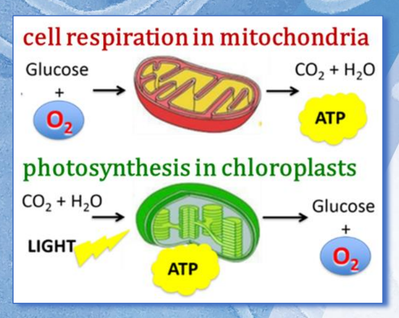
Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry

nora.went@oroboros.at

N. Went[1,2], M. Di Marcello[1], E. Gnaiger[1,3]

[1]Oroboros Instruments, Innsbruck, Austria; [2]MCI Management Ctr Innsbruck, Austria;
[3]Dept Visceral, Transplant Thoracic Surgery, D Swarovski Res Lab, Med Univ Innsbruck, Austria



Introduction

The bioenergetic crosstalk between mitochondria and chloroplasts plays a key role in maintaining metabolic integrity and controlling metabolite production for growth and regulation of cell concentration. We studied **dark respiration *DR*** and **net photosynthesis *NP*** in the green alga *Chlamydomonas reinhardtii* at varying oxygen concentrations and three cell concentrations. **Light-enhanced dark respiration *LEDR*** was measured after light-dark transitions [1]. Algae were grown photoautotrophically at 25 °C and a light intensity of 100 µmol·s^{-1}·m^{-2} (16:8 h L:D).

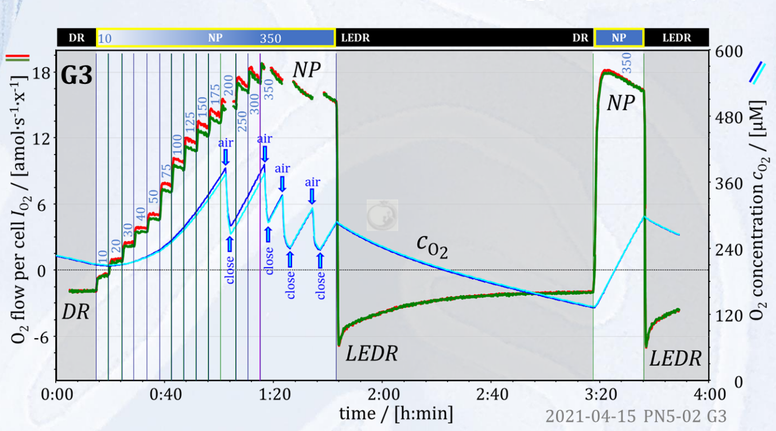
High-Resolution PhotoRespirometry

High-resolution respirometry based on the Oroboros O2k is extensively applied to the study of mitochondrial physiology in the biomedical field [2,3]. Real-time oxygen flux was measured using the NextGen-O2k, a two-chamber instrument with the PhotoBiology-Module (PB-Module), in growth medium TRIS at 25 °C.

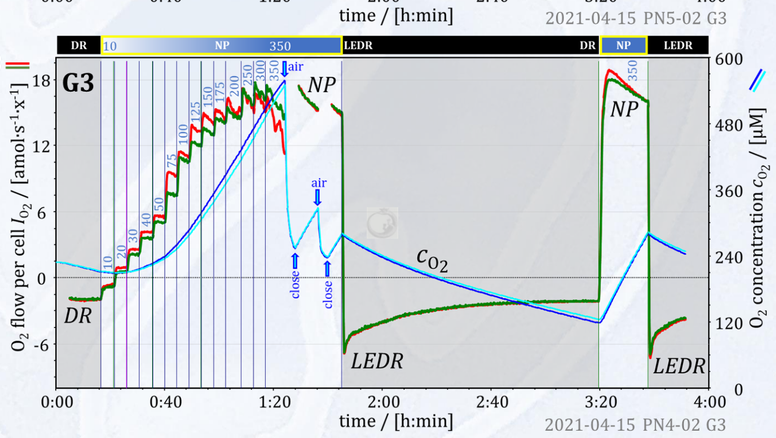
1. O_2 flow I_{O_2} as a function of the light regime and O_2 concentration c_{O_2}

Superimposed traces of two O2k-chambers. The net O_2 production rate (net photosynthesis *NP*) was stimulated from dark respiration *DR* at normoxia to a maximum by stepwise increments of light intensity (blue light; vertical numbers, 10 to 350 µmol·s^{-1}·m^{-2}). Light-enhanced dark respiration *LEDR* was a sharp peak of respiration immediately after switching off the light. (**1a**) O_2 concentration was prevented from reaching severe hyperoxia by intermittently opening the chambers (arrows, air). (**1b**) O_2 concentration increased in the closed chamber due to *NP*. The decline in maximum *NP* was reversed by lowering the O_2 concentration. (**1c**) *LEDR* was a sharp (negative) maximum at 30-60 s after light-dark transitions. Instrumental background *BG* indicated a small transient disturbance of the O_2 signal by switching off the light, which was corrected for as background O_2 flux.

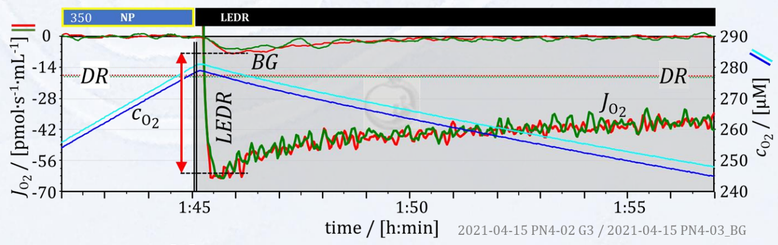
1a



1b



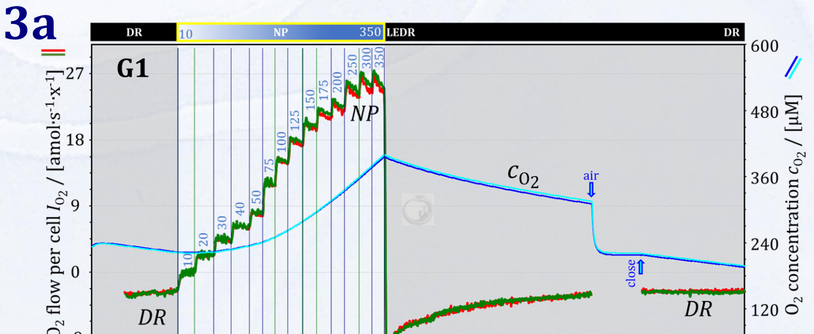
1c



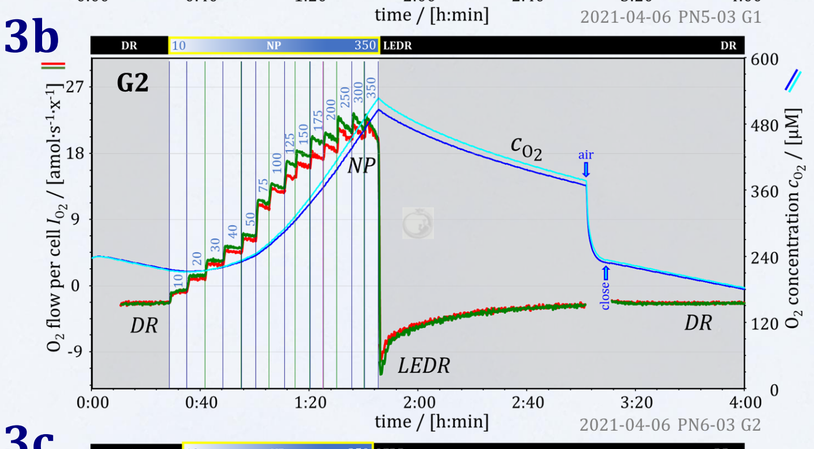
3. O_2 flow at different cell concentrations determines O_2 concentrations at increasing light intensities in the closed chamber

Superimposed traces of two O2k-chambers. A stepwise increase of light intensity (vertical numbers, 10 to 350 µmol·s^{-1}·m^{-2}) stimulated net photosynthesis *NP* to a maximum while O_2 concentration increased from 220 µM to 400, 520, and 550 µM in dilution groups **G1** to **G3** (Figure 2). Lower *NP* capacity at higher cell concentration was caused by hyperoxic inhibition of photo-synthesis (Figure 4).

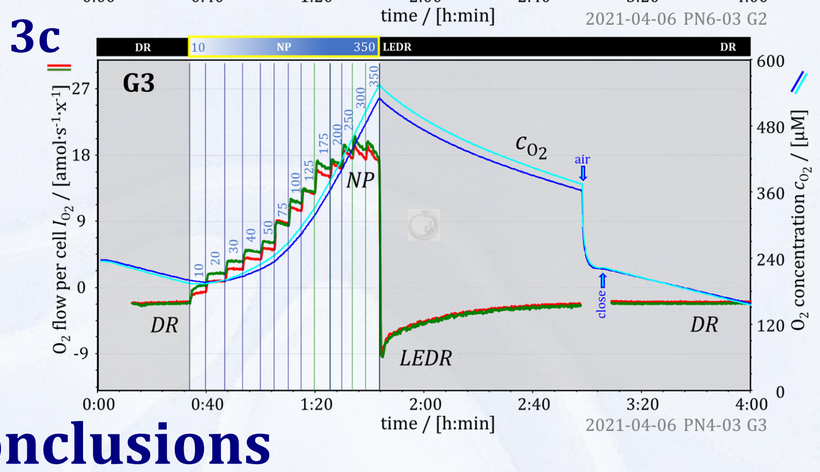
3a



3b



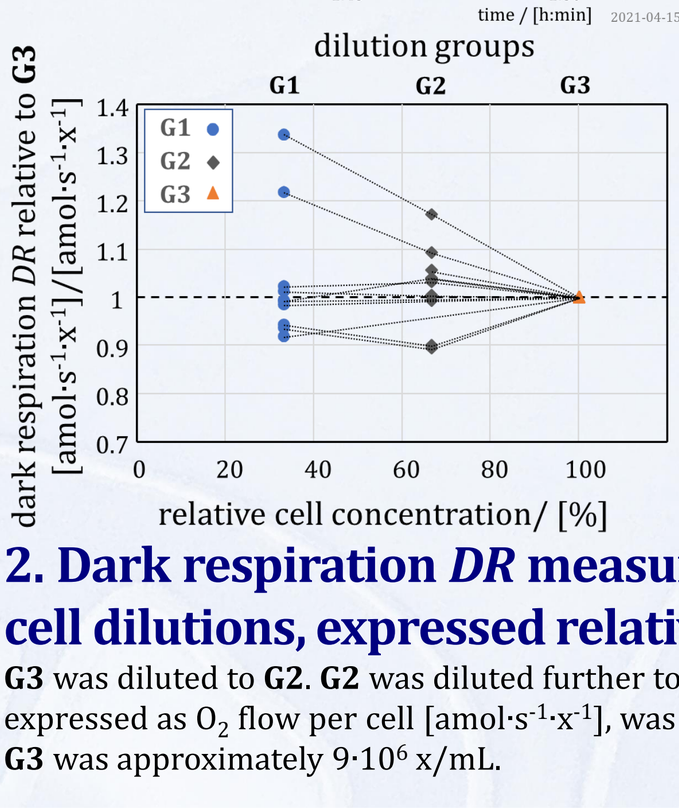
3c



2. Dark respiration *DR* measured simultaneously in three cell dilutions, expressed relative to dilution group G3

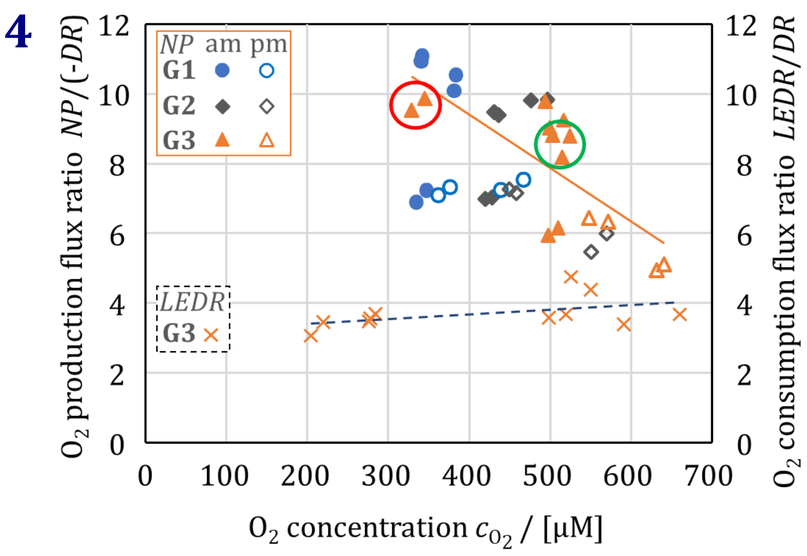
G3 was diluted to **G2**. **G2** was diluted further to **G1**. *DR*, measured initially at normoxia and expressed as O_2 flow per cell [amol·s^{-1}·x^{-1}], was independent of cell concentration C_{ce}. C_{ce} of **G3** was approximately 9·10^6 x/mL.

2



4. Oxygen dependence of net photosynthesis *NP* and light-enhanced dark respiration *LEDR*

4



O_2 flux ratios normalized for *DR*. Independent of cell concentration, *NP* was inhibited gradually from normoxia to severe hyperoxia by up to 40 %. In contrast, *LEDR* did not significantly depend on O_2 concentration. Red and green circles: data from Figure 1a and 1b [4].

Conclusions

- Light-enhanced dark respiration *LEDR* was 3.5- to 4-fold higher than steady-state *DR*.
- *LEDR* returned to *DR* after two hours of dark.
- The decline of net O_2 production *NP* under hyperoxia is not caused by compensatory light-enhanced photorespiration *LEPR*, if *LEDR* is proportional to *LEPR*, but by inhibition of photosynthesis at high oxygen concentrations.

References

1. Shimakawa G, Kohara A, Miyake C (2020) Int J Mol Sci 22:342. doi: 10.3390/ijms22010342
2. Doerrier C et al (2018) Methods Mol Biol 1782:31-70.
3. Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. Bioenerg Commun 2020.2:112 pp. doi:10.26124/bec:2020-0002
4. Went N, Di Marcello M, Gnaiger E (2021) Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry. MitoFit Prep 2021.5.

www.oroboros.at

The project NextGen-O2k has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.