# MODULAR, WIRELESS BIOELECTRONIC CELL CHIP SYSTEM

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**Abstract: A new intelligent mobile lab (IMOLA) system was deveoped for monitoring of metabolism of living cells. Parameters as the extracellular acidification, cellular respiration and adhesion / morphology can be determined. Such a device can be used in food quality analysis, chemosensitivity testing, pharmacology or for cellular research. The device has a modular set up and can be easily adapted to future devlopments. Determination of metabolism parameters of yeast cells is shown.**

## Introduction

For online monitoring of cell physiological parameters different devices and multiparametric measurement methods have been reported[1-3]. Parameters such as extracellular acidification, cellular respiration and changes in cell morphology/adhesion have been measured with different sensors. Measurement principles as pH – ISFETs[4], amperometric oxygen sensors[5], $O_2$-FETs[5;6], temperature diodes, Pt1000 and electric impedance (IDES)[7;8] were applied. Alternative sensor principles as metal-oxide based potentiometric pH sensors[9;10], optical sensors[5;11;12] or carbon nano tube based sensors[13] are currently under investigation. For a simple integration of different sensor chips (e.g. chips on silicon, glass and ceramic substrate) into a single standard type of measurement unit a new device was developed. A modular set up as shown in Figure 1 was designed and integrated into a new compact measurement device called intelligent mobile lab (IMOLA)[14]. Aspects for fluidics (1), sensor control (2), data management (3) and software (4) were considered. Common design rules as a shielding, guard lines or ground plains were incorporated. To fulfill the requirements a flexible modular set up was choosen. (1) Fluidics: To support the cells with cell culture media reservoir tanks, a pump, tubes and a chip adapter are needed. Additional a reference electrode has to be included and a protection against overfall had to be realized. (2) Sensor control: A seperate PCB board for the sensor control was necessary to achieve easy adaptability to the different sensor types. (3) Data management: A core module had to be designed to adjust the sensor control board, to digitize the gained sensor values, integrate a time stamp, buffer data and transfer data wireless. To allow the exchange of the sensor control board a standardized interface was designed. (4) Software: Due to the need for a mobile system the software was split into an embedded microcontroller part inside the mobile lab and into a standard personal computer application software. The software includes a user interface for measurement control, a data preprocessing and display unit for data analysis and a storage function.

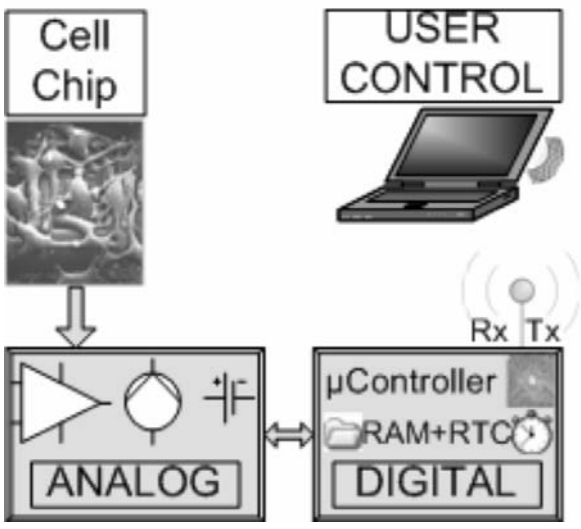


Figure 1: Modular, wireless bioelectronic cell chip system. Different sensor chips can be plugged in by adapting the analog module. The digital module which contains common interfaces as 16 bit A/D, D/A converters, $I^2C$ and RS232, a wireless transfer unit and the user control unit (Laptop or PC) with the application software is maintained.

## Materials and Methods

Generally the new device was inserted into a faradayic cage including the reservoir tanks and tubes to avoid electromagnetic disturbances (figure 2). Only the antenna for wireless data transfer was externally mounted. The reservoir tanks are connected at the back side of the device. They are included into an extra faradayic cage. This cage can be extended to meet specifications toward different or more reservoir tanks.
(1) Fluidics: To realize the life support system standard cell culture flasks and silicon tubes were used. As reference electrode a selfmade Ag/AgCl wire[15;16] and an electrolyte barrel (microelectrodes, inc., Bedford,


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USA) filled with 3M KCl saturated with AgCl was integrated into the outflow tube. For cell culture media transport a piezo micro pump (thinXXs, Mainz, Germany) was integrated.


Figure 2: Intelligent Mobile Lab device. The front part (size: 210mm x 105mm x 90mm) includes all the electronics, sensor chip, pump system and controls. The back part (size: 90mm x 105mm x 105mm) includes only the reservoir tanks.

A newly developed chip adapter which contains connectors for inflow, outflow (figure 3) and optional LED holding for investigation of photosynthesis of algae with a biosensor approach, is used. To avoid overflow of cell culture media the pump is driven in suction mode.

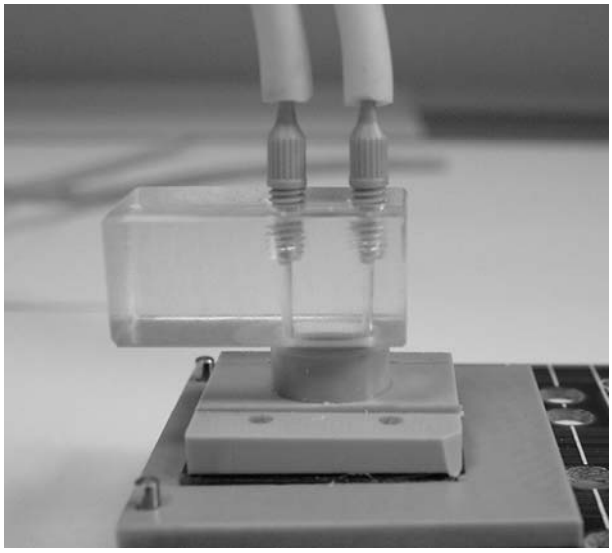

Figure 3: Connector, sensor chip, fluidic adapter and fluidic connectors. The sensor chip (middle) is set into the connector socket (height 3,0 mm) to contact the control PCB electronically. The fluidic adapter is pressed into the round cell culture well and seals it. Inflow and outflow tubes are plugged onto standard fluidic connectors. The transparent fluidic adapter has a mechanical extension for easier handling.

(2) Sensor control: Two versions of sensor control PCB were realized: (A) Silicon chip version with electronic circuits for four ISFETS, one Clark type oxygen sensor, one $O_2$-FET and one temperature diode. (B) Ceramic chip version with electronic circuits for two metal – oxide pH sensors, one Clark type oxygen sensor and one Pt1000. Both version are equipped with a mezzanine board for control of two IDES structures. To avoid crosstalking between the electrochemical active sensors the pH and oxygen sensors one both PCBs were galvanically isolated. As shown in figure 3 the control PCB has a holding for the chip socket where the sensor chip has to be placed. The connector area of the control PCB has no vias, the circuit path are made from NiAu and the borders are sealed with silicone. So a fluidic leakage can not affect any of the electronic circuitry besides the connection area. Furthermore it is possible to remove the chip connector without soldering and to clean the connection area with 70% Ethanol.
(3) Data management: Core of the IMOLA is the digital PCB. It is flexible with defined interfaces and can be used in different applications. The digital PCB (Figure 4) contains a microcontroller (PIC16F777), four 12 bit D/A converter (MAX5842), two 16 bit A/D converters (ADS8344), a real time clock (MAX6900), a 32kB EEPROM (24LC256), a wirelss transfer module (ALPS Japan) and additional electronics. The microcontroller is programmed to adjust the control parameters for the measurement, to convert analogue data, to buffer data and to transfer it wireless to a Labtop.

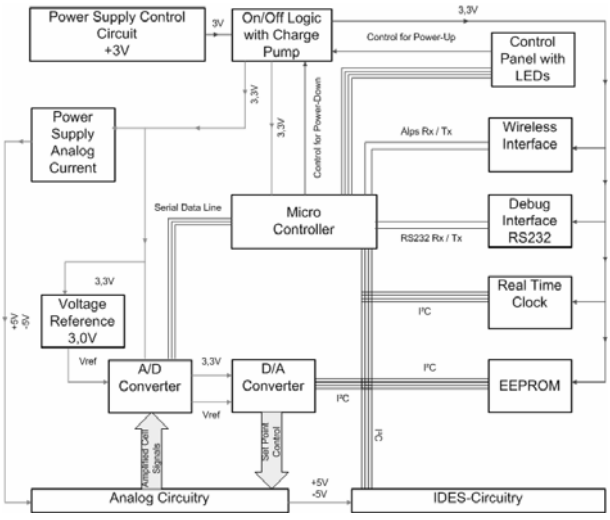


Figure 4: Architecture of digital data management module. Core is the PIC16F777 microcontroller. The control panells are connected to digital outputs. Two RS232 ports are implemented for connection of the wireless interface and for the debug plug. The D/A converter, the buffer, the clock and the IDES circuitry are connected via an $I^2C$ – bus. The A/D converters are connected directly via a fast serial bus.


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(4) Software: The software routine of the microcontroller is shown in figure 5. To optimize the lifetime of a wireless measurement the energy intensive wireless data transfer time was minimized by buffering the data in the EEPROM. If the EEPROM is filled with data a wireless connection is established, measurement data is transfered to the labtop (optional via a smartphone), new configuration values are received and the wireless connection is cut. The application software at the labtop has input fields for configuration data (i.e. pump and measurement cycle time, sensor control wave forms, etc.) a display unit for online monitoring of the measurement data, post processing tools and a data storage function. There are two software routines for the microcontroller available. (A) for the silicon chip version and (B) for the ceramic chip version. The labtop application software can be swiched via the properties menu. An IMOLA system can be switched from a silicon version to a ceramic version by exchanging the sensor control PCB and by additional adaption of the microcontroller software.

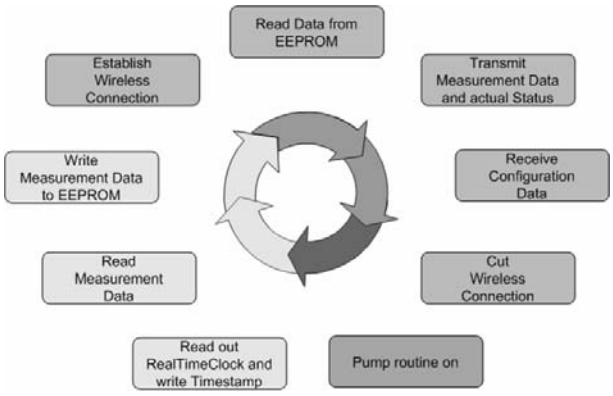


Figure 5: Software routine of the microcontroller. The dark grey fields are run thru only one time per measurement cycle. The bright grey fields are repeated until the buffer of the EEPROM is almost full (about 750 single readings of all sensors).

Due to the online access onto the sensor control parameters, aging processes or sensor drift can be compensated via the software and the sensor control parameter can be adapted. This is possible because after every pump cycle the cell culture media is defined and therefore can be used as a recalibration point. In case of an implemented reference sensor (for example an FET without an ion sensitive layer) fluctuations caused by changes in temperature or light can be compensated. Also a free programmable voltage cycle can be applied to the noble metal electrode of the $O_2$-FET.
At the current version two types of sensor chips can be used with the IMOLA. A well established high price silicon version (figure 6) and a new low price ceramic version (figure 7). Both chips include sensors for pH, $pO_2$, impedance and temperature.

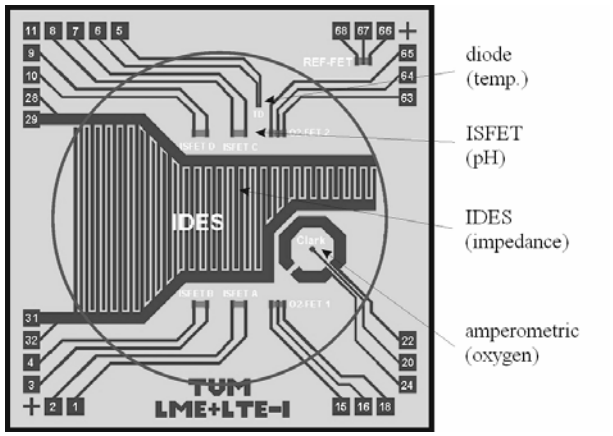


Figure 6: Silicon sensor chip with diode, ISFETs, IDES and Clark – type oxygen sensor realized at the Heinz Nixdorf department for Medical Electronics of the Technical University of Munich.

Both chips are bonded on standard PLCC68 carriers and encapsulated into a biocompatible plastic holding leaving just the active area (circle in figure 6) free for contact with the cell culture. A finished sensor chip is shown in figure 3. For measurements with different sensor chips only the analogue module has to be exchanged. Cells are grown directly on the surface of the sensor chip (figure 1). After cultivation of the cells the chip is inserted into the mobile device and the fluidic system is connected, providing a regular exchange of cell culture media and drug solutions. With the sensor device it is possible to monitor in real time cell physiologic conditions (cellular respiration, extracelluar acidification and changes in adhesion / morphology) and changes upon experimentally induced perturbations.

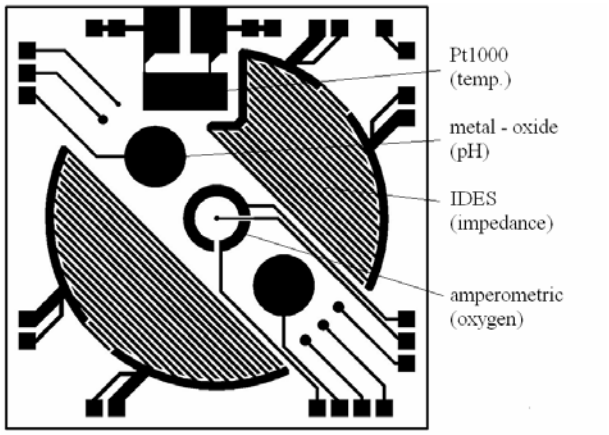


Figure 7: Ceramic sensor chip with Pt1000, metal – oxide pH sensor, IDES and Clark type oxygen sensor realized by Heraeus Sensor Technology GmbH.

The acquired data are digitized, preprocessed and transferred by wireless means to a laptop. With the IMOLA software application running at a laptop the cell signals can be analysed, displayed, and saved (figure 8). Also the control parameters for the used


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sensors and the control of the fluidic system is adjusted and processed by the IMOLA software.

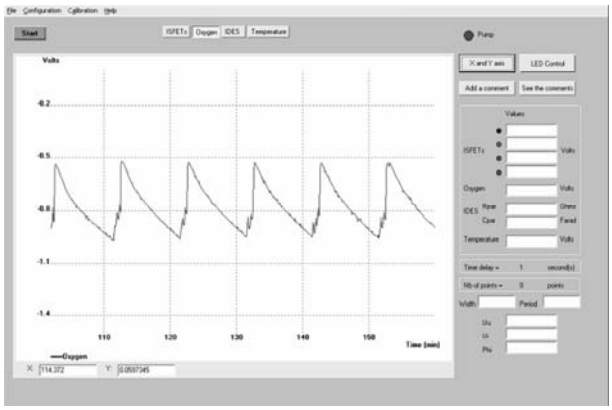

Figure 8: Screenshot of the software application: The data on cell physiology is displayed in the diagram. The right side is for adjustments of fluidics and sensor control. Calibration and configuration data can be changed in the menu on the top.

**Results**

Analysis of metabolism of continuous cell lines, human blood cells, yeast and bacteria were performed successfully. Changes in extracellular acidification, cellular respiration and adhesion/morphology can be monitored.

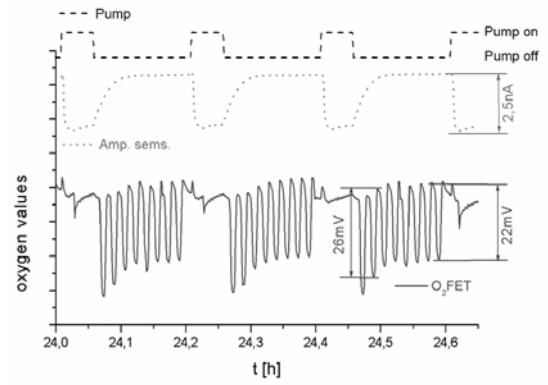


Figure 9: Monitoring of $O_2$ - consumption by yeast cells using the amperometric, and the $O_2$ - FET microsensor in parallel

To show the the ability of the device to measure the metabolism of living cells, the cellular respiration and extracellular acidification of yeast cells were monitored. In figure 9 three pump cycles of a long-term measurement with yeast cells is shown. RPMI - 1640 without glucose (Sigma - Aldrich Chemie, Taufkirchen, Germany) culture medium supplemented with 50 µg/ml Gentamycin to avoid bacterial growth was used. The amperometric sensor and the $O_2$ - FET show a return to base line values during the flow - on time. During the pump on mode the microsensors determine approximately the defined values of $pO_2$ and pH of the cell culture medium, i.e., large-scale sensor drift can be controlled and compensated. When the medium flow is stopped, the yeast cells start to consume oxygen (cellular respiration) and to excrete hydrogen ions (extracellular acidification) into the microenvironment of the cells. The amperometric sensor monitors relative changes in dissolved oxygen (in figure 9 a consumption of 100% of oxygen results in a current delta of 2.5nA). The $O_2$-FET also monitors the oxygen consumption which resulted in a voltage difference of (26mV – 22mV) 4mV. Additional the $O_2$-FET monitors the extracellular acidification which resulted in a difference of 6mV. The signals showed constant values after about 3 min, indicating that oxygen had been consumed completely. After the stationary phase, the pump started again and the signals returned to baseline values.

**Discussion**

With the newly developed IMOLA system it is possible to monitor changes in extracellular acidification, cellular respiration, cell adhesion and the surface grown with adherent cells, i.e. cell proliferation rate. Sensor-based monitoring avoids cell labelling and it allows to yield real time data. Measurements of absolute values of pH and oxygen are only possible with more elaborate calibration routines.
Thermostatting of the IMOLA is currently achieved by placing the device into an incubator. Certainly, an autonomous device useful for field applications will have to provide an exact temperature control for the cell culture media and, most important, for the cells. This temperature control system should work independently of the ambient temperature in a range between 0 $^oC$ and 37 $^oC$ and typically provide a temperature of 36,5 $^oC$ +/- 0.5 $^oC$. Technologies to meet this requirement are already existing.
Improvements of the sensor performances are expected from changing the sensor geometries/configurations or the electronic sensor control. Combinations of parameters, such as a pulsed amperometric sensor supply or the parallel recording of the nA current at the $O_2$-FET, will be investigated to reduce noise and to improve $pO_2$ sensitivity. Due to the modular design of the system future developments in sensor technology may be easily implemented by an exchange of the analogue module and by adaptation of the embedded software. Further efforts are made to implement self – organisation of more than one of such devices and to improve miniaturization. Furthermore the data transmission range of the device which is now about 10 meters shall be extended.

**Conclusion**

A new digital, modular, wireless and portable bioelectronic cell chip system was developed allowing optimal control of different sensor chips. The system can be operated with batteries for field applications. For easy adaption to different applications the system has a modular setup separated into (1) fluidics, (2) sensor control, (3) data management and (4) software application. Multiparametric metabolic profiling of


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yeast cells recorded with a silicon biosensor chip was shown.

Now a highly adaptable device for use in fields like toxicology (detection of toxins via their effect to living cells), chemosensitivity (measurement of the effectivity of cancer drugs), pharmacology (investigation of the effect of new substances), or environmental monitoring (measurement of metabolism of algae as an indicator for water quality) is available. The device has also applications as an alternative for animal experiments [6]. For example an irritation test can be performed with an in-vitro cell culture instead of using an animal.

Further efforts are made to implement self – organisation of more than one of such devices and to improve miniaturization. Furthermore the range of the device which is now about 10 meters shall be extended.

**Acknowledgment**

Authors would like to thank the 'Heinz Nixdorf Stiftung', the 'Bayrische Forschungsstiftung' and the 'Bund der Freunde der Technischen Universität München' for supporting the ongoing work.

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ISSN: 1727-1983 