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Single Camera System for Multi-wavelength Fruorescent Imaging in the Heart

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***Abstract*— Optical mapping has been a powerful method to measure the cardiac electrophysiological phenomenon such as membrane potential(V_m), intracellular calcium(Ca^{2+}), and the other electrophysiological parameters. To measure two parameters simultaneously, the dual mapping system using two cameras is often used. However, the method to measure more than three parameters does not exist. To exploit the full potential of fluorescence imaging, an innovative method to measure multiple, more than three parameters is needed. In this study, we present a new optical mapping system which records multiple parameters using a single camera. Our system consists of one camera, custom-made optical lens units, and a custom-made filter wheel. The optical lens units is designed to focus the fluorescence light at filter position, and form an image on camera's sensor. To obtain optical signals with high quality, efficiency of light collection was carefully discussed in designing the optical system. The developed optical system has object space numerical aperture(NA) 0.1, and image space NA 0.23. The filter wheel was rotated by a motor, which allows filter switching corresponding with needed fluorescence wavelength. The camera exposure and filter switching were synchronized by phase locked loop, which allow this system to record multiple fluorescent signals frame by frame alternately. To validate the performance of this system, we performed experiments to observe V_m and Ca^{2+} dynamics simultaneously (frame rate: 125fps) with Langendorff perfused rabbit heart. Firstly, we applied basic stimuli to the heart base (cycle length: 500ms), and observed planer wave. The waveforms of V_m and Ca^{2+} show the same upstroke synchronized with cycle length of pacing. In addition, we recorded V_m and Ca^{2+} signals during ventricular fibrillation induced by burst pacing. According to these experiments, we showed the efficacy and availability of our method for cardiac electrophysiological research.**

I. INTRODUCTION

Optical mapping has been a powerful method for cardiac electrophysiological research[1]. The principle of this method is based on fluorescence imaging that stains the specimen with dye, and record the fluorescent light using optical sensor such as high speed camera. Traditionally, the measurement of single signal is often performed, and recently dual mapping method became popular that uses dichroic mirror to separate fluorescence light and records each signal using two cameras[2]. Dual mapping method permits simultaneous measurement of membrane potential, intracellular calcium, NADH, and the other electrophysiological parameters[2][3][4].

However, current optical mapping method including dual mapping can measure only two parameters simultaneously, though cardiac arrhythmia is occured by complicated interaction of many parameters[5]. For the exploration of more details of mechanism of cardiac arrhythmia, an innovative method to measure multiple, more than three parameters is needed.

There are some attempt to measure multiple parameters using single camera. For example, Lee and his research groups reported a single sensor system with excitation light switching[6]. But their system used only one optical filter, so the choice of fluorescent dye should be strict. Threfore, there is no method to record arbitrary-chosen multiple parameters simultaneously.

In this study, we present a new innovative optical mapping method to measure multiple parameters using a single camera. For the proof of efficacy of our method, we measured membrane potential and intracellular calcium simultaneously at both normal and proarrhythmic condition.

II. SYSTEM CONSTRUCTION

A. System Overview

Figure 1 shows the overview of our system. The principle of our method is based on typical fruorescent imaging and

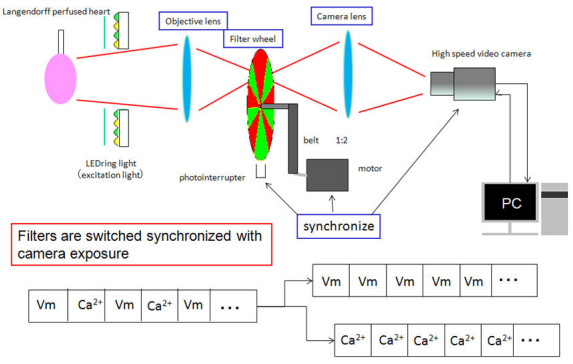

Fig. 1. System Overview

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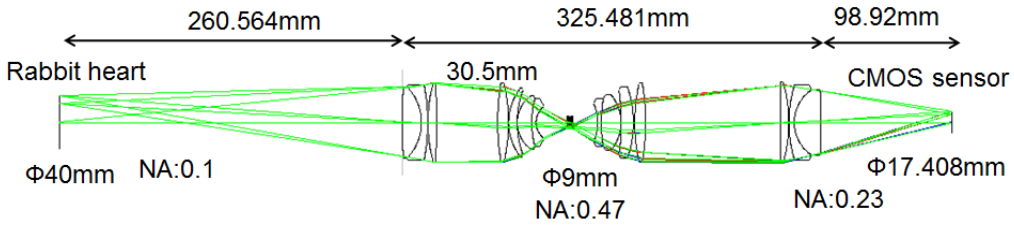

Fig. 2. Optical Lens System

TABLE I
REQUIRED SPECIFICATION

	required spec
object height	ϕ40mm
middle image height	ϕ10mm
image height	ϕ17.408mm
filter space	$\geq 30mm$
resolution	$MTF \geq 0.6$ at 3LP/mm on the object

optical mapping. Our system consists of an excitation light, custom-made optical lens units, fiter wheel, and one high speed camera. The fluorescent light from the specimen is focused at filter position by objective lens, and formed an final image on camera's sensor. Filter wheel is rotated by a motor synchronized with camera exposure signal. The camera records signals of membrane potential(V_m) and intracellular calcium(Ca^{2+}) frame by frame alternately.

B. Optical System Design

We designed custom-made optical lens units to focus a fluorescent light and form an image on the camera s sensor following the required specification listed in Table. I. The object height corresponds with the size of rabbit heart, and the image height corresponds with the size of camera s sensor. The middle image size is small enough so that the image on the optical filter can has large allowance in positioning during filter switching process. In addition, the resolution is determined to be Modular Transfer Function(MTF) 0.6 at 3LP/mm on the object. Figure. 2 shows our optical system. The objective lens form a small middle image at the filter position. Camera lens magnified this middle image and form the final image on camera s sensor. Fluorescent light from the specimen is so small that we gave an importance to lens brightness. As a result, we achive object space NA 0.1, image space NA 0.23. Figure. 3 and Fig. 4 show the simulation results of MTF using optical design software ZEMAX(ZEMAX Development Corporation, USA). The horizontal axis show spatial frequency[LP/mm], and the vertical axis shows MTF. The blue, green, and red lines in the image shows object height 0mm, 14mm, and 20mm respectively. 3LP/mm on the object corresponds with 6.9LP/mm on the image. According to the simulation result, MTF value exceed 0.6 at the center of the field of view, which meets the required specification.

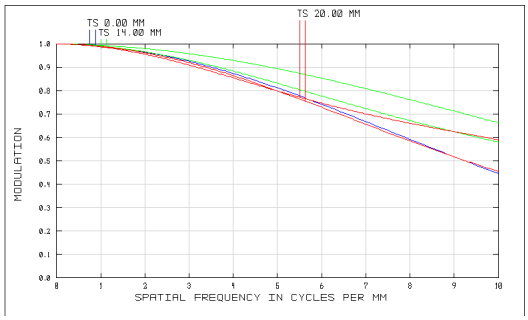

Fig. 3. MTF(V_m)

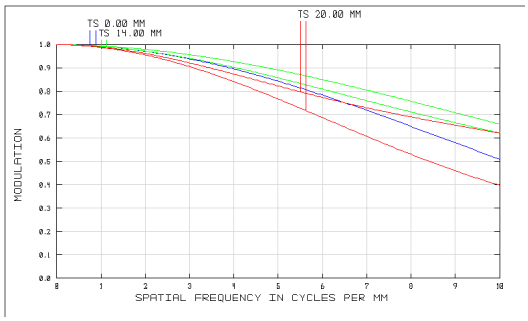

Fig. 4. MTF(Ca^{2+})

C. Filter Switching System

Accorcing to the other dual mapping method which uses voltage sensitive dye RH237 and calcium indicator Rhod-2, 710nm logn pass filter and 580nm 20nm band pass filter are popularly used[7]. However, in general, the characteristics of dielectric multilayer filter shifts to shorter wavelength depending on the light incident angle. Using our custom designed lens units, the light incidents at up to 30 degrees to the filter. So, we used custom designed optical filter which had preliminarily shifted to longer wavelength. The characteristics of each filter is shown in Fig.5, and Fig.6.

Filter wheel contains 5 long pass filters(for V_m), and 5 band pass filters(for Ca^{2+}) alternately. It was rotated by a motor and timing belt as shown in Fig. 7. Filter wheel has 10 small plates around its edge which pass across photo-interrupter near wheel and work as an encoder. Camera exposure signal and encoder signal were synchronized using phase-locked -loop techniques to avoid interruption of field of view by the metalic filter holder.

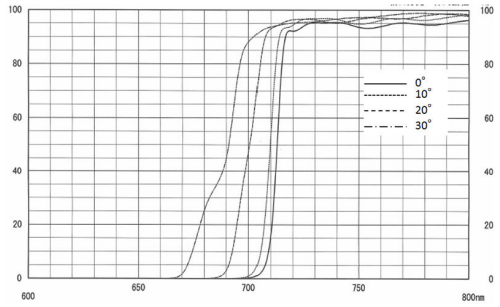

Fig. 5. Characteristic of Long Pass Filter

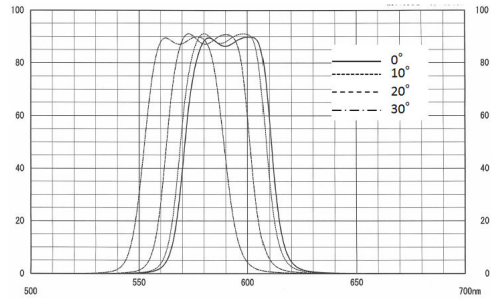

Fig. 6. Characteristic of Band Pass Filter

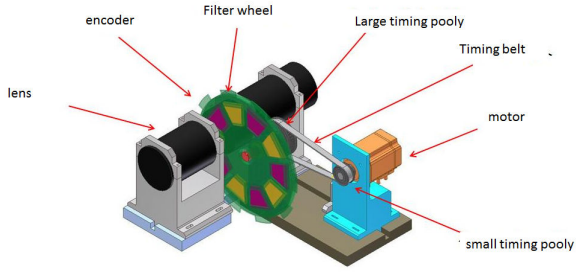

Fig. 7. Filter Switching System

III. EXPERIMENTS

A. Experimental Setup

Fig. 8 shows experimental setup. We used Langendorrf perfused rabbit hearts stained with voltage sensitive dye RH237 and calcium indicator Rhod-2. The temperature of perfusate was mentained at 27 to reduce bleaching. Cytochalasin-D was added to reduce motion artifact.

LED ring light was placed near the specimen as an excitation light. The ring light consisted of blue LEDs for RH237 and yellow LEDs for Rhod-2. The brightness of each LED was manually controlled. 570nm short pass filters(XF558, Asahi Spectra, Japan) was placed in front of the ring light to cut the residual wavelength.

Electrical stimuli for pacing were delivered from the electrode positioned on the apex of the heart. Firstly, we recorded V_m and Ca^{2+} simultaneously during planer wave. In addition, we recorded V_m and Ca^{2+} simultaneously during ventricular fibrillation(VF) induced by burst cycle length pacing.

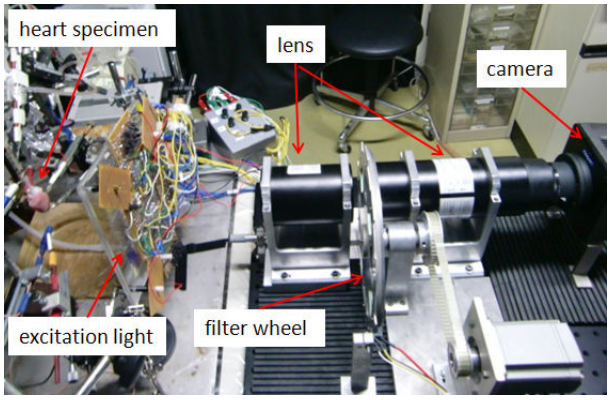

Fig. 8. Experimental Setup

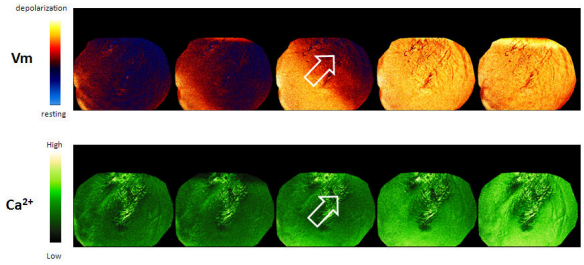

Fig. 9. Optical Mapping of V_m and Ca^{2+} during Pacing(every 16ms)

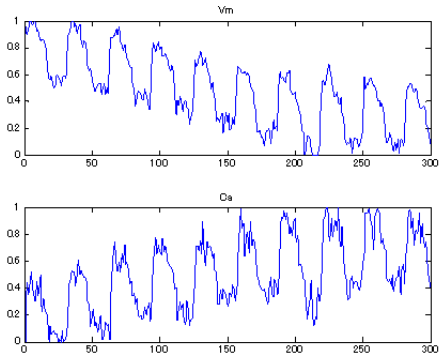

Fig. 10. Waveform of V_m and Ca^{2+} during Pacing(every 16ms)

B. Data Analysis

The aqcuired signal data from each filter was normalized respectively because the charasteristics of each optical filter were slightly different. The waveforms are aqcuired by averaging intensity of 3 3 pixel.

IV. RESULTS

A. Measurement of Normal Condition

Figure. 9 shows some snap shots of optical mapping images recorded during pacing recorded at camera speed 125fps, and spatial resolution 512 512 pixel. Fig. 10 shows normalized signal averaged in 3 3 pixel of mapped image. The horizontal axis shows camera frame, and the vertical axis

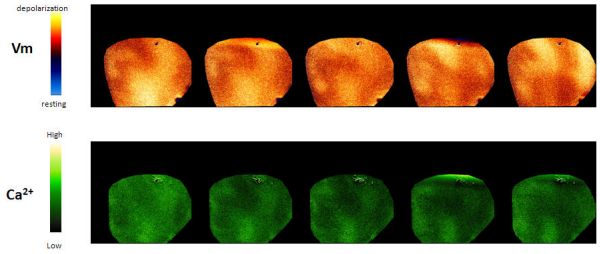

Fig. 11. Optical Mapping of V_m and Ca^{2+} during VF(every 16ms)

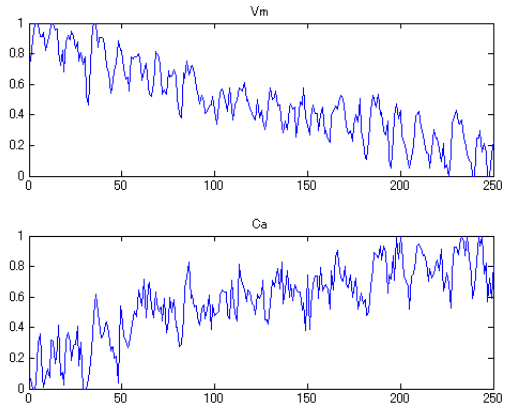

Fig. 12. Waveform of V_m and Ca^{2+} during VF(every 16ms)

shows the normalized value. Note that the waveforms of V_m and Ca^{2+} show the same upstroke synchronized with cycle length of pacing. This result is considered to be consistent compared with the other report[8].

B. Measurement of VF

Simultaneous measurement of V_m and Ca^{2+} was performed during VF induced by burst pacing recorded at camera speed 125fps, and spatial resolution 512 512 pixel... Figure. 11 shows optical mapping images during VF. Also, Fig. 12 shows waveforms. The waveform of V_m and Ca^{2+} did not match. However, previous research reported that the waveform of V_m and Ca^{2+} did not match during ventricular fibrillation[9]. Thus our result is considered to be electrophisiologically consistent.

V. DISCUSSION AND CONCLUSION

We presented an innovative optical mapping system to measure multiple electrophysiological parameters in the heart. System performance was achieved as follows:

- Camera speed : 125fps
- Resolution : 512 512pixel

The waveform acquired in the simultaneous measurement of V_m and Ca^{2+} is considered to be electrophysiologically consistent compared with the other reports. This result shows the validity and efficacy of our custom-made optical lens units and optical filters.

Simultaneous measurement of V_m and Ca^{2+} during proarrhythmic condition was successfully performed, which showed efficacy of our method for cardiac electrophysiological research.

The limitation of our measurement system is that V_m and Ca^{2+} signals are recorded different timing determined by interval between frames. However, by increasing its framerate and using appropriate interpolation, the two signals can be recorded as obtained simultaneouly.

Our system has superiority compared with other reported system. Key superiority points are wide spread of choices of fluorescent dye, easy image registration. high spatial resolution, and brightness of the lens

In principle, this method has potential to measure more than three parameters simultaneously by adding or changing fluorescent dye, optical filter and excitation light source. This method will contribute to detailed exploration of complicated cardiac electrophysiological phenomenon.

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