Real-time measurement of intracellular \( \text{O}_2 \) in mammalian cells

**Introduction**

In the majority of *in vitro* studies cells are cultured at ambient oxygen – ignoring the oxygen gradient between the atmosphere and the medium as well as the gradient between the medium and the intracellular cell environment. As the level of available oxygen significantly influences cell physiology any data obtained from cell-based *in vitro* studies should be related to the level of cellular oxygenation. A combination of a microplate reader with atmospheric control unit (ACU) and oxygen sensitive probes enables to run assays at defined oxygen environment and to monitor the oxygen experienced by the cells.

**Materials and Methods**

The oxygen sensitive probe MitoXpress\textsuperscript{®} Intra by Luxcel Biosciences reports on intracellular oxygenation as its phosphorescent signal is quenched by oxygen. Here, it was used in 3D and 2D culture of HepG2 cells to investigate the effect of environmental oxygen changes and mitochondrial manipulators on intracellular oxygen levels. Cells were loaded with the probe and exposed to either varying levels of oxygen created in the CLARIOstar\textsuperscript{®} microplate reader by the atmospheric control unit (ACU) or to the respective drugs, injected by the reader’s injectors. The CLARIOstar detected kinetic traces of time-resolved fluorescence that represented intracellular oxygenation.

**Results and Discussion**

The data in Figure 1 illustrate the effect of environmental oxygen and cell respiration on the \( [\text{O}_2] \) in liver cells cultured in 3D. The initial \( [\text{O}_2] \) set by the microplate reader ACU was ~19%; however, the measured \( [\text{O}_2] \) of cells was around 10%. This decrease in \( [\text{O}_2] \) was due to rapid local depletion of oxygen, caused by oxygen consumption through cellular respiration. At the levels of environmental oxygen typically used by researchers studying hypoxia (~5%), the local \( [\text{O}_2] \) of the cells was close to zero.

Addition of the mitochondrial inhibitor antimycin to a HepG2 monolayer increased the cellular oxygen to the ambient level of 18 % as respiration and, hence, oxygen consumption was blocked. In contrast, increasing the oxygen consumption rate through treatment with the mitochondrial uncoupler FCCP caused an acute and dramatic decrease in \( [\text{O}_2] \) to ~2–3 (Fig. 2).

**Conclusion**

The CLARIOstar\textsuperscript{®} equipped with an atmospheric control unit (ACU) is an excellent choice for measuring intracellular oxygen as it ensures temperature and gas control required for long-term cell-based assays.