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VIDEO**

Photometrics Customer Profile

Multi-Parametric Electrophysiological Imaging
of the Mammalian Heart *In Vivo*

“ For imaging particular fluorescent dyes in cardiac tissue, the camera has to have certain properties... The cameras from Photometrics fit the bill quite nicely.”

— Peter Lee, doctoral student at Oxford University

BACKGROUND

Cardiac arrhythmia is one of the most common diseases encountered in clinical cardiology. High-speed electrophysiological imaging using fluorescent probes has yielded tremendous insights into the basic mechanisms of arrhythmias and the effects of anti-arrhythmic drugs. However, optical mapping, as it is known to the cardiac research community, has remained relegated to the isolated (i.e. explanted) heart. Although useful, the traditional Langendorff-perfused isolated heart preparation is unphysiological in many respects. For instance, in this system, the heart is explanted from the animal, disconnecting neuro-humeral feedback, and is perfused with saline, a condition lethal to the live animal. Hence, in vivo optical mapping must be developed.

Two key parameters of interest are the cardiac action potential (AP) and intracellular calcium transient (CaT), which require the use of red-shifted dyes to image in vivo. Using a similar set-up as described in his human stem cell-derived heart tissue research, Peter Lee, a doctoral student in the Department of Physics at Oxford University, and a collaborator, Dr. Christopher Woods, M.D. Ph.D., cardiologist in the Division of Cardiovascular Medicine at Stanford University, demonstrated the first ever multi-parametric imaging of a mammalian heart in vivo through whole blood.¹

“The purpose of this in vivo study was to transition to imaging electrophysiological parameters with the heart still inside the animal through 100 percent blood,” said Lee. “We showed you can measure these two parameters that people care about in a live animal and you can do it quite simply with a single camera.” By simultaneously imaging AP and CaT inside the body in this proof-of-principle study, Lee and Woods hope to advance the clinical understanding, diagnosis, and treatment of arrhythmias.

¹ Manuscript under submission

RESEARCH TEAM

Peter Lee, a doctoral student in the Department of Physics at Oxford University and Dr. Christopher Woods, M.D. Ph.D., cardiologist in the Division of Cardiovascular Medicine at Stanford University.



OVERVIEW

Using the Photometrics Evolve™ 128, Oxford University doctoral student, Peter Lee, and his colleagues study cardiovascular disease and function using the first ever in vivo, multi-parametric imaging system.

PRODUCTS

Photometrics' high-speed Evolve™ 128 camera

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 of ~1000 fps at 64x64 pixel resolution

CHALLENGE

"All the experiments to this point in terms of imaging technology development have been in explanted hearts. Basically, you take the heart out of the animal, it beats on its own, and you feed it a clear physiologic-like saline solution," said Lee.

This Langendorff-perfused mammalian heart preparation has been used to study heart function for more than a century. In combination with optical mapping, this technique has given researchers insight into arrhythmia biology. However, the unphysiological nature of the heart preparation makes it difficult for researchers to apply this data in a clinically relevant setting.

"It might seem obvious that it is better to study the heart in the body where it lives, but it is even more important with cardiac disease. In particular, it is exactly the critical neuro-humeral and hormonal influence present in patients that we believe leads to important diseases like heart failure and arrhythmia, and we simply could not study this well in the explanted heart. But, what Peter has designed here has really been transformative. For the first time, we will be able to understand these disease processes where they happen over time," notes Dr. Woods.

Traditional methods of imaging the heart *in vivo*, such as magnetic resonance imaging (MRI) or ultrasound, provide relatively slow, low-resolution images that lack the ability to capture electrophysiological data. Thus far, only one *in vivo* optical mapping study has been reported. However, the research team was limited to studying a single parameter (voltage) using a low-resolution imaging system and complex illumination system based on lasers and acousto-optic deflectors. No further progress has been reported since that 1998 study.

Prior to Lee's research, multi-parametric imaging required the use of two cameras, making it costly and technically difficult for researchers to perform. Traditional light sources for such multi-color imaging require mechanical shuttering devices or filter wheels to quickly switch between excitation wavelengths, raising set-up complexity and component costs.

SOLUTION

Lee and his colleagues developed a simple and scalable single-camera-imaging and LED-illumination system that reduces the economic and technical hurdles associated with *in vivo* imaging. They also used a red-shifted calcium dye (blood does not absorb strongly in red wavelengths) and a new near-infrared (NIR) voltage-sensitive dye, both ideal for whole blood-perfused tissue. To eliminate the cross-talk between both dyes, Lee took advantage of the voltage dye's isosbestic point (a spectral property of ratiometric dyes) where no change in voltage-related emission occurs.

As part of his single camera, multi-parametric imaging design, Lee relied on Photometrics' high-performance Evolve™ 128 EMCCD camera. His system was also comprised of off-the-shelf optical

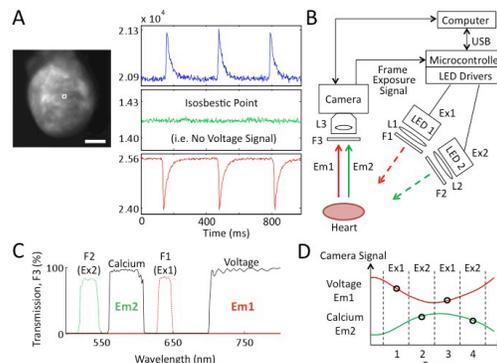


Figure 1. Schematic illustration of multi-parametric imaging approach.

A, di-4-ANBDQPG fluorescence in a Langendorff-perfused rat heart (sinus rhythm), excited with blue (blue LED, 470 ± 10 nm filter), green (green LED, 540 ± 12.5 nm filter) and red (red LED, 640 ± 10 nm filter) wavelengths. These fluorescence signals (taken from the 4×4 -pixel white-square region on the left-ventricle) were collected through a custom-made multi-band emission filter (F3 in B, C). The green trace ($[Ca^{2+}]_i$) shows negligible emission changes when di-4-ANBDQPG is excited at the emission-isosbestic point. Scale bar = 5 mm. **B**, Schematic outline of the imaging system, highlighting key components. Since only one camera is used, the system requires no challenging optical alignment. Excitation sources Ex1: red LED with a 640 ± 10 nm filter (F1), Ex2: green LED with a 540 ± 12.5 nm filter (F2). **C**, Transmission spectrum of the custom multi-band emission filter that passes both V_m (Em1) and $[Ca^{2+}]_i$ (Em2) emitted fluorescence signals. F1 and F2 excitation filter spectra are shown as dashed curves. **D**, Basic principle behind the single-camera multi-LED approach: During any frame exposure (occurring between the vertical dashed lines), the parameter being measured by the camera sensor is determined by the excitation source (either Ex1 or Ex2) that is switched on during that period. A sufficiently fast camera (compared to Em1 and Em2 signal dynamics), and interpolation between measured data points, provides simultaneous measures of multiple parameters, here V_m and $[Ca^{2+}]_i$.



Figure 2: Single-camera, multi-parametric imaging of human stem cell-derived heart tissue system.

Demonstration of the system components used by the research team, which features the Evolve 128 EMCCD camera.

filters and lenses and powerful LED chips which enabled the change from, according to Lee, a “single light source - multi-camera approach” to a “multi light source – single camera approach.”

“For imaging particular fluorescent dyes in cardiac tissue, the camera has to have certain properties, including high speed and a large well depth. The cameras from Photometrics fit the bill quite nicely,” said Lee.

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. 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.

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 excitation sources. Because of the established lack of crosstalk between the dyes, the emitted fluorescence at any time represents only one of the 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 re-create 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 the 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-based controller board.

RESULTS

Lee and Woods successfully demonstrated simultaneous AP and CaT imaging of a mammalian (rat) heart in vivo through 100 percent blood, thus proving its value in advancing arrhythmia imaging. Because the system is both scalable and technically less complex than traditional methods, it can also be applied to traditional Langendorff-perfused hearts. More importantly, it can be adapted to study arrhythmias and other diseases in vivo using endoscopic trans-catheter methods.

LOOKING FORWARD

Lee and Woods hope that their proof-of-concept imaging system will open the doors for clinically-relevant arrhythmia research. By adapting the system to minimally-invasive and clinically-compatible trans-catheter endoscopic methods for large animals and patients, such in vivo multi-parametric imaging will enable unprecedented high spatio-temporal electrophysiological studies of the heart in live animals, thus advancing arrhythmia treatment.

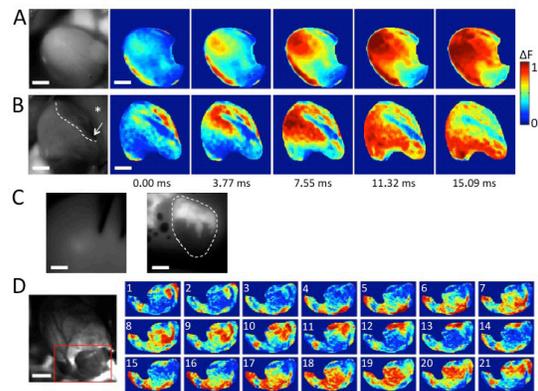


Figure 3. In vivo imaging of V_m and $[Ca^{2+}]_i$ dynamics in rat ventricles/atrium, during sinus rhythm and in atrial fibrillation.

A, Normalized V_m fluorescence intensity maps (colorbar shown) at progressive time points of the cardiac cycle with the heart in sinus rhythm. **B**, Normalized V_m fluorescence intensity maps at sequential time points of the heart in sinus rhythm, 1 hour after suture-ligation of the proximal LAD (to mimic myocardial infarction). In the left-most (raw grayscale) panel, the infarcted tissue can be recognized as the dark region (one side demarcated by dotted white line) in the top-right quadrant of the image; in the normalized fluorescence maps it is recognized as the persistently blue region in the same area. Suture location is indicated with a white arrow. The asterisk marks lung tissue. **C**, Endocardial view of same infarcted heart, after necropsy, submerged in blood (left) and with a coverslip gently placed on the tissue (right). The bright areas correspond to non-infarcted tissue, while the dark areas correspond to infarcted tissue where the dye is absent. The dashed white line demarcates the ventricular tissue in contact with the coverslip. Note the air bubbles on the left side of the panel at the coverslip-tissue interface. **D**, Normalized $[Ca^{2+}]_i$ fluorescence intensity maps at sequential camera frames in part of the left and right atria during atrial fibrillation, induced by global ventilatory hypoxia. Scale bar = 5 mm.

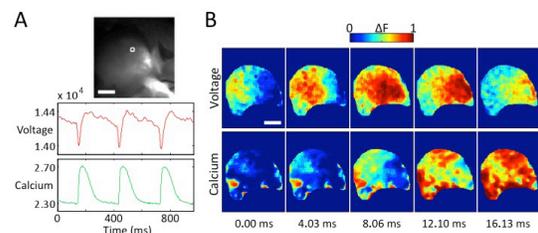


Figure 4. In vivo imaging of V_m and $[Ca^{2+}]_i$ dynamics in rat ventricles during sinus rhythm.

A, AP (red) and CaT (green) fluorescence signals taken from the 4x4-pixel white-square region highlighted in the top panel (left ventricle). **B**, Normalized fluorescence intensity maps (colorbar shown) for V_m and $[Ca^{2+}]_i$ during sinus rhythm; note the previously mentioned delay between V_m and $[Ca^{2+}]_i$ peaks. Scale bar = 5 mm.