New Optical Mapping Design and Automated Algorithms for Cardiac Electrophysiology

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Abstract

Optical mapping is an important tool for assessment of cardiac electrophysiology. We demonstrate a new system for quantification and measurement of electrophysiological parameters in isolated cardiac tissue. The system makes use of voltage sensitive fluorescent dyes that shift in spectral property in response to millivolt changes in potential across cell membranes. Automated analysis of the pixel-wise measurements yields information on action potential durations and isochronal maps allowing for high throughput of data analysis. The algorithms that we propose reliably describe activation sequences and allow for quantification of conduction velocities.

1 Introduction

Cardiovascular disease is the largest cause of death worldwide and atrial fibrillation (AF) is the most common arrhythmia. AF regularly causes strokes and cardiac death. Despite the progress made on the characterisation of the factors that lead to AF there is still a need to further understand disease mechanisms to improve therapies for prevention and management [2]. Genetic mouse models pose as an attractive tool for these studies [7] but their small size particularly the atria prove challenging for detailed assessment. Despite this, they allow for characterisation of functional molecular consequences of their genetic alterations.

Optical mapping with its high temporal and spatial resolution is a valuable technique in aiding our understanding of arrhythmias[1]. This method utilises voltage sensitive dyes;

these bind on to the lipid molecules on a cell membrane and will shift its spectral properties in response to a change in potential[6]. The motivation for development of these dyes arise where microelectrode measurements are unsuitable, and makes possible the measurement of spatial and temporal variations in membrane potential, hence it is it ideal for cardiac tissue[6]. High speed and high quantum efficient cameras are particularly well suited for imaging cardiac activation as events occur over the millisecond time scale and changes in fluorescence intensity are small. This is important, in particular for small animal preparations as heart rates are a magnitude faster than humans. These studies at high acquisition rates involve generating large data sets, in the range of hundreds of thousands of images, and hence the need for faster analysis methods. The use of semi-automated algorithms not only allows for signal processing but eliminates any user bias saving time and increasing reliability. One difficulty that arises from imaging cardiac muscle is its contractile properties causing movement artefacts; these greatly affect the emitted fluorescence but can be overcome with the use of mechanical uncouplers in the experimental setting which inhibit these muscle contractions.

There are several imaging techniques for the study of the heart such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound [3]. These are mostly limited to visualising diameters and contractile function but not electrical activation and repolarisation. Another method in which activation spread can be investigated is to use contact mapping [4] where an array of electrodes are placed on the surface, but these have limited use with the resolution determined by the number of electrodes that can be physically placed on the sample and there is no information on repolarisation.

In cardiac tissue, as the cells depolarise, they cause a sharp upstroke in membrane voltage. This is followed by a slower rate of repolarisation. In order to understand cardiac arrhythmias from optical mapping data we analyse the duration of these action potentials at 30, 50 and 70% of the repolarisation. These action potential durations (APD) are important in determining the membrane voltage as this is characteristic of ion channel activity. The different phases of an action potential are caused by ion channel currents. In addition to this, it is useful to image activation patterns from a sample by generating an isochronal map. This allows for determining spatial areas of slow or fast conduction, which is particularly important in evaluating disease models. In this paper we discuss the development of the optical mapping design along with the algorithms to assess the images produced.

2 Method

2.1 Optical mapping experimentation and design

Isolated murine hearts were perfused with the potentiometric dye Di-4-Anepps, with a peak Ex/Em of 502/703nm. This dye is fast responding and has been used in a multitude of other preparations not only murine hearts, but in larger animals and cells such as neurons[6]. After 5 minutes the atria were individually dissected and superfused with oxygenated solution and the excitation-contraction uncoupler blebbistatin. Preparations were continuously superfused and stimulated at twice the diastolic threshold with a 2ms pulse. The stimulation frequencies ranged from 3.33 Hz to 12.5 Hz. Samples were field illuminated by two twin 530nm LEDs and the emitted light was filtered at 630nm. Images were acquired at up to 2 kHz on the CMOS camera using WinFluor V3.4.9 (Dr John Dempster, University of Strathclyde, UK). From the raw images, time course information from regions of interest

was extracted into text format for APD measurements or an image series to tiff images for isochronal mapping.

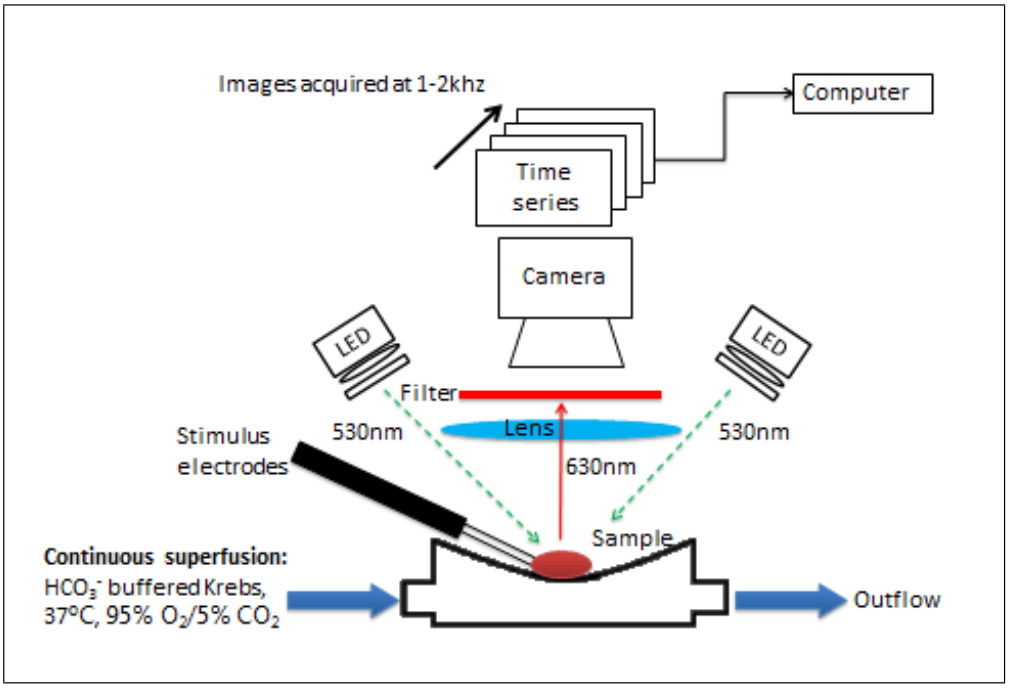

Figure 1: Optical imaging system. Samples were continuously superfused and stimulus electrodes were used to pace the heart at desired frequencies. Four LEDs were required to provide an even field illumination of the sample.Images acquired on high speed CMOS camera. (Figure adapted from submission to *Progress in Biophysics and Molecular Biology*, under review)

2.2 Optical action potential recordings

The fluorescence intensity obtained from the raw image data is inversely proportional to membrane voltage and the action potential properties can be measured pixel-wise from this time varying signal. The obtained optical traces often exhibit large shifts in baseline which is sometimes attributed to photobleaching and variations in solution level since the shifts do not follow a predetermined pattern. A linear top hat filter with a suitable structuring element was appropriate for flattening the signal baseline without affecting the action potential morphology. With high speed acquisition, noise is a strong contributing factor in image formation. To reduce noise, up to 25 action potentials were grouped together and averaged, but firstly each action potential was identified by using the 'findpeaks' detection algorithm in MATLAB. The next step was to identify the start of the action potential. This is conventionally chosen to be the fastest upstroke of the depolarisation. This was simply calculated by taking the peak of the signal derivative. The mean value of ten frames before the upstroke determined the baseline. This, along with the peak height, yields the absolute signal amplitude. From here it was possible to calculate the APDs at 30, 50, 70% repolarisation by linearly interpolating the data.

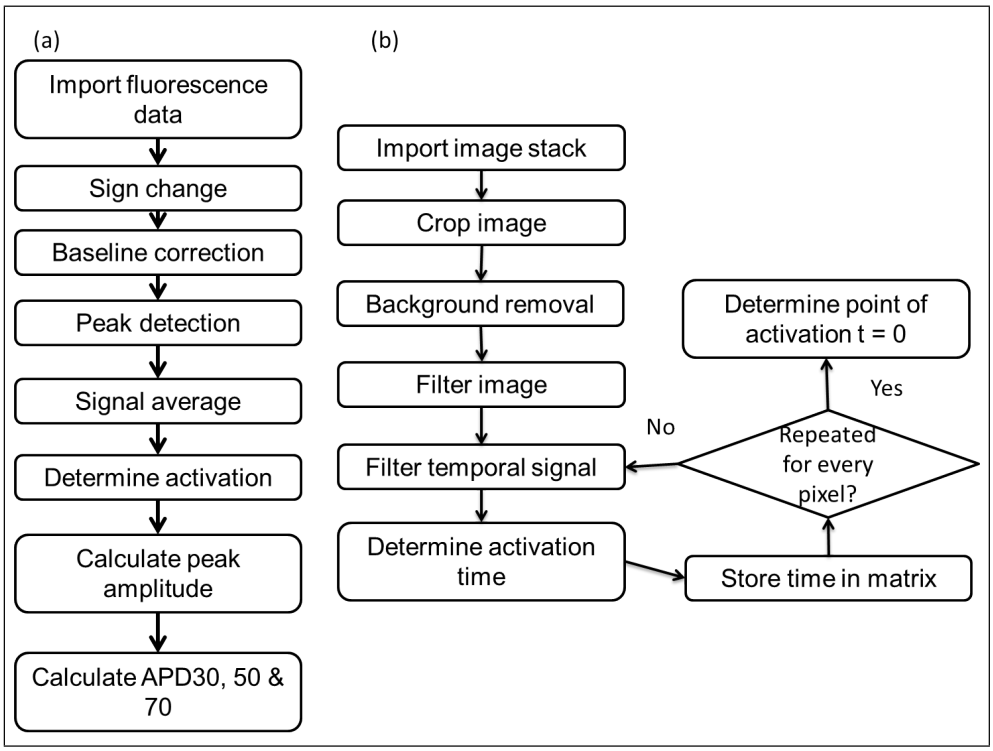

Figure 2: Outline of steps used to determine action potential durations (a) and generation of isochronal maps (b)

2.3 Isochronal maps

To create a map showing the different regions of activation the background must be first removed as this interferes with the subsequent processes. As the fluorescence signal tends to always be greater than the background, thresholding proved to be the most suitable method. Following this, a mean filter was applied to smooth the images. The next step was to determine the point of activation for every pixel. This was a similar process to that described in optical action potential recordings above, but signals were not averaged or did not have their durations measured. However, a Savitzky-Golay filter was applied to each pixel in the time series smoothing the data. This step was necessary to reduce noise amplification during signal differentiation. The peak of this derivative was chosen to be the activation time, repeating this process for every pixel generates the isochronal map. The origin of activation (t=0) was automatically determined by arranging the unique values of this array in ascending order. Each of these values represents a time determined by the exposure of the camera, and the second differential of these values should yield close to zero. The longest sequence of zeroes indicated the start point of activation spread. As each pixel represents a time value, the distance between two points of activation was used to represent a conduction velocity.

3 Results

An optical mapping system was built and used on small cardiac tissue samples. The automated algorithms described above accurately determined APD values from signal traces. The quality of these images also allowed for the generation of isochronal maps, which indicate

the changes in activation across a tissue. From Figure 3(a) the baseline is well defined, this is subtracted from the signal and allows for analysis of action potentials. From Figure 3(b) we can see that the averaging can accurately combine several action potentials as indicated by the small errors. This allows for APD measurements as indicated by Figure 3(c). Validation work has been completed using microelectrode and monophasic action potential (MAP) and is currently under review, but results remain consistent throughout all techniques. These traces are derived from the temporal data acquired from a selected region of interest typically a 4x4 area, an example of a raw image can be seen in Figure 4.

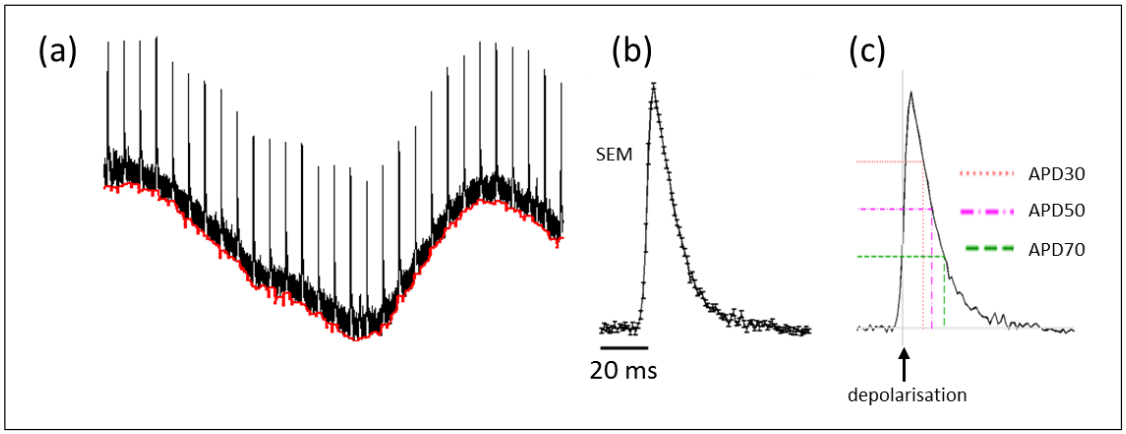

Figure 3: (a) An example of a drifting signal, the red line indicates the baseline detected. (b) An average of 10 action potentials: error bars indicates standard errors of the mean. (c) Displays the calculation of APD values and the point of activation indicated by the black arrow. (Figure (b) and (c) adapted from submission to *Progress in Biophysics and Molecular Biology*, under review)

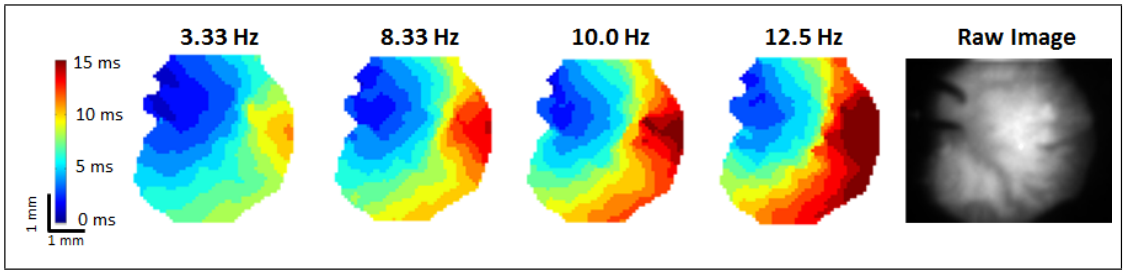

Figure 4: Isochronal maps showing activation patterns across an isolated left atrium and the raw image(left). These high resolution maps indicate that at higher stimulation frequencies the activation time increases across the entire tissue.

4 Discussion and Conclusion

This paper has presented a new optical mapping system capable of imaging isolated atria. The algorithms used have also been shown to perform APD calculations autonomously from fluorescence data, increasing signal quality by use of averaging. In addition to this, the baseline correction was proven to be robust and efficient. Other methods such as polynomial

fitting have limited use with signals that fluctuate greatly [5]. The isochrones were generated automatically, the only input was the user cropping the image to highlight the region of interest. As seen in Figure 4, the algorithms used to determine t=0, the activation start point, can be automated saving time, allowing for faster analysis. This differs from most current methods where manual selection is required, which can introduce subjectivity to results. This system was also sensitive enough to detect changes in activation at different stimulus frequencies. With these accurate measurements this system can be used in studies for characterising arrhythmias in mouse models. This is important in enhancing knowledge of treatments for those afflicted with cardiovascular disease.

References

[1] A Blana, S Kaese, L Fortmuller, S Laakmann, D Damke, K van Bragt, J Eckstein, I Piccini, U Kirchhefer, S Nattel, G Breithardt, P Carmeliet, E Carmeliet, U Schotten, S Verheule, P Kirchhof, and L Fabritz.

[2] A John Camm, Sana M Al-Khatib, Hugh Calkins, Jonathan L Halperin, Paulus Kirchhof, Gregory Y H Lip, Stanley Nattel, Jeremy Ruskin, Amitava Banerjee, Dan Blendea, Eduard Guasch, Matthew Needleman, Irina Savelieva, Juan Viles-Gonzalez, and Eric S Williams. A proposal for new clinical concepts in the management of atrial fibrillation. *American heart journal*, (3):292–302.e1, September . ISSN 1097-6744. doi: 10.1016/j.ahj.2012.05.017.

[3] L Fabritz, L Fortmuller, T Y Yu, M Paul, and P Kirchhof. Can preload-reducing therapy prevent disease progression in arrhythmogenic right ventricular cardiomyopathy? Experimental evidence and concept for a clinical trial. *Prog Biophys Mol Biol*, 110(2-3): 340–346, 2012. doi: 10.1016/j.pbiomolbio.2012.08.010.

[4] P Kirchhof, E Marijon, L Fabritz, N Li, W Wang, T Wang, K Schulte, J Hanstein, J S Schulte, M Vogel, N Mougenot, S Laakmann, L Fortmueller, J Eckstein, S Verheule, S Kaese, A Staab, S Grote-Wessels, U Schotten, G Moubarak, X H Wehrens, W Schmitz, S Hatem, and F U Muller. Overexpression of cAMP-response element modulator causes abnormal growth and development of the atrial myocardium resulting in a substrate for sustained atrial fibrillation in mice. *Int J Cardiol*. doi: 10.1016/j.ijcard.2011.10.057.

[5] Jacob I Laughner, Fu Siong Ng, Matthew S Sulkin, R Martin Arthur, and Igor R Efimov. Processing and analysis of cardiac optical mapping data obtained with potentiometric dyes. *American journal of physiology. Heart and circulatory physiology*, (7):H753–65, October . ISSN 1522-1539. doi: 10.1152/ajpheart.00404.2012.

[6] L M Loew. Potentiometric dyes: Imaging electrical activity of cell membranes. *Pure and Applied Chemistry*, 68(7):1405–1409, 1996. doi: DOI10.1351/pac199668071405.

[7] G Riley, F Syeda, P Kirchhof, and L Fabritz. An introduction to murine models of atrial fibrillation. *Front Physiol*, page 296. doi: 10.3389/fphys.2012.00296.