Time-frequency distribution analysis of heart rate and blood velocity variabilities in stage 24/34 chick embryos

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Time-Frequency Distribution Analysis of Heart Rate and Blood Velocity Variabilities in Stage 24/34 Chick Embryos

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the

MASTER OF SCIENCE in Mechanical Engineering

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Andrew J. Bonacci

Date
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Abstract

Time-frequency distribution (TFD) analysis is a relatively new process for decomposing a complex signal to understand its spectral content. Traditional signal spectral analysis examines both temporal and spectral contents distinctly. This method of analysis is suitable for signals where the spectral content is stationary and time-invariant. However, many naturally occurring signals are not only multicomponent, but are also highly time-variable, such as speech, heart rate variability, and other biological signals. Typically, the Fourier transform exposes sinusoidal frequencies present in a signal. It cannot, however, tell when these frequencies existed temporally. This is where time-frequency analysis excels over traditional spectral processing techniques.

Time-frequency analysis allows the spectral content of the signal to be determined as well as when these frequency components occurred. The process can be thought of as time dependent Fourier analysis. The following thesis explores the effectiveness of time-frequency analysis for examining heart rate and blood velocity variability of dorsal aortic blood flow in developing chick embryos. These hemodynamic data series are used to assess embryonic cardiovascular function.

It is hoped that this thesis aids in the creation of clinical tools for the early identification of functional heart defects in a developing human fetus. These heart defects can lead to serious heart disease later in life. Clinical treatments of morphological and functional heart defects are possible if they can be identified during early embryo/fetal development.

The time-frequency analysis performed, utilized the binomial distribution with a Hanning window as the input parameters. Through the use of Discrete Time Frequency Laboratory (DTFL™) software, TFD analysis appears to be an effective tool for functional assessment of cardiovascular health during development.
## Table of Contents

<table>
<thead>
<tr>
<th>Chapter 1. Introduction</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Overview</td>
<td>1</td>
</tr>
<tr>
<td>1.2. Physiologic Background</td>
<td>2</td>
</tr>
<tr>
<td>1.3. Technical/Application Background</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2. Methods</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Overview of Data Acquisition / Analysis</td>
<td>9</td>
</tr>
<tr>
<td>2.1.1. Rotterdam - Raw Data Acquisition</td>
<td>9</td>
</tr>
<tr>
<td>2.1.2. Rochester - Data Analysis</td>
<td>10</td>
</tr>
<tr>
<td>2.2. Time Series Analysis by TFD (Mathematics)</td>
<td>11</td>
</tr>
<tr>
<td>2.3. Time Series Analysis Test Applications</td>
<td>14</td>
</tr>
<tr>
<td>2.3.1. Test Cases</td>
<td>14</td>
</tr>
<tr>
<td>2.3.2. Example Output</td>
<td>16</td>
</tr>
<tr>
<td>2.3.3. Single Tone Test Signal</td>
<td>18</td>
</tr>
<tr>
<td>2.3.4. Dual Tone Test Signal</td>
<td>18</td>
</tr>
<tr>
<td>2.3.5. Spectral Chirp Test Signal</td>
<td>22</td>
</tr>
<tr>
<td>2.3.6. Frequency Hopper Test Signal</td>
<td>24</td>
</tr>
<tr>
<td>2.4. DTFL™ procedures</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 3. Results</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Stage 24</td>
<td>28</td>
</tr>
<tr>
<td>3.1.1. Controls</td>
<td>29</td>
</tr>
<tr>
<td>3.1.2. RA Treated</td>
<td>29</td>
</tr>
<tr>
<td>3.2. Stage 34</td>
<td>37</td>
</tr>
<tr>
<td>3.2.1. Controls</td>
<td>37</td>
</tr>
<tr>
<td>3.2.2. RA Treated / Normal</td>
<td>37</td>
</tr>
<tr>
<td>3.2.3. RA Treated / Abnormal</td>
<td>44</td>
</tr>
<tr>
<td>3.2.4. RA Treated / DORV</td>
<td>44</td>
</tr>
<tr>
<td>3.3. Comparative Summary</td>
<td>51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 4. Discussion</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Various TFD Pros and Cons</td>
<td>53</td>
</tr>
<tr>
<td>4.2. Windowing Pros and Cons</td>
<td>54</td>
</tr>
<tr>
<td>4.3. Limitations and Restrictions</td>
<td>56</td>
</tr>
<tr>
<td>4.4. Discussion of Data Trends</td>
<td>57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 5. Conclusion</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>63</td>
</tr>
<tr>
<td>Appendix A</td>
<td>65</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

Chapter 1.1 Overview

Time-frequency distribution analysis is a relatively new process for decomposing a complex signal to understand its spectral content. Traditional signal spectral analysis examines both temporal and spectral contents distinctly. This method of analysis is suitable for signals where the spectral content is stationary and time-invariant. However, many naturally occurring signals are not only multicomponent, but are also highly time-variable, such as speech, heart rate variability, and other biological signals. De-coupled time and frequency analyses are not sufficient to completely describe what is occurring in these signals. Typically, the Fourier transform exposes sinusoidal frequencies present in a signal. It cannot, however, tell when these frequencies existed temporally. This is where time-frequency analysis excels over traditional spectral processing techniques.

Time-frequency analysis allows the spectral content of the signal to be determined as well as when these frequency components occurred. The process can be thought of as time dependent Fourier analysis. The following thesis explores the effectiveness of time-frequency analysis for examining heart rate and blood velocity variability in developing chick embryos.

It is hoped that this thesis aids in the creation of clinical tools for the early identification of functional heart defects in the developing human fetus. These heart defects can lead to serious heart disease later in life. Clinical treatments of morphological
and functional heart defects are possible if they can be identified during early embryo/fetal development.

The remaining sections of this introduction will explain the physiologic background of the research and the technical framework of the time-frequency distributions chosen for use in this investigation. Chapter 2 will describe the methods segment of the research, pertaining to data acquisition, data processing and test applications of generated time series. The results are contained in Chapter 3 along with a comparative summary of embryonic research data. The data was acquired from chick embryos that were four and one half, and eight days old, respectively, of a 21-day incubation period. These periods correspond to stages 24 and 34, respectively, as defined by the Hamburger-Hamilton system of embryonic development (Hamburger, 1951). Chapter 4 will discuss the pros and cons of the various time-frequency distributions and windows used. Also, information on limitations and restrictions and a discussion of the data trends will be presented in this chapter. Finally, Chapter 5 will conclude this thesis, examining the effectiveness of time-frequency analysis to the research data.

Chapter 1.2 Physiologic Background

Embryonic cardiovascular development is a biomechanical process, with the heart being the first functioning organ. Many congenital and adult onset cardiovascular diseases may develop within the early stages of embryonic development. Hence, it is crucial to determine the control mechanisms of cardiovascular function and growth.
The embryonic heart begins to function at 29 - 33 hours of incubation in the chick, and immediately provides circulatory support to the developing embryo (Sissman, 1970). The heart performs this action while morphologically changing from a tubular structure into the familiar four-chambered organ (Marieb, 1992). In the adult, hemodynamic control is regulated by the autonomic nervous system. However, the autonomic nervous system is not developed during early cardiovascular development, but hemodynamic function is still precisely controlled. Afterload modulation has been hypothesized as a control mechanism preceding autonomic nervous system development and control (Kempski, 1993). Afterload modulation refers to changes in vascular loading of the heart due to vascular feedback, or changes in vascular resistance to blood flow.

Heart rate and amplitude variability of dorsal aortic blood flow are the hemodynamic data series used in this thesis to assess embryonic cardiovascular function. Dorsal aortic blood flow waveforms are recorded from the chick embryo, and the desired data series are generated from custom built algorithms. An example of how these parameters are captured from the dorsal aortic blood flow waveforms is shown in Figure 1.1. Heart rate threshold crossing is the criterion used to measure heart rate variability (HRV). It represents the fluctuation between consecutive heartbeats and the variations of consecutive instantaneous heart rates of a blood flow signal over time (Malik, 1996). Mean velocity variability (MVV) and peak velocity variability (PVV) are associated with amplitude variability (AMPV) and are determined from the blood flow signal as the mean blood flow and peak blood flow, respectively, in each cardiac cycle.
Chick embryos are analyzed in this study because of their ease in experimental setup, maintenance, and observation. However, the analyzed data is directly relevant to the overall goal of early detection of human cardiovascular diseases. The chick embryos produce specific and reproducible cardiac malformations when affected by teratogens, such as all-trans retinoic acid, which allows analysis of hemodynamics and heart morphology.

Chapter 1.3 Technical Application Background

The natural (i.e., biological) signals of particular interest to this thesis are often complex and non-stationary. Stationary signals fluctuate over a constant mean and fluctuate with a constant variance (Bowerman, 1993). Non-stationary signals do not fit well into the realm of Fourier analysis. Signal properties such as amplitude, phase, and
frequency that change with time cannot be adequately represented by a single Fourier transform (Kempski, 1995). Therefore, time-dependent Fourier analysis is often used to transform the data into a stationary signal using a windowing function (the short-time Fourier transform, see below).

However, even with time-dependent Fourier analysis, the signal spectral content cannot be readily discerned in the time domain due to technical limitations of this technique. Time-frequency analysis using non-Fourier based techniques allows the identification of signal spectral content and how it is distributed over time. The primary objective of time-frequency analysis is to create a distribution function that will describe the energy density of the signal in both time and frequency, concurrently.

As an example of time-frequency analysis, if we define audio frequencies existing in a musical composition, we are using Fourier analysis. While this does provide much valuable information on overall frequency content, one could not accurately reconstruct the audio frequencies into the original music. However, if we can define the magnitude, relative temporal location and temporal duration of each frequency component, the composition can be reconstructed as "sheet music" and performed by a musician. The sheet music is in itself a time-frequency plot. Time is represented on the horizontal axis, in terms of the meter of the song. The vertical axis represents the pitch of the note, which correlates to the frequency of an audible tone.

The short-time Fourier transform, as mentioned previously, utilizes a window function to look at just a small piece of the signal. Neglecting the rest of the signal and focusing on just the section the window covers, the Fourier transform is performed to find
what frequencies exist over the window time course. The short-time Fourier spectrum is a sum total of the different Fourier transforms at each time and results in a time-frequency distribution (TFD). This time-frequency distribution is commonly called the "spectrogram." The spectrogram is a robust tool in time-frequency analysis, and the prototype for a TFD.

Time-frequency analysis is composed of many distributions, each having its own advantages and disadvantages. This thesis utilizes three different TFD's, with the first being the spectrogram mentioned above. The other two TFD's employed are the Wigner-Ville distribution and the Binomial distribution. Both are discussed within the remainder of this section. A brief overview of the mathematics contained in these TFD's is presented in Chapter 2.2. The current section is meant to be an introduction to the time-frequency theory used in this work.

The Wigner-Ville distribution was the first distribution introduced which was qualitatively different from the spectrogram, and became the prototype for a new generation of TFD's (Cohen, 1995). Wigner originally developed this distribution to calculate a quantum mechanics problem, with Ville deriving and applying this joint distribution to spectral processing. Essentially, the Wigner-Ville distribution is the multiplicative summation of the signal at an equal time spacing, into the past and future, from a specific time point. In other words, the time signal is folded over itself at a particular time, $t$. The Fourier transform is then taken with respect to the time spacing for all points of interest in the signal. Occasionally though, the folding and overlap will result in a non-zero Wigner-Ville distribution for a point where the signal does not exist, in
either the temporal or spectral domains. This strange phenomenon can cause incorrect interpretations of data, and are referred to as “cross-terms” or “interference” (Cohen, 1995).

These cross-term artifacts are created by the mathematical implementation of the Wigner-Ville distribution and are evident in the distribution results (See Chapter 2.3.4 for an example of cross-term artifacts.) Thus, many researchers have developed new distributions to combat cross-term distribution artifacts. Choi (Choi, 1989), Jeong (Jeong, 1992), Papandreou (Papandreou, 1993), Zhao (Zhao, 1990), and Keselbrener (Keselbrener, 1996) are only a handful of researchers who have introduced new distributions that are superior to the Wigner-Ville distribution and the spectrogram in cross-term suppression.

The Binomial distribution used in this thesis is part of a larger group of distributions called RID’s, or reduced interference distribution’s. The RID was advanced from the exponential distribution developed by Choi and Williams (Choi, 1989). The exponential distribution overcomes shortcomings contained in the Wigner-Ville distribution and the spectrogram. The spectrogram cannot simultaneously optimize both the time and frequency resolutions, and the Wigner distribution exhibits negative values and cross-terms (Choi, 1989, and Jeong, 1992). These negative properties can lead to the misinterpretation of the temporal-spectral array generated by means of the TFD. RID’s utilize filtering techniques that are essentially smoothing functions applied in the instantaneous autocorrelation domain (DTFL™ manual, 1995). These functions suppress
terms more likely to be cross-terms and enhance terms more likely to be auto-terms (i.e.,
the 'true' spectral information of a signal.)

Discrete Time Frequency Laboratory (DTFL™) was the primary software used in
the TFD calculations. The software is in beta release for the UNIX environment from the
University of Michigan, and was used on a Hewlett-Packard Model 715/80 workstation.

DTFL™ is a tool that was developed to support the exploration of complex time-frequency
analysis methods and runs as an add-on module to MATLAB®, the commercial matrix
operating software from The Mathworks, Inc.
Chapter 2. Methods

Chapter 2.1 Overview of Data Acquisition

Chapter 2.1.1 Rotterdam - Raw Data Acquisition

The study group of this research consisted of Hamburger-Hamilton Stage 24 and 34 chick embryos, divided into control, sham, and treated groups. As mentioned previously, these stages represent 4½ and 8 days, respectively, in a 21-day incubation period. Hamburger-Hamilton Stages 24 and 34 correspond to time periods of 30-32 days and 46-48 days, respectively, in human gestational development (Sissman, 1970). Colleagues in the Netherlands (Erasmus University and the University of Leiden) have found evidence of cardiac morphological defects due to adverse retinoic acid effects during development (Broekhuizen, 1992). Embryos were subject to 1 μg all-trans retinoic acid (RA) dosage (topical bolus) at Stage 15 (comparable to 27-29 days human development), then re-incubated until either Stage 24 or Stage 34 (Broekhuizen, 1995). Between Stage 15 and 34 the heart undergoes morphologic changes in which it transforms from a curved tube into a four chambered pump. The RA treatment consistently produces cardiac anatomical defects.

In this thesis, Stage 24 data sets included control and RA treated groups. Stage 34 also included control and RA treated groups, but the treated group was subcategorized into normal, abnormal, and double-outlet right ventricle (DORV) clusters, based on morphometric observation (Broekhuizen, 1995). These clusters refer to the physical defects in the chick embryo. The control embryos were allowed to develop normally.
Dorsal aortic blood velocity was measured using a 20 MHz directional pulsed Doppler velocimeter (University of Iowa; Broekhuizen, 1993). Blood velocity data was sampled at 3.33 millisecond intervals for 100 seconds (nominal), and stored to magnetic media (Bernoulli Disks, Iomega Corp.) in binary file format using DATAPAC II (RUN Technologies, Laguna Hills, CA) software prior to shipment to the Rochester Institute of Technology for analysis. Upon arrival at the Rochester Institute of Technology, DATAPAC II software is used to edit and translate the data series to ASCII format for subsequent LabVIEW* (National Instruments, Inc., Austin TX) and DTFL™ analysis, as described below.

Chapter 2.1.2 Rochester - Data Analysis

A custom LabVIEW* virtual instrument first maps the chick embryo blood velocity from ASCII to LabVIEW* SGL format. DopW® software (developed at the Rochester Institute of Technology), written using LabVIEW* was used to compute heart rate variability and blood velocity amplitude variability time series from the pulse velocity data. The heart rate variability is determined using a velocity threshold crossing methodology. Here, a user-defined threshold allows for heart rate identification through the rising edge of the velocity waveform. The time at which the signal crosses the threshold is recorded for each cardiac cycle (See Figure 1.1). The time interval between crossings is computed and inverted to generate an instantaneous heart-rate (IHR) time series (also referred to as a “Beat series”). The mean HR is computed and subtracted from the IHR series to yield the variability in HR about its mean value.
The amplitude variability is divided into two subgroups, the mean velocity variability and the peak velocity variability. The mean velocity (MV) time series is determined from the original blood velocity waveform by computing the mean velocity in each cardiac cycle, as defined by successive threshold crossings (See Figure 1.1). The average of the mean velocity values is computed and then subtracted from the MV time series to define MVV, or the variability in mean velocity about its average value. The peak velocity (PV) time series is obtained by determining the peak velocity in each cardiac cycle. The average peak velocity is computed as the mean of successive PV values. PVV is determined by subtracting the average peak velocity from the PV time series. Hence, the PVV time series represents the variance in PV about its average value.

**Chapter 2.2 Time Series Analysis by TFD (Mathematics)**

The purpose of time-frequency analysis is to understand how the spectral content of a specific signal changes over time. The following description will aid in understanding the physical and mathematical ideas needed to interpret time-varying spectra. The subsequent mathematical presentation is meant to serve as an introduction to time-frequency analysis. Detailed presentations of time-frequency analysis can be found in related references listed at the end of this thesis.

Signals are the main focus of spectral processing and TFD analysis. Signals can be defined as a variation of a quantity in time, for example an electric field, pressure, or flow (Amin, 1995). Generally, signals are represented in complex form:
\[ s(t) = A(t)e^{j\omega(t)} = \text{Re} + j(\text{Im}) \]  \hspace{1cm} (2.1)

for ease of expression and simplicity.

The frequency description of a signal:

\[ S(\omega) = B(\omega)e^{j\phi(\omega)} \]  \hspace{1cm} (2.2)

is obtained from the Fourier transform of \( s(t) \). The Fourier transform of a signal is given by:

\[ S(\omega) = \frac{1}{\sqrt{2\pi}} \int s(t)e^{-j\omega t} dt, \]  \hspace{1cm} (2.3)

and aids in our physical understanding of the signal. In some instances, complex time domain signals will be easily distinguished in the frequency domain. In this thesis, the "analytic signal" was used in the DTFL computations. Real signals have a symmetrical power spectrum about zero frequency (i.e., DC). Through Fourier analysis of a real signal with a symmetrical frequency density, the average frequency is zero and the bandwidth is the distance between the positive and negative reflection components. The analytic signal redefines the real signal, producing only the positive half of the frequency spectrum. This produces a more accurate representation of the average frequency and bandwidth, and better reflects the physical situation presented by the real signal.

All time-frequency distributions belong to Cohen's class, represented by:

\[ C(t, \omega) = \frac{1}{4\pi^2} \iiint s^*(u - \frac{1}{2} \tau)s(u + \frac{1}{2} \tau)e^{-j(\omega - \omega_0 + \omega_0)} du d\theta, \]  \hspace{1cm} (2.4)

---

1 All limits of integration for those not given are from \(-\infty\) to \(+\infty\).
with the integration limits ranging from $-\infty$ to $\infty$ (Cohen, 1995). Here, $\phi(\theta, \tau)$ represents the kernel of the function, which essentially determines the TFD and its properties. A TFD is easily generated using a given kernel. An example of some TFD's and their kernels are shown in Table 2.1.

Table 2.1: Some Distributions and Their Kernels

<table>
<thead>
<tr>
<th>Name</th>
<th>Kernel: $\phi(\theta, \tau)$</th>
<th>Distribution: $C(\omega, \alpha)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wigner-Ville</td>
<td>$1$</td>
<td>$\int e^{-j2\pi\omega t^<em>} s^</em>(t - \frac{1}{2} \tau) s(t + \frac{1}{2} \tau) dt$</td>
</tr>
<tr>
<td>Wigner-Ville</td>
<td>$h(\tau)$</td>
<td>$\int e^{-j2\pi\omega t^<em>} h(\tau) s^</em>(t - \frac{1}{2} \tau) s(t + \frac{1}{2} \tau) dt$</td>
</tr>
<tr>
<td>(windowed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margenau-Hill</td>
<td>$\cos \pi \omega t^*$</td>
<td>$\text{Real } s(t) S^*(\omega)e^{j2\pi\omega t}$</td>
</tr>
<tr>
<td>Kirkwood-Rihaczek</td>
<td>$e^{j\pi \omega t^*}$</td>
<td>$-\frac{1}{\pi} \int \frac{e^{-j2\pi\omega t^<em>}}{-j\omega} dt^</em></td>
</tr>
<tr>
<td>sinc</td>
<td>$\sin 2\pi \omega t^* / 2\pi \omega t^*$</td>
<td></td>
</tr>
<tr>
<td>Page</td>
<td>$e^{j\pi</td>
<td>\tau</td>
</tr>
<tr>
<td>Choi-Williams</td>
<td>$e^{-\frac{j2\pi^2}{\sigma}}$</td>
<td>$\int \sqrt{\frac{\sigma}{\pi}} e^{-\sigma(u-\tau)^2 / 2\pi^2} du d\tau$</td>
</tr>
<tr>
<td>Spectrogram</td>
<td>$h^* (u - \frac{1}{2} \tau) e^{-j2\pi\omega t^*} h(u + \frac{1}{2} \tau) du$</td>
<td>$\left</td>
</tr>
<tr>
<td>Zhao-Atlas-Marks</td>
<td>$\frac{\delta_1 (\tau) \sin (2\pi \omega</td>
<td>\tau</td>
</tr>
</tbody>
</table>

$^2$ Adapted from Time-Frequency Signal Analysis, 1992; B. Boashash, ed.
As mentioned previously, the Wigner-Ville and Binomial (RID) distributions, and the spectrogram were used in this thesis, and are all members of Cohen's general class of time-frequency distributions.

Chapter 2.3 Time Series Analysis Test Application

Chapter 2.3.1 Test Cases

In order to validate the performance of DTFL™, test signals were created using MATLAB® and a Hewlett-Packard 15 MHz Function/Arbitrary Waveform Generator (Model 33120A). Four distinct types of test signals were used. Each test signal was run through DTFL™, and its output verified using programs written in LabVIEW® and MATLAB® (See Appendix A). Before presenting the output for the test cases, the following graphical glossary (See Figure 2.1) is presented to aid in the understanding of time-frequency analysis. This graphical glossary depicts the output that DTFL™ creates.

The labeling convention used for the axes is taken from the DTFL™ software manual (DTFL™ manual, 1995). However, for our purposes a non-sequential explanation of these labels is presented. Axis C on all of the following DTFL™ plots represents the real time series data, as read into DTFL™. The histogram to its left is a statistical frequency plot of the spread of the data. It can be used to describe the data in either Axis B or Axis C. For all of the processed chick data (not including the test cases) used in this thesis, the histogram describes the content of the time series depicted on Axis C. If the histogram

---

3 LabVIEW® and MATLAB® programs courtesy of Shrikant Kalegaonkar.
generally shows a normal distribution of data value versus the number of value occurrences, we may conclude that the data being analyzed is non-random.

**Figure 2.1: DTFL™ Graphical Glossary**

Axis B shows the absolute analytic FFT of the time signal. The analytic signal is formed from the real signal through a Hilbert transform. The analytic FFT is used in the analysis because it excludes the negative frequency components of the signal, which could contribute to the formation of cross-terms (DTFL™ manual, 1995). Axis A portrays the resultant TFD of the analytic signal. It is essentially a contour plot with the contour
variable being spectral energy density. The magnitude of the energy density is given by
the colormap located at the very top of the output.

Chapter 2.3.2 Example Output

An example output plot is shown in Figure 2.2 to introduce the reader to the
DTFL™ format. It is an arbitrary chick data file of 1024 in length that was not used in the
analysis and results of this thesis. The plot is in its raw format as generated from DTFL™,
and not configured for presentation as in the result's section of this thesis. Figure 2.3 is an
example of the MATLAB® program output and Figure 2.4 is an example of the
LabVIEW® program's output. These plots were generated along with the TFD output to validate DTFL™ and assist in interpreting the DTFL™ output. Note the y-axis unit differences between Axis B on Figure 2.2 and Figures 2.3 and 2.4.

![Figure 2.3: MATLAB® Example Output](image)

![Figure 2.4: LabVIEW® Example Output](image)
Figures 2.3 and 2.4 appear to show two different plots for the FFT of a signal. However, they are the same resultant FFT with different scaling on the plots. If one looks closely enough, the shapes of the plots are identical. However, the MATLAB® output has a peak magnitude of approximately 350 and the LabVIEW® output a peak magnitude of approximately 0.68. The MATLAB® output must be normalized to show that the magnitudes of the FFT's are the same. The FFT in Figure 2.3 is half of a double-sided FFT based upon the original data length of 1024 points. Therefore, we only concentrate on half of the plot. The peak magnitude is normalized by dividing 350 by 512 points, and we obtain a normalized magnitude of 0.6836. Thus, if analyzed signals are compared to one another, their respective magnitudes must be appropriately normalized prior to magnitude comparison.

Chapter 2.3.3 Single Tone Test Signal

The first set of the test cases consisted of six single tones, or single frequency sinusoids. The tones were generated through MATLAB® and stored in an m-file labeled tones.m (See Appendix A). Each signal was generated so 10 full cycles of 256 points were created, with frequencies ranging from 0.01 Hz to 1000 Hz in steps of an order of magnitude. For brevity, the results for these test cases are not presented.

Chapter 2.3.4 Dual Tone Test Case

Two tone signals comprised the second set of test signals used in this thesis. There are four different combinations used within this test case. All of the dual tone
combinations are described here, but only one of the combinations, "dualtonesC", is presented with plots, again for brevity. These dual tone signals were generated through MATLAB® and stored in the appropriate m-file (See Appendix A). The first combination, called "dualtones", consisted of a pair of sinusoids with a frequency of 0.1 Hz and a magnitude of 1, and a frequency of 2 Hz and a magnitude of 10, respectively. DualtonesA is the second sinusoid combination which contains the same frequencies as "dualtones", except that the magnitudes of the two sinusoids are reversed. The third combination, "dualtonesB", uses the same frequencies, but with equal magnitudes. The above mentioned signals were all originally generated using 4096 points. These signals were subsampled by a factor of 8 to produce 256 points. (The reason for this subsampling was due to initial hardware memory problems with the DTFL™ software running on the HP workstation, and will be explained later in Chapter 4.3: Limitations and Restrictions.) "dualtonesC" is the same as "dualtonesB" except that 512 points, instead of 4096, were generated in the MATLAB® output without subsampling.

Figure 2.5 shows dualtonesC's DTFL™ output. The first row of the set of plots used the Wigner-Ville distribution, the second row used the spectrogram, and the last row used the binomial distribution. The first column used a rectangular window and the second column a Hanning window. This convention is used for the remaining test signals as well. Notice how the magnitude of the 2 Hz component is not equal to that of the 0.1 Hz component, and seems to spread out around the main element. This is due to the fact that only 512 points were used to create the signal. The higher frequency component is
not accurately recreated due to its proximity to the Nyquist limit and spectral leakage phenomena. Nyquist states that the sampling rate must be at least twice as great as the highest frequency of the input signal to avoid aliasing. Originally, we had 4096 points for 100 seconds, which gives a sampling frequency of 40.96 Hz, and a Nyquist (or highest resolvable) frequency of 20.48 Hz. With 512 points, the sampling frequency becomes 5.12 Hz. This gives a Nyquist frequency of 2.56 Hz compared to our input signal of 2 Hz. We avoid aliasing and can recreate the signal, but we are close enough to the Nyquist frequency to show spectral leakage into adjacent frequency bins.

Focusing on Figure 2.5, the Wigner-Ville distribution creates cross-terms, at 1.05 Hz, between the two spectral components which is unacceptable for our work. The spectrogram, shows the two distinct spectral components accurately. Plot (c) is not missing the lower frequency component but is only a result of the color printer resolution. The two binomial distribution plots, (e) and (f), also accurately reproduces the two frequency components. There is some low-level noise associated with the damping of the cross-terms, but does not affect the representation of the results. Plot (e) also is not well represented by the resolution of the color printer.
Figure 2.5: DTFL™ Analysis of Dualtone C Signal Using TFD / Window
Chapter 2.3.5 Spectral Chirp Test Signal

The chirp signal is the third test case to be analyzed. The HP 33120A waveform generator was used because it has built-in capabilities to produce this chirp signal. The chirp signal is also referred to as a sweep sine test, because the signal linearly increases in frequency. The chirp function requires three input parameters. The start frequency of the signal was set to 10 mHz, and the stop frequency was set at 2 Hz. These frequencies were chosen because of their relationship to expected spectral behavior of the chick embryo variability time series. The sweep time was set at 60 seconds. A custom LabVIEW® data acquisition program (developed at the Rochester Institute of Technology) was linked to the waveform generator and collected 4096 points of data.

Figure 2.6 shows the chirp signal analyzed by the different TFD’s. The chirp signal is well represented by the Wigner-Ville distribution, but not perfectly. The frequency resolution is adequate with both rectangular and Hanning windows, but there appears to be noise in the signal as well as a possible cross-term. The spectrogram is insufficient, and lacks both frequency and time resolution. Notice the large spread of the chirp throughout the whole frequency range, for plot (c). The binomial distribution shows “feathering” of the chirp signal at the beginning and end of the time series, but is much better than the Wigner-Ville distribution since the contour plot is much smoother, with less emphasis of both noise and cross-term factors.
Figure 2.6: DTFL™ Analysis of Chirp Signal Using TFD / Window
Chapter 2.3.6  Frequency Hopper Test Signal

The last signal to be tested was a frequency hopper. A frequency hopper possesses step-changes (i.e., hops) between adjacent frequencies for a specified period of time. This signal was created by the Hewlett-Packard waveform generator, which required three input parameters. The “carrier frequency” is the starting frequency, and was set to 1 Hz. The “hop frequency” is the frequency to which the carrier changes, and was set to 0.1 Hz. The “frequency rate” was set to 1/0.01 Hz = 100 seconds. The frequency rate gives the overall time duration of the carrier-hop sequence before repeating. Hence, the carrier and hop frequencies each last for 50 seconds. For our test case signal, only one hop between the carrier frequency and the hop frequency was analyzed. The same LabVIEW® data acquisition program used for the chirp signal was also used to acquire 512 points of data for the frequency hopper.

Figure 2.7 depicts the DTFL” output for the frequency hopper. All of the plots show excellent frequency resolution. Plots (a) and (b) show the Wigner-Ville distribution results, using rectangular and Hanning windows, respectively. As evident from these plots, the Wigner-Ville distribution creates cross-terms and has poor temporal resolution, which is window dependent. The spectrogram eliminates the cross-terms, but still has poor temporal resolution. This poor temporal resolution relates to the difficulty discerning exactly where the change in frequency occurs. The binomial distribution shows excellent temporal resolution in comparison to the other two distributions. It shows the frequency
Figure 2.7: DTFL\textsuperscript{\textsuperscript{m}} Analysis of Frequency Hopper Signal Using TFD / Window
hop occurring at 50 seconds, and eliminates the cross-terms between the frequencies. The binomial distribution with the Hanning window appears to be improved over the rectangular window, in low-level noise suppression throughout the contour plot.

**Chapter 2.4 DTFL™ procedures**

This section documents the procedures used to run DTFL™ for the files processed in this thesis. There are many other variable fields and parameters in DTFL™ that are not mentioned in this description. This section reveals the inputs DTFL™ requires and the changeable parameters for computing a TFD. Descriptions of some of the parameters are included in this section.

The first step for pre-processing in DTFL™ is to import an ASCII file containing the data in one column. Subsampling can occur at this stage if necessary. This step was required for many of the data files processed in this thesis (See Chapter 4.3). A maximum subsampling of 8 was required for the analysis. A subsampling factor of 8 means that every eighth sample is taken and so on. The Nyquist rule was verified for these subsampled data files, as described in the test case dualtones section. For all of the data files, the Nyquist frequency was just under 4 Hz, but the spectral content of the data files rarely exceeded 2 Hz. Once the pre-processing is completed a vector object (*.vob) is created. The vector object is essentially a single column vector containing the magnitudes of the desired parameter.

The vector object is then manipulated, by defining the sampling rate. The sampling rate is the period of time between successive points in the data column, keeping the
subsampling in mind. Once the vector object is saved, the TFD calculations begin. The analytic signal option is checked to ignore the imaginary portion of the input signal. The distribution and window are chosen next. As mentioned in Chapter 1, the short-time Fourier Transform utilizes a window to temporally limit a signal in order to obtain a local spectral representation. Typically, a smoothing window that tapers the data is used during analysis to prevent spectral leakage, since non-stationary data is usually not periodic within the chosen window (Keselbrener, 1996). This window function is multiplied by the input signal and alters the true spectrum of that input signal. The length of the window is also an input to DTFL™, but defaults to a preset value. For a spectrogram, a short time window gives high temporal resolution, but poor frequency resolution. A long time window gives high frequency resolution, but poor temporal resolution. DTFL™ defaults to a window length that is twice the number of samples of the selected input vector object minus one. This default window length produces a TFD with equal number of samples on the horizontal axis (time) and the positive portion of the vertical axis (frequency) (DTFL™ manual, 1995).

The TFD is computed and an array object is created. Similar to a vector object, an array object is a discrete time series of two-dimensions. Once this array object is saved, everything is chosen and displayed on the axes in order to generate hardcopy output. Typically, the vector object is manipulated to produce the time signal on Axis C and the analytic frequency on Axis B. Axis A is reserved for array objects, and can be displayed in many forms. Refer to Figures 2.1 and 2.2 for, if necessary, for a visible explanation.
Chapter 3. Results

As mentioned previously, TFD analysis was used to identify functional abnormalities in retinoic acid (RA) treated chick embryos. The study group consisted of Hamburger-Hamilton Stages 24 and 34 embryos divided into control, sham, and treated classification groups (Broekhuizen, 1995). Specifically, the Stage 34 treated embryos were sub-categorized into normal, abnormal, and double-outlet right ventricle (DORV) groups. These sub-categories were based upon the physical morphometry, specifically the positioning of the great vessels, of the developing embryo. All of the treated embryos were subject to 1 µg RA dosage (topical bolus) at Stage 15, then re-incubated until Stage 24 or Stage 34. Dorsal aortic blood velocity was measured using a pulsed Doppler velocimeter (University of Iowa). This thesis only analyzes the control and treated groups.

Heart-rate variability, cardiac-cycle mean velocity variability, and peak velocity variability time series were analyzed through DTFL™ for every embryo. In the following sections, one representative embryo from each group is presented to summarize and confer the results. Each embryo presented, conveys the properties that are most typical of its parent group. For the two stages, with a total of six classification groups, eighteen plots are being presented. The plots follow one another in groups of three. Under each classification group heading, a characteristic description of the plots is given.
Chapter 3.1 Stage 24

Chapter 3.1.1 Controls

The heart-rate variability TFD plot (Figure 3.1) for the representative Stage 24 control embryo is characterized by medium intensity, short-duration chirp-like or ramping signals along with a few brief frequency bursts or "hot spots". These hot spots are greater in energy density magnitude and are of extremely short-duration, with a low frequency bandwidth, from 0.25 to 1.5 Hz. The MVV and PVV TFD's, in Figures 3.2 and 3.3 respectively, also contain these hot spots and medium intensity chirp signals, along with many medium intensity hot spots. However, their main components seem to be of long-duration (greater than 20 seconds) and very low frequency, about 0.1 Hz. The frequency content for all of the blood flow variability TFD's is concentrated at or below 1 Hz, consistent with power spectral analysis (See for instance the shape of the FFT plots shown along Axis B). The HRV FFT has a shape like that of a normal distribution centered about 1 Hz. In other words, it gradually increases in magnitude, peaks, and then decreases in magnitude. The two velocity variability FFT plots are dominated by very low frequency components between DC and 0.5 Hz.

Chapter 3.1.2 RA Treated

The Stage 24 treated embryo exhibits similar blood flow characteristics to the control embryo in terms of spectral content only. All of the analytic FFT plots (Axis B) for the treated embryo (Figures 3.4, 3.5, and 3.6) are very similar to the control embryo. The HRV plot has the normal distribution shape, and the velocity variabilities are
dominated by low frequency components. However, in terms of the TFD contour plots, subtle differences arise between control and treated. The treated heart-rate variability plot, Figure 3.4, has essentially the same spectral composition as the control (Figure 3.1) when color compensated. However, the treated TFD results exhibit "hot spots" of very short-duration with wide frequency bandwidth, ranging from 0.25 Hz to 1.75 Hz. The mean and peak velocity variability plots (Figures 3.5 and 3.6, respectively) are also very similar to the control embryo (Figures 3.2 and 3.3, respectively), but the long-duration, low frequency components evident in the control appear to be broken up into smaller time intervals of less than 20 seconds, in the treated TFD.
Figure 3.1: Stage 24 Control - Heart-rate Variability
Figure 3.2: Stage 24 Control - Mean Velocity Variability
Figure 3.3: Stage 24 Control - Peak Velocity Variability
Figure 3.4: Stage 24 Treated - Heart-rate Variability
Figure 3.5: Stage 24 Treated - Mean Velocity Variability
Figure 3.6: Stage 24 Treated - Peak Velocity Variability
Chapter 3.2  Stage 34

Chapter 3.2.1  Controls

Figures 3.7, 3.8, and 3.9 display the results for the Stage 34 control embryo. This later stage embryo still exhibits similar characteristics to the Stage 24 control chick embryo. The HRV FFT’s for the two stages are very similar in shape and spectral content (See Figures 3.7 and 3.1). Short time-duration chirp-like components and brief high frequency bursts characterize Figure 3.7. The MV and PV variability TFD’s are also similar to those at Stage 24, dominated by long-duration low frequency components. Figure 3.8 shows a frequency component of approximately 0.01 Hz that exists for more than 100 seconds. Again, all of the spectral components are concentrated at low frequencies, between 0 and 1.25 Hz.

Chapter 3.2.2  RA Treated / Normal

The treated normal embryos at Stage 34 have no apparent abnormal positioning of the great vessels. In general, the FFT plots (Axis B) have similar shapes and magnitudes between Stage 34 treated/normal and Stage 34 control for HRV, MVV, and PVV, inferring that these embryos are not diseased. Since there are no physical abnormalities present upon inspection, this result is not surprising. The TFD energy density plots between treated normals and controls, however, portray a different story. The treated /normal HRV plot (Figure 3.10) contains many short-duration, discrete broad-band frequency bursts. These frequency discharges vary from .5 to 1.5 Hz. Both the mean
Figure 3.7: Stage 34 Control - Heart-rate Variability
Figure 3.8: Stage 34 Control - Mean Velocity Variability
Figure 3.9: Stage 34 Control - Peak Velocity Variability
Figure 3.10: Stage 34 Treated / Normal - Heart-rate Variability
Figure 3.11: Stage 34 Treated / Normal - Mean Velocity Variability
Figure 3.12: Stage 34 Treated / Normal - Peak Velocity Variability
velocity and peak velocity variability FFT plots (Figures 3.11 and 3.12) have that same basic structure with a low dominant frequency. However, those dominant frequencies are of shorter duration (20 to 35 seconds) than the control embryo (Figures 3.8 and 3.9). Also, short-duration, greater broad-band frequency bursts, similar to those found in the heart-rate variability TFD plot (Figure 3.10), are apparent in Figures 3.11 and 3.12.

Chapter 3.2.3 RA Treated / Abnormal

Figures 3.13, 3.14, and 3.15 present the DTFL™ results for the treated abnormal chick embryo. Upon inspection, it is immediately evident that the TFD plots are very different from their control or RA normal counterparts. This abnormal embryo features very short-duration bursts of broad-band frequency content for HRV (Figure 3.13) and velocity variability (Figures 3.14 and 3.15), which are markedly different from the control embryo (Figures 3.7, 3.8, and 3.9, respectively). These frequency bursts range from 0 to 2.5 Hz. Analogously, they could be described as a mechanical impulse, in which all frequencies are excited. Remarkably, the analytic FFT plots are not at all dissimilar to the descriptions for the control or RA normal embryo variabilities. Here, the HRV has its familiar normal distribution shape, and the blood velocity variability profiles are still dominated by low frequency components.

Chapter 3.2.4 RA Treated / DORV

The DORV plots (Figures 3.16, 3.17, and 3.18) appear to be in a class of their own. They seem to exhibit characteristics of both the control and abnormal group
Figure 3.13: Stage 34 Treated / Abnormal - Heart-rate Variability
Figure 3.14: Stage 34 Treated / Abnormal -Mean Velocity Variability
Figure 3.15: Stage 34 Treated / Abnormal - Peak Velocity Variability
Figure 3.16: Stage 34 Treated / DORV - Heart-rate Variability
Figure 3.17: Stage 34 Treated / DORV - Mean Velocity Variability
Figure 3.18: Stage 34 Treated / DORV - Peak Velocity Variability
embryos. Chirp-like structures, and narrow-band (0 to 1 Hz) frequency hot spots are evident in all of the variability TFD plots. There is one long-duration low frequency components in the mean velocity plot (Figure 3.17), but no extremely intense broad-band frequency bursts in any of the plots like in the treated abnormal embryo (Figure 3.14). Most of the spectral content ranges from 0 to 1 Hz, which is consistent with the control embryos. It appears, however, that all of the variability TFD plots for the DORV embryo exhibit some mid-duration (5-10 seconds) broad-band frequency bursts (Figures 3.16, 3.17, and 3.18).

**Chapter 3.3 Comparative Summary**

The time-frequency representations of heart-rate variability and mean and peak velocity variabilities are noticeably different between control and abnormal embryos, which may indicate functional impairment of embryonic hemodynamics. In order to summarize the TFD analysis results of the chick embryos, Table 3.1 is presented. Table 3.1 is a comparative summary that depicts which characteristics, like chirp functions, are present in the embryonic groups.

<table>
<thead>
<tr>
<th>Stage - Group</th>
<th>Dominant Spectral Content</th>
<th>Respective Temporal Characteristics</th>
<th>Chirps?</th>
<th>Freq. Bursts</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 - Control</td>
<td>Very low</td>
<td>long-duration</td>
<td>Many</td>
<td>0 - 1.5 Hz</td>
</tr>
<tr>
<td>24 - Treated</td>
<td>Low to medium</td>
<td>medium-duration</td>
<td>Many</td>
<td>0 - 1.5 Hz</td>
</tr>
<tr>
<td>34 - Control</td>
<td>Very Low</td>
<td>long-duration</td>
<td>Many</td>
<td>0 - 1 Hz</td>
</tr>
<tr>
<td>34 - Treated / Normal</td>
<td>Low to medium</td>
<td>low to medium-duration</td>
<td>Some</td>
<td>0 - 1.4 Hz</td>
</tr>
<tr>
<td>34 - Treated / Abnormal</td>
<td>Low to high</td>
<td>very short-duration</td>
<td>Few</td>
<td>0 - 3 Hz</td>
</tr>
<tr>
<td>34 - Treated / DORV</td>
<td>Low to medium</td>
<td>Low to medium-duration</td>
<td>Few</td>
<td>0 - 1 Hz</td>
</tr>
</tbody>
</table>
With these results and the interpretation of the plots themselves, TFD analysis appears to be an effective tool for functional assessment of cardiovascular health during development.
Chapter 4. Discussion

Chapter 4.1 Various TFD Pros and Cons

The decision to use the binomial kernel was based solely upon the results of the test cases. The binomial kernel, a member of the RID group, proved to be the best suited at handling the non-stationary signals that were to be analyzed in this thesis. It provided excellent cross-term or interference reduction and accurately rendered the time and spectral signals on the contour plot.

The Wigner-Ville distribution could have provided the resolution needed for the analysis of the blood velocity data. However, due to its inherent nature of introducing cross-terms, the Wigner-Ville distribution results would have been difficult to interpret. With chirp signals and frequency bursts abounding throughout the data, cross-terms could easily have been interpreted as being part of the “true” temporal-spectral distribution.

The short-time Fourier transform, or spectrogram, was also insufficient in providing the results for this thesis. By virtue of its dependence upon a window function to determine its kernel, the spectrogram would have failed in supplying decent output. To get accurate temporal resolution, a short time window must be used. This, however, will provide poor frequency resolution. A long time window will provide excellent frequency resolution, but mediocre temporal resolution. Also, cross-terms can develop due to the bilinear nature of the spectrogram.

Unfortunately, TFD’s do not always behave as expected. Each TFD has its inherent advantages and disadvantages, which limits its usefulness in certain applications.
Therefore, the choice of a TFD should depend on the characteristics of the signal and the desired support properties of that TFD.

One of the subtle capabilities of TFD analysis is the accurate temporal-spectral discrimination of rapid transient signals. As noted in the frequency hopper test signal (Figure 2.7), the binomial distribution best depicted the sharp temporal change in sinusoidal frequencies, when compared to the Wigner-Ville distribution or the spectrogram. However, Figure 2.7(f) does indicate a short duration transition between the two distinct frequencies. This transition affects the energy density representation. The variability signals studied in this thesis undergo many rapid changes in signal frequency content. The TFD output from these signals may therefore reflect true energy density information as well as parasitic energy density data, resulting from rapid frequency transitions in these signals. The test data shown in Figure 2.7 would suggest that the binomial distribution minimizes the parasitic energy density data as compared to the spectrogram and the Wigner-Ville distribution.

**Chapter 4.2 Windowing Pros and Cons**

In terms of selecting a window, the hanning window was the easy choice over the rectangular. The hanning window provided more valuable frequency resolution in all of the test cases than the rectangular window. Even with the choice of windows being simplified, problems still arose during the analysis of the data files through DTFL™. The main parameter that must be decided in using a window for a TFD calculation is the
window size. This window size, essentially its length in samples, proved to be a nuisance during the data file processing.

The window size directly affected the frequency resolution seen in the DTFL output plots. As stated in the DTFL manual:

"The window length always determines the number of samples on the vertical axis of any resulting distribution...If you want to compute a distribution that has the same number of samples in the horizontal axis as on the positive part of the vertical axis, you must enter a window length that is twice the number of samples of the selected input vector minus one. This is the default case."

This proved not to be the case during the analysis of the data files. The first time DTFL is booted up and a file is processed, the default case holds true. Yet, when a successive file was processed, the window length always defaulted to 201 samples. Numerous data files were at this lesser resolution due to the shorter window length not being updated. Essentially, the contour plot with the shorter window length was a coarse or grainy representation of the contour plot with the default window length.

Another problem dealing with the window length was that the actual file size of the array objects was greatly affected. A file size of 880 Kb was generated from processing with the 201 sample window length. The default case always had a window length of 1023, which will be explained in the next section. This five to one ratio of window length, also became the ratio for the array object file size. To obtain the more accurate resolution, file sizes greater than 4.4 Mb were generated. Storing and processing many files of this size can be extremely difficult due to the inherent limitation of the software and hardware.
It was decided that the shorter window length files were acceptable, and all files were left in their present condition.

Chapter 4.3 Limitations and Restrictions

As mentioned previously, large file sizes are very difficult to handle and process. This problem, along with others, greatly affected this thesis. On the topic of file sizes, DTFL™ can produce 3-dimensional plots of Axis A. These plots can provide more thought and interpretation than the normal contour plots. However, high resolution must be used, which produced output files of 25 Mb in size. This was deemed too excessive to try to print and manipulate.

All files that were analyzed were kept at or subsampled to 512 points in length, as previously mentioned. This occurred due to the fact that DTFL™, running on the HP workstation, continually attained “out of memory” errors. These errors were associated with the length of the input signal as well as the window length. 512 samples with a window length of 201 points proved adequate enough for the analysis and interpretation of the data. Higher resolution output could have been obtained if these did not appear.

Another restriction that transpired due to the “out of memory” errors, was not being able to use a different type of distribution. The exponential distribution that was mentioned in Chapter 1, could also have provided valuable information, due to its alterable decay parameter. This decay parameter directly affects the cross-terms present within a signal. No combination of input signal size or window length would allow the exponential distribution to be calculated.
Chapter 4.4 Discussion of Data Trends

As presented in Chapter 3, the results show that time-frequency analysis identifies significant differences among the control and treated embryos. With similar spectral content among the different embryos, the contour plots reveal characteristics unique to the classification groups. The following discussion will present information and ideas on how an unclassified embryo (data) could be identified.

Regarding Stage 24 embryos, comparisons between heart-rate variability, mean velocity variability, and peak velocity variability are very subtle. The two velocity variabilities exhibit similar attributes. Only the heart-rate variability can comfortably distinguish between the control and treated groups. The treated embryo (Figure 3.4) displays dominant frequency bursts that are repetitive and much more impulsive in nature than the control embryo (Figure 3.1), even with comparable frequency ranges. This occurrence was consistent among all other Stage 24 control and treated embryos analyzed. The mean and peak velocity variabilities among the other analyzed data files were, again, too alike to discriminate between them.

The identification of Stage 34 embryos is somewhat more difficult to manage than Stage 24 embryos. This is due to subtle differences among some of the four different classification groups and the three variabilities. Table 4.1 shows how heart-rate variability, mean velocity variability, and peak velocity variability could each be used to identify a Stage 34, unclassified embryo. The blacked-out boxes indicate ambiguity among the variability contour plots for the embryos presented in this thesis.
Table 4.1: Stage 34 Embryo Identification Table

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated/Normal</th>
<th>Treated/Abnormal</th>
<th>Treated/DORV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV</td>
<td></td>
<td>Identifiable</td>
<td>Identifiable</td>
<td></td>
</tr>
<tr>
<td>MVV</td>
<td>Identifiable</td>
<td></td>
<td>Identifiable</td>
<td></td>
</tr>
<tr>
<td>PVV</td>
<td></td>
<td></td>
<td>Identifiable</td>
<td></td>
</tr>
</tbody>
</table>

Focusing on HRV, the treated / abnormal embryo (Figure 3.13) is easily identified due to the broad-band frequency impulses. No other embryo group contain these highly dominant frequency components. The treated / normal embryo (Figure 3.10) can also be identified from the others. Even though it contains the impulse frequency bursts similar to the treated / abnormal embryo, it also contains significant chirps, ramp signals, and other types of frequency bursts. The MVV can easily identify the treated / abnormal embryo, again, as well as the control embryo. The treated / abnormal embryo (Figure 3.14) is characterized by the same broad-band frequency impulses as by the HRV. The control embryo (Figure 3.8) contains a low-frequency, long duration component that is absent from the other embryo contour plots. The MVV contour plots are similar, thus not allowing a positive identification of either a treated / normal or a DORV embryo. However, remember that the treated / normal embryo can be identified by the HRV contour plot. If the HRV contour plot of an unidentified embryo does not fit the criteria for a treated / normal embryo, and if the MVV contour plot does not reveal characteristics of a control embryo, then the unidentified embryo is most likely a DORV.

Using these criteria, an unidentified embryo can be distinguished as to its classification group. A simple flowchart is presented in Figure 4.1, to aid in this task.
Incorporating this type of criteria into an analysis tool will allow medical clinicians to recognize embryonic cardiovascular disease and provide treatment.
Figure 4.1: Embryo Identification Flowchart
Chapter 5. Conclusion

Time-frequency analysis has proved to be an effective method for analyzing cardiovascular health in Stage 24 and 34 chick blood flow velocity data. Time-frequency analysis has the capacity to analyze highly non-stationary signals and discern relative temporal locations between signals with similar spectral content. Specifically, the reduced interference distribution (i.e. the Binomial distribution), in combination with a hanning window, accurately portrayed frequency impulses, chirps, frequency hops and frequency bursts while preserving inherent auto-terms and maintaining an effective reduction of cross-terms.

Discrete Time Frequency Laboratory™ is a robust time-frequency tool for the signals analyzed in this thesis. Though still in a beta-test format, DTFL™ possess a simple graphical user interface and is easily mastered. Several user interface items still are lacking, however. For instance, allowing a filename to be specified, by the user, for saving hardcopy output, would be helpful from a data management perspective.

Qualitatively, the differences among signals with similar spectral content were easily identified. Control and treated embryos, in both Stage 24 and 34, showed significant differences between their TFD contour plots for heart-rate variability, mean velocity variability, and peak velocity variability. Any embryo could easily be categorized if their group origin was unknown. Multiple iterations of time-frequency analysis during
embryonic development could be performed to obtain a "heart blood flow variability history" which could lead to identification of cardiovascular disease.

As a next step, a more in-depth interpretation of this data is required. Concentration on the quantitative aspects could reveal more insight into the temporal / spectral differences between control and treated groups. Also, minor changes to DTFL should be performed to allow faster processing times and smaller file sizes. Human data exists from the main research project sponsored by the National Institute of Health, and should be analyzed in DTFL. This would be a first step in identifying cardiovascular disease in humans.

Time-frequency analysis represents a significant step in providing a practical, ease-of-use analysis tool to assist medical clinicians. In terms of practical application, human fetal blood velocity data can readily and unobtrusively be gathered at regular intervals through Doppler ultrasound. "Watching" the developing embryo through time-frequency analysis, could become an effective means of pre-natal cardiovascular care.
References


Appendix A

tones.m

wl=0.01;
w2=0.1;
w3=1.0;
w4=10.;
w5=100.;
w6=1000.;
t1=linspace(0,10/wl,256);
t2=linspace(0,10/w2,256);
t3=linspace(0,10/w3,256);
t4=linspace(0,10/w4,256);
t5=linspace(0,10/w5,256);
t6=linspace(0,10/w6,256);
tone1=sin(2*pi*wl*t1);
tone2=10*sin(2*pi*w2*t2);
tone3=sin(2*pi*w3*t3);
tone4=sin(2*pi*w4*t4);
tone5=sin(2*pi*w5*t5);
tone6=sin(2*pi*w6*t6);
tone1=tone1';
tone2=tone2';
tone3=tone3';
tone4=tone4';
tone5=tone5';
tone6=tone6';
expvect('tone1.txt',tone1);
expvect('tone2.txt',tone2);
expvect('tone3.txt',tone3);
expvect('tone4.txt',tone4);
expvect('tone5.txt',tone5);
expvect('tone6.txt',tone6);

---
dualtones.m

wl=.1;
w2=2.0;
t1=linspace(0,10/wl,4096);
t2=linspace(0,10/wl,4096);
tone1=sin(2*pi*wl*t1);
tone2=10*sin(2*pi*w2*t2);
tone1=tone1';
tone2=tone2';
dualtone=tone1+tone2;
expvect('dualtone.txt',dualtone)
plot(dualtone)
Title('Dual Tones')
**dualtonesA.m**

```matlab
wl=1.1;
w2=2.0;
t1=linspace(0,(10/wl),4096);
t2=linspace(0,(10/wl),4096);
tone1=10*sin(2*pi*w1*t1);
tone2=sin(2*pi*w2*t2);
tone1=tone1';
tone2=tone2';
dualtone=tone1+tone2;
expvect('dualtoneA.txt', dualtone)
plot(dualtone)
Title('Dual Tones')
```

**dualtonesB.m**

```matlab
wl=1.1;
w2=2.0;
t1=linspace(0,(10/wl),4096);
t2=linspace(0,(10/wl),4096);
tone1=sin(2*pi*w1*t1);
tone2=sin(2*pi*w2*t2);
tone1=tone1';
tone2=tone2';
dualtone=tone1+tone2;
expvect('dualtoneB.txt', dualtone)
plot(dualtone)
Title('Dual Tones')
```

**dualtonesC.m**

```matlab
wl=1.1;
w2=2.0;
t1=linspace(0,(10/wl),512);
t2=linspace(0,(10/wl),512);
tone1=sin(2*pi*w1*t1);
tone2=sin(2*pi*w2*t2);
tone1=tone1';
tone2=tone2';
dualtone=tone1+tone2;
expvect('dualtoneC.txt', dualtone)
plot(dualtone)
Title('Dual Tones')
```
svkfft.m

clear all
fname, pname = uigetfile('*', 'Please select the data file to be analyzed.');
fname
path(pname,path);
load(fname);
len = length(fname);
fname(len-3:len) = [];
file = eval(fname);
time = file(:,1);
umpts = length(time);
tfinal = time(numpts);
delta_t = tfinal/numpts;
highestfreq = 1/delta_t;
peakvelocity = file(:,2);
fftpc = fft(peakvelocity);
magfftpc = abs(fftpc);
phsfftpc = unwrap(angle(fftpc));
freq = (0:length(fftpc)-1)' * highestfreq/length(fftpc);
figure; plot(freq(1:128), magfftpc(1:128),'c'); grid;
xlabel('Frequency (Hz)'); ylable('Magnitude');title('Peak Velocity FFT');
figure; plot(freq, phsfftpc); grid;
xlabel('Frequency (Hz)'); ylable('Phase');title('Peak Velocity FFT');