

Measuring oxygen consumption rate (OCR) in physiologically relevant conditions

Introduction

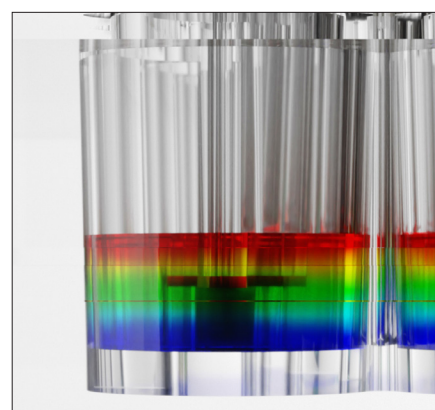
Traditionally, cells are cultured and studied using 5% CO₂ incubators and at 21% (atmospheric) oxygen. During the last half decade, more than 39,000 papers (PubMed)¹ have been published providing insight on how cells respond to lack of or excess of oxygen availability – many supporting the importance of creating consistent and physiologically relevant conditions for studying cells.²

Studying cells in physiologically relevant conditions can provide a better understanding of how cells behave in their natural environment, biological processes, and supports the development of effective treatments for diseases. Mimicking *in vivo* conditions in cell culture can help researchers to ‘bridge the gap’ between *in vitro* and *in vivo* research providing more reliable data that can be applied to animal and human models.

- **More accurate insight into cell behaviour:** Within the body, cells exist in a complex environment, interacting with other cells and the extracellular matrix. Studying cells in conditions that more accurately mimic *in vivo* conditions provides data that can be better applied to real-life systems.
- **Understanding complex biological processes:** By considering factors like oxygen levels, researchers can gain a better understanding of cellular processes like metabolism, immune responses, and signalling. All of these are key to learning more about disease progression.
- **More reliable drug discovery and screening:** Using cell models that more closely resemble *in vivo* conditions provides researchers with more reliable and applicable data to improve the predictability of drug efficacy and toxicity. It may also be useful in the development of personalised medicine for individuals and subpopulations.

Creating physiologically relevant conditions

Workstations from manufacturers such as Baker allow researchers to adapt and maintain the environment (O₂, CO₂, temperature, and humidity) to suit the cells under investigation enabling tasks such as media changes, cell passaging, treating or even imaging to be carried out under physiologically relevant conditions.



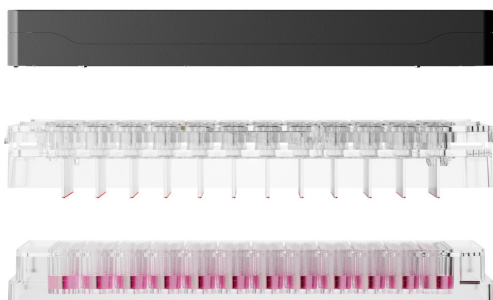
Exposure to ambient oxygen during cell culture processes can quickly reverse cellular hypoxic responses, compromising both reproducibility and data integrity.

Whilst the Lucid Scientific Resipher System turns a 96-well plate into a precise real-time oxygen consumption reader—continually streaming oxygen consumption rate (OCR) in cell cultures from within the hypoxia/physoxia workstation or incubator.



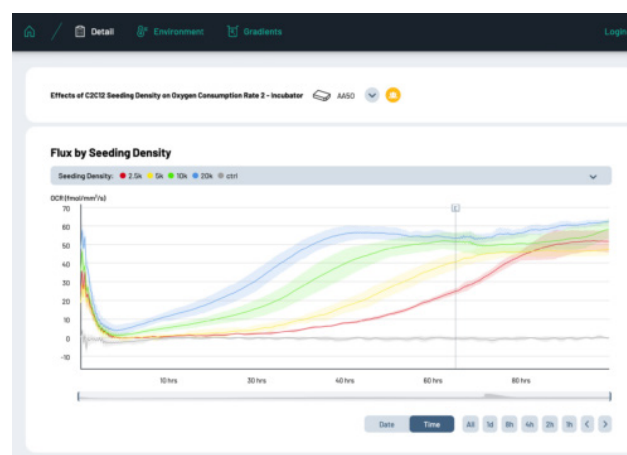
Lucid Scientific Resipher System

The **Resipher** is a non-invasive platform that continuously measures **oxygen consumption rate (OCR)** live, in 2D and 3D cell cultures, providing real-time metabolic data over hours, days, or even weeks. Designed for use inside standard incubators or hypoxia/physoxia workstations, the Resipher lets researchers capture the full metabolic profile of cells under physiological conditions—without interrupting their workflow.



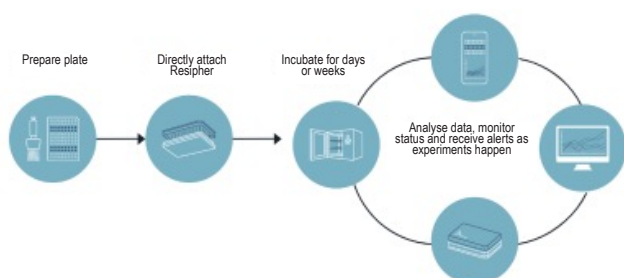
Unlike traditional endpoint assays, the Resipher enables:

- **Continuous, label-free metabolic monitoring.**
- **In situ data collection** without removing cultures from controlled environments.
- **Long-term profiling** for drug response, differentiation, or gene modulation studies.



Operational notes:

- Designed to be used with standard 96-well plates.
- The Resipher is placed directly on the 96-well plate, immediately after seeding cells.
- The Resipher is placed inside the physoxia workstation or incubator.
- The system continually analyses OCR and the oxygen environment of the cells, in real time.
- Quantitative data is continually streamed via Lucid's online platform.
- Up to 8 devices can be run from one hub.



Applications:

- Metabolic activity
- Cell proliferation
- Drug effects
- Mitochondrial function

Best practice

- **Start simple:** For the first Resipher experiment, start with a simple experiment, e.g., a cell density/seeding experiment. It allows users to get familiar with the Resipher system and confirms the general oxygen consumption behaviour of the cells before proceeding to more complex experiments. If the data is looking good during the first few days of the experiment, then consider applying treatments.
- **Let the cells settle:** It's always a good idea to allow cells to adhere or fall to the bottom of the wells before placing the sensing lids onto the well plates. This prevents cells from accidentally attaching to the sensing probes.
- **Temperature is important:** Sensors are cross-sensitive to temperature. Media should be pre-equilibrated to 37 °C before using to reduce initial stabilisation period. Temperature gradients may appear as a negative OCR.
- **Oxygen equilibration is essential:** Before initiating any cell culture experiment under defined oxygen conditions, it is crucial to pre-equilibrate the culture media to the target oxygen level. This step minimises the time required for equilibration during the experiment and ensures more consistent results. To facilitate this, we recommend placing the media in the hypoxia chamber the night before beginning a hypoxic experiment, allowing excess dissolved oxygen to dissipate effectively.
- **100 µl media recommended to start:** Commonly used media volumes range from 75 to 200 µl. Too little media (below 60 µl) may risk the probe moving out of the media. Due to the diffusion limit of oxygen in media, higher volumes mean lower theoretical maximum oxygen consumption rates (e.g., For example, 180 fmol/mm²/s for 100 µl and 100 fmol/mm²/s for 200 µl). Plus, the cells may experience a more hypoxic environment with greater media depths. Unless other volumes are required, the manufacturer typically recommends starting with 100 µl and make volume adjustments in future experiments.
- **Don't "lose" control:** At least one, possibly more, media only wells are recommended.
- **Limit evaporation:** Media or PBS can be added to unused wells to reduce media evaporation during experiments. Watch out for edge effects where evaporation rate is usually faster.
- **Data review:** Researchers can contact us to organise data review meetings—especially after initial experiments. Please reach out to: sales@labtech.com for any support and help from the experienced team.

Reducing the impact of media evaporation³

The Resipher system is commonly used for monitoring the OCR of cells over extended periods of time, ranging from days to weeks in many instances. With the Resipher, users can use most 96-well plates from various manufacturers.

The Resipher is uniquely designed to be an "open-air" setup, where oxygen is continuously supplied to the media and cells, thus allowing for long-term OCR monitoring. One possible challenge during long-term cell cultures is media evaporation which usually impacts edge wells to a greater extent. Limiting the evaporation impact will improve the accuracy and reliability of data. Here are some strategies researchers can employ to limit the impact of media evaporation.

Fill unused wells with PBS: When using a 32-channel Resipher system, the working wells are in columns 3, 4, 9, and 10. If the rest of the wells are not used for other analyses, it is always a good idea to fill them with PBS to help keep the air volume under the sensing lid as humid as possible.

Use well plates with surrounding moats: There are well plates that are engineered to reduce media evaporation, such as those with surrounding moats along the outer edges of the well plate. When filled with PBS, these moats can act as evaporation buffers during extended Resipher experiments.



Labtech

A CALIBRE SCIENTIFIC COMPANY

Baker Physoxia Workstations

Baker's controlled-atmosphere solutions enable a continuous physoxia workflow, where every step—from cell seeding to endpoint analysis—takes place under tightly regulated oxygen conditions. This seamless approach prevents reoxygenation artefacts and ensures that cell metabolism, differentiation, and signalling reflect the true *in vivo* microenvironment.

The Baker physoxia workstations provide precise, stable control of oxygen, CO₂, temperature, and humidity—giving researchers the ability to mimic physiological or pathological conditions *in vitro*. Unlike standard incubators, these workstations maintain tightly regulated environments down to <1% O₂, critical for studying cell behaviour under hypoxia or physoxia.

Baker workstations enable:

- **Consistent oxygen control** for extended culture under hypoxic or tissue-physiological conditions.
- **Seamless workflows** with full access to your cultures without exposing them to ambient air.
- **Reproducible results** ideal for metabolism, stem cell biology, cancer, and immunology studies.

Recommended workflow integration:

- **Culture initiation:** Establish cells directly within a Baker oxygen-controlled workstation to ensure early adoption to physiological O₂.
- **Maintenance and manipulation:** Perform all media changes and cell handling inside the workstation, maintaining stable oxygen levels throughout. Ensure all media has acclimatised. Media stored in the fridge will be at atmospheric oxygen, the diffusion of oxygen out of media takes time.
 - **Top tip:** Keep all media and plasticware inside the workstation and set the desired oxygen level for the system at least one hour before use.
- **Analysis and imaging:** Use integrated or connected instruments within the same controlled environment to prevent transient oxygen exposure.
- **Data interpretation:** Correlate biological responses with the exact oxygen tension used. This strengthens experimental reproducibility and translational value.

Applications:

- Cell function
- Cancer research
- Stem cells & cell therapy
- Cell biology



Labtech

A CALIBRE SCIENTIFIC COMPANY

Why combine the two?

Lucid Resipher and Baker InvivoO₂ 300

Laboratories combining the tightly controlled environment, provided by an anaerobic workstation, with the real-time monitoring of the Resipher system benefit from:

- **In situ metabolic profiling** without disturbing the culture environment.
- **True physiological relevance** for studies in cancer, immunology, and stem cell biology.
- **Improved reproducibility** by combining real-time measurement with tightly controlled conditions.
- **Reduced cost** compared to the alternative solutions, such as an XF metabolic analyser.

This combination empowers researchers to observe how cells adapt metabolically to their oxygen environment—in real time, over the full course of an experiment.



Applications – Case study

**Institute of Metabolic Science | Metabolic Research Laboratories
University of Cambridge | Dr Daniel Fazakerley**

Research Application:

Dr Fazakerley and his team research the effect of insulin on adipocyte metabolism. As part of ensuring that cell models most accurately recapitulate their *in vivo* counterparts, the team is interested in how cell culture conditions influence cell metabolism. They recently highlighted the importance of considering the oxygen tension experienced by cell monolayers in cell culture.⁴ This is distinct from atmospheric oxygen, since oxygen diffusion through media to cells can be limiting, even under standard culture conditions.

Reasons for choosing the Lucid Resipher and Baker InvivoO₂ 300

“The Resipher system allows us to monitor cellular oxygen consumption in standard media in the incubator. This means we can correlate oxygen use to other cellular phenotypes we measure under these exact same conditions. The system is very flexible and allows us to track oxygen use (and peri-cellular oxygen) over days, and in response to specific interventions. The Baker workstation provides an accurate, controlled oxygen environment. This allows us to measure real-time changes in oxygen levels/consumption by our cells without fluctuations in atmospheric oxygen levels. This is particularly helpful for conducting experiments in controlled hypoxic conditions, rather than having cells constantly adjust between high and low oxygen environments.” **Dr Daniel Fazakerley**

Benefits of combining the two products:

“The Baker workstations allow us to accurately control and limit atmospheric to cultured cells. However, atmospheric oxygen set-point does not reflect the oxygen tension experienced by cells in culture. Combining the Resipher and Baker technologies allowed us to precisely control and measure peri-cellular oxygen concentrations for our metabolic studies.” **Dr Daniel Fazakerley**

Other applications

Cancer metabolism

- Track how cancerous cells adapt and respond depending on oxygen levels over time.
- Monitor responses to stimuli metabolic inhibitors or chemotherapies under physiological O₂.

Neuroscience / stroke models

- Recapitulate ischemic conditions by precisely and rapidly reducing oxygen levels.
- Observe recovery or decline in neural cell types, post-hypoxia through tracking of cellular metabolism.
- Evaluate the efficacy of neuroprotective compounds under dynamic oxygen stresses.

Immunology and infection

- Record how immune cells shift their metabolism in physoxic microenvironments (e.g., tumour or inflamed tissues).
- Profile T cells, macrophages, or dendritic cells under different oxygen environments.
- Study pathogen-host interactions in O₂ limited niches.

Stem cell biology and differentiation

- Observe preserved metabolic phenotypes of stem cells under tissue specific oxygen levels.
- Monitor metabolic shifts during hypoxia-induced differentiation or reprogramming.

Drug discovery and screening

- Evaluate how, under physiologically relevant oxygen levels, metabolic response to drugs alters vs traditional culture methods metabolic response to drugs under different oxygen levels.
- Identify compounds with selective effects under hypoxia vs normoxia during chronic exposures.

Tissue engineering and organoids

- Cultivate 3D tissue models/organoids in physiologic oxygen and record 3D structure metabolism.
- Track 3D metabolic development or dysfunction over extended culture periods.

Acknowledgement

Dr Daniel Fazakerley, University of Cambridge

References

- [1] <https://pmc.ncbi.nlm.nih.gov/>
- [2] Bridging the Gap: Creating More Physiologically Relevant Human Cell Models: <https://www.technologynetworks.com/cell-science/articles/bridging-the-gap-creating-more-physiologically-relevant-human-cell-models-396896>
- [3] Lucid, Reducing the Impact of Media Evaporation: https://assets.ctfassets.net/kftvd9z34258/4qA1M2OsBvPJgc5HDE1vBz/e92fdaa972a40d92b3e9f543f53a8ebe/LucidScientific_Reducing_Impact_of_Media_Evaporation.pdf
- [4] Limited oxygen in standard cell culture alters metabolism and function of differentiated cells: <https://pubmed.ncbi.nlm.nih.gov/38580776/>

Relevant Articles

Limited oxygen in standard cell culture alters metabolism and function of differentiated cells: <https://www.embopress.org/doi/full/10.1038/s44318-024-00084-7>

Medium Depth Influences O₂ Availability and Metabolism in Human RPE Cultures: <https://iovs.arvojournals.org/article.aspx?articleid=2792989>

A Simple Cell Culture Intervention for Healthier Cells: <https://www.the-scientist.com/a-simple-cell-culture-intervention-for-healthier-cells-71996>