Electronic Supplementary Material (ESI) for Lab on a Chip


## SUPPORTING INFORMATION

# A MICROCHAMBER ARRAY FOR SINGLE CELL ISOLATION AND ANALYSIS OF INTRACELLULAR BIOMOLECULES

*K. Eyer[+], P. Kuhn[+], C. Hanke, P. S. Dittrich**

*ETH Zurich, Department of Chemistry and Applied Biosciences, CH-8093 Zurich (Switzerland)*

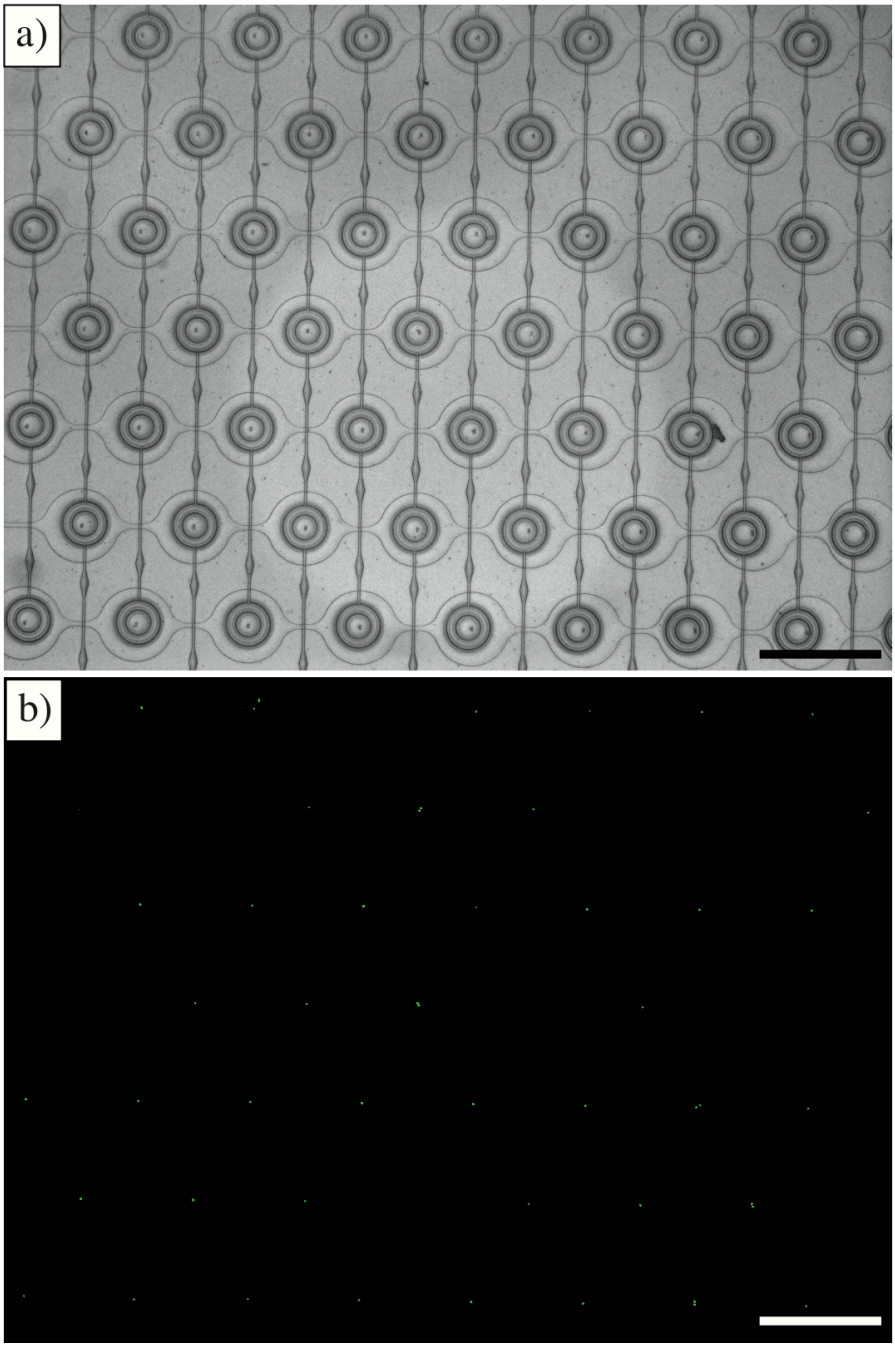


**Figure S1:** Images of single cells trapped and isolated in parallel. a) Brightfield image. b) Fluorescence image, each bright dot represents a single cell. Cells were stained with CellTracker™ Green from Invitrogen. Images were recorded with a Nikkon Multizoom AZ100M microscope equipped with a long distance objective, mercury lamp and optical filters (BrightLine Basic GFP BP Filter set). Scale bars: 1mm.

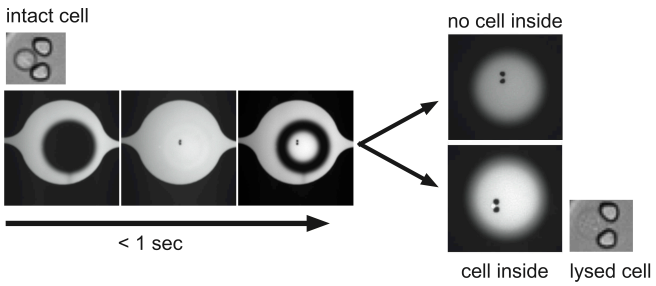


**Figure S2.** Images of a typical experiment. First, cells are trapped in the microchamber where they can be isolated, washed or incubated with further buffer solutions if required. The solution encapsulated inside the chamber is exchanged with lysis buffer in less than 1 sec by opening and closing. This image series was taken for a NAD(P)H determination. Here the lysis buffer contained the assay compounds, i.e. weakly fluorescent resaruzin and the enzyme diaphorase. If no cell was trapped in the microchamber, the weak fluorescence of resaruzin was visible. If a cell was trapped, fluorescent resorufin was produced which correlated to the amount of NAD(P)H. The morphological change from intact to lysed cells could also be detected via bright field imaging.

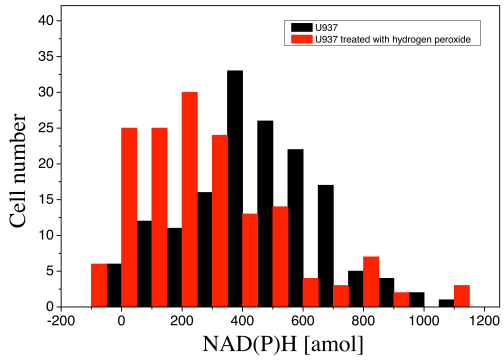


**Figure S3.** Determination of NAD(P)H in single cells. The histogram shows the analysis of 150 individual cells (not treated: black columns, treated with hydrogen peroxide: red columns, columns are displaced for clarity). Treated cells contain much less NAD(P)H than the untreated cells. However, few cells still maintain large NAD(P)H contents despite the exposure to oxidative stress.

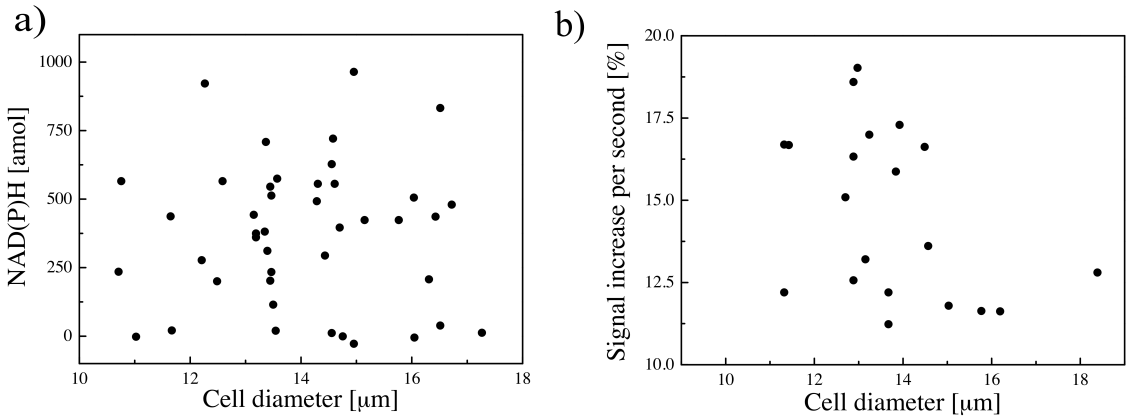


**Figure S4.** Correlation of cell diameter and measured concentration of a) NAD(P)H and b) glucose 6-phosphate dehydrogenase. Cell diameters were measured from brightfield images taken before the microchambers were opened to lyse the cell. The levels of the coenzymes as well as the enzyme were independent on the cell size.

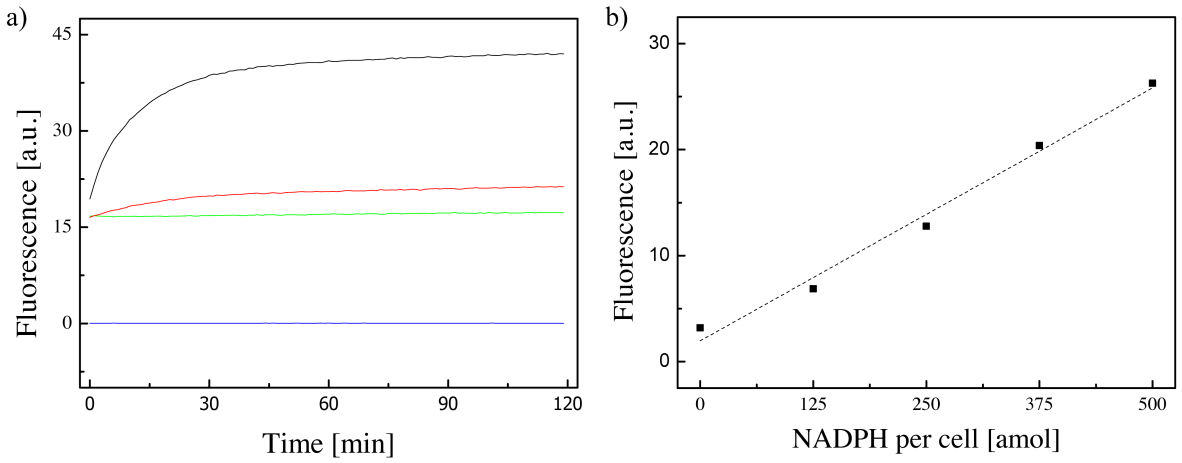


**Figure S5.** Plate reader results. a) Control experiments. If no dye was added, no fluorescence could be observed (blue). If cells were lacking in the lysis mixture, no increase in fluorescence was observable (green). If diaphorase was not added, a small increase in fluorescence was visible (red). For comparison, the signal for the complete assay was shown (black). For these experiments, 400,000 cells were measured in 200 µL lysis buffer. b) Calibration curve obtained from NADPH solutions in different concentrations from 0-1000 nM, here divided by 400,000 and multiplied with 200 µL to obtain the amount per cell (slope 0.05 ± 0.003, $r^2$=0.98). The findings support the on-chip measurements.

**Data correction for the G6PDH assay**

**1) Background correction and normalization of signal and bleaching data:**

Normalized, background-corrected signal with the measured fluorescence intensity inside ($I_S(t)$) and outside ($I_{S,BG}(t)$) the microchamber:

$$S_{norm}(t) = \frac{S(t)}{S_{max}} \qquad \text{with: } S(t) = I_S(t) - I_{S,BG}(t)$$

Normalized, background-corrected bleaching curve with the measured intensity inside ($I_{Bl}(t)$) and outside ($I_{S,BG}(t)$) the microchamber <u>after</u> completion of the assay:

$$Bl_{norm}(t) = \frac{Bl(t)}{Bl_{max}} \qquad \text{with: } Bl(t) = I_{Bl}(t) - I_{Bl,BG}(t)$$

**2) Bleaching correction and normalization**

Corrected signal curve: $S_{corr}(t) = S_{norm}(t) + \left(1 - Bl_{norm}(t)\right)$

Normalized (to 100), corrected signal curve: $S_{corr,norm}(t) = 100 \bullet \frac{S_{corr}(t)}{S_{corr,max}}$