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Title:

Microsystems for the electrochemical and optical monitoring
of bioenergetic activities of isolated mitochondria

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Mitochondria are known as central players in many cellular processes including oxidative phosphorylation, oxidative stress and signaling through the production of reactive oxygen species, or the activation of apoptosis by the cytochrome c release. Consequently, they play a key role in the progression of diseases linked to ageing, including cancers and neurodegenerative troubles. Thus, lots of efforts are currently devoted to develop innovative therapies based on the modulation of mitochondrial activity. This implies increasing demand for devices allowing the analysis of metabolic processes at the scale of isolated mitochondria. In this context, we developed the ElecWell (electrochemical microwell), based on the integration of ring nanoelectrodes (RNE) into silica microwell arrays made on glass substrates [1]. The new generation of ElecWell devices was adapted to a temperature-controlled microscopy platform. Two planar electrodes were integrated to obtain a complete electrochemical cell and allow experiments in closed-flow-through configuration (Figure 1A). Methods were developed to enhance the filling rate of microwells by mitochondria and to reduce biofouling (Figure 1B). First results were obtained with mitochondria isolated from rodent cardiomyocytes. Oxygen consumption was measured locally by cyclic voltammetry at the RNEs (Figure 1C) whereas individual variations of mitochondrial membrane potential were monitored by fluorescence microscopy (Figure 1D), [2]. Next steps consist in performing simultaneous measurements and to reach the electrochemical detection of the bioenergetic activity of single mitochondria with individually addressable microwells.

[1] Sékli Belaïdi F. et al., *Sensors Actuators B-Chemical*, **2016**, 232, 345

[2] Vajrала V.S. et al, *Integrative Biology*, **2016**, 8, 836.

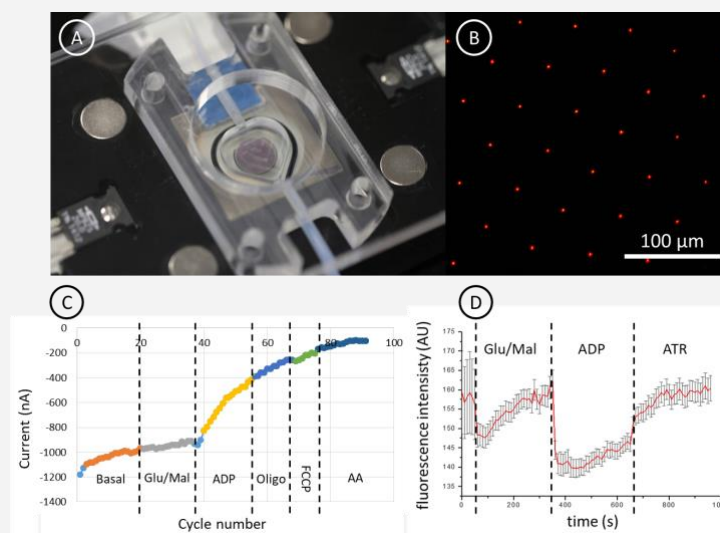


Figure 1: (A) the 2nd generation of the ElecWell device mounted on its microscopy platform; (B) mitochondria into microwells (TMRM fluorescence); (C) variations of dissolved oxygen concentration versus time; (D) variations of mitochondrial membrane potential versus time, both as function of activator/inhibitor additions.