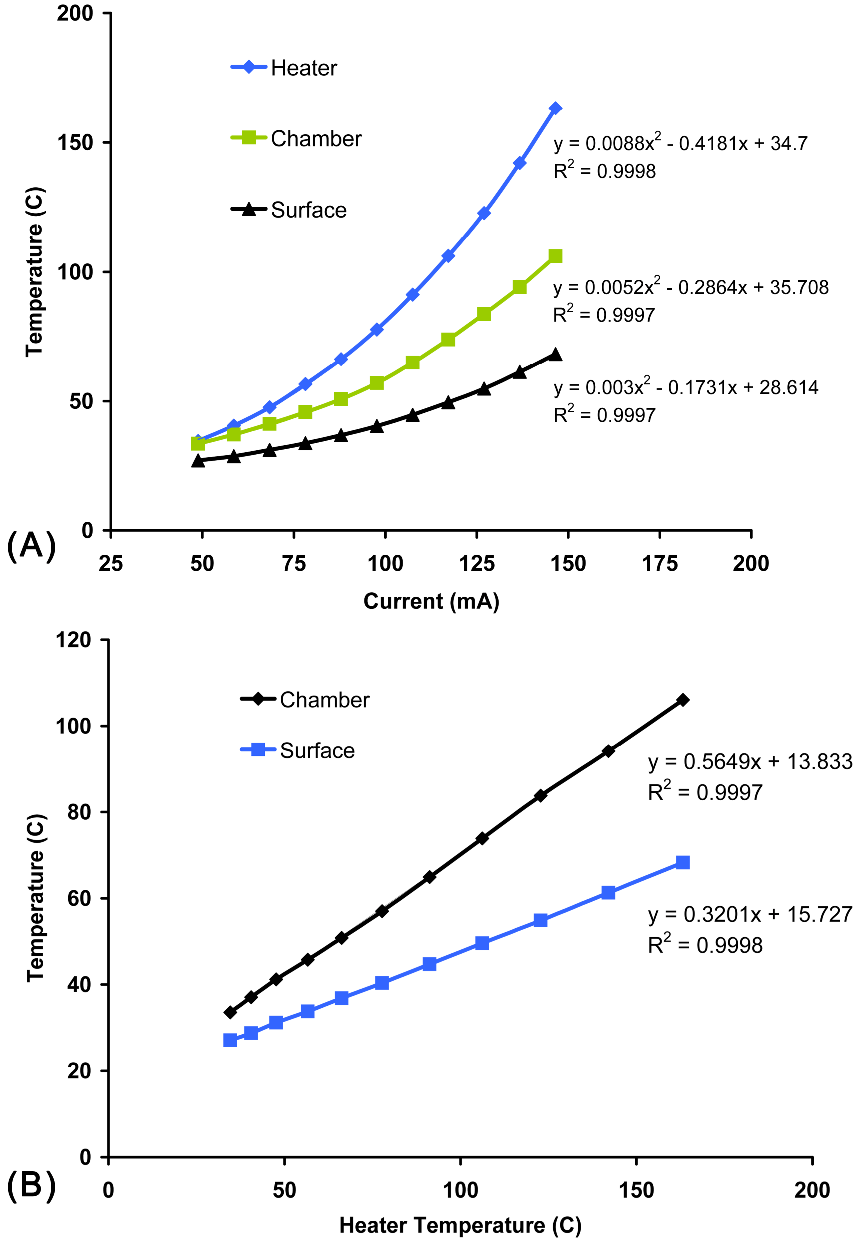


Figure S.3: Calibration of chamber temperature by measuring the air chamber surface temperature and the thin-film heater temperature. The heater temperature was measured by sensing resistance change [2]. The surface (TC device) and chamber temperatures were measured by thin-wire thermocouples. Data was acquired from averaging 3 chips (nominal resistance of 73 ohms) with standard deviations of less than a degree Celsius.

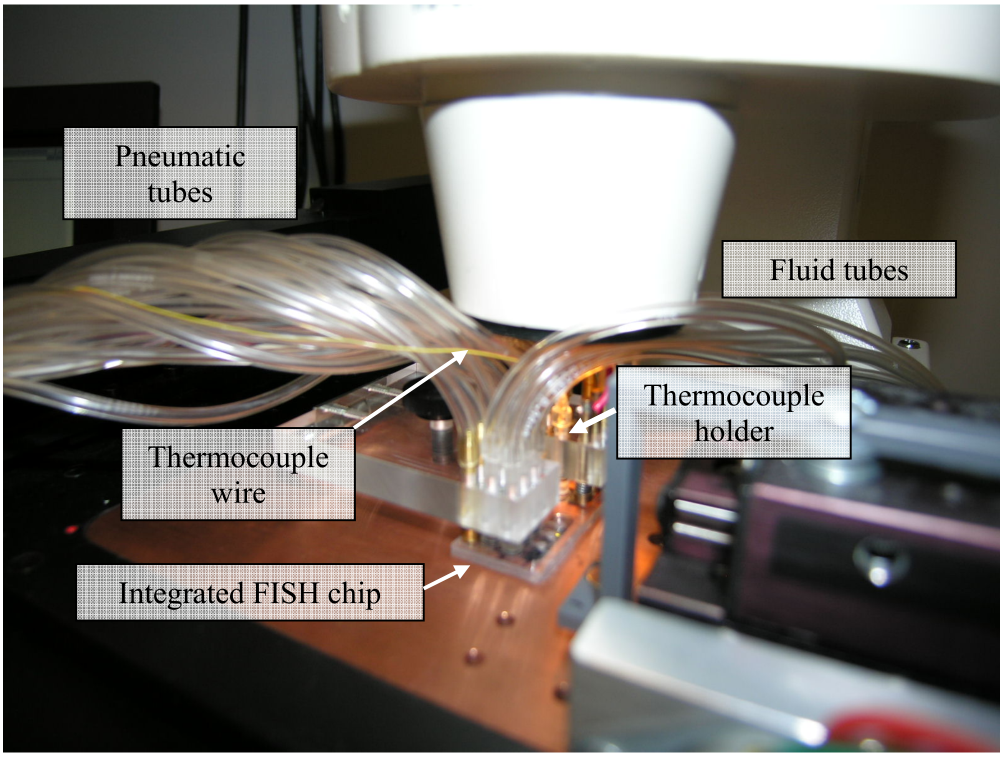


Figure S.3: This custom copper plate (heatsink[4] and physical support) and plexi-glass gantry (valve, fluid, and electronic coupling) was built that mounted onto the microscope stage in order to provide real-time images and videos while fluidic control and heater control were being applied to the microchip (please see supplementary information). A hole was made (7 mm diameter) and was centered underneath the FISH chamber to provide a viewing port for image acquisition. This custom chip interface, with the supporting electronics, operated the microfluidic chip (valves and heater) and was controlled via USB, connected to a personal computer. The left tubing connects to the pneumatic valves; the right tubing connects to the beakers containing large volumes of reagent. A motorized stage is visible in the lower right corner.

References:

1. G. V. Kaigala, V. N. Hoang, A. Stickel, J. Lauzon, D. Manage, L. M. Pilarski and C. J. Backhouse, *Analyst*, 2008, **133**, 331-338.
2. V. N. Hoang, G. V. Kaigala and C. J. Backhouse, *Lab Chip*, 2008, **8**, 484-487.
3. A. Iles, R. Fortt and A. J. de Mello, *Lab Chip*, 2005, **5**, 540-544.
4. V. N. Hoang, G. V. Kaigala, A. Atrazhev, L. M. Pilarski and C. J. Backhouse, *Electrophoresis*, 2008, **Accepted**.

## TLC video caption

The video shows the R37C1W TLC shifting from clear to red when the desired chamber temperature is 37 ºC. The video demonstrates the stability of temperature control and at the end of the video the thin-film heater is turned off and the TLC switches back to clear. The TLC (lot #: 80305-6) shifts are: 37.1ºC (red), 37.6ºC (green) and 38.1ºC (blue).