Programmatic Approach to Real-Time Monitoring and Analysis of Electrocardiogram Signals in Zebrafish

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Abstract

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Heart disease is the leading cause of mortality in the U.S. with approximately 610,000 people dying every year. The research into effective therapies for cardiac diseases is currently being held back due to the time and resources required to analyze raw test data for diagnostic purposes, such as electrocardiogram (ECG) readings. In this paper, a novel programmatic approach to expediting the process of analyzing raw ECG data and reporting the diagnostic results of the analysis to the user is presented. This program was initially designed to filter and diagnose a variety of heart conditions in zebrafish (Danio rerio) to facilitate heart disease research. However, as presented in this paper, the program was specifically designed so that future updates to expand its diagnostic capabilities beyond zebrafish would be relatively simple to complete with a basic understanding of the LabVIEW programming language. This solution holds promise to aid in the execution of numerous studies of heart disease, drug screening, stem cell-based therapy validation, and regenerative medicine.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

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DEDICATION

For my wife, Ariel: my love who has been supportive of all of my efforts and helped me to pursue them, regardless of how silly she thinks they are on occasion. Also, for my parents: without whose encouragement and help I wouldn't be here now, in more ways than one.

SECTION 1. INTRODUCTION

According to the Centers for Disease Control and Prevention the leading cause of mortality in the United States, with approximately 610,000 deaths a year between 1993 and 2015, is heart disease [1]. According to the World Health Organization heart disease is also the leading cause of death globally with an estimated 17.7 million deaths in 2015, which is 31% of all global deaths [2]. The National Institutes of Health alone funded approximately 2.169 billion dollars in research towards heart disease therapies in 2017 [3]. Even though there has been constant research into the subject, effective therapies for many cardiac diseases remain elusive.

To discover novel techniques for treating or curing heart disease, exploration into the regenerative capabilities of other species are being conducted in an attempt to find potential options. One of the species under investigation is the zebrafish *(Danio rerio)*. These have been used for a long time as a model for understanding cardiac development, disease, and regeneration due to their similar hearts and ease to work with [4-7]. Unlike humans, zebrafish hearts can fully regenerate after ventricular injury which provides a potential model to study heart regeneration [8, 9]. This model provides an approach to reveal the underlying mechanisms of numerous heart diseases along with potentially leading to treatments for those diseases. Given the currently poorly understood underlying genetic factors of heart disease [10], a model which allows for the study of the causative genes for heart diseases along with providing potential future therapeutic options is highly desirable.

In an effort to study the cardiac regeneration of zebrafish in real-time, electrocardiogram-based (ECG-based) techniques for monitoring the regenerative process of zebrafish recovering from heart injuries without requiring the test subjects to be removed from

the study for investigation have been explored for years [11-13]. Unfortunately, commercially available ECG systems have proven to be too difficult to work with primarily because of an inability to monitor the test subjects without anesthetizing them and to monitor more than one test subject at a time. In the last several years, the team I have worked with for my thesis has been developing a variety of systems using microelectrode array (MEA) membranes to acquire ECG signals from zebrafish [14]. The initial device was later improved upon with a flexible waterproof micro-sensor system which proved superior to the previous device, though the wired connection proved cumbersome and made taking readings from multiple test subjects simultaneously impossible [15]. This device was further improved with a wireless MEA ***"ECG jacket"*** which could be worn by the test subjects continuously for real-time ECG monitoring of multiple test subjects at the same time [14, 16-20].

The ***"ECG jacket"*** has proven an invaluable zebrafish ECG signal acquisition tool for the team I have been working with and has been utilized for several studies [14, 16-18]. Given that the ECG data acquired from the jacket still contained background electromagnetic noise which requires filtration before the signal can be properly analyzed, a tool was required to facilitate the filtering process. In addition, the real-time monitoring of a large population of zebrafish creates hours of analysis work to detect anomalies in even a few minutes' worth of data. To solve both problems at once, I developed a program which could perform the following functions on both real-time and pre-recorded data: Collect the data, filter out the background noise from the data, extract the ECG features from each heartbeat found in the data, detect anomalies in the ECG features found, save the acquired information, and generate a report on the detected anomalies. In this thesis, I will present the completed program's present functionality along with information on the specific steps taken to ensure that it will remain a valuable research tool for

years to come with the ability for future researchers to modify the program to meet their needs. The program presented in this thesis is the same program presented in [21].

SECTION 2. BACKGROUND

This section briefly outlines the anatomy and physiology of the heart (Section 2.1), the mechanics of electrocardiographs (Section 2.2), and the clinical uses for electrocardiographs when investigating heart disease (Section 2.3).

2.1 ANATOMY AND PHYSIOLOGY OF THE HEART

The heart's primary purpose within the body is to circulate blood to and from the tissues in an organism, delivering necessary materials for the tissues to function and removing waste produced by those tissues. The heart acts as a simple pump to circulate blood throughout the circulatory system. By contracting, the heart creates pressure to drive the blood. Without the blood flow provided by the heart, the organism would not be able to maintain homeostasis and the various tissues of the organism will expire due to lack of necessary nutrients [22].

To facilitate the flow of blood, the heart of a zebrafish is divided into two chambers: Ventricle and atrium [23]. The contractions of the chambers of the heart are controlled by cardiac muscles called myocardium, which are similar to skeletal muscles. The myocardium muscles are controlled by action potential signals similar to those that control skeletal muscles. An illustration of the anatomy of the heart is included in **Figure 1** [23].

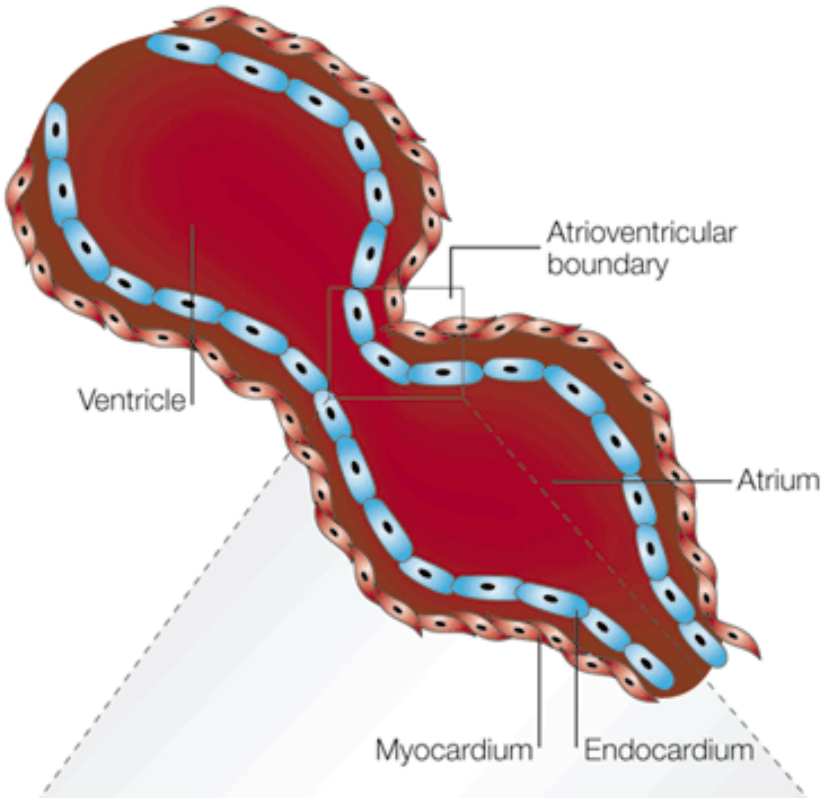


Figure 1. Cross-sectional view of the heart of a zebrafish [23].

Unlike the hearts of zebrafish, the human heart is divided into four chambers: Right atrium, left atrium, right ventricle, and left ventricle [24]. As with zebrafish, the contractions of the chambers of the heart are also controlled by cardiac muscles called myocardium. As with zebrafish as well, the myocardium are controlled by action potential signals similar to those that control skeletal muscles. An illustration of the anatomy of the heart is included in **Figure 2** [25].

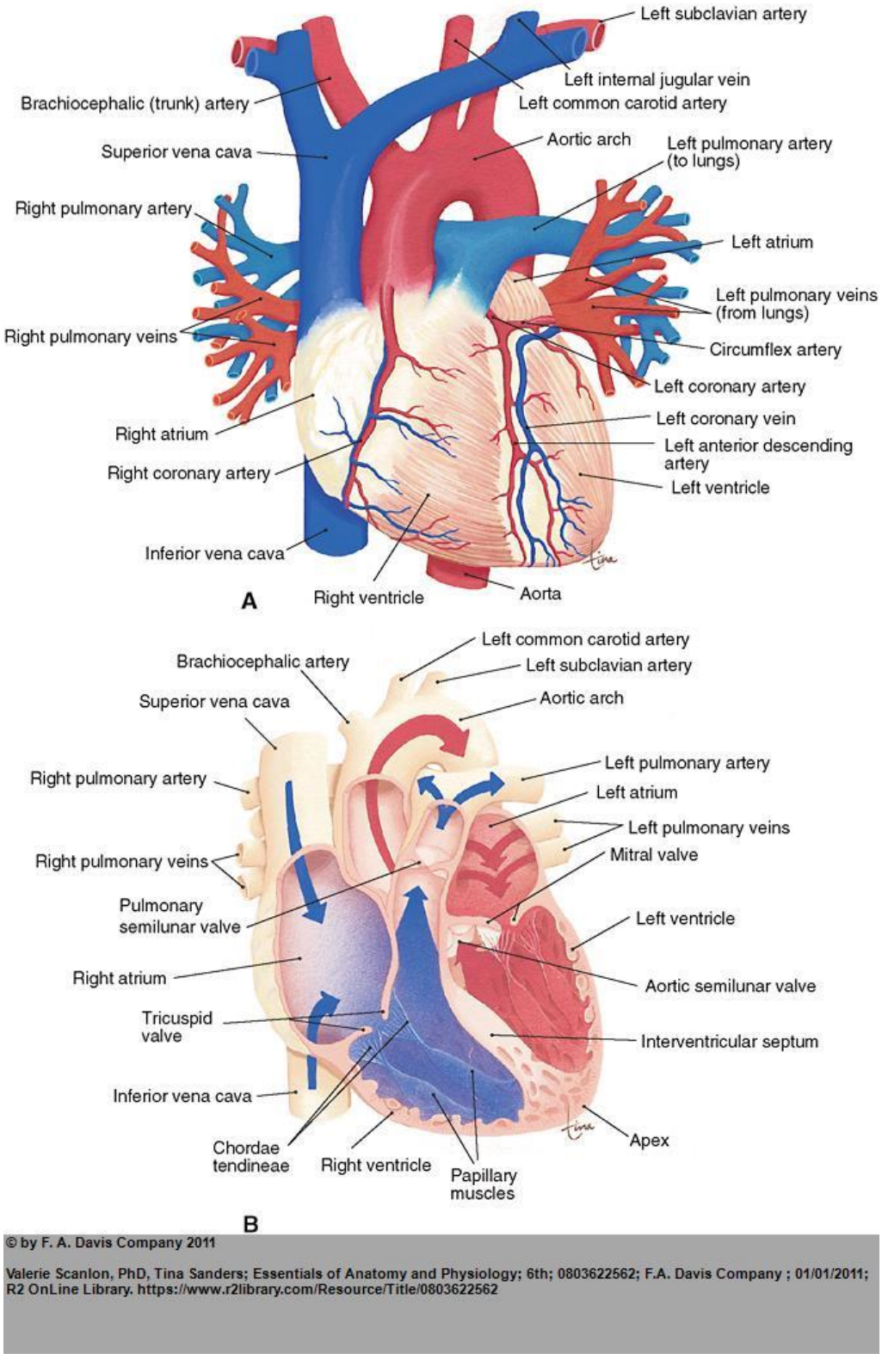


Figure 2. (A) Anterior view of the human heart. (B) Frontal section of the human heart in anterior view [25].

With each beat of the heart of a human, the following processes occur within that beat which circulates blood throughout the circulatory system:

1. “Oxygenated blood fills the left ventricle
2. Blood is ejected from the left ventricle into the aorta
3. Cardiac output is distributed among various organs
4. Blood flow from the organs is collected in the veins
5. Venous return to the right atrium
6. Mixed venous blood fills the right ventricle
7. Blood is ejected from the right ventricle into the pulmonary artery
8. Blood flow from the lungs is returned to the heart via the pulmonary vein” [22]

The circulatory process of zebrafish is similar to that of humans however the deoxygenated blood passes from the atrium, to the ventricle, and then the blood is distributed to the gills of the zebrafish for oxygenation and distribution to the rest of the body. Unlike the human heart, the hearts of zebrafish do not separate oxygenated and deoxygenated blood into separate sides of the heart since zebrafish hearts only have one atrium and ventricle pair [26].

Each step in the process of the blood circulation has a corresponding electrochemical signal controlling the myocardium of the chambers of the heart in both zebrafish and in humans. Each step requires the depolarization (A positive change in potential) and/or repolarization (A negative change in potential) of the chambers. When measured with an electrocardiograph (See Section 2.2), the electrochemical signals controlling the myocardium can be measured as a voltage change over time and that can related back to the physical process of the heart circulating blood. Those signals can be separated into five components of the measured voltage over time: P-wave, Q-wave, R-wave, S-wave, and T-wave (See **Figure 3** for an example of a human ECG

pattern with the ECG components labeled and see **Figure 4** for an example of a zebrafish ECG pattern without the ECG components labeled) [27, 28].

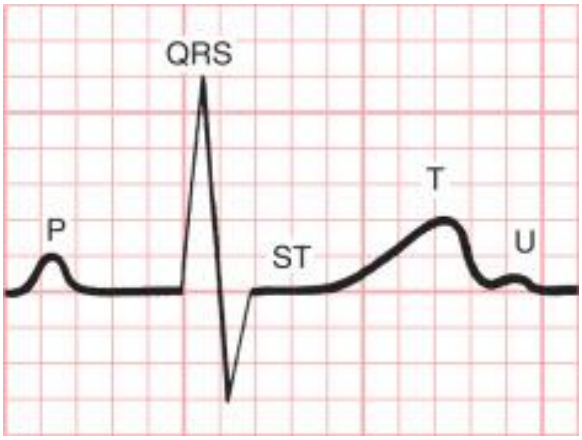


Figure 3. The five ECG components of an ordinary human heart signal, as measured by an electrocardiograph [27].

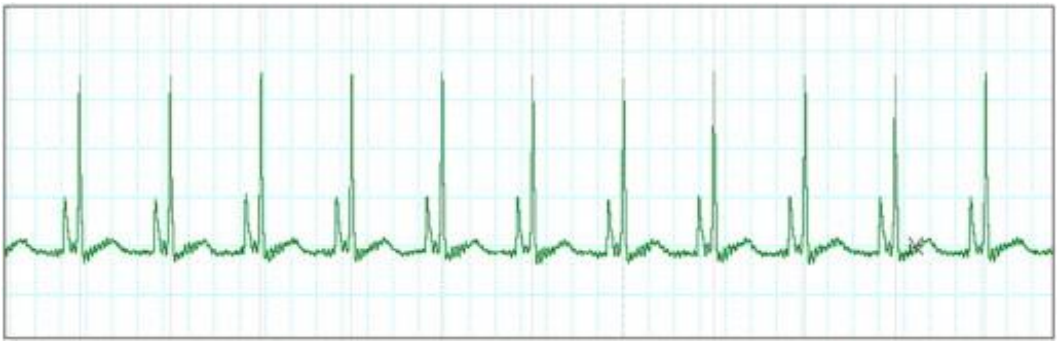


Figure 4. Example ECG pattern of a zebrafish, without the ECG components labeled [28].

The P-wave is the depolarization of the atria muscles corresponding to the contraction of the atria. The Q-wave, R-wave, and S-wave together correspond to the depolarization of the ventricles which corresponds to the contraction of the ventricles. The T-wave corresponds to the repolarization of the ventricles before the repeat of the process which corresponds to the ventricles returning to their resting state. The repolarization of the atria corresponding to the atria returning to their resting state are not normally observable via electrocardiograph due to the low

amplitude of the voltage required to repolarize the atria [27]. Together, the ECG components represent blood flowing from the atria, to the ventricles, throughout the body, and back.

Any anatomical or physiological anomalies which would cause the heart to pump blood throughout the body incorrectly will change the repolarization and depolarization of the chambers of the heart. These changes in the repolarization and depolarization can be detected with an electrocardiograph as a change in the relative positioning and amplitude of the ECG components (See Section 2.2 for additional details).

Though zebrafish and humans are quite different in terms of the rest of their physiology, "the genetic and molecular mechanisms behind cardiac physiologies are conserved throughout the vertebrate evolution from fishes to humans" [29]. There are molecular functions within zebrafish and humans which are unique to each species; however, both species share the same five ECG components with the same corresponding changes in atrial and ventricle polarization. This allows the zebrafish to serve as a model of the electrophysiology of humans [29, 30].

2.2 MECHANICS OF ELECTROCARDIOGRAPHS

The electrocardiograph is a specialized medical device which records electrical activity (Measured in terms of voltage) across the heart over a period. The electrical activity across the heart is measured as a voltage and can be charted over time to monitor the depolarization and repolarization of the chambers of the heart (See Section 2.1). Over time, the device has become a vital tool for the diagnosis of heart conditions through the interpretation of the ECG component measured by the device [27].

The first recorded instances of devices used obtain information about the health of a patient's heart using electrical data were conducted two separate individuals. Alexander Muirhead reportedly used wires attached to the wrists of patients to record their heartbeat in

1872, though the results from those recordings were never published [31]. On the other hand, Augustus Waller wrote an article in 1887 about his efforts to measure electrical changes across a patient's chest and correlates them to the beating of the patient's heart [32]. Waller's discovery was the first known instance of a recording of the electrical activity of the heart being recorded in real time.

After the initial efforts of Muirhead and Waller indicated a potential link between the heart's ability to pump blood through the body and a corresponding electrical signal measured across the heart, further attempts to create devices to more precisely measure the voltage were devised. The first device created that most closely resembles the electrocardiographs available today is the "string galvanometer" created by Willem Einthoven in 1901 [33]. This device was able to clearly distinguish all five of the components of ECG signal and allowed him to begin to investigate how changes in these components could be utilized to diagnose heart disorders, for which he earned a Nobel Prize in Medicine [34].

Today, electrocardiographs are utilized by doctors and researchers regularly to indirectly observe the behavior of a patient's heart. There are two commonly used types of electrocardiograph: The 12-lead ECG and cardiac monitors.

The 12-lead ECG is a system which uses 10 electrodes, with 12 leads connecting to those electrodes, to measure the voltage across different portions of the heart. This allows for the voltage changes across particular chambers of the heart to be observed. This, in turn, allows for anomalies in the electrophysiology of individual chambers to be assessed independently. The electrode placements for the 12-lead ECG on the limbs of the patient are shown in **Figure 5** and the electrode placements on the chest of the patient are shown in **Figure 6** [27].

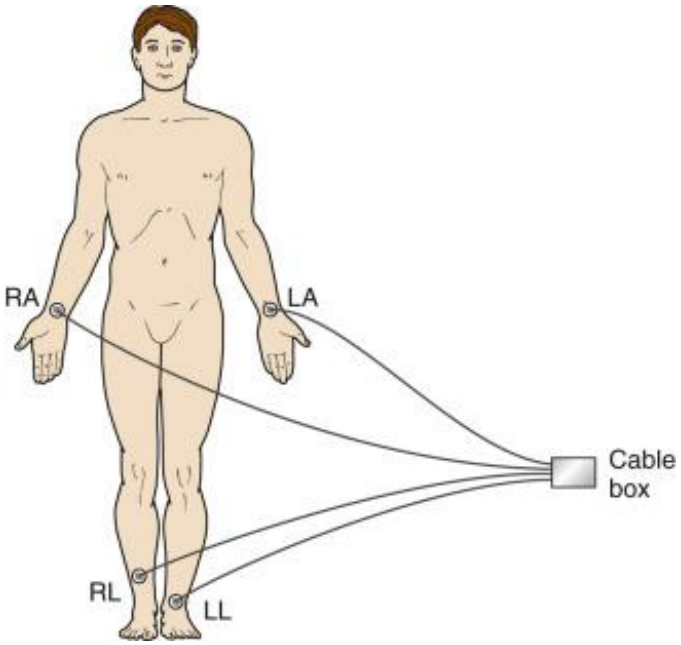


Figure 5. 12-lead ECG electrode placement on the limbs of patients [27].

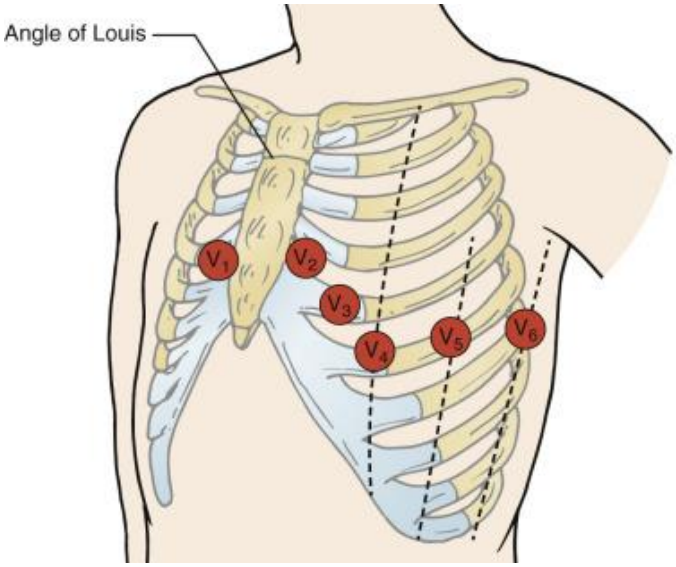


Figure 6. 12-lead ECG electrode placement on the chest of patients [27].

Though the 12-lead ECG allows for a greater level of detail in the electrophysiology of individual chambers of the heart, setting up the system for use on the patient can require more time that might be available to a doctor and would hinder a patient's ability to move freely. To mitigate these issues, cardiac monitors utilize a three-electrode system which is isolated to the

chest of the patient (See **Figure 7**). This system only provides a single ECG signal to analyze; however, by analyzing the changes in the ECG components relative to a "normal" ECG signal the single ECG signal can be used to preliminarily determine if an anomaly is present. If an anomaly is found in a patient's ECG signal when monitored with a cardiac monitor, their doctor can always utilize the 12-lead ECG to obtain a clearer picture of what anomalies are present in the ECG signals of the patient and what diagnosis that would correspond to [27].

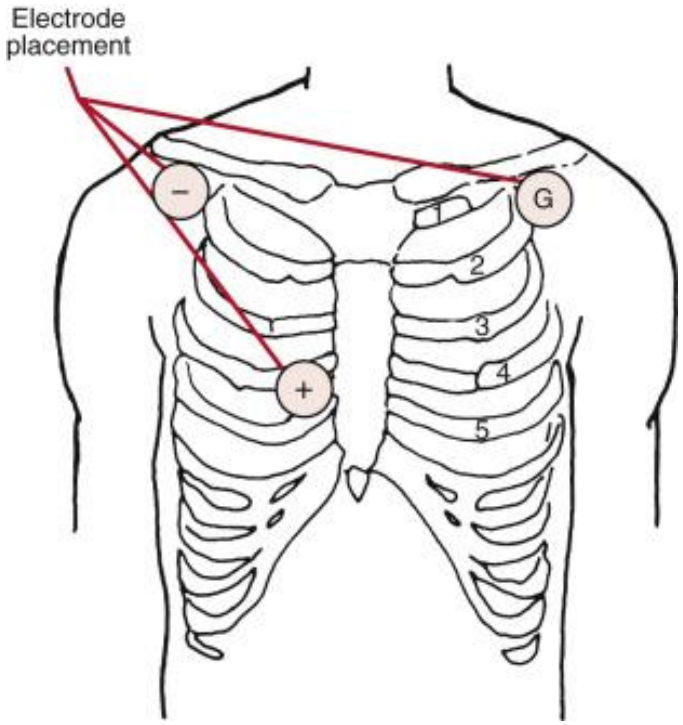


Figure 7. Three electrode configuration utilized with cardiac monitors [27].

Unlike ECG acquisition systems for humans, similar systems for measuring the ECG patterns for zebrafish were developed relatively recently. Single-lead microneedle electrodes systems were previously utilized to monitor ECG signals in zebrafish (See **Figure 8** for an example of microneedle electrode placement to measure zebrafish ECG) [35]; however, these systems provided relatively low signal stability and were unable to provide spatial resolution to allow for site-specific investigations [14]. To solve this issue, four-lead ECGs with flexible

microelectrode membranes were developed which allows the voltage across the heart of the zebrafish to be observed from a multitude of angles and distances to provide information relating to asymmetricity (See **Figure 9** for an example of a four-lead ECG monitoring system electrode implantation scheme for zebrafish and see **Figure 10** for a diagram of the relation between lead placement and observed ECG signal) [30]. The electrodes utilized for the ECG measurements in this thesis are based on the four-lead ECG flexible microelectrode membranes system.

Figure 8. Example of microneedle electrode placement to monitor ECG signals in zebrafish [35].

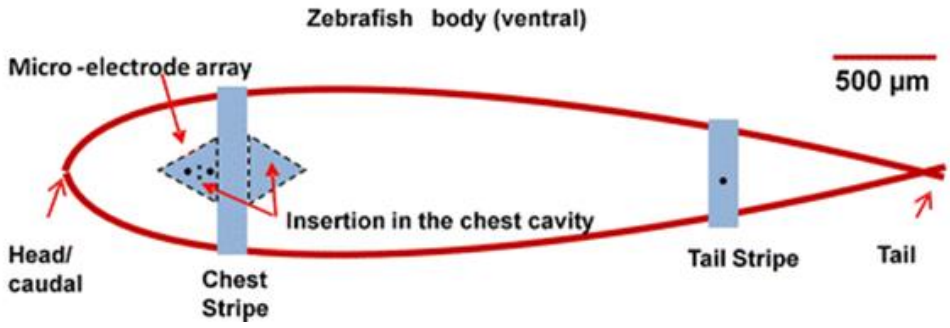


Figure 9. Example of a four-lead microelectrode array implantation scheme for monitoring zebrafish ECG signals [30].

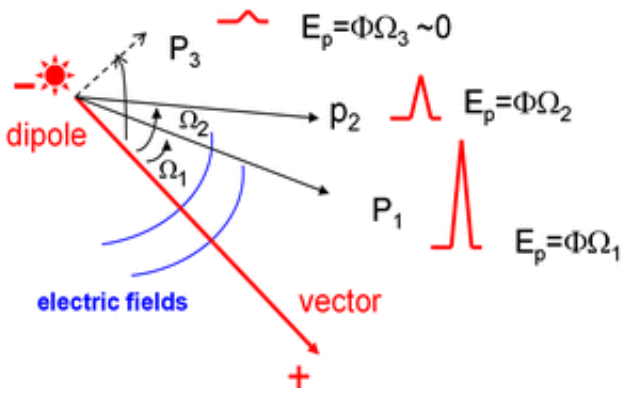


Figure 10. "Lead placements in relation to the vector direction. QRS amplitudes in P1 and P2 are dependent on the electrode lead position. E p denotes electric potential , Ω the solid angle , and Φ the strength of charge surface (Φ = voltage/unit of the solid angle)" [30].

2.3 Clinical Uses for Electrocardiographs in the Investigation of Heart Disease

Following the discoveries by Willem Einthoven [34] of potential methods for diagnosing heart disease through the detection of anomalies in the ECG signals measured from a patient, medically-accepted definitions for the anomalies in ECG signals that correspond to particular heart problems have been adopted with years of research. These definitions can be used, along with additional available medical information such as the medical history of the patient, to investigate potential indications of heart disease in patients. An earlier identification of heart disease in a patient allows for the issue to potentially be treated before a major cardiac event such as a heart attack or stroke occurs.

To identify anomalies in an ECG signal, a definition for a "normal" ECG pattern needs to be established to compare to. In humans, a "normal" heartrate is between 60 and 100 beats per minute with each beat being defined by the detection of an R-wave. These beats should be regularly spaced relative to one another. In addition, a "normal" heartrate has a P-R interval (The

time between the P-wave and R-wave) of less than 200 milliseconds. The ECG signal should appear similar to the one presented in **Figure 3**.

The five ECG anomalies with medically-accepted definitions that the program presented in this thesis is intended to diagnose (Sinus bradycardia, sinus tachycardia, sinus arrhythmia, sinus arrest, and first-degree AV block) are defined by how the measured ECG signal differs from a "normal" ECG signal. Sinus bradycardia and sinus tachycardia are defined by heartrates outside of the "normal" 60 to 100 beats per minute. Sinus bradycardia and sinus tachycardia are defined as a measured ECG signal with a heartrate lower than 60 beats per minute and a heartrate higher than 100 beats per minute, respectively [27]. Sinus arrhythmia and sinus arrest, however, are defined by irregularities in the R-R intervals of a measured ECG signal over time. Sinus arrhythmia and sinus arrest are defined as a measured ECG signal with a difference in R-R interval length over 10 beats of 0.16 seconds and a P-P interval greater than two times the previous R-R interval, respectively [36]. Finally, first-degree AV block is defined by a P-R interval longer than expected for a "normal" ECG signal. First-degree AV block is defined as a P-R interval greater than 200 milliseconds long [27].

Though the ECG signals for zebrafish are similar to those in humans, the "normal" heartrate for zebrafish is higher than that found in humans. Based on previous research performed with zebrafish [5, 14, 16-19, 21], the "normal" heartrate for zebrafish under test conditions is between 90 and 150 beats per minute. Therefore, the accepted definition currently being considered for use in the program for sinus bradycardia is a heartrate less than 90 beats per minute and the accepted definition for sinus tachycardia is greater than 150 beats per minute. The definitions sinus arrhythmia, sinus arrest, and first-degree AV block remain unchanged between humans and zebrafish.

Using these definitions, along with additional medical data such as patient history and further testing, medical personnel are able to utilize ECG in order to diagnose heart disease in patients accurately. In addition to using ECG for diagnosis, medical personnel are also able to use ECG to monitor the general health of a patient through observation of changes in the ECG signal over time. Monitoring a patient's ECG manually to diagnose heart disease or to observe changes in the health of the patient is currently a time-consuming process with the possibility of missing a transient change in the signal which only occurs for a brief period of time within hours of observation. With automated monitoring, such transient changes to the ECG signal can be identified and presented to medical personnel for follow-up investigation into the potential medical issues that are represented by those transient changes.

SECTION 3. DESIGNS, METHODS, AND IMPLEMENTATION

3.1 SIGNAL ACQUISITION HARDWARE SET-UP

This section provides details on two of the ECG acquisition devices that the program created for this thesis was designed to work with (See Sections 3.1.1 and 3.1.2) and the hardware methods of noise attenuation utilized to collect ECG data (See Section 3.1.3). These systems were utilized with the program presented in this thesis (See Section 3.2) to acquire data.

3.1.1 *Polymer-based MEA membranes*

The team I worked with for this thesis previously developed 4-channel MEA membranes for ECG acquisition in adult zebrafish based on parylene C [14]; here, the design is simplified and fabricated similar devices on a commercially-available polyimide film (125 μm Kapton, *Dupont, Wilmington, DE*). These newly-developed MEA membranes are cost-effective and suitable for acute measurements with anesthetized animals, providing favorable SNR and high

spatial resolution. The MEA membranes were fabricated by patterning sputtered metals (200 nm Au on 20 nm Cr) by wet etching followed by an encapsulation process by a 1-µm layer of hardened photoresist (S1813, *MicroChem, Westborough, MA*), leaving only the electrodes and contacts exposed (**Figure 11A)**. The working electrodes (WE) were designed in a circular shape with three sizes (200, 300, and 500 µm in diameter) while the reference electrode (RE) was designed much larger to maintain proper electrode-tissue interface. Silver epoxy was used to form electrical connections with thin Cu wires and then all contacts were protected by glue using a glue gun (**Figure 11B)**.

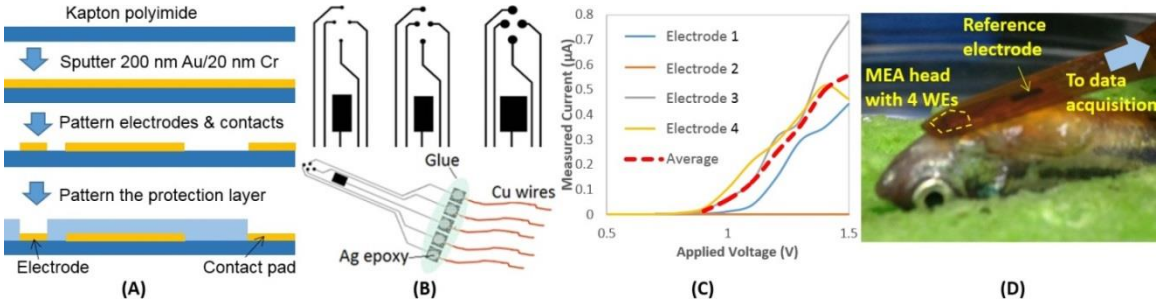


Figure 11. (A) Fabrication processes of the MEA membranes. **(B)** Different electrode sizes and the complete device. **(C)** Impedance curves of one 300-µm MEA membrane. **(D)** The MEA on the fish.

Prior to experiments, to validate connectivity and characterize the performance of the fabricated electrodes, each MEA membrane was immersed in a saline solution while applying a range of voltages between 0 V and 1.5 V across the WEs and RE. The resulting currents were then measured. A result from a 300-µm MEA membrane is showed in **Figure 11C**.

All experiments were in compliance with the Institutional Animal Care and Use Committee (IACUC) protocols (#4389-01) approved through the University of Washington to minimize the stress to animals. Adult zebrafish were anesthetized in a buffer solution with 150-200 mg/l tricaine methane sulfonate (Tricaine) and placed on a damp sponge with the ventral

side up and visualized under a stereomicroscope [14]. A 2-mm-long horizontal incision was created 0.5 mm caudally to the heart, and then the MEA membrane was located onto the incision placing the four WEs close to the heart and the RE onto the fish body (**Figure 11D**). Based on past experience, the MEA did not need to be inside in order to record ECG signals. Once an incision was made, though the wound was recovered, the MEA would be able to measure the signals for weeks. This finding helped ensure that there would not need to perform additional open-chest surgeries time to time which possibly cause infection and irritation during the investigation period.

The four electrodes were connected to 4 channels of a high-gain differential amplifier (*A-M Systems, Sequim, WA*) and an in-house LabVIEW (*National Instruments, Austin, TX*) program was used to display, analyze and record the data. The gain was set at 10,000 with filters including a bandpass of 0.1-500 Hz and a notch at 60 Hz to eliminate the unwanted interferences. All signals were then fed into a data acquisition device (DAQ 6000, *National Instruments, Austin, TX*), being digitized at a sampling rate of 1,000 Hz.

3.1.2 *PDMS Housing*

The in-house apparatus included a multiple-chamber housing made of polydimethylsiloxane (PDMS) using 3D-printed molds with embedded flexible electrodes. The molds were designed using the online-available software TinkerCAD (*Autodesk, Inc., Mill Valley, CA*) and then formed using a 3D printer. We used two 3D-printed parts, a rectangular tank with an inner length of 53 mm and inner width of 25 mm, and an oval-shape object with a length of 44 mm and a maximum width of 14.7 mm (**Figure 12**, **right panel**). First, PDMS (Sylgard 184, *Dow Corning, Midland, MI*) was cast into the rectangular object and then the oval mold was positioned to form the tapered housing. After PDMS was cured at room temperature in

24 hours, the molds were gently removed to make sure the apparatus remained the desired shape. To form the recording electrodes, two strips of 125 μm-thick polyimide (Kapton, *DuPont, Wilmington, DE*) with sputtered metals were inserted from the side of the apparatus through two thin-cut slits which were then sealed by applying PDMS. The electrode strips were positioned so that when the fish was loaded into the apparatus, the two electrodes would be securely in contact with the chest and abdominal areas, acting as recording and reference electrodes, respectively. Multiple apparatuses can be used simultaneously for measurement. **Figure 12 (lower right panel)** demonstrates a setup of four awake zebrafish ready for ECG recording.

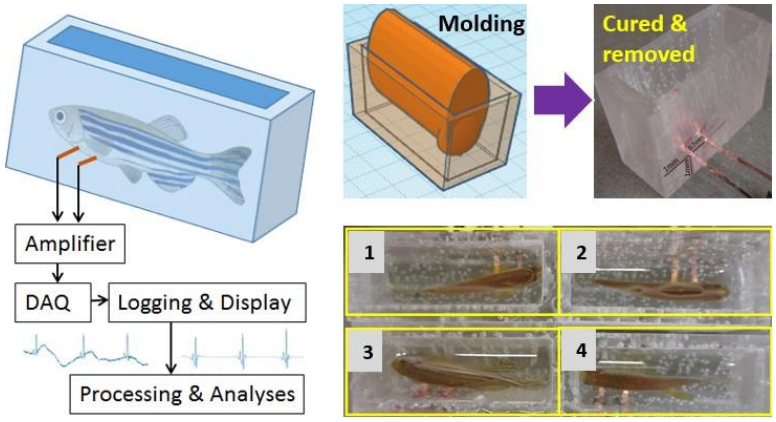


Figure 12. Apparatus formation and the measurement setup.

After the open-chest surgery, fish could be loaded into the chamber filled with system water for measurement. To maintain good recordings, the water level was lowered so that the fish body would make constant contacts with the electrodes. A reduced concentration of Tricaine can be used to minimize the stress caused to the fish, while still keeping them awake. The pair of electrodes in each chamber was connected to the high-gain differential amplifiers with settings described above.

3.1.3 *Noise Attenuation*

To reduce ambient electromagnetic interference (EMI), the entire setup was enclosed by an in-house Faraday cage (**Figure 13a**) and aluminum foil wrapped cables could be utilized. A Faraday cage was constructed of galvanized steel mesh with an aluminum foil lined pan acting as the bottom of the cage. When connected to the ground, this cage would help attenuate the 60-Hz interference from the 110-V AC power line. Its effectiveness was tested by measuring the noise level collected from a conductive wire with and without the Faraday cage covering the wire (The results for which are shown in **Figure 13b** and **Figure 13c**, respectively). In addition, the cables from the MEA device to the amplifier were wrapped in aluminum foil grounded to the amplifier chassis. The ambient noise and interferences will be also further taken care of by digital filters and our signal processing schemes, which will be described in the next sections.

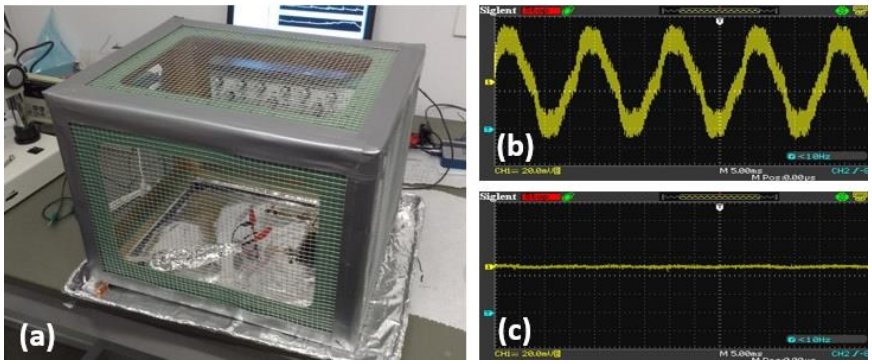


Figure 13. (**a)** The in-house Faraday cage. **(b-c)** Ambient EMI without (b) and with (c) the Faraday cage.

3.2 SOFTWARE STRUCTURE

In order to process and analyze data collected from the hardware set-up presented in Section 2.1, I created a LabVIEW *(National Instruments, Austin, TX)* program to perform the process automatically with data both collected in real-time and collected in previous tests. An overview flowchart of how the program functions is presented in **Figure 14**.

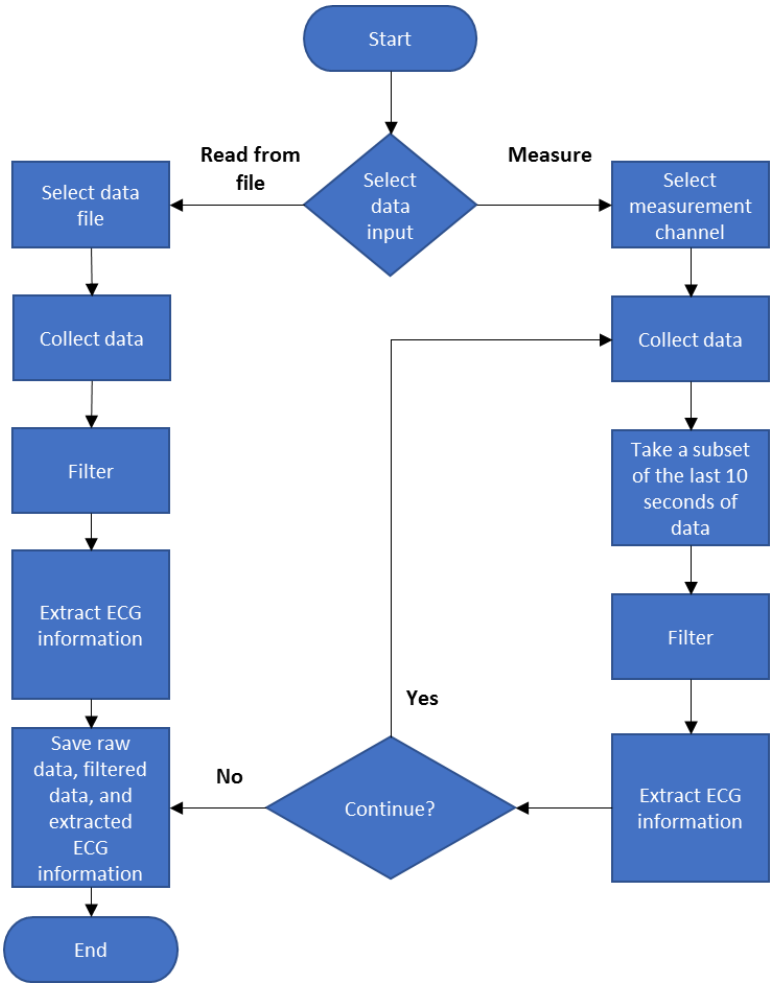


Figure 14. LabVIEW program flow chart.

The following sub-sections give specific details relating to how the user interfaces with the program (See Section 3.2.1), how the program gathers data (See Section 3.2.2), how the program filters acquired data (See Section 3.2.3), how the program detects heartbeats and extracts ECG features (See Section 3.2.4), how the program detects anomalies in the extracted ECG features (See Section 3.2.5), and how the program generates files containing processed data and a report on the detected anomalies in the data (See Section 3.2.6). The program also has tiered user access to depending on the amount and type of modifications that they need to make to the program (See Section 3.2.7).

3.2.1 *User Interface*

When first starting the program, the user is presented with the user interface in **Figure 15** with the text prompt to select the type of data being analyzed. The "Instructions for Use" text box is intended to provide indications to the user of what the expected actions required from them to progress forward properly are. This is to minimize the probability of potential errors when attempting to utilize the program.

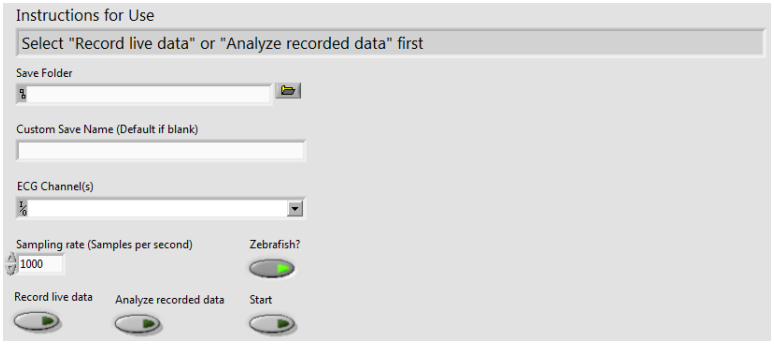


Figure 15. Initial user interface.

If the user selects "Analyze recorded data", then the interface changes to **Figure 16**.

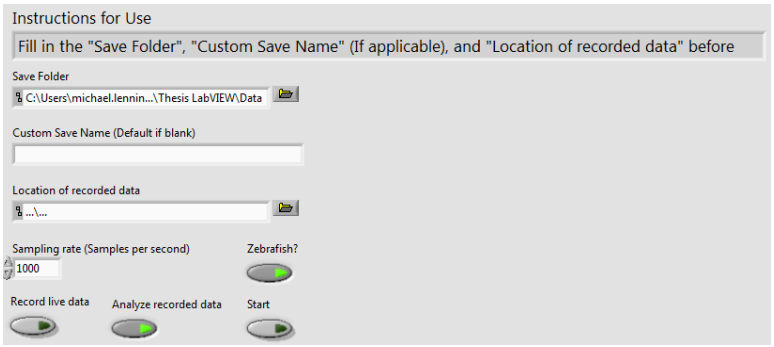


Figure 16. User interface after "Analyze recorded data" has been selected, with the text entry fields filled in.

After the user fills in all of the required text entry, as specified by the text prompt at the top of the user interface, the user can continue to processing the pre-recorded data (As presented in Sections 3.2.2, 3.2.3, 3.2.4, 3.2.5, and 3.2.6). This will change the interface to **Figure 17** when

the program has concluded processing the data which presents the filtered ECG with the identified features marked along with a basic summary of the detected heartrate and heartrate variability (HRV) for all of the data processed.

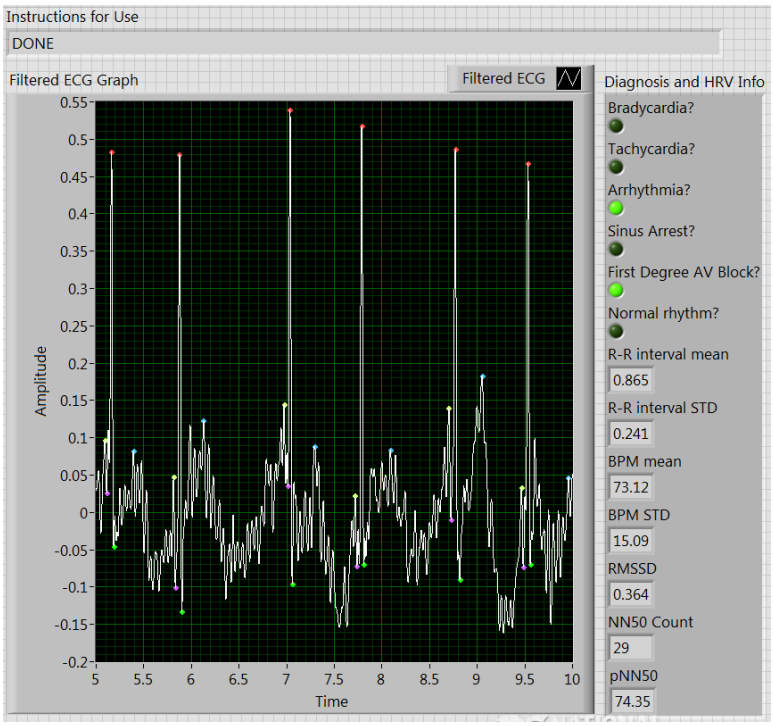


Figure 17. User interface with pre-recorded data after the program has concluded processing the data.

The test report and processed data can then be retrieved from the selected locations (See **Figure 22** in Section 3.2.6 for an example).

If the user selects “Record live data”, then the interface changes to **Figure 18**.

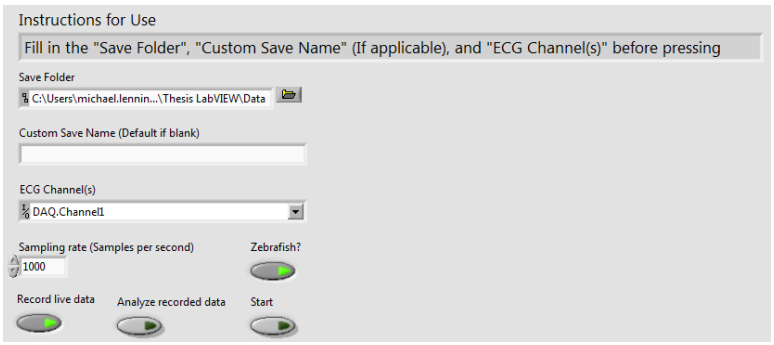


Figure 18. User interface after "Record live data" has been selected, with the text entry fields filled in.

After the user fills in all of the required text entry, as specified by the text prompt at the top of the user interface, the user can continue to collect real-time data using the next interface as shown in **Figure 19**.

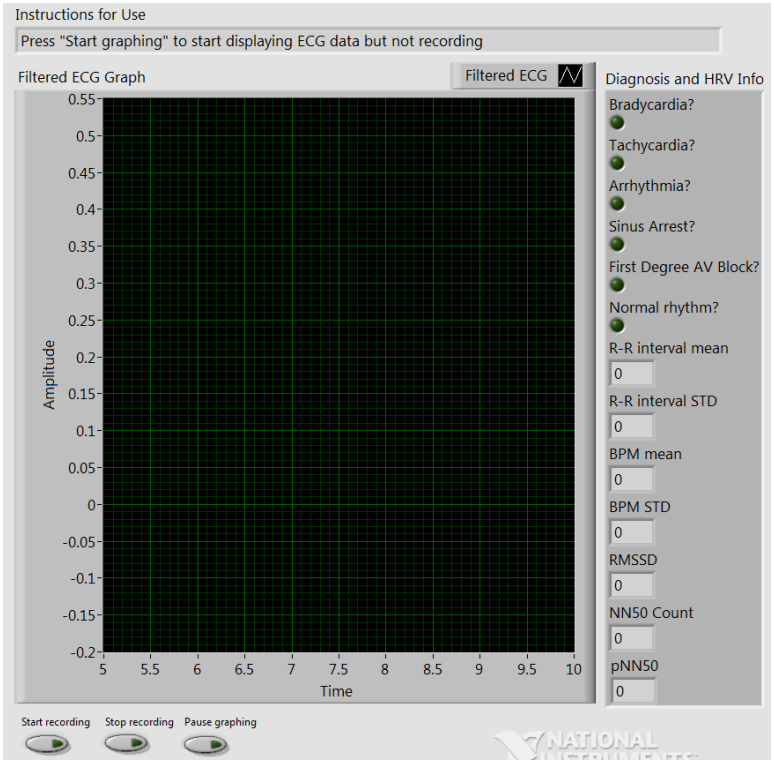


Figure 19. User interface with real-time data before the program has begun data collection.

After data collection has begun by following the text prompts at the top of the user interface, the filtered and analyzed data will begin to be presented to the user with the features identified along with a basic summary of the detected heartrate and HRV for the data currently being shown. Once all of the required data has been collected, the user can stop the recording by following the text prompts at the top of the user interface. The test report and processed data can then be retrieved from the selected locations (See **Figure 22** in Section 3.2.6 for an example).

3.2.2 *Signal Acquisition*

If the user selected to use pre-recorded raw data, the data from the file indicated by the user is extracted. The data is assumed to follow the format presented in Table 1 (See Section 3.2.6), which is the format that the program saves real-time recorded data in, and was collected at the sampling rate selected. The data is separated by recording channel, if four channels were recorded at the same time, and then the raw data is transferred to the filtration sub-VI to remove noise (See Section 3.2.3).

If the user selected to collect data in real-time, the data will be read from the input device selected by the user in one second blocks of data at the sampling rate selected. When processing the real-time collected data, the raw data is analyzed in blocks of the last ten seconds of collected data. The data is separated by recording channel, if four input recording channels were selected, and then the raw data is transferred to the filtration sub-VI to remove noise (See Section 3.2.3). The data is only processed and archived (See Section 3.2.6) once data collection has been concluded by the user.

3.2.3 *Filtration*

The collected data (See Section 3.2.2) is filtered within a sub-VI which is designed to remove noise from the data while still preserving the desired ECG signal. The filtering technique utilized by the sub-VI is based on the filtering technique described in a National Instruments article on using LabVIEW to process raw ECG signals [37]. The initial filters were designed based on the observed frequency range of ECG signals in humans of 1 to 20 Hz [38] due to the similarities between human and zebrafish ECG signals.

To remove high-frequency noise from the data, an initial Dolph-Chebyshev lowpass window filter is applied using the Biosignal Filtering VI, which is part of the Biomedical Toolkit for LabVIEW. The Dolph-Chebyshev window filter's Fourier transform is defined by [39, 40]:

$$W(\omega_k) = \frac{\cos\left\{M\cos^{-1}\left[\beta\cos\left(\frac{\pi k}{M}\right)\right]\right\}}{\cosh[M\cosh^{-1}(\beta)]} \tag{3.1}$$

$$M = Length\ of\ the\ window$$

$$k = 0,1,2,\ldots,M-1$$

$$\beta = \cosh\left[\frac{1}{M}\cosh^{-1}(10^{\alpha})\right]$$

$$\alpha = -\frac{Sidelobe\ attenuation}{20}$$

The current lowpass filter is configured to attenuate signals with a frequency greater than 40 Hz by a minimum of 40 dB (See **Figure 20** for the frequency response of the filter).

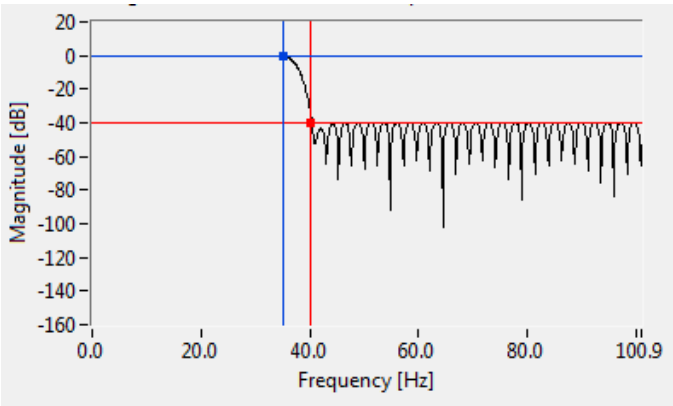


Figure 20. Frequency response of the Dolph-Chebyshev window filter.

The WA Detrend VI, which is part of the Digital Filter Design Toolkit for LabVIEW, is applied next with a Daubechies6 (db06) wavelet (See **Figure 21** for a graph of the wavelet function for the db06 wavelet) to remove baseline wander.

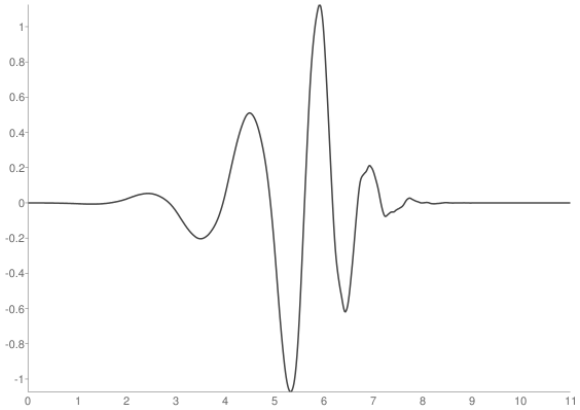


Figure 21. Wavelet function for the Daubechies6 (db06) wavelet.

This VI removes low frequency noise in the signal. To do this, the VI performs the following steps [41]:

- Applies a discrete wavelet transform to the signal using the db06 wavelet which returns a set of approximation coefficients and detail coefficients.
- The approximation coefficients are all set to 0.

- The signal is reconstructed using the db06 wavelet based on the detail coefficients from the discrete wavelet transform.

Then, the remainder of the wideband noise is removed using the Wavelet Denoise VI, which is also part of the Digital Filter Design Toolkit for LabVIEW, with a db06 wavelet (See **Figure 21** for a graph of the wavelet function for the db06 wavelet). To do this, the VI performs the following steps [42]:

- Applies a discrete wavelet transform to the signal using the db06 wavelet which returns a set of approximation coefficients and detail coefficients.
- Applies a soft threshold to the resulting coefficients to suppress coefficients which are smaller than the threshold.
- The signal is reconstructed using the db06 wavelet based on the new approximation coefficients and detail coefficients.

Finally, the filtered data is outputted from the sub-VI to be sent to the feature extraction sub-VI (See Section 3.2.4) for further processing. The filtration sub-VI was designed to allow for simple-to-implement modifications by future researchers if the data being collected contained noise outside of the range that the current filters are designed to handle (See Section 3.2.7).

3.2.4 *Feature Extraction*

The filtered data (See Section 3.2.3) is analyzed within a sub-VI which is designed to extract the ECG features (For example the location of the P-wave) from the data. The method for extracting ECG information from filtered zebrafish ECG data is based on the windowing algorithm presented in [43]. To extract the peaks of P, Q, R, S and T waves, the sub-VI for feature extraction was designed to look for these peaks within the area between R waves that they are expected to occur in. The R waves are found by using the Peak Detector VI within

LabVIEW to find peaks above a threshold of 50% of the maximum amplitude measured. This threshold is low enough that all R waves are detected and high enough that none of the other waves cross the threshold. Then, each R-R interval is divided into a set of 'windows' where each wave is detected by the sub-VI. The Q-wave peak and S-wave peak are searched for as the minimums 50 ms before and after the R-wave, respectively. The T-wave peak is the highest peak 15% to 55% of the R-R interval from the first R-wave in the interval. Finally, the P wave is the highest peak 65% to 95% of the R-R interval from the first R-wave in the interval. The locations and amplitudes of the peaks in each R-R interval are collected for each R-R interval.

In addition to finding the ECG components, the time and amplitude differences between various ECG components are also calculated as outputs of the sub-VI. The calculated time intervals collected are as follows: R-R interval, P-Q interval, Q-R interval, P-R interval, R-S interval, S-T interval, R-T interval, and T-P interval. As the longitudinal intervals are more desirable for ECG signals, the only amplitude information collected is the difference between the R-wave amplitude and the T-wave amplitude. Additionally, the data in the S-T interval are divided into values above and below the isoelectric line then the following information is also calculated as part of the sub-VI for the data points above and below the isoelectric line: The amount of data points, the average value of the data points, and the standard deviation of the data points. The data from this sub-VI is then taken by a sub-VI which detects the presence of ECG anomalies by comparing the data to currently accepted medical criteria for diagnosing them (See Section 3.2.5).

The feature extraction sub-VI was designed to allow for simple-to-implement modifications by future researchers to change the size of the 'windows' used for detecting the ECG components and to change the threshold voltage levels (See Section 3.2.7).

3.2.5 *Anomaly Detection*

The extracted ECG features (See Section 3.2.4) are analyzed within a sub-VI which is designed to detect anomalies within the data that could indicate the presence of heart disease. The method used for the sub-VI to detect ECG anomalies in the collected zebrafish ECG data was inherited from the diagnosis criteria reported in [27, 36, 44]. In our first-generation system, we aimed to diagnose the following ECG anomalies: Sinus bradycardia (A heartrate less than 90 beats per minute (BPM)), sinus tachycardia (A heartrate greater than 150 BPM), sinus arrhythmia (A difference in R-R interval length between the shortest and longest in the last 10 heartbeats of greater than 0.16 seconds), sinus arrest (A P-P interval greater than 2 R-R interval), and first-degree AV block (A P-R interval greater than 0.20 seconds long). The numbers chosen here were for zebrafish ECG, based on our past experience and observation. The sub-VI compares the ECG component data collected by the previous sub-VI (See Section 3.2.4) to the presented criteria for each R-R interval. The sub-VI then returns a diagnosis of whether each ECG anomaly is present for each R-R interval. The diagnosis information is then presented on the front panel of the VI, for the latest 10 seconds of data for the real-time data collection and for the entire data for the previously recorded data. The front panel will display "TRUE" for an anomaly if any of the R-R intervals being presented have been diagnosed with the anomaly by the sub-VI. The indicator for "Normal Rhythm" will only display "TRUE" if no anomalies were detected by the sub-VI for any of the R-R intervals.

The anomaly detection sub-VI was designed to allow for simple-to-implement modifications by future researchers to change the definitions of each ECG anomaly (See Section 3.2.7). This allows the anomaly definitions to be updated for use with test subjects which are not zebrafish, for example humans. In addition, the anomaly detection sub-VI was designed to allow

for integration with an ECG-anomaly library which can be designed in the future using machine learning techniques similar to those presented in Section 2.4 of [21].

3.2.6 *Data Archival and Report Generation*

After the raw data has been collected (See Section 3.2.2), filtered (See Section 3.2.3), the ECG features extracted (See Section 3.2.4), and any anomalies in the filtered data were detected and catalogued (See Section 3.2.5); all of data collected from those sub-VIs is collected and processed for archiving by a final sub-VI. This program saves the raw data, filtered data, the extracted ECG component data, and a summary of the test results in the folder selected by the user. The files containing the raw and filtered data are text files formatted to match Table 1, with tab separation between columns.

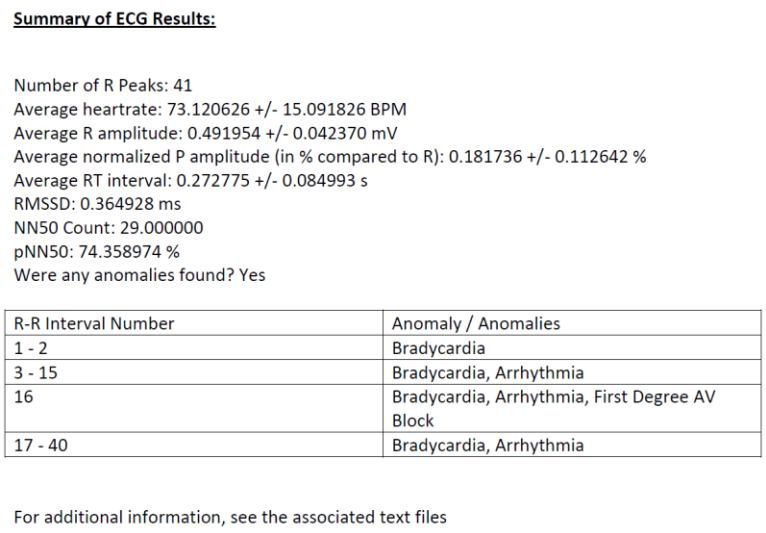
Table 1. Raw and filtered data text file format.

<First data point from channel 1>	<First data point from channel 2, if applicable>	<First data point from channel 3, if applicable>	<First data point from channel 4, if applicable>
<Second data point from channel 1>	<Second data point from channel 2, if applicable>	<Second data point from channel 3, if applicable>	<Second data point from channel 4, if applicable>
etc.	etc.	etc.	etc.

The file containing the extracted ECG component data is a text file formatted to match in a table, with tab separation between columns, which has the following data in separate columns with each subsequent row corresponding to the next R-R interval (See Section 3.2.4 for details): R Time, R Amplitude, S Time, S Amplitude, T Time, T Amplitude, P Time, P Amplitude, Q Time, Q Amplitude, R-R Interval Time, P-Q Interval Time, Q-R Interval Time, P-R Interval Time, R-S Interval Time, S-T Interval Time, R-T Interval Time, T-P Interval Time, R-T Amplitude Difference, T Wave Found?, P Wave Found?, Bradycardia?, Tachycardia?,

Arrhythmia?, Heart rate of last 10 beats, Heart rate STD of last 10 beats, Time with S-T Depressed, Mean Voltage with S-T Depressed, Voltage STD with S-T Depressed , Time with S-T Elevated, Mean Voltage with S-T Elevated, Voltage STD with S-T Elevated, Sinus Arrest?, First Degree AV Block?, P-P Interval Time. The first row of the text file labels the data associated with each column.

The summary of the test results is a Word document generated using the LabVIEW Report Generation toolkit. An example of a summary of ECG results is shown in **Figure 22**.



Summary of ECG Results:

Number of R Peaks: 41
Average heartrate: 73.120626 +/- 15.091826 BPM
Average R amplitude: 0.491954 +/- 0.042370 mV
Average normalized P amplitude (in % compared to R): 0.181736 +/- 0.112642 %
Average RT interval: 0.272775 +/- 0.084993 s
RMSSD: 0.364928 ms
NN50 Count: 29.000000
pNN50: 74.358974 %
Were any anomalies found? Yes

R-R Interval Number	Anomaly / Anomalies
1 - 2	Bradycardia
3 - 15	Bradycardia, Arrhythmia
16	Bradycardia, Arrhythmia, First Degree AV Block
17 - 40	Bradycardia, Arrhythmia

For additional information, see the associated text files

Figure 22. Example summary of ECG results.

The data archival and report generation sub-VI was not designed to allow for simple-to-implement modifications by future researchers. Modifying this sub-VI would require reprogramming on the block diagram level (See Section 3.2.7).

3.2.7 *Tiered User Access*

The program has also been implemented in such a way as to provide to tiered access to users depending on the amount and type of modifications that they need to be able to make. The two access tiers are first tier (Access to an executable file version of the program which cannot

be modified), second tier (Access to block diagram for the sub-VIs to allow the user to alter the structure of the sub-VI).

Users with first tier access are given the VI for the program along with the sub-VIs which can collect and analyze data but these users cannot modify the program because the sub-VIs and the block diagram for the main VI are password-protected. Users with second tier access are given the password to access the block diagram for the main VI file and all of the sub-VIs. For each sub-VI, the front panel contains variables which control abilities of the program and can be modified to make adjustments without potentially removing a vital part of the program when interacting with the block diagram.

The first tier is intended for individuals who only need to collect and analyze data and the second tier is intended for users who need to change the functionality of the program.

An example of what the front panel of one of the sub-VIs looks like and what variables can be adjust by second tier users is presented in **Figure 23**.

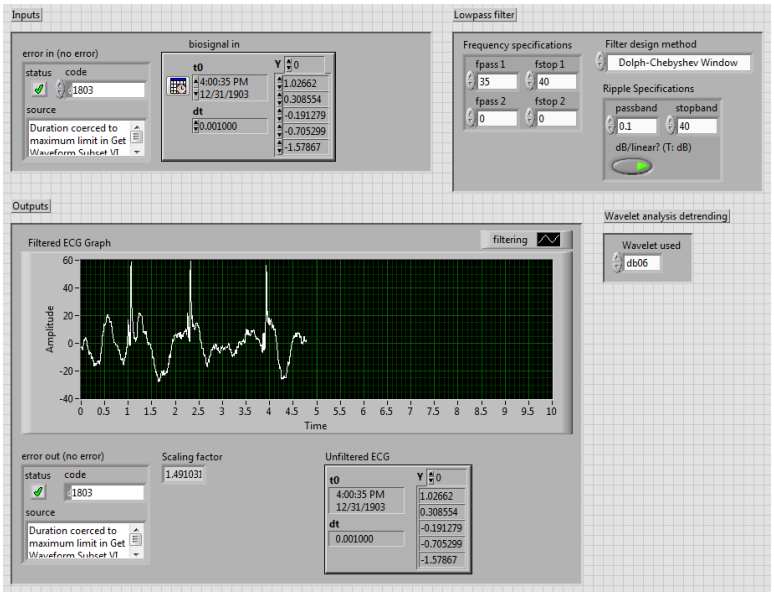


Figure 23. Front panel for the filtration sub-VI (See Section 3.2.3).

An example of what the block diagram for one of the sub-VIs looks like that can be adjusted by second tier users is presented in **Figure 24**.

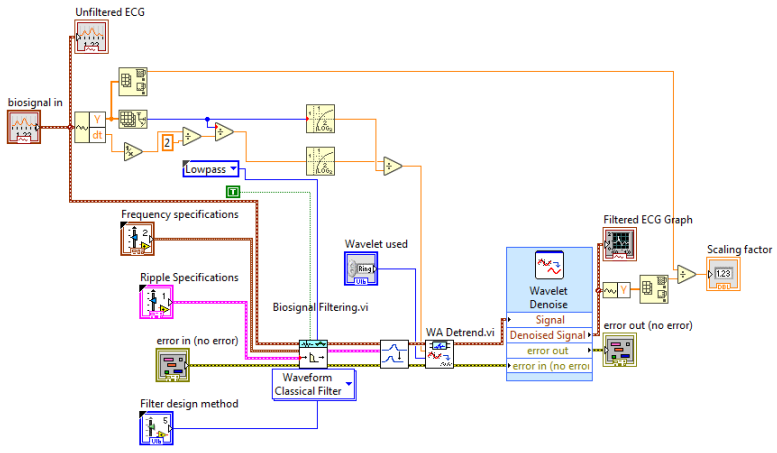


Figure 24. Block diagram for the filtration sub-VI (See Section 3.2.3).

SECTION 4. EXAMPLES OF USING THE PROGRAM TO PERFORM GENETIC SCREENING FOR ARRHYTHMIC GENES

In previous research related to the identification of the genes connected to cardiomyopathy (Diseases related to the muscles of the heart [45]) presented in [46-48], a line of zebrafish with particular genetic mutations were identified by the expression of the particular genes through the presence of related ECG anomalies. The zebrafish from that line could be further genetically screened through analysis of their ECG patterns over time to detect the presence of further anomalies. The next step from the research presented in [46-48] would be to extend into the identification of arrhythmic genes. However, manually screening through hours of ECG data for a single test subject to screen for the expression of arrhythmic genes would take days to perform. Repeating this process for the hundreds of test subjects required for a full genetic study would take years to analyze the data from only a few days of collection. Instead of manually assessing the data for each zebrafish, the program presented in this thesis could

potentially be utilized to assess hours of data for a single test subject for the presence of every anomaly under investigation in a few minutes.

To verify that the program in this thesis would be able to facilitate the identification of ECG anomalies related to those arrhythmic genes to allow for expedited genetic screening, the accuracy of the program was tested using the ECG data of a zebrafish from the ZIC (Zebrafish insertional cardiac) mutant line presented in [48] with the known phenotype related to sinus arrest to ensure that the program was able to accurately identify the sinus arrest that was found through manual analysis (See Section 4.1). Before testing the program on ECG data with a sinus arrest anomaly identified, the accuracy of the program was tested using the ECG data of a zebrafish from the control group without the known phenotype related to sinus arrest to ensure that the program was able to accurately identify that the signal does not include a sinus arrest as was verified through manual analysis (See Section 4.2). The ECG data of the zebrafish from the control group was also utilized to verify the filtering capabilities of the program's filtration sub-VI (See Section 3.2.3) in Section 4.2.

4.1 Verifying the Program's Ability to Identify ECG Data Without the Sinus Arrest Anomaly

Using the apparatus detailed in Section 3.1, ECG data of an awake zebrafish from the control group without the known phenotype related to sinus arrest was obtained. The raw collected signal (See Section 3.2.2) is shown in **Figure 25**.

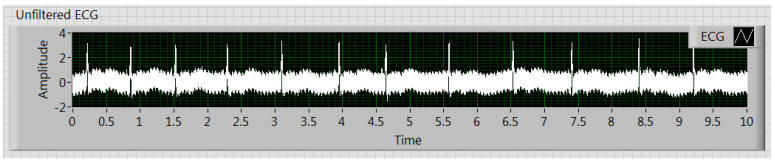


Figure 25. Raw ECG signal from an awake zebrafish from the control group.

The filtered signal after the filtration sub-VI (See Section 3.2.3) is shown in **Figure 26**.

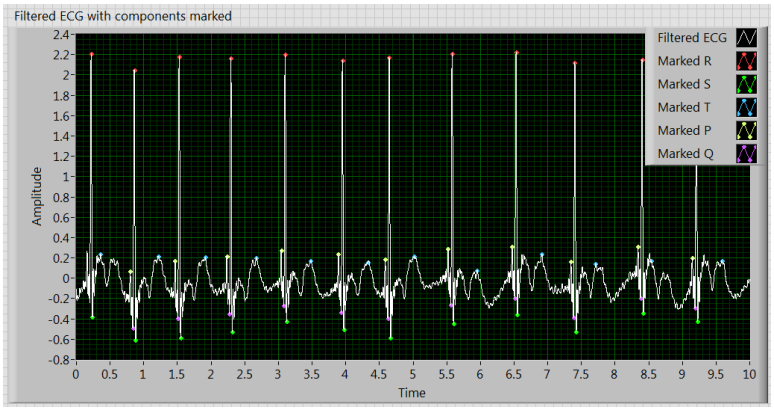


Figure 26. Filtered ECG signal from an awake zebrafish from the control group.

The signal-to-noise ratio for both signals is calculated using the following equation [49]:

$$SNR = \frac{\sqrt{\frac{1}{b_{s2}-b_{s1}+1}\sum_{k=b_{s1}}^{bs2}\frac{1}{t_{s2}-t_{s1}+1}\sum_{i=t_{s1}}^{t_{s2}}X_k^2(i)}}{\sqrt{\frac{1}{b_{n2}-b_{n1}+1}\sum_{k=b_{n1}}^{bn2}\frac{1}{t_{n2}-t_{n1}+1}\sum_{i=t_{n1}}^{t_{n2}}X_k^2(i)}} \quad (4.2)$$

$b_{s2} = End\ beat\ number\ used\ for\ calculating\ the\ signal$

$b_{s1} = Start\ beat\ number\ used\ for\ calculating\ the\ signal$

$t_{s2} = End\ time\ of\ the\ segment\ used\ for\ calculating\ the\ signal$

$t_{s1} = Start\ time\ for\ the\ segment\ used\ for\ calculating\ the\ signal$

$X_k(i) = Amplitude\ value\ of\ the\ data$

$b_{n2} = End\ beat\ number\ used\ for\ calculating\ the\ noise$

$b_{n1} = Start\ beat\ number\ used\ for\ calculating\ the\ noise$

$t_{n2} = End\ time\ of\ the\ segment\ used\ for\ calculating\ the\ noise$

$t_{n1} = Start\ time\ for\ the\ segment\ used\ for\ calculating\ the\ noise$

The signal value (The value from the numerator of Equation 4.2) was calculated using data from the R-wave portion of each heartbeat. The noise value (The value from the denominator of Equation 4.2) was calculated using data from the space between the T-wave and P-wave of each

heartbeat. The time intervals used for each of the signal and noise sections were the same for both the raw and filtered data.

The signal-to-noise ratio was increased from a ratio of 9-to-1, as seen in **Figure 25**, to a ratio of 120-to-1, as seen in **Figure 26**. This indicates that the filtration sub-VI is removing 92.5% of the noise from the ECG signal being analyzed. This improvement in the signal-to-noise ratio allows the program to clearly detect the ECG components other than the R-wave, which was already visible in the raw data.

The front panel of the main VI upon completion of analysis (See sections 3.2.4 and 3.2.5) with the ECG data of the awake zebrafish without the known phenotype related to sinus arrest is shown in **Figure 27**.

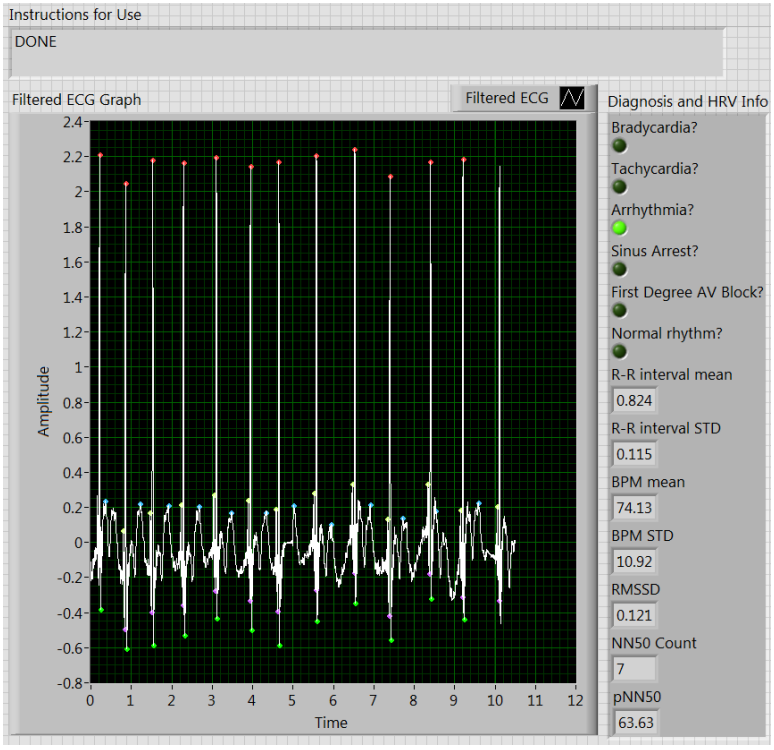


Figure 27. Main VI front panel upon completion of analysis of the raw ECG data from an awake zebrafish in the control group.

The “Filtered ECG Graph” in **Figure 27** shows the filtered signal with ECG components. The “Diagnosis and HRV Info” box shows diagnostic information about the presence of anomalies along with the following information related to heartrate and HRV: The mean BPM for all the R-R intervals (BPM Mean), the standard deviation of the BPMs of all the R-R intervals (BPM STD), the mean R-R interval length (R-R interval mean), the standard deviation of the R-R interval lengths (R-R interval STD), the square root of the mean of the squares of the differences between adjacent R-R interval lengths (RMSSD), the number of adjacent R-R intervals with a difference greater than 50 ms (NN50 Count), and the percentage of adjacent R-R intervals with a difference greater than 50 ms (pNN50). All of the P waves, QRS complexes, and T waves were successfully detected. The success rate for peak detection was 100% when compared to manual peak detection methods.

The indicator for “Sinus Arrest?” being turned off in **Figure 27** indicates that the program did not detect the presence of a sinus arrest in the ECG signal. This agrees with the manual assessment of the ECG data. This example verifies that the program can successfully screen for ECG signals that do not contain a sinus arrest and do not present the sinus arrest phenotype indicators.

4.2 Verifying the Program’s Ability to Identify ECG Data With the Sinus Arrest Anomaly

The ECG data of an awake zebrafish from the ZIC mutant line with the known phenotype related to sinus arrest was also obtained for verification of the program’s ability to identify the sinus arrest in the ECG signal. The front panel of the main VI upon completion of the analysis of the ECG data is shown in **Figure 28**.

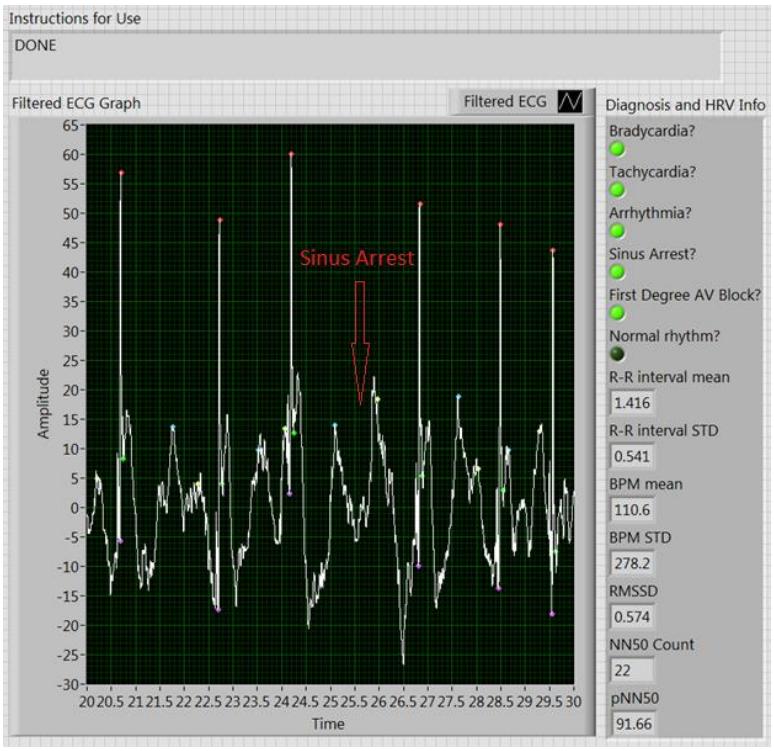


Figure 28. Main VI front panel upon completion of analysis of the raw ECG data from the ZIC mutant line zebrafish with the known phenotype related to sinus arrest

The indicator for "Sinus Arrest?" being turned on in **Figure 28** indicates that the program did detect the presence of a sinus arrest in the ECG signal. This agrees with the manual assessment of the ECG data (See the R-R interval greater than 2 seconds indicated by the red arrow in **Figure 28**). This example verifies that the program can successfully screen for ECG signals that do contain a sinus arrest and do present the sinus arrest phenotype indicators. The positive result from the testing verifies that the program is able to successfully screen for ECG anomalies which indicate the presence of the sinus arrest phenotype.

SECTION 5. FUTURE WORK AND CONCLUSIONS

This section is divided into the three groups of future uses for the program that it was designed for: the addition of new functionality (See Section 5.1), use in future research efforts (See Section 5.2), and use in potential future commercial products (See Section 5.3). Each of these subsections provides details on the specific programming choices implemented in the program presented in this thesis to facilitate each presented future use.

5.1 ADDITIONAL POTENTIAL FUNCTIONALITY

Three future functionality improvements were intended when designing this program: the integration of cloud-based data storage as a potential data archiving option (See Section 3.2.6 for the current data archival structure), the implementation of advanced pattern recognition methods (Such as machine learning) to enhance the diagnostic capabilities of the program (See Section 3.2.5 for the current diagnostic capabilities of the program), and the implementation of a motion detection system to automatically pause signal acquisition when the test subject is moving (See Section 3.2.2 for the current signal acquisition system of the program). These additional functions will allow for the transfer of data between research collaborators in disparate parts of the world, improve the precision of the diagnoses provided by the program, and reduce the effect of noise created by the motion of the test subject.

To implement cloud-based data storage with the current program, the primary tool considered for facilitating such a feature are the “Amazon Web Services” (A cloud computing and storage solution developed by Amazon.com, Inc.). The principal reason for designing the program for future implementation of data storage utilizing Amazon Web Services is that National Instruments (The company which developed LabVIEW) has a toolkit, the “LabVIEW

Cloud Toolkit for AWS by NI" [50], specifically designed to allow programs developed within LabVIEW to be easily updated to integrate Amazon Web Services data storage and retrieval without the need to drastically restructure the existing program to facilitate the modification. Data processed by the program can be saved to the cloud using the tools in this toolkit (See Section 3.2.6) and data saved to the cloud can be extracted for processing using the tools as well (See Section 3.2.2), Preliminary tests utilizing the toolkit to upload and download simple data have proved effective within a test program. To implement this feature within the program, an Amazon Web Services account for the project that the program is being utilized for would need to be created then the toolkit could be utilized on the data storage and retrieval portions of the program to allow for interaction with that account.

To implement advanced pattern recognition with the current program to improve the diagnostic capabilities of the program, the method that the program is designed to facilitate is machine learning. Machine learning is an approach to pattern recognition which is designed intended to create programs which can be "trained" to recognize patterns which are similar to those previously identified as well as being able learn and improved based on experience over time [51]. Research into the subject of utilizing machine learning to diagnose ECG anomalies in zebrafish has already been conducted by the research team that I was a part of and could be integrated into the current program [21]. National Instruments also has a toolkit, the "LabVIEW Analytics and Machine Learning Toolkit" [52], specifically designed to allow for the implementation of predictive analytics and machine learning algorithms within previously designed LabVIEW programs. The toolkit was designed with facilitating anomaly detection in mind and example programs are provided on the National Instruments website to aid future programmers. Preliminary tests utilizing the toolkit with human ECG data to diagnose

bradycardia and tachycardia have proved effective within a basic test program. To implement a machine learning feature with the program presented in this thesis, ECG data which contains known anomalies along with "normal" ECG data needs to be acquired for use as "training" data for the machine learning portion of the program along with modifying the program's diagnosis sub-VI (See Section 3.2.5) to utilize to generated algorithms for ECG anomaly detection.

In the current program, the system has difficulties with filtering the irregular and high amplitude noise that can be caused by occasional movements in the test subject during testing. To mitigate this issue, a potential option that could be explored in the future would motion detection using PIR (Passive Infra-red) motion sensors, or similar, to pause signal acquisition while motion is detected (See Section 3.2.2). Implementing the motion detection system would require the following steps: the signal acquisition would need to be updated to take readings from a motion detector, the ECG measurements would be paused while motion is detected, and the measurements would resume when motion is no longer detected (The motion detection method described in [53] could be used as an example for how a system similar to this could be created). The program was also designed to allow for modifications to the filtration system (See Section 3.2.3) which might be able to provide an alternative avenue to removing the noise due to muscle movement from the signal; however, the potential methods for modifying the filter setup to perform this task would require significant additional research to alter the sub-VI in such a way as to remove the noise related to motion without potentially significantly altering the ECG signal itself after filtration.

5.2 USE IN FUTURE RESEARCH

The current program was designed for zebrafish ECG, so it can continue to be utilized to collect and interpret ECG from zebrafish test subjects. As a model for humans, zebrafish have

been utilized for drug effectiveness and safety testing [54-56]. The program can be utilized to assess the health of zebrafish being tested by monitoring for the development of new ECG anomalies or the change in severity of a previously observed anomaly. The program can either be used to diagnose anomalies on its own or the observations of the program can be relayed to an expert in interpreting ECG signals to ensure the accuracy of the findings.

In addition to use in assessing anomalies found in zebrafish ECG data, the program's diagnostic sub-VI (See Section 3.2.5) has been designed to allow for the modification of the criteria used to assess if a particular anomaly from the front panel alone is present which allows for modification without the need to interact with the block diagram. If the diagnostic sub-VI is modified to utilize machine learning (See Section 5.1), then the program's diagnostic capabilities can be updated by teaching the machine learning portion using new "training" and "normal" data. Modifying the diagnostic sub-VI would allow the program to be used with species other than zebrafish. For example, using clinical data from humans the program can be updated to diagnose anomalies in humans. This would allow the program to monitor the cardiologic health of patients undergoing clinical trials with novel drugs to ensure that the desired effect of the medication is taking place without potentially creating new issues related to the heart of the patient. To utilize the program for stages of clinical trials in which other species are tested, the program's definitions can be updated to account for any changes in the medical definitions of particular cardiac anomalies between species.

5.3 Use in Future Commercial Products

Utilizing the program's ability to extract ECG features (See Section 3.2.4) and to diagnose anomalies in particular (See Section 3.2.5), this program can serve as the basis for the diagnostic portion of a variety of medical product types. Combining this program with available

ECG acquisition tools in hospitals, an automated diagnosis feature could be added to such a tool which is able to alert hospital personnel to potential indicators of heart disease found in the ECG patterns of patients connected to the device. When combined with an in-home ECG acquisition tool and the capability to store data in a cloud server (See Section 5.1), a distance care or e-care system could be developed which would allow patients to collect ECG data in their own homes and send alerts/data to the patient's doctor without requiring an in-person meeting unless an issue requiring medical attention presents itself. Finally, the program could be added to a fitness tracker device such as a Fitbit to alert patients if a particular ECG anomaly is detected (Such as early signs of a heart attack) along with alerting the appropriate medical personnel to the detected issue.

As the accuracy of the program continues to improve over time with the implementation of advanced pattern recognition such as machine learning (See Section 5.1), the program can progress from acting as an aid to identify potential ECG anomalies that indicate the possibility of a particular heart disease being present to being able to accurately diagnose heart disease with relatively minimal required outside assistance. Until then, the program can be utilized with both clinical and in-home devices to provide aid to individuals suffering from known heart disease symptoms as well as providing tools to diagnose for patients who demonstrate potential signs of heart disease but require long-term monitoring to search for infrequent anomalies.

Impressed with progress in machine learning and artificial intelligence, Elon Musk, the CEO of Tesla, recently even warned about the potential harm of artificial intelligence to the future society. However, so far they are mainly in the consumer and entertainment industries where regulations are lax. In the biomedical field, it is still immature. The work presented in this thesis can begin to pave the way for further developments in programmatic approaches to

diagnosing disease and monitoring patients with known medical conditions for signs of the need for medical intervention before the event occurs. With continued development, patients of the future will be able to treat any conditions that they may develop as early as they can be detected as well as being able to monitor those conditions to ensure that acute, severe events can be treated by the appropriate medical personnel in a timely manner.

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