Extraction of Periodic Multivariate Signals: Mapping of Voltage-Dependent Dye Fluorescence in the Mouse Heart

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Abstract—In many experimental circumstances, heart dynamics are, to a good approximation, periodic. For this reason, it makes sense to use high-resolution methods in the frequency domain to visualize the spectrum of imaging data of the heart and to estimate the deterministic signal content and extract the periodic signal from background noise in experimental data. In this paper, we describe the first application of a new method that we call cardiac rhythm analysis which uses a combination of principal component analysis and multitaper harmonic analysis to extract periodic, deterministic signals from high-resolution imaging data of cardiac electrical activity. We show that this method significantly increases the signal-to-noise ratio of our recordings, allowing for better visualization of signal dynamics and more accurate quantification of the properties of electrical conduction. We visualize the spectra of three cardiac data sets of mouse hearts exhibiting sinus rhythm, paced rhythm and monomorphic tachycardia. Then, for pedagogical purposes, we investigate the tachycardia more closely, demonstrating the presence of two distinct periodicities in the re-entrant tachycardia. Analysis of the tachycardia shows that cardiac rhythm analysis not only allows for better visualization of electrical activity, but also provides new opportunities to study multiple periodicities in signal dynamics.

Index Terms—Harmonic analysis, optical mapping, period averaging, signal processing.

I. INTRODUCTION

OPTICAL recordings of electrical activity using fluorescent dyes and high-speed imaging techniques in both cardiac [1], [2] and neural tissues have provided new opportunities to globally visualize electrical activity with unprecedented detail. These techniques have revolutionized our understanding of cardiac electrophysiology by providing important clues on basic mechanisms underlying cardiac arrhythmias, defibrillation, excitation, and conduction [3], [5], [6]. Although high-resolution imaging of cardiac excitation is a powerful approach, image processing techniques that can identify and extract small amplitude optical signals are needed to improve our understanding of the mechanisms that underlie the initiation and maintenance of cardiac arrhythmia.

We have used our method to analyze many datasets, including data of hearts beating in sinus rhythm, paced hearts, and hearts with induced tachycardias. The data that we analyze in this paper include an example of a mouse heart beating in sinus rhythm and in paced rhythm and a dataset of images of a mouse heart exhibiting a monomorphic re-entrant tachycardia. We acquire data at a rate of 914 frames/s. For the relatively short time that we image the heart (approximately 2 s), the approximation that the signal is periodic is especially good. The sinus and paced rhythms are the only rhythms found in their datasets. Therefore, their analysis is relatively straightforward. However, using visualization methods that we present, we found that there were actually two different periodicities in the tachycardia data: a 32-Hz tachycardia and also a slower 5-Hz atrial rhythm. In fact, the 32-Hz tachycardia induced movement in the heart at the half frequency of 16 Hz. However, we will refer to this signal as a 32-Hz signal, since the re-entrant wave rotated at 32 Hz.

There are advantages to analyzing periodic signals in frequency space, since a periodic signal then has distinctive features. The Fourier transform of such a signal is a set of discrete, equally spaced harmonics. We use multitaper harmonic analysis, a high-resolution, stable and accurate method of estimating deterministic sinusoids in noisy data, to extract this sequence of harmonics [7]. Only harmonics that are at multiples of the base pulsing frequency are extracted. Therefore, deterministic signal components that are not related to the heart’s action, for instance 60-cycle camera noise and other deterministic experimental noise, as well as any random-phase noise, can be removed and discarded resulting in a significantly denoised signal.

Mitra and Pesaran [8] have recently introduced high-resolution multitaper spectral and harmonic techniques for the analysis of dynamic brain imaging data with remarkable success. Multitaper methods, which are optimal in a well-defined sense (see Appendix), are the standard for analysis of geophysical data [9], and are becoming the standard in astrophysics [10]. By contrast, in the analysis of biomedical imaging data these methods are largely unexplored.

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Many readers may be familiar with windowing techniques. Multitaper analysis is an improvement on these methods in the following sense. Windowing a signal reduces the signal’s bias, but assumes that the probability distribution of the sampled data is identical to the probability distribution of the stationary process. This assumption is, in general, incorrect for data that is discretely sampled from a signal that is continuous in time and frequency. Multitaper analysis is different from data windowing in that the data is projected onto a set of tapers (sometimes called windows), then the statistics of the signal in frequency space are used to obtain estimates of frequency content. The multitaper approach is derived by considering the estimation of an orthogonal increment stationary process as an inverse problem [7]. The tapers onto which the data are projected have low bias, and the estimates are consistent (i.e., they converge) since statistics from a set of tapers are used. For these reasons, multitaper analysis is extremely robust.

In this paper, we present a new method that we call cardiac rhythm analysis that uses features of periodic signals in Fourier space for denoising imaging data. Although the method is generally applicable to any periodic signal, we will specialize the discussion to imaging data of a mouse heart that has been infused with voltage-sensitive fluorescent dye.

II. METHODS

A. Experimental Methods

We performed imaging studies on an adult mouse heart. The heart was surgically removed by performing a thoracotomy and placed in a custom-built perfusion/superfusion apparatus. While the heart was fully immersed in Tyrode’s solution, the aorta was cannulated and Langendorff perfused at a constant pressure of 68–74 mm Hg (1–2 ml/min). The heart was fluorescently labeled with the voltage-sensitive dye Di-4-ANEPPS. High-resolution optical mapping of the right ventricular free wall was performed on an upright microscope (BX50WI Olympus, Inc.) equipped with a high-speed charge-coupled device (CCD) camera (CA-D128 Dalsa, Inc.) using a 4× objective. Images were acquired at 914 frames/s for 2 s with a 64× 64-pixel array in the absence of any motion reduction techniques, as previously described [2]. Therefore, there were approximately 16 cycles of the rhythms in the sinus and paced data, 66 cycles of the tachycardia and ten cycles of the atrial rhythm in the tachycardia data. The re-entrant tachyarrhythmia was induced using a burst pacing protocol [3].

B. Data Analysis Methods: Cardiac Rhythm Analysis

Before examining spectral and harmonic content, it is necessary to bring the multivariate imaging data into a more manageable form. We always consider mean subtracted data. We can denote the heart data

\[ h = h(t, \vec{x}) \]  

(1)

where \( h(t, \vec{x}) \) is the activity (gray level) at time \( t \) and pixel \( \vec{x} \). Principal component analysis (PCA) furnishes an optimal representation of multivariate data such as ours in that it best compresses multivariate data [4]. As shown in the Appendix, we can express (1) as

\[ h = \sum_n \mu_n \alpha_n(t) \psi_n(\vec{x}) \]  

(2)

where the \( \{\alpha_n\} \) and \( \{\psi_n\} \) are each sets of orthonormal functions, and the \( \{\mu_n\} \) are constants. The eigenfunctions of the PCA are arranged in order of decreasing power. For typical mouse heart data, more than 99% of the signal power is retained in the first 50 principal components. In this form, we see that the dynamics is carried by the coefficients \( \alpha_n(t) \). It is, therefore, our goal to understand and extract the harmonic content from these coefficients.

Before extracting the harmonic content, we typically examine the frequency spectra of the principal components. This can be useful since often the structure of the data then becomes evident. For instance, the frequency spectrum of the tachycardia data that we analyze below show three sets of harmonic stacks associated with different periodicities: the 32-Hz re-entrant tachycardia, a 5-Hz atrial rhythm, and noise from the experimental apparatus with a base frequency of 60 Hz.

To determine harmonic content (i.e., to find amplitudes and phases of deterministic sinusoids in noisy data), the timecourses \( \alpha_n(t) \) are projected onto a set of low-bias tapers. Then a least-squares regression estimate is performed to find the best fitting complex amplitudes for sinusoids in the data. Due to the central limit theorem, in Fourier space the complex amplitude estimators are normally distributed. Under the null hypothesis that no sinusoid is present, a statistical F-test is used to determine the significance of the sinusoid. For a more complete description of multitaper harmonic analysis see the Appendix.

Using multitaper harmonic analysis, we identify and separately extract each periodic signal by extracting all statistically significant deterministic sinusoids that lie at multiples of the base frequencies in the principal component timecourses. We then reconstruct denoised datasets by combining the extracted deterministic periodic content with the spatial eigenfunctions. The denoised datasets resulting from our method can then be further analyzed using standard methods.

It is important to note that our method of extracting the periodic content is in some ways similar to the commonly used method of first, identifying the period by finding a peak in the autocorrelation function, then averaging periods to increase the signal-to-noise ratio (SNR). The period averaging method, however, retains all periodic information, including harmonic content that is not statistically significant. Our method, since it relies on an F-statistic to determine the statistical significance of individual harmonics, leaves out statistically insignificant harmonics, thereby reducing spurious noise in the signal. Furthermore, by visualizing the log-spectrum, it is easier to identify multiple periodicities in the data compared with the method of using the autocorrelation to identify periodic information.

III. RESULTS

Here, we present results from three datasets: a mouse heart in sinus rhythm, a mouse heart in paced rhythm and a mouse heart exhibiting a re-entrant tachycardia. We show only spectra
to visualize the periodicity and the results of our reconstruction for the first two datasets. For the tachycardia, we show spectra and give details on the reconstruction process and improvements in quantitative measurements on the reconstructed data.

Both the sinus and paced data are periodic with base frequencies of approximately 8 Hz. In Figs. 1 and 2, we plot log-spectra of principal components of the data as a function of frequency. The colorbar gives greyscale values in the plots for amplitudes of the log-spectrum. Fig. 1(a) shows the log-spectrum of the principal components of the raw data with principal component index on the abscissa and frequency in Hertz on the ordinate. The harmonic sequence includes a harmonic sequence with base frequency of approximately 8 Hz. There is also considerable background noise. Fig. 1(b) is the log-spectrum of the extracted sinus rhythm using cardiac rhythm analysis to extract the deterministic harmonic stacks described above. There is a general trend for power to lie at low frequencies for low principle component indexes and at higher frequencies for higher index principle components. This is seen in panel Fig. 1(b) where the high-amplitude peaks shift to the right at higher frequencies. In both the sinus rhythm and paced rhythm data, there are more than one discernible band of spectral power indicating the presence of wave behavior with a complex dispersion relation, perhaps arising from nonlinear wave behavior. Fig. 1(d) is the log-spectrum of the removed background noise. Note that there are some faint remaining harmonics in the background noise. These can arise from inaccuracies in the estimation of harmonic content. For the most part, however, the harmonic information has been well estimated, extracted and reconstructed.

In Fig. 2, we plot log-spectra of principal components of the paced rhythm. Again, Fig. 2(a) depicts the log-spectrum of the raw data, Fig. 2(b) depicts the log-spectrum of the reconstructed paced rhythm, and Fig. 2(c) depicts the log-spectrum of the residual background noise. Note that, for this data, the harmonic content also exhibits bands of power probably arising from nonlinear wave behavior. The power in the periodic signal extends to fewer principal components for this dataset because the signal was weaker.

From here on, we discuss and present results from the denoising of a dataset of a mouse heart exhibiting three separate periodic components: a 32-Hz monomorphic re-entrant tachycardia, a 5-Hz atrial rhythm and 60-Hz noise. We will discuss this dataset in detail because it exhibits the greatest complexity of the datasets that we have examined and demonstrates advantages of using cardiac rhythm analysis. The three signal components were unknown before we applied our analysis method.
Fig. 2. A multitaper spectral estimate of $\log_{10}$ of the spectrum of (a) PCA modes of raw mouse heart data imaged during pacing, (b) the periodic paced rhythm extracted from the raw data, and (c) the residual background noise. Note the clean extraction of the periodic signals, which can be seen as stacks of harmonics in the spectrum, from the background. Principal component index (PC Index) is indicated on the abscissa and frequency in Hertz on the ordinate.

The components were first identified visually by inspecting the multitaper estimated log-spectra of the principal components, then extracted using multitaper harmonic analysis. The periodic signals that one finds using our method, may or may not be of interest to the investigator. For instance, when we reconstructed the 60-Hz signal, it was clear that this signal represented common electrical noise in the camera. We have chosen not to include this reconstruction in the paper. The other periodicities that were identified and reconstructed were concluded to represent electrical activity originating from the atria and ventricles. With our approach, knowledge of the existence of the atrial and ventricular periodicities is brought out either by inspection of the spectra of the principal components or by inspection of the F-statistic as a function of frequency of the principal components.

Fig. 3 contains plots of the log-spectra of principal components of the data as a function of frequency. The colorbar gives greyscale values in the plots for amplitudes of the log-spectrum. Fig. 3(a) shows the $\log_{10}$-spectrum of the principal components of the raw data with principal component index on the abscissa and frequency in Hertz on the ordinate. The harmonic sequences, which include a 32-Hz monomorphic ventricular tachycardia, a 5-Hz atrial rhythm and 60-Hz noise (seen as horizontal lines) are evident to the eye in the raw data. As mentioned in the introduction, the 32-Hz tachycardia causes the heart to move mechanically at 16 Hz, which is half of the rotation frequency of the re-entrant wave, therefore, the base harmonic for this signal is actually at 16 Hz. There is also considerable background noise. Fig. 3(b) is the log-spectrum of the extracted tachycardia using the multitaper harmonic analysis method for extracting the deterministic harmonic stacks described above. The tachycardia along with the induced mechanical motion is periodic with a base frequency of 16-Hz and harmonic lines lie at multiples of this base frequency. There is a general trend for power to lie at low frequencies for low principle component indexes and at higher frequencies for higher index principle components. This is seen in panel Fig. 3(b) where the peaks shift to the right at higher frequencies. Fig. 3(c) is the log-spectrum of the low-frequency atrial rhythm. This represents a second electrical signal which is also present in the data set. The spectra show that this signal is periodic with a base frequency of 5 Hz. Just as in panel Fig. 3(b), all of the power shown is at multiples of this base frequency and is statistically significant. The spectral content of the atrial rhythm is primarily below 100 Hz. Fig. 3(d) is the log-spectrum of the removed background noise. Note that
there are some remaining harmonics in the background noise (notably a harmonic stack with base harmonic of 60 Hz). These harmonics are not part of the harmonic sequence of interest. They represent deterministic machine noise, due to the CCD camera, and are easily discernable since they do not lie on multiples of the base frequencies of the tachycardia or the atrial rhythm.

It is evident from the results shown in Fig. 3 that the periodic electrical signals in both the atria and ventricles were accurately identified and separated using our high-resolution methods. The accurate estimation of these signals enabled us to separately reconstruct these two signals with high precision.

Use of spectral analysis provides a useful window into the signal dynamics. Inspection of Figs. 1(b), 2(b), and 3(b), and to a lesser extent Fig. 3(c), indicates that the extracted signal contains power that peaks on parabolic branches. It has been observed that in two dimensions, the principal component index $n$ and spatial wavenumber $k$ are related as $n \propto k^2$ [13]. Therefore, since we find that frequency $\omega \propto k$, this means that, without resorting to visualization, we can already infer that there is wave propagation in the data.

To give the reader a sense for the harmonic analysis used to identify and extract harmonic content and how statistical significance is determined for the periodic content, in Fig. 4(a), we plot $-\log_{10}(1 - F_{\text{cdf}})$ versus frequency (in Hz). Here, $F_{\text{cdf}}$ is the value of the cumulative distribution for the F-statistic. (See the Appendix for the expression for calculating the F-statistic.) The horizontal line is set at $-\log_{10}(N)$ (we set our significance level at $-\log_{10}(N)$, see Appendix). The vertical dotted lines show the locations of the two harmonic sequences at 5 and 16 Hz. In Fig. 4(b), we plot $-\log_{10}(1 - F_{\text{cdf}})$ versus frequency (in Hz) for the residual time series. Note the accurate removal of the 16-Hz harmonic sequence. Note that there are some changes in the amplitudes of some of the 5-Hz harmonics in Fig. 4(b) due to overlaps in the bandwidths of the sinusoid estimates.

In Fig. 5, we plot two different estimates of the periodic content of one principal component timecourse of the 32-Hz tachycardia [Fig. 5(a)] and the 5-Hz atrial rhythm [Fig. 5(b)]. In this

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Fig. 3. A multitaper spectral estimate of $\log_{10}$ of the spectrum of (a) PCA modes of raw mouse heart data imaged during a tachycardia event, (b) the periodic monomorphic re-entrant tachycardia (and induced mechanical motion at half of the tachycardia frequency) extracted from the raw data, (c) an atrial rhythm in the data, and (d) the residual background noise. Note the clean extraction of the periodic signals, which can be seen as stacks of harmonics in the spectrum, from the background. Principal component index (PC Index) is indicated on the abscissa and frequency in Hertz on the ordinate. Arrows indicate the base frequency of the periodic signals found at 5 Hz (atrial rhythm), 32 Hz (ventricular tachycardia), the induced mechanical motion at 16 Hz and 60 Hz (CCD camera noise, on right hand side) in the data.
reconstruction, and for the rest of Section III, we retained only the harmonics at multiples of 32 Hz, effectively averaging over the mechanical motion. In light grey, with one-sigma error bars, we plot a period averaged timecourse. In dark grey, also with one-sigma error bars, we plot the timecourse estimated with our method. Note that the error bars from our estimate are substantially smaller compared with those of the period averaged estimate. The reason for this is that, with our estimate, statistically insignificant harmonics are not retained. Therefore, spurious noise is discarded and the error is smaller with our method. Although it is not typically thought of in this way, in a period averaged estimate, all harmonics that are periodic with a base frequency commensurate with the period that is averaged are retained, including those that are not statistically significant. Therefore, spurious noise is retained in period averaged estimates.

In Fig. 6, we compare error bars estimated with our method as a function of principal component index. x’s are the amplitude of the error estimated with period averaging, and dots are the amplitude of the error estimated with our method. Note that the error bars from our estimate are everywhere less with our method relative to period averaging. This is for the same reason as mentioned above: statistically insignificant spurious noise is retained with period averaged estimates, but not with our method. One way to think of this result is that our method accurately identifies the frequency subspace occupied by the multivariate cardiac signal. Fig. 6 shows the significant increase in SNR that can be obtained in cardiac imaging data with our method.

In Fig. 7, we compare images of the spatio-temporal evolution of one cycle of the extracted tachycardia (left panel) with one cycle from the raw data (right panel). The left ventricular free wall (lower left) and the left atrial appendage (upper right) are in view. Light shades of grey represent depolarized tissue, while darker regions are at rest. Approximately one full period of the tachycardia (26.2 ms) is presented with images separated by equal time increments of 2.2 ms, (i.e., two frames). Note the clean extraction and depiction of the periodic wave travelling in a counter-clockwise direction around the ventricle. The raw data is contaminated both by shot noise and also by the beginning of one period of the low-frequency atrial rhythm.

In Fig. 8, we compare activation maps made from a reconstruction of the tachycardia using our method (left panel) and using a period averaged reconstruction (right panel). Greyscales indicate time in milliseconds as the wave propagates counter-clockwise on the ventricle. Activation times were taken to be the times when the potential measured in a pixel reached half
Fig. 5. Superimposed plots of a period averaged principal component timecourse (here, we have chosen principal component 2) with (a) base frequency 32 Hz (this is the re-entrant tachycardia) plotted with one-sigma error bars (light grey) and the multitaper extracted harmonic content with one-sigma error bars (dark grey) and (b) base frequency 5 Hz (this is the atrial rhythm). The SNR in the tachycardia timecourse extracted using our method is about half that of the period averaged timecourse and the SNR in the atrial rhythm is about one third that of the period averaged timecourse.

height during the rise of the action potential [2]. The estimated SNR using our method is roughly twice that of the period averaging method. Therefore, particularly in areas at the edges of the sweeping wave of re-entrant electrical activity such as areas near the wave core (i.e., the center of rotation of the wave depicted in the inset panels) and near the edges of the heart, the activation time estimate is more accurate, giving rise to a less noisy activation map.

In Fig. 9, we compare images of the spatio-temporal evolution of one cycle from the extracted atrial rhythm (left panel) with one cycle from the raw data (right panel). Slightly more than one full period of the atrial rhythm is presented with images separated by equal time increments of 9.1 ms. Notice that the raw data is contaminated with high-frequency shot noise and the voltage signal originating from the ventricular tachycardia, which is faintly visible in this image series, but would be more evident with a contracted color axis. Also apparent in some of the raw images is darkening near the border of the ventricle which is due to mechanical motion. Mechanical motion induces darkening since we have subtracted the mean from the data, therefore, when the heart wall moves, the pixel values relative to the average decrease and look darker. The atrial component of the signal that we separately reconstruct and present in this figure is the second of two periodic signals which were present in the raw data set. That we can identify and separately reconstruct these signals demonstrates the versatility of our approach.

In Fig. 10, we compare activation maps from our reconstruction (left panel) and a period averaged reconstruction (right panel). The maps were calculated in the same way as the above tachycardia activation maps. Activation maps were generated from the initial 5-ms segment of the periodic atrial signal which contained the voltage-dependent fluorescence. The remainder of the period contained the mechanical activity induced by the electrical signal. Electrical activity begins as a broad breakthrough along the upper right border of the image and rapidly spreads downward to activate the entire field in less than 5 ms. Similar to our findings with the activation map of the ventricular tachycardias, the reconstructed atrial activation map shows smoother and more uniform activation times.

In Fig. 11, we compare an array of 6 × 6 pixels taken from the atrial rhythm reconstructed using our method and using period averaging. The voltage amplitude as a function of time reconstructed using our method is plotted in black and the reconstruction using period averaging is plotted in grey. The lower left axis is labeled for reference. These traces show an initial relatively flat diastolic phase followed by a rapid rise in voltage due to electrical activation. The electrical activation is followed by a slow decline caused by the repolarization of the cell mem-
brane. In addition, the fluorescence may change due to motion of the heart, which, in principle can cause an increase or a decline. In this case, the sum of these two can cause either an increase or a decrease [11]. Recent methods based on ratiometry [12] avoid this problem by isolating the voltage-dependent part of the signal from mechanical motion. Note that the high-frequency activation phase of the optical action potential is accurately captured while high-frequency noise not associated with the periodic electrical signal is selectively eliminated.

IV. LIMITATIONS

In our method, we make use of multitaper harmonic analysis to identify and extract sinusoids from noisy data. Because the data is projected onto a set of tapers with bandwidth $2W$, and the analysis assumes that only one harmonic exists within this width, this sets a limit on how well two harmonics with small separation may be distinguished. For the dataset presented here, for example, the smallest separation between harmonics is $5 \text{ Hz}$. Therefore, $W$ must be set to $2.5$ in order to avoid overlap of harmonic estimates. In general, this argument leads to the conclusion that datasets with more periods of oscillation lead to better sinusoid estimates, than datasets with fewer periods of oscillation.

Due to the above bandwidth considerations, difficulties arise when harmonic separations in two harmonic sequences are not separated by more than $2W$. For instance, if the tachycardia were at $30 \text{ Hz}$, half of the harmonics would be indistinguishable from the $60\text{-Hz CCD}$ noise artefact from the camera. Since the tachycardia in our data was at $32 \text{ Hz}$, this problem was avoided,
Fig. 9. A comparison of the reconstructed spatio-temporal evolution of the atrial rhythm (left panel) with one cycle from the raw data (right panel). Slightly more than one full period of the atrial rhythm is presented with images separated by equal time increments of 9.1 ms. Images are 3.4 x 3.4 mm². The order of the images is from left to right, top to bottom.

Fig. 10. Activation maps made from a reconstruction of the initial electrical component (5 ms) of the atrial rhythm using our method (left panel) and from a period averaged reconstruction (right panel). Only the upper right region, containing the atria, of the activity map is shown. Activation times were taken to be the times when the potential measured in a pixel reached half height during the rise of action potential.

Since the second harmonic at 68 Hz was more than 2.5 Hz away from the 60-Hz noise. In the discussion of Fig. 4, we pointed out changes in the F-statistic of one of the 5-Hz harmonics when a nearby 16-Hz harmonic that lay within the bandwidth of the 5-Hz harmonics was removed.

As is evident in the title of our paper, our method is limited to periodic signals. We only obtain good estimates of electrical activity when the heart (in our case, the mouse heart) dynamics are very close to being periodic. However, typically, in adult mouse hearts, heart dynamics are periodic only over times of a few seconds. It is for this reason that we focused on finding good estimates for periodic activity in finite datasets. In the large cycle limit, our method gives the same result as period averaging, but there are seldom enough periods in actual data for the limit to be reached. In developing hearts, we have found the approximation that the signal is periodic to be less appropriate than in adult hearts. Here, heart dynamics can only be described as pseudoperiodic. With a pseudoperiodic signal, if the activation pattern is similar from beat to beat, but the interval length varies, the dataset can be artificially adjusted, forcing the activation intervals to be periodic. We then use our method on the artificial dataset. When electrical activity changes from beat to beat, i.e., the heart dynamics is nonstationary, our analysis fails and a more general approach becomes necessary. Our laboratory is currently engaged in developing approaches to signal detection in nonstationary heart data.

V. DISCUSSION AND CONCLUSION

The analysis techniques which we have described in this study have identified two distinct periodic electrical signals in the tachycardia data: one was the result of re-entrant ventricular activation (and some associated mechanical motion) and the other was due to electrical activity within the right atrium. The cycle length of the atrial signal (5 Hz) was similar to the spontaneous rhythm prior to induction of the arrhythmia (6.5 Hz). Thus, it is likely that the fast ventricular rate suppressed conduction within the atrioventricular (AV) node allowing for the sinus node to drive electrical activation of the atria normally.

Using multitaper log-spectra to visualize the data aided us in detecting multiple periodicities in our data that can be overlooked by other methods such as using the autocorrelation function to identify periodic content in a signal.
Cardiac rhythm analysis is a new method for denoising periodic multivariate signals in imaging data of the mouse heart. The method makes use of PCA to compress the multivariate image set down to a manageable set of images and timecourses. The timecourses are then analyzed using multitaper harmonic analysis and the periodic content is extracted. A new, denoised dataset is then reconstructed by recombining the principal component images with the extracted periodic content.

The reason our extraction method gives increased SNR relative to simply averaging together successive periods of the response is that we make use of characteristics of a periodic signal in frequency space. The use of multitaper techniques to estimate sinusoid amplitudes and phases at multiples of the base pulsing frequency accurately extracts only the statistically significant portion of the signal at harmonic frequencies. Thus, we discard spurious noise in the signal by accurately detecting the frequency and principal component subspace where the signal lies.

In data taken from the mouse heart, we estimate that the denoised signal has a SNR approximately 2 to 3 times that of period averaged estimates. As can be seen in the comparison of activation maps, our method shows a significant improvement in the reconstruction and estimation of the dynamics of the core region of the re-entrant wave.

Our method is applicable, in general, to periodic multivariate signals, and, as such, is applicable to cardiac data taken of normal hearts beating regularly. These data include those collected while hearts are beating spontaneously in sinus rhythm, during epicardial pacing, and during stable atrial and ventricular rhythms including, atrial flutter, junctional ventricular tachycardias and monomorphic ventricular tachycardias. Greater SNRs will provide new opportunities for detailed analysis of low-amplitude optical signals including studies of electrical activity in early embryonic hearts, cell cultures, studies of passive membrane properties where subthreshold stimuli are delivered to cardiac tissue, studies of electrical signals recorded near the center of rotation of spiral waves, conduction studies in the sinus node and other specialized conduction tissues including the AV node and the His-Purkinje tissues [2], [3], [14], [15].

APPENDIX

In this appendix, we give an introduction to PCA [13]. We also outline some of the mathematical background of the spectral and harmonic analysis methods that we use to reconstruct periodic signals from imaging data [7]. Then, we describe our method and how to calculate the SNR of the extracted signal.
A. PCA and Multivariate Data

PCA (also known as singular-value decomposition and Karhunen-Loéve decomposition) is a method for compressing an image set and also for separating out uncorrelated components. Since images in our data are 64 × 64 pixels, a brute force approach would leave us with around 4000 pixel timecourses to analyze. We use PCA to compress the data into a small set of images and timecourses resulting in significantly fewer timecourses to analyze.

The dataset $h(t, x)$ is decomposed as

$$ h(t, x) = \sum_{n} \mu_{n} \alpha_{n}(t) \psi_{n}(x) $$

(3)

where $t$ is the time index and $x$ is pixel number. We seek a decomposition where $\alpha_{n}(t)$ and $\psi_{n}(x)$ are orthonormal. It follows that:

$$ \alpha_{n} = \frac{1}{\mu_{n}} (\psi_{n}, h)_{x} $$

(4)

and

$$ \psi_{n} = \frac{1}{\mu_{n}} (\alpha_{n}, h)_{t}. $$

(5)

To obtain the $\alpha_{n}(t)$s and the $\psi_{n}(t)$s, we may solve one of two problems

$$ \sum_{y} K(x, y) \psi_{n}(y) = \lambda_{n} \psi_{n}(x) $$

(6)

where

$$ K(x, y) = (h(t, x), h^{\dagger}(t, y)) $$

(7)

or equivalently

$$ \sum_{n} C(t, s) \alpha_{n}(s) = \lambda_{n} \alpha_{n}(t) $$

(8)

where

$$ C(t, s) = (h(t, x), h^{\dagger}(s, x))_{x}. $$

(9)

Only one of these eigenvalue problems need be solved, since the above expressions for $\alpha_{n}$ and $\psi_{n}$ can be used to furnish the complementary eigenfunctions. The problem that is solved in practice is that which gives the smaller matrix to diagonalize. In our case, this is $C(t, s)$.

The principal component decomposition is optimal in the sense that it is the best term by term approximation to the data. Therefore, truncating the decomposition gives an optimally compressed dataset. Often truncation is possible since many of the principal components that contain little of the signal power are associated with noise.

B. Multitaper Harmonic and Spectral Methods

Multitaper methods have been successful because they have the smallest bias and variance among the spectral and harmonic analysis methods that have been developed to date [7]. Also, there is a very accurate and useful nonparametric multitaper harmonic analysis technique for extracting deterministic sinusoids from noisy data. This technique is useful both for the extraction of a deterministic signal from noisy data, as well as for better characterization of spectra that include harmonic lines.

It has long been known that “tapering” or windowing a signal can improve the bias characteristics of the signal’s estimated spectrum. Bias is a measure of how much power at one frequency in a signal influences the estimate of power at other frequencies. Multitaper methods achieve low bias through the use of orthogonal tapers (sometimes also called windows), which are solutions to an optimization problem in which frequency concentration is optimized within a certain bandwidth. Eigenfunctions of the optimization problem that are associated with large eigenvalues have extremely good frequency concentration properties, i.e., low bias. Therefore, a set of the eigenfunctions with good frequency concentration is selected and projected onto the data to obtain a set of (approximately orthogonal) estimates of the spectral content. The estimates are then averaged to decrease the variance of spectral estimates or regressed upon to determine harmonic content.

1) Frequency Concentration: Multitaper spectral and harmonic analysis make use of a set of tapers with optimal frequency concentration properties. These tapers are referred to as discrete prolate spheroidal sequences (DPSSs) or also simply as Slepian sequences. The DPSSs are eigenfunctions of a frequency concentration problem, which can be sketched as follows: we define frequency concentration $\beta$ with the expression

$$ \beta^{2}(W) \equiv \frac{\int_{-W}^{W} |G(f)|^{2} df}{\int_{-\infty}^{\infty} |G(f)|^{2} df}. $$

(10)

This is a measure of the amount of power found in a frequency interval with bandwidth $2W$, relative to the amount of power in the entire signal. We wish to find functions $G$ with frequency maximally concentrated within the frequency interval (bandwidth) $2W$. $G$ is the Fourier transform of the discretely sampled time sequence $g(t)$ to be solved for

$$ G(f) = \sum_{t=0}^{N-1} g(t) e^{-2\pi ft}. $$

(11)

Here, $t$ is the time coordinate in the sequence and $f$ is the frequency. Inserting this expression into the expression for $\beta^{2}(W)$ gives

$$ \beta^{2}(W) = \frac{\sum_{t=0}^{N-1} \sum_{t'=0}^{N-1} g(t) s \sin \frac{\pi N}{2} \frac{t'-t}{N} g(t')}{\sum_{t=0}^{N-1} |g(t)|^{2}}. $$

(12)

We now maximize $\beta^{2}(W)$ resulting in the eigenvalue problem

$$ \sum_{t'=0}^{N-1} \sin 2\pi W(t'-t) g_k(t') = \lambda_k(N, W) g_k(t). $$

(13)

The solution of this eigenvalue problem results in a set of eigenfunctions $\{ g_k(t) \}$ that are parametric in $N$, the number of points in the time series, and $W$ the bandwidth within which power is concentrated. They are ordered according to their frequency concentration characteristics. The eigenfunction corresponding to the largest eigenvalue has the best concentration properties, and so on in decreasing order.

2) Spectral Analysis: We use multitaper spectral estimates primarily as a means of inspecting our data. Multitaper harmonic analysis, explained next, is the technique used in our reconstruction method. A spectral estimate only provides information concerning the power at a given frequency, and gives no information concerning the phase of the signal, however, the
spectrum can be extremely useful in visualizing the distribution of power in various components of a signal.

The spectrum is calculated by tapering the signal (here assumed to be stationary) with a set of low-bias DPSS tapers, giving a set of individual estimates of the spectrum

\[ E_k \{ S(f) \} = \sum_{l=0}^{N-1} g_k(t)a(t)e^{-i2\pi ft} \]  \hspace{1cm} (14)

where \( k = 1, \ldots, K \) denotes the tapered set used to calculate the estimate. These estimates, each having good bias properties, are averaged to give a low-variance spectral estimate

\[ E \{ S(f) \} = \frac{1}{K} \sum_{k=0}^{K-1} E_k \{ S(f) \}. \] \hspace{1cm} (15)

To retain low bias in the spectral estimate, a good first choice for \( K \) is \( K = 2NW - 1 \). For a spectral analysis of a periodic signal, \( W \) should be at most half the frequency separation of successive multiples of the base frequency.

3) Harmonic Analysis: Multitaper harmonic analysis is one of the two main techniques used in our analysis method. Harmonic analysis provides information on both amplitude and phase of deterministic sinusoids in noisy data.

The procedure here is to begin with the assumption that the time series \( a(t) \) has a deterministic sinusoidal component at frequency \( f \) with complex amplitude \( c \) (i.e., amplitude and phase) accompanied by noise \( \eta(t) \)

\[ a(t) = c(f)e^{i2\pi ft} + c(f)^*e^{-i2\pi ft} + \eta(t). \] \hspace{1cm} (16)

The Fourier transform \( A \) of \( a \) tapered with the \( k \)th DPSS taper at frequency \( f \) is, to a good approximation

\[ A_k(k) = c(f)\frac{G_k(0)}{(\Delta t)^{1/2}} + \epsilon_k \] \hspace{1cm} (17)

where \( \epsilon_k \) is the tapered estimate of the noise at frequency \( f \), \( \Delta t \) is the increment in time between samples (assumed fixed), and \( G_k \) is the Fourier transform of the \( k \)th DPSS. At this point, we have \( k = 1, \ldots, K \) estimates of the sinusoidal amplitude at frequency \( f \). We can now use linear regression (i.e., a least squares fit) to obtain an estimate for \( c \)

\[ \hat{c}(f) = (\Delta t)^{1/2} \sum_{k=0}^{K-1} \frac{A_k(f)G_k(0)}{\sum_{k=0}^{K-1} G_k^2(0)}. \] \hspace{1cm} (18)

As a consequence of the central limit theorem, the estimates \( \hat{c}(f) \) are normally distributed. With the estimate \( \hat{c} \) in hand, we construct the ratio of the estimated variance of the distribution of the complex signal amplitude \( c \) with an estimate of variance of the distribution of the noise \( \epsilon_k \). The ratio is F-distributed with 2 and \( 2K - 2 \) degrees of freedom under the null hypothesis (that there is no sinusoid present) and takes the form

\[ \frac{(K - 1)\hat{c}^2(f)\sum_{k=0}^{K-1} G_k^2(0)}{\Delta t \sum_{k=0}^{K-1} \left| A_k(f) - \hat{A}_k(f) \right|^2} = F_{2, 2K - 2} \] \hspace{1cm} (19)

where

\[ \hat{A}_k(f) \equiv \hat{c}(f)\frac{G_k(0)}{(\Delta t)^{1/2}} \] \hspace{1cm} (20)

and \( = \) signifies “equal” in the distributional sense. With the above expression for an F-distribution, we can test whether a sinusoid exists in the data. We take as null hypothesis that there is no sinusoid. We then ask the question, is the data consistent with the null hypothesis. Mitra and Pesaran [8] suggest using a significance level for the F-test of \( 1 - 1/N \). At this level, a Gaussian noise process would, on average, result in only one spuriously identified sinusoid in the time series.

It should be emphasized here that this method gives excellent results, even for sinusoids in substantial background noise.

C. Method for Extracting the Periodic Signal From Imaging Data

Our method, which we also describe verbally in the main text, is as follows.

We begin by compressing the image set using PCA

\[ f(t, x) = \sum_{n} \mu_n \alpha_n(t) \psi_n(x) \] \hspace{1cm} (21)

here, \( t \) is time, or often just frame number, and \( x \) is pixel number. We determine where to truncate the expansion by either examining the spectrum visually for the location of the end of the harmonic sequences due to periodic content and the spectra become smooth, or by examining the locations of significant harmonics to determine where the harmonic sequences end. We also check the amount of power in the principal components and ascertain that we are not discarding more than 1% of the power in the signal. We have found in the mouse heart data analyzed here that 50 principal components are usually sufficient.

We then use multitaper harmonic analysis to remove statistically significant sinusoids that are located at multiples of the base pulsing frequency. We choose the bandwidth \( 2W \) of the Slepian sequences used in the estimates to be 90% of the width between harmonics in the harmonic sequence. The harmonic analysis results in a set \( \{ \hat{a}_n^h(t) \} \) of estimated periodic time courses associated with the set of eigenimages \( \{ \psi_n(x) \} \).

Finally, we reconstruct an estimate of the harmonic content in the image set \( f^h(t, x) \) by recombining the estimated periodic timecourses with the eigenimages from the PCA.

\[ f^h(t, x) = \sum_{n} \mu_n \hat{a}_n^h(t) \psi_n(x). \] \hspace{1cm} (22)

\( f^h(t, x) \) contains all of the statistically significant periodic content of the original raw data.

D. SNR of the Extracted Periodic Component

The results from the harmonic regression analysis may be used to calculate error bars for each extracted harmonic. The variance of an estimated harmonic is

\[ Var(\hat{\epsilon}(f)) = \frac{SN(f)}{\sum_{k=1}^{K} G_k^2(0)} \] \hspace{1cm} (23)

where the local continuous part of the spectrum may be estimated from the regression analysis as

\[ SN(f) = \frac{1}{K} \sum_{k=1}^{K} G_k^2(0) \] \hspace{1cm} (24)

One-sigma error bars for a single harmonic, for example, may then be estimated as \( Var(\hat{\epsilon}(f))^{1/2} \). When multiple significant
harmonics exist in a timeseries, global error bars may be calculated for the extracted timecourse as
\[
\left( \sum_j \text{Var}(\hat{c}(f_j)) \right)^{1/2}
\]
where \(j\) counts the significant harmonics.

REFERENCES