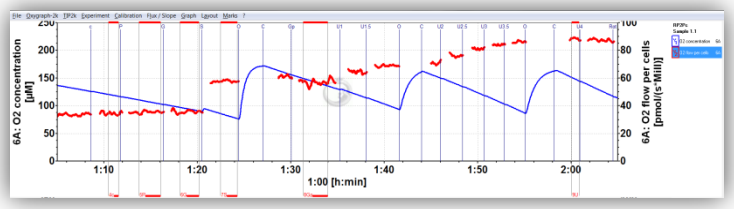
Mitochondrial Physiology Network 19.18(C04):23-38 (2016)
Version C03: 2016-08-08 DatLab 7 ©2014-2016 OROBOROS
C: http://wiki.oroboros.at/index.php/MiPNet19.18C_DatLab_Guide

DatLab guide



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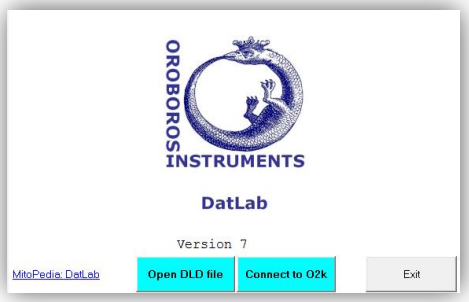
Section Page

C

This guide through features of DatLab presents an integral component of high-resolution respirometry. For specific applications of DatLab, see:

» MiPNet19.18A O2k start
» MiPNet19.18D DatLab O2k calibration
» MiPNet19.18E DatLab O_2 flux analysis: real-time
» MiPNet12.10 Titration-Injection microPump, TIP2k
» MiPNet17.05 O2k-Fluo LED2-Module
» MiPNet15.03 O2k-MultiSensor-ISE
» MiPNet15.05 NO-Manual



1. File

1.1. Open

Ctrl+O L🖱 Left mouse click Open DLD file to open a previously saved **D**at**L**ab **D**ata file. It is not possible to open a second file simultaneously. You can do this in an additionally opened DatLab programme. If DatLab is connected to

the O2k for recording, another DatLab file cannot be opened before disconnecting the O2k and closing the currently recorded file.

1.2. Close **Ctrl+F4** Close a DLD file. A window Save changes to file? pops up offering the options to close the file after saving the changes, or close the file without saving any modifications on the presently open file.

1.3. Save **Ctrl+S** When disconnected from the O2k, save any changes made under the identical file name overwriting the previous file. Such changes do not affect the raw data of the experiment, but relate to calibrations, experimental protocol, marks, events, and layout.

Temporary backup files are generated by DatLab in the current user's temp directory, 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.

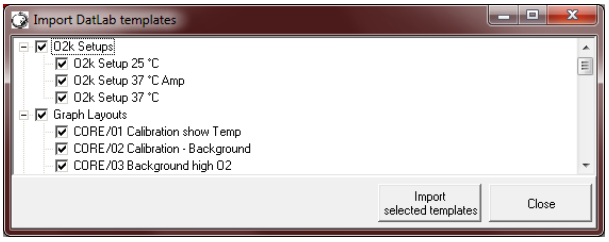
1.4. Save and disconnect **Ctrl+F4** Stop data acquisition and disconnect from the O2k (MiPNet19.18A).

1.5. Save as When disconnected from the O2k, save the file under a different file name, optionally in a different directory.

1.6. File search **Ctrl+F** yields a list of all files labelled by the experimental code in a selected directory (see Experiment \ Edit F3). Click on the file name to preview the protocol.

1.7. Delete The decision to delete a file containing no useful data can be made most easily when viewing the traces. Only available when disconnected from the O2k.

1.8. Import **DatLab templates** can be imported for O2k setups, graph layouts, mark names, TIP2k setups and marks statistics configurations.



DatLab templates can be copied into the programme subdirectory \DatLab\DLTemplates from:

http://www.bioblast.at/index.php/DatLab_templates

1.9. Export **Data to text file (*.csv)** exports plots and events to a text file for further use in Excel and other programs.

Events to text file (*.csv) exports all information in Events to a text file (*.csv). This file may be used as a protocol, including the comments in the Events.

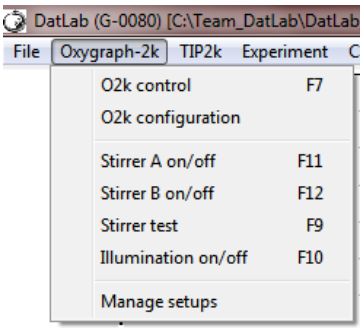
One channel to DatLab 2 analysis exports the O2 raw signal to a DatLab 2 file (*.DLR); e.g. for O_2 kinetics in DatLab 2.

1.10. Change user Enter the name to update the user code.

1.11. Manage users Rename or delete users.

1.12. Exit Exit DatLab.

2. Oxygraph-2k



2.1. O2k control F7 Control the O2k operation mode. » MiPNet19.18A O2k start

2.2. O2k configuration Select or deselect channels that are not actually used, enter sensor numbers and edit channel labels. » MiPNet19.18A O2k start

2.3. Stirrer A (B) on/off F11 (F12) Stirrers in chamber A or B are switched on/off.

2.4. Stirrer test F9 Stirrers are stopped intermittantly (default: 30 s) for a stirrer test. » MiPNet06.03

2.5. Illumination on/off F10 The illumination in both chambers is switched on/off.

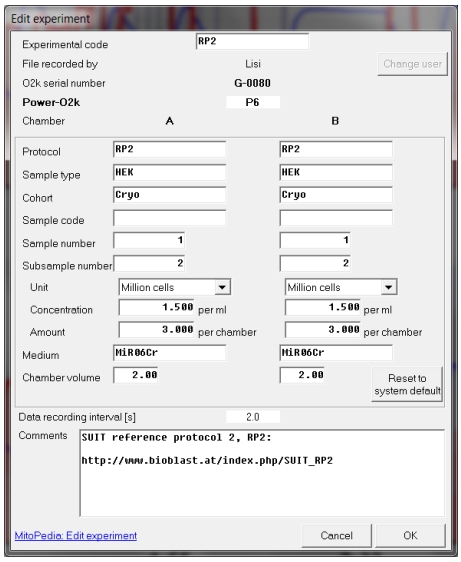
2.6. Manage setups Setups can be renamed or deleted.

3. TIP2k

» MiPNet12.10 Titration-Injection microPump, TIP2k

4. Experiment

4.1. Edit F3 Information on the experimental protocol can be edited at any time during or after the experiment, and all related results are re-calculated instantaneously with the new parameters. Initially, the Edit experiment window displays information from the last file recorded and saved while connected to the O2k.



Reset to system default L🖱 to reset values to system default.

Cancel L🖱 to proceed quickly with the experiment, and edit any time later.

Experimental code Up to 10 digits. The File search function Ctrl+F lists all files with identical experimental code within a selected directory.

File recorded by (*read only*) shows the user who recorded the file. While connected to the O2k the User code can be changed by L🖱 Change user.

O2k serial number (*read only*) automatically recorded.

Power-O2k (*read only*) as defined in the menue Oygraph-2k \ O2k configuration.

Chamber The following entries are entered separately for the left (A) and right (B) O2k chamber.

Protocol Enter the protocol name.

Sample Enter information about sample used in each chamber. No sample is added in O_2 calibration experiments.

- **Sample type, Cohort, Sample code, Sample number, Subsample number**
  » http://www.bioblast.at/index.php/Edit_experiment_in_DatLab
- **Sample concentration and amount**

Unit ▼ Select a unit to express the concentration or amount of sample in the HRR assay.

Million cells ▼ - Flow: cell number
mg ▼ - Flux: mg of protein, wet weight or dry weight.
Unit ▼ - Flux: units of another marker of sample size.

Concentration Enter the sample concentration (e.g. Million cells/ml, mg W_w/ml, mg mt-protein/ml). The corresponding amount of sample is calculated on the basis of the O2k chamber volume.

Amount Alternatively, enter the sample amount (e.g. biopsy W_w) if a known amount of sample is added into the chamber. The corresponding sample concentration is calculated on the basis of the chamber volume.

Medium Name of the incubation medium in the O2k chamber.

Chamber volume The default is 2.00 ml. It is important to define the actually used effective volume of the O2k chamber for further calculations of oxygen flux.

Data recording interval [s] (*read only*) is selected in the window O2k control F7.

Comments For display and printing in the window Experimental log.

4.2. Experimental log Ctrl+F3

The experimental log is generated automatically with information on O2k settings and calibrations, the Edit experiment window and various events. Time-dependent information can be viewed for chambers A, B or both. A filter is selected for viewing minimum, intermittent (default), or all information. L Preview to view the protocol, and Save as PDF file for quality control.

4.3. 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 at the top of the graph. A short comment can be entered to describe the event in detail.

Set events F4 Real-time: Press F4 to set an event quickly at the current time of the experiment (e.g. to indicate a manual titration into the chamber). The Edit event window pops up after setting a new event. Pressing F4 defines the time point of the event. Full attention can then be paid to the experiment. Edit the event later.

Ctrl+L Insert an event at any chosen moment of the plotted record of the experiment by placing the cursor anywhere in the graph at the selected time point, press Ctrl and click the left mouse button Ctrl+L.

Edit event L Left click on the name of an existing event to open the Edit event window to edit or Delete event.

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

Comment Further information can be entered into the text field.

Select O2k chamber A, B or both. The Event will be shown on plots for both or one selected chamber.

5. Calibration

Oxygen, potentiometric and amperomtric channels

Select chamber and channel to open Calibration.

» MiPNet19.18D O2k-Calibration

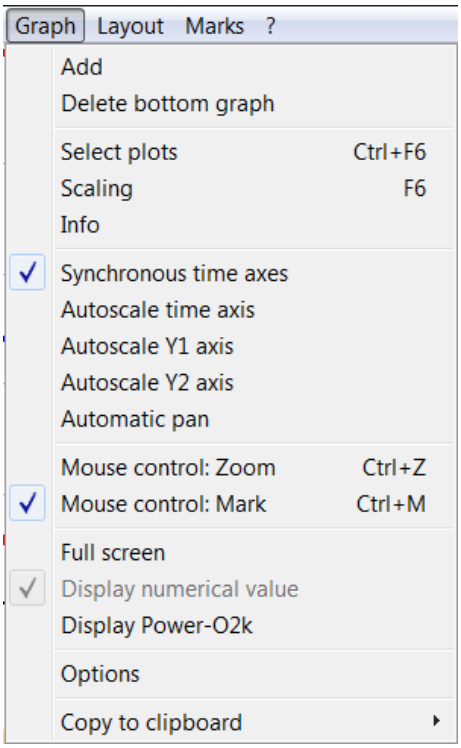
6. Flux/Slope

Oxygen, potentiometric and amperomtric channels

Select chamber and channel to open Slope configuration.

» MiPNet19.18E O2 Flux Analysis

7. Graph



L🖱 The active graph is selected by a left click into the graph. The active graph is highlighted and indicated by the OROBOROS logo.

7.1. Graph \ Add

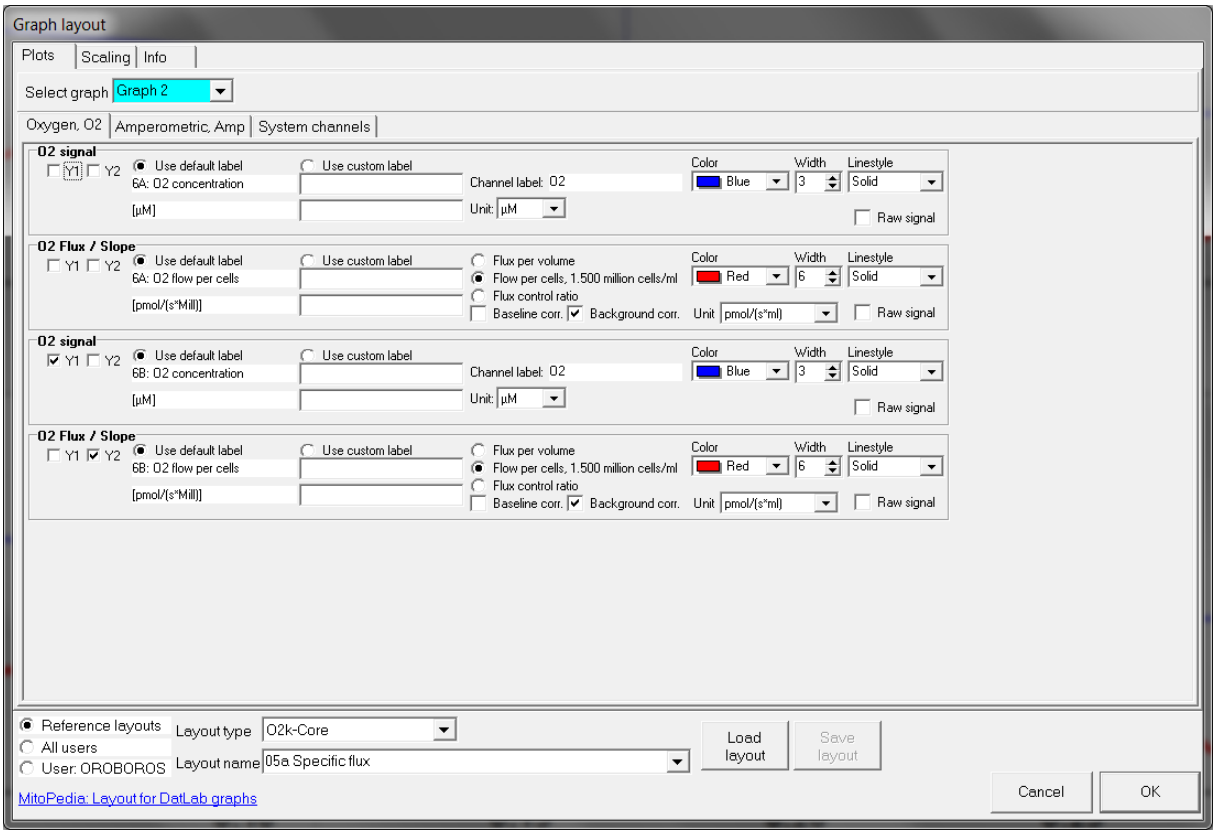
A new graph is added at the bottom of the screen. Select plots for display in the new graph, Ctrl+F6.

7.2. Graph \ Delete bottom graph

The bottom graph is deleted, which reappears with the same layout by Add.

7.3. Graph \ Select plots Ctrl+F6

7.3.1. Tab: Oxygen, O2



Select graph ▼ Pull down to select one of the displayed graphs.

Tabs Oxygen O2, Amperometric Amp, Potentiometric pX, System channels: L🖱 Left click on a channel type to select plots from this channel.

☑ **Y1, Y2** Select the left (Y_1) or right axis (Y_2) for a plot.
⊙ **Use default label** selects the default axis label.
⊙ **Use custom label** To define a different axis label, select and enter or edit a custom label.

Channel label..Edit (for Amp and pX channels).
▼ **Color** Defines the color of the plot.

▼ **Width** Defines the line width of the plot.
▼ **Line style** Defines the type of line (solid, dash, dot) of the plot.
▼ **Unit** Pull down to select a different unit (for Amp and pX).

☑ **Raw signal** displays the non-calibrated raw signal.

⊙ **Flux per volume**
⊙ **Normalization**
⊙ **Flux control ratio**
☑ **Baseline correction**
☑ **Background correction**

Further details:
http://www.bioblast.at/index.php/Select_plots_in_DatLab

Load layout Select layout category (⊙ Reference, ⊙ All users, ⊙User: Name), select Layout type: O2k-Core ▼, O2 & Amp ▼', O2 & pX ▼ , Other ▼ (depending on the channel selection in O2k configuration), and Layout name ▼.

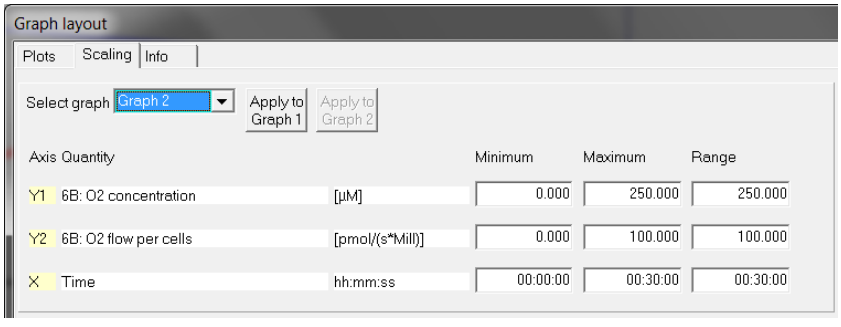
Save layout A layout can be saved with any layout name under the category ⊙ All users or ⊙ User: Name.

7.3.2. Tab: System channels

Block temperature The continuously measured temperature of the copper block, housing the two glass chambers [°C].
Barometric pressure The continuously recorded absolute (local) barometric pressure [kPa].
Ext temp Signal from external PT1000 temperature sensor (optional), "50.000" if no sensor connected [°C].
Env temp Temperature from internal temperature sensor recording the environmental (room) temperature [°C].
Peltier power for regulation of block temperature, continuously recorded [% of maximum power].

7.4. Scaling

7.4.1. Graph \ Scaling F6



L Left mouse click on the X-, Y_1- or Y_2-axis opens the window Graph layout Scaling. F6 provides flexibility to vary the display of the plots and create Graph layouts. Viewing plots

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. Different ranges for the axes change the appearance of data dramatically.

Choose a Graph reference layout for a standard layout.

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

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.

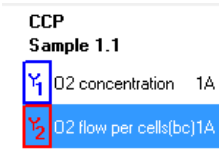
Range Define the range of the Y_1 axis (left), Y_2 axis (right) and X axis (Time).

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

Apply to Graph 1 The scaling defined for Graph 2 is applied to Graph 1.

7.4.2. Arrow keys

Use arrow keys to scrol (data on Y-axes), pan (time on X-axis), or zoom (expansion or compression: Ctrl+arrow key), independet of the F6 window.



L🖱 Select the active plot in a graph by a left click onto the label of the plot in the figure legend on the right. The active plot is highlighted. Scrolling and setting marks apply to the active plot.

↑ or ↓ Scroll up and down the Y axis, with a shift of 50% each time of the active plot.

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

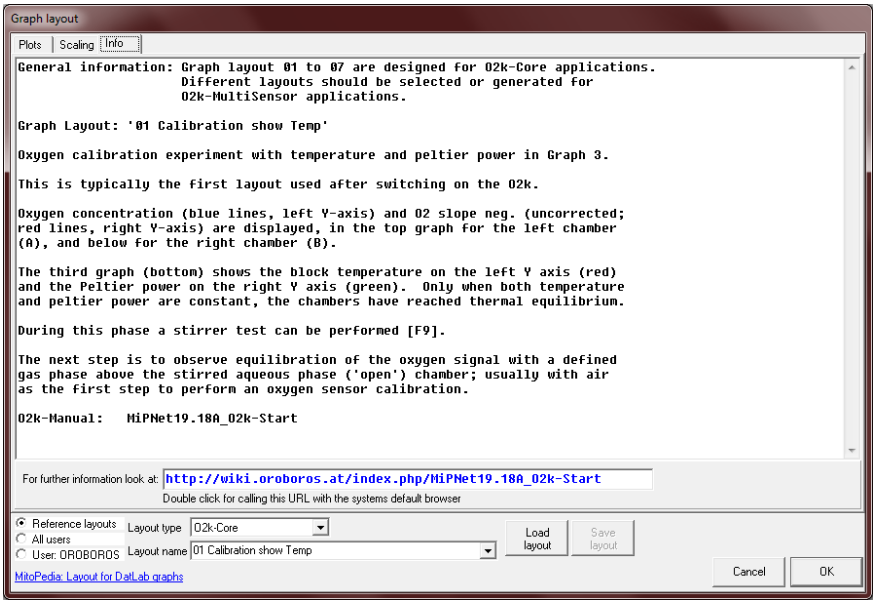
Ctrl+↓ Cover a larger range on the screen for overview (twice the signal range is displayed).

→ or ← Panning, shift 50% of the time axis to the right or left. During data acquisition, switch off automatic panning to pan 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. The upper limit of the time range is fixed.

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 right during zooming in and out.

7.5. Graph Info



View and edit information, and load or save a graph layout (Section 8).

A quick selection of the tabs Plots Ctrl+F6 and Scaling (F6) is possible.

7.6. Snchronous time axes

☑ Sets the time axes of all graphs at an identical range and offset, which is particularly useful while panning.

7.7. Autoscale time axis

Autoscale the entire experimental time scale.

7.8. Autoscale Y1 (Y2) axis

Autoscale the full data range.

7.9. Automatic pan

☑ Toggles automatic panning on/off, » MiPNet19.18A, Status line.

7.10. Mouse control: Zoom Ctrl+Z

Zoom in Select ☑ Mouse control: Zoom in the menu or press Ctrl+Z. L 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.

7.11. Mouse control: Mark Ctrl+M

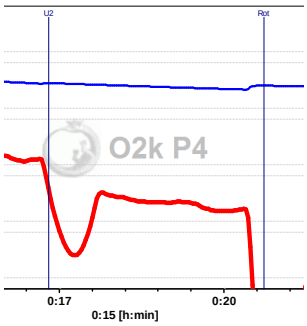
☑ The Mark mode is active by default, or can be selected in the menu or by Ctrl+M. Specific sections of the experiment can be marked on each plot. Usually, marks are set on the plot for oxygen concentration for calibration (MiPNet19.18D), whereas marks on the plot for oxygen flux are set for exporting the median or average of flux to a table.

7.12. Full screen ☑ On/Off

☑ On The selected graph may be shown alone on the full screen ☑, or together with the other defined graphs Off. Full screen is particularly useful for a single channel overview and for Copy to clipboard ALT+G B.

7.13. Display numerical value

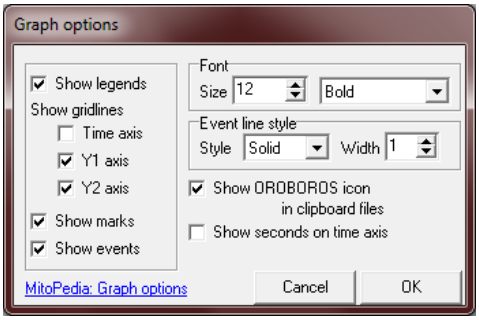
☑ On The current numerical values are displayed in the graph for the active plots on the Y_1 axis and Y_2 axis (during data acquisition only).



7.14. Display Power-O2k

☑ On The Power-O2k number, set in Oxygraph-2k \ O2k configuration, is shown in the active graph.

7.15. Graph options



☑ Show legends ☑**On/Off** Shows the quantities plotted on each *Y* axis.

Show gridlines gives options to show or hide vertical and horizontal gridlines.

☑ Show marks ☑ **On/Off** optionally shows or hides marks in all graphs, without deleting the marks.

☑ Show events ☑ **On/Off** optionally shows or hides events in all graphs, without deleting the events.

Font Change font size and style of axes labels and numbers.

Event line style can be modified.

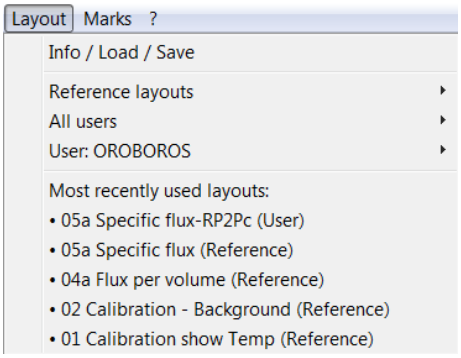
☑ Show OROBOROS icon in clipboard files *Yes!*

☐ **Show seconds on time axis** gives an option to exclude or ☑ include the display of seconds on the labels of the time axis.

7.16. Copy to clipboard

Select the active graph. In the Graph menu, L Copy to clipboard, and select the WMF or BMP format (Graph\Copy to clipboard\WMF); Alt+G B W. Open the target file (DatLab-Excel template, Word, PowerPoint, Paint, etc.), and paste the image Ctrl+V.

8. Layout



Graph layouts are selected from the Layout menu for standardized display of graphs, plots and scaling of axes.

8.1. Info / Load / Save

Open this window (Section 7.5) to view and edit information, and load or save a graph layout.

Types Four types of layout can be selected depending on the O2k configuration:

- O2k-Core: Oxygen channel ony.
- O2 & Amp: Oxygen and amperometric channel.
- O2 & pX: Oxygen and potentiometric channel.
- Other

8.2. Reference layouts – O2k-Core

In many Reference layouts, plots are shown for the left chamber in Graph 1 (top), and the right chamber in Graph 2 (below). During data acquisition, a 30 min time range is frequently used.

01 Calibration show Temp The layout used after switching on the O2k, for performing oxygen calibration. Oxygen concentration (blue lines, left *Y*-axis) and O2 slope negative (red lines, right *Y*-axis) are displayed in the top graph for the left chamber, and below for the right chamber. Graph 3 (bottom) shows the block temperature on the left *Y* axis and the Peltier power on the right *Y* axis. The chambers reach thermal equilibrium

when Peltier power stabilizes. Next observe equilibration of the oxygen signal with air in the gas phase above the stirred aqueous phase ('open' chamber), to perform an oxygen calibration (MiPNet19.18D).

02 Calibration - Background for recording O2 sensor calibration and instrumental O_2 background test. 'O2 Slope neg.' 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 O_2 background flux, $J°_{O2}$. 'O2 Slope neg.' is plotted on the right *Y*-axis with a scaling to display ±10 pmol·s^{-1}·ml^{-1} and zero in the middle of the Y_2 axis. Zero slope in the 'open' chamber at air calibration indicates stability of the oxygen signal. After closing the chamber, the slope 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, which is the first point in the instrumental O_2 background test (MiPNet19.18E).

03 Background high O2 for recording an instrumental O_2 background test at high oxygen from 150 to 450 µM.

04a Flux per volume displays background-corrected oxygen flux per volume, which is most relevant to evaluate experimental details, i.e. flux per volume is optimally in the range of 20 to 200-500 pmol·s^{-1}·ml^{-1}. This plot is also chosen when measurements on sample density are available only at a later stage (MiPNet19.18E). Total O_2 flux is corrected for instrumental O_2 background, $J°_{O2,V}$, to obtain sample oxygen flux per chamber volume, $J_{O2,V}$(sample):

$$J_{O2,V}(\text{sample}) = J_{O2,V}(\text{total}) - J°_{O2,V}$$

04b Flux per volume overlay is similar to the layout '04a Flux per volume'. Graph 1 shows the background-corrected oxygen flux per volume [pmol·s^{-1}·ml^{-1}] superimposed from both chambers. Graph 2 shows oxygen concentration [µM].

05a Specific flux for plotting background-corrected oxygen flux per unit sample. The unit as a marker for the amount of sample is defined in the F3 window. Example: Select Unit mg ▼ for the amount (mass) of sample added to the chamber. The mass-concentration is automatically calculated (division by chamber volume, typically 2 ml). Then mass-specific flux [pmol·s^{-1}·mg^{-1}] is displayed as

volume-specific flux [pmol·s^{-1}·ml^{-1}] divided by mass-concentration [mg/ml]:

$$J_{O2,mass} = J_{O2,V} / \text{mass-concentration}$$

05b Specific flux overlay is similar to '05a Specific flux'. Graph 1 shows the background-corrected oxygen flux per unit sample superimposed from both chambers. Graph 2 shows oxygen concentration [µM].

06a Specific flux high O2 is same as '05a Specific flux' with the scaling adapted to the high oxygen concentration used for permeabilized muscle fibers (150 to 450 µM; range 300 µM).

06b Specific flux high 02 overlay as '06a Specific flux high O2', but oxygen flux (graph 1) and oxygen concentration (graph 2) superimposed from both chambers.

07a Flux control ratios shows the flux control ratio (*FCR*) and the oxygen concentration for the left chamber in graph 1 and for the right chamber in graph 2. First, the reference and baseline metabolic states are marked and the marks are named (Marks \ Names). Then the marks are selected in the menu Flux/Slope \ O2 slope.

07b Flux control ratios overlay as '07a Flux control ratios', but *FCR* (graph 1) and oxygen concentration (graph 2) superimposed from both chambers.

» Layouts for O2k-MultiSensor applications are explained in the specific sections of the O2k-Manual.

8.3. All users

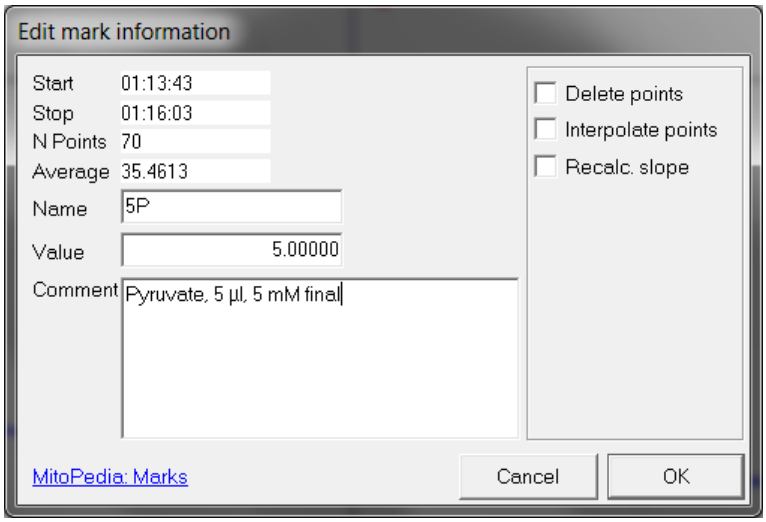
A reference layout or any other layout can be modified and saved under All users, which is recommended for a team using project-specific layouts.

8.4. User: Name

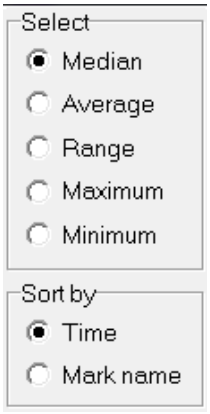
A reference layout or any other layout can be modified and saved under a specific user name, which is recommended to distinguish individual layouts from standard layouts used by a team.

Further details:
http://www.bioblast.at/index.php/Select_plots_in_DatLab

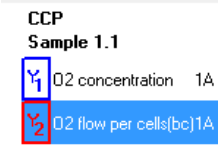
9. Marks



Set Marks to obtain the median, average and range of the data within the mark, for calibration of the oxygen signal (MiPNet19.18D) and flux analysis (MiPNet19.18E). Marks define a section (period of time) in a selected plot (column) and contain the data of the selected plot within the defined section of time. Marks are shown by a horizontal bar in the active plot. Several marks can be set on any plot, but marks cannot overlap within a plot and are separated by one or more data points which are not marked.



L **Mark** In the Graph menu select ☑ Mouse control: Mark, or press Ctrl+M.



L Select the active plot in a graph by a L left click onto the label of the plot in the figure legend on the right. The active plot is highlighted.

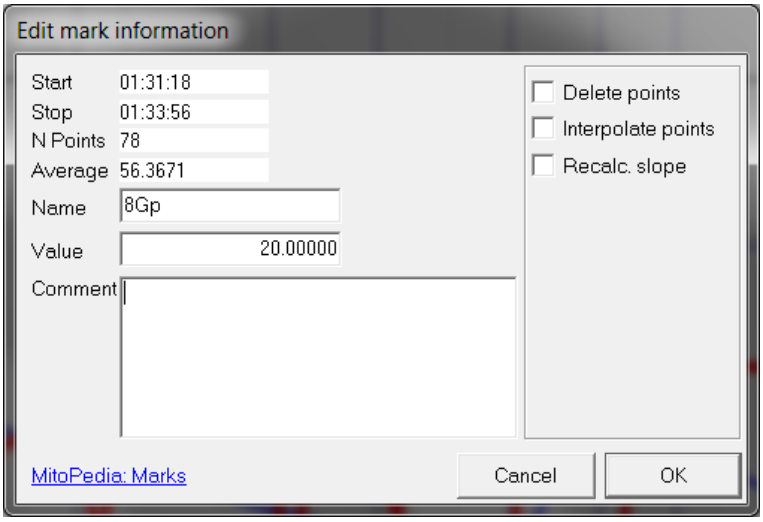
Shift+L Set the cursor on the starting position of the mark, hold Shift and press the left mouse button Shift+L, while moving the cursor along the *X*-axis.

Shift+R 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 Marks are labelled with consecutive numbers by default, starting from 01, independent of the sequence of position of the marks. When deleting a mark Shift+R, then this mark number is missing on the mark list. Marks >99 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 information L click into the top or bottom bar of the mark:

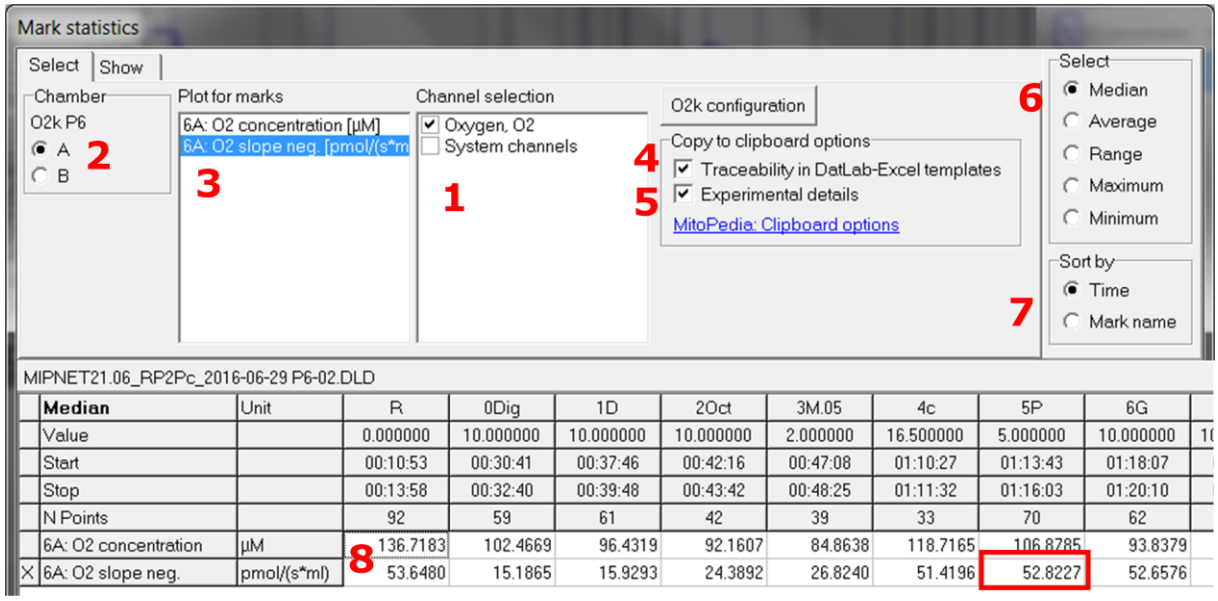
Name: Enter the new name. Mark names are R1 and R0 for air and zero O_2 calibration, respectively.



Value: optional. In SUIT protocols, the value is entered as the volume of a titration, for calculation of the dilution of sample in DatLab-Excel templates. The value is used as a concentration in the Amp calibration window.

Comment: optional.

9.1. Marks \ Statistics F2



1. ☑ Select channels for which plots should be displayed.
2. ⊙ Select the O2k chamber for which plots should be displayed in the marks statistics table.
3. L🖱 click on the source plot on which the marks are set.
4. ☑ Traceability in DatLab-Excel templates: Experimental details are copied to clipboard, where they can be edited further. Instrumental O_2 background correction and normalization of flux are calculated in the DatLab-Excel template, from O2 slope neg. This allows for traceability of instrumental settings, normalization and baseline correction of flux in the spreadsheets. Instrumental background tests and analyses on the amount of sample can be completed after an experimental series, and updated corrections of flux can thus be finalized in the spreadsheet.

5. ☑ Experimental details are copied to clipboard. This option is de-selected for export of data as displayed in the graph windows, particularly for using DatLab-Excel templates without traceability.
6. ⊙ Select the statistical value calculated over the sections defined by marks. Default: ⊙ Median.
7. ⊙ Select the sequence of marks, sorted as a ⊙ Time sequence or ⊙ Mark name in alphanumerical order.
8. Values are displayed for selected plots. The source plot for marks is indicated by an "X".

L click to select a single data cell of the table, copy by Ctrl+C, and paste into a Windows™ file by Ctrl+V.

Copy to clipboard: L Copy to clipboard to copy the mark statistics table into an Excel or SigmaPlot file. It is important to carefully evaluate which set of data rows is relevant.

More details » MiPNet19.18E O2 flux analysis.

Show system channels in statistics: Select or deselect sytem channels (barometric pressure, block temperature etc.) to be shown in Statistics.

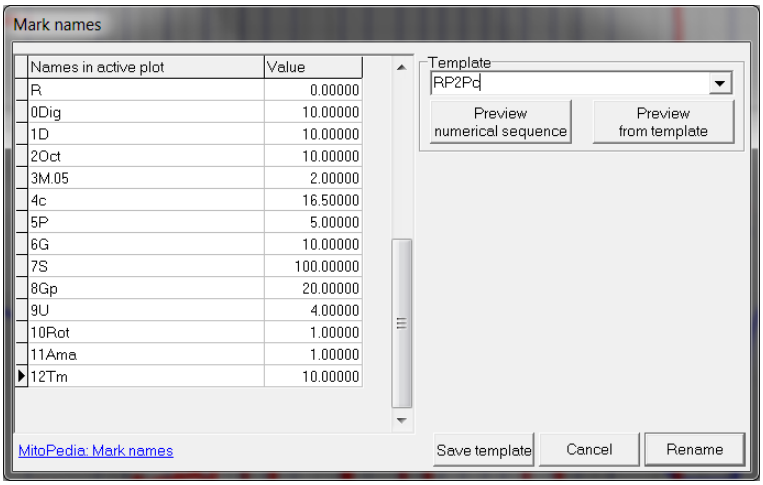
9.2. Manage mark statistics setup

Rename or delete.

9.3. Copy marks from

a selected plot to the active plot.

9.4. Names



Mark names are shown from the active plot. Define the Template Name ▼ and Save template.

Select a Template ▼, L click on Preview from template, and Rename all marks of the plot, including the Values.

9.5. Manage mark name templates

Rename or delete mark name templates in this window.

Updates »http://wiki.oroboros.at/index.php/MiPNet19.18C_DatLab_Guide
»http://wiki.oroboros.at/index.php/MiPNet19.18A_O2k-Start

Next step **– O2k-Core Manual D** »MiPNet19.18D O2k-Calibration

Supplement: 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).