Troubleshooting

Avoid problems

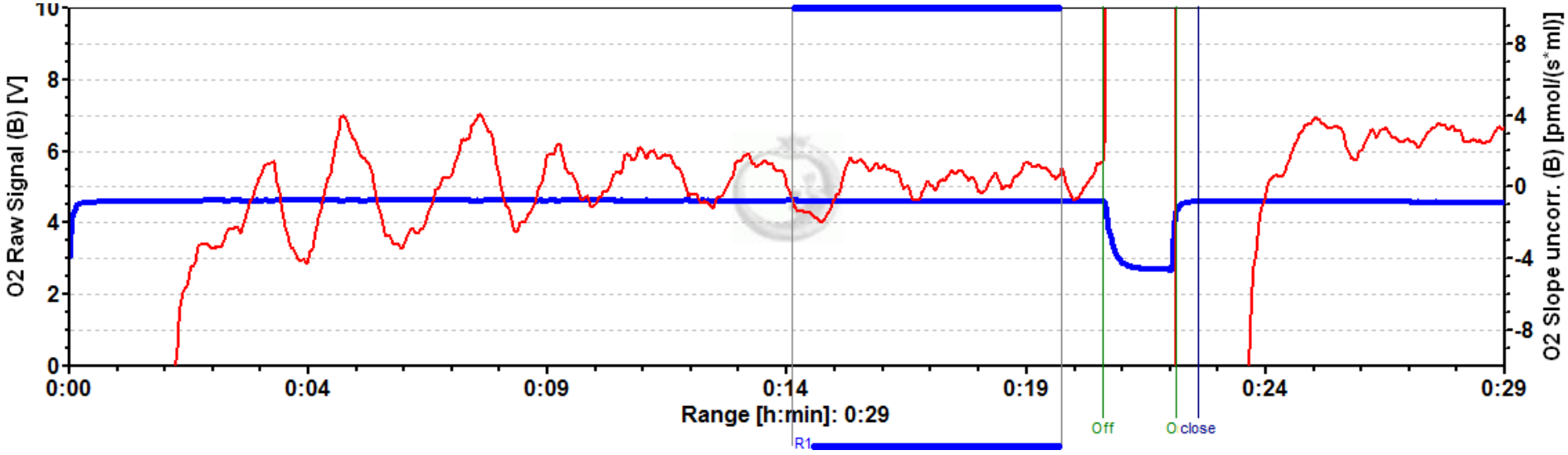
Localize an error

Solve problems on your own

Support

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

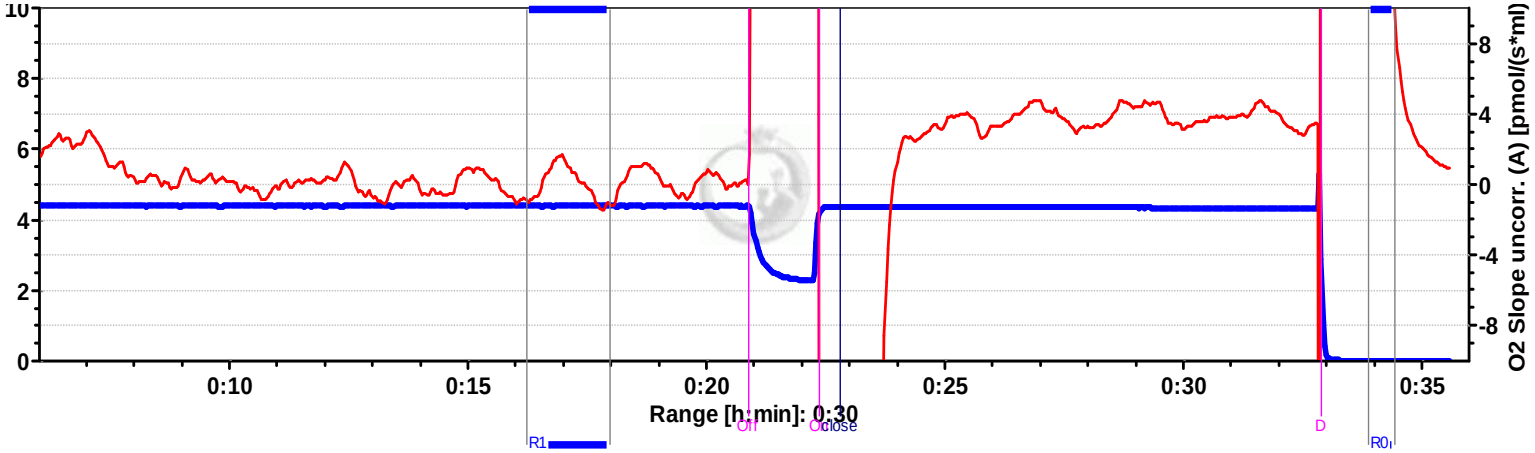
When?

- after sensor service, new membrane, ….
- routine check
- trouble shooting

Info:

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

How to do a sensor test:



Parameters:

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

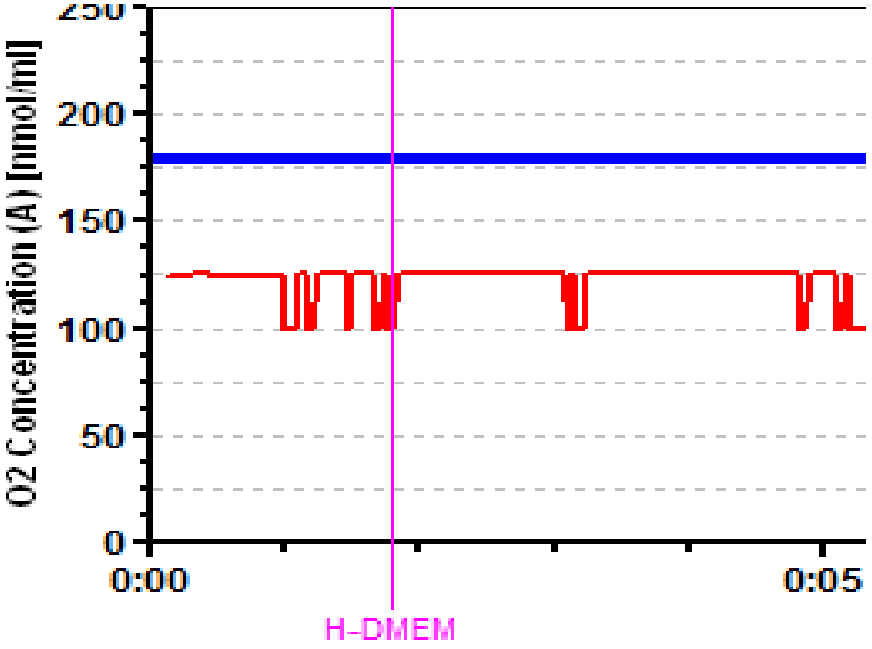
Procedure:

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)

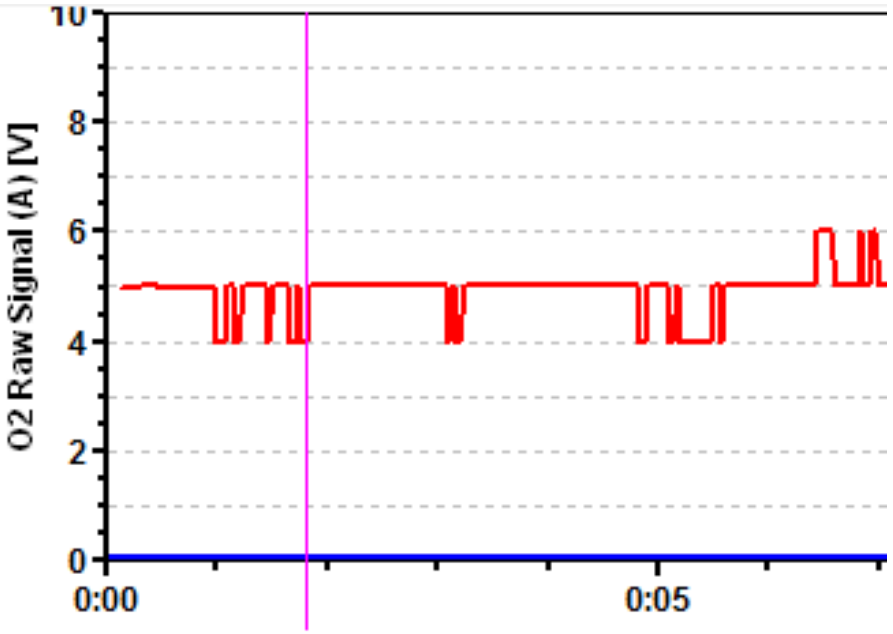
O2k trouble shooting – Examples

Why to look at the raw signal ?

A strange signal

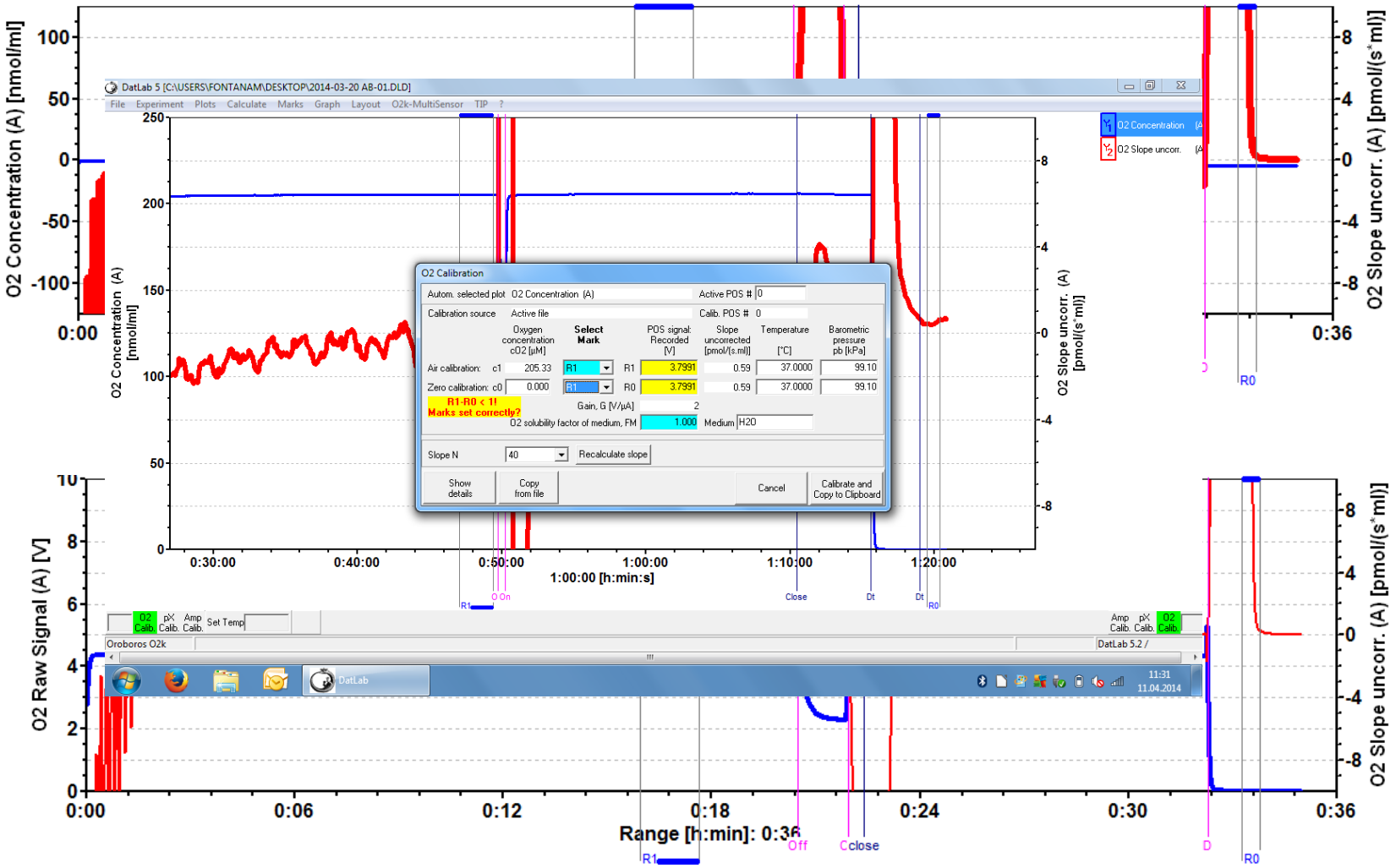


is actually no signal !



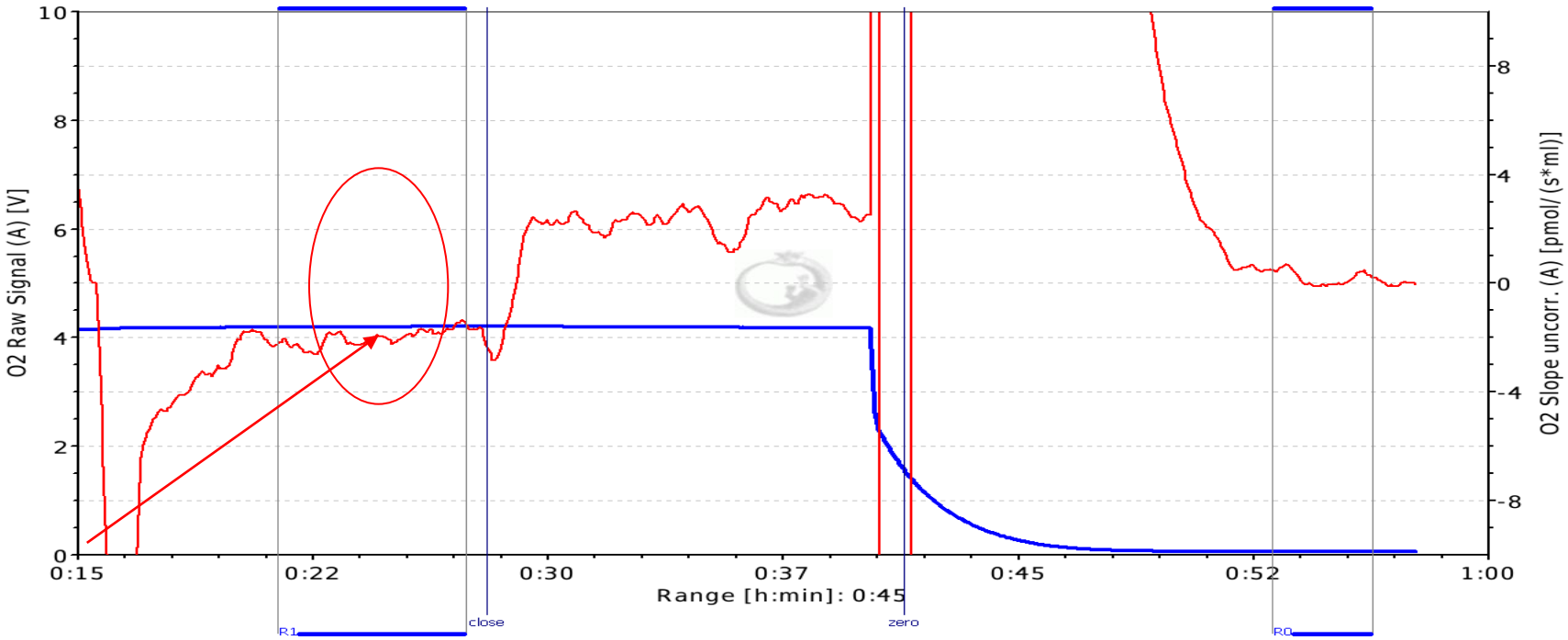
O2k trouble shooting – calibration

No zero calibration



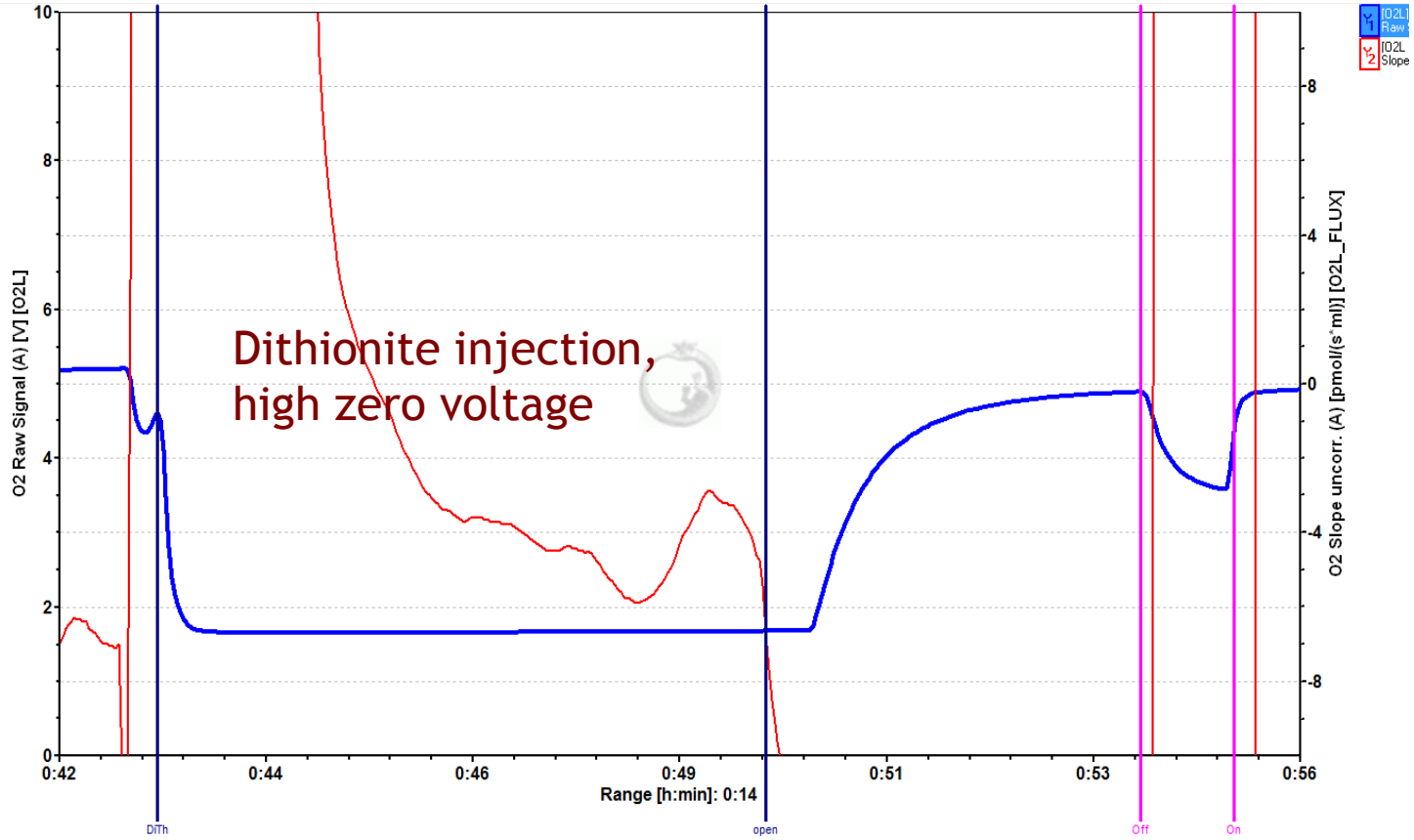
O2k trouble shooting - Sensor

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

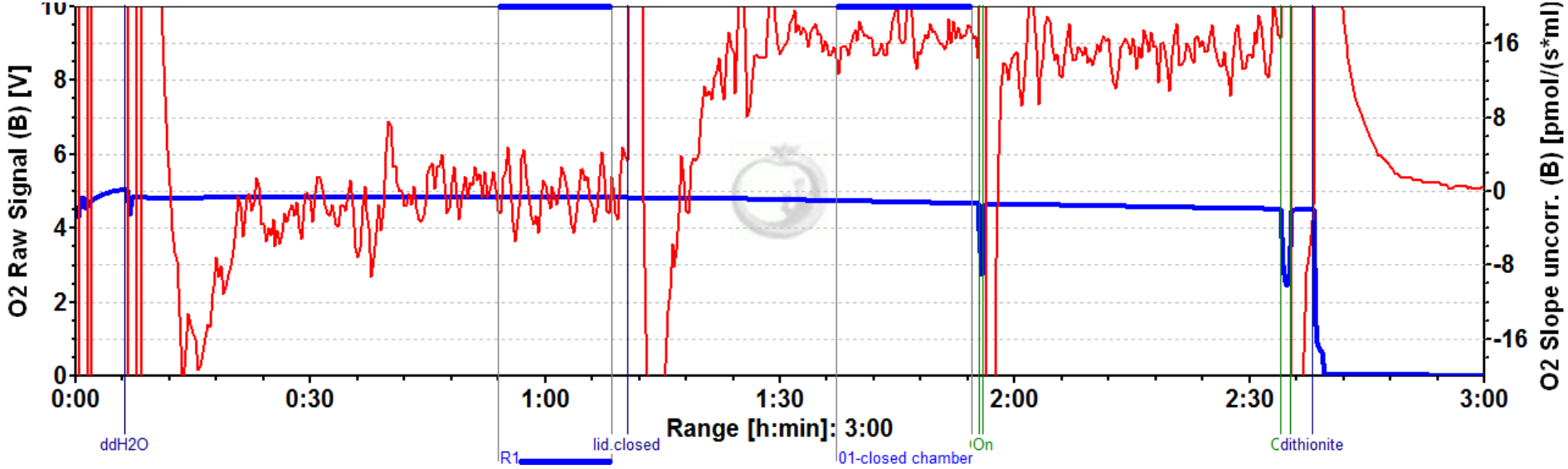


O2k trouble shooting - Sensor

Zero current is high (>2.5 %)
Slow response



O2k trouble shooting - biological contamination



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

higher values – medium or chamber is contaminated

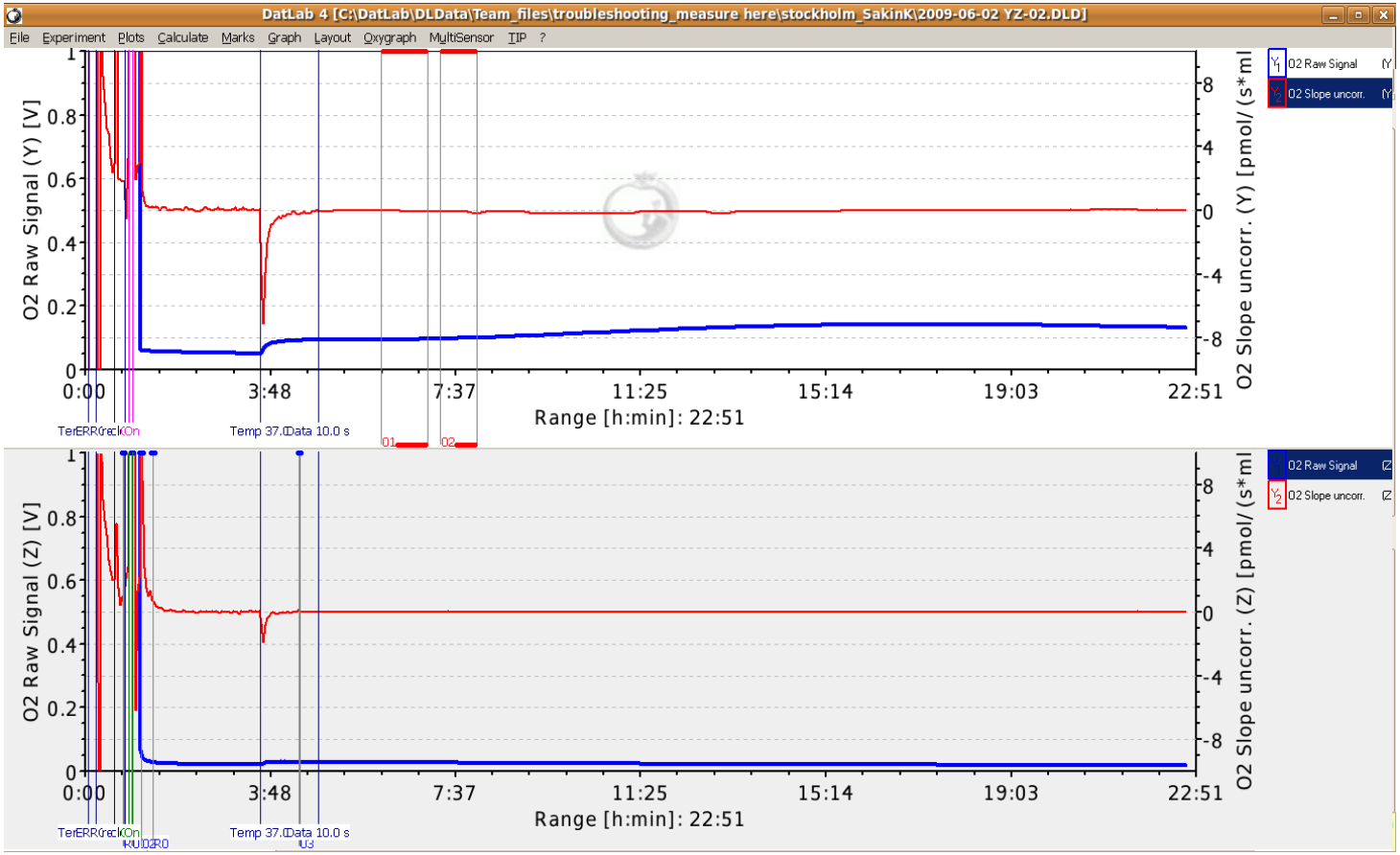
check if problem persists in water:

NO: new medium

YES: intensiv cleaning of the system with 70 % EtOH

clean glass chamber with 10N HCl

O2k trouble shooting – POS conector



High zero current with drift in the left chamber

Problem was located on sensor connector

Localization of a Problem

www.bioblast.at “Troubleshooting”

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

O2k trouble shooting

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 or EtOH abs.)
Apply contact oil

MiPNet 08.04 Service of the Polarographic Oxygen Sensor

www.bioblast.at Troubleshooting

O2k trouble shooting – 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 oxygene range of your experiment
- at the experimental temperature

O2k trouble shooting – syringes

TIP – syringes

- TIP2k-Manual: **MiPNet12.10**
- Rinse the outside with water immediately after use
- Wash 3 x 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: 3 x solvent, 3 x EtOH 100%
- Storage: dry
- http://www.bioblast.at/index.php/Titration_Set

O2k trouble shooting – 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
- optional: wash with remaining cell suspension/isolated mitochondria or tissue to get rid e.g. of sodium azide
- fill with 70 % EtOHand insert the stopper making sure that the ethanol fills up the receptacle and cover with perspex cover , leave for 5 min – repeat 2 more times
- fill with EtOH absolute and leave for 20 min
- store in 70 % ethanol

O2k trouble shooting – summary

Recommendations:

Keep your system clean

During troubleshooting: Discharge yourself, especially before touching the connector - NO croc

Try to **localize your problem**

Perform sensor test for

- your troubleshooting
- support by OROBOROS Instrument
- send us the .DLD file (no screenshoot)

www.bioblast.at Troubleshooting

O2k trouble shooting – summary

Troubleshooting team

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HAPPY TROUBLESHOOTING