**Electronic Supplementary Information**

# Towards Single Cell Heat Shock Response by Accurate Control on Thermal Confinement with an On-Chip Microwire Electrode

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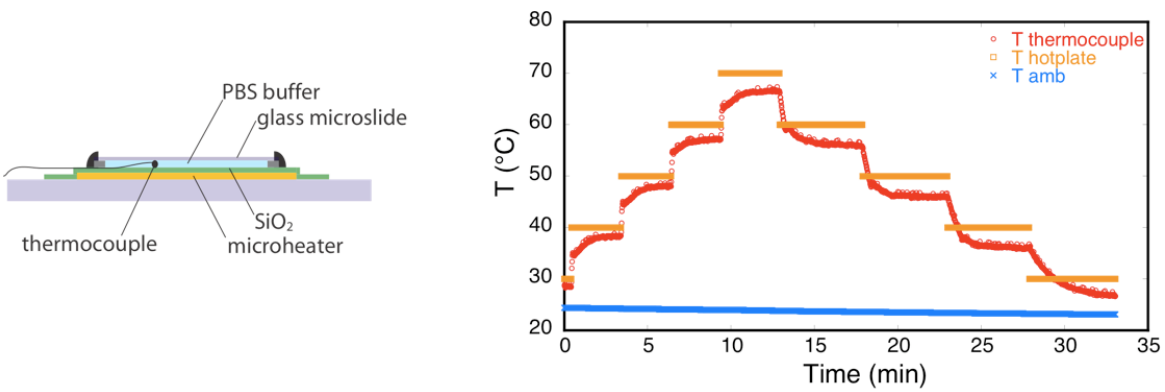
## 1-Calibration of rhodamine B

Calibration of rhodamine B fluorescence emission was done on microheater devices without applying any voltage but using a thermoplate (MATS-1002RO, Tokai Hip Co., Ltd., Japan) to set the temperature between 30 and 70˚C. As a preliminary study, the thermal dynamics of the calibration system was verified by inserting a thermocouple between the microheaters and the glass microslide (Figure S1) and applying a thermal cycle from 30˚C to 70˚C through the thermoplate. The results are presented in Figure S1.




(a) (b)

Figure S1. Characterization of the thermal dynamics of the calibration system, (a) sample configuration, (b) results for a 30˚C-70˚C cycle

The shift between the thermoplate temperature and the thermocouple temperature can be explained by the intermediate position of the latter in the PBS-filled space between glass substrate and glass microslide. These results show that the time constant to reach the thermal equilibrium in the liquid surrounding the microheater is approximately 2 min when increasing the temperature and 3 min during decrease. In further calibration experiments we consequently waited for 2 min and 3 min respectively to stabilize temperature before fluorescence measurement when increasing and decreasing from 30˚C to 70˚C. A 2 s exposure time was used for the acquisition of the

fluorescence images. In order to investigate the repeatability, each calibration point was measured 20 times among several 30˚C-70˚C temperature cycles. The average calibration values and the corresponding standard deviations are presented in Figure S2.

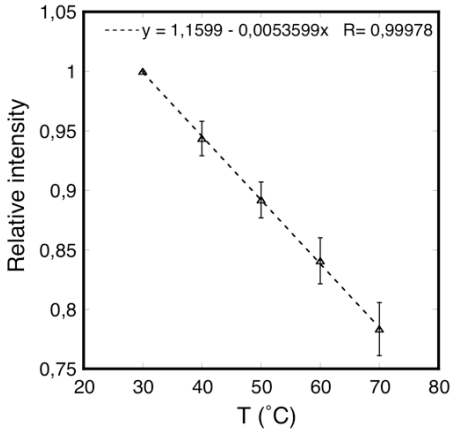



Figure S2. Calibration of the temperature dependence of rhodamine B fluorescence

One may notice that, because in the following fluorescence-based thermal measurements the fluorescence is always compared to the reference one measured at 30˚C for each device and experiment, all the relative intensities have been normalized to 1 at 30˚C. Consequently only the slope calculated and reported in Figure S2 is used in further thermal measurements on microheaters. Even though all the calibration curves were obtained from independent experiments with different devices and separately prepared rhodamine B solutions, the uncertainty on the absolute temperature did not exceed ± 5˚C at high temperatures and decreased at lower temperatures. This uncertainty can be notably explained by the slight difference in rhodamine B density on the silica surface resulting from each particular surface preparation.

## 2-Canceling PhotoBleaching

Neutral density filters (ND6 & ND25) were used in order to avoid strong photobleaching of rhodamine B as shown in Figure S3 where their effects have been investigated for a 300 s exposure time.

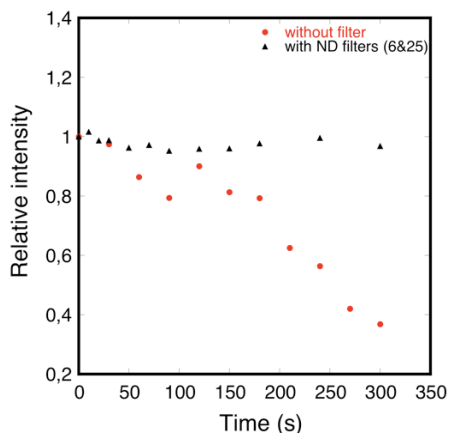



Figure S3. Comparison of the photobleaching of rhodamine B with and without neutral density filters

## 3-Fluorescent image processing

Figure S4 describes the image processing used to provide the best temperature signal and accuracy.

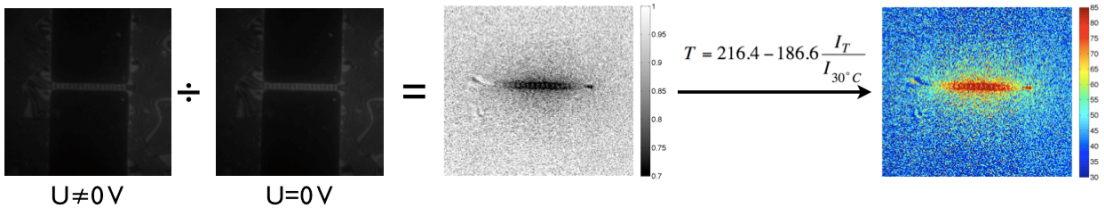



Figure S4. Temperature mapping reconstruction from fluorescence images

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