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Signal Decomposition of Transmembrane Voltage-Sensitive Dye Fluorescence Using a Multiresolution Wavelet Analysis

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Abstract

Fluorescence imaging of transmembrane voltage-sensitive dyes is used to study electrical activation in cardiac tissue. However, the fluorescence signals, typically, have low SNRs and may be contaminated with motion artifact. In this report, we introduce a new processing approach for fluoresced transmembrane potentials (fTmps) that is based upon a discrete wavelet transform. We show how fTmp signals can be decomposed and reconstructed to form three subsignals that contain signal noise (noise signal), the early depolarization phase of the action potential (rTmp signal), and motion artifact (rMA signal). A coiflet4 wavelet is used for fTmp decomposition and reconstruction of these subsignals. Results using fTmp signals that are contaminated with motion artifact indicate that the approach is a useful processing step to remove baseline drift, reduce noise, and reveal wavefronts. It streamlines the preprocessing of fTmps for the subsequent measurement of activation times and conduction velocities. It is a promising approach for studying wavefronts without aggressive mechanical tissue constraint or electromechanical uncoupling agents and is, useful for single-camera systems that do not provide for ratiometric imaging.

Index Terms

Discrete wavelet transform (DWT); fluorescence; motion artifact; optical mapping; transmembrane voltage-sensitive dye

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I. Introduction

Cardiac arrhythmias are studied in great detail by recording the fluorescence of living myocardial tissue stained with a voltage-sensitive dye. Local changes in transmembrane potential (Tmp) are represented as changes in dye fluorescence at each recording site and are usually referred to as optical action potentials (OAPs) [1]. A limitation is that spatial registration between the imaging device and tissue is distorted by contraction. As a result, OAPs become contaminated with “motion artifact” [2], [3]. While “artifact” may have negative connotation, motion artifact is actually the result of the fundamental function of the heart. In fact, patterns of cardiac motion may be derived from motion artifact signals to show the effect of ischemia or alterations in excitation–contraction coupling [4].

Motion artifact usually occurs within the OAP plateau and repolarization phases. This is because of the delay between the action potential upstroke (phase-zero depolarization) and the onset of contraction. Typically, phase-zero depolarization is not affected by motion and can be used to identify excitation wavefronts. This can be done using the first derivative of fluoresced Tmp (fTmp) signals [2], [5]. However, noise is amplified by differentiation so that high signal to noise levels are needed or spectral properties of fTmp signals must allow for aggressive filtering to reduce noise levels [6].

The impact of contraction on OAPs must be minimized to record the plateau and repolarization phases. This can be done before or after data acquisition. Preacquisition approaches include mechanical constraint or pharmacological immobilization of the heart using an electromechanical uncoupler such as blebbistatin [7] or 2,3-butanedione monoxime (BDM) [8]. These uncouplers disrupt the normal sequence of excitation–contraction coupling to reduce contractile motion. Postacquisition approaches include ratiometry [9], [10] and image registration [11], [12]. Ratiometry also minimizes the effect of photo-bleaching, yet it does not always completely remove motion artifact. Coupling ratiometry with gentle mechanical constraint or using an uncoupler at a low concentration can improve removal of motion artifact [9], [13]. A modified subtraction method based upon a linear model has also been applied to ratiometric data for improving the removal of motion artifact [11], [13].

In this report, we describe a new postacquisition nonratiometric approach to minimize fTmp distortions caused by motion. A main objective was to separate phase-zero depolarization sequences from signal noise and motion artifact to clearly reveal wavefront propagation. We hypothesized that this could be done using a wavelet analysis since phase-zero depolarization and motion artifact occur at different temporal scales. The hypothesis was tested using a discrete wavelet transform (WDT) of fTmps to reconstruct a subsignal that contains motion artifact (rMA signal) and another subsignal that contains phase-zero depolarization plus early repolarization of OAPs (rTmp signal). In one processing step, fTmp signals were denoised, baseline drift was removed, and OAP phase-zero depolarization was separated from motion artifact. The result was reconstructed signals (rTmps) from which measurements of activation times (ATs) and conduction velocities could be easily derived.

II. Methods

A. Heart Preparation and General Protocol

Adult Sprague-Dawley rats (300–400 g) were injected with sodium heparin and anesthetized with sodium pentobarbital. The heart was quickly excised and the aorta was cannulated. The heart was stained with the potentiometric dye RH237 (10 μ M) by a bolus injection (5 mL) to the aorta and transferred to a Langendorff perfusion system where it was retrograde perfused

at constant pressure (50 mmHg) with oxygenated Tyrode's solution. After a stabilization period of at least 15 min the fluorescence of RH237 was imaged during both endogeneous and paced rhythms. Pacing was at twice diastolic threshold (700 and 350 ms cycle lengths) using a bipolar electrode placed on the anterior epicardial surface. Blebbistatin (10 μ M) was then added to the perfusate and the fluorescence of RH237 was imaged again at periodic intervals during both endogeneous and paced rhythms. This general protocol was used to conduct additional studies using excised hearts from New Zealand white rabbits. All experimental procedures were approved by The George Washington University Institutional Animal Care and Use Committee.

B. Fluorescence Imaging System

RH237 was energized by illuminating the anterior epicardium with light from two LEDs (LumiLEDs, 530/35 nm). Emitted light passed through a single-lens system (Cosmicar 6 mm, F/1.0 with +27 closeup lenses), a dichroic mirror (610 nm), a long-pass filter (680 nm), and was imaged with a charge-coupled device camera (Andor IXON DV860). Images were acquired at frame rates of at least 650 frames/s. Background fluorescence was subtracted and signals were normalized at each pixel. Images were typically filtered with a 3×3 point spatial conical filter using a slope of one.

C. Multiresolution Wavelet Analysis

Fluorescence signals (fTmps) at each pixel [$x(t)$] were decomposed using the discrete wavelet transform (DWT). Wavelets are mathematical constructs used to analyze signals according to scale or, equivalently, according to resolution (i.e., the reciprocal of scale). According to the theory of multiresolution [14], the difference of information between the approximation of a signal at different resolutions can be extracted by decomposing a signal with finite energy $x(t) \in L^2(R)$ using the DWT. This decomposition defines a set of basis functions [$\psi_{m,n}(t)$] obtained by translation (characterized by shift parameter n) and dilation (characterized by scale parameter m) of a single function $\psi(t)$, which is also referred to as a mother wavelet. The shifts and scales take values from discrete sets. A common choice for DWT is the dyadic DWT. Using the dyadic grid, a wavelet is obtained from the mother wavelet by scaling (i.e., dilating) by a factor 2^m and translated by a factor $2^m n$ as

$$\psi_{m,n}(t) = \frac{1}{\sqrt{2^m}} \psi\left(\frac{t - 2^m n}{2^m}\right). \quad (1)$$

To obtain a signal approximation at scale m , the scaling functions [$\phi_{m,n}(t)$] are used. They are defined as

$$\phi_{m,n}(t) = \frac{1}{\sqrt{2^m}} \phi\left(\frac{t - 2^m n}{2^m}\right) \quad (2)$$

where $\phi(t)$ is the prototype scaling function (also called the father wavelet).

For a signal $x(t)$, the orthogonal wavelet series expansion is

$$x(t) = \sum_{n=-\infty}^{\infty} a_{m,n} \phi_{m,n}(t) + \sum_{m=-\infty}^M \sum_{n=-\infty}^{\infty} d_{m,n} \psi_{m,n}(t) \quad (3)$$

where a_{Mn} are the approximation coefficients that represent the coarse information of the signal at maximum scale M and shift $2^M n$. The detail coefficients d_{mn} represent the detailed features of the signal $x(t)$ at scale m and shift parameter n . a_{Mn} and d_{mn} are defined as

$$a_{Mn} = \langle \varphi_{Mn}, x \rangle = \int_{-\infty}^{\infty} \varphi_{Mn}(t) x(t) dt \quad (4)$$

and

$$d_{mn} = \langle \psi_{mn}, x \rangle = \int_{-\infty}^{\infty} \psi_{mn}(t) x(t) dt. \quad (5)$$

A multiresolution analysis for a basis function is implemented numerically using a hierarchical algorithm. The original full length signal $x(t)$ is filtered using a combination of scaling (low pass H_0) and wavelet (high pass H_1) filters (see Fig. 1). This is also referred to as octave filter banks. The frequency content of the signal is divided into two halves representing the low and high frequencies. The downsampled output of the high-pass filter provides the detail coefficients (at each level m) while the downsampled output of the low-pass filter provides the approximation coefficients. Downsampling removes signal redundancy since bandwidth is twice smaller than that of the input signal at each stage. The process of filtering and downsampling continues until the desired level of decomposition is achieved.

The reconstruction process follows a reverse path, where coefficients are upsampled and filtered using the synthesis counterparts of the decomposition filters described earlier. An extended discussion of wavelet theory and its application to multiresolution analysis for signal decomposition is provided in [15]. In this report, all signals were processed and analyzed using MATLAB (Mathworks, Natick, MA) and wavelet analyses were implemented using functions from the MATLAB wavelet toolbox.

D. Test Signal Synthesis

fTmp decomposition using a DWT was tested using idealized fTmp signals that represent those recorded from ventricular epicardial tissue. One signal represented rat fTmps and another represented rabbit fTmps. In this way, the reconstruction of OAP phase-zero depolarization could be tested for OAPs having two different morphologies: rat OAPs have an exponentially decaying repolarization phase following depolarization while rabbit OAPs have a substantial plateau phase [see Fig. 2(A1) and (A2)]. The idealized fTmps had three components: an idealized OAP (IOAP), simulated motion artifact, and simulated noise. The IOAP was generated by the ensemble average of fTmp signals acquired from the epicardial surface after the administration of blebbistatin [see Fig. 2(A1) and (A2)]. IOAPs had no motion artifact. Motion artifact was simulated using a half-wave rectified sine wave [see Fig. 2(B1) and (B2)] and was added to the IOAP [see Fig. 2(C1) and (C2)]. White noise was added to the total signal using the MATLAB function `awgn.m` [see Fig. 2(D1) and (D2)]. The SNR of the final idealized fTmp signal was 20 dB (rat) and 30 dB (rabbit). This signal was used for optimizing the multiwavelet resolution analysis of experimental fTmp signals.

E. Decomposition of fTmp Signals

fTmp signals were decomposed to scale level M using the MATLAB function `wavedec.m`. M , ranged from 1 to 10. A wavelet decomposition to level $M=10$ of the rat idealized fTmp signal using the `coiflet4` wavelet is shown in Fig. 3. The detail coefficients d_1 to d_m at levels

1 to m were then used to reconstruct the signals shown on the left while the approximation coefficients a_m were used to reconstruct the signals shown on the right. Signals were reconstructed using the MATLAB function `waverec.m`.

We identified the optimal level of decomposition (M) for the idealized fTmp signals that provided the best reconstruction of the simulated motion artifact. This was done by finding the scale level with the lowest root-mean-squared error (RMSE) between the signal reconstructed using the approximation coefficients a_m and the simulated motion artifact [see Fig. 2(B1) and (B2)]. For the idealized fTmp signals, this corresponded to level $M=7$ for both the rat (see Fig. 3) and rabbit. A signal with phase-zero depolarization and early repolarization of the IOAP could then be reconstructed using detail coefficients from levels 1 to 7, although still contaminated with noise. Detail coefficients at low scale levels (1 to 3) mainly contained noise information. Thus, a denoised version of the IOAP could be obtained by performing a reconstruction using detail coefficients from levels 4 to 7 (see Fig. 3). Signals reconstructed using detail coefficients only (rTmp signals) and approximation coefficients only (rMA signals) are shown in Fig. 4.

Reconstructions using detail and approximation coefficients above level 7 did not provide distinct separation of motion artifact and phase-zero depolarization (see Fig. 3).

F. Identifying an Optimal Wavelet

The idealized fTmp signals [see Fig. 2(D1) and (D2)] were analyzed using 35 wavelets of different classes using the decomposition and reconstruction approach described earlier. The wavelets included orthogonal bases such as Daubechies, symlets, coiflets, digital Meyer wavelets, and biorthogonal wavelets (using `bior.m` and `rbio.m` from the MATLAB wavelet toolbox). At each level m , rTmp and rMA signals were compared (by computing RMSEs) to the IOAP and simulated motion artifact, respectively. Results for six wavelets that provided the lowest RMSEs for both rTmp and rMA signals (rat) are listed in Table I and include Daubechie, symlet, and coiflet wavelets. The scaling and wavelet functions for these wavelets are shown in Fig. 5.

The lowest RMSE for rTmp signals for idealized fTmps was provided by Daubechies (db14 and db17) wavelets. However, when applied to raw fTmp signals, these wavelets introduced ripples of significant amplitude in the reconstructed subsignals. This is because higher order Daubechies functions are more oscillatory than the symlet and coiflet wavelets that were tested (see Fig. 5). Thus, db14 or db17 wavelets were not used in our analyses.

The difference in RMSEs for reconstructed signals using the symlet (sym10 and sym15) and coiflet (coif4 and coif5) wavelets was small (see Table I). The RMSE of rTmp signals using the coiflet4 wavelet was less than that provided by the symlet wavelets. Ultimately, we chose to use the coiflet4 wavelet for rat fTmps because the scaling and wavelet functions are more symmetric compared to the symlet functions (see Fig. 5). Asymmetric functions such as symlets have a centroid shifted to the right. This resulted in an undesired shift in the detected location of phase-zero depolarization as well as the peak of the OAP. When compared to coiflet5, coiflet4 had lower RMSEs for rMA but slightly higher RMSEs for the rTmp signal. The length L of the coif4 wavelet ($L=11$) is also shorter than the coif5 ($L=14$), which improves computational speed. Analysis of rabbit fTmps also indicated that the coiflet4 wavelet would provide a low error for reconstructed signals.

G. Frequency-Domain Analyses

The spectral content of fTmp signals and reconstructed signals was measured using Fourier analyses. The power spectral density was computed using Welch's averaged modified periodogram method, as implemented in the `pwelch.m` MATLAB function. Typical

parameters were: window size = 428 samples, overlap = 214 samples, NFFT = 2048. Temporal changes in spectral content within OAPs were measured using a discrete short-time Fourier transform analysis, as implemented in the MATLAB spectrogram.m function. Typical parameters were: window size = 22 samples, overlap = 21 samples, NFFT = 1024.

H. AT and Conduction Velocity Measurements

ATs and longitudinal and transverse conduction velocities (V_L and V_T) were measured before and after the administration of blebbistatin to determine the relative accuracy of computing these values using rTmps. ATs were measured using the maximum upstroke of rTmps, which conceptually corresponds to $dV_m/dt_{\max}$ [16]. ATs and conduction velocities were also measured from fTmps using the maximum upstroke. A two-way analysis of variance (ANOVA) was used to reveal any significant effects ($p < 0.05$) of blebbistatin and/or signal (rTmp or fTmp) on conduction velocity measurements.

Conduction velocities were measured perpendicular to the long (V_L) and short axis (V_T) of elliptical wavefronts that were initiated using point stimulation. Speeds were computed following the general strategy described by Knisley and Hill [17].

III. Results

Unless indicated otherwise, results pertain to studies using fTmp signals recorded from the epicardium of rat ventricles.

A. fTmp Decomposition and Reconstruction of Subsignals

fTmp signals at each pixel in fluorescence datasets were decomposed using the multiresolution wavelet analysis described earlier. Datasets were fTmp movies 4–5 s in duration (650 frames/s). First, an appropriate level of reconstruction for rTmp and rMA subsignals was determined using the fTmp signal from one pixel. That signal was decomposed and reconstructed in the same way as the idealized fTmp signals (see Fig. 3). Using those reconstructions, it was possible to identify the level at which all motion artifact was detected in the approximation coefficients without leakage of motion artifact into rTmp signals. Similarly, the detail coefficients cutoff level for signal noise was identified as the level at which no information from the early phases of the OAP was present in reconstructed noise signals. The same levels were then used to reconstruct subsignals at all other pixels in the dataset. Overall, we found that appropriate selection of the levels of reconstruction at one pixel provided adequate separation of motion artifact and the early phases of OAPs for all pixels.

The reconstruction of subsignals for one fluorescence dataset is shown in Fig. 6 (pacing, no blebbistatin). fTmp SNR was 15.3 dB. Detail coefficients at levels 4–6 provided denoised rTmp signals at each pixel that revealed the early phases of OAPs with minimal distortions due to motion artifact. rMA at each pixel was reconstructed using approximation coefficients at level 6. The rMA signal also contained the baseline drift that was present in the fTmp signal (see Fig. 8 for additional information regarding baseline drift removal). Signal noise was reconstructed using detail coefficients at levels 1–3.

A reconstruction of each image in the dataset using rTmp signals at each pixel revealed a characteristic elliptical pattern of wavefront propagation emanating from a pacing electrode (see Fig. 6, right). The multiresolution analysis was repeated for five other studies (six total). Reconstruction levels for each of the three components of fluorescence signals (noise, early phases of the OAP, and motion artifact) are summarized in Table II.

B. Spatial Variation of Motion Artifact, Baseline Drift, and Unsteady Rhythms

Motion artifact in fTmp signals is the result of local tissue deformation, rigid body motion, and spatial variations of background fluorescence caused by anatomic features such as vessels and fat deposits. As such, the duration, magnitude, and morphology of motion artifact will vary across the epicardial surface. Motion artifacts from six different epicardial sites in a typical dataset were reconstructed and compared to the original fTmp signals to determine whether motion artifact of different morphologies could be properly reconstructed. As shown in Fig. 7, rMAs adequately represented the lower frequency components that were characteristic of motion artifacts of multiple morphologies.

Approximation coefficients at higher scale levels (>7) mainly contained information related to fTmp baseline drift. An example of how a signal reconstructed using approximation coefficients at a scale level of 10 can be used to remove baseline drift is shown in Fig. 8.

Even during unsteady rhythms, phase-zero depolarization of OAPs could be successfully reconstructed. This was true mainly when motion artifacts were comprised of low-frequency components (16 Hz and below). A typical rTmp for an unstable rhythm (2 S1s followed by a premature S2 and another premature S3) is shown in Fig. 9(A1) and (A2). Well-defined wavefronts were observed in the fTmp movies. An example of an unsuccessful reconstruction is shown in Fig. 9(B1) and (B2), where motion artifacts were comprised of higher frequency components. When present, this type of signal usually originated from the edges of the heart and could be removed using an appropriate image mask. An example of successful reconstruction during complex wave propagation is shown in Fig. 10 for a reentrant wave. ATs measured from rTmps revealed the reentrant pathway and a slowly conducting zone near the base of the heart.

C. Frequency-Domain Analyses

fTmp signal decomposition using the DWT captures content in both time and frequency. This provides for improved temporal localization of signal content in comparison to Fourier-based analyses. As we have shown, the DWT can be used to separate fTmp signals into rMA, rTmp, and noise subsignals. These subsignals also have specific frequency content. rMA is comprised of lower frequencies, rTmp contains higher frequencies, and the highest frequencies typically contribute to signal noise.

It may be possible to separate fTmp signals into each subsignal using a Fourier-based approach, if the frequency band of motion artifact does not significantly overlap with other subsignals. To determine whether this is true, we measured temporal changes in frequency content for fTmp signals with motion (before administration of blebbistatin) and for fTmp signals without motion (after administration of blebbistatin) using a discrete short-time Fourier transform. Results revealed a significant overlap in the frequency content of the two signals (see Fig. 11). Phase-zero depolarization contained frequencies from 22 Hz and below. In the absence of motion artifact, frequencies in the OAP quickly dropped after the completion of phase zero (see Fig. 11A). In the presence of motion artifact, OAP spectral power remained elevated at 16 Hz and below (see Fig. 11B), indicating significant overlap in the frequency content of motion artifact and the early phases of the OAP. Therefore, the selection of frequency bands for optimal separation of motion artifact from fTmps using a Fourier approach will be difficult.

We then measured the spectral content of wavelet reconstructed subsignals (see Fig. 12A) to determine if our wavelet approach could provide optimal filter frequencies for Fourier-based signal separation. We observed distinct differences in the frequency content of rMA signals (3.4 Hz and below) and rTmp signals (3.4 to 50 Hz). We also found that frequencies above 40 Hz had largest contributions to the noise subsignal. Based on this information, we applied

the digital filtering scheme shown in Fig. 12B to raw fTmp signals. Signals were first low pass filtered (Butterworth, passband cutoff 30 Hz, stopband cutoff 55 Hz) to remove noise. Signals were then high pass filtered (Butterworth, stopband cutoff 3 Hz, passband cutoff 7 Hz) to extract signal components that corresponded to early phases of the OAP. The latter was then subtracted from the denoised signal to obtain the motion artifact subsignal. The results shown in Fig. 6 and 12B indicate good agreement between the two approaches (wavelet-based and Fourier-based). We note that Fourier-based signal separation was successful primarily because the filter frequencies were determined from information provided by the wavelet analysis. An important advantage of the wavelet-based approach is that no *a priori* knowledge of distinct frequency content for each subsignal is required for decomposing raw fTmp signals into subsignals.

D. AT and Conduction Velocity Analyses

ATs were measured using idealized fTmps and rTmps reconstructed from idealized fTmps to show how wavelet-based processing may be used for measurements of AT in signals with noise and motion artifact. Noise level was varied in idealized fTmps [see Fig. 2(D1)] and ATs were computed at each noise level. Summary results for filtered fTmps and rTmps are shown in Table III. We found that for no noise, rTmp provided accurate ATs while fTmp filtered at 20 Hz provided an AT error of 1.5 ms. Up to a SNR of 10 dB, rTmps provided accuracy of AT measurements similar to that of filtered fTmps. For low SNR (10–20 dB), all AT errors were small, ranging from almost zero to 3.1 ms.

The accuracy of finding activations (ACTs) within experimental datasets was studied using fTmps recorded without blebbistatin. Summary results for filtered fTmps and rTmps are shown in Table IV. We found that for individual beats, ACTs could be identified with similar success using either rTmps or filtered fTmps. However, the success of identifying all ACTs within individual pixels was clearly higher for rTmps (see Table IV, column 4).

AT maps before and after administering blebbistatin were used to measure V_L and V_T , as shown in Fig. 13. Velocities were measured from fTmps (after a 3×3 point spatial conical filter) and rTmps from eight datasets recorded from two rat hearts. Before blebbistatin, AT maps generated using rTmps were of higher quality, with fewer pixels having missing ATs (see Fig. 13A and B). This improved the ability to measure conduction velocities in those datasets. After blebbistatin, AT maps were similar for fTmps and rTmps (see Fig. 13C and D). We found no statistically significant differences between V_L and V_T measured using fTmps or rTmps. A small reduction in V_L after administering blebbistatin (two-way ANOVA, $p = 0.03$) could be attributed to edema caused by smooth muscle relaxation. This finding should be validated in future studies where postblebbistatin velocity measurements are corrected for changes in tissue dimension.

IV. Discussion

In this paper, we have shown how a wavelet analysis of epicardial Tmp (transmembrane potential) fluorescence is a useful postacquisition processing step. It may be particularly useful for studying wavefronts using single-camera fluorescence imaging systems that do not provide for ratiometric imaging. It could be used instead of conventional Fourier-based filtering to remove noise and baseline drift and as an alternative to signal differentiation to reveal wavefronts.

A. fTmp Signal Analysis Using Wavelets

A wavelet is a basis function used in signal processing to extract information and important signal descriptors. Wavelet analysis is a powerful mathematical tool for the decomposition and synthesis of signals [15] in a variety of disciplines, such as physics, geophysics, medical

signal processing, statistics, and computer graphics. To date, the application of wavelet theory to cardiac electrophysiology has primarily focused on ECG signal processing and classification [18]. It has also been used for ECG denoising [19], to remove baseline drift, and to extract ECG signal parameters [20]–[22].

In this paper, we show how wavelet theory can be used to denoise fTmps, remove baseline drift, and separate OAP phase-zero depolarization from motion artifact in one processing step. Unlike the Fourier transform, signal analysis using wavelets is localized in both time and frequency (or scale) and often reveals features that might not be revealed by Fourier analysis, such as distinct morphological components and nonperiodic components [21], [23]. As our results show, the separation of fTmp signal components using a wavelet analysis is relatively straight forward while separation using a Fourier analysis can be difficult due to overlapping spectral content.

B. Reconstructed Signals

Wavelet-based decomposition of fTmps reduced noise and removed baseline drift. Denoising was achieved when reconstructing fTmp signals by discarding the detail coefficients of smaller scale levels. This increased SNR without aggressively smoothing fTmp signals using average or Fourier-based filters. Thresholding the detail coefficients (soft and hard thresholding) could further reduce noise. However, we found that this alters rTmp morphology because the shape of the wavelet becomes more apparent in the reconstructed signal.

rTmps did not contain baseline drift because drift was represented by approximation coefficients at larger levels. It is possible to remove only the baseline drift when reconstructing the fTmp signals by discarding the approximation coefficients at larger scale levels (i.e., $m \geq 10$). In this way, signal reconstruction is convenient in that information at multiple scales can be discarded during a single reconstruction step. Phase-zero depolarization of the OAP was preserved in rTmps while motion artifact was greatly reduced. rTmp signals could then be used to create activation maps and compute local conduction velocities using automated algorithms [24], [25], as the results indicate. This objective was achieved with accuracy because, when compared to the original signal, the RMSE in the phase-zero depolarization and early phase one components of rTmp signals was typically less than 1%. Such low error was augmented by selecting an optimal wavelet basis function (coiflet4) that best fit phase zero of the OAP. By design, this approach attributes most of the repolarization phase of the OAP to the motion artifact subsignal. This limitation is discussed next.

Information provided by rMA signals could be useful for quantifying the effect of electromechanical uncoupling agents or alterations that influence contractility, such as ischemia. Error within rMA signals was mainly the result of overlap of motion artifact with the repolarization phase of the OAP (see Fig. 4B and D). The wavelet basis function (coiflet4) was specifically chosen as a best fit for the depolarization phase of the rat OAP. As a result, the repolarization phase was represented by the approximation coefficients, along with motion artifact. Motion artifact and repolarization could potentially be separated using a wavelet basis function that mimics more closely the shape of the entire OAP, thereby minimizing leakage of repolarization phase into the approximation coefficients. Overall, this error was small but may have resulted in slight elevations in estimates of motion artifact amplitudes.

C. Reduction of Motion Artifact in fTmp Signals

There are several approaches to minimize motion artifact in fTmp signals. One is to add blebbistatin to the perfusate solution. This reversible inhibitor of myosin II isoforms [26] has been shown to have minimal effects on action potentials, ion currents, and activation patterns [7]. While the side effects of blebbistatin are less pronounced than other mechanical uncouplers, such as cytochalasin D [27] and BDM [8], the most important effect is the loss of contraction and the metabolic processes associated with contraction. Such processes influence membrane ion pumps and other cellular events that require adenosine triphosphate. Because of this, mechanical uncouplers have limited utility in fluorescence imaging studies aimed at understanding the metabolic underpinnings of conduction disturbances.

Ratiometric imaging can be used to reduce motion artifact without uncouplers and it requires multiple detectors [9], [10], [13]. Typically, gentle mechanical constraint, a low concentration of a mechanical uncoupler, and/or postacquisition signal processing is still required for complete removal of motion artifact [11], [13]. Another way to minimize motion artifact is by postacquisition image registration. In this method, a sequence of fluorescence images is spatially aligned such that each location in the images represents a fluorescence measurement from the same tissue location throughout the recording episode [11], [12].

In contrast to ratiometric imaging, wavelet-based processing can be applied to fTmps acquired from one camera. Although gentle mechanical constraint is required to minimize rigid body motion during contraction, wavefronts can be clearly identified without using a mechanical uncoupler. This allows cardiac metabolism to be studied using typical dual-camera fluorescence imaging systems. One camera could be used to image fTmps to reveal wavefront propagation while the second camera could be used to image metabolic parameters such as nicotinamide adenine dinucleotide fluorescence [28]–[30]. Local motion artifact amplitudes could also be measured using the rMA subsignals. The synergistic combination of these datasets could be useful for studying arrhythmia mechanisms in working heart preparations during acute local ischemia, reperfusion, and fibrillation.

V. Limitations

One limitation is that, in the presence of motion, fluorescence is recorded throughout the trajectory that each pixel traces over the surface of the tissue. This is also a limitation of ratiometric imaging. The wavelet analysis explained here will minimize motion artifact associated with this trajectory but will not remove fluorescence components that are associated with the OAP. Because of this, trajectory lengths should be minimized by applying gentle mechanical constraint to the tissue.

Another limitation is the inability to reconstruct the repolarization phase of the OAP. Signal components corresponding to the repolarization phase are represented by the approximation coefficients, along with motion artifact. In our studies, fTmp signals were recorded from rat ventricles, which have little or no plateau phase and an exponentially decaying repolarization phase. In other species, such as rabbit, much longer plateau phases may confound the accurate reconstruction of motion artifact subsignals. Motion artifact and complete OAPs could potentially be separated using a wavelet basis function that mimics more closely the shape of the entire OAP, thereby minimizing parameterization of plateau and repolarization phases with approximation coefficients.

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Fig. 1. Octave band filter of M stages. The scheme for implementing a DWT using low-pass (H_0) and high-pass (H_1) filters is shown. *Top:* Decomposition of signal x into detail coefficients (d_1 to d_M) and approximation coefficients (a_M). *Bottom:* Reconstruction of signal $\hat{x}$ using detail and approximation coefficients. x , d_i and a_i are arrays with entries in temporal order.

Fig. 2.

Idealized fTmp signals. IOAP: ensemble averaged fTmp signal acquired from the epicardial surface of a rat heart (A1) and rabbit heart (A2) after the administration of blebbistatin (B1 and B2). Simulated motion artifact: a half-wave rectified sine wave (C1 and C2).

Summation of signals in A and B. D1 and D2 Idealized fTmp signal: white noise was added to the signal in C. SNR is 20 dB and 30 dB (D1 and D2), respectively.

Fig. 3.

Decomposition of idealized fTmp signal. At each level m signals were reconstructed from details coefficients d_1 to d_m (left panel) and approximation coefficients a_m (right panel). Lower level details represent the noise signal ($m = 1-3$). Detail coefficients at levels 4-7 contain information relating to phase zero and early phase one of the action potential. Approximation coefficients at levels 6 or 7 represent the motion artifact. The RMSE of rMA to the simulated motion artifact [see Fig. 2(B)] was computed at each level m and are listed on the far right.

Fig. 4. Reconstruction using the idealized fTtmp signal. (A) rat IOAP [dotted gray, see Fig. 2(A1)] and rTtmp from detail coefficients at levels 4–7 (solid black). (B) Simulated motion artifact (dotted gray) and rMA (rat) from approximation coefficients at level 7 (solid black). (C) Rabbit IOAP [dotted gray, see Fig. 2(A2)] and rTtmp from detail coefficients at levels 4–7 (solid black). (D) Simulated motion artifact (dotted gray) and rMA (rabbit) from approximation coefficients at level 7 (solid black).

Fig. 5.

Wavelet functions. Scaling and wavelet functions are shown for wavelets having the lowest RMSEs for rTmps and rMAs. (A) Daubechies 14 (db14), (B) Symlet 10 (sym10) and (C) Coiflet4 (coif4).

Fig. 6.

Pixel by pixel decomposition and reconstruction of transmembrane voltage-sensitive dye fluorescence. A typical raw fTtmp signal from one pixel in a dataset is shown on the left. The signal was decomposed and three subsignals were reconstructed. The rTtmp signal was reconstructed using detail coefficients at levels 4–6. The rMA signal was reconstructed using approximation coefficients at level 6. The noise signal was reconstructed using detail coefficients at levels 1–3. Signals from all other pixels in the dataset were decomposed and reconstructed in the same way. rTtmp images at times $t = 0, 10$ and 25 ms from the application of stimulating current to the anterior epicardial surface of a rat heart surface are shown on the right.

Fig. 7.

Morphology of motion artifact: motion artifacts were reconstructed using approximation coefficients at level 6 from six different epicardial sites. (A) An image of the heart during diastole. The locations of signals shown in B are labeled. (B) rMA (gray) is shown on top of original fTmp signals (black) from the six locations marked in A.

Fig. 8. Baseline drift reconstruction at $m = 10$. (A) Spatially averaged fTmp signal using a 3 pixel radius. Baseline drift (gray line) was reconstructed using approximation coefficients at level 10. (B) fTmp signal after subtracting the reconstructed baseline drift shown in A.

Fig 9. Successful and unsuccessful reconstructions of OAP phase zero using wavelet analysis. fTmps and rTmps are shown for unsteady rhythms. Wavelet-based reconstruction of OAP phase zero is most successful when motion artifacts are comprised of low frequency components (panels A1 and A2) and typically fails when motion artifacts have high frequency components (panels B1 and B2).

Fig 10.

Measurement of ATs during reentrant activity using rTmps. ATs were successfully measured from rTmps reconstructed from fTmps measured during reentry without the use of blebbistatin.

Fig. 11.

Discrete short-time Fourier transform analysis of fTtmp signals before and after the application of blebbistatin. Spatially averaged fTtmp signals (3 pixel radius) are shown above the spectrogram for each signal. (A) Spectrogram of an fTtmp signal after the administration of blebbistatin (no motion artifact). (B) Spectrogram of an fTtmp signal before the administration of blebbistatin (with motion artifact). Analysis parameters were window size = 32, overlap = 31, and NFFT = 1024.

Fig. 12.

Frequency-domain analysis. (A) Spectral power for typical subsignals (rTtmp, rMA, and noise). rMA had power below 3.4 Hz and rTtmp had power from 3.4 to 38.4 Hz. The noise subsignal had power above 38.4 Hz. (B) Fourier-based filter scheme to extract subsignals is shown (see text for details). The raw fTtmp signal shown here was also used for the wavelet decomposition shown in Fig. 6.

Fig. 13.

Measurement of ATs and conduction velocities before and after administering blebbistatin. Before blebbistatin, AT maps generated using rTmps were of higher quality, with fewer pixels having missing ATs, thereby improving the ability to measure conduction velocities. After blebbistatin, AT maps were similar, thereby validating AT measurements using rTmps. Box plots are shown for V_L and V_T measurements for each dataset (A:D). Each dataset consisted of four total measurements from two rat hearts.

TABLE I

Summary of RMSE Results for Optimal Choice of Wavelet

Wavelet Family	Wavelet	M	RMSE of rMA signal	RMSE of rTmp signal
<i>Daubechie</i>	db14	7	0.217	0.036
	dc17	7	0.226	0.039
<i>Symlet</i>	sym10	7	0.196	0.064
	sym15	7	0.214	0.063
<i>Coiflet</i>	coif4	7	0.207	0.052
	coif5	7	0.214	0.048

Wavelets having the lowest RMSEs for rTmps and rMAs. 35 total wavelets were tested. M is the maximum level of decomposition as well as the level of approximation coefficients used to reconstruct motion artifacts. rTmp signals were reconstructed using detail coefficients at level 4 to M .

TABLE II

Summary of Reconstruction Levels for Six Studies

Study	Nosie	rTmp signal	rMA signal
1	m=1 to 3	m=4 to 6	m=6
2	m=1 to 3	m=4 to 7	m=7
3	m=1 to 3	m=4 to 6	m=6
4	m=1 to 3	m=4 to 7	m=7
5	m=1 to 3	m=4 to 7	m=7
6	m=1 to 3	m=4 to 6	m=6

Reconstruction levels for noise, rTmp and rMA signals are indicated for six different rat heart studies. For signal noise and rTmps, m represents the detail coefficients levels. For rMA, m represents the approximation coefficient level.

TABLE III

Effect of Noise on Activation Time Measurements

SNR (dB)	Raw fTmp	fTmp after 30Hz LPF	fTmp after 20Hz LPF	rTmp
N/A*	32.3	32.3	30.8	32.3
20	702	32.2	30.8	32.2
15	37.0	33.9	33.9	29.3
10	642	33.9	32.3	35.4
5	542	20.0	26.2	645

Effect of noise level in idealized rat fTmps [see Fig. 2(D1)] on measured AT (ms). ATs were measured using maximum upstroke of the idealized fTmp (columns 2–4) then measured using the maximum upstroke of rTmps (column 5).

The asterisk indicates that no noise was added.

TABLE IV

Accuracy of Detecting Activations Within Individual Signals

Level of filtering	% sites with error type A	% sites with error type B	Ave # ACTs not properly detected
<i>Raw fTmps</i>	22.8	81.6	26.1
<i>fTmps 120Hz LPF</i>	0.3	54.9	10.8
<i>fTmps 60Hz LPF</i>	0	33.3	11.6
<i>fTmps 32Hz LPF</i>	0	18.7	13.8
<i>fTmps 10Hz LPF</i>	0.9	29.1	10.7
<i>rTmps</i>	0.3	30.2	7.3

Comparison of the success of finding activations (ACTs) at each pixel in fTmp datasets using raw fTmps, filtered fTmps, and rTmps. All ACTs were determined using the upstroke of the respective signal. Column 2 (Error type A): activation at a site was not detected for a particular beat. Column 3 (Error type B): the number of activations at a site not properly counted for a sequence of 7 paced beats. Column 4 shows the average # of ACTs found for all pixels with type B errors. Numbers closest to 7 (the number of paced beats) indicate greater success in finding ACTs.