Mitochondrial Physiology Network 17.16(02):1-4 (2015)

Updates: http://wiki.oroboros.at/index.php/MiPNet17.16_O2k-Spectrophotometry

O2k-Spectrophotometry – A *MitoCom* Project

Harrison DK[1], Fasching M[1], Gnaiger E[1,2]

[1]Oroboros Instruments
O2k High-Resolution FluoRespirometry (HRFR)
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: instruments@oroboros.at; www.oroboros.at

[2]D. Swarovski Research Lab
Dept of Visceral, Transplant and Thoracic Surgery
Medical Univ Innsbruck, Austria
www.mitofit.org

1. Introduction

The principal thrust of the technical development of the O2k high-resolution respirometer within the *MitoCom* project is towards the O2k-FluoRespirometer. However, simultaneously, a parallel project is working towards the integration of spectrophotometry into the O2k in order to measure the redox state of cytochromes – particularly cytochromes c and *aa*3, but also cytochrome b.

2. Methods

Initially, a specially designed stopper was used that incorporated two lightguide fibres (see Figure 1): one transmitting light from a quartz halogen (QH) lamp into the cuvette, the other collecting the emergent light scattered from the medium [1]. This stopper, however, prevents the use of other multi parameter probes, so the concept of using the chamber window for both fluorometry and spectrophotometry [2] was developed. Again, a pair of lightguides was used to transmit and receive light through the chamber window.

Experiments were carried out to determine the optimal configuration and spacing for the lightguides. The most recent approach uses a white light emitting diode (LED) built into into the O2k itself opposite the window. In all cases, the analysis of cytochrome absorbance difference spectra (reduced minus oxidised) recorded from 1.5 mg/ml bakers' yeast using the dual wavelength absorption differences for cytochromes c (550-540 nm) and aa3 (444-465 nm) were used to quantify the signal-to-noise (S/N) ratios.

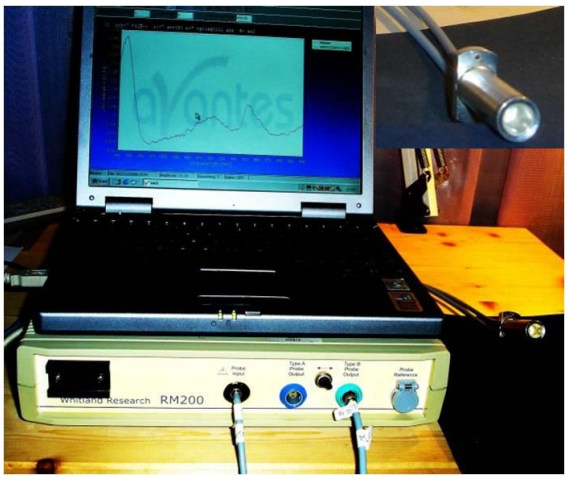

Figure 1: Whitland Research RM200 lightguide spectrophotometer and the LEA stopper (enlarged top right).

3. Results

The results of measurements of cytochrome difference spectra at different separations are shown in Figure 2 and corresponding S/N ratios for cytochromes *c* and *aa*3 are shown in Figure 3.

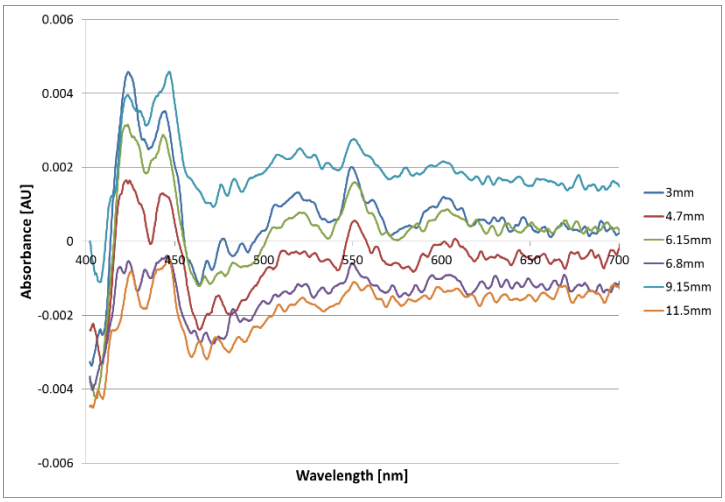

Figure 2: Reduced minus oxidised cytochrome difference spectra measured in yeast through the Oroboros O2k window for various source-receiving lightguide fibre separations.

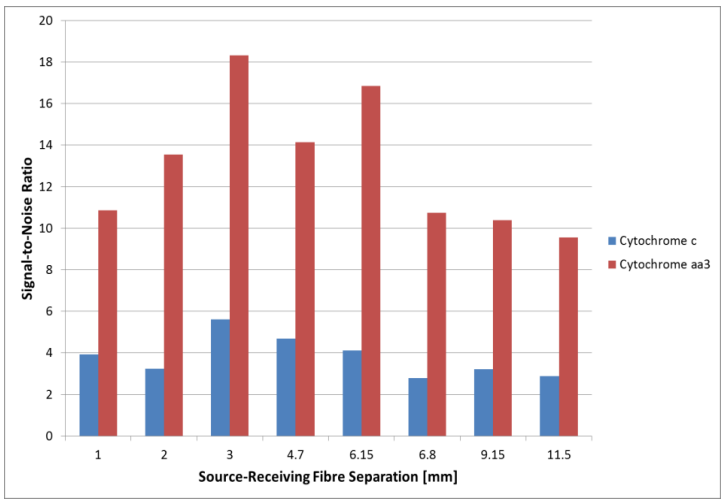

Figure 3: Signal-to-noise ratios for cytochromes c and *aa*3 at various source-receiving lightguidefibre separations.

Similarly, Figures 4 and 5 compare results using the LEA cuvette, the optimal 3mm window lightguide separation and the in-built LED light source.

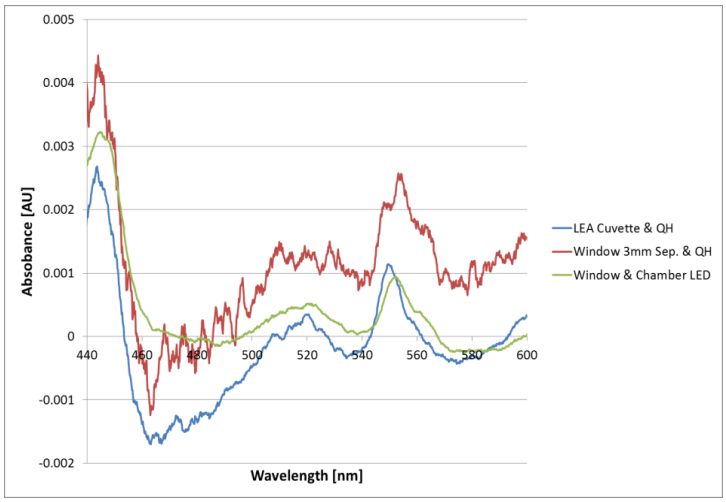

Figure 4: Comparison of cytochrome difference spectra measured using different configurations of lightguides and light sources; 1.5 mg/ml yeast.

The above studies have only used fully reduced cytochrome spectra for comparisons, so recently cytochrome difference spectra were recorded at predetermined levels of O_2 flux achieved using TIP. Levels were defined relative to the initial fully oxygenated flux, J_0.

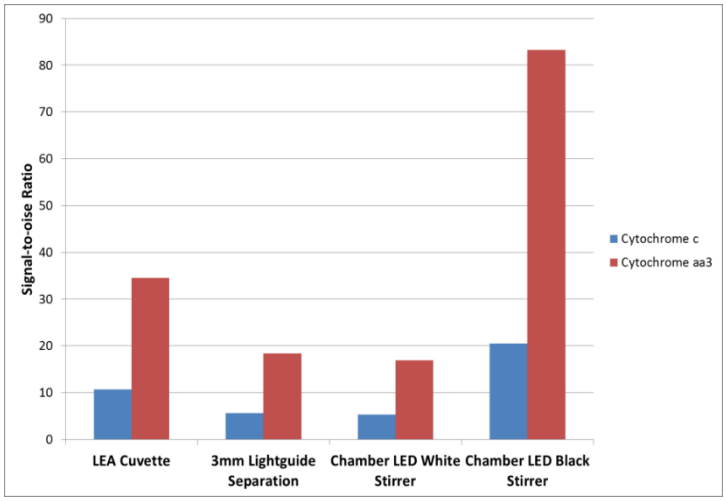

Figure 5: Comparison of signal-to-noise ratios for the LEA cuvette, window 3 mm lightguide separation and measurements through the window using the in-built LED in the presence of white and black stirrers.

Figures 6 and 7 show resultant average cytochrome difference spectra and percentage of cytochrome reduction compared with fully reduced (anoxic) which corresponds to 0% J_0.

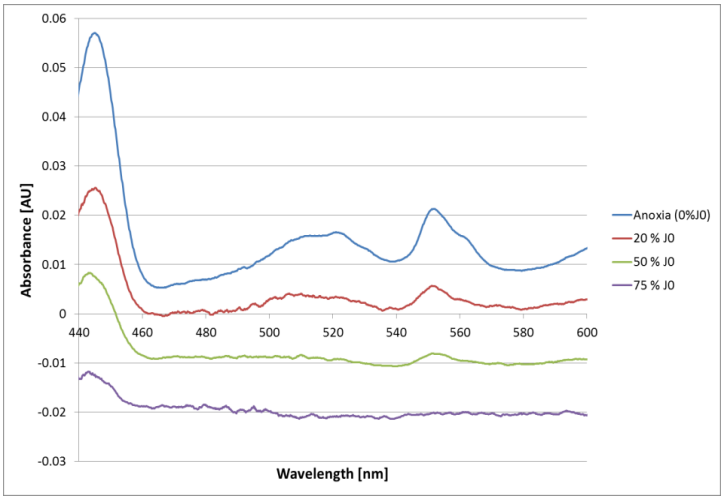

Figure 6: Average cytochrome difference spectra at 0%, 20%, 50% and 75% J_0. The spectra have been offset for clarity.

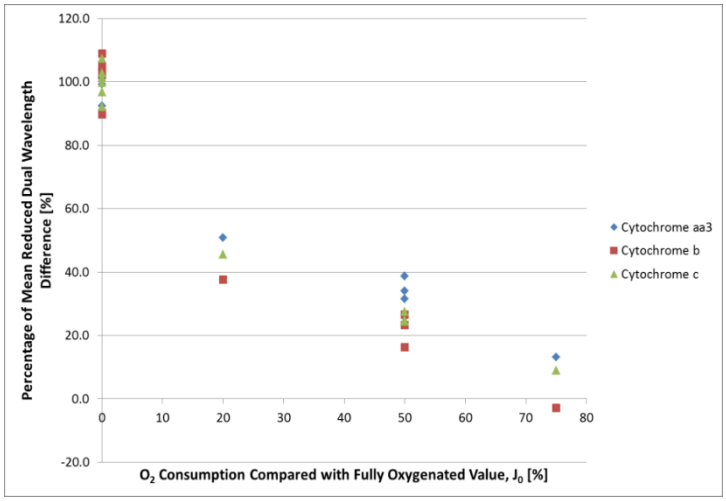

Figure 7: Percentage reduction of cytochromes *aa*3, *b* and *c*, as evaluated using the dual wavelength difference method, as compared with 100% reduction during anoxia (0% J_0) at 20%, 50% and 75% J_0.

4. Conclusions

- The built-in LED configuration provides the best S/N ratios.
- Difference spectra can still be recorded and analysed at 75% J_0.
- The optimal spectral characteristics for the LED are being investigated.

5. References

1. Sommer N, Pak O, Schörner S, Derfuss T, Krug A, Gnaiger E, Ghofrani HA, Schermuly RT, Huckstorf C, Seeger W, Grimminger F, Weissmann N (2010) Mitochondrial cytochrome redox states and respiration in acute pulmonary oxygen sensing. Eur Respir J 36: 1056-66. »Bioblast link«
2. 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. »Bioblast link«