OROBOROS INSTRUMENTS
high-resolution respirometry

Oxygraph-2k Manual

DatLab Guide Through the Menus
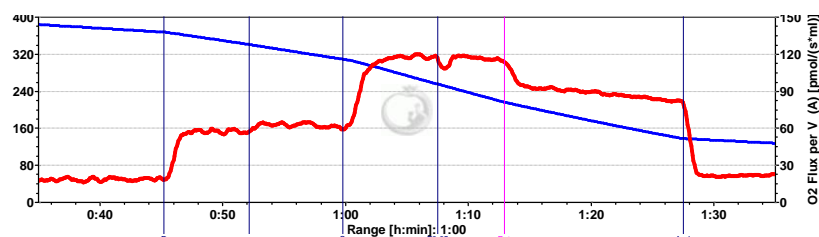
Erich Gnaiger, Mario Fasching, Lukas Gradl

OROBOROS INSTRUMENTS Corp
high-resolution respirometry
Schöpfstr 18, 6020 Innsbruck, Austria
Email: erich.gnaiger@oroboros.at
www.oroboros.at

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This guide through the DatLab menus offers an overview of the options of the software forming an integral component of high-resolution respirometry and its application. This horizontal introduction to DatLab follows the menu structure. A complimentary vertical approach to introducing DatLab is presented in descriptions of specific Oxygraph-2k applications:

O2k-Start	**MiPNet12.06**
DatLab Oxygen and pX (pH) Calibration	**MiPNet12.08**
DatLab O₂ Flux Analysis: On-Line	**MiPNet12.09**
Titration-Injection microPump, TIP2k	**MiPNet12.10**
O2k-Fluorescence LED2-Module	**MiPNet17.05**
O2k-MultiSensor-ISE	**MiPNet15.03**

1. File

1.1. Open [Ctrl+O]

A previously recorded DatLab file is opened. The default file type is DatLab (*.DLD) for DatLab files.

It is not possible to open a second file simultaneously (you can do this in separately opened DatLab programmes). If the Oxygraph-2k is connected for DatLab recording, another DatLab file cannot be opened before disconnecting the Oxygraph-2k [F7] and closing the currently recorded file.
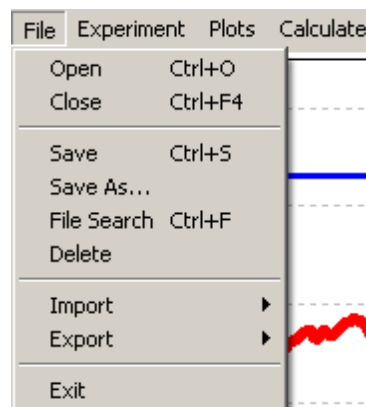
1.2. Close [Ctrl+F4]

Close a data file. A window Save changes to file? pops up offering the options to close the file after saving any changes, or close the file without saving the modifications on the presently open file.

1.3. Save [Ctrl+S]

Save changes made on a file in the off-line mode under the identical file name by overwriting the old file. Such changes do not affect the raw data of the experiment, but relate to calibrations, experimental protocol, marks and events.

1.4. Save As

Save the information contained in the present state of a file under a different file name, and optionally in a different directory. This option is available in the off-line mode only.

1.5. File Search [Ctrl+F]

A file search yields a list of all files which are categorized by a defined experimental code in a selected directory (see Experiment - Edit [F3]). Click on the file to preview the protocol, which aids in selecting a file for opening.

1.6. Delete

Delete is available off-line only when viewing an opened file. In this case the decision to delete a file not containing useful data can be made most clearly.

1.7. Import   **DatLab File**- w*ill be installed.*

Import DatLab Templates
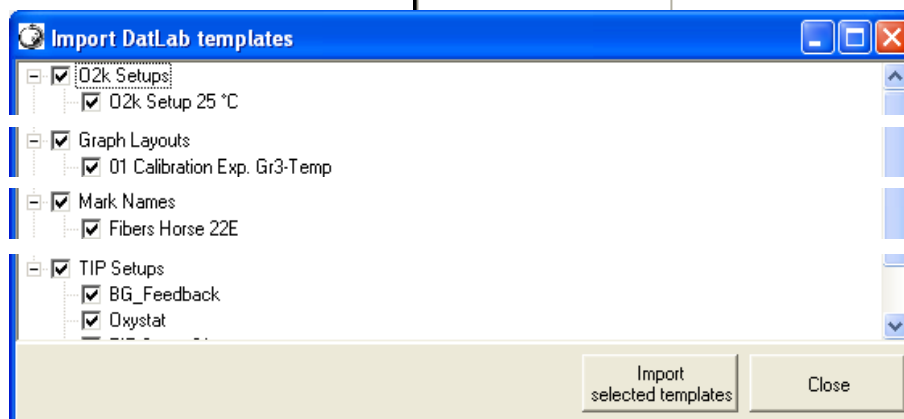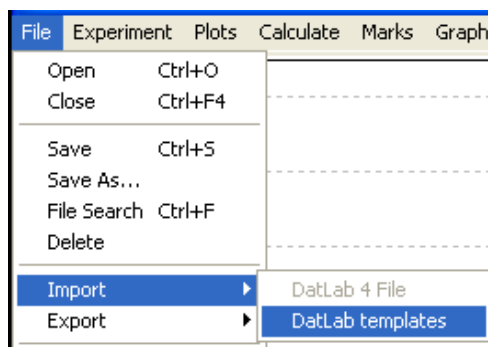
Default templates are generated automatically during installation of DatLab. Specific DatLab Templates can be imported for O2k-Setups, Graph Layouts, Mark Names, and TIP2k-Setups.

DLTemplates*.dlt are installed automatically in C:\DatLab\DLTemplates. Backup files are on the OROBOROS USB-Stick under O2k\DatLab-Install\DL-Templates.

1.8. Export   Text File (*.csv)

Export plots and events to a text file for further use in Excel and other compatible programs.

Events to Text File:
Export all information in Events to a text file (*.csv). This file may be used as a protocol, including the comments in the Events.
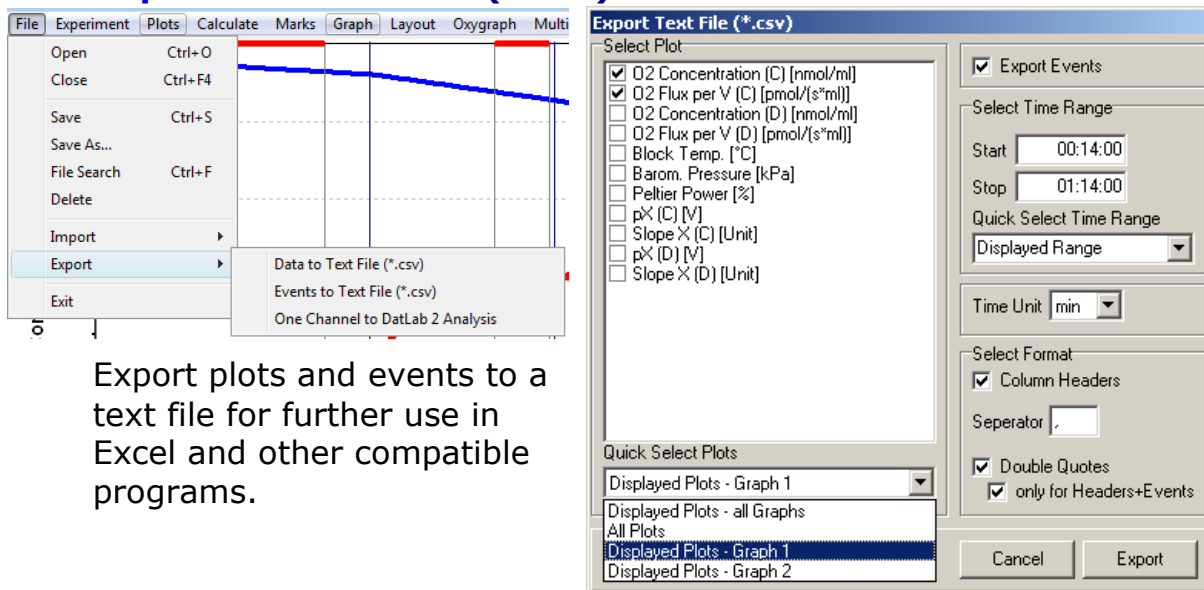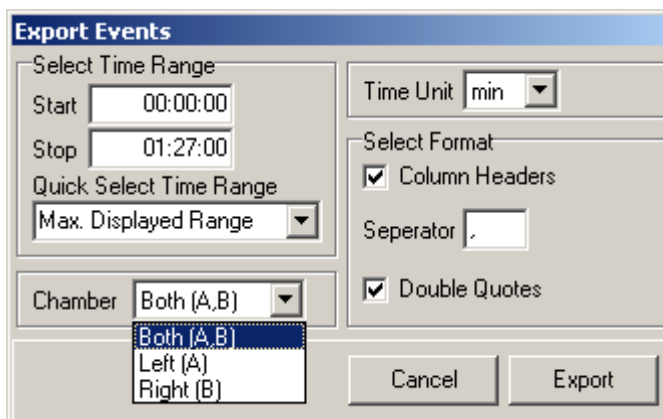
One plot to DatLab 2 Analysis:
Export a single plot (data column) to a DatLab 2 file (*.DLR) for analysis in DatLab 2. Select a single plot and confirm.
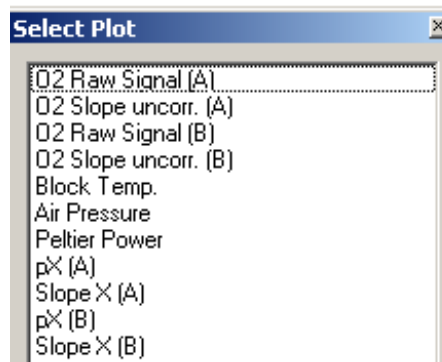
O2 Raw Signal (A): Oxygen signal of left chamber [V].

O2 Slope uncorr. (A): Oxygen flux for the left chamber, not corrected for background.

O2 Raw Signal (B): Oxygen signal of right chamber [V].

O2 Slope uncorr. (B): Oxygen flux for the right chamber, not corrected for background.

Block Temp: Block temperature of the O2k-Main Unit, continuously recorded [°C].

Air Pressure: Barometric pressure, continuously recorded [kPa].

Peltier Power: Peltier power, for regulation of block temperature, continuously recorded [% of maximum power].

pX (A): Signal of an electrode connected to the left side of the Oxygraph-2k.

Slope X (A): Slope calculated for the signal of electrode (A).

pX (B): Signal of an electrode connected to the right side of the Oxygraph-2k.

Slope X (B): Slope calculated for the signal of electrode (B).

For standard DatLab 2-Analysis, export only the oxygen signals (O2 Raw Signal), since fluxes will be re-calculated in DatLab 2 Analysis.
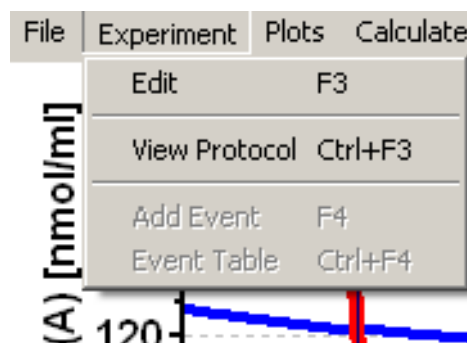
1.9. Exit   Exit DatLab.

Temporary backup files: Are generated by DatLab in the off-line mode in the active subdirectory, indicated by adding tmp.$$$ to the file name. These files are retained only if the PC has failed during data analysis. During data acquisition, the data are written continuously onto the file, hence backup files are not necessary under these conditions.

2. Experiment

For every DatLab file, a protocol is generated automatically in part by communication with the Oxygraph-2k, whereas other information is entered on-line or off-line. Important features of the protocol include information on the experimental sample, the calibration, and the control settings. The protocol can be viewed and saved (pdf), as can the results for various marked sections of the experiment.

2.1. Edit [F3]   For details see MiPNet12.06.

2.2. View Protocol [Ctrl+F3]

The experimental protocol can be viewed, saved as a pdf file, or printed as a hard copy. This protocol is generated automatically by DatLab, and displays information about the Oxygraph settings, Experiment Edit and Calibration windows, and various Events. Time-dependent information can be viewed for both, or a single chamber, and the filter can be selected for viewing minimum information, intermittent by default,

or all information. Click on Preview to view the protocol, and save as a pdf file.
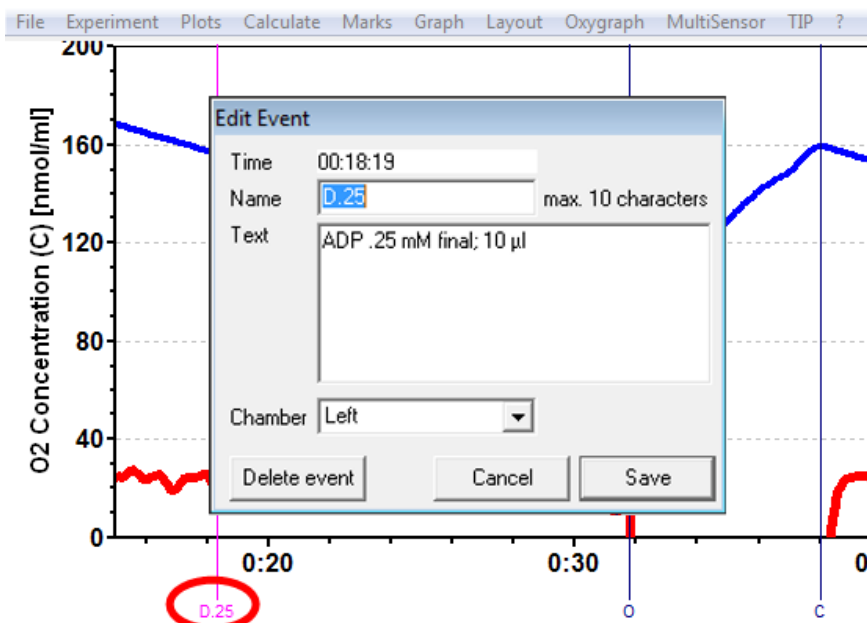
2.3. Add Event [F4]

Events:
An event is a defined point in time, labeled by a name (1 to 10 characters). The event is shown by a vertical line in the graph (line style can be modified under 'Graph Options') and the label of the event is shown on the bottom. A short comment can be entered to describe the event in detail.

Set Events [F4]:

[F4]:
On-line: To set an event quickly at the current time of the experiment (e.g. to indicate a manual titration into the chamber), simply press [F4]. This defines the time at which the event is set. Full attention can then be paid to the experiment. The event can be edited later.

[Ctrl+L⌐]:
Insert an event at any chosen moment of the plotted record of the experiment by placing the cursor at the selected moment in the graph, pressing [Ctrl] and clicking the left mouse button [Ctrl+L⌐].

Edit Event:

Name:
Enter an event name of 1 to 10 characters. Short names (e.g. O instead of Open) are recommended.

Text:
Further information can be entered into the text window.

Chamber:
In the pull-down box ▼, both, left or right can be selected for the event, which then will be shown selectively for both chambers (navy blue), left (pink) or right (green).
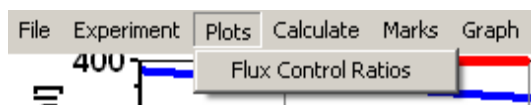
The Edit event window pops up after setting a new event at the current time by pressing [F4] or after clicking on any past time point with [Ctrl] and the left mouse button [Ctrl+L⊕].
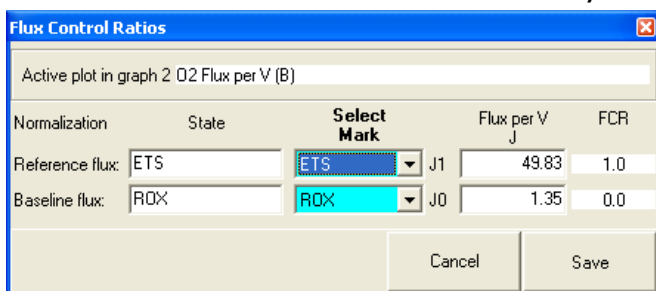
⊕   Once an event has been set, editing or Delete event is possible after clicking on the name of the event and thus opening the Edit event window.

2.4. Event Table [Ctrl+F4] *This window will become functional in one of the next updates.* For exporting events, see File \ Export.

3.   Plots – Flux Control Ratios, FCR

Flux Control Ratios (*FCR*) can be plotted after defining the (maximum) reference flux, J_1 (e.g. ETS capacity, non-coupled), and a (minimum) baseline flux, J_0 (e.g. flux after inhibition by rotenone and myxothiazole, ROX) in the window Plots \ Flux Control Ratios. After selecting the appropriate marks, the corresponding fluxes per volume [pmol·s^{-1}·ml^{-1}] are shown, which will be 1.0 and 0.0 in the plot of FCR. The flux control ratio, FCR$_t$ is then, at any time of the experiment, *t*, plotted as,

Eq.(1)
$$FCR_t = \frac{J_t - J_0}{J_1 - J_0}$$

4.   Calculate *Various options will become available.*

5. Marks
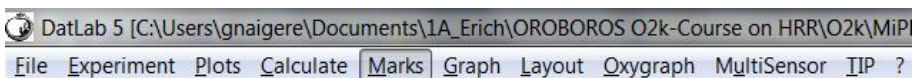
Marks are set for obtaining the average of the data within the mark, for calibration (MiPNet12.08) and data analysis (MiPNet12.09).

Set Marks: A mark is set to define a section (period of time) in a selected plot (column). Marks are shown by a horizontal bar in the active plot. One or several sections of an experiment can be marked. For a given plot, the marks cannot overlap and must be separated by one or more data points which are not marked. The marked section contains the data of the selected plot within the period of time defined by the mark.

🖱 **Mark:**   In the Graph Menu select "Mouse Control: Mark", or press [Ctrl+M]. Select a plot on a graph, by clicking on the column label in the figure legend on the right side of the graph.
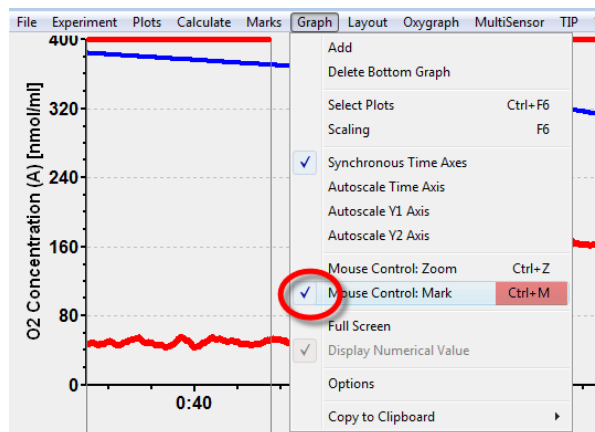
The active column is highlighted. Set the curser on the starting position of the mark, hold [Shift] and press the left mouse button [Shift+L🖱], while moving the cursor along the *X*-axis.

The period of a mark may be reduced or the entire mark deleted by holding [Shift] and pressing the right mouse button [Shift+R🖱], while moving the cursor along the *X*-axis over the marked section.

Default mark names and edit mark names: see Section 5.2

5.1. Statistics [F2]
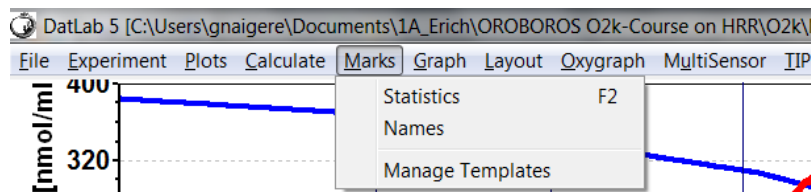
⊙   Averages, maximum or minimum values are displayed for marks defined on a selected plot. The active plot is indicated from which the marks over time sections are taken. The active plot can be selected by a mouse click on the ▶ name of the plot in the upper panel. Values are then shown for all available plots for sections defined by the marks of the selected plot.
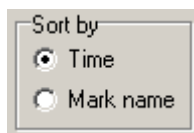
Sort by 'Time' or 'Mark name'. You can select the sequence of marks to be listed according to a time sequence (Time) or in alphanumerical order (Mark name).

Any single data field of the table can be selected by a left mouse click, copied by Ctrl+C, and pasted into a Windows™ file by Ctrl+V.

Mark Statistics

Select Marks in:

Plot
O2 Flux per V (A)
O2 Concentration (B)
▶ O2 Flux per V (B)
Block Temp.
Barom. Pressure

Select:
- ◉ Averages
- ○ Maximum
- ○ Minimum

Sort by:
- ◉ Time
- ○ Mark name

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| Averages	Unit	PGM	PGM+D	PGMc+D	PGMSc+D	PGMSc+u	S(Rot)c+u	Azd
Start		00:38:41	00:47:53	00:54:54	01:03:43	01:09:30	01:21:59	01:32:06
Stop		00:43:42	00:49:52	00:58:27	01:06:48	01:11:49	01:25:41	01:37:44
N Points		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
O2 Concentration (A)	nmol/ml	374.1556	353.8741	322.0682	271.7690	232.6622	156.0967	127.8469
O2 Flux per V (A)	pmol/(s*ml)	18.6840	57.6745	62.6937	117.9906	117.0354	85.3870	22.0974
O2 Concentration (B)	nmol/ml	371.3489	348.9256	319.7391	280.6017	253.8285	196.3220	180.3078
✕ O2 Flux per V (B)	pmol/(s*ml)	31.1614	55.7507	55.2544	80.5934	79.3549	56.8724	8.6126
Block Temp.	°C	37.0000	37.0001	37.0001	37.0001	36.9999	37.0001	36.9999
Barom. Pressure	kPa	87.9000	87.9000	87.9000	87.9000	87.9000	87.9000	87.9000
Peltier Power	%	-42.6487	-42.7782	-42.9898	-43.5464	-43.7752	-44.2473	-44.2408
pX (A)	V	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900
Slope X (A)	Unit	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
pX (B)	V	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900	-9.9900
Slope X (B)	Unit	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Copy to Clipboard   Close

Copy to Clipboard: After a mouseclick on the Copy to Clipboard button, the mark statistics table can be copied into an Excel or SigmaPlot file. It is important to carefully evaluate which set of data rows is relevant. For example, with marks selected on "O2 Flux per V (B)", oxygen concentration and flow are also shown for chamber (A), but for plots in chamber (A) the marks on (B) do not necessarily reflect properly chosen sections of the experiment in (A). For demonstration, an analysis with Excel is shown in 'Oxygen flux analysis: DatLab On-Line' (MiPNet12.09).

5.2. Mark Names

Default mark names: Marks are labelled with consecutive numbers, starting from 01, independent of the sequence of position of the marks. When deleting a mark [Shift+R🖱] other than the highest number, then this mark number is missing on the mark list. For any number <99, the consecutive automatic mark name will be the next higher number. >99, all marks are named as 00. Extending the default mark names, e.g. from '03' to '03-TD', maintains the numerical sequence for default names in subsequently generated marks.

Edit Mark Name: To rename a mark, click into the top bar of the mark and enter the new name. Marks for calibration may be named R1 and R0, for air and zero calibration, respectively.
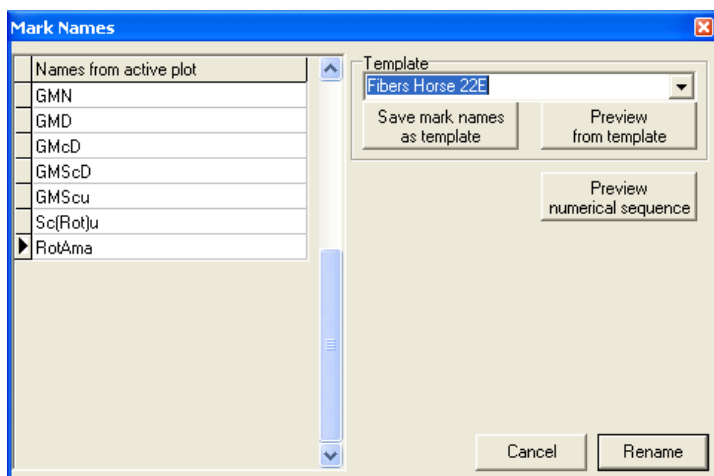
Mark Name window: Names for the active plot are listed and can be edited in this overview window. These mark names can be saved as a template for automatically renaming all marks of another plot in any experiment. All marks can also be renamed in numerical sequence.

6. Graph
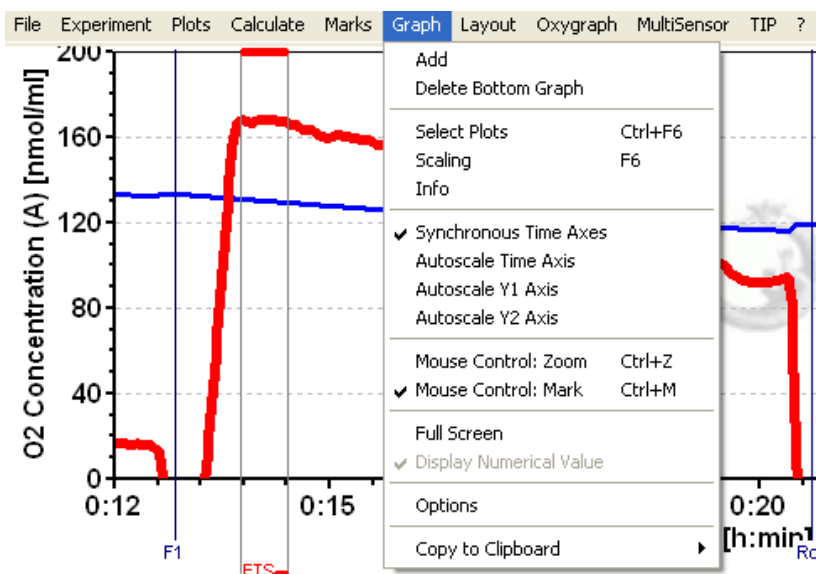
☞   The active graph is highlighted and indicated by the OROBOROS logo. The active graph is selected by clicking into the graph.

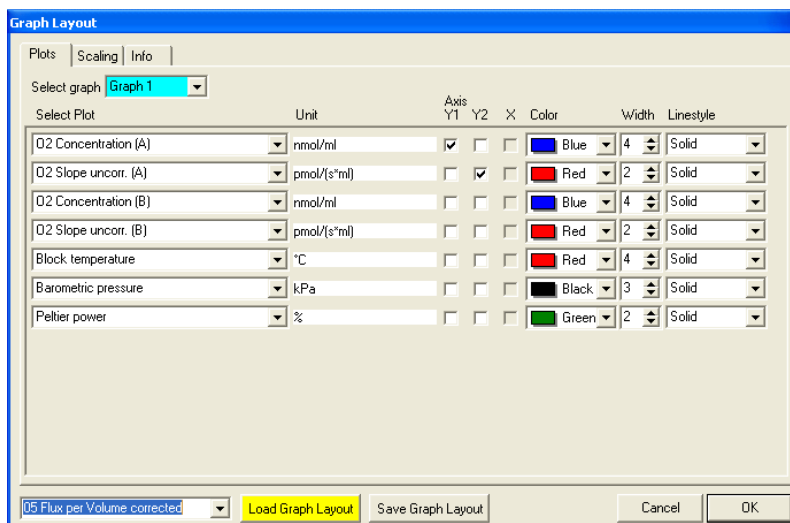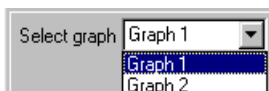**6.1. Add**   A new graph is added at the bottom of the screen. For the new graph, you have to select plots for display [Ctrl+F6].

6.2. Delete Bottom Graph

The bottom graph is deleted, which re-appears with the same layout by Add.

6.3. Graph Layout – Select Plots [Ctrl+F6]

▼ Pull down to select any of the defined graphs and load Graph Layout for defining the plots in a graph.

Select Plot – ▼: Select the specific plot, the units of which are displayed in the next column.

O$_2$ Concentration (A and B) ▼:
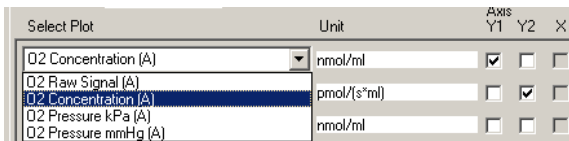
O2 Raw Signal [V]
O2 Concentration [nmol/ml]*
O2 Pessure [kPa]*
O2 Pressure [mmHg]*

O$_2$ Flux (A and B) ▼:
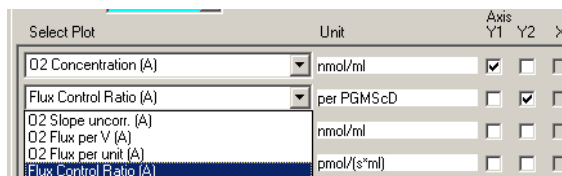
O2 Slope uncorrected [pmol·s^{-1}·ml^{-1}]*
O2 Flux per Volume [pmol·s^{-1}·ml^{-1}]**
O2 Flow per cells [pmol·s^{-1}·10^{-6}]^{***a}
O2 Flux per mass [pmol·s^{-1}·mg^{-1}]^{***b}

O2 Flux per unit [pmol·s^{-1}·ml^{-1}·Unit^{-1}]^{***c}
Flux Control Ratio [per reference state]^{***d}

* Accurate results depend on oxygen calibration; edit in [F5].
** In addition, accurate results depend on correction for instrumental background oxygen flux; edit in [F3].
*** Accurate results depend further on information about sample concentration; edit in [F3].
^{a} Sample unit in [F3] selected as <u>Million cells</u>▼.
^{b} Sample unit in [F3] selected as <u>mg   </u> ▼.
^{c} Sample unit in [F3] selected as <u>Units   </u> ▼.

d This option is available after defining a reference state in the window Plots \ Flux Control Ratios.

Note: Oxygen flux is expressed in moles of oxygen, O_2, independent of choosing a plot of oxygen pressure or concentration.

Block Temperature [°C]: The continuously measured temperature of the copper block, housing the two glass chambers.

Barometric Pressure: The continuously recorded absolute (local) barometric pressure [kPa].

Peltier Power: For regulation of block temperature, continuously recorded [% of maximum power].

pX (A):   Signal of an electrode connected to the left side of the Oxygraph-2k.

Slope pX (A): Negative slope [mpX/s] calculated for the signal of electrode (A).

pX (B):   Signal of an electrode connected to the right side of the Oxygraph-2k.

Slope pX (B): Negative slope [mpX/s] calculated for the signal of electrode (B).

Axis, Y1, Y2: Define the left (Y_1) or right axis (Y_2) for a plot to be shown by setting or deleting the arrow in the appropriate box.

Color: Define the color of the plot.
Width: Define the line width of the plot.
Line style: Define the line style of the plot. This is only possible when the width is defined as 1.

Save Graph Layout: After editing the settings in the [Ctrl+F6] and [F6] windows, the layout may be saved under the old or a new name. Layout files can be renamed and deleted using the Layout menu.

Load Graph Layout: Loads a Graph layout selcted with the drop-down window on the left of the button.

6.4. Graph Layout – Scaling [F6]

Left mouse click on the X-axis, Y_1- or Y_2-axes opens the F6 window. The F6 window provides flexibility to vary the display of the plotted data according to specific needs. Viewing plotted data in differently scaled graphs, zooming the signal and time scales, and scrolling along the axes of the graph provide maximum information on the current experiment, but do not influence the format of stored data.

👆 Choosing standardized Graph layouts with suitable ranges for the axes is recommended for visual evaluation of the results, since the selected ranges for the axes changes the appearance of the data dramatically.

Select graph ▼: Pull down to select any of the defined graphs for Scaling.

Minimum: Defines the minimum axis position (suppression) for the display of data at a constant range.

Maximum: Defines the maximum axis position for the display of data at a constant range.

Range: The Y_1 axis (left), Y_2 axis (right) and X axis (Time [s]; bottom) display data in the ranges defined in the Scaling window.

Select a predefined Graph layout from the options in the pull down menu. Edit the Graph layout name by clicking on the name, and click on Save to save the entire graph layout.

Example: Y_1-axis: Concentration [nmol/ml=µM]; A range of 200 and Start at 0 is chosen after calibration to show the oxygen concentration in the full range (0-200 µM) without amplification.
Y_2-axis: Flux [pmol·s^{-1}·ml^{-1}]; Range of 60 and Start at 0 shows flux from 0 to 60 pmol·s^{-1}·ml^{-1} when the oxygen signal is calibrated [µM].
X-axis: Range 1:00 h and Start at 35 min presents data starting at 35 min, over a 1:00 h time interval from 35 to a maximum of 1:35 h.

Apply to Graph 2: The chosen scaling is applied to Graph 2.

Standard DatLab Graph Layouts: Any defined Layout may be selected from the ▼ pull-down menu for defining initial settings for the Plot [Ctrl+F6] and Scaling [F6] windows (Sec. 6.3).

Save Graph Layout: After editing the settings in the [Ctrl+F6] and [F6] windows, the Graph layout may be saved under the old or a new name (Sec. 3.1).

Load Graph Layout: Loads a Graph layout selected with the drop-down window on the left side of the button.

OK: Activates the Graph layout settings.

Arrow keys: Independet of the [F6] window, zooming (expansion or compression), panning (time on *X*-axis) and scrolling (data on *Y*-axes) are possible by using the arrow keys.

[→] or [←]:   Panning, shift 50 % of the time axis to the right or left. During on-line operation, you may have to switch the automatic panning into off-mode [F6], to view the data backwards without changing the time range.

[Ctrl+→]:   Magnify the time resolution on the screen (decreasing the time range). [Ctrl+→] expands the data, half the original time range is displayed.

[Ctrl+←]:   Cover a larger time range on the screen. [Ctrl+←] compresses the data, twice the original time range is displayed. The reference time point is fixed on the origin (left) during zooming in and out.

Scaling of *Y*-Axes:
  ✆   Select a single plot by clicking onto the label of the plot in the figure legend on the right of the graph. The active plot is highlighted. Operations (setting marks, scrolling) generally apply to the active plot.

Arrow keys: Independent of the [F6] window, zooming (expansion or compression) and scrolling (data on *Y*-axes) are possible by using the arrow keys after selecting a plot. The reference point is fixed on the bottom of the graph. Scale expansion or compression are effected by holding [Ctrl] while pressing the up/down arrow key. Only the active plot is thus modified, while the plot on the other *Y*-axis, and all plots on the other graph remain unchanged.

[↑] or [↓]:   Scroll up and down the *Y* axis, with a shift of 50% each time.

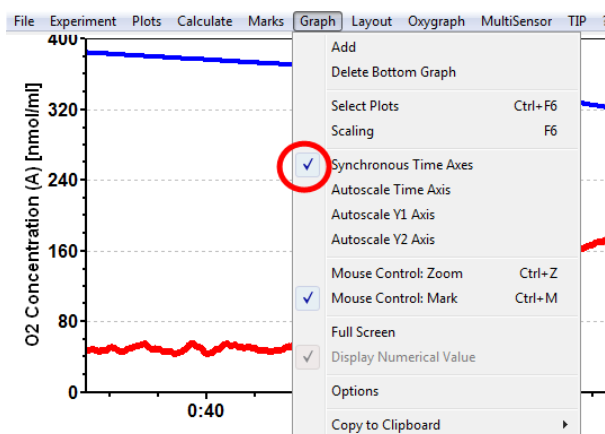[Ctrl+↑]:   Magnify the signal (half the signal range is displayed).

[Ctrl+↓]:   Cover a larger range on the screen for overview (twice the signal range is displayed).

6.5. Graph Layout – Info
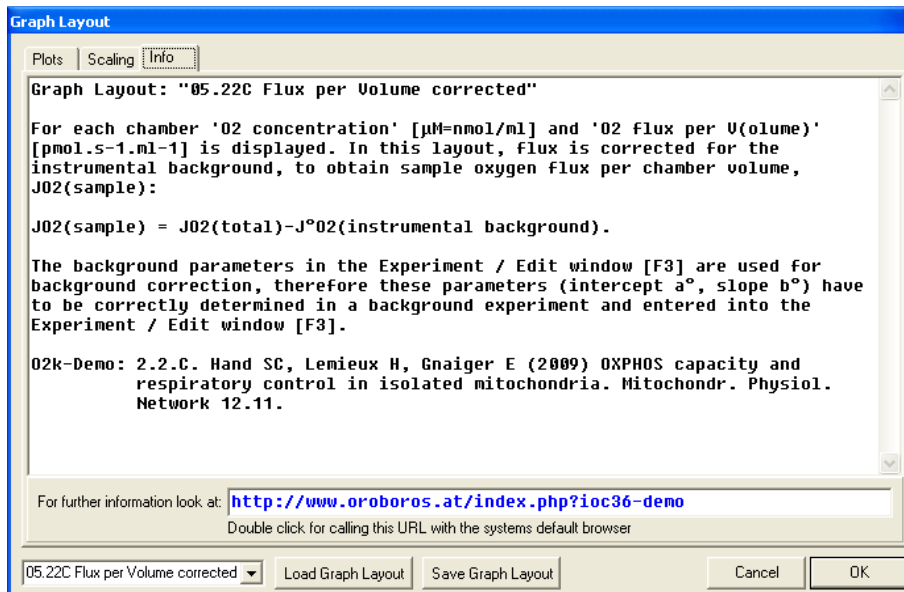
6.6. Synchronous Time Axes

✓   This option sets the time axes of all graphs at an identical range and offset, which is particularly useful while panning.

6.7. Autoscale Time Axes

Autoscaling overview the entire experimental period.

6.8. Mouse Control: Zoom [Ctrl+Z]

🖰   **Zoom in:** Select 'Mouse Control: Zoom' in the menu or press [Ctrl+Z]. Click into the graph where you want to zoom in. Place the cursor at the upper lefthand corner of the field for zooming. Hold [Shift],press the left mouse button [Shift+L🖰] and slide the cursor to the lower righthand corner to define the field for zooming in. The text for all axes now indicates the respective range (full scale).
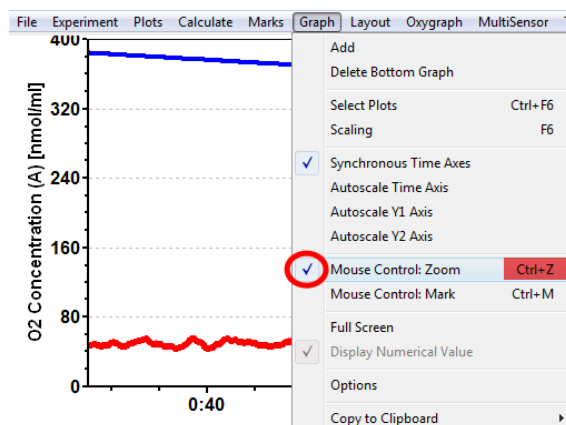
 ⊕   **Zoom out:** To return to the original scaling (as defined in the Graph layout), hold [Shift], press the left mouse button and slide the cursor anywhere to the left [Shift+L⊕].

6.9. Mouse Control: Mark [Ctrl+M]

The Mark mode is active by default, or can be selected in the menu or by [Ctrl+M]. Specific sections of the experiment can be marked on each plot. Usually, marks are set on the plot for oxygen concentration for calibration ([MiPNet12.08](#)), whereas marks on the plot for oxygen flux are set for exporting average values of flux to a table.

6.10. Full screen [on/off]

 ✓   The selected graph may be shown alone on the full screen [on], or together with the other defined graphs [off]. Full screen [on] is particularly useful for a single channel overview and for Copy to clipboard [ALT−G and C].

6.11. Display numerical values

 ✓ **On:**   The current numerical values are displayed in the graph for the active plots on the Y_1 axis and Y_2 axis (on-line option only).

6.12. Graph Options

 ✓ **Show Legend:** Lists the quantities plotted on each *Y* axis.
  Show Legend: Is changed to the option *off* with a left mouse click. In this case, the names of plots are not shown in the legend.
  Show Gridlines: Gives an option to show or hide vertical and horizontal gridlines.
  Show Marks [on/off]: The display of marks can be switched on or off for all graphs, without deleting the marks.
 **Show events [on/off]:** The display of events can be switched on or off for all graphs, without deleting the events.
  Font: Provides options to change the font size and style of axes labels and numbers.
  Event Line Style: Can be modified.
 ✓ **Show OROBOROS identiy in clipboard files:** *Yes!*
 ✓ **Show seconds on time axis:** Gives an option to include (bottom graph) or exclude (top graph) the display of seconds on the labels of the time axis.
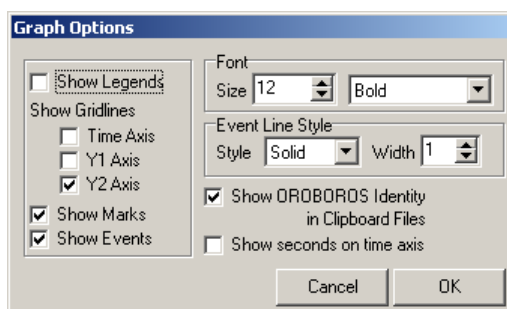
Copy to Clipboard
>   Select the active graph. In the Graph menu, click on Copy to Clipboard, and select the WMF or BMP format (Graph\Copy to clipboard\WMF); [Alt+G C W]. Open the target file (Word, PowerPoint), and paste [Ctrl+V], or open Microsoft Photo Editor (BMP format), select Edit and Insert as new Graph, and save the Graph in JPG format.

7. Layout

DatLab Graph Layouts: Any defined Layout can be selected from the Layout menu for defining initial settings for the Plots [Ctrl+F6] and Scaling [F6]. Typical standard DatLab graph layouts yield plots for the
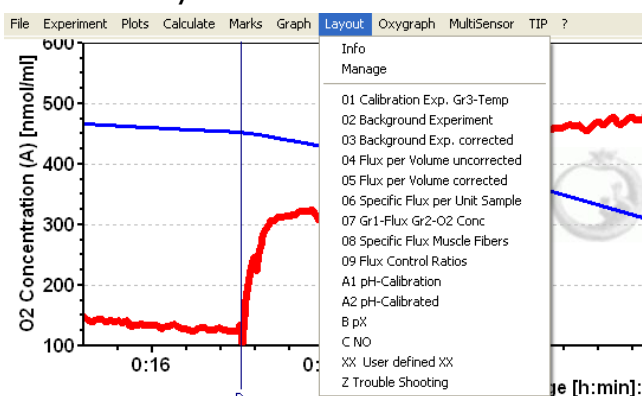

left chamber in Graph 1 (top), and for the right chamber in Graph 2 (below). For on-line display, a 30 min time range is frequently used, but adjusted during the experiment [F6]. The standard option recommended at start is (MiPNet12.06):

01 Calibration Exp. Gr3-Temp : Y_2 is the (negative) slope of O_2 concentration over time [pmol·s^{-1}·ml^{-1}]. In Graph 3 (bottom), the Oxygraph-2k block temperature is plotted on the Y_1 axis, and the relative Peltier power for thermostatic regulation is plotted on the Y_2 axis.

02 Background Experiment: The uncorrected O2 slope is plotted with zero in the middle of the Y_2 axis to observe stabilization of the signal (MiPNet12.09).

03 Background Exp. Corrected: Displays the background-corrected volume-specific oxygen flux (MiPNet12.09).

04 Flux per Volume uncorrected: This layout displays total oxygen flux per volume, without background correction.
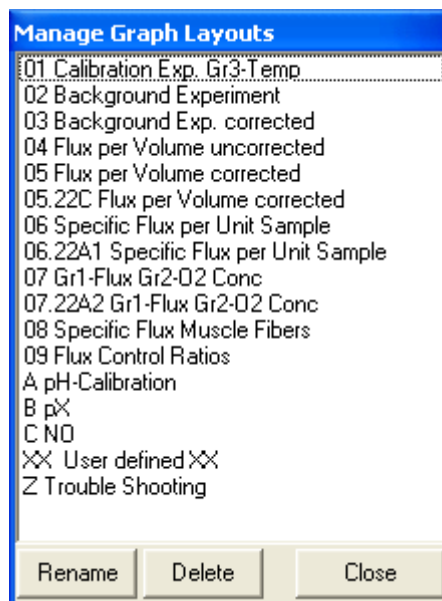
05 Flux per Volume corrected: This layout displays background-corrected oxygen flux per volume, which is most relevant to evaluate experimental details. This plot is also chosen when measurements on sample density are available only at a later stage (MiPNet12.09).

06 Specific Flux per Unit Sample: Used for plotting background-corrected oxygen flux per unit sample. The unit depends on the choice made in the F3 window in the pull-down menu Sample Unit.

07 Gr1-Flux Gr2-Conc: This provides an example of arranging oxygen flux for both chambers in the top graph, and oxygen concentration in the bottom graph.

A pH Calibration: Displays raw signals of the pX channel for chamber A and B in graph 1 and 2. After calibration, the calibrated signal (e.g pH value) is diplayed.

B pX: As layout 01, but instead of oxygen concentrations raw voltages are displayed. This layout should be used for trouble shooting (too high or too low voltage, thermal instability, etc).

C NO: For experiments with additional NO sensors.

XX user defined XX: By editing and saving plots and scaling information in in the Plots/Scaling menus, the user can generate specific layout files.
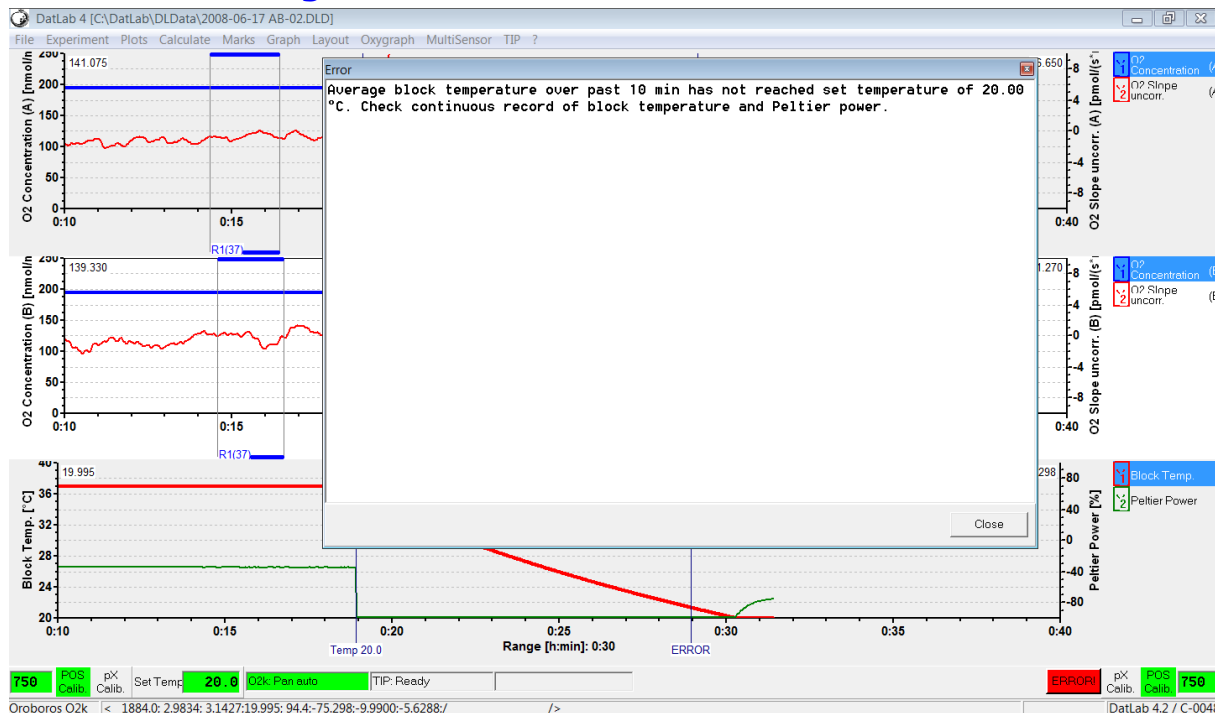
Z Trouble Shooting: For critical evaluation of the oxygen sensor performance.

Info: Layouts are explained, links are provided to related chapters of the O2k-Manual or O2k-Protocols with links to O2k-Demofiles (for examples, see Appendix).
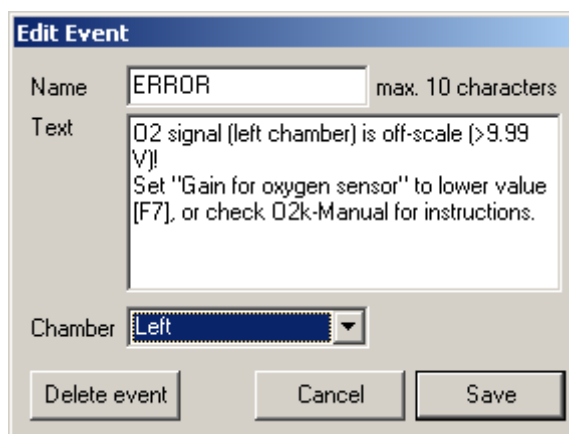
Manage Layouts: Predefined as well as user defined layouts can be renamed or deleted.

8. General Information

8.1. Error Messages

If an error message appears (on-line only), click on the ERROR signal (red), and note the information in the Error window. Click on Close to remove the ERROR signal from the O2k Status Line.

An error message is additionally recorded as an event if the oxygen signal is above 9.99 V for more than 60 s.

9. Some features of Windows™

Print:	The entire screen is 'printed' to clipboard.
Alt+Print:	The active window is 'printed' to clipboard.
Ctrl+C:	Copy to clipboard.
Ctrl+V:	The contents of the clipboard is pasted into the page of a Windows™ program (Word; Excel; PowerPoint).

Appendix: Graph Layouts

Graph Layout: **01 Calibration Exp. Gr3-Temp**

Calibration experiment with temperature and Peltier power in Graph 3.

This is typically the first layout used after switching on the O2k. Oxygen concentration (blue lines, left Y-axis) and O2 slope uncorrected (red lines, right Y-axis) are displayed on the top graph for the left chamber, and below for the right chamber. The third graph (bottom) shows the block temperature on the left Y axis and the Peltier power on the right Y axis. Only when both temperature and Peltier power are constant, the chambers have reached thermal equilibrium. The next step is to observe equilibration of the oxygen signal with a defined gas phase above the stirred aqueous phase ('open' chamber; usually with air as the first step) to perform an oxygen calibration.

If anything unusual is observed (always zero flux, jumping signals), the layout "Z Trouble Shooting" should be used.

Manual: Oxygraph-2k: Start High-Resolution Respirometry. MiPNet12.06.

Info: www.oroboros.at/?O2k-Manual

Graph Layout: **02 Background Experiment**

For recording O2 sensor calibration and a test for instrumental background oxygen flux.

For each chamber, 'O2 Concentration' and 'O2 Slope uncorrected' are displayed on the left and right Y-axis, respectively. 'O2 Slope uncorrected' is the negative slope of oxygen concentration (multiplied by 1000 to convert to units [pmol/ml]) over time [s]. No correction is applied for instrumental background oxygen flux. Zero flux in the 'open' chamber at air calibration indicates stability of the oxygen signal. After closing the chamber, 'O2 Slope uncorrected' deviates from zero as a function of the oxygen consumption of the polarographic oxygen sensor and of oxygen diffusion into or out of the chamber.

Manual: Oxygen and pX (pH) calibration by DatLab. MiPNet12.08.
                Oxygen Flux Analysis: DatLab On-Line. MiPNet12.09.

Ref: Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 128: 277-297.

Info: www.oroboros.at/?O2k-Manual