



APPLICATION NOTE

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# EPI-FLUORESCENCE MITOCHONDRIAL IMAGING IN LIVE SINGLE CELLS

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## INTRODUCTION

Mitochondria are deeply involved both in cell life and death mechanisms. In addition to serving as the major source of ATP production, mitochondria also function as one of the major buffer for calcium, that regulates enzymes activities. In addition, ROS generated from the electron transport chains of mitochondria may cause oxidative damage to the cells. For these reasons, mitochondria staining with fluorescent dyes, antibodies or naturally fluorescent molecules contributes to the study of their structure and function in normal physiological and pathophysiological states. MitoGreen (PromoKine, PromoCell) is a green fluorescent mitochondrial dye with properties similar to those of MitoTracker Green FM. It diffuses across the plasma membrane of live or fixed cells equally, afterwards accumulating in the mitochondria. The dye is non-fluorescent until it partitions into the mitochondrial membrane. The staining relies on mitochondrial mass, not on mitochondria membrane potential. CELLviewer system enables high-content, time-lapse fluorescence imaging of live cells grown in suspension inside a disposable cartridge, while dispensing drugs solutions to the sample chamber in order to visualize their biological effects at single cell level. In this application note, MitoGreen stained Jurkat single cells are isolated inside CELLviewer disposable cartridge and time-lapse imaged both in Bright-field and GFP channels. MitoGreen is spectrally similar to FITC, making it optimally excitable at 488 nm.

## MATERIALS AND METHODS

- Jurkat cells (ATCC)
- RPMI culture medium (Gibco, Life Technologies, Thermo Fisher Scientific)
- MitoGreen (PromoKine, PromoCell)
- CELLviewer imaging system and disposable cartridge
- CELLviewer 50 ml DOCK

Jurkat cells were grown at 37 °C and 5% CO<sub>2</sub> in RPMI 1640 medium, supplemented with 2 mM L-glutamine, 10% FBS, 100 units/mL penicillin and 100 mg/mL streptomycin. Before the experiments, Jurkat cells were washed and suspended at a final concentration of 5 x 10<sup>5</sup> cells/ml in a 5% FBS culture medium. Then, sample was incubated for 20 minutes at the dark at 37°C with MitoGreen 200 mM (PromoKine, PromoCell).

After the incubation, cells were centrifuged at 2000 rpm for 5 minutes to remove excess MitoGreen and resuspended in 5% culture medium at the CELLviewer working concentration of 5 x 10<sup>3</sup> cells/ml. Sample is then pipetted inside a 50 mL Falcon tube closed with a CELLviewer 50 ml DOCK. After single cell isolation and fluidic adaptive autofocusing, CELLviewer automatically acquires sample images in Brightfield channel and GFP channel at 0.5 fps with 20X magnification.

ImageJ software was used for image analysis using (i) Measure function to calculate single cell diameter; (ii) Plot profile plugin to create the graphic of fluorescence intensity along a straight line passing across the cell; (iii) 3D Surface plot plugin to 3D visualize spatial fluorescence intensity distribution. ImageJ software was used for image analysis using (i) Measure function to calculate single cell diameter; (ii) Plot profile plugin to create the graphic of fluorescence intensity along a straight line passing across the cell; (iii) 3D Surface plot plugin to 3D visualize spatial fluorescence intensity distribution.

## RESULTS

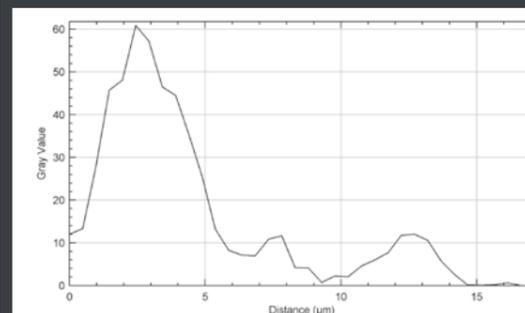
A single Jurkat cell is isolated in the microfluidic cartridge and time-lapse imaged for 4 hours. It shows green staining signal around nuclear region and inside plasmatic membrane borders, that conceptually overlaps mitochondrial region. Both immortalized and primary cell lines stained with MitoGreen (PromoKine, PromoCell) are suitable for testing on CELLviewer.

### SINGLE JURKAT CELL MITOGREEN LABELLED ANALYSIS

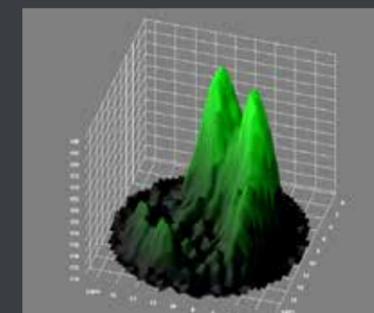
Scale bar: 20 µm



Acquisition in Bright-field channel. Acquisition in GFP-channel.



Plot profile of MitoGreen (Promokine, Promocell) fluorescence signal along a straight line crossing the cell.



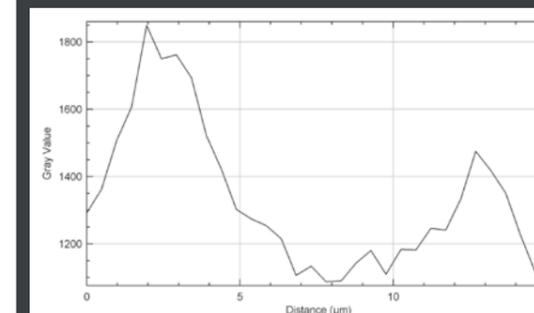
3D surface plot of MitoGreen (Promokine, Promocell) fluorescence signal with ImageJ software.

### SINGLE JURKAT CELL MITOGREEN LABELLED ANALYSIS

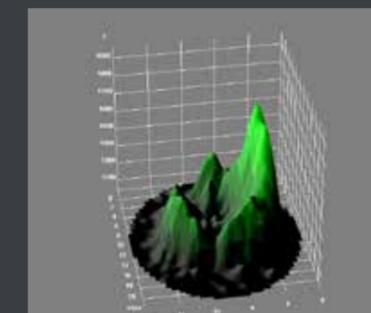
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