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# Optical Mapping Design for Murine Left Atrial Electrophysiology

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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 located on the left atrium. 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.

**Keywords:** optical mapping; cardiovascular disease; voltage imaging; electrophysiology; action potential duration

## 1. Introduction

### 1.1 *Cardiovascular Disease*

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 Camm, Al-Khatib, et al. (2012). Genetic mouse models pose as an attractive tool for these studies Riley, Syeda, et al. (2012) 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.

### 1.2 *Optical Mapping*

Optical mapping with its high temporal and spatial resolution is a valuable technique in aiding our understanding of arrhythmiasBlana, Kaese, et al. (2010) and cardiac physiologyEfimov, Nikolski and Salama (2004). 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 potentialLoew (1996). 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 tissueLoew (1996). High speed and high quantum efficient cameras are particularly well suited for imaging cardiac activation as events occur over the mil-

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lisecond 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 Swift, Asfour, et al. (2012). Here we show that with simple image processing techniques it is possible to generate and analyse optical mapping data.

### 1.3 *Other Imaging Modalities*

There are several imaging techniques for the study of the heart such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound Fabritz, Fortmuller, et al. (2012). 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 Kirchhof, Marijon, et al. (2011) 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.

### 1.4 *Action Potentials*

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. It is possible to measure these signals from an optical mapping system aong with other techniques such as microelectrode and monophasic action potential electrodesYu, Syeda, et al. (2014). 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 using the Langendorff preparation with the potentiometric dye Di-4-Anepps as seen on Figure 1. This dye is fast responding with a peak Ex/Em of 502/703nm and has been used in a multitude of other preparations not only murine hearts, but in larger animals and cells such as neuronsLoew (1996). 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 witha 2ms pulse at twice the minimum voltage required to induce an activation. The stimulation frequencies used were 3.33 Hz, 8.33 Hz, 10Hz and 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 a tiff stack for isochronal mapping. All analysis measurements were performed using algorithms developed in MATLAB.

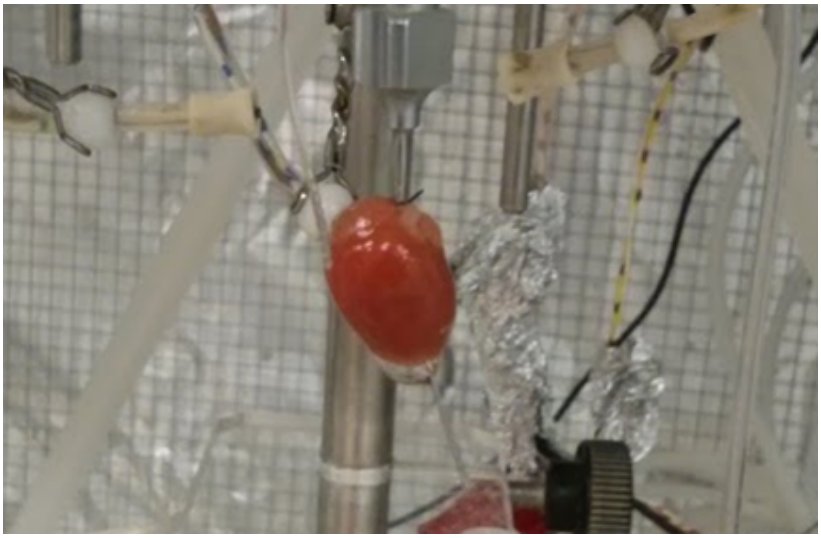

Figure 1. Image of the mouse whole heart sustained on the Langendorff preparation before dye infusion

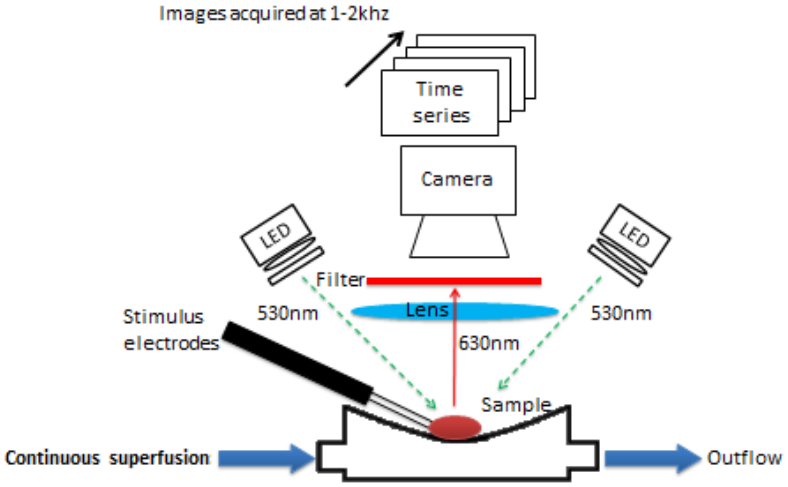


Figure 2. Optical imaging system. Atrial samples were continuously superfused and paced using stimulus electrodes desired frequencies. Four LEDs were required to provide an even field illumination of the sample.Images acquired on high speed CMOS camera

## 2.2 *Optical action potential recordings*

The fluorescence intensity obtained from the raw image data is inversely proportional to membrane voltage, this is due to the properties of the dye and the range of the optical filter used reducing the number of photons reaching the detector. As a result 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 attributed to photobleaching and variations in the buffer solution level. Since the shifts do not follow a predetermined pattern, a linear top hat filter with a suitable

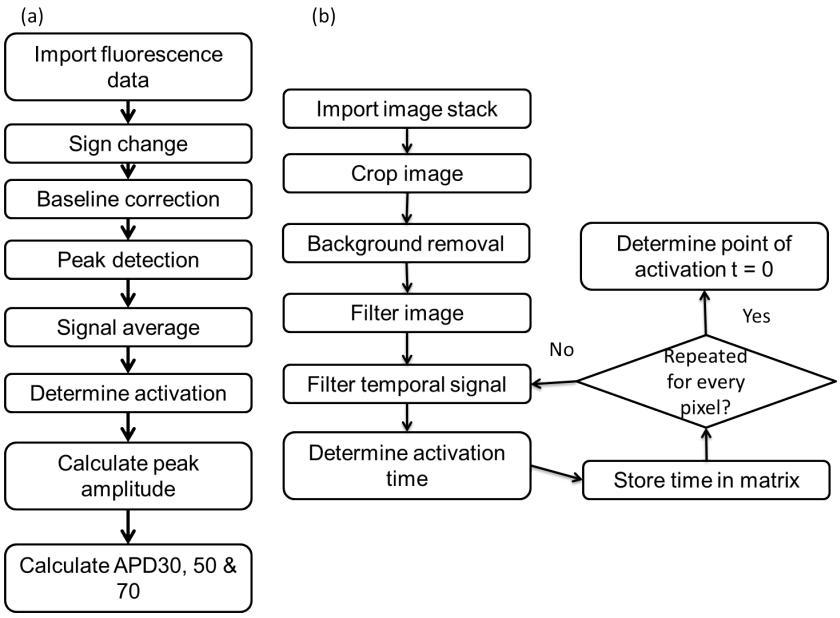


Figure 3. Outline of steps used to determine action potential durations (a) and generation of isochronal maps (b)

structuring element was appropriate for flattening the signal baseline without affecting the action potential morphology, see Figure 4. With high speed acquisition it is very difficult to capture strong signals combined with the relatively small changes in fluorescence, noise is a strong contributing factor in image formation. To reduce this, up to 25 action potentials were grouped together and averaged. In order to perform this averaging step, each individual action potential was identified. The 'findpeaks' Matlab function locates local maxima within a signal provided criteria is met. Here we set the algorithm to look for peaks that were above over half of the magnitude of the highest value and a minimum peak distance of 50ms. This was sufficient as the fastest pacing frequency of 12.5Hz equates to an action potential every 80ms. Each signal is 'overlaid' into a matrix and each point the standard error on the mean can be calculated to see the efficacy of the averaging see Results. The next step was to determine where the action potential starts. This is conventionally chosen to be the fastest upstroke of the depolarisation. This was simply calculated as the peak of the signal derivative (dF/dt). In order to calculate the duration of the signal, the baseline was needed. This marks the resting state or phase 4 of the action potential and was calculated to be the mean of ten points before the beginning of the upstroke. 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. In order to calculate the time for each duration, the closest two points to 30% of the repolarisation tail were determined. One point above and below this time, from this we can perform a simple linear interpolation using the equation of a straight line to find a more accurate intersecting time point. This is repeated for durations at 50% and 70%.

### 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. For an analysable image stack the fluorescent signal must be sufficiently high, this allows for a segmentation using a basic thresholding 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 as this is not required for measuring the activation time. However, a Savitzky-Golay filter was

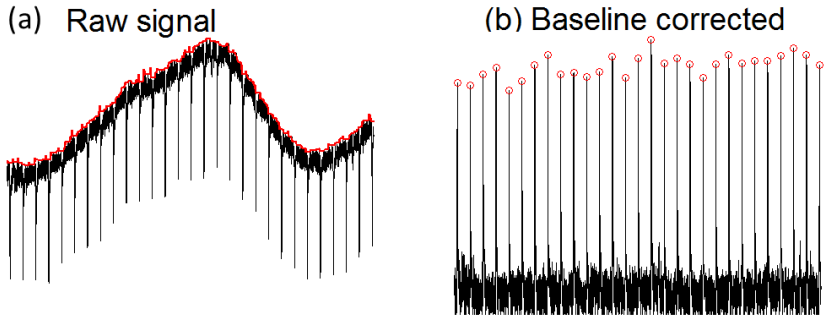


Figure 4. (a) Raw fluorescence data of multiple action potentials with the baseline highlighted in red. (b) These signals have been inverted with the drift corrected. Each individual peak is highlighted and prepared for signal averaging.

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.

As the start of every activation occurs at a arbitrary frame within the image series, it is necessary to determine the origin of the depolarisation (t=0). From Figure 5 we can see the distribution of activation times for every pixel within the image. In this example the first action potential occurs at 25ms, this was the offset of our map. This origin can be 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 yielded a series of zeroes. The longest sequence of zeroes indicated the start point of activation spread. From this we scaled the maps to a more suitable range. As a result of each pixel representing a time value, the distance between two points of activation was used to represent the speed at which the tissue transmitted an action potential also known as the conduction velocity.

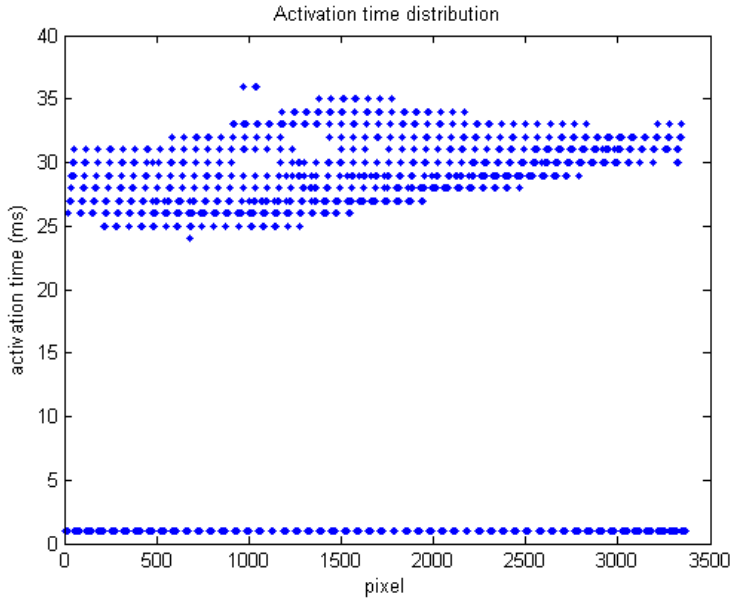


Figure 5. This plot displays the point of activation for every pixel in the image stack. This can be used to determine which group of pixels are first to be conducting across the tissue. Note that there are many values at 1 ms due to the number of background pixels.

## 3. Results

Here we show an effective method for analysing optical mapping data from TIFF stack images from a new second generation CMOS detector.This system has been built to study the left atrium in mouse samples but can be easily used in other minute cardiac tissues. The algorithms have effectively measured durations of action potentials 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 6(a) and (b) we can see that the averaging has accurately combined multiple signals from the trace as indicated by the small variations on the errors. This suggests that the baseline correction and the peak detection parameters are suitable for these measurements. This allows for APD measurements as indicated by Figure 6(c). 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 8(a) and its corresponding raw isochronal map at (b). Whilst it is possible to determine the conduction velocity without correcting for the start of the propagating action potential, it is wise to be able to visualise the map with ease hence the automated offset shown in 6(c).

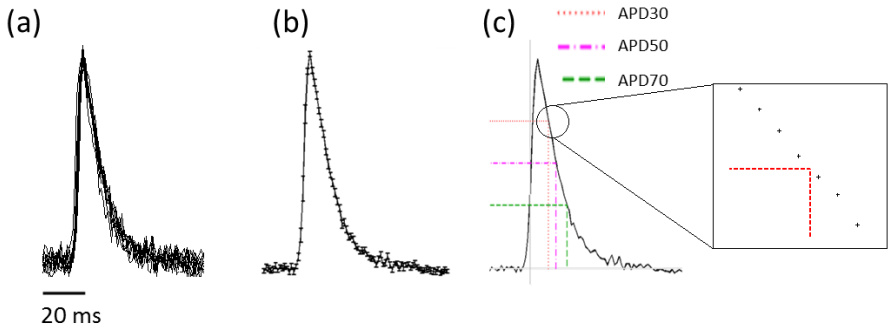


Figure 6. (a) An example of 10 signals that have been superimposed to illustrate the variation in action potentials. (b) The Averaged signal with error bars indicates standard error on the mean. (c) Displays the calculation of APD values at 30, 50 and 70 % repolarisation and a magnified section to show the value is often interpolated between two points.

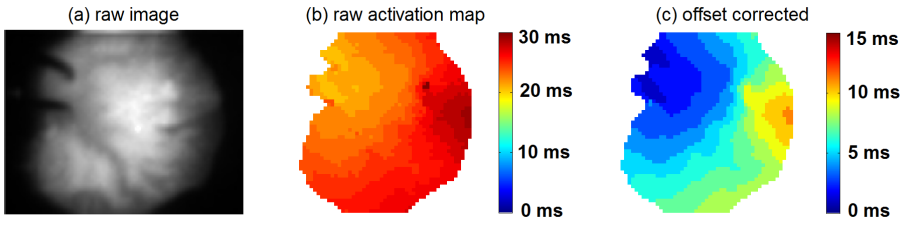


Figure 7. (a) shows the raw image acquired directly from the camera. (b) is the activation map generated by the algorithms without having the offset to t=0. (c) is the corrected map to show the starting point of activation taking a maximum time of approximately 15 milliseconds

## 4. Discussion and Conclusion

This paper has presented a new optical mapping system capable of imaging isolated atria. The use of the TIFF image format allows for more open access for others to develop an imaging system compared to those which often uses proprietary file types. The algorithms used have also been shown to perform APD calculations autonomously from fluorescence data, increasing signal quality

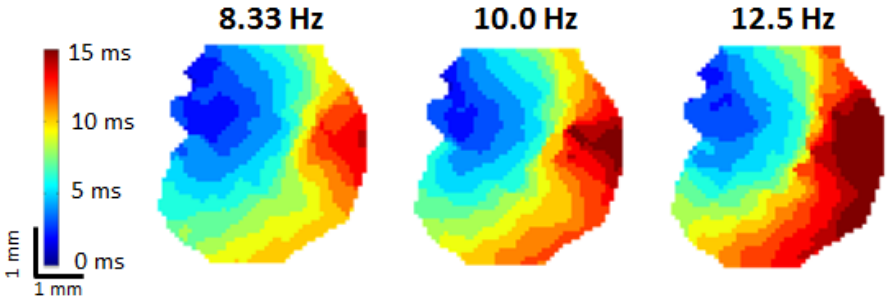


Figure 8. Isochronal maps showing activation patterns across an isolated left atrium. These high resolution maps indicate that at higher stimulation frequencies the activation time increases across the entire tissue.

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 Laughner, Ng, et al. (2012). The isochrones were generated automatically, the only input was the user cropping the image to highlight the region of interest. As seen in Figure 8, 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. One limitation of this method is the measurement of the conduction velocity which is measured from two points at different 'time zones' and the speed calculated. This introduces user bias but remains a fast method to determine the velocity at a particular direction. 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.

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