Simultaneous Optical Mapping System of Endocardial and Epicardial Excitation

T. Harada, T. Arafune, M. Yamazaki, H. Honjo, N. Shibata and I. Sakuma

***Abstract*— The cardiac excitation propagation during arrhythmia shows a three-dimensional complex excitation behavior. Numerous optical measurements of the propagation and action potentials of the cardiac muscles have been made to elucidate the detailed arrhythmia phenomenon. The conventional optical measurement system mainly observes the action potential signal of the epicardium, and the endocardial signal measurement without incising the heart is difficult. In addition, an incised heart no longer exhibits the natural excitation behavior. Therefore, we constructed a simultaneous measurement system that integrates the conventional epicardial measurement system and the endocardial measurement system by using an endoscope for an intact heart. Then, we proposed a line-laser registration method that can match correspondence between the epicardial and endocardial images for a short period. We demonstrated that this registration method has a sub-millimeter accuracy. Subsequently, we succeeded in simultaneous optical measurement of the excitation propagation of the epicardium and endocardium of the right heart wall by using an isolated rabbit heart.**

I. Introduction

Optical mapping, a technique that can measure the action potential with high spatial and temporal resolution using voltage-sensitive fluorescent dyes whose fluorescence changes with change in membrane potential, is an effective technique for elucidation of anomalous excitation propagation that occurs in arrhythmia, and many studies have been performed in this area. Measurement is limited to a few potential activities of the myocardial tissue surface because of the penetration depth of the excitation light in the optical mapping. However, the actual cardiac excitation propagation is a complex and three-dimensional propagation. Based on this, various attempts have been made to capture the excitation of the endocardium and heart wall [1][2][3].

A common method of measuring the endocardial excitation is to expose a sample piece of the endocardium [4][5]. Recently, studies that measured the excitation using an endoscope that was inserted through an incision of the apex from inside the atrium were reported [6][7]. However, there is a concern that these studies do not show the natural excitement behavior. Spiral re-entry could not be observed because the excitement is lost at the edges in the specimen piece, and any incision to the specimen blocks the excitement.

Although there is no effective method for measuring the excitation of the heart wall, simulation studies using heart myocardial models constructed in three dimensions have been conducted [8]. Some studies are trying to estimate the local excitation generation site of the heart wall from the surface information of the epicardial and endocardial sides using a sample piece.

Therefore, systems capable of simultaneous measurements from both sides of the endocardium–epicardium, that is, non-invasive are desired that can reveal, new findings such as locating the excitation producing site of the heart wall and measurement of the natural excitation behavior.

II. Optical Mapping Method

Optical mapping method uses a voltage-sensitive dye whose spectra shifts to shorter wavelengths almost linearly with change in the membrane potential. When irradiated with a blue-green excitation, the dye emits fluorescence at 600 nm (Fig. 1). The fluorescence intensity of the long wavelength side is measured using a high-speed camera through a long-pass filter that has a cutoff wavelength of 600 nm near the emission fluorescence intensity peak. A brightness value change from 5 to 10% is measured as a 100 mV change in the membrane potential. As a result, a waveform that inverts the action potential waveform vertically is obtained because the luminance value measured by the camera decreases with the excitation. The desired action potential waveform is obtained by an inverting normalization process for each pixel of the camera image.

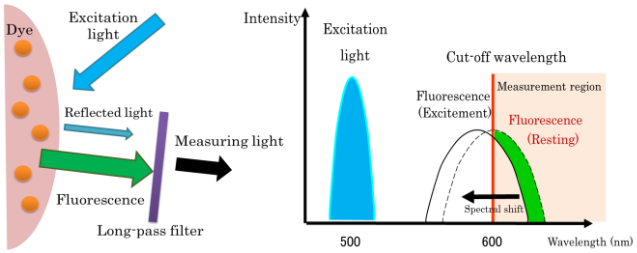

Fig. 1 Property of voltage sensitive dye

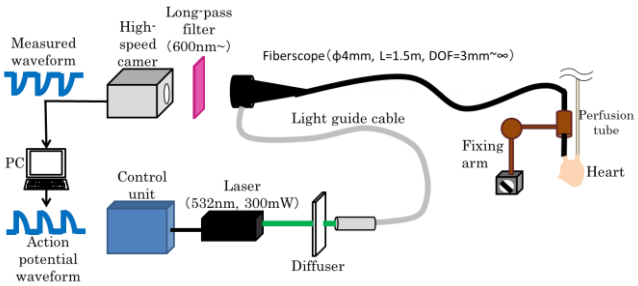

Fig. 2 Endocardial optical mapping system

Resrach supported by JSPS.

T. Harada and I.Sakuma is with School of Engineering, the University of Tokyo, Hongo, 113-8654 Japan (corresponding author to provide phone: +81-3-5841-7480; fax: +81-3-5841-6446; e-mail: harada@bmpe.t.u-tokyo.ac.jp).

T. Arafune is with School of Science and Engineering, Tokyo Denki University, Hiki-gun, 350-0394 Japan (e-mail: arafune.t@mail.dendai.ac.jp).

M.Yamazaki and H.Honjo are with the Research Institute of Environmental Medicine, Nagoya University, Nagoya, 464-8601, (e-mail: honjo@riem.nagoya-u.ac.jp, yamazaki@riem.nagoya-u.ac.jp).

N. Shibata is with Shinjuku Mitsui Building Clinic, Nishishinjuku, 163-0804 Japan (e-mail: shibata@isk-smbc.org).

III. ENDOCARDIAL OPTICAL MAPPING SYSTEM

A. Instruments

The endocardial measurement system is limited to approaches from the vessel such that there is no incisionon the inside of the heart at the time of measurement and that requires a diameter less than 5mm because the diameter of the rabbit aorta and vena cava is 5mm. In order to measure the anatomy of the atrium and ventricle of the heart, there should be approximately 3mm depth of field because of the very narrow chamber of the rabbit heart, and the system should be bendable after insertion. We proposed and constructed a flexible endocardial optical mapping system that satisfies the required specifications (Fig. 2). The system is composed of a high-speed camera (Photron, FASTCAM SA-4), a green semiconductor laser (BWTEC, BWI-532-300-E), a 4.1mm diameter fiberscope (Olympus, IF4D5) and a long-pass filter.

B. Animal Preparation

The evaluation experiments were performed using this system. Subjects were Japanese white rabbits (1.7–2.0 kg).

After heparin administration and anesthetization with sodium pentobarbital (10–15 mg/kg), the chest was dissected and the heart removed quickly. An isolated rabbit heart was perfused a modified Krebs-Ringer solution (95% O_2, 5% CO_2, pH 7.3–7.4) at a constant flow rate (35–45 ml/min) at 37°C using the Langendorff perfusion apparatus. The specimen was stained with the voltage-sensitive dye, di-4-ANEPPS (2 μmol/L). During the experiment, 0.2 μmol/L of di-4-ANEPPS and 15mmol 2,3-butanedione monoxime were added to the perfusate to minimize motion artifacts.

C. Results & Discussion

An endoscope was inserted from the superior vena cava of the isolated rabbit heart after the specimen preparation; the specimen was then irradiated with excitation light from the interior of the heart. Spontaneous excitation propagation on the inner wall of the left ventricle was measured with a field of view of 6 mm diameter and recording speed of 1000 fps. Anatomical information of the left ventricular papillary muscle and boundary between the left ventricular heart wall and the septum was confirmed by endoscopic images, and the optical excitation propagation on these areas was measured (Fig. 3). The excitation propagation from the septum to the heart wall was observed after image processing. This propagation process from the septum to the heart wall was found to be cardioelectrophysiologically accurate.

The fluorescence change rate is used as an index for evaluating the signal of the measured action potential waveform. The fluorescence change rate of di-4-ANEPPS due to a membrane potential change of 100 mV is 5–10%; a good signal typically has a higher value. The fluorescence change rate of the waveform obtained using this system was approximately 7%, which is a good signal (Fig. 4) to conventional epicardial measuring systems, which have approximately an 8% fluorescence change rate.

IV. REGISTRATION

A. Requirements

One problem in constructing a simultaneous measurement system is registration of the epicardial and the endocardial

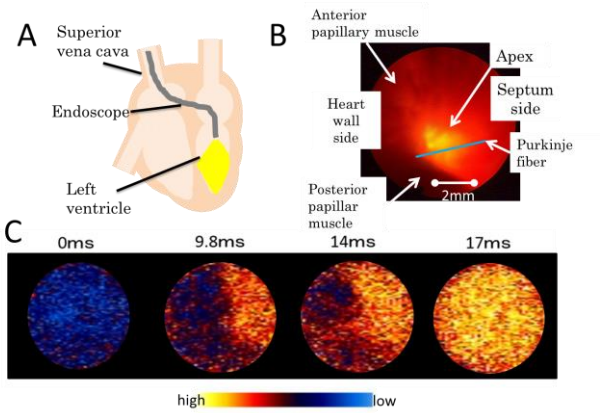

Fig. 3 Endocardial action potential propagation
A: Insertion path of the endoscope. B: Endoscopic image.
C: Action potential propagation

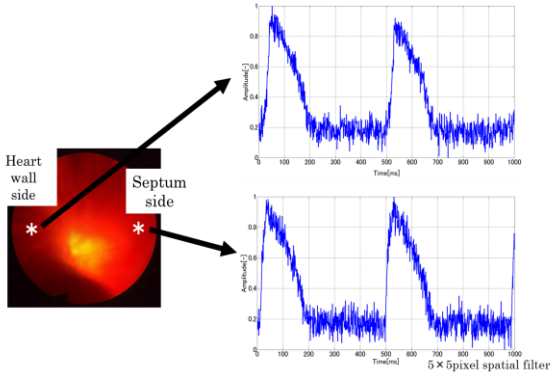

Fig. 4 Endocardial action potential waveform

images. Positioning is difficult and the endocardial measurement system cannot be seen by the naked eye. Moreover, the registration must be of submillimeter accuracy, considering the size of the rabbit heart and observation region. There is experimental background that using this extraction of hearts a good signal can be obtained for approximately 1 h owing to survival time, and all the measurements must be completed within this time. Considering that this technique is in basic research widely by physicians, a rapid and simple technique is required.

B. Proposed method

To meet the abovementioned requirements, we proposed a registration method using transmitted light (Fig. 5). The method uses a line laser. The reflected light of the scanning line laser from outside the heart is measured by the epicardial measurement system and the transmitted light is measured by the endocardial system simultaneously. Subsequently, a scan is performed by rotating the line laser by 90°. This process is measured by the epicardial-endocardial measurement system simultaneously in synchronization. The correspondence between the scale and the upper, lower, left and right epicardial and endocardial measurement is matched by associating the line that has been measured. This approach has the advantage in that it can be performed quickly without the need to change the system such that wavelength larger than 600 nm dominant wavelength of the line laser is required and this approach will require only two scans.

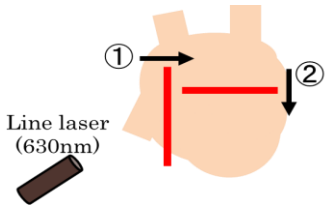

Fig. 5 Line laser registration method

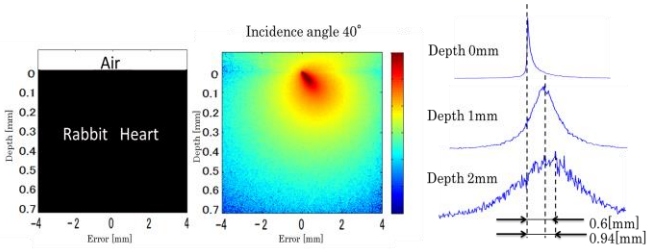

Fig. 6 Result of Monte Carlo simulation with incidence angle of 40°

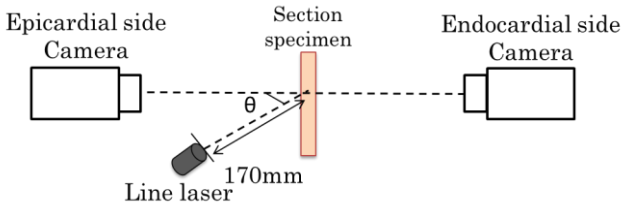

Fig. 7 Evaluation experiment setup

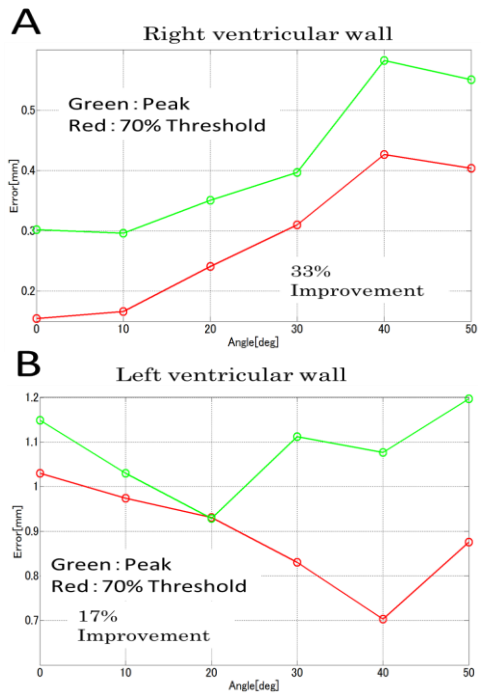

Fig. 8 Error between the incident light and transmitted light
A: Right ventricular wall. B: Left ventricular wall

The precision of the system should meet the required specification. Generally, forward scattering is dominant in biological tissues. The brightness peak at the laser incident point in the epicardial side is inclined with respect to the tissue in this procedure, but since it is the point where the laser is straight, deviation in the forward scattering in the endocardial side occurs, which depends on the incident angle.

C. *Monte Carlo Simulation*

We examined the accuracy using Monte Carlo simulations to evaluate the deviation (Fig. 6). The source codes of the Monte Carlo simulations have been published and we used a modified code of the Monte Carlo simulation code published by the Oregon Medical Laser Center (http://omlc.ogi.edu/software/mc/). Simulations were performed by a photon irradiation of 4 million using a scattering coefficient $\mu_s = 22[mm^{-1}]$, absorption coefficient $\mu_a = 0.1[mm^{-1}]$, anisotropic scattering parameter g = 0.96, and a wavelength of 690 nm for the isolated rabbit heart stained with di-4-ANEPPS. From the simulation result, it was observed that scattered light will be isotropic at a depth of 1–2 mm from the tissue surface of the incident point and not forward scattered, at a greater depth, light propagates concentrically and the position of the intensity peak does not shift significantly. The deviation of the peak position was found to be approximately 1 mm even at 70° incidence. In the actual experiment, the actual error would be approximately 0.8 mm because the incident angle would be at most 40°.

D. *The evaluation experiment using specimen section*

We also evaluated the registration method in the myocardial tissue by using the right and left heart wall of the prepared rabbit heart. Two cameras were installed on the endocardial and epicardial sides, and the fixed specimen was irradiated from the epicardial side by the laser at 10° intervals from 0–50°, and the light intensity of the reflected and transmitted lights were observed (Fig. 7). Two cameras were used to perform the registration in advance. The error was less than 0.4 mm at the peak on average in the case of the 50° incident angle at the right heart, which has a thin wall of approximately 2 mm thickness, but there was also approximately 1.1 mm error locally. On average, there was an error of approximately 0.5mm in the thick left ventricular wall of 5 mm with a local error of 2.1mm. A significant portion of the local error was found at anatomically thick structures such as when the papillary muscle tissue crosses the laser line. Using the center position of the range that had brightness greater than 70% instead of using the luminance peak registration was thought to reduce the local error. We were able to site indicating the error of 2mm locally by which also reduced to about 0.3mm. We have found that registration with sub-millimeter accuracy is possible even in the myocardial tissue (Fig. 8).

V. SIMULTANEOUS MAPPING

We simultaneously measured the endocardial and epicardial sides using the registration techniques described in the previous section. After inserting the endoscope into the right ventricle through the right atrium from the superior vena cava in the isolated rabbit heart, the endoscope was bend to 90° to observe the right heart wall, and we measured the same site at the epicardial side using the epicardial measurement system equipped with a macro lens (Nikon, Nikkor 60mm). We were then able to measure the spontaneous excitation propagation of the right heart wall (Fig. 9).

Results of the endocardial and epicardial sides excitation

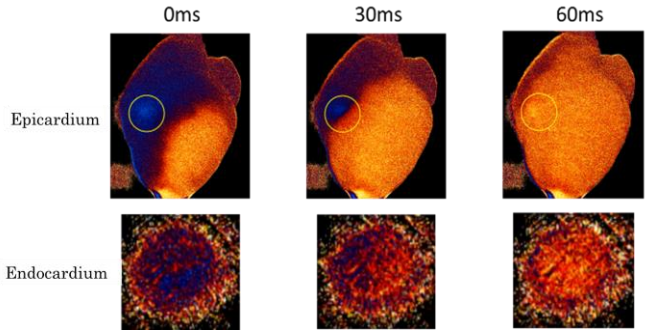

Fig. 9 Right ventricular action potential propagation of simultaneous mapping result

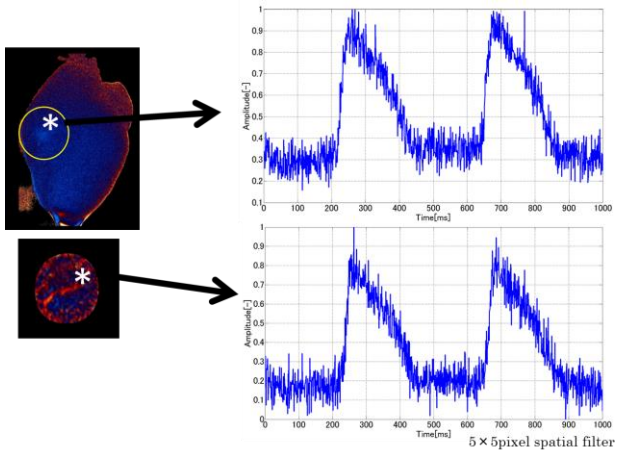

Fig. 10 Right ventricular action potential waveform of simultaneous mapping result

propagation and action potential (Fig. 10) were nearly the same. Since the right heart wall was thin, these results were electrophysiologically reasonable.

VI. CONCLUSION

We constructed an endocardial measurement system intended for a non-incision-based investigation of the heart. Good action potential signals and a 7% change in the fluorescence ratio were obtained using the endoscope inserted from the superior vena cava of the perfused rabbit heart. Further, we proposed a registration method using transmitted light that could be rapidly and easily performed with respect to the registration of the measurement result endocardial and epicardial coincidence measurement, which is a problem. Alignment at submillimeter accuracy is possible, as shown with the accuracy evaluation using tissue pieces and simulations. We performed an endocardial–epicardial simultaneous measurement for the right heart wall and obtained excitation propagation results that were reasonable in terms of cardiac electrophysiology. However, there are challenges in the precision of the registration because of the narrow field of view of the endoscope. This can be improved by optimizing the luminance distribution, intensity, and wavelength of the line laser. Noise removal and high-speed recording are also problems for comparing the difference between the action potential duration and the difference between the rise-time in the action potential waveform by using this measurement system.

ACKNOWLEDGMENT

This study is performed partly by support from JSPS, Grant-in-Aid for Scientific Research B.

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