O2k-Workshop IOC79
## Fluorometry and high-resolution respirometry

2013 September 19-23
Obergurgl, Tyrol, Austria

The **79th** O2k-Workshop on High-Resolution Respirometry (HRR) is a pre-conference event to **MiP2013**. High-level mitochondrial experts interested in fluorometry join with participants who will experience first steps in HRR combined with O2k-MultiSensor approaches. Following a short introduction to HRR, the O2k-Fluorescence LED2-Module is presented in overview lectures and hands-on sessions.

O2k-Fluorescence LED2-Module

Discussions cover protocols for the measurement of hydrogen peroxide production (Amplex red), mt-membrane potential (Safranin), ATP production (Mg green®), and Ca^{2+} (Ca green®).

Particular emphasis will be placed on selecting a mitochondrial preparation (isolated mitochondria, Imt; tissue homogenate, Tho; permeabilized fibres, Pfi; permeabilized cells, Pc; or intact cells), incubation media, experimental temperature, oxygen regime and substrate-uncoupler-inhibitor titration (SUIT) protocols.

These topics will be covered in some introductory lectures, leading towards hands-on experiments, and in open discussions. The programme will evolve according to main interests, with parallel sessions for different groups. An international team of experienced tutors guides small working groups step-by-step through the approach of fluorometry and HRR. Six O2k (12 chambers) are available for practical applications of both hardware and software. Combined with some introductions and demo experiments, it is best to put the O2k into action yourself.
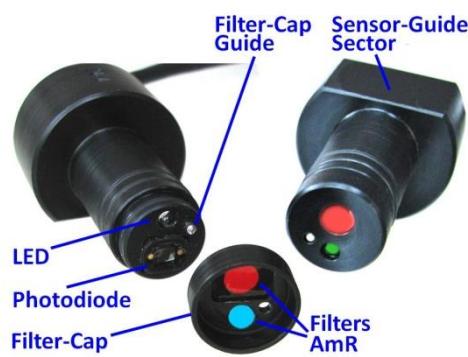
The **O2k-Fluorescence LED2-Module** is an amperometric add-on module to the O2k-Core, adding a new dimension to HRR. Optical sensors are inserted through the front window of the O2k-glass chambers, for measurement of the optical signal simultaneously with and without disturbance of the oxygen measurement. Numerous applications have been tested and are open for O2k-user innovation.

The O2k-Fluorescence LED2-Module consists of **optical sensors** for both O2k-

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Chambers. Each optical sensor is equipped with a removable **Filter-Cap** for exchange of optical filters, which is possible independently for the optical pathway from the LED and to the photodiode. Two optical sensors are supplied with LEDs for green excitation, and two sensors with LEDS for blue excitation. The Fluorescence-Control Unit is an add-on module to the O2k-Core. Light intensity and amplification of the signals are regulated through the DatLab software. Calibrated concentrations and metabolic fluxes (rates, flux control ratios) are displayed simultaneously, in flexible displays in separate graphs or within the graph for oxygen concentration and oxygen flux.

Lecturers and tutors

Gnaiger Erich, Prof. PhD	OROBOROS INSTRUMENTS
Fasching Mario, PhD	OROBOROS INSTRUMENTS
Fontana-Ayoub Mona, BMA	OROBOROS INSTRUMENTS
Laner Verena, MSc	OROBOROS INSTRUMENTS

Guest Tutors

Garcia-Roves Pablo, PhD	Hospital Clinic de Barcelona, ES
Pesta Dominik, Mag. PhD	Department of Cellular and Molecular Physiology, Yale University School of Medicine, US

Programme IOC78
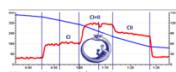
O2k-Workshop Obergurgl, Austria IOC78

2013-September-19 to 23

1   Thursday, September 19

	Arrival	
18:30	Welcome reception University Center Obergurgl	
19:00	*Dinner*	
20:45	**Get-together: Introduction of participants and their research interests - Groups: 2 introductory; 4 expert groups**	

2   Friday, September 20

	Workshop	Weblink
08:30	**Introduction to high-resolution respirometry coupled with O2k-Fluorometry (E Gnaiger)**	MiPNet 17.05
09:30-10:00	**Introduction to a DemoExperiment with Amplex red (AmR)**	MiPNet 18.05
10:00	*Coffee / Tea*	
10:30-12:00	**DemoExperiment: Mouse heart homogenate, simple SUIT protocol, AmR**	
12:00	*Lunch - Walk & Talk*	
15:00-16:30 Intro	**Hands on with the O2k: Calibration of the oxygen and fluorescence signals**	_
15:00-16:30 Experts	**Hands on experiment: Mouse heart homogenate, simple SUIT protocol, AmR**	_
16:30	*Coffee / Tea*	
17:15-18:45	**Experimental results – discussion**	_
19:00	*Dinner*	_
	Hot MiP*topics*	
20:45-20:55	Shabalina IG, Edgar D, Gibanova N, Cannon B, Nedergaard J (2013) Enhanced Complex I but low Complex II substrate-derived ROS production rates in mice, prematurely aging due to enhanced mtDNA mutation rate.	_
21:00-21:10	Bristot IJ, Lopes FM, de Medeiros LM, Lisboa da Motta L, de Oliveira Porciuncula L, Klamt F (2013) Differences in basal mitochondrial respiratory parameters and reactive species generation between the cholinergic, dopaminergic and tumoral phenotypes of SH-SY5Y human neuroblastoma cell line.	_
21:15-21:25	Larsen FJ (2013) Mitochondrial determinants of exercise performance in hypoxia.	_
21:30-21:40 +5 min	Cavalcanti de Albuquerque JP, Salvador IC, Martins EG, Jardim-Messeder D, Werneck de Castro JPS, Carvalho DP, Galina A (2013) Skeletal muscle mitochondrial function in ovariectomized rats: A time course study and the role of estrogen replacement.	_

3   Saturday, September 21

09:00-10:30	**O2k-MultiSensor overview and some O2k-Fluorometry applications (M Fasching)**	_
10:30	*Coffee / Tea*	
10:30-12:00 Intro	**DatLab overview with DemoExperiment (E Gnaiger)**	MiPNet 17.17
10:30-12:00 Experts	**O2k-Fluorometry: Technical details (M Fasching)**	
12:00	*Lunch break – sports*	_
16:00-17:00	**The PBI-Shredder (Brian A Irving)**	MiPNet 17.15
17:00	*Coffee / Tea*	

17:15-18:45	**Protocol design: mt-prep - tissues, limitations imposed by fluorometry - controls parallel to fluorometry (E Gnaiger)**	
19:00	*Dinner*	-
	Hot MiP*topics*	
20:45-20:55	Pablo Garcia-Roves (2013) Mitochondrial function and central control of energy homeostasis.	-
21:00-21:10	Bajzikova M, Vondrusova M, Neuzil J (2013) Mitochondrially targeted vitamin E succinate and its effects on the mitochondrial oxidative phosphorylation system.	-
21:15-21:25	Le CH, deMooy AB, Mulligan CM, Heuberger AL, Prenni JE, Chicco AJ (2013) Substrate-specific impairment of oxidative phosphorylation in Tafazzin-deficient cardiac mitochondria: potential role of CoA deficiency.	-
21:30-21:40 +5 min	Kressig F, Pak O, Weissmann N, Sommer N (2013) Mechanism of increased pulmonary vascular remodeling in UCP2 k/o mice.	-

4   Sunday, September 22 -

09:00-10:30 Intro	**Basics: HRR-protocols, DatLab**	-
09:00-10:30 Experts	**Experiments with safranin, Amplex red, …**	MiPNet 18.06
10:30	*Coffee / Tea*	-
11:00-12:00		-
12:00	*Lunch break – sports*	-
16:00-17:00	**Safranin a challenge for simultaneous measurement of respiration and mt-membrane potential (E Gnaiger)**	
17:00	*Coffee / Tea*	
17:15-18:45	**Special interest groups**	
19:00	*Dinner*	-
21:00	**Summary - Feedback - IOC Party**	-

5   Monday, September 23

MiP2013 or Departure

Participants

Name	Lab
Bajzikova Martina	**CZ_Prague_Neuzil J:** Institute of Biotechnology, Academy of Sciences, Prague. - cancer cells, VE analogs, CII
Bir Aritri	**IN_Kolkata_Chakrabarti S:** Department of Biochemistry, Institute of Post-graduate Medical Education & Research. - Respiration study - ROS production - membrane potential - in different models http://www.bioblast.at/index.php/Bir_2013_Abstract_MiP2013
Breen Laura	**IE_Dublin_O Gorman D:** National Institute for Cellular Biotechnology (NICB), Dublin City University.
Cavalcanti de Albuquerque João Paulo	**BR_Rio de Janeiro_Galina A:** Institute of Medical Biochemistry Federal University of Rio de Janeiro. - brain, hypothalamus, synaptosome, mitochondria, hexokinase, ROS. www.bioblast.at/index.php/Cavalcanti_de_Albuquerque_2013_Abstract_MiP2013
Chicco Adam J	**US_CO Fort Collins_Chicco AJ:** Department of Health and Exercise Science, Colorado State University. - cardiolipin, heart failure, diabetes, polyunsaturated fatty acids, comparative biology. http://www.bioblast.at/index.php/Chicco_2013_Abstract_MiP2013
Chung Dillon	**CA_Vancouver_Richards JG:** Department of Zoology, University of British Columbia. - Fundulus heteroclitus, oxygen limited thermal tolerance, pheotypic plasticity http://www.bioblast.at/index.php/Schulte_2013_Abstract_MiP2013
Crandall Amy	**US_UT Provo_Hancock C:** Eyring Science Center, Brigham Young University.
Fontana-Ayoub Mona (team tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS** http://www.bioblast.at/index.php/Krumschnabel_2013_Abstract_MiP2013
Fasching Mario (team tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS** http://www.bioblast.at/index.php/Krumschnabel_2013_Abstract_MiP2013
Galli Gina L	**UK_Manchester_Galli G:** Faculty of Life Sciences, University of Manchester. - Heart, anoxia, ischemia, heart failure
Garcia-Roves Pablo (guest tutor)	**ES_Barcelona_Garcia-Roves PM:** Hospital Clinic de Barcelona. http://www.bioblast.at/index.php/Garcia-Roves_2013_Abstract_MiP2013
Gnaiger Erich (team tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS**: [1]D Swarovksi Research Laboratory, Dept Visceral, Transpl Thoracic Surgery, Medical Univ Innsbruck; [2]OROBOROS INSTRUMENTS, Innsbruck, Austria. http://www.bioblast.at/index.php/Gnaiger_2013_Abstract_MiP2013
Heidler Juliana	**DE_Frankfurt_Heidler J:** Molecular Hematology, JW-Goethe University, Frankfurt. - subsarcolemmal mitochondria, interfibrillar mitochondria, hypobaric oxygen, hyperbaric oxygen. http://www.bioblast.at/index.php/Heidler_2013_Abstract_MiP2013
Hey-Mogensen Martin	**DK_Malov_Hey-Mogensen M:** Diabetes NBEs & Obesity Biology, Novo Nordisk A/S. - Regulation of cellular bioenergetics including cellular redox state
Hostrup Morten	**DK_Copenhagen_Bangsbo J:** Department of Nutrition, Exercise and Sports, University of Copenhagen. - Beta2-agonists, salbutamol, terbutaline, beta2-adrenergic receptors
Irving Brian A (guest lecturer)	**US_PA Danville_Irving BA:** Obesity Institute, Geisinger Health System. http://www.bioblast.at/index.php/Irving_2013_Abstract_MiP2013

Klamt Fabio	**BR_Porto Alegre_Klamt F:** Instituto de Ciências Básicas da Saúde, Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul. - tumor cell metabolism, lung cancer, in vitro neuronal model, neuronal differentiation
Kutschke Maria	**DE_Munich_Jastroch M:** Institute for Diabetes and Obesity Helmholtz Zentrum München. - Assessment of mitochondrial function in metabolic diseases
Kressig Franz	**DE_Giessen_Weissmann N:** ECCPS, Justus-Liebig-University, Giessen. - pulmonary hypertension(ph), chronic hypoxia, pulmonary vascular remodeling, UCP2k/o, disease model, PASMC, mmp
Laner Verena (team tutor)	**AT_Innsbruck_OROBOROS INSTRUMENTS** http://www.bioblast.at/index.php/Laner_2013_Mitochondr_Physiol_Network_MiP2013
Larsen Filip J	**SE_Stockholm_Weitzberg E:** Department of Physiology and Pharmacology, Karolinska Institute, Stockholm. http://www.bioblast.at/index.php/Larsen_FJ_2013_Abstract_MiP2013
Meszaros Andras	**HU_Szeged_Boros M:** Institute of Surgical Research, School of Medicine, University of Szeged. - hypoxia, reoxygenation, electrophyl methyl groups (EMGs), methane, long-chain fatty acids
Nowikovsky Karin	**AT_Vienna_Nowikovsky K:** Medizinische Universität Wien. - mitochondrial dynamics, & energy metabolism
Oelkrug Rebecca	**DE_Bonn_Pfeifer A:** Institute of Pharmacology and Toxicology, Biomedical Center, University Bonn.
Roy Chowdhury Subir K	**CA_Winnipeg_Fernyhough P:** Department of Pharmacology and Therapeutics, University of Manitoba. - nerve, AMPK, sensor neuron, nutrient stress, diabetes, neuropathy.
Pesta Dominik (guest tutor)	**US_CT New Haven_Pesta D:** Department of Cellular and Molecular Physiology, Yale University School of Medicine.
Scott Graham	**CA_Hamilton_Scott GR:** Department of Biology, McMaster University, Hamilton. - integrative respiratory adaptations to hypoxia in vertebrates http://www.bioblast.at/index.php/Scott_2013_Abstract_MiP2013
Shabalina Irina G	**SE_Stockholm_Nedergaard J:** The Wenner-Gren Institute, Stockholm University. - uncoupling proteins, mitochondria, brown and white adipose tissue, mtDNA mutation, mitochondrial ROS production http://www.bioblast.at/index.php/Shabalina_2013_Abstract_MiP2013
Sonkar Vijay K	**IN_Varanasi_Dash D:** Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi. - Platelets, hemostsis, APP, Aβ. http://www.bioblast.at/index.php/Sonkar_2013_Abstract_MiP2013
Sparks Lauren	**US_FL Orlando_Smith SR:** Translational Research Institute for Metabolism and Diabetes.

MiPNet Abstracts IOC78: 10+5 min
Hot topics in Mitochondrial Physiology

Online: www.bioblast.at/index.php/MiPNet18.07_IOC78_Abstracts
Continue the discussion:
www.bioblast.at/index.php/Talk:MiPNet18.07_IOC78_Abstracts

Accommodation and Location
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Further information

O2k-Manual – www.oroboros.at/?O2k-Manual
O2k-Fluorescence LED2-Module - www.oroboros.at/?O2k-FluorescenceLED2Module
O2k-Protocols – www.oroboros.at/?O2k-Protocols
**O2k-Publications** – www.oroboros.at/?O2k-Publications

Recommended Reading

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial Dysfunction in Drug-Induced Toxicity (Dykens JA, Will Y, eds) John Wiley: 327-352. – *A methodological introduction into high-resolution respirometry.*

Hickey AJ, Renshaw GM, Speers-Roesch B, Richards JG, Wang Y, Farrell AP, Brauner CJ (2012) A radical approach to beating hypoxia: depressed free radical release from heart fibres of the hypoxia-tolerant epaulette shark (Hemiscyllum ocellatum). J Comp Physiol B 182: 91-100.

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopisies of human muscle. Methods Mol Biol 810: 25-58.

Tretter L, Adam-Vizi V (2012) High Ca^{2+} load promotes hydrogen peroxide generation via activation of α-glycerophosphate dehydrogenase in brain mitochondria. Free Radic Biol Med 53: 2119-21130

Acknowledgements
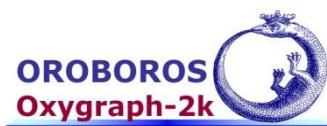
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The O2k-Fluorescence LED2-Module

Description

The **O2k-Fluorescence LED2-Module** is an amperometric add-on module to the [O2k-Core](#), adding a new dimension to [HRR](#). Optical sensors are inserted through the front window of the O2k-glass chambers, for measurement of **hydrogen peroxide production** ([Amplex red](#)), **ATP production** ([Magnesium green](#)), **mt-membrane potential** ([Safranin](#)), **Ca^{2+}** ([Calcium green](#)), and numerous other applications open for O2k-user innovation.

The O2k-Fluorescence LED2-Module consists of optical sensors for both [O2k-Chambers](#) (LEDs for green and blue excitation), optical filters, [Fluorescence-Control Unit](#) for regulation of light intensity, data input into the [O2k-Main Unit](#), and the updated **DatLab** software.

Product ID 12100-01

Type O2k, O2k-Module, MultiSensor, Catalogue

Link [O2k-Fluorescence@OROBOROS](#), [Oxygraph-2k](#), [G MiPNet17.05 O2k-Fluorescence LED2-Module](#)

Application-specific settings

The light intensity of the LED ([LED-intensity](#)) and the signal amplification ([Gain](#)) can be adjusted in a wide range. The table suggests initial values, which may be optimised for specific applications.

Application	Sensor	Filter set	Light intensity (polarization voltage) - *Note a*	Gain
[Amplex® UltraRed](#)	[Fluorescence-Sensor Green](#)	[AmR](#)	100 - 500	1000 (at light intensity = 100)
[Safranin](#)	[Fluorescence-Sensor Blue](#)	[Saf](#)	200 for [safranin] > 2 µM; 500 or higher for [safranin] < 2 µM	1000
[Magnesium green](#)	[Fluorescence-Sensor Blue](#)	[MgG / CaG](#)	300	*Note b*
[Calcium green](#)	[Fluorescence-Sensor Blue](#)	[MgG / CaG](#)		*Note b*

- *Note a*: Set the polarization voltage [mV] for the amperometric channel (Amp) in the DatLab menu [O2k-MultiSensor \ O2k Control \ Amp polarisation voltage]. Divide the polarisation voltage [mV] by 100 to obtain the current [mA] through the LED. For simple operation instructions, it is sufficient to refer to the polarization settings selected in DatLab.
- *Note b*: The amplification for Magnesium green(R) and Calcium green(R) depends on the concentration of the fluorophore, which varies drastically between different applications. Therefore, no general recommendation is given for the appropriate gain.