Simultaneous Visual and Electro-Cardiogram Measurements of Zebrafish Embryos

by

Elizabeth A. Ellingson

Submitted to the Department of Mechanical Engineering in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Mechanical Engineering at the Massachusetts Institute of Technology June 2001

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ABSTRACT

An experimental study was performed to determine a simultaneous visual and electro-cardiogram measurement of zebrafish embryos. One zebrafish embryo was placed between two electrodes and the electrical signal was amplified 100 times, then a computer recorded the data. The visual reading of the zebrafish heart rate was obtained by viewing the embryo under a microscope. A variety of approaches were investigated to determine the heart rate including amplification, noise filtering and data manipulation. Noise was a significant obstacle in determining the zebrafish embryo’s heart rate. Therefore, the signal was smoothed, digitally filtered, and a system transfer function was determined to extract the heart rate from the noisy signal. After the data manipulation, the electrical signal appeared to correspond to the visual reading of the heart rate. Providing a simultaneous visual and electrical measurement of the heart rate can lead to a better understanding of cardiological genetic mutations. This method of measuring the heart rate can supply information on the strength and pattern of the heartbeat, and also detect irregularities in the beat, which could lead to further understanding of cardiological genetic mutations and other related health problems in the future.

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Acknowledgements

This thesis would not have been possible without the help from the people in the BioInstrumentation Lab at MIT. I would like to give a special thanks to Professor Ian Hunter, Patrick Anquetil, and Aimee Angel for their guidance and support. Also, Calum MacRae and Amy Siddons from the MGH Cardiovascular Research Center have my gratitude for their assistance in this project and the weekly supply of zebrafish embryos.
1. Introduction

Since the 1970’s, zebrafish have been used to study vertebrate development and genetics. As simple vertebrates, the information gained through the study of zebrafish can be applied to more complex vertebrates, such as human beings. Zebrafish make excellent test animals because they are transparent through young development and have a fast development cycle. One important area of investigation is cardiological genetic mutations. In order to identify specific cardiological mutations, the heart rate of individual zebrafish embryos must be measured within 48 hours of fertilization. Current methods of measuring the heart rate are both primitive and time intensive. The existing techniques include looking at the specimen under a microscope and using a stopwatch; therefore, a more advanced method of measuring the heart rate is needed. The remainder of this thesis describes the procedure of simultaneously taking visual and electrical readings of the zebrafish heart rate. By placing the embryo between two electrodes, an electrical reading can be recorded. Additionally, a visual representation of the zebrafish’s heart rate is obtained using a microscope. This method will aid in the detection of cardiological genetic mutations in zebrafish and provide a step into an easier and faster method of measuring the heart rate of zebrafish embryos.
2. Background

2.1. Reasons for Zebrafish Research

In the early 1970's, a scientist, Dr. George Streisinger, determined that zebrafish were an excellent model for studying vertebrate development and genetics. Since he began using them in his research, zebrafish embryos have become a popular means of understanding how not only fish, but all vertebrates, develop from the moment of fertilization. The rapid development, short inter-generation time (3-4 months), high fecundity (mature females lay several hundred eggs at weekly intervals), small size, and easy maintenance make zebrafish an excellent candidate to study. Zebrafish are visibly transparent through young development, which allow scientists to watch zebrafish eggs grow from an embryo to a newly formed fish under a microscope. Scientists observe the cells form different body parts over a development span of 2-4 days.

A zebrafish's genetic make-up can be altered by a variety of manipulations. These include rearranging or moving cells to different locations, inducing genetic mutations, or by simply destroying cells. Observing the results of these manipulations can lead to better comprehension of genetic mutations in zebrafish and other vertebrates, such as humans. Zebrafish provide information on how all vertebrates grow and give an understanding of development and how it relates to birth defects and other health problems. By understanding why these birth defects (i.e. mutations) occur and which original cells are involved, scientists try to find ways to prevent them. The understanding and knowledge gained from this type of zebrafish research may play a prominent role in overcoming these defects and other related health problems in the future.

2.2. Application

The current method of measuring zebrafish heart rates is rudimentary, time intensive, and inaccurate. Providing an electrical measurement of the heart rate would not only give scientists information on the regularity and rhythm of the beat, but also the strength of the heartbeat. An electrical signal along with a visual confirmation of the heart rate could provide a means of identifying cardiac mutations by displaying any irregularities. In addition, it could provide information on blood flow rates and will also supply a faster means of measuring the heart rate opposed to the lengthy stopwatch approach that is currently in use.

2.3. Relevant Development Stages of Zebrafish Embryos

The primary period of cardiac development in zebrafish occurs during the pharyngula period (24-48 hours). Their hearts start beating at the end of the segmentation period, which occurs 24 hours after fertilization. During the pharyngula period, the heart is first visible as a cone-shaped tube attached at the base of the brain, as shown in Figures 2.1 and 2.2. At first, there is no apparent regularity to the beat, and the rhythm may be interrupted. Scientists are particularly interested in studying zebrafish during this time period because many of the cardiological genetic mutations either cause the zebrafish to die or fundamentally change within 48-72 hours of development. Further information of zebrafish development can be found in Appendix A.
Figure 2.1: Zebrafish embryo at 24 hours of development (taken from Edwards, http://zfin.org).

Figure 2.2: Zebrafish embryo at 48 hours of development (taken from Edwards, http://zfin.org).
3. Method of Study

3.1. Measurement Difficulties

A zebrafish heart rate is difficult to measure for several reasons. First, the size of a zebrafish’s heart makes measuring the rhythm of the organ a challenging prospect. A zebrafish embryo diameter is around 1.2 mm; subsequently the heart is a fraction of that length. The signal size is predicted to be 50 μV. A zebrafish is also a complex organism. Second, it is a vertebrate; therefore, other electrical stimulations occurring from various internal organs influence the electrical measurement. Finally, the electrodes and electrode wiring introduce additional sources of inaccuracy (e.g. noise). The embryo is placed between the two electrodes for an external measurement of the heart rate; therefore the electrodes add a large amount of outside noise to the signal. In addition, the electrode size prevents placing them directly on or near the heart. As a result, they pick up the electrical stimulations from internal and external sources.

3.2. Method to Extract the Zebrafish Heart Rate from a Noisy Signal

3.2.1. Description of the Method

The zebrafish heart rate measurement is buried in a large amount of outside noise. Data manipulation including smoothing and digital filtering using Mathcad (http://www.mathcad.com) were attempted to extract the signal from the noise. The digital filtering technique eliminated some of the noise, but the signal was still unrecognizable.

On the other hand, the heartbeat can be monitored visually with a microscope. A Morse code apparatus was used to transfer the visual heartbeat to an electrical signal, where each pulse corresponds to a heartbeat. A transfer function was then found to relate the noisy data and the idealized ‘Morse code’ data. By deconvolving the visual signal from ‘the system’ transfer function, the heartbeat was extracted from the noisy data. A description of the experimental setup can be found in Section 4. In addition, Appendix B displays more details on the various Mathcad operations performed throughout this section.

The ‘Morse code’ data provided by the visual representation of the heart rate is the desired shape of the signal and the desired output of the filter. By finding the transfer function that relates the noisy data and the ‘Morse code’ data, it can then be applied to other sets of data to determine the heart rate signal. The following block diagram shows the transfer function relation between the noisy data and the ‘Morse code’ data.

![Figure 3.1: Transfer function relation.](image-url)
3.2.2. Correlations

Correlations are extremely useful in signal analysis and pattern recognition. An auto-correlation is computed by correlating the signal with itself for various lags or shifts. The signal is multiplied by itself (square each value) and then the average is computed. As a result, the noise is filtered out, and the signal is easier to identify. If two different signals are correlated with each other, it is referred to as a cross-correlation whereas when one signal is correlated with itself, it is an auto-correlation. In the case of zebrafish heart rate, an auto-correlation was performed individually on both the noisy data and the ‘Morse code’ data, and a cross-correlation was performed using both sets of data. The following information and equations illustrate the steps in correlating the two sets of data (Hunter, 2.131 Class Notes).

An auto-correlation was first performed individually on both sets of data. The auto-correlation function is shown in Equation 1 where \( c_{xx} \) is the correlated data, \( x \) is the data being correlated (noisy voltage data), \( m \) is the number of correlations, and \( i \) is the number of data points. The \( lcorr \) function in Mathcad was used to perform this calculation.

\[
c_{xx} = \frac{1}{n-j+1} \sum_{i=j}^{n} x_{i-j} \cdot x_{i} \tag{1}
\]

The input-output cross-correlation was then determined using both sets of data as shown in Equation 2 where \( x \) is the noisy data and \( y \) is the idealized ‘Morse code’ data.

\[
c_{xy} = \frac{1}{n-j+1} \sum_{i=j}^{n} x_{i-j} \cdot y_{i} \tag{2}
\]

3.2.3. Transfer Function

To solve for the transfer function \( h \) that relates the noisy data and the ‘Morse code’ data as shown in Figure 3.1, the input auto-correlation function is deconvolved from the cross-correlation function via the Toeplitz matrix inversion. The Toeplitz matrix is formed from the input auto correlation function as shown in Equation 3.

\[
C_{xx_{j,k}} = c_{xx_{|j-k|}} \tag{3}
\]
The transfer function, $h$, can then be found using the Toeplitz matrix inversion and the cross-correlation (found in Equation 2) function where $\Delta t$ is the sampling frequency.

$$h = \frac{1}{\Delta t} (Cxx^{-1} \cdot cxy)$$  \hspace{1cm} (4)

3.2.4. Application

Once the transfer function is known, it can be applied to other noisy data sets to determine the heart rate. The output ($y_2$) is found using the convolution of the new noisy input data ($x_2$) and the transfer function $h$, as shown in Equation 5. The Mathcad operation *convol* was used for this calculation.

$$y_{2_i} = \Delta t \cdot \sum_{j=0}^{i \leq m, l, m} h_j \cdot x_{2_{i-j}}$$  \hspace{1cm} (5)
4. Experimental Setup

There were many considerations that defined the specifications for the experimental apparatus. It was necessary for the system to detect 50 μV signal at a frequency of 1-3 Hz, which was the predicted signal of a zebrafish embryo. In addition, there needed to be a holding fixture for the 1.2 mm diameter embryo so that the fish would not dry out and also keep it contained for electrical measurement purposes. Finally, the setup needed to provide a simultaneous visual and electrical recording of the zebrafish embryo heart rate.

4.1. Design Apparatus

A clear visual representation of the zebrafish heart rate can be viewed under the microscope, and an electrical signal of the heart rate is recorded by placing the embryo between two electrodes. Figure 4.1 shows the overall experimental setup, excluding the Digital to Analog Converter (D/A) and computer.

![Figure 4.1: Experimental apparatus.](image)
The zebrafish was placed in the holding fixture between two electrodes. Because of the small signal size, noise was a large issue; therefore, the electrodes were soldered directly to two coaxial cables where the outer mesh is used as an electromagnetic shield. This helped to both simplify the system and remove any noise arising from electromagnetic phenomena. Figure 4.2 shows the overall block diagram of the system. The coaxial cables were connected to the Tektronix Differential Preamplifier (Tektronix ADA 400A, Beaverton, OR, USA) where the signal was amplified 100 times. The differential preamplifier also filtered out frequencies greater than 100 Hz, which assisted in reducing noise interference. The preamplifier was then connected to the Tekprobe Power Supply (Tektronix 1103, Beaverton, OR, USA), which was needed to adapt the preamplifier to the remainder of the system. The Tekprobe Power Supply was then connected to both an oscilloscope and an Allios Digital to Analog Converter (D/A) (MIT BioInstrumentation Lab), which along with a Visual Basic Program recorded the data to the computer at a sampling frequency of 200 Hz throughout the experiments.

For the visual recording of the heartbeat, the zebrafish was viewed under the microscope (Zeiss Stemi SV 11, West Germany) at 66x magnification, and each heartbeat was manually tapped out using a Morse code practice oscillator (CPO). A DC power supply (Hewlett Packard E3631 Triple Output DC Power Supply) was input into the Morse CPO unit, and the CPO was connected to both the oscilloscope and the D/A where the results were recorded to the computer. Section 4.3 provides additional information on the Morse code apparatus.
The expected signal size of the zebrafish heart rate is small, around 50 μV at a frequency of 1-3 Hz. This prediction was based on previous experiments and the heart rate signals of other embryos, such as a water flea, which has approximately a 30 μV signal size (Hunter and MacCrae, personal communication).

A variety of electrodes including copper, gold, and stainless steel were tested to determine which would be the best option. The final result was platinum iridium wire (Alpha Aesar #10056, 90:10 wt %) for several reasons. Platinum iridium wire has excellent conductivity, it is less likely to erode than tungsten or stainless steel, and it is also biocompatible.

4.2. Zebrafish Embryo Holding Fixture

The zebrafish embryo holding fixture was manufactured from a small piece of delrin (acetal). The dimensions of the fixture were not critical; therefore, all dimensions mentioned hereafter are approximations. The figure below shows a three-dimensional drawing of the holding fixture. The overall delrin block was 25 × 10 × 15 mm. A 2.5 mm diameter hole was drilled approximately 4 mm deep through the top of the delrin. This hole was where embryo was placed during experimentation. Two holes of diameter 0.75 mm were drilled perpendicular on either side of the larger hole, as shown in Figure 3.3. The electrodes were placed through these smaller holes where they were in contact with the embryo to measure the heart rate.

![Diagram of zebrafish embryo holding fixture.](image-url)
The platinum iridium wires were connected to coaxial cables as shown in Figure 4.4. They were directly soldered to the cables in an effort to decrease noise interference by reducing exposed wires and unnecessary cable connections.

![Figure 4.4: Zebrafish embryo holding fixture and connecting cables.](image)

For testing purposes, two additional electrode holes of diameter 0.6 mm were drilled at a 45° angle to the holding fixture face as shown in Figure 4.5. These holes were placed in the holding fixture for two reasons. The electrodes placed in these holes were to simulate a potential difference similar to the zebrafish heartbeat (e.g. a 50 μV signal). It also provided the possibility to stimulate the zebrafish electrically in future experiments.

![Figure 4.5: Modified zebrafish embryo holding fixture.](image)
4.3. **Morse Code Apparatus**

A Morse code practice oscillator (CPO), as shown in Figure 4.6, was used to manually tap out each heartbeat as viewed through the microscope. When a heartbeat was visually detected, the CPO switch was simultaneously pressed down creating a peak in the data where the heartbeats were occurring. The DC power supply connected to the Morse CPO unit was set at one volt. When the CPO was closed or the switch was pressed down, the circuit would be completed causing a one-volt signal to appear on the oscilloscope screen. When open or the switch was not pressed down, the signal would be at zero volts.

![Figure 4. 6: Morse Code Practice Oscillator (CPO).](image)

The graph below shows a typical set of ‘Morse code’ data a range of ten seconds.

![Figure 4. 7: ‘Morse code’ data.](image)
5. System Characterization

A variety of tests were performed to determine various characteristics of the system. The influence of noise on various components of the system was the first set of tests. The next set of experiments was to ensure that the system setup could detect a 50 μV signal, which was the predicted signal size of the zebrafish embryo heart rate. Because the electrodes were not placed directly on the zebrafish heart, it was necessary to determine if the electrodes could detect a potential voltage signal. Therefore, a test to simulating a potential difference test was executed. In addition, the Mathcad algorithms were also investigated for their reliability and accuracy.

The Hewlett Packard 3245A Universal Source was used to create a 50 μV DC signal, a 10 μV DC signal, or a 100 mV sinusoidal signal. This signal was input into the differential preamplifier and was amplified 100 times. The tests were performed by manually adjusting the input signal using the HP Universal Source. Figure 5.1 shows a block diagram of the equipment set up for the following tests.

![Figure 5.1: Block diagram for system verification tests.](image-url)
5.1. **System Noise Characterization**

5.1.1. Digital to Analog Converter Detection

The DC input signal from the HP Universal Source was 50 μV and amplified by 100 times; therefore, it was necessary for the D/A to detect a 5 mV signal. For this test, a DC input was directly connected to the preamplifier. Figure 5.2 shows a clear jump when the signal was varied from 0 to 50 μV proving that the D/A could detect such a small signal.

![Figure 5.2: D/A verification of a 5 mV signal detection.](image)

5.1.2. Noise Added by the Oscilloscope

In the second test, the oscilloscope was then placed into the system to determine if it was contributing noise. The differential amplifier was connected to both the oscilloscope and the D/A using a T-connector. As shown in Figure 5.3, the data was similar to that in the previous test. The jump was still evident; therefore, the oscilloscope did not add any additional noise.

![Figure 5.3: Oscilloscope noise factor.](image)
5.1.3. Holding Fixture Noise

The next test was to see how much noise the zebrafish embryo holding fixture was adding to the signal. The holding fixture was filled with the embryonic salt solution and was connected to the preamplifier without a DC input signal. It was found that it contributed ± 5 mV of noise as shown in Figure 5.4. The noise density level was found to be ± 0.354 V/√Hz.

![Figure 5.4: Holding fixture noise.](image)

5.2. System Detection without Mathcad

5.2.1. Fifty μV DC Step

To verify that the system could detect a 50 μV signal, the holding fixture was filled with the embryonic salt solution and was connected to the preamplifier. A varying input signal of 50 μV was applied directly to the electrode, amplified 100 times by the differential preamplifier, and finally, recorded by the D/A. The signal jump was apparent as shown in Figure 5.5; therefore, it was confirmed that the system could measure a 50 μV signal.

![Figure 5.5: Holding fixture with signal.](image)
5.2.2. Ten μV DC Step

The signal size of the zebrafish heart rate was predicted to be around 50 μV, but if the signal size were smaller, the system would need to be able to measure it. For that reason, tests were performed to determine the smallest signal that the system could detect. The tests were similar to the ones performed for the system verification only the voltage input changed. Figure 5.6 shows a 10 μV signal amplified 100 times to 1 mV. There is a slight change in the signal amplitude; however, it is obscured by noise. This shows that a 10 μV is the lower limit on signal size detection.

![Figure 5.6: Ten μV signal.](image)

5.2.3. Potential Difference

Figure 4.5 depicts the holding fixture for the potential difference tests. Platinum iridium wires were placed in the 45° angle holes. The electrodes placed in these holes were to simulate a potential difference similar to the zebrafish heartbeat (e.g. a 50 μV signal). The wires were situated approximately 1 mm apart. A 100 mV AC signal with a frequency of 3 Hz was input to the wires, thus creating a potential field. The other two electrodes picked up the signal and it was amplified by 100 times through the differential preamplifier, and the data was recorded to the computer. Although the signal was amplified 100 times, the potential loss through the solution essential eliminated the amplification and allowed for a 180 mV signal detection, as shown in Figure 5.7.

![Figure 5.7: Potential difference test.](image)
5.3. **System Detection with Mathcad Digital Lowpass Filter**

The 10 μV signal shown in Figure 5.6 has a slight amplitude change when the DC input voltage was varied from 0 to 50 μV. The signal becomes more evident with the aid of a digital lowpass filter. Mathcad was used to smooth the data and filter out frequencies greater than 10 Hz, and it made the signal more identifiable as shown in Figure 5.14 (refer to Appendix B for more Mathcad information).

![Figure 5.14: Ten μV signal with digital filter.](image)

Tests were performed to ensure that the digital filter was working correctly. The data taken from the potential difference test (refer to Figure 5.7), was run through the Mathcad digital filter that eliminates frequencies greater than 10 Hz. Figure 5.15 is the frequency plot determined from the data. As shown in the figure below, there is a peak at 3 Hz, which was the data input frequency, and there was a drop at the 10 Hz mark indicating that the filter was working correctly.

![Figure 5.15: Frequency plot.](image)
5.4. Mathcad Algorithm Verification

To ensure that the Mathcad operations were working successfully, two sets of 'Morse code' data were entered into the program. One set of 'Morse code' data had a varying 0 and 50 µV signal generated from the HP Universal Source. This set of data simulated the zebrafish embryo heart rate. The Morse CPO device was not used because it was found to contribute too much noise at small signals. The zebrafish-simulated signal was manually changed from 0 to 50 µV, and then it was amplified through the differential preamplifier, while D/A and the computer recorded the data.

For reasons of consistency, the other set of 'Morse code' data was also generated through the HP Universal Source. The Universal Source was directly connected to the D/A, and the signal was set at one volt to simulate the actual setup of manually tapping out the heart rate using the Morse CPO unit.

The following diagrams show the two sets of data. Figure 5.8 shows the 50 µV signal, and Figure 5.9 is the one-volt signal generated from the HP Universal Source.

![Figure 5.8: 50 µV signal (simulated fish heartbeat).](image_url)

![Figure 5.9: One-volt signal (simulated 'Morse code' signal).](image_url)
To eliminate some of the noise in the 50 μV signal, data was first smoothed, and frequencies greater than 10 Hz were eliminated using a Mathcad digital filter. Smoothing the data entails averaging the voltage over 91 points. Both sets of data were then reduced to 7 seconds of data in order to make the computer computation faster. Figure 5.10 shows the reduced data sets: A) the filtered 50 μV signal and B) the one-volt signal.

![Figure 5.10: A) Filtered 50 μV signal. B) One volt signal with 0 mean.](image)

The data were then correlated, the Toeplitz matrix was found, and the transfer function, \( h \), was determined using Equations 1-5. The transfer function that resulted is shown in the figure below.

![Figure 5.11: Computed transfer function, \( h \).](image)
The original smoothed and filtered 50 μV signal along with the transfer function, \( h \), were entered into Equation 5, and the output, \( y_{est} \), was found from the convolution of those two elements. The diagram of this convolution is shown in the figure below. Figure 5.12 possesses the same basic shape of the one volt ‘Morse code’ data shown in Figure 5.10B (Note ±0.5 V step signal). This similarity verifies that the correlations are performing the correct operations. The noisiness of \( y_{est} \) comes from the fact that the data sets used were quite small.

![Figure 5.12: Output response from filtered 50 μV signal with signal smoothing.](image)

5.5. **Summary**

The experimental setup and the Mathcad operations were analyzed to determine the characteristics of both systems. A variety of tests were performed to determine the influence of noise on the electrical system. It was found that the oscilloscope was not a contributing factor. The holding fixture was bringing ±5 mV of noise into the system and the noise density level was found to be ± 0.354 V/√Hz. The system can easily detect a 50 μV signal and can measure a signal down to 10 μV with the aid of a digital filter. It was also determined that the electrodes can detect a potential voltage signal. A 100 mV AC signal with a frequency of 3 Hz was input into the electrodes, thus creating a potential field. The electrical system detected this signal; however, a potential loss occurred through the solution medium. In addition, the Mathcad algorithms were investigated for verification. It was found that they were operating correctly.
6. Measurement of Zebrafish Heart Rate

6.1. Procedure

The zebrafish embryo heart rate was measured through two platinum iridium wires as described in Section 4. To eliminate any unnecessary noise, all excessive connections were removed from the system. First, the embryo was placed in the holding fixture between the electrodes. Then the embryo was viewed under the microscope, and the Morse CPO device was pressed down each time the heart beat. Both the voltage and ‘Morse code’ signals were sent to the Data Acquisition Card. The D/A transmitted both sets of data to a computer where the data were recorded at a sampling frequency of 200 Hz during the length of the experiment, which was approximately 30 seconds. A Mathcad program then processed the data where the voltage data were manipulated through digital filtering, smoothing, and correlating as described in Section 3. A transfer function relating the noisy signal and the ‘Morse code’ data was determined and was then applied to other data sets to acquire the zebrafish embryo heart rate signal.

6.2. Results

To determine the electrical signal of the zebrafish heart rate, it was necessary to collect two sets of data from the same embryo. The first set was used to determine the filter transfer function. That transfer function was then convolved with the second set of data to find the zebrafish heart rate signal, as discussed in Section 3.

6.2.1. Determining the Transfer Function

The first set of data includes the noisy voltage data shown in Figure 6.1, and the ‘Morse code’ data that was tapped out for each heartbeat, as displayed in Figure 6.2. By inspection of the ‘Morse code’ data, the frequency of the heartbeat was found to be 1.3 Hz.

![Figure 6.1: Raw data from the embryo cell.](image-url)
The noisy data were smoothed as described in Section 5.4 and frequencies greater than 10 Hz were filtered out of the signal. Figure 6.3 shows the smoothed and filtered data, and it appears that there is a correlation with the peaks of the ‘Morse code’ data in Figure 6.2.
The transfer function between the noisy signal and the ‘Morse code’ data were found via Equation 4 and resulted in the following graph.

![Graph showing the transfer function](image)

**Figure 6.4:** Transfer function calculated between the noisy signal and the ‘Morse code’ data.

### 6.2.2. Verifying the Transfer Function

To ensure that the transfer function was operating correctly, a convolution was performed between the smoothed and filtered data, as shown in Figure 6.3, and the transfer function, $h$, in Figure 6.4. The result of this convolution is shown in Figure 6.5. Inspection of the graph shows definite peaks similar to the ‘Morse code’ data shown in Figure 6.2, verifying that the transfer function is working correctly.

![Graph showing the verified transfer function](image)

**Figure 6.5:** Verifying the transfer function.
6.2.3. Application using Second Data Set

The second set of heartbeat data were recorded, and then the raw data was smoothed and filtered. The result of these operations is shown in the figure below.

![Second smoothed and filtered data set.](image)

Figure 6.6: Second smoothed and filtered data set.

The data shown in Figure 6.6 were convolved with the previously calculated transfer function, \( h \), shown in Figure 6.4. The following graph shows the zebrafish heart rate signal after the Mathcad data manipulation.

![Zebrafish heart rate signal after data manipulation.](image)

Figure 6.7: Zebrafish heart rate signal after data manipulation.
'Morse code' data were also simultaneously collected with the second set of noisy data. The 'Morse code' data are displayed in the figure below for comparison with the manipulated data shown in Figure 6.7. It appears that there is a correlation between the visually determined 'Morse code' signal and the manipulated electrical signal.

A FFT (Fast Fourier Transform) algorithm was applied to the estimated heart rate data as shown in Figure 6.6 and a peak at 1.3 Hz was found. Inspection of the Morse data in Figure 6.7 shows a heart rate frequency of the same value, which supported the correlation between the heart rate signal and the 'Morse code' data.
6.3. Discussion

6.3.1. Sources of Error

It was demonstrated in Section 5.2.3 that the system can detect a potential field of 100 mV. Therefore, it is believed that the source of error does not lie in the instrumentation but arise from an electrochemical phenomenon. The potential loss through the medium can vary based on salt concentration in the solution and solution stirring, which changes the ionic concentration gradient (Na⁺, Cl⁻) around the electrodes. This means that a single movement of the fish disturbs these concentration gradients resulting in a potential signal error.

There were many uncertainties in the data acquisition process. The human influence in the 'Morse code' data certainly brought in a source of error to the system. The Mathcad algorithms were subject to human influence because the transform function relating the noisy and 'Morse code' data was manually 'tapped out', which allowed for discrepancies in the data manipulation process. While Figure 6.7 appears to show a correlation between the 'Morse code' data and the manipulated data, it is possible that the Mathcad algorithms distorted the data.

6.4. Suggestions for Improvement

Noise was a large problem in measuring the zebrafish embryo's heart rate. Because the signal was so small, it was lost within the outside noise arising from various sources, such as the 60 Hz signal due to the lights. Performing these experiments in a controlled environment to eliminate as much outside noise as possible would assist in obtaining more accurate results.

The electrodes were placed externally on the embryo; therefore, there was a potential loss through the solution medium, which made the signal difficult to detect. The possibility of placing the electrodes directly on the heart may have rendered better results, but this would have run the risk of killing the embryo.

Future versions of this setup should include higher amplification and better noise elimination. The current setup amplifies the signal by 100 times and eliminates frequencies greater than 100 Hz. However, having a larger amplification and better noise elimination would make it easier to detect the small heart rate signal.

6.5. Conclusions

The creation of a method for zebrafish heart rate detection has the potential to greatly enhance the understanding of cardiological genetic mutations. This technique of simultaneously visually and electrically measuring the heart rate could provide information on the regularity and rhythm of the beat. Further work should be done to improve the system with reduced noise and higher amplification in order to obtain more straightforward results.
References


Appendix A: Stages of Development

A.1. The Zygote period (0 – ¼ hour)

The first cell cycle of the embryo is the zygote period. The newly fertilized egg is in the zygote period until the first cleavage occurs (Appendix A, Figure 1), about 40 minutes after fertilization. The zygote is about 0.7 mm in diameter at the time of fertilization.

![Figure A1: The Zygote period. A: the zygote a few minutes after fertilization. B: The zygote about 10 min after fertilization. Scale bar: 250 μm (taken from Edwards, http://zfin.org).]

A.2. The Cleavage Period (0.7- 2.2 h)

The embryo begins its division in the cleavage period (Figure A2-A). The cells divide symmetrically at 15-minute intervals. The rapid developing time allows for active viewing of the division stages.

A.3. The Blastula Period (2 1/4 - 5 1/4 h)

The blastula period encompasses the late divisions of cleavage and continues on until the beginning stages of gastrula. During the early stages of blastoderm, the cells continue to divide rapidly at 15-minute intervals. The blastomeres lie against the yolk and remain cytoplasmically connected to it throughout cleavage. During the blastula stage, these cells release their cytoplasm and nuclei together into the immediately adjoining cytoplasm of the yolk cell, producing the yolk syncytial layer. Beginning in the late blastula stage, the yolk syncytial layer and the blastodisc spread over the yolk cell in a process known as epiboly, see Figure A2-B.

A.4. The Gastrula Period (5 1/4 - 10 h)

The appearance of the germ layer (see Figure A2-C) marks the beginning of the gastrula period. This occurs at 50%-epiboly. As a consequence, within minutes of reaching 50%-epiboly a thickened marginal region termed the germ rim appears around the blastoderm rim. The first occurrence of differentiating cells transpires in the gastrula. The older cells proceed to form the spinal cord, brain, and other organs close to the axial midline of the embryo, as shown in Figures A2-C & D.
A.5. The Segmentation Period (10-24 h)

Body parts develop and become identifiable, as shown in Figures A2-E & F. The tail bud becomes more prominent and embryo elongates. The eye and ear appear, and the segmentation of the brain is evident. At the completion of the segmentation period there are between 8,000 and 10,000 cells and the major systems of the embryo are laid out. The embryos are touch sensitive and their hearts start beating.

A.6. The Pharyngula Period (24-48 h)

The heart is first visible as a cone-shaped tube attached at the base of the brain (refer to Figure A3). The heart begins to beat just prior to this stage. At first there is no apparent direction to the beat, and the rhythm may be interrupted. The embryo exhibits spontaneous side-to-side contractions involving the trunk and tail, pigmentation becomes prominent in the eye, and blood cells begin to occupy the yolk ball.

A.7 The Hatching Period (48-72 h)

During the hatching period, the embryo continues to grow at about the same rate as in the previous period. Development of most of the organ fundamentals is now mostly complete and, with some notable exceptions including the gut and its associated organs. Visually, it is easy to see the rapidly developing rudiments of the pectoral fins, the jaws, and the gills (Figure A3).

![Diagram of zebrafish development](image-url)

Figure A2: Overview of zebrafish development during the first 24 hours (taken from Haffter et al., 1996).
Figure A3: Embryos at 29 hours, 48 hours, and 5 days of development (taken from Haffter et al., 1996).
Appendix B: Mathcad Algorithms

Data taken from the text file and places them into matrices

```mathcad
raw_data := D:\..\fish8a.txt
morse_data := D:\..\fish8a.txt
time_data := D:\..\fish8a.txt
```

Sets the time data to start at zero
```
time_data := time_data - time_data_0
```
```
x := raw_data
```
Graphs of the noisy voltage data and the morse code data

Redefine the data variables
```
x := raw_data          y := morse_data          t := time_data
```
Smoothing the Data

Smoothing takes the average voltage reading over the span of 91 points

\[ i := 0.. \text{last}(x) \]
\[ \mu_x := \text{mean}(x) \quad \mu_y := \text{mean}(y) \]
\[ \sigma_x := \text{Stdev}(x) \quad \sigma_y := \text{Stdev}(y) \]
\[ x := x - \mu_x \quad y := y - \mu_y \]
\[ \text{mean}(x) = 0 \quad \text{mean}(y) = 0 \]

\[ \Delta t := 0.005 \]

![Graph of x and y plots](image)

\[ x := \text{medsmooth}(x, 91) \]

Smoothed data

![Graph of smoothed data](image)
\[ m := 1400 \] This value can be changed to make the computation go faster
\[ n := 500 \] starting point in computing

Places all the data into proper length matrices for calculations

\[
x := \text{submatrix}(x, n, m+n, 0, 0) \quad y := \text{submatrix}(y, n, m+n, 0, 0) \quad t := \text{submatrix}(t, n, m+n, 0, 0)
\]

Removing the DC component by subtracting the mean from the data

Digital Filter Filter out frequencies greater than 10 Hz

Set the cutoff frequency and number of coefficients.
\[
f := 0.05 \quad N := 200
\]

Calculate the coefficients with rectangular and Blackman windows.

\[
\text{filter} := \text{lowpass}(f, N) \quad \text{AfterFilter} := \text{convol}(x, \text{filter}) \quad \text{AfterFilterb} := \text{Re}(\text{AfterFilter})
\]
Produce Frequency Plot

Caf := cfft(AfterFilterb)

\[ j := 0 \ldots \frac{N}{2} \quad N = 200 \]
\[ N := \text{last(Caf)} \]

Magnitude \( j := \text{endtime} \frac{|\text{Caf}|_j}{\sqrt{N}} \)

\[ s := \text{last}(t) \quad s = 1.4 \times 10^3 \]
\[ \text{endtime} := t_s \quad \text{endtime} = 9.5 \]

Frequency \( j := \frac{j}{\text{endtime}} \)

Correlations

Use normalized covariance function lcorr

Auto correlation

\[ cxx := \text{lcorr}(x, x) \]

\[ cyy := \text{lcorr}(y, y) \]
Cross correlation

cxy := Icorr(x, y)

Calculation of the transfer function, h

\[ C_{x,j,k} := c_{x,j-k} \]

Forming the Toeplitz matrix

\[ \text{inv}C_{xx} := C_{xx}^{-1} \]

Inverse of the Toeplitz matrix

\[ h := \frac{1}{\Delta t} (\text{inv}C_{xx}c_{xy}) \]

Transfer Function
Found from the convolution of the input data and the transfer function

\[ y_{est} := \text{convol}(h, x) \]

Second data set convolved with the transfer function