From Eye to Insight

DEVELOPING HEART PACEMAKER CELLS FROM CARDIAC SPHEROIDS

Imaging 3D cell culture with clarity and speed

Authors

Christopher Murphy[1], Christoph Greb, Dr.[1], James DeRose PhD.[1]
Leica Microsystems

Abstract

During the last decade, 3D cell culture has been established as a more realistic model compared to classical 2D culture systems. Cells can develop into miniature 3D objects, so called spheroids, which resemble organs in function and development better than 2D cell cultures. This fact makes them a great tool for the study of diseases in vitro. In addition, it is imaginable to use 3D cell culture to produce "spare parts" which ultimately can be implanted into a living organism for the sake of curing disease.

Introduction

Arrhythmias (irregular heartbeats) are experienced by millions of people. Frequently brought on by age, congenital defects, or heart disease, arrhythmia has traditionally been mediated medically by implanting artificial pacemakers which impact the patient's quality of life.

For children, these electronic devices are too large, so for this reason there is a research laboratory striving to develop a "hardware free" solution, i.e., producing functional pacemaker cells from ordinary heart muscle. This lab research requires the use of 3D cell culture and spheroids which present several challenges for high resolution observation with microscopy.

Fig. 1: The strategy of the research lab at Department of Biomedical Engineering, Georgia Tech and Emory University is to use 3D cell culture and spheroids to develop functional pacemaker cells from ordinary heart muscle

Challenges

While widefield microscopy offers advantages for speed, viability, and cost, imaging large spheroids with up to four fluorescent channels using traditional light microscopy methods creates significant out-of-focus haze which limits the ability to document the growth and function of the specimens.

Methods

Spheroids were derived from neonatal rat ventricular myocytes (NRVM) which were grown on special plates. Afterwards, spheroids were covered with a monolayer cell culture. The spheroids were stained with alpha-actin and vimentin antibodies. A Z-stack of approximately 60 µm was acquired with a THUNDER Imager Live Cell which also applied the opto-digital technique Instant Computational Clearing (ICC). For comparison, a maximum projection of raw widefield and THUNDER ICC images was produced.

MICROSYSTEMS

Results

The THUNDER images processed with Computational Clearing show more details of the spheroids compared to the classical widefield images. Moreover, the images shown in figure 2 below enable more of the dynamics between the monolayer cells and those in the spheroid to be explored.

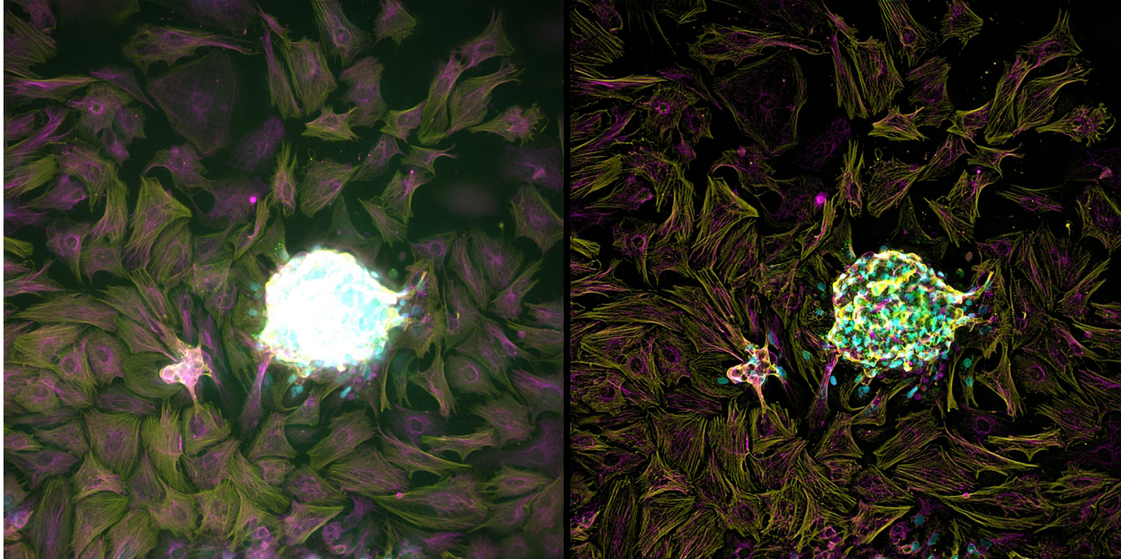

Fig. 2: Spheroid shown here as a maximum projection of the raw widefield image data (left) and THUNDER image after Instant Computational Clearing (right). The images are derived from approximately 60 µm Z stacks. Different stains (alpha actin and vimentin) are used to help identify the various cell types. Images courtesy of Sandra Grijalva, Wallace H. Coulter Department of Biomedical Engineering, Georgia Tech and Emory University, Atlanta, USA.

References

> S.I. Grijalva, J.-M. Gu, J. Li, N. Fernandez, J. Fan, J. Hoon Sung, S. Yup Lee, C. Herndon, E.M. Buckley, S-J. Park, F.H. Fenton, H. Cheol Cho, Engineered Cardiac Pacemaker Nodes Created by TBX18 Gene Transfer Overcome Source–Sink Mismatch, Advanced Science (2019) vol. 6, iss. 22, 1901099, DOI: 10.1002/advs.201901099.

> C. Greb, Introduction to Mammalian Cell Culture: Morphology and Cell Types & Organization, Science Lab (2017) Leica Microsystems.

> J. Schumacher, L. Bertrand, THUNDER Technology Note: THUNDER Imagers: How Do They Really Work?, Science Lab (2019) Leica Microsystems.

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Leica Microsystems CMS GmbH | Ernst-Leitz-Strasse 17–37 | D-35578 Wetzlar (Germany)
Tel. +49 (0) 6441 29-0 | F +49 (0) 6441 29-2599

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