



HOW MUCH O₂ IS INSIDE YOUR CELLS?

Dmitri B. Papkovsky
Luxcel Biosciences Ltd.
University College Cork, Ireland



Hypoxia Set-Ups

- Macro:
 - » CO₂/O₂ incubator
 - » Hypoxia stations/incubators

- Mini:
 - » Micro-chambers
 - » Perfusion and climate control systems



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Contactless pO₂ Measurement

- pO₂ range - 0.00 – 25.0 %
- Disposable sensors,
- Handheld, calibration-free
- T and P compensation

OpTech™ System

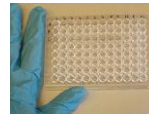


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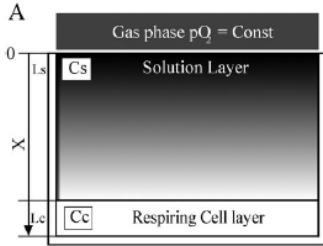
O₂ Control *In Situ*

- Tissue culture
- Sample preparation, bioassays
- Perfusion systems, microfluidic biochips
- *Ex vivo* and *in vivo* experiments ...



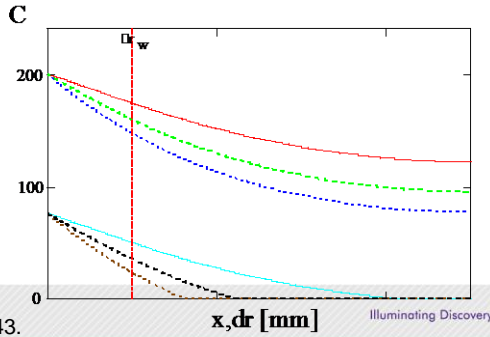
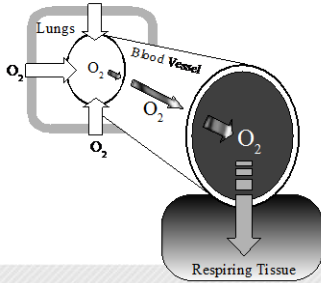
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O₂ Gradients in Cell Culture



$$C_s = C_0 - \frac{L_c}{D_s} k(X + L_s) \quad (1)$$

$$C_c = \frac{k}{2D_c} X^2 - k \frac{L_c}{D_c} X + H \left(C_0 - \frac{L_c L_s}{D_s} k \right) \quad (2)$$



Zhdanov AV et al, *Integr. Biol.* 2010, 2, 443.



Measurement Approach



O₂-Sensing Probes
(Reagent Vials)



Standard 96/384 WP
Other substrates



Standard Readers
(TR-F)

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Phosphorescent O₂ Probes

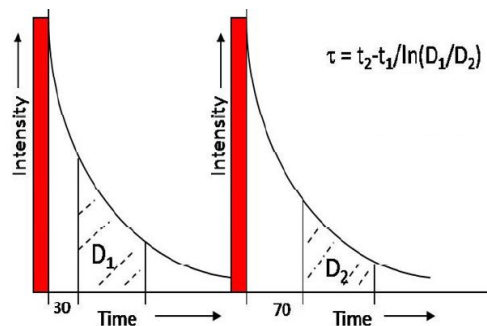
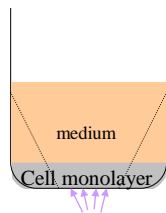
- Extracellular: **MitoXpress™**
 - » Analytical tasks:
 - Control of medium oxygenation
 - Cellular oxygen consumption (OCR)
 - Multiplexed metabolic assays:
 - OCR, lactate, CO₂, ATP, MMP
- Intracellular:
 - » MitoXpress™ - requires transfection (phased out)
 - » Peptide based probes - self-loading, different localisation
 - » **Nanoparticle probe – self loading, super-bright**

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O₂ Quantification by TR-F

- Two Intensity reads: D₁, D₂
- Lifetime calculation
- LT conversion into [O₂]

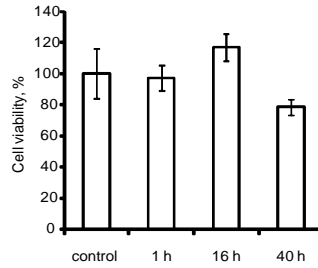
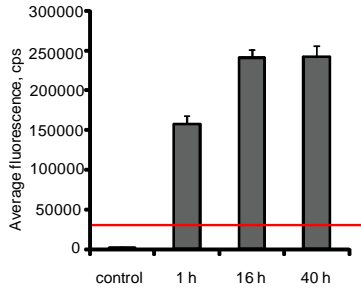


O'Riordan TC et al, *Anal. Chem.*, 2007,79(24):9414

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Probe Performance



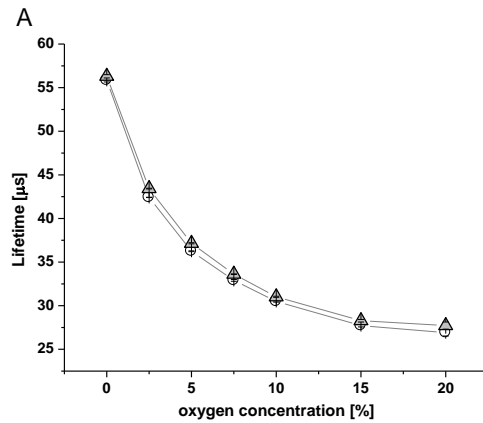
Dmitriev RI et al, *Anal. Biochem.*, 2010 Mar 1;398(1):24-33

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O₂ Calibration

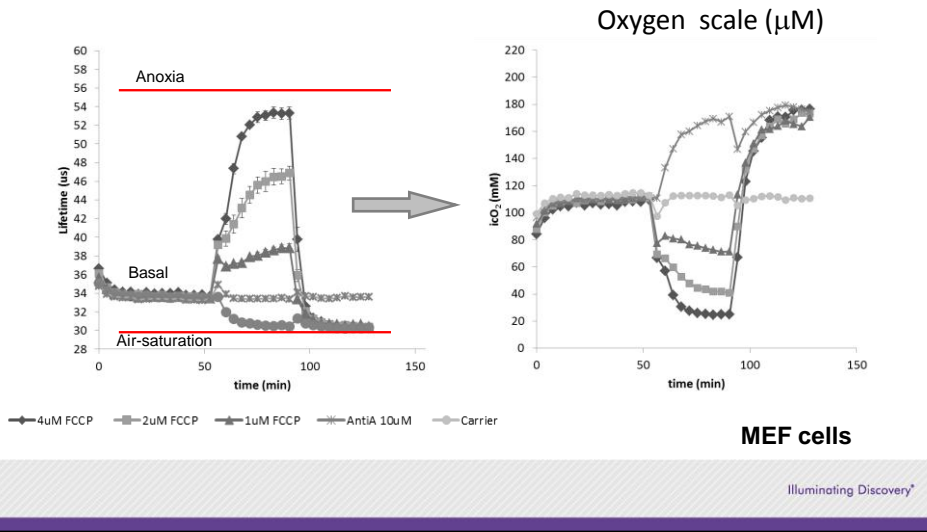
- Loading cells with probe
- Blocking cell respiration (Ant A)
- Measuring LT at different pO₂



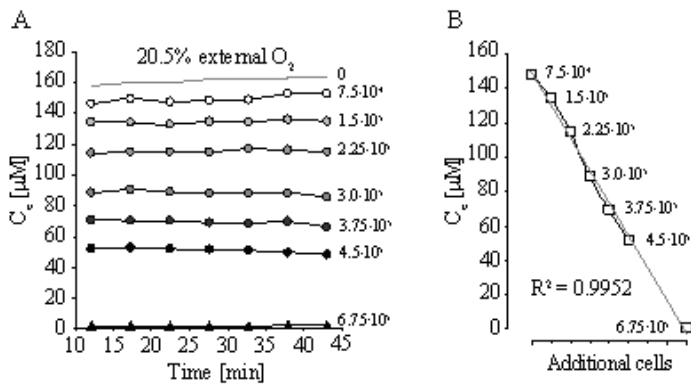
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Applications - icO₂ probes



PC12 Cells in a 96WP, 21%

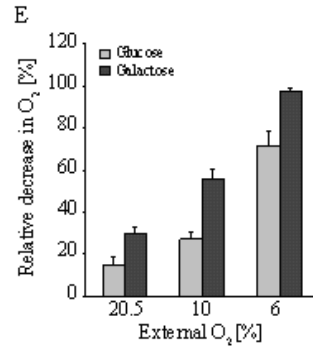
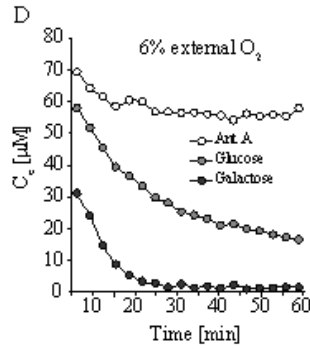


Zhdanov AV at al, *Integr. Biol.* 2010, 2, 443.

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PC12 Cells in a 96WP - Hypoxia



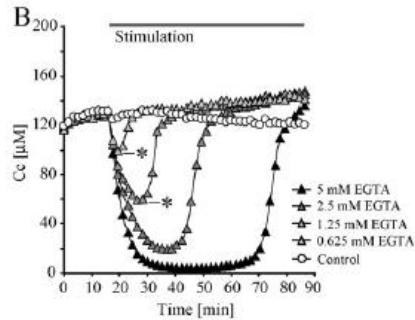
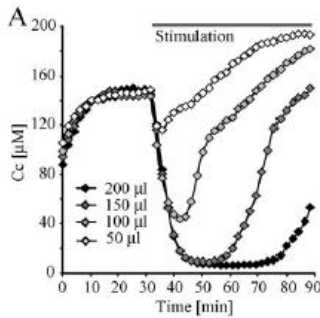
Main determinants of cellular O₂:

- » External pO₂, T, mass exchange
- » **Cell resp. activity / numbers,**
- » Barrier layer (medium): height, D, viscosity

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Effect of Metabolic Stimulation



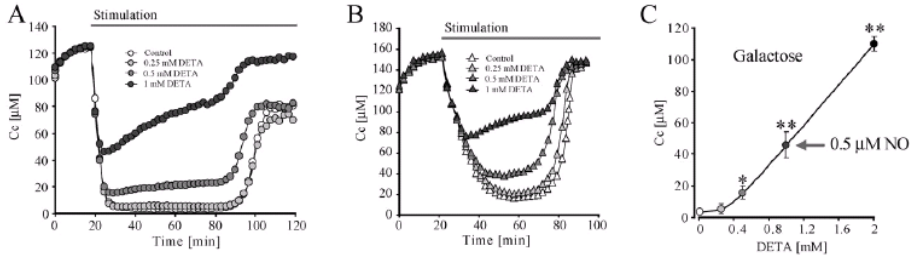
Parameters affecting the response:

- » **Mechanism of action**
- » Biological (cell No, activity, ...)
- » Physical (barrier, viscosity, thickness, O₂, T, ...)

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NO, HIF-1 α and Cellular O₂

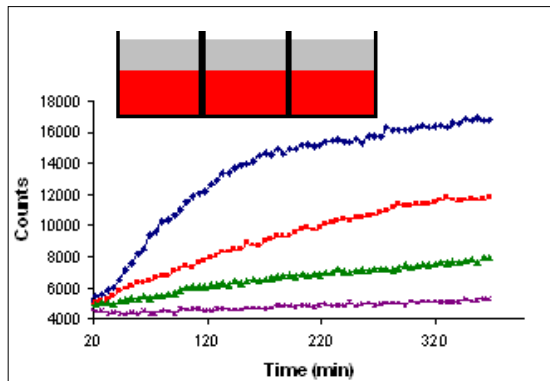


- NO protects the cells from hypoxia
- HIF responses can occur under normoxia

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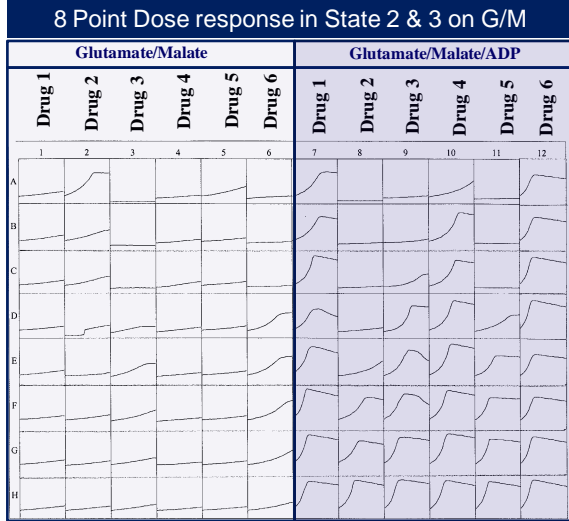


Applications: Extracellular O₂ probe



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Mitochondrial Toxicity - Dose Responses



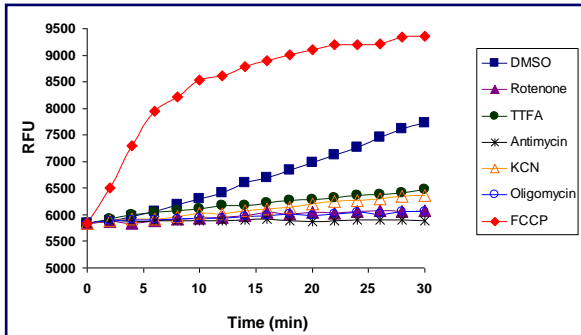
Will Y. et al, *Nature Protocols*, 2006, v.1(6), p.2563-2572

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Cellular O₂ Consumption



Effect of Classical Inhibitors on HepG2 Respiration



- Rotenone:** Complex I Inhibitor
- TTFA:** Complex II Inhibitor
- Antimycin:** Complex III Inhibitor
- KCN:** Complex IV Inhibitor
- FCCP:** Uncoupler
- Oligomycin:** ATPase Inhibitor

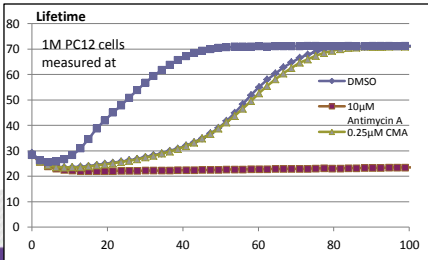
Hynes J. et al, *Curr Protoc Toxicol*, (2009), Ch. 2, v.2, p.16

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Sealed Cuvettes

- A set of 4 quartz micro-cuvettes with Teflon caps (<1 mL)
- Suspension cells
- Measurement of:
 - » Absolute OCR
 - » Changes in respiration at low O₂



Luxcel O₂ Sensing Platform

- Operates on standard TR-F readers and 96/384 well plates
- Provides real-time, minimally invasive monitoring of IC and EC [O₂]
- Flexible and versatile, stable to interferences (TR-F)
- Can operate in hypoxia chambers



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What we can measure...



Assessing Cell Metabolism

- Isolated Mitochondria
- Whole Cells
- Intracellular Oxygen
- Mitochondrial Dysfunction
- Glycolytic Inhibition
- Mito Tox Screening
- Bacterial Metabolism
- Hypoxia
- etc, etc...



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Imaging Cellular O₂

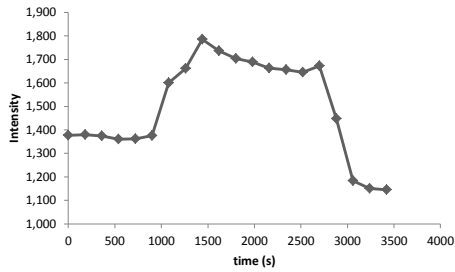


Fercher A et al, In: **Live Cell Imaging**, D.B.Papkovsky (Ed), *Methods in Molecular Biology*, 2010, v.591, p.257-73

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Imaging in Intensity Mode



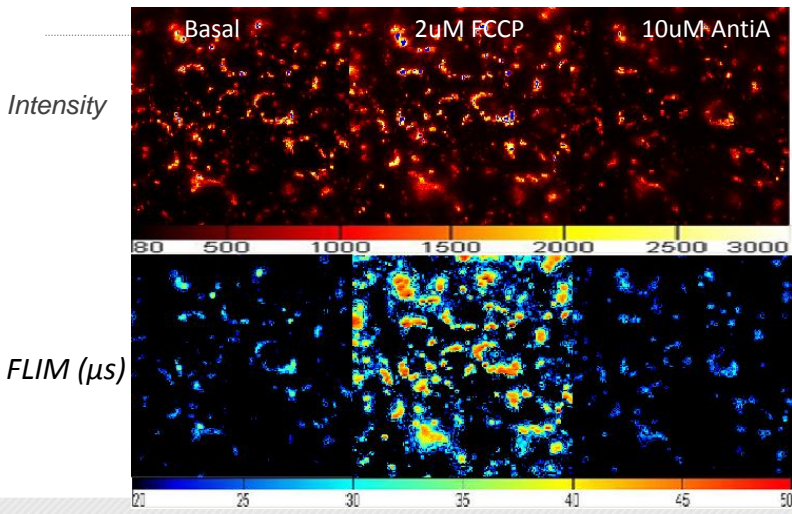
- High signals, short exposure times
- Photostable probe
- Relative changes in O₂

Basal *2μM FCCP* *10 μM AntiA*


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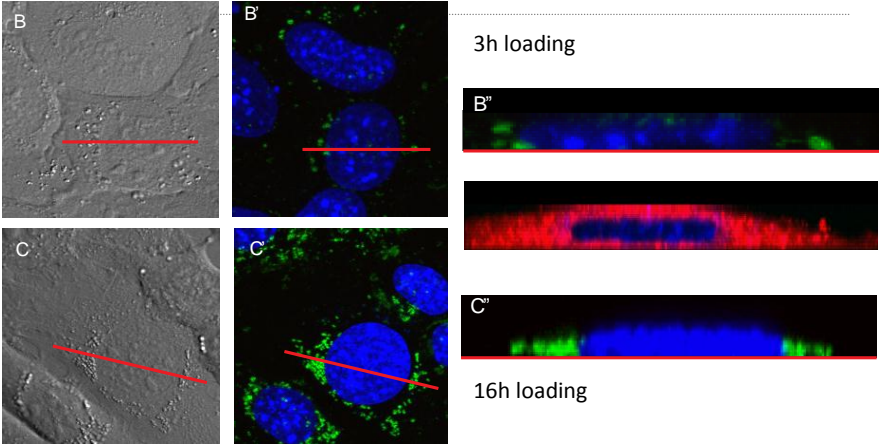
FLIM Imaging



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Probe Localisation (NP)



3h loading

16h loading

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Other Relevant Publications

Dmitriev RI et al. – *FEBS J.*, 2010, 277(22):4651-61.
 Zhdanov AV et al., *Cell. Mol. Life Sci.* 2010, DOI 10.1007/s00018-010-0502-8
 O’Flaherty L et al, *Human Molec Genetics*, 2010 Oct 1;19(19):3844-51.
 Zhdanov AV et al, *Biochim. Biophys Acta*, 2010, 1797(9):1627-1637
 Favre C et al, *Oncogene* 2010, 29(27): 3964-76
 Fercher A et al, *Anal. Bioanal. Chem.* 2010, Jan 10, PMID: 20063150.
 Hynes J et al, *Anal Biochem.* 2009, v. 390, p. 21-28.
 Hagan KA et al, *Proc Natl Acad Sci U S A*, 2009 Feb 17;106(7):2188-93
 Zhdanov AV et al, *J. Biol. Chem.*, 2008; 283(9):5650-61

- Contact us : www.luxcel.com
info@luxcel.com

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