Automation in 3D cellular system in Live-Imaging with Microfluidic Technology CELLviewer®

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Automation in 3D cellular system in Live-Imaging with Microfluidic Technology CELLviewer®

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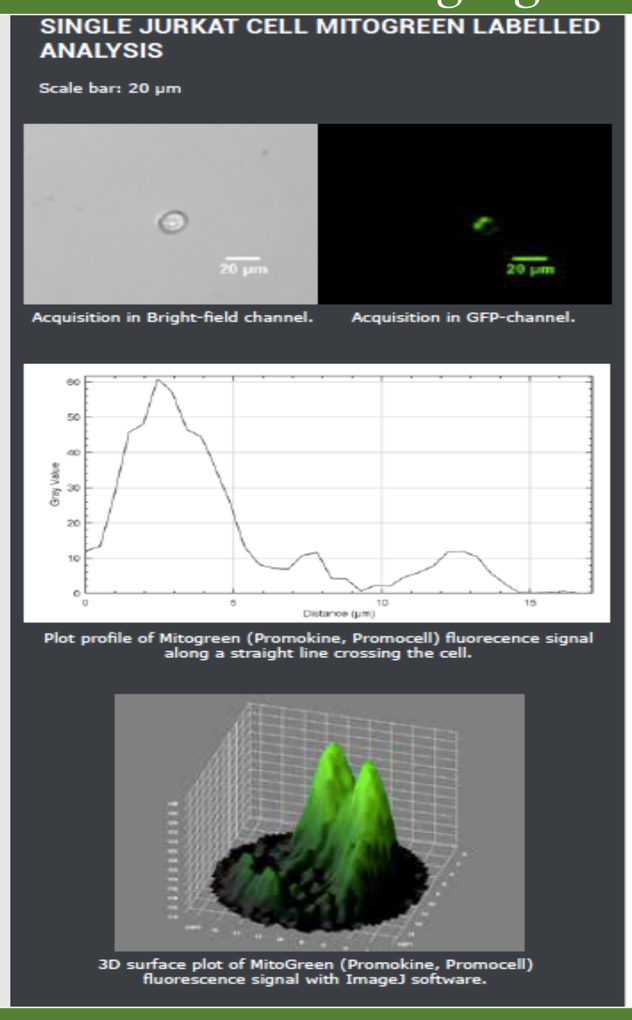
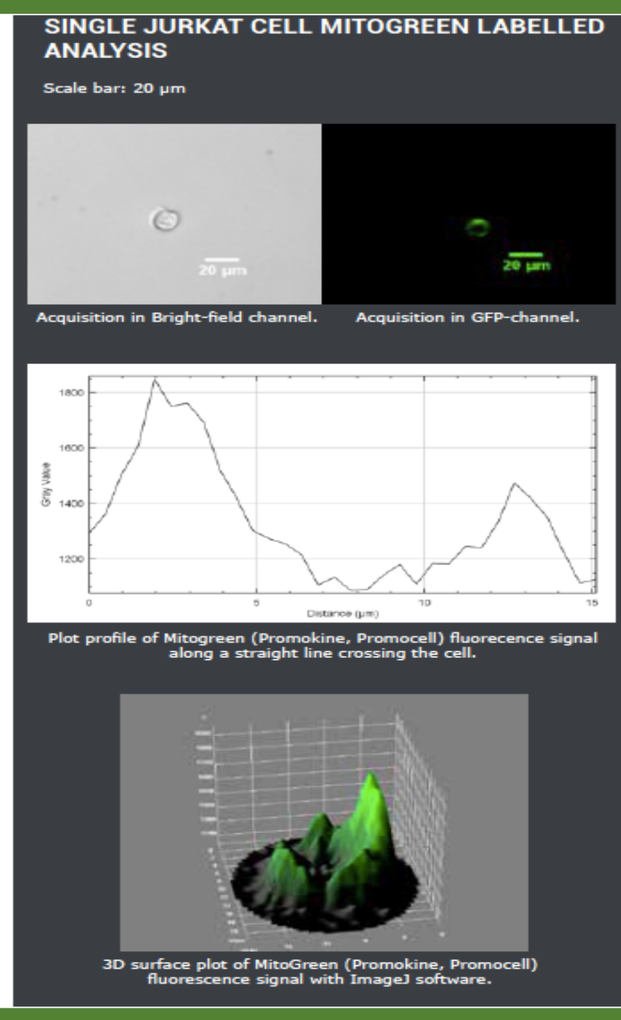
Introduction

- Differences observed when comparing cell cultures in 2D and 3D is morphological dissimilarity and their evolution over time. Cells grown in a monolayer tend to flatten on the bottom of the plate by adhering and spreading on the horizontal plane without expanding into the vertical dimension;
- Mitochondria are involved in crucial cellular tasks controlling the cell cycle and growth such as cell signaling, differentiation, and death. Damage to and subsequent dysfunction of mitochondria play a role in various diseases like diabetes, myopathy and other systemic disorders;
- **CELLviewer® enables the simultaneous 3D cell culture and live cell imaging as well, featuring microfluidics and time-lapse multicolour epifluorescence microscopy;**
- **Single cell tracking in 3D** space is now possible and is combined with subsequent biochemical analyses of individually tracked cells, keeping their identity traceable with CELLviewer® system;

Epi-Fluorescence mitochondrial in live- imaging

- ❑ Jurkat (ATCC) Cells grown at 37°C and 5% CO2;
- ❑ Medium RPMI 1640 soil (Gibco, Life Technologies, Thermo Fisher Scientific), with 2 mM of L-glutamine, 10% FBS, 100 units/mL of penicillin and 100 mg/mL of streptomycin;
- ❑ MitoGreen (PromoKine, PromoCell) incubated for 20 minutes in the dark at 37°C with MitoGreen 200 mM;
- ❑ The sample is then piped inside a 50ml Falcon tube closed with a 50ml CELLviewer® DOCK and flowed inside the cartridge chamber;
- ❑ CELLviewer® automatically captures sample images in Brightfield channel and GFP channel;
- ❑ ImageJ software was used for image analysis using the Measure function to calculate the diameter of a single cell;
- ❑ 3D surface plot plug-in to display in 3D the distribution of the intensity of spatial fluorescence;



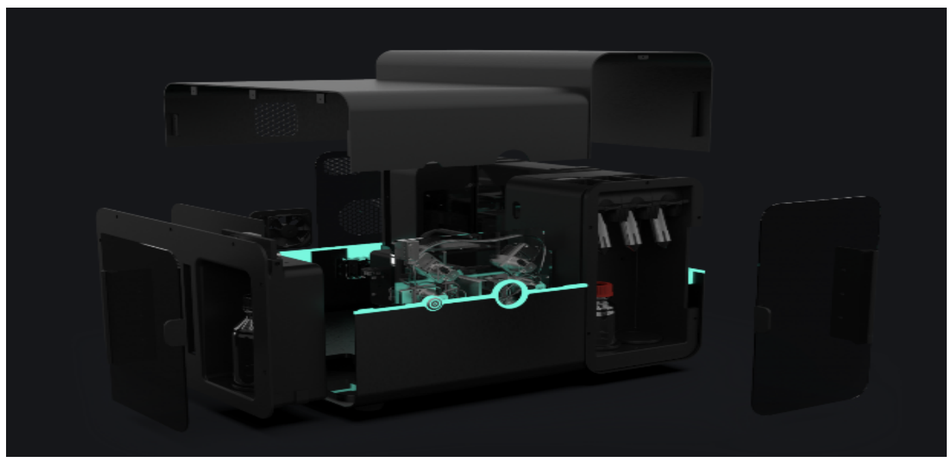


Results

Single-cell Jurkat cells was isolated and imaged for 4 and 7 hours respectively and intensified labelling of the mitochondria and fluidic transport were observed over time. **CELLviewer® can obtain detailed images of current cellular morphology with resolution and high-quality data;** employing time-lapse imaging can be achieved, the evolution of cells and their 3D morphology.

Conclusions

Staining of mitochondria with fluorescent dyes, antibodies or fluorescent molecules can greatly facilitate studies of their function and distribution and the viability of cells in healthy and diseased individuals. The preliminary experience conducted with CELLviewer indicates that this equipment responds to the needs of individual operators as it consists of a synthesis of different integrated tools, which works both with manual and automated control. **A microfluidic system has been developed and demonstrated that the 3D model can locate the 3D model spatially, it's possible to carry out experiments in direct time in terms of physiology, toxicology and clinical pharmacology.**
The entire automated system allows full autonomy and protocol management thanks to the software making the operator free to conduct other work, thus increasing the productivity of his project. In summary, the proposed microfluidic technology can serve as a new platform approach, which has the potential to advance studies at the cellular level.



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