

Optical mapping of electromechanics in intact organs

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Impact statement

Electrical and mechanical functions in the heart are bidirectionally coupled, yet are usually studied separately because of the different instrumentation technologies that are used in the two areas. Optical mapping is a powerful and widespread tool for imaging electrical propagation, but has traditionally required mechanical function to be arrested. Recently new methods have been devised that enable optical mapping to be performed in beating hearts and also to simultaneously quantify mechanical function. These new technologies promise to yield new information about electromechanical interactions in normal and pathological settings. They are also beginning to find application in other organ systems such as the gastrointestinal tract where they may provide new insight into motility disorders.

Abstract

Optical mapping has become a widely used and important method in cardiac electrophysiology. The method typically uses voltage-sensitive fluorescent dyes and high-speed cameras to image propagation of electrical waves. However, signals are highly susceptible to artifact caused by motion of the target organ. Consequently, cardiac optical mapping is traditionally performed in isolated, perfused organs whose contraction has been pharmacologically arrested. This has prevented optical mapping from being used to study interactions between electrical and mechanical motion. However, recently, a number of groups have developed methods to implement cardiac optical mapping in the presence of motion. These methods employ two basic strategies: (1) compensate for motion by measuring it or (2) ratiometry. In ratiometry, two signals are recorded from each site. The signals have differing sensitivity to membrane potential, but common motion artifact, which can be cancelled by taking the ratio of the two signals. Some methods use both of these strategies. Methods that measure motion have the additional advantage that this information can be used to quantify the organ's mechanical function. Doing so enables combined "electromechanical mapping," which allows optical study of electromechanical interactions.

By allowing recording in the presence of motion, the new methods open the door to optical recording in in-vivo preparations. In addition, it is possible to implement electromechanical optical mapping techniques in organ systems other than the heart. For example, it was recently shown that optical mapping of slow wave propagation in the swine stomach is feasible. Such studies have the potential to uncover new information on the role of dysrhythmic slow wave propagation in gastric motility disorders.

Keywords: Voltage sensitive dye, strain, electrophysiology, arrhythmias, gastroenterology, cardiac

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Introduction

Electrical excitation and mechanical function are integral parts of the physiological activity of organs such as the heart, stomach, and intestine.^{1,2} Over the last century, technological innovations have enabled researchers to more completely describe the independent roles of electrophysiology and mechanical strain in normal and pathological physiology. However, electrical and mechanical functions are coupled bidirectionally. In the heart, electrical excitation regulates mechanical contraction (excitation–contraction coupling),¹ and mechanical deformation can modulate electrical activity, potentially triggering ectopic electrical excitation (mechanosensitivity).³ Similar mechanisms

operate in the gastrointestinal tract: the rhythmic slow wave regulates peristaltic contraction⁴ and the interstitial cells of Cajal respond to stretch.⁵

In recent decades, clinical observations, animal experiments, and computational models have suggested that cardiac mechanosensitivity may play a role in cardiac arrhythmia onset.^{6–9} Until recently, the experimental study of such mechanisms in intact organs was restricted by technical limitations. For example, optical mapping is a powerful method for imaging electrical wave propagation. However, because wall motion creates artifacts that obscure signals, contraction is typically abolished by electromechanical uncoupling agents such as blebbistatin.¹⁰

This practice eliminates the possibility of using optical mapping to investigate mechanical modulation of electrical activity. However, by taking advantage of solid state lighting, high speed imaging, and image processing techniques, optical mapping of electromechanics in intact organs is emerging as a tool for simultaneous, high resolution, quantification of electrical and mechanical activity.^{11–15} In this article, we will briefly review electromechanical optical mapping by first discussing optical mapping, then mechanical mapping techniques, and finally integrated electromechanical optical mapping.

Optical mapping

Optical mapping is a high-resolution tool for imaging physiological parameters such as membrane potential and intracellular calcium concentration. First developed in the late 1970s as a non-contact method of measuring membrane potential at higher resolution than practical with microelectrodes, the technique has since greatly influenced the fields of neuroscience and cardiac electrophysiology, driving greater understanding of cellular mechanisms and organ level physiology.^{16,17} Current methods typically utilize high-speed cameras to measure fluorescence emitted by reporters that transduce the parameter of interest.

Electrophysiology

Potentiometric dyes

We will focus on optical mapping of electrical propagation. Optical mapping usually utilizes fast response potentiometric probes to quantify variations in spatial and temporal

membrane potentials across cells and tissue. First developed over 30 years ago, these dyes exhibit a shift in fluorescent spectra in response to a change in membrane potential via an electrochromic mechanism.¹⁸ Although the spectral shifts are slight, with appropriate choice of excitation and emission bands, voltage-dependent emission light intensity change is measurable and large enough for researchers to obtain signals proportional to membrane potential.¹⁸ It has also become possible to genetically encode potentiometric fluorescent proteins, a powerful technique emerging in some experimental settings.¹⁹

For optical mapping, the dyes have been designed to align parallel to the electric field across the cell membrane to maximize sensitivity to membrane potential. Dyes within the styryl family, specifically di-4-ANEPPS and di-8-ANEPPS, have emerged as popular voltage sensitive dyes, especially for blood free preparations. Styryl dyes are typically anchored in the cell membrane by hydrocarbon chains and oriented by a hydrophilic group that aligns the fluorophore perpendicular to the membrane interface and parallel to the electric field across the cell membrane.²⁰ When the fluorophore is excited by a photon of excitation light, an electric charge moves across the molecule. Potentiometric shifts of the excitation and emission spectra result because the energy required to move this charge depends on the transmembrane potential.²⁰ This family of dyes also tends to have a Stokes shift greater than 200 nm, helping improve the signal-to-noise ratio.²⁰ Figure 1 illustrates how this type of dye transduces membrane potential. A limitation of di-4-ANEPPS and di-8-ANEPPS has been the excitation wavelength range of 450–550 nm, in which endogenous molecules, such as hemoglobin, interfere

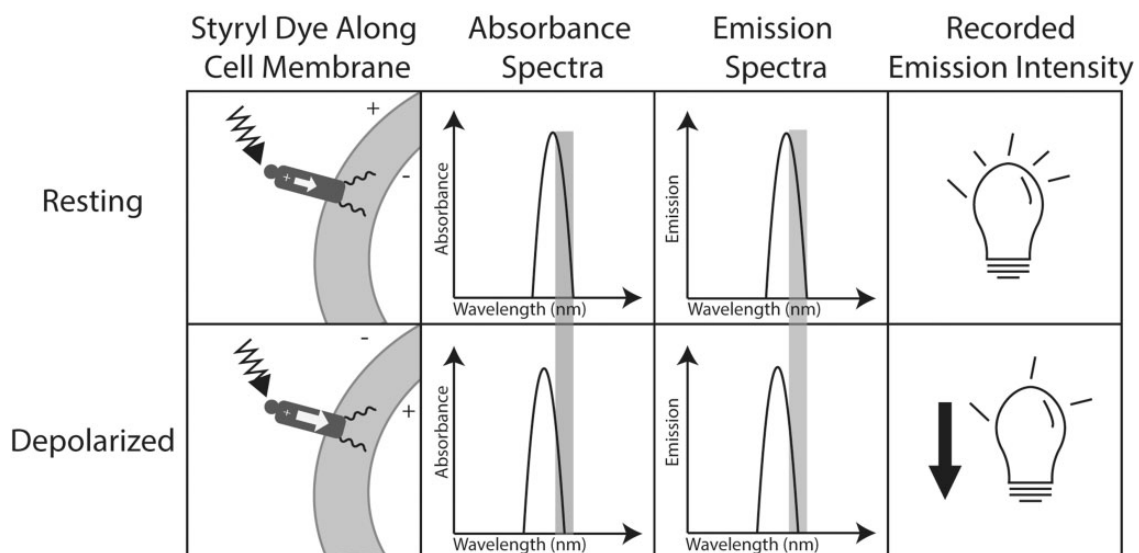


Figure 1. Schematic mechanism of dye voltage sensitivity. Column 1: fluorophores orient parallel to the membrane potential. When the fluorophore is excited by absorbing a photon, a positive charge moves across the molecule. This requires a higher energy photon when the cell is depolarized because the fluorophore's excited state is no longer stabilized by the membrane potential. Column 2: because more energy is required to excite the fluorophore when cells are depolarized, the absorbance spectrum shifts to the left (shorter, more energetic wavelengths). The wavelength of excitation light is often chosen to lie on the right flank of the absorbance spectrum. Therefore, when the spectrum shifts, less excitation light is absorbed by the stained tissue. Column 3: the Stokes shift is fixed, so the emission spectrum also shifts to shorter wavelengths when cells are depolarized. The band of emission light that is recorded is also typically chosen to lie on the right flank of the emission spectrum. Column 4: because both absorbance and emission spectra shift "out from under" the respective excitation and emission bands, less light reaches the photodetector during depolarization. A fluorescence signal recorded from a single site will therefore have inverted action potentials.

with excitation light absorption.²¹ Investigators have introduced variants of the styryl fluorophore with large Stokes shifts and excitation wavelengths greater than 700 nm.^{21–23} New dye development has enabled deeper tissue probing and optical mapping of blood-perfused preparations, including *in vivo* studies.²⁴

Mitigating motion artifact

While optical mapping offers advantages relative to electrical mapping (an older technique in which extracellular potentials are recorded from arrays of electrodes), the technique is susceptible to motion-induced artifacts when recording from tissue that moves or deforms. In optical mapping's simplest form, transmembrane potential is indirectly measured by observing the change in fluorescence relative to background fluorescence. This change is proportional to membrane potential. However, the background fluorescence varies spatially due to heterogeneities of effective dye concentration or excitation light intensity. Consequently, when tissue moves beneath a photodetector pixel, background fluorescence is not constant and simply subtracting background is insufficient to determine the potentiometric fluorescence change. Traditionally, this issue is avoided simply by arresting the movement of the heart. For this purpose, the electromechanical uncoupling agent blebbistatin is frequently used. It prevents the heart from contracting, but the electrophysiology of the heart is largely maintained.¹⁰ Other pharmacological agents, such as 2,3-butanedione monoxime, have also been used to arrest the heart's movement, but are known to alter the electrophysiology.²⁵ Although this method is useful for studying electrophysiology in isolation, the interaction between mechanical and electrical activity cannot be studied using this method. Furthermore, the use of electromechanical uncoupling agents precludes optical mapping *in vivo* unless complex methods such as cardiopulmonary bypass are used to support the animal.²⁶

A few motion artifact mitigation techniques have been developed to study the beating heart. Image registration performs post-processing on images to correct for motion artifact. To track the heart's deformation while beating, each frame of a video is transformed to match a selected reference frame. Rohde *et al.* employed affine transformation, a technique that uses linear transformations like translation, scaling, and rotation,²⁷ while others have used a variety of elastic methods to transform the target image to match the reference.²⁸ Ratiometry attempts to minimize the effects of motion artifact by taking the ratio of two signals acquired using either two excitation wavelengths (excitation ratiometry) or two emission wavelengths (emission ratiometry).^{29,30} Such schemes are designed so that the two signals have markedly different sensitivity to membrane potential. However, motion artifacts are common to both signals, so taking the ratio should preserve the voltage signal, while canceling out artifacts. In a typical excitation ratiometry design, the two bands of excitation light are on opposite flanks of the absorbance spectrum and are switched with every camera frame. A single emission band is recorded and the two signals from the two

excitation bands are temporally interleaved. In emission ratiometry, there is one excitation band, but two emission wavelengths are recorded—often with two carefully aligned cameras. The emission bands are usually chosen to be on opposite flanks of the emission spectrum. Recently Bourgeois *et al.* combined image marker tracking with excitation ratiometry to not only eliminate motion artifact but also enable the simultaneous study of bidirectional coupling of electrical and mechanical functions of the heart.¹¹ Zhang *et al.* later extended this method from tracking a limited number of discrete sites to mapping membrane potential as well as strain across a continuous region.¹⁵

Mechanics

Mechanical organ mapping has been studied in parallel to electrophysiological mapping to more deeply understand the differences in structure and dynamics between normal and abnormal hearts. Over the past century, advancement in high-speed imaging technologies permitted high spatio-temporal assessment of cardiac mechanical deformation. In the 1980s and 1990s, a series of animal experiments were carried out to quantify the mechanical deformation of the heart using bi-plane X-ray.^{31–33} The adoption of the concept of finite strain provided a powerful mathematical tool for quantifying myocardial deformation.^{32–34} Finite strain is a concept from continuum mechanics that precisely characterizes deformation in three dimensions. It requires knowledge of the motion of material points.³⁵

Currently, ultrasound tissue-Doppler speckle tracking and tagged magnetic resonance imaging (MRI) are the most prevalent techniques for evaluating cardiac mechanical function. Tissue-Doppler speckle tracking tracks speckles, formed from ultrasound beam interference, as tissue material markers while the heart is deforming over time. Speckle displacement is used to determine strain.³⁶ Tagged MRI tracks material markers created by specialized pulse-sequences and calculates strain in three-dimensions.³⁷ While both methods have high spatial resolution, they are limited by temporal resolution of 10–35 ms.³⁸ Recently, a variant of speckle tracking with higher temporal resolution has emerged. Electromechanical wave imaging, an ultrasound-based technique, divides the heart into segments that are imaged on consecutive heart beats and uses radio frequency speckle cross correlation to create strain maps with temporal resolution fine enough to track cardiac waves (~2 ms), provided the rhythm is repeatable.³⁹ Laughner *et al.* attempted to improve the spatial and temporal resolution of mechanical imaging using structured light imaging, a technique capable of 1 ms temporal resolution and spatial resolution of 87 μm .³⁸ While structured light offers higher resolution than the other methods, it does not directly measure the motion of material points, and instead uses a geometric registration approach to approximate this information. In addition, the technique does not provide transmural information while tissue-Doppler speckle tracking and tagged-MRI can.

Electromechanics

While the tools discussed above have been foundational for our knowledge of the heart as both an electrical and mechanical system, there is much to be learned about the effects of bidirectional electromechanical coupling in the heart during pathological states. In 1993, Evans *et al.* performed an early study to simultaneously image electrical and mechanical functions by combining biplane x-ray and a dense array of epicardial electrodes.¹³ More recently, developments in camera technology, motion tracking techniques, and ratiometry for motion artifact mitigation have inspired a plethora of new methods, several of which have incorporated motion tracking along with optical mapping.^{11,14,15,40} Seo *et al.* identified the relationship between strain heterogeneity and the initiation of focal excitation by applying known stretch to ventricular tissue preparations and whole hearts.¹⁴ They tracked deformation with fiducial markers and mapped electrophysiology with potentiometric dye and emission ratiometry.¹⁴ Bourgeois *et al.* built on this technique by developing a method in which membrane potential was optically acquired from tissue encircled by ring-shaped markers.¹¹ At the same time, epicardial strain was measured by tracking the relative motion of the markers.¹¹ This method used excitation ratiometry to suppress motion artifact and was implemented in whole isolated large animal hearts.¹¹ Zhang *et al.* extended Bourgeois *et al.*'s methods to increase spatial resolution by two orders of magnitude.¹⁵ Markers were tracked with a binocular camera arrangement, which enabled strain to be computed without artifact caused by motion perpendicular to the camera plane. They used a subpixel marker tracking algorithm and interpolated deformation between markers to produce continuous, high resolution maps of strain and electrical propagation. The method corrects motion sufficiently to be used in vigorously beating working hearts and has sufficient spatiotemporal resolution to image electromechanical function during complex arrhythmias.¹⁵ Figure 2 shows an example of electromechanical mapping data acquired with this system. A key limitation of these methods is an inability to measure strain and membrane potential transmurally. In 2018, Christoph *et al.* utilized ultrasound speckle tracking to image strain transmurally in isolated swine hearts.⁴¹ At the same time, they optically mapped electrical propagation on the epicardial surface using a marker-free motion correction algorithm to suppress motion artifact. They studied reentrant waves during ventricular fibrillation and using the two modalities were able to correlate mechanical "vortex filaments" within the heart wall with electrical phase singularities on the epicardium. Phase singularities are the epicardial manifestation of the intersection of a vortex filament with the heart's surface.⁴¹

Future directions

Electromechanical mapping of the intact heart has seen great advancements over the past decade. With innovations in motion artifact mitigation and the development of longer wavelength potentiometric dyes, researchers are initiating

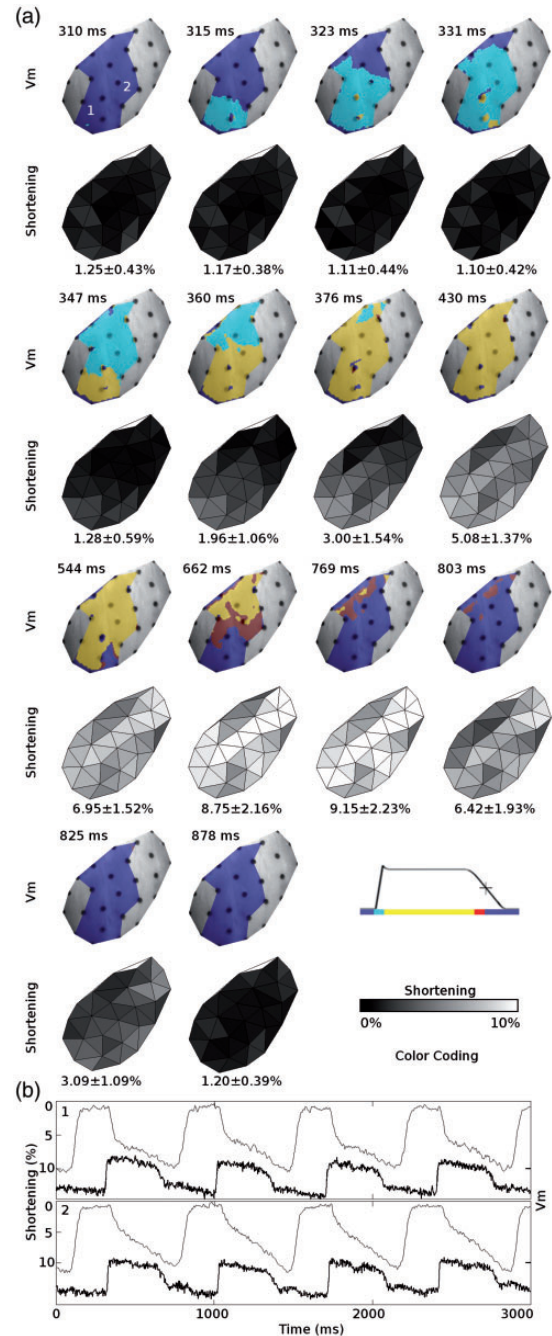


Figure 2. Electrical and mechanical functions mapped simultaneously in a beating *ex-vivo* pig heart using the method of Zhang *et al.*¹⁵ (a: upper rows) Propagation of membrane depolarization and repolarization for a single apically paced beat. (a: lower rows) The resulting contraction. The mapped region includes the anterior left ventricle. The left anterior descending coronary artery is along the top-left edge of each image and the apex is at the bottom. (b) Membrane potential (Vm, bold line) and mechanical shortening (fine line) recorded from the two sites indicated in (a). Shortening is defined as the most negative eigenvalue of the stretch tensor. Reproduced from Zhang *et al.*¹⁵ with permission.

in vivo studies as a potential platform for studying neural and endocrine influences on the heart. Lee *et al.* performed one of the first *in vivo* optical mapping studies earlier this year.²⁴ During ventricular fibrillation, when cardiac motion is not pronounced, they were able to image cardiac wavefronts using excitation ratiometry without any additional

motion tracking. Future *in-vivo* studies could incorporate mechanical mapping as well, offering a more physiologically relevant model. Many investigators have implemented dual optical mapping of membrane potential and intracellular calcium concentration.⁴² It is possible in principle to combine this with mechanical measurements. We can also expect to see electromechanical mapping applied to other electromechanical organ systems such as the gastrointestinal tract. Zhang *et al.* optically mapped gastric slow wave propagation for the first time in 2019, setting the stage for a better understanding gastric motility disorders through optical mapping.⁴³ In this study, they mapped *in vivo* swine stomach. Dye was delivered to a local region of the stomach wall through a cannula placed in the gastropiploic artery. To record in the presence of stomach wall motion, which was not suppressed, they used a variation of Zhang *et al.*'s cardiac mapping method¹⁵ that employed both fiducial marker tracking and excitation ratiometry to minimize motion artifact. We expect further developments will be made to simultaneously map the electromechanics of *in vivo* models and in additional organ systems.

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