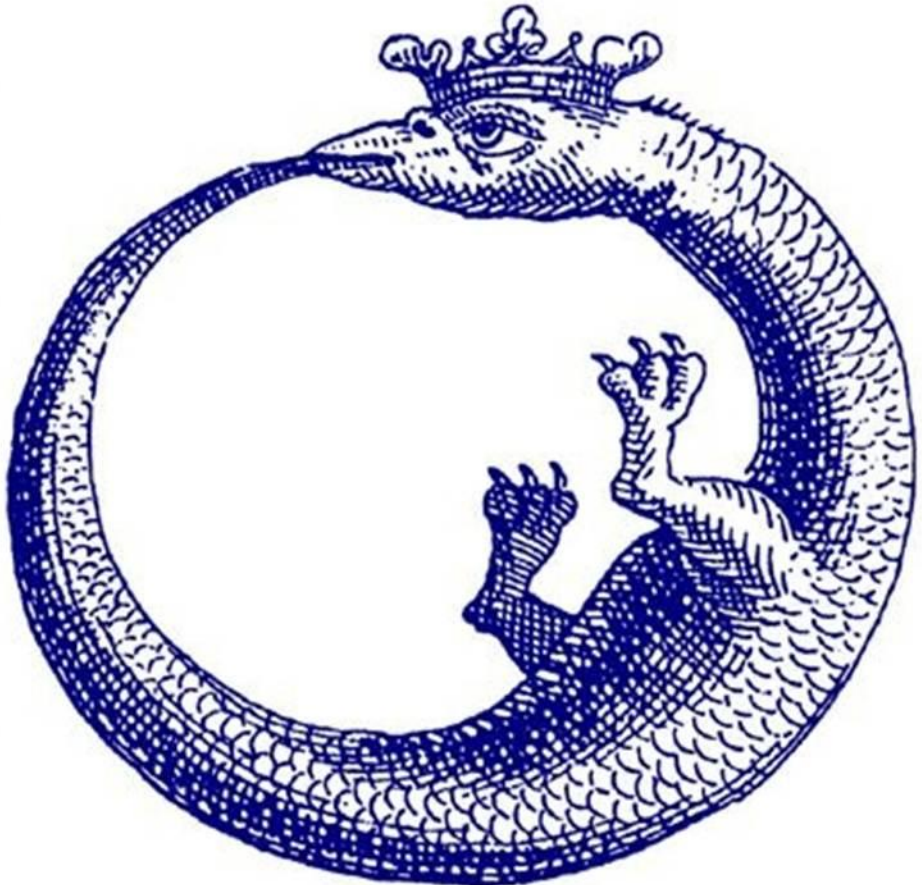
OROBOROS



SUPPORT

O2k technical support

- O2k-Workshops
- Videosupport » *new!*
- wiki.oroboros.at
- Technical support

Technical support team

Ondrej Capek

Gerhard Krumschnabel

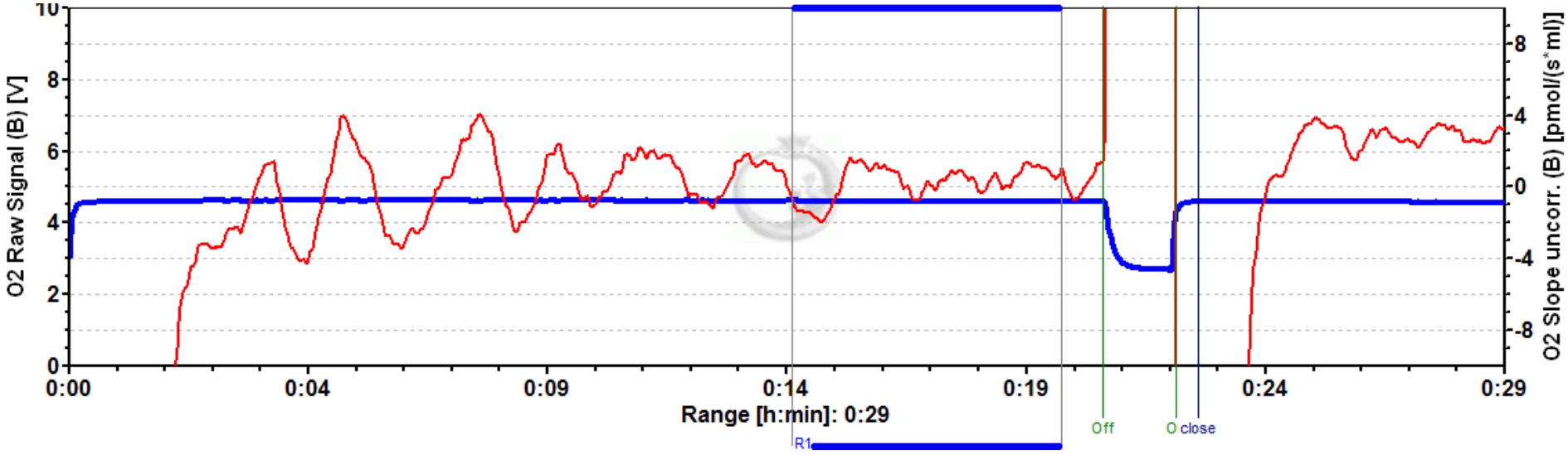
Mona Fontana-Ayoub

Email us: instruments@oroboros.at

- Avoid problems ;-)
- Try to localize the problem
- Solve them on your own
- Support by OROBOROS

Daily routine before starting an experiment:

- air calibration
- stirrer test
- “medium test”: closed chamber without sample



Regular tests:

- sensor test
- instrumental oxygen background

Sensor test

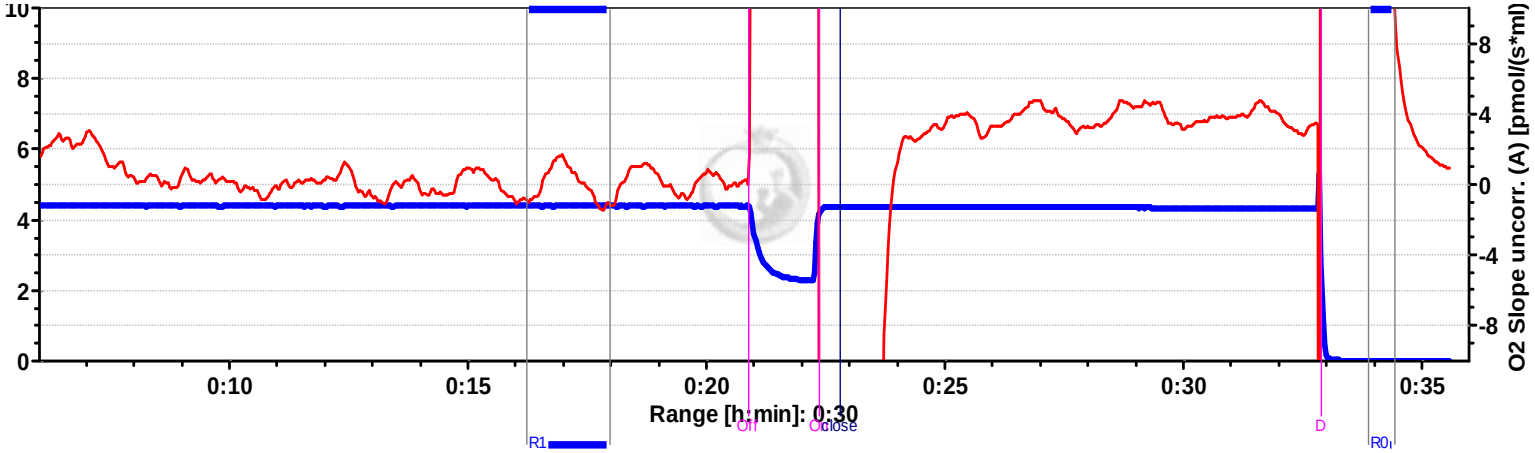
When?

- after sensor service, new membrane, ….
- routine check
- technical support

Info:

- **wiki.oroboros.at** „sensor test“
- Demo File: -link from wiki.oroboros.at
  -USB
  -DatLab directory: DLDemo

How to do a sensor test:



Parameters:

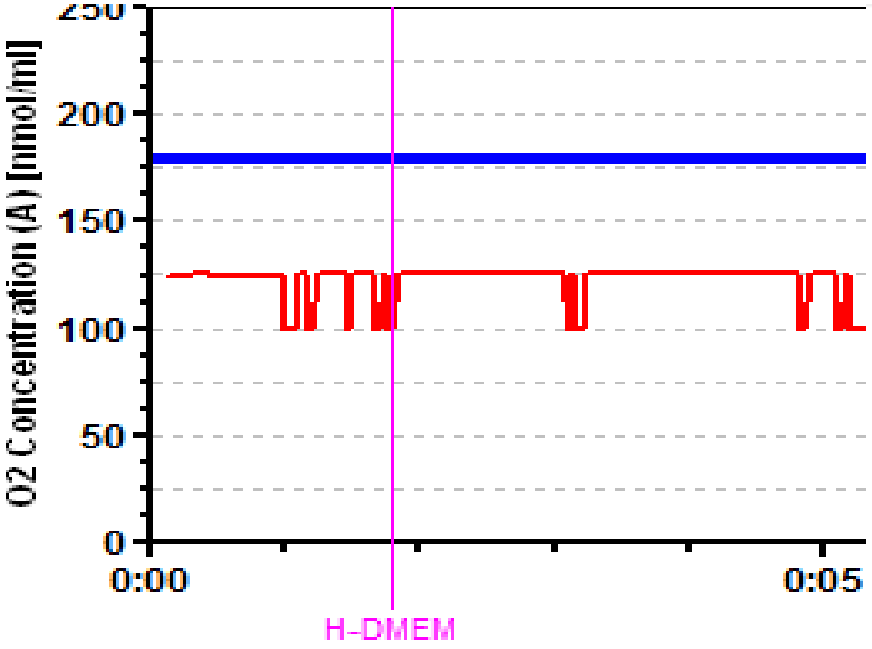
water, T= 37°C, gain = 1, stirring = 750 rpm

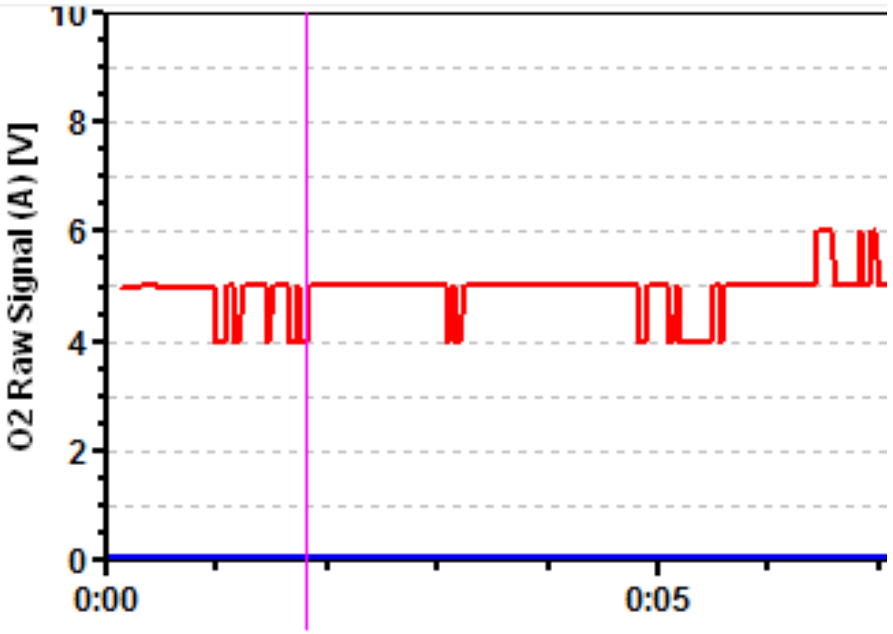
Procedure:

1. use layout “Z Troubleshooting” (raw signal)
2. Air calibration: open chamber, wait for thermal equilibration (stable peltier power)
3. Stirrer test
4. Close chamber (flux up to ± 4 pmol/(s*ml))
5. Zero calibration (with “Zero solution powder“- dithionite)

O2k technical support – examples

Why to look at the raw signal ?



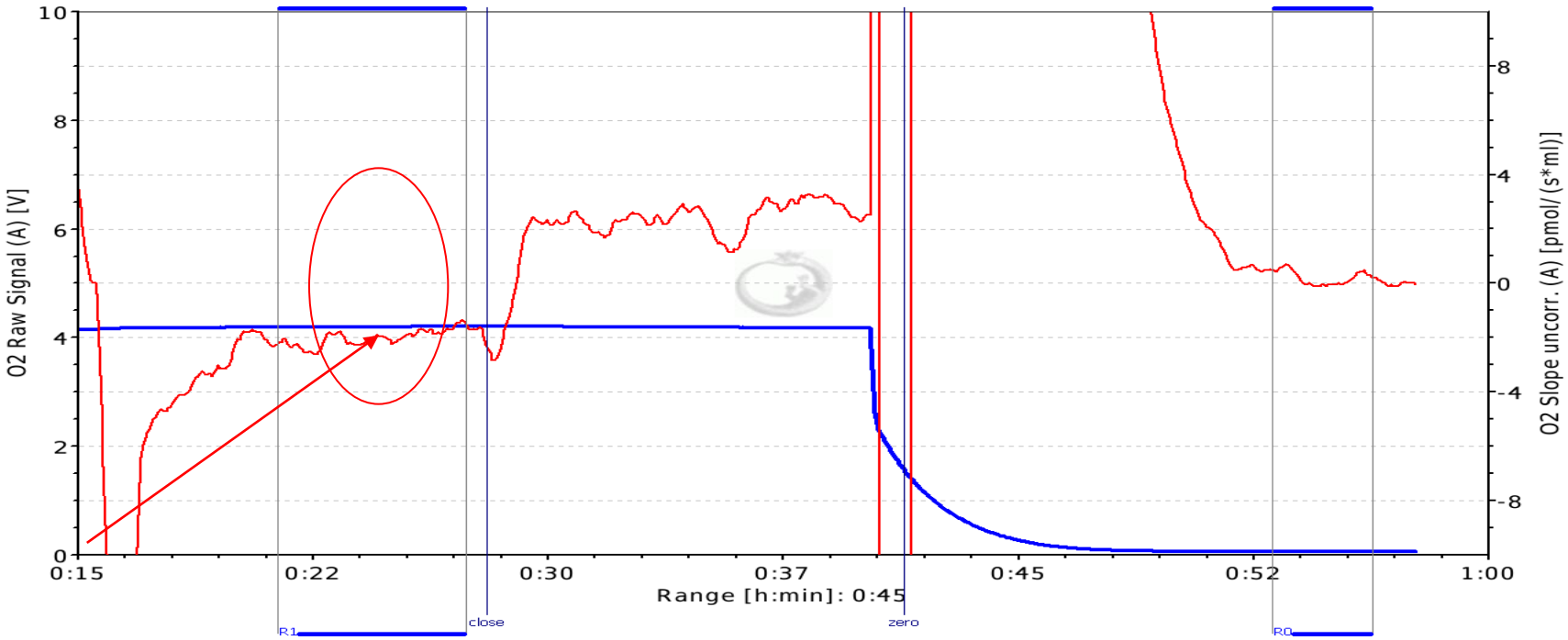


A strange signal

is actually no signal !

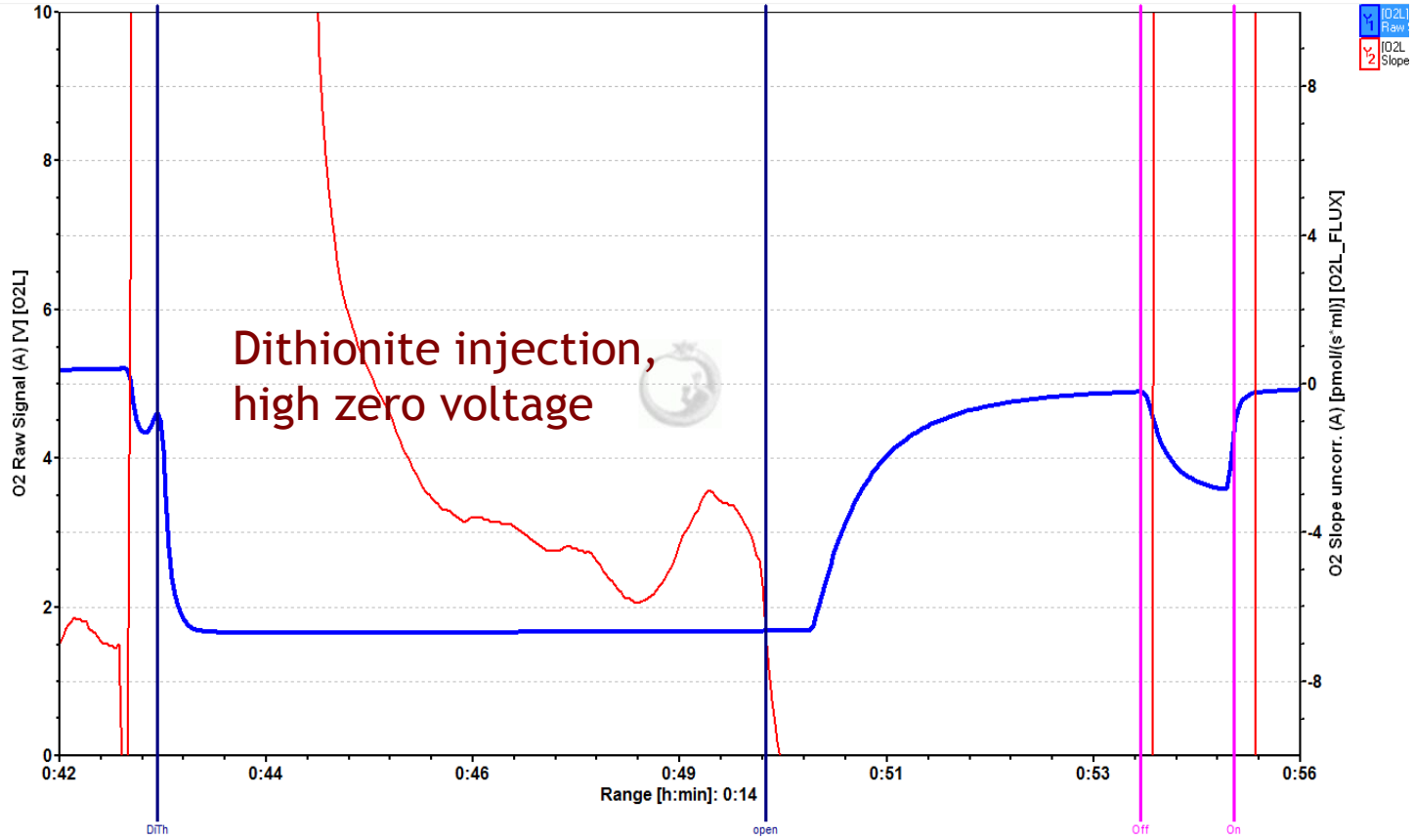
O2k technical support - sensor

1. Drift of the oxygen signal during calibration – bubbles (sensor, capillary)
2. Slow response

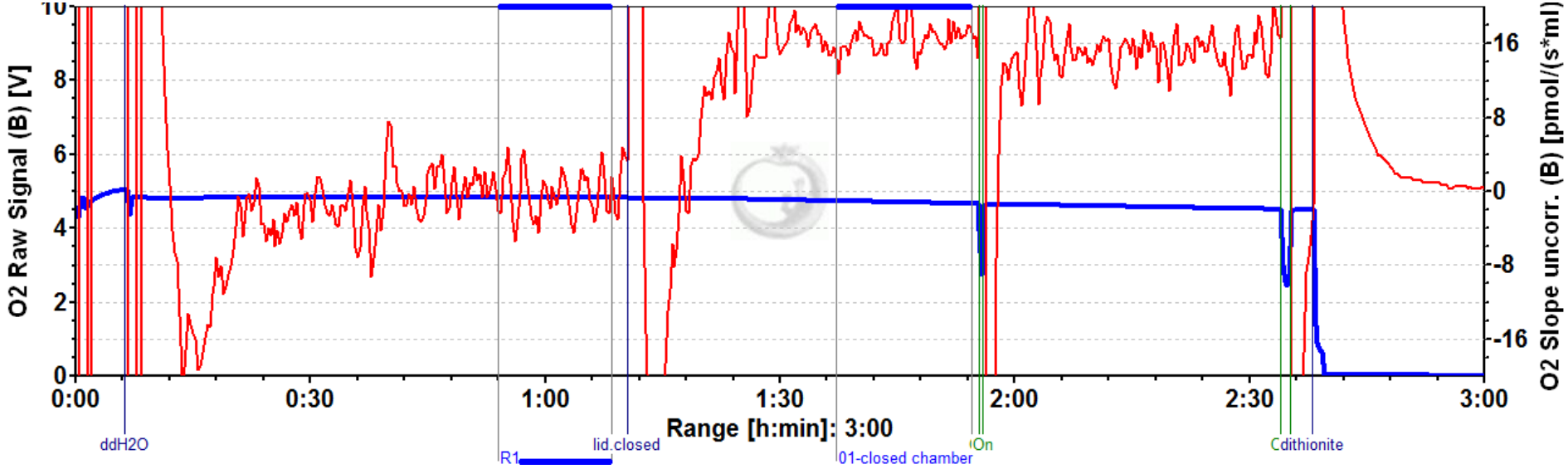


O2k technical support - sensor

Zero current is high (>2.5 %)
Slow response



O2k technical support - biological contamination



normal flux after closing the chamber - up to 4 pmol/(s*ml)

higher values – medium or chamber is contaminated

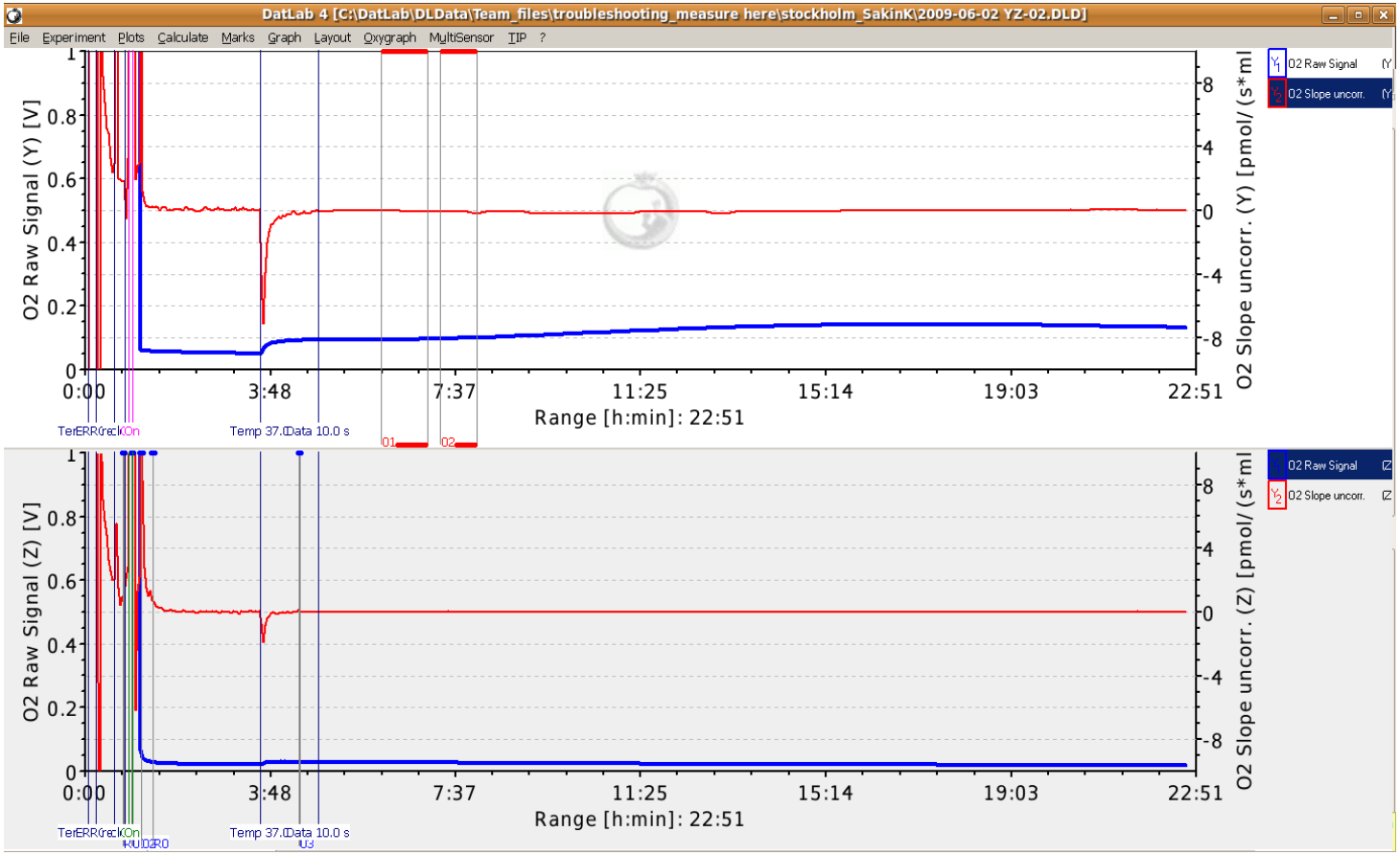
check if problem persists in water:

NO: new medium

YES: intensiv cleaning of the system with 70 % EtOH

clean glass chamber with 10N HCl

O2k technical support – POS connector



High zero current with drift in the left chamber

Problem was located on sensor connector

Localization of a Problem

intern.wiki.oroboros “O2k-technical support”

IN GENERAL

Make a sensor test

Change single components between chambers

- sensors
- POS holder
- glass chamber
- stirrers

Run protocol of the sensor test again after switching one component between chambers

If problem occurs now in the other chamber – problem located

Problems with the sensor:

Change membrane
Sensor service:
cathode and anode cleaning
long ammonia service (over night): apply membrane
run over night in water before a new test run!

Sensor connector:

Clean the gold pin and threads (water and Methanol/EtOH abs.)
Apply contact oil

MiPNet 08.04 Service of the polarographic oxygen sensor OroboPOS

O2k technical service – FAQ/problems

Stirrer sticks/jumps

- exchange stirrers between chambers
- remove chamber, control for small glass pieces
- clean stirrer and clean chamber and with 10 N HCl

Instrumental Background

- the ultimate instrument test!
- after a new chamber assembly
- before or after a series of experiments (e.g. diagnostics)
- performed in MiR05
- in the oxygen range of your experiment (normoxia, hypoxia…)
- at the experimental temperature

O2k technical service – syringes

TIP – syringes

- TIP2k-Manual: **MiPNet12.10_TIP2k-Manual.pdf**
- Rinse the outside with water immediately after use
- Wash 3x with last used solvent, rinse with EtOH
- Storage: dry
- Rinse with pure solvent before use

Hamilton syringes

- Separate uncoupler and inhibitors from substrates
- Between two runs during the day : rinse outside with water
- End of the day: 3x solvent, 3 x EtOH 100%
- Storage: dry
- **MiPNet19.14 SOP Hamilton microsyringes**
- http://wiki.oroboros.at/index.php/Titration_Set

O2k technical service – chamber cleaning

Chamber cleaning:

- Siphon off the cell/mitochondrial suspension
- Rinse the stoppers and chamber with distilled water five times (fill up to the rim)
- Clean bottom of the stopper and stirrer bar with Kimwipe and rinse with water
- Pptional: wash with remaining cell suspension/isolated mitochondria or tissue to get rid e.g. of sodium azide
- Fill with 70 % EtOH and insert the stopper making sure that the ethanol fills up the receptacle and cover with perspex cover, leave for 5 min – repeat two more times
- Fill with EtOH absolute and leave for 15 min
- Store in 70 % ethanol
- MiPNet19.03_O2k-cleaning_and_ISS

O2k technical support – summary

Recommendations:

- Keep your system clean
- During troubleshooting: Discharge yourself, especially before touching the connector - NO Crocs
- Try to **localize your problem**

Perform sensor test for:

- your 'troubleshooting'
- When support by OROBOROS Instruments required
- send us the .DLD file (no screenshot!)

wiki.oroboros.at **Technical Support**