APPLICATION NOTE

Evaluation of mitochondrial integrity and mitochondria membrane potential using automated cell imaging and analysis

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Benefits

- **Evaluate mitochondrial integrity and membrane potential in response to compound treatment**
- **Rapidly produce multiple readouts to assess mitochondrial toxicity**
- **Generate assay results efficiently with predefined analysis protocols**

Introduction

Mitochondrial function, a key indicator of cell health, can be assessed by monitoring changes in mitochondrial membrane potential (MMP)[1,2]. Mitochondrial depolarization is an early signal for hypoxic damage or oxidative stress. Cationic fluorescent dyes are commonly used tools to assess MMP. We performed a short-term (60 min) compound treatment with two known inhibitors of oxidative phosphorylation, Antimycin A and CCCP. Compound treatment was followed by staining with MitoTracker Orange and a nuclear stain. The assay allows examination of the immediate effects of compounds on MMP.

Methods

U20S cells (ATCC) were seeded at 6,500 cells per well in 384-well microplates (Greiner, black clear-bottom plates) and

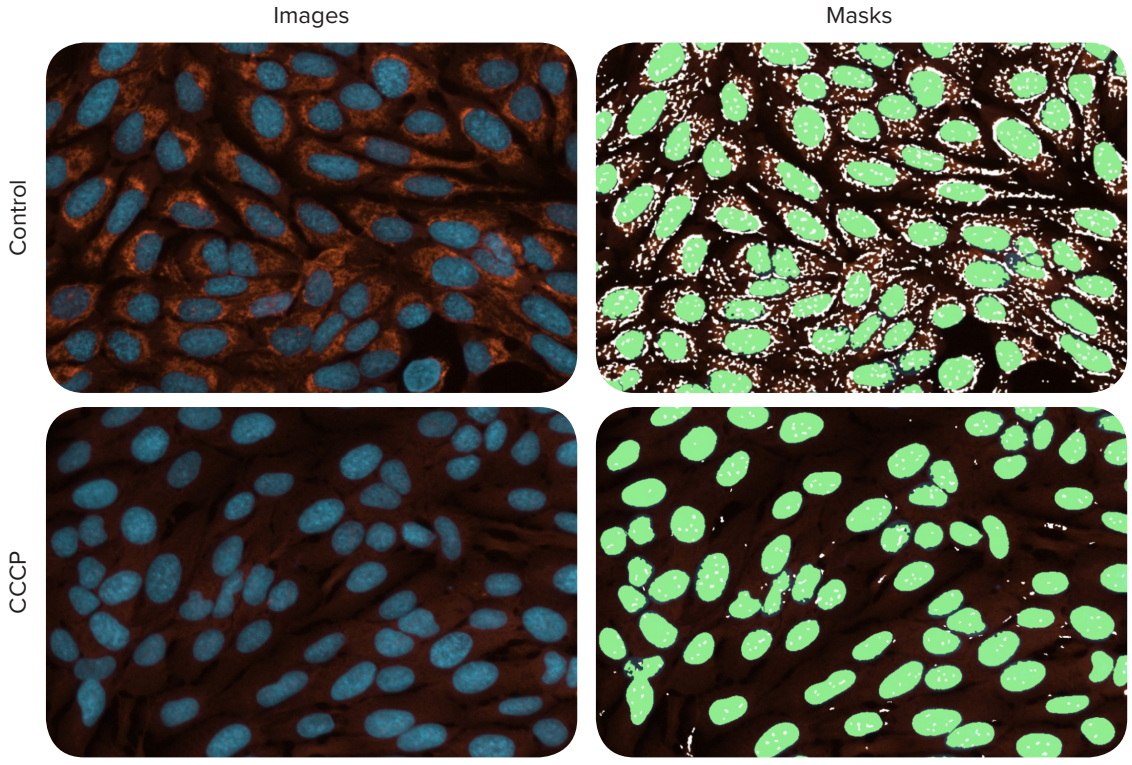

Figure 1. Evaluation of mitochondrial integrity and membrane potential by automated imaging. 20X images of control U2OS cells and cells treated for 60 min with CCCP were imaged with the ImageXpress Pico system and analyzed using the CellReporterXpress software. **Left:** Images of control cells and cells treated with 1 µM of CCCP, stained with MitoTracker Orange (orange) and Hoechst nuclear dye (blue). **Right:** Image analysis masks showing the nuclei (green) and mitochondria particles (white). Treatment with inhibitor of oxidative phosphorylation CCCP resulted in loss of membrane potential.

treated the following day with different concentrations of Antimycin A and CCCP (Sigma) for 60 min. The treatments were done in triplicates, and the compounds were diluted 1:3 starting from 100 µM Antimycin A and 50 µM CCCP. After 30 minutes into the compound treatment, the cells were stained with a mix of MitoTracker Orange and Hoechst 33342 dyes (Thermo Fisher, Carlsbad, CA) in PBS, for final 0.1 µM and 6 µM concentrations, respectively. Cells were stained for 30 min at 37°C and 5% CO_2. Cells can be imaged live or after fixing with 4% para-formaldehyde. The cells were fixed with a 4% para-formaldehyde solution for 30 min at room temperature. After washing 2 times with PBS buffer, cells were imaged using the ImageXpress® Pico Automated Cell Imaging System with the 20X objective. Images were acquired at one site per well using DAPI and TRITC channels with 20 ms and 300 ms exposure times, respectively.

Multi-parametric analysis of mitochondrial integrity and membrane potential

Figure 1 shows images of control cells and cells treated with inhibitors of oxidative phosphorylation CCCP. A dramatic difference in the content of intact mitochondria (orange stain) is observed. The Mitochondria analysis protocol of the CellReporterXpress™ Image Analysis Software was used to assess mitochondrial damage. The analysis finds granules (mitochondria) per individual cell, defined by the nuclear stain (Figure 1), and allows characterization of multiple parameters such as total number of granules, total granule area, number of granules per cell, and average intensity and integrated intensity of the granules. The resulting concentration–response curves for indicated readouts and measured EC_{50} values are shown in Figure 2 and Table 1.

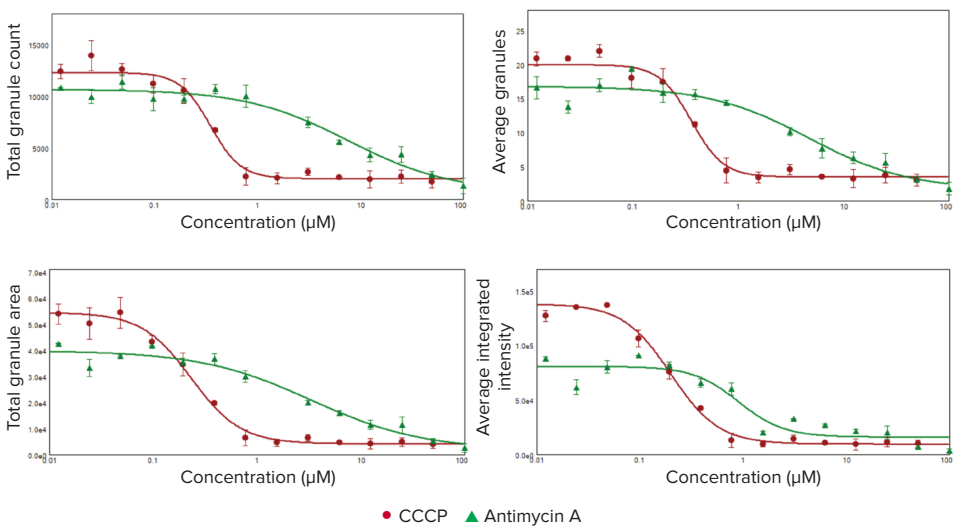

Figure 2. Concentration-response curves for two compounds CCCP (red) and Antimycin A (green) presented for different readouts derived from the mitochondria analysis. The Mitochondria analysis algorithm in CellReporterXpress software was used to analyze and quantitate the effects of these two compounds on mitochondrial integrity and membrane potential. The EC_{50} values for different readouts are presented in Table 1.

Compound	Analysis readout	EC_{50} ± standard deviation
Antimycin A	Total granules	7.649 ± 3.286
	Average granules per cell	4.692 ± 2.131
	Total granule area	3.359 ± 1.426
	Average granule integrated intensity	0.906 ± 0.227
CCCP	Total granules	0.349 ± 0.035
	Average granules per cell	0.334 ± 0.028
	Total granule area	0.233 ± 0.019
	Average granule integrated intensity	0.200 ± 0.017

Table 1. EC_{50} values for the analysis readouts detailed in Figure 2. Several readouts can be selected from the Mitochondria analysis algorithm that represented the effects of Antimycin A and CCCP on mitochondrial integrity and MMP.

Conclusion

Mitochondrial dysfunction has been implicated in the pathogenesis of a variety of disorders including neurodegenerative and cardiovascular diseases, as well as toxicity effects of some pharmaceuticals and exposure to various environmental compounds. This assay demonstrates the efficiency of the ImageXpress Pico system and CellReporterXpress software for evaluation of mitochondria integrity and mitochondria membrane potential for numerous cell-based assays and applications.

References

1. Sakamuru, S., Attene-Ramos, M. S., & Xia, M. (2016). Mitochondrial Membrane Potential Assay. *Methods in Molecular Biology* (Clifton, N.J.), 1473, 17–22. http://doi.org/10.1007/978-1-4939-6346-1_2
2. Attene-Ramos MS1, Huang R, Michael S, Witt KL, Richard A, Tice RR, Simeonov A, Austin CP, Xia M. (2015). Profiling of the Tox21 chemical collection for mitochondrial function to identify compounds that acutely decrease mitochondrial membrane potential. *Environ Health Perspect.* 123(1):49-56.

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The ImageXpress Pico system features optics by Leica Microsystems.