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Automated Masking of Voltage-Sensitive Dye Imaging Data

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Abstract— **This paper discusses the development of an algorithm to mask poor quality data in fluorescence videos of cardiac tissue stained with voltage-sensitive dye. The aim was to simplify further analysis by eliminating the step of manually masking areas of poor signal quality and areas outside the preparation of interest. Our algorithm estimates signal to noise ratio (SNR) from the power spectral density (PSD) for each pixel. This information is combined with information about the fluorescence intensity in each pixel, according to a user-selectable weighting factor. A threshold is then applied to the resulting combined measure. This approach resulted in an effective algorithm that is capable of automatically creating a "mask" that can be applied to the data to exclude parts of the data from further analysis. The algorithm is sufficiently efficient to allow interactive use, allowing the user to adjust the parameters of the algorithm and instantly view the resulting mask. This tool will be useful as a technique to simplify further analysis of voltage-sensitive dye imaging data.**

I. Introduction

Voltage-sensitive dye imaging is becoming a commonly used technique for studying action potential propagation in the heart [1-2]. One problem that this can present is that often the videos generated via this means will have pixels which are not on the preparation or for some other reason have weak signal. Thus, analysis of this data requires a tedious manual step of masking out undesired data prior to further processing. For example, masking of irrelevant data is a necessary predecessor to any processing which seeks to locate the exact times of depolarization for each time series extracted from the video, to avoid erroneous detections resulting from background noise. This is especially true since the data are normalized on a pixel-by-pixel basis, and the background noise may therefore have steep enough transitions that any algorithm looking for steep rises in amplitude to indicate action potentials might be affected by the background noise. Thus, the ability to reliably and repeatably generate a mask, which would select only those pixels which are relevant, is a very valuable tool for post-processing voltage-sensitive dye imaging data. Assuming that the algorithm can be made sufficiently efficient, it may even be applied during an ongoing experiment to provide feedback on data quality. Another important reason to make this algorithm fast enough to be interactive is to allow the user to quickly change the mask by adjusting parameters. The objective of this study was to develop an interactive algorithm for this automated masking operation.

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II. Methods

A. Data Acquisition

This tool was designed to analyze data generated by voltage-sensitive dye imaging of mouse atria as described by Nygren *et al* [2]. Briefly, preparations consisting of the two mouse atria in isolation were stained with the voltage sensitive dye di-4-ANEPPS, so as to generate a fluorescence video containing membrane potential information. The data in this video is sampled at a rate of approximately 950 Hz, using a CCD camera. These data were then processed by removal of baseline drift, inversion, and normalization on a pixel-by-pixel basis [2]. These processing steps are necessary because the raw data that is generated by the CCD camera has a high baseline, and only small amplitude changes corresponding to an action potential. The raw data is useful for getting a picture of the overall fluorescence intensity, but not useful as an indication of membrane potential. It is necessary to normalize the data on a pixel-by-pixel basis in order to be able to process it further. This normalized data is the data to be processed by the masking software tool.

B. Signal Characteristics

To begin, it is useful to look at the basic properties inherent to our signal. The signal of interest is the fluorescence signal of an action potential of a mouse atrium, typically occurring periodically at a frequency lower than 10 Hz. This is seen in Fig. 1, which depicts a typical signal from a pixel with good signal quality. It can be seen from Fig. 1 that the signal consists of a strong low-frequency component (action potential) and high-frequency noise. An estimate of the power spectral density (PSD) of the noise-free signal was obtained as follows: 1) a relatively noise-free action potential waveform was obtained by signal-averaging a series of subsequent action potentials from a single typical recording, 2) this noise-free AP was repeated 125 times at a cycle length of 200 ms, 3) the PSD of this periodic signal

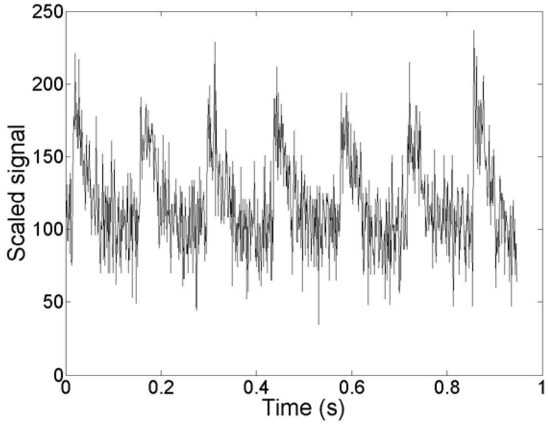

Fig. 1. Typical signal seen in a pixel which contains a good action potential signal. Note that the signal consists of a strong, relatively low-frequency periodic signal (action potential) and high-frequency noise.

was computed using the Welch algorithm [3], implemented in Matlab, using a Hamming window. Figure 2 shows the resulting PSD. Note that the power spectrum decays rapidly, with the majority of power found in the low-frequency end of the spectrum.

C. Criterion I: The Power Spectral Ratio (PSR)

We hypothesized that one could distinguish between a pixel containing a valid signal (action potential data) and a pixel containing only noise, by taking the ratio of the low-frequency power over the high frequency power as a rough estimate of signal to noise ratio (SNR). This "power spectral ratio" (PSR) should be small for pixels containing only noise, and large for pixels containing a strong low-frequency signal. The PSR can be computed by summing the values (P_i) in the PSD below and above a user specified frequency threshold (T), then taking the ratio of these sums, where M is the bin corresponding to the Nyquist frequency, as follows:

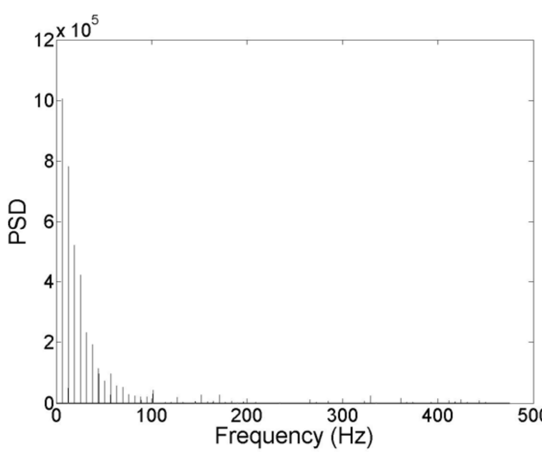

Fig. 2. Power spectral density (PSD) of a noise-free action potential signal (see text).

$$\text{PSR} = \sum_{i=0}^{T} P_i \Bigg/ \sum_{i=T+1}^{M} P_i \tag{1}$$

These calculations yield an estimated SNR value for each pixel. The PSD was calculated based on a record of 1500 data points, using the Welch algorithm [3], implemented in Matlab and a Hamming window.

D. Criterion II: Raw Fluorescence Intensity

A high PSR value (high SNR) does not necessarily mean that a pixel contains valid action potential data. Movement of stimulation electrodes outside the area covered by the preparation, as well as occasional air bubbles introduced by the perfusion apparatus, can cause transient large-amplitude and low-frequency events in the signal in some pixels. The PSR algorithm will estimate a high SNR for a pixel affected by these artifacts. These effects were partially suppressed by clipping SNR values at a level corresponding to the mean SNR plus three times the standard deviation (SNR values above this threshold were set to the threshold value). Furthermore, since these artifacts occur outside the preparation, the overall fluorescence intensity in these pixels will generally be low. Raw fluorescence intensity was therefore added to the algorithm as a secondary criterion. A user-selectable parameter, Q, determines the relative importance of the PSR and the raw fluorescence intensity in generating the final mask:

$$D = (R \times Q) + (J \times (1 - Q))\,, \tag{2}$$

where D is the decision map value, R is the PSR value, and J is the raw fluorescence intensity value. After a decision map of numbers had been generated according to (2), the final map was obtained by applying a user-selectable threshold to the decision map (D). Pixels with decision map values above this threshold are considered part of the accepted data in the mask, to be included in further analysis.

E. Smoothing of Masks

The two criteria described above often yielded a mask with a few isolated rejected pixels inside, and a few isolated accepted pixels outside the preparation (Fig. 3). This was easily corrected by implementing a smoothing algorithm that was able to change the status of a pixel based on the number of opposite pixels around it. The smoothing algorithm examines all eight of the pixels bordering a given pixel. If the number of pixels with opposite status (included/excluded) from the pixel under consideration exceeds five, the status of the pixel is changed. The changes are not implemented until all the pixels have been examined. Alternative thresholds of more than four and more than six adjacent pixels of opposite status were considered and tested. However, a threshold of five pixels was found to represent the best compromise between removing isolated pixels of opposite status, and retaining the overall shape of the mask.

F. Development Environment

Algorithm development and testing was carried out on a 3.2GHz Pentium 4 processor with 1GB of RAM running Windows XP SP2 and Matlab 7.

III. RESULTS

A. Performance of the PSR Algorithm

Extensive testing showed that the PSR was a useful estimate of the SNR for a given pixel, and also useful to make a mask using the previously described algorithm. Comparisons between manually generated masks using previously existing tools [2] and the masks generated by the PSR algorithm were in general very similar. As discussed in the METHODS, high-amplitude low-frequency artifacts are occasionally present in the signal (e.g. from bubbles introduced by the perfusion apparatus). The PSR algorithm based on Criterion I (PSR thresholding) alone was found to be sensitive to these artifacts as the algorithm would classify such a pixel as one with a high quality signal. However, this problem was successfully alleviated by the addition of Criterion II (Raw Fluorescence Intensity), in combination with the suppression of outliers above three standard deviations above the mean.

The PSR algorithm first calculates the power spectrum of each pixel, which takes approximately 12 to 13 seconds for 1500 frames of a 60x60 pixel recording, and then stores this data. When a new mask is subsequently required in response to user adjustment of the parameter settings, the algorithm can generate a new mask in approximately 0.15 seconds. This performance fulfilled our requirement for the software to be interactive.

B. Accuracy of the PSR as an SNR estimate

The PSR algorithm introduces a degree of foreseeable error in the SNR estimate. Typical noise power spectra (data not shown) demonstrate that noise does contribute to the low-frequency domain. Similarly, due to the abruptness of the upstroke of any normal action potential, there is some high frequency content present in a valid action potential signal. Thus, the simple estimate of SNR employed here (PSR) results in some noise being counted as signal, and some signal being counted as noise. To some extent, this effect will cancel itself out, but there will still be some error introduced by the very nature of this algorithm. To quantify this error, a synthetic noise-free "action potential" was generated by summing Boltzman curves fit to a typical action potential obtained by signal averaging real data. Noise (gaussian white noise) was then added to this signal, with the power of both the original signal and the original noise being previously calculated. The PSR technique was then applied to calculate an SNR estimate. This estimate was compared to the known SNR value, found by taking the ratio of the known signal power over the known noise power. This was repeated with different random noise being added for each trial. It was found that for a signal with the true SNR of 0.5, the PSR technique, with a frequency cutoff of 50 Hz, yielded an SNR which was 0.05±0.03 (n=30) higher than the theoretical value. For a true SNR of 0.25, the PSR technique yielded a value 0.10±0.03 (n=20) higher than the theoretical value. This effect is demonstrated by Fig. 4, showing theoretical SNR values and estimated SNR values based on the PSR. As seen in Fig. 4, the PSR tends to overestimate the SNR for low true SNR values, and underestimate SNR for higher values. The majority of our data have SNR of less than 0.7, meaning that in practice the PSR algorithm tends to overestimate SNR in our application.

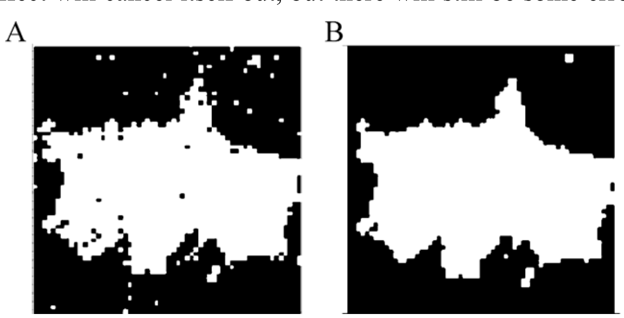

Fig. 3. Typical mask before (A) and after (B) the smoothing algorithm is applied.

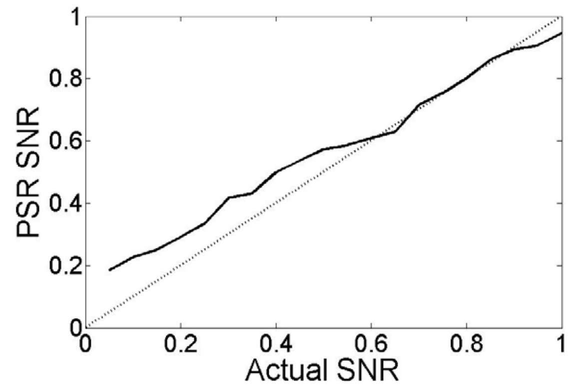

Fig. 4. Estimated SNR based on the PSR, plotted versus theoretical SNR values. The solid line is the PSR values and the dotted line is the theoretical values. Note that the PSR estimate tends to overestimate SNR for low SNR values, and underestimate SNR for high SNR values (see text).

IV. DISCUSSION

In this study, we have developed an automated and interactive algorithm for the purpose of masking data in voltage-sensitive dye recordings of cardiac tissue. The algorithm is based on an estimate of the signal to noise ratio (SNR), and therefore also provides an objective assessment of the quality of the data across the preparation. The data being manipulated by the algorithm consists of post-processed (see METHODS) files which contain voltage-sensitive dye action potential data generated by a CCD camera. The motivation for developing this tool was to give researchers the ability to automatically mask out irrelevant data, rather than relying on a tedious manual masking process.

A. Choice of Algorithm

The observation that the power spectrum of a pixel with a noise-free action potential signal had a strong weighting towards low-frequency power was a key consideration in the design of the masking algorithm. We defined the ratio of two portions of the power spectrum (PSR) as an estimate of SNR, and used this estimate as the primary decision criterion in generating the mask.

Testing of this approach uncovered problems with pixels containing large-amplitude low-frequency deflections (see METHODS), which yielded very large estimated SNRs. This observation led us to implement clipping of SNR values above mean plus 3 standard deviations. The resulting SNR estimate on its own proved to be relatively effective as a decision criterion for generating a mask. However, there may be times when a researcher wants to include pixels that have relatively noisy signal in the mask as long as they are part of the preparation. The manual masking procedure currently used in the lab [2] relies solely on the raw fluorescence intensity (prior to background subtraction and other processing, see METHODS) to construct a mask. Since the dye preferentially stains tissue, pixels that are part of the preparation have higher fluorescence intensity than background pixels. Thus, we included a parameter which allowed the user to also consider intensity information. By adjusting a weighting parameter, the user can set the relative importance of signal quality (PSR) and intensity in generating the final mask.

The final algorithm developed was sufficiently fast for interactive use, and had only 3 user-adjustable parameters. The first parameter allows the user to set the cutoff between frequencies classified as noise and those classified as signal. The second parameter lets the user control the relative influence of the SNR estimate versus the fluorescence intensity information in generating a decision criterion for the mask. The final parameter lets the user set a threshold for the decision criterion. Any pixel whose decision criterion value is above the threshold is classified as part of the preparation and included for further processing, whereas those below the threshold are not.

B. Assessment of Signal Quality

The choice of an algorithm based on an estimate of SNR (PSR) offers advantages beyond the creation of a mask. Often a researcher may be interested in obtaining an overview of the signal quality in their recordings. Since the PSR algorithm involves the calculation of an estimate of the SNR for each pixel, it can easily be extended to generate a map of SNR across the entire field of view. Such a map provides a concise overall view of the quality of data being recorded.

It is important to note, however, that this estimated SNR contains an error introduced by the nature of the algorithm. This error was quantified through simulations, and it was estimated that a typical SNR estimate from our PSR algorithm would be approximately 0.075 higher than the correct SNR for the given pixel. There are two sources of this error: 1) the high frequency content of the signal which is counted as noise, and 2) the low frequency content of the noise which is counted as signal. As a result, the PSR algorithm produces an SNR estimate that is slightly above the true SNR for data with low SNR, and significantly below true SNR for data with high SNR. When considering why this might be, we looked at the shape of the signal power spectrum (see Fig. 2) and the noise spectrum, which is approximately uniform across the frequency spectrum (white noise). It is understandable that when the true SNR is low the SNR estimate is slightly high, as this is evidence of the existence of noise in the low frequency spectrum being counted as signal. Similarly, when applied to synthetically generated noise-free signals, the PSR algorithm yields an SNR estimate that can be as low as half of the true SNR value. This can be explained by the existence of significant signal power existing in the frequency band above 50 Hz (our cutoff between signal and noise for this comparison). This error introduced by the PSR algorithm does not pose a large problem for our algorithm for the purpose of generating a mask. However, if the estimated SNR is used to assess data quality, these limitations must be kept in mind when interpreting the results.

C. Alternative Algorithms

A relatively simple filtering and amplitude comparison approach [4] was initially considered for this work. However, testing indicated that this approach was less reliable than the PSR algorithm. In addition, it is worth noting that this alternative approach provided no estimate of the SNR of the given data. The PSR approach developed here, while adequate for our current purposes, could be refined by using a more accurate estimate of SNR. Possible approaches include using wavelet denoising techniques to separate the signal and noise components for this purpose.

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