Confocal-based Platform for Screening Mitochondrial Morphological and Functional Changes in the AC16 Cardiac Cell Line

Hsu-Ting Kuo[1], Yi-Ju Lee[2], Chan-Min Hsu[2], Ching-Hsiang Chu[2], An-Chi Wei[2]

[1]Department of Biomedical Engineering, National Taiwan University, Taipei, Taiwan [2]Department of Electrical Engineering, National Taiwan University, Taipei, Taiwan

INTRODUCTION

Mitochondria generate the vast majority of energy powering cellular activities and participate in crucial cellular processes. Many drugs and chemicals have been reported to affect mitochondrial function and morphology, causing cardiac toxicity and side effects. In this study, we build a toxicity screening platform using confocal microscopy to quantify dose-response changes in the mitochondrial morphology and the mitochondrial membrane potential in the AC16 human cardiomyocyte cell line.

MATERIALS & METHODS

The protocol established in this study is as shown below (Fig. 1), which covers sample preparation, drug perturbation, microscopy imaging, imaging preprocessing, and data analysis.

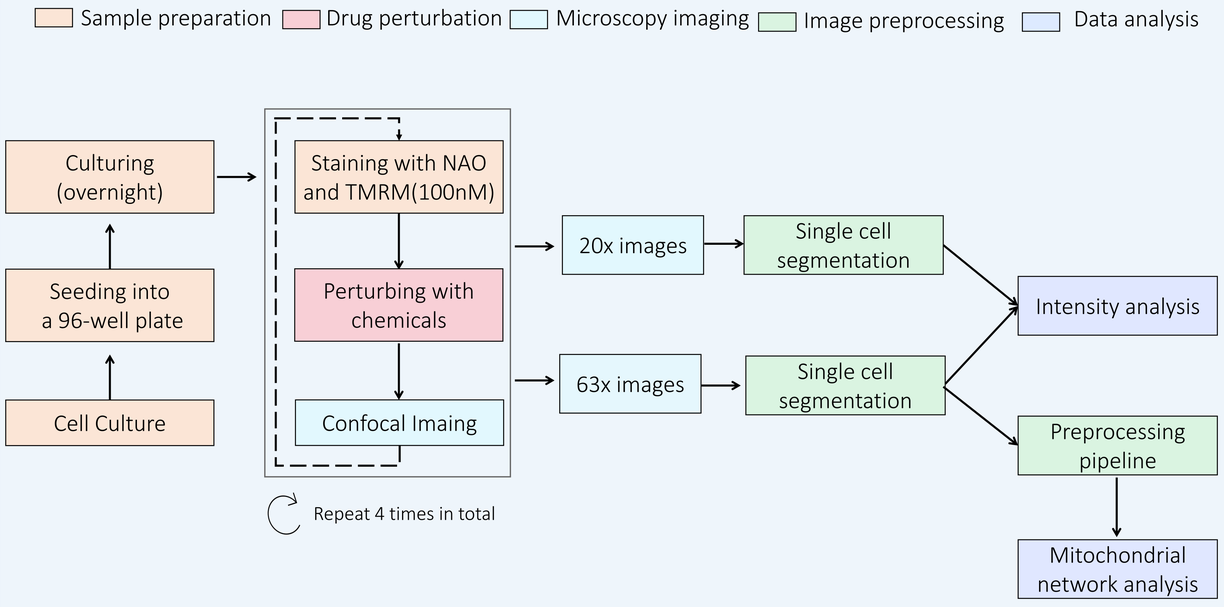

Fig. 1: The protocol designed for our confocal-based toxicity screening platform

1. Sample preparation

   The cell line used in this study is AC16 Human Cardiomyocyte Cell Line (AC16). To monitor the mitochondrial membrane potential and the morphology, we seeded cells in 96-well glass-bottom plates(Fig. 2) and stained cells with tetramethylrhodamine, methyl ester (TMRM), and nonyl acridine orange (NAO).

2. Drug perturbation

   To validate the results obtained through our platform, well-characterized compounds were used to perturb AC16 cells

   - **0-10 μM Oligomycin**: an ATP synthase inhibitor.
   - **0-5 μM FCCP**: an uncoupler.
   - **0-10 μM Rotenone**: a complex I inhibitor.

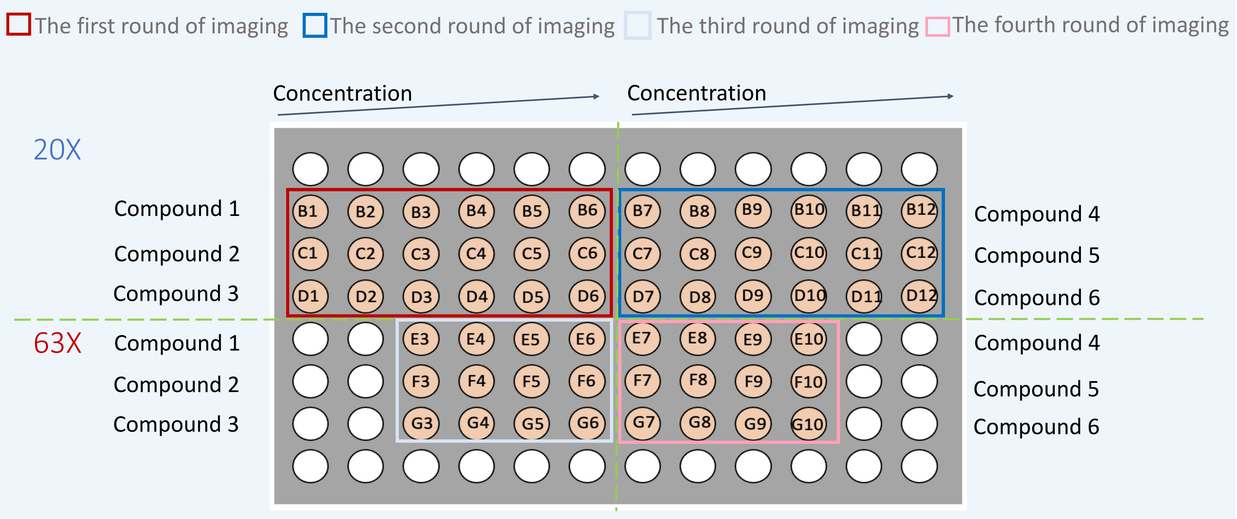

Fig. 2: The 96-well plate design

3. Microscopy imaging

   Zeiss's laser scanning confocal microscope was used to acquire fluorescent and transmitted light images.

   - 1.40-NA, 20x air objective is used to acquire 20x images (512 * 512 pixels) for TMRM intensity analysis.
   - 1.40-NA, 63x oil objective is used to acquire 63x images (1987 * 1987 pixels) for mitochondrial network analysis.
   - Imaging channel: Transmitted Light, orange channel (561nm laser), and green channel (488nm laser).

4. Image processing and analysis

   The ImageJ image analysis pipeline is built to quantify the mitochondrial morphology and the membrane potential.

   - Intensity analysis: The average pixel intensities of images in each group are calculated and compared. (Fig. 3)
   - Mitochondrial network analysis: "Particle analysis" and "skeleton analysis", the two signature mitochondrial features are analyzed. (Fig. 4)

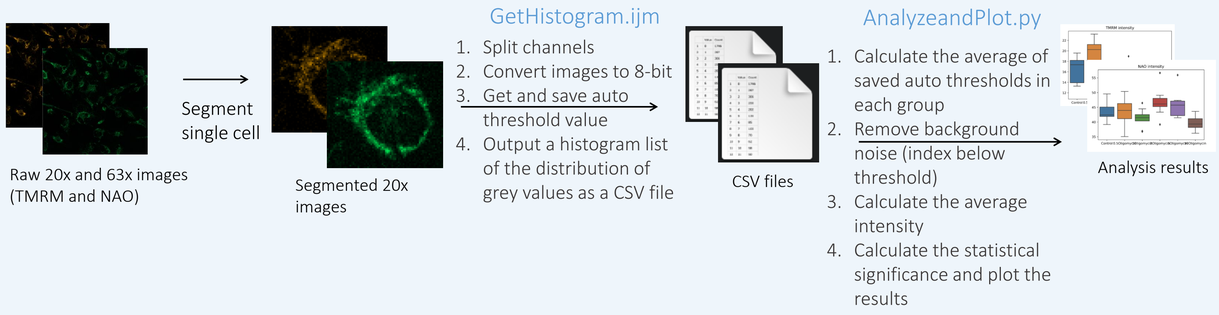

Fig. 3: The pipeline for fluorescence intensity analysis

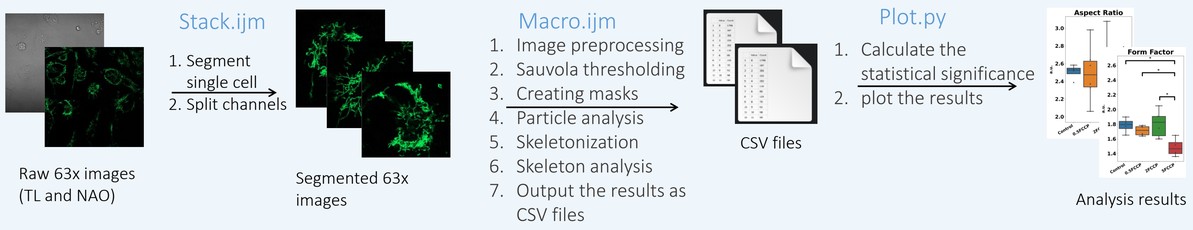

Fig. 4: The pipeline for mitochondrial network analysis

RESULTS

1. Comparison of changes in TMRM intensities induced by FCCP, Oligomycin and Rotenone
   a. The intensity analysis results:
      - FCCP : TMRM intensity is decreased when the concentration increases; NAO intensity is decreased by high-concentration FCCP.
      - Oligomycin: TMRM intensity is increased by 0.5μM Oligomycin, and decreased by higher concentration.
      - Rotenone: TMRM intensity tends to be decreased by Rotenone

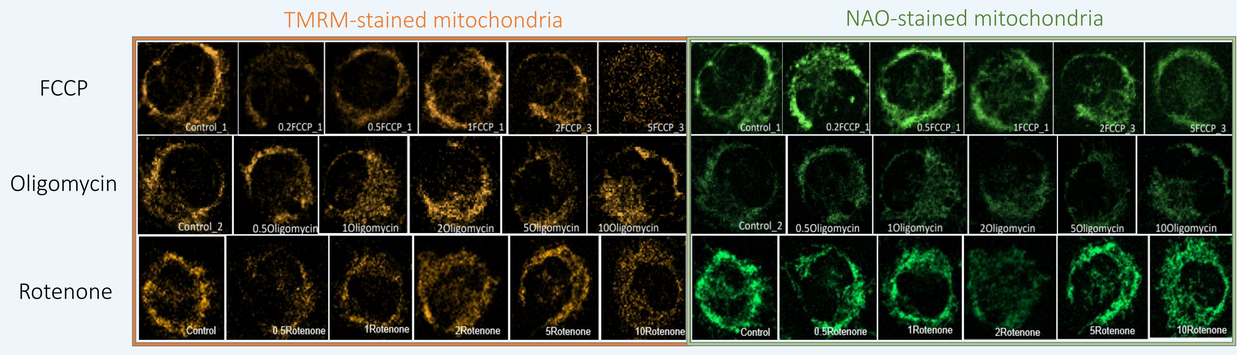

Fig. 5: 20x confocal images of mitochondria in AC16 cells treated with FCCP, Oligomycin and Rotenone

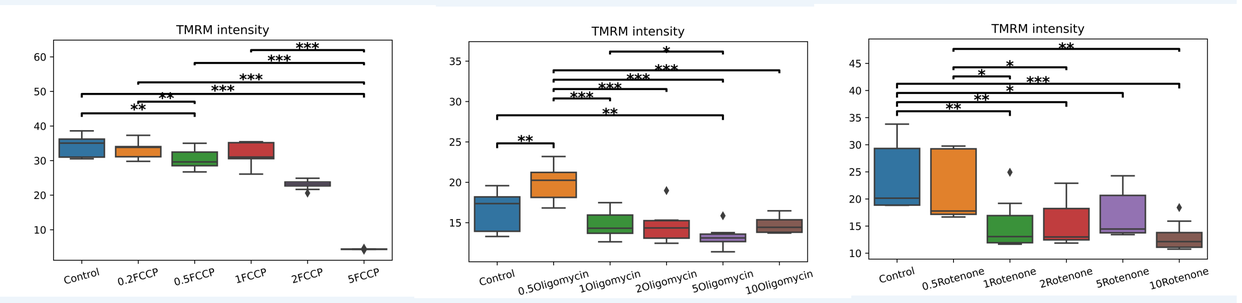

Fig. 6: Results of image analysis

2. Comparison of changes in mitochondrial network induced by FCCP, Oligomycin and Rotenone
   a. The mitochondrial network analysis results:
      - FCCP: induces mitochondrial fragmentation.
      - Oligomycin: induces mitochondrial fragmentation.
      - Rotenone: results in mitochondrial morphology toward network-like structures.

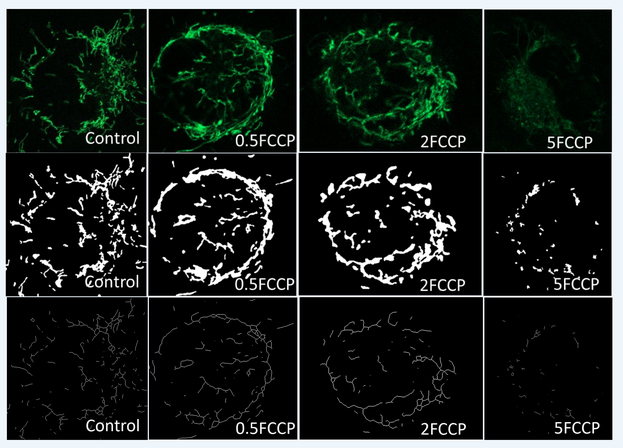

Fig. 7: (Top row) 63x confocal images of FCCP-treated mitochondria
(Middle row) Processed images for the particle analysis
(Bottom row) Processed images for the skeleton analysis

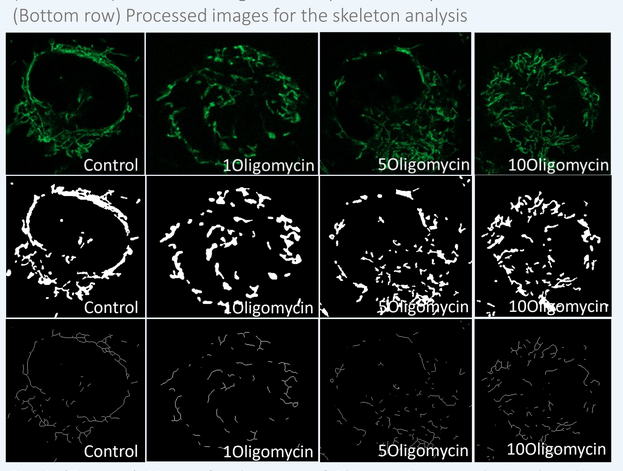

Fig. 8: (Top row) 63x confocal images of Oligomycin-treated mitochondria
(Middle row) Processed images for the particle analysis
(Bottom row) Processed images for the skeleton analysis

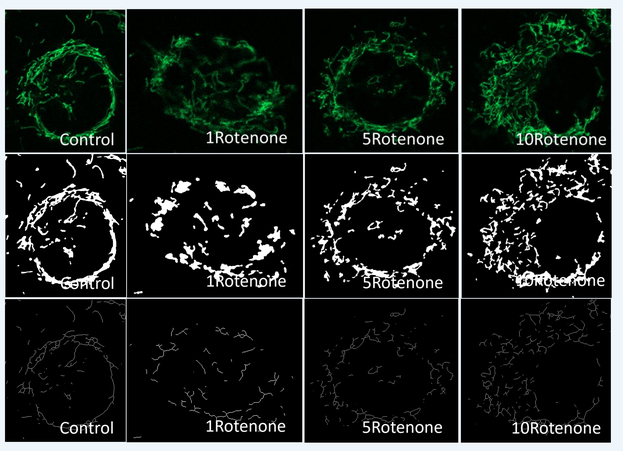

Fig. 9: (Top row) 63x confocal images of Rotenone-treated mitochondria
(Middle row) Processed images for the particle analysis
(Bottom row) Processed images for the skeleton analysis

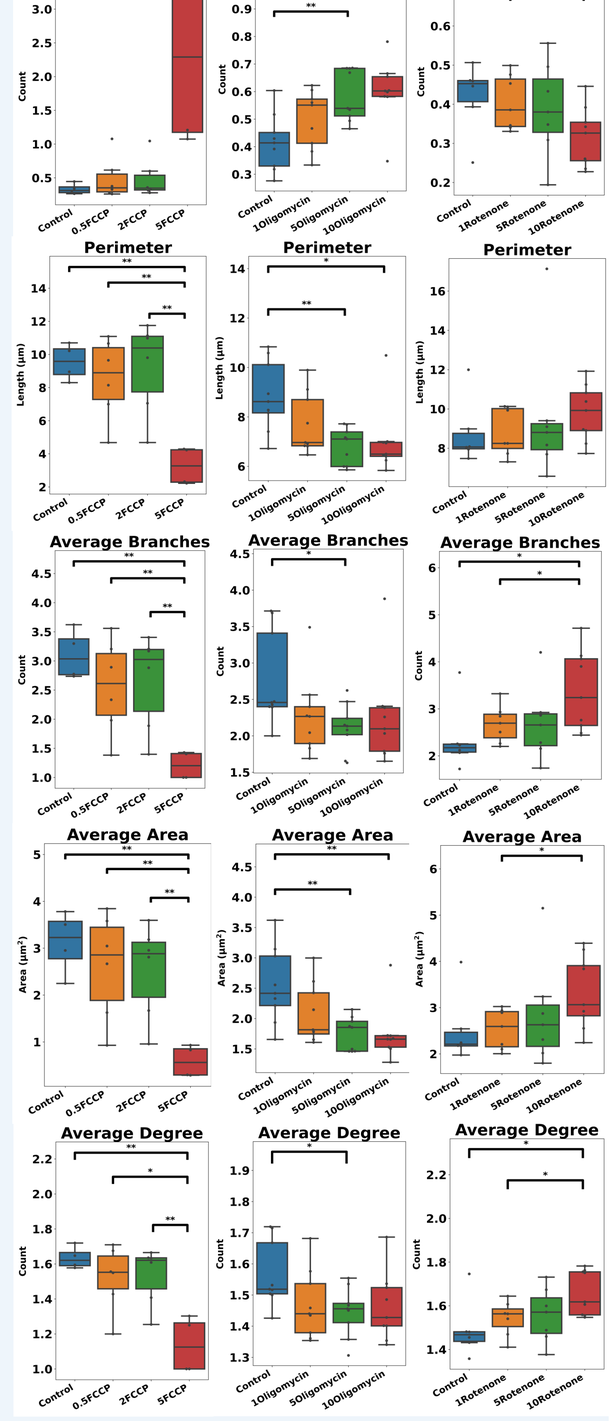

Fig. 10: The results of mitochondrial network analysis for FCCP-treated mitochondria (left column), Oligomycin-treated mitochondria (middle column), and Rotenone-treated mitochondria (right column).

CONCLUSIONS

We have successfully built a confocal-based toxicity screening platform to quantify dose-response changes in the mitochondrial morphology and the mitochondrial membrane potential in the AC16 human cardiomyocyte cell line. With this multi-well mitochondrial toxicity screening platform, we can reduce the time for sample preparation and imaging, decrease the cost of experiments, and obtain more accurate results for mitochondrial functional and morphological assessments.

REFERENCES and ACKNOWLEDGEMENTS

1. Iannetti, E. F. et al. (2016). Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1857, e124. https://doi.org/10.1016/j.bbabio.2016.04.264
2. Tseng, W. W. et al. (2021). BioRxiv. https://doi.org/10.1101/2021.10.04.462897
3. Leonard, A. P. (2015). Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1853(2), 348–360. https://doi.org/10.1016/j.bbamcr.2014.11.002

This research is supported by Ministry of Science and Technology of the Republic of China (MOST) under MOST-110-2636-B-002-0017-.