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Single-Camera, Multi-Parametric Imaging of Human Stem Cell-Derived Heart Tissue

> "This method presents a new optical mapping technique for quantitative measurement of AP and calcium wave propagation at multiple levels of cardiac tissue organization from cellular monolayers to whole hearts."
>
> **– Peter Lee,** doctoral student at Oxford University

BACKGROUND

The ability to create heart tissue from induced pluripotent stem cells (iPS) has generated excitement in many fields of biomedical research including regenerative cardiovascular research. However, the utility of iPS cell technology for personalized cardiac therapy and disease modeling has only recently been realized. It was not until 2009 that human iPS cardiomyocytes (iPS-CMs) were shown to be a viable alternative to human embryonic stem (ES) cells as a source of human cardiomyocytes (i.e. heart muscle cells) for research applications studying cardiac disease mechanisms, developmental biology and drug development for cardiac repair.

Using iPS cell-derived heart tissue to screen for potential therapeutic compounds offers a personalized approach to patient care. Before drug companies can rely on this new cell source, researchers need to develop a method to measure their electrophysiological function. Standard multi-parameter imaging has proven to be too costly and labor intensive. That is until Peter Lee, a doctoral student in the Department of Physics at Oxford University and Todd Herron, Ph.D., assistant research professor in the Department of Molecular & Integrative Physiology and Center for Arrhythmia Research at the University of Michigan, developed a scalable and simple single-camera, multi-parametric functional measurement system. Using human cardiac tissue created with Cellular Dynamics International's iCell™ Cardiomyocytes iPS-CM technology, the system was designed to quantify action potential (AP) and calcium transient and wave propagation characteristics.

Using a single high-performance EMCCD camera and off-the-shelf components, Lee and Herron were able to demonstrate for the first time simultaneous voltage (i.e. AP) and intracellular-calcium transient and propagation measurements of large human iPS cardiomyocyte monolayers. This proof-of-concept study was also applied to rat monolayers, ventricular tissue slices and whole hearts to demonstrate its general applicability to increasing levels of tissue complexity[i].

OVERVIEW

Using Photometrics Evolve™ 128 EMCCD camera, Peter Lee of Oxford University, and Todd Herron of University of Michigan, study electrophysiological properties of human stem cell-derived heart tissue using a single-camera, multi-parametric imaging system.

FACILITIES

Oxford University
University of Michigan

CAMERAS

Photometrics Evolve™ 128

KEY FEATURES

- *Clear, ultrafast image capture (highest quantum efficiency and lowest read noise of any EMCCD camera at 10MHz readout speed)*
- *High camera frame rate ~1000 fps at 64x64 pixel resolution*

CHALLENGE

Optical mapping, as it is referred to in the cardiac research community, of electrophysiological parameter-sensitive dyes has contributed to a better understanding of AP generation and conduction dynamics in multi-cellular cardiac preparations. Fluorescence imaging has become a standard tool for functional research in cardiac tissue; however, the technical complexity of simultaneously measuring more than one parameter and the associated cost has prevented researchers from broadly taking up this approach. Using multiple cameras to collect emission from each parameter appeared to be the only feasible, albeit technically challenging and costly solution. Traditional cardiac imaging systems can cost upwards of $70,000 per parameter. For a set-up that includes three parameters such as Lee's, a multi-camera approach was not an option.

Aside from the cost, this type of set-up presents several difficulties, including matching camera pixels, loss of intensity due to extended light paths and positioning in a restricted space. For physiologists looking to reproduce this type of arrangement, the challenge of aligning even two cameras can prove daunting.

"Even if one divides a single camera sensor into separate areas, dedicated to the collection of separate parameters, optical alignment remains challenging if more than two parameters are involved," said Lee.

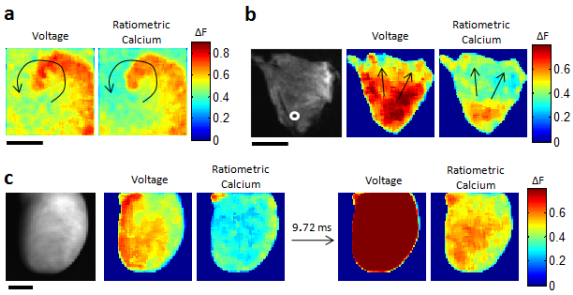
SOLUTION

To demonstrate a single camera, multi-parameter imaging approach, Lee relied on Photometrics' high-performance Evolve™ 128 EMCCD camera. His system also comprised of off-the-shelf optical filters, lenses, LEDs (from UV to visible) and custom-built electronics.

"The first thing we considered when selecting the Evolve 128 was the speed needed to capture the rapid electrical wave propagation patterns in cardiac tissue. The other key function was the camera's well-depth. When imaging voltage dyes, you need a camera that can capture large volumes of light at a low noise level."

With a 10-MHz readout, the Evolve 128 was designed for high-speed image visualization. It also has the lowest read noise available for EMCCD cameras. While his initial research functioned as a proof-of-concept, Lee knew he needed a camera that would also serve his low-light, single-cell research. Because the Evolve is adaptable to a range of scenario conditions, it can be applied to different imaging applications and requirements. Photometrics' reputation for providing researcher's with custom support allowed Lee to build an imaging system based on his growing needs.

Figure 1. Simultaneous voltage and intracellular-calcium imaging in rat monolayers, tissue-slices and whole-hearts.



(a) Neonatal rat ventricular myocyte monolayer: Normalized fluorescence intensity maps (colorbar shown) at a time point during spontaneous and sustained rotor (~3Hz) activity. The black circular arrow shows the direction of rotation.

(b) Rat ventricular tissue-slice (350µm thick; left ventricle): Normalized fluorescence intensity maps (colorbar shown) at a time point after point (white circle) electrical excitation. Black arrows show the progression of the activation wave.

(c) Rat whole-heart (mostly left ventricle view): Two sets of normalized fluorescence intensity maps (colorbar shown), separated by 9.72ms in time, during sinus rhythm. Since activation of the left-ventricle surface occurred almost simultaneously, two sets of intensity maps were needed to show the delayed calcium activity.

Scale bar: 5mm.

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When dealing with commonly desired cardiac physiological parameters, achieving very high speeds (as can be acquired with the Evolve 128) is not as much an issue as with other set-ups. Therefore, Lee was able to manipulate the speed by sharing the frames between samples, allowing him to conduct his research with only a single camera. In doing so, he completely eliminated the need to align multiple cameras, which improves reproducibility, minimizes set-up time and significantly reduces equipment costs.

The fluorescence emission is then passed through a multi-band emission filter and camera lens. During any frame exposure period of the camera, the tissue is illuminated with only one of the three excitation sources. Because of the established lack of crosstalk between the dyes, the emitted fluorescence at any time represents only one of the three parameters. The multi-band filter was essential in allowing Lee to image multiple parameters along the same optical path. Because high-speed LEDs were used for illumination, he did not have to worry about constantly having to switch through the filters in a filter wheel. Therefore, there were no moving parts, making it easy to recreate and alter the set-up.

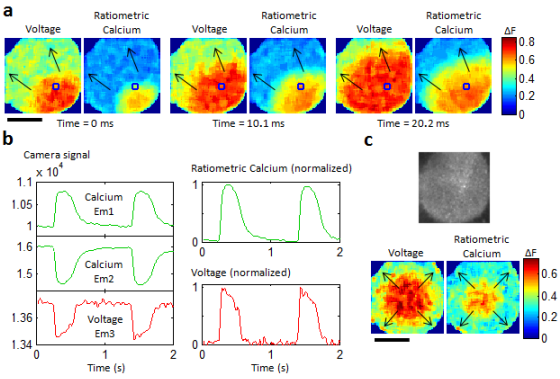
Due to the exceedingly high camera frame rate of ~1000 fps at 64x64 pixel resolution and smooth signal dynamics, with interpolation, one can measure all three parameters in a straightforward fashion. A high-speed microcontroller coordinates the fast-response-time LEDs with the frame exposure signal from the camera. A standard desktop computer is used to support the camera system and to communicate with the microcontroller.

RESULTS

"This method presents a new optical mapping technique for quantitative measurement of AP and calcium wave propagation at multiple levels of cardiac tissue organization from cellular monolayers to whole hearts," said Lee.

Sample results from initial tests in rat cardiac tissue are shown in Figure 1[i]. The same imaging system was applied to three different levels of tissue complexity. Figure 2 shows examples of simultaneous voltage and ratiometric calcium imaging of large human iPS-CM monolayers. Such confluent monolayers of cardiac cells has been established as a simple and controlled experimental model for the study of re-entrant arrhythmias in the heart.

Figure 2. Simultaneous fluorescence imaging of voltage and ratiometric calcium in human iPS-CM monolayers.



(a) Normalized fluorescence intensity maps (colorbar shown) at 3 time points after a spontaneous activation. Black arrows show the propagation direction of the activation wave, which originates from the bottom-right corner of the monolayer.

(b) Left panel: Camera signals (on a 16-bit scale) of Em1, Em2 and Em3 fluorescence from the blue-squared-region in over the course of 2 seconds. Right panel: Normalized ratiometric calcium signal (obtained by dividing Em1 by Em2, followed by normalization) and normalized voltage signal (Em3 normalization).

(c) Normalized fluorescence intensity maps (colorbar shown) at a single time point after point electrical stimulation at the monolayer center. Black arrows show the progression of the activation wave.
Scale bar: 5mm.

LOOKING FORWARD

With the recent commercialization of human iPS-CMs, extensive experiments using human cells are imminent. Lee's innovative multi-parametric imaging method will be integral in unraveling the underlying mechanisms of cardiac arrhythmias and will be pivotal for validating new cell-based therapies that will emerge from the biomedical field of cardiovascular regenerative medicine.

Lee and Herron are currently working on cell-culture technologies for application to human iPS-CMs that will rely on their optical mapping method to assess, at high-throughput, electrophysiological properties of the tissues they generate.

[i] T Herron, P Lee, J Jalife. Optical Imaging of Voltage and Calcium in Cardiac Cells & Tissues. Circulation Research. 2012; 110: 609-623. (February 17, 2012).

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