A MULTICHANNEL FIBER-OPTIC MAPPING SYSTEM FOR INTRAMURAL RECORDING OF CARDIAC ACTION POTENTIALS

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Abstract: Measuring cardiac action potentials at many sites within the ventricular wall is important for understanding cardiac arrhythmias; however, recording in the depth of the heart wall presents many difficulties. We have developed a multichannel optical mapping system for recording cardiac action potentials transmurally. Each channel uses a single small-diameter optical fiber to transmit and collect light from the cardiac tissue. Excitation light is supplied by low-power green lasers. Wavelength separation is performed with a dichroic mirror, and fluorescence is detected with a photodiode. We have recorded action potentials with an unfiltered signal-to-noise ratio (SNR) as high as 60:1 and a temporally filtered SNR as high as 200:1. The collection of fluorescence is optimized so that low excitation light intensity can be used, which increases the available recording time. Channels are modular and compact, and the system can be easily expanded to include additional channels, ratiometry or dual-dye mapping. In addition, the system is highly flexible and can be used for virtually any experiment from single cell recording to surface and transmural mapping of the whole heart.

Introduction

Measuring cardiac action potentials throughout the heart is a very useful tool for understanding the mechanisms of cardiac arrhythmias. Cardiac tissue is not a uniform electrical syncytium, but is instead heterogeneous. There is evidence that differences in intramural recovery time may play a role in the initiation of reentry in several models of disease states [1, 2]. For this reason it is important to understand both activation and recovery in cardiac cells throughout the ventricular wall. However, there are many difficulties involved in measuring action potentials within cardiac tissue without damaging the tissue in the process.

Extracellular electrode arrays have been used to map the ventricle in three dimensions [3, 4, 5]. However, extracellular signals represent the integration of currents over a volume of tissue and therefore can only provide an estimate of cellular activation and repolarization [6].

Transmembrane potential can be directly recorded with glass microelectrodes, but they can only record from a limited depth in cardiac tissue. More recently, optical fluorescence mapping has been used, a method that utilizes voltage-sensitive dyes that fluoresce with intensity proportional to transmembrane potential. This method has many advantages over microelectrode recordings, including the ability to record for long periods of time and from multiple simultaneous sites.

In optical fluorescence mapping, fluorescence is typically collected from the tissue surface with CCD cameras or photodiode arrays. Alternatively, optical fibers can be used to record throughout the myocardium. Optrodes have been developed that consist of bundles of fibers that can be inserted into the myocardium [7, 8]. These optrodes use a single fiber for both delivery of excitation light and collection of fluorescence. Because a single fiber is used for two wavelengths of light, separation of wavelengths is necessary. This has previously been accomplished with either a fused-fiber coupler for each fiber [9] which is expensive for a multichannel system, or with a dichroic mirror to separate light from the entire bundle which requires precise image alignment [7, 8].

We introduce a low-cost, modular, fiber-optic system with 1:1 fiber-detector coupling for recording cardiac action potentials at multiple sites within the myocardial wall. Single recording fibers can be positioned on the tissue surface or can be inserted into the tissue for transmural recording.

Materials and Methods

A multichannel optical mapping system was built as seen in the schematic in Figure 1.

Excitation Light Delivery. Excitation light was provided by inexpensive 5 mW diode-pumped, frequency-doubled neodymium YAG lasers (532 nm, JLM-5, OnPoint Lasers, Minneapolis, MN, USA) encased in copper heat sinks. A single laser was used for four channels at a time by focusing the beam onto the surface of a custom-made 4-fiber bundle. To make the bundle, four 125 μm diameter fused silica multimode fibers (105 μm core, NA=0.22, AFS105/125Y, ThorLabs Inc., Newton, NJ, USA) were bonded with optical epoxy into the ferrule of a custom FC connector (Fiber Instrument Sales, Oriskany, NY,

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USA). The 310 µm diameter center hole in the ferrule allows a square packing arrangement of the four fibers.

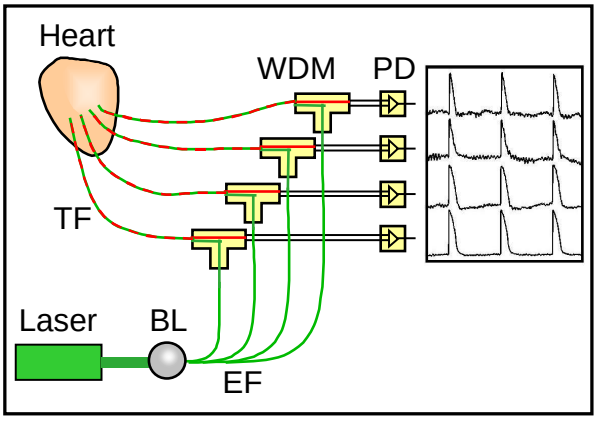

Figure 1: Schematic of 4 channels of the optical mapping system. Excitation (green) light is coupled into four excitation fibers (EF) with a ball lens (BL). The tissue fibers (TF) deliver excitation light and collect fluorescence (red). Wavelength division multiplexers (WDM) separate green and red light and direct red light to photodiodes and amplification circuits (PD). Inset shows action potentials recorded simultaneously from four channels.

Laser light was focused onto the polished surface of the fiber bundle as shown in Figure 2. A 5 mm ball lens (BK-7, Edmund Optics, Barrington, NJ, USA) was bonded with epoxy to the end of a 3.2 mm diameter bronze alignment sleeve (Fiber Instrument Sales). This centers the lens with respect to the long axis of the sleeve. Then the sleeve was slid over the ferrule containing the fiber bundle. The tight fit of the sleeve on the ferrule ensures that the ball lens is also aligned with respect to the surface of the bundle. The ball lens alignment was completed by leaving a 1.02 mm gap between the ball lens and the surface of the bundle. This places the fiber bundle inside the focal point of the ball lens at the point where the beam diameter is equal to the bundle diameter. The desired gap size is determined by the laser beam diameter (2.4 mm), the fiber bundle diameter (310 µm), and the ball lens properties.

Even with perfect alignment, not all laser light will be coupled into the fibers. The two sources of light loss are the packing fraction of the fiber cores in the bundle and the expected loss due to reflection at each optical surface. With a packing fraction of 0.46 and 3 optical surfaces, it is expected that 42.4% of the laser light will be coupled into the fibers. Excitation coupling efficiency was tested directly by measuring the laser intensity with a photodiode sensor (Model 2031, New Focus, San Jose, CA, USA), and then coupling the laser into the fiber bundle and measuring the output of each of the four fibers with the same photodiode sensor.

Wavelength Separation. In order to transmit excitation (green) and emission (red) light in a single

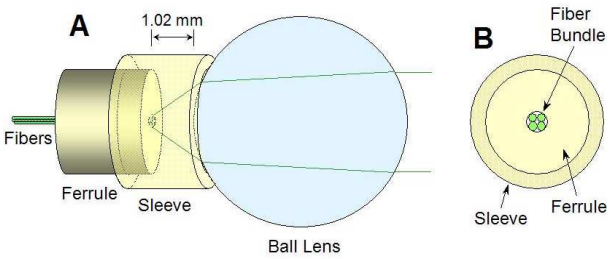

Figure 2: Coupling of excitation light into fiber bundle. (A) Alignment mechanism for ball lens and ferrule containing 4-fiber bundle, with lines representing ray traces from laser. (B) View of the face of the fiber bundle with the ball lens removed. The relative diameter of the fibers is exaggerated to show the packing arrangement.

tissue fiber, wavelength separation and filtering is necessary. We designed and built high-efficiency wavelength division multiplexers that use a dichroic mirror to couple green light into the tissue fiber and direct the returning red fluorescence onto the surface of a photodiode (S1226-5BK, Hamamatsu, Bridgewater, NJ, USA). Each multiplexer is housed in a hollowed-out TNC (Threaded Neill-Concelman) RF 'Tee' connector (see Figure 3). The excitation and tissue fibers connect to two ends of the 'Tee' connector, and the photodiode fits into the third end. Inside the 'Tee' connector, both fibers were terminated with ball lenses similar to Figure 2 to couple light into and out of the fibers. A longpass filter (OG590, Edmund Optics) positioned in front of the photodiode substrate removes scattered excitation light. Two channels are packaged together in a single shielded module (EFP204A97, Vector Inc., North Hollywood, CA, USA) as shown in Figure 3.

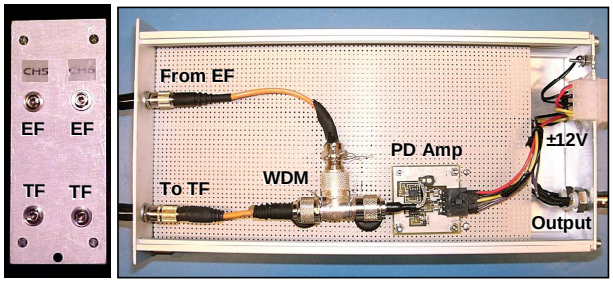

Figure 3: Two-channel module. On the left is a picture of the front panel with four FC mating sleeves for connecting excitation (EF) and tissue (TF) fibers for the two channels. On the right is a side view of the module with the side panel removed to show the interior of one channel. The wavelength division multiplexer (WDM), photodiode amplification circuit (PD Amp), ±12 V input, and signal output are labeled. The second channel in this module is located on the opposite side of the perforated board. Module dimensions are 25.0 x 5.2 x 11.7 cm.

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Fluorescence Detection and Signal Amplification. The current output of the photodiode is converted to a voltage with a 2-stage high-gain current-to-voltage converter as shown in Figure 4. The first stage is a transimpedance amplifier with a 100 MΩ feedback resistor (International Resistive Company Inc, Boone, NC, USA) resulting in a gain of $1x10^8$ V/A. A capacitive 'Tee' network with a variable capacitor (0.4-10 pF, BC Components, Malvern, PA, USA) is placed across the feedback resistor to allow compensation for stray capacitance in order to maximize the frequency response [10]. The second stage is a noninverting amplifier with a gain of 11. This circuit was custom-made on a 38 x 38 mm surface-mount circuit board as shown in Figure 4.

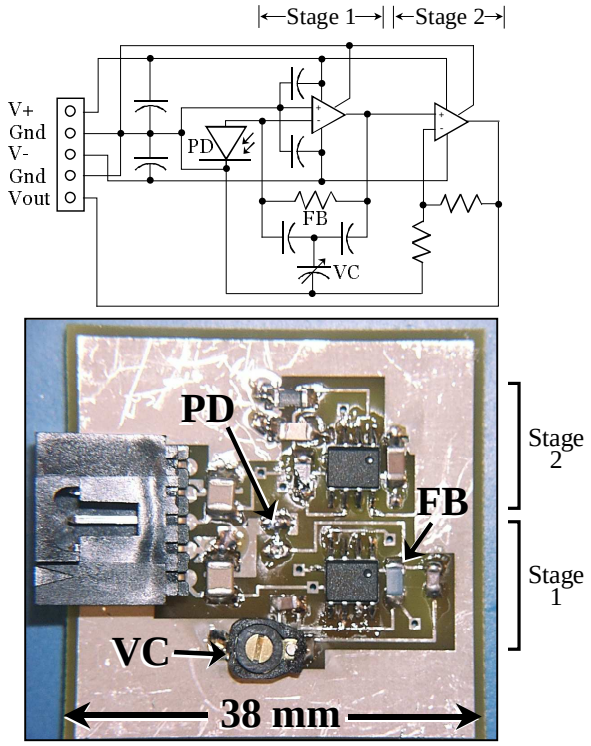

Figure 4: Two-stage photodiode amplification circuit. Above is a diagram of the circuit with the photodiode (PD), 100 MΩ feedback resistor (FB), and variable capacitor (VC) labeled. Below is an image of the component side of the surface-mount circuit board. The photodiode terminals are labeled because the photodiode itself is inside the wavelength division multiplexer.

Testing with Experimental Preparation. With the modular design of this mapping system, cardiac action potentials can be recorded at any site within the myocardium or on a surface. This was tested using a rabbit left ventricular wedge preparation [11] that was submerged in a bath and perfused with 38°C oxygenated Tyrode's solution. The excitation-contraction uncoupler 2,3-butanedione monoxime (15 mM) was added to the perfusate to prevent motion artifacts in the optical signals. The tissue was stained with the voltage sensitive dye di-4-ANEPPS (5 µM, Molecular Probes Inc., Eugene, OR, USA) and paced from the endocardium with a bipolar stimulus electrode.

Figure 5 shows the tissue preparation with six optical recording fibers. For recording within the ventricular wall, four bare, cleaved fibers stripped of the outer jacket were inserted 3 mm into the myocardium. A fiber holder was made that allows fibers to be individually advanced into the tissue and assists the placement of the fibers in an evenly spaced row spanning from sub-epicardium to sub-endocardium. For epicardial and endocardial surface recording, the end of the tissue fiber was epoxied into a ferrule, polished, and then placed against the tissue surface. Excitation intensity was set to 100 µW per tissue fiber. The tissue bath and optical mapping system were enclosed in a dark cage, and the excitation laser was shuttered between recordings to reduce unnecessary photobleaching.

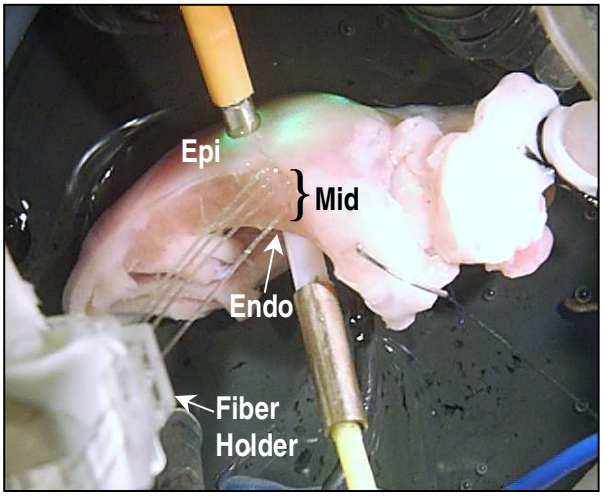

Figure 5: Rabbit left ventricular wedge preparation with six optical recording fibers. Four bare fibers are inserted into the transmurally cut edge of the ventricle (labeled Mid; includes sub-epicardium, mid-wall, and sub-endocardium), one fiber encased in a ferrule rests on the epicardial surface (Epi), and a second fiber in a ferrule rests on the endocardial surface (Endo). The thickness of the myocardial wall is approximately 5 mm.

Optical signals were highpass filtered at 0.01 Hz to remove DC offset, low-pass filtered at 500 Hz, and amplified 100x before being digitized at 12 bit resolution and sampled at a rate of 1 kHz per channel. Post-processing was done using MATLAB software (MathWorks, Inc., Natick, MA, USA). Optical action potentials were digitally filtered with a 21-point median filter. The signal-to-noise ratio (SNR) of optical action potentials was computed as the ratio of action potential amplitude to RMS noise. The rise time of optical action potentials was measured from 10% to 90% of the action potential amplitude. Data are expressed as mean ± standard deviation.

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Results

The measured excitation coupling efficiency into the 4-fiber bundle was 37%, which is only 5% less than the expected efficiency. The RMS dark noise of the photodiode amplification circuit was 355 µV.

The SNR of unfiltered optical action potentials was as high as 60:1 and could increase up to 200:1 with a 21-point median filter. Intramural and surface action potential measurements were of equal quality. Figure 6 shows filtered action potentials recorded at five locations spanning the rabbit left ventricular wall from epicardium to sub-endocardium. The mean SNR over a three minute recording interval (from which Figure 6 is a subset) ranged from 82:1 to 144:1 across the five channels.

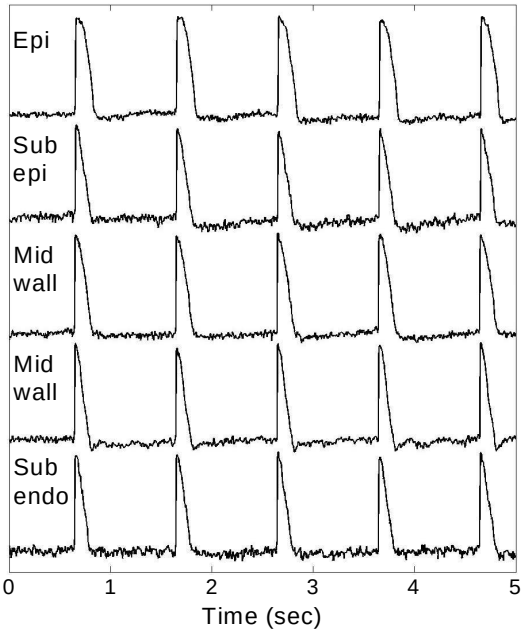

Figure 6: Median-filtered action potentials from five optical channels recorded from the epicardium, sub-epicardium, mid-wall, and sub-endocardium. Excitation intensity was 100 µW per tissue fiber. Mean SNR for the action potentials shown is 120:1.

Long-term continuous recording was possible with minimal signal degradation. Figure 7 shows the change in SNR over a three-minute recording interval. Mean change in SNR over the three-minute period was 9.3 ± 16.4%. To measure photobleaching, the action potential signal was DC coupled and change in baseline fluorescence with time was measured. After 6 minutes of recording with 126 µW of excitation light, baseline fluorescence had decreased by 15.5%.

The rise time of the optical action potentials ranged from 1.7 ± 0.9 ms to 5.4 ± 2.0 ms. Overall mean rise time was 3.8 ± 1.5 ms.

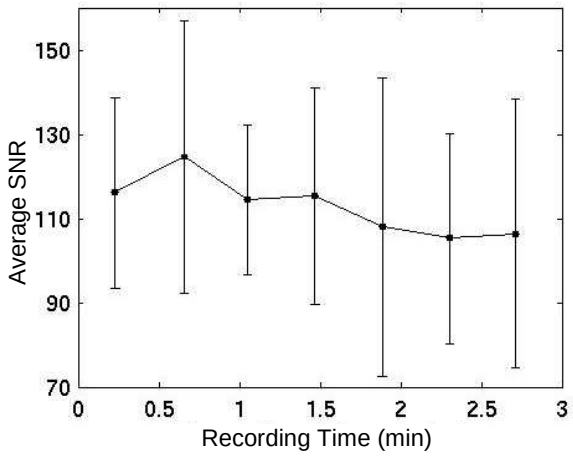

Figure 7: SNR of median-filtered action potentials averaged from five optical channels over a three-minute recording interval. Excitation intensity was 100 µW per tissue fiber. SNR does not change markedly over this interval (mean change = 9.3 ± 16.4%). Overall mean SNR was 112.0 ± 25.9.

Conclusions

We show that high-quality optical action potentials can be obtained from the tissue surfaces and transmurally in the rabbit ventricle using our fiber-optic mapping system. The signal quality is sufficient for accurate measurements of action potential duration. The rise times of the optical action potentials demonstrate that the upstrokes are also well-preserved. Microelectrode recordings have a mean rise time of 2 ms [12], while rise times recorded with a previous optrode ranged from 5.82 – 9.03 ms [7]; our mean rise time of 3.8 ms lies between the two. Blurring of the upstrokes of optical action potentials is thought to be caused by spatial averaging [12]. The small fiber core diameter (105 µm) that we used reduces the area over which fluorescence is collected. This may account for our improved rise times compared with previously published values. In addition, the lowpass filter cutoff of 500 Hz is well above the frequency content of the action potential, most of which is band-limited to 150 Hz [12]. Because of the overall signal quality and the well-preserved upstrokes, conduction velocity could also be measured accurately with this system.

Our system also addresses many common problems encountered with fiber optic action potential recording. Intramural recording is necessarily invasive, but our tissue fibers minimize injury to the tissue in two ways. First, the fiber diameter is smaller than that used in other optrodes [7, 8, 9], and second, the method of inserting single fibers instead of a fiber bundle keeps the total diameter at 125 µm, which is on the order of the length of a single cardiac cell.

We were able to use fiber with a smaller diameter without diminishing signal quality because we optimized the collection of the picowatt-intensity action potential signal. Our use of custom-built single-channel wavelength division multiplexers improves fluorescence

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detection by directly coupling each tissue fiber to a photodiode. In addition to using smaller diameter fiber, we were also able to use lower excitation light intensity without sacrificing signal quality. With lower excitation intensity, longer periods of recording are possible without excessive photobleaching, which both reduces signal quality and releases toxins to the tissue. Previous optrodes have required 100 times the excitation power that was used in this study [7].

Because our excitation light needs are low, we are able to use low-power lasers to supply excitation light for multiple channels at a time. Our design for coupling excitation light into four fibers using a ball lens reduces the cost per channel. While a 1:1 coupling of laser to fiber would be more efficient, our method requires fewer alignments and still supplies ample excitation light intensity per channel.

This optical mapping system can be used for a variety of experiments. We have shown that the tissue fibers can record from surfaces or transmurally, but it may also be possible to record from single cell preparations because of the small fiber core diameter and the high efficiency of fluorescence detection. Design features such as the use of standard TNC connectors for the wavelength division multiplexers also add flexibility to this system. Because of their modular design, the multiplexers could be used for ratiometry or for dual calcium and action potential recordings with only small modifications such as filter choices.

Acknowledgements

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