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Development of Microwelll arrays: Studying the metabolic responses of single Mitochondrial

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Mitochondria play a central role in cellular respiration, ageing process and also in the onset of degenerative diseases. These duties are performed by the collections of mitochondria (within a cell) that are heterogeneous, dynamic, and subject to fusion and fission to form a network [1]. Since the valuable information regarding mitochondrial physiology is missing during averaged analysis of a large population of these organelles, development of methods to probe the properties of individual mitochondria simultaneously at single organelle level is, therefore, a challenge for analyticians. In this context, we developed microwell array platforms based on fiber optic [2] and PDMS micro-structuration [3] that allow the screening of a large number of individual mitochondria simultaneously using fluorescence microscopy.We used either a chemical etching procedure to create a high-density array of femtoliter (fL) containers from optical fiber bundles, or puncturing method to create milli to micrometric wells in PDMS- polymer thin layers. Mitochondria organelles were entrapped in these wells and independently imaged via reflecting optical signals. We developed experimental techniques which are required for the immobilization of mitochondria and also the screening of whole population and individual responses within those populations using optical fiber based microwell arrays and PDMS wells.We studied the endogenous NADH (auto-fluorescence) variation of populations of individual mitochondria under activation with EtOH (substrate) and inhibition with Antimycin A (respiratory inhibitor). Statistical studies of mitochondrial NADH value distributions evidenced three different kinds of responses within the mitochondrial population, the metabolic performance being not related to the size of the mitochondrion. This individual mitochondrion analysis approach is now extended to the monitoring of different mitochondrial metabolic parameters, including mitochondrial membrane potential [4] (oxidative phosphorylation activity) and oxidative stress markers for the detection of superoxide radical ($0_2^{\cdot-}$) [5].

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