Single-sensor system for spatially resolved, continuous, and multiparametric optical mapping of cardiac tissue

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BACKGROUND Simultaneous optical mapping of multiple electrophysiologically relevant parameters in living myocardium is desirable for integrative exploration of mechanisms underlying heart rhythm generation under normal and pathophysiologic conditions. Current multiparametric methods are technically challenging, usually involving multiple sensors and moving parts, which contributes to high logistic and economic thresholds that prevent easy application of the technique.

OBJECTIVE The purpose of this study was to develop a simple, affordable, and effective method for spatially resolved, continuous, simultaneous, and multiparametric optical mapping of the heart, using a single camera.

METHODS We present a new method to simultaneously monitor multiple parameters using inexpensive off-the-shelf electronic components and no moving parts. The system comprises a single camera, commercially available optical filters, and light-emitting diodes (LEDs), integrated via microcontroller-based electronics for frame-accurate illumination of the tissue. For proof of principle, we illustrate measurement of four parameters, suitable for ratiometric mapping of membrane potential (di-4-ANBDQPQ) and intracellular free calcium (fura-2), in an isolated Langendorff-perfused rat heart during sinus rhythm and ectopy, induced by local electrical or mechanical stimulation.

RESULTS The pilot application demonstrates suitability of this imaging approach for heart rhythm research in the isolated heart. In addition, locally induced excitation, whether stimulated electrically or mechanically, gives rise to similar ventricular propagation patterns.

CONCLUSION Combining an affordable camera with suitable optical filters and microprocessor-controlled LEDs, single-sensor multiparametric optical mapping can be practically implemented in a simple yet powerful configuration and applied to heart rhythm research. The moderate system complexity and component cost is destined to lower the threshold to broader application of functional imaging and to ease implementation of more complex optical mapping approaches, such as multiparametric panoramic imaging. A proof-of-principle application confirmed that although electrically and mechanically induced excitation occur by different mechanisms, their electrophysiologic consequences downstream from the point of activation are not dissimilar.

KEYWORDS Arrhythmia; Electrophysiology; Fluorescence; Mechano-electric coupling; Optical mapping

ABBREVIATIONS **AP** = action potential; **$[Ca^{2+}]_i$** = intracellular free calcium; **CaT** = Ca^{2+} transient; **EMCCD** = electron-multiplied charge-coupled device; **LED** = light-emitting diode; **UV** = ultraviolet; **V_m** = membrane potential

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Introduction

Optical imaging has had a profound impact on understanding excitable tissue physiology. In particular, optical mapping of electrophysiologic parameter-sensitive dyes has contributed to a better understanding of action potential (AP) generation and conduction dynamics in multicellular preparations so that fluorescence imaging has become a gold standard for functional research (for review see Efimov et al[1]). However, the technical complexity of measuring more than one parameter and the associated cost have impeded broad uptake of multiparametric imaging as a standard research tool.

Modern fluorescent probes can selectively report membrane potential (V_m), pH, and ion concentrations such as

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intracellular free calcium ($[Ca^{2+}]_i$), and it is possible to combine certain probes in one preparation.[2] For heart rhythm research, combined V_m and $[Ca^{2+}]_i$ monitoring is arguably of particular relevance, as these parameters underlie electromechanical integration. Normal excitation–contraction coupling involves V_m depolarization, transsarcolemmal Ca^{2+} influx, bulk Ca^{2+} release from the sarcoplasmic reticulum, resulting in increased $[Ca^{2+}]_i$ forming a Ca^{2+} transient (CaT). Via interaction with troponin-C, CaT enables cross-bridge interactions for myocardial force generation/shortening. Excitation–contraction coupling–related interactions between V_m and $[Ca^{2+}]_i$ are complemented by mechano-electric feedback,[3] where the mechanical environment affects Ca^{2+} buffering[4] and fluxes across sarcolemmal[5] and intracellular membranes in cardiomyocytes[6] and nonmyocytes,[7] with consequences for electrical behavior, including induction of premature ventricular beats.[8]

Thus, V_m and $[Ca^{2+}]_i$ interactions are dynamic and complex. Underlying mechanisms are modulated by pathologies such as ischemia, with relevance for cardiac mechanical and electrical performance, including arrhythmogenesis.[9–11] Therefore, simultaneous V_m and CaT mapping in one and the same sample is desirable for probing spatiotemporal relationships of these key (patho-)physiologically relevant parameters.

Simultaneous V_m and CaT measurements have been achieved in myocardial preparations, including whole heart.[12–15] Utilizing appropriate probe/filter combinations, V_m and CaT have been imaged separately, using two cameras. Although multiparametric optical mapping is still relatively uncommon, the insight gleaned from such simultaneous measurements, particularly at high spatiotemporal resolution, is extremely promising, and wider application in cardiac research appears desirable.[16]

Technical limitations of currently available optical mapping approaches include uneven dye loading and/or illumination, photobleaching, and imaging artifacts induced by contraction (so-called "motion artifacts"). Emission ratiometry with V_m-sensitive dyes, such as di-4-ANEPPS,[17] or nonratiometric Rh237 combined with calcium probes such as fluo-4 or Oregon-green BAPTA-1,[18] have been used to reduce or partially correct these effects. Second-generation ratiometric calcium probes (fura-2, indo-1) have been used to characterize absolute $[Ca^{2+}]_i$ levels,[19,20] although $[Ca^{2+}]_i$ calibration and motion tracking remain challenging in multicellular preparations (and are outside the scope of the present methods development; for review see Entcheva and Bien[21]).

Ratiometric dyes display a spectral shift (e.g., on Ca^{2+} binding),[22] which changes with exposure to different excitation wavelengths on opposite sides of the "emission isosbestic point" (the excitation wavelength at which a change in reported parameters produces no change in emission). On either side of this isosbestic point, alterations in emission intensity are of opposite polarity. The ratio of these two emission intensities is independent of fluorescence intensity so that some of the above-stated limitations (e.g., uneven dye loading) can be addressed. Inherent to multiwavelength mapping is that light of different wavelengths penetrates tissue to varying degrees, which may affect recorded light intensities. This need not be a restriction because it can be used to explore depth-weighted behavior.[23,24]

To exploit the full potential of ratiometric techniques for multiparametric optical mapping, simple, robust, and scalable approaches to multiwavelength imaging are needed. Thus far, dedicated cameras have generally been used to collect light at different wavelengths. This makes multiparametric mapping challenging. Difficulties include detector alignment (i.e., matching camera pixels), intensity loss due to extended light paths and additional components (e.g., beam splitters), and positioning in a restricted space. Even if one divided a single sensor into separate areas, dedicated to collection of separate wavelengths, optical alignment remains challenging if more than two parameters are involved.

That said, modern camera frame rates permit multicolor imaging using a single detector if combined with suitable excitation timing and emission filtering. In this context, multiband emission filtering[25] offers exciting possibilities for cardiac optical mapping.

Traditional wide-field imaging light sources include xenon, halogen, or mercury lamps in combination with mechanical shutters and filter wheels to switch excitation wavelengths. This imposes limitations on wavelength switching and scalability. In addition, altering individual wavelength intensity in broad-band sources is nontrivial, requiring neutral density filters. These limitations can be overcome by light-emitting diodes (LEDs), which provide stable, flexible, and economical alternatives to previous lighting methods. For example, unlike traditional sources, LED light intensities can be modulated exceedingly fast (microsecond domain; e.g., Figure 3C). Powerful LED chips are available now from ultraviolet (UV) to infrared, making them attractive for multiwavelength imaging, such as of V_m and CaT in myocardium, where deep UV-LED illumination has unexplored potential.[26]

Using standard mapping approaches, the study of normal and pathophysiologically disturbed interactions of V_m and $[Ca^{2+}]_i$ would require at least two, potentially four, cameras for ratiometric measurements of single-excitation/dual-emission dyes. Here, we present a method to simultaneously measure V_m and CaT using two excitation wavelengths for each parameter and only a single camera. This is combined with readily available filters and LEDs, integrated by custom-made microcontroller-based electronics, using off-the-shelf components. We used the novel V_m-sensitive di-4-ANBDQPQ[27] and the calcium dye fura-2 to demonstrate the applicability of this method to multiparametric studies in Langendorff-perfused rat hearts. As proof of principle, we illustrate that ectopic excitation following local electrical or mechanical stimulation gives rise to similar propagation patterns.