104th Workshop on O2k high-resolution respirometry & O2k-Fluorometry

2015 August 09-10
Greenville, NC, USA

Venue:
East Carolina Diabetes & Obesity Institute
East Carolina University
East Carolina Heart Institute Building at ECU

Host:
P. Darrell Neufer, Ph.D., Professor, Director ECDOI
David A. Brown, Associate Professor
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Lecturers and tutors:
Erich Gnaiger, Ao.Univ.-Prof. PhD
Verena Laner, Mag.
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This O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is held in cooperation with one of our prominent O2k-Network Labs in Greenville. The O2k-Workshop includes a basic introduction to quality control of instrumental performance of the OROBOROS O2k with integrated on-line analysis, introducing new features of DatLab 6.

The workshop will include a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations, to evaluate coupling efficiencies and OXPHOS capacities with carbohydrate versus fatty acid substrates, and to diagnose defects in respiratory complexes of the electron transfer system and phosphorylation system. Novel developments are presented on substrate-uncoupler-inhibitor titration (SUIT) protocols in HRR using the O2k-Fluorescence LED2-Module for simultaneous measurement of hydrogen peroxide production (Amplex red®). Discussions are extended on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TPP⁺ or TPMP⁺ (potentiometric), and on perspectives of HRR in mitochondrial physiology.
Program IOC

Sunday, August 09:

08:45 Registration
09:00 – 09:15 Welcome
09:15 – 09:30 Introduction of participants: who is who?
09:30 – 10:30 Erich Gnaiger: Get started with the O2k.
10:30 Coffee break – Registration ctn.
11:00 – 12:15 Pro’s and con’s of mt-preparations: Coupling and substrate control of O_2 consumption and H_2O_2 production in homogenate, permeabilized fibres – or isolated mitochondria?
12:15 – 12:30 Permeabilized fibre preparation – what to take care of?
12:30 Lunch
13:15 – 14:00 Phosphorylation protocol for intact cells.
14:00 – 15:00 Comprehensive OXPHOS analysis:
A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.
15:00 – 15:30 Experimental setup 1:
OroboPOS - sensor quality control, calibration.
15:30 Coffee Break
16:00 – 17:00 Experimental setup 2:
Calibration of O2k-Fluo Sensors
17:00 – 17:30 Neufer P. Darrel: Sharing our experience as an O2k-Network Lab.
17:30 – 18:00 Q&A session on HRR and OXPHOS analysis: Design of experimental protocol - day 2.
18:30 O2k-Workshop dinner

Monday, August 10:

08:30 – 10:30 Experiment: HRR and O2k-Fluorometry with intact cells – respiration and extracellular H_2O_2 production.
10:30 Coffee break
11:00 – 12:00 Experiment continued
12:00 Lunch
12:45 – 15:30 Data analysis
15:30 Coffee break
16:00 – 16:40 Technical support
16:40 – 18:00 Feedback – conclusions – stay connected as an O2k-Network Lab
Recommended reading

O2k-Core Manual

»O2k-Core Manual«

SUIT protocols for O2k high-resolution respirometry


HRR and O2k-Fluorometry

»Manual: O2k-Fluorescence LED2-Module«


Mitochondrial pathways