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## **O<sub>2</sub>-sensitive microcavity arrays: A new platform for oxygen measurements in 3D cell cultures**

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### **Abstract:**

Oxygen concentration plays a crucial role in (3D) cell culture. However, the oxygen content in vitro is usually not comparable to the in vivo situation, which is partly due to the fact that most experiments are performed under ambient atmosphere supplemented with 5% CO<sub>2</sub>, which can lead to hyperoxia. Cultivation under physiological conditions is necessary, but also fails to have suitable measurement methods, especially in 3D cell culture. Current oxygen measurement methods rely on global oxygen measurements (dish or well) and can only be performed in 2D cultures. In this paper, we describe a system that allows the determination of oxygen in 3D cell culture, especially in the microenvironment of single spheroids/organoids. For this purpose, microthermoforming was used to generate microcavity arrays from oxygen-sensitive polymer films. In these oxygen-sensitive microcavity arrays (sensor arrays), spheroids cannot only be generated but also cultivated further. In initial experiments we could show that the system is able to perform mitochondrial stress tests in spheroid cultures to characterize mitochondrial respiration in 3D. Thus, with the help of sensor arrays, it is possible to determine oxygen label-free and in real-time in the immediate microenvironment of spheroid cultures for the first time.

Keywords: oxygen measurement, spheroid organoid, fluorescence quenching, mito stress test, 3D cell culture, microcavity array