

## Multiparametric, six (+n) channel, online analysis of MCF-7 cells

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### Introduction

Long time monitoring of cell viability and response to changes in environmental conditions play a crucial role in applications such as chemosensitivity [1] or environmental monitoring [2]. The presented six channel IMOLA – which is a sensor-chip based approach [2] - was used to monitor the kinetic of cellular respiration and extracellular acidification of MCF-7 cells for 48 hours in six channels parallel. Previously parameters as number of seeded cells per chip, pre-incubation time before measurement and reproducibility between the six channels and between two different experiments were investigated.

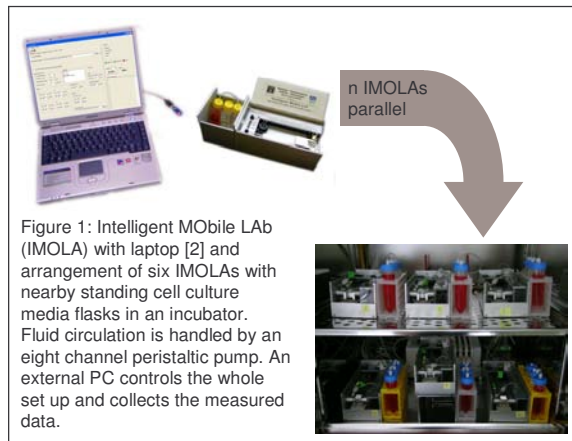


Figure 1: Intelligent MOBILE Lab (IMOLA) with laptop [2] and arrangement of six IMOLAs with nearby standing cell culture media flasks in an incubator. Fluid circulation is handled by an eight channel peristaltic pump. An external PC controls the whole set up and collects the measured data.

### Materials and Methods

- Setup:** Six IMOLAs were combined in one incubator. The fluidic life support system was managed by one 8 channel peristaltic pump (see Figure 1). The pump was set up to a stop (7 min) and go (3 min) mode and a pump rate of 60 µl/min.
- Chips:** Ceramic based multiparametric sensor chips with an amperometric dissolved oxygen sensor, two metal oxide sensors for pH-measurement, one Pt1000 temperature sensor (only for temperature surveillance) and two interdigitated electrode structures for impedance measurement (not used) were utilized. The active chip surface for cell growth has a diameter of 6 mm. The cell culture volume is adjusted to 7µl via a spacer ring.
- Cells:** The adherent cell line MCF-7 (human mamma carcinoma cells) were seeded directly on the chips and incubated at 37 °C before the measurement was started.
- Media:** For subculture and prior incubation on the chip, Dulbeccos Modified Eagle Medium (DMEM) with 5 % FCS was used. During measurement DMEM with 5% FCS and 50 µg/ml Gentamycin was used. For sensor calibration phosphat buffered saline (PBS) with different pH values was used.
- Cell numbers:** Different cell numbers between  $2 \cdot 10^4$  and  $10 \cdot 10^4$  cells were seeded on chips and incubated for 1 to 3 days. Growth area coverage was determined optically via reflected light microscopy.

### Results

#### a) Determination of initial cell number:

In Figure 2 reflected light microscopy images are shown where the cover of the chip surface with a cell monolayer was evaluated. Table 1 shows data where the threshold toward 100 % cover was detected.

#### b) Reproducibility:

The reproducibility of the metal oxide sensors between the IMOLAs (n = 12) was 55,3 mV/pH with a standard deviation of 7,4 in a first experiment. In a second experiment (with different PBS; n = 12) it was 58,0 mV/pH with a standard deviation of 7,6.

#### c) Monitoring of cell vitality:

In figure 3 the monitoring of cellular respiration and extracellular acidification of MCF-7 cells during a period of 1 h is shown. In figure 4 the kinetic of cellular vitality for 48 h (MCF-7) and the response to an addition of 0,2% Triton X-100 solution is shown.

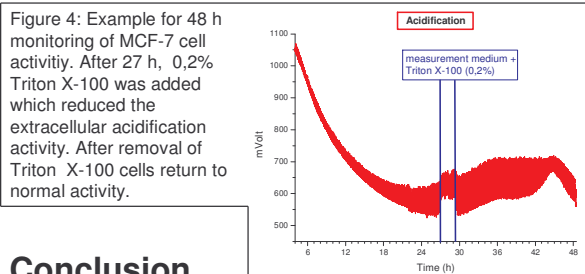


Figure 4: Example for 48 h monitoring of MCF-7 cell activity. After 27 h, 0,2% Triton X-100 was added which reduced the extracellular acidification activity. After removal of Triton X-100 cells return to normal activity.

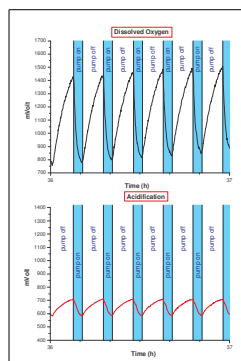


Figure 3: Example for acidification and oxygen consumption of MCF-7 cells on the Chip.

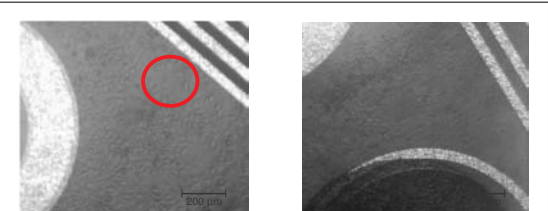


Figure 2: Surface covered with cell monolayer. Left:  $3 \cdot 10^4$  MCF-7 cells after 2 days of incubation. About 90% of the chip cell growth area is covered. The red circle indicates (e.g.) a not completely covered area. Right:  $4 \cdot 10^4$  MCF-7 cells after 2 days of incubation. 100% of the chip cell growth area is covered. Cells start to grow stacked on top of each other.

Table 1: Area covered with cells dependent on initial cell number

Number of seeded cells	Incubation period	Sample size	Average cell growth area covered
$3 \cdot 10^4$	2 days	n = 7	79,3 %
$4 \cdot 10^4$	2 days	n = 6	> 100 %

### References

- [1] B. Wolf, M. Kraus, M. Brischwein, R. Ehret, W. Baumann, M. Lehmann: Biofunctional hybrid structures - cell-silicon hybrids for applications in biomedicine and bioinformatics. Bioelectrochemistry and Bioenergetics. 1998, 46(2), 215-225.
- [2] J. Wiest, T. Stadthagen, M. Schmidhuber, M. Brischwein, J. Ressler, U. Raeder, H. Grothe, A. Melzer, B. Wolf: Intelligent Mobile Lab for Metabolics in Environmental Monitoring, Analytical Letters, 2006, 39(8), 1759 - 1771

### Conclusion

Optimal number of seeded cells was determined to be  $3 \cdot 10^4$  cells which should be incubated for a period of 48 hours previous to the experiment. Incubation periods of 28 h and less showed poor cell metabolism, as did cell numbers of  $2 \cdot 10^4$ . Higher cell numbers ( $4 \cdot 10^4$ ) and longer incubation periods (72 h) showed declining metabolism signals after 24 h (data not shown). Reproducibility experiments showed good results both between the six channels and in time separated runs. Long time monitoring of cell vitality for a period of 48 h and response to changes in environmental conditions could be achieved. Advantages of the n-channel IMOLA system are flexibility, no cross-contamination, non-invasive test and low-cost one-way chips

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Heraeus

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