Electronic Supplementary Material (ESI) for Lab on a Chip.


# Dynamic microfluidic nanocalorimetry system for measuring *Caenorhabditis elegans* metabolic heat

## Supplementary Information

**Roger Krenger, Thomas Lehnert and Martin A. M. Gijs**

*Laboratory of Microsystems, Ecole Polytechnique Fédérale de Lausanne, Switzerland*

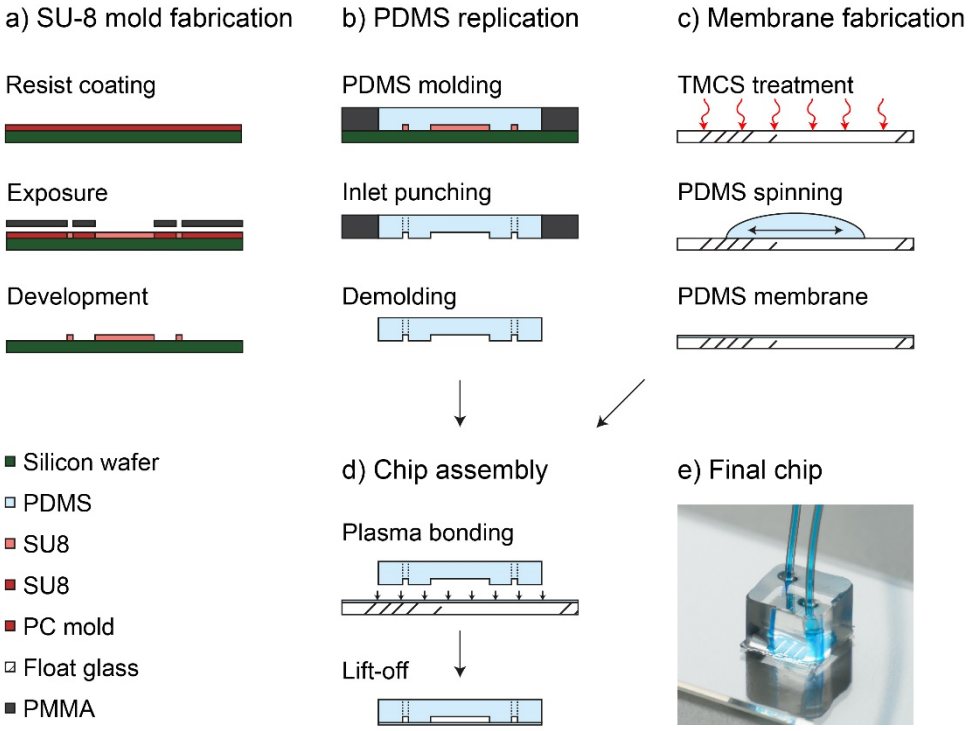


Figure S1: Fabrication steps of the PDMS microfluidic chips. a) SU-8 photolithography process on a 4 inch Si wafer. b) PDMS replication using a micromilled PMMA mold to define the outer shape of the individual PDMS chips. c) PDMS membrane fabrication on a 4 inch float glass wafer (thickness 20 µm, spin speed 3000 rpm, prepolymer to curing agent ratio 10:1). d) Oxygen plasma bonding of the demolded PDMS chip to the PDMS membrane. e) Photograph of a microfluidic chip (8 x 8 $mm^2$, height ~5 mm) filled with a dye solutionon a glass slide.

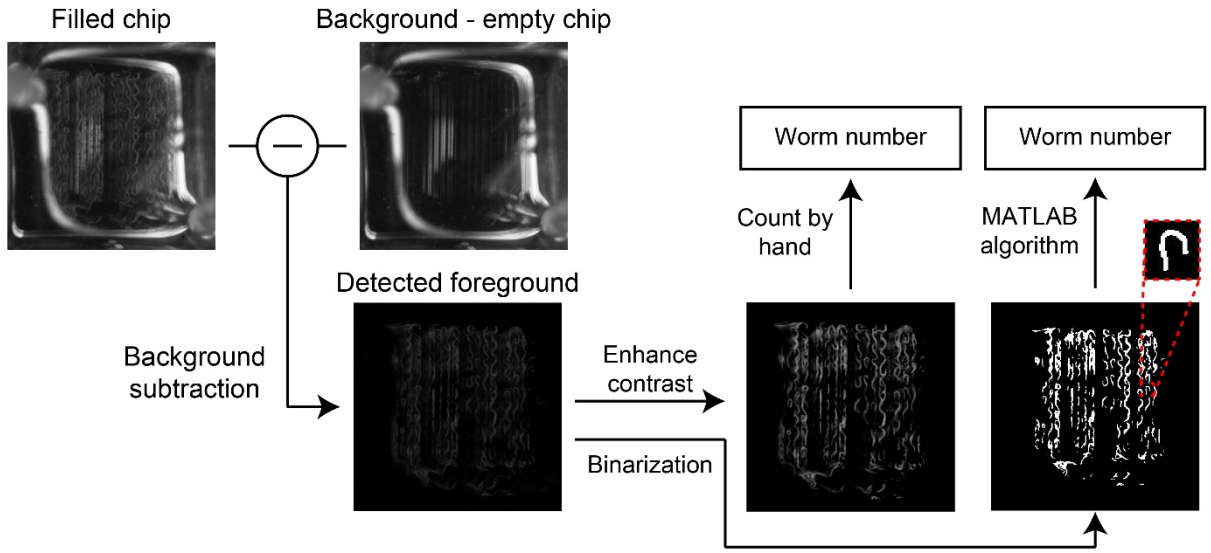


Figure S2: Image processing for counting on-chip worm populations. Images of the microfluidic chips were taken after worm loading and compared to background images before worm loading (filled with buffer solution). MATLAB was used to subtract the background image from the image of a filled chip, yielding only the contribution of the worms (foreground). MATLAB was also applied to enhance the contrast and binarize foreground images (using MATLABs adaptive thresholding function with sensitivity = 0.05). On the processed images, worm populations can either be counted manually or estimated using MATLAB. In the later case, a rectangular area is manually drawn around a worm to find the number of white pixels making up single organism. This number of pixels is then divided by the number of white pixels in the whole binarized foreground image to estimate the total number of worms on-chip.

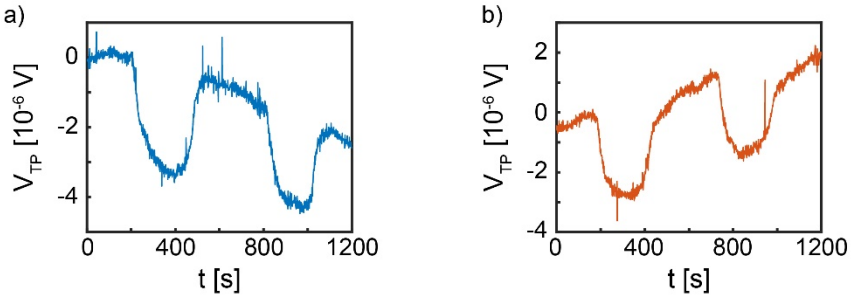


Figure S3: Long-term control experiment with L3 worms (for population information see Table S1, assay 4). a) $V_{TP}(t)$ response of the oscillatory flow profile (2 cycles) after worm loading and 60 min thermal stabilization. $V_{pop}$ values of 3.4 µV (cycle 1) and 3.1 µV (cycle 2) were calculated. b) $V_{TP}(t)$ response of the oscillatory flow profile of the same untreated population after on-chip incubation for 8 hours in buffer without bacterial food supply. $V_{pop}$ values decreased slightly to 2.5 µV (cycle 1 and cycle 2). No baseline correction was applied in these cases.

This control assay shows that the measured increase in $P_{pop/FCCP}$ after FCCP treatment compared to the initial $P_{pop}$ values (Fig. 4 and 5, after a total experimental time span of 3-4 hours) is not due to a shift caused by long experiment durations, but by the FCCP treatment indeed. The slight decrease of $V_{pop}$ values may possibly be attributed to starvation and/or arrested development of the on-chip worm population.

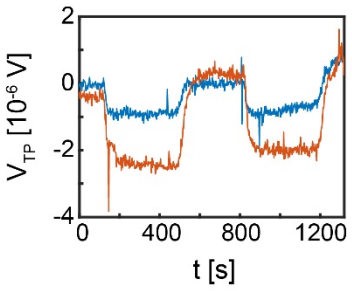


Figure S4: Representative $V_{TP}(t)$ response of a L2 worm population, before (blue curve) and after (red curve) FCCP treatment (for population information see Table S1, assay 1).

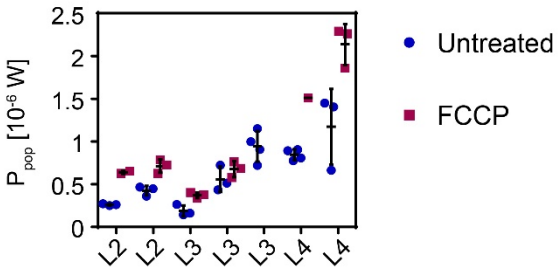


Figure S5: Individual data for each metabolic assay indicated in SI Table 1: Total metabolic heat power $P_{pop}$ ($P_{pop}$ = $V_{pop}$/PS with PS = 3.4 V/W) generated by an on-chip worm population before and after FCCP treatment. Data points represent $P_{pop}$ values obtained by a typical loading/unloading cycle ($n$ = 3), error bars represent mean ±SD.

| Assay | Larval stage | Worm age (hours) | Number of worms | FCCP treatment |
|---|---|---|---|---|
| 1 | L2 | 12 | 170 | Yes |
| 2 | L2 | 12 | 100 | Yes |
| 3 | L2 | 12 | 100 | Yes |
| 4 | L3 | 24 | 135 | No |
| 5 | L3 | 24 | 60 | Yes |
| 6 | L4 | 48 | 220 | Yes |
| 7 | L4 | 48 | 100 | Yes |

Table S1: Metabolic heat assays performed in this work, using *C. elegans* populations of different larval stages. The worm count was done on processed stereomicroscope images (see ESI Fig. S2).

| Larval stage | Worm volume [$10^6$ µm$^3$] (Worm age [hours]) | mtDNA copies |
|---|---|---|
| L2 | 0.21 (12 h) | 22000 |
| L3 | 0.25 (24 h) | 29000 |
| L4 | 1.92 (48 h) | 130000 |

Table S2: *C. elegans* larval body volume at different stages (courtesy of B. Atakan, EPFL-LMIS2) and the total number of mtDNA copies per larva (N2 wild type worms). Values for the worm length and area were extrapolated from 2D images at the indicated age, then the volume was calculated using the formula proposed in [2]. mtDNA numbers for a specific larval stage was taken from [3] (exact age in hours was not reported).

| Larval stage | *p*-value | *t*-ratio | *df* | $P_{worm}$ and $P_{vol}$ increase by FCCP treatment |
|---|---|---|---|---|
| L2 | 0.006 | 3.185 | 15 | 95% |
| L3 | 0.003 | 4.110 | 8 | 63% |
| L4 | 0.026 | 2.662 | 9 | 55% |

Table S3: Student's *t*-test with a 95% confidence limit ($p < 0.05$) of metabolic heat data and percentage of increase of metabolic heat production $P_{worm}$ and $P_{vol}$ upon FCCP treatment.

## REFERENCES


1 R. F. Lama and B. C.-Y. Lu, *J. Chem. Eng. Data*, 1965, **10**, 216–219.
2 B. T. Moore, J. M. Jordan and L. R. Baugh, *PLOS ONE*, 2013, **8**, e57142.
3 W. Y. Tsang and B. D. Lemire, *Biochemical and Biophysical Research Communications*, 2002, **291**, 8–16.