Optical Mapping of Embryonic Heart Electrophysiology
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**ABSTRACT**

Optical Mapping is a powerful technique for mapping conduction in the heart using a voltage-sensitive fluorescent dye to visualize changes in membrane potential. Optical mapping signals are inherently noisy due the short temporal sampling times and the need to measure small changes in fluorescent intensity. This challenge is exacerbat-
ed in the embryonic heart, which is only several cell layers thick. To increase the SNR of the signals we normalize and apply both temporal and spatial filters to the data. The processed data allows us to extract various measurements, including activation times and action potential durations. Using these measurements we create various representations of action potential propagation including isochronal activation maps and propagation velocity vectors fields. These representations allow efficient and effec-tive analysis of optical mapping data.

**MOTIVATION**

- We are studying heart development in an embryonic quail model to better under-
stand congenital heart defects.
- Previously, we have used Optical Coherence Tomography (OCT) and histology to study the structure, genetics and hemodynamics of the developing heart.
- Optical Mapping (OM) is a technique that allows us to characterize the propaga-
tion of electrical signals in the heart by visualizing changes in membrane potential using a membrane-bound, voltage-sensitive fluorescent dye (setup in Figure 1).
- OM collects extremely small and noisy signals, and the image processing scheme de-
veloped here is needed in order to be able to make meaningful measurements.

**METHODS**

- To collect an OM data set, we capture a series of images in time with our EMCCD camera.
- Data pre-processing increases apparent SNR. Steps include normalization, temporal filtering, photobleaching correction and spatial filtering (shown in Figures 2 and 3).
- After pre-processing, we fit a polynomial to the rising and falling edge of the action potential (AP) and use this fit to determine the activation point and AP duration.
- Using the information calculated for the action potential curve on each pixel, we can generate activation maps, duration maps and conduction propagation vector fields.

**RESULTS**

Data pre-processing increases SNR and improves data analysis outcomes. SNR improvement is typically one order of magnitude. This improvement does come at some cost to realizable spatial resolution, but accomplishes the goal of preparing the data for curve-fitting in subsequent steps.

**CONCLUSION**

- Accurate prediction of action potential characteristics. Our rising/falling edge split polynomial fitting method consistently predicts activation points and durations for a variety of action potential curve shapes, as shown in Figure 4. While this method is generally robust given the variety of curve shapes encountered, there are occasional errors and further refinement using other types of non-linear curve fits has the po-tential to improve the detection method.
- Various representations of conduction propagation in the heart are demonstrated. Isochronal activation maps, action potential duration maps and conduction propaga-
tion vector fields are shown in Figures 5 and 6. Comparison of isochronal maps and vector maps suggests a consistent result between the different representations.

**REFERENCES**


**Figure 1.** Picture of an Optical Mapping Sys-
tem. Light travels from the light source to the sample through the microscope and is reflect-
ed back into the EMCCD through various filters.

**Figure 2.** Flowchart of data processing. Data collection is followed by pre-processing, AP detection and various measurements.

**Figure 3.** (A-D) provide representative ex-
damples of data through each of the pre-pro-
cessing steps. (A) Data normalization using a small averaged section of the data to offset. (B) Temporal filtering with a Butterworth low pass filter, cutoff of 200Hz. (C) Photobleaching correc-
tion (affine fit). (D) Spatial Filtering with a 3-pixel radius disc filter.

**Figure 4.** (A-D) Four examples of the effec-
tiveness of the fitting method. Red lines intersect on the 50%-max point on the rising-
ing edge of the action potential (Activation Point). Blue lines intersect on the 20%-max point on the falling edge. The difference be-
tween these to be the action potential dura-
tion. All curves are from the same data set.

**Figure 5.** Various representations of an alcohol-treated embryonic quail heart. The units displayed on the side of all images are in pixels. (A) Averaged composite image of an entire data set. (B) Isochronal activation map of the heart (in sec). Each contour shows 0.02 sec of time. (C) Contour map of action potential durations across the heart (in msec). Each con-
tour shows 20 msec in time. (D) Conduction propagation vector map of the heart. Vector magnitudes are displayed on a logarithmic scale.

**Figure 6.** Various representations of an saline-treated embryonic quail heart. The units dis-played on the side of all images are in pixels. (A) Averaged composite image of an entire data set. (B) Isochronal activation map of the heart (in sec). Each contour shows 0.02 sec of time. (C) Contour map of action potential durations across the heart (in msec). Each con-
tour shows 20 msec in time. (D) Conduction propagation vector map of the heart. Vector magnitudes are displayed on a logarithmic scale.