

# NOVEL APPROACH TOWARDS TRAPPING AND IMAGING OF INDIVIDUAL MITOCHONDRIA

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## ABSTRACT

Here we are presenting a novel platform for interrogation of single isolated mitochondria. Individual mitochondria are physically immobilized along arrays of PDMS fluidic channels with a cross section of 500 nm x 2 μm. Fluorescence microscopy of JC-1 stained mitochondrial reveals that trapped mitochondrial maintain their membrane potential; and indicate that they remain vital and functional in this trapped state. There is growing interest in study of mitochondrial dysfunction due to its role in a multitude of pathological conditions. This chip enables fluorescence imaging of individual mitochondria in a controlled environment and will be a very useful tool in investigation of mitochondrial bioenergetics.

**KEYWORDS:** Mitochondria, Lab on a chip, Nanofluidics, Microfluidics, Trapping, Membrane potential

## INTRODUCTION

While there has been extensive research to assay and analyze individual cells using microfluidic devices, analysis of individual sub-cellular organelles is a new field. Here we report a simple, economic device for trapping and imaging individual mitochondria. Mitochondrial functions are essential to cell life. Through oxidative phosphorylation coupled with the electrochemical gradient established by the electron transport chain, mitochondria convert dietary calories to ATP, the energy currency for almost all cellular processes. Mitochondria also play key roles in other physiological processes. Studies have shown that mitochondrial dysfunction leads to a variety of illnesses and has a significant role in the aging process[1]. It has been shown recently that mitochondria are morphologically and functionally heterogeneous[2]. It is important to study this heterogeneity in order to understand the mechanisms of mitochondrial functions. Current technologies are mostly suitable for investigating the average behavior of a large number of mitochondria and the dynamics and behavior of individual mitochondria is lost. Therefore there is a need for development of novel technologies for single mitochondria studies. Microfluidic and nanofluidic technologies make it possible to control fluids in small volumes and enable assays requiring significantly less sample size. This makes the microfluidics technology ideal for studying the functional heterogeneity of mitochondria under different chemical conditions.

## Experimental

Devices reported here were fabricated by soft lithography of polydimethylsiloxane (PDMS) over a patterned silicon mold. Briefly, to make the mold, using photolithography technic the channel pattern is created on Piranha (3:1 mixture of sulfuric acid and hydrogen peroxide) cleaned 4" silicon wafer with Microposit SC1827 positive photoresist. During the developing process, the wafer is left a few seconds longer than the standard develop time in MF-319 developer and is slightly overdeveloped so that channels with a smaller width also have a smaller height. After the photolithography the mold is salinized to prevent sticking of the PDMS to the mold. Silicon elastomer and curing agent (Sylgard® 184, Dow Corning Co.) are mixed thoroughly at a 10:1 weight ratio. PDMS is degassed for 30 minutes in a vacuum desiccator and poured over salinized mold to a thickness of 3 mm. The mold is placed in a 70° C curing oven overnight. After PDMS is cured it is cut and peeled off from the mold and two 0.63 mm fluidic access holes are punched for fluid inlet and outlet. The PDMS channel is treated with 70 W Oxygen plasma for 20 s to make the surface hydrophilic. When placed over a Piranha cleaned glass slide, the hydrophilic PDMS bonds well with the glass surface.

The geometry of the trap channels are shown in figure 1. The trapping area consists of 10 parallel channels. Each channel has a semi-circular cross section with a maximum height of around 500nm and average width of 2  $\mu\text{m}$ .

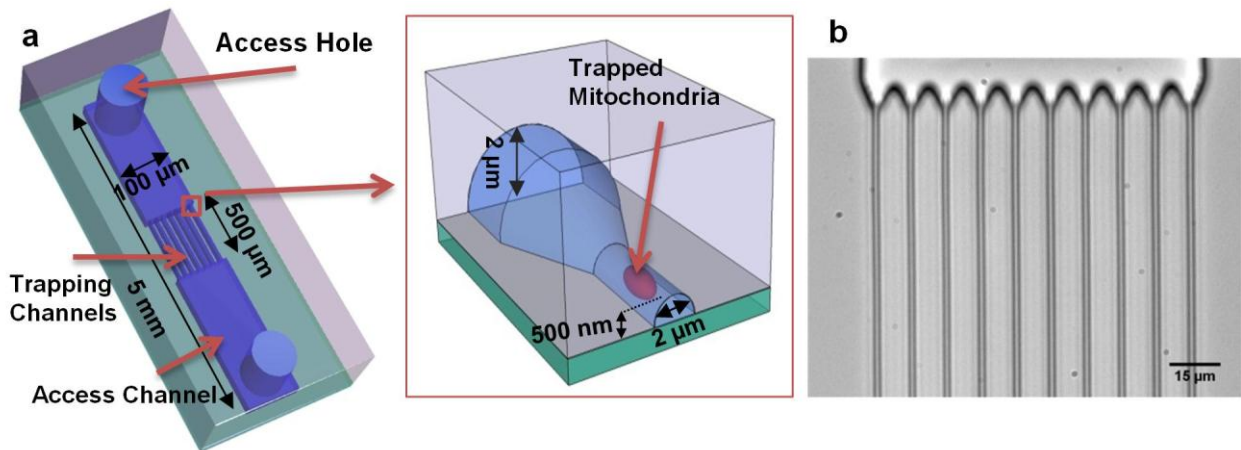


Figure 1: a) Schematic of the device and trapping mechanism. b) Bright field microscope image of the device.

On average, mitochondria have cigar shaped morphology with a width of 200nm to 1  $\mu\text{m}$ . Since the trap channel height is around 500 nm, mitochondria that are around 500 nm or larger in diameter will be immobilized along the channels. The trap channels are connected to two 100  $\mu\text{m}$  wide, 2  $\mu\text{m}$  high access channels. Although the mold is patterned in a single lithography step, due to the overdeveloping, the narrow trap channels also have a smaller height.

To test the channels, the chip is connected to the syringe pump and a buffer (225 mM Mannitol, 75 mM Sucrose, 10 mM KCl, 10 mM Tris-HCl, 5 mM KH<sub>2</sub>PO<sub>4</sub>, pH adjusted to 7.2 with 1M KOH) containing isolated mitochondria from the human cervical cancer cell line HeLa (ATCC, CCL-2) is pumped into the channels. Mitochondria are stained with JC-1 prior to introduction into the channel. JC-1 is a fluorescent dye sensitive to mitochondria membrane potential. Figure 2 shows JC-1 stained mitochondria that are trapped in the channels. The red fluorescence of JC-1 stained mitochondria indicates that they sustain a high membrane potential[3] in the trapped state and therefore shows that they remain vital.

## Conclusion

We present the first device for trapping and fluorescence imaging of individual isolated mitochondria. The device has a simple and cost effective fabrication process and will have variety of application in study of heterogeneity of isolated mitochondria.

## References

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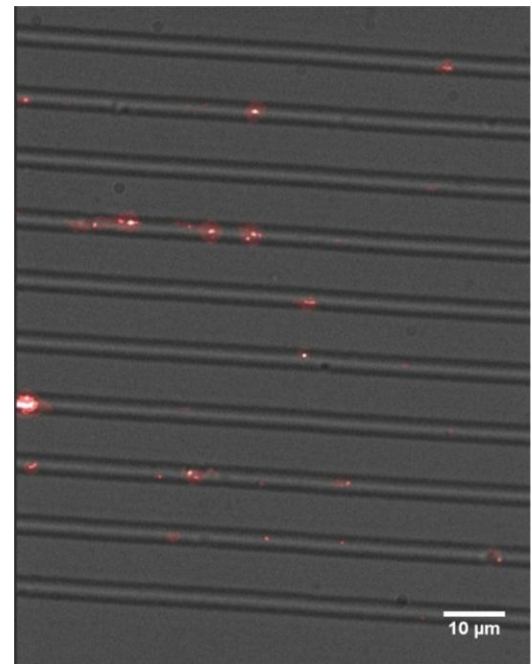


Figure 2: Individual JC-1 stained mitochondria trapped along the channels. The fluorescence image of mitochondria and the bright field image of the channels is taken separately and merged.