Using Image Registration to Reconstruct Spatiotemporal Electrical Activity in Cardiac Optical Mapping Studies

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Abstract

Optical mapping using membrane potential-sensitive fluorescent dyes provides a means of recording electrical activation on the heart surface with high spatial resolution. However, in order to interpret these data, it is necessary to correct for artifact introduced by heart wall motion. This paper describes a novel image registration technique that enables electrical activity on the heart surface to be reconstructed in the absence of pharmacological uncouplers commonly used to minimize heart wall motion. Image registration substantially reduced motion artifact and enabled activation times and action potential duration to be reconstructed accurately with respect to well-defined material points on the heart surface.

1. Introduction

Optical mapping is widely used in experimental studies of cardiac electrophysiology. The approach utilizes cell membrane-bound dyes, which emit fluorescence that is modulated by small changes in transmembrane potential.

Optical mapping has a number of advantages over conventional extracellular recording. Optical signals are acquired without physical contact, are directly affected by local transmembrane potential and may be recorded at high spatial density and in the presence of defibrillation-strength shocks[1]. However, optical signal quality is degraded by motion artifact, photo-bleaching and high frequency noise.

In practice, it is necessary to minimize motion artifact in order to record reliable action potentials using cardiac optical mapping. Typically, this is achieved by restraining heart wall motion physically or, more commonly, by employing pharmacological agents such as 2,3-BDM or Cytochalasin D to block excitation-contraction coupling. Although widely used for this purpose, these uncoupling agents directly affect cardiac electrophysiology at the concentrations necessary to block contraction. An alternate approach has been to utilize the fact that, in ratiometric dyes such as di-4-ANEPPS, voltage-dependent modulation occurs in opposite directions on either side of the isosbestic wavelength, whereas changes due to motion and photo-bleaching do not. By recording such fluorescence signals across two wavelength windows, it is possible to minimize motion and photo-bleaching artifacts in the absence of pharmacological uncouplers using ratiometry [2] or subtraction techniques [3]. However, while these methods remove motion signal they do not correct for the loss of spatial registration that occurs during contraction.

In this study, we seek to investigate image-based transformations that can be used to reconstruct spatiotemporal electrical activity.

2. Methods

Isolated heart preparation

Rats were anesthetized and their hearts were rapidly excised and immersed in cold (4°C) saline. The aorta was cannulated, and the heart mounted on a Langendorff system and perfused with modified Krebs-Hensleit solution containing (in mM NaCl 118, KCl 4.75, MgSO4 1.18, KH2PO4 1.18, NaHCO3 24.8, Glucose 10, CaCl2 2.5) and perfused at room temperature at a constant pressure ~80mmHg. The hearts were stabilized for 30 minutes. The coronary circulation was loaded with voltage sensitive dye di-4-ANEPPS (Molecular Probes) at ~100 μM for 5 minutes.

Optical system

The heart was illuminated with 2 blue LEDs (Luxen V Star, LHXL-LB5C), each equipped with a small reflector, short pass filter (505 nm) and cooling fan. The LEDs were ~80 mm from the heart surface. The LEDs were

driven at 700 mA using a power supply (POWERTECH, MP3087) in current control mode and were allowed to stabilize for at least 60 minutes before measurements were made.

The heart was imaged using a cooled EMCCD camera (Cascade 128+, Photometrics) at 128x128 pixels, 16bit resolution and 326 frame/s. The camera was equipped with a zoom lens (Navitar NAV DO-5095), x2 close up lens (B+W, NL-2) and long pass filter (600nm, Marumi MC-R2). The camera was positioned ~220mm from the heart providing a field of view of 20 by 20 mm at a spatial resolution of 156 μm^2 per pixel. Camera acquisition was controlled with a laboratory PC using V++ software (Digital Optics).

Data processing

All data were recorded at full resolution in "TIFF" format. Time series records for each pixel were processed as follows. Signals were averaged across 3 successive "periods" each synchronized with the action potential (AP) [4]

$$y(i) = \frac{1}{M} \sum_{j\ 0}^{j=M-1} \left[x_j(i) \right], i = 0,1,2,.., N \tag{1}$$

where the window M = 3 and N is the period length.

Signals were separated into a series of "periods" each containing an AP and synchronized across all pixels from the AP upstroke detected at one location.

Image registration

Motion artifacts are caused in part by physical translation of heart regions with respect to the photodetector. Planar transformation of successive images to preserve the registration of key structural features provides a means of correcting for this [5]. That is, if we have separate images of the heart surface B and A that differ as a result of contraction, it may be possible to correct for the effects of heart wall motion using established image registration methods that transform A to best match B.

This process is described by the equation

$$\alpha_0 = \arg\max_{\alpha} .c\left(B(x_B), A'(T_\alpha(x_A))\right) \tag{3}$$

where the geometric transform Tα, controlled by the parameter vector α, is used to map image A onto A'. Criterion c defines a measure of image similarity and the vector α_0 maximizes that criterion [8].

Rohde et al. [1] used mutual information [6] as their measure of image similarity, but we use the cross-correlation coefficient. This criterion is reliable and robust in the presence of variable image intensity [7]. The cross-correlation coefficient is estimated [8] as

$$\frac{\sum_i (a_i - \bar{a})(b_i - \bar{b})}{\sqrt{\sum_i (a_i - \bar{a})^2 \sum_i (b_i - \bar{b})^2}} \tag{4}$$

where

$$\bar{a} = \frac{1}{N} \sum_i a_i \quad and \quad \bar{b} = \frac{1}{N} \sum_i b_i$$

and N is the number of pixels.

In this study, we adopted a two stage image registration process, as illustrated in Figure 1. Firstly, structural features within the image were tracked using a simple transformation that allowed translation in two axes. Small regions of interest (ROI) uniformly distributed throughout the initial image were used to define marker points that were tracked through successive frames. To increase tracking resolution, images were interpolated to 512 x 512 pixels.

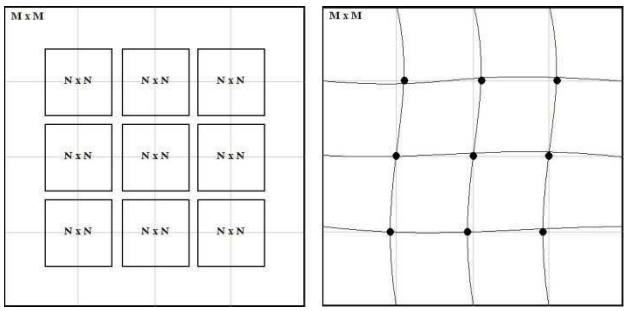

Figure 1: This picture on the right depicts the grid (M x M) of marker points defined by (N x N pixel) ROI on the left. The movement of marker points is used to reconstruct motion across the frame by bilinear interpolation from marker points (shown as filled circles at right). An initially uniform grid is deformed as shown.

Secondly, for each frame, pixel motion was reconstructed from the tracked marker points employing bilinear interpolation and this was used to define a nonrigid transformation that preserved image registration. The spatiotemporal trajectory of marker points was smoothed with a linear averaging filter (n=3) prior to interpolation. Frames were registered across a 112 x 112 pixel subregion of the 128 x 128 pixel source image.

For analysis purposes, a graphics user interface (GUI) application was designed in the computing environment MATLAB (ver. R2007B). All data were processed using this GUI.

3. Results

In this study, fluorescence signals were acquired from isolated beating hearts in the absence of pharmacological uncouplers or mechanical restraint. Image intensities are represented in pseudocolour with red indicating maximum and blue minimum intensities.

Figure 2 shows grid of marker points and associated ROI across the processed frame.

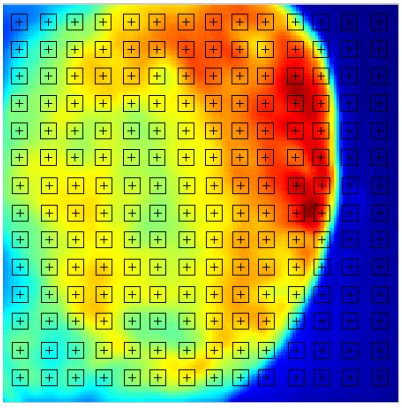

Figure 2: Grid (14 x 14) of the marker points defined ROI (8x8 pixels).

The image registration techniques outlined in the Methods section were used to define frame-to-frame motion of features within a specified ROI. In the upper panel of Figure 3, the movement of a readily identified feature is tracked through a cycle of contraction in frames separated by 46 ms. There is evident deformation of the image throughout this period. In the lower panel of Figure 3, corresponding frames are shown after rigid image registration.

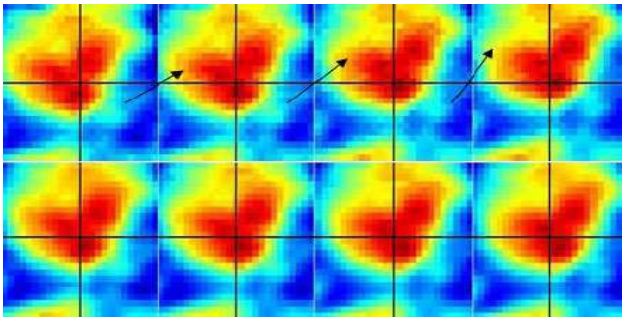

Figure 3. Suppression of cardiac motion in ROI (N=25) using rigid image registration. Upper panel: Frames at 46 ms intervals through cardiac cycle without image registration. Arrow tracks the registered path on the heart surface. Lower panel: Corresponding frames after image registration. The cross hairs provide a fixed spatial reference.

Spatiotemporal electrical activity was reconstructed using non-rigid registration based on the motion of all marker points. Figure 4 compares frames from the original and reconstructed (registered) sequences for a typical cardiac cycle from the beginning of activation.

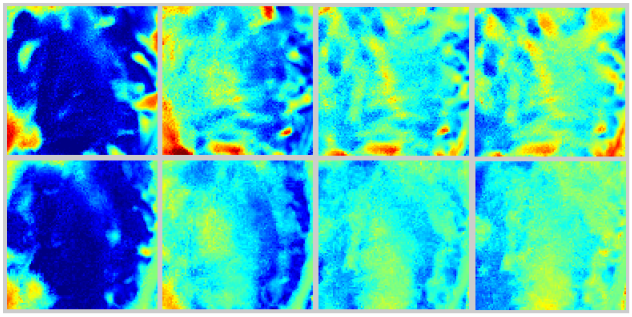

Figure 4: Spatiotemporal electrical activity. Upper panel: Frames at 46 ms intervals through cardiac cycle without image registration. Lower panel: Corresponding frames after image registration.

Image registration suppresses motion artifact and enables spatiotemporal electrical activity to be visualized. This is evident in Figure 5 depicted where signals from 25 locations across the heart surface are compared before and after image registration.

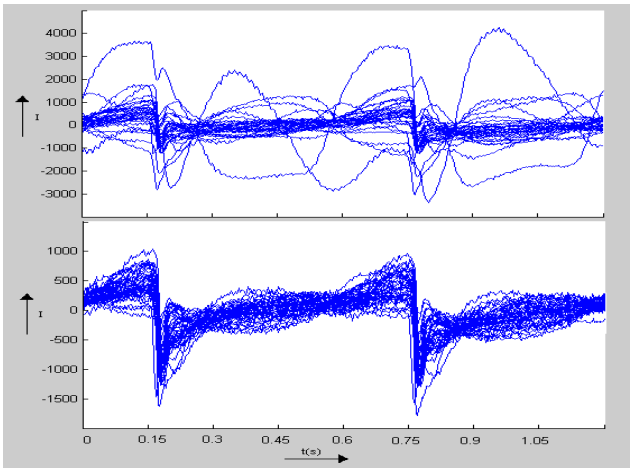

Figure 5: Signals from 25 locations across the heart surface from unregistered (top) and registered sequences (bottom). The vertical axes shows absolute signal intensity without DC offset (the scale is 16 bit unsigned integer).

4. Discussion and conclusions

In this study, we have shown that spatiotemporal electrical activity can be reconstructed from optical maps acquired from the heart surface in the absence of electromechanical uncouplers or physical restraint, using appropriate image registration techniques. We have used a simple linear transformation (see Figure 3) to track the

motion of characteristic features within subregions of the image field. The movement of this array of marker points was then used to identify nonlinear transformations that provide image registration across the whole frame. Using this approach it is possible to stabilize regions to which measures of electrical activity are referred and to minimize motion artifact, substantially. As a result it is possible to estimate action potential duration for instance with much greater reliability than would otherwise be the case (see Figure 5.).

A strength of this approach is that background information only is used to track regional heart wall motion and image registration is achieved by mapping nonuniformly stained regions so that they match each other on a frame-to-frame basis. We argue that this provides an efficient means of maintaining image registration in the beating heart. This should enable reliable measurements of electrical activity to be made in more physiological conditions than is the case at present.

Acknowledgements

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References

[1] Efimov RI, Nikolski PV, Salama G. Optical Imaging of the Heart, Circulation Research 2004;95:21-33.
[2] Kinsley SB, et al. Ratiometry of transmembrane voltage-sensitive fluorescent dye emission in hearts. American Journal of Physiology Heart and Circulatory Physiology 2000;279:1421-1433.
[3] Tai DCS, et al. Correction of motion artifact in transmembrane voltage sensitive fluorescent dye emission in hearts. American Journal of Physiology Heart and Circulatory Physiology 2004;287:985-993.
[4] Jan J. Digital Signal Filtering, Analysis and Restoration. Institution of Electrical Engineers 2000; ISBN 0852967608.
[5] Rhode GK, Dwant BM, Lin S. Correction of motion artifact in cardiac optical mapping using image registration. IEEE Transaction on Biomedical Engineering 2005;52:338-341.
[6] Maes F, et al. Multimodality Image Registration by Maximization of Mutual Information. IEEE Transactions of Medical Imaging 1997;16:187-198.
[7] Jähne B. Digital Image Processing, 6th revised and extended edition. Springer-Verlag Berlin Heidelberg 2005; ISBN 3-540-24035-7.
[8] Jan J. Medical Image Processing Reconstruction and Restoration, Concepts and Methods. CRC Press 2006; ISBN 0-8247-5849-8.

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