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WAVELENGTH-DEPENDENT PROPERTIES OF MOTION ARTIFACTS IN ACTION POTENTIALS ACQUIRED WITH DUAL WAVELENGTH CARDIAC OPTICAL MAPPING IMPACT THE PERFORMANCE OF RATIOMETRY

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INTRODUCTION

Cardiac optical mapping has been an influential research technique for the study of cardiac electrophysiology in healthy and disease states [1]. The technique allows the analysis of several parameters of interest across the cardiac surface imaged. One of the main advantages of the technique is the possibility of performing the analysis in whole heart preparations, as opposed to tissue. Cardiac optical mapping is a high temporal resolution tool that facilitates the study of the rapid change of cardiac electrical activity.

Action potentials (APs) are acquired optically in cardiac optical mapping experiments, the technique allows the acquisition of multiple APs simultaneously across the heart. A voltage sensitive dye is perfused using a Langendorff apparatus in order to map the electrical activity of the heart. The dye responds to changes in the transmembrane potential, converting those changes into variations of fluorescence intensity. The dye is excited using a halogen lamp; the excitation light is filtered to achieve the excitation window of the dye.

The datasets acquired with cardiac optical mapping correspond to a fluorescence video. Optical APs are retrieved following the change in light intensity of a single pixel across the duration of the video. A major disadvantage of cardiac optical mapping is the presence of motion artifacts in the recorded APs. Motion artifacts create a distortion of the shape of the APs affecting their repolarization phase. Motion artifacts have diverse origins, including the mechanical motion due to the contraction of the heart during the experiments. Figure 1 presents an example of action potential with and without

Cardiac contraction causes the sensor pixels to image different areas of the heart across the duration of the video adding artifacts to the signals recorded. However, there are additional sources of motion artifacts as presented by Brandes et al in [2], i.e. reorientation of the tissue with regards to excitation light and change of volume of the organ during the experiments.

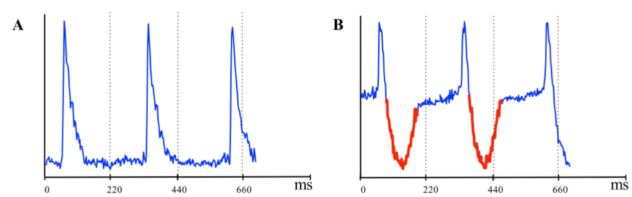

Figure. 1. Example of motion artifact in a recorded optical AP. (A) Example of an AP not affected by motion artifacts. (B) Example of an optical AP affected by motion artifacts, the motion artifact is presented in red.

Dual wavelength optical mapping allows the utilization of new techniques for the control of motion artifacts including ratiometry. Ratiometry have been investigated during the last few decades leading to favorable results for the control of motion artifacts and requires the recording of two APs per pixel to perform the ratio [3]. Dual wavelength optical mapping collects two fluorescence videos from the same field of view at two different wavelengths simultaneously and provides the datasets required by ratiometry. It has been assumed by several studies that motion artifacts are common to both wavelengths [2][3] in shape and direction.

This paper presents early evidence that assumptions of similarity for shape, amplitude and direction of motion artifacts do not hold for a considerable number of pixels in a typical dual wavelength recoding. Examples are presented for several types and amount of artifacts in the recordings analyzed.

METHODS

Animal experiments

The animal protocol, animal care and experimental procedures were approved by the University of Calgary health science animal care committee.

The results presented in this paper were obtained from datasets recorded using Sprague-Dawley rats between 200 and 300 g. Rats were injected with Heparin prior the extraction of the heart. Ten minutes later the animals were administered inhaled anesthesia (Isoflurane). The aorta was severed to separate the heart from the body. The heart was immediately immersed in cold Krebs solution before being transported to the Langendorff perfusion system.

Dual wavelength experiments

Recordings were acquired at two wavelengths simultaneously. Two identical high resolution cameras were used to record each wavelength. The first camera will be referred to as camera A, and it was placed in front of the preparation. Camera A acquired recordings at wavelengths larger than 590 nm. APs extracted from datasets acquired with camera A will be presented in red through the document. The second camera, camera B, imaged the same field of view that camera A. Camera B acquired recordings at a lower wavelength range (518 to 558 nm). APs extracted from recordings acquired with camera B will be shown in green throughout the paper.

RESULTS

The effectiveness of techniques such as ratiometry for the correction of motion artifacts highly relies on similarities of motion artifacts in direction, amplitude and shape when comparing APs between corresponding pixels from both cameras. As it was mentioned before, several studies were performed under the assumption of similarity. Some studies, however, presented evidence of slight differences between corresponding APs, mainly in terms of amplitude [4][5][6].

Two main circumstances were observed regarding motion artifacts in our experimentally recorded datasets. The first type refers to corresponding APs, extracted from the same pixel in camera A and camera B, that present motion artifacts with similar direction, even though the amplitude of the artifact and the shape may slightly differ between corresponding APs. The second situation refers to motion artifacts that present opposite directions.

Figure 2 depicts several examples of corresponding APs that present motion artifacts with similar directions, however several levels of artifacts are observed. Figure 2 (A) shows an example of corresponding AP slightly affected by motion artifacts. This is a case close to the centre of the heart, where gentle pressure was mechanically applied to the preparation in order to reduce the mechanical motion of the heart. Figure 2 (B) presents an example of motion artifacts that exhibit similar directions, however the signal acquired from camera A is clearly more affected by artifacts than its corresponding signal. Figure 2 (C)–(D) illustrate the case of both signals greatly affected by artifacts. Figure 2 (E) exemplifies the case when one of the signals is barely affected by motion artifacts, camera A, while the corresponding signal presents a moderate motion artifact. Finally, figure 2 (F) presents a case of similar motion artifacts in shape, directions and amplitude.

Even though Figure 2, illustrates examples of corresponding APs affected with motion artifacts that present similar direction. The figure also exhibits differences that can affect the result of correction algorithms. Different types and levels of artifacts are present in the same recordings. This observation highlights the fact that a single parameter of correction cannot be applied to all the pixels in a dual wavelength recording.

Figure 3 presents examples of corresponding signals affected by motion artifacts with opposite direction, levels of amplitudes and shape.

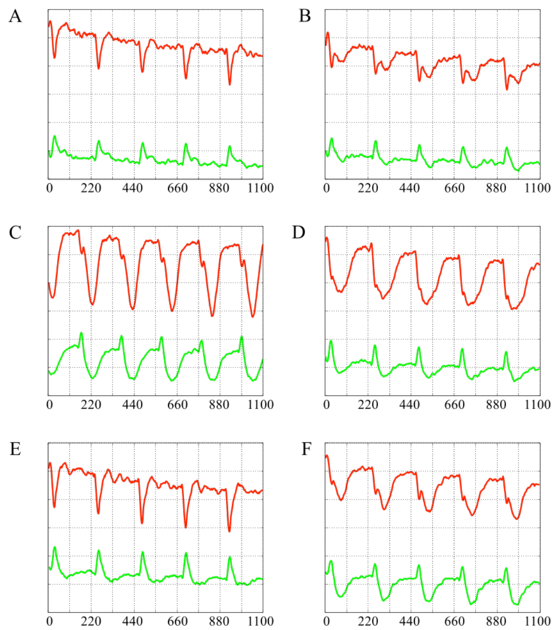

Figure 2. Examples of APs that exhibit same direction in corresponding APs. (A) shows an example of low levels of artifacts in both signals. (B) – (D) depicts examples of larger artifacts distorting the signals. (E) illustrates a case of one signal being largely affected by artifacts (green) while the corresponding signal is clearly distorted by the artifact. (F) presents an example of signals affected by very similar artifacts.

Figure 3 (A) shows the example were the amplitude of the artifacts are similar in both channels, however the shape of artifacts differs in both signals. Figure 3 (B) – (C) exemplifies artifacts with similar shape in corresponding APs, although the amplitude of such artifacts is visible different between signals. Figure 3 (D) illustrates the case of APs affected with motion artifacts that present opposite directions, different amplitudes and a slight difference in shape. Figure 3 (E) – (F) present a clear example of motion artifacts with opposite direction but with very similar shape.

Motion artifacts presenting different amplitudes, similar directions and slight differences in shape are the most common cases of corresponding APs found in the datasets analyzed. Approximately 15 % of the pixels over the surface of the heart presented opposite direction and difference in shapes.

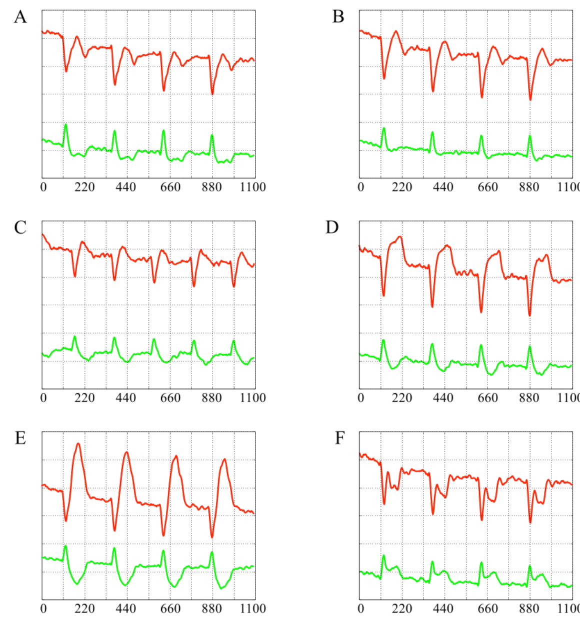

Figure 3. Examples of APs exhibiting motion artifacts with opposite direction. (A) motion artifacts with similar amplitude and different shape. (B) and (C) similar shapes (E)-(F) motion artifacts with similar shapes

Differences in amplitudes and shapes affect the calculation of ratio signals and therefore interfere with the effectiveness of the technique to correct for such artifacts. Figure 3 presents examples on how the ratiometry technique is unable to correct motion artifacts that are significantly different in corresponding signals at the wavelengths acquired. The original APs acquired are presented in red and green, the mathematical ratio between the original APs is shown in blue and the pink signal corresponds ratio between the original signals that have been scaled to each other in order to match the amplitude of the motion artifacts. Figure 4 (A) and (B) present examples of motion artifacts with similar shapes and different amplitudes in the corresponding signals. The simple ratio of the signals is unable to correct for the artifacts given the difference in amplitude (blue signals). However when the original APs are scaled to match the amplitude of the motion artifacts, better corrections are observed (pink signals). Figure 4 (B) illustrates the case of motion artifacts with opposite direction. In this case either of the ratio techniques is able to correct for the artifacts.

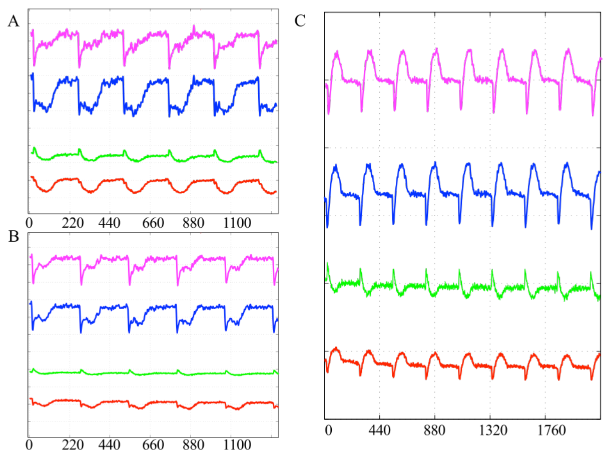

Figure 4. Example of ratio calculation in the presence of motion artifacts that exhibit different amplitude and direction in the corresponding wavelengths.

DISCUSSION

Ratiometric techniques constitute a valuable tool for the correction of motion artifacts. However, the effectiveness of the technique relies on similarities of the motion artifacts affecting the APs extracted from corresponding pixels at two different wavelengths.

Several prior publications have reported differences in motion artifacts acquired at shorter and longer wavelengths [2][7][8]. This paper presented specific examples of differences that reported by other authors as well as important difference in the shape between motion artifacts in corresponding pixels for a considerable number of pixels.

The differences between motion artifacts presented in this document must be considered for the implementation of motion artifact correction algorithms that use dual wavelength optical mapping data. These differences, for example, explain the inability of ratiometry to completely correct for motion artifacts.

The results presented seem to indicate that motion artifacts are wavelength dependent. This means that motion artifacts affect the signals acquired by cameras at shorter and longer wavelengths in different ways. This contradicts the mathematical models proposed to describe motion artifacts presented by other teams [2][8] in which motion artifacts were independent of the acquisition wavelength.

A typical mathematical ratiometry technique is unable to account for differences in motion artifacts in corresponding pixels at two wavelengths. An improved ratiometry technique involves the scaling of the APs acquired in one wavelength with respect to their corresponding AP at a different wavelength, the technique accounts for difference in amplitudes (see Figure 4 (B), however large differences in shape cannot be corrected (see Figure 4 (C)).

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