



Oxygraph-2k

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DatLab 4: The Fourth Dimension of High-Resolution Respirometry

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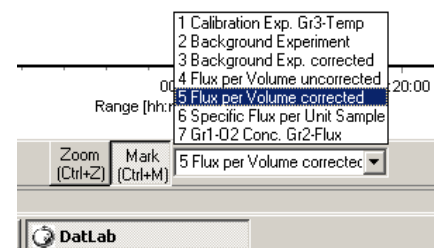


The new features of DatLab 4: A new dimension to high-resolution respirometry

- **Why?** - Small changes in cellular respiration, minor alterations in respiratory control ratios, and subtle differences in respiratory effects of inhibitors may indicate significant mitochondrial defects, reflecting injuries of mitochondrial proteins or membranes, defects of mtDNA, or alterations in mitochondrial signalling cascades. The high resolution and accuracy required to meet these challenges in biomedical and clinical studies is provided by the OROBOROS Oxygraph-2k, based on the unique concept now known as high-resolution respirometry.

- **How?** - The fourth dimension of high-resolution respirometry is speed of analysis, now combining high-resolution of the OROBOROS Oxygraph-2k with instant diagnostic information.

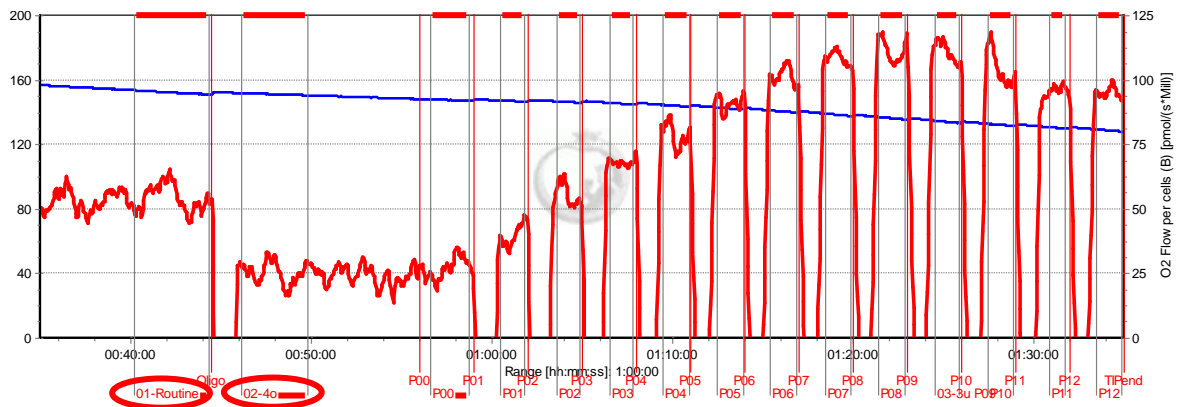
On-line respirometric analysis with DatLab 4 is illustrated by an experiment with 0.14 million fibroblasts per ml (Chamber B). In the experimental regime of a "phosphorylation control titration", oligomycin and rotenone were titrated manually, while the OROBOROS TIP2k was applied for FCCP titrations.



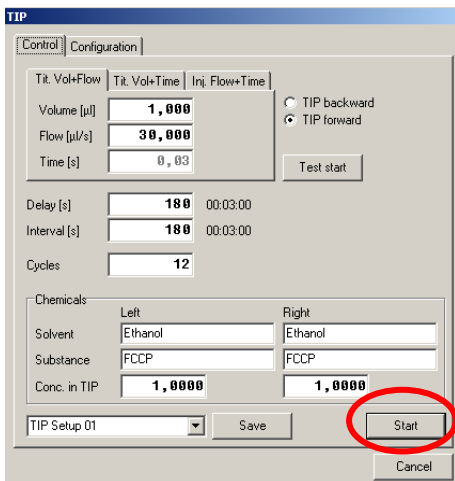
Edit Experiment	
Experimental code	Fibro-FCCP
Chamber label	A B
Sample	PFFp21+NAC PFFp21-NAC
Unit	Million cells Million cells
Concentration	0,105 per ml 0,141 per ml
Amount	0,210 per chamber 0,282 per chamber
Medium	DMEM DMEM

Enter the cell concentration for each chamber in the "Edit Experiment" window. Then select a graph layout to plot respiration per million cells (per unit sample), automatically corrected for instrumental background.

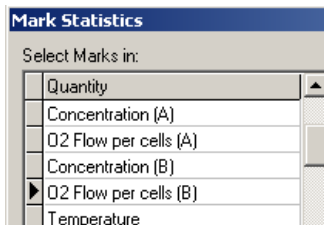
The following instructions provide a guideline for the application of DatLab 4.



Mark sections of the experiment corresponding to selected metabolic states, while the experiment proceeds: Mark **01-Routine**: Respiration in culture medium DMEM. Mark **02-4o**: Oligomycin-induced state 4o (LEAK).



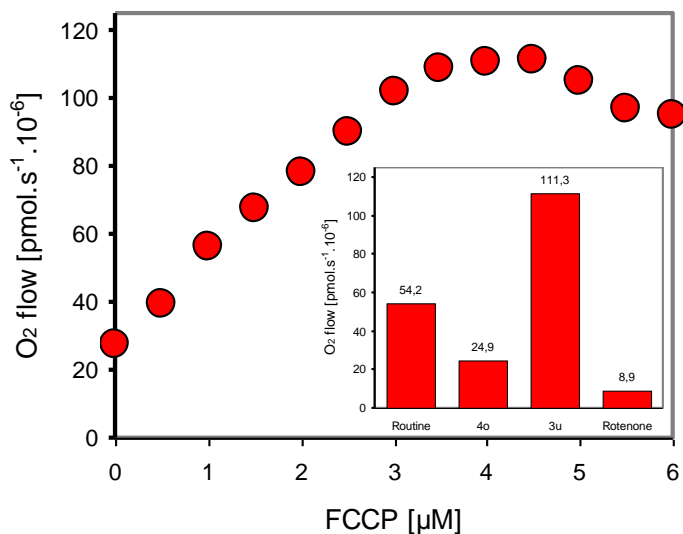
Start the TIP2k with a selected titration regime. TIP-events are added automatically to the plot. You merely mark the corresponding steady states as the titration proceeds. Marks **P00** to **P12** correspond to respiration at 0 to 6 μM FCCP.



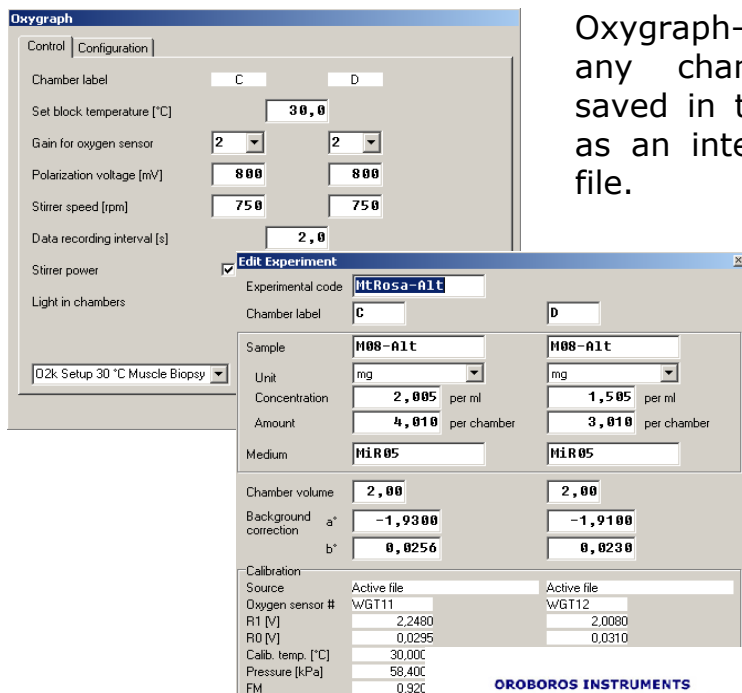
Open the Mark Statistics window, copy to clipboard, and paste into Excel or SigmaPlot.

Graphs of the FCCP titration for maximum flux and on metabolic states in the phosphorylation control titration (inset) may be available already at the end of the Oxygraph-2k experiment.

Analysis can be performed on-line or off-line.



The fourth dimension of DatLab 4 is easily accessible, with several additional features marking the state-of-the-art of a scientific software.



Oxygraph-2k control settings and any changes are automatically saved in the experimental protocol as an integrative part of the data file.

In addition, sample specifications and concentration, medium, and calibration parameters applied in the latest stage of analysis are listed in the protocol. The protocol can be saved as a pdf file.

OROBOROS INSTRUMENTS
high-resolution respirometry



Oxygraph-2k

DatLab 4	File protocol	
File name:	2004-08-30 CD-01 M08alt.DLD	
Path:	C:\DataDL\Monte Rosa\Monte Rosa	
Experimental code:	MtRosa-Alt	
Chamber label:	(C)	(D)
Sample:	M08-Alt	M08-Alt
Sample concentration:	2,00 mg/ml	1,50 mg/ml
Medium:	MiR05	MiR05
Chamber volume [ml]:	2,00	2,00
Background corr. a*:	-1,9300	-1,9100
b*:	0,0256	0,0230
Calibration Source:	Active file	Active file
Sensor #:	WGT11	WGT12
R1 [V]:	2,2480	2,0080
R0 [V]:	0,0295	0,0310
Block temp. [°C]:	30,0000	30,0000
Barom. pressure [kPa]:	58,4000	58,4000
FM:	0,9200	0,9200

Users of a highly automatic software must be aware of the fact that results will be accurate only if the parameters used by DatLab are based on corresponding calibration procedures. Such calibrations and experimental background control tests have to be performed at intervals chosen according to the aims regarding resolution, precision and accuracy, and on instrumental maintenance.

References

- Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dykens JA, Will Y, eds) John Wiley: 327-352.
- Steinlechner-Maran R, Eberl T, Kunc M, Margreiter R, Gnaiger E (1996) Oxygen dependence of respiration in coupled and uncoupled endothelial cells. *Am. J. Physiol.* 271: C2053-C2061.