Robust Framework for Quantitative Analysis of Optical Mapping Signals without Filtering

Ilija Uzelac[1], Flavio H Fenton[1,2,3]

[1]School of Physics, Georgia Institute of Technology, Atlanta, GA, USA
[2]School of Physiology, Georgia Institute of Technology, Atlanta, GA, USA
[3]School of Biology, Georgia Institute of Technology, Atlanta, GA, USA

Abstract

Experimental studies with in-vitro isolated hearts using optical mapping techniques have had a significant impact on our understanding of cardiac electrophysiology. The trans-membrane voltage V_m, and intracellular free calcium concentration $[Ca_i]^{+2}$ signals obtained from optical mapping experiments can often be corrupted with noise. This is mostly due to the small light intensities and very short exposure times when high speed cameras are used at frames rates of 500-1000 fps. In addition, for small preparations or recordings of small areas, the noise floor levels are even greater and can be comparable to the amplitude of the signal (S/N ≈1). In general strong spatial and temporal filtering is necessary to remove the noise at the expenses of signal degradation and loss of critical information especially at high frequencies that are of a particular interest. In this paper we present and analyze an oversampling image processing technique where due to the cycling processes in cardiac activity during steady state dynamics we are able to stack (sum up) the images recorded at specific equidistant time intervals. The stacking process reduces the noise effectively as the square root of the number of stacked images used. We show that no spatial or temporal filtering is needed to obtain useful data with the stacking technique that allows us to resolve information on a time scale only limited with a sampling rate.

1. Introduction

Experimental optical mapping technique have proven to be a powerful tool for research and have been widely used at the leading edge of research especially in the fields of neurology [1–2] and cardiology [3-5]. In particular for cardiac electrophysiology, trans-membrane voltage (V_m) sensitive dyes have made important contributions to our understanding of many electro-physiological properties that exhibit spatial-temporal complexity such as: the development of alternans [6-7] and its dependence to frequency and temperature [8]; the dynamics of spiral waves and their role as drivers of tachycardia [9] and fibrillation[10]; more recently in studies of defibrillation using drugs [11] and shocks [12-14]; and the mechanisms of virtual electrode formation [12-13,15]. Optical mapping techniques are based on the use of fluorescent dyes sensitive to changes of a physiological parameter. For example, the change of trans-membrane voltage in cardiac cells is measured with membrane voltage sensitive dyes (such as Di-4-ANNEPS, and JPW-6003) which absorption and emission spectra shifts a few nanometers as a function of voltage changes across the cardiac cell membrane. Therefore, the shift in the emitted fluorescence can be linearly related to voltage changes. Similarly, the intracellular free calcium concentration $[Ca_i]^{+2}$ can be measured using fluorescent sensitive dyes such as Rhod-2 and Rhod-4. In optical mapping experiments, the emitted florescence is recorded with high speed cameras typically at the rates of 500-1000 fps in order to capture fast dynamic of V_m and $[Ca_i]^{+2}$. At such high frame rates, exposure time for each image is very short, and in addition the fractional change in intensity of the emitted fluorescence light is typically <10%. Even in the "best" experiments, the S/N ratio is rarely greater than 10, and often noise levels can be comparable to the signal levels of V_m and $[Ca_i]^{+2}$. Furthermore, there can be variation of signal levels among the different regions of the heart due to the staining procedure in which some regions of the heart became more stained than others. Simultaneous measurements of V_m and $[Ca_i]^{+2}$ are often performed to study the development of arrhythmias in so called restitution protocols where pacing interval is slowly decreased from a slow pacing cycle length to a faster one. Typically from 1 second to 120 ms depending on a specie. In restitution protocols it is of a particular interest to measure the action potential duration (APD) adaption to new cycle lengths as an indicator of possible complex cardiac dynamics. It is of a special interest to have recordings at high pacing rates to detect the development of alternans [6-8,10], a period doubling bifurcation, where the response to a stimulus is a large action potential (AP), followed by a short action potential before repeating. Unfortunately with the increased pacing rate, the amplitudes of V_m and $[Ca_i]^{+2}$ often also become smaller. Therefore, detecting small variations of APD in time or variations of APD along space (dispersion) from beat to beat can be difficult as it is often indistinguishable among

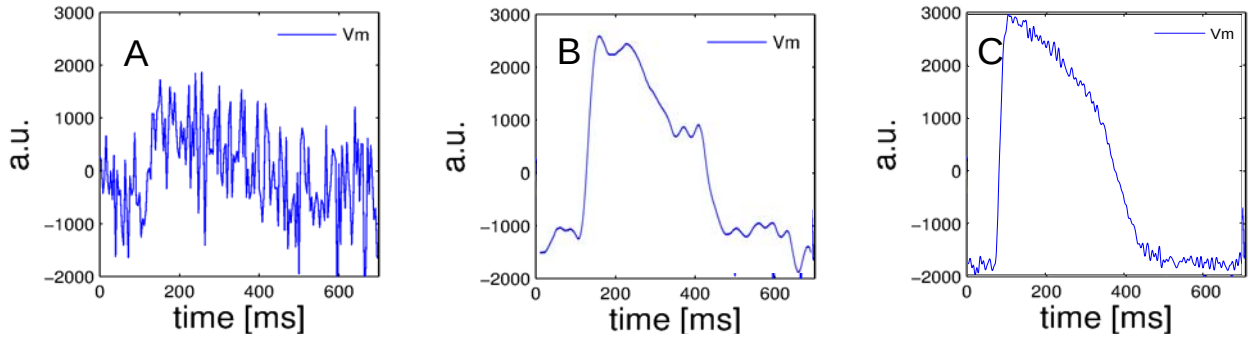

Figure 1. Voltage signal from one pixel measured in the right ventricle of a Zebra fish heart. A) Raw signal of an action potential obtained from a pacing at constant cycle length of 700ms. B) Signal from (A) after being filtered in time and space. C) Low noise action potential signal obtained after pacing for 1000 times and stacking of the signals.

noise in unprocessed images. Traditionally image processing is performed with various spatial and temporal filters (essentially low-pass filters). Although filtering process cleans and removes noise substantially, on its down side filtering degrades the signal, especially at high frequencies, and effectively decreases the resolution of the florescence images in both time and space. When beat to beat alternation in APD develops, their difference are typically less than 10ms, and the filtering process limits the ability to resolve and quantify these small APD changes in time and space, throughout the tissue.

Our stacking technique is based on oversampling where in a controlled environment we are able to stimulate (pace) isolated hearts at precise time intervals controlled with a micro-controller. Due to the cycling process we are able to record hundreds of pacing period and stack (sum up) images (spatiotemporal signals) that are apart in time by the pacing interval.

2. Experimental procedure

All Experiments conformed to the current Guide for care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication No. 85-23, revised 1996), and approved by the Office of Research and Integrity Assurance at Georgia Institute of Technology. New Zealand White Rabbits where anesthetized with ketamine/ xylazine/ acempromazine (17/9/0.9 mg/kg) and then injected with heparin (300 U/Kg). After five minutes euthanasia was induced with 120 mg/kg of pentobarbital. Hearts were then quickly excised via a left thoracotomy and perfused retrogradely via the aorta with a cardioplegic solution, gassed with 95% O_2 and 5% CO_2. Then, the heart was immersed in a chamber kept at 37.0±0.3ºC and perfused at a pressure of about 60 mmHg with a peristaltic pump and Tyrode's solution gassed with 95% O2 and 5% CO2 and also kept at 37.0±0.3ºC. Zebra fish hearts where obtained by first euthanizing the fish by immersion in tricaine methanesulfonate (MS222) solution (250 mg/L; 25-30 ºC). For imagining the rabbit hearts are stained with the JPW-6003 voltage sensitive dye (1mg dissolved in 40µL of ethanol) and Rhod-2 calcium-sensitive dye (1 mg dissolved in 1 mL of DMSO). The staining of the fish hearts was done by immersion in oxygenated Tyrode's solution for 10 min, containing the voltage dye in the concentration of 0.15mM. As a contraction decoupler we used Blebbistatin in the concentration of 3-5 µM (dissolved in DMSO at the ratio of 5 mg/mL). Images are recorded with a single Photometric Evolve 128 EMCCD camera that provides high quantum efficiency (peak QE > 90%). The signals were digitized with a 16-bit A/D converter at a frame rate ranging 500-1000 fps and spatial resolution of 128x128 at 500 fps and 64x64 at 1000fps. The PC interface provided high-bandwidth uninterrupted data transfer. An acquisition toolbox using C and Java was developed and used for experimental control, display, and data analysis, together with custom-made drivers for camera control and readout developed using C and OpenGL. The optical mapping system used here is described in more detail in [12-13].

3. Results

For dual V_m-Ca measurements, images are recorded at 500-1000 fps using alternating LEDs excitation lights for the voltage and calcium dyes for every even and odd frames respectively. Afterward the software splits even and odd image frames and processes them separately as 3D matrices where the third dimension represents time. Figure 1a shows the action potential of a Zebra fish heart (ventricle) plotted as a time trace from one pixel, where the signal to noise ratio is close to one, resulting in almost no recognizable signal. This is due to the size of a Zebra fish heart (approximately 1x1 mm), which leads to a very weak fluorescence intensity when recorded at 500 fps. Figure 1b shows the AP obtained after filtering in time and space as performed routinely in optical mapping studies with Gaussian filters. In contrast, by performing a stacking using a recording from 1000 activations at a constant pacing interval of 700 ms, a clear low-noise and not smoothed AP signal can be obtained, Figure 1c. In

Figure 2 we show how even in a larger heart such as a rabbit heart where more light can be collected, the raw images are not with a high enough S/N ratios to detect precisely detailed wave fronts (and wave backs). When using a stack of 100 images obtained at the same constant pacing cycle length of 350 ms, the resulting signal is clean enough to clearly detect the wave fronts (and backs) allowing quantitative measurements of APD and conduction velocities in a timescale that is only limited by a sampling rate.

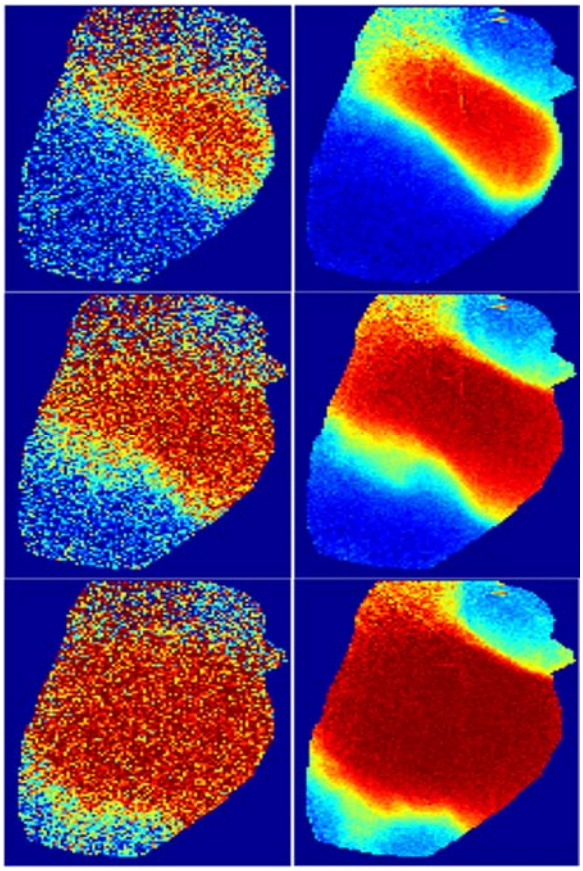

Figure 2. 2D images of the activation wavefront of the transmembrane voltage action potential propagating through a rabbit heart paced at a cycle length of 350 ms. Left column represent three sequential images of raw data (separated every 8 ms). Right column shows the same propagating wave but obtained from a stacking of 100 images obtained during steady state.

This method can be applied even for complex dynamics of wave propagation such as during alternans or even during higher order periods [7] as long as there is a periodicity. Figure 3 shows an example during alternans where waves do not only propagate differently beat to beat but also can follow complex patterns. The stacking method leads to clear signals as long as the system is in steady state with a constant repetition after every two activations. The left panel of Figure 3, displays even beats while the right odd beats. The stacking shows clear differences between the two activations that are displayed at the same time relative to the stimulation for even and odd stimulus respectively (cycle length of 160ms). Figure 4 shows the voltage and calcium signal from one pixel obtained during the episode of discordant alternans from a rabbit heart shown in Figure 3. The top traces show the signal after being filtered as in Figure 1b. Notice that while the period of stimulation is constant of 160 ms the signal alternates in duration and height (particularly for calcium), however it repeats at every two beats as indicated with the green lines.

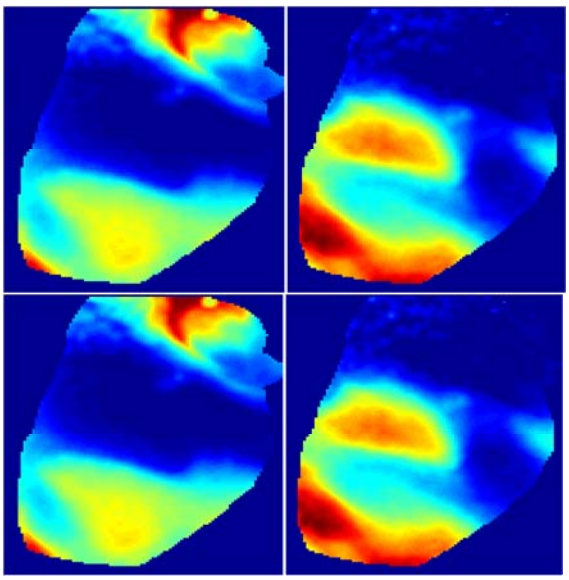

Figure 3. Propagation of an action potential wave along the epicardium of a rabbit heart paced at 160ms showing discordant alternans in action potential. During discordant alternans, one section of the heart can display a long action potential while other a short action potential. This leads to a wave that propagates unevenly with regions that remain activated while others are de-activated. Therefore, period doubling results in two different activations despite a constant pacing interval as shown by the left panels corresponding to odd beats and the right for even beats.

3. Conclusions

We have shown how high resolution images/signals with a substantially increased S/N ratio can be reconstructed from optical mapping data, even when very high noise exists in the raw signal, by a relatively simple technique when the system is periodic therefore identical repetitive measurement can be obtained. Oversampling increases the S/N ratio as a square root of the number of measurements which in this case are repeated excitations

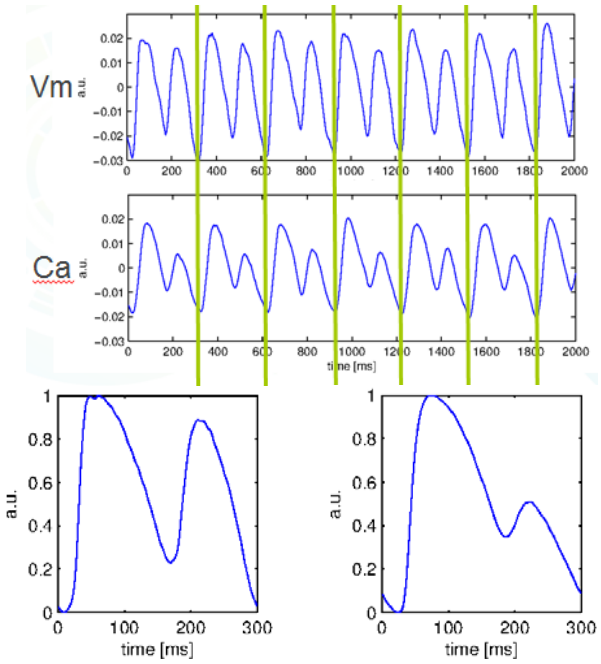

Figure 4. Voltage and calcium signals (from one pixel) during constant pacing cycle length of 160ms in a rabbit heart. At this pacing cycle length discordant alternans develop, shown as a clear difference in duration and height between odd/even activations every two periods (denoted with the green lines). Top panels show the signals after filtering displaying small variations artifacts due to the filtering. Lower two panels show the low noise signals obtained only with the stacking method.

of the whole heart at a constant pacing time interval. Therefore, for this method it is required that timing of a pacing stimulus is precisely controlled by a microcontroller in order to know the exact times for the stacking. Additionally it is necessary that with each pacing stimulus the dynamic of the heart is the same, that is, it repeats itself in both space and time. To reach this, it is necessary first to pace the heart until it reaches steady state dynamics, which in practice ranges from few to tens of seconds. Therefore, this method is easy to implement in optical mapping recordings where repetitive and deterministic measurement can be performed. The limitations of this technique are in recordings that are non-deterministic, or of unknown periodicity, such as those occurring during ventricular fibrillation (VF). However this technique can be of great use to obtain with great precision the bifurcation frequencies at which alternans in cardiac tissue appear. Also the amplitude and duration of an action potential as function of a period can be used to accurately detect wave velocities and dispersion of APD through the tissue. All these are important characteristics that can help determine and explore the cardiac dynamics and stability.

Acknowledgements

We thank support from NSF under grant #1413037

References

[1] S. Antic, D. Zecevic, “Optical signals from neurons with internally applied voltage-sensitive dyes”, J. Neuroscience, vol. 15, 1995, pp. 1392-1405

[2] B. J. Baker et al., “Imagining brain activity with voltage and calcium sensitive dyes”, Cell Mol Neurobiology, vol. 25, 2005, pp. 245–282.

[3] G. Salama, “Optical Mapping–Cardiac Excitation and Arrhythmias”, Wiley, 2001

[4] T. Sakai, K. Kamino, “Optical mapping approaches to cardiac electrophysiological functions”. Jpn. J. Physiology, vol. 51, 2001, pp. 1-18

[5] I. R. Efimov, V. P. Nikolski, G. Salama, “Optical mapping of the heart”, Circ. Res, vol 95, 2004, pp. 21-33

[6] Pastore JM, Girouard SD, Laurita KR, Akar, FG, Rosenbaum DS. “Mechanism Linking T-Wave Alternans to the Genesis of Cardiac Fibrillation” Circulation. 99: 1385-1394. 1999

[7] A. Gizzi, E. M. Cherry, R. F. Gilmour, S. Luther, F. H. Fenton, “Effects of pacing site and stimulation history on alternans dynamics and the development of complex spatiotemporal patterns in cardiac tissue”, Frontiers in Physiology, vol. 4, 2013, pp. 71

[8] F. H. Fenton, A. Gizzi, C. Cherubini, N. Pomella, S. Filippi, “Role of temperature on nonlinear cardiac dynamics”, Physical Review E, vol. 87(4), 2014, pp. 424-434

[9] A. M. Pertsov, J. M. Davidenko, R. Salomonsz, W. T. Baxter, J. Jalife, “Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle”, Circulation Research, vol. 72, 1993, pp. 631-650

[10] E. M. Cherry, F. H Fenton, “Visualization of spiral and scroll waves in simulated and experimental cardiac tissue”, IOP Science, vol. 10, 2008, pp. 1367-2630

[11] Fenton FH, Cherry EM, Kornreich BG. Termination of equine atrial fibrillation by quinidine: an optical mapping study”, J Vet Cardiol. 2008 Dec;10(2):87-103.

[12] S. Luther, F. H. Fenton, B. G. Kornreich, A. Squires, P. Bittihn. D. Hournung, “Low-energy control of electrical turbulence in the heart”, Nature, vol. 475(7355), 2011, pp. 235-239

[13] Fenton FH, Luther S, Cherry EM, Otani NF, Krinsky V, Pumir A, Bodenschatz E, Gilmour RF Jr. “Termination of atrial fibrillation using pulsed low-energy far-field stimulation”. Circulation. 2009 Aug 11;120(6):467-76

[14] Li W, Janardhan AH, Fedorov VV, Sha Q, Schuessler RB, Efimov IR. “Low-energy multistage atrial defibrillation therapy terminates atrial fibrillation with less energy than a single shock”. Circ Arrhythm Electrophysiol. 2011 Dec; 4(6):917-25.

[15] Boukens BJ1, Gutbrod SR, Efimov IR. “Imaging of Ventricular Fibrillation and Defibrillation: The Virtual Electrode Hypothesis”. Adv Exp Med Biol. 2015; 859:343-65