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Russell K. Fung

Jack M. Rogers

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Russell K. Fung, Jack M. Rogers
Department of Biomedical Engineering
The University of Alabama at Birmingham

Abstract

Optical technology has been incorporated in cardiac studies, utilizing intracellular dyes and photo detectors to image physiological parameters. However, one major challenge in optical mapping is motion artifact, produced by the physical beating of the heart. To this end, it is known that deformation of the heart can be captured and quantified to eliminate motion artifact without using chemical agents. However, we have planned to develop this technique further to simultaneously quantify membrane potential, calcium transients, and mechanical contractions in isolated hearts. Hearts are double stained with voltage-sensitive dye di-4-ANEPPS and calcium-sensitive dye x-rhod-1. Ring markers are attached to the epicardium and tracked in software, quantifying wall motion concurrently with membrane potential and calcium transient signals. Emitted fluorescence is elicited on alternating frames by 450, 505, and 580 nm LEDs, captured by a camera integrated with a 605/25 nm emission filter. Interlaced signals from 450, 505 and 580 nm fluorescence are collected from pixels encircled by the ring marker. De-interlacing of the raw signal results in 3 individual signals: 450 nm signal contains non-voltage sensitive and non-calcium sensitive signal, consisting of only motion artifact. 505 nm signal contains V_m with motion artifact, while the 580 nm signal contains C_{am} with motion artifact. A ratio of the 505 and 580 nm to the 450 nm signal will estimate V_m and C_{am} respectively. This instrumentation design will be the first to simultaneously map three fundamental parameters of heart contraction.

Introduction

Ventricular fibrillation (VF) is a lethal heart condition characterized by the uncoordinated contraction of the ventricles. Despite successful defibrillation, the heart may still exhibit post-resuscitation contractile dysfunction - a depressed contractile motion of heart which prevents the heart from perfusing sufficient blood to sustain life.[1] Little progress has been made in the understanding of the complex phenomenon of VF and its mechanical and chemical behavior. Previous studies have approached the understanding of VF by focusing on various stages of the cardiac contraction mechanism (See Figure 1). Because the electrical and mechanical functions of the heart are coupled, researchers have performed qualitative studies to evaluate each stage. Spatiotemporal mapping of electrical activity in the heart is widely performed and is traditionally accomplished by attaching multiple electrodes to a specific region of the myocardium.[2]

A more current approach is to employ optical techniques with intracellular dyes. To optically map membrane potential, pig hearts are isolated using a Langendorff preparation and stained with voltage-sensitive fluorescent dye. When the heart is illuminated with excitation light, the emitted fluorescence from the heart transmits a signal proportional to the transmembrane potential.[3] The same method can be used to measure intracellular calcium transient using an ester form of a calcium indicator dye. As the dye enters the cell, intracellular esterase enzymes will cleave the esters allowing for the Ca^{+2} to bind, thus trapping the dye. Calcium concentration is then measured by recording the intensity of the emitted fluorescence.[4] One method to optically map membrane potential and calcium transients is to stain the heart with both di-4-ANEPPS voltage sensitive dye and x-rhod-2 calcium sensitive dye. Both dyes absorb excitation light of different wavelength, but emit at the same wavelengths such that the same detectors can capture the alternating signal.[5] Previous studies have recorded calcium transients and membrane potential and epicardial wall motion sequentially 6,but spatiotemporal mapping of these three parameters has not been performed. The primary advantage of optical mapping is the ability to map multiple regions of the heart without attaching various electrodes to the regions of interest as is done with the traditional method of electrical mapping. As a result, the lack of electrical stimulation will also leave the acquired signals free from its associated artifact.

While optical mapping may appear seemingly sound, it has a major disadvantage when imaging beating hearts. The contraction of the myocardium causes physical displacement of heart, resulting in a loss of correspondence between the photodetector pixel and the imaged tissue. This movement produces a data set of the desired signal combined with motion artifact. Conventional studies have utilized electromechanical uncoupling agents (butanedione monoxime) to chemically arrest heart motion. However, recent investigations have dealt with this problem that by taking an additional dataset containing only artifact signals, removing motion artifact by taking a ratio of the two datasets.[7]

In this study, we want to expand and implement optical technology to improve the understanding of contractile dysfunction following defibrillation, with the goal of improving success rates for resuscitation due to prolonged VF. The development process will specifically investigate the usage of optical mapping techniques to simultaneously map membrane potential, calcium transient, and wall motion of the myocardium. This instrumentation design will

be the first of its kind, capable of measuring these three essential parameters of contraction.

Aims

To implement a new optical mapping method designed to capture membrane potential, intracellular calcium, and epicardial wall motion simultaneously from multiple sites in an isolated heart preparation. Using swine hearts, we plan to investigate the feasibility of using optical methods to simultaneously characterize the electrical and mechanical processes from selected sites on the myocardium.

Methods

Preparation of Heart

Domestic swine hearts of either sex was used. An isolated heart (Langendorff) preparation was performed to allow optimal access to the heart. The aortic root was cannulated and perfused with 2 liters of warm (37°C) Tyrode solution to wash out blood and metabolites. The heart was then connected to a constant-flow (200 ml/min) Langendorff apparatus. Two 22 gauge needles were inserted into the ventricles to minimize the beating heart motion. During perfusion, droplets of perfusate were collected within the ring markers, diffracting emitted fluorescence and ultimately affect the acquired signal of interest. To minimize this effect, the heart was blotted with absorbent gauze immediately before each optical recording.

Material Point Markers

4-mm diameter plastic rings with 2.5 mm diameter holes composed of polyethylene plastic material were used. The markers were placed on the epicardial surface with black cyanoacrylic gel (Loctite #426) to serve as a reference point for recording various signals. Multiple markers was placed on the epicardial surface of the left ventricle and tracked by software.

Dual Staining

x-rhod-1 and di-4-ANEPPS were fluorescent dyes that are calcium and voltage sensitive respectively. The dyes have separate excitation bands, but overlapping emission bands (See Figure 2). The entire heart was stained with di-4-ANEPPS through the perfusion system while x-rhod-1 dye will be applied by locally injecting small amounts in the center of the applied markers. 2 LED lights were used to stimulate each respective dye: cyan (di-4-ANEPPS), and amber (x-rhod-1). A third color, royal blue, elicits fluorescence that is insensitive to both membrane potential and calcium transients. This LED served as a reference signal used to remove motion artifact by employing a ratiometry technique. 5

Software Integration of LED Lights

A counter board was used to integrate the delivery of the 5-V pulses to the LEDs and the camera capturing software. The heart was illuminated by the three different excitation colors on successive camera frames (See Figure 3). Signals was captured by the same CCD camera set to 86 x 128 pixel resolution and fitted with a 605/25 bandpass filter positioned 10 cm from the heart. The resulting signal was interlaced in time. Fluorescence elicited by cyan, amber, and blue are deinterlaced in software after data acquisition.

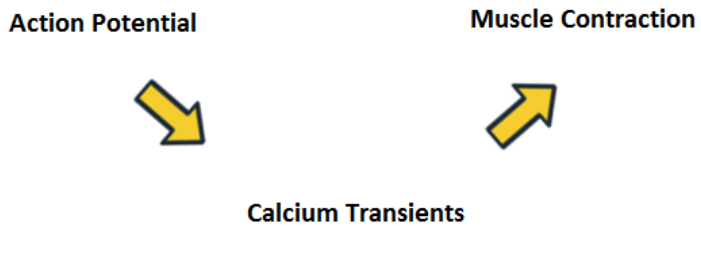


Figure 1. Cardiac Contraction Model. Each stage is dependent on the previous stage to ultimately elicit muscle contraction.

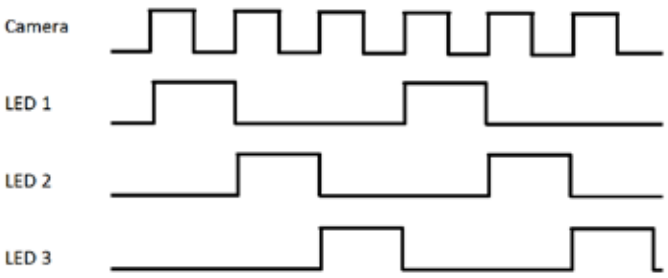


Figure 3. Pulse train configuration interlacing camera control and LED activation in time. Each high edge represents a 5V pulse which activates the camera to capture a frame and the LED to flash.

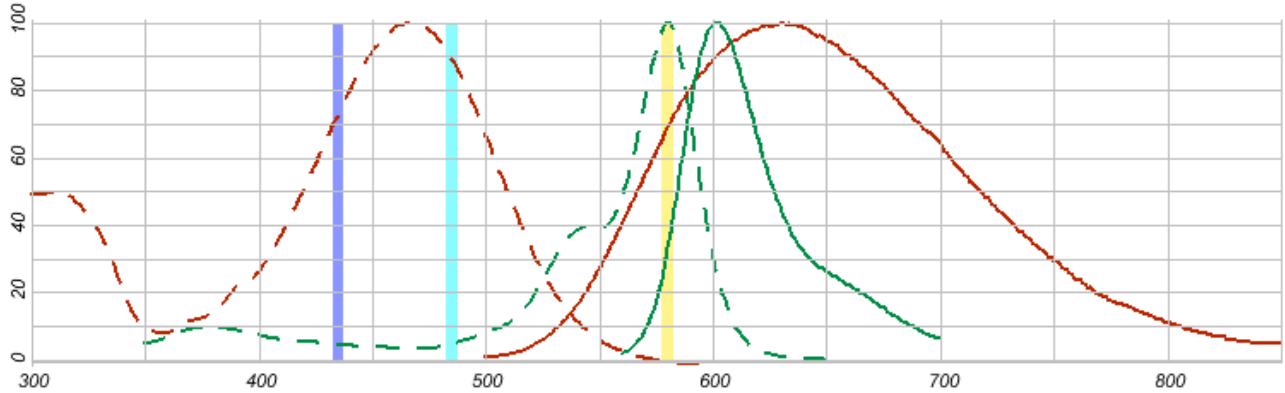


Figure 2. Excitation (Dotted) and Emission Spectra of x-rhod-1 (Green) and di-4-ANEPPS (Red)

Validation Studies and Cross-Talk Analysis

Separate hearts was stained with di-4-ANEPPS, x-rhod-1 alone to ensure that only the signal emitted by each respective dye was present. Membrane potential, calcium transient, and epicardial wall motion will be modulated with drugs to test the sensitivity of the mapping system.

Results and Conclusion

We successfully mapped each of the three fundamental parameters individually. By collecting signals from the marked surface, we were able to obtain inverted raw signals of calcium transient, membrane potential and motion from pilot studies (See Figure 4). These signals were all replicated utilizing the equipment available in our laboratory. To test instrumentation simultaneity, we effectively produced an interlaced signal of membrane potential and its corresponding motion artifact (See Figure 5). Cross-talk analysis between signals acquired from di-4-ANEPPS and x-rhod-1 were performed to further ensure signal quality. Though the dyes may have separate excitation bands, they still possess an overlapping region. As a result, it is possible that the signal from one parameter can be detected by the stimulation of the non-corresponding LED. Although this may produce additional artifact, the effect is greatly minimized by equipping LEDs with filters to narrow the wavelength of the excitation light.

We are currently investigating the feasibility of examining a double stained heart and employing ratiometry techniques to optimally eliminate motion artifact from the raw signal. Ultimately, this developmental study will show the interaction of various mechanisms during cardiac contraction. When applied to different cardiac conditions such as VF and post resuscitation cardiac dysfunction, it will allow a better understanding of the underlying mechanisms behind cardiac diseases.

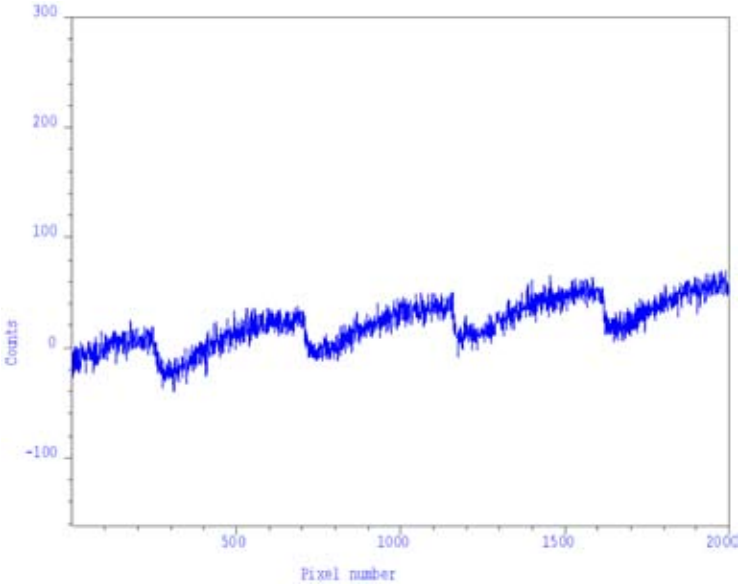
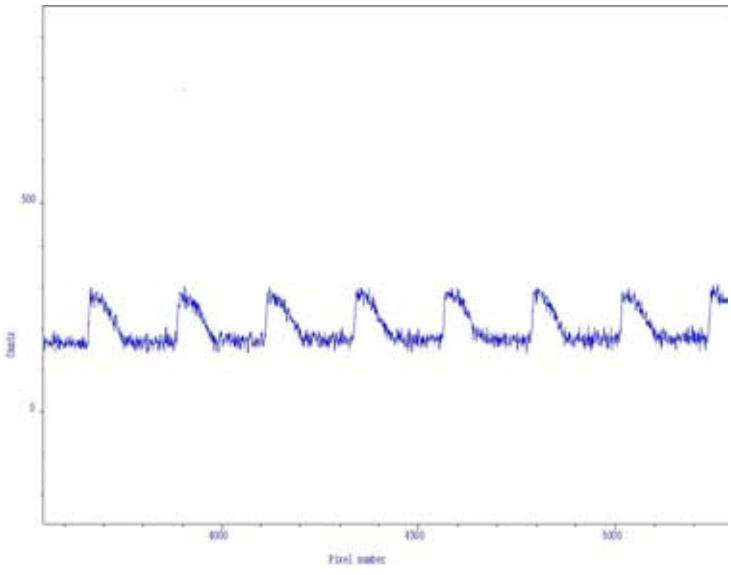
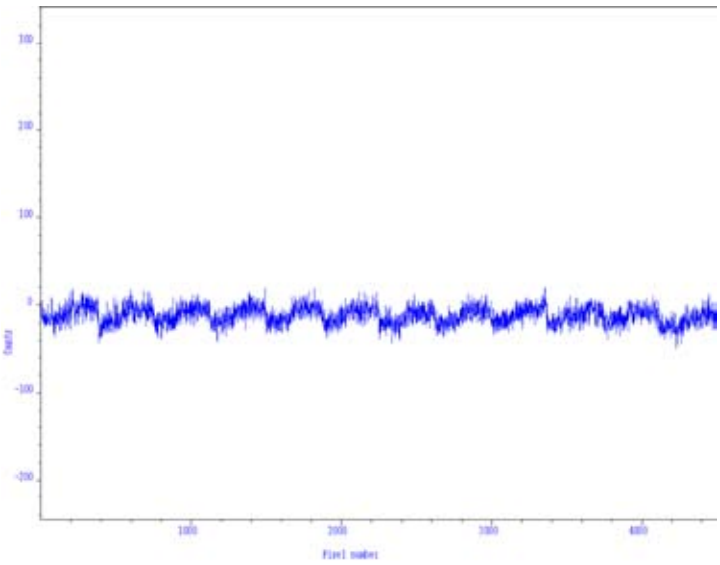


Figure 4. a) Fluorescent calcium transient signal containing motion artifact.



4.b) Fluorescent action potential signal containing motion artifact.



4.c) Signal containing only motion artifact.

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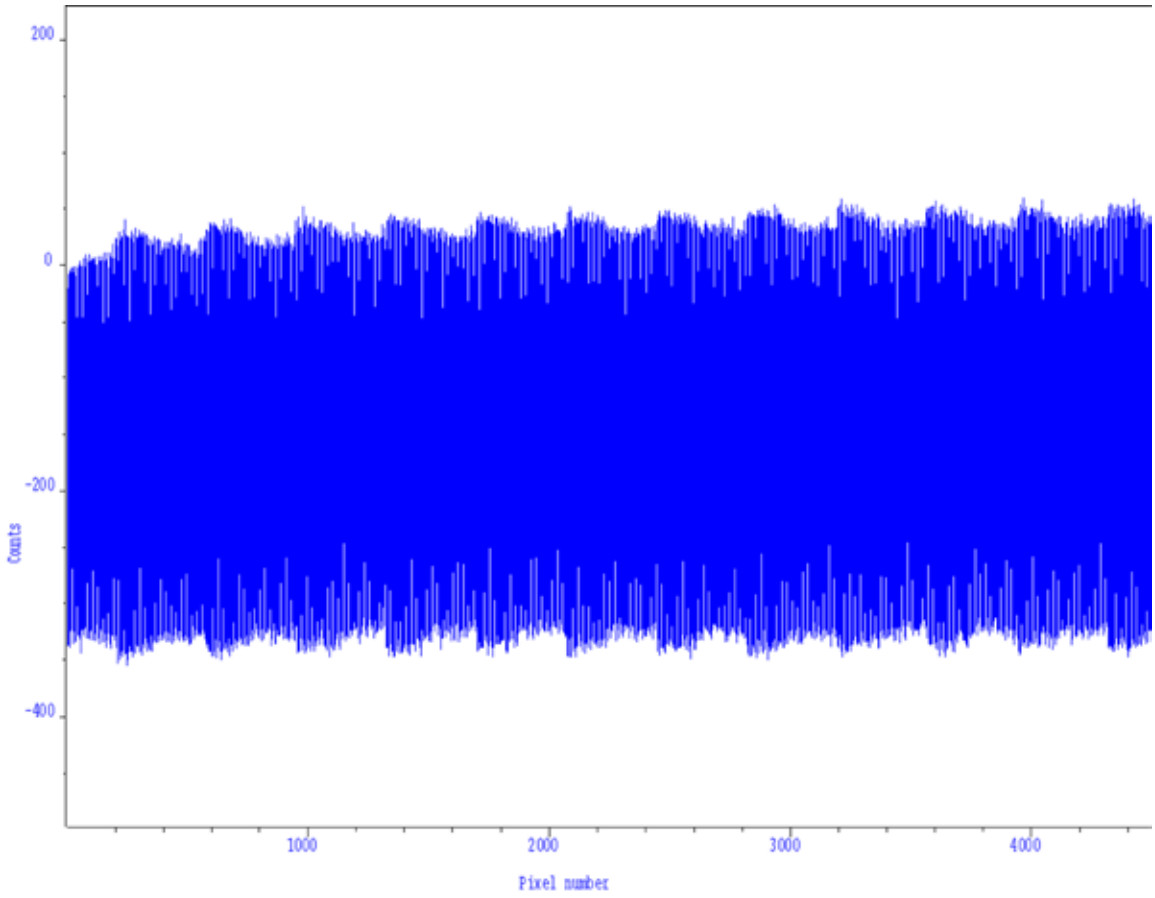

Figure 5. Interlaced signal of membrane potential dataset with its corresponding motion artifact only data set. The data points on top of the graph represent the dataset of membrane potential polluted with motion artifact. The data points on the bottom are the corresponding motion artifact only dataset.

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