Optical mapping system with real-time control capability

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Iravanian S, Christini DJ. Optical mapping system with real-time control capability. Am J Physiol Heart Circ Physiol 293: H2605–H2611, 2007. First published July 20, 2007; doi:10.1152/ajpheart.00588.2007.—Real-time, closed-loop intervention is an emerging experiment-control method that promises to provide invaluable new insight into cardiac electrophysiology. One example is the investigation of closed-loop feedback control of cardiac activity (e.g., alternans) as a possible method of preventing arrhythmia onset. To date, such methods have been investigated only in vitro using microelectrode systems, which are hindered by poor spatial resolution and are not well suited for atrial or ventricular tissue preparations. We have developed a system that uses optical mapping techniques and an electrical stimulator as the sensory and effector arms, respectively, of a closed-loop, real-time control system. The system consists of a 2,048 × 1 pixel line-scan charge-coupled device camera that records optical signals from the tissue. Custom-image processing and control software, which is implemented on top of a hard real-time operation system (RTAI Linux), process the data and make control decisions with a deterministic delay of <1 ms. The system is tested in two ways: 1) it is used to control, in real time, simulated optical signals of electrical alternans; and 2) it uses precisely timed, feedback-controlled initiation of antitachycardia pacing to terminate reentrant arrhythmias in an arterially perfused swine right ventricle stained with voltage-sensitive fluorescent dye 4[β-2-(di-n-butylamino)-6-naphthylvinyl]pyridinium (di-4-ANEPPS). Thus real-time control of cardiac activity using optical mapping techniques is feasible. Such a system is attractive because it offers greater measurement resolution than the electrode-based systems with which real-time control has been used previously.

cardiac arrhythmias; nonlinear systems; antitachycardic pacing; charge-coupled device camera

This work focuses on methods of investigating real-time control of tissue-level activity. As previously reported, we have developed a hard real-time software system [originally known as RTLab; now known as real-time experimental interface (RTXI); www.rtxi.org] that accomplishes the stringent control requirements (e.g., one that is capable of acquiring electrophysiological signals and measuring APD with a high degree of accuracy) (6). This system has been used for APD alternans control in Purkinje fibers using multiple glass microelectrodes (5). Although microelectrodes are capable of acquiring high-fidelity transmembrane voltage measurements, their use is hindered by a number of factors, including the inherent difficulty in placing them in close proximity to one another and the difficulty in using them in tissue preparations due to their fragility. Thus it is impractical to use microelectrodes to obtain high spatial resolution data in cardiac tissue preparations.

On the other hand, optical mapping techniques using fluorescent voltage-sensitive dyes allow a dense, high-resolution mapping of cardiac electrical activity and have greatly contributed to our understanding of the dynamics of arrhythmias, especially regarding the role of APD alternans (3, 4, 13, 18, 21, 22, 27) (for a recent technical review, see Ref. 10). In this article, we describe a system that uses optical mapping techniques and an electrical stimulator as the sensory and effector arms, respectively, of a closed-loop, real-time control system. Whereas optical mapping control systems using custom-made synchronization hardware have been used previously for both functional cortical mapping and feedback stimulation in heart (25, 26), to our knowledge, this is the first report of a software-driven “hard” real-time control system for electrophysiological (cardiac or neuronal) optical mapping with a deterministic delay of <1 ms that is necessary to achieve alternans control.

One example of the utility of such a system is to test various control algorithms designed to suppress APD alternans. Although the efficacy of control at a short distance from the stimulating electrode has been well established (3, 4), open questions remain regarding the feasibility of control farther from the electrode (9). Characterizing the efficacy of such distant control requires quantifying electrophysiological properties, such as conduction velocity restitution and repolarization heterogeneities, at finite spatial resolution over the entire spatial control region. Because of the spatial resolution limitations inherent to the microelectrode approach, real-time optical mapping would be invaluable for such an application.

This article is divided into three main parts. First, we discuss the technical design and characteristics of the system. Second, we test the ability of the system to control, in real time, simulated optical signals of electrical alternans. Such simulations (1) establish a lower limit on alternans detectability and
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REAL-TIME OPTICAL MAPPING SYSTEM

controllability, 2) provide information necessary to interpret the results of future experiments, and 3) can aid the design and verification of control algorithms by enabling quantification of noise sensitivity. Third, we demonstrate the feasibility of real-time control in a real optical mapping environment by implementing an antitachycardia pacing (ATP) system and testing it in vitro porcine experiments. The ATP system is used as a proof of principle since it requires acquisition of action potential data and real-time calculation (as a function of the acquired action potential data) of the delivery time of the next stimulus. (Note that the alternans control was not tested in vitro here because the available porcine tissue is relatively resistant to alternans occurrence.)

DESIGN AND IMPLEMENTATION

Technical challenges. In developing such a system, we were faced with the following challenges. First, traditionally, optical mapping data are acquired in discrete buffers. A buffer is processed only after it is recorded in its entirety. Moreover, the signal processing algorithms commonly used for optical mapping must be applied postacquisition (i.e., the data segment must be available before processing). For application of control, we need causal online algorithms that require only the data available at each point in time, within a predetermined delay.

Second, considering the large size of the two-dimensional images, communication between the charge-coupled device (CCD) camera and computer memory can act as a bottleneck in high-speed signal acquisitions.

Third, commonly used operating systems, such as Microsoft Windows or Linux, are not capable of providing deterministic timing with a well-defined upper limit on interrupt latencies, as is required for real-time control applications. Latency in excess of 10 ms is not uncommon with either operating system.

Fourth, for control applications, we require mapping over a relatively large area (4 to 5 cm). Using a standard, single-lens optical setup over such a large area would necessitate a large distance between the front of the lens and the sample, which, in turn would require a high-intensity excitation light, causing excessive photobleaching and phototoxicity and limiting the recording duration and sampling frequency (due to low-light intensity reaching the CCD).

To address these challenges, we have used a hard real-time operating system (RTAI Linux) to achieve accurate timing. To solve the latency problem while preserving the high sampling frequency, we have chosen a line-scan CCD camera (also called a push-broom camera). Line-scan cameras have a one-dimensional light sensor (2,048 × 1 pixels in our case) that greatly reduces the size of each frame (a few kilobytes instead of hundreds of kilobytes) and therefore decreases the transfer latency. In addition to low latency, our line-scan CCD camera had a long sensor (28 mm) compared with a typical area-scan CCD (a few millimeters), allowing us to use a tandem-lens assembly optical setup that greatly reduced the distance between the lens and the sample.

Imaging hardware. The main components of the optical mapping system were a line-scan CCD camera connected to a tandem-lens assembly (10,23) and a light-emitting diode (LED) -based light source. We used an AVIIVA M2 CL 2014 line-scan camera (Atmel, San Jose, CA) with a 28.6 mm-long sensor and 2,048 × 1 pixels. Each pixel is 14 × 14 μm in size and has a well-size capacity of ~300 kiloelectrons. The camera could read up to 28,700 lines/s, although it was used mainly at 500–1,500-Hz sampling rates. The light intensity was digitized at 12 bits and transmitted to the computer via a CameraLink interface and a 60-MHz framegrabber (microEnable III, Silicon Software, Mannheim, Germany). Supplement 1 (note: supplemental material may be found with the online version of this article) shows the CCD noise characteristics (1). The predominant noise in our operational range (i.e., sampling rate and dynamic range) was shot noise, with dark noise and readout noise contributing to <20% of the total noise level. Therefore, using a cooled-CCD camera would not have reduced the total noise to any significant degree (as it primarily affects the dark noise), whereas improving the light-gathering capability by using a tandem-lens assembly drastically improved the signal-to-noise ratio.

The lens assembly was composed of a 105 mm f/1.8 lens (Nikon) reverse mounted on a 50 mm f/1.4 lens (Nikon), providing ×0.47 magnification at a working distance of 40 mm. With a consideration of the pixel pitch of 14 μm, this magnification is translated into a theoretical resolution of 30 μm/pixel, though in practice the optical system point-spread function (PSF) limited the resolution to ~0.5 mm. A 600-nm emission filter (R60, Nikon) was placed between the two lenses (more details in supplement 2).

We used a solid-state illumination system (high-power LEDs) in place of standard Quartz Tungsten Halogen lamps (11, 17). This decision simplified the design by removing the excitation filter and the mechanical shutter. Eight 5-W high-power Luxeon V LEDs (Lumileds, San Jose, CA) with a peak output at 505 nm provided the emission light. No excitation filter was used; the light leakage through the emission filter was measured as <0.1%. The lack of an excitation filter and a mechanical shutter allowed us to mount the LEDs between the front end of the lens assembly and the sample at a distance of 15–20 mm from the tissue. Nevertheless, these LEDs suffer from initial light output instability because of high thermal load since their light output is temperature dependent, which complicates the signal processing (see Signal processing).

A National Instruments PCI-6254 analog-to-digital board provided the link between the RTXI kernel module and the ECG recorder and the stimulator.

Software. We chose the Linux operating system (kernel 2.4.24) augmented by RTAI patch (version 3.1) as our hard real-time platform. The software system was written around RTXI signal acquisition and real-time control software, which was previously used to implement the aforementioned electrode-based control system (5). The software was composed of three kernel space and two user space modules: RTXI and the optical mapping interface in the user space and the framegrabber driver, helper driver, and RTXI kernel modules (Fig. 1).

Signal processing. The main goal of processing the transmembrane potential recording obtained from the optical mapping technique is twofold: to improve signal-to-noise ratio and to remove baseline drift resulting from dye photobleaching and light-source instability. The former goal is usually achieved through convolution of the signal with various temporal or spatial smoothing kernels, whereas in a typical optical mapping application, the latter goal is realized by subtracting a trend line, or in some cases a higher-order polynomial, from the signal.
19). The time integration is performed using the Runge-Kutta method modeled as a one-dimensional cable of Noble Purkinje cells (9, realistic noise and distortion information. The cardiac tissue is recorded by the line-scan camera. This model incorporates spatial resolution to improve the signal-to-noise ratio. In effect, this filter reduced B11015/H9262.

APD at 80% repolarization was determined before presenting it to the control loop. Hence, we developed a model for the optical signals to introduce error into APD measurement. Hence, we developed a method to remove the baseline is prone to oscillation (ringing) that can be solved by adjusting the pacing interval (BCL) fast enough to produce alternans. Control is unstable.

In our system, we used a boxcar spatial filter of width 16 to improve the signal-to-noise ratio. In effect, this filter reduced spatial resolution to \( \approx 480 \, \mu m \), which is comparable with the optical system PSF.

Detrending the signal proved more complicated. Not only does a LED light source with electronic shutter follow an exponential decay rather than a linear one (Fig. 2A), the restriction imposed by the real-time nature of the control application precluded the use of an off-line polynomial detrending algorithm. As an additional practical constraint, the algorithm should have been simple and robust and preferably using only fixed-point arithmetic to be implemented as a real-time Linux kernel module.

Under these conditions, a simple linear IIR high-pass filter to remove the baseline is prone to oscillation (ringing) that can introduce error into APD measurement. Hence, we developed a nonlinear recursive high-pass filter to achieve efficient detrending with minimum ringing artifact as described in supplement 3 (Fig. 2B). APD at 80% repolarization was determined using a threshold algorithm. Upstroke was defined as crossing 0.5 of the (previous) action potential amplitude level, whereas the end of action potential was defined as crossing 0.2 action potential amplitude level.

VERIFICATION: SIMULATED OPTICAL REAL-TIME ALTERNANS CONTROL

In this section, we develop a model for the optical signals recorded by the line-scan camera. This model incorporates realistic noise and distortion information. The cardiac tissue is modeled as a one-dimensional cable of Noble Purkinje cells (9, 19). The time integration is performed using the Runge-Kutta method, whereas the finite difference technique is employed to solve the reaction-diffusion partial differential equation (see supplement 4). The transmembrane potential is converted into a simulated optical signal as follows:

First, transmembrane potential is inverted and added to a background based on the ratio of change in fluorescent signal due to action potentials to the baseline fluorescence (\( \Delta F/\Delta F \)) as measured from the tissue experiments (see VERIFICATION: IN VITRO OPTICAL REAL-TIME ANTITACHYCARDIA PACING).

Second, different sources of optical distortion are simulated. Multiplying the signal by a spatial Hanning (cosine) window simulates Vignetting (darkening near the edges). The blurring effect of PSF is modeled by convoluting the resulting signal with a Gaussian kernel. All the parameters (width of the Hanning window and the kernel) are derived from actual experimental data.

Third, an exponential decay function models the LED light instability.

Fourth, Gaussian random noise is added to the signal. In supplement 1, we showed that the primary source of noise in the system is shot noise, which is proportional to the square root of the signal level.

Afterward, the simulated optical signals are processed by the routines designed to remove various sources of noise and distortion as described in Signal processing. Figure 3A demonstrates an example of such a processed signal in the presence of a typical amount of noise. Vertical axis is time (top to bottom), whereas the horizontal axis corresponds to the line-scan sensor. The data are color coded, such that blue corresponds to the baseline and red to the action potential upstroke.

We checked the effect of the detrending algorithms on the ability of the system to accurately measure APD alternans. Figure 4A shows the strong correlation that existed between the measured alternans (the difference between consecutive APDs) and the actual alternans. Hence, although the detrending algorithm may potentially result in an underestimation of APDs, its effect on alternans, which is the main interest for us, is minimal.

To test the feasibility of such a system for real-time control of APD alternans, the simulated tissue is paced with a basal cycle length (BCL) fast enough to produce alternans. Control is realized by adjusting the pacing interval (\( T_a \)) using (5)

\[ A \] raw (unprocessed except for inversion) optical signal vs. time. Note the nonlinear baseline drift and a gradual reduction in action potential amplitude toward the end of the trace. B: filtered signal using the real-time algorithm as described in the main text. The baseline drift has been removed, and the action potentials are of the same amplitude. Note the introduction of some processing artifacts, especially undershoots at the end of action potentials (best seen after the first and fifth action potentials). \( \Delta F/\Delta F \), ratio of the signal to the baseline fluorescence; \( t \), time. Horizontal bar shows 200 ms.
where

$$\Delta T_n = (\gamma/2)(A_n - A_{n-1}),$$

$$T_n = \begin{cases} T_n + \Delta T_n & \text{if } \Delta T_n < 0 \\ T_n & \text{if } \Delta T_n \geq 0 \end{cases}$$

(1)

Fig. 3. A: simulated action potentials while control algorithm is off. Cycle length is 275 ms, and noise level (see text) is 2.0. Note the obvious action potential duration (APD) alternans. B: simulated action potentials while control algorithm is on. The baseline cycle length is still 275 ms, but it is perturbed by the control algorithm. Note that the alternans control is achieved.

Fig. 4. A: the relationship between the measured alternans (after addition of noise and passing through the signal processing pipeline) and the actual alternans (measured from raw, unprocessed simulate transmembrane potentials). Data from 1,624 points are plotted. The slope of the regression line is 0.95 with $r^2 = 0.90$. More than 93% of the measured alternans fall within 1 ms of the actual alternans.

B: residual alternans vs. noise for 3 different basic cycle lengths (BCLs: 225, 250, and 300 ms) during control of alternans. Noise is scaled such that noise 1 corresponds to the level obtained from a freshly stained tissue. For each BCL, residual alternans increases linearly with additional noise. The slope of the line is dependent on BCL. C: an example of alternans control. The control algorithm is turned on at the first beat. BCL is 300 ms. Two cases are shown: noise = 0 (●) and noise = 2.5 (○). APD, duration of the n-th action potential.
curve at this BCL. The final result is that the effect of noise on alternans is proportional to $\gamma_s$. In Fig. 4B, a shorter BCL operates in a steeper portion of the restitution curve, and, consequently, it results in a larger residual alternans.

**VERIFICATION: IN VITRO OPTICAL REAL-TIME ANTITACHYCARDIA PACING**

Isolated arterially perfused right ventricular preparation.

The research protocol was approved by the Institutional Animal Care and Use Committee of the Weill Medical College of Cornell University. Yorkshire pigs (40–50 kg) of either sex were used in the study. Isolated arterially perfused porcine right ventricles were prepared according to standard techniques (16, 20). After premedication with an intramuscular injection of ketamine (20 mg/kg), acepromazine (0.5 mg/kg), and atropine (0.05 mg/kg), the pigs were anesthetized with 20 mg/kg of intravenous thiopental sodium, intubated, and ventilated. After euthanasia, the heart was removed through a median sternotomy and the right coronary artery was rapidly perfused with 60 ml of cold crystalloid cardioplegia solution containing (in mmol/l) 137 NaCl, 8.0 KCl, 1.0 MgCl$_2$, 1.8 CaCl$_2$, 0.4 NaHPO$_4$, 16.0 NaHCO$_3$, and 5.6 glucose. The heart was then placed into cold cardioplegia and transferred to the experimental setup within 5 min.

The right ventricular free wall (Fig. 5) was removed, and the right coronary artery was cannulated using a 6-Fr (2 mm) catheter after its distal end was securely closed by 4-0 silk sutures. Any transected epicardial coronary artery was closed to maintain perfusion pressure. Afterward, the isolated right ventricle was transferred to a custom-made recording chamber and perfused by warm oxygenated Tyrode solution containing (in mmol/l) 137 NaCl, 2.7 KCl, 1.0 MgCl$_2$, 1.8 CaCl$_2$, 0.4 NaHPO$_4$, 16.0 NaHCO$_3$, and 5.6 glucose at a perfusion pressure of 40–60 mmHg. A bipolar pacing electrode was attached to the endocardial surface. A continuous electrogram was recorded using two silver chloride electrodes in the bath. After spontaneous contraction was noted in the perfused right ventricle, it was paced at twice the threshold at a rate of 0.5 Hz. The pacing interval was progressively shortened, while maintaining 1:1 capture, until the pacing interval equaled 500 ms. If at any time during the experiment, except when the ATP protocol was active, reentrant tachycardia was noted, the tissue was defibrillated at 2–5 J to terminate the arrhythmia. After pacing at 500 ms for at least 15 min to achieve equilibrium, the tissue was stained by an injection of 200 $\mu$l of 5 mmol/l solution of voltage-sensitive dye (di-4-ANEPPS; Molecular Probes, Eugene, OR) in dimethyl sulfoxide into the perfusate over a 5-min period. At the same time, the perfusion solution was changed to one containing an electromechanical decoupler: either 5 mmol/l of 2,3-butanedione monoxime or 5 mmol/l of cytochalasin-D in Tyrode solution (both from Sigma, St. Louis, MO) (29). Any residual contraction was stopped by gently pressing a transparent cover over the tissue.

**Experimental results.** Figure 6 shows a stack diagram of the optical signal recorded during pacing at a cycle length of 400 ms. The optical signal is processed, normalized, and color coded, such that red corresponds to action potential peak and blue corresponds to the baseline. The y-axis is time, increasing from top to bottom. The x-axis depicts various points on the line-scan CCD sensor. White horizontal lines show the stimulation times. The wavy white vertical lines are the calibration lines placed on the coverslip above the tissue (10 mm apart); waviness is caused by image processing.

**Fig. 5.** Typical right ventricular preparation showing the epicardial surface of the right ventricular free wall. Dashed line demarcates the atrioventricular groove and the course of the right coronary artery. Right atria and appendix (RA) is below this line, whereas the right ventricle (RV) is above it. The imaging line (dotted yellow rectangle) is located 1–1.5 cm below and parallel to the atrioventricular groove. The stimulating electrode is placed close to the imaging line.

**Fig. 6.** Stack diagram during pacing at a cycle length of 400 ms. The optical signal is processed, normalized, and color coded, such that red corresponds to action potential peak and blue corresponds to the baseline. The y-axis is time, increasing from top to bottom. The x-axis depicts various points on the line-scan CCD sensor. White horizontal lines show the stimulation times. The wavy white vertical lines are the calibration lines placed on the coverslip above the tissue (10 mm apart); waviness is caused by image processing.
In fact, the activation wavefront forms a curved shape in Fig. 6. This curvature is the result of the distance between the stimulation electrode and the imaging line (5–10 mm) and tissue anisotropy. Moreover, the wavefront encodes enough information to derive conduction velocity, direction, and anisotropy ratio (see supplement 5).

We induced arrhythmia in the tissue by rapid pacing. The most likely mechanism for the arrhythmia was reentry, since 1) burst pacing was the initiation mechanism and 2) the arrhythmias were fast (cycle length down to 100 ms) and usually regular and monomorphic, although we occasionally observed polymorphic arrhythmias (Fig. 7B).

The arrhythmias were very stable and did not terminate spontaneously as long as the tissue was perfused. However, a direct-current shock of 2–5 J readily terminated them.

Figure 7 shows the stack diagram of three different reentries. In this situation, the shape of the wavefront is different from simple pacing and cannot be predicted from the model presented in supplement 5, since the assumption that all waves diverge from a single point no longer holds.

To test the real-time control system, we applied a variant of commonly used ATP algorithms to reentry occurrences. ATP compromises a group of techniques employed in some implantable cardiac devices to terminate reentrant tachyarrhythmias by overdrive pacing (14). Our algorithm is based on a closed-loop control system (in contrast to open-loop ATP algorithms common in clinical settings) that monitors cardiac electrical activity through an optical channel and then, based on analyses of this activity, systematically triggers precisely timed feedback-controlled stimuli to entrain the reentry (see supplement 6).

Figure 8 shows an example. The different shape of the fourth and fifth action potential in the map (fusion complexes), combined with the timing information (e.g., 5th action potential is early, whereas the interval between the 4th and 6th is twice the baseline cycle length), strongly suggests that entrainment of the fifth stimulus into the reentry loop occurred. Hence, the real-time control system is capable of detecting the action potentials and synchronizing the firing of the stimuli accordingly.

DISCUSSION

In this article, we have shown the feasibility of a real-time cardiac control application using optical mapping techniques. Although technically challenging, such a hybrid system demonstrates some of the best characteristics of both optical mapping and real-time control systems. It is capable of providing a dense mapping with accurate reconstruction of the action potentials and the ability to measure APDs over a large area. Furthermore, it can process the optical signal and generate control interventions within a short, deterministic delay, as was the case with the ATP algorithm.

We used the data obtained from this system to develop a realistic model for the optical signals. Applying APD control to the model, we were able to show that control is achievable over a wide range of noise levels encountered in real experiments.
Nonetheless, noise sets a lower limit on the realizable level of alternans control. Having this knowledge will help with the design and interpretation of future experiments, when the system is used for APD control.

Such a hybrid system also possesses some of the problems it inherits from the base technologies. Similar to traditional optical mapping methods, photobleaching and phototoxicity remain problematic. Additionally, the need to use electromechanical decouplers potentially interferes with the shape and duration of the action potentials (15, 24). Also, the timing and processing-load restrictions currently imposed by real-time control algorithms and available hardware limited us to use a linear CCD, rather than a full two-dimensional area-scan camera.

Nevertheless, as we showed in the EXPERIMENTAL RESULTS, the signal acquired from a linear CCD can be processed to extract global information (including anisotropy ratio) about the tissue under study. We expect that technological advances in CCD cameras, processors, and data transfer hardware will allow construction of a real-time optical mapping system using area-scan cameras in the near future. Such a system should be useful in implementing and testing complex control algorithms to modulate or terminate cardiac arrhythmias.

GRANTS

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