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Computational Modeling of Cardiac Dual Calcium-Voltage Optical Mapping

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Abstract—**Optical imaging allows mapping the complex spatiotemporal dynamics of transmembrane potential and intracellular calcium in cardiac tissue. Several studies have shown that the epi-fluorescent optical action potential contains contributions from the sub-epicardium, owing to scattering of photons in tissue. Hybrid electro-optical models have allowed careful quantification of these scattering effects and have lead to a better interpretation of the optical action potential. However, until now, these effects have not been investigated for optically recorded calcium transients. Here, we develop a hybrid model of cardiac dual calcium-voltage epi-fluorescence mapping. This model allows simulating both optical action potentials and optical calcium transients and investigating the effects of photon scattering on their synthesis. We find that optical calcium transients contain contributions from sub-epicardial layers up to 0.8 mm below the epicardium. These lead to significant differences in rise time and activation times between the optically acquired calcium signal and the epicardial intracellular calcium concentration. As has been the case with optically recording action potentials, these results should be taken into account in the interpretation of experimental optical measurements of intracellular calcium.**

I. Introduction

Every contraction of the heart is triggered by a propagating action potential. Intracellular calcium cycling plays a crucial role in excitation-contraction coupling in cardiac cells and changes in intracellular calcium (Ca_i^{2+}) and transmembrane potential difference (V_m) are intricately related [1]: on the one hand, the action potential triggers a calcium-influx leading to a release of calcium from the sarcoplasmic reticulum resulting in contraction, whereas on the other hand, Ca_i^{2+} affects the time course of V_m through various calcium-sensitive ionic currents. Abnormalities in spatiotemporal dynamics of excitation and Ca_i^{2+} handling have been implicated in life-threatening arrhythmias, such as ventricular fibrillation [1, 2]. Measurement of V_m and Ca_i^{2+} in the heart is therefore important in understanding normal cardiac excitation and mechanisms underlying arrhythmias.

Optical imaging utilizing fluorescent probes allows for mapping cardiac activity over large areas of the heart [3]. Although initially primarily developed for the visualization of propagating action potentials using voltage-sensitive dyes, it has also been applied to the study of intracellular calcium using a variety of fluorescent calcium indicators. More recently, significant efforts have been dedicated to the simultaneous use of voltage-sensitive and calcium dyes excited at a common wavelength, but whose fluorescence can be well separated [4]. This allows mapping the complex interplay between V_m and Ca_i^{2+} during various conditions and several experimental studies based on this approach have shed new light on the mechanisms underlying arrhythmias (e.g. [5]).

Cardiac dual voltage-calcium optical mapping has so far been used in epi-fluorescence mode, where the epicardial, surface of the heart is uniformly illuminated at the excitation wavelength and the fluorescence is collected at the appropriate wavelengths from this surface using CCD-cameras or photodiode arrays [3, 4]. In epi-fluorescence mapping of electrical activity using voltage-sensitive dyes, it has been known for over a decade that the optical signal not only originates from the epicardium, but from a sub-surface volume that can extend as much as 1 mm below the epicardial surface depending on the optical scattering and absorption properties of cardiac tissue [6].

Optical scattering was found to have profound implications on the shape of the optical action potential, especially its upstroke [7]. Hybrid computational models, coupling bio-photonic to electrophysiological models of cardiac tissue, allowed to carefully quantify the depth averaging effects on the optical action potential and significantly improved our interpretation of experimental optical mapping data [8]. However, although similar effects are to be expected for optically recorded calcium transients, these have not yet been quantified.

The main aim of this study was therefore to investigate the optical scattering effects on optical calcium transient recorded in epi-fluorescence mode from slabs of ventricular tissue. To achieve this aim we developed a novel hybrid computational model of dual voltage-calcium mapping and simulated optical action potentials and calcium transients for the voltage-sensitive dyes RH-237 and two different calcium indicators (Rhod-2 and Rhod-FF), respectively.

II. Methods

A. Bio-photonic model

Cardiac tissue displays spectral sensitivity to light due to absorption and scattering of photons: shorter wavelengths penetrate less deep than longer wave lengths. Absorption and scattering not only reduce the depth penetration of light, but also result in optical blurring, especially in wide-field imaging: the optical signal recorded at a single surface location is a weighted average of contributions from a sub-

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surface volume, extending both laterally and in depth from the recording site. Optical diffusion theory has been used in several recent studies to calculate light penetration depths at various wavelengths [8]. Using the diffusion approximation one can derive analytical expressions for the excitation fluence inside tissue Φ_e and the fluorescence point-spread function Γ in the slab geometry and for given boundary conditions. Φ_e describes how well excitation light penetrates inside cardiac tissue, whereas Γ represents the fluorescence recorded from the surface of interest for a point source inside the tissue. As in our previous studies, we considered uniform illumination of the epicardial surface (z=0) and we used Robin boundary conditions for a tissue-saline interface. The excitation fluence Φ_e and the point-spread function Γ can then be derived analytically and will depend on the optical attenuation lengths at excitation and emission wavelengths.

B. Electrophysiological model

Electrical wave propagation in cardiac tissue was simulated using a well-established rabbit electrophysiological model [9]. This was achieved by solving following equation for the transmembrane potential V_m:

$$\partial_t V_m(\vec{r},t) = -\frac{I_{ion}}{C_m} + \vec{\nabla}\cdot D_E \vec{\nabla} V_m(\vec{r},t) \quad (1)$$

where C_m is the membrane capacitance, and D_E is the electrical diffusivity tensor. I_{ion} represents the total transmembrane ionic current and is obtained through the solution of several additional differential equations as described in the rabbit electrophysiological model used in this study [9]. Electrical waves propagate faster along than across muscle fibers. Therefore, we scaled the diffusivity tensor D_E to produce conduction velocities of 60 cm/s in longitudinal and 20 cm/s in transverse direction. The fibers were assumed to rotate at a linear rate of 24°/mm with depth, thus yielding a total fiber rotation of 120° in a 5 mm thick slab of ventricular tissue. Electrical activity following twice threshold point stimuli in the centre of the epicardium at a basic pacing rate of 2Hz was simulated. Equation (1) was intergrated using an explicit finite difference scheme using an adaptive time step scheme (0.001-0.005 ms) and a space step of 0.1 mm. Simulations were carried out on a parallel cluster consisting of 20 dual AMD Athlon MP2200+ processors running at 1.8 GHz. We used the MPI library and a "domain slicing" algorithm to parallelize the code.

C. Hybrid model of cardiac dual optical mapping

Optical signals can be calculated by coupling the bio-photonic and electrophysiological models described above. In the case of electrical activity, the fluorescence optical signal V_F recorded from the surface point (x,y) can then be obtained by convolving V_m with Φ_e and Γ as follows [8, 10]:

$$V_F(x,y,t) = \int_V V_m(\vec{r}',t)\cdot\Phi_e(\vec{r}')\cdot\Gamma(z',\rho)\cdot d\vec{r}' \quad (2)$$

with ρ the radial coordinate on the imaged xy surface, centered around (x',y'), and where V is the volume of the cardiac tissue. Equation (2) relies on the observation that the fluorescence of voltage-sensitive dyes depends linearly on changes in transmembrane potential.

In the case of calcium imaging however, the fluorescence does not depend linearly on Ca_i^{2+}, but rather on the bound calcium-dye concentration, CaD. Using a simple model of chemical equilibration, one can find the following relationship between CaD and the actual intracellular calcium concentration [4]:

$$CaD = \frac{Ca_i^{2+}\cdot D_T}{K_d + Ca_i^{2+}} \quad (3)$$

where D_T is the total dye concentration and K_d is the dye's dissociation constant. Hence, the wide-field calcium optical signal can be obtained as a two-step process: (i) calculate CaD from Ca_i^{2+} given by the electrophysiological model, and (ii) convolution of CaD with Φ_e and Γ over the volume of the slab, to yield the fluorescent calcium transient Ca_F:

$$Ca_F(x,y,t) = \int_V CaD(\vec{r}',t)\cdot\Phi_e(\vec{r}')\cdot\Gamma(z',\rho)\cdot d\vec{r}' \quad (4)$$

Optical signals were simulated for the voltage-sensitive dye RH-237 and two calcium indicators (Rhod-2 and Rhod-FF) in order to assess the role of the calcium dye's affinity on the optically recorded calcium transient. Rhod-2 is a high-affinity dye with a dissociation constant of 0.57 µM, whereas Rhod-FF is a low-affinitiy dye with K_d = 17 µM. The total dye concentration D_T was 10 µM in all simulations. Excitation of the dyes was assumed to be at 532nm, whereas fluorescence for RH-237 was at wavelengths >700nm and for the Rhod-dyes at ~600nm. The optical attenuation length at these wavelengths was set to 0.48 mm (532nm), 1.65 mm (600nm), and 3.31 mm (715nm) [8, 11].

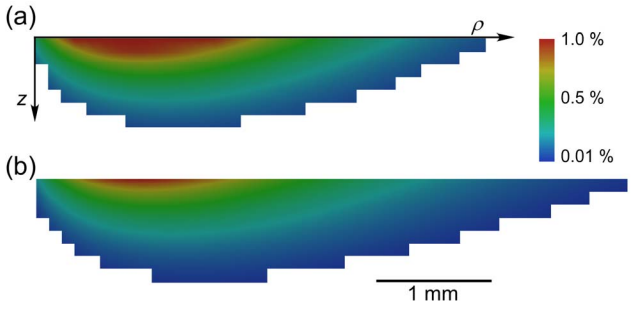

Fig. 1: Optical integration volume for the calcium indicators Rhod-2 and Rhod-FF (a) and the voltage-sensitive dye RH-237 (b). A transmural cross section through a slab of cardiac tissue is shown in which the relative contribution of each voxel is color coded.

III. Results

A. Optical Integration Volume

The optical properties of tissue for both excitation and emission wavelengths determine the sub-surface volume that contributes to the optical signal in a single pixel. We investigated the size and shape of this so-called optical integration volume (OIV) for the voltage-sensitive and calcium dye used in this study. As in our previous work [8],

we calculated the OIV by considering a uniform transmembrane potential and intracellular calcium-dye distribution (V_m=CaD=1) in (2) and (4) and calculated $\Phi_e \cdot \Gamma / V_F(0,0)$ and $\Phi_e \cdot \Gamma / Ca_F(0,0)$ for each voxel. Because of the cylindrical symmetry with respect to the optical axis, we further integrated all contributions within annular regions around the optical axis and at constant depths z. Fig. 1 shows the OIV's defined as the sub-surface volume that accounts for 75% of the epicardial optical signal. Fig. 1(a) shows the OIV for the Rhod calcium indicators, whereas Fig. 1(b) depicts the OIV for the voltage-sensitive dye RH-237. The calcium OIV extends to 0.8mm below the epicardial surface and up to 3.7mm in the radial direction. It is about 70% of the voltage OIV, which extends to 0.9mm in depth and to 4.8mm laterally.

B. *Optical calcium transients*

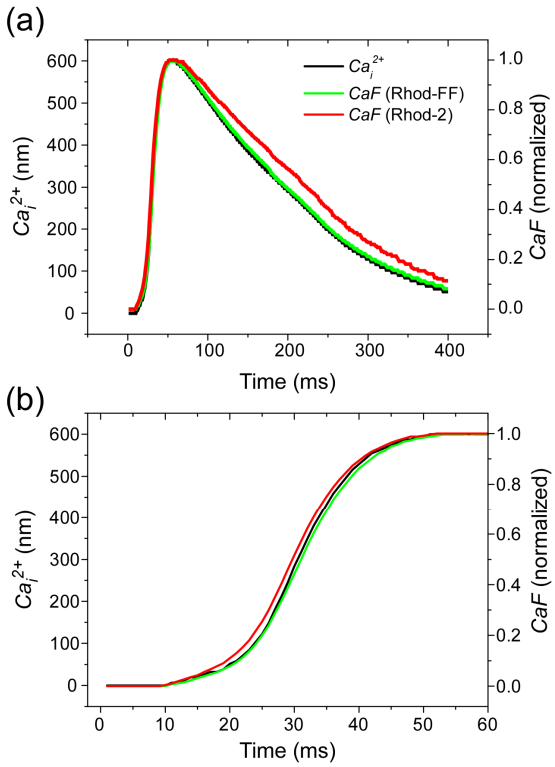

Fig. 2: Comparison of intracellular calcium transients (black) with its optical recording using Rhod-FF (green) or Rhod-2 (red). Panel (a) shows the complete transients, whereas panel (b) focuses on the rising phase.

Fig. 2(a) shows the optical traces recorded from an epicardial location (indicated in Fig. 3 – see below), compared to the actual calcium transient in the same location. As expected, the optical signals obtained using the low-affinity dye Rhod-FF closely follows the intracellular calcium time course, whereas the signal obtained with Rhod-2 shows a much slower calcium recovery. In these simulations, the calcium transient had a duration of 283.1 ms, whereas the optical calcium transient had a duration of 312.5 ms in the case of Rhod-2 and 283.2 ms in the case of Rhod-FF. For comparison, the action potential had a duration of 237.5 ms, whereas its optical counterpart had a duration of 239.5 ms.

It is worth noting that the rising phase of the calcium optical signal is also slowest for Rhod-2 as shown in Fig. 2(b), although the Rhod-FF signal shows a slightly slower rise compared to the intracellular calcium transient as well. These slow kinetics may not only be due to dyes' non-linear response to changes in Ca_i^{2+}, but also to sub-surface optical integration. This is verified by comparison of the rise times in the epi-fluorescent signal CaF vs. the local calcium-dye concentration CaD: it is 20.1 vs. 21.7 ms respectively for Rhod-2, and 19.2 vs. 21.2 ms respectively for Rhod-FF. This increase in rise time is similar, but smaller in magnitude, to the increase observed in optical action potential upstroke duration (1.1 vs. 4.7 ms respectively – not shown).

C. *Dual calcium-voltage optical activation maps*

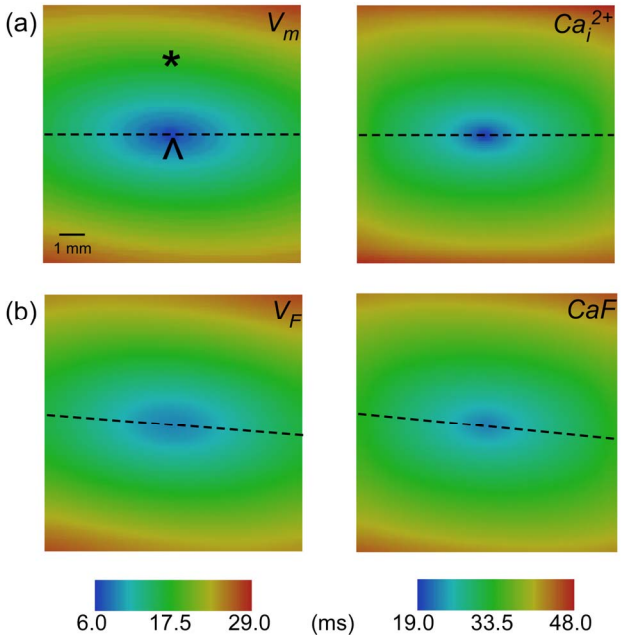

Fig. 3: Epicardial activation maps calculated from the propagating action potentials and intracellular calcium transients (a) and their corresponding optical recordings (b).

Fig. 3(a) shows simulated V_m and Ca_i^{2+} epicardial activation maps. These were obtained by measuring the time from stimulation to reach 50% of maximal V_m or Ca_i^{2+} in each point of the surface. The stimulus was applied in the middle of the field of view. Note that epicardial fibers ran horizontally. As expected, the wave shows an ellipsoidal shape with long axis oriented along the epicardial fiber direction (dashed line). Fig. 3(b) depicts the corresponding optical V_F and CaF activation maps for RH-237 and Rhod-2 respectively (similar results were obtained for Rhod-FF). Comparison with panel (a) indicates two key differences between the optical signals and the true epicardial activity, both for the transmembrane potential and the intracellular calcium concentration: (i) optical activation is delayed, and (ii) the direction of fast propagation inferred from the optical signal (dashed line) is at an angle with the epicardial fiber orientation (horizontal). We further quantified the

differences in activation times between V_m and V_F on one hand, and Ca_i^{2+} and CaF on the other hand, by performing a linear regression analysis. The results are presented in Table I. From this, it is clear that the optical activation times as defined above show a linear correlation to the actual epicardial activation time, but consistently underestimate the latter.

TABLE I
LINEAR REGRESSION ANALYSIS ON ACTIVATION MAPS

	Slope (ms)	Intercept (ms)	R
V_m *vs.* V_F	0.91	1.52	0.99
Ca_i^{2+} *vs.* CaF (Rhod-2)	0.88	4.03	0.99
Ca_i^{2+} *vs.* CaF (Rhod-FF)	0.88	2.91	0.99

IV. DISCUSSION

Significant efforts have been devoted in the last decade to simultaneously map transmembrane potential and intracellular calcium optically from the heart's surface [4]. Optical imaging offers the possibility to investigate cardiac activity from large regions of intact hearts and which has led to a significant improvement of our understanding of normal heart activity and of mechanisms underlying arrhythmias [3].

The optical action potential is known to be different from the underlying electrical action potential due to scattering of photons in tissue [6, 7]. This scattering results in so-called "optical blurring", i.e. the optical signal recorded at a particular location on the epicardium originates not only from that point, but from a sub-surface volume which dimensions depend on the optical properties of the tissue. This effect has been well characterized in the case of optical action potentials using voltage-sensitive dyes, where it was found to yield information on the sub-surface wave front orientation [7,8].

In the case of optical calcium transients, however, the effects of optical scattering in tissue remained unknown. In this study we have utilized a detailed hybrid electrophysiological-optical model to investigate the optical averaging effects in epi-fluorescence on the optical calcium signal. The validity of these hybrid models has been discussed in detail previously [7,8,10].

We found that the sub-surface volume contributing to the optical calcium signal is smaller than its equivalent in the case of optical mapping of transmembrane potential, consistent with the longer (and thus less scattered) emission wavelength used for the voltage-sensitive dye. Nevertheless, sub-surface scattering remains an important determinant in the synthesis of optical calcium signal. As is the case for voltage mapping, sub-surface scattering results in a modest, yet noticeable, increase in rise time, on top of any effects due to the calcium dyes' affinities (Fig. 2). Furthermore, sub-surface scattering is also responsible for significant discrepancies between optical activation patterns and the underlying epicardial activity. This is most easily observed in the rotation of the apparent direction of rapid propagation inferred from the optical signals, owing to the transmural rotational anisotropy and optical integration from a sub-surface volume (Fig. 3).

V. CONCLUSION

Our study is the first to characterize optical scattering effects in the synthesis of optical calcium signals using detailed bio-photonic models coupled to electrophysiological models of ventricular activity. Our results indicate that photon scattering effects can considerable affect the optical calcium transients and should be taken into consideration when interpreting epi-fluorescence optical calcium signals.

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