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

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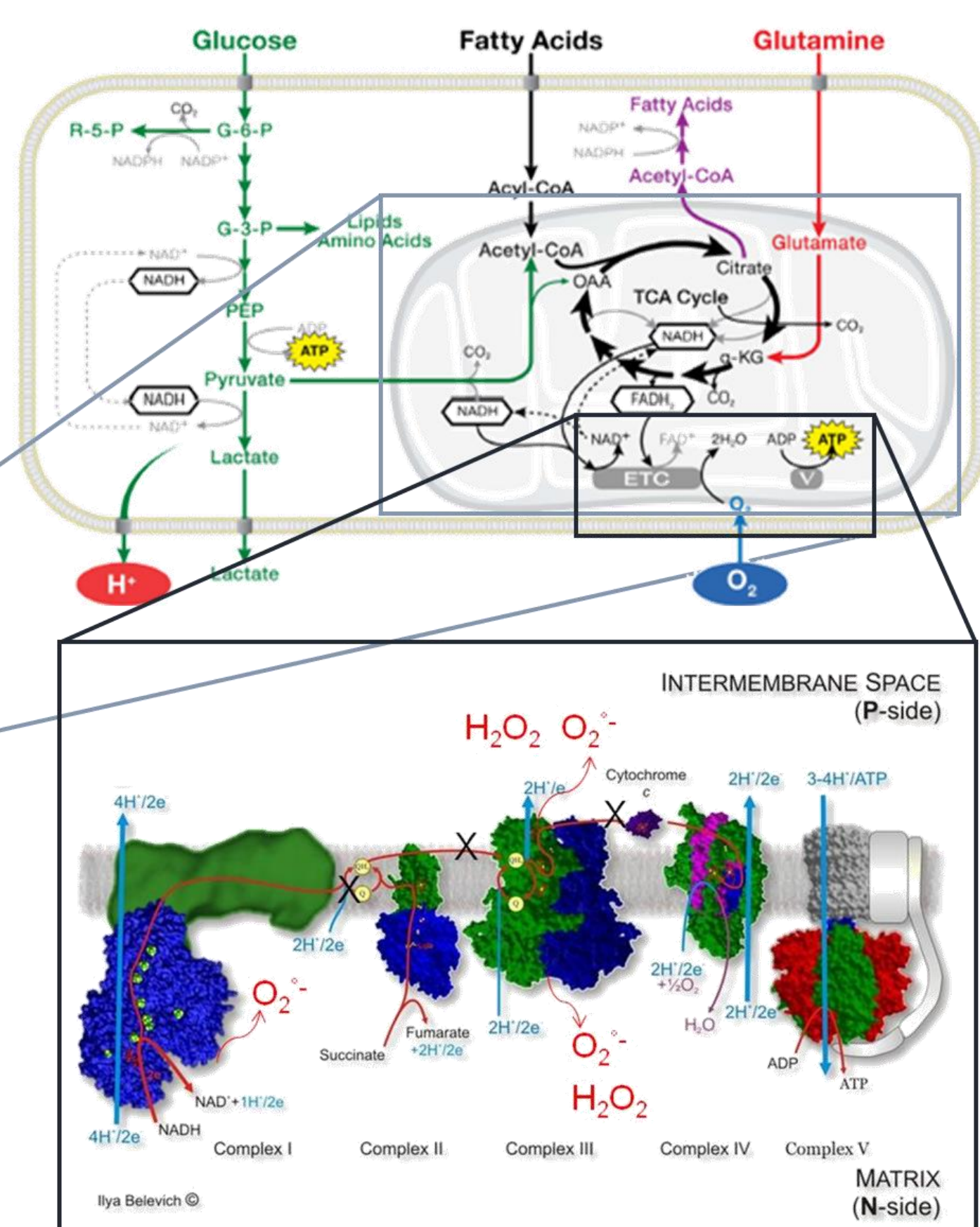
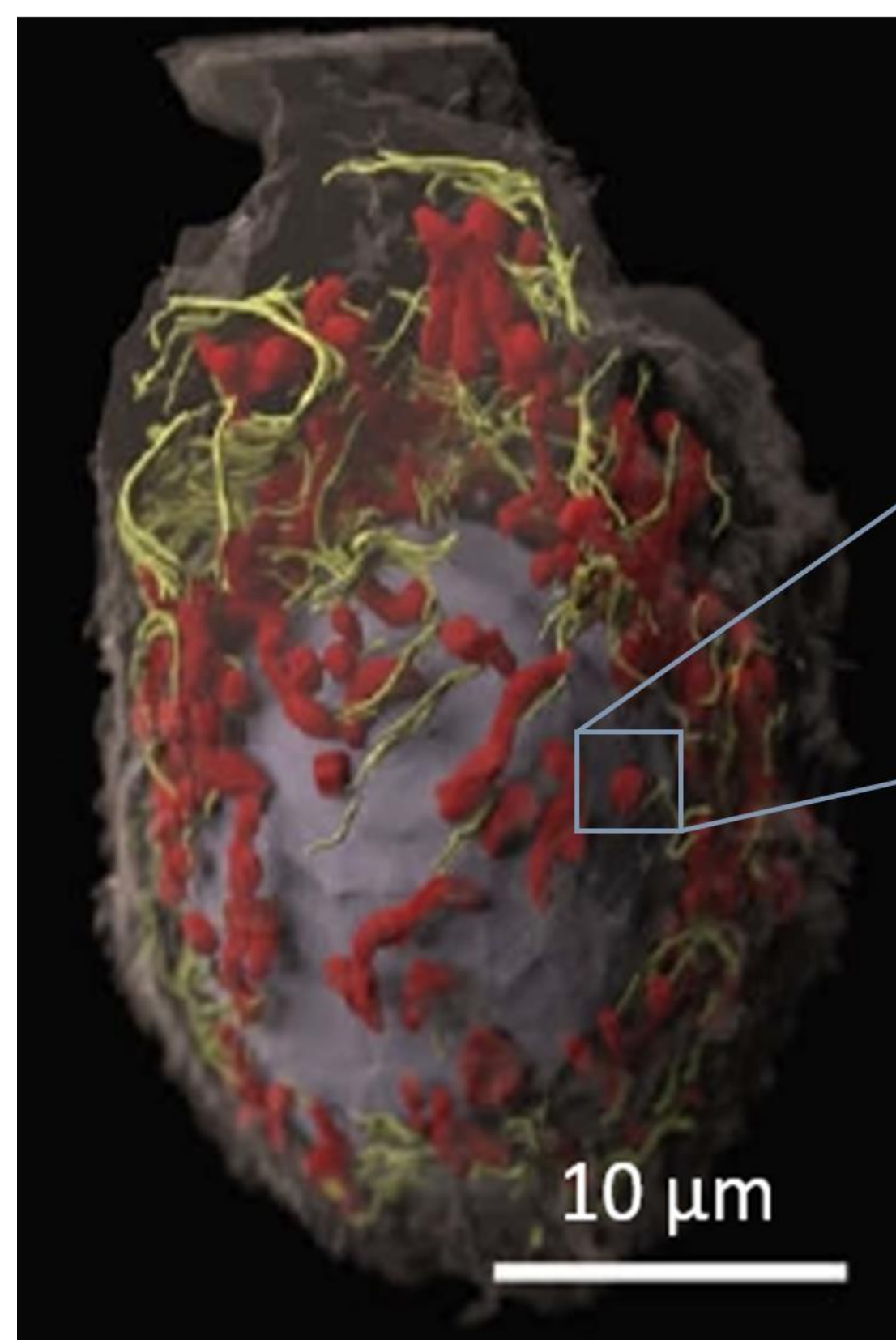
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Rational

Mitochondria are known as central players in many cellular processes including oxidative phosphorylation, oxidative stress and signaling through the production of reactive oxygen species (ROS), 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. The objective of this work is to develop solutions allowing the real time monitoring of bioenergetic activities of isolated mitochondria, at the single mitochondrion scale.



Key biomarkers of mitochondrial activity:

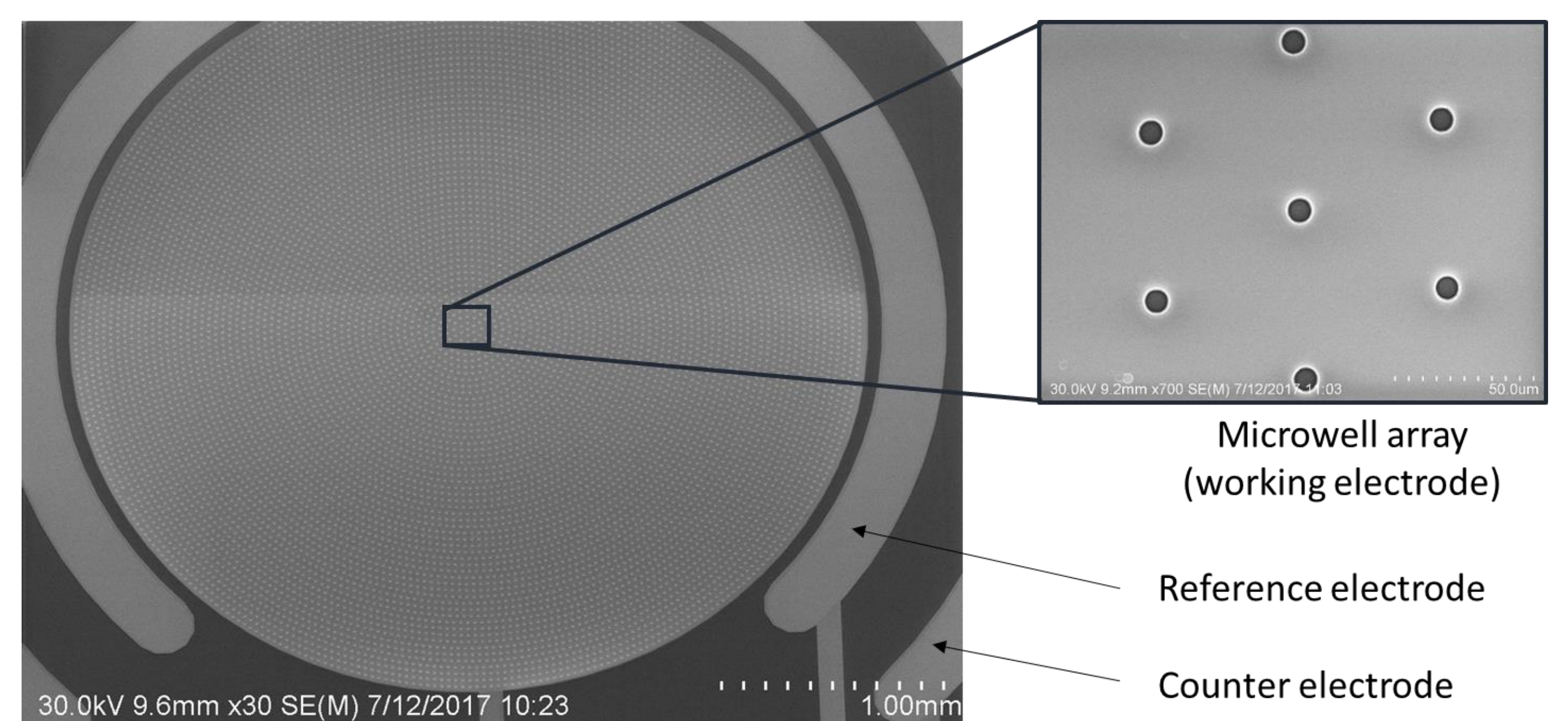
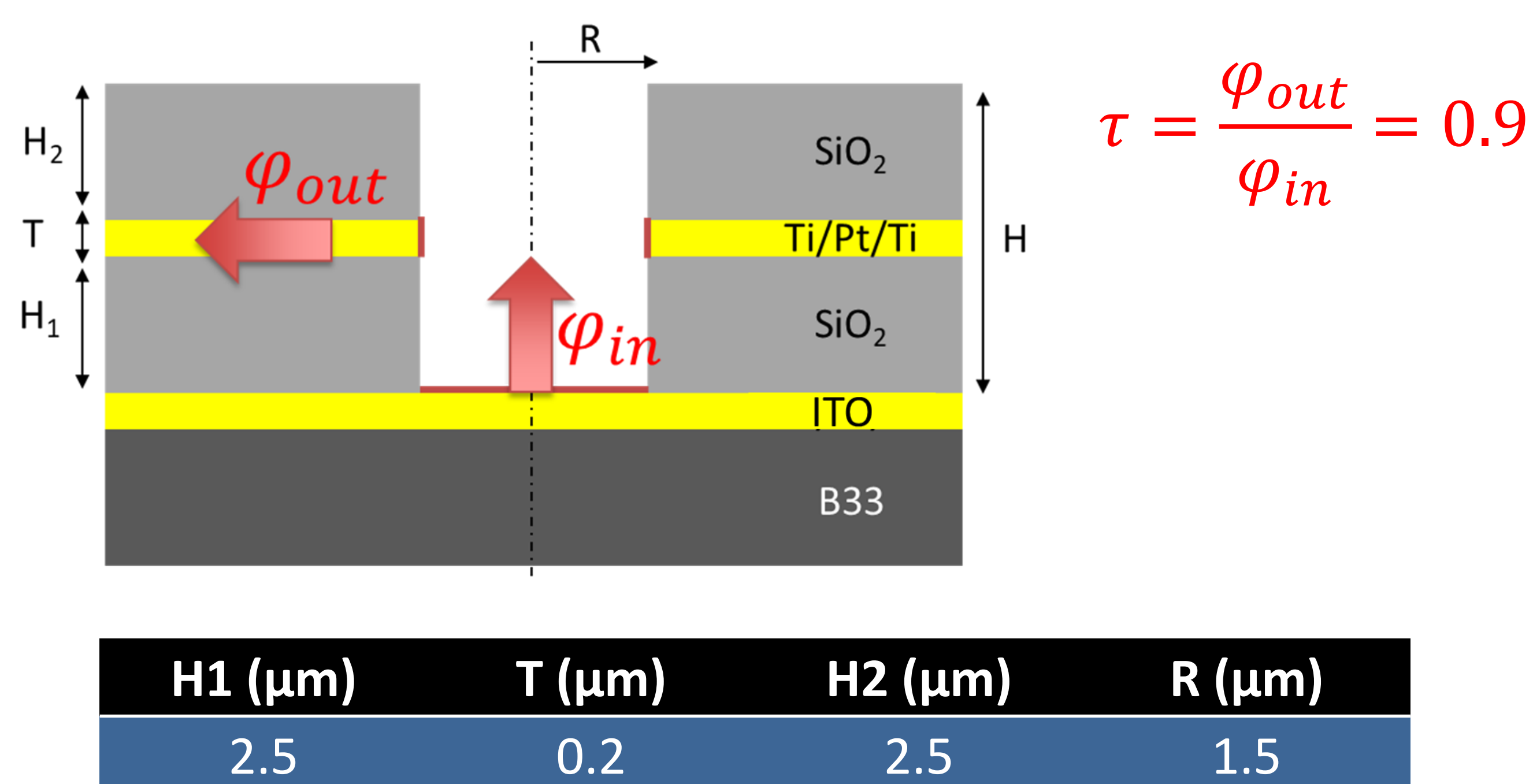
- O₂ consumption
- ROS production (H₂O₂)
- Membrane potential (ΔΨ_m)

	Optical analysis	Electrochemical analysis
Advantages	<ul style="list-style-type: none"> ■ Analysis at the scale of single mitochondrion ■ Sensitivity ■ Diversity of probes 	<ul style="list-style-type: none"> ■ Selectivity ■ Kinetic and quantitative data
Drawbacks	<ul style="list-style-type: none"> ■ Selectivity ■ Kinetic and quantitative data 	<ul style="list-style-type: none"> ■ Number of detectable species ■ Hardly applicable to single mitochondrion analysis

J. A. W. Heymann et al. "3D Imaging of mammalian cells with ion-abrasion scanning electron microscopy," *J. Struct. Biol.*, 2009
L. S. P. Winer and M. Wu, "Rapid analysis of glycolytic and oxidative substrate flux of cancer cells in a microplate," *PLoS One*, 2014

Microwell arrays: design and manufacturing

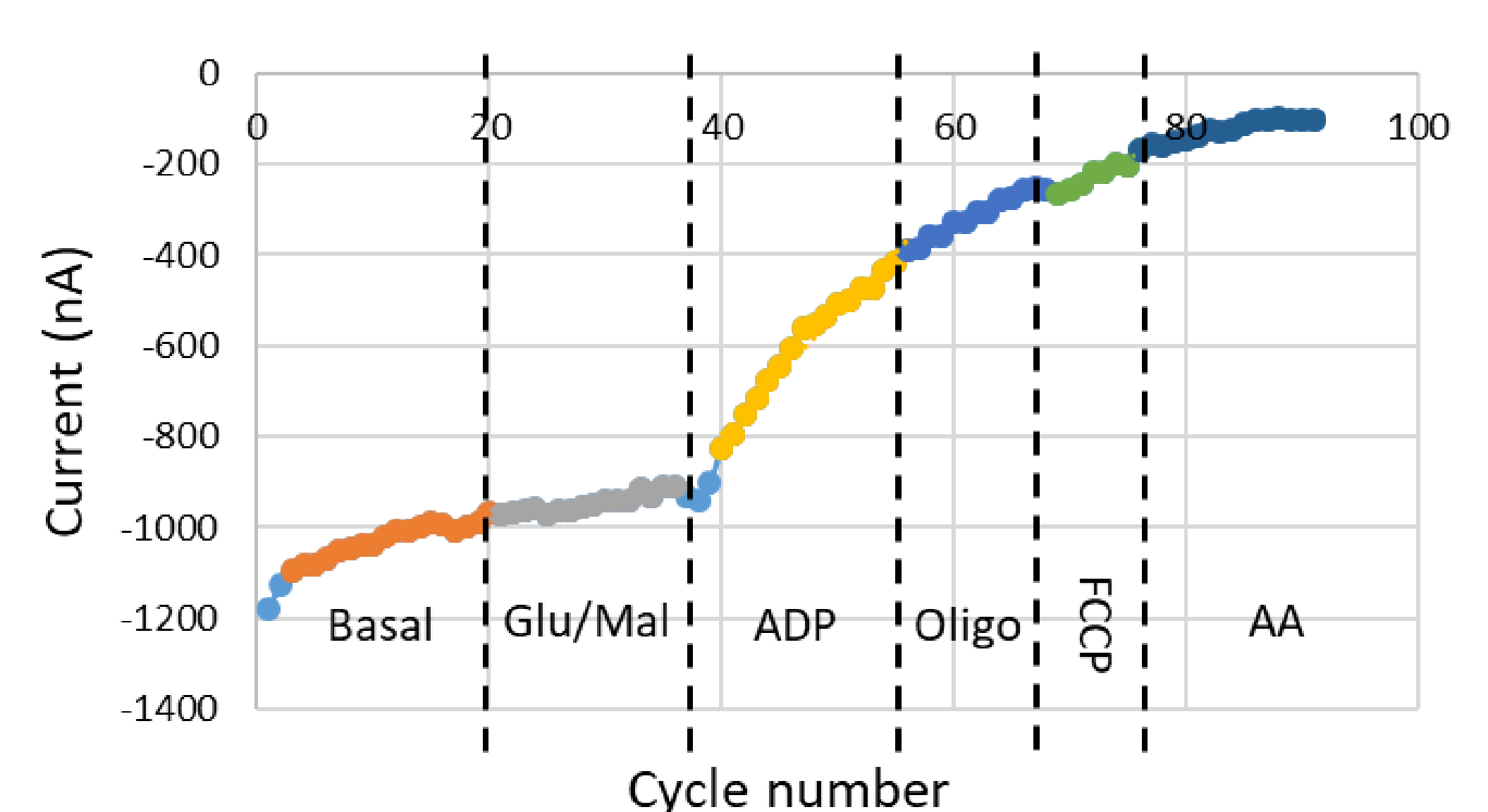
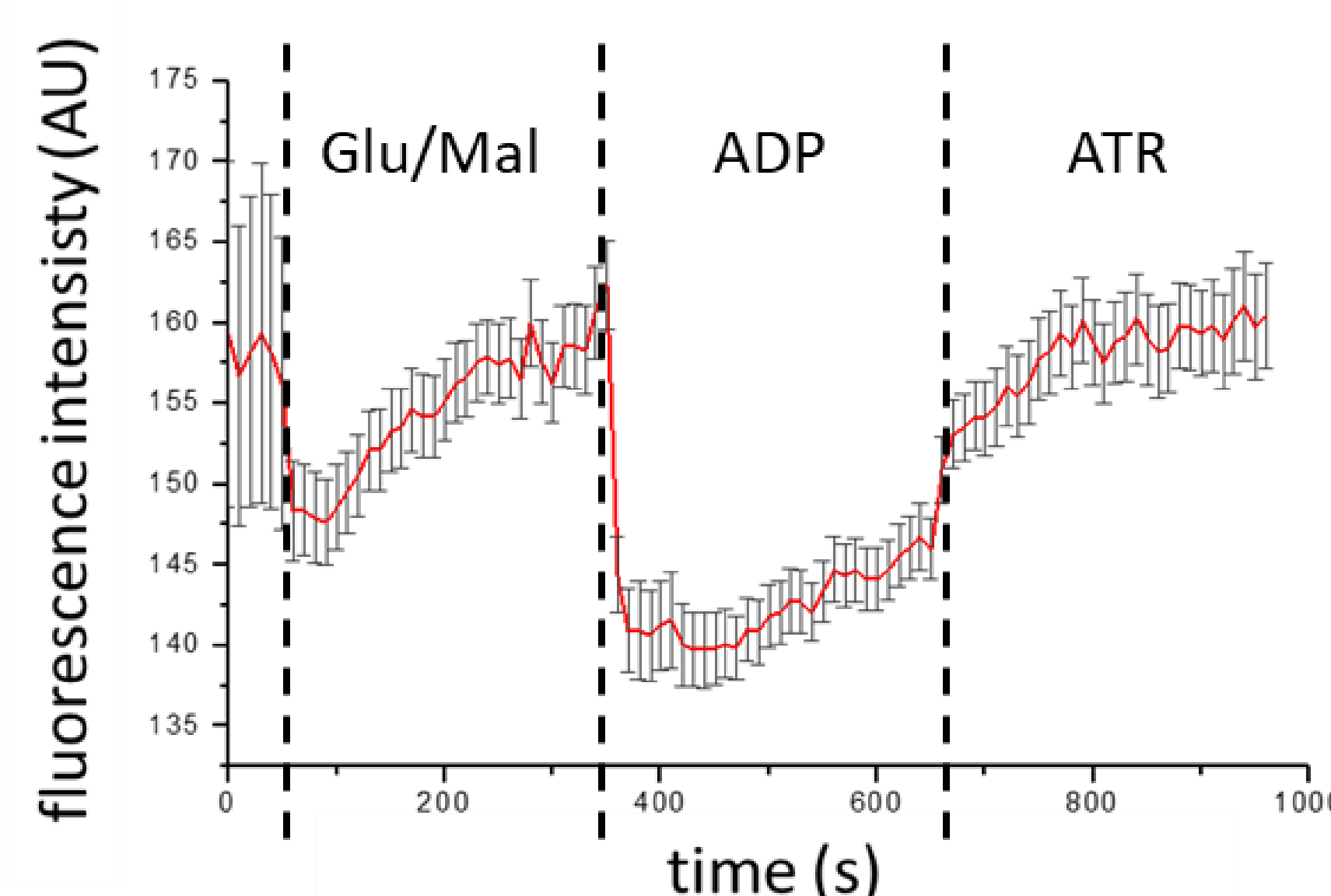
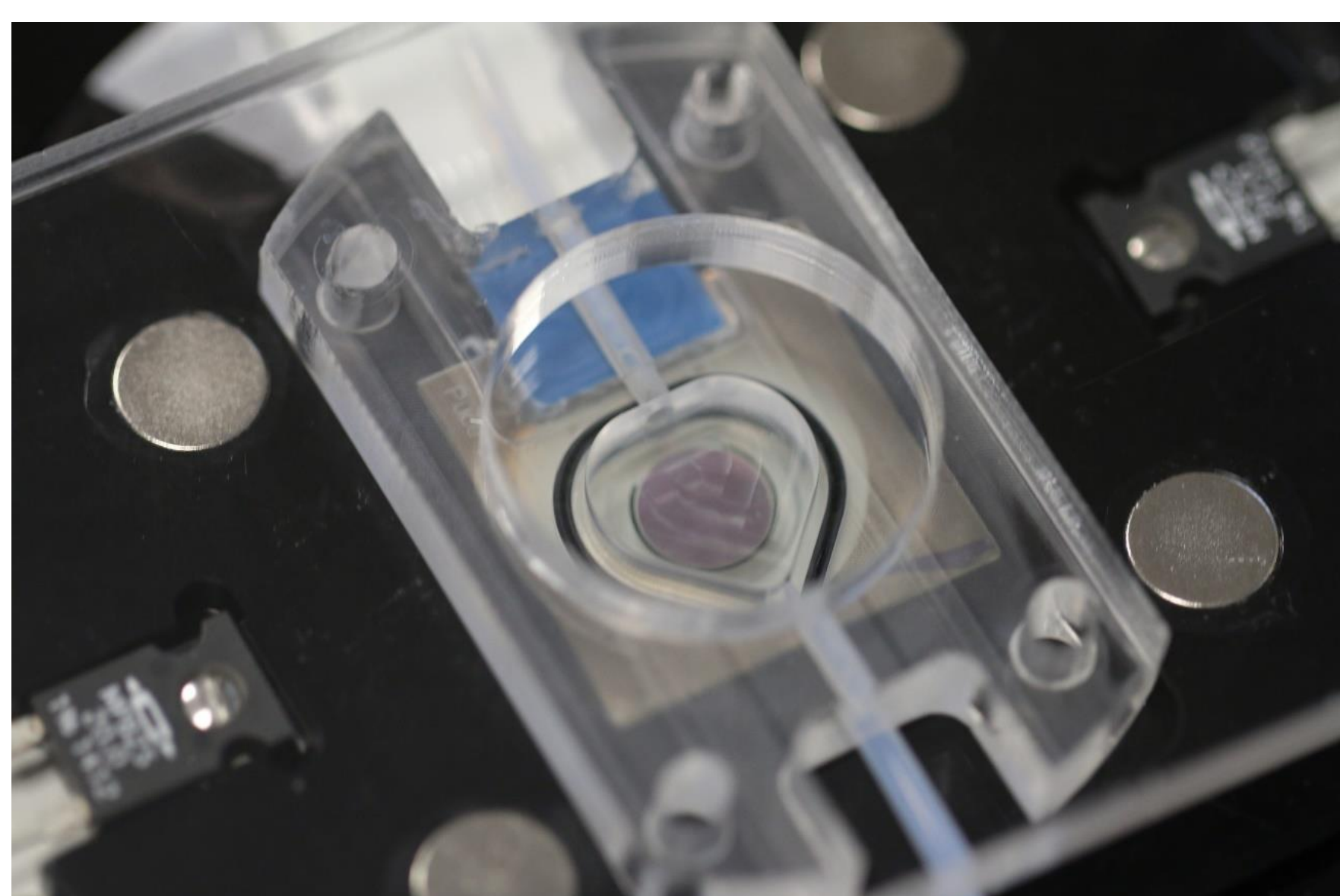
Integrate ring nanoelectrode into microwell arrays is one solution to separate mitochondria one from each other and reach analysis at the single scale. Moreover, the geometrical structure of microwells provide the confinement of the emitted species, leading to the maximization of the collection ratio (τ). Built on glass substrates, the ElecWell device offers the coupling of two complementary techniques: electrochemistry and optics. Designed in order to ensure compatibility with a microscopic platform thermally regulated, the second generation of the ElecWell device provides a perfect coupling with microscopy and fluidics.



Analysis of isolated mitochondria activities

Activators and inhibitors of the electron transport chain are added in a becher under magnetic agitation. The fluid is constantly circulating within a closed circuit passing by the ElecWell microfluidic chamber thanks to a peristaltic pump. Fluorescence microscopy (ex: ΔΨ_m) and electrochemistry (ex: O₂ consumption) can be performed simultaneously.

Variations of ΔΨ_m (single mitochondrion scale) Variations of O₂ consumption (10⁴ microwell network)



Next step: get reproducibility and progressively reduce the number of microwells forming the network, from 10⁴ to 1.