The closest to physiology
State-of-the-art cell culture employs biosensors to study biological changes arising from drugs or different genetic backgrounds in real-time. The aim is to conduct experiments that resemble physiological conditions as close as possible. This is achieved by addition of growth factors or by choosing particular culturing methods such as 3D cell culture. However, one major aspect is mostly neglected: oxygen (O₂) tension.
Within a human body, cells are exposed to O₂ concentrations that vary between 1-14 %, depending on the organ or tissue. In contrast, in vitro experiments are usually conducted at ambient O₂, i.e. approximately 20 %. These hyperoxic conditions affect cellular behaviour detrimentally. In fact, the O₂ environment impacts on gene and protein expression, alters the energy metabolism and secretion of soluble factors. The use of tissue-specific O₂ conditions will result in more reliable in vitro data that better translate to in vivo situations.

A model for ischaemia/reperfusion
Disturbance of tissue-specific and stable O₂ conditions is often related to life-threatening diseases. Reduced O₂ and nutrient supply due to impaired blood flow occurs during stroke, myocardial infarction, shock, atherosclerosis and cancer.
Despite being essential for survival, reperfusion and sudden re-oxygenation of tissues upon ischaemia induces significant cell damage through the formation of reactive oxygen species and activation of inflammatory responses. To date, in vitro solutions to study cellular reactions in response to ischaemia and reperfusion are not amenable to real-time monitoring and are limited in throughput. Investigating these pathologies in vitro requires an experimental set-up capable of rapid deoxygenation and reoxygenation.
The CLARIOstar® with Atmospheric Control Unit (ACU) was engineered to not only provide stable atmospheric O₂ and CO₂ tensions, but also to run user-definable protocols of O₂ deprivation and reoxygenation.
For the first time you can fully manipulate the environment within the microplate reader, by mimicking in vitro hypoxia, ischaemia/reperfusion and much more.
Hypoxic and situational conditions affect cellular behaviour detrimentally. In fact, alterations in oxidative metabolism and secretion of soluble factors derived from rapid deoxygenation and reperfusion.

For the first time, you can fully manipulate the environment within the microplate reader, by mimicking in vitro hypoxia conditions. This is achieved by addition of growth factors or by choosing particular culturing methods such as 3D cell culture. State-of-the-art cell culture employs biosensors to study biological changes arising from drugs or different genetic conditions. In vitro solutions to study cellular reactions in response to pathologies in vitro requires an experimental set-up capable of rapid deoxygenation, rapid re-oxygenation of tissues upon ischaemia induces significant tension.

Despite being essential for survival, reperfusion and sudden hypoxic and reperfusion can be reproduced in vitro in a microplate reader. By using the Atmospheric Control Unit (ACU) module, you can regulate CO2 tensions, but also to run user-definable protocols of O2 gas ramps. For instance, the ACU can deprive O2 and then rapidly re-oxygenate back to physiological conditions, keeping mean steady CO2 levels. This capability enhances live cell-based assays, as disease models such as ischaemia/reperfusion can be reproduced in vitro in a microplate reader.

The CLARIOstar microplate reader equipped with software- focus adjustment, the ACU provides an ideal ‘walk-away’ solution for any long-term cell-based assay.

The ACU features include:
- O2 and CO2 control range: 0.1 - 20%
- O2 and CO2 gas ramps
- Gas value trackability in MARS data analysis software
- Intuitive interface with touchscreen, up to 10 gas pre-sets and gas concentration curve display
- Altitude correction for accurate gas regulation

**Fig. 1:** Example of ischaemia/reperfusion conditions mimicked in the CLARIOstar with ACU.

**Gas ramping**
As a unique feature within plate readers, the CLARIOstar offers the capability to run O2 and CO2 gas ramps. For instance, the ACU can deprive O2 and then rapidly re-oxygenate back to physiological conditions, keeping meanwhile steady CO2 levels. This capability enhances live cell-based assays, as disease models such as ischaemia/reperfusion can be reproduced in vitro in a microplate reader.

**Applications include:**
- Proliferation - Cell viability - Bacterial growth - Migration and invasion - Hypoxia - Angiogenesis - Ischaemia/reperfusion - Cytotoxicity studies - Viral uptake - Intracellular pH
The CLARIOstar with ACU exposes cells to ischaemia/reperfusion conditions and monitors their oxygenation

C. Carey, J. Hynes (Luxcel Biosciences Ltd., Cork, Ireland), M. Schwalfenberg, R. Kettenhofen (Axiogenesis AG, Cologne, Germany)

Introduction
The lack of oxygen supply is associated with a number of life-threatening diseases whereby cells are temporarily deprived of O₂ and nutrient (ischaemia). Significant cell damage can also occur during the reperfusion phase through oxidative stress and inflammation. Investigating these pathologies in vitro requires an experimental set-up capable of rapid deoxygenation, rapid reperfusion, and parallel monitoring of critical biological parameters including cellular oxygenation and Reactive Oxygen Species (ROS).

Assay principle
The ischaemia/reperfusion model presented here uses the CLARIOstar plate reader with programmable O₂ and CO₂ regulation in combination with MitoXpress®-Intra, [Luxcel Biosciences] which enables real-time monitoring of cellular oxygenation. Data are presented using HepG2 cells and iPS derived cardiomyocytes [Cor.4U®, Axiogenesis]. Mitochondrial membrane potential (MMP) was determined with the JC-1 fluorescent indicator. Induction of ROS was reported by the redox-sensitive fluorophore dihydroethidium (DHE).

Results & discussion
The CLARIOstar microplate reader equipped with software-controlled programmable O₂ and CO₂ regulation achieves precise atmospheric control, with O₂ reduced to 1%, maintained low for 50 min and then rapidly increased to 18% [fig.2]. Real-time monitoring of oxygenation reveals the impact of cellular respiration and ambient O₂ on O₂ concentrations at the cell monolayer. Antimycin treated HepG2 cells [no respiration], reflect instrument conditions (ACU). However, respiring cells experience much lower resting O₂ concentrations and deeper more sustained hypoxia. This disparity between atmospheric and cellular O₂ increases further when respiration is increased through FCCP treatment [uncoupled cells].

The approach was also evaluated using iPS-derived cardiomyocytes (Cor.4U) with parallel monitoring of MMP and ROS using the CLARIOstar’s convenient multiplexing function. Respiring cells experience significantly reduced O₂ concentrations while antimycin treated cells reflected ACU conditions [fig. 3A] and also causing MMP dissipation as well as increased ROS production [fig. 3B].

Conclusion
- O₂ ramping of ACU facilitates control of ischemic and reperfusion insults in cells
- Intracellular probe tracks cellular oxygenation during ischaemia/reperfusion cycle
- Parallel monitoring of ROS and MMP probes allow detailed metabolic characterization of ischemia-reperfusion
Mitochondrial oxidant generation follows oxygen deprivation and re-oxygenation

Daniel Pastor-Flores and Tobias Dick, German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction
Yeast is a popular eukaryotic model organism because it is easy to genetically modify and robust to differing environments. Plated on agar plates, it can be studied under aerobic conditions. However, studying transition of agar-plated yeast to anaerobic conditions requires atmospheric control to reduce O2 tension. The Singer Instruments ROTOR device pins colonies of yeast onto agar plates in 96, 384 or 1536 plate format and enables high-throughput studies. The CLARIOstar plate reader with ACU exposes yeast colonies to a desired O2 and CO2 atmosphere and detects their fluorescence.

Assay principle
The dependence of oxidant formation on ambient O2 was measured in yeast clones expressing mito-roGFP2-Tsa2DCR, a fluorescent mitochondrial redox-sensitive sensor. In addition, yeast cells expressed a citrate synthase 2 (Cit2) fusion-protein with mCherry. Cit2 is upregulated upon activation of the retrograde pathway, a common marker of mitochondrial dysfunction. Auto-fluorescence of yeast NAD(P)H corrected for the growth of yeast colonies. Yeast was pinned onto agar plates resembling the layout of a 384 well plate. Changes in O2 pressure were achieved by the ACU.

![Graphs showing oxygen and carbon dioxide levels](image)

Fig. 4: Oxygen and carbon dioxide levels in the course of the experiment.

Results & discussion
Yeast was exposed to varying levels of O2 (fig. 4) and the redox state of mitochondrial roGFP2 was investigated. During the period of O2 decrease [0 –1.2 h] from 18 % to 12 %, the redox state of mitochondrial H2O2 probe was not influenced by the decreasing O2. This points to a favoured O2 supply to mitochondria over the cytosol in case of fluctuating O2 concentrations. At lower O2 saturation, the amount of the reduced probe increased as reported by lower roGFP2 ratios. While the O2 is kept at 1 % (5-6.5 h), the probe persists in its reduced form and gets oxidized only in the phase of re-oxygenation. The retrograde pathway is induced upon inhibition of mitochondrial respiration and reports mitochondrial failure to the nucleus. A protein synthesized as a result of pathway activation is Cit2. During O2 deprivation, Cit2 expression is slightly reduced whereas it remarkably increases during reperfusion (fig. 5). Whether the Cit2 increase is due to reperfusion or is a delayed response to hypoxic conditions, remains to be elucidated.

![Graphs showing oxygen and carbon dioxide levels](image)

Fig. 5: The influence of O2 availability on the redox state of mitochondrial peroxidase-1 based probe (A) and Cit2 expression (C). O2 pressure modulates the redox state of a genomic-integrated Mito-roGFP2-Tsa2ΔCR probe. roGFP2 oxidation is represented by 400nm/480nm ratio and indicative of H2O2 generation (B). Cit2 expression is reported by CIT2-mCherry fusion protein corrected for growth by NAD(P)H autofluorescence (C).

Conclusion
- Measurement of fluorescent yeast colonies on agar
- Investigation of normoxic and hypoxic conditions using the CLARIOstar with atmospheric control unit
- Measurement of multiple parameters employing the flexibility of the LVF-monochromators™
Due to the modularity of BMG LABTECH’s instruments, all, or combinations of the features below can be installed at purchase or upgraded at any time. Please contact your local representative for more details or a quote.

### Detection modes
- Fluorescence intensity - including FRET
- Fluorescence polarization/anisotropy
- AlphaScreen®, AlphaLISA®, AlphaPlex™
- Luminescence (flash and glow) - including BRET
- Time-Resolved Fluorescence - including TR-FRET
- UV/vis absorbance

### Measurement modes
- Top and bottom reading
- Endpoint and kinetic
- Sequencal multi-excitation
- Sequential multi-emission
- Spectral scanning (fluorescence, luminescence, absorbance)
- Ratiometric measurements
- Well scanning

### Microplate formats
- 6- to 1536-well plates, user-definable
- LVis Plate with 16 low volume microspots (2 µL)

### Microplate carrier
- Robot compatible

### Light sources
- High energy xenon flash lamp
- Dedicated laser for AlphaScreen®, AlphaLISA®, AlphaPlex™

### Detectors
- Low-noise photomultiplier tube
- CCD spectrometer

### Wavelength selection
- Dual Linear Variable Filter (LVF) Monochromators™
- Linear Variable Dichroic Mirror: separates ex & em LVF Monochromators
- Optical filters: Ex and em slides hold 4 filters each
- LVF Monochromators + optical filters: Use one for ex and the other for em
- UV/vis absorbance spectrometer: Full spectrum or 8 distinct wavelengths in < 1 sec/well

### Optical filters
- Excitation and emission slides for 4 filters each

### Optical path guides
- Top and bottom: free air optical light path guided by motor-driven mirrors and dichroics

### Z-Adjustment
- Automatic focal height adjustment (0.1 mm resolution)

### Spectral range
- Filters: 240 - 750 nm or 240 - 900 nm for FI, FP, TRF
- LVF Monochromators™: 320 - 850 nm for FI, 320 - 750 nm for LUM
- Linear Variable Dichroic Spectrometer: 340 - 740 nm for FI, 400 - 750 nm for FLU

### Sensitivity
- FI Filters (top): < 0.15 pM fluorescein (< 3 amol/well, 384sv, 20 µL)
- FI Filters (bottom): < 1.0 pM fluorescein (< 50 amol/well, 384g, 50 µL)
- FI Monochromator (top): < 0.35 pM fluorescein (< 7 amol/well, 384sv, 20 µL)
- FI Monochromator (bottom): < 3.0 pM fluorescein (< 150 amol/well, 384g, 50 µL)
- FP: < 0.5 mP SD at 1 nM fluorescein (384sv, 20 µL)
- TRF: < 20 I m europium, 384, 80 µL

### THERMOstar Microplate incubator and shaker
- Incubation +3 °C above ambient up to 45 °C or 65 °C
- Moisture control: 10% to 50% relative humidity at 30 °C and 90% RH at 45 °C
- For optimal temperature control, please ensure to select the correct ambient temperature.

### Optical path guides
- Top and bottom: free air optical light path guided by motor-driven mirrors and dichroics

### Z-Adjustment
- Automatic focal height adjustment (0.1 mm resolution)

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- FP: < 0.5 mP SD at 1 nM fluorescein (384sv, 20 µL)
- TRF: < 20 I m europium, 384, 80 µL

### HTRF®
- (black and white microplates)
- Reader Control Kit (Eu) after 18h (384sv, 20 µL)
- Delta F = (100 % (High Calibrator)
- Delta F = (1 % (Low Calibrator)
- Dynamic Range: 9 decades

### LUM
- (black and white microplates)
- 0.4 pM ATP (< 8 amol/well, 384sv, 20 µL)
- Dynamic Range: 9 decades

### Abs
- Reader Control Kit (Eu) after 18h (384sv, 20 µL)
- Delta F = (100 % (High Calibrator)
- Delta F = (1 % (Low Calibrator)
- Dynamic Range: 9 decades

### LUM
- (black and white microplates)
- 0.4 pM ATP (< 8 amol/well, 384sv, 20 µL)
- Dynamic Range: 9 decades

### Abs
- Reader Control Kit (Eu) after 18h (384sv, 20 µL)
- Delta F = (100 % (High Calibrator)
- Delta F = (1 % (Low Calibrator)
- Dynamic Range: 9 decades

### Read times
- Flying mode (1 flash)
- 8 s (96), 15 s (384), 28 s (1536)
- 10 flashes
- 19 s (96), 57 s (384), 3 min 4 s (1536)

### Reagent injection
- Up to 2 built-in reagent injectors
- Individual injection volumes for each well: 3 to 500 µL (optionally up to 2 mL)
- Variable injection speed up to 420 µL/s

### Shaking
- Linear, orbital, and double-orbital with user-definable time and speed

### Incubation
- 7 to 95 °C, 5% CO2 to 50% O2
- The incubation chamber operates at 0.5 °C more than the lower plate
- This prevents condensation build-up on the lid or sealers

### Software
- Integrated fluorophore library
- Multi-user reader Control and MARS data analysis software included
- FDA 21 CFR part 11 compliant

### Dimensions
- Width: 45 cm, depth: 51 cm, height: 40 cm, weight: 32 kg

### Optional accessories
- LVis Plate
- Sixteen separate microdrop wells for 2 µL samples; standard cuvette position
- Quality control internal standards (optional)

### Atmospheric Control Unit
- Actively regulates O2 and CO2 - 0.1-20%
- *Limit of detection (sensitivity) was calculated according to the IUPAC standard: 3x(SDblank) / slope

### Made in Germany