

## Micro system comprising 96 micro valves on a titer plate

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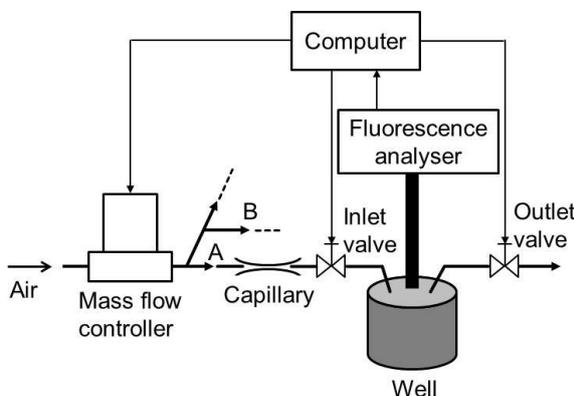
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**Abstract.** A system of 96 micro valves has been developed and mounted on top of a 48-well micro titer plate providing two valves for each well controlling its air inlet and outlet. Testing of the valve system showed that all valves are working and are opened and closed reliably. A pneumatic system is switching inlet and outlet valves independently of each other. The geometry of the feed channels ensures an equal air flow through all wells, when the valves are open. Between the micro valves, one optical fibre was inserted through the lid of each well allowing measuring the oxygen partial pressure in the enclosed air volume by fluorescence sensor spots. *Escherichia coli* bacteria were grown inside the wells and their metabolism was observed by the oxygen partial pressure change due to respiration. In all 48 wells, the same oxygen transfer rate was observed within an averaged standard deviation of 1 mmol/L/h. The oxygen transfer rate differences compared to a macroscopic standard shake flask system were overall compatible within their uncertainties.

### 1. Introduction

The metabolism of cells in bio reactors is often observed by measuring their oxygen consumption. A macroscopic commercially available device providing such measurements is the Respiratory Activity Monitoring System (RAMOS) presented in [1] and [2]. It determines the respiratory activity of aerobic microorganisms by measuring their oxygen transfer rate in eight shaking flasks, each with an inner volume of 0.125 to 1 L. The capacity of RAMOS now has been enlarged from the parallel observation of 8 vessels to the 48 wells of a micro titer plate [3]. This new device is called  $\mu$ RAMOS.

To achieve this, each well was equipped with an inlet and an outlet valve (cf. Fig. 1). The oxygen consumption in the wells was determined within measurement circles. Each circle started with the closing of both valves. Then the change of the oxygen partial pressure was measured for 3 minutes. After that time first the outlet and then the inlet valves of the wells were opened. A time difference of 1 s between switching of the two valves made sure that no overpressure in the wells could occur. After venting the wells for 17 minutes, a new



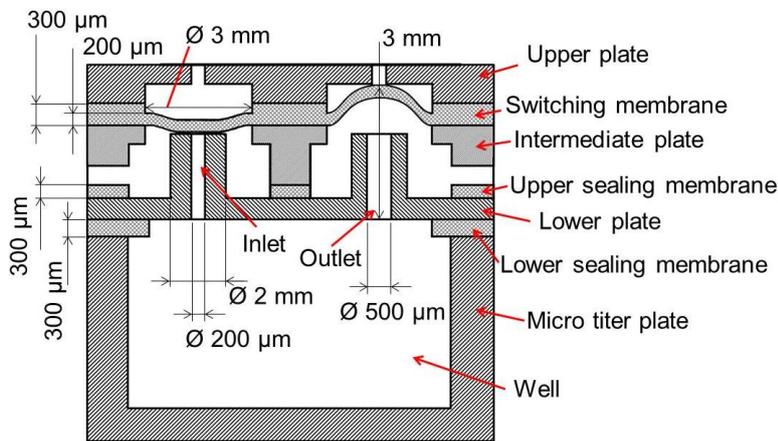
**Figure 1.** Schematic drawing of the setup realized for each of the 48 wells of the  $\mu$ RAMOS system.

measurement cycle was started.

To provide such a measurement cycle, it was necessary to equip every well with two valves, which can be switched independently of each other and with an oxygen sensor. The oxygen sensor was provided by Pyro Science GmbH in Aachen, Germany. It consists of a polymer whose fluorescence is a function of the oxygen partial pressure in the well. The oxygen sensor was glued on the lid of the wells and was excited to fluorescence and read out by an optical fibre, 2 mm in diameter. All these components need to fit into a circle, 12.4 mm in diameter, available at the top of each well. In this paper, there are described the design and testing of the valve system.

## 2. Design of the micro valve system

The micro valve system consists of three plates milled from poly methyl methacrylate (PMMA) and three casted silicone membranes with micro structures (Fig. 2). This stack of plates and membranes is pressed onto a micro titer plate by screws and an aluminium holder. The upper and the bottom plates comprise separate fluidic systems. The upper plate again consists of two pneumatic microfluidic systems separately switching outlet and inlet valves. The bottom part provides an equal air flow to each of the 48 wells.

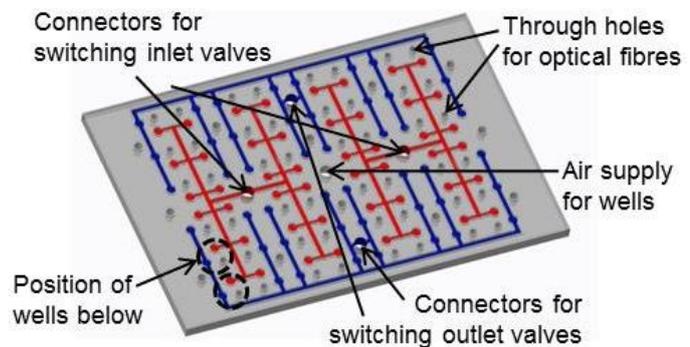


**Figure 2.** Schematic drawing of the two valves on top of each well of the 48-well micro titer plate. Dimensions are not to scale.

it is necessary to pull the switching membrane up with 35 kPa underpressure when the valve shall be opened. On the other hand, when the valves shall be closed, an overpressure of 65 kPa is applied making sure that all micro valves are tightly closed.

### 2.1. Pneumatic switching system

The upper PMMA plate contains four micro fluidic channel systems. Two of them are employed for switching all inlet valves and the other two for switching all outlet valves. The respective two fluidic systems are merged by tubes and tube connectors above the valve system. The connector for the air flow supply into the wells is located in the centre. All channels are 1.5 and 2 mm in width and depth, respectively. They widen up to circles, 3 mm in diameter, in the valve areas. The thickness of the switching membrane is 200 μm where it needs being deflected. To focus the pressure provided by the screws onto the sealing areas of the membrane, this area was



**Figure 3.** Drawing of the upper PMMA plate comprising the valve systems for switching the inlet (red) and outlet (blue) valves.

valve chambers. In the sealing areas the thickness of the switching membrane is 300  $\mu\text{m}$  and where it is neither employed as a valve membrane nor for sealing, the membrane thickness is 500  $\mu\text{m}$ .

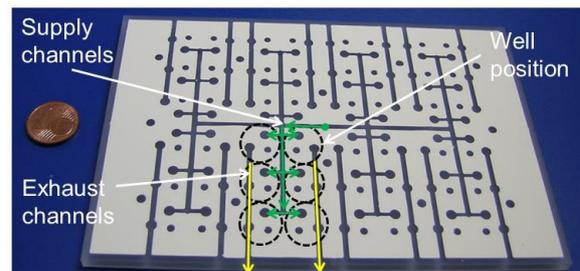
### 2.2. Air supply system

The micro fluidic system providing the air flow into and out of the wells is formed by intermediate and lower PMMA plate and the upper sealing membrane. All channels are of the same size as the channels of the switching system (1.5 x 2 mm<sup>2</sup> and widening to circles in the valve areas).

To ensure an equal flow through all wells, it was defined by the dimensions of the orifices in the inlet valves. Diameter and length of these orifices are 200  $\mu\text{m}$  and 3 mm, respectively. The length of the orifices in the outlet valves is the same as for the inlet valves but their diameter is 500  $\mu\text{m}$ . This way, it is made sure that the flow resistance of the entire system is defined by the size of the inlet orifices alone. The flow resistances of the orifices of inlet and outlet valves and the feed channels at 37 °C are 94, 2.4, and 5 Pa s/mL, respectively, as calculated with the Hagen Poiseuille equation (see, e.g., [4]).

The exhaust channels lead to the rim of the intermediate plate disposing the used air to the environment.

The upper sealing membrane is 300  $\mu\text{m}$  thick. Its thickness also defines the distance of the valve seats to the non-deflected switching membrane which is 100  $\mu\text{m}$ . In an open valve, the switching membrane is deflected 1.2 mm off the valve seat.



**Figure 4.** Photo of the lower PMMA plate covered by the upper sealing membrane from silicone.

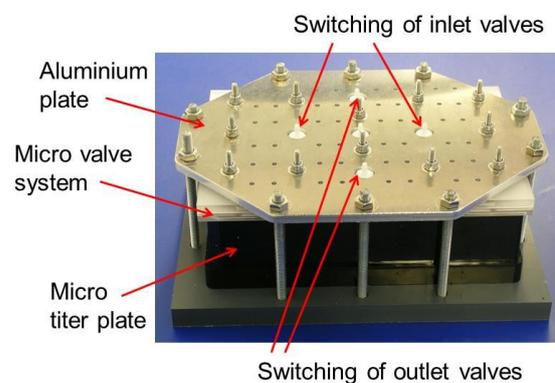
### 3. Fabrication

The PMMA plates as well as the moulds for the silicone membranes and the aluminium holder were milled by the milling machine M7HP from Datron AG, Mühltal, Germany. The milling tools were purchased also from Datron AG. The drilling heads, 200  $\mu\text{m}$  and 500  $\mu\text{m}$  in diameter, required for drilling the orifices of the valves were purchased from vhf camfacture AG, Ammerbuch, Germany.

The drilling parameters for the 200  $\mu\text{m}$  holes were a feed rate of 400 mm/s and 10,000 rotations per minute while cooling with air at 300 kPa. The parameters for the 500  $\mu\text{m}$  wide holes differed only in the feed rate of 500 mm/s.

Milling and drilling was performed in extruded PMMA plates, 4 mm in thickness. Optimum fabrication parameters are a strong function of the material used and its processing (casting or extrusion). The membranes were cast from the silicone Elastosil RT 625 A mixed at a 9 to 1 ratio with its hardening component RT 625 B from Wacker Chemie AG, Munich, Germany, in an open air process at room temperature. As moulds PMMA plates were employed which had been milled as described above. After casting the silicone into the moulds they were closed with a PMMA plate and pressed together by tongs squeezing out surplus silicone. After hardening the membranes were removed from the moulds and cleaned.

Four micro systems each with 96 valves have been fabricated up to now and all of their micro valves are opening and closing tightly. One micro titer plate with the micro valve system mounted on top is shown in Fig. 5.

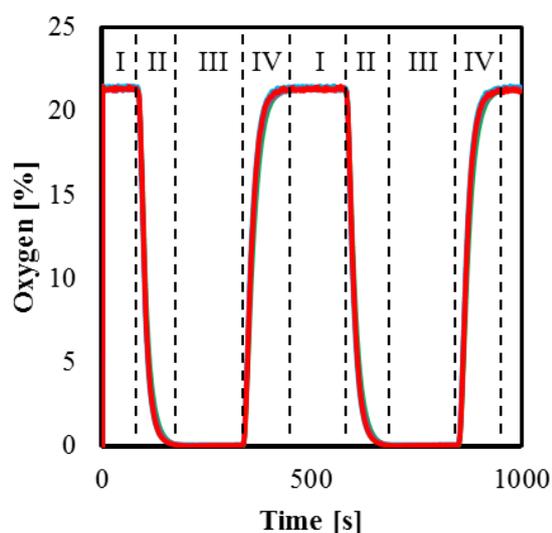


**Figure 5.** Photo of the micro valve system mounted on top of a micro titer plate.

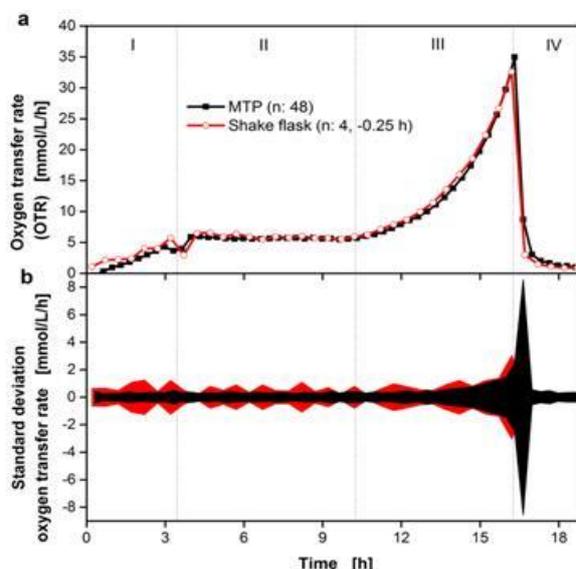
#### 4. Testing

For testing, the gas flow through the wells was changed from air to  $N_2$  and back while the valves were switched and the oxygen partial pressure was measured in the wells. In Fig. 6, the converted oxygen concentrations in four wells are drawn. In phase I, the wells were filled with air and then all valves were closed. Then (phase II), the outlet and 1 s later the inlet valves opened and  $N_2$  was flowing in. In phase III, first the inlet and 1 s later the outlet valves closed. In phase IV, first the outlet and then the inlet valves opened again and air was flooding the wells. During phases I and III no leakage was observed although the  $N_2$  and the air pressure, respectively, already had been applied to the inlet channels. The average difference of oxygen concentration in the wells was 0.15 %.

Mean values of the measured oxygen transfer rates of 48 wells and 4 shake flask cultivations are shown in Fig. 7a [3]. No significant deviations of the mean oxygen transfer rates are found between RAMOS and  $\mu$ RAMOS (Fig. 7b).



**Figure 6.** Oxygen concentration in four wells measured during two switching cycles with air and  $N_2$ .



**Figure 7.** Comparison of *E. coli* BL21 (DE3) pRotHi-YFP cultivations using RAMOS shake flask system and  $\mu$ RAMOS MTP system.

#### 5. Conclusions

Micro valve systems each with 96 micro valves have been manufactured and successfully tested. The measured oxygen transfer rates in all 48 wells deviate by less than 1 mmol/L/h and, therefore, show that reliable investigations of cell cultures can be performed in such devices. These results were achieved by conventional milling of PMMA plates and casting of silicone membranes.

#### References

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