Optical Imaging of the Heart

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Abstract—Optical techniques have revolutionized the investigation of cardiac cellular physiology and advanced our understanding of basic mechanisms of electrical activity, calcium homeostasis, and metabolism. Although optical methods are widely accepted and have been at the forefront of scientific discoveries, they have been primarily applied at cellular and subcellular levels and considerably less to whole heart organ physiology. Numerous technical difficulties had to be overcome to dynamically map physiological processes in intact hearts by optical methods. Problems of contraction artifacts, cellular heterogeneities, spatial and temporal resolution, limitations of surface images, depth-of-field, and need for large fields of view (ranging from $2 \times 2$ mm$^2$ to $3 \times 3$ cm$^2$) have all led to the development of new devices and optical probes to monitor physiological parameters in intact hearts. This review aims to provide a critical overview of current approaches, their contributions to the field of cardiac electrophysiology, and future directions of various optical imaging modalities as applied to cardiac physiology at organ and tissue levels. (Circ Res. 2004; 94:21-33.)

Key Words: optical mapping • fluorescent probes • electrophysiology • arrhythmia • defibrillation

Mammalian physiology has an ingrained hierarchy with molecular and cellular physiology at its base, followed by the interactions of large populations of cells and organ systems, and finally the integration of multiple organ functions of an entire animal. For the past 4 decades, cardiovascular physiology has been dominated by a “reductionist” approach, focusing on cellular mechanisms. Major strides have been accomplished in our understanding of cellular mechanisms, including metabolism, intracellular signaling, trafficking, ion channel structure, function, and expression. With a greater understanding of cellular mechanisms came the growing realization that organs such as the heart are composed of several types of interacting cells with significant and important heterogeneities of properties, cell-to-cell coupling, and function within each group. Thus, an understanding of molecular and cellular mechanisms must still be integrated to explain the more complex organ system while taking into account spatial and temporal heterogeneities of cell functions throughout the organ.

Unfortunately, experimental methodologies available for studies at the organ level are not as abundant as at the cellular scale. Nonoptical imaging modalities, including positron emission tomography, magnetic resonance, and ultrasound imaging have only started to bridge molecular and organ physiology using novel contrast agents. On the other hand, optical modes of imaging, in combination with parameter-sensitive probes have already demonstrated their ability to overcome the problem of spatiotemporal resolution in two dimensions for a wide range of applications from single molecular events to in vivo whole animal physiology.

Fluorescence has been used to measure a wide range of physiological parameters in cells and tissues. For instance, cellular metabolic state can be monitored through (1) the intrinsic fluorescence changes of NADH or flavoproteins;
(2) the differential absorption changes of mitochondrial cytochromes, which report on the oxidative phosphorylation redox state, or (3) the oxygen content of blood and cardiac muscle through the absorption changes of oxy to deoxy hemoglobin and myoglobin, respectively. More recent has been the development of probes to selectively measure functional parameters such as membrane potential, intracellular concentrations of free calcium, magnesium, sodium and potassium, pH, nitric oxide, oxygen tension, and sulfhydryl redox sate. Of these, optical probes of membrane potential and intracellular free calcium (\( [Ca^{2+}] \)) indicators have had the most impact in cardiovascular physiology. The development of optical recordings of membrane potential was driven by the need to overcome many obstacles in electrophysiology and the promise of a technology “for measuring membrane potential in systems where, for reasons of scale, topology, or complexity, the use of electrodes is inconvenient or impossible.” Specific to cardiac electrophysiology, there was also the need to record transmembrane voltage changes during or immediately after the firing of electric shocks used for defibrillation. Optical mapping techniques and potentiometric probes have now made major contributions to our understanding of nerve network behavior and cardiac electrophysiology in ways that could not have been accomplished by other approaches. Advances in neuroscience driven by optical mapping have been extensively reviewed. Although there is considerable overlap in the instruments used for optical mapping of neuronal and cardiac tissues, there are also important differences, and this review will focus on instrumentation, optical probes, major findings, and future directions of optical imaging as it applies to the heart.

**General Principles**

Optical imaging modalities are based on physical principles of wavelength-dependent light-tissue interaction, including photon scattering, total internal reflection, absorption, reflectance, and fluorescence. The physical interactions of photons with tissue, namely, intrinsic tissue absorption and light scattering, limit the depth of penetration and spatiotemporal resolution of images that can be obtained from bulk tissue. However, recent advances in optical contrast agents, optical probes of physiological parameters, light transport theory, light sources, and optical detectors have created conditions for major breakthroughs in optical imaging at the organ level. Novel imaging modalities with expanding areas of application have emerged, including optical diffuson tomography, optical coherence tomography, and various confocal fluorescence imaging approaches. These new technologies have encouraged the development of new fluorescence or absorption contrast agents and probes of physiological parameters, and the combination of new instruments and probes will most likely propel a new era of multidimensional and multiparametric imaging in organ system physiology.

The ionic basis of cellular electrophysiology, intercellular coupling, and electrical propagation are understood with considerable detail. Although intracellular and patch-clamp microelectrode techniques have been used to elucidate the physiology of excitable cells, an understanding of the behavior of cardiac tissues have been more difficult to attain by conventional electrode techniques. More than 30 years ago, investigators discovered molecular probes that bind to the plasma membrane of neuronal and cardiac cells and exhibited changes in fluorescence and/or absorption that tracked changes in transmembrane potential.

Several optical properties of membrane-bound dyes have been used to measure membrane potential changes; namely, fluorescence, absorption, dichroism, birefringence, fluorescence resonance energy transfer, nonlinear second harmonic generation, and resonance Raman absorption. However, most studies in cardiac cells or tissues have relied on the fluorescence mode that tends to yield higher fractional changes in signal compared with the other modes and because fluorescence signals tend to be considerably less sensitive to movement artifacts generated by muscle contractions.

Several mechanisms have been proposed to explain voltage-dependent changes in fluorescence and/or absorption of dye molecules based on interactions of the electric field with dye molecules resulting in intra- and extramolecular rearrangements of the dye in the membrane. Cohen and Salzberg introduced a simple classification of voltage-sensitive dyes into two groups, fast and slow dyes, based on their response times and presumed molecular mechanism of voltage sensitivity. Only the fast probes are used in cardiac electrophysiology, due to their ability to follow voltage changes on a time scale of microseconds. The precise mechanisms underlying the voltage-dependent spectroscopic properties of fast voltage-sensitive dyes are still not fully understood. Spectral shift in the properties of chromophore is thought to be related to the changes in excitation-induced intramolecular reorientation of electronic charge along the electric field gradient (electrochromic theory) or to electric field-induced reorientation of the dye molecule in the plasma membrane (solvatochromic theory).

**New Molecular Probes for Optical Recordings of Electrical Activity**

In tests of over 1500 different compounds, several useful classes of chromophores have emerged, including merocyanine, oxonol, and styryl dyes. Styryl dyes represent the most popular family of dyes; RH-421 and di-4-ANEPPS being the most important members of this family. The spectroscopic properties of these dyes have been shown to linearly change with membrane potential changes in the normal physiological range of transmembrane voltages.

Initial studies were performed with a class of molecules called merocyanine dyes, which exhibited 1% fractional changes in fluorescence in cardiac tissue. As shown in Figure 1A, action potentials were simultaneously recorded through the fluorescence changes of the potentiometric dye and with an intracellular microelectrode. The fluorescence action potentials were recorded from frog ventricular tissue stained with merocyanine 540 from a 2-mm diameter excitation spot and compared with microelectrode recording from one of the cells excited by the incident light beam. The two techniques show excellent correlation between the two signals. In the intact heart, surface or volume electrograms are also in excellent correlation with optical recordings. But unlike electrode recordings, optical signals can be easily...
recorded from different regions of the heart by simply displacing the optical paths. Figure 1B shows examples of optical action potentials from different regions of atrial and ventricular rabbit myocardium.

Early studies faced marked phototoxic effects of potentiometric probes in some preparations. These initial obstacles were resolved through the development of better probes with reduced phototoxic effects and greater sensitivity to potential changes. A.S. Waggoner, L.A. Ernst, and G. Salama (unpublished data, 2003) have recently developed and tested new potentiometric dyes in search of probes with greater fractional fluorescence changes per action potential and longer peak excitation and emission wavelengths, with large Stokes shifts. The longer wavelengths would be particularly desirable for optical recordings from deep inside the myocardial wall as light absorption and scattering diminishes with longer wavelengths. One of these new dyes, PGH1 (Pittsburgh 1), was found to yield excellent signal-to-noise ratio and stability when applied to the whole heart with an excitation wavelength of 690 nm and an emission at 850 nm.

Although initial tests of PGH1 by several groups were highly positive in heart (B.-R. Choi, University of Pittsburgh, PA, and R.A. Gray, University of Alabama), experiments on isolated cells reported up to 30% fractional fluorescence change per action potential, but at a high level of photobleaching or phototoxicity (E. Entcheva, Stony Brook University, G. Smith, University of Glasgow, personal communication). Further modifications of successful potentiometric probes and better staining procedures are likely to yield improvements in voltage sensitivity, depth penetration of the signals, and the opportunity to detect increasingly smaller potential changes from smaller areas of membrane stained with dye.

Another significant limitation of optical mapping of the heart is artifact introduced by the muscle contractions. These “movement” artifacts distort optical action potentials, preventing accurate recordings of repolarization phase. Several methods have been used in the past to minimize the effect of movement artifact. Mechanical restriction of the movement can successfully restrict the artifact without affecting physiology of the heart. This method works particularly well with small hearts such as mice, rats, and guinea pig. Alternatives include various pharmacological approaches, such as calcium channel blockers, 2,3-butanedione monoxime, or cytochalasin D. Another approach to reduce movement artifacts is to apply a ratiometric technique. This method is based on simultaneous measurements of fluorescent signal at two different wavelength ranges: one where the dye exhibits a largest voltage-dependent response and the other at a wavelength where the potentiometric dye exhibits an inverted or no voltage-dependent response. The ratio or difference of these two signals would then result in an optical signal free of, or with significantly reduced, motion artifacts. Unfortunately, this method works well with relatively weak contractions and with biphasic action spectra of fluorescent and/or absorption dyes.

Calibration of Optical Recordings

It is important to note that fast voltage-sensitive dyes do not provide an absolute measurement of transmembrane potential but merely track the changes in membrane potential with high temporal fidelity. Initial studies calibrated optical action potential by simultaneously recording fluorescence and microelectrode signals from the same region of the heart. The linearity and kinetics of the voltage-dependent merocyanine dye signals were characterized in heart muscles under sucrose-gap voltage clamp conditions. More recent studies have concentrated on application of ratiometry approach for quantitative measurements of transmembrane potential with newer and better dyes. Initially these methods were developed on neuroblastoma cells. The early experiments have not been repeated to confirm the linearity of these dyes in artificial planar bilayers or cardiac cells. Simultaneous ratiometric optical and microelectrode recordings of transmembrane potential in perfused hearts confirmed excellent correlation and linearity of optical recordings.

Need for Optical Mapping

The spread of electrical activity is important for our understanding of the mechanisms responsible for the normal cardiac rhythm and for the initiation and maintenance of arrhythmias. Although much has been learned regarding the ionic basis of the cardiac action potential using intracellular microelectrodes, single cell impalements cannot be practically used to simultaneously record action potentials from hundreds of recording sites. The generally used methods to map activation and repolarization are based on surface unipolar and bipolar electrograms measured with arrays of electrodes. Although surface electrodes can describe the spread of excitation and repolarization, interpretation of data in some cases is uncertain. For instance, activation sequences were difficult to interpret during rapid synchronous depolarization, as after electric shock application, and during slowly changing, low-level depolarization, as in ischemia. Repolarization measured with an electrogram often does...
not coincide with the actual repolarization at the recording site.31,32

Progress in Imaging Technology: Advances in Light Detectors

Current imaging technology presents several approaches for fast imaging, including photomultipliers (PMT), laser scanning,33 charge-coupled device (CCD) cameras, and photodiode arrays (PDA). Currently, only CCD cameras and PDA detectors are predominantly used in heart imaging applications.19,34–37 Complementary metal-oxide semiconductor (CMOS) cameras represent another emerging candidate. Combination of PMT devices with a laser scanning technique failed to overcome spatiotemporal limitations imposed by sequential nature of recordings.38,39

PDA technology was first developed by Centronix.40,41 Now PDAs are available from Hamamatsu. WuTech42 produces custom arrays of arbitrary size and shape made of individual photodiodes.43

CCD technology has a significant advantage of higher spatial resolution due to the large number of pixels on a CCD sensor. However, the rate of data acquisition is usually lower. This can be increased by pixel binning; however, this defeats the major advantage of CCD technology because binning reduces spatial resolution. For example, a camera from DALSA has 128×128 pixels at 490 frames/sec.

Theoretically, CCD technology should provide significant advantages with respect to signal-to-noise ratio, particularly at low-light applications, due to the right combination of high quantum efficiency and low background noise level. It is important to emphasize that an optical mapping experiment on a whole heart or a multicellular preparations tends not to be light-limited but deals with conditions where the greater the fluorescence intensity from the preparation, the greater the absolute amplitude of the fractional fluorescence change. The dynamic range of CCD cameras is constrained by the accuracy of A/D conversion and the saturation of the sensor at light levels readily detected from heart tissue; a dynamic range of 10$^3$ is not easily achieved. Thus, for practical purposes the majority of single cell and cell culture optical mapping studies has been done using PDA.43–45

Improvements in CMOS technology produced a family of novel image sensors with high-speed image acquisition while retaining the quantum efficiency of CCD. MiCAM Ultima CMOS camera from SciMedia was recently tested on a perfused guinea pig heart at 10 000 frames/sec and 100×100 pixels. High signal-to-noise ratio allowed detection of activation times from the first derivative of optical signals during ventricular fibrillation without spatial averaging. CMOS cameras are more costly than CCD or PDA cameras. However, due to clear advantages these CMOS cameras will become competitive soon.

Design of Optical Mapping System

Any optical imaging system consists of a 2D optical sensor and a stable light source, such as a laser or DC-powered tungsten-halogen lamp, mercury source, or light-emitting diodes. Figure 2 describes a typical single-lens design. It consists of a PDA, 256-channel signal conditioner/amplifier, analog-to-digital converter, and a computer. An excitation beam is passed through 520±30-nm interference filters and focused on the surface of a perfused preparation placed in a tissue chamber. A camera lens collects the fluorescence light, directs it to a 610-nm long pass filter and focuses an image of a chosen region of the heart on the surface of PDA.

Mapping of Activation and Repolarization

Figure 3 shows a map of action potentials optically recorded from the anterior surface of a Langendorff-perfused guinea pig heart. An optical trace at the right illustrates that the quality of optical recordings approaches that of the gold standard: microelectrode recordings. When movement artifacts are a concern, activation and repolarization times can be still be determined by calculating the maximum first derivative of the action potential upstroke and the maximum second derivative of the downstroke, respectively19 (Figure 3). Alternatively, these times could be determined from the time point at which the upstroke reaches 50% or recovers to 90% of its maximum amplitude,66–47 respectively. Recently, phaselocking techniques were introduced for an analysis of quasiperiodic electrical activity in the heart.48 These techniques determine the specific phase of electrical activity that corresponds to each moment of the action potential. The algorithms used are based on analysis of phase-space trajectories, which result from a plot of a measured variable (optical action potential) against a transform of the same variable (time-delay,48 first derivative, Hilbert transform49). Activation and repolarization data are usually presented as isochronal or isochronal maps (see Figure 3) or as a vector field of conduction in the cardiac muscle (see Figure 3).53,54 allowing quantitative assessment of spread of activation and repolarization in highly anisotropic heart muscle under normal and pathological conditions.55–56
Conventional high-resolution multielectrode mapping was successful in assessing activation sequences. However, repolarization was generally beyond the capabilities of electrode mapping. Only a monophasic action potential (MAP) technique was able to reliably measure repolarization and action potential duration. However, MAP cannot be used for multisite mapping. Optical mapping provides a tool with the unique ability to overcome limitations of electrode-based techniques and to reliably and faithfully record action potentials. However, it typically requires the use of mechanical or pharmacological immobilization techniques to suppress the motion artifacts.

In particular, optical mapping has made a significant contribution to our understanding of the role of intrinsic myocardial heterogeneities in the normal repolarization processes in normal heart; of dynamic concordant and discordant alternans in the onset of ventricular tachyarrhythmias; and the role of the slope of the restitution curve in the transition from ventricular tachycardia to fibrillation.

Mapping of Stimulation and Defibrillation
Optical mapping is an especially powerful tool in studies of electrostimulation therapy. Due to overwhelming stimulus-induced artifacts, the conventional electrode techniques are not able to record electrical activity during and after stimuli. In contrast, optical recordings provide an accurate account of transmembrane potential changes during stimulation and defibrillation. Dillon demonstrated the prolongation of action potential duration by strong electric shocks applied during the refractory period. Optical mapping explained the mechanisms of epicardial unipolar and bipolar pacing. Optical mapping also provided the experimental basis for the new theory of stimulus-induced arrhythmogenesis and the related theory of success and failure of defibrillation based on virtual electrode effects, which are also known as secondary source effects. Figure presents experimental evidence of a virtual electrode-induced phase singularity mechanism of shock-induced arrhythmogenesis. Optical recordings for the first time allowed faithful dynamic registration of maps of transmembrane potential before, during,
and after shock application. Figure 4B illustrates the typical pattern of virtual electrode polarization mapped as a distribution of transmembrane potential seen immediately after the application of a shock. Virtual electrode patterns are characterized by the presence of both positive and negative polarizations, which are induced by the virtual cathode and anode, respectively. As a result, a shock-induced wavefront of break-excitation can form a reentrant pattern around the virtual electrode-induced phase singularity.66,69,70

Mapping of the Impulse Generation and Conduction System of the Heart

Optical mapping has made a significant contribution to functional studies of the fundamental mechanisms of impulse generation in the SA and AV nodes, and impulse propagation in the conduction system of the heart. Kamino’s group pioneered optical mapping techniques in the brain and heart, investigating the genesis of spontaneous electrical activity in the embryonic heart.71–74 Optical mapping was also instrumental in the identification of nonradial spread of activation via preferential pathways from the SA node toward the AV node.75,76 Our groups applied optical mapping to studies of AV nodal conduction,22,76,77 AV nodal reentrant arrhythmias,78,79 and AV junctional rhythm.80 Finally, Morley and colleagues54,56 pioneered the application of optical mapping to investigate mechanisms of conduction in the Purkinje system, which was unattainable by electrode-based techniques.

In addition to contributing to our understanding of mechanisms of impulse generation and conduction, these studies demonstrated the ability of optical techniques to assess electrical activity in three dimensions. Figure 5 presents an example of optical (OAP) and microelectrode (MAP) action potential recordings and histology from the distal AV junctional area of the rabbit heart. Figure 5B shows histology of this area in another preparation from the apical area of the triangle of Koch,80 which clearly shows multilayered morphology of the AV junction. One can see the thin layer of AN transitional tissue (○), the compact AV node (●), and loosely coupled deeper layer of NH transitional cells. Optical action potentials (OAPs) recorded during conducted beats contained two components, representing electrical activity in the two layers. OAPs recorded during AV block contained only one component. Microelectrode recordings conducted simultaneously provides evidence that the first component of optical recording corresponds to the superficial transitional AN layer (see ○, in Figure 5A and 5B) of the AV junction, whereas the second component represents electrical activity of the deeper NH layer (● in Figure 5A and 5B).

Mapping of Atrial and Ventricular Tachyarrhythmias

Optical mapping techniques presented a unique opportunity to study the mechanisms of supraventricular and ventricular
arrhythmias because of unprecedented spatiotemporal resolution and the ability to map all phases of electrical activity, including activation and repolarization.

Jalife’s group pioneered the application of optical mapping to study arrhythmogenesis and made numerous significant contributions to our understanding of mechanisms of both atrial and ventricular arrhythmias. Since that time, many groups presented optical mapping data supporting reentrant nature of ventricular tachycardia and fibrillation. Furthermore, optical mapping presented evidence for 3D nature of ventricular reentry, which is sustained by scroll waves.

Despite the success of dynamic optical imaging of wavefronts and phase singularities during arrhythmias, there is still no agreement on the mechanisms that induce and sustain ventricular and atrial fibrillation. Two competing dominant theories are being tested: the so-called “mother rotor” and “break-up” hypotheses. According to the mother rotor hypothesis, ventricular fibrillation is maintained by a single or limited number of leading centers of reentrant nature, ie, mother rotors. These centers occupy regions of myocardium that are capable of sustaining the highest possible frequency of reentrant activity. Disorganized activity observed throughout the heart, in regions beyond those of the mother rotor(s) results from conduction blocks between the regions encompassed by the mother rotor and regions with longer refractory periods. In contrast, the break-up theory proposes that fibrillation is perpetuated by dynamic or anatomic heterogeneities in the myocardium sustained through constant creation and annihilation of waves. Profound complexity of conduction patterns during fibrillation makes quantitative analysis of wavefronts and wavebreaks a formidable task. Therefore, frequency analysis of optical data was adopted following the pioneering work of Wiggers.

As presented in Figure 6A, according to one school of thought, during fibrillation, dominant frequencies of electrical activity are distributed in a stationary pattern. This appears to support the mother rotor hypothesis. On the other hand, as presented in Figure 6B and 6C, another group of investigators presents evidence of constantly changing pattern of frequencies of electrical activity without clear dominant frequency. This issue remains controversial as evidence in support of both theories continues to appear in the literature.

Multiparametric Optical Mapping: Imaging of Voltage and Intracellular Calcium

Calcium cycling is arguably the single most important component of cardiac excitation-contraction coupling. The action potential elicits an influx of Ca²⁺ through the activation of L-type voltage-gated Ca²⁺ channels that trigger a release of Ca²⁺ from intracellular stores called the sarcoplasmic reticulum (SR) resulting in a contraction. Ca²⁺ release from the SR occurs via Ca²⁺ release channels or ryanodine receptors that are activated by a local elevation of intracellular Ca²⁺ ([Ca²⁺]i) by a process called Ca²⁺-induced Ca²⁺ release (CICR). Normally, depolarization triggers [Ca²⁺]i transients, but in pathological conditions, abnormalities in [Ca²⁺]i handling may activate Ca²⁺-dependent currents that influence the time course of the AP and trigger a spontaneous membrane depolarization. Abnormalities in [Ca²⁺], handling have been implicated as the underlying mechanism in a number of pathologies that promote arrhythmias such as ischemia, reperfusion-arrhythmias, the generation of early and delayed afterdepolarizations, and torsades de pointes that occurs in patients with the long QT syndrome. [Ca²⁺] overload has been implicated in triggering electromechanical alternans and in increasing the steepness of APD restitution curves, which are both associated with promotion of arrhythmias. Thus, one cannot overstate the importance of simultaneous measurements of APs and [Ca²⁺], transients in intact hearts to address fundamental questions regarding the spatiotemporal relationship of voltage and [Ca²⁺], and their interplay in arrhythmias.

The first techniques for measuring changes in cytosolic [Ca²⁺], concentration of coronary-perfused hearts involved
19F nuclear magnetic resonance,\(^{106,107}\) fluorescent Ca\(^{2+}\) indicator Indo-1,\(^{108}\) and a bioluminescent Ca\(^{2+}\) indicator aequorin.\(^{109}\) Later, other fluorescent Ca\(^{2+}\) indicators (Fluo 3\(^{110}\) and Rhod 2\(^{111,112}\)) were successfully used for heart imaging. Simultaneous Ca\(^{2+}\) and voltage imaging of the heart has also been achieved by costaining with RH421 and Rhod-2,\(^{113}\) RH237,\(^{59,114}\) with RH237 and Fluo-4/Oregon Green BARTA 1,\(^{25}\) with di-4-ANEPPS and Fluor3/4,\(^{115}\) and with di-4-ANEPPS and Indo-1.\(^{116}\)

Figure 7 shows the optical system for simultaneous recording voltage and [Ca\(^{2+}\)]\(_i\) signals from perfused hearts stained with RH237 and Rhod-2.\(^{59}\) It uses two PDAs (Figure 7A). RH237 and Rhod-2 dyes can be excited at the same wavelength but fluoresce at different wavelengths, allowing the separation of the \(V_m\) and [Ca\(^{2+}\)]\(_i\) signals. Rhod-2 was found to be an excellent Ca\(^{2+}\) indicator for perfused hearts because of its rapid association/dissociation with Ca\(^{2+}\), fast loading into myocytes of perfused hearts, and long-term stability or low levels of exocytosis.\(^{59}\) The independence of \(V_m\) and [Ca\(^{2+}\)]\(_i\) recordings is illustrated in Figure 7C through 7E. First, signals from both \(V_m\) and [Ca\(^{2+}\)]\(_i\) arrays were recorded when the heart was stained with one of the two dyes but not both dyes. Second, after staining with both dyes, \(V_m\) and [Ca\(^{2+}\)]\(_i\) signals were recorded before and after the addition of ryanodine, known to markedly inhibit [Ca\(^{2+}\)]\(_i\) transients.

Multiparametric optical mapping is still in its infancy. Other probes are being synthesized and evaluated for simultaneous recordings from the same tissue, stained with multiple fluorescent probes. This exciting progress is likely to bring about a better understanding of cellular physiology at a tissue and organ level.

Mapping Transgenic Mouse Models
Molecularly engineered mice are increasingly used to genetically alter a specific component of a complex signaling process and to develop models of human diseases. Transgenic and knockout mice are used as models for various cardiac diseases and offer an effective strategy to elucidate the mechanisms underlying arrhythmias, metabolic diseases, the pathology of heart failure, and altered ion channel and gap junction expression.\(^{117}\) A limitation of mouse models is the rapid heart rate and small size of the heart, which makes it difficult to study changes in contractility, electrophysiology, and arrhythmias vulnerability. The challenge of studying cardiac phenotypes in mice has been effectively tackled by optical mapping of action potentials and [Ca\(^{2+}\)]\(_i\) transients.

Dominant-negative transgenic mice that overexpress an N-terminal fragment of the K\(^+\) channel Kv1.1 were shown to exhibit prolonged action potential duration due to the loss of a slowly inactivating 4-aminopyridine sensitive current, \(I_{slow}\).
which is likely to be encoded by Kv1.5. These mice were found to have long-QT intervals, spontaneous nonsustained ventricular tachycardia (VT) during ambulatory telemetry monitoring and inducible polymorphic VT during programmed stimulation in anesthetized open chest preparations. Optical mapping demonstrated a 2-fold increase in action potential durations and enhanced dispersion of repolarization from apex to base in dominant-negative transgenic mice. In dominant-negative transgenic mice, a premature impulse applied at the apex of the heart produced sustained reentrant VT that did not occur with stimulation at any location in controls hearts. Direct injection of adenoviral vectors expressing wild-type Kv1.5 (AV-Kv1.5) in the myocardium of these Kv1 dominant-negative transgenic mice resulted in the overexpression of Kv1.5, a shortening of QT interval, decreased dispersion of repolarization, and increased the heart rate. These changes were consistent with a physiological reversal of the arrhythmogenic phenotype by the adenoviral induced expression of Kv1.5 in Kv1 dominant-negative mice. Transgenic mice overexpressing the inflammatory cytokine tumor necrosis factor α (TNF-α) develop a progressive heart failure (HF) phenotype characterized by biventricular dilatation, decreased ejection fraction, and ventricular arrhythmias on ambulatory telemetry and decreased survival compared with control litter mates. Optical maps of action potentials and [Ca^{2+}]i transients showed that TNF-α hearts had prolonged action potential durations, no change in dispersion of repolarization, elevated diastolic, depressed systolic, and prolonged [Ca^{2+}]i, compared with controls. Premature beats had lower action potential amplitudes, slower conduction velocities, and elicited reentrant beats. Increasing heart rate produced [Ca^{2+}]i alternans in TNF-α but not in control hearts. In this model, anomalies of both AP and [Ca^{2+}]i contributed to arrhythmias.

Gap junction channels are essential for cell-to-cell coupling and impulse propagation. Changes in channel conductance may underlie the development and maintenance of lethal arrhythmias in pathological conditions. Null mutations of connexin 43 (Cx43) in mice, the predominant gap junction channel proteins in ventricular tissue, was lethal in homozygous (Cx43−/−) mice but seemingly normal in heterozygous (Cx43+/−) mice. Measurements of conduction velocity from the same line of Cx43+/− mice produced conflicting results depending on the optical mapping technique. Using a CCD camera, Morley et al. found no significant difference in conduction velocity between heterozygous Cx43+/− mice and controls, whereas Eloff et al. found a 23% to 35% slowing of conduction velocity using a photodiode array (PDA) to map action potentials. The different findings perhaps reflect the differences in temporal resolution of the CCD and PDA technology because the same line of Cx43+/− mice was used in both studies.

Mapping Developing Myocardium
In embryonic hearts and neonatal cell cultures, the ability to optically record simultaneously from multiple sites is making important contributions to our understanding of the developing heart. Optical mapping was applied to study the patterning and organization of the conduction system and working myocardium in the developing avian and mammalian hearts. Optical mapping of action potentials and [Ca^{2+}]i, in combination with patterned cultured neonatal rat mouse and genetically modified mice is bound to make major contributions to our understanding of propagation in the adult and developing heart. Optical mapping has contributed to investigation of the important role of mechano-electrical coupling in shaping the working myocardium and the conduction system of chick heart. Combination of optical mapping of electrical activity with structural 3D mapping of developing hearts is especially exciting new opportunity.

Emerging Optical Imaging Modalities
Imaging with voltage-sensitive probes has several limitations. Pharmacological effects of the dye include phototoxicity138 and increased contractility. Yet, arguably, the major limitation of organ-level optical imaging is its restricted depth of penetration.

To overcome the depth limitation, Hooks et al. developed an elegant optrode-based method. This method extends the idea of a plunge needle electrode. Plunge optrodes present a unique possibility for multisite intramural recordings of transmembrane potential, yet cause damage of the myocardium.

Depth restriction is due to light absorption and scattering on intrinsic differences of tissue optical properties (endogenous contrast). Absorption occurs primarily at endogenous chromophores of hemoglobin, melanin, fat, and water. Scattering is usually due to refractive index differences of extracellular and intracellular structures. These properties are strongly wavelength-dependent. Baxter et al. proposed an original method that takes advantage of this wavelength dependence to visualize excitation waves within the cardiac muscle. Light in the 700- to 900-nm range is known as the “therapeutic window,” due to low intrinsic tissue absorbance and high scattering of photons dominates in this range of wavelengths. This window opened an opportunity for tomographic reconstruction of 3D structures using novel biophotonics imaging modalities, such as optical coherence tomography.

Optical coherence tomography (OCT) exploits the heart tissue scattering heterogeneity and at present can reconstruct through 2- to 3-mm depth of cardiac tissue with up to 1-µm resolution. The latter characteristics make tomography a method of choice for in situ analysis of embryonic heart morphology. AV nodal multilayer structures (Figure 8), complex 3D geometry of trabeculated structures of myocardium and the Purkinje network could directly resolve the 2D limitations of conventional optical mapping. Alternatively, 3D limitations could potentially be addressed with confocal, Nipkow disk, or Ronchi grating approaches. Another promising direction relies on the possibility of solving the inverse problem for light diffusion/scattering in the tissue. This could numerically separate the optical signal collected from the heart surface into individual components related to signals from the different layers of the tissue.
Conclusion

Optical imaging of cardiac electrical activity at the tissue and organ levels has emerged as powerful novel approach during the last decade and has made a significant contribution to cardiovascular research. Exciting new developments in biophotonics suggest that the best is yet to come. The next decade is likely to yield (1) novel optical molecular probes for multiparametric optical sensing of various biological parameters, processes, molecules, proteins, and their functional states in real time with submillisecond resolution and (2) novel optical imaging modalities for 3D optical interrogation of molecular probes with precise anatomical localization of the signal origin with subcellular spatial resolution.

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